SYNTHESE UND BIOLOGISCHE EVALUIERUNG VON INHIBITOREN DER CARBOANHYDRASE II

DISSERTATION

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Vorwort

Diese vorliegende wissenschaftliche Arbeit wurde im Zeitraum von Mai 2021 bis Juni 2025 am Institut für Chemie im Bereich für Organische Chemie der Martin-Luther-Universität Halle-Wittenberg in der Arbeitsgruppe von Prof. Dr. René Csuk angefertigt.

Die Dissertation wurde in kumulativer Form verfasst und die Forschungsergebnisse wurden bereits in internationalen Fachzeitschriften publiziert.

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Für meinen Opa

Inhaltsverzeichnis

Abkürzungsverzeichnis

[*I*] ..... Inhibitorkonzentration
[*S*] ..... Substratkonzentration
4-NA 4-Nitrophenylacetat
4-NP 4-Nitrophenol
AAZ ..... Acetazolamid
AcBS ..... acetylierte Betulinsäure
BA ..... Betulinsäure
BN ..... Betulin
CA ..... Carboanhydrasen
CAI ..... Carboanhydraseinhibitor
ESK ..... Enzym-Substrat-Komplex
GS ..... Glycyrrhetinsäure
hCA ..... humane Carboanhydrase
K_i ..... Inhibierungskonstante
K_m ..... Michaelis-Menten-Konstante
Me ..... Methylrest
n ..... Kettenlänge
OMe ..... Methoxyrest
R ..... Rest
SAR ..... Structure Activity Relationship
SI ..... Selektivitätsindex
^{t}Bu ..... tert-Butyl
v ..... Reaktionsgeschwindigkeit
V_{max} ..... maximale Reaktionsgeschwindigkeit
ZBG ..... Zinkbindende Gruppe

Einleitung

Die Carboanhydrase (CA) ist ein essenzielles Enzym, welches u.a. an der Regulation des Säure-Basen-Haushalts[1], des CO_2-Bicarbonat-Gleichgewichtes[2] und der Ionenhomöostase beteiligt ist. Aufgrund dieser fundamentalen Funktionen spielen die CAs nicht nur in physiologischen Prozessen eine wichtige Rolle, sondern sind auch an pathologischen Zuständen wie Glaukomen[3], Tumorprogressionen[3b] und epileptischen Erkrankungen[2]. In den letzten Jahrzehnten haben CA-Inhibitoren deshalb erhebliche wissenschaftliche und therapeutische Aufmerksamkeit erlangt, insbesondere die Entwicklung selektiver Inhibitoren, die unterschiedliche CA-Isoformen gezielt inhibieren können.

Der Fokus der vorliegenden Arbeit ist die Synthese und Optimierung von CA-Inhibitoren unter Verwendung natürlicher Leitstrukturen. Naturstoffe wurden bereits seit Jahrhunderten in der Medizin eingesetzt[4], lange vor der modernen synthetischen Chemie[5]. Ihre langjährige Anwendung in der traditionellen Heilkunde spiegelt den unschätzbaren Wert dieser Verbindungen als Ausgangsbasis für die Entwicklung neuer Therapeutika wider. Insbesondere pflanzliche Naturstoffe wie pentazyklische Triterpene und Diterpene haben sich aufgrund ihrer strukturellen Vielfalt und ihrer pharmakologischen Aktivitäten als vielversprechende Stoffgruppe erwiesen[5-6]. Die bereits vorhandenen Forschungsergebnisse bestätigen, dass die Kombination von jahrhundertelanger Erfahrung mit Naturstoffen und moderner Synthesechemie ein vielversprechender Ansatz zur Identifikation und Optimierung von therapeutisch wirksamen Enzyminhibitoren darstellt[7].

Carboanhydrasen – Biologische Relevanz und Funktion

Carboanhydrasen gehören zu einer Klasse weit verbreiteter Metalloenzyme, welche in Bakterien, Pflanzen und Tieren vorkommen[8]. Im aktiven Zentrum des Enzyms befindet sich ein am Katalysezyklus beteiligtes Metallion. Diese Enzymfamilie spielt dabei eine essenzielle Rolle in der Regulierung des Stoffwechsels durch die reversible Hydratation von CO_2 zu Hydrogencarbonat und einem Proton (Gleichung 1).

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

Gleichung 1: Katalysierte Reaktion der CA

Somit wirken sie auch auf weitere physiologische Prozesse wie die Säure-Base-Regulierung[9], den Gasaustausch[10], den Transport von Metaboliten[11] und die Photosynthese[12] bei Pflanzen ein.

Die Carboanhydrasen besitzen acht unterschiedliche genetische Familien; die α-, β-, γ-, δ-, ζ-, η-. θ-, ι-Carboanhydrasen[1], welche sich in ihrer Aminosäuresequenz, ihrer Tertiärstruktur und z.T. ihrem Zentralion im aktiven Zentrum unterscheiden[8]. Zu der wichtigsten Enzymfamilie gehören die α-Carboanhydrasen. Sie sind für wesentliche Stoffwechselprozesse in Wirbeltieren verantwortlich und stellen somit die wichtigste Gruppe dieser Enzyme dar[1]. Im Gegensatz dazu kommen β-, γ-, δ-, ζ-Carboanhydrasen vor allem in Bakterien, Algen, Pflanzen, Pilzen und einigen Archaeen vor[8]. Alle humanen Carboanhydrasen (hCA) gehören zu den α-Carboanhydrasen und können zusätzlich noch in 15 Isoformen[1] (CA I bis CA XIV) unterteilt werden, von denen 12 katalytisch aktiv sind.

Tabelle 1 Übersicht über die 12 katalytisch aktiven Spezies der humanen α-CA Isoenzyme[8, 13]

Enzym	Katalytische Aktivität	Subzelluläre Lokalisation	Gewebeverteilung	Korrelierte Krankheiten
CA I	niedrig	Zytosol	Erythrozyten, Auge, Gastrointestinaltrakt	retinale und cerebrale Ödeme
CA II	hoch	Zytosol	Erythrozyten, Gastrointestinaltrakt, Niere, Lunge, Gehirn, Auge, Osteoklasten, Hoden	Glaukome, Ödeme, Epilepsie, Höhenkrankheit
CA III	Sehr niedrig	Zytosol	Adipozyten, Skelettmuskulatur	oxidativer Stress
CA IV	mittel	Membran gebunden	Niere, Lunge, Pankreas, Gehirn, Darm, Herzmuskel, Auge	Glaukome, Schlaganfälle, retinitis pigmentosa
CA VA	niedrig	Mitochondrien	Leber	Fettleibigkeit
CA VB	hoch	Mitochondrien	Herz – und Skelettmuskel, Pankreas, Niere, Rückenmark, Gastrointestinaltrakt	Fettleibigkeit
CA VI	niedrig	Milch – und Speichelsekret	Speichel – und Milchdrüsen	Kariogenese
CA VII	hoch	Zytosol	Zentrales Nervensystem	Epilepsie, oxidativer Stress
CA IX	hoch	Membran gebunden	Tumore, Gastrointestinalschleimhäute	Krebs
CA XII	niedrig	Membran gebunden	renale, intestinale und reproduktives Epithel, Auge, Tumore	Krebs, Glaukome
CA XIII	niedrig	Zytosol	Niere, Gehirn, Lunge, Darm, Reproduktionsorgane	Unfruchtbarkeit
CA XIV	niedrig	Membran gebunden	Gehirn, Leber, Auge, Skelettmuskel	Epilepsie, Retinopathie

Die verschiedenen Isoformen können weiterhin in 4 Untergruppen unterteilt werden, je nach Vorkommen in subzellulären Raum[14]. Somit sind diese Enzyme in einer Vielzahl von Geweben und Organen vertreten und spielen dabei eine wichtige Funktion in verschiedenen physiologischen Prozessen. Durch eine Störung in ihrer eigentlichen Funktion durch Überexpression oder sonstiger abnormaler Aktivitäten treten somit auch diverse Krankheitsbilder auf[15].

Molekularer Aufbau der Carboanhydrase – Strukturmerkmale und aktive Zentren

Die humanen α-Carboanhydrasen besitzen eine ca. 15 Å tiefe, konisch geformte Enzymtasche[8]. Dabei besteht ein Teil der Innenseite dieser Tasche aus einem Cluster von hydrophoben Aminosäuren und der andere aus einem Cluster hydrophiler Aminosäuren[16]. Es entsteht somit eine amphiphile Umgebung, welche für die Katalyse unabdingbar ist. Die hydrophobe Region dient dabei für den Transport des CO_2 -Substrats zum aktiven Zentrum und sorgt für eine Vororientrierung des Kohlenstoffs für die katalytische Reaktion. Die hydrophile Region spielt wiederum eine wichtige Rolle bei der Abgabe der Reaktionsprodukte HCO_3^- und H^+ an das Lösemittel[8]. Im aktiven Zentrum befindet sich ein Zink(II)- Ion, welches von drei Histidinresten (His94, 96 und 119) und einem Wassermolekül bzw. Hydroxidion tetraedrisch koordiniert ist[8]. Dieses Wassermolekül bzw. Hydroxidion ist dabei über eine Wasserstoffbrückenbindung mit der Hydroxid-Funktion des Threonins 199 zusätzlich gebunden (Vgl. Abbildung 4). Neben dem an Zink koordinierten Wasser gibt es zusätzlich noch zwei weiter Wassermoleküle: zum einen in der hydrophoben Region der Enzymtasche, dem sogenannten *DEEP WATER*, und zum anderen in der hydrophilen Region in der Nähe des aktiven Zentrums[8]. Dieses Netzwerk aus Wasserstoffbrücken sorgt für eine Erhöhung der Nucleophilie des Zn^{2+}-Zentrums und ermöglicht somit die dort stattfindende katalytische Reaktion[8].

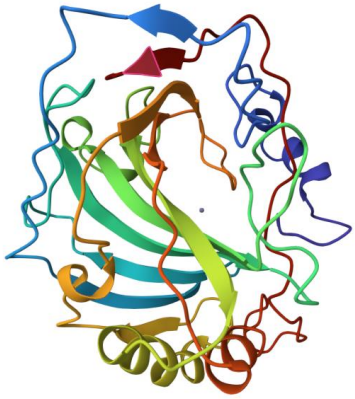


Abbildung 1: Struktur der hCA II (aus PBD, 1CA2)[17]

Katalytischer Mechanismus der Carboanhydrasen[13]

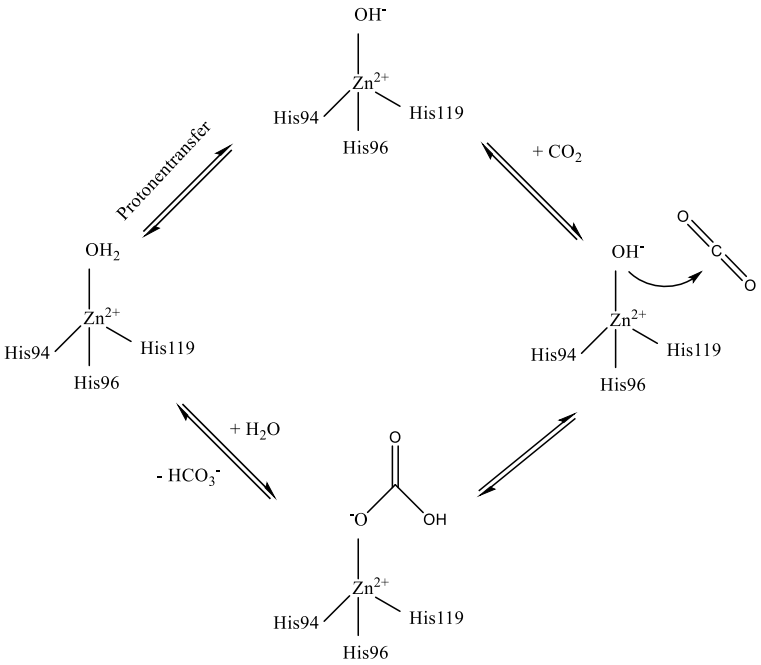


Abbildung 2: Vereinfachter Katalysemechanismus der CA[13]

Der katalytische Zyklus beginnt durch einen nucleophilen Angriff des an Zink gebundenen Hydroxidions an Kohlenstoffdioxid. Das dadurch entstandene Intermediat ist nicht sehr stabil, wodurch durch die Anlagerung von Wasser das gebundene Hydrogencarbonat freigesetzt wird. Im letzten Schritt wird die katalytisch inaktive Form des Enzyms durch einen Protonentransfer in die aktive Form zurückgeführt. Im ersten Schritt der Reaktion, der Bindung von CO_2, wird dieses Substrat in der hydrophoben Tasche bestehend aus Val121, Val143, Leu198

und Trp209 gebunden und für einen nucleophilen Angriff vororientiert. Ein Sauerstoffatom des CO_2 nimmt dabei den Platz des zuvor erwähnten *DEEP WATERS* ein. Bei dem zweiten Schritt der Reaktion, der Deprotonierung des gebundenen Wassers, handelt es sich um den Geschwindigkeit bestimmenden Schritt der Reaktion[8]. Ein Proton wird dabei meistens von Histidin (His64) aufgenommen. Dieser Prozess wird zudem noch über ein Wasserstoffbrückennetzwerk in der hydrophilen Region der Enzymtasche unterstützt. Die wichtige Rolle des Histidins für die Effizienz des katalytischen Mechanismus spiegelt sich im Vergleich verschiedener Isoformen wider. Die CA III besitzt beispielsweise an Stelle des Histidins einen Lysinrest und zeigt die geringste katalytische Effizienz der Isoformen. Im Vergleich dazu stellt die CA II eines der schnellsten bekannten Enzyme mit einer Reaktionsgeschwindigkeit von bis zu 1,40 x 10^6 s^{-1} [18]dar.

Inhibitoren der Carboanhydrase – Grundlagen und Wirkprinzipien

Die Aktivität von Enzymen kann durch verschiedenste Substanzen beeinflusst werden. So kann es sein, dass das Enzym reversibel oder irreversibel inhibiert wird. Ein irreversibler Inhibitor bindet entweder kovalent oder nicht kovalent an das Protein und dissoziiert nur langsam oder gar nicht wieder von diesem ab. Dies führt zu einer starken Inhibierung, da der Inhibitor-Enzym-Komplex über lange Zeit stabil bleibt. Im Gegensatz dazu kann bei einer reversiblen Inhibierung der Inhibitor von dem Enzym mehr oder minder schnell dissoziieren und dessen biologische Funktion kann so wieder hergestellt werden. Bei der reversiblen Inhibierung unterscheidet man in vier verschiedene Fälle[19]: a.) kompetitive Inhibierung, b.) nicht-kompetitive Inhibierung, c.) unkompetitive Inhibierung, d.) gemischte Inhibierung.

Bei der kompetitiven Inhibierung ähnelt der Inhibitor dem Substrat und konkurriert somit um die Bindungsstelle im aktiven Zentrum des Enzyms (Abbildung 3 B). Durch einen Überschuss des eigentlichen Substrats kann der Inhibitor wieder von der Bindungsstelle verdrängt werden, wodurch die Reaktion reversibel wird. Bei der nicht-kompetitiven Inhibierung verringert sich die Enzymaktivität durch die Bindung des Inhibitors an einer anderen Stelle als dem aktiven Zentrum. Das Substrat kann zwar weiterhin an das Enzym binden, jedoch wird durch die induzierte Konformationsänderung das Reaktionsprodukt nicht mehr freigesetzt. Der Inhibitor weist bei dieser Inhibierungsform die gleiche Affinität sowohl zum Enzym als auch zum Enzym-Substrat-Komplex (ESK) auf (Abbildung 3 C). Bei der unkompetitiven Inhibierung bindet der Inhibitor nur am Enzym-Substrat-Komplex und nicht am freien Enzym. In diesem eher seltenen Fall bindet ein Inhibitor außerhalb des aktiven

Zentrums an den Enzym-Substrat-Komplex. Der Inhibitor stabilisiert somit diesen Komplex und verhindert eine Umwandlung von dem Substrat zum Produkt (Abbildung 3 D). Ein Spezialfall ist die gemischte Inhibierung. Hier kann der Inhibitor unabhängig von der Bindung des Substrats binden. Es handelt sich somit um eine Kombination aus kompetitiver und unkompetitiver Inhibierung

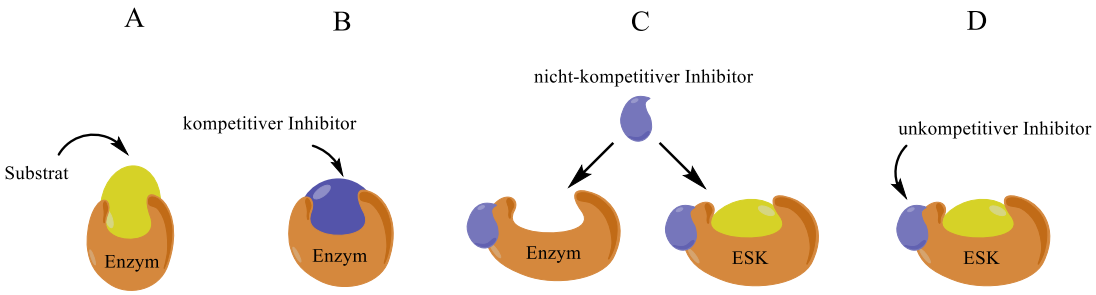


Abbildung 3: Schematische Darstellung der Funktion reversibler Inhibitoren (A = ohne Inhibitor, B = kompetetiver Inhibitor, C = nicht-kompetetiver Inhibitor, D = unkompetetiver Inhibitor)

Generell können Carboanhydraseinhibitoren in fünf verschiedene Klassen unterteilt werden[20]:

I Verbindungen, welche direkt an das aktive Zentrum binden, z.B. Sulfonamide[8], Sulfamate[8], Dithiocarbamate[21], Hydroxamate[22])

II Verbindungen, welche an das Zink koordinierte Wassermolekül binden, z.B. Phenole[23], Polyamine[24]

III Verbindungen, welche die Öffnung zur aktiven Seite des Enzyms blockieren, z.B. Coumarine und Thiocoumarine[25]

IV Verbindungen, welche außerhalb des aktiven Zentrums binden, z.B. 2-Benzylsulfonyl)benzoesäure[26]

V Verbindungen, welche auf bisher unbekannte Weise binden, z.B. sekundäre und tertiäre Sulfonamide[27]

Der Fokus dieser Arbeit soll dabei auf die Klasse I der Carboanhydraseinhibitoren liegen. Da das Zn(II)-Ion im aktiven Zentrum von drei neutralen Histidinresten und einem Wassermolekül bzw. Hydroxidion koordiniert ist, ergibt sich formal eine positive Ladung am Zentralatom, was die Triebkraft für die Anlagerung von Anion darstellt (Abbildung 4). Die Bindung primärer Sulfonamide ($R\text{-}SO_2NH_2$) erfolgt dabei durch Koordination des deprotonierten Stickstoffs am katalytisch wirksamen

Zn^{2+} und dem damit einhergehenden Austausch des gebundenen Wassers[8]. Zusätzlich fungiert das verbleibende H-Atom am koordinierten Stickstoff als Wasserstoffbrückendonor für das in der Nähe befindliche Thr199, was die Effizienz der Sulfonamide als CA-Inhibitoren verstärkt[8] (Abbildung 4). Neben der direkten Koordination an das Zentralion spielen noch weitere Wechselwirkungen mit den hydrophilen und hydrophoben Regionen des aktiven Zentrums eine wichtige Rolle bei der Inhibierung. Es kommt somit zu stabilisierenden oder destabilisierenden Interaktionen durch den Rest am Sulfonamid. Somit stehen bei der Entwicklung neuer Wirkstoffe eine Vielzahl an Möglichkeiten zur Verfügung, um möglichst gute Interaktionen zwischen Enzym und Inhibitor zu schaffen. Ein Ansatzpunkt hierzu ist das Gerüst des Inhibitors mit den Aminosäureseitenketten der Enzymtasche wechselwirken zu lassen, um so den Enzym-Inhibitor-Komplex stärker zu stabilisieren.

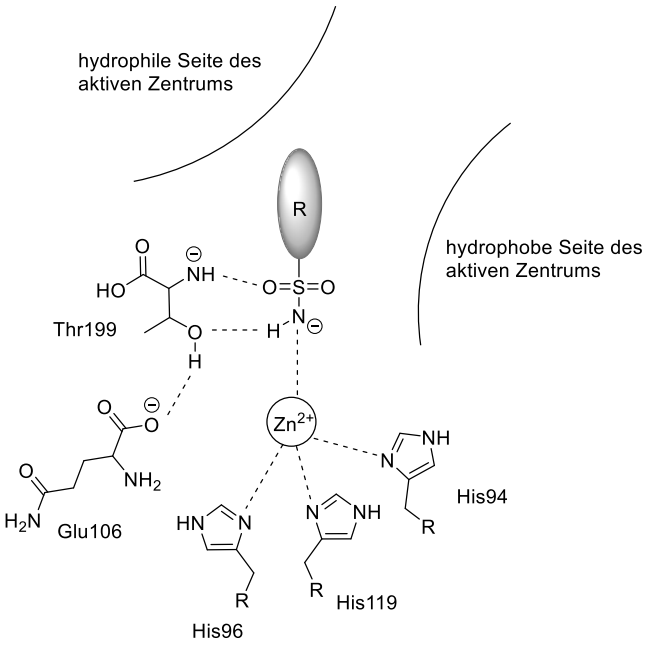


Abbildung 4: Schematische Darstellung der Wechselwirkungen eines Sulfonamids am aktiven Zentrum der hCA-II[8]

Sulfonamide stellen somit die wichtigste Gruppe an Inhibitoren dar, mit einer Vielzahl an Verbindungen, welche sich bereits im klinischen Einsatz befinden. Prominente Vertreter darunter sind u.a. Acetazolamid[3b], Benzolamid[28] und Ethoxolamid[29], welche oftmals als Leitstruktur für die Entwicklung neuartiger Inhibitoren wirken (Abbildung 5). Diese erste Generation an Inhibitoren wirken als potente Inhibitoren, jedoch weisen diese Verbindungen nur eine geringe

Isoformenselektivität auf[30]. Dadurch können neben der angestrebten auch mehrere verschiedene CA beeinflusst werden, was wiederum zu unerwünschten Nebenwirkungen führen kann[31]. Es kam somit die Notwendigkeit hervor neue und optimierte Inhibitoren mit möglichst hoher Aktivität und Selektivität zu erschaffen. Neben den strukturspezifischen Wechselwirkungen spielt auch die Permeabilität über die Zellmembran eine wichtige Rolle, um die Isoformenselektivität zu steuern[28]. So können beispielsweise Inhibitoren mit großen, membranundurchdringlichen Resten nur membranständige Carboanhydrasen wie die CA IV inhibieren[32].

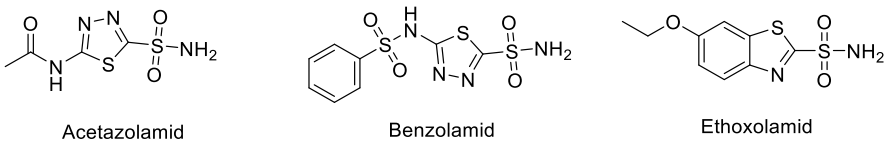
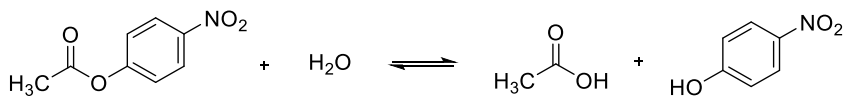


Abbildung 5: Klassische Vertreter der CAI

Bestimmung der Inhibierungskonstante K_i mittels enzymkinetischer Methoden

Ein zentraler Parameter zur Bewertung der Wirksamkeit synthetisierter Inhibitoren ist, neben der prozentualen Hemmung der Enzymaktivität, die Inhibierungskonstante K_i. Sie beschreibt die Affinität eines Inhibitors zu seinem Zielenzym und erlaubt somit quantitative Rückschlüsse auf die Stärke der Inhibierung: Je kleiner der K_i -Wert, desto höher die Affinität und damit die Wirksamkeit des Inhibitors.

Carboanhydrasen verfügen neben der bisher beschriebenen biologischen Aktivität zusätzlich eine Esteraseaktivität[8]. Diese ermöglicht die enzymatische Spaltung des farblosen Substrats 4-Nitrophenylacetat (4-NA) zu 4-Nitrophenol (4-NP) (Abbildung 6), dass eine charakteristische Absorptionsbande bei $\lambda = 405$ nm zeigt. Diese Reaktion dient als Grundlage für einen kolorimetrischen Esterase-Assay, der die Enzymaktivität über zeitabhängige Absorptionsmessungen quantifizierbar macht.



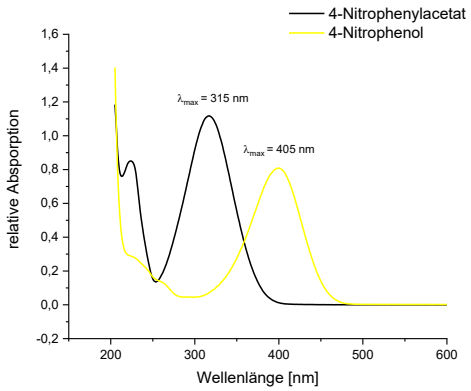


Abbildung 6: Hydrolyse von 4-NA zu 4-NP mit entsprechenden Absorptionsspektrum

Durch die Zugabe von bekannten Inhibitoren, wie etwa Acetazolamid, lässt sich die Enzymaktivität gezielt untersuchen. Die Auswertung solcher Inhibitionsversuche erlaubt nicht nur Aussagen über die Inhibierung der Esterasefunktion, sondern in begrenztem Maße auch Rückschlüsse auf die Inhibierung der physiologischen Funktionen der Carboanhydrase.

Zur Bestimmung von K_i eignen sich klassische graphische Verfahren auf Basis der Michaelis-Menten-Kinetik. Eine verbreitete Methode ist der Dixon-Plot, bei dem die reziproke Reaktionsgeschwindigkeit $1/v$ gegen die Inhibitorkonzentration $[I]$ aufgetragen wird, wobei die Substratkonzentration $[S]$ konstant bleibt[33]. Je nach Inhibitionstyp ergeben sich charakteristische Kurvenschnitte:

- **kompetitive Inhibierung:** Die Geraden schneiden sich nicht auf der x-Achse bei $-K_i$. Da Substrat und Inhibitor um das aktive Zentrum konkurrieren, lässt sich der Inhibitor bei steigender Substratkonzentration zunehmend verdrängen – die Inhibierung wird also schwächer.
- **nichtkompetitive Inhibierung:** Der Schnittpunkt der Geraden liegt auf der x-Achse, da der Inhibitor an eine vom aktiven Zentrum unabhängige Stelle (allosterisches Zentrum) bindet und somit sowohl das freie Enzym als auch den Enzym-Substrat-Komplex inhibiert. Der Effekt bleibt unabhängig von der Substratkonzentration bestehen, stattdessen wird vor allem V_{max} reduziert. Der Anstieg der Geraden ist somit nahezu gleich, der Ordinatenabschnitt jedoch unterschiedlich.

- **unkompetitive Inhibierung:** Die Geraden verlaufen parallel zueinander und schneiden sich daher nicht. Sowohl K_m als auch V_{max} werden im gleichen Verhältnis reduziert, weshalb die Steigung der Linien konstant bleibt.

- **gemischte Inhibierung:** Da bei dieser Art der Inhibierung der Inhibitor sowohl am freien Enzym (K_i), als auch am Enzym-Substrat-Komplex (K_i') binden kann, ist dessen Wirkung eine Kombination aus kompetitiver und unkompetitiver Inhibierung. Je nach Aussehen des Graphen bzw. durch den Vergleich von K_i und K_i' können Rückschlüsse daraus gezogen werden, welcher Typ der Inhibierung dominiert. Für den Fall, dass $K_i < K_i'$ bindet der Inhibitor stärker an das freie Enzym und der Schnittpunkt der Geraden liegt oberhalb der x-Achse (stärkere kompetitive Inhibierung) und umgekehrt (stärkere unkompetitive Inhibierung). Für den Fall, dass $K_i = K_i'$ scheiden sich die Geraden in allen Graphen auf der x-Achse (reine nichtkompetitive Inhibierung).

Um dieses Verhalten gezielt zu untersuchen, braucht es neben dem Dixon-Plot noch den Cornish-Bowden-Plot, um K_i' bestimmen zu können.

Ausgangspunkt ist die klassische Michaelis-Menten-Gleichung[34]:

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

Diese beschreibt die Geschwindigkeit einer enzymatischen Reaktion als Funktion der Substratkonzentration [S], wobei V_{max} die maximale Geschwindigkeit und K_m die Michaelis-Konstante ist.

Bei kompetitiver Inhibition konkurriert der Inhibitor mit dem Substrat um das aktive Zentrum des Enzyms. Dies führt zu einer Erhöhung von K_m, während V_{max} unbeeinflusst bleibt. Die modifizierte Michaelis-Menten-Gleichung lautet daher[34]:

$$v = \frac{V_{max}[S]}{K_m\left(1 + \frac{[I]}{K_i}\right) + [S]}$$

Um die Auswirkung des Inhibitors sichtbar zu machen, wird diese Gleichung für eine konstante Substratkonzentration [S] umgestellt, und das Reziprok genommen:

$$\frac{1}{v} = \frac{K_m}{V_{max}[S]K_i}[I] + \frac{K_m + [S]}{V_{max}[S]}$$

Diese Gleichung entspricht der Geradengleichung y = mx + n und bildet die Grundlage des Dixon-Plots. Der y-Achsenwert $1/v$ wird gegen die

Inhibitorkonzentration [I] aufgetragen. Für verschiedene [S]-Werte ergeben sich Geraden mit unterschiedlichen Steigungen. Der gemeinsame Schnittpunkt auf der x-Achse entspricht -K_i[33].

Für den Cornish-Bowden-Plot wird die ursprüngliche Michaelis-Menten-Gleichung nach [S]/v umgestellt[35]:

$$\frac{[S]}{v} = \frac{K_m}{V_{max}} \left(1 + \frac{[I]}{K_i}\right) + \frac{[S]}{V_{max}} \left(1 + \frac{[I]}{K_i'}\right)$$

Durch diese Umstellung wird wieder eine Geradengleichung erhalten, wobei hier y = [S]/v und x = [I] ist. Die daraus resultierenden Geraden schneiden sich ebenfalls bei -K_i'.

Graphisch ergibt sich der Nutzen dieser Umformungen dadurch, dass durch Regressionsanalysen diese Plots einfach ausgewertet werden können. Die charakteristische Form der Linien erlaubt Rückschlüsse auf den Inhibitionstyp (kompetitiv, nicht-kompetitiv, unkompetitiv) und die Berechnung von K_i durch Ermittlung des Schnittpunkts.

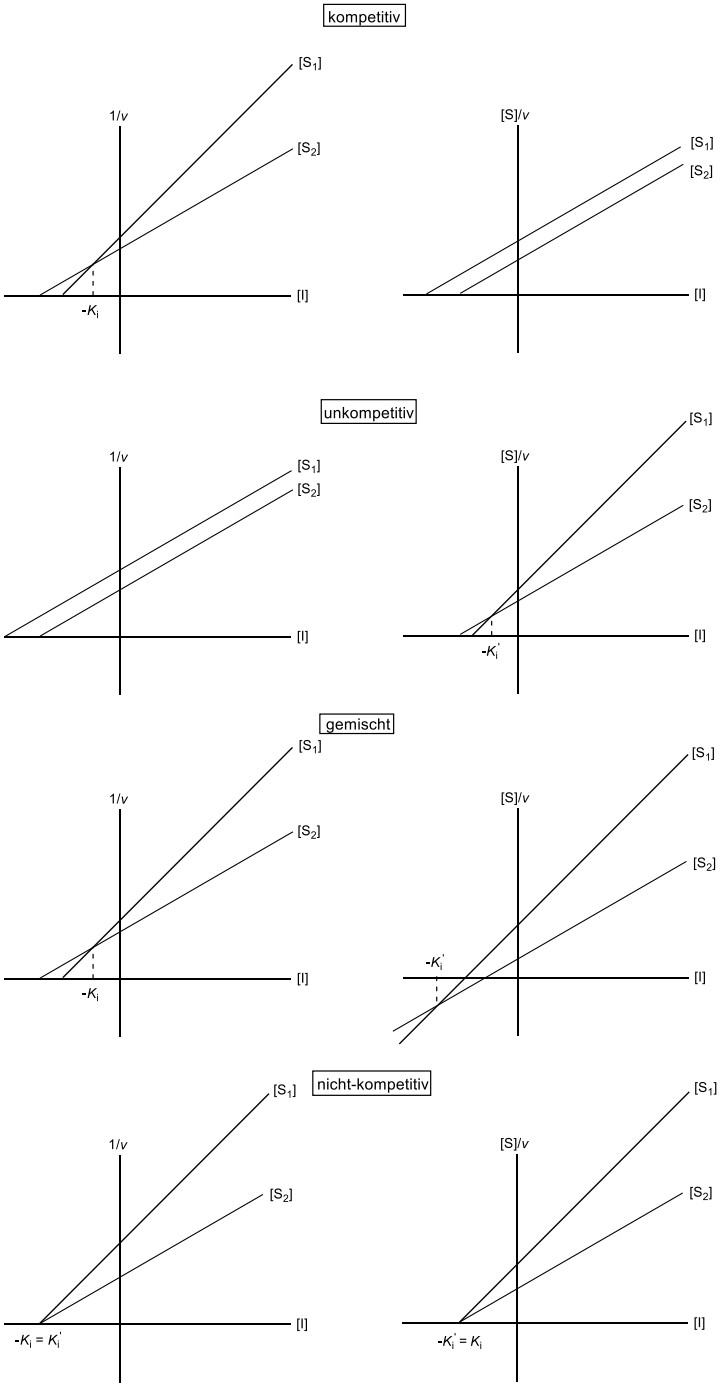


Abbildung 7: Dixon-Plot ($1/v$ gegen $[I]$) und Cornish-Bowden Plot ($[S]/v$ gegen $[I]$) zur Charakterisierung verschiedener Inhibitionstypen. Der Schnittpunkt der Geraden bei verschiedenen Substratkonzentrationen $[S_1]$ und $[S_2]$ erlaubt die Bestimmung der Inhibierungskonstanten K_i im Dixon-Plot und K_i' im Cornish-Bowden Plot[35].

Die Gleichungen resultieren aus der Anwendung stationärer Näherungen (STEADY STATE) auf das enzymatische Reaktionsschema mit Inhibitor[36]. Die Annahme dabei ist, dass sich das Enzym-Inhibitor-Gleichgewicht schnell einstellt, sodass es im Verlauf der Reaktion als konstant betrachtet werden kann. Die resultierenden Gleichungen stellen Näherungen dar, die im Rahmen experimenteller Genauigkeit

gute Ergebnisse liefern können, wenn die Voraussetzungen, wie etwa stabile Enzymaktivität, erfüllt sind.

Die Wahl der Substratkonzentration ist hierbei von zentraler Bedeutung: Nur bei Verwendung mehrerer [*S*]-Werte – idealerweise unter- und oberhalb von K_m – lassen sich Inhibitionstypen klar unterscheiden. Beispielsweise zeigt sich die kompetitive Hemmung deutlich bei niedrigen [*S*], während unkompetitive Effekte bei höheren Konzentrationen sichtbar werden.

Ein weiterer kritischer Aspekt ist die Enzymstabilität. Da sowohl der Dixon- als auch der Cornish-Bowden-Plot auf der Annahme stabiler Enzymaktivität beruhen, führen Denaturierung oder zeitabhängiger Aktivitätsverlust zu systematischen Fehlern: Die Reaktionsgeschwindigkeit erscheint niedriger als sie ist, was zu verschobenen oder falsch interpretierten Schnittpunkten führt. Daher ist es essenziell, ausschließlich Initialraten zu analysieren, welche sich noch im linearen Bereich der Kinetik befinden.

Carboanhydrasen als Schlüsselfaktoren der Tumorprogression

In metastasierenden Tumoren führt die schnelle Vermehrung von Krebszellen zu einer extrazellulären Umgebung, die durch einen stark reduzierten Sauerstoffgehalt (≤ 1,0 %) und eine erhöhte Säurebildung gekennzeichnet ist[37]. Dieser Zustand, bekannt als Tumorhypoxie, resultiert aus einem Ungleichgewicht zwischen dem Sauerstoffbedarf der proliferierenden Zellen und der Versorgungskapazität des umgebenden Gefäßsystems[38]. Im Zuge der Hypoxie stellen Tumorzellen ihren Energiestoffwechsel von der mitochondrialen oxidativen Phosphorylierung auf eine überwiegend anaerobe Glykolyse im Zytosol um – ein Phänomen, das als Warburg-Effekt bezeichnet wird[37, 39]. Trotz aerober Bedingungen wird das in der Glykolyse entstehende Pyruvat nicht vollständig dem Citratzyklus zugeführt, sondern zu Laktat reduziert, was zu einer Ansäuerung des extrazellulären pH-Werts auf etwa 6,5 führt[39b]. Diese Umstellung verursacht einen ausgeprägten Protonengradienten, der in entgegengesetzter Richtung zum Sauerstoffgradienten verläuft. Die resultierende Azidose bewirkt eine Degradierung des extrazellulären Gewebes und erleichtert somit das invasive Wachstum der Tumorzellen[39b]. Durch die Lockerung des Zellverbandes wird es den malignen Zellen ermöglicht, sich vom Primärtumor zu lösen, was die Entstehung von Metastasen begünstigt[40]. Trotz der ausgeprägten extrazellulären Azidose bleibt der intrazelluläre pH-Wert der Tumorzellen im leicht

alkalischen Bereich zwischen 7,2 und 7,5 erhalten[41]. Dies ist essenziell, da viele zelluläre Enzymfunktionen pH-sensitiv sind und eine stabile intrazelluläre Umgebung voraussetzen. Zur Aufrechterhaltung dieser pH-Gradienten tragen verschiedene pH-Regulationsmechanismen bei, unter denen die Bicarbonattransportsysteme eine zentrale Rolle spielen. Diese Systeme werden durch tumorassoziierte, membranständige Carboanhydrasen gesteuert, die durch die reversible Umwandlung von Kohlendioxid in Bicarbonat und Protonen die pH-Homöostase aktiv regulieren[37, 42].

In hypoxischen Tumorregionen kommt es dabei häufig zu einer Überexpression der Carboanhydrase-Isoformen CA IX[37] und CA XII[8]. Diese Isoenzyme katalysieren die Bildung von Bicarbonat (HCO_3^-), das über gekoppelte Transportmechanismen in das Zellinnere eingeschleust wird, wodurch der intrazelluläre pH-Wert basisch wird, während gleichzeitig der extrazelluläre Raum weiter acidifiziert[37] . Zusätzlich wirkt die zytoplasmatische Carboanhydrase II unterstützend, indem sie das intrazelluläre Verhältnis von Protonen und Bicarbonat feinstimmt und so die intrazelluläre pH-Stabilität weiter absichert[37, 42].

Isoformen der Carboanhydrase – Struktur, Verteilung und physiologische Funktion

Im Rahmen dieser Arbeit wurde die Wirkung verschiedener Inhibitoren auf verschiedene Isoformen der Carboanhydrase untersucht. Im folgenden Kapitel sollen diese Isoformen noch etwas näher betrachtet werden.

CA II

Die CA II gehört wohl mit zu den bekanntesten und am besten untersuchten Isoformen der Carboanhydrasen. Dies ist unter anderem dem Umstand zu schulden, dass diese Isoform bereits vor über 80 Jahren in Erythrozyten entdeckt wurde[43]. Seitdem zeigten sich weitere Fundorte in Zellen, Geweben und Organen in fast allen Wirbeltieren[44]. Dabei werden diverse essenzielle Funktionen, wie z.B. der CO_2-Transport[45], die Harnbildung[46] und die Aufrechterhaltung des Augeninnendrucks reguliert[47]. Zudem zeigt diese Isoform mit eine der höchsten bekannten katalytischen Aktivitäten mit einer Geschwindigkeitskonstante von $k_{kat} = 1{,}40 \times 10^6\ s^{-1}$ [18]. Damit verbunden stehen auch einige Erkrankungen, wie z.B. die Bildung von Glaukomen durch die Einlagerung von Kammerwasser im Glaskörper,

was zu einer Erhöhung des Augeninnendrucks[48] führt oder die Entstehung von Krebs[42].

CA V

Die CA V kommt im Organismus in zwei Untertypen vor: der CA VA und der CA VB. Beide Formen sind in den Mitochondrien und Adipozyten zu finden, jedoch jeweils in unterschiedlichen Organen. So wurde beispielsweise die CA VA in den Mitochondrien der Leber und den Nieren[49] nachgewiesen, wohingegen die CA VB im Rückenmark, der Bauchspeicheldrüse, dem Herz – und Skelettmuskel vorkommt[50]. Da der Subtyp VB nicht in der Leber vorkommt, lässt sich darauf schließen, dass beide Isoformen unterschiedliche physiologische Funktionen erfüllen und gewebespezifisch exprimiert werden. Die CA V übernimmt in der Leber die Bereitstellung von Hydrogencarbonat für mitochondriale Enzyme, insbesondere die Pyruvatcarboxylase und die Carbamoylphosphatsynthase[51]. Über diese Funktion ist sie indirekt an der Regulation des Harnstoffzyklus sowie der Gluconeogenese beteiligt[52]. Neben diesen physiologischen Funktionen wird die CA V auch mit der Regulierung des Insulinspiegels in Verbindung gebracht. Dies konnte im Tierversuch durch die Inhibierung der Insulinausschüttung in der Bauchspeicheldrüse durch den CA-Inhibitor Acetazolamid beobachtet werden[51]. Die Inhibierung von CA VA und CA VB wird außerdem mit einem signifikanten Gewichtsverlust in Verbindung gebracht, somit stellen Inhibitoren für diese Enzyme potenzielle Wirkstoffe gegen Fettleibigkeit dar[53]. Im Gegensatz zu der CA II besitzen die CA VA und VB nur eine niedrige katalytische Aktivität (CA VA k_{kat} = 2,9 x 10^5 s^{-1}, CA VB k_{kat} = 9,5 x 10^5 s^{-1} [18]), wobei die CA VB aktiver ist als ihr anderer Subtyp. Strukturell unterscheiden sich diese beiden Isoformen zudem durch die Aminosäuren im aktiven Zentrum. So ist beispielsweise His64 in der CA II in der CA V durch Tyr64 ausgetauscht. Weiterhin spielen bei der CA V weitere Aminosäuren wie Lys91, Tyr131 und Lys132 am äußeren Bereich der Enzymtasche eine Rolle bei dem Protonentransfer, dem Geschwindigkeitsbestimmenden Schritt und der Katalysereaktion[54].

CA IX

Neben der CA II stellt die CA IX eine der Isoformen mir der höchsten Aktivität (k_{kat} = 1,1 x 10^6 s^{-1} [18]) dar. Diese hohe Aktivität kann durch die Proteoglycan-Domäne, welche unter den CA nur die CA IX besitzt, erklärt werden[55]. Durch diese Domäne kann diese membranständige Isoform auch noch bei niedrigen pH-Werten gut funktionieren, was bei den anderen CA nicht der Fall ist[56]. Dadurch ist es möglich die biologische Funktion des Enzyms auch unter diesen Bedingungen noch zu erhalten, wie sie in vielen hypoxischen Tumoren zu finden ist[37, 39b, 42]. Da die CA IX in nur wenigen gesunden Geweben vorkommt[57], dafür jedoch in Tumoren z.T überexprimiert wird[58], macht dies dieses Enzym zu einem interessanten Ziel für eine Tumortherapie. Durch die hohe Aktivität und hohe Expression dieser Isoform wird es tumorösen Gewebe ermöglicht eine hohe Zellproliferation und dadurch ein invasives Wachstum zu haben[59]. CA IX liegt unter physiologischen Bedingungen als Trimer, in einigen Fällen auch als Dimer vor[56, 60]. Die inter– und intramolekularen Wechselwirkungen zwischen den Domänen der einzelnen Monomere werden dabei durch Disulfidbrücken von drei Cysteinresten vermittelt[55].

Sulfonamide als klassische CA-Inhibitoren

Sulfonamide gehören zu den frühesten synthetischen Wirkstoffklassen mit weitreichender therapeutischer Anwendung[61]. Charakteristisch ist die Sulfonamidgruppe (R-SO_2NH_2), die als zentraler pharmakophorer Bestandteil fungiert. Neben ihrer klassischen Anwendung als Antibiotika[61], z. B. in der Behandlung von Harnwegsinfekten[61], wurden Sulfonamide im Laufe der Jahre auch als Inhibitoren der Carboanhydrase (CA) identifiziert. Ein prominentes Beispiel ist Acetazolamid (Vgl. Abbildung 5), das als nicht-selektiver CA-Inhibitor breite therapeutische Verwendung, wie bei Glaukomen oder pulmonale Hypertension[62] Anwendung findet. Trotz ihrer Wirksamkeit zeigen klassische Sulfonamide Nebenwirkungen[63] und eine eingeschränkte Isoformenselektivität[64]. Dies begründet das Interesse an der Entwicklung neuer, selektiver CA-Inhibitoren mit optimierter Pharmakodynamik und -kinetik. In der hier vorgestellten Arbeit dient Acetazolamid als Referenzverbindung zur Evaluierung der Inhibierung neu entwickelter Verbindungen auf unterschiedliche CA-Isoformen, insbesondere auf CA II.

Diterpene in der Inhibitorentwicklung: Isosteviol als modifizierbares Grundgerüst

Diterpene, bestehend aus vier Isopreneinheiten (C_{20}), sind meist pflanzlichen Ursprungs und zeichnen sich durch eine ausgeprägte strukturelle Diversität und funktionelle Heterogenität aus. Diese Merkmale machen sie zu attraktiven Ausgangsverbindungen in der medizinisch-chemischen Forschung. Klassische Vertreter wie Cafestol, dass eine antitumoraktive Wirkung[65] zeigt, oder Forskolin, ein Aktivator der Adenylatcyclase mit Relevanz für kardiovaskuläre[66] und neurologische[67] Erkrankungen, verdeutlichen das breite pharmakologische Spektrum dieser Naturstoffklasse[68]. Taxol, das bereits als Arzneistoff in der Therapie maligner Tumore eingesetzt wird[69], verdeutlicht das Potential dieser Wirkstoffe zusätzlich.

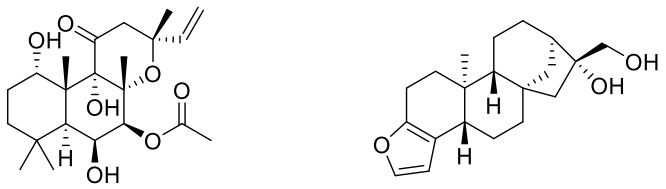


Abbildung 8: Struktur von den Diterpenen Forskolin (links) und Cafestol (rechts).

Ein besonderes Augenmerk gilt hier dem tetracyclischen Diterpen Isosteviol (Abbildung 9), das durch saure Hydrolyse aus dem aus STEVIA REBAUDIANA isolierten Glykosid Steviosid gewonnen werden kann[68a, 70] (Vgl. Abbildung 28). Studien belegen eine Vielzahl biologischer Aktivitäten von Isosteviol, insbesondere im kardiovaskulären Bereich[68c, 71]. Zusätzlich wurden entzündungshemmende[68b, 71], neuroprotektive[68c] und antitumoraktive[72] Eigenschaften beschrieben. Der lipophile Charakter des Moleküls erleichtert darüber hinaus die Zellmembranpassage, was für die pharmakologische Bioverfügbarkeit von Vorteil sein kann. In Kombination mit modifizierenden Substituenten lassen sich gezielt amphiphile Derivate erzeugen, die eine verbesserte Löslichkeit und Rezeptoraffinität aufweisen.

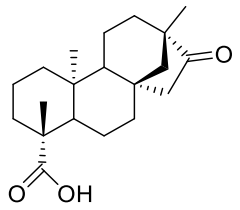


Abbildung 9: Struktur von Isosteviol

Triterpene als Naturstoffbasierte Leitstrukturen für die CA-Inhibition

Terpene sind eine Klasse von Naturstoffen meist pflanzlichen Ursprungs, die sich durch ihren Aufbau aus Isopren-Einheiten auszeichnen[73]. Sie werden basierend auf der Anzahl dieser Einheiten und den daraus resultierenden Kohlenstoffatomen in verschiedene Gruppen unterteilt: Hemiterpene (C_5), Monoterpene (C_{10}), Sesquiterpene (C_{15}), Diterpene (C_{20}), Sesterterpene (C_{25}), Triterpene (C_{30}), Tetraterpene (C_{40}) und Polyterpene ($>C_{40}$)[73a, 74]. Innerhalb dieser Gruppen unterscheidet man weiter zwischen azyklischen und zyklischen Verbindungen. Zu den wichtigsten Pionieren auf diesem Gebiet gehören die Chemiker OTTO WALLACH[74] und LEOPOLD RUŽIČKA[73b]. Wallach begann bereits 1885 mit der systematischen Untersuchung von Terpenen und legte durch seine Arbeiten die Grundlage für die Identifikation von Isopren als zentrale Untereinheit der Terpene. Diese Erkenntnis bildete später die Basis für Ružičkas "Isopren-Regel"[73b], die er im Rahmen seiner Forschungen aufstellte. Für seine Arbeiten erhielt WALLACH 1910 den Nobelpreis für Chemie. RUŽIČKA setzte die Arbeiten fort und konnte erstmals eine Verbindung zwischen Terpenen und Steroiden aufzeigen[73b]. Seine bedeutendste Entdeckung war 1930 die Isolierung von Androsteron[75], welches er erfolgreich in Testosteron umwandelte, wofür auch er 1939 mit dem Nobelpreis für Chemie geehrt wurde. Die Grundlagenforschung von WALLACH und RUŽIČKA ebnete den Weg für nachfolgende Studien, die zunehmend auch pharmakologische Eigenschaften von Terpenen in den Mittelpunkt stellten.

Eine wichtige Untergruppe der Terpene sind die pentazyklischen Triterpene, die aufgrund ihres pharmakologischen Potenzials intensiv erforscht wurden[76]. Besonders verbreitete Grundgerüste dieser Triterpene sind das Lupan-, Oleanan- und Ursan-Gerüst. Ein prominenter Vertreter ist die Betulinsäure, die für ihre zytotoxischen[77] und apoptoseinduzierenden[78] Eigenschaften bekannt ist, auch wenn der genaue Wirkmechanismus noch nicht vollständig aufgeklärt ist. Neben der Betulinsäure haben auch andere pentazyklische Triterpene wie Ursolsäure,

Oleanolsäure und Maslinsäure großes Interesse geweckt[79]. Diese Naturstoffe wurden ebenfalls auf ihre biologischen Aktivitäten hin untersucht, wobei insbesondere antimikrobielle[80], antivirale[81] und antitumoraktive[82] Effekte nachgewiesen wurden.

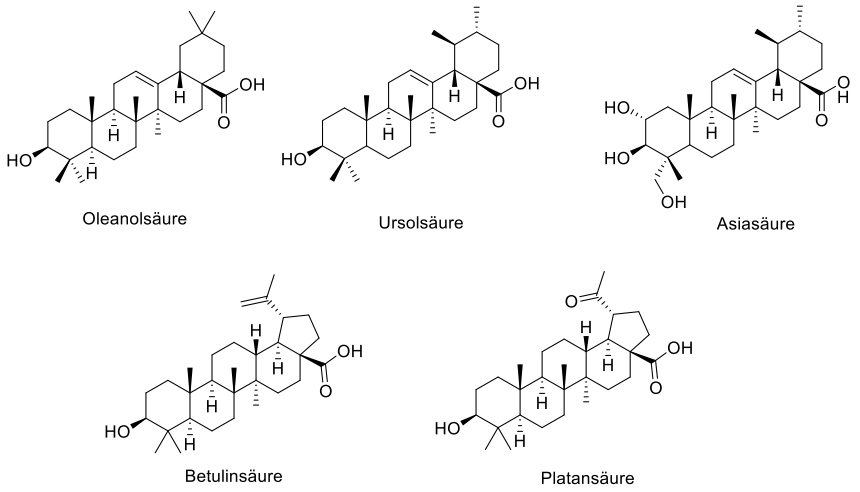


Abbildung 10: Strukturen einiger pentacyklischer Triterpene

Ein weiteres, weniger erforschtes pentazyklisches Triterpen ist die Asiasäure, die beispielsweise in der Pflanze CENTELLA ASIATICA (indischer Wassernabel) vorkommt[83]. In über 50 Pflanzenarten wurde dieses Triterpen bereits als bioaktiver Bestandteil nachgewiesen. Für die Isolierung und Extraktion gibt es mehrere Ansätze, wobei moderne Verfahren wie die Extraktion mit superkritischen Fluiden zunehmend an Bedeutung gewinnen und eine Alternative zu herkömmlichen Lösungsmittelmethoden darstellen[84]. Die Asiasäure weist ein breites pharmakologisches Wirkspektrum auf, das von antioxidativen[85] und antiinflammatorischen[83a] über cardio[86]-, neuro[83a]- und gastroprotektive[83a] bis hin zu antitumoraktiven[87] Eigenschaften reicht.

Zielstellung

Sulfonamide und Sulfamate stellen durch ihre hohe Affinität für Carboanhydrasen, ihre Stabilität und ihre vergleichsweise geringe Toxizität[88] ideale Verbindungen für die Forschung an neuartigen Inhibitoren dar. Trotz ihrer vielseitigen Vorteile und hoher Aktivität fehlt es oftmals an einer hohen Selektivität, v.a. zwischen den verschiedenen Isoformen der CA. Es ist somit erforderlich Modifikationen an den bekannten Leitstrukturen durchzuführen, um zusätzliche Interaktionen in der aktiven

Seite des Enzyms hervorzurufen, um so die Isoformenselektivität zu verbessern und ohne dabei die Inhibitoraktivität zu verschlechtern oder diese sogar zu optimieren. Zudem ist bei dieser Arbeit noch der Einfluss des Charakters und v.a. der Größe des Grundgerüsts von Interesse. Um dies zu untersuchen, wurden verschiedene Sulfamate zum einen mit kleinen, einfachen Grundgerüst bis hin zu großen, komplexeren Grundgerüsten synthetisiert. Innerhalb dieser Gruppen wurden außerdem die Art des Sulfamats und der damit verbundenen Spacers variiert, um gleichzeitig deren Einflüsse zu untersuchen. Mittels dieser Ergebnisse soll schließlich eine SAR abgeleitet werden.

Diskussion der Forschungsergebnisse

In diesem Abschnitt der Arbeit sollen die wichtigsten Forschungsergebnisse der einzelnen Publikationen zusammenfassend dargestellt werden. Weitere Informationen wie die Synthesebedingungen, spektroskopischen Charakterisierungen und Durchführung der biologischen Assays als auch deren gesamte Ergebnisse können in den jeweiligen Publikationen entnommen werden. Für eine bessere Übersichtlichkeit werden die Verbindungen abschnittsweise neu nummeriert. Die einzelnen Publikationen werden mit P1 bis P8 abgekürzt.

Publikationen

P1
Synthesis and Enzymatic Evaluation of a Small Library of Substituted Phenylsulfonamido-Alkyl Sulfamates towards Carbonic Anhydrase II

Um einen ersten Einblick in die Struktur-Wirkungsbeziehungen für den Aufbau von Sulfonamiden als Inhibitoren der CA II zu untersuchen, wurde eine Verbindungsreihe von 79 substituierten Phenylsulfonamidoalkylsulfamaten (**1b**–**79b**) synthetisiert (Abbildung 11). Die Synthese begann mit Arylsulfonylchloriden und Aminoalkoholen, die sich in der Anzahl der Methylengruppen zwischen der Hydroxyl- und der Aminogruppe unterschieden. Diese Reaktion führte zu Zwischenprodukten (**1a**–**79a**), die anschließend mit Sulfamoylchlorid zur Bildung der Endverbindungen (**1b**–**79b**) umgesetzt wurden. Ein allgemeines Syntheseschema ist in Abbildung 12 dargestellt.

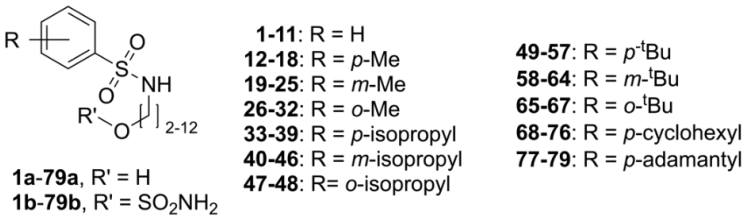


Abbildung 11: Übersicht über der in P1 verwendeten Substituenten

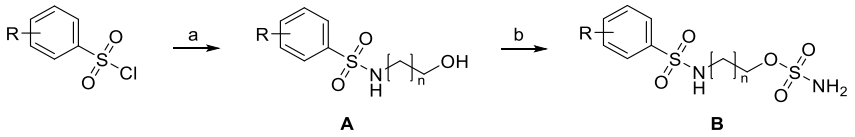


Abbildung 12: Allgemeines Syntheseschema zur Herstellung der Phenylsulfonamidoalkylsulfamaten

Die synthetisierten Verbindungen wurden auf ihre Aktivität gegenüber CA II getestet. Die Zwischenstufen (**1a**–**79a**) zeigten keine Inhibierung, während die Sulfamate (**1b**–**79b**) das Enzym inhibieren konnten.

Tabelle 2: Inhibierungsprozent der in P1 untersuchten Sulfamate mit einer Inhibitorkonz. von 1 µM

Verbindung	Inhibierung [%]	Verbindung	Inhibierung [%]	Verbindung	Inhibierung [%]
AAZ	99.2 ± 0.2	**27**	50.0 ± 0.8	**54**	28.4 ± 0.4
1	59.2 ± 0.8	**28**	37.9 ± 0.7	**55**	21.3 ± 1
2	39.9 ± 0.4	**29**	57.2 ± 0.7	**56**	10.0 ± 0.6
3	32.2 ± 0.3	**30**	68.1 ± 0.3	**57**	20.6 ± 0.6
4	51.8 ± 0.7	**31**	55.6 ± 0.7	**58**	54.0 ± 0.9
5	56.0 ± 0.5	**32**	56.2 ± 0.8	**59**	53.9 ± 0.3
6	56.0 ± 0.8	**33**	63.2 ± 0.4	**60**	43.3 ± 0.6
7	60.2 ± 0.7	**34**	60.3 ± 0.9	**61**	39.2 ± 0.1
8	55.4 ± 0.7	**35**	43.7 ± 0.4	**62**	41.7 ± 0.8
9	10.7 ± 0.4	**36**	40.8 ± 0.6	**63**	33.3 ± 0.3
10	7.2 ± 0.9	**37**	45.9 ± 0.7	**64**	16.0 ± 0.4
11	5.9 ± 0.9	**38**	30.3 ± 0.1	**65**	56.2 ± 0.8
12	75.8 ± 0.6	**39**	28.1 ± 0.5	**66**	34.5 ± 0.4
13	63.8 ± 0.8	**40**	63.7 ± 0.1	**67**	27.5 ± 0.3
14	42.9 ± 0.1	**41**	50.1 ± 0.9	**68**	52.1 ± 1.2
15	58.9 ± 0.2	**42**	35.3 ± 0.5	**69**	43.1 ± 1.5
16	40.3 ± 0.7	**43**	44.4 ± 0.3	**70**	34.7 ± 2.5
17	42.8 ± 0.5	**44**	45.5 ± 0.7	**71**	18.6 ± 1.0
18	54.7 ± 0.6	**45**	37 ± 0.3	**72**	8.6 ± 0.6
19	59.5 ± 0.1	**46**	29.5 ± 0.9	**73**	14.8 ± 0.4
20	48.4 ± 0.1	**47**	38.4 ± 0.2	**74**	14.2 ± 1.1
21	32.2 ± 0.6	**48**	34.2 ± 0.7	**75**	32.1 ± 0.8
22	49.1 ± 0.2	**49**	75.6 ± 0.4	**76**	19.7 ± 1.6
23	51.8 ± 0.7	**50**	63.4 ± 0.6	**77**	32.6 ± 0.4
24	27.1 ± 0.9	**51**	44.9 ± 0.4	**78**	36.1 ± 1.8
25	38.8 ± 0.1	**52**	35.9 ± 0.4	**79**	40.1 ± 0.8
26	69.1 ± 0.7	**53**	29.1 ± 0.9		

Die Aktivität der Sulfamate wurde durch die Art des Substituenten, die Position des Substituenten am Phenylring (*ortho*, *meta*, *para*) und die Länge des Spacers zwischen der Sulfonamidgruppe und dem Sulfamat beeinflusst. *Para*-substituierte Verbindungen zeigten im Allgemeinen eine bessere Inhibierung im Vergleich zu ihren *meta*- oder *ortho*-substituierten Analoga. Dieser Trend war bei verschiedenen

Substitutionsmustern konsistent. Verbindungen mit voluminöseren Substituenten, wie *tert*-Butyl (^{t}Bu) in *para*-Position, zeigten eine stärkere Inhibierung als solche mit kleineren Substituenten. Jedoch führten übermäßig sperrige Gruppen (wie Cyclohexyl oder Adamantyl) zu einer verringerten Inhibierung, selbst in der *para*-Position. Für die meisten Substitutionsmuster wiesen Verbindungen mit kürzeren Spacern (n = 2) zwischen der Sulfonamid- und der Sulfamatgruppe eine stärkere Inhibierung auf. Verbindungen mit längeren Spacerlängen zeigten im Allgemeinen eine verringerte Aktivität.

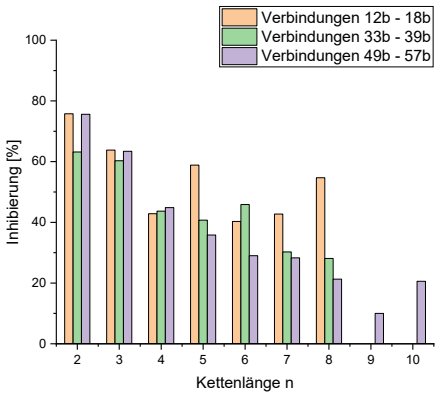


Abbildung 13: Veranschaulichung der Inhibierungsprozent der *para*-substituierten Verbindungen **12b**–**18b** (Methyl), **33b**–**39b** (*iso*-Propyl), und **49b**–**57b** (*tert*-Butyl) in Abhängigkeit von der Kettenlänge.

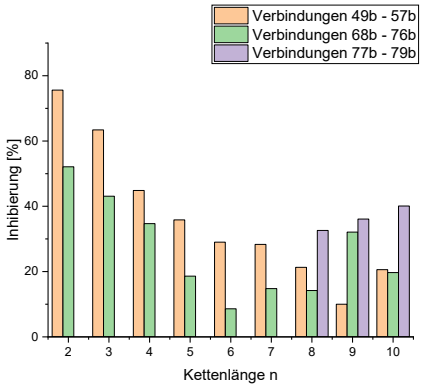


Abbildung 14: Veranschaulichung der Inhibierungsprozent der sterisch anspruchsvollsten Substituenten in *para* Position **49b**–**57b** (^{t}butyl (orange)), **68b**–**76b** cyclohexyl (grün), und **77b**–**79b** (adamantyl (violett)) in Abhängigkeit von der Kettenlänge.

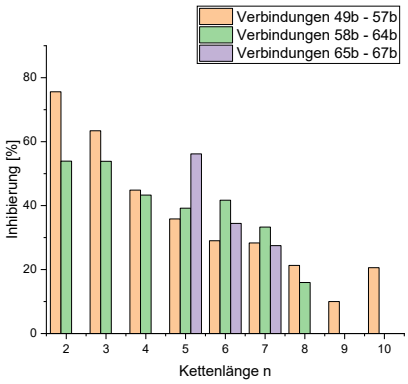


Abbildung 15: Veranschaulichung der Inhibierungsprozent der *tert*-Butyl substituierten Verbindungen in Abhängigkeit von der Kettenlänge

Unter den 79 Sulfamaten stachen mehrere Verbindungen als potente CA II Inhibitoren hervor, darunter **12b**, **49b**, **26b**, **30b**, **13b**, **40b**, **50b**, **33b** und **34b**. Um einen genaueren Einblick in die Inhibierungseffekte zu erhalten, wurden von diesen Verbindungen kinetische Untersuchungen durchgeführt. Der stärkste Inhibitor der gesamten Serie war Verbindung **49b** (mit einem *tert*-Butylsubstituenten in *para*-Position und einem kurzen Spacer), der eine Inhibierung der CA II von 75,6 % mit einem K_i-Wert von 0,67 µM erreichte. Um diese Ergebnisse weiter zu untersuchen, wurde molecular modeling an den Verbindungen durchgeführt. Die hohe Flexibilität der Moleküle im aktiven Zentrum des Enzyms sowie die Größe des aktiven Zentrums begrenzten die Präzision der Berechnungen. Nichtsdestotrotz bestätigten die Ergebnisse, dass eine weitere Substitution am Spacer, insbesondere an der α-Position, zu einer besseren Orientierung der Inhibitoren im aktiven Zentrum des Enzyms führen könnte. Diese Erkenntnis wurde in P3 aufgegriffen. Verbindungen mit *para*-substituierten *tert*-Butylgruppen und kürzeren Spacerlänge zeigten durchweg die beste Inhibierung. Die Ergebnisse der biologischen Evaluierung bestätigten, dass die Inhibierung von CA II durch Phenylsulfonamidoalkylsulfamate von den strukturellen Merkmalen der Verbindungen abhängt, insbesondere von der Art des Substituenten, seiner Position am Phenylring und der Spacerlänge.

Tabelle 3: K_i-Werte der aktivsten Phenylsulfonamidoalkylsulfamate

Verbindung	**12b**	**26b**	**30b**	**33b**	**40b**	**49b**
K_i [µM]	0.76 ± 0.03	1.1 ± 0.03	1.58 ± 0.02	0.84 ± 0.02	1.64 ± 0.03	0.67 ± 0.05

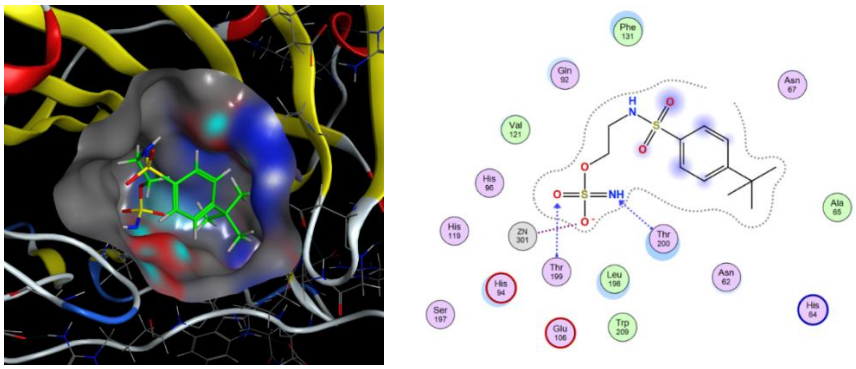


Abbildung 16: 3D - und 2D-Modeling Verbindung **49b** im aktiven Zentrum der CA II

P2

Arylsulfonamido-alkyl-sulfamates act as inhibitors of bovine carbonic anhydrase II

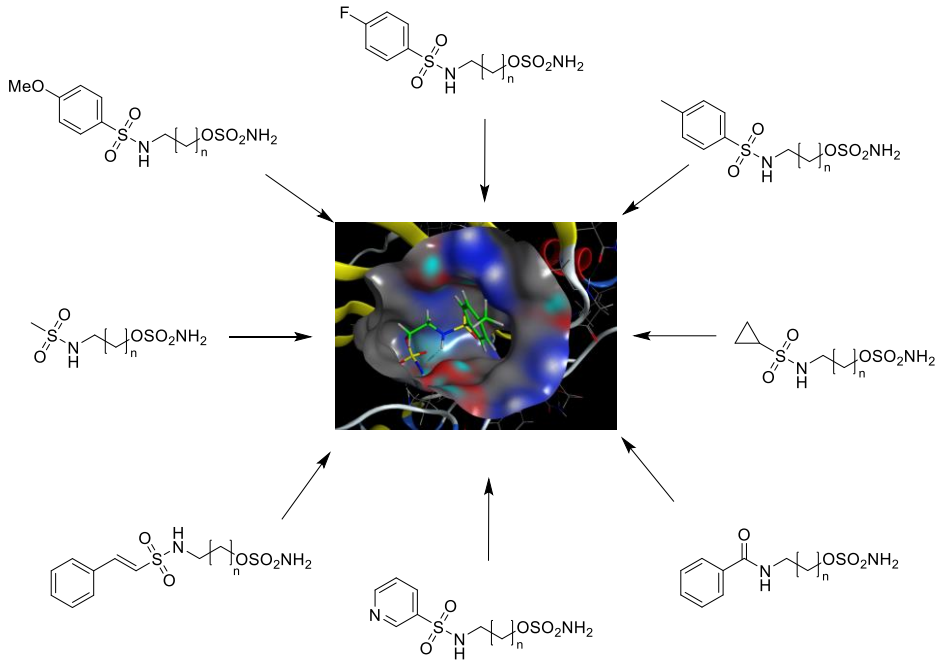


Abbildung 17: Grundstrukturen der in P2 verwendeten Sulfamate

Analog zu **P1** wurde eine Reihe an Verbindungen synthetisiert, um eine SAR abzuleiten. Bei diesen Verbindungen lag der Fokus jedoch auf dem Einfluss von Heteroatomen und weiteren Modifikationen am Phenylring. Wie bei der vorangegangenen Serie an Inhibitoren zeigt sich, dass die inhibitorische Aktivität stark durch das Substitutionsmuster Aromaten sowie durch die Länge des Spacers, der diese mit der Sulfamatgruppe verbindet, beeinflusst wurde. Verbindungen mit kleinen Substituenten erleichterten π-π-Wechselwirkungen mit Resten im aktiven Zentrum wie His94, was die Bindung des Inhibitors verstärkte.

Elektronendonierende Gruppen wie Methyl (-Me) und Methoxy (-OMe) in *para*-Position erwiesen sich als besonders effektiv, wahrscheinlich aufgrund ihrer Fähigkeit, Wechselwirkungen innerhalb der hydrophoben Taschen des Enzyms zu stabilisieren. Im Gegensatz dazu waren elektronenziehende Gruppen wie Fluor (-F) zwar ebenfalls wirksam, jedoch weniger potent als elektronendonierende Gruppen. Dies deutet darauf hin, dass elektronreiche Aromaten für die Interaktion mit dem Enzym vorteilhafter sind. Die Länge des Alkylspacers zwischen dem Arylsulfonamid und der Sulfamatgruppe hatte ebenfalls einen erheblichen Einfluss auf die Inhibierung: Kürzere Spacer (n = 2) führten wieder zu den Verbindungen mit der höheren Aktivität. Diese kürzeren Spacer ermöglichten es der Sulfamatgruppe, effizienter mit dem Zink(II)-Ion im aktiven Zentrum des Enzyms zu interagieren, während der Arylring gleichzeitig für optimale π-π-Wechselwirkungen mit den Resten im aktiven Zentrum positioniert wurde. Längere Spacer (n = 8) waren zwar in einigen Fällen noch aktiv, zeigten jedoch einen allgemeinen Trend zu verminderter Aktivität. Dies könnte auf eine geringere Interaktion zwischen dem Arylring und den wichtigen Resten im Enzym sowie auf eine suboptimale Ausrichtung der Sulfamatgruppe innerhalb des aktiven Zentrums zurückzuführen sein. Für Analoga mit Cyclopropyl- oder Methylsulfonyl-Gruppen konnte die beobachtete Aktivität auf den geringeren sterischen Anspruch dieser kleineren Substituenten zurückgeführt werden. Diese kleineren Substituenten passten vermutlich besser in das aktive Zentrum des Enzyms und führten zu einer verbesserten Inhibierung. Über das molecular modeling konnten diese Überlegungen wieder untermauert werden.

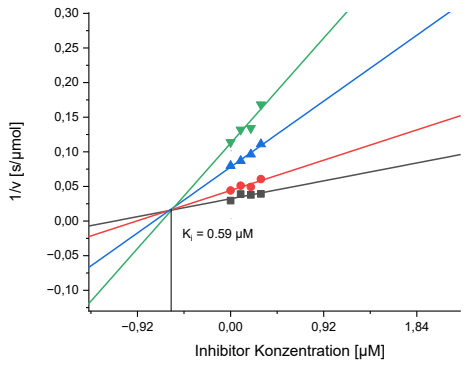


Abbildung 18: Dixon-Plot für Verbindung **47b** (mit den Inhibitorkonzentrationen: 0,1; 0,2; 0,3 µM); K_i = 0.59 ± 0.02 µM

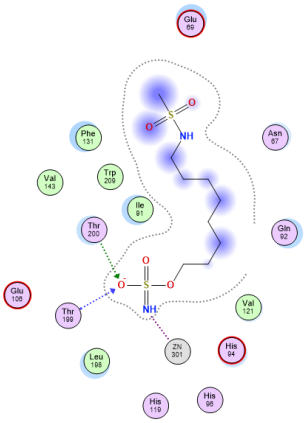


Abbildung 19: 2D-Modeling von Verbindung **47b** im aktiven Zentrum der CA-II und entsprechenden Wechselwirkungen

P3

Stereochemistry matters: inhibition of carbonic anhydrase II by amino acid derived sulfamates depends on their absolute configuration

Um den Einfluss der Konfiguration der Reste eines Inhibitors zu untersuchen, wurde eine Reihe von Aminosäuren abgeleiteter Verbindungen synthetisiert. Diese unterscheiden sich in Größe und Art ihrer Reste, um somit ein möglichst breites Spektrum an Variationen abzudecken. Die Synthese der enantiomeren Phenylsulfonamid-Sulfamate begann mit der Reaktion von Benzolsulfonylchlorid und Ethanolamin, was zur Bildung von Sulfonamid **1a** führte. Dieses wurde mit Sulfamoylchlorid zu Modellverbindung **1b** umgesetzt und bereits in **P1** untersucht. Durch ein vorläufiges molecular modelling konnte daraus geschlossen werden, dass Substituenten in der α-Position die Inhibierung beeinflussen können. Basierend auf diesen Erkenntnissen wurden verschiedene Aminoalkohole, die von α-Aminosäuren abgeleitet sind, wie Alanin, Leucin, Prolin, Valin und Tryptophan, verwendet, um eine Reihe von Sulfonamiden (**2a**-**16a**) zu synthetisieren, die anschließend zu den entsprechenden Sulfamaten (**2b**-**16b**) umgesetzt wurden.

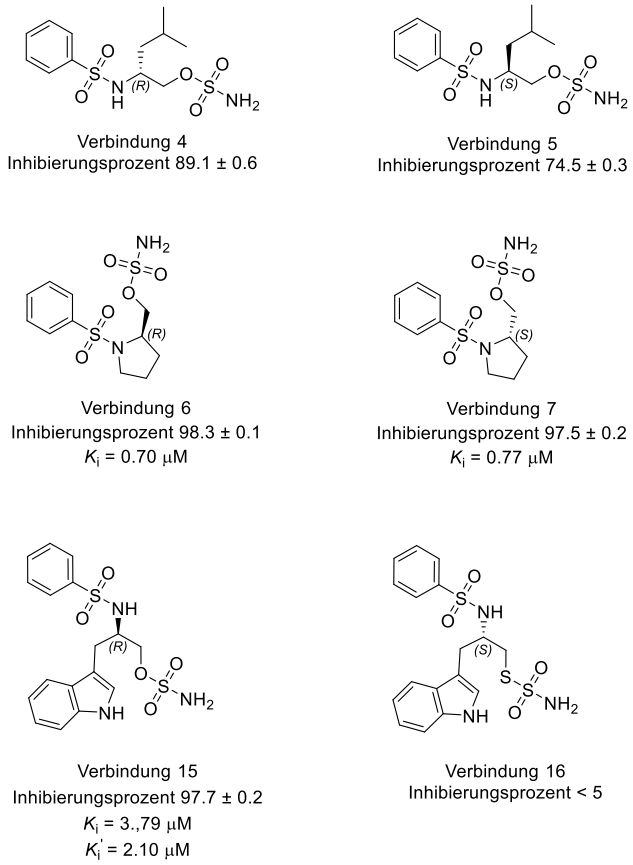


Abbildung 20: Übersicht einiger in P3 verwendeten Aminosäurederivate

Im CA II Assay konnte gezeigt werden, dass die Sulfamate, die von Aminosäuren wie Alanin, Leucin, Prolin und Valin abgeleitet wurden, Inhibierungsprozente von 50-98 % bei einer Konzentration von 10 µM aufwiesen. Besonders bemerkenswert war, dass das Produkt **16b**, abgeleitet von (*S*)-Tryptophan, keine Aktivität zeigte, während das (*R*)-konfigurierte Produkt **15b** zu den stärksten Inhibitoren gehörte und eine Inhibierung von 97,9 % erreichte. Im Vergleich dazu zeigte die Referenzverbindung AAZ eine Inhibierung von 99,3 % bei derselben Konzentration.

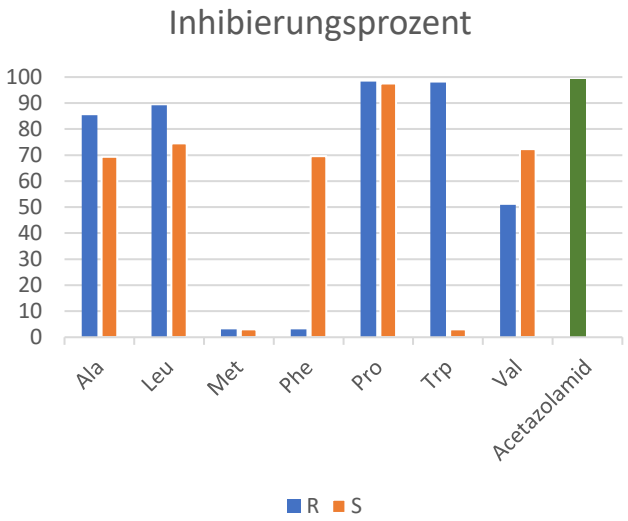


Abbildung 21: Aktivität der verschiedenen Aminosäurederivate in Abhängigkeit der Konfiguration

Zur weiteren Analyse wurden die Inhibitionskonstanten K_i für ausgewählte Verbindungen ermittelt, wobei bei den aktivsten Verbindungen Werte von bis zu 0.70 µM erreicht wurden. Das molecular modelling zeigte für die von Tryptophan abgeleiteten Verbindungen **15b** und **16b**, dass die Aminogruppe des Sulfamats von **15b** stark an das Zn^{2+} im aktiven Zentrum der CA II bindet und zusätzliche stabilisierende Wechselwirkungen mit Aminosäuren wie Thr199 und Phe131 eingeht. Im Gegensatz dazu war die Bindung von **16b** durch fehlende stabilisierende Wechselwirkungen schwächer, was die fehlende Aktivität erklärte.

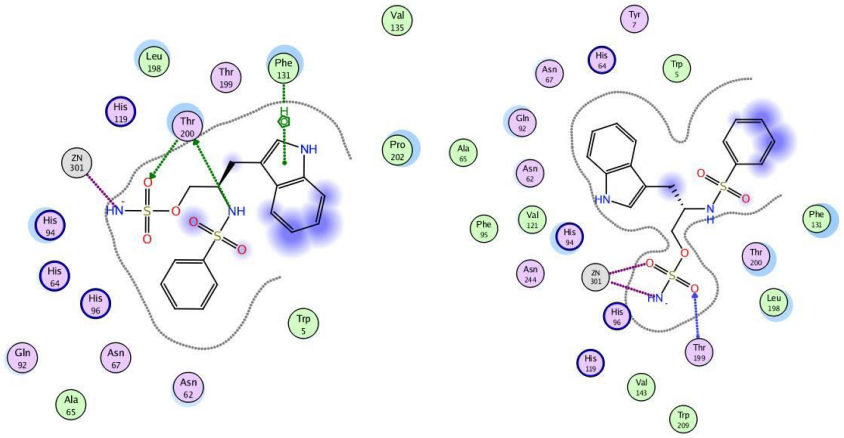


Abbildung 22: 2D-Modeling von Verbindung **15b** und **16b** im aktiven Zentrum der CA-II und entsprechenden Wechselwirkungen

Insgesamt wurde gezeigt, dass Verbindungen mit (*R*)-Konfiguration, insbesondere solche mit der Fähigkeit stabilisierende Wechselwirkungen auszubilden eine stärkere Aktivität auf CA II aufweisen als ihre (*S*)-konfigurierten Gegenstücke. Die Ergebnisse dieser Publikation belegen somit den Einfluss der Konfiguration des Inhibitors. Mittels dieser Erkenntnisse kann nun der Fokus auf Inhibitoren mit komplexerem Aufbau und z.T. mehreren Stereozentren erfolgen.

P4

Ureidobenzenesulfonamides as selective carbonic anhydrase I, IX, and XII inhibitors

Es ist bereits bekannt, dass CA-Inhibitoren mit Ureido-Funktionalität wie das SLC-0111 Ziele für die Entwicklung neuartiger Therapeutika darstellen[89]. In Folge dieser Erkenntnis wurden 15 neuartige Sulfonamide synthetisiert, indem Sulfanilamid mit Isocyanaten umgesetzt wurde. Ein besonderer Fokus wurde dabei auf Benzylphenyl- und Biphenyl-Derivate gelegt, da frühere Studien auf eine hohe Bindungsaffinität dieser Strukturen zu verschiedenen CA-Isoformen hingewiesen[90] hatten. Die Aktivität und Selektivität der synthetisierten Verbindungen wurde gegen vier menschliche CA-Isoformen (hCA I, hCA II, hCA IX und hCA XII) untersucht.

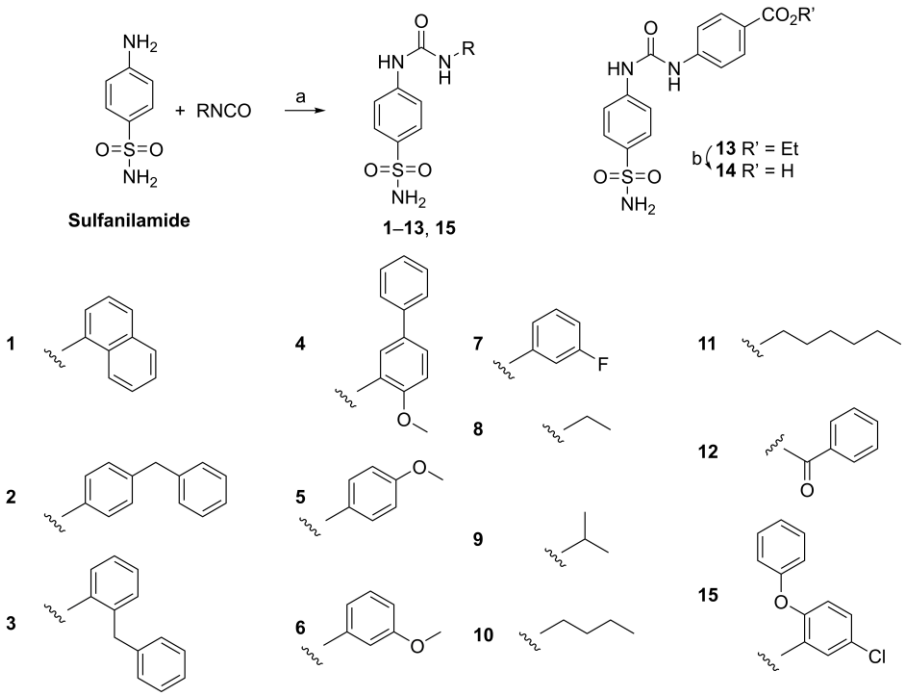


Abbildung 23: Übersicht über die in P4 verwendeten Ureidobenzenesulfonamide

Die Ergebnisse zeigten, dass die synthetisierten Verbindungen in der Lage waren, alle getesteten Isoformen zu inhibieren. Als besonders aktiv zeigten sich die Verbindungen **3** mit 2-Benzylphenylrest und **10** mit *n*-Butylrest mit sehr niedrigem K_i-Werten gegenüber allen Isoformen. Verbindung **3** erwies sich mit einem K_i-Wert von 1.0 nM für hCA XII und 8.2 nM für hCA IX als der stärkste Inhibitor und übertrifft damit den klinisch getesteten Inhibitor SLC-0111 mit einem K_i-Wert von 4.5 nM gegenüber hCA XII. Verbindung **2** zeigte ebenfalls eine sehr gute Aktivität gegenüber hCA IX und hCA XII, mit K_i-Werten von 15.2 nM bzw. 6.4 nM.

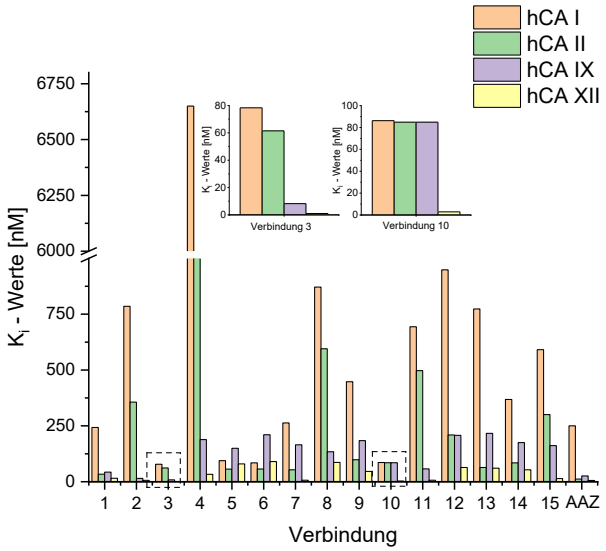


Abbildung 24: K_i-Werte der einzelnen Inhibitoren gegenüber den Isoformen hCA I, hCA II, hCA IX und hCA XI

Die phenylbenzyl-substituierten Verbindungen zeigten insgesamt eine starke Inhibierung auf alle getesteten Isoenzyme, wobei besonders niedrige K_i-Werte bei den Tumor-assoziierten Isoformen hCA IX und hCA XII gemessen wurden. Durch die Änderung des Substitutionsmusters von Verbindung **2** zu **3** konnte gezeigt werden, dass bereits geringfügige strukturelle Änderungen im Aufbau des Inhibitors Einfluss auf die Wirksamkeit der Aktivität haben. Die Röntgenstrukturanalyse von Verbindungen **8** und **10** belegt dies weiterhin, da die Verlängerung der Alkylkette, eine signifikante Auswirkung auf die Inhibierung hat. Diese Modifikationen verstärkten vor allem hydrophobe Interaktionen im aktiven Zentrum der Enzyme.

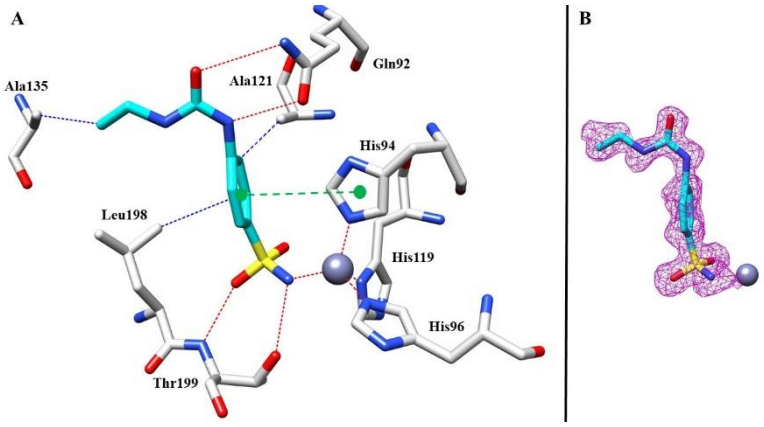


Abbildung 25: (A) Darstellung der Bindung von Verbindung **8** im aktiven Zentrum der hCA I. Hydrophobe (blau), hydrophile (rot) sowie π-stapelnde (grün) Wechselwirkungen sind farblich hervorgehoben. (B) Elektronendichtekarte (2Fo–Fc) der an Zink koordinierten Verbindung 8 im aktiven Zentrum von hCA I; dargestellt bei einem Konturierungsniveau von 1,0 σ.

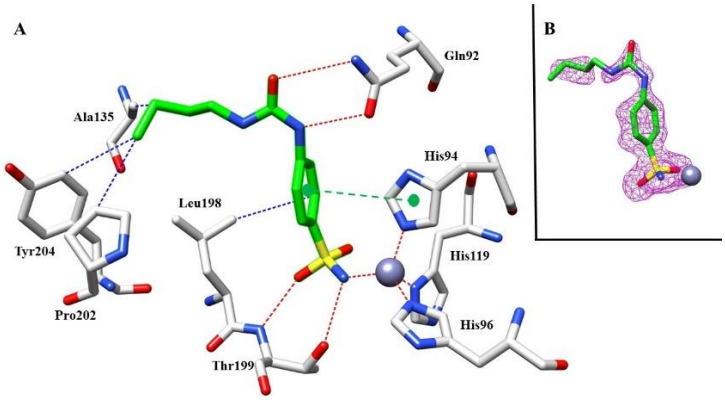


Abbildung 26: (A) Darstellung der Bindung von Verbindung **10** im aktiven Zentrum der hCA I. Hydrophobe (blau), hydrophile (rot) sowie π-stapelnde (grün) Wechselwirkungen sind farblich hervorgehoben. (B) Elektronendichtekarte (2Fo–Fc) der an Zink koordinierten Verbindung 10 im aktiven Zentrum von hCA I; dargestellt bei einem Konturierungsniveau von 1,0 σ.

Neben der Aktivität der Inhibitoren stellt auch die Isoformenselektivität einen bedeuteten Aspekt der biologischen Evaluierung dar. So zeigt Verbindung **3** moderrate SI-Werte in Bezug auf sie Selektivität von hCA I zu hCA XII mit 78 und 62 von hCA II zu hCA XII. Die SI-Werte sind in folgender Abbildung zusammengefasst.

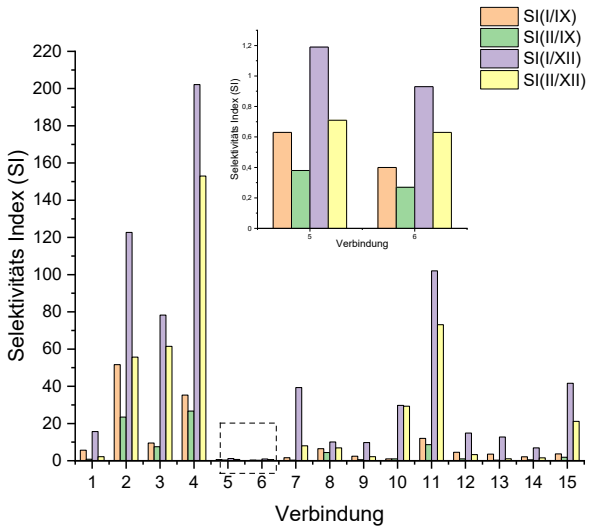


Abbildung 27: SI-Werte der synthetisierten Verbindungen in Bezug auf die einzelnen Isoformen.

P5

Isosteviol – A new scaffold for the synthesis of carbonic anhydrase II inhibitors

Zur Untersuchung des Einflusses größerer und komplexerer Strukturelemente wurde Isosteviol, ein Diterpen, als Ausgangsmaterial für die Synthese einer Reihe von Inhibitoren herangezogen, um deren Fähigkeit zur Inhibierung der Carboanhydrase II zu evaluieren. Zur Bewertung der Inhibierungsaktivität wurden sowohl Isosteviol als auch, zum Vergleich, Steviol in verschiedene Sulfamate mit und ohne Spacer derivatisiert. Isosteviol diente hierbei als ein kleines Naturstoffmolekül, welches als Vergleich für die später folgenden größeren Triterpene verwendet wurde.

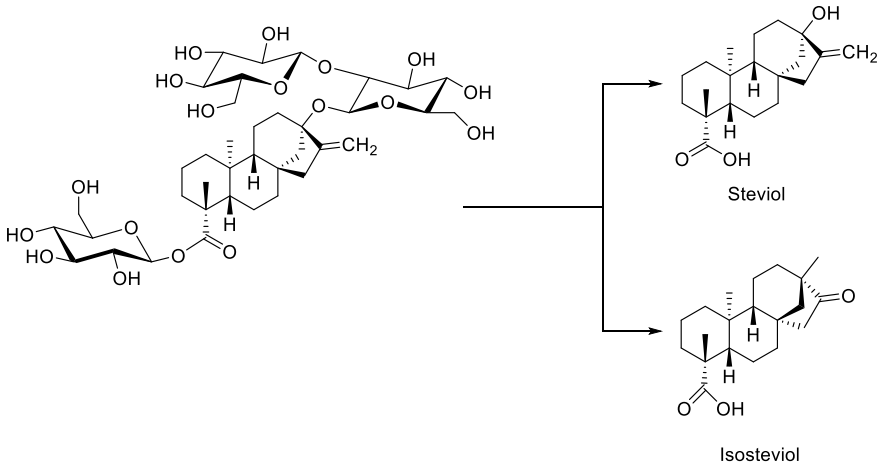


Abbildung 28: Gewinnung von Isosteviol bzw. Steviol aus dem Steviosid

Im ersten Schritt wurde Steviosid hydrolytisch gespalten, um Isosteviol zu erhalten. Dessen Struktur wurde anschließend durch NMR-spektroskopische Untersuchungen analysiert und bestätigt. Isosteviol wurde daraufhin mit Natriumborhydrid zu Dihydro-Isosteviol reduziert, während eine weitere Reduktion mit Lithiumaluminiumhydrid zum Diol führte. Beide Verbindungen dienten als Zwischenprodukte für nachfolgende Reaktionen.

Zu den wesentlichen Derivatisierungen gehörte die Succinoylierung von Diol unter Bildung von Verbindung **6** sowie die Anbringung eines bisher noch nicht betrachteten 5-Amino-1,3,4-thiadiazol-2-yl-amino-Substituenten über einen Spacer, was zu Verbindung **7** führte. Die Acetylierung von Diol **5** ergab ein Gemisch aus den Acetaten **8** und **9**, die durch säulenchromatographische Aufreinigung getrennt wurden. Diese Acetate wurden anschließend weiter modifiziert, um Sulfamate wie Verbindung **10** herzustellen und zusätzliche Sulfamate wurden direkt durch Reaktion der Alkohole mit Sulfamoylchlorid synthetisiert.

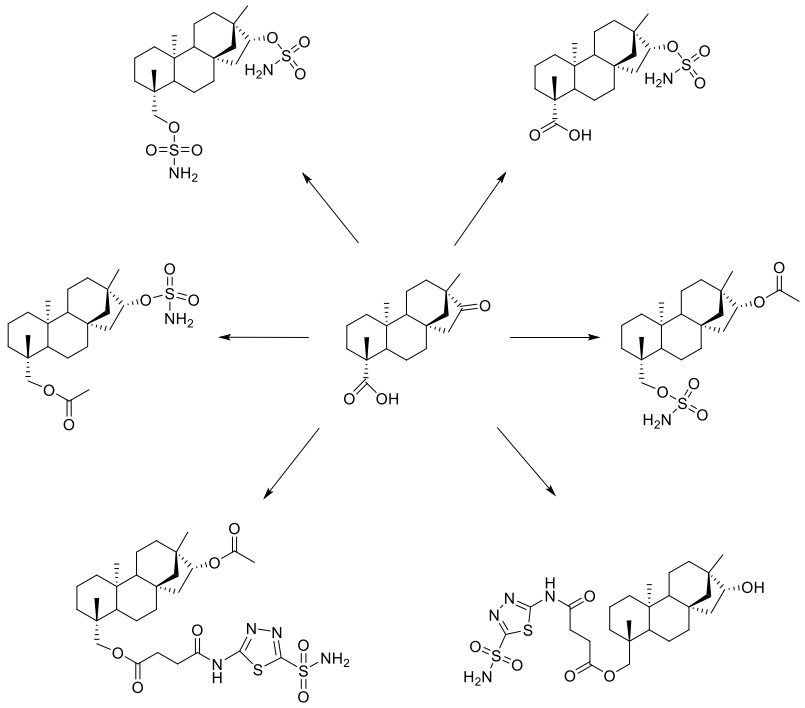


Abbildung 29: Struktureller Vergleich von isosteviolbasierten Derivaten mit funktionellen Gruppen an unterschiedlichen Positionen

Neben den unterschiedlichen Spacer-Typen und Sulfamatgruppen wurde auch der Einfluss der Position der Sulfamatgruppe auf die Inhibierungsaktivität untersucht. Unter den getesteten Verbindungen zeigten die Derivate **11**, **12** und **15** die höchste Aktivität. Verbindung **11**, die eine Acetylgruppe beibehielt, inhibierte das Enzym fast vollständig, vermutlich aufgrund verbesserter Bindungsinteraktionen.

Verbindung **12** zeigte eine Inhibierung von 94,5 %, was die synergistische Wirkung der Sulfamatgruppe an C-16 und der Acetylgruppe an C-18 unterstreicht. Im Gegensatz dazu zeigte Verbindung **13** eine geringere Inhibierung von 73,5 %, was die Bedeutung der Positionierung der funktionellen Gruppen verdeutlicht. Verbindung **15** zeigte ebenfalls eine starke Inhibierung mit einer Sulfamatgruppe an C-16. Die Verbindungen **14** und **16** zeigten dagegen nur eine minimale Aktiviät, was darauf hindeutet, dass eine doppelte Sulfamoylierung oder die Verwendung des Steviol-Grundgerüsts die Aktivität negativ beeinflussen kann.

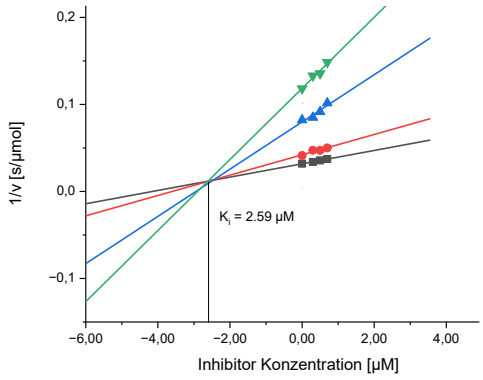


Abbildung 30: Dixon-Plot von Verbindung **11**

Mit den aktivsten Verbindungen wurde das molecular modeling durchgeführt und es bestätigte diese Ergebnisse und zeigte, wie die Verbindungen **11**, **12** und **15** im aktiven Zentrum des Enzyms interagierten. Acetylgruppen schienen die Bindung durch Wasserstoffbrückenbindungen oder hydrophobe Wechselwirkungen zu verstärken, was zu einer erhöhten Aktivität führte. Zusätzlich bestätigten kinetische Messungen, dass diese Verbindungen als kompetitive Inhibitoren von CA II wirkten.

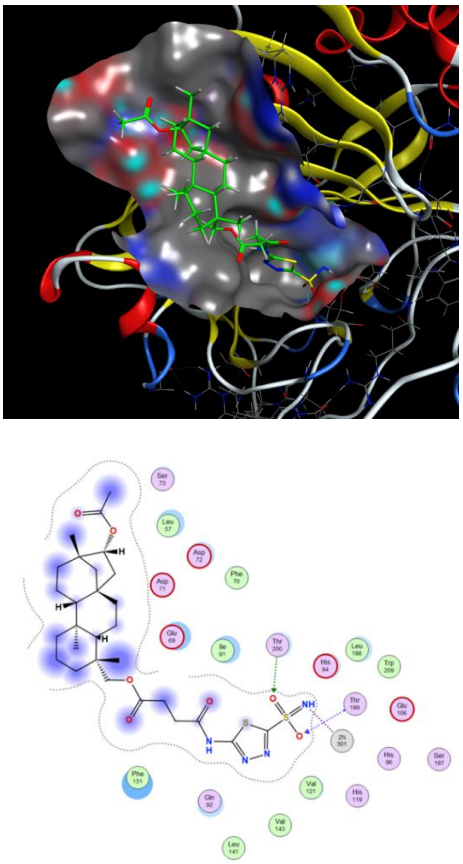


Abbildung 31: 3D - und 2D-Modeling von Verbindung **11** im aktiven Zentrum der CA II

P6

Lupane acetates in small molecule drug hybrids: Probing their inhibitory activity for carbonic anhydrase II

Um die Erkenntnisse aus den vorangegangenen Untersuchungen weiter zu vertiefen, wurden Strukturen mit Substituenten gesucht, die gezielte Wechselwirkungen mit dem aktiven Zentrum der Carboanhydrase II ermöglichen. Untersuchungen zeigten bereits[91], dass pentacyclische Triterpene vielversprechende Grundstrukturen für die Entwicklung neuartiger Inhibitoren der CA II darstellen. Betulin, Betulinsäure, Glycyrrhetinsäure, Ursolsäure und Oleanolsäure wurden als beispielhafte Musterverbindungen ausgewählt. Diese Verbindungen wurden acetylisiert und über verschiedene Spacer mit Taurinamid oder de-acetyliertem Acetazolamid umgesetzt.

Die Synthese begann mit der Acetylierung von Betulinsäure (BS), was zu dem entsprechenden Acetat (AcBS) führte. Durch weitere Reaktionen mit Oxalylchlorid sowie Taurinamid oder de-acetyliertem Acetazolamid wurde eine Reihe von Konjugaten hergestellt.

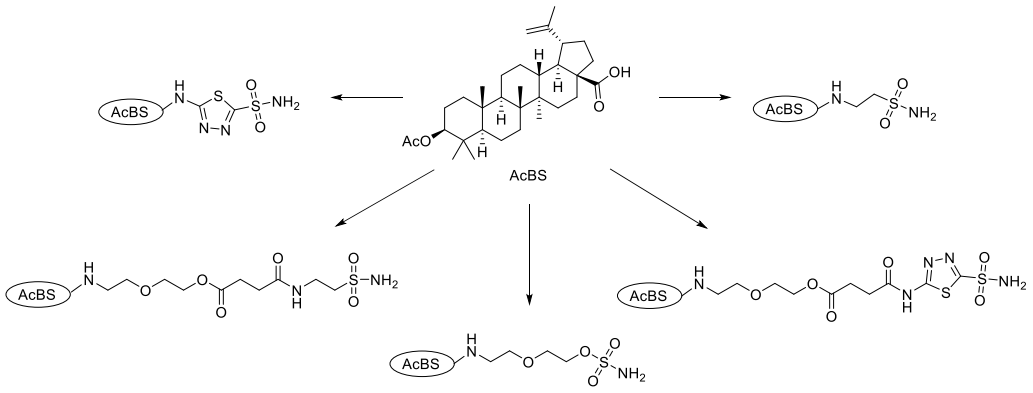


Abbildung 32: Syntheseschema der in P6 vorgenommen Derivatisierungen am Beispiel der acetylierten Betulinsäure (AcBS)

Im Fokus stand zudem eine Struktur-Wirkungsbeziehung durch Variation der Spacer zwischen der Sulfamatgruppe und dem Triterpengrundgerüst zu bestimmen. Verbindungen mit längeren Spacern zeigten eine stärkere CA II-Inhibierung, wahrscheinlich aufgrund der besseren Positionierung der Sulfamatgruppe, ohne dass das sperrige Triterpen in das aktive Zentrum des Enzyms eindringen musste.

Von den getesteten Substanzen erwiesen sich Verbindung **8** und **18**, abgeleitet von Betulinsäure bzw. Betulin, als die stärksten kompetitiven Inhibitoren der CA II. Verbindung **8** hatte einen K_i-Wert von 1,27 µM, während Derivat **18** mit einem noch besseren K_i-Wert von 0,129 µM zeigte.

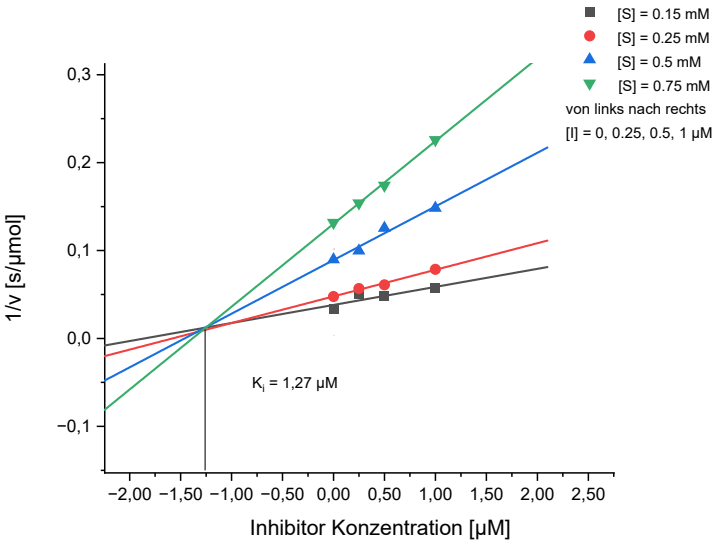


Abbildung 33: Dixon-Plot von Verbindung **8**

Die anderen pentacyclischen Triterpene Glycyrrhetin-, Ursol- und Oleanolsäure wurden ebenfalls in die entsprechenden Acetate umgewandelt und über einen

ähnlichen Syntheseweg mit Taurinamid oder de-acetyliertem Acetazolamid verknüpft. Ihre Inhibierung auf CA II war jedoch weniger stark als die der lupanbasierten Verbindungen.

Das molecular modeling unterstützte diese Ergebnisse und zeigte, dass Verbindungen mit längeren Spacern die Sulfamatgruppe effektiv im aktiven Zentrum des Enzyms positionieren konnten, was die Inhibierungswirkung verstärkte.

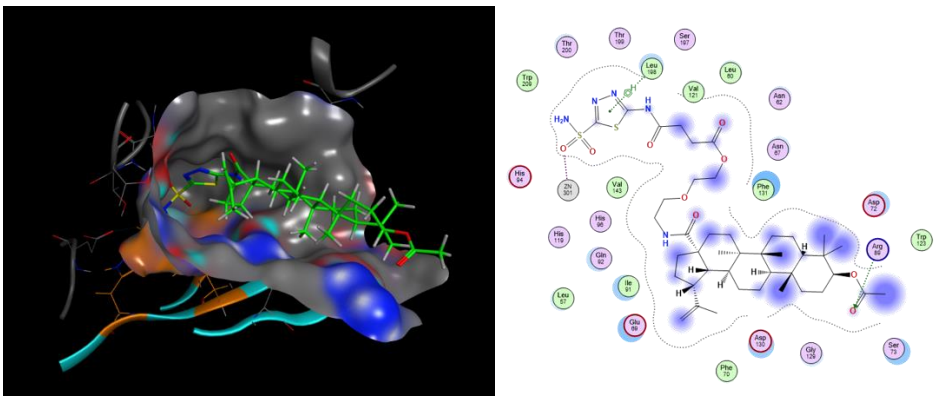


Abbildung 34: 3D - und 2D-Modeling von Verbindung 8 im aktiven Zentrum der CA II

P7

An asiatic acid derived trisulfamate acts as a nanomolar inhibitor of human carbonic anhydrase VA

Um die Wirkung der Triterpene als Inhibitoren weiter zu untersuchen, wurden sulfamoylierte Benzylamide, die aus fünf ausgewählten Triterpenen abgeleitet wurden, synthetisiert: Oleanolsäure, Maslinsäure, Betulinsäure, Platansäure und Asiasäure. Diese Verbindungen repräsentieren drei verschiedene triterpenoide Grundgerüste: Oleanan, Lupanan und Ursan. Die Synthese begann mit der Acetylierung der Ausgangstriterpene, wodurch ihre entsprechenden Acetate erhalten wurden. Anschließend wurden diese Acetate mit Oxalylchlorid und anschließend mit Benzylamin zu den entsprechenden Benzylamiden umgesetzt. Nach diesem Schritt wurden die Amide de-acetyliert, was zu den Zwischenprodukten **16**–**20** führte. Im letzten Schritt wurden die Hydroxylgruppen dieser Zwischenprodukte mit Hilfe von Natriumhydrid deprotoniert, was die Reaktion mit Sulfamoylchlorid ermöglichte und schließlich zu den endgültigen sulfamoylierten Benzylamiden (**21**–**25**) führte.

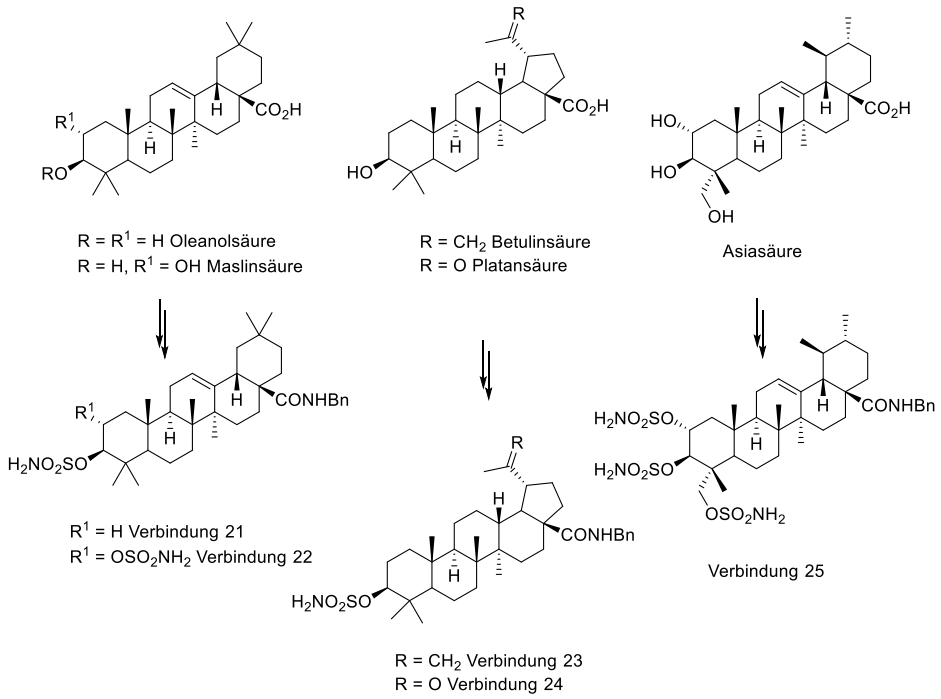


Abbildung 35: Derivatisierungen am den Triterpengrundgerüsten in P7

Die Endverbindungen wurden anschließend auf ihre Fähigkeit hin getestet verschiedene Isoformen der humanen Carboanhydrase (hCA I, hCA II, hCA VA und hCA IX) zu inhibieren.

Bei der biologischen Evalurierung zeigte das Derivat **23**, das auf Betulinsäure basiert, bereits eine starke Inhibierung der hCA VA mit einem K_i-Wert von 88,1 nM. Das Asiasäurederivat **25** wies jedoch eine noch größere Aktivität auf und erreichte einen K_i-Wert von 36,2 nM für hCA VA. Somit zeigt Verbindung **25** sogar einen besseren Wert als der Standardinhibitor Acetazolamid (AAZ), welches einen K_i-Wert von 63,0 nM für dieselbe Isoform aufweist.

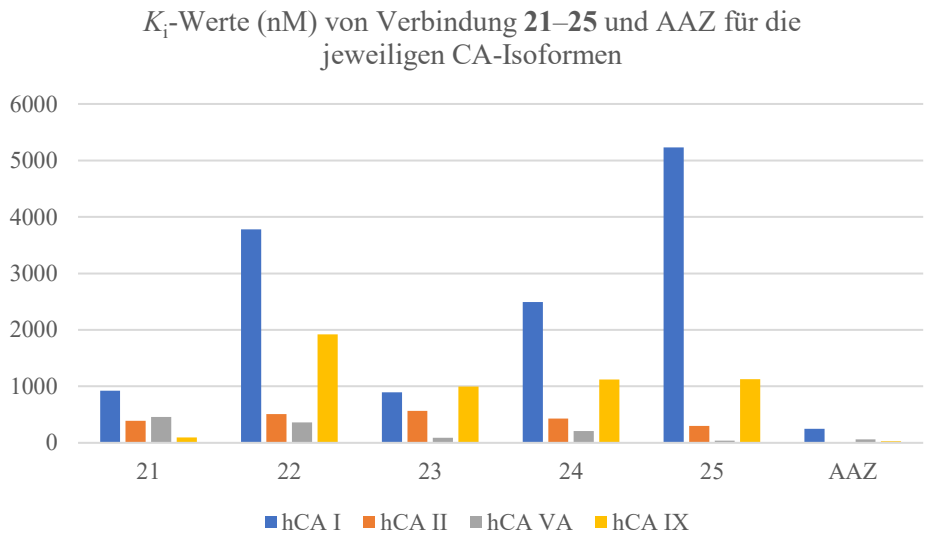


Abbildung 36: Inhibierungsprozente der Endverbindungen gegen verschiedene CA-Isoformen

Die Selektivitätsindizes (SI) zeigten, dass Verbindung **25** einen SI von 144,5 für hCA VA im Vergleich zu hCA I aufwies, was auf eine hohe Isoformspezifität hinweist. Im Gegensatz dazu war Verbindung **23**, obwohl sie eine moderate Selektivität für hCA VA zeigte, weniger wirksam gegen hCA IX, was darauf hindeutet, dass kleine strukturelle Unterschiede im triterpenoiden Grundgerüst einen großen Einfluss auf die Isoformselektivität haben.

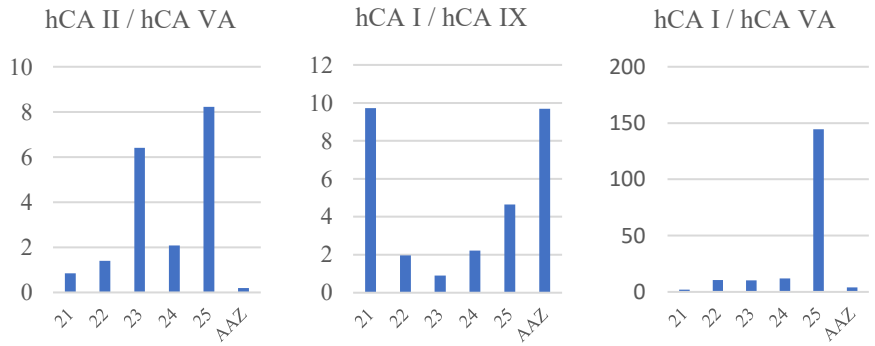


Abbildung 37: Vergleich der SI-Werte der Endverbindungen in P7

Darüber hinaus zeigte Verbindung **21**, die auf Oleanolsäure basiert, eine moderate Inhibierung der hCA IX mit einem K_i-Wert von 95,2 nM.

Für die Bewertung der synthetisierten Inhibitoren ist zudem relevant, inwieweit eine Aufnahme durch den Körper möglich ist. Daher wurde die Wasserlöslichkeit von dem Asiasäurederivat **25** eingehender untersucht. Während die

Ausgangsverbindungen kaum in Wasser löslich sind (mit einer Löslichkeit zwischen 0,01 und 0,02 µg/mL), zeigte Verbindung **25** eine 20-fach verbesserte Löslichkeit, was ihr Potenzial für therapeutische Anwendungen erhöht. Eine Toxizitätsschätzung mittels ProTox II ergab einen LD_{50}-Wert von über 3 g/kg, was darauf hindeutet, dass Verbindung **25** für weitere biologische Tests geeignet sein könnte.

Unter den getesteten Verbindungen erwies sich das auf Asiasäure basierende Derivat **25** als der potenteste Inhibitor von hCA VA, mit einem K_i-Wert von 36,2 nM und einer guten Selektivität auf die Isoform hCA VA im Vergleich zur der Isoform hCA I und zum Standardinhibitor Acetazolamid. Verbindung **25** zeigte zudem eine verbesserte Löslichkeit und ein günstiges Toxizitätsprofil, was sie zu einem vielversprechenden Kandidaten für die weitere therapeutische Erforschung macht, insbesondere zur Behandlung von Fettleibigkeit und verwandten Erkrankungen. Verbindung **21** zeigte sich hingegen als potenzieller Ansatzpunkt zur Behandlung von Hirnödemen und neurodegenerativen Erkrankungen wie Alzheimer, aufgrund ihrer Selektivität für hCA I.

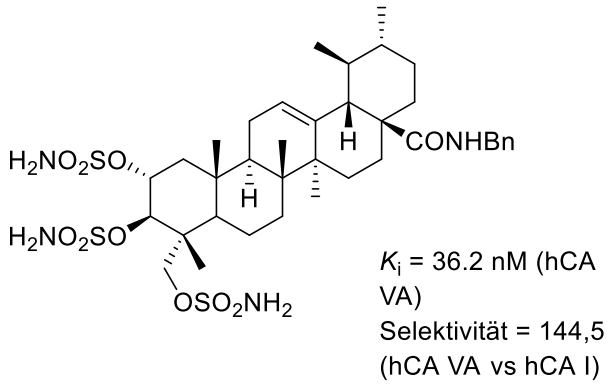


Abbildung 38: Das Asiasäurederivat als aktivste Verbindung aus der Reihe

P8

Small structural differences govern the carbonic anhydrase II inhibition activity of cytotoxic triterpene acetazolamide conjugates

Die Synthese der neuartigen CA II Inhibitoren begann mit der Auswahl der vier pentazyklischen Triterpenen Betulin (BN), Oleanolsäure, Ursolsäure und Glycyrrhetinsäure (GS) als Ausgangsstoffe. BN wurde nach bekannten Verfahren in das Diacetat umgewandelt, dessen selektive Mono-Deacylierung mit CaH_2 das 3-*O*-Acetat lieferte. Die Reaktion von dem Betulinderivat mit Bernsteinsäureanhydrid in Pyridin in Gegenwart von DMAP führte zum Derivat **3**. Die Deacetylierung von Acetazolamid mit konzentrierter Salzsäure unter Rückfluss ergab das 5-Amino-1,3,4-thiadiazol-2-sulfonamid (Verbindung **5**), welches

anschließend mit den Zwischenverbindungen umgesetzt werden konnte. Die Kupplung von Verbindung **3** mit Verbindung **5** zur Bildung des Konjugats **6** stellte sich zunächst als schwierig heraus. Versuche mit in situ generierten Carbonsäurechloriden sowie die Verwendung von Kupplungsreagenzien wie EDC, DCC, PyBOP oder T_3P schlugen fehl. Schließlich führte die Reaktion von Verbindung **3** mit Ethylchloroformiat in Gegenwart von 4-Methylmorpholin in THF zur Bildung eines gemischten Anhydrids, dessen anschließende Reaktion mit Verbindung **5** und einer Ausbeute von 88 % zu Verbindung **6** führte.

Für die Synthese der entsprechenden Analoga aus OS, US und GS mussten OS und US zunächst mit $LiAlH_4$ reduziert werden, was deren Diol-Derivate in guten Ausbeuten lieferte. Diese wurden in die entsprechenden Diacetate umgewandelt, deren selektive Deacetylierung die Monoacetate **11** und **12** ergab. Nach den oben beschriebenen Bedingungen wurden die Verbindungen **11** und **12** zu den Bernsteinsäure-Derivaten **13** und **14** umgewandelt. Da die Reduktion von GS mit $LiAlH_4$ keine guten Ausbeuten lieferte, wurde GS zunächst in das Acetat **15** überführt, das mit Ethylchloroformiat/TEA zu einem unisolierten gemischten Anhydrid reagierte, das anschließend mit $NaBH_4$ bei Raumtemperatur reduziert wurde und Verbindung **16** in guten Ausbeuten ergab. Die Reaktion von **16** mit Bernsteinsäureanhydrid führte zu Verbindung **17**. Die Kupplung der Verbindungen **13**, **14** und **17** mit **5** ergab die Produkte **18**-**20**.

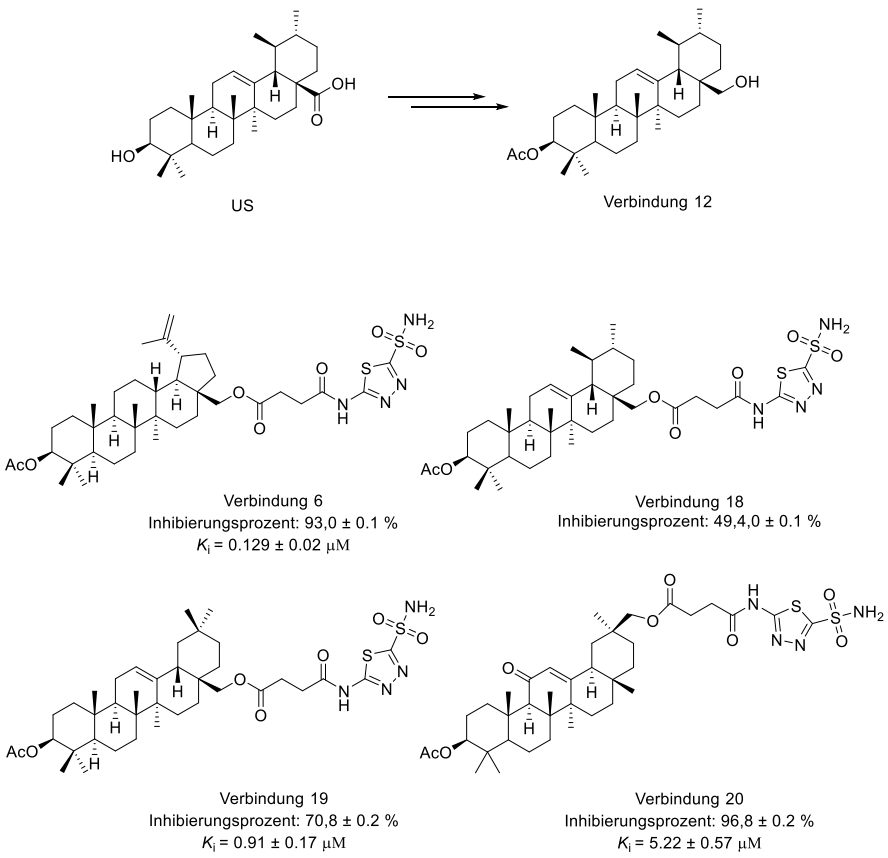


Abbildung 39: Endverbindungen und bestimme Inhibierungsprozente und Inhibierungskonstanten in P8

Verbindungen **6** und **18**-**20** wurden auf ihre Aktivität gegenüber CA II getestet. Diese Tests zeigten, dass das von der Glycyrrhetinsäure abgeleitete Konjugat **20** die stärkste Aktivität aufwies, gefolgt von dem Betulin-Derivat **6**. Die von Oleanolsäure und Ursolsäure abgeleiteten Konjugate zeigten eine geringere Inhibierung auf CA II. Für die Verbindungen mit der höchsten Aktivität, d.h. **6**, **19** und **20**, wurden zusätzliche Messungen durchgeführt, um ihre jeweiligen Inhibitionskonstanten K_i zu bestimmen. Die Ergebnisse dieser Experimente sind in Abbildung 39 zusammengefasst; Abbildung 40 zeigt den Dixon-Plot für Verbindung **6**, die als kompetitiver Inhibitor des Enzyms mit einem niedrigen K_i-Wert von 0.129 μM wirkt.

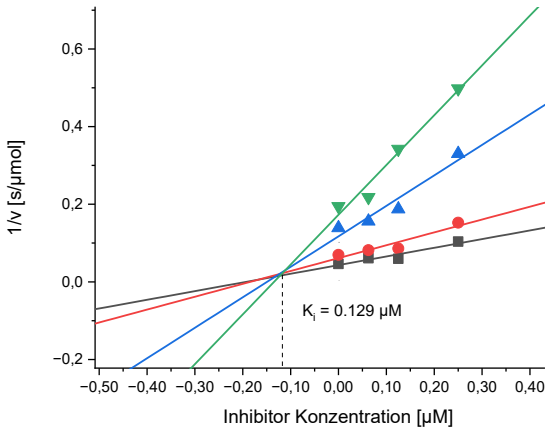


Abbildung 40: Dixon-Plot von Betulin-Derivat Verbindung **6**

Zudem wurde molecular modelling durchgeführt, um Einblicke in den Wirkungsmechanismus der Verbindungen zu gewinnen. Diese Berechnungen lieferten jedoch keine schlüssige Erklärung für die unterschiedliche Fähigkeit der Konjugate, das Enzym zu inhibieren. Während es plausibel erscheint, dass die Acetazolamid-Einheit ähnlich wie das Ausgangs-Acetazolamid mit dem aktiven Zentrum des Enzyms interagiert, kann nicht ausgeschlossen werden, dass die Konjugate auch als Nicht-Zink-bindende Inhibitoren wirken, was frühere Erkenntnisse über strukturell ähnliche pentazyklische Triterpenoide wie Arjunolsäure widerspiegelt[92].

Die Verbindungen **6** und **20** zeigten die höchste Zytotoxizität gegenüber humanen Tumorzelllinien, was mit ihrer Inhibierung gegenüber CA II korrelierte. Eine geringere Zytotoxizität wurde für die von Oleanolsäure oder Ursolsäure abgeleiteten Verbindungen **18** und **19** festgestellt. Die Selektivität zwischen malignen und nicht-malignen Zellen war jedoch für alle Verbindungen gering (Vgl. Tabelle 4).

Tabelle 4: Zytotoxizitätergebnisse von Acetazolamid (**4**) sowie der Konjugate **6** und **18**–**20**, bestimmt mittels SRB-Assay (EC_{50}-Werte [µM] nach 72 h Inkubation). Untersuchte humane Tumorzelllinien: A375 (epitheliales Melanom), HT29 (kolorektales Adenokarzinom), MCF-7 (Mammakarzinom), A2780 (Ovarialkarzinom), HeLa (Zervixkarzinom); nicht-malign: NIH 3T3 (Fibroblasten). n.d.: nicht bestimmt; positive Kontrolle: Doxorubicin (**DX**).

	A375	**HT29**	**MCF-7**	**A2780**	**NIH 3T3**
4	> 30	> 30	> 30	> 30	> 30
6	8.5±0.7	10.2±1.3	8.9±0.7	9.3±1.2	9.5±1.0
18	10.1±0.8	14.2±1.4	10.6±1.4	11.8±1.4	14.0±1.5
19	13.7±1.1	15.0±0.6	12.4±0.8	12.5±1.7	14.8±1.5
20	9.2±0.5	13.0±1.3	10.5±1.2	9.8±0.8	11.9±1.7
DX	n.d.	0.25±0.02	0.1±0.01	0.1±0.01	0.01±0.001

Zusammenfassung und Ausblick

In der vorliegenden Dissertation wurden neuartige Inhibitoren der Carboanhydrase II basierend auf Sulfonamiden, Aminosäurederivaten, Diterpenen und Triterpenen entwickelt und umfassend charakterisiert. Ziel war es, über die gezielte Analyse von Struktur-Wirkungs-Beziehungen (SAR) Grundlagen für die Optimierung potenter und selektiver Enzyminhibitoren zu schaffen.

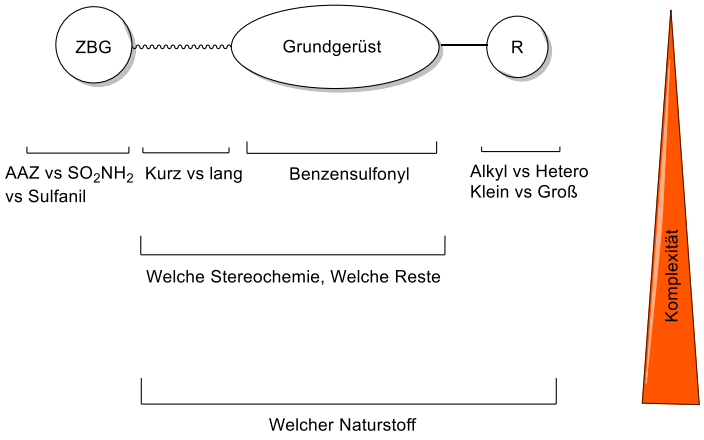


Abbildung 41: Modifikationen an einem allgemeinen CAI für die Durchführung einer SAR

Im Rahmen der Arbeiten P1 und P2 wurden zunächst verschiedene Phenylsulfonamidoalkylsulfamate synthetisiert und hinsichtlich ihrer Inhibierungsaktivität gegenüber CA II untersucht. Im Mittelpunkt stand dabei die Frage, inwieweit strukturelle Variationen, wie beispielsweise Alkylsubstituenten unterschiedlicher Größe, der Einbau von Heteroatomen sowie die Position der Substituenten am aromatischen Ringsystem in Kombination mit der Länge des Spacers die Inhibierung der CA II beeinflussen. Besonders para-substituierte Verbindungen mit *tert*-Butylgruppen und kurzen Spacern (z. B. P1, Verbindung **49b**) erreichten hohe Inhibierungsraten mit einem K_i-Wert von 0,67 µM. Das molecular modeling belegte, dass diese Substitutionen stabile π-Wechselwirkungen mit His94 im aktiven Zentrum eingehen und die Sulfamatgruppe optimal am katalytischen Zink(II)-Ion positioniert wird. Gleichzeitig zeigte sich, dass übermäßig sperrige Gruppen wie Adamantyl (P1, Verbindung **55b**) oder Cyclohexyl (P1, Verbindung **53b**) die Inhibierung durch sterische Hinderung verschlechtern, da sie eine effiziente Orientierung im aktiven Zentrum behindern.

Die Arbeiten P3 und P4 erweiterten die Untersuchungen um Aminosäure-abgeleitete Sulfonamide und Ureidobenzensulfonamide. Hierbei zeigte sich, dass die absolute

Konfiguration der Inhibitoren einen entscheidenden Einfluss auf die Affinität zur CA II hat. (*R*)-konfigurierte Derivate, wie P3, Verbindung **15b** (vom Tryptophan abgeleitet), wiesen eine fast vollständige Inhibierung von 97,9 % bei einer Inhibitorkonzentration von 10 µM auf, während die (*S*)-konfigurierte Analoga kaum Aktivität zeigten. Die modeling zeigte, dass die (*R*)-Konfiguration zusätzliche Wasserstoffbrückenbindungen sowie eine optimale Anpassung an die Enzymstruktur ermöglicht. In P4 wurden außerdem Ureidobenzensulfonamide entwickelt, die eine selektive Inhibierung tumorassoziierter Isoformen wie hCA IX und hCA XII ermöglichen konnten (z. B. P4, Verbindung **3** mit einem K_i von 1,0 nM für hCA XII).

Ein weiterer Schwerpunkt der Arbeit war die gezielte Nutzung von Naturstoffen, deren strukturelle Merkmale zentrale Aspekte der zuvor untersuchten Substituenteneffekte und stereochemischen Einflüsse vereinen. Neben den bisher betrachteten Endgruppen (ZBG), wird nun auch die vom Acetazolamid abgeleitete 5-Amino-1,3,4-thiadiazol-2-sulfonamid-Endgruppe betrachtet. In P5 wurde Isosteviol als Ausgangsstruktur für neue CA II-Inhibitoren verwendet. Durch gezielte Funktionalisierungen mit Sulfamat- und Acetylgruppen konnten mehrere aktive Derivate generiert werden, welche mit deren weniger aktiven Derivaten verglichen werden konnten. Neben der getesteten biologischen Aktivität belegt das molecular modeling, dass sowohl die Platzierung der Sulfamatgruppe zur direkten Koordination an das Zinkion als auch hydrophobe Wechselwirkungen entscheidend für die Bindung und somit eine effektive Inhibierung sind. Verbindungen wie P5, Verbindung **14**, bei denen diese Ausrichtung nicht optimal gelang, zeigten hingegen eine signifikant reduzierte Inhibierungsaktivität.

Im Bereich der Triterpene (P6–P8) wurden Betulin, Betulinsäure, Ursolsäure, Oleanolsäure, Maslinsäure, Glycyrrhetinsäure und Asiasäure als Grundstrukturen untersucht. In P6 wurden Konjugate aus Betulinsäure und de-acetyliertem Acetazolamid mit unterschiedlich langen Spacern synthetisiert. Besonders Verbindung **18** aus P6 zeigte mit einem K_i von 0,129 µM die höchste Inhibierung von CA II. Hier zeigte sich, dass längere und flexible Spacer entscheidend sind, um die aktive Endgruppe effizient ins aktive Zentrum der CA II einzuführen, während das voluminöse Triterpengrundgerüst außerhalb der Bindungstasche verbleibt. Zu kurze oder starre Spacer (z. B. P6, Verbindung **12**) behinderten die Platzierung der aktiven Gruppe und führten zu reduzierter Inhibierung.

Auch die Arbeiten aus P7 und P8 bestätigten diese Ergebnisse. So konnte das Asiasäurederivat, P7 Verbindung **25**, eine hochselektive Inhibierung der

mitochondrialen Isoform hCA VA mit einem K_i von 36,2 nM erreichen. Durch gezielte Platzierung von drei Sulfamatgruppen und geeignete Spacerlängen wurde eine hohe Affinität bei gleichzeitig verbesserter Löslichkeit und günstiger Toxizitätsprofilierung erzielt. In P8 gelang es, durch Derivatisierung von Glycyrrhetinsäure mit Acetazolamid (P8, Verbindung **20**) eine starke CA II Inhibierung zu erzielen, wobei strukturelle Modifikationen an den Alkylspacern entscheidenden Einfluss auf die Aktivität hatten.

Die Auswertung der Arbeiten P1 bis P8 erlaubt die Ableitung zentraler Strukturmerkmale für eine effiziente Inhibierung der Carboanhydrase II. Wesentliche Einflussgrößen stellen die Zink-chelatierende Endgruppe, das molekulare Trägergerüst sowie die Spacerstruktur dar.

Unter den Endgruppen zeigten Sulfamat-, Sulfanilamid- und Acetazolamidmotive hohe Wirksamkeit. Sulfamate bewährten sich insbesondere in Kombination mit aromatischen Einheiten (z. B. P1, **49b**), während Acetazolamid, eingebunden in triterpenoide Konjugate (z. B. P8), durch eine hohe Affinität überzeugte. Sulfanilamidstrukturen (z. B. P4) ermöglichten bei gezielter Substitution eine hohe Isoformenselektivität. Die Sulfamatgruppe erscheint aufgrund ihrer geringen Größe und guten Koordinationseigenschaften als besonders vielseitig einsetzbar.

Als Trägergerüste erwiesen sich lupanbasierte Triterpene wie Betulin oder Betulinsäure (P6, **18**) als besonders geeignet, gefolgt von Asiasäure (P7, **25**). Die Wahl des Gerüsts sollte mit Blick auf die Spacerwahl erfolgen: kleine aromatische Systeme profitieren von kurzen Alkylketten (n = 2), während bei voluminösen Naturstoffen längere und flexible Spacer wie Succinylspacer günstiger sind, um die Endgruppe besser zu positionieren.

Substituenten wie *para*-positionierte tert-Butylgruppen (P1, **49b**) oder Methyl-oder Methoxygruppen (P2) unterstützten die Bindung über hydrophobe oder elektronendonierende Effekte. Zu große Gruppen (z. B. Adamantyl) sowie überladene Mehrfachsulfamoylierungen (P5, **14**/**16**) verringerten die Aktivität. Acetylgruppen (P5, **11**/**12**) der Benzylgruppen (P7, **25**) verbesserten die Bindung durch zusätzliche Wechselwirkungen. Auch die Stereochemie spielte bei einfachen Strukturen eine Rolle: (*R*)-konfigurierte Aminosäurederivate (P3, **15b**) zeigten eine signifikant höhere Inhibierung als ihre (*S*)-Analoga.

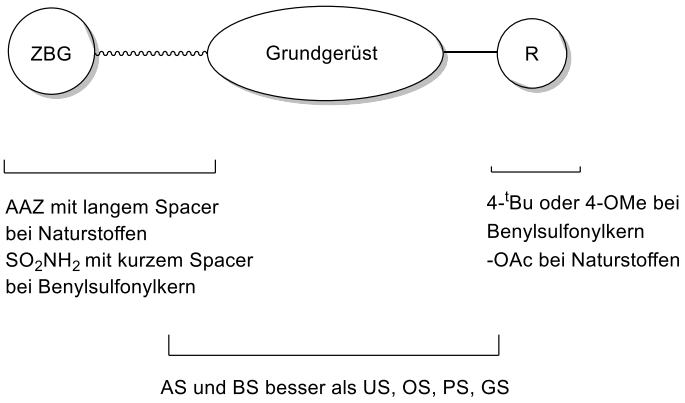


Abbildung 42: Basierend auf den Ergebnissen von P1 bis P8 optimaler Inhibitor der CA II

Nichtsdestotrotz lassen sich die Ergebnisse nicht pauschalisieren, da es stets strukturbezogene Vor- und Nachteile gibt, wie beispielsweise hinsichtlich der Isoformenselektivität oder Membrandurchlässigkeit. Vielmehr müssen einzelne Strukturelemente stets im Kontext des jeweiligen Inhibitordesigns bewertet werden, da deren Einfluss stark von der Kombination mit dem Grundgerüst, der Endgruppe sowie dem Spacer abhängt.

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Abbildungsverzeichnis

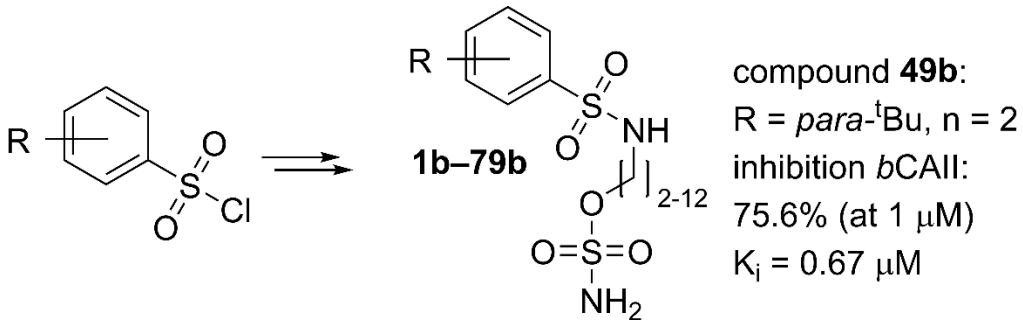
Anhang

Publikationen

Die dieser Dissertation zugrundeliegenden Publikationen sind nachfolgend aufgelistet:

Publikation P1: „*Synthesis and Enzymatic Evaluation of a Small Library of Substituted Phenylsulfonamido-Alkyl Sulfamates towards Carbonic Anhydrase II*“ *Denner, T.C.; Heise, N.V.; Al-Harrasi, A.; Csuk, R. Synthesis and Enzymatic Evaluation of a Small Library of Substituted Phenylsulfonamido-Alkyl Sulfamates towards Carbonic Anhydrase II. Molecules* ***2024****, 29, 3015.*

Graphical Abstract



Abstract

A small library of 79 substituted phenylsulfonamidoalkyl sulfamates, 1b–79b, was synthesized starting from arylsulfonyl chlorides and amino alcohols with different numbers of methylene groups between the hydroxyl and amino moieties yielding intermediates 1a–79a, followed by the reaction of the latter with sulfamoyl chloride. All compounds were screened for their inhibitory activity on bovine carbonic anhydrase II. Compounds 1a–79a showed no inhibition of the enzyme, in contrast to sulfamates 1b–79b. Thus, the inhibitory potential of compounds 1b–79b towards this enzyme depends on the substituent and the substitution pattern of the phenyl group as well as the length of the spacer. Bulkier substituents in the *para* position proved to be better for inhibiting CAII than compounds with the same substituent in the *meta* or *ortho* position. For many substitution patterns, compounds with shorter spacer lengths were superior to those with long chain spacers. Compounds with shorter spacer lengths performed better than those with longer chain spacers for a variety of substitution patterns. The most active compound held inhibition constant as low as K_i = 0.67 μM (for 49b) and a tert-butyl substituent in *para* position and acted as a competitive inhibitor of the enzyme.

Keywords

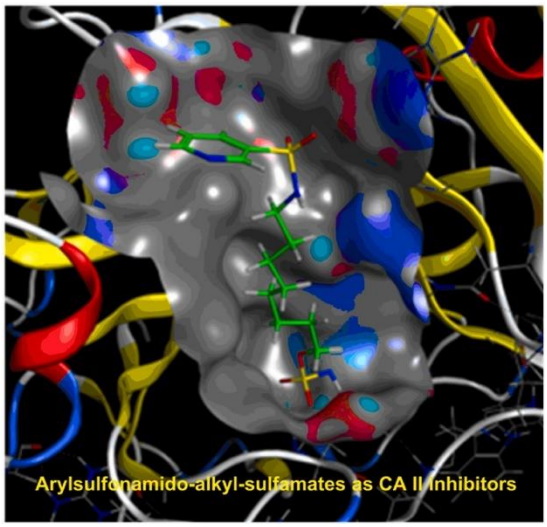
sulfamates; carbonic anhydrase II; inhibitor

DOI

https://doi.org/10.3390/molecules29133015

Publikation P2: „*Arylsulfonamido-alkyl-sulfamates act as inhibitors of bovine carbonic anhydrase II*“

Graphical Abstract



Abstract

A small library of arylsulfonamido-alkyl sulfamates was prepared by a two-step synthesis from readily available starting materials. The compounds were tested for their ability to inhibit bovine carbonic anhydrase II. Several of them were found as good competitive inhibitors holding K_i values as low as $K_i = 0.9$ μM (compound **47b**). The activity was influenced by the substitution pattern of the arylsulfonamide moiety as well as the length of the spacer to the distal sulfamate group. Molecular docking studies were used to substantiate these findings. For the aryl-substituted analogues, the increase in inhibitory activity for compounds with a shorter spacer can be explained by stabilization via aromatic π-interactions. For the cyclopropyl or methylsulfonyl substituted analogues, their inhibitory activity can be attributed to their reduced steric hindrance. These results provide a basis for designing effective CA II inhibitors.

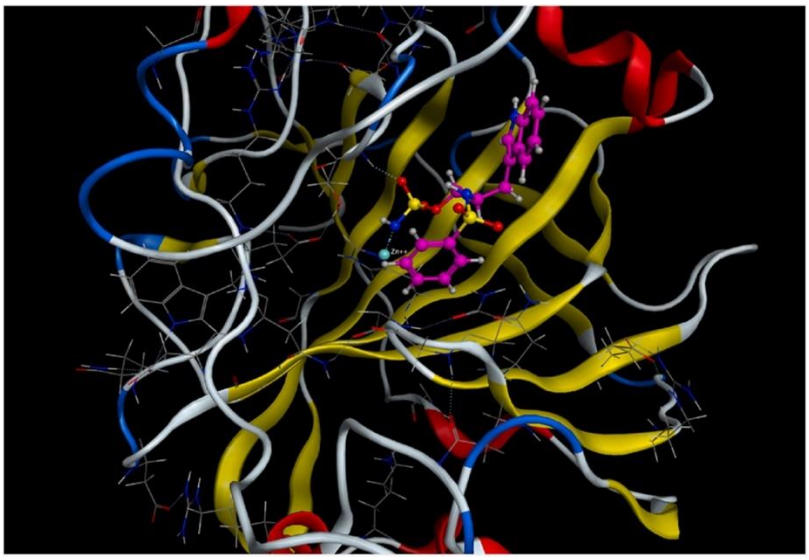
Keywords

Sulfamates, Carbonic anhydrase II, Inhibitor

DOI: https://doi.org/10.1016/j.ejmcr.2024.100177

Publikation P3: „*Stereochemistry matters: inhibition of carbonic anhydrase II by amino acid derived sulfamates depends on their absolute configuration"*

Graphical Abstract



Abstract

Aminoalcohols were converted into the corresponding enantiomeric phenylsulfonamide sulfamates. These compounds proved to be inhibitors of carbonic anhydrase II. Interestingly, a sulfamate derived from (*S*)-tryptophan was no inhibitor at all while its (*R*) configurated enantiomer was an excellent inhibitor of carbonic anhydrase II (CA II). A rationale can be deduced from molecular modeling studies. The sulfamates derived from (*R*) or (*S*) proline held very low inhibition constants for this enzyme as K_i = 0.77 µM and 0.70 µM, respectively.

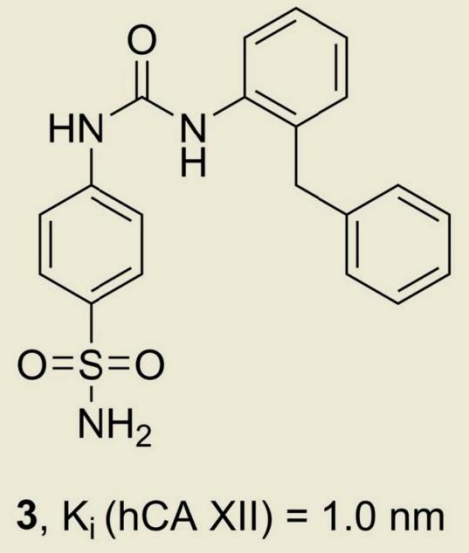
Keywords

Carbonic anhydrase II, Inhibitor, Sulfamates, Sulfonamides

DOI: https://doi.org/10.1016/j.ejmcr.2024.100162

Publikation P4: „*Ureidobenzenesulfonamides as selective carbonic anhydrase I, IX, and XII inhibitors"*

Graphical Abstract



Abstract

Sulfonamides remain an important class of drugs, especially because of their inhibitory effects on carbonic anhydrases. Herein, we have synthesized several sulfonamides and tested them for their inhibitory activity against carbonic anhydrases hCA I, hCA II, hCA IX, and hCA XII, respectively. Thereby, biphenyl- and benzylphenyl-substituted sulfonamides showed high selectivity against hCA IX and hCA XII; these enzymes are common targets in the treatment of hypoxic cancers, and noteworthy inhibitory activity was observed for several compounds toward hCA I that might be of interest for future applications to treat cerebral edema. Compound 3 (4-[3-(2-benzylphenyl)ureido]benzenesulfonamide) held an exceptionally low Ki value of 1.0 nM for hCA XII.

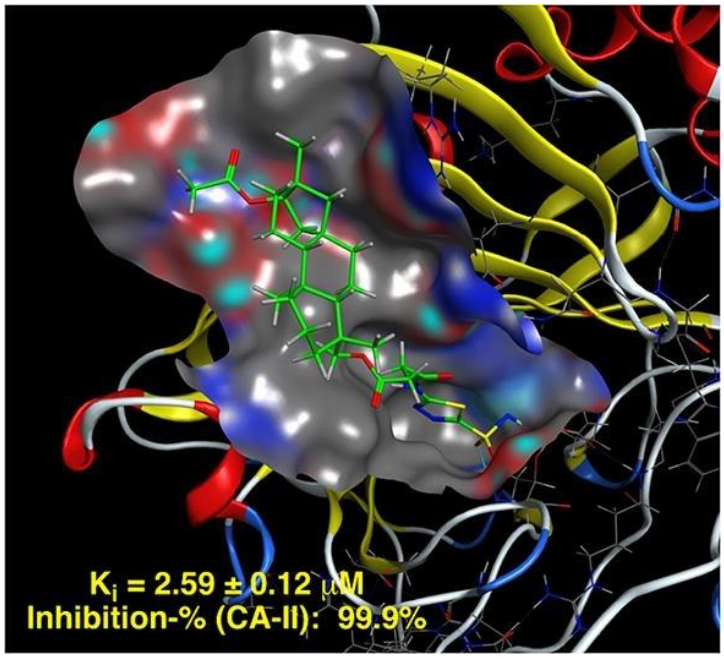
Keywords

carbonic anhydrase; inhibitor; ureidobenzenesulfonamides

DOI: https://doi.org/10.3390/molecules28237782

Publikation P5: „*Isosteviol – A new scaffold for the synthesis of carbonic anhydrase II inhibitors"*

Graphical Abstract



Abstract

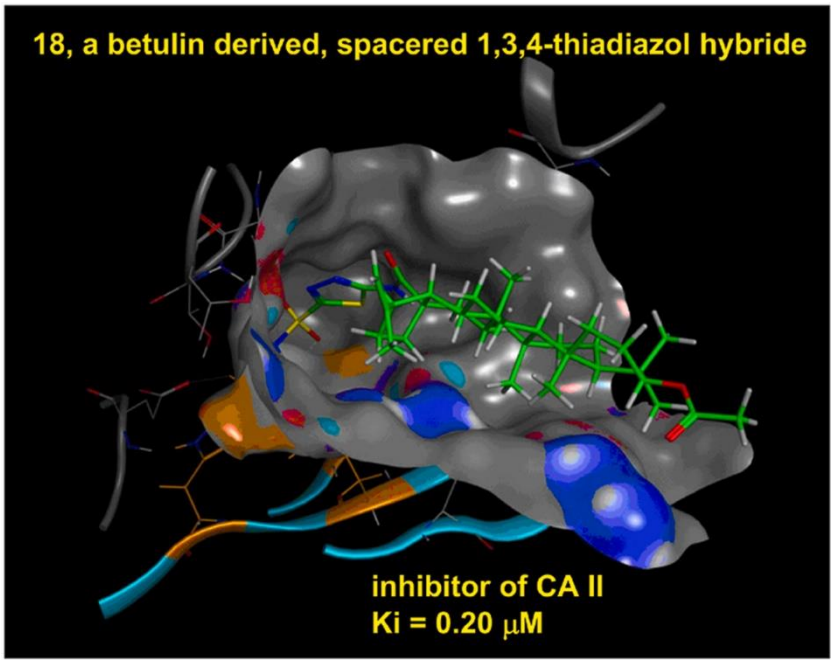
The diterpene isosteviol can easily be synthesized via hydrolysis from stevioside, a renewable resource adding particular utility to its potential for drug synthesis. Of late, there has been an increase in scientific studies focusing on inhibitors of the enzyme carbonic anhydrase II, CA II, since this enzyme is not used just in glaucoma treatment but also in mitigating the side effects of Alzheimer's disease antibody therapy. The presence of a sulfamate or a sulfonamide group is a key structural feature in many CA II inhibitors. Thus, isosteviol was transformed into sulfamates, either spacered or unspacered, to scrutinize their ability to perform as CA II inhibitors. Three particular derivatives were discovered to be effective inhibitors. As a competitive inhibitor with a K_i = 2.59 µM, a 16-*O*-acetyl-isosteviol compound holding a 5-amino-1,3,4-thiadiazol-2-yl-amino substituent attached to a succinoyl ester nearly entirely inhibited CA II.

Keywords

DOI: https://doi.org/10.1016/j.rechem.2024.101426

Publikation P6: „*Lupane acetates in small molecule drug hybrids: Probing their inhibitory activity for carbonic anhydrase II"*

Graphical Abstract



Abstract

Earlier studies had shown the potential of modified pentacyclic triterpenes as possible inhibitors of carbonic anhydrase II (CA II). In an extension of our earlier studies, betulin, betulinic acid and, for comparison purposes, glycyrrhetinic acid, ursolic acid and oleanolic acid were therefore converted into the respective acetates and linked to either taurinamide or de-acetylated acetazolamide via a variable linker. In particular, the derivatives **8** and **18** derived from betulinic acid or betulin and provided with a long spacer were found to be strong competitive inhibitors of CA II, thereby holding K_i = 1.27 and 0.20 μM, respectively.

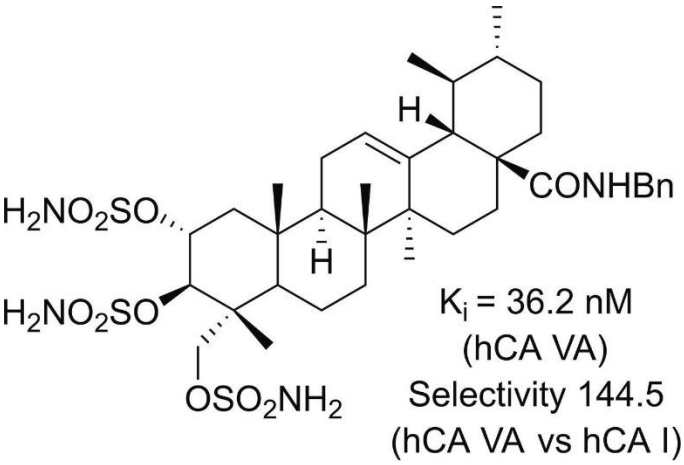
Keywords

Betulin, Betulinic acid, Pentacyclic triterpene, Carbonic anhydrase II, Inhibitor

DOI: https://doi.org/10.1016/j.ejmcr.2024.100139

Publikation P7: „*An asiatic acid derived trisulfamate acts as a nanomolar inhibitor of human carbonic anhydrase VA"*

Graphical Abstract



Abstract

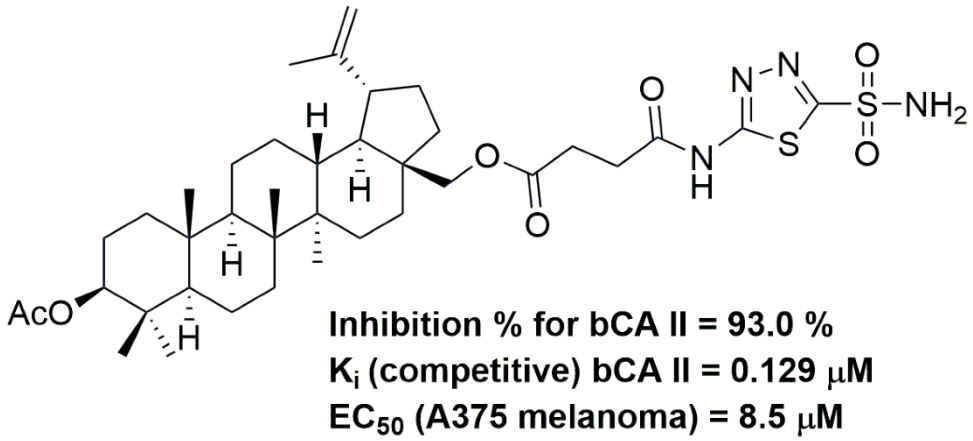
This investigation delves into the inhibitory capabilities of a specific set of triterpenoic acids on diverse isoforms of human carbonic anhydrase (hCA). Oleanolic acid (1), maslinic acid (2), betulinic acid (3), platanic acid (4), and asiatic acid (5) were chosen as representative triterpenoids for evaluation. The synthesis involved acetylation of parent triterpenoic acids 1–5, followed by sequential reactions with oxalyl chloride and benzylamine, de-acetylation of the amides, and subsequent treatment with sodium hydride and sulfamoyl chloride, leading to the formation of final compounds 21–25. Inhibition assays against hCAs I, II, VA, and IX demonstrated noteworthy outcomes. A derivative of betulinic acid, compound 23, exhibited a Ki value of 88.1 nM for hCA VA, and a derivative of asiatic acid, compound 25, displayed an even lower Ki value of 36.2 nM for the same isoform. Notably, the latter compound displayed enhanced inhibitory activity against hCA VA when compared to the benchmark compound acetazolamide (AAZ), which had a Ki value of 63.0 nM. Thus, this compound surpasses the inhibitory potency and isoform selectivity of the standard compound acetazolamide (AAZ). In conclusion, the research offers insights into the inhibitory potential of selected triterpenoic acids across diverse hCA isoforms, emphasizing the pivotal role of structural attributes in determining isoform-specific inhibitory activity. The identification of compound 25 as a robust and selective hCA VA inhibitor prompts further exploration of its therapeutic applications.

Keywords carbonic anhydrase VA, Inhibitor, Sulfamates, Triterpenes, Asiatic acid

DOI https://doi.org/10.1016/j.steroids.2024.109381

Publikation P8: „*Small structural differences govern the carbonic anhydrase II inhibition activity of cytotoxic triterpene acetazolamide conjugates"*

Graphical Abstract



Abstract

Acetylated triterpenoids betulin, oleanolic acid, ursolic acid, and glycyrrhetinic acid were converted into their succinyl-spacered acetazolamide conjugates. These conjugates were screened for their inhibitory activity onto carbonic anhydrase II and their cytotoxicity employing several human tumor cell lines and non-malignant fibroblasts. As a result, the best inhibitors were derived from betulin and glycyrrhetinic acid while those derived from ursolic or oleanolic acid were significantly weaker inhibitors but also of diminished cytotoxicity. A betulin-derived conjugate held a Ki = 0.129 μM and an EC50 = 8.5 μM for human A375 melanoma cells.

Keywords

acetazolamide conjugate; carbonic anhydrase II; cytotoxicity; triterpenoic acid.

DOI: 10.3390/molecules28031009

Erklärung über den Autorenteil

Publikation P1: „*Synthesis and Enzymatic Evaluation of a Small Library of Substituted Phenylsulfonamido-Alkyl Sulfamates towards Carbonic Anhydrase II“*

T.C. Denner, N.V. Heise, A. Al-Harrasi, R. Csuk

Die Synthese der Verbindungen sowie die Auswertung der spektroskopischen Daten und die biologische Evaluierung wurde von mir vorgenommen. Das molecular modelling wurde von N.V. Heise durchgeführt. R. Csuk und A. Al-Harrasi betreuten praktische sowie theoretische Aspekte der Arbeit.

Publikation P2: „*Arylsulfonamido-alkyl-sulfamates act as inhibitors of bovine carbonic anhydrase II“*

T.C. Denner, N.V. Heise, R. Csuk

Die Synthese der Verbindungen sowie die Auswertung der spektroskopischen Daten und die biologische Evaluierung wurde von mir vorgenommen. Das molecular modelling wurde von N.V. Heise durchgeführt. R. Csuk betreute praktische sowie theoretische Aspekte der Arbeit.

Publikation P3: „*Stereochemistry matters: inhibition of carbonic anhydrase II by amino acid derived sulfamates depends on their absolute configuration”*

T.C. Denner, E.L. Klett, N.V. Heise, R. Csuk

Die Synthese der Verbindungen sowie die Auswertung der spektroskopischen Daten und die biologische Evaluierung wurde von mir vorgenommen. E.L. Klett stellte einen Teil der Ausgangsmaterialen her. Das molecular modelling wurde von N.V. Heise durchgeführt. R. Csuk betreute praktische sowie theoretische Aspekte der Arbeit.

Publikation P4: „*Ureidobenzenesulfonamides as selective carbonic anhydrase I, IX, and XII inhibitors”*

T.C. Denner, A. Angeli, C.T. Supuran, R. Csuk

Die Synthese der Verbindungen sowie die Auswertung der spektroskopischen Daten wurde von mir vorgenommen. A. Angeli führte die biologische Evaluierung durch. R. Csuk und C.T. Supuran betreuten praktische sowie theoretische Aspekte der Arbeit.

Publikation P5: „*Isosteviol – A new scaffold for the synthesis of carbonic anhydrase II inhibitors*"

T.C. Denner, N.V. Heise, R. Csuk

Die Synthese der Verbindungen sowie die Auswertung der spektroskopischen Daten und die biologische Evaluierung wurde von mir vorgenommen. Das molecular modelling wurde von N.V. Heise durchgeführt. R. Csuk betreute praktische sowie theoretische Aspekte der Arbeit.

Publikation P6: „*Lupane acetates in small molecule drug hybrids: Probing their inhibitory activity for carbonic anhydrase II*"

T.C. Denner, N.V. Heise, J. Zacharias, R. Csuk

Die Synthese der Verbindungen sowie die Auswertung der spektroskopischen Daten und die biologische Evaluierung wurde von mir vorgenommen. N.V. Heise und J. Zacharias stellten einen Teil der Ausgangsmaterialen her. Das molecular modelling wurde von N.V. Heise durchgeführt. R. Csuk betreute praktische sowie theoretische Aspekte der Arbeit

Publikation P7: „*An asiatic acid derived trisulfamate acts as a nanomolar inhibitor of human carbonic anhydrase VA*"

T.C. Denner, N.V. Heise, I. Serbian, C.T. Supuran, R. Csuk

Die Synthese der Verbindungen sowie die Auswertung der spektroskopischen Daten und ein Teil der biologischen Evaluierung wurde von mir vorgenommen. I. Serbian stellte einen Teil der Ausgangsmaterialen her. R. Csuk und C.T. Supuran betreuten praktische sowie theoretische Aspekte der Arbeit. C.T. Supuran stellte zudem einen Teil der biologischen Evaluierung zur Verfügung.

Publikation P8: „*Small structural differences govern the carbonic anhydrase II inhibition activity of cytotoxic triterpene acetazolamide conjugates*"

T.C. Denner, N.V. Heise, J. Zacharias, O. Kraft, S. Hoenke, R. Csuk

Die Synthese der Verbindungen sowie die Auswertung der spektroskopischen Daten und ein Teil der biologischen Evaluierung wurde von mir vorgenommen. J. Zacharias, O. Kraft und N.V. Heise stellten einen Teil der Ausgangsmaterialen her. Ein Teil der biologischen Evaluierung wurde von S. Hoenke durchgeführt. R. Csuk betreute praktische sowie theoretische Aspekte der Arbeit.

Lebenslauf

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2019	Lehrgang zum staatl. geprüften Pyrotechniker
03.2017 bis 11.2017	**Dozent** DAA Dessau-Roßlau

Publikationen

Denner, T. C.; Hoenke, S.; Kraft, O.; Deigner, H.; Al-Harrasi, A.; Csuk, R. Hydroxyethylamide substituted triterpenoic acids hold good cytotoxicity for human tumor cells. *Results in Chemistry* **2022**, *4*, 100371.

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Heise, N. V.; Denner, T. C.; Becker, S. W.; Hoenke, S.; Csuk, R. Developing an Amide-Spacered Triterpenoid Rhodamine Hybrid of Nano-Molar Cytotoxicity Combined with Excellent Tumor Cell/Non-Tumor Cell Selectivity. *Molecules* **2023**, *28* (17), 6404.

Denner, T. C.; Angeli, A.; Supuran, C. T.; Supuran, C. T.; Csuk, R. Ureidobenzenesulfonamides as selective carbonic anhydrase I, IX, and XII inhibitors. *Molecules* **2023**, *28* (23), 7782.

Denner, T. C.; Heise, N. V.; Serbian, I.; Supuran, C. T.; Supuran, C. T.; Csuk, R. An asiatic acid derived trisulfamate acts as a nanomolar inhibitor of human carbonic anhydrase VA. *Steroids* **2024**, 109381.

Denner, T. C.; Heise, N. V.; Zacharias, J.; Csuk, R. Lupane acetates in small molecule drug hybrids: Probing their inhibitory activity for carbonic anhydrase II. *European Journal of Medicinal Chemistry Reports* **2024**, 100139.

Denner, T. C.; Heise, N. V.; Csuk, R. Isosteviol – A new scaffold for the synthesis of carbonic anhydrase II inhibitors. *Results in Chemistry* **2024**, 101426

Denner, T. C.; Klett, E. L.; Heise, N. V.; Csuk, R. Stereochemistry matters: Inhibition of carbonic anhydrase II by amino acid derived sulfamates depends on their absolute configuration. *European Journal of Medicinal Chemistry Reports* **2024**, *11*, 100162

Denner, T. C.; Heise, N. V.; Hoenke, S.; Csuk, R. Synthesis of Rhodamine-Conjugated lupane type triterpenes of enhanced cytotoxicity. *Molecules* **2024**, *29* (10), 2346.

Denner, T. C.; Heise, N. V.; Csuk, R. Arylsulfonamido-alkyl-sulfamates act as inhibitors of bovine carbonic anhydrase II. *European Journal of Medicinal Chemistry Reports* **2024**, *12*, 100177.

Denner, T. C.; Heise, N. V.; Al-Harrasi, A.; Csuk, R. Synthesis and Enzymatic Evaluation of a Small Library of Substituted Phenylsulfonamido-Alkyl Sulfamates towards Carbonic Anhydrase II. *Molecules* **2024**, *29* (13), 3015.

Selbständigkeitserklärung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Arbeit selbstständig und nur unter Verwendung der von mir angegebenen Quellen und Hilfsmittel verfasst habe. Die aus den benutzten Werken, wörtlich oder inhaltlich, entnommenen Stellen wurden als solche kenntlich gemacht. Die Arbeit wurde bisher in gleicher oder ähnlicher Form an keiner anderen Universität oder Hochschule zur Erlangung eines akademischen Grades eingereicht.

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Toni Christopher Denner

Halle (Saale), den 08.07.2025

Angehangene Publikationen

Im nachfolgenden sind die Publikationen P1 bis P8 angefügt

molecules

MDPI

Article

Synthesis and Enzymatic Evaluation of a Small Library of Substituted Phenylsulfonamido-Alkyl Sulfamates towards Carbonic Anhydrase II

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Abstract: A small library of 79 substituted phenylsulfonamidoalkyl sulfamates, **1b**–**79b**, was synthesized starting from arylsulfonyl chlorides and amino alcohols with different numbers of methylene groups between the hydroxyl and amino moieties yielding intermediates **1a**–**79a**, followed by the reaction of the latter with sulfamoyl chloride. All compounds were screened for their inhibitory activity on bovine carbonic anhydrase II. Compounds **1a**–**79a** showed no inhibition of the enzyme, in contrast to sulfamates **1b**–**79b**. Thus, the inhibitory potential of compounds **1b**–**79b** towards this enzyme depends on the substituent and the substitution pattern of the phenyl group as well as the length of the spacer. Bulkier substituents in the *para* position proved to be better for inhibiting CAII than compounds with the same substituent in the *meta* or *ortho* position. For many substitution patterns, compounds with shorter spacer lengths were superior to those with long chain spacers. Compounds with shorter spacer lengths performed better than those with longer chain spacers for a variety of substitution patterns. The most active compound held inhibition constant as low as K_i = 0.67 μM (for **49b**) and a *tert*-butyl substituent in *para* position and acted as a competitive inhibitor of the enzyme.

Keywords: sulfamates; carbonic anhydrase II; inhibitor

Citation: Denner, T.C.; Heise, N.V.; Al-Harrasi, A.; Csuk, R. Synthesis and Enzymatic Evaluation of a Small Library of Substituted Phenylsulfonamido-Alkyl Sulfamates towards Carbonic Anhydrase II. *Molecules* **2024**, *29*, 3015. https://doi.org/10.3390/molecules29133015

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1. Introduction

The scientific interest in enzymes from the family of carbonic anhydrases has increased significantly in recent years, as it has been recognized that these enzymes are involved in a large number of metabolic reactions in a wide variety of organisms. Recently, inhibitors of the enzyme CA II have again become the focus of scientific interest [1–14].

Zinc-containing protein CA II (among other CAs) is involved in acid–base homeostasis [15–24], gluconeogenesis [22–24], lipogenesis [25–31], osteoclast development [32–37], and consequently in calcification. Several studies have revealed that CA II promotes the synthesis of calcium carbonate, and—as a result—the addition of a CA inhibitor. For example, acetazolamide (Figure 1) resulted in a notable reduction in calcium carbonate. This indicates that the synthesis of calcium carbonate depends on CA isoenzymes, which are acetazolamide-sensitive. Proton generation in osteoclasts is facilitated by CA II, too. This causes the resorption lacunae to become acidified and eventually dissolve the bone. Additionally, compared to normal artery tissue from the same person, human atheromatous plaques were shown to overexpress CA II. As a consequence, CA II seems highly interesting in future therapies for osteoporosis and atherosclerosis [11].

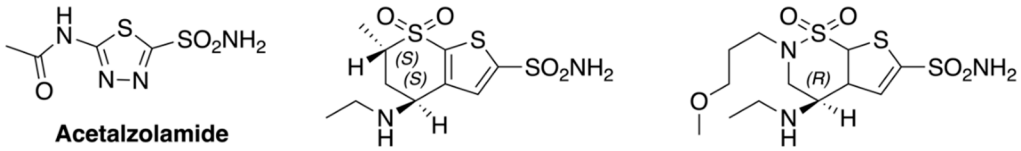


Figure 1. Structures of established CA inhibitors acetazolamide, dorzolamide, and brinzolamide.

Furthermore, out of all the organs studied, the mammalian central nervous system (CNS) possesses the greatest number of CA isoforms (at least 9). Isoforms I, VB, VII, VIII, X, XI, XII, and XIV are also found, with *h*CA II being the most prevalent. Carbonic anhydrase inhibitors have been used therapeutically in a number of brain pathologies because of the broad expression range of CA isoforms in the brain. In epilepsy and idiopathic intracranial hypertension, where acetazolamide [38–41] is one of the medications now in clinical use, inhibition by CAIs has been shown to be clinically beneficial. Moreover, migraine, neuropathic pain, diabetes-induced blood–brain barrier failure, and amyloid β-induced mitochondrial dysfunction characteristic of Alzheimer's disease are possible therapeutic uses of CAIs targeting CNS isoforms.

However, the treatment of primary open-angle glaucoma (POAG), a multifactorial optic neuropathy linked to progressive retinal ganglion cell death and visual field loss, is one of the primary uses of CA II inhibitors [42–47]. Elevated intraocular pressure is the primary risk factor for POAG, although there are numerous other related variables. Because of the unsatisfactory side effect profile of oral carbonic anhydrase inhibitors, topical CAIs took a long time to develop but have been shown to be a valuable adjunct to the treatment of primary open-angle glaucoma. They work by preventing the ciliary epithelium's CA II from functioning. As a result, fewer bicarbonate ions are formed, which decreases intraocular pressure and fluid transfer. Inhibitor drugs such as dorzolamide or brinzolamide (Figure 1) have been in use for many years [48–53].

Furthermore, the treatment of cerebral oedema has once again become the focus of scientific interest, since brain swelling is a known side effect of some of the new drugs (e.g., lecanemab [54–60] and donanemab [61–64]) recently approved for the treatment of Alzheimer's disease. Treatment of cerebral edema could include intravenous injection of a CA inhibitor.

2. Results

During the last few decades, numerous sulfamates have been described as inhibitors of CAs because the sulfamate group is ideally suited to interact with the central atom zinc in the active site of the enzyme [65–72]. Sulfamates are bio-isosteres to sulfonamides; the latter constitute one of the main classes of CA inhibitors. For several of them, very low inhibition constants in the range 3.1–4.8 nm have been reported [73].

Figure 2 depicts the Zn(II) ion coordination in the CA II active site by three histidine ligands (His94, His96, and His119) and gate-keeping residues (Thr199, Glu106) [73].

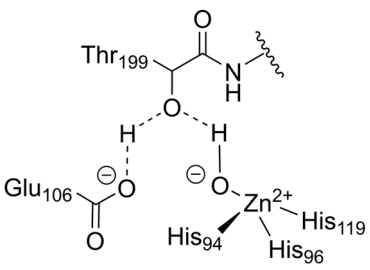
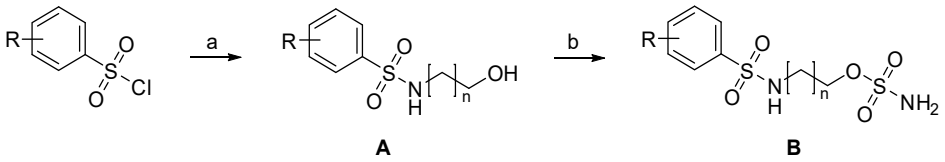


Figure 2. Zn(II) ion coordination in the CA II active site.

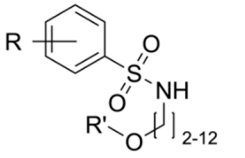
Evaluation of literature data on the potential of sulfamates but also our own studies showed, to our surprise, that compounds of structure **B** (Scheme 1) have not been investi-

gated as CA inhibitors so far [65–71]. Very recently, we could present results showing that similar compounds derived from enantiomerically pure amino-alcohols might be efficient inhibitors of CA II [72].



Scheme 1. Synthesis of target sulfamates (structure **B**) from arylsulfonylchlorides via precursors **A**; (a) CH_2Cl_2, NEt_3, 20 °C 3–24 h; (b) CH_2Cl_2, NEt_3, sulfamoyl chloride, 0 °C → 20 °C, 3–24 h.

Target structure **B** can be easily synthesized by the reaction of 1,ω-aminoalcohols with arylsulfonyl chlorides, Furthermore, many arylsulfonyl chlorides are commercially available. This makes the synthesis of a small library of compounds immensely easier. Their reaction with corresponding amino alcohols led to type **A** compounds (Scheme 1), **1a**–**79a**. The compounds **1a**–**79a** (Figure 3) were reacted with sulfamoyl chloride and triethylamine in dichloromethane, resulting in the desired target compounds of type **B**, **1b**–**79b**. The latter compounds resemble a small library of substituted phenylsulfonamido alkyl sulfamates; they differ in the nature and position of substituents on the aromatic ring (*ortho*, *meta*, and *para*, as well as substituents: hydrogen, methyl, *iso*-propyl, *tert*-butyl, cyclohexyl, and adamantyl), and also in terms of the length of the spacer (n = 2 up to 10 methylene groups).



1a-79a, R' = H
1b-79b, R' = SO_2NH_2

1-11: R = H
12-18: R = *p*-Me
19-25: R = *m*-Me
26-32: R = *o*-Me
33-39: R = *p*-isopropyl
40-46: R = *m*-isopropyl
47-48: R= *o*-isopropyl

49-57: R = *p*-^{t}Bu
58-64: R = *m*-^{t}Bu
65-67: R = *o*-^{t}Bu
68-76: R = *p*-cyclohexyl
77-79: R = *p*-adamantyl

Figure 3. Structure of compounds **1a**–**79a** and **79b**–**79b**.

All of compounds **1a**–**79a** as well as **1b**–**79b** were fully characterized and subjected to biological testing employing *b*CA II. While no inhibitory activity for this enzyme was observed for compounds **1a**–**79a**, compounds **1b**–**79b** proved to be inhibitors of this enzyme. The results from the assays are summarized in Table 1 and the figures.

As a result, compounds **12b**, **49b**, **26b**, **30b**, **13b**, **40b**, **50b**, **33b**, and **34b** proved to be the best inhibitors of *b*CA II in this series of compounds.

With the exception of compounds **1b**–**11b**, derivatives with a shorter spacer length (preferably n = 2) show higher inhibitory activity than their longer-chain analogues. Only in group **1**–**11** is compound **7** (n = 8) the most active compound.

For the alkyl-substituted compounds **12**–**79**, an alkyl substituent in the *para* position proves to be superior to comparable compounds holding a substituent in the *meta* or *ortho* position (Figures 4–6).

The large space occupancy of the *para* substituent alone is only of limited benefit in achieving higher inhibitory activity. For example, compound **12b** is significantly better than **1b**, and the activity decreases for **33b** (with the same chain length of the spacer), while ^{t}Bu-substituted compound **49b** proves to be the strongest inhibitor of the whole series with I = 75.6%. Even bulkier substituents (as in **68b**–**76b** and **77b**–**79b**) with a cyclohexyl or adamanyl residue again drop significantly and prove to be poorer inhibitors for CA II (Figure 7).

Table 1. Inhibition (I in %) of *b*CA II by compounds **1b–79b** (at 1 μM concentration of the inhibitor); acetazolamide (**AAZ**) was used as a positive standard. All experiments were performed in triplicate with three technical replicas.

Cmp.	Inhibition [%]	Cmp.	Inhibition [%]	Cmp.	Inhibition [%]
AAZ	99.2 ± 0.2	27	50.0 ± 0.8	54	28.4 ± 0.4
1	59.2 ± 0.8	28	37.9 ± 0.7	55	21.3 ± 1
2	39.9 ± 0.4	29	57.2 ± 0.7	56	10.0 ± 0.6
3	32.2 ± 0.3	30	68.1 ± 0.3	57	20.6 ± 0.6
4	51.8 ± 0.7	31	55.6 ± 0.7	58	54.0 ± 0.9
5	56.0 ± 0.5	32	56.2 ± 0.8	59	53.9 ± 0.3
6	56.0 ± 0.8	33	63.2 ± 0.4	60	43.3 ± 0.6
7	60.2 ± 0.7	34	60.3 ± 0.9	61	39.2 ± 0.1
8	55.4 ± 0.7	35	43.7 ± 0.4	62	41.7 ± 0.8
9	10.7 ± 0.4	36	40.8 ± 0.6	63	33.3 ± 0.3
10	7.2 ± 0.9	37	45.9 ± 0.7	64	16.0 ± 0.4
11	5.9 ± 0.9	38	30.3 ± 0.1	65	56.2 ± 0.8
12	75.8 ± 0.6	39	28.1 ± 0.5	66	34.5 ± 0.4
13	63.8 ± 0.8	40	63.7 ± 0.1	67	27.5 ± 0.3
14	42.9 ± 0.1	41	50.1 ± 0.9	68	52.1 ± 1.2
15	58.9 ± 0.2	42	35.3 ± 0.5	69	43.1 ± 1.5
16	40.3 ± 0.7	43	44.4 ± 0.3	70	34.7 ± 2.5
17	42.8 ± 0.5	44	45.5 ± 0.7	71	18.6 ± 1.0
18	54.7 ± 0.6	45	37 ± 0.3	72	8.6 ± 0.6
19	59.5 ± 0.1	46	29.5 ± 0.9	73	14.8 ± 0.4
20	48.4 ± 0.1	47	38.4 ± 0.2	74	14.2 ± 1.1
21	32.2 ± 0.6	48	34.2 ± 0.7	75	32.1 ± 0.8
22	49.1 ± 0.2	49	75.6 ± 0.4	76	19.7 ± 1.6
23	51.8 ± 0.7	50	63.4 ± 0.6	77	32.6 ± 0.4
24	27.1 ± 0.9	51	44.9 ± 0.4	78	36.1 ± 1.8
25	38.8 ± 0.1	52	35.9 ± 0.4	79	40.1 ± 0.8
26	69.1 ± 0.7	53	29.1 ± 0.9		

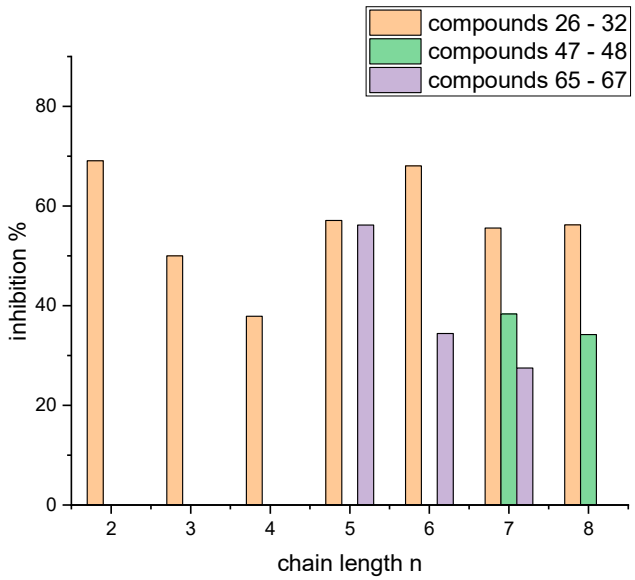


Figure 4. Inhibition (in %) of *ortho*-substituted compounds **26b–32b** (methyl), **47b–48b** (*iso*-propyl) and **65b–67b** (*tert*-butyl), respectively.

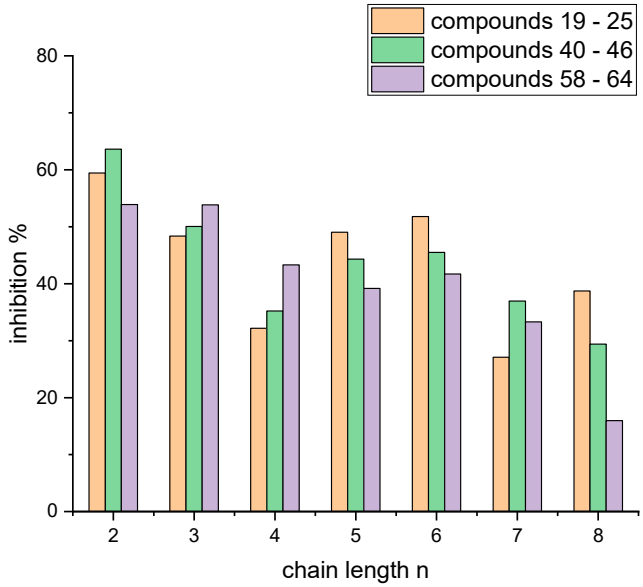


Figure 5. Inhibition (in %) of *meta*-substituted compounds **19b**–**25b** (methyl), **40b**–**46b** (*iso*-propyl), and **58b**–**64b** (*tert*-butyl), respectively.

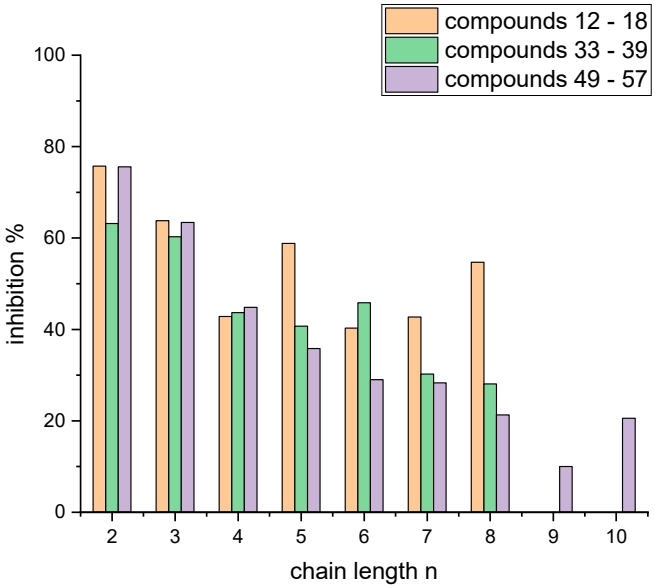


Figure 6. Inhibition (in %) of *para*-substituted compounds **12b**–**18b** (methyl), **33b**–**39b** (*iso*-propyl), and **49b**–**57b** (*tert*-butyl), respectively.

For ^{t}Bu-substituted compounds **49b**–**67b**, a correlation between the position of the substituent and chain length is depicted (vide supra). As already mentioned, compounds with a *para*- substituent are superior to those with *ortho*- or *meta*-terminal substituents (Figures 7–9). At the same time, the dependence on the chain length of the spacer is also evident (Figure 9).

A more detailed illustration of the ^{t}Bu-substituted derivatives **49b**–**67b** can be found in Figure 8.

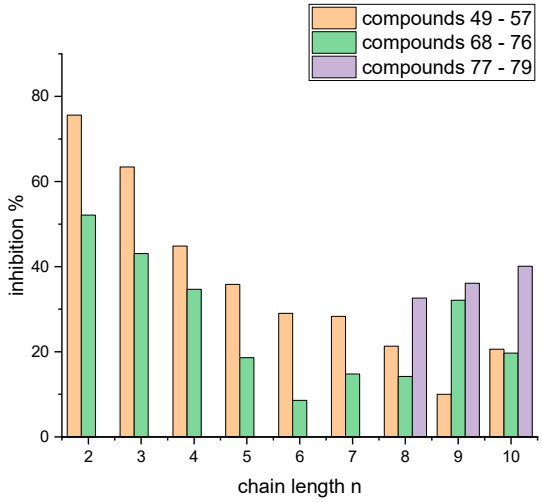


Figure 7. Inhibition (in %) of compounds holding the bulkiest substituents in the *para* position (^{t}butyl (orange), cyclohexyl (green), adamantyl (violet)).

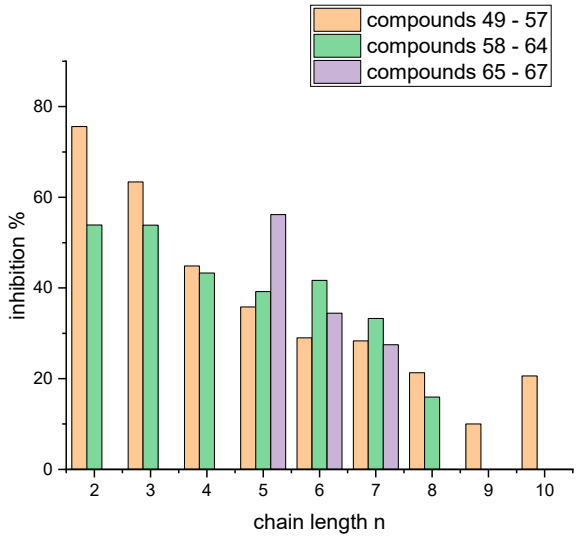


Figure 8. Inhibition (in %) of ^{t}butyl-substituted compounds **49b–67b**.

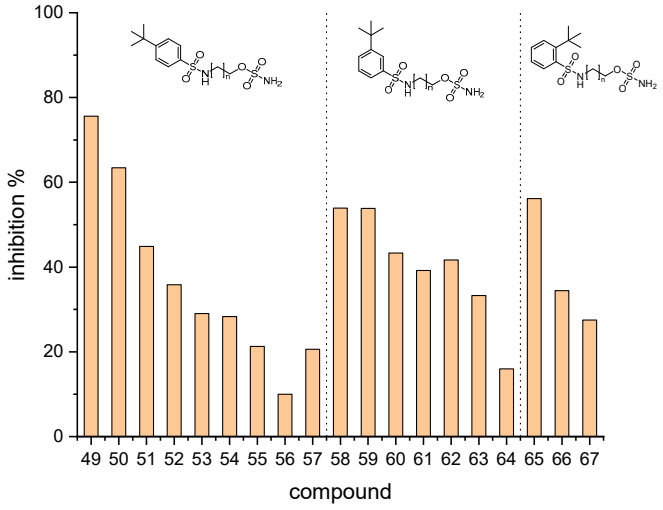


Figure 9. Inhibition (in %) of ^{t}butyl-substituted compounds depends on position of the ^{t}Bu group and on the chain length of the alkyl spacer.

The isopropyl-substituted compounds also prove to be effective with a chain length of n = 2 (compounds **33b** and **40b**), and a decrease in inhibition can be observed with longer chains (Figure 10).

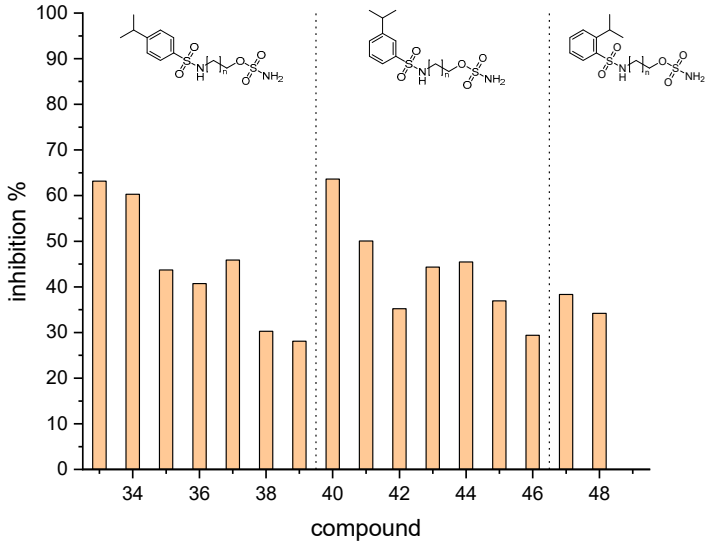


Figure 10. Inhibition (in %) of isopropyl-substituted compounds.

The same is generally true for the methyl-substituted compounds (Figure 11), although with these, the inhibition force usually increases again with longer chains (from n = 5), whereas for the unsubstituted compounds **1b**–**11b** (Figure 12), with the exception of chain lengths 3, 4 and 10–12, a fairly constant inhibition of the enzyme was observed.

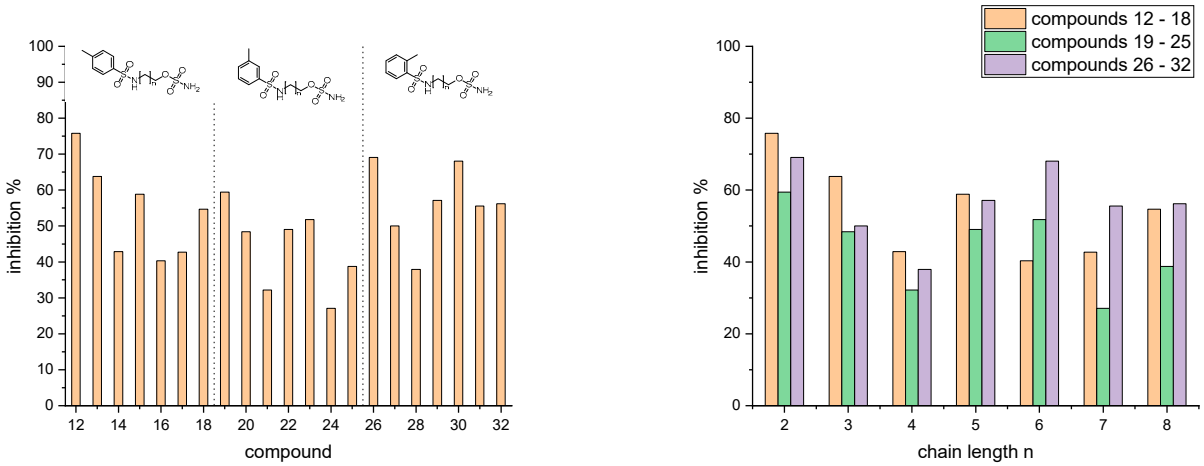


Figure 11. Inhibition (in %) of methyl-substituted compounds **12b**–**32b**.

Although we carried out many molecular modelling calculations attempting to better rationalize these empirical findings, these calculations were only partially helpful. Figure 13 depicts the calculated modeling scores for some of the compounds. By and large, the calculated scores agree with the measured biological activity (cf. Figure 11).

However, the limitations of calculations (especially for fine-tuning) are due to the fact that the molecules have relatively high degrees of (translational and rotational) freedom within the active site and the active site being relatively large. The calculations, however, confirm our earlier findings that further substitution of the spacer (especially in an α position, adjacent to the sulfonamide) should allow for better orientations of the inhibitor in the enzyme pocket, so that stronger (or weaker) interactions can be expected, depending on the absolute configuration. A depiction of the results of the modeling calculations (for

compounds **12b**, **26b**, **30b**, **33b**, **40b**, and **49b**) is shown in the Supplementary Materials File. A depiction of the scores is given in Figure 13.

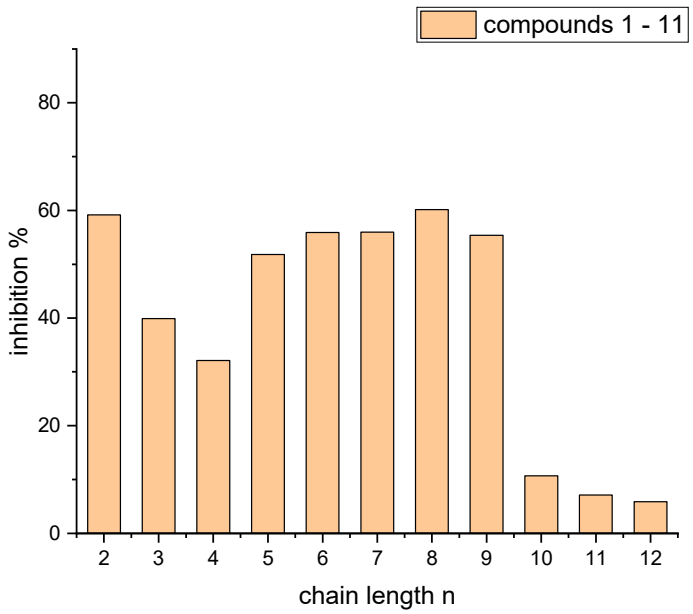


Figure 12. Inhibition (in %) of unsubstituted compounds **1b**–**11b**.

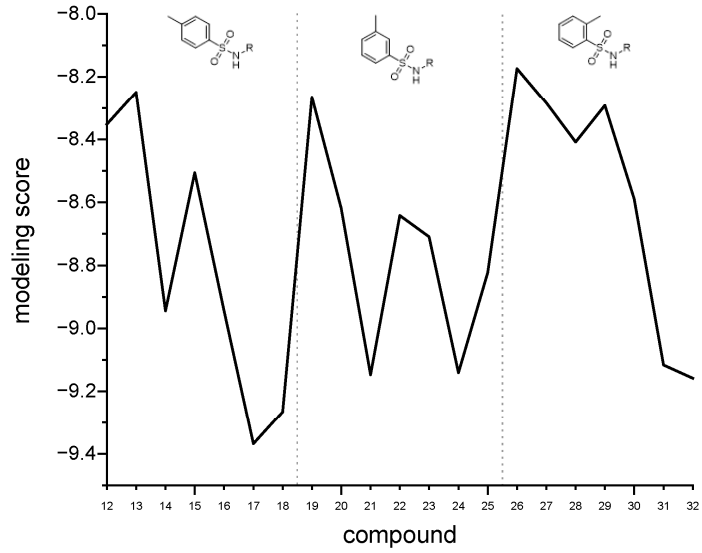


Figure 13. Modeling scores of the methyl-substituted compounds **12b**–**32b**.

Some extra measurements gave an insight into the mode of action of the most active compounds, and inhibition constants K_i were determined. The results from these experiments are summarized in Table 2 and depicted as Dixon plots in Figure 14.

Table 2. Inhibition constants (K_i) for the most active compounds **12b**, **26b**, **30b**, **33b**, **40b**, and **49b** and CA II.

Cmp	12b	26b	30b	33b	40b	49b
K_i [μM]	0.76 ± 0.03	1.1 ± 0.03	1.58 ± 0.02	0.84 ± 0.02	1.64 ± 0.03	0.67 ± 0.05

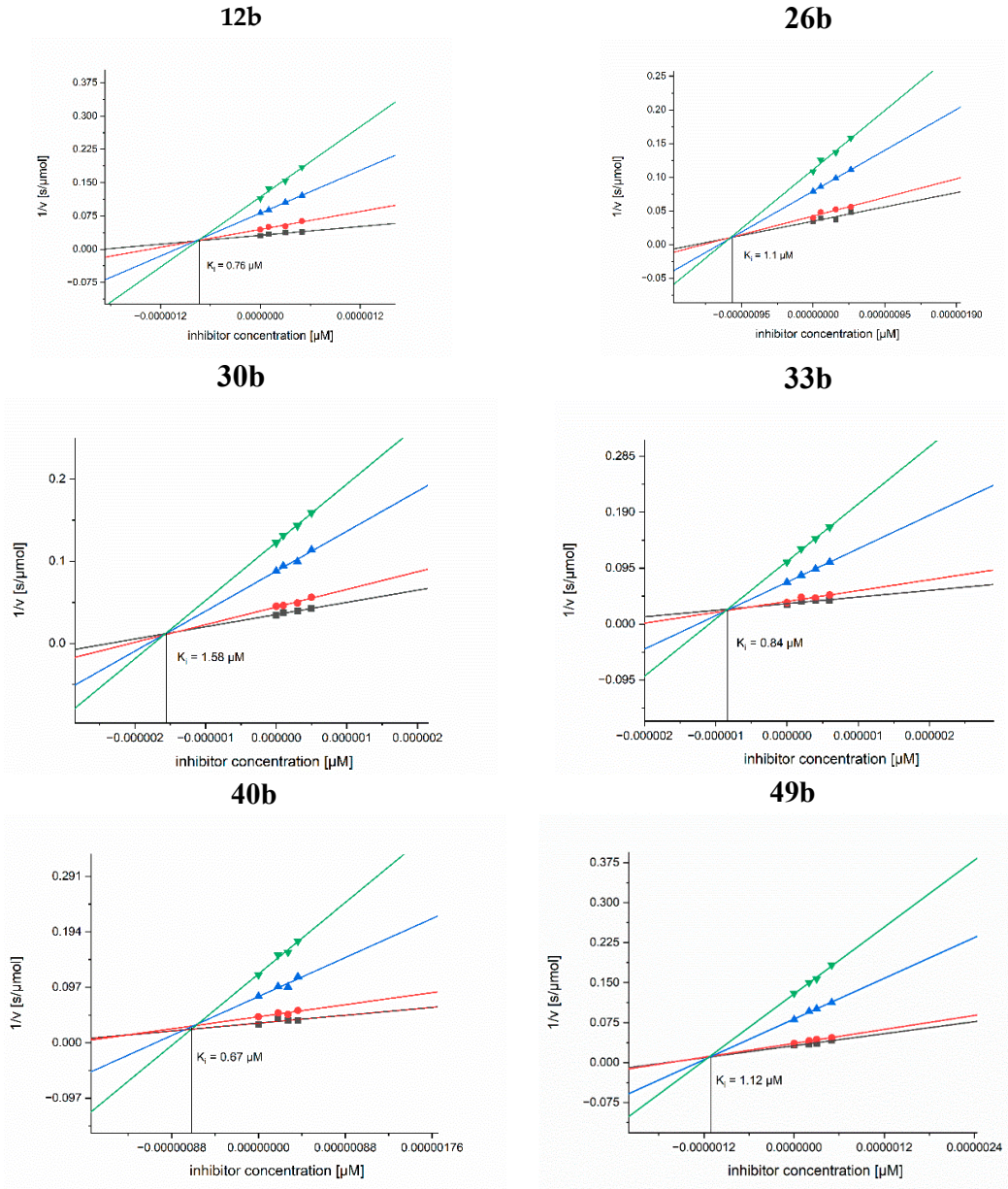


Figure 14. Dixon plots for compounds **12b**, **26b**, **30b**, **33b**, **40b**, and **49b**, respectively. The concentration of the inhibitor: for **12b**: 0.1, 03, 0.5 µM; for **26b**: 0.1, 0.3, 0.5 µM; for **30b**: 0.1, 0.3 0.5 µM; for **33b**: 0.2, 0.4, 0.6 µM; for **40b**: 0.1, 0.3, 0.5 µM; for **49b**: 0.2, 0.3, 0.4 µM.

As a result, all these compounds acted as efficient inhibitors for CA II with K_i values as low as 0.76 µM (for **12b**) or K_i = 0.67 µM (for **49b**).

Each of the sulfamates showed inhibitory activity towards *b*CA II. For every series of substituents, it is noticeable that the inhibitory activity decreases with an increase in the length of the spacer. Furthermore, substituents in the *para* position proved to be better inhibitors compared to their counterparts holding the substituent either in the *ortho* or in the *meta* position. Regarding the type of substituent, methyl and *tert*-butyl proved to be superior to the other substituents. For a better insight into the differences in the inhibition of the most active compounds, their respective inhibition constants were determined. Again, the *para* methyl and tert-butyl-substituted compounds with shorter chain lengths proved to be superior, with inhibition constants of K_i = 0.76 µM and 0.67 µM, respectively. With these additional measurements, the *tert*-butyl substituent proved to be the most active compound. To gain more insight into the advantages of bulkier substituents, another series of cyclohexyl- and adamantyl-substituted compounds was synthesized. However, even

with short chain lengths, the inhibitory activity proved to be significantly worse than that of the *tert*-butyl or even methyl-substituted compounds. In conclusion, compounds with *tert*-butyl substituents in the *para* position and chain lengths around n = 2 proved to be the best inhibitors for *b*CA II in this library of compounds.

3. Conclusions

Starting with arylsulfonyl chlorides and amino alcohols with varying numbers of methylene groups between the hydroxyl and amino moieties (leading to **1a**–**79a**), a small library of 79 substituted phenylsulfonamido alkyl sulfamates **1b**–**79b** has been synthesized. These substances were tested using carbonic anhydrase II to determine whether they were inhibitive. Sulfamates **1b**–**79b** gave inhibition of the enzyme, but compounds **1a**–**79a** did not. In conclusion, the substituent, the phenyl group's substitution pattern, and the spacer's length all affect how inhibitive compounds **1b**–**79b** are of this enzyme. Compounds containing the same substituent in a *para* position demonstrated superior CAII inhibition than those containing the same substituent in a *meta* or *ortho* position. Compounds with shorter spacer lengths performed better than those with longer chain spacers for a variety of substitution patterns. The most active compounds held inhibition constants as low as K_i = 0.67 μM (for **49b**) and a *tert*-butyl substituent in a *para* position.

4. Experimental Procedure

4.1. General

Starting materials were obtained from local vendors; solvents were dried under the usual conditions; equipment and assays were as previously reported [72]. The absorbance was measured with a 96-well plate reader from BMG Labtech (BMG Labtech, Ortenberg, Germany).^{1}H and ^{13}C NMR spectra of all compounds as well as representative HRMS spectra can be found in the Supplementary Materials File. Chromatography was performed on silica gel; IR spectra were recorded as ATR spectra; UV/vis spectra were measured in MeOH. ^{1}H NMR was measured in DMSO-d_6 at 400 MHz; ^{13}C NMR spectra were measured in DMSO-d_6 at 101 MHz, if not stated otherwise. MS spectra were taken as ESI-MS in MeOH; for UV–Vis spectra, λ_{max} (log ε) values are reported.

4.2. Syntheses

4.2.1. Preparation of **1a**–**79a** (General Procedure A, GPA)

Reaction of the amino-alcohol (1.5 equiv.) in dry CH_2Cl_2 (15 mL), with dry NEt_3 (2 equiv.) and the sulfonyl chloride (1 equiv.) at 22 °C for 3 hours followed by evaporation of the solvents and chromatography gave **1a**–**79a**. For long-chain amino-alcohols (n = 8–12), the solvent composition was modified, and a 1:1 mixture of CH_2Cl_2 and acetonitrile was used instead.

4.2.2. Preparation of **1b**–**79b** (General Procedure B, GPB)

Reaction of **1a**–**79a** (1 equiv.) in dry CH_2Cl_2 (6 mL), NEt_3 (3 equiv.) with sulfamoyl chloride (3 equiv.) at 22 °C until completion of the reaction followed by evaporation and column chromatography gave **1b**–**79b**.

4.2.3. N-(2-Hydroxyethyl)benzene Sulfonamide (**1a**) [59724-42-4]

Applying GPA: from benzenesulfonyl chloride (500 mg, 2.83 mmol) and 2-aminoethanol (260 mg, 4.25 mmol): **1a** [73–80] (542 mg, 94%); white solid; R_f = 0.07 (petrolether/EtOAc, 2:3); m.p. = 78–79 °C (lit.: [73,74] 79–80 °C); spectral data as previously reported [72].

4.2.4. 2-(Phenylsulfonamido]ethyl Sulfamate (**1b**)

Applying GPB: from **1a** (175 mg, 0.87 mmol): **1b** (202 mg, 82%); white solid; R_f = 0.38 ($CHCl_3$/EtOAc, 2:3); m.p. = 71–73 °C; spectral data as previously reported [72].

4.2.5. N-(3-Hydroxypropyl)benzene Sulfonamide (**2a**) [3351-94-8]

Applying GPA: from benzenesulfonyl chloride (500 mg, 2.83 mmol) and 3-aminopropanol (319 mg, 4.25 mmol): **2a** [81–83] (594 mg, 97%); oil; R_f = 0.09 (petrolether/EtOAc, 2:3); UV–Vis: 221 nm (4.02); IR: ν = 3500*w*, 3276*m*, 2943*w*, 2881*w*, 1478*w*, 1447*m*, 1309*s*, 1154*vs*, 1092*s*, 1070*s*, 1007*w*, 959*w*, 871*w*, 754*m*, 720*m*, 689*s*, 586*s*, 469*w* cm^{-1}; ^{1}H NMR:δ = 7.81–7.76 (*m*, 2H, 2-H, 2′-H), 7.66–7.56 (*m*, 3H, 3-H, 3′-H, 4-H), 7.52 (*s*, 1H, NH), 4.40 (*s*, 1H, OH), 3.39–3.32 (*m*, 2H, 7-H), 2.79 (*t*, *J* = 7.3 Hz, 2H, 5-H), 1.57–1.46 (*m*, 2H, 6-H); ^{13}C NMR:δ = 140.5 (C-1), 132.3 (C-4), 129.2 (C-3), 126.4 (C-2), 58.0 (C-7), 40.0 (C-5), 32.3 (C-6) ppm; MS: *m*/*z* = 238.1 (100%, $[M + Na]^+$); anal. calcd. for $C_9H_{13}NSO_3$ (215.27): C 50.22, H 6.09, N 6.51; found: C 49.97, H 6.34, N 6.38.

4.2.6. 3-(Phenylsulfonamido]propyl Sulfamate (**2b**)

Applying GPB: from **2a** (200 mg, 0.93 mmol): **2b** (241 mg, 88%); white solid; R_f = 0.40 ($CHCl_3$/EtOAc, 2:3); m.p. = 64–66 °C; UV–Vis: 221 nm (3.78); IR: ν = 3344*m*, 3264*m*, 3255*m*, 1449*w*, 1373*s*, 1353*m*, 1311*s*, 1177*s*, 1155*s*, 1113*w*, 1090*m*, 1074*w*, 1052*m*, 1003*m*, 950*s*, 917*m*, 896*m*, 886*m*, 836*s*, 754*m*, 727*s*, 688*s*, 673*m*, 627*m*, 599*m*, 586*s*, 562*s*, 546*vs*, 503*m*, 490*m*, 476*w*, 441*w* cm^{-1}; ^{1}H NMR:δ = 7.82–7.77 (*m*, 2H, 2-H, 2′-H), 7.72–7.58 (*m*, 4H, 3-H, 3′-H, 4-H, NH), 7.41 (*s*, 2H, NH_2), 4.02 (*t*, *J* = 6.3 Hz, 2H, 7-H), 2.85–2.78 (*m*, 2H, 5-H), 1.76 (*dt*, *J* = 13.2, 6.5 Hz, 2H, 6-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 140.2 (C-1), 132.4 (C-4), 129.3 (C-3), 126.4 (C-2), 66.5 (C-7), 39.2 (C-5), 28.7 (C-6) ppm; MS: *m*/*z* = 316.9 (100%, $[M + Na]^+$); anal. calcd. for $C_9H_{14}N_2S_2O_5$ (294.34): 36.73, H 4.79, N 9.52; found: C 36.50, H 4.98, N 9.36.

4.2.7. N-(4-Hydroxybutyl)benzene Sulfonamide (**3a**) [842146-77-4]

Applying GPA: from benzenesulfonyl chloride (500 mg, 2.83 mmol) and 4-aminobutanol (378 mg, 4.25 mmol): **3a** (621 mg, 96%); oil; R_f = 0.12 (petrolether/EtOAc, 2:3); UV–Vis: 221 nm (4.07); IR: ν = 3506*br*, 3278*br*, 2940*w*, 2872*w*, 1478*w*, 1447*m*, 1318*s*, 1309*s*, 1152*vs*, 1092*s*, 1055*m*, 1027*w*, 999*w*, 952*w*, 909*w*, 867*w*, 754*m*, 719*m*, 688*s*, 583*s*, 567*s*, 483*w* cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.82–7.77 (*m*, 2H, 2-H, 2′-H), 7.65–7.53 (*m*, 4H, 3-H, 3′-H, 4-H, NH), 4.37 (*t*, *J* = 5.1 Hz, 1H, OH), 3.35–3.30 (*m*, 2H, 8-H), 2.74 (*q*, *J* = 6.0 Hz, 2H, 5-H), 1.44–1.33 (*m*, 4H, 6-H, 7-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 140.6 (C-1), 132.3 (C-4), 129.2 (C-3), 126.4 (C-2), 60.2 (C-8), 42.6 (C-5), 29.5 (C-7), 25.8 (C-6) ppm; MS: *m*/*z* = 243.2 (90%, $[M-H]^-$); anal. calcd. for $C_{10}H_{15}NSO_3$ (229.29): C 50.22, H 6.09, N 6.51; found: C 49.97, H 6.31, N 6.37.

4.2.8. 4-(Phenylsulfonamido]butyl Sulfamate (**3b**)

Applying GPB: from **3a** (200 mg, 0.87 mmol): **3b** (215 mg, 80%); oil; R_f = 0.43 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 221 nm (3.95); IR: ν = 3345*m*, 3258*m*, 3257*m*, 1442*w*, 1370*s*, 1350*m*, 1316*s*, 1187*s*, 1157*s*, 1110*w*, 1093*m*, 1072*w*, 1056*m*, 1001*m*, 956*s*, 914*m*, 894*m*, 889*m*, 840*s*, 751*m*, 729*s*, 685*s*, 670*m*, 621*m*, 595*m*, 582*s*, 565*s*, 544*vs*, 501*m*, 496*m*, 470*w*, 440*w* cm^{-1}; ^{1}H NMR:δ = 7.83–7.76 (*m*, 2H, 2-H, 2′-H), 7.68–7.56 (*m*, 4H, 3-H, 3′-H, 4-H, NH), 7.38 (*s*, 2H, NH_2), 3.96 (*t*, *J* = 6.3 Hz, 2H, 8-H), 2.76 (*q*, *J* = 6.6 Hz, 2H, 5-H), 1.66–1.56 (*m*, 2H, 7-H), 1.49–1.40 (*m*, 2H, 6-H) ppm; ^{13}C NMR:δ = 140.5 (C-1), 132.4 (C-4), 129.2 (C-3), 126.4 (C-2), 68.6 (C-8), 42.0 (C-5), 25.6 (C-7), 24.7 (C-6) ppm; MS: *m*/*z* = 331.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{10}H_{16}N_2S_2O_5$ (308.37): C 38.95, H 5.23, N 9.08; found: C 38.70, H 5.51, N 8.84.

4.2.9. N-(5-Hydroxypentyl)benzene Sulfonamide (**4a**) [191529-31-4]

Applying GPA: from benzenesulfonyl chloride (500 mg, 2.83 mmol) and 5-aminopentanol (438 mg, 4.25 mmol): **4a** [84,85] (647 mg, 94%); oil; R_f = 0.12 (petrolether/EtOAc, 2:3); UV–Vis: 221 nm (3.91); IR: ν = 3503*w*, 3281*m*, 2937*w*, 2865*w*, 1478*w*, 1447*m*, 1320*s*, 1309*s*, 1153*vs*, 1092*s*, 1071*s*, 1040*w*, 999*w*, 880*w*, 754*s*, 719*m*, 689*s*, 583*s*, 567*s*, 464*w* cm^{-1}; ^{1}H NMR:δ = 7.82–7.76 (*m*, 2H, 2-H, 2′-H), 7.66–7.56 (*m*, 3H, 3-H, 3′-H, 4-H), 7.54 (*t*, *J* = 5.8 Hz, 1H, NH), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.36–3.28 (*m*, 2H, 9-H), 2.71 (*td*, *J* = 7.0, 5.7 Hz, 2H, 5-H), 1.40–1.27 (*m*, 4H, 6-H, 8-H), 1.27–1.16 (*m*, 2H, 7-H) ppm; ^{13}C NMR:δ = 140.6 (C-1), 132.2 (C-4), 129.1 (C-3), 126.4 (C-2), 60.5 (C-9), 42.6 (C-5), 32.0 (C-8), 28.8 (C-6), 22.6 (C-7) ppm;

MS: m/z = 243.2 (90%, $[M-H]^-$); anal. calcd. for $C_{11}H_{17}NSO_3$ (243.32): C 54.30, H 7.04, N 5.76; found: C 54.11, H 7.23, N 5.43.

4.2.10. 5-(Phenylsulfonamido]pentyl Sulfamate (**4b**)

Applying GPB: from **4a** (200 mg, 0.82 mmol): **4b** (250 mg, 94%); oil; R_f = 0.46 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 221 nm (3.91); IR: ν = 3344*w*, 3307*m*, 3232*w*, 2953*w*, 2933*w*, 2854*w*, 1555*w*, 1469*w*, 1442*w*, 1421*w*, 1399*vw*, 1363*s*, 1311*s*, 1295*w*, 1276*w*, 1172*s*, 1153*vs*, 1092*m*, 1078*m*, 1067*w*, 1043*w*, 1025*w*, 993*m*, 945*m*, 922*s*, 911*s*, 808*m*, 766*m*, 750*m*, 722*s*, 693*s*, 599*s*, 570*s*, 553*vs*, 502*m*, 482*m*, 435*w* cm^{-1}; ^1H NMR:δ = 7.82–7.76 (*m*, 2H, 2-H, 2′-H), 7.67–7.55 (*m*, 4H, 3-H, 3′-H, 4-H, NH), 7.39–7.35 (*m*, 2H, NH_2), 3.96 (*t*, *J* = 6.5 Hz, 2H, 9-H), 2.73 (*td*, *J* = 6.7, 5.8 Hz, 2H, 5-H), 1.55 (*p*, *J* = 6.7 Hz, 2H, 6-H), 1.44–1.34 (*m*, 2H, 8-H), 1.33–1.23 (*m*, 2H, 7-H) ppm; ^{13}C NMR:δ = 140.6 (C-1), 132.2 (C-4), 129.1 (C-3), 126.4 (C-2), 60.5 (C-9), 42.6 (C-5), 32.0 (C-8), 28.8 (C-6), 22.6 (C-7) ppm; MS: m/z = 345.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{11}H_{18}N_2S_2O_5$ (322.39): C 40.98, H 5.63, N 8.69; found: C 40.72, H 5.87, N 8.57.

4.2.11. N-(6-Hydroxyhexyl)benzene Sulfonamide (**5a**) [195003-60-2]

Applying GPA: from benzenesulfonyl chloride (500 mg, 2.83 mmol) and 6-amino-hexanol (438 g, 4.25 mmol): **5a** (701 mg, 96%); white solid; R_f = 0.14 (petrolether/EtOAc, 2:3); m.p. = 40–42 °C; UV–Vis: 221 nm (3.97); IR: ν = 3506*br*, 3260*br*, 2934*w*, 2860*w*, 1447*m*, 1320*m*, 1309*m*, 1153*vs*, 1092*m*, 1071*m*, 1025*w*, 1000*w*, 754*m*, 719*m*, 689*s*, 584*s*, 568*s*, 475*w* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.81–7.76 (*m*, 2H, 2-H, 2′-H), 7.65–7.56 (*m*, 3H, 3-H, 3′-H, 4-H), 7.54 (*t*, *J* = 5.8 Hz, 1H, NH), 4.30 (*t*, *J* = 5.2 Hz, 1H, OH), 3.36–3.31 (*m*, 2H, 10-H), 2.72 (*td*, *J* = 7.0, 5.7 Hz, 2H, 5-H), 1.38–1.30 (*m*, 4H, 6-H, 9-H), 1.38–1.14 (*m*, 4H, 7-H, 8-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 140.6 (C-1), 132.2 (C-4), 129.1 (C-3), 126.4 (C-2), 60.6 (C-10), 42.5 (C-5), 32.3 (C-9), 29.0 (C-6), 25.9 (C-8), 25.0 (C-7) ppm; MS: m/z = 280 (100%, $[M + Na]^+$); anal. calcd. for $C_{12}H_{19}NSO_3$ (257.35): C 56.01, H 7.44, N 5.44; found: C 55.76, H 7.69, N 5.21.

4.2.12. 6-(Phenylsulfonamido]hexyl Sulfamate (**5b**)

Applying GPB: from **5b** (230 mg, 0.89 mmol): **5b** (202 mg, 67%); white solid; R_f = 0.51 ($CHCl_3$/EtOAc, 2:3); m.p. = 64–66 °C; UV–Vis: 221 nm (3.98); IR: ν = 3346*w*, 3306*m*, 3238*w*, 2955*w*, 2931*w*, 2858*w*, 1554*w*, 1465*w*, 1447*w*, 1421*w*, 1390*vw*, 1366*s*, 1314*s*, 1291*w*, 1279*w*, 1171*s*, 1155*vs*, 1095*m*, 1074*m*, 1063*w*, 1046*w*, 1025*w*, 993*m*, 946*m*, 926*s*, 912*s*, 808*m*, 761*m*, 751*m*, 720*s*, 690*s*, 598*s*, 571*s*, 550*vs*, 507*m*, 481*m*, 436*w* cm^{-1}; ^1H NMR:δ = 7.81–7.77 (*m*, 2H, 2-H, 2′-H), 7.66–7.53 (*m*, 4H, 3-H, 3′-H, 4-H, NH), 7.37 (s, 2H, NH_2), 3.97 (*t*, *J* = 6.5 Hz, 2H, 10-H), 2.77–2.69 (*m*, 2H, 5-H), 1.56 (*p*, *J* = 6.7 Hz, 2H, 9-H), 1.35 (*p*, *J* = 6.9 Hz, 2H, 6-H), 1.30–1.17 (*m*, 4H, 7-H, 8-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 140.6 (C-1), 132.3 (C-4), 129.2 (C-3), 126.4 (C-2), 68.9 (C-10), 42.4 (C-5), 28.8 (C-9), 28.2 (C-6), 25.5 (C-8), 24.6 (C-7) ppm; MS: m/z = 359.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{12}H_{20}N_2S_2O_5$ (336.42): C 42.84, H 5.99, N 8.33; found: C 42.57, H 6.17, N 8.04.

4.2.13. N-(7-Hydroxyheptyl)benzene Sulfonamide (**6a**) [2773002-10-9]

Applying GPA: from benzenesulfonyl chloride (500 mg, 2.83 mmol) and 7-amino-heptanol (558 mg, 4.25 mmol): **6a** (740 mg, 96%); oil; R_f = 0.19 (petrolether/EtOAc, 2:3); UV–Vis: 221 nm (3.97); IR: ν = 3504*br*, 3282*br*, 2931*m*, 2858*w*, 1447*m*, 1320*m*, 1310*m*, 1153*vs*, 1093*s*, 1071*m*, 1058*w*, 1025*m*, 1000*w*, 755*m*, 719*m*, 689*s*, 584*s*, 568*s*, 469*w* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.81–7.77 (*m*, 2H, 2-H, 2′-H), 7.65–7.56 (*m*, 3H, 3-H, 3′-H, 4-H), 7.54 (*t*, *J* = 5.7 Hz, 1H, NH), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.37–3.32 (*m*, 3H, 11-H), 2.72 (*q*, *J* = 6.7 Hz, 2H, 5-H), 1.40–1.30 (*m*, 4H, 6-H, 10-H), 1.23–1.11 (*m*, 6H, 7-H, 8-H, 9-H) ppm; ^{13}C NMR:δ = 140.7 (C-1), 132.2 (C-4), 129.1 (C-3), 126.4 (C-2), 60.7 (C-11), 42.5 (C-5), 32.4 (C-10), 28.9 (C-7), 28.4 (C-6), 26.0 (C-9), 25.3 (C-8) ppm; MS: m/z = 294.2 (100%, $[M + Na]^+$); anal. calcd. for $C_{13}H_{21}NSO_3$ (271.38): C 57.54, H 7.80, N 5.16; found: C 57.26, H 8.03, N 4.97.

4.2.14. 7-(Phenylsulfonamido]heptyl Sulfamate (**6b**)

Applying GPB: from **6a** (300 mg, 1.11 mmol): **6b** (315 mg, 81%); white solid; R_f = 0.60 ($CHCl_3$/EtOAc, 2:3); m.p. = 59–61 °C; UV–Vis: 221 nm (3.99); IR: ν = 3377*w*, 3273*m*, 2959*w*, 2938*m*, 2924*w*, 2860*w*, 1545*w*, 1477*w*, 1449*m*, 1423*m*, 1397*w*, 1374*s*, 1310*s*, 1291*m*, 1229*vw*, 1188*s*, 1152*vs*, 1115*vw*, 1091*s*, 1063*m*, 1049*w*, 1007*m*, 998*m*, 969*s*, 908*m*, 852*vw*, 820*s*, 754*m*, 722*s*, 686*s*, 590*s*, 567*s*, 555*vs*, 524*vs*, 473*w*, 448*w*, 441*w* cm^{-1}; ^1H NMR:δ = 7.81–7.77 (*m*, 2H, 2-H, 2′-H), 7.66–7.52 (*m*, 4H, 3-H, 3′-H, 4-H, NH), 7.37 (*s*, 2H, NH_2), 3.98 (*t*, *J* = 6.5 Hz, 2H, 11-H), 2.76–2.69 (*m*, 2H, 5-H), 1.62–1.53 (*m*, 2H, 10-H), 1.39–1.30 (*m*, 2H, 6-H), 1.28–1.15 (*m*, 6H, 7-H, 8-H, 9-H) ppm; ^{13}C NMR:δ = 140.7 (C-1), 132.3 (C-4), 129.2 (C-3), 126.4 (C-2), 68.9 (C-11), 42.5 (C-5), 28.8 (C-10), 28.2 (C-7), 28.0 (C-6), 25.8 (C-9), 24.9 (C-8) ppm; MS: *m*/*z* = 373.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{13}H_{22}N_2S_2O_5$ (350.45): C 44.56, H 6.33, N 7.99; found: C 44.47, H 6.50, N 7.76.

4.2.15. N-(8-Hydroxyoctyl)benzene Sulfonamide (**7a**) [2771470-62-1]

Applying GPA: from benzenesulfonyl chloride (500 mg, 2.83 mmol) and 8-amino-octanol (617 mg, 4.25 mmol): **7a** (566 mg, 70%); white solid; R_f = 0.21 (petrolether/EtOAc, 2:3); m.p. = 59–60 °C; UV–Vis: 221 nm (3.99); IR: ν = 3280*m*, 2929*m*, 2854*m*, 1478*w*, 1447*m*, 1428*m*, 1326*s*, 1266*w*, 1158*vs*, 1092*s*, 1053*m*, 1031*w*, 982*w*, 905*m*, 755*m*, 720*m*, 687*s*, 596*s*, 563*s*, 530*m*, 500*w*, 481*w*, 455*w* cm^{-1}; ^1H NMR:δ = 7.81–7.76 (*m*, 2H, 2-H, 2′-H), 7.66–7.56 (*m*, 3H, 3-H, 3′-H, 4-H), 7.54 (*t*, *J* = 5.8 Hz, 1H, NH), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.36 (*td*, *J* = 6.5, 5.1 Hz, 2H, 12-H), 2.72 (*q*, *J* = 6.7 Hz, 2H, 5-H), 1.42–1.27 (*m*, 4H, 6-H, 11-H), 1.27–1.09 (*m*, 8H, 7-H, 8-H, 9-H, 10-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 140.7 (C-1), 132.2 (C-4), 129.1 (C-3), 126.4 (C-2), 60.7 (C-12), 42.5 (C-5), 32.5 (C-11), 28.9 (C-9), 28.7 (C-6), 28.5 (C-8), 25.9 (C-7), 25.4 (C-10) ppm; MS: *m*/*z* = 308.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{14}H_{23}NSO_3$ (285.40): C 58.92, H 8.12, N 4.91; found: C 58.76, H 8.37, N 4.63.

4.2.16. 8-(Phenylsulfonamido]octyl Sulfamate (**7b**)

Applying GPB: from **7a** (300 mg, 1.05 mmol): **7b** (203 mg, 53%); white solid; R_f = 0.64 ($CHCl_3$/EtOAc, 2:3); m.p. = 77–78 °C; UV–Vis: 221 nm (3.95); IR: ν = 3338*w*, 3267*m*, 2934*w*, 2916*w*, 2856*w*, 1577*w*, 1469*w*, 1450*w*, 1427*w*, 1395*w*, 1381*s*, 1311*m*, 1179*s*, 1158*vs*, 1116*vw*, 1092*m*, 1065*w*, 1058*m*, 1039*w*, 1025*vw*, 997*m*, 965*s*, 931*m*, 898*m*, 858*m*, 821*s*, 794*m*, 753*s*, 726*s*, 688*s*, 588*vs*, 566*s*, 549*s*, 526*s*, 480*w*, 468*w*, 440*w* cm^{-1}; ^1H NMR:δ = 7.82–7.76 (*m*, 2H, 2-H, 2′-H), 7.67–7.56 (*m*, 3H, 3-H, 3′-H, 4-H), 7.40 (*s*, 3H, NH, NH_2), 3.99 (*t*, *J* = 6.5 Hz, 2H, 12-H), 2.72 (*t*, *J* = 7.0 Hz, 2H, 5-H), 1.65–1.54 (*m*, 2H, 11-H), 1.39–1.23 (*m*, 4H, 6-H, 10-H), 1.23–1.14 (*m*, 6H, 7-H, 8-H, 9-H) ppm; ^{13}C NMR:δ = 140.7 (C-1), 132.2 (C-4), 129.1 (C-3), 126.4 (C-2), 69.0 (C-12), 42.5 (C-5), 28.9 (C-11), 28.3 (C-9), 28.3 (C-6), 28.3 (C-8), 25.9 (C-7), 24.9 (C-10) ppm; MS: *m*/*z* = 387.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{14}H_{24}N_2S_2O_5$ (364.48): C 46.14, H 6.64, N 7.69; found: C 45.87, H 6.84, N 7.55.

4.2.17. N-(9-Hydroxynonyl)benzene Sulfonamide (**8a**)

Applying GPA: from benzenesulfonyl chloride (500 mg, 2.83 mmol) and 9-amino-nonanol (676 mg, 4.25 mmol): **8a** (745 mg, 88%); white solid; R_f = 0.23 (petrolether/EtOAc, 2:3); m.p. = 40–41 °C; UV–Vis: 221 nm (3.97); IR: ν = 3465*br*, 3263*br*, 2930*m*, 2917*w*, 2892*w*, 2849*w*, 1475*w*, 1445*m*, 1433*w*, 1313*s*, 1157*s*, 1094*s*, 1058*s*, 1051*s*, 1036*m*, 1023*w*, 974*m*, 928*w*, 904*m*, 878*m*, 824*m*, 752*m*, 726*m*, 689*s*, 665*m*, 595*w*, 571*s*, 560v*s*, 522*s*, 470*w*, 460*w*, 439*w* cm^{-1}; ^1H NMR:δ = 17.81–7.76 (*m*, 2H, 2-H, 2′-H), 7.66–7.56 (*m*, 3H, 3-H, 3′-H, 4-H), 7.54 (*s*, 1H, NH), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.36 (*td*, *J* = 6.5, 4.7 Hz, 2H, 13-H), 2.72 (*t*, *J* = 7.0 Hz, 2H, 5-H), 1.43–1.28 (*m*, 4H, 6-H, 12-H), 1.28–1.11 (*m*, 10H, 7-H, 8-H, 9-H, 10-H, 11-H) ppm; ^{13}C NMR:δ = 140.7 (C-1), 132.2 (C-4), 129.1 (C-3), 126.4 (C-2), 60.7 (C-13), 42.5 (C-5), 32.5 (C-12), 28.9 (C-9), 28.9 (C-10), 28.8 (C-6), 28.5 (C-8), 26.0 (C-7), 25.4 (C-11) ppm; MS: *m*/*z* = 322.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{25}NSO_3$ (299.43): C 60.17, H 8.42, N 4.68; found: C 59.87, H 8.70, N 4.39.

4.2.18. 9-(Phenylsulfonamido]nonyl Sulfamate (**8b**)

Applying GPB: from **8a** (300 mg, 1.00 mmol): **8b** (267 mg, 70%); white solid; R_f = 0.68 ($CHCl_3$/EtOAc, 2:3); m.p. = 82–84 °C; UV–Vis: 221 nm (3.92); IR: ν = 3378*w*, 3273*m*, 2937*w*, 2922*m*, 2855*w*, 1545*w*, 1476*w*, 1449*w*, 1424*w*, 1397*w*, 1375*s*, 1309*s*, 1286*w*, 1275*w*, 1188*s*, 1153*vs*, 1118*vw*, 1092*m*, 1063*m*, 1033*m*, 994*w*, 969*s*, 910*m*, 883*m*, 820*s*, 776*w*, 753*m*, 723*s*, 686*s*, 589*s*, 568*s*, 555*vs*, 534*m*, 524*m*, 500*w*, 489*m*, 465*w*, 453*w* cm^{-1}; ^1H NMR:δ = 7.81–7.76 (*m*, 2H, 2-H, 2′-H), 7.66–7.55 (*m*, 3H, 3-H, 3′-H, 4-H), 7.41 (*s*, 3H, NH, NH_2), 4.00 (*t*, *J* = 6.5 Hz, 2H, 13-H), 2.72 (*t*, *J* = 7.0 Hz, 2H, 5-H), 1.65–1.56 (*m*, 2H, 12-H), 1.37–1.27 (*m*, 4H, 6-H, 11-H), 1.25–1.13 (*m*, 8H, 7-H, 8-H, 9-H, 10-H) ppm; ^{13}C NMR:δ = 140.7 (C-1), 132.2 (C-4), 129.1 (C-3, C-3′), 126.4 (C-2, C-2′), 69.0 (C-13), 42.5 (C-5), 28.9 (C-6), 28.7 (C-8), 28.4 (C-9), 28.4 (C-10), 28.3 (C-12), 25.9 (C-7), 25.0 (C-11) ppm; MS: *m*/*z* = 401.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{26}N_2S_2O_5$ (378.50): C 47.60, H 6.92, N 7.40; found: C 47.40, H 7.11, N 7.06.

4.2.19. N-(10-Hydroxydecyl)benzene Sulfonamide (**9a**)

Applying GPA: from benzenesulfonyl chloride (220 mg, 1.25 mmol) and 10-amino-decanol (324 mg, 4.25 mmol): **9a** (350 mg, 90%); white solid; R_f = 0.28 (petrolether/EtOAc, 2:3); m.p. = 67–69 °C; UV–Vis: 221 nm (3.95); IR: ν = 3416*m*, 3279*m*, 2920*w*, 2889*w*, 2849*m*, 1465*m*, 1447*m*, 1426*m*, 1350*m*, 1318*s*, 1287*w*, 1153*vs*, 1094*m*, 1059*m*, 1033*w*, 1009*w*, 973*w*, 909*m*, 750*m*, 722*s*, 684*s*, 596v*s*, 581*s*, 531*w*, 515*m*, 429*w*, 475*w* cm^{-1}; ^1H NMR:δ = 7.80–7.76 (*m*, 2H, 2-H, 2′-H), 7.66–7.56 (*m*, 3H, 3-H, 3′-H, 4-H), 7.53 (*t*, *J* = 5.8 Hz, 1H, NH), 4.30 (*s*, 1H, OH), 3.40–3.34 (*m*, 2H, 15-H), 2.71 (*td*, *J* = 7.0, 5.8 Hz, 2H, 5-H), 1.43–1.28 (*m*, 4H, 6-H, 14-H), 1.27–1.10 (*m*, 12H, 7-H, 8-H, 9-H, 10-H, 11-H, 12-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 140.7 (C-1), 132.2 (C-4), 129.1 (C-3), 126.4 (C-2), 60.7 (C-15), 42.5 (C-5), 32.5 (C-14), 29.0 (C-6), 28.9 (C-8, C-11), 28.8 (C-9), 28.5 (C-10), 26.0 (C-7), 25.5 (C-12) ppm; MS: *m*/*z* = 336.4 (100%, $[M + Na]^+$); anal. calcd. for $C_{16}H_{27}NSO_3$ (313.46): C 61.31, H 8.68, N 4.47; found: C 61.07, H 8.95, N 4.24.

4.2.20. 10-(Phenylsulfonamido]decyl Sulfamate (**9b**)

Applying GPB: from **9a** (185 mg, 0.59 mmol): **9b** (172 mg, 74%); white solid; R_f = 0.71 ($CHCl_3$/EtOAc, 2:3); m.p. = 89–91 °C; UV–Vis: 221 nm (4.02); IR: ν = 3339*w*, 3266*m*, 2962*vw*, 2933*w*, 2917*m*, 2849*w*, 1576*vw*, 1470*w*, 1450*w*, 1428*w*, 1395*w*, 1381*s*, 1336*vw*, 1313*m*, 1308*m*, 1287*w*, 1266*vw*, 1235*vw*, 1178*m*, 1159*vs*, 1118*vw*, 1093*m*, 1078*w*, 1059*m*, 1042*w*, 1020*w*, 990*m*, 965*s*, 932*m*, 918*m*, 892*m*, 855*w*, 822*s*, 764*w*, 753*m*, 725*s*, 688*s*, 590*s*, 567*s*, 549*s*, 534*s*, 511*w*, 483*w*, 458*w* cm^{-1}; ^1H NMR:δ = 7.81–7.76 (*m*, 2H, 2-H, 2′-H), 7.66–7.56 (*m*, 3H, 3-H, 3′-H, 4-H), 7.53 (*t*, *J* = 5.8 Hz, 1H, NH), 7.37 (*s*, 2H, NH_2), 4.00 (*t*, *J* = 6.5 Hz, 2H, 15-H), 2.72 (*td*, *J* = 7.0, 5.8 Hz, 2H, 5-H), 1.65–1.57 (*m*, 2H, 14-H), 1.39–1.27 (*m*, 4H, 6-H, 12-H), 1.27–1.12 (*m*, 10H, 7-H, 8-H, 9-H, 10-H, 11-H) ppm; ^{13}C NMR:δ = 140.7 (C-1), 132.2 (C-4), 129.1 (C-3), 126.4 (C-2), 69.0 (C-15), 42.5 (C-5), 28.9 (C-6), 28.8 (C-9, C-14), 28.5 (C-8, C-11), 28.3 (C-10), 25.9 (C-7), 25.0 (C-12) ppm; MS: *m*/*z* = 415.2 (100%, $[M + Na]^+$); anal. calcd. for $C_{16}H_{28}N_2S_2O_5$ (392.53): C 48.96, H 7.19, N 7.14; found: C 48.75, H 7.35, N 6.87.

4.2.21. N-(11-Hydroxyundecyl)benzene Sulfonamide (**10a**)

Applying GPA: from benzenesulfonyl chloride (500 mg, 2.83 mmol) and 11-amino-undecanol (795 mg, 4.25 mmol): **10a** (901 mg, 97%); white solid; R_f = 0.33 (petrolether/EtOAc, 2:3); m.p. = 58–59 °C; UV–Vis: 221 nm (3.97); IR: ν = 3464*m*, 3265*m*, 2918*m*, 2848*m*, 1475*m*, 1466*m*, 1445*m*, 1432*w*, 1353*m*, 1314*s*, 1287*w*, 1158*vs*, 1094*s*, 1052*s*, 1008*m*, 928*w*, 894*w*, 885*m*, 819*m*, 753*s*, 725*s*, 689*s*, 664*m*, 595*m*, 560*s*, 529*w*, 510*m*, 493*w*, 410*w* cm^{-1}; ^1H NMR:δ = 7.81–7.76 (*m*, 2H, 2-H, 2′-H), 7.66–7.56 (*m*, 3H, 3-H, 3′-H, 4-H), 7.53 (*t*, *J* = 5.8 Hz, 1H, NH), 4.30 (*td*, *J* = 5.2, 1.0 Hz, 1H, OH), 3.37 (*td*, *J* = 6.5, 5.1 Hz, 2H, 15-H), 2.71 (*td*, *J* = 7.0, 5.8 Hz, 2H, 5-H), 1.45–1.28 (*m*, 4H, 6-H, 14-H), 1.28–1.11 (*m*, 14H, 7-H, 8-H, 9-H, 10-H, 11-H, 12-H, 13-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 140.7 (C-1), 132.2 (C-4), 129.1 (C-3), 126.4 (C-2), 60.7 (C-15), 42.5 (C-5), 32.5 (C-14), 29.0 (C-6), 28.9 (C-12), 28.9 (C-11), 28.9 (C-10), 28.8 (C-9), 28.5 (C-8), 25.9 (C-7), 25.5 (C-13) ppm; MS: *m*/*z* = 350.2 (100%, $[M + Na]^+$); anal. calcd. for $C_{17}H_{29}NSO_3$ (327.48): C 62.35, H 8.93, N 4.28; found: C 62.17, H 9.16, N 4.01.

4.2.22. 11-(Phenylsulfonamido]undecyl Sulfamate (**10b**)

Applying GPB: from **10a** (300 mg, 0.92 mmol): **10b** (321 mg, 86%); white solid; R_f = 0.74 ($CHCl_3$/EtOAc, 2:3); m.p. = 95–97 °C; UV–Vis: 221 nm (3.99); IR: ν = 3392*w*, 3269*m*, 2963*w*, 2936*w*, 2917*m*, 2852*w*, 1558*w*, 1476*m*, 1447*w*, 1429*w*, 1395*w*, 1369*s*, 1314*s*, 1258*vw*, 1183*m*, 1153*s*, 1120*w*, 1093*m*, 1066*m*, 1051*m*, 1028*w*, 991*m*, 970*s*, 928*m*, 903*m*, 869*w*, 824*s*, 755*m*, 719*s*, 690*m*, 639*m*, 590*s*, 565*s*, 555*vs*, 530*s*, 525*s*, 500*w*, 470*w*, 452*m*, 432*m*, 413*vw*, 511*w*, 483*w*, 458*w* cm^{-1}; ^1H NMR:δ = 7.82–7.75 (*m*, 2H, 2-H, 2′-H), 7.67–7.54 (*m*, 3H, 3-H, 3′-H, 4-H), 7.40 (*s*, 3H, NH, NH_2), 4.00 (*t*, *J* = 6.5 Hz, 2H, 15-H), 2.72 (*t*, *J* = 6.9 Hz, 2H, 5-H), 1.67–1.57 (*m*, 2H, 14-H), 1.39–1.11 (*m*, 16H, 6-H, 7-H, 8-H, 9-H, 10-H, 11-H, 12-H, 13-H) ppm; ^{13}C NMR:δ = 140.7 (C-1), 132.2 (C-4), 129.1 (C-3), 126.4 (C-2), 69.0 (C-15), 42.5 (C-5), 28.9 (C-6), 28.9 (C-9), 28.8 (C-10, C-11, C-13), 28.5 (C-8), 28.3 (C-14), 26.0 (C-12), 25.0 (C-7) ppm; MS: *m*/*z* = 429.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{17}H_{30}N_2S_2O_5$ (406.56): C 50.22, H 7.44, N 6.89; found: C 49.96, H 7.69, N 6.58.

4.2.23. N-(12-Hydroxydodecyl)benzene Sulfonamide (**11a**)

Applying GPA: from benzenesulfonyl chloride (500 mg, 2.83 mmol) and 12-amino-dodecanol (855 mg, 4.25 mmol): **11a** (944 mg, 98%); white solid; R_f = 0.37 (petrolether/EtOAc, 2:3); m.p. = 76–77 °C; UV–Vis: 221 nm (3.95); IR: ν = 3412*m*, 3350*m*, 3278*s*, 2920*s*, 2848*m*, 1477*m*, 1464*m*, 1447*m*, 1425*w*, 1359*m*, 1320*s*, 1266*w*, 1154*vs*, 1095*s*, 1064*m*, 1048*s*, 1053*w*, 999*m*, 909*m*, 750*m*, 722*s*, 684*s*, 596*s*, 561*s*, 530*s*, 489*w*, 459*m* cm^{-1}; ^1H NMR:δ = 7.81–7.76 (*m*, 2H, 2-H, 2′-H), 7.66–7.56 (*m*, 3H, 3-H, 3′-H, 4-H), 7.53 (*t*, *J* = 5.8 Hz, 1H, NH), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.37 (*td*, *J* = 6.5, 5.1 Hz, 2H, 16-H), 2.71 (*q*, *J* = 6.7 Hz, 2H, 5-H), 1.44–1.28 (*m*, 4H, 6-H, 15-H), 1.28–1.12 (*m*, 16H, 7-H, 8-H, 9-H, 10-H, 11-H, 12-H, 13-H, 14-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 140.7 (C-1), 132.2 (C-4), 129.1 (C-3), 126.4 (C-2), 60.7 (C-16), 42.5 (C-5), 32.5 (C-15), 29.0 (C-12), 29.0 (C-11), 28.9 (C-9), 28.9 (C-10, C-13), 28.8 (C-6), 28.5 (C-8), 25.9 (C-7), 25.5 (C-14) ppm; MS: *m*/*z* = 364.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{18}H_{31}NSO_3$ (341.51): C 63.31, H 9.15, N 4.10; found: C 63.00, H 9.43, N 3.97.

4.2.24. 12-(Phenylsulfonamido]dodecyl Sulfamate (**11b**)

Applying GPB: from **11a** (300 mg, 0.88 mmol): **11b** (229 mg, 62%); white solid; R_f = 0.77 ($CHCl_3$/EtOAc, 2:3); m.p. = 102–103 °C; UV–Vis: λ_{max} (log ε) =; 221 nm (3.47); IR: ν = 3338*m*, 3265*m*, 2962*vw*, 2917*m*, 2849*m*, 1576*w*, 1470*w*, 1450*w*, 1428*w*, 1396*w*, 1381*s*, 1313*s*, 1289*w*, 1178*m*, 1158*vs*, 1094*m*, 1060*m*, 1043*m*, 1024*vw*, 996*w*, 983*m*, 964*s*, 931*m*, 905*m*, 880*m*, 854*w*, 838*m*, 821*s*, 794*w*, 768*w*, 753*s*, 726*s*, 688*s*, 589*s*, 567*s*, 550*s*, 535*vs*, 504*w*, 482*vw*, 433*m* cm^{-1}; ^1H NMR:δ = 7.79–7.74 (*m*, 2H, 2-H, 2′-H), 7.64–7.54 (*m*, 3H, 3-H, 3′-H, 4-H), 7.51 (*t*, *J* = 5.8 Hz, 1H, NH), 7.35 (*s*, 2H, NH_2), 3.98 (*t*, *J* = 6.5 Hz, 2H, 16-H), 2.69 (*td*, *J* = 7.0, 5.8 Hz, 2H, 5-H), 1.64–1.55 (*m*, 2H, 15-H), 1.36–1.09 (*m*, 18H, 6-H, 7-H, 8-H, 9-H, 10-H, 11-H, 12-H, 13-H, 14-H) ppm; ^{13}C NMR:δ = 140.7 (C-1), 132.2 (C-4), 129.1 (C-3), 126.4 (C-2), 69.0 (C-16), 42.5 (C-5), 28.9 (C-9), 28.9 (C-6, C-11, C-12), 28.9 (C-15), 28.5 (C-8), 28.3 (C-13), 26.0 (C-14), 25.1 (C-7) ppm; MS: *m*/*z* = 443.4 (100%, $[M + Na]^+$); anal. calcd. for $C_{18}H_{32}N_2S_2O_5$ (420.58): C 51.40, H 7.67, N 6.66; found: C 51.15, H 7.93, N 6.42.

4.2.25. N-(2-Hydroxyethyl)-4-methylbenzene Sulfonamide (**12a**) [914083-49-1]

Applying GPA: from 4-methylbenzenesulfonyl chloride (300 mg, 1.57 mmol) and 2-amino-ethanol (144 mg, 2.36 mmol): **12a** [86–92] (315 mg, 93%); white solid; R_f = 0.54 ($CHCl_3$/EtOAc, 2:3); m.p. = 55–57 °C; UV–Vis: 227 nm (3.68); IR: ν = 3497*br*, 3273*br*, 2926*w*, 2881*w*, 1598*w*, 1495*w*, 1424*m*, 1402*m*, 1318*s*, 1305*s*, 1290*m*, 1152*vs*, 1093*s*, 1055*s*, 1019*w*, 948*m*, 880*w*, 814*s*, 706*w*, 661*s*, 550*s*, 462*w* cm^{-1}; ^1H NMR:δ = 7.72–7.64 (*m*, 2H, 2-H, 2′-H), 7.48 (*s*, 1H, NH), 7.42–7.37 (*m*, 2H, 3-H, 3′-H), 4.65 (*s*, 1H, OH), 3.35 (*t*, *J* = 6.7 Hz, 2H, 7-H), 2.76 (*t*, *J* = 6.4 Hz, 2H, 6-H), 2.38 (*s*, 3H, 5-H) ppm; ^{13}C NMR:δ = 142.5 (C-1), 137.7 (C-4), 129.6 (C-3), 126.5 (C-2), 59.9 (C-7), 45.0 (C-6), 20.9 (C-5) ppm; MS: *m*/*z* = 238 (80%, $[M + Na]^+$); anal. calcd. for $C_9H_{13}NSO_3$ (215.27): C 50.22, H 6.09, N 6.51; found: C 49.87, H 6.30, N 6.33.

4.2.26. 2-[(4-Methylphenyl)sulfonamido]ethyl Sulfamate (**12b**) [914083-49-1]

Applying GPB: from **12a** (250 mg, 1.16 mmol): **12b** (330 mg, 96%) [93]; white solid; R_f = 0.49 ($CHCl_3$/EtOAc, 2:3); m.p. = 79–81 °C; UV–Vis: 224 nm (3.91); IR: ν = 3358*w*, 3332*w*, 3265*m*, 1480*w*, 1448*w*, 1425*w*, 1377*m*, 1366*s*, 1322*s*, 1305*m*, 1238*w*, 1227*w*, 1178*s*, 1160*s*, 1148*s*, 1117*w*, 1098*m*, 1083*s*, 1033*m*, 1011*s*, 954*m*, 931*s*, 913*m*, 880*w*, 869*w*, 818*m*, 803*m*, 783*s*, 769*s*, 702*m*, 684*s*, 659*m*, 598*m*, 589*m*, 574*s*, 548*vs*, 529*m*, 509*m*, 487*m*, 471*s*, 448*m*, 431*w*, 411*w* cm^{-1}; ^{1}H NMR:δ = 7.82 (s, 1H, NH), 7.72–7.65 (*m*, 2H, 2-H, 2′-H), 7.58–7.46 (*m*, 2H, NH_2), 7.44–7.37 (*m*, 2H, 3-H, 3′-H), 3.99 (*t*, *J* = 5.8 Hz, 2H, 7-H), 3.02 (*t*, *J* = 5.8 Hz, 2H, 6-H), 2.39 (*s*, 3H, 5-H) ppm; ^{13}C NMR:δ = 142.8 (C-4), 137.3 (C-1), 129.7 (C-3), 126.5 (C-2), 67.5 (C-7), 41.5 (C-6), 21.0 (C-5) ppm; MS: *m*/*z* = 317.1 (100%, $[M + Na]^+$); anal. calcd. for $C_9H_{14}N_2S_2O_5$ (294.34): C 36.73, H 4.79, N 9.52; found: C 36.41, H 4.97, N 9.35.

4.2.27. N-(3-Hydroxypropyl)-4-methylbenzene Sulfonamide (**13a**) [13379-98-1]

Applying GPA: from 4-methylbenzenesulfonyl chloride (200 mg, 1.05 mmol) and 3-amino-propanol (118 mg, 1.57 mmol): **13a** (225 mg, 94%) [94–99]; white solid; R_f = 0.12 (petrolether/EtOAc, 2:3); m.p. = 55–57 °C (lit.: [98] 55–56 °C); UV–Vis: 227 nm (4.14); IR: ν = 3492*br*, 3274*br*, 2945*w*, 2879*w*, 1598*m*, 1495*w*, 1423*m*, 1318*s*, 1305*s*, 1296*m*, 1185*w*, 1153*vs*, 1091*s*, 1070*s*, 1019*w*, 1006*w*, 959*m*, 872*w*, 815*s*, 706*w*, 662*s*, 570*w*, 550*s*, 515*w* cm^{-1}; ^{1}H NMR:δ =7.69–7.64 (*m*, 2H, 2-H, 2′-H), 7.46–7.35 (*m*, 3H, 3-H, 3′-H, NH), 4.39 (*t*, *J* = 5.1 Hz, 1H, OH), 3.40–3.30 (*m*, 2H, 8-H), 2.76 (*td*, *J* = 7.6, 3.3 Hz, 2H, 6-H), 2.38 (*s*, 3H, 5-H), 1.50 (*p*, *J* = 6.4 Hz, 2H, 7-H) ppm; ^{13}C NMR:δ = 142.5 (C-4), 137.6 (C-1), 129.6 (C-3), 126.5 (C-2), 58.1 (C-8), 40.0 (C-6), 32.3 (C-7), 20.9 (C-5) ppm; MS: *m*/*z* = 252.3 (90%, $[M + Na]^+$); anal. calcd. for $C_{10}H_{15}NSO_3$ (229.29): C 52.38, H 6.59, N 6.11; found: C 52.09, H 6.89, N 5.87.

4.2.28. 3-[(4-Methylphenyl)sulfonamido]propyl Sulfamate (**13b**)

Applying GPB: from **13a** (300 mg, 1.31 mmol): **13b** (260 mg, 64%); white solid; R_f = 0.49 ($CHCl_3$/EtOAc, 2:3); m.p. = 86–87 °C; UV–Vis: 227 nm (3.72); IR: ν = 3352*w*, 3317*w*, 3272*w*, 2959*vw*, 2902*vw*, 1597*vw*, 1554*w*, 1472*w*, 1434*vw*, 1409*w*, 1392*w*, 1362*m*, 1321*m*, 1309*m*, 1292*w*, 1239*vw*, 1211*vw*, 1181*m*, 1160*s*, 1122*w*, 1093*m*, 1047*m*, 1020*vw*, 980*w*, 956*s*, 919*m*, 893*w*, 854*m*, 841*m*, 818*m*, 799*w*, 773*m*, 706*w*, 667*s*, 590*m*, 573*m*, 547*vs*, 494*m*, 476*m*, 444*w*, 413*vw*, 511*w*, 483*w*, 458*w* cm^{-1}; ^{1}H NMR:δ = 7.71–7.64 (*m*, 2H, 2-H, 2′-H), 7.57–7.35 (*m*, 5H, 3-H, 3′-H, NH, NH_2), 4.02 (*t*, *J* = 6.3 Hz, 2H, 8-H), 2.79 (*t*, *J* = 7.1 Hz, 2H, 6-H), 2.39 (*s*, 3H, 5-H), 1.75 (*p*, *J* = 6.7 Hz, 2H, 7-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 142.7 (C-4), 137.4 (C-1), 129.7 (C-3), 126.5 (C-2), 66.5 (C-8), 39.2 (C-6), 28.7 (C-7), 20.9 (C-5) ppm; MS (ESI, MeOH) *m*/*z* = 331.2 (90%, $[M + Na]^+$); anal. calcd. for $C_{10}H_{16}N_2S_2O_5$ (308.37): C 38.95, H 5.23, N 9.08; found: C 38.77, H 5.54, N 8.78.

4.2.29. N-(4-Hydroxybutyl)-4-methylbenzene Sulfonamide (**14a**) [78521-69-4]

Applying GPA: from 4-methylbenzenesulfonyl chloride (400 mg, 2.1 mmol) and 4-amino-butanol (318 mg, 3.15 mmol): **14a** (477 mg, 93%); white solid; R_f = 0.12 (petrolether/EtOAc, 2:3); m.p. = 50–51 °C (lit.: [100] 50–52 °C); UV–Vis: 227 nm (4.18); IR: ν = 3502*br*, 3279*br*, 2940*w*, 2871*w*, 1597*m*, 1475*m*, 1454*m*, 1433*m*, 1314*s*, 1305*s*, 1289*m*, 1150*vs*, 1121*w*, 1091*s*, 1061*s*, 1027*m*, 1019*w*, 941*m*, 848*w*, 817*s*, 753*m*, 721*m*, 709*m*, 662*s*, 576*s*, 551*s*, 484*m*, 456*w* cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.69–7.65 (*m*, 2H, 2-H, 2′-H), 7.48–7.43 (*m*, 1H, NH), 7.40–7.36 (*m*, 2H, 3-H, 3′-H), 4.36 (*t*, *J* = 5.1 Hz, 1H, OH), 3.32 (*q*, *J* = 5.5 Hz, 2H, 9-H), 2.73–2.67 (*m*, 2H, 6-H), 2.37 (*s*, 3H, 5-H), 1.43–1.32 (*m*, 4H, 7-H, 8-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 142.4 (C-4), 137.7 (C-1), 129.6 (C-3), 126.5 (C-2), 60.2 (C-9), 42.5 (C-6), 29.6 (C-8), 25.8 (C-7), 20.9 (C-5) ppm; MS: *m*/*z* = 266.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{11}H_{17}NSO_3$ (243.32): C 54.30, H 7.04, N 5.76; found: C 54.11, H 7.32, N 5.55.

4.2.30. 4-[(4-Methylphenyl)sulfonamido]butyl Sulfamate (**14b**)

Applying GPB: from **14a** (100 mg, 0.41 mmol): **14b** (90 mg, 68%); white solid; R_f = 0.52 ($CHCl_3$/EtOAc, 2:3); m.p. = 66–68 °C; UV–Vis: 227 nm (3.81); IR: ν = 3354*w*, 3314*w*, 3275*w*, 2955*vw*, 2902*vw*, 1597*vw*, 1552*w*, 1474*w*, 1434*vw*, 1410*w*, 1398*w*, 1362*m*, 1321*m*, 1309*m*,

1294*w*, 1235*vw*, 1211*vw*, 1180*m*, 1162*s*, 1120*w*, 1098*m*, 1046*m*, 1020*vw*, 982*w*, 957*s*, 917*m*, 894*w*, 854*m*, 840*m*, 820*m*, 799*w*, 774*m*, 704*w*, 665*s*, 596*m*, 571*m*, 547*vs*, 495*m*, 473*m*, 444*w*, 413*vw*, 511*w* cm^{-1}; ^1H NMR:δ = 7.70–7.65 (*m*, 2H, 2-H, 2′-H), 7.52 (*t*, *J* = 5.9 Hz, 1H, NH), 7.45–7.36 (*m*, 4H, 3-H, 3′-H, NH_2), 3.96 (*t*, *J* = 6.3 Hz, 2H, 9-H), 2.73 (*q*, *J* = 6.8 Hz, 2H, 6-H), 2.38 (*s*, 3H, 5-H), 1.65–1.55 (*m*, 2H, 8-H), 1.49–1.39 (*m*, 2H, 7-H) ppm; ^{13}C NMR:δ = 142.6 (C-4), 137.7 (C-1), 129.6 (C-3), 126.5 (C-2, C-2), 68.6 (C-9), 42.0 (C-6), 25.6 (C-8), 25.4 (C-7), 21.0 (C-5) ppm; MS: *m*/*z* = 345.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{11}H_{18}N_2S_2O_5$ (322.39): C 40.98, H 5.63, N 8.69; found: C 40.78, H 5.89, N 8.43.

4.2.31. N-(5-Hydroxypentyl)-4-methylbenzene Sulfonamide (**15a**) [16780-44-2]

Applying GPA: from 4-methylbenzenesulfonyl chloride (500 mg, 2.62 mmol) and and 5-amino-pentanol (405 mg, 3.93 mmol): **15a** (522 mg, 77%) [101–108]; white solid; R_f = 0.11 (petrolether/EtOAc, 2:3); m.p. = 60–61 °C; UV–Vis: 227 nm (3.99); IR: ν = 3456*m*, 3109*br*, 2920*w*, 2862*w*, 1597*m*, 1475*m*, 1454*m*, 1433*m*, 1314*s*, 1305*s*, 1289*m*, 1150*vs*, 1121*w*, 1091*s*, 1061*s*, 1027*m*, 1019*w*, 941*m*, 848*w*, 817*s*, 753*m*, 721*m*, 709*m*, 662*s*, 576*s*, 551*s*, 484*m*, 456*w* cm^{-1}; ^1H NMR:δ = 7.69–7.63 (*m*, 2H, 2-H, 2′-H), 7.44 (*t*, *J* = 5.9 Hz, 1H, NH), 7.41–7.36 (*m*, 2H, 3-H, 3′-H), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.35–3.29 (*m*, 2H, 10-H), 2.68 (*td*, *J* = 7.0, 5.9 Hz, 2H, 6-H), 2.38 (*s*, 3H, 5-H), 1.39–1.27 (*m*, 4H, 7-H, 9-H), 1.27–1.17 (*m*, 2H, 8-H) ppm; ^{13}C NMR:δ = 142.4 (C-4), 137.7 (C-1), 129.5 (C-3), 126.5 (C-2), 60.5 (C-10), 42.5 (C-6), 32.0 (C-9), 28.8 (C-7), 22.6 (C-8), 20.9 (C-5) ppm; MS: *m*/*z* = 280.0 (100%, $[M + Na]^+$); anal. calcd. for $C_{12}H_{19}NSO_3$ (257.35): C 56.01, H 7.44, N 5.44; found: C 55.76, H 7.78, N 5.16.

4.2.32. 5-[(4-Methylphenyl)sulfonamido]pentyl Sulfamate (**15b**)

Applying GPB: from **15a** (200 mg, 0.78 mmol): **15b** (232 mg, 89%); white solid; R_f = 0.53 ($CHCl_3$/EtOAc, 2:3); m.p. = 94–95 °C UV–Vis: 227 nm (3.92); IR: ν = 3351*w*, 3301*m*, 3250*w*, 2957*vw*, 2940*w*, 2930*w*, 2852*w*, 1554*w*, 1466*w*, 1422*w*, 1395*vw*, 1361*s*, 1316*s*, 1301*m*, 1294*w*, 1281*w*, 1177*s*, 1150*vs*, 1096*m*, 1074*w*, 1063*w*, 1044*w*, 1027*vw*, 1021*w*, 992*m*, 965*vw*, 947*m*, 915*s*, 870*w*, 815*s*, 810*s*, 749*w*, 722*w*, 708*w*, 671*s*, 630*w*, 598*m*, 574*m*, 550*vs* cm^{-1}; ^1H NMR:δ = 7.70–7.63 (*m*, 2H, 2-H, 2′-H), 7.47 (*t*, *J* = 5.9 Hz, 1H, NH), 7.42–7.34 (*m*, 4H, 3-H, 3′-H, NH_2), 3.96 (*t*, *J* = 6.4 Hz, 2H, 10-H), 2.70 (*q*, *J* = 6.6 Hz, 2H, 6-H), 2.38 (*s*, 3H, 5-H), 1.55 (*p*, *J* = 6.7 Hz, 2H, 9-H), 1.43–1.33 (*m*, 2H, 7-H), 1.33–1.22 (*m*, 2H, 8-H) ppm; ^{13}C NMR:δ = 142.5 (C-4), 137.7 (C-1), 129.6 (C-3), 126.5 (C-2), 68.8 (C-10), 42.3 (C-6), 28.5 (C-9), 27.8 (C-7), 22.2 (C-8), 20.9 (C-5) ppm; MS: *m*/*z* = 359.4 (100%, $[M + Na]^+$); anal. calcd. for $C_{12}H_{20}N_2S_2O_5$ (336.42): C 42.84, H 5.99, N 8.33; found: C 42.57, H 6.26, N 8.14.

4.2.33. N-(6-Hydroxyhexyl)-4-methylbenzene Sulfonamide (**16a**) [385369-83-5]

Applying GPA: from 4-methylbenzenesulfonyl chloride (500 mg, 2.62 mmol) and 6-amino-hexanol (461 mg, 3.93 mmol): **16a** (551 mg, 77%) [104,109–112]; white solid; R_f = 0.15 (petrolether/EtOAc, 2:3); m.p. = 49–51 °C; UV–Vis: 227 nm (4.10); IR: ν = 3423*w*, 3364*w*, 3290*m*, 2936*m*, 2891*w*, 2860*w*, 1589*w*, 1495*w*, 1476*w*, 1422*m*, 1385*w*, 1319*m*, 1303*w*, 1290*w*, 1154*vs*, 1091*m*, 1067*m*, 1036*m*, 983*w*, 905*m*, 817*s*, 734*w*, 707*w*, 666*s*, 573*s*, 549*s*, 523*m*, 484*w*, 430*w* cm^{-1}; ^1H NMR:δ = 7.70–7.63 (*m*, 2H, 2-H, 2′-H), 7.47–7.41 (*m*, 1H, NH), 7.41–7.35 (*m*, 2H, 3-H, 3′-H), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.38–3.30 (*m*, 2H, 11-H), 2.73–2.65 (*m*, 2H, 6-H), 2.37 (*s*, 3H, 5-H), 1.41–1.27 (*m*, 4H, 7-H, 10-H), 1.22–1.14 (*m*, 4H, 8-H, 9-H) ppm; ^{13}C NMR:δ = 142.4 (C-4), 137.8 (C-1), 129.6 (C-3, C-3′), 126.5 (C-2, C-2′), 60.6 (C-11), 42.5 (C-6), 32.4 (C-10), 29.0 (C-7), 25.9 (C-9), 25.0 (C-8), 20.9 (C-5) ppm; MS: *m*/*z* = 294 (100%, $[M + Na]^+$); anal. calcd. for $C_{13}H_{21}NSO_3$ (271.38): C 57.54, H 7.80, N 5.16; found: C 57.24, H 8.02, N 4.96.

4.2.34. 6-[(4-Methylphenyl)sulfonamido]hexyl Sulfamate (**16b**)

Applying GPB: from **16a** (200 mg, 0.74 mmol): **16b** (206 mg, 80%); white solid; R_f = 0.55 ($CHCl_3$/EtOAc, 2:3); m.p. = 49–50 °C; UV–Vis: 227 nm (4.03); IR: ν = 3350*w*, 3300*m*, 3251*w*, 2959*vw*, 2939*w*, 2932*w*, 2854*w*, 1556*w*, 1464*w*, 1422*w*, 1391*vw*, 1363*s*, 1317*s*, 1308*m*, 1291*w*, 1280*w*, 1178*s*, 1151*vs*, 1094*m*, 1075*w*, 1062*w*, 1043*w*, 1027*vw*, 1020*w*, 992*m*, 968*vw*, 945*m*, 917*s*,

868*w*, 817*s*, 810*s*, 749*w*, 722*w*, 708*w*, 670*s*, 634*w*, 595*m*, 576*m*, 549*vs*, 513*m*, 487*m*, 473*m* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.69–7.64 (*m*, 2H, 2-H, 2′-H), 7.46 (*t*, *J* = 5.8 Hz, 1H, NH), 7.41–7.35 (*m*, 4H, 3-H, 3′-H, NH_2), 3.97 (*t*, *J* = 6.5 Hz, 2H, 11-H), 2.70 (*q*, *J* = 6.6 Hz, 2H, 6-H), 2.38 (*s*, 3H, 5-H), 1.59–1.52 (*m*, 2H, 10-H), 1.35 (*p*, *J* = 6.9 Hz, 2H, 7-H), 1.27–1.17 (*m*, 4H, 8-H, 9-H) ppm; ^{13}C NMR:δ = 142.5 (C-4), 137.7 (C-1), 129.6 (C-3), 126.5 (C-2), 68.9 (C-11), 42.4 (C-6), 28.8 (C-10), 28.2 (C-7), 25.5 (C-9), 24.6 (C-8), 20.9 (C-5) ppm; MS: *m*/*z* = 373.7 (100%, $[M + Na]^+$); anal. calcd. for $C_{13}H_{22}N_2S_2O_5$ (350.45): C 44.56, H 6.33, N 7.99; found: C 44.34, H 6.51, N 7.65.

4.2.35. N-(7-Hydroxyheptyl)-4-methylbenzene Sulfonamide (**17a**) [1669425-24-4]

Applying GPA: from 4-methylbenzenesulfonyl chloride (500 mg, 2.62 mmol) and 7-amino-heptanol (516 mg, 3.93 mmol): **17a** (557 mg, 74%) [113] oil; R_f = 0.19 (petrolether/EtOAc, 2:3); UV–Vis: 227 nm (4.15); IR: ν = 3503*w*, 3279*w*, 2930*m*, 2858*w*, 1598*w*, 1495*vw*, 1429*w*, 1320*m*, 1305*m*, 1289*m*, 1152*vs*, 1120*w*, 1092*s*, 1056*m*, 1019*w*, 814*m*, 723*w*, 707*m*, 660*s*, 635*w*, 571*m*, 549*vs*, 466*w* cm^{-1}; ^1H NMR:δ = 7.68–7.65 (*m*, 2H, 2-H, 2′-H), 7.44 (*t*, *J* = 5.8 Hz, 1H, NH), 7.40–7.36 (*m*, 2H, 3-H, 3′-H), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.37–3.34 (*m*, 2H, 12-H), 2.69 (*td*, *J* = 7.0, 5.7 Hz, 2H, 6-H), 2.37 (*s*, 3H, 5-H), 1.40–1.29 (*m*, 4H, 7-H, 11-H), 1.25–1.10 (*m*, 6H, 8-H, 9-H, 10-H) ppm; ^{13}C NMR:δ = 142.4 (C-4), 137.8 (C-1), 129.5 (C-3), 126.5 (C-2), 60.7 (C-12), 42.5 (C-6), 32.4 (C-11), 28.9 (C-7), 28.4 (C-9), 26.1 (C-8), 25.3 (C-10), 20.9 (C-5) ppm; MS: *m*/*z* = 308.2 (100%, $[M + Na]^+$); anal. calcd. for $C_{14}H_{23}NSO_3$ (285.40): C 58.92, H 8.12, N 4.91; found: C 58.69, H 8.33, N 4.65.

4.2.36. 7-[(4-Methylphenyl)sulfonamido]heptyl Sulfamate (**17b**)

Applying GPB: from **17a** (200 mg, 0.7 mmol): **17b** (94 mg, 37%); white solid; R_f = 0.60 ($CHCl_3$/EtOAc, 2:3); m.p. = 85–86 °C; UV–Vis: 227 nm (4.07); IR: ν = 3351*w*, 3298*m*, 3252*w*, 2960*w*, 2940*w*, 2921*w*, 2853*w*, 1598*vw*, 1558*w*, 1477*vw*, 1466*w*, 1457*w*, 1439*w*, 1420*w*, 1392*w*, 1362*s*, 1319*s*, 1308*m*, 1292*w*, 1281*w*, 1180*s*, 1150*vs*, 1137*m*, 1094*m*, 1080*m*, 1048*w*, 1020*w*, 1009*w*, 996*w*, 982*vw*, 950*m*, 930*s*, 904*s*, 834*w*, 818*s*, 798*w*, 782*m*, 737*w*, 723*w*, 707*w*, 668*s*, 597*m*, 577*m*, 548*vs*, 519*m*, 495*m*, 474*m*, 448*w*, 404*vw* cm^{-1}; ^1H NMR:δ = 7.69–7.64 (*m*, 2H, 2-H, 2′-H), 7.45 (*t*, *J* = 5.8 Hz, 1H, NH), 7.41–7.35 (*m*, 4H, 3-H, 3′-H, NH_2), 3.98 (*t*, *J* = 6.5 Hz, 2H, 12-H), 2.69 (*q*, *J* = 6.7 Hz, 2H, 6-H), 2.38 (*s*, 3H, 5-H), 1.62–1.54 (*m*, 2H, 11-H), 1.38–1.30 (*m*, 2H, 7-H), 1.30–1.14 (*m*, 6H, 8-H, 9-H, 10-H) ppm; ^{13}C NMR:δ = 142.4 (C-4), 137.8 (C-1), 129.5 (C-3), 126.5 (C-2), 68.9 (C-12), 42.4 (C-6), 28.8 (C-11), 28.2 (C-7), 28.0 (C-9), 25.9 (C-10), 24.9 (C-8), 20.9 (C-5) ppm; MS: *m*/*z* = 387.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{14}H_{24}N_2S_2O_5$ (364.48): C 46.14, H 6.64, N 7.69; found: C 45.87, H 6.92, N 7.46.

4.2.37. N-(8-Hydroxyoctyl)-4-methylbenzene Sulfonamide (**18a**) [2772203-84-4]

Applying GPA: from 4-methylbenzenesulfonyl chloride (400 mg, 2.1 mmol) and 8-amino-octanol (457 mg, 3.15 mmol): **18a** (442 mg, 70%); white solid; R_f = 0.23 (petrolether/EtOAc, 2:3); m.p. = 87–88 °C; UV–Vis: 227 nm (4.00); IR: ν = 3418*w*, 3278*m*, 2933*m*, 2854*m*, 1598*w*, 1478*w*, 1466*w*, 1425*m*, 1384*w*, 1364*w*, 1333*m*, 1324*m*, 1305*m*, 1290*w*, 1157*vs*, 1109*w*, 1092*m*, 1064*m*, 1052*s*, 1031*w*, 1020*w*, 992*w*, 982*m*, 905*m*, 817*s*, 733*w*, 707*w*, 668*s*, 571*s*, 551*vs*, 530*s*, 500*m*, 493*m*, 465*w* cm^{-1}; ^1H NMR:δ = 7.69–7.65 (*m*, 2H, -H, 2′-H), 7.44 (*t*, *J* = 5.8 Hz, 1H, NH), 7.41–7.36 (*m*, 2H, 3-H, 3′-H), 4.31 (*t*, *J* = 5.2 Hz, 1H, OH), 3.40–3.34 (*m*, 2H, 13-H), 2.69 (*q*, *J* = 6.8 Hz, 2H, 6-H), 2.38 (*s*, 3H, 5-H), 1.42–1.28 (*m*, 4H, 7-H, 12-H), 1.26–1.12 (*m*, 8H, 8-H, 9-H, 10-H, 11-H) ppm; ^{13}C NMR:δ = 142.9 (C-4), 138.3 (C-1), 130.0 (C-3), 127.0 (C-2), 61.2 (C-13), 43.0 (C-6), 33.0 (C-12), 29.4 (C-7), 29.3 (C-9), 29.0 (C-10), 26.4 (C-8), 25.9 (C-11), 21.4 (C-5).ppm; MS: *m*/*z* = 322 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{25}NSO_3$ (299.43): C 60.17, H 8.42, N 4.68; found: C 59.87, H 8.69, N 4.55.

4.2.38. 8-[(4-Methylphenyl)sulfonamido]octyl Sulfamate (**18b**)

Applying GPB: from **18a** (200 mg, 0.67 mmol): **18b** (140 mg, 55%); white solid; R_f = 0.64 ($CHCl_3$/EtOAc, 2:3); m.p. = 98–100 °C; UV–Vis: 227 nm (4.00); IR: ν = 3344*m*, 3311*m*, 3250*m*, 2944*w*, 2928*w*, 2857*w*, 2844*w*, 1476*w*, 1430*m*, 1362*s*, 1332*w*, 1319*m*, 1309*m*, 1183*m*, 1147*vs*,

1119*w*, 1095*m*, 1074*w*, 1065*w*, 1054*m*, 1040*w*, 963*s*, 927*s*, 902*m*, 818*s*, 724*w*, 707*w*, 666*s*, 591*m*, 561*s*, 550*vs*, 526*m*, 511*s*, 499*s*, 474*m*, 405*w* cm^{-1}; ^1H NMR:δ = 7.68–7.63 (*m*, 2H, 2-H, 2′-H), 7.44 (*t*, *J* = 5.9 Hz, 1H, NH), 7.41–7.35 (*m*, 4H, 3-H, 3′-H. NH_2), 3.99 (*t*, *J* = 6.5 Hz, 2H, 13-H), 2.69 (*td*, *J* = 7.0, 5.8 Hz, 2H, 6-H), 2.38 (s, 3H, 5-H), 1.64–1.55 (*m*, 2H, 12-H), 1.38–1.31 (*m*, 2H, 7-H), 1.30–1.12 (*m*, 8H, 8-H, 9-H, 10-H, 11-H) ppm; ^{13}C NMR:δ = 142.4 (C-4), 137.8 (C-1), 129.5 (C-3), 126.5 (C-2), 69.0 (C-13), 42.4 (C-6), 28.9 (C-12), 28.4 (C-7), 28.3 (C-10), 28.3 (C-9), 25.9 (C-8), 24.9 (C-11), 20.9 (C-5) ppm; MS: *m*/*z* = 401.7 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{26}N_2S_2O_5$ (378.50): C 47.60, H 6.92, N 7.40; found: C 47.37, H 7.19, N 7.38.

4.2.39. N-(2-Hydroxyethyl)-3-methylbenzene Sulfonamide (**19a**) [1082883-27-9]

Applying GPA: from 3-methylbenzenesulfonyl chloride (500 mg, 2.62 mmol) and 2-amino-ethanol (240 mg, 3.93 mmol): **19a** (542 mg, 96%); oil; R_f = 0.1 (petrolether/EtOAc, 2:3); UV–Vis: 224 nm (3.92); IR: ν = 3493*w*, 3274*w*, 2927*w*, 2882*w*, 1601*vw*, 1478*w*, 1425*w*, 1321*s*, 1303*s*, 1220*w*, 1148*vs*, 1097*m*, 1087*m*, 1054*s*, 999*w*, 949*m*, 866*w*, 785*m*, 687*s*, 580*vs*, 524*m*, 492*w*, 459*m*, 435*w* cm^{-1}; ^1H NMR:δ = 7.63–7.61 (*m*, 1H, 6-H), 7.61–7.58 (*m*, 1H, 2-H), 7.52 (*t*, *J* = 5.8 Hz, 1H, NH), 7.50–7.42 (*m*, 2H, 4-H, 5-H), 4.66 (*t*, *J* = 5.6 Hz, 1H, OH), 3.37 (*q*, *J* = 6.3 Hz, 2H, 9-H), 2.79 (*q*, *J* = 6.2 Hz, 2H, 8-H), 2.39 (*s*, 3H, 7-H) ppm; ^{13}C NMR:δ = 140.5 (C-1), 138.8 (C-3), 132.9 (C-4), 129.0 (C-5), 126.7 (C-6), 123.6 (C-2), 59.9 (C-9), 45.1 (C-8), 20.8 (C-7) ppm; MS: *m*/*z* = 238.4 (40%, $[M + Na]^+$); anal. calcd. for $C_9H_{13}NSO_3$ (215.27): C 50.22, H 6.09, N 6.51; found: C 49.97, H 6.34, N 6.27.

4.2.40. 2-[(3-Methylphenyl)sulfonamido]ethyl Sulfamate (**19b**)

Applying GPB: from **19a** (73 mg, 0.4 mmol): **19b** (72 mg, 72%); white solid; R_f = 0.52 ($CHCl_3$/EtOAc, 2:3); m.p. = 36–38 °C; UV–Vis: 224 nm (3.65); IR: ν = 3341*m*, 3259*m*, 3100*w*, 2988*w*, 1566*w*, 1473*w*, 1444*w*, 1421*w*, 1389*w*, 1371*vs*, 1345*m*, 1325*m*, 1303*s*, 1226*w*, 1174*s*, 1172*s*, 1147*vs*, 1078*m*, 1062*m*, 1001*m*, 951*s*, 932*s*, 905*m*, 879*m*, 865*w*, 837*s*, 791*m*, 765*m*, 701*s*, 683*s*, 639*s*, 591*s*, 571*s*, 546*vs*, 504*m*, 452*m*, 429*w*, 544*vs*, 526*m* cm^{-1}; ^1H NMR:δ = 7.86 (*t*, *J* = 5.9 Hz, 1H, NH), 7.64–7.57 (*m*, 2H, 2-H, 6-H), 7.53–7.44 (*m*, 4H, 4-H, 5-H, NH_2), 3.99 (*t*, *J* = 5.7 Hz, 2H, 9-H), 3.04 (*q*, *J* = 5.6 Hz, 2H, 8-H), 2.39 (*s*, 3H, 7-H) ppm; ^{13}C NMR:δ = 140.1 (C-1), 139.0 (C-2), 133.2 (C-4), 129.1 (C-5), 126.7 (C-6), 123.6 (C-3), 67.5 (C-9), 41.6 (C-8), 20.9 (C-7) ppm; MS: *m*/*z* = 317.1 (100%, $[M + Na]^+$); anal. calcd. for $C_9H_{14}N_2S_2O_5$ (294.34): C 36.73, H 4.79, N 9.52; found: C 36.55, H 5.00, N 9.35.

4.2.41. N-(3-Hydroxypropyl)-3-methylbenzene Sulfonamide (**20a**) [1082805-58-0]

Applying GPA: from 3-methylbenzenesulfonyl chloride (500 mg, 2.62 mmol) and 3-amino-propanol (295 mg, 3.93 mmol): **20a** (522 mg, 87%) [114] oil; R_f = 0.09 (petrolether/EtOAc, 2:3); UV–Vis: 224 nm (3.91); IR: ν = 3502*w*, 3276*w*, 2947*w*, 2882*w*, 1477*w*, 1423*w*, 1320*m*, 1303*s*, 1222*w*, 1148*vs*, 1096*m*, 1085*s*, 1068*m*, 1008*w*, 998*w*, 959*w*, 879*w*, 787*m*, 688*s*, 579*vs*, 524*m*, 498*w*, 463*m*, 434*w* cm^{-1}; ^1H NMR:δ = 7.62–7.56 (*m*, 2H, 2-H, 6-H), 7.50–7.41 (*m*, 3H, 4-H, 5-H, NH), 4.40 (*t*, *J* = 5.1 Hz, 1H, OH), 3.40–3.35 (*m*, 2H, 10-H), 2.78 (*q*, *J* = 7.0 Hz, 2H, 8-H), 2.39 (*s*, 3H, 7-H), 1.52 (*p*, *J* = 6.4 Hz, 2H, 9-H) ppm; ^{13}C NMR:δ = 140.4 (C-1), 138.8 (C-3), 132.9 (C-4), 129.0 (C-5), 126.7 (C-6), 123.6 (C-2), 58.1 (C-10), 40.0 (C-8), 32.4 (C-9), 20.9 (C-7) ppm; MS: *m*/*z* = 252.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{10}H_{15}NSO_3$ (229.29): C 52.38, H 6.59, N 6.11; found: C 52.03, H 6.88, N 5.94.

4.2.42. 3-[(3-Methylphenyl)sulfonamido]propyl Sulfamate (**20b**)

Applying GPB: from **20a** (300 mg, 1.31 mmol): **20b** (305 mg, 76%); white solid; R_f = 0.52 ($CHCl_3$/EtOAc, 2:3); m.p. = 73–74 °C; UV–Vis: 224 nm (3.88); IR: ν = 3351*m*, 3256*m*, 3108*w*, 2983*w*, 2921*vw*, 1566*w*, 1473*w*, 1444*w*, 1421*w*, 1399*w*, 1373*vs*, 1352*m*, 1318*m*, 1303*s*, 1241*w*, 1226*w*, 1218*w*, 1176*s*, 1170*s*, 1147*vs*, 1113*w*, 1085*m*, 1056*m*, 1004*m*, 951*s*, 929*s*, 905*m*, 887*m*, 881*m*, 865*w*, 837*s*, 792*m*, 759*m*, 703*s*, 685*s*, 638*s*, 591*s*, 573*s*, 545*vs*, 508*m*, 486*m*, 450*m*, 426*w*, 548*vs*, 519*m*, 495*m*, 474*m*, 448*w*, 404*vw* cm^{-1}; ^1H NMR:δ = 7.67–7.57 (*m*, 3H, 2-H, 6-H, NH), 7.52–7.37 (*m*, 4H, 4-H, 5-H, NH_2), 4.02 (*t*, *J* = 6.3 Hz, 2H, 10-H), 2.81 (*t*, *J* = 7.1 Hz, 2H, 8-H), 2.40 (*s*, 3H, 7-H), 1.76 (*p*, *J* = 6.7 Hz, 2H, 9-H) ppm; ^{13}C NMR:δ = 140.2

(C-1), 138.9 (C-3), 133.0 (C-4), 129.1 (C-5), 126.7 (C-6), 123.6 (C-2), 66.5 (C-10), 39.2 (C-8), 28.7 (C-9), 20.8 (C-7) ppm; MS: m/z = 331.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{10}H_{16}N_2S_2O_5$ (308.37): C 38.95, H 5.23, N 9.08; found: C 38.77, H 5.52, N 8.85.

4.2.43. N-(4-Hydroxybutyl)-3-methylbenzene Sulfonamide (**21a**) [1082889-69-7]

Applying GPA: from 3-methylbenzenesulfonyl chloride (500 mg, 2.62 mmol) and 4-amino-butanol (351 mg, 3.93 mmol): **21a** (589 mg, 92%); oil; R_f = 0.56 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 224 nm (3.90); IR: ν = 3502*w*, 3279*w*, 2940*w*, 2871*w*, 1598*w*, 1428*w*, 1319*m*, 1305*m*, 1289*m*, 1152*vs*, 1120*w*, 1091*s*, 1054*m*, 1020*m*, 814*m*, 706*w*, 659*s*, 571*m*, 549*vs*, 491*w*, 469*w* cm^{-1}; ^1H NMR:δ = 7.62–7.56 (*m*, 2H, 2-H, 6-H), 7.52–7.41 (*m*, 3H, 4-H, 5-H, NH), 4.35 (*t*, *J* = 5.1 Hz, 1H, OH), 3.35–3.29 (*m*, 2H, 11-H), 2.72 (*q*, *J* = 6.7 Hz, 2H, 8-H), 2.39 (*s*, 3H, 7-H), 1.45–1.31 (*m*, 4H, 9-H, 10-H) ppm; ^{13}C NMR:δ = 140.5 (C-1), 138.8 (C-3), 132.8 (C-4), 129.0 (C-5), 126.7 (C-6), 123.6 (C-2), 60.2 (C-11), 42.5 (C-8), 29.5 (C-10), 25.8 (C-9), 20.8 (C-7) ppm; MS: m/z = 266.2 (100%, $[M + Na]^+$); anal. calcd. for $C_{11}H_{17}NSO_3$ (243.32): C 54.30, H 7.04, N 5.76; found: C 54.00, H 7.31, N 5.52.

4.2.44. 4-[(3-Methylphenyl)sulfonamido]butyl Sulfamate (**21b**)

Applying GPB: from **21a** (250 mg, 1.03 mmol): **21b** (317 mg, 96%); white solid; R_f = 0.55 ($CHCl_3$/EtOAc, 2:3); m.p. = 62–64 °C; UV–Vis: 225 nm (3.84); IR: ν = 3351*w*, 3272*m*, 1474*w*, 1428*w*, 1375*s*, 1339*w*, 1315*m*, 1303*s*, 1198*w*, 1171*s*, 1153*vs*, 1097*m*, 1091*m*, 1065*m*, 1014*w*, 970*s*, 930*m*, 897*m*, 888*m*, 823*m*, 793*m*, 746*w*, 701*s*, 697*s*, 688*s*, 609*m*, 594*s*, 582*s*, 553*s*, 525*m*, 491*m*, 436*w* cm^{-1}; ^1H NMR:δ = 7.59–7.50 (*m*, 3H, 4-H, 5-H, NH), 7.49–7.39 (*m*, 2H, 2-H, 6-H), 7.36 (*s*, 2H, NH_2), 3.94 (*t*, *J* = 6.3 Hz, 2H, 11-H), 2.72 (*q*, *J* = 6.7 Hz, 2H, 8-H), 2.36 (*s*, 3H, 7-H), 1.63–1.54 (*m*, 2H, 10-H), 1.47–1.38 (*m*, 2H, 9-H) ppm; ^{13}C NMR:δ = 140.5 (C-1), 138.9 (C-3), 132.9 (C-4), 129.1 (C-5), 126.7 (C-6), 123.6 (C-2), 68.6 (C-11), 42.0 (C-8), 25.6 (C-10), 24.7 (C-9), 20.9 (C-7) ppm; MS: m/z = 345.6 (90%, $[M + Na]^+$); anal. calcd. for $C_{11}H_{18}N_2S_2O_5$ (322.39): C 40.98, H 5.63, N 8.69; found: C 40.76, H 8.92, N 8.38.

4.2.45. N-(5-Hydroxypentyl)-3-methylbenzene Sulfonamide (**22a**) [1986639-01-3]

Applying GPA: from 3-methylbenzenesulfonyl chloride (500 mg, 2.62 mmol) and 5-amino-pentanol (406 mg, 3.93 mmol): **22a** (620 mg, 92%); oil; R_f = 0.13 (petrolether/EtOAc, 2:3); UV–Vis: 224 nm (3.89); IR: ν = 3454*w*, 3108*w*, 2954*w*, 2919*w*, 2880*w*, 2862*w*, 1597*vw*, 1474*w*, 1455*w*, 1437*w*, 1313*s*, 1305*m*, 1289*m*, 1245*w*, 1150*s*, 1122*m*, 1108*w*, 1091*s*, 1061*m*, 1041*w*, 1027*m*, 1019*w*, 940*m*, 849*w*, 817*m*, 801*w*, 755*m*, 721*m*, 709*m*, 662*s*, 636*w*, 577*s*, 551*vs*, 484*m*, 458*w* cm^{-1}; ^1H NMR:δ = 7.62–7.60 (*m*, 1H, 6-H), 7.60–7.56 (*m*, 1H, 2-H), 7.51–7.41 (*m*, 3H, 4-H, 5-H, NH), 4.31 (*t*, *J* = 5.1 Hz, 1H, OH), 3.36–3.30 (*m*, 2H, 12-H), 2.71 (*q*, *J* = 6.5 Hz, 2H, 8-H), 2.39 (*s*, 3H, 7-H), 1.41–1.29 (*m*, 4H, 9-H, 11-H), 1.28–1.18 (*m*, 2H, 10-H) ppm; ^{13}C NMR:δ = 141.0 (C-1), 139.3 (C-3), 133.3 (C-4), 129.5 (C-5), 127.1 (C-6), 124.1 (C-2), 61.0 (C-12), 43.1 (C-8), 32.5 (C-11), 29.4 (C-9), 23.1 (C-10), 21.3 (C-7) ppm; MS: m/z = 280.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{12}H_{19}NSO_3$ (257.35): C 56.01, H 7.44, N 5.44; found: C 55.76, H 7.69, N 5.20.

4.2.46. 5-[(3-Methylphenyl)sulfonamido]pentyl Sulfamate (**22b**)

Applying GPB: from **22a** (300 mg, 1.17 mmol): **22b** (378 mg, 96%); white solid; R_f = 0.60 ($CHCl_3$/EtOAc, 2:3); m.p. = 67–68 °C; UV–Vis: 224 nm (3.75); IR: ν = 3371*w*, 3265*m*, 2978*vw*, 2933*w*, 2867*vw*, 1536*vw*, 1475*w*, 1462*w*, 1439*w*, 1423*w*, 1404*w*, 1377*m*, 1358*s*, 1316*m*, 1302*s*, 1281*w*, 1227*w*, 1179*s*, 1150*vs*, 1138*m*, 1097*w*, 1085*m*, 1061*m*, 1049*m*, 1037*w*, 997*w*, 976*s*, 960*s*, 931*m*, 911*s*, 878*w*, 856*w*, 823*s*, 785*m*, 749*m*, 713*s*, 699*s*, 685*s*, 598*s*, 581*s*, 566*s*, 551*s*, 538*m*, 525*s*, 489*m*, 466*s*, 449*w*, 448*w*, 404*vw* cm^{-1}; ^1H NMR:δ = 7.61–7.59 (*m*, 1H, 6-H), 7.58–7.56 (*m*, 1H, 2-H), 7.54–7.42 (*m*, 3H, 4-H, 5-H, NH), 7.37 (*s*, 2H), 3.96 (*t*, *J* = 6.5 Hz, 2H, 12-H), 2.72 (*q*, *J* = 6.6 Hz, 2H, 8-H), 2.39 (*s*, 3H, 7-H), 1.56 (*p*, *J* = 6.7 Hz, 2H, 11-H), 1.44–1.35 (*m*, 2H, 9-H), 1.33–1.25 (*m*, 2H, 10-H) ppm; ^{13}C NMR:δ = 140.5 (C-1), 138.8 (C-3), 132.9 (C-4), 129.0 (C-5), 126.6 (C-6), 123.6 (C-2), 68.8 (C-12), 42.3 (C-8), 28.5 (C-11), 27.8 (C-9), 22.2 (C-10),

20.8 (C-7) ppm; MS: m/z = 359.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{12}H_{20}N_2S_2O_5$ (336.42): C 42.84, H 5.99, N 8.33; found: C 42.62, H 6.13, N 8.17.

4.2.47. N-(6-Hydroxyhexyl)-3-methylbenzene Sulfonamide (**23a**) [1916290-41-9]

Applying GPA: from 3-methylbenzenesulfonyl chloride (500 mg, 2.62 mmol) and 6-amino-hexanol (461 mg, 3.93 mmol): **23a** (649 mg, 91%); oil; R_f = 0.15 (petrolether/EtOAc, 2:3); UV–Vis: 224 nm (3.90); IR: ν = 3503*w*, 3279*w*, 2933*m*, 2860*w*, 1477*w*, 1428*w*, 1321*m*, 1303*s*, 1221*w*, 1148*vs*, 1097*m*, 1086*m*, 1072*m*, 1054*m*, 882*w*, 787*m*, 688*s*, 591*s*, 581*vs*, 525*m*, 490*m*, 459*w*, 453*w*, 435*w* cm^{-1}; ^1H NMR:δ = 7.61–7.59 (*m*, 1H, 6-H), 7.59–7.56 (*m*, 1H, 2-H), 7.50–7.41 (*m*, 3H, 4-H, 5-H, NH), 4.30 (*t*, *J* = 5.2 Hz, 1H, OH), 3.38–3.31 (*m*, 2H, 13-H), 2.75–2.67 (*m*, 2H, 8-H), 2.39 (*s*, 3H, 7-H), 1.40–1.28 (*m*, 4H, 9-H, 12-H), 1.24–1.13 (*m*, 4H, 10-H, 11-H) ppm; ^{13}C NMR:δ = 140.6 (C-1), 138.8 (C-3), 132.8 (C-4), 129.0 (C-5), 126.7 (C-6), 123.6 (C-2), 60.6 (C-13), 42.5 (C-8), 32.4 (C-12), 29.0 (C-9), 25.9 (C-10), 25.0 (C-11), 20.8 (C-7) ppm; MS: m/z = 294.4 (100%, $[M + Na]^+$); anal. calcd. for $C_{13}H_{21}NSO_3$ (271.38): C 57.54, H 7.80, N 5.16; found: C 57.26, H 8.01, N 4.97.

4.2.48. 6-[(3-Methylphenyl)sulfonamido]hexyl Sulfamate (**23b**)

Applying GPB: from **23a** (300 mg, 1.11 mmol): **23b** (364 mg, 94%); white solid; R_f = 0.64 ($CHCl_3$/EtOAc, 2:3); m.p. = 74–75 °C; UV–Vis: 224 nm (3.84); IR: ν = 3371*w*, 3261*m*, 2975*vw*, 2958*vw*, 2943*w*, 2910*vw*, 2897*vw*, 2852*w*, 1556*w*, 1479*w*, 1472*w*, 1455*w*, 1431*w*, 1398*w*, 1369*s*, 1341*w*, 1323*s*, 1308*m*, 1282*w*, 1230*vw*, 1221*vw*, 1161*vs*, 1112*vw*, 1099*w*, 1086*w*, 1062*w*, 1053*w*, 1042*vw*, 1007*m*, 988*vw*, 963*s*, 940*m*, 912*m*, 881*w*, 861*vw*, 818*s*, 801*w*, 791*w*, 720*m*, 685*s*, 596*vs*, 580*s*, 550*m*, 529*m*, 505*w*, 494*m*, 457*vw*, 436*w*, 404*vw* cm^{-1}; ^1H NMR:δ = 7.62–7.55 (*m*, 2H, 2-H, 6-H), 7.52–7.42 (*m*, 3H, 4-H, 5-H), 7.37 (*s*, 2H, NH_2), 3.97 (*t*, *J* = 6.5 Hz, 2H, 13-H), 2.72 (*q*, *J* = 6.6 Hz, 2H, 8-H), 2.39 (*s*, 3H, 7-H), 1.56 (*p*, *J* = 6.7 Hz, 2H, 12-H), 1.36 (*p*, *J* = 6.9 Hz, 2H, 9-H), 1.28–1.18 (*m*, 4H, 10-H, 11-H) ppm; ^{13}C NMR:δ = 140.5 (C-1), 138.8 (C-3), 132.8 (C-4), 129.0 (C-5), 126.6 (C-6), 123.6 (C-2), 68.9 (C-13), 42.4 (C-8), 28.8 (C-12), 28.2 (C-9), 25.5 (C-10), 24.6 (C-11), 20.8 (C-7) ppm; MS: m/z = 373.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{13}H_{22}N_2S_2O_5$ (350.45): C 44.56, H 6.33, N 7.99; found: C 44.26, H 6.58, N 8.19.

4.2.49. N-(7-Hydroxyheptyl)-3-methylbenzene Sulfonamide (**24a**)

Applying GPA: from 3-methylbenzenesulfonyl chloride (500 mg, 2.62 mmol) and 7-amino-heptanol (516 mg, 3.93 mmol): **24a** (648 mg, 87%); oil; R_f = 0.19 (petrolether/EtOAc, 2:3); UV–Vis: 224 nm (3.78); IR: ν = 3474*m*, 3128*m*, 2930*m*, 2889*w*, 2850*m*, 1483*w*, 1465*m*, 1447*m*, 1402*w*, 1372*w*, 1317*m*, 1309*m*, 1298*m*, 1289*m*, 1222*m*, 1143*vs*, 1099*s*, 1083*s*, 1072*s*, 1020*m*, 1001*m*, 957*m*, 906*m*, 870*w*, 862*w*, 835*w*, 790*s*, 755*w*, 709*s*, 689*s*, 584*vs*, 555*m*, 545*m*, 524*m*, 483*s*, 472*m*, 439*m*, 408*w* cm^{-1}; ^1H NMR:δ = 7.61–7.56 (*m*, 2H, 2-H, 6-H), 7.50–7.41 (*m*, 3H, 4-H, 5-H, NH), 4.30 (*td*, *J* = 5.2, 1.1 Hz, 1H, OH), 3.38–3.33 (*m*, 2H, 14-H), 2.71 (*td*, *J* = 7.0, 5.8 Hz, 2H, 8-H), 2.39 (*s*, 3H, 7-H), 1.40–1.28 (*m*, 4H, 9-H, 13-H), 1.26–1.11 (*m*, 6H, 10-H, 11-H, 12-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 140.6 (C-1), 138.8 (C-3), 132.8 (C-4), 129.0 (C-5), 126.6 (C-6), 123.6 (C-2), 60.7 (C-14), 42.5 (C-8), 32.4 (C-13), 28.9 (C-9), 28.4 (C-11), 26.0 (C-12), 25.3 (C-10), 20.8 (C-7) ppm; MS: m/z = 308.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{14}H_{23}NSO_3$ (285.40): C 58.92, H 8.12, N 4.91; found: C 58.78, H 8.34, N 4.65.

4.2.50. 7-[(3-Methylphenyl)sulfonamido]heptyl Sulfamate (**24b**)

Applying GPB: from **24a** (300 mg, 1.05 mmol): **24b** (279 mg, 73%) a white waxy solid; R_f = 0.77 ($CHCl_3$/EtOAc, 2:3); m.p. = 55–57 °C; UV–Vis: 224 nm (3.80); IR: ν = 3361*w*, 3265*m*, 2961*w*, 2937*w*, 2906*w*, 2895*w*, 2856*w*, 1566*w*, 1477*w*, 1429*m*, 1396*w*, 1370*s*, 1317*s*, 1301*m*, 1226*w*, 1183*s*, 1150*vs*, 1114*w*, 1096*w*, 1085*m*, 1061*m*, 1049*w*, 1000*m*, 972*s*, 927*m*, 922*m*, 904*m*, 878*w*, 825*s*, 783*m*, 741*w*, 723*w*, 702*s*, 685*s*, 665*m*, 597*s*, 578*s*, 556*vs*, 528*m*, 521*m*, 495*s*, 478*w*, 450*w*, 429*w*, 505*w*, 494*m*, 457*vw*, 436*w*, 404*vw* cm^{-1}; ^1H NMR:δ = 7.62–7.56 (*m*, 2H, 2-H, 6-H), 7.51–7.42 (*m*, 3H, 4-H, 5-H, NH), 7.37 (*s*, 2H, NH_2), 3.98 (*t*, *J* = 6.5 Hz, 2H, 14-H), 2.72 (*td*, *J* = 7.0, 5.8 Hz, 2H, 8-H), 2.39 (*s*, 3H, 7-H), 1.63–1.54 (*m*, 2H, 13-H), 1.39–1.15 (*m*, 8H, 9-H, 10-H, 11-H, 12-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 140.6 (C-1), 138.8 (C-3),

132.8 (C-4), 129.0 (C-5), 126.6 (C-6), 123.6 (C-2), 68.9 (C-14), 42.5 (C-8), 28.9 (C-9), 28.2 (C-13), 28.0 (C-11), 25.9 (C-10), 24.9 (C-12), 20.8 (C-7) ppm; MS: m/z = 387.2 (100%, $[M + Na]^+$); anal. calcd. for $C_{14}H_{24}N_2S_2O_5$ (364.48): C 46.14, H 6.64, N 7.69; found: C 45.97, H 6.90, N 7.47.

4.2.51. N-(8-Hydroxyoctyl)-3-methylbenzene Sulfonamide (**25a**)

Applying GPA: from 3-methylbenzenesulfonyl chloride (500 mg, 2.62 mmol) and 8-amino-octanol (571 mg, 3.93 mmol): **25a** (702 mg, 89%); oil; R_f = 0.25 (petrolether/EtOAc, 2:3); UV–Vis: 224 nm (3.90); IR: ν = 3508*w*, 3282*w*, 2928*m*, 2856*m*, 1477*w*, 1458*w*, 1429*w*, 1322*s*, 1303*s*, 1221*w*, 1149*vs*, 1097*m*, 1086*m*, 999*w*, 883*w*, 786*m*, 689*s*, 592*s*, 581*vs*, 525*m*, 492*m*, 436*w* cm^{-1}; ^1H NMR:δ = 7.61–7.59 (*m*, 1H, 6-H), 7.59–7.56 (*m*, 1H, 2-H), 7.50–7.41 (*m*, 3H, 4-H, 5-H, NH), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.39–3.32 (*m*, 2H, 15-H), 2.71 (*td*, *J* = 7.0, 5.8 Hz, 2H, 8-H), 2.38 (*s*, 3H, 7-H), 1.42–1.28 (*m*, 4H, 9-H, 14-H), 1.26–1.11 (*m*, 8H, 10-H, 11-H, 12-H, 13-H) ppm; ^{13}C NMR:δ = 140.6 (C-1), 138.8 (C-3), 132.8 (C-4), 129.0 (C-5), 126.7 (C-6), 123.6 (C-2), 60.7 (C-15), 42.5 (C-8), 32.5 (C-14), 28.9 (C-9), 28.8 (C-11), 28.6 (C-12), 26.0 (C-10), 25.4 (C-13), 20.8 (C-7) ppm; MS: m/z = 322.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{25}NSO_3$ (299.43): C 60.17, H 8.42, N 4.68; found: C 59.87, H 8.70, N 4.45.

4.2.52. 8-[(3-Methylphenyl)sulfonamido]octyl Sulfamate (**25b**)

Applying GPB: from **25a** (300 mg, 1.00 mmol): **25b** (284 mg, 75%); white solid; R_f = 0.83 ($CHCl_3$/EtOAc, 2:3); m.p. = 54–56 °C; UV–Vis: 224 nm (3.84); IR: ν = 3369*w*, 3254*m*, 2970*w*, 2938*m*, 2920*m*, 2859*m*, 2853*w*, 1562*w*, 1473*m*, 1458*w*, 1440*m*, 1431*w*, 1397*w*, 1324*s*, 1303*s*, 1217*w*, 1164*s*, 1150*vs*, 1116*w*, 1099*m*, 1087*m*, 1057*m*, 1053*m*, 1039*w*, 994*m*, 960*vs*, 920*m*, 896*s*, 864*w*, 849*vw*, 832*m*, 800*w*, 787*m*, 731*w*, 701*s*, 688*vs*, 602*m*, 578*s*, 569*s*, 524*m*, 517*m*, 493*s*, 478*m*, 446*w*, 431*w*, 415*w*, 457*vw*, 436*w*, 404*vw* cm^{-1}; ^1H NMR:δ = 7.61–7.58 (*m*, 2H, 6-H), 7.58–7.56 (*m*, 1H, 2-H), 7.50–7.42 (*m*, 3H, 4-H, 5-H, NH), 7.37 (*s*, 2H, NH_2), 3.99 (*t*, *J* = 6.5 Hz, 2H, 15-H), 2.71 (*td*, *J* = 7.0, 5.8 Hz, 2H, 8-H), 2.39 (*s*, 3H, 7-H), 1.65–1.55 (*m*, 2H, 14-H), 1.40–1.24 (*m*, 2H, 9-H, 13-H), 1.23–1.14 (*m*, 6H, 10-H, 11-H, 12-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 140.6 (C-1), 138.8 (C-3), 132.8 (C-4), 129.0 (C-5), 126.6 (C-6), 123.6 (C-2), 69.0 (C-15), 42.5 (C-8), 28.9 (C-14), 28.4 (C-9), 28.3 (C-11), 28.3 (C-12), 25.9 (C-10), 24.9 (C-13), 20.8 (C-7) ppm; MS (ESI, MeOH) m/z = 401.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{26}N_2S_2O_5$ (378.50): C 47.60, H 6.92, N 7.40; found: C 47.36, H 7.17, N 7.15.

4.2.53. N-(2-Hydroxyethyl)-2-methylbenzene Sulfonamide (**26a**) [19829-14-2]

Applying GPA: from 2-methylbenzenesulfonyl chloride (500 mg, 2.62 mmol) and 2-amino-ethanol (3.93 mg, 3.93 mmol): **26a** (420 mg, 74%) [115–117]; white solid; R_f = 0.11 (petrolether/EtOAc, 2:3); m.p. = 73–74 °C (lit.: [76,116] 73–75 °C [(4)]); UV–Vis: 222 nm (3.87); IR: ν = 3452*m*, 3189*m*, 3067*w*, 2959*w*, 2939*w*, 2867*w*, 2687*vw*, 1592*vw*, 1459*m*, 1421*m*, 1399*w*, 1381*w*, 1349*w*, 1301*s*, 1285*m*, 1261*m*, 1207*w*, 1154*vs*, 1133*s*, 1094*s*, 1070*m*, 1059*s*, 1037*m*, 995*w*, 963*s*, 899*w*, 880*vw*, 839*m*, 803*w*, 760*s*, 710*m*, 687*s*, 590*s*, 580*vs*, 542*s*, 511*m*, 491*m*, 468*s*, 443*w*, 415*m* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.81 (*dd*, *J* = 7.9, 1.4 Hz, 1H, 3-H), 7.63–7.58 (*m*, 1H, 6-H), 7.51 (*td*, *J* = 7.5, 1.4 Hz, 1H, 4-H), 7.41–7.35 (*m*, 2H, 5-H, NH), 4.65 (*t*, *J* = 5.5 Hz, 1H, OH), 3.36–3.32 (*m*, 2H, 9-H), 2.81 (*t*, *J* = 6.5 Hz, 2H, 8-H), 2.57 (*s*, 3H, 7-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 138.8 (C-1), 136.5 (C-2), 132.4 (C-4), 132.3 (C-3), 128.3 (C-6), 126.1 (C-5), 59.9 (C-9), 44.7 (C-8), 19.7 (C-7) ppm; MS: m/z = 238.2 (100%, $[M + Na]^+$); anal. calcd. for $C_9H_{13}NSO_3$ (215.27): C 50.22, H 6.09, N 6.51; found: C 49.98, H 6.37, N 6.39.

4.2.54. 2-[(2-Methylphenyl)sulfonamido]ethyl Sulfamate (**26b**)

Applying GPB: from **26a** (150 mg, 0.70 mmol): **26b** (185 mg, 90%); white solid; R_f = 0.52 ($CHCl_3$/EtOAc, 2:3); m.p. = 70–72 °C; UV–Vis: 270 nm (3.08); IR: ν = 3394*w*, 3359*w*, 3311*m*, 3288*m*, 3256*m*, 1566*vw*, 1539*w*, 1479*vw*, 1455*w*, 1434*w*, 1417*w*, 1398*w*, 1370*s*, 1358*vs*, 1320*m*, 1309*vs*, 1290*m*, 1237*w*, 1190*m*, 1174*s*, 1155*vs*, 1127*m*, 1112*m*, 1091*w*, 1076*m*, 1026*m*, 962*s*, 933*s*, 909*s*, 872*w*, 845*w*, 803*m*, 759*vs*, 753*vs*, 708*m*, 691*m*, 590*s*, 567*m*, 548*vs*, 538*s*,

526*s*, 514*m*, 481*m*, 467*m*, 454*m*, 424*m*, 410*w* cm^{-1}; ^1H NMR:δ = 7.97 (*t*, *J* = 6.0 Hz, 1H, NH), 7.82 (*dd*, *J* = 7.8, 1.4 Hz, 1H, 3-H), 7.56–7.46 (*m*, 3H, 4-H, NH_2), 7.44–7.35 (*m*, 2H, 5-H, 6-H), 3.97 (*t*, *J* = 5.8 Hz, 2H, 9-H), 3.08 (*q*, *J* = 5.7 Hz, 2H, 8-H), 2.58 (*s*, 3H, 7-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 138.4 (C-1), 136.6 (C-2), 132.6 (C-4), 132.5 (C-3), 128.3 (C-6), 126.2 (C-5), 67.5 (C-9), 41.2 (C-8), 19.8 (C-7) ppm; MS: *m*/*z* = 317.2 (100%, $[M + Na]^+$); anal. calcd. for $C_9H_{14}N_2S_2O_5$ (294.34): C 36.73, H 4.79, N 9.52; found: C 36.54, H 4.44, N 9.38.

4.2.55. N-(3-Hydroxypropyl)-2-methylbenzene Sulfonamide (**27a**) [1082811-80-0]

Applying GPA: from 2-methylbenzenesulfonyl chloride (500 mg, 2.62 mmol) and 3-amino-propanol (240 mg, 3.93 mmol): **27a** (420 mg, 74%); oil; R_f = 0.15 (petrolether/EtOAc, 2:3); UV–Vis: 222 nm (3.84); IR: ν = 3498*w*, 3294*w*, 2939*w*, 2882*w*, 1472*w*, 1457*w*, 1311*s*, 1290*m*, 1153*vs*, 1131*m*, 1065*s*, 1006*w*, 957*w*, 872*w*, 806*w*, 759*m*, 710*m*, 689*m*, 591*vs*, 575*s*, 541*m*, 481*m*, 444*w*, 425*w*, 420*w* cm^{-1}; ^1H NMR:δ = 7.79 (*dd*, *J* = 7.8, 1.4 Hz, 1H, 3-H), 7.61–7.51 (*m*, 1H, 6-H), 7.48 (*td*, *J* = 7.5, 1.4 Hz, 1H, 4-H), 7.40–7.32 (*m*, 2H, 5-H, NH), 4.38 (*s*, 1H, OH), 3.36–3.30 (*m*, 2H, 10-H), 2.80 (*t*, *J* = 7.2 Hz, 2H, 8-H), 2.56 (*s*, 3H, 7-H), 1.55–1.44 (*m*, 2H, 9-H) ppm; ^{13}C NMR:δ = 138.7 (C-1), 136.5 (C-2), 132.5 (C-4), 132.3 (C-3), 128.4 (C-6), 126.2 (C-5), 58.1 (C-10), 39.8 (C-8), 32.4 (C-9), 19.8 (C-7) ppm; MS: *m*/*z* = 252.2 (100%, $[M + Na]^+$); anal. calcd. for $C_{10}H_{15}NSO_3$ (229.29): C 52.38, H 6.59, N 6.11; found: C 52.04, H 6.80, N 5.96.

4.2.56. 3-[(2-Methylphenyl)sulfonamido]propyl Sulfamate (**27b**)

Applying GPB: from **27a** (300 mg, 1.31 mmol): **27b** (328 mg, 81%); white solid; R_f = 0.54 ($CHCl_3$/EtOAc, 2:3); m.p. = 68–70 °C; UV–Vis: 224 nm (3.90); IR: ν = 3314*m*, 3238*m*, 3115*w*, 2942*vw*, 1570*w*, 1470*w*, 1439*w*, 1418*w*, 1396*w*, 1361*s*, 1315*s*, 1281*w*, 1216*vw*, 1199*vw*, 1171*m*, 1155*vs*, 1134*m*, 1111*m*, 1095*m*, 1066*m*, 1056*w*, 1005*w*, 940*s*, 886*m*, 843*s*, 805*w*, 763*s*, 711*m*, 691*s*, 643*w*, 589*vs*, 574*s*, 549*vs*, 543*vs*, 508*m*, 486*m*, 457*m*, 436*w*, 415*w* cm^{-1}; ^1H NMR:δ = 7.85–7.77 (*m*, 1H, 3-H), 7.71 (*t*, *J* = 5.8 Hz, 1H, 6-H), 7.56–7.47 (*m*, 1H, 4-H), 7.44–7.34 (*m*, 4H, 5-H, NH, NH_2), 4.01 (*t*, *J* = 6.3 Hz, 2H, 10-H), 2.84 (*td*, *J* = 7.2, 5.8 Hz, 2H, 8-H), 2.57 (*s*, 3H, 7-H), 1.76 (*p*, *J* = 6.6 Hz, 2H, 9-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 138.5 (C-1), 136.5 (C-2), 132.5 (C-4), 132.4 (C-3), 128.3 (C-6), 126.2 (C-5), 66.5 (C-10), 39.0 (C-8), 28.9 (C-9), 19.8 (C-7) ppm; MS: *m*/*z* = 331.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{10}H_{16}N_2S_2O_3$ (308.37): C 38.95, H 5.23, N 9.08; found: C 38.78, H 5.51, N 8.76.

4.2.57. N-(4-Hydroxybutyl)-2-methylbenzene Sulfonamide (**28a**) [1082772-69-7]

Applying GPA: from 2-methylbenzenesulfonyl chloride (500 mg, 2.62 mmol) and 4-amino-butanol (351 mg, 3.93 mmol): **28a** (546 mg, 86%); oil; R_f = 0.15 (petrolether/EtOAc, 2:3); UV–Vis: 224 nm (3.85); IR: ν = 3502*w*, 3296*w*, 2939*w*, 2872*w*, 1472*w*, 1456*w*, 1311*s*, 1153*vs*, 1131*m*, 1065*s*, 1034*m*, 952*vw*, 867*w*, 808*w*, 760*m*, 711*m*, 688*m*, 591*vs*, 541*m*, 488*m*, 451*w*, 444*w* cm^{-1}; ^1H NMR:δ = 7.80 (*dd*, *J* = 7.8, 1.4 Hz, 1H, 3-H), 7.65–7.55 (*m*, 1H, 6-H), 7.50 (*td*, *J* = 7.5, 1.4 Hz, 1H, 4-H), 7.41–7.33 (*m*, 2H, 5-H, NH), 4.35 (*s*, 1H, OH), 3.30 (*t*, *J* = 6.0 Hz, 2H, 8-H), 2.76 (*t*, *J* = 6.7 Hz, 2H, 11-H), 2.57 (*s*, 3H, 7-H), 1.43–1.29 (*m*, 4H, 9-H, 10-H) ppm; ^{13}C NMR:δ = 138.8 (C-1), 136.4 (C-2), 132.4 (C-4), 132.3 (C-3), 128.3 (C-6), 126.1 (C-5), 60.2 (C-11), 42.3 (C-8), 29.5 (C-10), 25.9 (C-9), 19.8 (C-7) ppm; MS: *m*/*z* = 266.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{11}H_{17}NSO_3$ (243.32): C 54.30, H 7.04, N 5.76; found: C 53.99, H 7.34, N 5.31.

4.2.58. 4-[(2-Methylphenyl)sulfonamido]butyl Sulfamate (**28b**)

Applying GPB: from **28a** (300 mg, 1.23 mmol): **28b** (312 mg, 79%); oil; R_f = 0.55 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 224 nm (3.72); IR: ν = 3317*m*, 3240*m*, 3112*w*, 2940*vw*, 1574*w*, 1471*w*, 1438*w*, 1417*w*, 1393*w*, 1360*s*, 1317*s*, 1280*w*, 1214*vw*, 1198*vw*, 1170*m*, 1154*vs*, 1130*m*, 1110*m*, 1096*m*, 1068*m*, 1050*w*, 1001*w*, 945*s*, 884*m*, 845*s*, 801*w*, 760*s*, 711*m*, 690*s*, 646*w*, 590*vs*, 572*s*, 548*vs*, 543*vs*, 508*m*, 484*m*, 456*m*, 433*w*, 415*w* cm^{-1}; ^1H NMR:δ = 7.82–7.78 (*m*, 1H, 3-H), 7.66 (*t*, *J* = 5.9 Hz, 1H, 6-H), 7.51 (*td*, *J* = 7.5, 1.4 Hz, 1H, 4-H), 7.42–7.35 (*m*, 4H, 5-H, NH, NH_2), 3.95 (*t*, *J* = 6.4 Hz, 2H, 11-H), 2.79 (*q*, *J* = 6.7 Hz, 2H, 8-H), 2.57 (*s*, 3H, 7-H), 1.64–1.55

(*m*, 2H, 10-H), 1.50–1.39 (*m*, 2H, 9-H) ppm; ^{13}C NMR:δ = 139.2 (C-1), 136.9 (C-2), 132.9 (C-4), 132.8 (C-3), 128.7 (C-6), 126.6 (C-5), 69.0 (C-11), 42.2 (C-8), 26.0 (C-10), 26.0 (C-9), 20.2 (C-7) ppm; MS: *m*/*z* = 345.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{11}H_{18}N_2S_2O_5$ (322.39): C 40.98, H 5.63, N 8.69; found: C 40.77, H 5.90, N 8.41.

4.2.59. N-(5-Hydroxypentyl)-2-methylbenzene Sulfonamide (**29a**) [1965509-68-5]

Applying GPA: from 2-methylbenzenesulfonyl chloride (500 mg, 2.62 mmol) and 5-amino-pentanol (406 mg, 3.93 mmol): **29a** (396 mg, 59%); oil; R_f = 0.17 (petrolether/EtOAc, 2:3); UV–Vis: 222 nm (3.87); IR: ν = 3501*w*, 3295*w*, 2937*w*, 2864*w*, 1472*w*, 1457*w*, 1313*s*, 1154*vs*, 1131*m*, 1066*m*, 1046*m*, 1039*m*, 877*w*, 806*w*, 760*m*, 733*w*, 711*m*, 688*m*, 592*vs*, 541*m*, 488*m*, 423*w*, 418*w* cm^{-1}; ^{1}H NMR:δ = 7.80 (*dd*, *J* = 7.8, 1.4 Hz, 1H, 3-H), 7.62–7.56 (*m*, 1H, 6-H), 7.50 (*td*, *J* = 7.5, 1.4 Hz, 1H, 4-H), 7.41–7.33 (*m*, 2H, 5-H, NH), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.30 (*td*, *J* = 6.4, 5.1 Hz, 2H, 12-H), 2.74 (*td*, *J* = 7.0, 5.5 Hz, 2H, 8-H), 2.57 (*s*, 3H, 7-H), 1.39–1.25 (*m*, 4H, 9-H, 11-H), 1.25–1.15 (*m*, 2H, 10-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 138.9 (C-1), 136.4 (C-2), 132.4 (C-4), 132.2 (C-3), 128.3 (C-6), 126.1 (C-5), 60.5 (C-12), 42.3 (C-8), 31.9 (C-11), 29.0 (C-9), 22.5 (C-10), 19.8 (C-7) ppm; MS: *m*/*z* = 280.2 (100%, $[M + Na]^+$); anal. calcd. for $C_{12}H_{19}NSO_3$ (257.35): C 56.01, H 7.44, N 5.44; found: C 55.76, H 7.69, N 5.16.

4.2.60. 5-[(2-Methylphenyl)sulfonamido]pentyl Sulfamate (**29b**)

Applying GPB: from **29a** (300 mg, 1.17 mmol): **29b** (282 mg, 72%); oil; R_f = 0.64 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 224 nm (4.02); IR: ν = 3277*w*, 2941*w*, 2868*vw*, 1567*w*, 1472*w*, 1458*w*, 1360*m*, 1312*s*, 1177*s*, 1154*vs*, 1130*m*, 1082*m*, 1066*m*, 1048*w*, 1034*w*, 919*s*, 813*m*, 761*m*, 729*w*, 710*m*, 689*m*, 592*s*, 551*s*, 542*s*, 494*m*, 480*m* cm^{-1}; ^{1}H NMR:δ = 7.83–7.78 (*m*, 1H, 3-H), 7.62 (*t*, *J* = 5.9 Hz, 1H, 6-H), 7.51 (*td*, *J* = 7.5, 1.4 Hz, 1H, 4-H), 7.42–7.34 (*m*, 4H, NH, 5-H, NH_2), 3.94 (*t*, *J* = 6.5 Hz, 2H, 12-H), 2.76 (*q*, *J* = 6.8 Hz, 2H, 8-H), 2.57 (*s*, 3H, 7-H), 1.57–1.48 (*m*, 2H, 11-H), 1.42–1.33 (*m*, 2H, 9-H), 1.33–1.22 (*m*, 2H, 10-H) ppm; ^{13}C NMR:δ = 138.8 (C-1), 136.4 (C-2), 132.5 (C-4), 132.3 (C-3), 128.3 (C-6), 126.2 (C-5), 68.8 (C-12), 42.0 (C-8), 28.6 (C-11), 27.8 (C-9), 22.2 (C-10), 19.8 (C-7) ppm; MS: *m*/*z* = 359.4 (100%, $[M + Na]^+$); anal. calcd. for $C_{12}H_{20}N_2S_2O_5$ (336.42): C 42.84, H 5.99, N 8.33; found: C 42.56, H 6.23, N 8.04.

4.2.61. N-(6-Hydroxyhexyl)-2-methylbenzene Sulfonamide (**30a**) [1914655-85-8]

Applying GPA: from 2-methylbenzenesulfonyl chloride (500 mg, 2.62 mmol) and 6-amino-hexanol (461 mg, 3.93 mmol): **30a** (666 mg, 94%); oil; R_f = 0.19 (petrolether/EtOAc, 2:3); UV–Vis: 223 nm (3.97); IR: ν = 3503*w*, 3296*w*, 2934*m*, 2860*w*, 1471*w*, 1458*w*, 1314*s*, 1154*vs*, 1131*m*, 1066*m*, 807*w*, 759*m*, 728*w*, 711*m*, 688*m*, 592*vs*, 541*m*, 490*m* cm^{-1}; ^{1}H NMR:δ = 7.80 (*dd*, *J* = 7.8, 1.4 Hz, 1H, 3-H), 7.59 (*t*, *J* = 5.7 Hz, 1H, 6-H), 7.50 (*td*, *J* = 7.5, 1.4 Hz, 1H, 4-H), 7.41–7.33 (*m*, 2H, 5-H, NH), 4.29 (*t*, *J* = 5.1 Hz, 1H, OH), 3.36–3.29 (*m*, 2H, 13-H), 2.75 (*td*, *J* = 7.1, 5.8 Hz, 2H, 8-H), 2.57 (*s*, 3H, 7-H), 1.38–1.26 (*m*, 4H, 9-H, 12-H), 1.21–1.10 (*m*, 4H, 10-H, 11-H) ppm; ^{13}C NMR:δ = 138.9 (C-1), 136.4 (C-2), 132.4 (C-4), 132.2 (C-3), 128.3 (C-6), 126.1 (C-5), 60.6 (C-13), 42.2 (C-8), 32.3 (C-12), 29.1 (C-9), 25.9 (C-10), 25.0 (C-11), 19.8 (C-7) ppm; MS: *m*/*z* = 294.2 (100%, $[M + Na]^+$); anal. calcd. for $C_{13}H_{21}NSO_3$ (271.38): C 57.54, H 7.80, N 5.16; found: C 76.26, H 8.03, N 4.76.

4.2.62. 6-[(2-Methylphenyl)sulfonamido]hexyl Sulfamate (**30b**)

Applying GPB: from **30a** (300 mg, 1.11 mmol): **30b** (333 mg, 86%); oil; R_f = 0.71 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 223 nm (3.05); IR: ν = 3277*w*, 3113*vw*, 2938*w*, 2863*w*, 1564*w*, 1471*w*, 1459*w*, 1360*s*, 1312*s*, 1177*s*, 1154*vs*, 1131*m*, 1082*m*, 1066*m*, 1048*w*, 922*s*, 807*m*, 761*m*, 726*w*, 710*m*, 689*m*, 592*s*, 551*s*, 543*s*, 492*m* cm^{-1}; ^{1}H NMR:δ = 7.80 (*dd*, *J* = 7.8, 1.4 Hz, 1H, 3-H), 7.60 (*t*, *J* = 5.8 Hz, 1H, 6-H), 7.50 (*td*, *J* = 7.4, 1.4 Hz, 1H, 4-H), 7.41–7.34 (*m*, 4H, 5-H, NH, NH_2), 3.96 (*t*, *J* = 6.5 Hz, 2H, 13-H), 2.76 (*q*, *J* = 6.6 Hz, 2H, 8-H), 2.57 (*s*, 3H, 7-H), 1.59–1.47 (*m*, 2H, 12-H), 1.40–1.28 (*m*, 2H, 9-H), 1.26–1.15 (*m*, 4H, 10-H, 11-H) ppm; ^{13}C NMR:δ = 139.3 (C-1), 136.9 (C-2), 132.9 (C-4), 132.7 (C-3), 128.8 (C-6), 126.6 (C-5), 69.3 (C-13), 42.6 (C-8), 29.4 (C-12), 28.6 (C-9), 25.9 (C-10), 25.0 (C-11), 20.2 (C-7) ppm; MS: *m*/*z* = 373.4

(100%, $[M + Na]^+$); anal. calcd. for $C_{13}H_{22}N_2S_2O_5$ (350.45): C 44.56, H 6.33, N 7.99; found: C 44.31, H 6.68, N 7.69.

4.2.63. N-(7-Hydroxyheptyl)-2-methylbenzene Sulfonamide (**31a**)

Applying GPA: from 2-methylbenzenesulfonyl chloride (500 mg, 2.62 mmol) and 7-amino-heptanol (516 mg, 3.93 mmol): **31a** (394 mg, 53%); oil; R_f = 0.20 (petrolether/EtOAc, 2:3); UV–Vis: 223 nm (3.90); IR: ν = 3504*w*, 3295*w*, 3064*vw*, 2931*m*, 2858*w*, 1458*w*, 1314*s*, 1154*vs*, 1131*m*, 1066*m*, 871*w*, 806*w*, 760*m*, 725*w*, 711*m*, 688*m*, 592*vs*, 541*m*, 491*m*, 419*vw* cm^{-1}; ^{1}H NMR:δ = 7.80 (*dd*, *J* = 7.8, 1.4 Hz, 1H, 3-H), 7.62–7.56 (*m*, 1H, 6-H), 7.50 (*td*, *J* = 7.5, 1.4 Hz, 1H, 4-H), 7.40–7.33 (*m*, 2H, 5, NH), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.37–3.31 (*m*, 2H, 14-H), 2.78–2.70 (*m*, 2H, 8-H), 2.57 (*s*, 3H, 7-H), 1.40–1.26 (*m*, 4H, 9-H, 13-H), 1.23–1.07 (*m*, 6H, 10-H, 11-H, 12-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 138.9 (C-1), 136.4 (C-2), 132.4 (C-4), 132.2 (C-3), 128.3 (C-6), 126.1 (C-5), 60.7 (C-14), 42.2 (C-8), 32.4 (C-13), 29.0 (C-9), 28.4 (C-11), 26.0 (C-10), 25.3 (C-12), 19.8 (C-7) ppm; MS: *m*/*z* = 308.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{14}H_{23}NSO_3$ (285.40): C 58.92, H 8.12, N 4.91; found: C 58.76, H 8.34, N 4.78.

4.2.64. 7-[(2-Methylphenyl)sulfonamido]heptyl Sulfamate (**31b**)

Applying GPB: from **31a** (300 mg, 1.05 mmol): **31b** (294 mg, 77%); oil; R_f = 0.74 (SiO_2 $CHCl_3$/EtOAc, 2:3); UV–Vis: 269 nm (3.16); IR: ν = 3504*w*, 3295*w*, 3064*vw*, 2931*m*, 2858*w*, 1458*w*, 1314*s*, 1154*vs*, 1131*m*, 1066*m*, 871*w*, 806*w*, 760*m*, 725*w*, 711*m*, 688*m*, 592*vs*, 541*m*, 491*m*, 419*vw* cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.80 (*dd*, *J* = 7.8, 1.4 Hz, 1H, 3-H), 7.60 (*t*, *J* = 5.8 Hz, 1H, 6-H), 7.50 (*td*, *J* = 7.5, 1.4 Hz, 1H, 4-H), 7.41–7.34 (*m*, 4H, 5-H, NH, NH_2), 3.98 (*t*, *J* = 6.5 Hz, 2H, 14-H), 2.75 (*td*, *J* = 7.0, 5.8 Hz, 2H, 8-H), 2.57 (*s*, 3H, 7-H), 1.60–1.53 (*m*, 2H, 13-H), 1.37–1.28 (*m*, 2H, 9-H), 1.27–1.20 (*m*, 2H, 12-H), 1.19–1.13 (*m*, 4H, 10-H, 11-H) ppm; ^{13}C NMR:δ = 138.9 (C-1), 136.4 (C-2), 132.4 (C-4), 132.3 (C-3), 128.3 (C-6), 126.1 (C-5), 68.9 (C-14), 42.2 (C-8), 28.9 (C-13), 28.2 (C-9), 27.9 (C-11), 25.8 (C-12), 24.9 (C-10), 19.8 (C-7) ppm; MS: *m*/*z* = 387.4 (100%, $[M + Na]^+$); anal. calcd. for $C_{14}H_{24}N_2S_2O_5$ (364.48): C 46.14, H 6.64, N 7.69; found: C 45.96, H 6.90, N 7.36.

4.2.65. N-(8-Hydroxyoctyl)-2-methylbenzene Sulfonamide (**32a**)

Applying GPA: from 2-methylbenzenesulfonyl chloride (500 mg, 2.62 mmol) and 8-amino-octanol (571 mg, 3.93 mmol): **32a** (766 mg, 98%); oil; R_f = 0.24 (petrolether/EtOAc, 2:3); UV–Vis: 223 nm (3.91); IR: ν = 3504*w*, 3295*w*, 2929*m*, 2856*m*, 1458*m*, 1315*s*, 1154*vs*, 1131*m*, 1066*m*, 876*vw*, 807*w*, 759*m*, 723*w*, 711*m*, 688*m*, 592*vs*, 541*m*, 489*m* cm^{-1}; ^{1}H NMR:δ = 7.80 (*dd*, *J* = 7.8, 1.4 Hz, 1H, 3-H), 7.62–7.55 (*m*, 1H, 6-H), 7.50 (*td*, *J* = 7.5, 1.4 Hz, 1H, 4-H), 7.41–7.34 (*m*, 2H, 5-H, NH), 4.29 (*t*, *J* = 5.1 Hz, 1H, OH), 3.35 (*td*, *J* = 6.6, 5.0 Hz, 2H, 15-H), 2.74 (*t*, *J* = 7.0 Hz, 2H, 8-H), 2.57 (*s*, 3H, 7-H), 1.41–1.26 (*m*, 4H, 9-H, 14-H), 1.25–1.07 (*m*, 8H, 10-H, 11-H, 12-H, 13-H) ppm; ^{13}C NMR:δ = 139.4 (C-1), 136.9 (C-2), 132.9 (C-4), 132.7 (C-3), 128.8 (C-6), 126.6 (C-5), 61.2 (C-15), 42.7 (C-8), 32.9 (C-14), 29.4 (C-9), 29.2 (C-11), 29.0 (C-12), 26.3 (C-10), 25.8 (C-13), 20.2 (C-7) ppm; MS: *m*/*z* = 322.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{25}NSO_3$ (299.43): C 60.17, H 8.42, N 4.68; found: C 59.84, H 8.66, N 4.32.

4.2.66. 8-[(2-Methylphenyl)sulfonamido]octyl Sulfamate (**32b**)

Applying GPB: from **32a** (300 mg, 1.00 mmol): **32b** (283 mg, 75%); oil; R_f = 0.76 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 270 nm (3.16); IR: ν = 3280*w*, 3114*vw*, 2931*w*, 2857*w*, 1563*w*, 1460*w*, 1361*m*, 1314*m*, 1178*s*, 1154*vs*, 1131*m*, 1066*m*, 1048*w*, 924*s*, 807*w*, 761*m*, 723*w*, 711*m*, 689*m*, 593*s*, 551*s*, 542*s*, 491*m* cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.80 (*dd*, *J* = 7.8, 1.4 Hz, 1H, 3-H), 7.59 (*t*, *J* = 5.7 Hz, 1H, 6-H), 7.50 (*td*, *J* = 7.5, 1.4 Hz, 1H, 4-H), 7.40–7.34 (*m*, 4H, 5-H, NH, NH_2), 3.99 (*t*, *J* = 6.5 Hz, 2H, 15-H), 2.75 (*td*, *J* = 7.0, 5.8 Hz, 2H, 8-H), 2.57 (*s*, 3H, 7-H), 1.62–1.54 (*m*, 2H, 14-H), 1.36–1.22 (*m*, 4H, 9-H, 13-H), 1.21–1.09 (*m*, 6H, 10-H, 11-H, 12-H) ppm; ^{13}C NMR:δ = 138.9 (C-1), 136.4 (C-2), 132.4 (C-4), 132.3 (C-3), 128.3 (C-6), 126.1 (C-5), 69.0 (C-15), 42.2 (C-8), 29.0 (C-14), 28.3 (C-9), 28.3 (C-11), 28.3 (C-12), 25.8

(C-13), 24.9 (C-10), 19.8 (C-7) ppm; MS: *m*/*z* = 401.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{26}N_2S_2O_5$ (378.50): C 47.60, H 6.92, N 7.40; found: C 47.42, H 7.18, N 7.20.

4.2.67. N-(2-Hydroxyethyl)-4-isopropylbenzene Sulfonamide (**33a**) [117867-88-6]

Applying GPA: from 4-isopropylbenzenesulfonyl chloride (500 mg, 2.29 mmol) and 2-amino-ethanol (209 mg, 3.43 mmol): **33a** (441 mg, 79%) [115–117] oil; R_f = 0.16 (petrolether/EtOAc, 2:3); UV–Vis: 228 nm (4.08); IR: ν = 3516*m*, 3168*w*, 2955*m*, 2891*w*, 2870*w*, 1599*w*, 1494*w*, 1464*w*, 1453*w*, 1433*w*, 1411*m*, 1354*vw*, 1323*s*, 1282*w*, 1261*w*, 1211*w*, 1187*w*, 1158*vs*, 1107*w*, 1090*m*, 1070*m*, 1051*s*, 1018*w*, 956*s*, 905*w*, 851*w*, 845*w*, 824*m*, 777*s*, 738*m*, 724*m*, 646*m*, 632*w*, 580*s*, 562*s*, 532*w*, 496*w*, 484*w*, 462*m*, 451*w* cm^{-1}; ^1H NMR:δ = 7.73–7.69 (*m*, 2H, 2-H, 2′-H), 7.53–7.43 (*m*, 3H, 3H-, 3′-H, NH), 4.66 (*s*, 1H, OH), 3.37 (*t*, *J* = 6.4 Hz, 2H, 8-H), 2.97 (*hept*, *J* = 6.9 Hz, 1H, 5-H), 2.77 (*t*, *J* = 6.4 Hz, 2H, 7-H), 1.22 (*d*, *J* = 6.9 Hz, 6H, 6-H, 6′-H) ppm; ^{13}C NMR:δ = 153.0 (C-4), 138.0 (C-1), 127.0 (C-2), 126.6 (C-3), 59.9 (C-8), 45.1 (C-7), 33.3 (C-5), 23.5 (C-6) ppm; MS: *m*/*z* = 266.2 (100%, $[M + Na]^+$); anal. calcd. for $C_{11}H_{17}NSO_3$ (243.32): C 54.30, H 7.04, N 5.76; found: C 54.03, H 7.28, N 5.44.

4.2.68. 2-[(4-Isopropylphenyl)sulfonamido]ethyl Sulfamate (**33b**)

Applying GPB: from **33a** (300 mg, 1.23 mmol): **33b** (241 mg, 61%); white solid; R_f = 0.59 ($CHCl_3$/EtOAc, 2:3); m.p. = 81–82 °C; UV–Vis: 228 nm (4.03); IR: ν = 3276*m*, 2963*w*, 1598*w*, 1559*w*, 1411*w*, 1364*s*, 1319*s*, 1284*w*, 1179*s*, 1157*vs*, 1091*m*, 1054*w*, 1015*m*, 924*s*, 832*m*, 775*m*, 753*m*, 649*s*, 632*w*, 549*s*, 488*w*, 436*w* cm^{-1}; ^1H NMR:δ = 7.83 (*t*, *J* = 6.0 Hz, 1H, NH), 7.76–7.70 (*m*, 2H, 2-H, 2′-H), 7.53–7.44 (*m*, 4H, 3-H, 3′-H, NH_2), 4.01 (*t*, *J* = 5.7 Hz, 2H, 8-H), 3.03 (*q*, *J* = 5.4 Hz, 2H, 7-H), 3.00–2.92 (*m*, 1H, 5-H), 1.22 (*d*, *J* = 6.9 Hz, 6H, 6-H, 6′-H) ppm; ^{13}C NMR:δ = 153.3 (C-4), 137.7 (C-1), 127.2 (C-2), 126.6 (C-3), 67.6 (C-8), 41.6 (C-7), 33.4 (C-5), 23.5 (C-6) ppm; MS: *m*/*z* = 345.2 (100%, $[M + Na]^+$); anal. calcd. for $C_{11}H_{18}N_2S_2O_5$ (322.39): C 40.98, H 5.63, N 8.69; found: C 40.71, H 5.98, N 8.43.

4.2.69. N-(3-Hydroxypropyl)-4-isopropylbenzene Sulfonamide (**34a**) [920113-99-1]

Applying GPA: from 4-isopropylbenzenesulfonyl chloride (500 mg, 2.29 mmol) and 3-amino-propanol (258 mg, 3.43 mmol): **34a** (558 mg, 95%); oil; R_f = 0.16 (petrolether/EtOAc, 2:3); UV–Vis: 228 nm (4.22); IR: ν = 3501*w*, 3278*w*, 2962*w*, 2874*w*, 1598*w*, 1464*w*, 1410*m*, 1386*w*, 1364*w*, 1317*s*, 1283*m*, 1156*vs*, 1091*s*, 1053*s*, 1016*w*, 1008*w*, 959*w*, 833*m*, 774*m*, 648*s*, 632*m*, 579*s*, 565*s*, 486*w* cm^{-1}; ^1H NMR:δ = 7.73–7.68 (*m*, 2H, 2-H, 2′-H), 7.48–7.40 (*m*, 3H, 3-H, 3′-H, NH), 4.40 (*t*, *J* = 5.1 Hz, 1H, OH), 3.37 (*td*, *J* = 6.2, 5.0 Hz, 2H, 9-H), 2.97 (*hept*, *J* = 7.0 Hz, 1H, 5-H), 2.77 (*td*, *J* = 7.3, 5.8 Hz, 2H, 7-H), 1.57–1.49 (*m*, 2H, 8-H), 1.22 (*d*, *J* = 6.9 Hz, 6H, 6-H, 6′-H) ppm; ^{13}C NMR:δ = 153.0 (C-4), 137.9 (C-1), 127.0 (C-2), 126.6 (C-3), 58.1 (C-9), 40.0 (C-7), 33.3 (C-5), 32.4 (C-8), 23.5 (C-6) ppm; MS: *m*/*z* = 280.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{12}H_{19}NSO_3$ (257.35): C 56.01, H 7.44, N 5.44; found: C 55.76, H 7.62, N 5.18.

4.2.70. 3-[(4-Isopropylphenyl)sulfonamido]propyl Sulfamate (**34b**)

Applying GPB: from **34a** (200 mg, 0.78 mmol): **34b** (226 mg, 86%); white solid; R_f = 0.60 ($CHCl_3$/EtOAc, 2:3); m.p. = 83–84 °C; UV–Vis: 228 nm (4.11); IR: ν = 3359*m*, 3285*m*, 3262*m*, 2963*w*, 1599*w*, 1565*w*, 1474*w*, 1468*w*, 1435*m*, 1402*m*, 1372*vs*, 1337*w*, 1311*s*, 1283*m*, 1255*w*, 1176*s*, 1157*vs*, 1111*w*, 1091*m*, 1068*m*, 1054*m*, 1039*m*, 1017*w*, 943*s*, 920*s*, 887*m*, 835*s*, 827*s*, 774*m*, 756*m*, 733*w*, 676*s*, 635*m*, 596*m*, 579*s*, 561*s*, 546*vs*, 520*s*, 486*m*, 426*m* cm^{-1}; ^1H NMR:δ = 7.73–7.68 (*m*, 2H, 2-H, 2′-H), 7.60 (*t*, *J* = 5.9 Hz, 1H, NH), 7.49–7.44 (*m*, 2H, 3-H, 3′-H), 7.41 (*s*, 2H, NH_2), 4.03 (*t*, *J* = 6.3 Hz, 2H, 9-H), 2.98 (*hept*, *J* = 7.0 Hz, 1H, 5-H), 2.81 (*td*, *J* = 7.1, 5.8 Hz, 2H, 7-H), 1.77 (*p*, *J* = 6.7 Hz, 2H, 8-H), 1.22 (*d*, *J* = 6.9 Hz, 6H, 6-H, 6′-H) ppm; ^{13}C NMR:δ = 153.2 (C-4), 137.7 (C-1), 127.3 (C-2), 126.6 (C-3), 66.5 (C-9), 39.2 (C-7), 33.3 (C-5), 28.8 (C-8), 23.5 (C-6) ppm; MS: *m*/*z* = 359.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{12}H_{20}N_2S_2O_5$ (336.42): C 42.84, H 5.99, N 8.33; found: C 42.67, H 6.20, N 8.02.

4.2.71. N-(4-Hydroxybutyl)-4-isopropylbenzene Sulfonamide (**35a**) [1082772-50-6]

Applying GPA: from 4-isopropylbenzenesulfonyl chloride (500 mg, 2.29 mmol) and 4-amino-butanol (306 mg, 3.43 mmol): **35a** (540 mg, 87%); oil; R_f = 0.14 (petrolether/EtOAc, 2:3); UV–Vis: 228 nm (4.32); IR: ν = 3501*w*, 3281*w*, 2961*m*, 2871*w*, 1598*w*, 1463*w*, 1410*m*, 1386*w*, 1364*w*, 1318*s*, 1283*w*, 1156*vs*, 1091*s*, 1053*s*, 1017*w*, 991*vw*, 833*m*, 773*w*, 735*w*, 648*s*, 632*m*, 581*s*, 565*s*, 488*w*, 471*w* cm^{-1}; ^{1}H NMR:δ = 7.72–7.68 (*m*, 2H, 2-H, 2′-H), 7.48–7.43 (*m*, 3H, 3-H, 3′-H, NH), 4.39–4.31 (*m*, 1H, OH), 3.37–3.27 (*m*, 2H, 10-H), 2.97 (*hept*, *J* = 6.9 Hz, 1H, 5-H), 2.75–2.67 (*m*, 2H, 7-H), 1.44–1.31 (*m*, 4H, 8-H, 9-H), 1.22 (*d*, *J* = 6.9 Hz, 6H, 6-H, 6′-H) ppm; ^{13}C NMR:δ = 152.9 (C-4), 138.1 (C-1), 127.0 (C-2), 126.6 (C-3), 60.2 (C-10), 42.5 (C-7), 33.3 (C-5), 29.5 (C-9), 25.8 (C-8), 23.5 (C-6) ppm; MS: *m*/*z* = 294.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{13}H_{21}NSO_3$ (271.38): C 57.54, H 7.80, N 5.16; found: C 57.21, H 8.04, N 4.87.

4.2.72. 4-[(4-Isopropylphenyl)sulfonamido]butyl Sulfamate (**35b**)

Applying GPB: from **35a** (200 mg, 0.74 mmol): **35b** (233 mg, 90%); white solid; R_f = 0.61 ($CHCl_3$/EtOAc, 2:3); m.p. = 95–97 °C; UV–Vis: 228 nm (4.16); IR: ν = 3356*m*, 3286*m*, 3262*m*, 2965*w*, 2879*w*, 1558*w*, 1481*w*, 1476*w*, 1431*w*, 1409*w*, 1398*w*, 1384*w*, 1366*s*, 1337*m*, 1320*s*, 1284*w*, 1203*w*, 1175*s*, 1157*vs*, 1143*m*, 1093*m*, 1063*m*, 1047*w*, 969*s*, 942*w*, 922*s*, 901*s*, 840*m*, 824*s*, 773*m*, 750*w*, 732*w*, 666*s*, 632*m*, 588*s*, 580*s*, 552*vs*, 513*m*, 505*m*, 415*w* cm^{-1}; ^{1}H NMR:δ = 7.73–7.68 (*m*, 2H, 2-H, 2′-H), 7.53 (*t*, *J* = 5.9 Hz, 1H, NH), 7.48–7.43 (*m*, 2H, 3-H, 3′-H), 7.38 (*s*, 2H, NH_2), 3.96 (*t*, *J* = 6.3 Hz, 2H, 10-H), 2.98 (*hept*, *J* = 6.9 Hz, 1H, 5-H), 2.75 (*td*, *J* = 6.9, 6.0 Hz, 2H, 7-H), 1.66–1.56 (*m*, 2H, 9-H), 1.50–1.41 (*m*, 2H, 8-H), 1.22 (*d*, *J* = 6.9 Hz, 6H, 6-H, 6′-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 153.0 (C-4), 138.0 (C-1), 127.1 (C-2), 126.6 (C-3), 68.5 (C-10), 42.0 (C-7), 33.3 (C-5), 25.6 (C-8), 25.4 (C-9), 23.5 (6) ppm; MS: *m*/*z* = 373.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{13}H_{22}N_2S_2O_5$ (350.45): C 44.56, H 6.33, N 7.99; found: C 44.27, H 6.68, N 7.65.

4.2.73. N-(5-Hydroxypentyl)-4-isopropylbenzene Sulfonamide (**36a**) [1925596-35-5]

Applying GPA: from 4-isopropylbenzenesulfonyl chloride (500 mg, 2.29 mmol) and 5-amino-pentanol (354 mg, 3.43 mmol): **36a** (604 mg, 93%); oil; R_f = 0.18 (petrolether/EtOAc, 2:3); UV–Vis: 228 nm (4.42); IR: ν = 3503*w*, 3278*w*, 2960*w*, 2936*m*, 2869*w*, 1598*w*, 1460*w*, 1410*m*, 1386*w*, 1364*w*, 1318*s*, 1283*w*, 1156*vs*, 1091*s*, 1053*m*, 1016*w*, 833*m*, 774*w*, 733*w*, 648*s*, 632*m*, 581*s*, 565*s* cm^{-1}; ^{1}H NMR:δ = 7.72–7.67 (*m*, 2H, 2-H, 2′-H), 7.48–7.42 (*m*, 3H, 3-H, 3′-H, NH), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.35–3.29 (*m*, 2H, 11-H), 2.97 (*hept*, *J* = 7.0 Hz, 1H, 5-H), 2.70 (*td*, *J* = 6.8, 6.0 Hz, 2H, 7-H), 1.40–1.27 (*m*, 4H, 8-H, 10-H), 1.27–1.16 (*m*, 8H, 6-H, 6′-H, 9-H) ppm; ^{13}C NMR:δ = 152.9 (C-4), 138.1 (C-1), 127.0 (C-2), 126.6 (C-3), 60.5 (C-11), 42.6 (C-7), 33.3 (C-5), 32.0 (C-10), 28.9 (C-8), 23.5 (C-6), 22.6 (C-9) ppm; MS: *m*/*z* = 308.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{14}H_{23}NSO_3$ (285.40): C 58.92, H 8.12, N 4.91; found: C 58.76, H 8.47, N 4.59.

4.2.74. 5-[(4-Isopropylphenyl)sulfonamido]pentyl Sulfamate (**36b**)

Applying GPB: from **36a** (200 mg, 0.7 mmol): **36b** (227 mg, 89%); white solid; R_f = 0.66 ($CHCl_3$/EtOAc, 2:3); m.p. = 95–97 °C; UV–Vis: 228 nm (4.14); IR: ν = 3350*w*, 3280*m*, 2963*w*, 2946*w*, 2869*w*, 2859*vw*, 1475*vw*, 1432*w*, 1407*w*, 1400*w*, 1386*w*, 1366*s*, 1328*s*, 1310*m*, 1286*w*, 1191*w*, 1174*m*, 1157*vs*, 1142*m*, 1112*w*, 1094*m*, 1068*w*, 1058*w*, 1038*m*, 963*s*, 922*m*, 900*m*, 843*w*, 825*s*, 771*w*, 737*w*, 733*w*, 661*s*, 632*m*, 584*s*, 552*vs*, 525*m*, 517*m*, 501*w*, 444*w* cm^{-1}; ^{1}H NMR:δ = 7.72–7.68 (*m*, 2H, 2-H, 2′-H), 7.51–7.43 (*m*, 3H, 3-H, 3′-H, NH), 7.37 (*s*, 2H, NH_2), 3.96 (*t*, *J* = 6.5 Hz, 2H, 11-H), 2.97 (*hept*, *J* = 7.0 Hz, 1H, 5-H), 2.72 (*q*, *J* = 6.5 Hz, 2H, 7-H), 1.55 (*p*, *J* = 6.7 Hz, 2H, 10-H), 1.43–1.34 (*m*, 2H, 8-H), 1.34–1.26 (*m*, 2H, 9-H), 1.22 (*d*, *J* = 6.9 Hz, 6H, 6-H, 6′-H) ppm; ^{13}C NMR:δ = 153.0 (C-4), 138.0 (C-1), 127.1 (C-2), 126.6 (C-3), 68.8 (C-11), 42.3 (C-7), 28.5 (C-10), 27.8 (C-8), 23.5 (C-6), 22.2 (C-9) ppm; MS: *m*/*z* = 387.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{14}H_{24}N_2S_2O_5$ (364.48): C 46.14, H 6.64, N 7.69; found: C 45.84, H 6.99, N 7.44.

4.2.75. N-(6-Hydroxyhexyl)-4-isopropylbenzene Sulfonamide (**37a**) [1912844-40-6]

Applying GPA: from 4-isopropylbenzenesulfonyl chloride (500 mg, 2.29 mmol) and 6-amino-hexanol (402 mg, 3.43 mmol): **37a** (612 mg, 89%); oil; R_f = 0.20 (petrolether/EtOAc, 2:3); UV–Vis: 228 nm (4.08); IR: ν = 3505*w*, 3282*w*, 2960*w*, 2933*m*, 2864*w*, 1598*w*, 1462*w*, 1410*m*, 1386*w*, 1364*w*, 1319*s*, 1283*w*, 1157*vs*, 1092*s*, 1073*m*, 1053*m*, 1016*w*, 891*vw*, 833*m*, 773*w*, 727*w*, 649*s*, 633*m*, 581*s*, 565*s*, 493*w*, 456*w* cm^{-1}; ^{1}H NMR:δ = 7.73–7.68 (*m*, 2H, 2-H, 2′-H), 7.48–7.43 (*m*, 3H, 3-H, 3′-H, NH), 4.32–4.27 (*m*, 1H, OH), 3.34 (*td*, *J* = 6.3, 2.9 Hz, 2H, 12-H), 2.97 (*hept*, *J* = 6.9 Hz, 1H, 5-H), 2.71 (*td*, *J* = 7.0, 5.9 Hz, 2H, 7-H), 1.38–1.29 (*m*, 4H, 8-H, 11-H), 1.22 (*d*, *J* = 6.9 Hz, 6H, 6-H, 6′-H), 1.20–1.15 (*m*, 4H, 9-H, 10-H) ppm; ^{13}C NMR:δ = 152.9 (C-4), 138.2 (C-1), 127.0 (C-2), 126.6 (C-3), 60.6 (C-12), 42.5 (C-7), 33.3 (C-5), 32.3 (C-11), 29.0 (C-8), 25.9 (C-10), 25.0 (C-9), 23.5 (C-6) ppm; MS: *m*/*z* = 322.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{25}NSO_3$ (299.43): C 60.17, H 8.42, N 4.68; found: C 59.76, H 8.72, N 4.43.

4.2.76. 6-[(4-Isopropylphenyl)sulfonamido]hexyl Sulfamate (**37b**)

Applying GPB: from **37a** (300 mg, 1.00 mmol): **37b** (260 mg, 69%); white solid; R_f = 0.72 ($CHCl_3$/EtOAc, 2:3); m.p. = 102–103 °C; UV–Vis: 228 nm (4.19); IR: ν = 3384*w*, 3278*m*, 2962*m*, 2933*w*, 2860*w*, 1600*w*, 1544*w*, 1477*w*, 1469*w*, 1420*m*, 1396*w*, 1374*s*, 1320*s*, 1312*s*, 1283*w*, 1179*s*, 1157*vs*, 1146*s*, 1112*w*, 1092*m*, 1065*m*, 1054*m*, 1003*s*, 977*s*, 951*w*, 924*m*, 907*m*, 873*m*, 845*w*, 830*m*, 814*s*, 802*s*, 774*m*, 757*w*, 697*s*, 647*m*, 633*m*, 579*s*, 564*s*, 551*vs*, 537*s*, 505*m*, 484*w*, 456*w* cm^{-1}; ^{1}H NMR:δ = 7.72–7.67 (*m*, 2H, 2-H, 2′-H), 7.49–7.43 (*m*, 3H, 3-H, 3′-H, NH), 7.37 (*s*, 2H, NH_2), 3.97 (*t*, *J* = 6.5 Hz, 2H, 12-H), 2.97 (*hept*, *J* = 6.9 Hz, 1H, 5-H), 2.71 (*q*, *J* = 6.7 Hz, 2H, 7-H), 1.56 (*p*, *J* = 6.6 Hz, 2H, 11-H), 1.35 (*p*, *J* = 6.8 Hz, 2H, 8-H), 1.28–1.19 (*m*, 10H, 6-H, 6′-H, 9-H, 10-H) ppm; ^{13}C NMR:δ = 153.0 (C-4), 138.1 (C-1), 127.0 (C-2), 126.6 (C-3), 68.9 (C-12), 42.4 (C-7), 33.3 (C-5), 28.8 (C-11), 28.2 (C-8), 25.5 (C-10), 24.6 (C-9), 23.5 (C-6) ppm; MS: *m*/*z* = 401.2 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{26}N_2S_2O_5$ (378.50): C 47.60, H 6.92, N 7.40; found: C 47.36, H 7.20, N 7.05.

4.2.77. N-(7-Hydroxyheptyl)-4-isopropylbenzene Sulfonamide (**38a**)

Applying GPA: from 4-isopropylbenzenesulfonyl chloride (500 mg, 2.29 mmol) and 7-amino-heptanol (450 mg, 3.43 mmol): **38a** (658 mg, 92%); oil; R_f = 0.26 (petrolether/EtOAc, 2:3); UV–Vis: 228 nm (4.11); IR: ν = 3503*w*, 3281*w*, 2960*w*, 2931*m*, 2859*m*, 1598*w*, 1495*vw*, 1463*w*, 1410*m*, 1386*w*, 1364*w*, 1319*s*, 1283*w*, 1157*vs*, 1092*s*, 1053*m*, 1016*w*, 891*vw*, 833*m*, 773*w*, 724*w*, 648*s*, 633*m*, 581*s*, 565*s*, 493*w*, 485*w* cm^{-1}; ^{1}H NMR:δ = 7.70–7.66 (*m*, 2H, 2-H, 2′-H), 7.45–7.40 (*m*, 3H, 3-H, 3′-H, NH), 3.97 (*s*, 1H, OH), 3.33 (*t*, *J* = 6.6 Hz, 2H, 13-H), 2.94 (*hept*, *J* = 6.9 Hz, 1H, 5-H), 2.69 (*td*, *J* = 7.0, 5.9 Hz, 2H, 7-H), 1.39–1.27 (*m*, 4H, 8-H, 12-H), 1.19 (*d*, *J* = 6.9 Hz, 6H, 6-H, 6′-H), 1.17–1.10 (*m*, 6H, 9-H, 10-H, 11-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 152.9 (C-4), 138.2 (C-1), 127.0 (C-2), 126.6 (C-3), 60.7 (C-13), 42.5 (C-7), 33.3 (C-5), 32.4 (C-12), 28.9 (C-8), 28.4 (C-10), 26.1 (C-9), 25.3 (C-11), 23.5 (C-6) ppm; MS: *m*/*z* = 336.2 (100%, $[M + Na]^+$); anal. calcd. for $C_{16}H_{27}NSO_3$ (313.46): C 61.31, H 8.68, N 4.47; found: C 61.00, H 8.95, N 4.18.

4.2.78. 7-[(4-Isopropylphenyl)sulfonamido]heptyl Sulfamate (**38b**)

Applying GPB: from **38a** (200 mg, 0.67 mmol): **38b** (230 mg, 90%); white solid; R_f = 0.75 ($CHCl_3$/EtOAc, 2:3); m.p. = 70–72 °C; UV–Vis: 228 nm (4.10); IR: ν = 3382*w*, 3274*m*, 2966*w*, 2921*m*, 2911*w*, 2852*w*, 1605*w*, 1543*w*, 1475*w*, 1467*w*, 1420*m*, 1392*w*, 1376*vs*, 1320*s*, 1281*w*, 1181*s*, 1159*s*, 1110*w*, 1090*m*, 1065*w*, 1056*m*, 1040*m*, 1018*w*, 1000*m*, 978*s*, 945*w*, 926*m*, 892*m*, 852*w*, 843*w*, 831*m*, 815*s*, 776*m*, 762*w*, 733*m*, 698*s*, 645*m*, 639*w*, 580*s*, 566*s*, 551*vs*, 530*s*, 510*m*, 486*w*, 477*w*, 445*w* cm^{-1}; ^{1}H NMR:δ = 7.72–7.68 (*m*, 2H, 2-H, 2′-H), 7.48–7.43 (*m*, 3H, 3-H, 3′-H, NH), 7.37 (*s*, 2H, NH_2), 3.98 (*t*, *J* = 6.5 Hz, 2H, 13-H), 2.97 (*hept*, *J* = 6.9 Hz, 1H, 5-H), 2.71 (*q*, *J* = 6.8 Hz, 2H, 7-H), 1.63–1.53 (*m*, 2H, 12-H), 1.38–1.31 (*m*, 2H, 8-H), 1.22 (*d*, *J* = 6.9 Hz, 6H, 6-H, 6′-H), 1.30–1.14 (*m*, 6H, 9-H, 10-H, 11-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 153.0 (C-4), 138.1 (C-1), 127.0 (C-2), 126.6 (C-3), 68.9 (C-13), 42.5 (C-7), 33.3 (C-5), 28.9 (C-12), 28.2 (C-8), 28.0 (C-10), 25.8 (C-11), 24.9 (C-9), 23.5 (C-6) ppm; MS:

m/*z* = 415.3 (100%, [M + Na]$^+$); anal. calcd. for $C_{16}H_{28}N_2S_2O_5$ (392.53): C 48.96, H 7.19, N 7.14; found: C 48.73, H 7.41, N 6.87.

4.2.79. N-(8-Hydroxyoctyl)-4-isopropylbenzene Sulfonamide (**39a**)

Applying GPA: from 4-isopropylbenzenesulfonyl chloride (500 mg, 2.29 mmol) and 8-amino-octanol (498 mg, 3.43 mmol): **39a** (721 mg, 96%); oil; R_f = 0.28 (petrolether/EtOAc, 2:3); UV–Vis: 228 nm (4.23); IR: ν = 3500*w*, 3281*w*, 2929*m*, 2857*m*, 1599*w*, 1463*w*, 1410*w*, 1364*w*, 1320*m*, 1283*m*, 1158*s*, 1092*m*, 1053*m*, 1017*w*, 924*w*, 892*w*, 833*m*, 809*w*, 773*w*, 722*m*, 649*m*, 582*w*, 566*m*, 514*w*, 488*w* cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.71–7.68 (*m*, 2H, 2-H, 2′-H), 7.47–7.43 (*m*, 3H, 3-H, 3′-H, NH), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.36 (*td*, *J* = 6.5, 5.1 Hz, 2H, 14-H), 2.97 (*hept*, *J* = 6.9 Hz, 1H, 5-H), 2.71 (*td*, *J* = 7.0, 5.8 Hz, 2H, 7-H), 1.41–1.29 (*m*, 4H, 8-H, 13-H), 1.21 (*d*, *J* = 6.9 Hz, 6H, 6-H, 6′-H), 1.20–1.11 (*m*, 8H, 9-H, 10-H, 11-H, 12-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 152.9 (C-4), 138.2 (C-1), 127.0 (C-2), 126.6 (C-3), 60.7 (C-14), 42.5 (C-7), 33.3 (C-5), 32.5 (C-13), 28.9 (C-8), 28.8 (C-12), 28.6 (C-9), 25.9 (C-11), 25.4 (C-10), 23.5 (C-6) ppm; MS: *m*/*z* = 350.2 (100%, [M + Na]$^+$); anal. calcd. for $C_{17}H_{29}NSO_3$ (327.48): C 62.35, H 8.93, N 4.28; found: C 62.03, H 9.22, N 3.93.

4.2.80. 8-[(4-Isopropylphenyl)sulfonamido]octyl Sulfamate (**39b**)

Applying GPB: from **39a** (200 mg, 0.61 mmol): **39b** (220 mg, 89%); white solid; R_f = 0.80 ($CHCl_3$/EtOAc, 2:3); m.p. = 69–70 °C; UV–Vis: 229 nm (4.08); IR: ν = 3385*w*, 3278*m*, 2961*w*, 2929*m*, 2915*w*, 2853*w*, 1600*w*, 1543*w*, 1477*w*, 1468*w*, 1419*m*, 1396*w*, 1376*vs*, 1319*s*, 1283*w*, 1182*s*, 1158*s*, 1112*w*, 1093*m*, 1069*w*, 1055*m*, 1038*m*, 1016*w*, 1001*m*, 977*s*, 945*w*, 925*m*, 894*m*, 858*w*, 845*w*, 830*m*, 814*s*, 775*m*, 762*w*, 731*m*, 699*s*, 648*m*, 633*w*, 582*s*, 564*s*, 550*vs*, 532*s*, 513*m*, 485*w*, 477*w*, 443*w* cm^{-1}; ^{1}H NMR:δ = 7.72–7.66 (*m*, 2H, 2-H, 2′-H), 7.47–7.43 (*m*, 3H, 3-H, 3′-H, NH), 7.37 (*s*, 2H, NH_2), 3.99 (*t*, *J* = 6.5 Hz, 2H, 14-H), 2.97 (*hept*, *J* = 6.9 Hz, 1H, 5-H), 2.75–2.66 (*m*, 2H, 7-H), 1.64–1.54 (*m*, 2H, 13-H), 1.40–1.11 (*m*, 10H, 8-H, 9-H, 10-H, 11-H, 12-H), 1.22 (*d*, *J* = 6.9 Hz, 6H, 6-H, 6′-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 153.0 (C-4), 138.2 (C-1), 127.0 (C-2), 126.6 (C-3), 69.0 (C-14), 42.5 (C-7), 33.3 (C-5), 28.9 (C-13), 28.4 (C-11), 28.3 (C-8), 28.3 (C-10), 25.9 (C-9), 24.9 (C-12), 23.5 (C-6) ppm; MS: *m*/*z* = 429.4 (100%, [M + Na]$^+$); anal. calcd. for $C_{17}H_{30}N_2S_2O_5$ (406.56): C 50.22, H 7.44, N 6.89; found: C 49.93, H 7.71, N 6.65.

4.2.81. N-(2-Hydroxyethyl)-3-isopropylbenzene Sulfonamide (**40a**)

Applying GPA: from 3-isopropylbenzenesulfonyl chloride (500 mg, 2.29 mmol) and 2-amino-ethanol (209 mg, 3.43 mmol): **40a** (548 mg, 98%); oil; R_f = 0.16 (petrolether/EtOAc, 2:3); UV–Vis: 222 nm (3.94); IR: ν = 3492*w*, 3275*w*, 2962*w*, 2874*w*, 1478*w*, 1461*w*, 1428*m*, 1386*w*, 1365*w*, 1323*s*, 1306*s*, 1217*w*, 1156*vs*, 1144*s*, 1090*m*, 1052*m*, 998*vw*, 949*m*, 902*w*, 798*m*, 695*s*, 623*m*, 586*vs*, 564*m*, 542*m*, 517*m*, 470*m*, 460*w* cm^{-1}; ^{1}H NMR:δ = 7.69–7.66 (*m*, 1H, 5-H), 7.64–7.60 (*m*, 1H, 6-H), 7.55 (*t*, *J* = 5.9 Hz, 1H, NH), 7.53–7.48 (*m*, 2H, 2-H, 4-H), 4.67 (*t*, *J* = 5.6 Hz, 1H, OH), 3.38 (*td*, *J* = 6.3, 5.5 Hz, 2H, 10-H), 2.98 (*hept*, *J* = 6.9 Hz, 1H, 7-H), 2.80 (*q*, *J* = 6.2 Hz, 2H, 9-H), 1.22 (*d*, *J* = 6.9 Hz, 6H, 8-H, 8′-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 149.6 (C-3), 140.7 (C-1), 130.4 (C-4), 129.1 (C-5), 124.1 (C-2), 124.0 (C-6), 59.9 (C-10), 45.1 (C-9), 33.3 (C-7), 23.6 (C-8) ppm; MS: *m*/*z* = 266.2 (100%, [M + Na]$^+$); anal. calcd. for $C_{11}H_{17}NSO_3$ (243.32): C 54.30, H 7.04, N 5.76; found: C 53.99, H 7.36, N 5.41.

4.2.82. 2-[(3-Isopropylphenyl)sulfonamido]ethyl Sulfamate (**40b**)

Applying GPB: from **40a** (200 mg, 0.82 mmol): **40b** (238 mg, 90%); oil; R_f = 0.64 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 269 nm (2.95); IR: ν = 3275*m*, 2963*w*, 2873*vw*, 1558*w*, 1479*w*, 1461*w*, 1430*w*, 1364*s*, 1325*s*, 1307*s*, 1179*s*, 1157*vs*, 1101*m*, 1091*m*, 1070*w*, 1021*m*, 998*w*, 924*s*, 798*m*, 757*m*, 694*s*, 624*m*, 585*vs*, 549*s*, 491*w*, 445*w*, 432*w* cm^{-1}; ^{1}H NMR:δ = 7.88 (*t*, *J* = 6.0 Hz, 1H, NH), 7.69–7.66 (*m*, 1H, 5-H), 7.63 (*dt*, *J* = 6.7, 2.0 Hz, 1H, 6-H), 7.56–7.47 (*m*, 4H, 2-H, 4-H, NH_2), 4.01 (*t*, *J* = 5.7 Hz, 2H, 10-H), 3.05 (*q*, *J* = 5.0, 4.3 Hz, 2H, 9-H), 3.03–2.94 (*m*, 1H, 7-H), 1.23 (*d*, *J* = 6.9 Hz, 6H, 8-H, 8′-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 149.7 (C-3), 140.3 (C-1), 130.6 (C-4), 129.3 (C-5), 124.1 (C-2), 124.0 (C-6), 67.6 (C-10), 41.6 (C-9), 33.3

(C-7), 23.6 (C-8) ppm; MS: *m*/*z* = 345.3 (100%, [M + Na]$^+$); anal. calcd. for $C_{11}H_{18}N_2S_2O_5$ (322.39): C 40.98, H 5.63, N 8.69; found: C 40.66, H 6.01, N 8.40.

4.2.83. N-(3-Hydroxypropyl)-3-isopropylbenzene Sulfonamide (**41a**)

Applying GPA: from 3-isopropylbenzenesulfonyl chloride (500 mg, 2.29 mmol) and 3-amino-propanol (258 mg, 3.43 mmol): **41a** (562 mg, 95%); oil; R_f = 0.16 (petrolether/EtOAc, 2:3); UV–Vis: 222 nm (3.91); IR: ν = 3496*w*, 3279*w*, 2962*m*, 2875*w*, 1478*w*, 1462*w*, 1426*m*, 1386*w*, 1365*w*, 1323*m*, 1305*s*, 1217*w*, 1156*vs*, 1144*s*, 1084*m*, 1068*s*, 1008*w*, 998*w*, 960*w*, 904*w*, 871*w*, 799*m*, 696*s*, 623*m*, 587*vs*, 530*m*, 488*w* cm^{-1}; ^{1}H NMR:δ = 7.67–7.64 (*m*, 1H, 5-H), 7.62–7.58 (*m*, 1H, 6-H), 7.54–7.45 (*m*, 3H, 2-H, 4-H, NH), 4.39 (*t*, *J* = 5.1 Hz, 1H, OH), 3.36 (*td*, *J* = 6.3, 5.0 Hz, 2H, 11-H), 2.99 (*hept*, *J* = 6.9 Hz, 1H, 7-H), 2.79 (*td*, *J* = 7.1, 5.2 Hz, 2H, 9-H), 1.57–1.47 (*m*, 2H, 10-H), 1.23 (*d*, *J* = 6.9 Hz, 6H, 8-H, 8′-H) ppm; ^{13}C NMR:δ = 149.6 (C-3), 140.5 (C-1), 130.4 (C-4), 129.1 (C-5), 124.1 (C-2), 124.0 (C-6), 58.1 (C-11), 40.0 (C-9), 33.3 (C-7), 32.3 (C-10), 23.6 (C-8) ppm; MS: *m*/*z* = 280.2 (100%, [M + Na]$^+$); anal. calcd. for $C_{12}H_{19}NSO_3$ (257.35): C 56.01, H 7.44, N 5.44; found: C 55.71, H 7.73, N 5.18.

4.2.84. 3-[(3-Isopropylphenyl)sulfonamido]propyl Sulfamate (**41b**)

Applying GPB: from **41a** (200 mg, 0.78 mmol): **41b** (176 mg, 67%); oil; R_f = 0.64 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 269 nm (2.90); IR: ν = 3273*m*, 2964*w*, 2874*w*, 1560*w*, 1478*w*, 1464*w*, 1427*w*, 1364*s*, 1324*s*, 1306*s*, 1217*w*, 1177*s*, 1156*vs*, 1145*s*, 1090*m*, 1070*w*, 1051*w*, 940*s*, 824*w*, 798*m*, 738*w*, 695*s*, 623*m*, 585*vs*, 550*s*, 494*m* cm^{-1}; ^{1}H NMR:δ = 7.69–7.63 (*m*, 2H, 5-H, NH), 7.63–7.59 (*m*, 1H, 6-H), 7.56–7.49 (*m*, 2H, 2-H, 4-H), 7.41 (*s*, 2H, NH_2), 4.03 (*t*, *J* = 6.3 Hz, 2H, 11-H), 3.00 (*hept*, *J* = 6.9 Hz, 1H, 7-H), 2.83 (*q*, *J* = 6.7 Hz, 2H, 9-H), 1.80–1.72 (*m*, 2H, 10-H), 1.23 (*d*, *J* = 6.9 Hz, 6H, 8-H, 8′-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 149.7 (C-3), 140.3 (C-1), 130.5 (C-4), 129.2 (C-5), 124.1 (C-2), 124.0 (C-6), 66.5 (C-11), 39.2 (C-9), 33.3 (C-7), 28.7 (C-10), 23.6 (C-8) ppm; MS: *m*/*z* = 359.3 (100%, [M + Na]$^+$); anal. calcd. for $C_{12}H_{20}N_2S_2O_5$ (336.42): C 42.84, H 5.99, N 8.33; found: C 42.57, H 6.21, N 8.02.

4.2.85. N-(4-Hydroxybutyl)-3-isopropylbenzene Sulfonamide (**42a**)

Applying GPA: from 3-isopropylbenzenesulfonyl chloride (500 mg, 2.29 mmol) and 4-amino-butanol (306 mg, 3.43 mmol): **42a** (593 mg, 95%); oil; R_f = 0.18 (petrolether/EtOAc, 2:3); UV–Vis: 223 nm (3.91); IR: ν = 3500*w*, 3277*w*, 2961*w*, 2871*w*, 1478*w*, 1462*w*, 1427*m*, 1386*w*, 1365*w*, 1323*m*, 1305*s*, 1217*w*, 1156*s*, 1144*s*, 1087*m*, 1067*m*, 1054*m*, 1034*w*, 998*w*, 904*w*, 866*vw*, 798*m*, 737*w*, 696*s*, 623*m*, 587*vs*, 564*m*, 517*m*, 493*w*, 477*w*, 464*w* cm^{-1}; ^{1}H NMR:δ = 7.66–7.64 (*m*, 1H, 5-H), 7.62–7.58 (*m*, 1H, 6-H), 7.54–7.47 (*m*, 3H, 2-H, 4-H, NH), 4.35 (*t*, *J* = 5.1 Hz, 1H, OH), 3.35–3.28 (*m*, 2H, 12-H), 2.99 (*hept*, *J* = 6.9 Hz, 1H, 7-H), 2.78–2.69 (*m*, 2H, 9-H), 1.44–1.31 (*m*, 4H, 10-H, 11-H), 1.22 (*d*, *J* = 6.9 Hz, 6H, 8-H, 8′-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 149.6 (C-3), 140.7 (C-1), 130.3 (C-4), 129.1 (C-5), 124.0 (C-2), 124.0 (C-6), 60.2 (C-12), 42.5 (C-9), 33.3 (C-7), 29.5 (C-11), 25.8 (C-10), 23.6 (C-8) ppm; MS: *m*/*z* = 294.3 (100%, [M + Na]$^+$); anal. calcd. for $C_{13}H_{21}NSO_3$ (271.38): C 57.54, H 7.80, N 5.16; found: C 57.27, H 8.05, N 4.92.

4.2.86. 4-[(3-Isopropylphenyl)sulfonamido]butyl Sulfamate (**42b**)

Applying GPB: from **42a** (200 mg, 0.68 mmol): **42b** (240 mg, 93%); oil; R_f = 0.64 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 269 nm (3.01); IR: ν = 3274*m*, 2963*w*, 2874*w*, 1562*w*, 1478*w*, 1463*w*, 1452*w*, 1428*w*, 1364*m*, 1324*s*, 1306*s*, 1268*w*, 1217*vw*, 1178*s*, 1156*vs*, 1145*s*, 1089*m*, 1069*m*, 1051*w*, 997*w*, 922*s*, 800*m*, 735*w*, 696*s*, 626*m*, 587*vs*, 551*s* cm^{-1}; ^{1}H NMR:δ = 7.67–7.64 (*m*, 1H, 5-H), 7.63–7.56 (*m*, 2H, 6-H, NH), 7.55–7.48 (*m*, 2H, 2-H, 4-H), 7.38 (*s*, 2H, NH_2), 3.95 (*t*, *J* = 6.3 Hz, 2H, 12-H), 2.99 (*hept*, *J* = 6.9 Hz, 1H, 7-H), 2.81–2.72 (*m*, 2H, 9-H), 1.66–1.55 (*m*, 2H, 11-H), 1.49–1.39 (*m*, 2H, 10-H), 1.23 (*d*, *J* = 7.0 Hz, 6H, 8-H, 8′-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 149.6 (C-3), 140.6 (C-1), 130.4 (C-4), 129.2 (C-5), 124.0 (C-2), 123.9 (C-6), 68.5 (C-12), 42.0 (C-9), 33.3 (C-7), 25.6 (C-10), 25.4 (C-11), 23.6 (C-8) ppm; MS: *m*/*z* = 373.3 (100%, [M + Na]$^+$); anal. calcd. for $C_{13}H_{22}N_2S_2O_5$ (350.45): C 44.56, H 6.33, N 7.99; found: C 44.18, H 6.67, N 7.68.

4.2.87. N-(5-Hydroxypentyl)-3-isopropylbenzene Sulfonamide (**43a**)

Applying GPA: from 3-isopropylbenzenesulfonyl chloride (500 mg, 2.29 mmol) and 5-amino-pentanol (354 mg, 3.43 mmol): **43a** (611 mg, 94%); oil; R_f = 0.20 (petrolether/EtOAc, 2:3; UV–Vis: 223 nm (4.03); IR: ν = 3503*w*, 3279*w*, 2960*w*, 2936*w*, 2868*w*, 1478*w*, 1460*w*, 1427*w*, 1386*w*, 1365*w*, 1323*m*, 1305*s*, 1217*w*, 1156*s*, 1144*s*, 1087*m*, 1069*m*, 1050*m*, 998*w*, 905*w*, 799*m*, 730*w*, 696*s*, 623*m*, 587*vs*, 564*m*, 526*m*, 500*m*, 469*w*, 465*w*, 458*w*, 449*w* cm^{-1}; ^1H NMR:δ = 7.66–7.64 (*m*, 1H, 5-H), 7.62–7.58 (*m*, 1H, 6-H), 7.53–7.47 (*m*, 3H, 2-H, 4-H, NH), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.34–3.29 (*m*, 2H, 13-H), 2.99 (*hept*, *J* = 6.9 Hz, 1H, 7-H), 2.77–2.67 (*m*, 2H, 9-H), 1.39–1.27 (*m*, 4H, 10-H, 12-H), 1.22 (*d*, *J* = 6.9 Hz, 8H, 8-H, 8′-H, 11-H) ppm; ^{13}C NMR:δ = 149.6 (C-3), 140.7 (C-1), 130.3 (C-4), 129.1 (C-5), 124.0 (C-2), 124.0 (C-6), 60.5 (C-13), 42.6 (C-9), 33.3 (C-7), 32.0 (C-12), 28.8 (C-10), 23.6 (C-8), 22.6 (C-11) ppm; MS: *m*/*z* = 308.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{14}H_{23}NSO_3$ (285.40): C 58.92, H 8.12, N 4.91; found: C 58.66, H 8.32, N 4.63.

4.2.88. 5-[(3-Isopropylphenyl)sulfonamido]pentyl Sulfamate (**43b**)

Applying GPB: from **43a** (200 mg, 0.70 mmol): **43b** (160 mg, 63%); oil; R_f = 0.65 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 269 nm (2.99); IR: ν = 3275*m*, 2962*w*, 2871*w*, 1561*w*, 1478*w*, 1463*w*, 1428*w*, 1363*s*, 1323*s*, 1305*s*, 1217*vw*, 1176*s*, 1156*vs*, 1089*m*, 1070*m*, 1032*w*, 998*w*, 919*s*, 816*m*, 799*m*, 769*w*, 728*w*, 696*s*, 624*m*, 587*vs*, 551*s* cm^{-1}; ^1H NMR:δ = 7.66–7.64 (*m*, 1H, 5-H), 7.63–7.57 (*m*, 1H, 6-H), 7.56–7.47 (*m*, 3H, 2-H, 4-H, NH), 7.37 (*s*, 2H, NH_2), 3.95 (*t*, *J* = 6.5 Hz, 2H, 13-H), 2.99 (*hept*, *J* = 7.0 Hz, 1H, 7-H), 2.73 (*q*, *J* = 6.6 Hz, 2H, 9-H), 1.55 (*p*, *J* = 6.7 Hz, 2H, 12-H), 1.43–1.34 (*m*, 2H, 10-H), 1.33–1.26 (*m*, 2H, 11-H), 1.23 (*d*, *J* = 6.9 Hz, 6H, 8-H, 8′-H) ppm; ^{13}C NMR:δ = 149.6 (C-3), 140.6 (C-1), 130.4 (C-4), 129.2 (C-5), 124.0 (C-2), 124.0 (C-6), 68.8 (C-13), 42.3 (C-9), 33.3 (C-7), 28.5 (C-12), 27.8 (C-10), 23.6 (C-8), 22.2 (C-11) ppm; MS: *m*/*z* = 387.4 (100%, $[M + Na]^+$); anal. calcd. for $C_{14}H_{24}N_2S_2O_5$ (364.48): C 46.14, H 6.64, N 7.69; found: C 45.86, H 6.98, N 7.41.

4.2.89. N-(6-Hydroxyhexyl)-3-isopropylbenzene Sulfonamide (**44a**)

Applying GPA: from 3-isopropylbenzenesulfonyl chloride (500 mg, 2.29 mmol) and 6-amino-hexanol (402 mg, 3.43 mmol): **44a** (658 mg, 96%); oil; R_f = 0.24 (petrolether/EtOAc, 2:3); UV–Vis: 223 nm (3.88); IR: ν 3502*w*, 3281*w*, 2960*w*, 2933*m*, 2863*w*, 1598*vw*, 1478*w*, 1462*w*, 1427*w*, 1386*w*, 1365*w*, 1323*m*, 1305*s*, 1217*vw*, 1156*s*, 1144*s*, 1087*m*, 1069*m*, 1051*m*, 999*w*, 904*w*, 798*m*, 726*w*, 696*s*, 624*m*, 587*vs*, 563*m*, 529*m*, 454*w* cm^{-1}; ^1H NMR:δ = 7.66–7.64 (*m*, 1H, 5-H), 7.62–7.57 (*m*, 1H, 6-H), 7.53–7.46 (*m*, 3H, 2-H, 4-H, NH), 4.29 (*t*, *J* = 5.1 Hz, 1H, OH), 3.33 (*td*, *J* = 6.5, 5.1 Hz, 2H, 14-H), 2.99 (*hept*, *J* = 6.9 Hz, 1H, 7-H), 2.77–2.69 (*m*, 2H, 9-H), 1.38–1.28 (*m*, 4H, 10-H, 13-H), 1.22 (*d*, *J* = 6.9 Hz, 6H, 8-H, 8′-H), 1.21–1.14 (*m*, 4H, 11-H, 12-H) ppm; ^{13}C NMR:δ = 149.5 (C-3), 140.7 (C-1), 130.3 (C-4), 129.1 (C-5), 124.0 (C-2), 124.0 (C-6), 60.6 (C-14), 42.5 (C-9), 33.3 (C-7), 32.3 (C-13), 29.0 (C-10), 25.9 (C-12), 25.0 (C-11), 23.6 (C-8) ppm; MS: *m*/*z* = 322.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{25}NSO_3$ (299.43): C 60.17, H 8.42, N 4.68; found: C 59.84, H 8.76, N 4.37.

4.2.90. 6-[(3-Isopropylphenyl)sulfonamido]hexyl Sulfamate (**44b**)

Applying GPB: from **44a** (300 mg, 1.00 mmol): **44b** (260 mg, 69%); oil; R_f = 0.66 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 269 nm (2.99); IR: ν = 3276*m*, 2961*w*, 2866*w*, 1561*w*, 1478*w*, 1463*w*, 1428*w*, 1363*s*, 1323*s*, 1306*s*, 1217*vw*, 1177*s*, 1156*vs*, 1145*s*, 1088*m*, 1069*m*, 1050*w*, 922*s*, 799*m*, 725*w*, 696*s*, 624*m*, 587*vs*, 551*s* cm^{-1}; ^1H NMR:δ = 7.66–7.64 (*m*, 1H, 5-H), 7.63–7.58 (*m*, 1H, 6-H), 7.54–7.47 (*m*, 3H, 2-H, 4-H, NH), 7.37 (*s*, 2H, NH_2), 3.97 (*t*, *J* = 6.5 Hz, 2H, 14-H), 2.99 (*hept*, *J* = 6.9 Hz, 1H, 7-H), 2.77–2.69 (*m*, 2H, 9-H), 1.55 (*p*, *J* = 6.7 Hz, 2H, 13-H), 1.34 (*p*, *J* = 7.0 Hz, 2H, 10-H), 1.22 (*d*, *J* = 6.9 Hz, 10H, 8-H, 8′-H, 11-H, 12-H) ppm; ^{13}C NMR:δ = 149.6 (C-3), 140.7 (C-1), 130.4 (C-4), 129.1 (C-5), 124.0 (C-2), 124.0 (C-6), 68.9 (C-14), 42.4 (C-9), 33.3 (C-7), 28.8 (C-13), 28.2 (C-10), 25.5 (C-12), 24.6 (C-11), 23.6 (C-8) ppm; MS: *m*/*z* = 401.4 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{26}N_2S_2O_5$ (378.50): C 47.60, H 6.92, N 7.40; found: C 47.29, H 7.18, N 7.35.

4.2.91. N-(7-Hydroxyheptyl)-3-isopropylbenzene Sulfonamide (**45a**)

Applying GPA: from 3-isopropylbenzenesulfonyl chloride (500 mg, 2.29 mmol), and 7-amino-heptanol (450 mg, 3.43 mmol): **45a** (667 mg, 93%); oil; R_f = 0.23 (petrolether/EtOAc, 2:3); UV–Vis: 223 nm (3.99); IR: ν = 3504*w*, 3280*w*, 2960*w*, 2930*m*, 2859*w*, 1478*w*, 1462*w*, 1427*w*, 1385*w*, 1365*w*, 1324*m*, 1306*s*, 1217*vw*, 1157*s*, 1144*s*, 1088*m*, 1069*m*, 999*w*, 904*w*, 798*m*, 696*s*, 624*m*, 587*vs*, 564*m*, 527*m*, 462*w* cm^{-1}; ^1H NMR:δ = 7.66–7.64 (*m*, 1H, 2-H), 7.63–7.57 (*m*, 1H, 6-H), 7.52–7.46 (*m*, 3H, 4-H, 5-H, NH), 4.29 (*t*, *J* = 5.1 Hz, 1H, OH), 3.37–3.32 (*m*, 2H, 15-H), 2.98 (*hept*, *J* = 6.9 Hz, 1H, 7-H), 2.72 (*q*, *J* = 6.9 Hz, 2H, 9-H), 1.40–1.27 (*m*, 4H, 10-H, 14-H), 1.22 (*d*, *J* = 6.9 Hz, 6H, 8-H, 8′-H), 1.20–1.11 (*m*, 6H, 11-H, 12-H, 13-H) ppm; ^{13}C NMR:δ = 149.5 (C-3), 140.8 (C-1), 130.3 (C-4), 129.1 (C-5), 124.0 (C-2), 124.0 (C-6), 60.7 (C-15), 42.5 (C-9), 33.3 (C-7), 32.4 (C-14), 28.9 (C-10), 28.4 (C-12), 26.0 (C-11), 25.3 (C-13), 23.6 (C-8) ppm; MS: *m*/*z* = 336.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{16}H_{27}N_2S_2O_5$ (313.46): C 61.31, H 8.68, N 4.47; found: C 61.07, H 8.97, N 4.29.

4.2.92. 7-[(3-Isopropylphenyl)sulfonamido]heptyl Sulfamate (**45b**)

Applying GPB: from **45a** (200 mg, 0.67 mmol): **45b** (227 mg, 90%); oil; R_f = 0.68 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 269 nm (2.93); IR: ν = 3276*m*, 2961*w*, 2933*w*, 2862*w*, 1561*w*, 1478*w*, 1464*w*, 1428*w*, 1363*s*, 1323*s*, 1306*s*, 1217*vw*, 1178*s*, 1157*vs*, 1145*s*, 1089*m*, 1069*m*, 1052*w*, 997*w*, 923*s*, 799*m*, 724*w*, 696*s*, 624*m*, 587*vs*, 551*s* cm^{-1}; ^1H NMR:δ = 7.66–7.64 (*m*, 1H, 5-H), 7.62–7.58 (*m*, 1H, 6-H), 7.53–7.47 (*m*, 3H, 2-H, 4-H, NH), 7.37 (*s*, 2H, NH_2), 3.98 (*t*, *J* = 6.5 Hz, 2H, 15-H), 2.99 (*hept*, *J* = 6.9 Hz, 1H, 7-H), 2.76–2.69 (*m*, 2H, 9-H), 1.63–1.52 (*m*, 2H, 14-H), 1.39–1.28 (*m*, 2H, 10-H), 1.23 (*d*, *J* = 6.9 Hz, 6H, 8-H, 8′-H), 1.29–1.13 (*m*, 6H, 11-H, 12-H, 13-H) ppm; ^{13}C NMR:δ = 149.6 (C-3), 140.7 (C-1), 130.3 (C-4), 129.1 (C-5), 124.0 (C-2), 124.0 (C-6), 68.9 (C-15), 42.5 (C-9), 33.3 (C-7), 28.8 (C-14), 28.2 (C-11), 28.0 (C-10), 25.8 (C-13), 24.9 (C-12), 23.6 (C-8) ppm; MS: *m*/*z* = 415.4 (100%, $[M + Na]^+$); anal. calcd. for $C_{16}H_{28}N_2S_2O_5$ (392.53): C 48.96, H 7.19, N 7.14; found: C 48.75, H 7.34, N 6.86.

4.2.93. N-(8-Hydroxyoctyl)-3-isopropylbenzene Sulfonamide (**46a**)

Applying GPA: from 3-isopropylbenzenesulfonyl chloride (500 mg, 2.29 mmol) and 8-amino-octanol (498 mg, 3.43 mmol): **46a** (649 mg, 87%); oil; R_f = 0.30 (petrolether/EtOAc, 2:3; UV–Vis: 223 nm (3.88); IR: ν = 3504*vw*, 3281*w*, 2960*w*, 2929*m*, 2857*m*, 1478*w*, 1462*w*, 1428*w*, 1385*w*, 1365*w*, 1324*m*, 1306*m*, 1217*vw*, 1157*s*, 1144*s*, 1088*m*, 1069*m*, 1051*m*, 998*vw*, 904*w*, 798*m*, 722*w*, 696*s*, 624*m*, 587*vs*, 564*m*, 511*m*, 456*w* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.66–7.64 (*m*, 1H, 2-H), 7.62–7.58 (*m*, 1H, 6-H), 7.52–7.47 (*m*, 3H, 4-H, 5-H, NH), 4.29 (*td*, *J* = 5.2, 0.8 Hz, 1H, OH), 3.35 (*td*, *J* = 6.6, 5.1 Hz, 2H, 16-H), 2.99 (*hept*, *J* = 6.9 Hz, 1H, 7-H), 2.72 (*td*, *J* = 7.0, 5.8 Hz, 2H, 9-H), 1.41–1.28 (*m*, 4H, 10-H, 15-H), 1.22 (*d*, *J* = 6.9 Hz, 6H, 8-H, 8′-H), 1.20–1.11 (*m*, 8H, 11-H, 12-H, 13-H, 14-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 149.5 (C-3), 140.8 (C-1), 130.3 (C-4), 129.1 (C-5), 124.0 (C-2), 124.0 (C-6), 60.7 (C-16), 42.5 (C-9), 33.3 (C-7), 32.5 (C-15), 28.9 (C-10), 28.8 (C-12), 28.6 (C-13), 25.9 (C-11), 25.4 (C-14), 23.6 (C-8) ppm; MS: *m*/*z* = 350.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{17}H_{29}NSO_3$ (327.48): C 62.35, H 8.93, N 4.28; found: C 62.17, H 9.20, N 3.97.

4.2.94. 8-[(3-Isopropylphenyl)sulfonamido]octyl Sulfamate (**46b**)

Applying GPB: from **46a** (500 mg, 2.29 mmol): **46b** (196 mg, 79%); oil; R_f = 0.73 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 268 nm (3.00); IR: ν = 3276*m*, 2961*w*, 2930*m*, 2859*w*, 1561*w*, 1478*w*, 1464*w*, 1428*w*, 1363*s*, 1324*m*, 1306*s*, 1217*vw*, 1178*s*, 1157*vs*, 1145*s*, 1089*m*, 1070*m*, 1051*w*, 998*w*, 922*s*, 799*m*, 723*w*, 696*s*, 624*m*, 587*vs*, 552*s*, 461*w* cm^{-1}; ^1H NMR:δ = 7.66–7.64 (*m*, 1H, 5-H), 7.61–7.58 (*m*, 1H, 6-H), 7.53–7.48 (*m*, 3H, 2-H, 4-H, NH), 7.37 (*s*, 2H, NH_2), 3.99 (*t*, *J* = 6.5 Hz, 2H, 17-H), 2.99 (*hept*, *J* = 6.9 Hz, 1H, 7-H), 2.73 (*td*, *J* = 7.0, 5.8 Hz, 2H, 9-H), 1.63–1.55 (*m*, 2H, 16-H), 1.38–1.10 (*m*, 10H, 10-H, 11-H, 12-H, 13-H, 14-H), 1.23 (*d*, *J* = 6.9 Hz, 6H, 8-H, 8′-H) ppm; ^{13}C NMR:δ = 149.6 (C-3), 140.7 (C-1), 130.3 (C-4), 129.1 (C-5), 124.0 (C-2), 124.0 (C-6), 69.0 (C-17), 42.5 (C-9), 33.3 (C-7), 28.8 (C-16), 28.4 (C-13), 28.3 (C-10), 28.3 (C-12), 25.9 (C-11), 24.9 (C-14), 23.6 (C-8) ppm; MS: *m*/*z* = 429.5 (100%, $[M + Na]^+$); anal. calcd. for $C_{17}H_{30}N_2S_2O_5$ (406.56): C 50.22, H 7.44, N 6.89; found: C 49.97, H 7.70, N 6.54.

4.2.95. N-(7-Hydroxyheptyl)-2-isopropylbenzene Sulfonamide (**47a**)

Applying GPA: from 2-isopropylbenzenesulfonyl chloride (500 mg, 2.29 mmol) and 7-amino-heptanol (450 mg, 3.43 mmol): **47a** (634 mg, 88%); oil; R_f = 0.32 (petrolether/EtOAc, 2:3); UV–Vis: 224 nm (4.02); IR: ν = 3502*w*, 3292*w*, 2931*m*, 2859*m*, 1474*m*, 1461*w*, 1444*m*, 1385*w*, 1363*w*, 1316*s*, 1204*w*, 1148*vs*, 1115*m*, 1056*s*, 1031*m*, 878*w*, 763*s*, 725*w*, 686*m*, 595*vs*, 565*s*, 545*s*, 462*w*, 448*w* cm^{-1}; ^{1}H NMR:δ = 7.80–7.76 (*m*, 1H, 3-H), 7.66 (*t*, *J* = 5.7 Hz, 1H, NH), 7.60–7.53 (*m*, 2H, 6-H, 4-H), 7.33 (*ddd*, *J* = 8.0, 5.7, 2.9 Hz, 1H, 5-H), 4.29 (*t*, *J* = 5.1 Hz, 1H, OH), 3.85 (*hept*, *J* = 6.8 Hz, 1H, 7-H), 3.35 (*td*, *J* = 6.5, 5.2 Hz, 2H, 15-H), 2.79 (*td*, *J* = 7.0, 5.7 Hz, 2H, 9-H), 1.40–1.30 (*m*, 4H, 10-H, 14-H), 1.25–1.11 (*m*, 12H, 8-H, 8′-H, 11-H, 12-H, 13-H) ppm; ^{13}C NMR:δ = 147.7 (C-2), 138.1 (C-1), 132.5 (C-4), 128.0 (C-6), 127.9 (C-4), 125.8 (C-5), 60.7 (C-15), 42.4 (C-9), 32.4 (C-14), 29.2 (C-10), 28.4 (C-12), 28.3 (C-7), 26.0 (C-11), 25.3 (C-13), 23.9 (C-8, C-8′) ppm; MS: *m*/*z* = 336.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{16}H_{27}NSO_3$ (313.46): C 61.31, H 8.68, N 4.47; found: C 61.07, H 9.02, N 4.16.

4.2.96. 7-[(2-Isopropylphenyl)sulfonamido]heptyl Sulfamate (**47b**)

Applying GPB: from **47a** (200 mg, 0.64 mmol): **47b** (131 mg, 52%); oil; R_f = 0.76 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 224 nm (4.04); IR: ν = 3283*m*, 2933*w*, 2862*w*, 1593*vw*, 1570*w*, 1474*w*, 1444*w*, 1361*s*, 1315*s*, 1202*w*, 1179*s*, 1162*s*, 1148*vs*, 1115*m*, 1083*m*, 1071*m*, 1056*m*, 1031*w*, 924*s*, 815*m*, 764*s*, 724*w*, 687*m*, 596*s*, 563*s*, 550*vs*, 444*w* cm^{-1}; ^{1}H NMR:δ = 7.80–7.75 (*m*, 1H, 3-H), 7.68 (*t*, *J* = 5.7 Hz, 1H, NH), 7.61–7.54 (*m*, 2H, 4-H, 6-H), 7.37 (*s*, 2H, NH_2), 7.34 (*ddd*, J = 7.9, 5.9, 2.8 Hz, 1H, 5-H), 3.98 (*t*, *J* = 6.5 Hz, 2H, 15-H), 3.84 (*hept*, *J* = 6.9 Hz, 1H, 7-H), 2.79 (*td*, *J* = 7.0, 5.7 Hz, 2H, 9-H), 1.63–1.52 (*m*, 2H, 14-H), 1.42–1.31 (*m*, 2H, 10-H), 1.21 (*d*, *J* = 6.8 Hz, 6H, 8-H, 8′-H), 1.31–1.13 (*m*, 6H, 11-H, 12-H, 13-H) ppm; ^{13}C NMR:δ = 147.7 (C-2), 138.1 (C-1), 132.6 (C-4), 128.0 (C-3), 127.9 (C-6), 125.8 (C-5), 68.9 (C-15), 42.3 (C-9), 29.1 (C-10), 28.3 (C-7), 28.2 (C-14), 28.0 (C-12), 25.8 (C-11), 24.9 (C-13), 23.9 (C-8) ppm; MS: *m*/*z* = 415.6 (100%, $[M + Na]^+$); anal. calcd. for $C_{16}H_{28}N_2S_2O_5$ (392.53): C 48.96, H 7.19, N 7.14; found: C 48.75, H 7.37, N 6.87.

4.2.97. N-(8-Hydroxyoctyl)-2-isopropylbenzene Sulfonamide (**48a**)

Applying GPA: from 2-isopropylbenzenesulfonyl chloride (500 mg, 2.29 mmol) and 8-amino-octanol (498 mg, 3.43 mmol): **48a** (720 mg, 96%); oil; R_f = 0.34 (petrolether/EtOAc, 2:3); UV–Vis: 224 nm (3.90); IR: ν = 3498*w*, 3294*w*, 2929*m*, 2856*m*, 1474*m*, 1444*m*, 1385*w*, 1363*w*, 1316*s*, 1204*w*, 1148*vs*, 1115*m*, 1080*m*, 1071*m*, 1056*s*, 1031*m*, 890*w*, 763*s*, 723*w*, 686*m*, 595*vs*, 565*vs*, 545*s*, 450*w*, 419*vw* cm^{-1}; ^{1}H NMR:δ = 7.80–7.75 (*m*, 1H, 3-H), 7.66 (*s*, 1H, NH), 7.60–7.54 (*m*, 2H, 4-H, 6-H), 7.33 (*ddd*, *J* = 8.0, 5.8, 2.8 Hz, 1H, 5-H), 4.30 (*t*, *J* = 5.2 Hz, 1H, OH), 3.84 (*hept*, *J* = 6.8 Hz, 1H, 7-H), 3.38–3.33 (*m*, 2H, 16-H), 2.78 (*t*, *J* = 7.1 Hz, 2H, 9-H), 1.41–1.29 (*m*, 4H, 10-H, 15-H), 1.21 (*d*, *J* = 6.8 Hz, 6H, 8-H, 8′-H), 1.18–1.12 (*m*, 8H, 11-H, 12-H, 13-H, 14-H) ppm; ^{13}C NMR:δ = 147.7 (C-2), 138.1 (C-1), 132.5 (C-4), 128.0 (C-3), 127.9 (C-6), 125.8 (C-5), 60.7 (C-16), 42.4 (C-9), 32.5 (C-15), 29.2 (C-13), 28.8 (C-10), 28.5 (C-12), 28.3 (C-7), 25.9 (C-11), 25.4 (C-14) 23.9 (C-8) ppm; MS: *m*/*z* = 350.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{17}H_{29}NSO_3$ (327.48): C 62.35, H 8.93, N 4.28; found: C 62.6, H 9.24, N 3.96.

4.2.98. 8-[(2-Isopropylphenyl)sulfonamido]octyl Sulfamate (**48b**)

Applying GPB: from **47a** (200 mg, 0.61 mmol): **47b** (202 mg, 82%); oil; R_f = 0.82 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 224 nm (3.86); IR: ν = 3285*w*, 2931*m*, 2858*w*, 1593*vw*, 1570*w*, 1474*w*, 1444*w*, 1362*s*, 1315*s*, 1202*w*, 1179*s*, 1161*s*, 1149*vs*, 1115*m*, 1056*m*, 1031*w*, 925*s*, 819*w*, 764*s*, 724*w*, 687*m*, 596*s*, 564*s*, 550*s*, 450*w*, 446*w* cm^{-1}; ^{1}H NMR:δ = 7.80–7.75 (*m*, 1H, 3-H), 7.67 (*t*, *J* = 5.7 Hz, 1H, NH), 7.60–7.54 (*m*, 2H, 4-H, 6-H), 7.37 (*s*, 2H, NH_2), 7.34 (*ddd*, *J* = 7.9, 5.9, 2.8 Hz, 1H, 5-H), 3.99 (*t*, *J* = 6.5 Hz, 2H, 16-H), 3.84 (*hept*, *J* = 6.8 Hz, 1H, 7-H), 2.79 (*td*, *J* = 7.0, 5.7 Hz, 2H, 9-H), 1.63–1.55 (*m*, 2H, 15-H), 1.39–1.31 (*m*, 2H, 10-H), 1.30–1.24 (*m*, 2H, 14-H), 1.21 (*d*, *J* = 6.8 Hz, 6H, 8-H, 8′-H), 1.22–1.14 (*m*, 6H, 11-H, 12-H, 13-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 147.7 (C-2), 138.1 (C-1), 132.5 (C-4), 128.0 (C-3), 127.9 (C-6), 125.8 (C-5), 69.0 (C-16), 42.3 (C-9), 29.2 (C-15), 28.4 (C-10), 28.3 (C-7, C-13), 28.3 (C-12),

25.9 (C-11), 24.9 (C-14), 23.9 (C-8) ppm; MS: m/z = 429.6 (100%, $[M + Na]^+$); anal. calcd. for $C_{17}H_{30}N_2S_2O_5$ (406.56): C 50.22, H 7.44, N 6.89; found: C 49.96, H 7.70, N 6.55.

4.2.99. 4-(tert-Butyl)-N-(2-hydroxyethyl)benzene Sulfonamide (**49a**) [477483-08-2]

Applying GPA: from 4-(tert-butyl)benzenesulfonyl chloride (500 mg, 2.15 mmol) and 2-amino-ethanol (197 mg, 3.22 mmol): **49a** (435 mg, 79%); white solid; R_f = 0.20 (petrolether/EtOAc, 2:3); m.p. = 75–77 °C; UV–Vis: 228 nm (4.10); IR: ν = 3522*w*, 3162*w*, 3072*vw*, 2952*w*, 2903*w*, 2891*w*, 2868*w*, 1596*w*, 1463*w*, 1450*w*, 1432*w*, 1400*w*, 1364*w*, 1354*vw*, 1325*s*, 1308*m*, 1291*w*, 1261*w*, 1207*vw*, 1198*w*, 1161*vs*, 1115*m*, 1087*m*, 1071*m*, 1057*s*, 1016*w*, 955*m*, 905*w*, 844*w*, 831*vw*, 823*m*, 763*s*, 726*m*, 624*s*, 580*vs*, 550*s*, 515*vw*, 498*vw*, 473*w*, 457*w*, 421*w*, 411*w* cm^{-1}; ^1H NMR:δ = 7.75–7.70 (*m*, 2H, 2-H, 2′-H), 7.63–7.58 (*m*, 2H, 3-H, 3′-H), 7.50 (*s*, 1H, NH), 4.66 (*t*, *J* = 5.5 Hz, 1H, OH), 3.37 (*q*, *J* = 6.0 Hz, 2H, 8-H), 2.77 (*t*, *J* = 6.4 Hz, 2H, 7-H), 1.30 (*s*, 9H, 6-H, 6′-H, 6′-H) ppm; ^{13}C NMR:δ = 155.2 (C-4), 137.7 (C-1), 126.4 (C-2), 125.9 (C-3), 59.9 (C-8), 45.1 (C-7), 34.8 (C-5), 30.8 (C-6) ppm; MS: m/z = 280.4 (100%, $[M + Na]^+$); anal. calcd. for $C_{12}H_{19}NSO_3$ (257.35): C 56.01, H 7.44, N 5.44; found: C 55.76, H 7.72, N 5.16.

4.2.100. 2-[(4-(tert-Butyl)phenyl)sulfonamido]ethyl Sulfamate (**49b**)

Applying GPB: from **49a** (200 mg, 0.78 mmol): **49b** (228 mg, 87%); white solid; R_f = 0.60 ($CHCl_3$/EtOAc, 2:3); m.p. = 92–93 °C; UV–Vis: 228 nm (4.02); IR: ν = 3392*w*, 3292*m*, 3267*m*, 3075*vw*, 2951*w*, 2902*w*, 2865*w*, 1596*w*, 1542*w*, 1462*w*, 1445*m*, 1434*w*, 1401*w*, 1375*s*, 1325*s*, 1309*m*, 1292*w*, 1268*w*, 1228*vw*, 1197*w*, 1181*s*, 1159*s*, 1115*m*, 1088*s*, 1021*m*, 954*s*, 923*s*, 853*s*, 841*s*, 833*m*, 786*m*, 773*m*, 751*m*, 656*m*, 621*m*, 601*vs*, 578*m*, 549*vs*, 487*m*, 461*w*, 427*w*, 413*w* cm^{-1}; ^1H NMR:δ = 7.84 (*t*, *J* = 6.0 Hz, 1H, NH), 7.77–7.71 (*m*, 2H, 2-H, 2′-H), 7.64–7.60 (*m*, 2H, 3-H, 3′-H), 7.50 (*s*, 2H, NH_2), 4.01 (*t*, *J* = 5.7 Hz, 2H, 8-H), 3.03 (*q*, *J* = 5.6 Hz, 2H, 7-H), 1.31 (*s*, 9H, 6-H, 6′-H, 6′-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 155.9 (C-1), 137.8 (C-4), 126.8 (C-2), 126.5 (C-3), 68.0 (C-8), 42.0 (C-7), 35.3 (C-5), 31.3 (C-6) ppm; MS: m/z = 359.4 (100%, $[M + Na]^+$); anal. calcd. for $C_{12}H_{20}N_2S_2O_5$ (336.42): C 42.84, H 5.99, N 8.33; found: C 42.55, H 6.25, N 8.03.

4.2.101. 4-(tert-Butyl)-N-(3-hydroxypentyl)benzene Sulfonamide (**50a**) [1017436-75-7]

Applying GPA: from 4-(tert-butyl)benzenesulfonyl chloride (500 mg, 2.15 mmol) and 3-amino-propanol (242 mg, 3.22 mmol): **50a** (530 mg, 91%); white solid; R_f = 0.20 (petrolether/EtOAc, 2:3); m.p. = 63–65 °C; UV–Vis: 228 nm (4.15); IR: ν = 3311*m*, 3241*m*, 2963*m*, 2870*w*, 1597*w*, 1472*w*, 1426*m*, 1402*m*, 1363*w*, 1334*m*, 1313*s*, 1292*m*, 1270*w*, 1203*w*, 1160*vs*, 1112*m*, 1088*m*, 1069*m*, 1027*m*, 1008*m*, 960*m*, 908*w*, 847*w*, 834*m*, 826*m*, 756*s*, 704*m*, 625*s*, 578*vs*, 550*s*, 512*m*, 487*m*, 475*w*, 461*w* cm^{-1}; ^1H NMR:δ = 7.73–7.68 (*m*, 2H, 2-H, 2′-H), 7.63–7.58 (*m*, 2H, 3-H, 3′-H), 7.43 (*s*, 1H, NH), 4.40 (*s*, 1H, OH), 3.37 (*td*, *J* = 6.2, 2.8 Hz, 2H, 9-H), 2.77 (*t*, *J* = 7.3 Hz, 2H, 7-H), 1.58–1.49 (*m*, 2H, 8-H), 1.30 (*s*, 9H, 6-H, 6′-H, 6′-H) ppm; ^{13}C NMR:δ = 155.2 (C-4), 137.6 (C-1), 126.4 (C-2), 126.0 (C-3), 58.1 (C-9), 40.0 (C-7), 34.8 (C-5), 32.4 (C-8), 30.8 (C-6) ppm; MS: m/z = 294.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{13}H_{21}NSO_3$ (271.38): C 57.54, H 7.80, N 5.16; found: C 57.21, H 8.03, N 5.96.

4.2.102. 3-[(4-(tert-Butyl)phenyl)sulfonamido]propyl Sulfamate (**50b**)

Applying GPB: from **50a** (200 mg, 0.74 mmol): **50b** (251 mg, 97%); white solid; R_f = 0.61 ($CHCl_3$/EtOAc, 2:3); m.p. = 85–86 °C; UV–Vis: 228 nm (4.18); IR: ν = 3338*w*, 3293*w*, 3252*m*, 3121*vw*, 2964*w*, 2906*w*, 2870*vw*, 1597*w*, 1571*w*, 1476*w*, 1463*w*, 1437*w*, 1421*vw*, 1401*w*, 1373*s*, 1363*s*, 1329*m*, 1318*s*, 1294*m*, 1270*w*, 1242*vw*, 1169*s*, 1156*vs*, 1133*w*, 1111*m*, 1085*m*, 1060*w*, 1016*vw*, 982*s*, 949*s*, 891*m*, 877*w*, 839*m*, 827*s*, 757*s*, 678*m*, 631*m*, 595*m*, 589*m*, 573*s*, 564*s*, 548*vs*, 501*w*, 491*m*, 463*w*, 413*vw* cm^{-1}; ^1H NMR:δ = 7.74–7.69 (*m*, 2H, 2-H, 2′-H), 7.64–7.58 (*m*, 3H, 3-H, 3′-H, NH), 7.41 (*s*, 2H, NH_2), 4.03 (*td*, *J* = 6.7, 5.0 Hz, 2H, 9-H), 2.81 (*td*, *J* = 7.1, 5.8 Hz, 2H, 7-H), 1.78 (*p*, *J* = 6.7 Hz, 2H, 8-H), 1.31 (*s*, 9H, 6-H, 6′-H, 6′-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 155.4 (C-4), 137.4 (C-1), 126.3 (C-2), 126.1 (C-3), 66.5 (C-9), 39.2 (C-7), 34.8 (C-5), 30.8 (C-6), 28.8 (C-8) ppm; MS: m/z = 373.4 (100%, $[M + Na]^+$);

anal. calcd. for $C_{13}H_{22}N_2S_2O_5$ (350.45): C 44.56, H 6.33, N 7.99; found: C 44.21, H 6.65, N 7.68.

4.2.103. 4-(tert-Butyl)-N-(4-hydroxybutyl)benzene Sulfonamide (**51a**) [1082935-75-8]

Applying GPA: from 4-(tert-butyl)benzenesulfonyl chloride (500 mg, 2.15 mmol) and 4-amino-butanol (287 mg, 3.22 mmol): **51a** (518 mg, 84%) [118]; white solid; R_f = 0.20 (petrolether/EtOAc, 2:3); m.p. = 58–60 °C; UV–Vis: 228 nm (4.15); IR: ν = 3452*w*, 3248*w*, 3115*w*, 2962*w*, 2944*m*, 2867*w*, 1594*w*, 1471*w*, 1463*w*, 1435*w*, 1400*w*, 1364*w*, 1316*s*, 1291*m*, 1267*w*, 1197*w*, 1154*s*, 1111*m*, 1084*m*, 1065*m*, 1038*m*, 1018*w*, 988*w*, 909*w*, 839*m*, 753*m*, 735*w*, 685*w*, 627*s*, 581*vs*, 549*s*, 514*w*, 489*w*, 472*w*, 464*w* cm^{-1}; ^1H NMR:δ = 7.73–7.68 (*m*, 2H, 2-H, 2′-H), 7.62–7.58 (*m*, 2H, 3-H, 3′-H), 7.47 (*s*, 1H, NH), 4.35 (*s*, 1H, OH), 3.35–3.29 (*m*, 2H, 10-H), 2.75–2.69 (*m*, 2H, 7-H), 1.44–1.33 (*m*, 4H, 8-H, 9-H), 1.30 (*s*, 9H, 6-H, 6′-H, 6′-H) ppm; ^{13}C NMR:δ = 155.1 (C-4), 137.8 (C-1), 126.3 (C-2), 125.9 (C-3), 60.2 (C-10), 42.5 (C-7), 34.8 (C-5), 30.8 (C-6), 29.5 (C-9), 25.8 (C-8) ppm; MS: *m*/*z* = 308.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{14}H_{23}NSO_3$ (285.40): C 58.92, H 8.12, N 4.91; found: C 59.76, H 8.32, N 4.67.

4.2.104. 4-[(4-(tert-Butyl)phenyl)sulfonamido]butyl Sulfamate (**51b**)

Applying GPB: from **51a** (200 mg, 0.7 mmol): **51b** (218 mg, 85%); white solid; R_f = 0.63 ($CHCl_3$/EtOAc, 2:3); m.p. = 100–102 °C; UV–Vis: 228 nm (4.12); IR: ν = 3358*m*, 3288*m*, 3262*w*, 2965*w*, 1597*vw*, 1558*w*, 1483*vw*, 1476*w*, 1427*w*, 1399*w*, 1383*w*, 1368*s*, 1338*m*, 1321*s*, 1310*m*, 1292*w*, 1269*w*, 1205*w*, 1176*s*, 1158*vs*, 1122*w*, 1114*m*, 1108*m*, 1089*m*, 1065*m*, 1044*w*, 970*s*, 941*w*, 919*s*, 902*s*, 838*m*, 826*s*, 809*w*, 754*m*, 750*m*, 660*s*, 628*s*, 580*s*, 552*vs*, 533*m*, 514*w*, 507*m*, 413*w* cm^{-1}; ^1H NMR:δ = 7.74–7.68 (*m*, 2H, 2-H, 2′-H), 7.63–7.58 (*m*, 2H, 3-H, 3′-H), 7.54 (*t*, *J* = 5.9 Hz, 1H, NH), 7.38 (*s*, 2H, NH_2), 3.96 (*t*, *J* = 6.3 Hz, 2H, 10-H), 2.79–2.71 (*m*, 2H, 7-H), 1.66–1.56 (*m*, 2H, 9-H), 1.50–1.40 (*m*, 2H, 8-H), 1.30 (*s*, 9H, 6-H, 6′-H, 6′-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 155.2 (C-4), 137.7 (C-1), 126.3 (C-2), 126.0 (C-3), 68.5 (C-10), 42.0 (C-7), 34.8 (C-5), 30.8 (C-6), 25.6 (C-9), 25.4 (C-8) ppm; MS: *m*/*z* = 387.4 (100%, $[M + Na]^+$); anal. calcd. for $C_{14}H_{24}N_2S_2O_5$ (364.48): C 46.14, H 6.64, N 7.69; found: C 45.86, H 6.98, N 7.42.

4.2.105. 4-(tert-Butyl)-N-(5-hydroxypentyl)benzene Sulfonamide (**52a**) [1925550-01-1]

Applying GPA: from 4-(tert-butyl)benzenesulfonyl chloride (500 mg, 2.15 mmol) and 5-amino-pentanol (332 mg, 3.22 mmol): **52a** (603 mg, 93%); white solid; R_f = 0.20 (petrolether/EtOAc, 2:3); m.p. = 53–54 °C; UV–Vis: 228 nm (4.02); IR: ν = 3286*m*, 2933*m*, 2863*w*, 1597*w*, 1473*w*, 1462*w*, 1424*m*, 1399*w*, 1362*w*, 1331*m*, 1318*s*, 1292*m*, 1268*w*, 1199*w*, 1158*s*, 1112*m*, 1088*m*, 1048*s*, 1016*w*, 887*m*, 843*m*, 826*m*, 755*m*, 735*w*, 689*m*, 638*m*, 630*s*, 573*vs*, 552*s*, 525*m*, 502*w* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.72–7.68 (*m*, 2H, 2-H, 2′-H), 7.62–7.58 (*m*, 2H, 3-H, 3′-H), 7.46 (*t*, *J* = 5.7 Hz, 1H, NH), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.34–3.29 (*m*, 2H, 11-H), 2.73–2.68 (*m*, 2H, 7-H), 1.39–1.31 (*m*, 4H, 8-H, 10-H), 1.30 (*s*, 9H, 6-H, 6′-H, 6′-H), 1.26–1.15 (*m*, 2H, 9-H) ppm; ^{13}C NMR:δ = 155.2 (C-4), 137.8 (C-1), 126.3 (C-2), 125.9 (C-3), 60.5 (C-11), 42.6 (C-7), 34.8 (C-5), 32.0 (C-10), 30.8 (C-6), 28.9 (C-8), 22.6 (C-9) ppm; MS: *m*/*z* = 322.4 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{25}NSO_3$ (299.43): C 60.17, H 8.42, N 4.68; found: C 59.87, H 8.70, N 4.39.

4.2.106. 5-[(4-(tert-Butyl)phenyl)sulfonamido]pentyl Sulfamate (**52b**)

Applying GPB: from **52a** (500 mg, 2.15 mmol): **52b** (196 mg, 78%); white solid; R_f = 0.65 ($CHCl_3$/EtOAc, 2:3); m.p. = 88–89 °C; UV–Vis: 228 nm (4.17); IR: ν = 3356*m*, 3278*m*, 3254*m*, 2968*w*, 2954*w*, 2860*w*, 1597*w*, 1557*w*, 1475*w*, 1463*w*, 1427*w*, 1398*w*, 1363*s*, 1326*s*, 1311*m*, 1292*w*, 1269*w*, 1175*s*, 1156*vs*, 1124*w*, 1111*m*, 1089*m*, 1068*w*, 1032*m*, 1000*m*, 965*s*, 918*s*, 900*s*, 852*w*, 840*m*, 821*s*, 775*m*, 754*m*, 738*w*, 627*s*, 579*vs*, 552*vs*, 533*s*, 520*m*, 501*m*, 443*w*, 412*w* cm^{-1}; ^1H NMR:δ = 7.73–7.68 (*m*, 2H, 2-H, 2′-H), 7.63–7.57 (*m*, 2H, 3-H, 3′-H), 7.49 (*t*, *J* = 5.9 Hz, 1H, NH), 7.37 (*s*, 2H, NH_2), 3.96 (*t*, *J* = 6.4 Hz, 2H, 11-H), 2.72 (*q*, *J* = 6.6 Hz, 2H, 7-H), 1.55 (*p*, *J* = 6.7 Hz, 2H, 10-H), 1.44–1.35 (*m*, 2H, 8-H), 1.30 (*s*, 11H, 6-H, 6′-H, 6′-H, 9-H) ppm; ^{13}C NMR:δ = 155.2 (C-4), 137.7 (C-1), 126.3 (C-2), 126.0 (C-3), 68.8 (C-11), 42.3 (C-7), 34.8 (C-5),

30.8 (C-6), 28.5 (C-8), 27.8 (C-10), 22.2 (C-9) ppm; MS: m/z = 401.6 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{26}N_2S_2O_5$ (378.50): C 47.60, H 6.92, N 7.40; found: C 47.32, H 7.24, N 7.09.

4.2.107. 4-(tert-Butyl)-N-(6-hydroxyhexyl)benzene Sulfonamide (**53a**) [1787706-38-0]

Applying GPA: from 4-(tert-butyl)benzenesulfonyl chloride (500 mg, 2.15 mmol) and 6-amino-hexanol (378 mg, 3.22 mmol): **53a** (635 mg, 94%) [119,120]; white solid; R_f = 0.45 (petrolether/EtOAc 1: 1); m.p. = 53–54 °C; UV–Vis: 229 nm (4.21); IR: ν = 3504*br*, 3281*br*, 2935*m*, 2864*w*, 1596*w*, 1475*w*, 1462*w*, 1398*w*, 1365*w*, 1321*s*, 1269*w*, 1198*w*, 1157*vs*, 1112*s*, 1087*s*, 1056*m*, 1026*w*, 1014*w*, 835*m*, 754*m*, 726*w*, 734*w*, 626*s*, 581*s*, 549*s*, 455*w* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.72–7.68 (*m*, 2H, 2-H, 2′-H), 7.62–7.58 (*m*, 2H, 3-H, 3′-H), 7.49–7.44 (*m*, 1H, NH), 4.29 (*t*, *J* = 5.1 Hz, 1H, OH), 3.36–3.32 (*m*, 2H, 12-H), 2.71 (*td*, *J* = 7.2, 3.7 Hz, 2H, 7-H), 1.37–1.31 (*m*, 4H, 8-H, 11-H), 1.30 (*s*, 9H, 6-H, 6′-H, 6′-H), 1.22–1.13 (*m*, 4H, 9-H, 10-H) ppm; ^{13}C NMR:δ = 155.2 (C-4), 137.8 (C-1), 126.3 (C-2), 125.9 (C-3), 60.6 (C-12), 42.5 (C-7), 34.8 (C-5), 32.3 (C-11), 30.8 (C-6), 29.0 (C-8), 25.9 (C-10), 25.0 (C-9) ppm; MS: m/z = 336.2 (100%, $[M + Na]^+$); anal. calcd. for $C_{16}H_{27}NSO_3$ (313.46): C 61.31, H 8.68, N 4.47; found: C 59.97, H 8.98, N 4.16.

4.2.108. 6-[(4-(tert-Butyl)phenyl)sulfonamido]hexyl Sulfamate (**53b**)

Applying GPB: from **53a** (196 mg, 0.62 mmol): **53b** (152 mg, 62%); white solid; R_f = 0.69 ($CHCl_3$/EtOAc, 2:3); m.p. = 89–90 °C; UV–Vis: 228 nm (4.12); IR: ν = 3364*w*, 3292*m*, 3253*m*, 2964*w*, 2952*w*, 2941*w*, 2863*w*, 1596*w*, 1571*w*, 1481*w*, 1427*w*, 1399*w*, 1364*s*, 1338*m*, 1323*s*, 1308*m*, 1293*w*, 1285*w*, 1270*w*, 1158*vs*, 1110*m*, 1088*m*, 1062*m*, 1023*m*, 1011*w*, 963*m*, 942*m*, 897*m*, 884*m*, 826*s*, 805*w*, 755*m*, 733*w*, 658*s*, 633*m*, 577*s*, 568*s*, 552*m*, 536*m*, 528*m*, 504*w* cm^{-1}; ^1H NMR:δ = 7.70–7.65 (*m*, 2H, 2-H, 2′-H), 7.60–7.55 (*m*, 2H, 3-H, 3′-H), 7.45 (*t*, *J* = 5.9 Hz, 1H, NH), 7.34 (*s*, 2H, NH_2), 3.94 (*t*, *J* = 6.5 Hz, 2H, 12-H), 2.69 (*q*, *J* = 6.7 Hz, 2H, 7-H), 1.57–1.48 (*m*, 2H, 11-H), 1.37–1.29 (*m*, 2H, 8-H), 1.28 (*s*, 9H, 6-H, 6′-H, 6′-H), 1.25–1.17 (*m*, 4H, 9-H, 10-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 155.2 (C-4), 137.8 (C-1), 126.3 (C-2), 125.9 (C-3), 68.9 (C-12), 42.4 (C-7), 34.8 (C-5), 30.8 (C-6), 28.8 (C-11), 28.1 (C-8), 25.5 (C-10), 24.6 (C-9) ppm; MS: m/z = 415.6 (100%, $[M + Na]^+$); anal. calcd. for $C_{16}H_{28}N_2S_2O_5$ (392.53): C 48.96, H 7.19, N 7.14; found: C 48.71, H 7.32, N 6.91.

4.2.109. 4-(tert-Butyl)-N-(7-hydroxyheptyl)benzene Sulfonamide (**54a**)

Applying GPA: from 4-(tert-butyl)benzenesulfonyl chloride (500 mg, 2.15 mmol) and 7-amino-heptanol (423 mg, 3.22 mmol): **54a** (688 mg, 98%); white solid; R_f = 0.27 (petrolether/EtOAc, 2:3); m.p. = 51–52 °C; UV–Vis: 228 nm (4.20); IR: ν = 3296*w*, 3248*m*, 2927*m*, 2856*m*, 1597*w*, 1475*w*, 1464*w*, 1436*w*, 1401*m*, 1364*w*, 1319*s*, 1292*m*, 1269*w*, 1202*w*, 1156*s*, 1113*m*, 1089*m*, 1059*s*, 1030*m*, 1015*w*, 1006*w*, 891*w*, 843*w*, 831*m*, 758*m*, 695*m*, 627*m*, 573*vs*, 551*s*, 524*w*, 509*w* cm^{-1}; ^1H NMR:δ = 7.73–7.68 (*m*, 2H, 2-H, 2′-H), 7.62–7.57 (*m*, 2H, 3-H, 3′-H), 7.49–7.43 (*m*, 1H, NH), 4.29 (*t*, *J* = 5.1 Hz, 1H, OH), 3.34 (*td*, *J* = 6.8, 5.3 Hz, 2H, 13-H), 2.71 (*q*, *J* = 6.4 Hz, 2H, 7-H), 1.40–1.31 (*m*, 4H, 8-H, 12-H), 1.30 (*s*, 9H, 6-H, 6′-H, 6′-H), 1.23–1.13 (*m*, 6H, 9-H, 10-H, 11-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 155.1 (C-4), 137.8 (C-1), 126.3 (C-2), 125.9 (C-3), 60.6 (C-13), 42.5 (C-7), 34.8 (C-5), 32.4 (C-12), 30.8 (C-6), 28.9 (C-8), 28.4 (C-10), 26.0 (C-9), 25.3 (C-11) ppm; MS: m/z = 350.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{17}H_{29}NSO_3$ (327.48): C 62.35, H 8.93, N 4.28; found: C 62.06, H 4.55, N 3.96.

4.2.110. 7-[(4-(tert-Butyl)phenyl)sulfonamido]heptyl Sulfamate (**54b**)

Applying GPB: from **54a** (300 mg, 0.92 mmol): **54b** (269 mg, 72%); white solid; R_f = 0.75 ($CHCl_3$/EtOAc, 2:3); m.p. = 95–96 °C; UV–Vis: 228 nm (4.10); IR: ν = 3337*w*, 3248*m*, 3125*vw*, 2953*w*, 2931*m*, 2856*w*, 1596*w*, 1572*w*, 1476*w*, 1463*w*, 1432*w*, 1397*w*, 1373*s*, 1366*s*, 1324*s*, 1315*s*, 1311*s*, 1291*m*, 1269*w*, 1202*vw*, 1169*s*, 1158*vs*, 1121*w*, 1111*m*, 1088*m*, 1074*w*, 1057*m*, 1047*w*, 1015*w*, 1000*m*, 972*m*, 949*s*, 895*m*, 854*vw*, 833*m*, 824*s*, 775*w*, 760*s*, 726*m*, 699*s*, 626*m*, 590*m*, 575*vs*, 564*s*, 550*s*, 535*m*, 529*m*, 517*m*, 502*w*, 477*w*, 411*vw* cm^{-1}; ^1H NMR:δ = 7.72–7.68 (*m*, 2H, 2-H, 2′-H), 7.62–7.58 (*m*, 2H, 3-H, 3′-H), 7.47 (*t*, *J* = 5.8 Hz, 1H, NH), 7.37 (*s*, 2H, NH_2), 3.98 (*t*, *J* = 6.5 Hz, 2H, 13-H), 2.71 (*td*, *J* = 7.0, 5.9 Hz, 2H, 7-H),

1.63–1.54 (*m*, 2H, 12-H), 1.30 (*s*, 9H, 6-H, 6′-H, 6′-H), 1.39–1.16 (*m*, 8H, 8-H, 9-H, 10-H, 11-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 155.2 (C-4), 137.8 (C-1), 126.3 (C-2), 125.9 (C-3), 68.9 (C-13), 42.5 (C-7), 34.8 (C-5), 30.8 (C-6), 28.9 (C-8), 28.2 (C-12), 28.0 (C-10), 25.8 (C-11), 24.9 (C-9) ppm; MS: *m*/*z* = 429.2 (100%, [M + Na]$^+$); anal. calcd. for $C_{17}H_{30}N_2S_2O_5$ (406.56): C 50.22, H 7.44, N 6.89; found: C 49.96, H 7.77, N 6.53.

4.2.111. 4-(tert-Butyl)-N-(8-hydroxyoctyl)benzene Sulfonamide (**55a**)

Applying GPA: from 4-(tert-butyl)benzenesulfonyl chloride (500 mg, 2.15 mmol) and 8-amino-octanol (468 mg, 3.22 mmol): **55a** (653 mg, 89%); white solid; R_f = 0.30 (petrolether/EtOAc, 2:3); m.p. = 69–71 °C; UV–Vis: 228 nm (4.13); IR: ν = 3263*m*, 2927*m*, 2854*m*, 1597*w*, 1479*w*, 1467*w*, 1428*m*, 1401*w*, 1363*w*, 1325*s*, 1292*m*, 1270*w*, 1202*w*, 1156*vs*, 1122*w*, 1111*m*, 1088*m*, 1057*m*, 1046*m*, 1016*w*, 999*w*, 950*vw*, 896*w*, 881*w*, 841*w*, 827*m*, 757*s*, 736*w*, 723*w*, 675*m*, 634*m*, 575*vs*, 550*s*, 535*w*, 525*w*, 511*m* cm^{-1}; ^{1}H NMR:δ = 7.72–7.68 (*m*, 2H, 2-H, 2′-H), 7.62–7.58 (*m*, 2H, 3-H, 3′-H), 7.48–7.43 (*m*, 1H), 4.30 (*t*, *J* = 5.2 Hz, 1H), 3.35 (*td*, *J* = 6.6, 5.1 Hz, 2H, 14-H), 2.71 (*q*, *J* = 6.5 Hz, 2H, 7-H), 1.41–1.32 (*m*, 4H, 8-H, 13-H), 1.30 (*s*, 9H, 6-H, 6′-H, 6′-H), 1.26–1.12 (*m*, 8H, 9-H, 10-H, 11-H, 12-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 155.1 (C-4), 137.8 (C-1), 126.3 (C-2), 125.9 (C-3), 60.7 (C-14), 42.5 (C-7), 34.8 (C-5), 32.5 (C-13), 30.8 (C-6), 28.9 (C-8), 28.7 (C-11), 28.6 (C-10), 25.9 (C-9), 25.4 (C-12) ppm; MS: *m*/*z* = 364.4 (100%, [M + Na]$^+$); anal. calcd. for $C_{18}H_{31}NSO_3$ (341.51): C 63.31, H 9.15, N 4.10; found: C 63.11, H 4.40, N 3.86.

4.2.112. 8-[(4-(tert-Butyl)phenyl)sulfonamido]octyl Sulfamate (**55b**)

Applying GPB: from **55a** (200 mg, 0.59 mmol): **55b** (240 mg, 98%); white solid; R_f = 0.79 ($CHCl_3$/EtOAc, 2:3); m.p. = 79–81 °C; UV–Vis: 228 nm (4.10); IR: ν = 3334*w*, 3256*m*, 2950*w*, 293*m*, 2856*w*, 1596*w*, 1476*w*, 1463*w*, 1432*w*, 1392*w*, 1370*s*, 1360*s*, 1328*s*, 1314*s*, 1312*s*, 1285*m*, 1285*w*, 1169*s*, 1154*vs*, 1120*w*, 1110*m*, 1090*m*, 1060*m*, 1041*w*, 1011*w*, 1005*m*, 970*m*, 949*s*, 891*m*, 830*m*, 825*s*, 778*w*, 762*s*, 732*m*, 698*s*, 626*m*, 591*m*, 576*vs*, 562*s*, 555*s*, 531*m*, 530*m*, 514*m* cm^{-1}; ^{1}H NMR:δ = 7.72–7.68 (*m*, 2H, 2-H, 2′-H), 7.62–7.58 (*m*, 2H, 3-H, 3′-H), 7.46 (*t*, *J* = 5.8 Hz, 1H, NH), 7.37 (*s*, 2H, NH_2), 3.99 (*t*, *J* = 6.5 Hz, 2H, 14-H), 2.71 (*td*, *J* = 7.0, 5.8 Hz, 2H, 7-H), 1.59 (*dt*, *J* = 8.2, 6.5 Hz, 2H, 13-H), 1.39–1.24 (*m*, 4H, 8-H, 12-H), 1.30 (*s*, 9H, 6-H, 6′-H, 6′-H), 1.26–1.12 (*m*, 6H, 9-H, 10-H, 11-H) ppm; ^{13}C NMR:δ = 155.2 (C-4), 137.8 (C-1), 126.3 (C-2), 125.9 (C-3), 68.9 (C-14), 42.5 (C-7), 34.8 (C-5), 30.8 (C-6), 28.9 (C-13), 28.4 (C-11), 28.3 (C-8), 28.3 (C-10), 25.9 (C-12), 24.9 (C-9) ppm; MS: *m*/*z* = 443.8 (100%, [M + Na]$^+$); anal. calcd. for $C_{18}H_{32}N_2S_2O_5$ (420.58): C 51.40, H 7.67, N 6.66; found: C 51.17, H 7.90, N 6.38.

4.2.113. 4-(tert-Butyl)-N-(9-hydroxynonyl)benzene Sulfonamide (**56a**)

Applying GPA: from 4-(tert-butyl)benzenesulfonyl chloride (500 mg, 2.15 mmol) and 9-amino-nonanol (513 mg, 3.22 mmol): **56a** (636 mg, 83%); white solid; R_f = 0.78 ($CHCl_3$/EtOAc, 2:3); m.p. = 69–70 °C; UV–Vis: 228 nm (4.07); IR: ν = 3459*vw*, 3285*m*, 2963*w*, 2925*m*, 2856*m*, 1598*w*, 1484*vw*, 1471*m*, 1438*w*, 1400*w*, 1363*w*, 1323*vs*, 1294*m*, 1270*w*, 1243*vw*, 1199*vw*, 1154*vs*, 1120*w*, 1110*m*, 1090*s*, 1072*s*, 1045*m*, 1037*m*, 1013*m*, 973*w*, 902*w*, 863*m*, 836*m*, 820*m*, 776*w*, 754*m*, 723*w*, 681*m*, 627*m*, 574*vs*, 551*s*, 534*m*, 521*m*, 473*vw*, 461*w*, 438*w* cm^{-1}; ^{1}H NMR:δ = 7.73–7.67 (*m*, 2H, 2-H, 2′-H), 7.62–7.57 (*m*, 2H, 3-H, 3′-H), 7.46 (*t*, *J* = 5.8 Hz, 1H, NH), 4.29 (*td*, *J* = 5.2, 1.0 Hz, 1H, OH), 3.36 (*td*, *J* = 6.6, 5.1 Hz, 2H, 15-H), 2.71 (*td*, *J* = 7.0, 5.8 Hz, 2H, 7-H), 1.43–1.35 (*m*, 2H, 14-H), 1.35–1.31 (*m*, 2H, 8-H), 1.30 (*s*, 9H, 6-H, 6′-H, 6′-H), 1.27–1.11 (*m*, 10H, 9-H, 10-H, 11-H, 12-H, 13-H) ppm; ^{13}C NMR:δ = 155.1 (C-4), 137.8 (C-1), 126.3 (C-2), 125.9 (C-3), 60.7 (C-15), 42.5 (C-7), 34.8 (C-5), 32.5 (C-14), 30.8 (C-6), 28.9 (C-8), 28.9 (C-12), 28.8 (C-10), 28.5 (C-11), 26.0 (C-9), 25.4 (C-13) ppm; MS: *m*/*z* = 378.1 (100%, [M + Na]$^+$); anal. calcd. for $C_{19}H_{33}NSO_3$ (355.54): C 64.19, H 9.36, N 3.94; found: C 63.76, H 9.51, N 3.68.

4.2.114. 9-[(4-(tert-Butyl)phenyl)sulfonamido]nonyl Sulfamate (**56b**)

Applying GPB: from **56a** (200 mg, 0.56 mmol): **56b** (187 mg, 76%); white solid; R_f = 0.88 ($CHCl_3$/EtOAc, 2:3); m.p. = 106–107 °C; UV–Vis: 228 nm (4.06); IR: ν = 3358*w*, 3270*m*, 3124*vw*, 2963*w*, 2918*w*, 2853*w*, 1597*w*, 1571*w*, 1475*w*, 1466*w*, 1432*w*, 1397*w*, 1376*s*, 1314*s*, 1295*m*, 1272*w*, 1201*vw*, 1181*m*, 1157*vs*, 1111*m*, 1090*m*, 1062*w*, 1033*m*, 1015*vw*, 991*w*, 968*m*, 933*m*, 904*w*, 882*w*, 837*w*, 818*s*, 778*vw*, 758*m*, 733*vw*, 721*w*, 692*m*, 628*w*, 590*m*, 576*s*, 566*s*, 551*m*, 537*m*, 529*w*, 513*w*, 484*m*, 456*vw*, 411*vw* cm^{-1}; ^1H NMR:δ = 7.72–7.68 (*m*, 2H, 2-H, 2′-H), 7.62–7.58 (*m*, 2H, 3-H, 3′-H), 7.46 (*t*, *J* = 5.8 Hz, 1H, NH), 7.37 (*s*, 2H, NH_2), 3.99 (*t*, *J* = 6.5 Hz, 2H, 15-H), 2.71 (*td*, *J* = 7.0, 5.8 Hz, 2H, 7-H), 1.65–1.55 (*m*, 2H, 14-H), 1.39–1.25 (*m*, 4H, 8-H, 13-H), 1.30 (*s*, 9H, 6-H, 6′-H, 6′-H), 1.26–1.12 (*m*, 8H, 9-H, 10-H, 11-H, 12-H) ppm; ^{13}C NMR:δ = 155.16 (C-4), 137.82 (C-1), 126.31 (C-2), 125.92 (C-3), 68.96 (C-15), 42.48 (C-7), 34.78 (C-5), 30.80 (C-6), 28.92 (C-14), 28.71 (C-8), 28.43 (C-10), 28.39 (C-12), 28.29 (C-11), 25.94 (C-9), 25.01 (C-13) ppm; MS: *m*/*z* = 457.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{19}H_{34}N_2S_2O_5$ (434.61): C 52.51, H 7.89, N 6.45; found: C 52.31, H 8.02, N 6.15.

4.2.115. 4-(tert-Butyl)-N-(10-hydroxydecyl)benzene Sulfonamide (**57a**)

Applying GPA: from 4-(tert-butyl)benzenesulfonyl chloride (300 mg, 1.29 mmol) and 10-amino-decanol (335 mg, 1.93 mmol): **57a** (415 mg, 87%); white solid; R_f = 0.77 ($CHCl_3$/EtOAc, 2:3); m.p. = 75–76 °C; UV–Vis: 228 nm (4.13); IR: ν = 3497*w*, 3131*w*, 2961*w*, 2915*s*, 2876*w*, 2849*m*, 1596*w*, 1476*w*, 1465*m*, 1447*w*, 1441*w*, 1398*m*, 1363*w*, 1317*s*, 1289*m*, 1267*w*, 1198*w*, 1154*vs*, 1110*s*, 1091*m*, 1074*s*, 1043*w*, 1031*w*, 1020*m*, 915*m*, 843*m*, 826*m*, 756*m*, 721*w*, 635*s*, 580*vs*, 554*m*, 521*m*, 505*w*, 468*m* cm^{-1}; ^1H NMR:δ = 7.72–7.68 (*m*, 2H, 2-H, 2′-H), 7.62–7.57 (*m*, 2H, 3-H, 3′-H), 7.46 (*t*, *J* = 5.7 Hz, 1H, NH), 4.30 (*td*, *J* = 5.2, 1.0 Hz, 1H, OH), 3.36 (*td*, *J* = 6.5, 5.1 Hz, 2H, 16-H), 2.74–2.67 (*m*, 2H, 7-H), 1.43–1.35 (*m*, 2H, 15-H), 1.36–1.26 (*m*, 2H, 8-H), 1.30 (*s*, 9H, 6-H, 6′-H, 6′-H), 1.27–1.11 (*m*, 12H, 9-H, 10-H, 11-H, 12-H, 13-H, 14-H) ppm; ^{13}C NMR:δ = 155.1 (C-4), 137.9 (C-1), 126.3 (C-2), 125.9 (C-3), 60.7 (C-16), 42.5 (C-7), 34.8 (C-5), 32.5 (C-15), 30.8 (C-6), 29.0 (C-8), 28.9 (C-10, C-13), 28.8 (C-11), 28.5 (C-12), 26.0 (C-9), 25.5 (C-14) ppm; MS: *m*/*z* = 457.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{20}H_{35}NSO_3$ (369.56): C 65.00, H 9.55, N 3.79; found: C 64.76, H 9.76, N 3.46.

4.2.116. 10-[(4-(tert-Butyl)phenyl)sulfonamido]decyl Sulfamate (**57b**)

Applying GPB: from **57a** (180 mg, 0.48 mmol): **57b** (140 mg, 64%); white solid; R_f = 0.87 ($CHCl_3$/EtOAc, 2:3); m.p. = 92–93 °C; UV–Vis: 228 nm (4.11); IR: ν = 3354*w*, 3294*w*, 3264*m*, 2920*m*, 2852*m*, 1475*w*, 1466*w*, 1433*w*, 1393*w*, 1366*s*, 1319*s*, 1292*w*, 1158*vs*, 1113*m*, 1089*m*, 1055*m*, 1025*w*, 937*m*, 895*w*, 835*m*, 824*m*, 756*m*, 721*w*, 670*m*, 628*m*, 578*s*, 552*s*, 532*m* cm^{-1}; ^1H NMR:δ = 7.72–7.67 (*m*, 2H, 2-H, 2′-H), 7.62–7.57 (*m*, 2H, 3-H, 3′-H), 7.45 (*t*, *J* = 5.8 Hz, 1H, NH), 7.37 (*s*, 2H, NH_2), 4.00 (*t*, *J* = 6.5 Hz, 2H, 16-H), 2.74–2.67 (*m*, 2H, 7-H), 1.61 (*p*, *J* = 6.6 Hz, 2H, 15-H), 1.37–1.26 (*m*, 2H, 8-H), 1.30 (*s*, 9H, 6-H, 6′-H, 6′-H), 1.27–1.11 (*m*, 12H, 9-H, 10-H, 11-H, 12-H, 13-H, 14-H) ppm; ^{13}C NMR:δ = 155.2 (C-4), 137.8 (C-1), 126.3 (C-2, 2′), 125.9 (C-3, 3′), 69.0 (C-16), 42.5 (C-7), 34.8 (C-5), 30.8 (C-6, 6′, 6′), 28.9 (C-8), 28.8 (C-11), 28.8 (C-15), 28.5 (C-10), 28.5 (C-13), 28.3 (C-12), 26.0 (C-14), 25.0 (C-9) ppm; MS: *m*/*z* = 457.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{20}H_{36}N_2S_2O_5$ (448.64): C 53.54, H 8.09, N 6.24; found: C 53.34, H 8.37, N 5.99.

4.2.117. 3-(tert-Butyl)-N-(2-hydroxyethyl)benzene Sulfonamide (**58a**)

Applying GPA: from 3-(tert-butyl)benzenesulfonyl chloride (500 mg, 2.15 mmol) and 2-amino-ethanol (197 mg, 3.22 mmol): **58a** (522 mg, 94%); oil; R_f = 0.20 (petrolether/EtOAc, 2:3); UV–Vis: 224 nm (4.04); IR: ν = 3500*w*, 3276*w*, 2962*w*, 2872*w*, 1481*m*, 1460*w*, 1416*m*, 1398*w*, 1366*w*, 1325*m*, 1307*s*, 1267*w*, 1205*vw*, 1156*vs*, 1125*m*, 1096*m*, 1057*m*, 998*vw*, 949*m*, 896*w*, 865*w*, 796*m*, 775*w*, 696*s*, 678*m*, 625*m*, 586*vs*, 545*m*, 534*m*, 524*m*, 481*w*, 456*w* cm^{-1}; ^1H NMR:δ = 7.82–7.80 (*m*, 1H, 2-H), 7.67 (*ddd*, *J* = 7.8, 2.0, 1.1 Hz, 1H, 6-H), 7.62 (*ddd*, *J* = 7.8, 1.8, 1.1 Hz, 1H, 4-H), 7.58 (*s*, 1, NH), 7.51 (*td*, *J* = 7.8, 0.5 Hz, 1H, 5-H), 4.67 (*t*, *J* = 5.5 Hz, 1H, OH), 3.37 (*q*, *J* = 6.1 Hz, 2H, 10-H), 2.79 (*t*, *J* = 6.4 Hz, 2H, 9-H), 1.31 (*s*, 9H, 8-H, 8′-H, 8′-H) ppm; ^{13}C NMR:δ = 151.9 (C-3), 140.4 (C-1), 129.3 (C-4), 128.9 (C-5), 123.7 (C-2), 122.9

(C-6), 59.9 (C-10), 45.1 (C-9), 34.7 (C-7), 30.9 (C-8) ppm; MS: *m*/*z* = 280.3 (100%, [M + Na]$^+$); anal. calcd. for $C_{12}H_{19}NSO_3$ (257.35): C 56.01, H 7.44, N 5.44; found: C 55.76, H 7.69, N 55.16.

4.2.118. 2-[(3-(tert-Butyl)phenyl)sulfonamido]ethyl Sulfamate (**58b**)

Applying GPB: from **58a** (200 mg, 0.78 mmol): **58b** (194 mg, 74%); oil; R_f = 0.68 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 224 nm (4.02); IR: ν = 3276*w*, 2963*w*, 2871*w*, 1560*w*, 1482*w*, 1460*w*, 1417*w*, 1365*s*, 1326*m*, 1309*m*, 1288*w*, 1267*w*, 1179*s*, 1156*vs*, 1125*m*, 1097*m*, 1021*m*, 998*w*, 925*s*, 867*w*, 797*w*, 775*m*, 753*m*, 695*m*, 678*m*, 626*m*, 585*s*, 549*s*, 493*w*, 434*w* cm^{-1}; ^{1}H NMR:δ = 7.94–7.87 (*m*, 1H, NH), 7.81–7.78 (*m*, 1H, 2-H), 7.73–7.67 (*m*, 1H, 6-H), 7.65–7.61 (*m*, 1H, 4-H), 7.56–7.47 (*m*, 3H, 5-H, NH_2), 4.00 (*t*, *J* = 5.7 Hz, 2H, 10-H), 3.08–2.99 (*m*, 2H, 9-H), 1.32 (s, 9H, 8-H, 8′-H, 8′-H) ppm; ^{13}C NMR:δ = 152.1 (3), 140.0 (1), 129.6 (4), 129.0 (5), 123.7 (2), 122.8 (6), 67.5 (10), 41.5 (9), 34.7 (7), 30.9 (8, 8′, 8′) ppm; MS: *m*/*z* = 359.6 (100%, [M + Na]$^+$); anal. calcd. for $C_{12}H_{20}N_2S_2O_5$ (336.42): C 42.84, H 5.99, N 8.33; found: C 42.63, H 6.24, N 8.01.

4.2.119. 3-(tert-Butyl)-N-(3-hydroxypropyl)benzene Sulfonamide (**59a**)

Applying GPA: from 3-(tert-butyl)benzenesulfonyl chloride (500 mg, 2.15 mmol) and 3-amino-propanol (242 mg, 3.22 mmol): **59a** (572 mg, 98%); oil; R_f = 0.23 (petrolether/EtOAc, 2:3); UV–Vis: 224 nm (4.15); IR: ν = 3501*w*, 3276*w*, 2961*m*, 2873*w*, 1481*m*, 1460*w*, 1416*w*, 1398*w*, 1366*w*, 1324*m*, 1307*s*, 1267*w*, 1178*w*, 1155*vs*, 1125*m*, 1084*m*, 1069*m*, 1008*w*, 998*w*, 960*w*, 876*w*, 798*m*, 774*m*, 696*s*, 678*m*, 625*m*, 586*vs*, 534*m*, 491*m*, 460*w* cm^{-1}; ^{1}H NMR:δ = 7.79 (*td*, *J* = 1.9, 0.5 Hz, 1H, 2-H), 7.67 (*ddd*, *J* = 7.8, 2.0, 1.1 Hz, 1H, 6-H), 7.61 (*ddd*, *J* = 7.7, 1.8, 1.2 Hz, 1H, 4-H), 7.52 (*td*, *J* = 7.5, 1.8 Hz, 2H, 5-H, NH), 4.39 (*t*, *J* = 5.1 Hz, 1H, OH), 3.36 (*td*, *J* = 6.2, 4.5 Hz, 2H, 11-H), 2.79 (*td*, *J* = 7.5, 3.6 Hz, 2H, 9-H), 1.55–1.46 (*m*, 2H, 10-H), 1.31 (*s*, 9H, 8-H, 8′-H, 8′-H) ppm; ^{13}C NMR:δ = 151.9 (C-3), 140.3 (C-1), 129.3 (C-4), 128.9 (C-5), 123.7 (C-2), 122.9 (C-6), 58.0 (C-11), 40.0 (C-9), 34.7 (C-7), 32.3 (C-10), 30.9 (C-8) ppm; MS: *m*/*z* = 294.1 (100%, [M + Na]$^+$); anal. calcd. for $C_{13}H_{21}NSO_3$ (271.38): C 57.54, H 7.80, N 5.16; found: C 57.16, H 8.03, N 4.91.

4.2.120. 3-[(3-(tert-Butyl)phenyl)sulfonamido]propyl Sulfamate (**59b**)

Applying GPB: from **59a** (500 mg, 2.15 mmol): **59b** (212 mg, 82%); oil; R_f = 0.68 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 224 nm (3.85); IR: ν = 3274*w*, 2964*w*, 1482*w*, 1417*w*, 1364*s*, 1325*m*, 1308*m*, 1177*s*, 1156*vs*, 1125*m*, 1096*m*, 939*m*, 798*m*, 772*m*, 696*m*, 678*m*, 625*m*, 585*s*, 550*s*, 536*m*, 496*m* cm^{-1}; ^{1}H NMR:δ = 7.79 (*t*, *J* = 1.9 Hz, 1H, 2-H), 7.72–7.59 (*m*, 3H, 4-H, 6-H, NH), 7.53 (*t*, *J* = 7.8 Hz, 1H, 5-H), 7.41 (*s*, 2H, NH_2), 4.02 (*t*, *J* = 6.3 Hz, 2H, 11-H), 2.81 (*t*, *J* = 7.2 Hz, 2H, 9-H), 1.75 (*p*, *J* = 6.7 Hz, 2H, 10-H), 1.31 (*s*, 9H, 8-H, 8′-H, 8′-H) ppm; ^{13}C NMR:δ = 152.1 (C-3), 140.1 (C-1), 129.5 (C-4), 129.0 (C-5), 123.7 (C-2), 122.8 (C-6), 66.5 (C-11), 39.2 (C-9), 34.7 (C-7), 30.9 (C-8), 28.7 (C-10) ppm; MS: *m*/*z* = 373.5 (100%, [M + Na]$^+$); anal. calcd. for $C_{13}H_{22}N_2S_2O_5$ (350.45): C 44.56, H 6.33, N 7.99; found: C 44.16, H 6.68, N 7.51.

4.2.121. 3-(tert-Butyl)-N-(4-hydroxybutyl)benzene Sulfonamide (**60a**)

Applying GPA: from 3-(tert-butyl)benzenesulfonyl chloride (500 mg, 2.15 mmol) and 4-amino-butanol (287 mg, 3.22 mmol): **60a** (589 mg, 96%); oil; R_f = 0.20 (petrolether/EtOAc, 2:3); UV–Vis: 224 nm (3.90); IR: ν = 3499*w*, 3280*w*, 2959*m*, 2870*w*, 1481*m*, 1460*w*, 1416*w*, 1398*w*, 1366*w*, 1324*m*, 1307*m*, 1267*w*, 1156*vs*, 1125*m*, 1088*m*, 1056*m*, 1034*w*, 998*w*, 872*w*, 797*m*, 773*w*, 696*s*, 678*m*, 626*m*, 586*vs*, 535*m*, 520*m*, 496*w*, 464*w* cm^{-1}; ^{1}H NMR:δ = 7.81–7.78 (*m*, 1H, 2-H), 7.66 (*ddd*, *J* = 7.8, 2.0, 1.1 Hz, 1H, 6-H), 7.61 (*ddd*, *J* = 7.8, 1.8, 1.1 Hz, 1H, 4-H), 7.56–7.48 (*m*, 2H, 5-H, NH), 4.35 (*s*, 1H, OH), 3.37–3.27 (*m*, 2H, 12-H), 2.77–2.70 (*m*, 2H, 9-H), 1.43–1.32 (*m*, 4H, 10-H, 11-H), 1.31 (*s*, 9H, 8-H, 8′-H, 8′-H) ppm; ^{13}C NMR:δ = 151.9 (C-3), 140.5 (C-1), 129.2 (C-4), 128.9 (C-5), 123.7 (C-2), 122.9 (C-6), 60.2 (C-12), 42.5 (C-9), 34.7 (C-7), 30.9 (C-8), 29.5 (C-11), 25.7 (C-10) ppm; MS: *m*/*z* = 308.4 (100%, [M + Na]$^+$); anal. calcd. for $C_{14}H_{23}NSO_3$ (285.40): C 58.92, H 8.12, N 4.91; found: C 58.68, H 8.33, N 4.78.

4.2.122. 4-[(3-(tert-Butyl)phenyl)sulfonamido]butyl Sulfamate (**60b**)

Applying GPB: from **60a** (183 mg, 0.64 mmol): **60b** (206 mg, 88%); oil; R_f = 0.69 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 225 nm (3.85); IR: ν = 3276*w*, 2962*w*, 2872*w*, 1481*w*, 1460*w*, 1438*w*, 1415*w*, 1366*m*, 1329*m*, 1308*m*, 1287*w*, 1266*w*, 1180*m*, 1157*vs*, 1125*m*, 1098*m*, 1089*m*, 1068*m*, 1010*w*, 996*w*, 923*m*, 865*w*, 799*m*, 778*m*, 741*w*, 698*s*, 677*s*, 631*m*, 588*vs*, 552*s*, 535*m*, 458*vw* cm^{-1}; ^1H NMR:δ = 7.78 (*t*, *J* = 1.9 Hz, 1H, 2-H), 7.74–7.58 (*m*, 3H, 4-H, 6-H, NH), 7.52 (*t*, *J* = 7.7 Hz, 1H, 5-H), 7.38 (*s*, 2H, NH_2), 3.95 (*t*, *J* = 6.3 Hz, 2H, 12-H), 2.76 (*q*, *J* = 6.6 Hz, 2H, 9-H), 1.66–1.56 (*m*, 2H, 11-H), 1.48–1.39 (*m*, 2H, 10-H), 1.32 (*s*, 9H, 8-H, 8′-H, 8′-H) ppm; ^{13}C NMR:δ = 152.0 (C-3), 140.4 (C-1), 129.3 (C-4), 129.0 (C-5), 123.7 (C-6), 122.8 (C-2), 34.7 (C-7), 30.9 (C-8), 25.6 (C-11), 25.4 (C-10) ppm; MS: *m*/*z* = 387.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{14}H_{24}N_2S_2O_5$ (364.48): C 46.14, H 6.64, N 7.69; found: C 45.85, H 6.99, N 7.41.

4.2.123. 3-(tert-Butyl)-N-(5-hydroxypentyl)benzene Sulfonamide (**61a**)

Applying GPA: from 3-(tert-butyl)benzenesulfonyl chloride (500 mg, 2.15 mmol) and 5-amino-pentanol (332 mg, 3.22 mmol): **61a** (610 mg, 95%); oil; R_f = 0.23 (petrolether/EtOAc, 2:3); UV–Vis: 224 nm (3.90); IR: ν = 3503*w*, 3278*w*, 2939*m*, 2868*w*, 1481*m*, 1459*w*, 1416*w*, 1398*w*, 1366*w*, 1325*m*, 1307*m*, 1267*w*, 1156*vs*, 1125*m*, 1087*m*, 1040*m*, 998*w*, 898*w*, 797*w*, 774*w*, 697*s*, 678*m*, 626*m*, 586*vs*, 534*m*, 501*w*, 478*w*, 461*w* cm^{-1}; ^1H NMR:δ = 7.79 (*td*, *J* = 1.9, 0.5 Hz, 1H, 2-H), 7.66 (*ddd*, *J* = 7.8, 2.0, 1.1 Hz, 1H, 6-H), 7.61 (*ddd*, *J* = 7.7, 1.8, 1.2 Hz, 1H, 4-H), 7.55–7.48 (*m*, 2H, 5-H, NH), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.35–3.27 (*m*, 2H, 13-H), 2.72 (*q*, *J* = 6.2 Hz, 2H, 9-H), 1.38–1.31 (*m*, 4H, 10-H, 12-H), 1.31 (*s*, 9H, 8-H, 8′-H, 8′-H), 1.26–1.17 (*m*, 2H, 11-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 151.9 (C-3), 140.5 (C-1), 129.2 (C-4), 128.9 (C-5), 123.7 (C-2), 122.8 (C-6), 60.5 (C-13), 42.6 (C-9), 34.7 (C-7), 32.0 (C-12), 30.9 (C-8), 28.8 (C-10), 22.6 (C-11) ppm; MS: *m*/*z* = 322.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{25}NSO_3$ (299.43): C 60.17, H 8.42, N 4.68; found: C 59.86, H 8.85, N 4.32.

4.2.124. 5-[(3-(tert-Butyl)phenyl)sulfonamido]pentyl Sulfamate (**61b**)

Applying GPB: from **61a** (200 mg, 0.67 mmol): **61b** (150 mg, 59%); oil; R_f = 0.69 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 224 nm (4.15); IR: ν = 3420*w*, 3368*w*, 3287*m*, 2920*m*, 2859*w*, 2869*w*, 1478*w*, 1410*m*, 1388*w*, 1332*m*, 1315*w*, 1299*w*, 1152*vs*, 1078*m*, 1062*m*, 1013*m*, 963*w*, 900*m*, 817*s*, 735*w*, 701*w*, 668*s*, 570*s*, 552*s*, 516*m*, 464*w*, 415*w* cm^{-1}; ^1H NMR:δ = 7.91–7.83 (*m*, 2H, 2-H, NH), 7.66 (*dd*, *J* = 8.1, 1.4 Hz, 1H, 6-H), 7.52 (*ddd*, *J* = 8.1, 7.2, 1.5 Hz, 1H, 4-H), 7.43–7.40 (*m*, 1H, 5-H), 7.39–7.37 (*m*, 2H, NH_2), 3.99 (*t*, *J* = 6.5 Hz, 2H, 13-H), 2.86–2.78 (*m*, 2H, 9-H), 1.65–1.55 (*m*, 2H, 12-H), 1.51 (*s*, 9H, 8-H, 8′-H, 8′-H), 1.49–1.45 (*m*, 2H, 10-H), 1.41–1.30 (*m*, 2H, 11-H) ppm; ^{13}C NMR:δ = 148.9 (C-3), 140.6 (C-1), 131.8 (C-4), 129.4 (C-5), 129.0 (C-2), 126.4 (C-6), 68.9 (C-13), 42.6 (C-9), 36.7 (C-7), 31.8 (C-8), 28.7 (C-12), 27.9 (C-10), 22.3 (C-11) ppm; MS: *m*/*z* = 401.6 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{26}N_2S_2O_5$ (378.50): C 47.60, H 6.92, N 7.40; found: C 47.38, H 7.17, N 7.36.

4.2.125. 3-(tert-Butyl)-N-(6-hydroxyhexyl)benzene Sulfonamide (**62a**)

Applying GPA: from 3-(tert-butyl)benzenesulfonyl chloride (500 mg, 2.15 mmol) and 6-amino-hexanol (378 mg, 3.22 mmol): **62a** (621 mg, 92%); oil; R_f = 0.25 (petrolether/EtOAc, 2:3); UV–Vis: 225 nm (3.94); IR: ν = 3505*vw*, 3278*w*, 2935*m*, 2864*w*, 1597*vw*, 1481*w*, 1460*w*, 1416*w*, 1397*w*, 1366*w*, 1325*m*, 1307*m*, 1287*w*, 1267*w*, 1156*vs*, 1125*m*, 1097*m*, 1086*m*, 1073*m*, 1055*m*, 899*w*, 869*w*, 797*m*, 773*w*, 697*s*, 678*m*, 626*m*, 587*vs*, 534*m*, 500*w*, 469*w*, 457*w*, 445*w* cm^{-1}; ^1H NMR:δ = 7.80–7.78 (*m*, 1H, 2-H), 7.66 (*ddd*, *J* = 7.8, 2.0, 1.1 Hz, 1H, 6-H), 7.60 (*ddd*, *J* = 7.7, 1.8, 1.2 Hz, 1H, 4-H), 7.55–7.48 (*m*, 2H, 5-H, NH), 4.29 (*t*, *J* = 5.1 Hz, 1H, OH), 3.36–3.30 (*m*, 2H, 14-H), 2.72 (*td*, *J* = 7.0, 5.7 Hz, 2H, 9-H), 1.37–1.32 (*m*, 4H, 10-H, 13-H), 1.31 (*s*, 9H, 8-H, 8′-H, 8′-H), 1.22–1.12 (*m*, 4H, 11-H, 12-H) ppm; ^{13}C NMR:δ = 151.9 (C-3), 140.5 (C-1), 129.2 (C-4), 128.9 (C-5), 123.7 (C-2), 122.8 (C-6), 60.6 (C-14), 42.5 (C-9), 34.7 (C-7), 32.3 (C-13), 30.9 (C-8), 28.9 (C-10), 25.9 (C-11), 25.0 (C-12) ppm; MS: *m*/*z* = 336.2 (100%, $[M + Na]^+$); anal. calcd. for $C_{16}H_{27}NSO_3$ (313.46): C 61.31, H 8.68, N 4.47; found: C 61.07, H 9.01, N 4.15.

4.2.126. 6-[(3-(tert-Butyl)phenyl)sulfonamido]hexyl Sulfamate (**62b**)

Applying GPB: from **62a** (200 mg, 0.64 mmol): **62b** (160 mg, 64%); oil; R_f = 0.73 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 225 nm (3.84); IR: ν = 3276*w*, 2960*w*, 2867*w*, 1561*vw*, 1481*w*, 1462*w*, 1417*w*, 1364*s*, 1325*m*, 1307*m*, 1267*w*, 1177*s*, 1156*vs*, 1125*m*, 1098*m*, 1088*m*, 923*s*, 798*m*, 773*w*, 725*w*, 697*m*, 678*m*, 626*m*, 587*vs*, 551*s*, 535*m*, 465*w* cm^{-1}; ^{1}H NMR:δ = 7.80–7.78 (*m*, 1H, 2-H), 7.67 (*ddd*, *J* = 7.8, 2.1, 1.2 Hz, 1H, 6-H), 7.62–7.58 (*m*, 1H, 4-H), 7.57–7.48 (*m*, 2H, 5-H, NH), 7.36 (*s*, 2H, NH_2), 3.96 (*t*, *J* = 6.5 Hz, 2H, 14-H), 2.73 (*q*, *J* = 6.6 Hz, 2H, 9-H), 1.60–1.50 (*m*, 2H, 13-H), 1.39–1.28 (*m*, 2H, 10-H), 1.31 (*s*, 9H, 8-H, 8′-H, 8′-H), 1.27–1.17 (*m*, 4H, 11-H, 12-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 151.9 (C-3), 140.5 (C-1), 129.2 (C-4), 128.9 (C-5), 123.7 (C-2), 122.8 (C-6), 68.9 (C-14), 42.4 (C-9), 34.7 (C-7), 30.9 (C-8), 28.8 (C-13), 28.2 (C-10), 25.5 (C-12), 24.6 (C-11) ppm; MS: *m*/*z* = 415.6 (100%, $[M + Na]^+$); anal. calcd. for $C_{16}H_{28}N_2S_2O_5$ (392.53): C 48.96, H 7.19, N 7.14; found: C 48.71, H 7.32, N 6.84.

4.2.127. 3-(tert-Butyl)-N-(7-hydroxyheptyl)benzene Sulfonamide (**63a**)

Applying GPA: from 3-(tert-butyl)benzenesulfonyl chloride (500 mg, 2.15 mmol) and 7-amino-heptanol (422 mg, 3.22 mmol): **63a** (635 mg, 90%); oil; R_f = 0.25 (petrolether/EtOAc, 2:3); UV–Vis: 225 nm (4.00); IR: ν = 3503*vw*, 3279*w*, 2932*m*, 2860*w*, 1481*w*, 1461*w*, 1416*w*, 1397*w*, 1366*w*, 1325*m*, 1308*m*, 1267*w*, 1156*vs*, 1125*m*, 1098*m*, 1087*m*, 1056*m*, 999*w*, 897*w*, 797*w*, 773*w*, 724*w*, 697*s*, 678*m*, 626*m*, 587*vs*, 534*m*, 500*w*, 461*w*, 445*w* cm^{-1}; ^{1}H NMR:δ = 7.80–7.77 (*m*, 1H, 2-H), 7.66 (*ddd*, *J* = 7.8, 2.0, 1.1 Hz, 1H, 6-H), 7.60 (*ddd*, *J* = 7.7, 1.8, 1.1 Hz, 1H, 4-H), 7.55–7.47 (*m*, 2H, 5-H, NH), 4.29 (*t*, *J* = 5.1 Hz, 1H, OH), 3.40–3.31 (*m*, 2H, 15-H), 2.76–2.68 (*m*, 2H, 9-H), 1.40–1.32 (*m*, 4H, 10-H, 14-H), 1.31 (*s*, 9H, 8-H, 8′-H, 8′-H), 1.24–1.09 (*m*, 6H, 11-H, 12-H, 13-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 151.8 (C-3), 140.5 (C-1), 129.2 (C-4), 128.9 (C-5), 123.7 (C-2), 122.8 (C-6), 60.7 (C-15), 42.5 (C-9), 34.7 (C-7), 32.4 (C-14), 30.9 (C-8), 28.8 (C-10), 28.4 (C-12), 26.0 (C-11), 25.3 (C-13) ppm; MS: *m*/*z* = 350.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{17}H_{29}NSO_3$ (327.48): C 62.35, H 8.93, N 4.28; found: C 62.03, H 9.21, N 3.96.

4.2.128. 7-[(3-(tert-Butyl)phenyl)sulfonamido]heptyl Sulfamate (**63b**)

Applying GPB: from **63a** (200 mg, 0.61 mmol): **63b** (134 mg, 54%); oil; R_f = 0.78 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 225 nm (4.10); IR: ν = 3277*w*, 2937*w*, 2862*w*, 1562*w*, 1481*w*, 1417*w*, 1364*s*, 1325*m*, 1307*m*, 1267*w*, 1177*s*, 1156*vs*, 1125*m*, 1098*m*, 1088*m*, 923*s*, 799*m*, 773*m*, 724*w*, 697*m*, 678*m*, 626*m*, 587*vs*, 551*s*, 495*w*, 460*w*, 443*vw* cm^{-1}; ^{1}H NMR:δ = 7.79 (*td*, *J* = 1.9, 0.5 Hz, 1H, 2-H), 7.67 (*ddd*, *J* = 7.8, 2.0, 1.1 Hz, 1H, 6-H), 7.60 (*ddd*, *J* = 7.7, 1.8, 1.2 Hz, 1H, 4-H), 7.56–7.49 (*m*, 2H, 5-H, NH), 7.37 (*s*, 2H, NH_2), 3.98 (*t*, *J* = 6.5 Hz, 2H, 15-H), 2.73 (*td*, *J* = 6.9, 5.8 Hz, 2H, 9-H), 1.61–1.53 (*m*, 2H, 14-H), 1.31 (*s*, 9H, 8-H, 8′-H, 8′-H), 1.35–1.28 (*m*, 2H, 10-H), 1.27–1.14 (*m*, 6H, 11-H, 12-H, 13-H) ppm; ^{13}C NMR:δ = 151.9 (C-3), 140.5 (C-1), 129.2 (C-4), 128.9 (C-5), 123.7 (C-2), 122.8 (C-6), 68.9 (C-15), 42.5 (C-9), 34.7 (C-7), 30.9 (C-8), 28.8 (C-14), 28.2 (C-11), 28.0 (C-10), 25.8 (C-13), 24.9 (C-12) ppm; MS: *m*/*z* = 429.7 (100%, $[M + Na]^+$); anal. calcd. for $C_{17}H_{30}N_2S_2O_5$ (406.56): C 50.22, H 7.44, N 6.89; found: C 49.96, H 7.79, N 6.65.

4.2.129. 3-(tert-Butyl)-N-(8-hydroxyoctyl)benzene Sulfonamide (**64a**)

Applying GPA: from 3-(tert-butyl)benzenesulfonyl chloride (500 mg, 2.15 mmol) and 8-amino-octanol (468 mg, 3.22 mmol): **64a** (703 mg, 96%); oil; R_f = 0.30 (petrolether/EtOAc, 2:3); UV–Vis: 225 nm (3.92); IR: ν = 3503*vw*, 3279*w*, 2930*m*, 2857*m*, 1481*w*, 1461*w*, 1416*w*, 1397*w*, 1366*w*, 1325*m*, 1308*m*, 1267*w*, 1157*vs*, 1125*m*, 1098*m*, 1086*m*, 998*vw*, 898*w*, 797*m*, 774*w*, 722*w*, 697*s*, 678*m*, 626*m*, 587*vs*, 534*m*, 515*m* cm^{-1}; ^{1}H NMR:δ = 7.79 (*td*, *J* = 1.9, 0.5 Hz, 1H, 2-H), 7.66 (*ddd*, *J* = 7.8, 2.0, 1.1 Hz, 1H, 6-H), 7.60 (*ddd*, *J* = 7.7, 1.8, 1.1 Hz, 1H, 4-H), 7.54–7.48 (*m*, 2H, 5-H, NH), 4.29 (*t*, *J* = 5.1 Hz, 1H, OH), 3.38–3.33 (*m*, 2H, 16-H), 2.72 (*td*, *J* = 6.9, 2.7 Hz, 2H, 9-H), 1.41–1.32 (*m*, 4H, 10-H, 15-H), 1.31 (*s*, 9H, 8-H, 8′-H, 8′-H), 1.25–1.09 (*m*, 8H, 11-H, 12-H, 13-H, 14-H) ppm; ^{13}C NMR:δ = 151.9 (C-3), 140.6 (C-1), 129.2 (C-4), 128.9 (C-5), 123.7 (C-2), 122.9 (C-6), 60.7 (C-16), 42.5 (C-9), 34.7 (C-7), 32.5 (C-15), 30.9 (C-8), 28.8 (C-10), 28.8 (C-12), 28.6 (C-13), 25.9 (C-11), 25.4 (C-14) ppm; MS: *m*/*z* = 364.4

(100%, $[M + Na]^+$); anal. calcd. for $C_{18}H_{31}NSO_3$ (341.51): C 63.31, H 9.15, N 4.10; found: C 63.07, H 9.42, N 3.91.

4.2.130. 8-[(3-(tert-Butyl)phenyl)sulfonamido]octyl Sulfamate (**64b**)

Applying GPB: from **64a** (200 mg, 0.59 mmol): **64b** (221 mg, 90%); white solid; R_f = 0.83 ($CHCl_3$/EtOAc, 2:3); m.p. = 57–59 °C; UV–Vis: 225 nm (3.99); IR: ν = 3364*w*, 3279*m*, 2967*w*, 2928*m*, 2858*w*, 1558*w*, 1481*w*, 1475*w*, 1466*w*, 1428*w*, 1399*w*, 1378*s*, 1322*m*, 1310*s*, 1289*m*, 1270*w*, 1178*s*, 1170*s*, 1158*vs*, 1127*m*, 1099*w*, 1070*w*, 1057*m*, 1037*m*, 999*w*, 959*vs*, 909*s*, 896*s*, 820*s*, 793*m*, 775*m*, 761*w*, 725*m*, 706*m*, 692*vs*, 667*m*, 628*m*, 621*m*, 588*vs*, 554*s*, 535*s*, 510*s*, 485*m* cm^{-1}; ^1H NMR:δ = 7.79 (*td*, *J* = 1.9, 0.5 Hz, 1H, 2-H), 7.67 (*ddd*, *J* = 7.8, 2.0, 1.1 Hz, 1H, 6-H), 7.60 (*ddd*, *J* = 7.7, 1.8, 1.2 Hz, 1H, 4-H), 7.56–7.48 (*m*, 2H, 5-H, NH), 7.37 (*s*, 2H, NH_2), 3.99 (*t*, *J* = 6.5 Hz, 2H, 16-H), 2.72 (*td*, *J* = 7.0, 5.8 Hz, 2H, 9-H), 1.63–1.54 (*m*, 2H, 15-H), 1.36–1.21 (*m*, 4H, 10-H, 14-H), 1.31 (*s*, 9H, 8-H, 8′-H, 8′-H), 1.22–1.11 (*m*, 6H, 11-H, 12-H, 13-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 151.9 (C-3), 140.5 (C-1), 129.2 (C-4), 128.9 (C-5), 123.7 (C-2), 122.8 (C-6), 68.9 (C-16), 42.5 (C-9), 34.7 (C-7), 30.9 (C-8), 28.8 (C-15), 28.4 (C-13), 28.3 (C-10), 28.3 (C-12), 25.9 (C-11), 24.9 (C-14) ppm; MS: *m*/*z* = 443.8 (100%, $[M + Na]^+$); anal. calcd. for $C_{18}H_{32}N_2S_2O_5$ (420.58): C 51.40, H 7.67, N 6.66; found: C 51.17, H 7.96, N 6.45.

4.2.131. 2-(tert-Butyl)-N-(5-hydroxypentyl)benzene Sulfonamide (**65a**)

Applying GPA: from 2-(tert-butyl)benzenesulfonyl chloride (330 mg, 1.42 mmol) and 5-amino-pentanol (219 mg, 2.13 mmol): **65a** (352 mg, 83%); oil; R_f = 0.23 (petrolether/EtOAc, 2:3); UV–Vis: 221 nm (3.83); IR: ν = 3493*vw*, 3294*w*, 3060*vw*, 2938*m*, 2867*w*, 1463*w*, 1432*w*, 1400*w*, 1366*w*, 1314*s*, 1260*w*, 1243*w*, 1199*vw*, 1175*w*, 1152*vs*, 1128*m*, 1111*m*, 1082*m*, 1057*m*, 1044*m*, 934*vw*, 874*vw*, 841*vw*, 760*m*, 734*m*, 662*m*, 592*vs*, 546*s*, 475*w*, 434*vw* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.89 (*dd*, *J* = 8.0, 1.5 Hz, 1H, 3-H), 7.83 (*t*, *J* = 5.6 Hz, 1H, NH), 7.66 (*dd*, *J* = 8.2, 1.4 Hz, 1H, 6-H), 7.51 (*td*, *J* = 7.6, 1.5 Hz, 1H, 4-H), 7.40 (*td*, *J* = 7.6, 1.4 Hz, 1H, 5-H), 4.32 (*t*, *J* = 5.1 Hz, 1H, OH), 3.36 (*td*, *J* = 6.4, 5.0 Hz, 2H, 13-H), 2.81 (*q*, *J* = 6.6 Hz, 2H, 9-H), 1.53–1.50 (*m*, 9H, 8-H, 8′-H, 8′-H), 1.48–1.25 (*m*, 6H, 10-H, 11-H, 12-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 148.9 (C-2), 140.7 (C-1), 131.7 (C-4), 129.4 (C-3), 128.9 (C-6), 126.4 (C-5), 60.5 (C-13), 42.8 (C-9), 36.7 (C-7), 32.0 (C-12), 31.8 (C-8), 29.1 (C-10), 22.6 (C-11) ppm; MS: *m*/*z* = 322.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{25}NSO_3$ (299.43): C 60.17, H 8.42, N 4.68; found: C 59.82, H 8.69, N 4.32.

4.2.132. 5-[(2-(tert-Butyl)phenyl)sulfonamido]pentyl Sulfamate (**65b**)

Applying GPB: from **65a** (120 mg, 0.4 mmol): **65b** (88 mg, 58%); oil; R_f = 0.66 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 221 nm (3.98); IR: ν = 3283*w*, 2941*w*, 2867*w*, 1566*w*, 1464*w*, 1432*w*, 1362*s*, 1313*s*, 1260*w*, 1243*w*, 1176*s*, 1152*vs*, 1128*m*, 1111*m*, 1083*w*, 1059*w*, 923*s*, 839*w*, 822*w*, 761*s*, 733*m*, 662*m*, 593*vs*, 550*vs* cm^{-1}; ^1H NMR:δ = 7.80–7.77 (*m*, 1H, 6-H), 7.67 (*ddd*, *J* = 7.8, 2.0, 1.1 Hz, 1H, 3-H), 7.60 (*ddd*, *J* = 7.7, 1.8, 1.2 Hz, 1H, 5-H), 7.56 (*t*, *J* = 5.9 Hz, 1H, NH), 7.52 (*t*, *J* = 7.8 Hz, 1H, 4-H), 7.37 (*s*, 2H, NH_2), 3.95 (*t*, *J* = 6.5 Hz, 2H, 13-H), 2.73 (*q*, *J* = 6.6 Hz, 2H, 9-H), 1.58–1.50 (*m*, 2H, 12-H), 1.42–1.33 (*m*, 2H, 10-H), 1.31 (*s*, 9H, 8-H, 8′-H, 8′-H), 1.34–1.22 (*m*, 2H, 11-H) ppm; ^{13}C NMR:δ = 151.9 (C-2), 140.4 (C-1), 129.3 (C-4), 128.9 (C-3), 123.7 (C-6), 122.8 (C-5), 68.8 (C-13), 42.3 (C-9), 34.7 (C-7), 30.9 (C-8), 28.4 (C-12), 27.8 (C-10), 22.2 (C-11) ppm; MS: *m*/*z* = 401.6 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{26}N_2S_2O_5$ (378.50): C 47.60, H 6.92, N 7.40; found: C 47.26, H 7.17, N 7.13.

4.2.133. 2-(tert-Butyl)-N-(6-hydroxyhexyl)benzene Sulfonamide (**66a**)

Applying GPA: from 2-(tert-butyl)benzenesulfonyl chloride (330 mg, 1.42 mmol) and 6-amino-hexanol (249 mg, 2.13 mmol): **66a** (419 mg, 94%); oil; R_f = 0.30 (petrolether/EtOAc, 2:3); UV–Vis: 221 nm (3.78); IR: ν = 3497*vw*, 3295*w*, 2934*m*, 2863*w*, 1463*w*, 1432*w*, 1400*w*, 1366*w*, 1314*s*, 1260*w*, 1242*w*, 1199*vw*, 1175*w*, 1152*vs*, 1128*m*, 1111*m*, 1056*m*, 842*vw*, 760*m*, 734*m*, 662*m*, 592*vs*, 546*m*, 476*w* cm^{-1}; ^1H NMR:δ = 7.89 (*dd*, *J* = 8.0, 1.5 Hz, 1H, 3-H), 7.82 (*t*, *J* = 5.8 Hz, 1H, NH), 7.66 (*dd*, *J* = 8.1, 1.3 Hz, 1H, 6-H), 7.51 (*ddd*, *J* = 8.0, 7.2, 1.5 Hz, 1H,

4-H), 7.43–7.37 (*m*, 1H, 5-H), 4.31 (*t*, *J* = 5.2 Hz, 1H, OH), 3.36 (*td*, *J* = 6.5, 5.2 Hz, 2H, 14-H), 2.81 (*td*, *J* = 7.1, 5.8 Hz, 2H, 9-H), 1.51 (*s*, 9H, 8-H, 8′-H, 8′-H), 1.48–1.33 (*m*, 4H, 10-H, 13-H), 1.31–1.19 (*m*, 4H, 11-H, 12-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 148.8 (C-2), 140.7 (C-1), 131.7 (C-4), 129.4 (C-3), 128.9 (C-6), 126.4 (C-5), 60.6 (C-14), 42.8 (C-9), 36.7 (C-7), 32.4 (C-13), 31.8 (C-8), 29.3 (C-10), 26.0 (C-12), 25.1 (C-11) ppm; MS: *m*/*z* = 336.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{16}H_{27}NSO_3$ (313.46): C 61.31, H 8.68, N 4.47; found: C 61.04, H 8.94, N 4.11.

4.2.134. 6-[(2-(tert-Butyl)phenyl)sulfonamido]hexyl Sulfamate (**66b**)

Applying GPB: from **66a** (120 mg, 0.38 mmol): **66b** (141 mg, 94%); oil; R_f = 0.70 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 221 nm (4.00); IR: ν = 3283*w*, 2941*w*, 2867*w*, 1566*w*, 1464*w*, 1432*w*, 1362*s*, 1313*s*, 1260*w*, 1243*w*, 1176*s*, 1152*vs*, 1128*m*, 1111*m*, 1083*w*, 1059*w*, 923*s*, 839*w*, 822*w*, 761*s*, 733*m*, 662*m*, 593*vs*, 550*vs* cm $^{-1}$; ^{1}H NMR:δ = 7.89 (*dd*, *J* = 8.0, 1.5 Hz, 1H, 3-H), 7.84 (*t*, *J* = 5.8 Hz, 1H, NH), 7.66 (*dd*, *J* = 8.1, 1.4 Hz, 1H, 6-H), 7.51 (*ddd*, *J* = 7.9, 7.2, 1.5 Hz, 1H, 4-H), 7.44–7.39 (*m*, 1H, 5-H), 7.38 (*s*, 2H, NH_2), 3.99 (*t*, *J* = 6.5 Hz, 2H, 14-H), 2.81 (*td*, *J* = 7.0, 5.8 Hz, 2H, 9-H), 1.64–1.54 (*m*, 2H, 13-H), 1.51 (*s*, 9H, 8-H, 8′-H, 8′-H), 1.49–1.41 (*m*, 2H, 10-H), 1.33–1.25 (*m*, 4H, 11-H, 12-H) ppm; ^{13}C NMR:δ = 148.9 (C-2), 140.7 (C-1), 131.8 (C-4), 129.4 (C-3), 129.0 (C-6), 126.4 (C-5), 68.9 (C-14), 42.7 (C-9), 36.7 (C-7), 31.8 (C-8), 29.1 (C-13), 28.2 (C-10), 25.6 (C-12), 24.6 (C-11) ppm; MS: *m*/*z* = 415.6 (100%, $[M + Na]^+$); anal. calcd. for $C_{16}H_{28}N_2S_2O_5$ (392.53): C 48.96, H 7.19, N 7.14; found: C 48.75, H 7.43, N 6.87.

4.2.135. 2-(tert-Butyl)-N-(7-hydroxyheptyl)benzene Sulfonamide (**67a**)

Applying GPA: from 2-(tert-butyl)benzenesulfonyl chloride (330 mg, 1.42 mmol) and 7-amino-heptanol (279 mg, 2.13 mmol): **67a** (411 mg, 89%); oil; R_f = 0.34 (petrolether/EtOAc, 2:3); UV–Vis: 221 nm (4.03); IR: ν = 3496*vw*, 3295*w*, 2931*m*, 2859*w*, 1463*w*, 1432*w*, 1400*w*, 1366*w*, 1315*s*, 1260*w*, 1242*w*, 1199*vw*, 1175*vw*, 1152*s*, 1128*m*, 1111*m*, 1057*m*, 866*vw*, 840*vw*, 760*m*, 734*m*, 662*m*, 593*vs*, 547*m*, 477*w*, 428*vw* cm^{-1}; ^{1}H NMR:δ = 7.90 (*dt*, *J* = 8.0, 1.3 Hz, 1H, 3-H), 7.82 (*s*, 1H, NH), 7.66 (*dt*, *J* = 8.1, 1.3 Hz, 1H, 6-H), 7.54–7.47 (*m*, 1H, 4-H), 7.44–7.37 (*m*, 1H, 5-H), 4.23 (*s*, 1H, OH), 3.40–3.33 (*m*, 2H, 15-H), 2.85–2.76 (*m*, 2H, 9-H), 1.56–1.48 (*m*, 9H, 8-H, 8′-H, 8′-H), 1.47–1.33 (*m*, 4H, 10-H, 14-H), 1.30–1.16 (*m*, 6H, 11-H, 12-H, 13-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 148.8 (C-2), 140.7 (C-1), 131.7 (C-4), 129.5 (C-3), 128.9 (C-6), 126.4 (C-5), 60.7 (C-15), 42.7 (C-9), 36.7 (C-7), 32.4 (C-14), 31.8 (C-8), 29.2 (C-10), 28.5 (C-12), 26.1 (C-11), 25.4 (C-13) ppm; MS: *m*/*z* = 350.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{17}H_{29}NSO_3$ (327.48): C 62.35, H 8.93, N 4.28; found: C 62.02, H 9.25, N 3.96.

4.2.136. 7-[(2-(tert-Butyl)phenyl)sulfonamido]heptyl Sulfamate (**67b**)

Applying GPB: from **67a** (150 mg, 0.46 mmol): **67b** (165 mg, 89%); oil; R_f = 0.76 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 221 nm (3.87); IR: ν = 3283*w*, 2936*w*, 2862*w*, 1566*w*, 1464*w*, 1432*w*, 1362*s*, 1314*s*, 1177*s*, 1152*vs*, 1128*m*, 1111*m*, 1083*w*, 1058*w*, 1000*w*, 924*s*, 838*w*, 814*w*, 762*s*, 734*m*, 662*m*, 593*vs*, 550*vs*, 500*m* cm^{-1}; ^{1}H NMR:δ = 7.89 (*dd*, *J* = 8.0, 1.5 Hz, 1H, 3-H), 7.83 (*t*, *J* = 5.8 Hz, 1H, NH), 7.66 (*dd*, *J* = 8.1, 1.3 Hz, 1H, 6-H), 7.51 (*td*, *J* = 8.1, 7.7, 1.5 Hz, 1H, 4-H), 7.44–7.38 (*m*, 1H, 5-H), 7.37 (*s*, 2H, NH_2), 3.99 (*t*, *J* = 6.5 Hz, 2H, 15-H), 2.81 (*td*, *J* = 7.0, 5.8 Hz, 2H, 9-H), 1.64–1.55 (*m*, 2H, 14-H), 1.51 (*s*, 9H, 8-H, 8′-H, 8′-H), 1.48–1.39 (*m*, 2H, 10-H), 1.35–1.21 (*m*, 6H, 11-H, 12-H, 13-H) ppm; ^{13}C NMR:δ = 148.8 (C-2), 140.7 (C-1), 131.8 (C-4), 129.5 (C-3), 129.0 (C-6), 126.4 (C-5), 69.0 (C-15), 42.7 (C-9), 36.7 (C-7), 31.8 (C-8), 29.1 (C-14), 28.2 (C-11), 28.0 (C-10), 25.9 (C-13), 25.0 (C-12) ppm; MS: *m*/*z* = 429.6 (100%, $[M + Na]^+$); anal. calcd. for $C_{17}H_{30}N_2S_2O_5$ (406.56): C 50.22, H 7.44, N 6.89; found: C 49.94, H 7.76, N 6.53.

4.2.137. 4-Cyclohexyl-N-(2-hydroxyethyl)benzene Sulfonamide (**68a**) [919974-61-1]

Applying GPA: from 4-cyclohexylbenzenesulfonyl chloride (500 mg, 1.93 mmol) and 2-amino-ethanol (177 mg, 2.90 mmol): **68a** (453 mg, 83%); oil; R_f = 0.56 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 229 nm (4.19); IR: ν = 3514*vw*, 3264*w*, 3146*w*, 2920*m*, 2848*m*, 1597*w*, 1496*vw*, 1483*vw*, 1461*w*, 1451*m*, 1427*w*, 1409*w*, 1348*w*, 1319*s*, 1278*w*, 1260*w*, 1216*vw*, 1188*w*, 1156*s*,

1134*w*, 1093*s*, 1059*m*, 1036*m*, 1018*w*, 996*w*, 953*m*, 890*w*, 864*vw*, 842*w*, 826*s*, 802*w*, 782*vw*, 731*w*, 701*s*, 631*w*, 597*s*, 574*vs*, 527*m*, 504*w*, 491*w*, 479*w*, 458*m* cm^{-1}; ^1H NMR:δ = 7.72–7.68 (*m*, 2H, 2-H, 2′-H), 7.51–7.46 (*m*, 1H, NH), 7.45–7.40 (*m*, 2H, 3-H, 3′-H), 4.66 (*t*, *J* = 5.6 Hz, 1H, OH), 3.37 (*q*, *J* = 6.3 Hz, 2H, 10-H), 2.77 (*q*, *J* = 5.9 Hz, 2H, 9-H), 2.64–2.55 (*m*, 1H, 5-H), 1.84–1.74 (*m*, 4H, 6-H_a, 6′-H_a, 7-H_a, 7′-H_a), 1.74–1.66 (*m*, 1H, 8-H_a), 1.50–1.30 (*m*, 4H, 6-H_b, 6′-H_b, 7-H_b, 7′-H_b), 1.30–1.17 (*m*, 1H, 8-H_b) ppm; ^{13}C NMR:δ = 152.1 (C-4), 138.0 (C-1), 127.4 (C-2), 126.6 (C-3), 59.9 (C-10), 45.1 (C-9), 43.6 (C-5), 33.5 (C-6), 26.2 (C-7), 25.4 (C-8) ppm; MS: *m*/*z* = 305.9 (90%, $[M + Na]^+$); anal. calcd. for $C_{14}H_{21}NSO_3$ (283.39): C 59.34, H 7.47, N 4.94; found: C 59.11, H 7.85, N 4.65.

4.2.138. 2-[(4-Cyclohexylphenyl)sulfonamido]ethyl Sulfamate (**68b**)

Applying GPB: from **68a** (200 mg, 0.71 mmol): **68b** (232 mg, 91%); white solid; R_f = 0.77 ($CHCl_3$/EtOAc, 2:3); m.p. = 109–110 °C; UV–Vis: 229 nm (4.28); IR: ν = 3358*m*, 3256*w*, 2922*m*, 2849*w*, 1599*w*, 1549*w*, 1462*w*, 1452*w*, 1412*m*, 1363*s*, 1314*s*, 1279*w*, 1252*vw*, 1181*s*, 1154*vs*, 1095*m*, 1078*w*, 1010*m*, 998*m*, 957*s*, 903*m*, 828*m*, 781*m*, 748*s*, 723*m*, 702*s*, 634*w*, 610*s*, 563*s*, 549*vs*, 525*m*, 501*m*, 465*m*, 449*m* cm^{-1}; ^1H NMR:δ = 7.82 (*t*, *J* = 6.0 Hz, 1H, NH), 7.73–7.69 (*m*, 2H, 2-H, 2′-H), 7.50 (*s*, 2H, NH_2), 7.47–7.42 (*m*, 2H, 3-H, 3′-H), 4.00 (*t*, *J* = 5.7 Hz, 2H, 10-H), 3.03 (*q*, *J* = 5.8 Hz, 2H, 9-H), 2.64–2.55 (*m*, 1H, 5-H), 1.84–1.76 (*m*, 4H, 6-H_a, 6′-H_a, 7-H_a, 7′-H_a), 1.75–1.67 (*m*, 1H, 8-H_a), 1.49–1.30 (*m*, 5H, 6-H_b, 6′-H_b, 7-H_b, 7′-H_b), 1.30–1.18 (*m*, 1H, 8-H_b) ppm; ^{13}C NMR:δ = 152.4 (C-1), 137.7 (C-4), 127.5 (C-2), 126.6 (C-3), 67.6 (C-10), 43.6 (C-5), 41.5 (C-9), 33.5 (C-6), 26.2 (C-7), 25.4 (C-8) ppm; MS: *m*/*z* = 385.5 (100%, $[M + Na]^+$); anal. calcd. for $C_{14}H_{22}N_2S_2O_5$ (362.46): C 46.39, H 6.12, N 7.73; found: C 46.16, H 6.38, N 7.41.

4.2.139. 4-Cyclohexyl-N-(3-hydroxypropyl)benzene Sulfonamide (**69a**) [919974-63-3]

Applying GPA: from 4-cyclohexylbenzenesulfonyl chloride (500 mg, 1.93 mmol) and 3-amino-propanol (218 mg, 2.90 mmol): **69a** (470 mg, 82%); white solid; R_f = 0.53 ($CHCl_3$/EtOAc, 2:3); m.p. = 78–79 °C; UV–Vis: 229 nm (4.13); IR: ν = 3471*w*, 3167*w*, 2929*m*, 2851*m*, 1598*w*, 1494*vw*, 1472*w*, 1463*w*, 1448*w*, 1424*w*, 1407*w*, 1375*vw*, 1351*vw*, 1310*s*, 1283*w*, 1230*vw*, 1185*w*, 1157*vs*, 1096*m*, 1075*s*, 1005*m*, 964*m*, 943*w*, 876*m*, 853*w*, 838*w*, 827*m*, 803*w*, 781*w*, 740*m*, 698*m*, 595*vs*, 574*s*, 507*m*, 481*w*, 455*w* cm^{-1}; ^1H NMR:δ = 7.72–7.66 (*m*, 2H, 2-H, 2′-H), 7.47–7.37 (*m*, 3H, NH, 3-H, 3′-H), 4.39 (*t*, *J* = 5.1 Hz, 1H, OH), 3.39–3.33 (*m*, 2H, 11-H), 2.80–2.72 (*m*, 2H, 9-H), 2.59 (*td*, *J* = 9.4, 4.7 Hz, 1H, 5-H), 1.83–1.75 (*m*, 4H, 6-H_a, 6′-H_a, 7-H_a, 7′-H_a), 1.74–1.67 (*m*, 1H, 8-H_a), 1.52 (*dt*, *J* = 13.3, 6.3 Hz, 2H, 10-H), 1.48–1.31 (*m*, 4H, 6-H_b, 6′-H_b, 7-H_b, 7′-H_b), 1.29–1.18 (*m*, 1H, 8-H_b) ppm; ^{13}C NMR:δ = 152.1 (C-4), 137.9 (C-1), 127.4 (C-2), 126.6 (C-3), 58.1 (C-11), 43.6 (C-5), 40.0 (C-9), 33.5 (C-6), 32.4 (C-10), 26.2 (C-7), 25.4 (C-8) ppm; MS: *m*/*z* = 319.9 (90%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{23}NSO_3$ (297.41): C 60.58, H 7.80, N 4.71; found: C 60.44, H 8.02, N 4.54.

4.2.140. 3-[(4-Cyclohexylphenyl)sulfonamido]propyl Sulfamate (**69b**)

Applying GPB: from **69a** (200 mg, 0.67 mmol): **69b** (210 mg, 83%); white solid; R_f = 0.74 ($CHCl_3$/EtOAc, 2:3); m.p. = 113–114 °C; UV–Vis: 229 nm (4.27); IR: ν = 3351*w*, 3283*m*, 2929*m*, 2851*w*, 1598*w*, 1567*w*, 1474*w*, 1438*w*, 1403*w*, 1371*vs*, 1323*m*, 1307*s*, 1281*w*, 1256*w*, 1177*vs*, 1152*vs*, 1093*m*, 1070*m*, 1039*m*, 999*w*, 944*s*, 921*m*, 888*m*, 836*s*, 824*s*, 778*w*, 756*m*, 729*m*, 706*s*, 654*m*, 630*m*, 599*s*, 593*s*, 568*vs*, 548*s*, 527*m*, 515*m*, 506*m*, 486*w*, 428*w* cm^{-1}; ^1H NMR:δ = 7.74–7.67 (*m*, 2H, 2-H, 2′-H), 7.57–7.36 (*m*, 5H, 3-H, 3′-H, NH_2, NH), 4.02 (*t*, *J* = 6.3 Hz, 2H, 11-H), 2.80 (*t*, *J* = 7.1 Hz, 2H, 9-H), 2.64–2.55 (*m*, 1H, 5-H), 1.84–1.67 (*m*, 7H, 6-H_a, 6′-H_a, 7-H_a, 7′-H_a, 8-H_a, 10-H), 1.49–1.31 (*m*, 4H, 6-H_b, 6′-H_b, 7-H_b, 7′-H_b), 1.29–1.18 (*m*, 1H, 8-H_b) ppm; ^{13}C NMR:δ = 152.2 (C-1), 137.7 (C-4), 127.5 (C-2), 126.6 (C-3), 66.5 (C-11), 43.5 (C-5), 39.2 (C-9), 33.5 (C-6), 28.8 (C-10), 26.2 (C-7), 25.4 (C-8) ppm; MS: *m*/*z* = 399.5 (100%, $[M + Na]^+$); anal. calcd. for $C_{15}H_{24}N_2S_2O_5$ (376.49): C 47.85, H 6.43, N 7.44; found: C 47.57, H 6.61, N 7.21.

4.2.141. 4-Cyclohexyl-N-(4-hydroxybutyl)benzene Sulfonamide (**70a**) [1976415-75-4]

Applying GPA: from 4-cyclohexylbenzenesulfonyl chloride (500 mg, 1.93 mmol) and 4-amino-butanol (258 mg, 2.90 mmol): **70a** (487 mg, 81%); white solid; R_f = 0.50 ($CHCl_3$/EtOAc, 2:3); m.p. = 58–60 °C; UV–Vis: 229 nm (4.17); IR: ν = 3477*m*, 3101*w*, 2950*w*, 2922*m*, 2849*m*, 1446*m*, 1429*w*, 1396*w*, 1312*s*, 1257*w*, 1150*vs*, 1097*m*, 1081*m*, 1030*s*, 994*m*, 941*m*, 916*w*, 821*m*, 812*w*, 786*s*, 771*m*, 735*m*, 694*w*, 601*s*, 570*vs*, 537*m*, 508*w*, 485*w*, 438*w*, 490*m*, 476*w*, 441*w* cm^{-1}; ^{1}H NMR:δ = 7.71–7.66 (*m*, 2H, 2-H, 2′-H), 7.48–7.40 (*m*, 3H, NH, 3-H, 3′-H), 4.34 (*s*, 1H, OH), 3.33–3.28 (*m*, 2H, 12-H), 2.71 (*t*, *J* = 6.5 Hz, 2H, 9-H), 2.63–2.54 (*m*, 1H, 5-H), 1.83–1.75 (*m*, 4H, 6-H_a, 6′-H_a, 7-H_a, 7′-H_a), 1.75–1.66 (*m*, 1H, 8-H_a), 1.47–1.30 (*m*, 8H, 6-H_b, 6′-H_b, 7-H_b, 7′-H_b, 10-H, 11-H), 1.29–1.20 (*m*, 1H, 8-H_b) ppm; ^{13}C NMR:δ = 152.0 (C-1), 138.1 (C-4), 127.4 (C-2), 126.5 (C-3), 60.2 (C-12), 43.6 (C-5), 42.5 (C-9), 33.5 (C-6), 29.5 (C-11), 26.2 (C-7), 25.8 (C-10), 25.4 (C-8) ppm; MS: *m*/*z* = 334.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{16}H_{25}NSO_3$ (311.44): C 61.71, H 8.09, N 4.50; found: C 61.53, H 8.24, N 4.16.

4.2.142. 4-[(4-Cyclohexylphenyl)sulfonamido]butyl Sulfamate (**70b**)

Applying GPB: from **70a** (200 mg, 0.64 mmol): **70b** (224 mg, 89%); white solid; R_f = 0.69 ($CHCl_3$/EtOAc, 2:3); m.p. = 103–105 °C; UV–Vis: 229 nm (4.25); IR: ν = 3365*w*, 3308*w*, 3266*w*, 2923*m*, 2850*w*, 1598*w*, 1543*w*, 1466*w*, 1453*w*, 1411*m*, 1388*w*, 1366*s*, 1315*s*, 1279*w*, 1272*w*, 1180*m*, 1157*s*, 1132*m*, 1095*m*, 1065*w*, 1051*m*, 1019*m*, 997*w*, 958*m*, 921*m*, 872*w*, 827*m*, 790*s*, 737*m*, 710*s*, 656*m*, 631*m*, 597*m*, 588*m*, 571*s*, 550*vs*, 532*m*, 508*w*, 503*w*, 489*w*, 478*w*, 453*w*a cm^{-1}; ^{1}H NMR:δ = 7.73–7.67 (*m*, 2H, 2-H, 2′-H), 7.53 (*t*, *J* = 5.9 Hz, 1H, NH), 7.46–7.41 (*m*, 2H, 3-H, 3′-H), 7.38 (*s*, 2H, NH_2), 3.96 (*t*, *J* = 6.3 Hz, 2H, 12-H), 2.75 (*q*, *J* = 6.7 Hz, 2H, 9-H), 2.64–2.55 (*m*, 1H, 5-H), 1.84–1.76 (*m*, 4H, 6-H_a, 6′-H_a, 7-H_a, 7′-H_a), 1.75–1.67 (*m*, 1H, 8-H_a), 1.66–1.56 (*m*, 2H, 11-H), 1.51–1.31 (*m*, 6H, 6-H_b, 6′-H_b, 7-H_b, 7′-H_b, 10-H), 1.30–1.17 (*m*, 1H, 8-H_b) ppm; ^{13}C NMR:δ = 152.1 (C-1), 138.0 (C-4), 127.4 (C-2), 126.5 (C-3), 68.5 (C-12), 43.6 (C-5), 42.0 (C-9), 33.5 (C-6), 26.2 (C-7), 25.6 (C-10), 25.4 (C-8, C-11) ppm; MS: *m*/*z* = 413.7 (100%, $[M + Na]^+$); anal. calcd. for $C_{16}H_{26}N_2S_2O_5$ (390.51): C 49.21, H 6.71, N 7.17; found: C 48,97, H 6.97, N 6.86.

4.2.143. 4-Cyclohexyl-N-(5-hydroxypentyl)benzene Sulfonamide (**71a**)

Applying GPA: from 4-cyclohexylbenzenesulfonyl chloride (500 mg, 1.93 mmol) and 5-amino-pentanol (299 mg, 2.90 mmol): **71a** (520 mg, 83%); oil; R_f = 0.49 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 229 nm (4.20); IR: ν = 3498*vw*, 3280*w*, 2924*m*, 2852*m*, 1598*w*, 1495*vw*, 1448*m*, 1410*w*, 1314*s*, 1283*w*, 1188*vw*, 1153*vs*, 1093*s*, 1040*m*, 999*w*, 920*vw*, 894*w*, 868*w*, 826*m*, 781*w*, 732*w*, 699*s*, 594*s*, 574*s*, 506*m*, 443*vw*, 508*w*, 485*w*, 438*w*, 490*m*, 476*w*, 441*w* cm^{-1}; ^{1}H NMR:δ = 7.71–7.66 (*m*, 2H, 2-H, 2′-H), 7.46–7.40 (*m*, 3H, 3-H, 3′-H, NH), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.34–3.28 (*m*, 2H, 13-H), 2.70 (*td*, *J* = 7.0, 3.2 Hz, 2H, 9-H), 2.63–2.54 (*m*, 1H, 5-H), 1.83–1.76 (*m*, 4H, 6-H_a, 6′-H_a, 7-H_a, 7′-H_a), 1.74–1.66 (*m*, 1H, 8-H_a), 1.48–1.15 (*m*, 11H, 6-H_b, 6′-H_b, 7-H_b, 7′-H_b, 8-H_b, 10-H, 11-H, 12-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 152.0 (C-4), 138.1 (C-1), 127.4 (C-3), 126.5 (C-2), 60.5 (C-13), 43.6 (C-5), 42.6 (C-9), 33.5 (C-6), 32.0 (C-12), 28.9 (C-10), 26.2 (C-7), 25.4 (C-8), 22.6 (C-11) ppm; MS: *m*/*z* = 348.4 (100%, $[M + Na]^+$); anal. calcd. for $C_{17}H_{27}NSO_3$ (325.47): C 62.74, H 8.36, N 4.30; found: C 62.51, H 8.63, N 4.17.

4.2.144. 5-[(4-Cyclohexylphenyl)sulfonamido]pentyl Sulfamate (**71b**)

Applying GPB: from **71a** (200 mg, 0.61 mmol): **71b** (240 mg, 96%); white solid; R_f = 0.62 ($CHCl_3$/EtOAc, 2:3); m.p. = 112–114 °C; UV–Vis: 229 nm (3.95); IR: ν = 3343*w*, 3277*m*, 3240*w*, 2934*m*, 2867*w*, 2848*w*, 1599*w*, 1471*w*, 1449*w*, 1444*w*, 1433*w*, 1404*w*, 1371*s*, 1356*m*, 1309*s*, 1278*w*, 1179*s*, 1154*vs*, 1093*m*, 1077*w*, 1052*m*, 968*s*, 920*s*, 886*m*, 866*w*, 821*vs*, 782*w*, 777*w*, 731*w*, 703*s*, 661*m*, 631*m*, 599*s*, 584*m*, 571*vs*, 558*s*, 537*s*, 521*m*, 504*m*, 478*w* cm^{-1}; ^{1}H NMR:δ = 7.71–7.67 (*m*, 2H, 2-H, 2′-H), 7.48 (*t*, *J* = 5.9 Hz, 1H, NH), 7.46–7.41 (*m*, 2H, 3-H, 3′-H), 7.37 (*s*, 2H, NH_2), 3.96 (*t*, *J* = 6.5 Hz, 2H, 13-H), 2.72 (*q*, *J* = 6.6 Hz, 2H, 9-H), 2.64–2.55 (*m*, 1H, 5-H), 1.83–1.76 (*m*, 4H, 6-H_a, 6′-H_a, 7-H_a, 7′-H_a), 1.75–1.67 (*m*, 1H, 8-H_a), 1.60–1.51 (*m*, 2H, 12-H), 1.49–1.34 (*m*, 6H, 6-H_b, 6′-H_b, 7-H_b, 7′-H_b, 10-H), 1.34–1.21 (*m*, 3H, 8-H_b,

11-H) ppm; ^{13}C NMR:δ = 152.1 (C-1), 138.0 (C-4), 127.4 (C-2), 126.5 (C-3), 68.8 (C-13), 43.5 (C-5), 42.3 (C-9), 33.5 (C-6), 28.5 (C-12), 27.8 (C-10), 26.2 (C-7), 25.4 (C-8), 22.2 (C-11) ppm; MS: *m*/*z* = 427.8 (100%, [M + Na]$^+$); anal. calcd. for $C_{17}H_{28}N_2S_2O_5$ (404.54): C 50.47, H 6.98, N 6.92; found: C 50.10, H 7.13, N 6.76.

4.2.145. 4-Cyclohexyl-N-(6-hydroxyhexyl)benzene Sulfonamide (**72a**)

Applying GPA: from 4-cyclohexylbenzenesulfonyl chloride (500 mg, 1.93 mmol) and 6-amino-hexanol (340 mg, 2.90 mmol): **72a** (589 mg, 90%); white solid; R_f = 0.48 ($CHCl_3$/EtOAc, 2:3); m.p. = 87–88 °C; UV–Vis: 229 nm (4.14); IR: ν = 3424*w*, 3365*vw*, 3261*m*, 2963*vw*, 2926*m*, 2855*m*, 1599*w*, 1479*w*, 1475*w*, 1462*vw*, 1451*w*, 1429*m*, 1411*w*, 1358*w*, 1316*s*, 1279*w*, 1187*w*, 1157*s*, 1103*w*, 1092*m*, 1063*m*, 1035*m*, 1016*w*, 996*w*, 984*vw*, 904*w*, 878*w*, 865*vw*, 844*w*, 827*m*, 800*w*, 780*w*, 735*m*, 709*s*, 679*m*, 598*s*, 571*vs*, 534*m*, 513*w*, 505*w*, 497*m* cm^{-1}; ^{1}H NMR:δ = 7.71–7.66 (*m*, 2H, 2-H, 2′-H), 7.47–7.39 (*m*, 3H, NH, 3-H, 3′-H), 4.29 (*s*, 1H, OH), 3.36–3.29 (*m*, 2H, 14-H), 2.75–2.66 (*m*, 2H, 9-H), 2.63–2.55 (*m*, 1H, 5-H), 1.82–1.75 (*m*, 4H, 6-H_a, 6′-H_a, 7-H_a, 7′-H_a), 1.74–1.67 (*m*, 1H, 8-H_a), 1.48–1.29 (*m*, 8H, 6-H_b, 6′-H_b, 7-H_b, 7′-H_b, 10-H, 13-H), 1.28–1.21 (*m*, 1H, 8-H_b), 1.21–1.14 (*m*, 4H, 11-H, 12-H) ppm; ^{13}C NMR:δ = 152.0 (C-4), 138.1 (C-1), 127.4 (C-3), 126.5 (C-2), 60.6 (C-14), 43.6 (C-5), 42.5 (C-9), 33.5 (C-6), 32.3 (C-13), 29.0 (C-10), 26.2 (C-7), 25.9 (C-12), 25.4 (C-8), 25.0 (C-11) ppm; MS: *m*/*z* = 361.9 (100%, [M + Na]$^+$); anal. calcd. for $C_{18}H_{29}NSO_3$ (339.49): C 63.68, H 8.61, N 4.13; found: C 63.58, H 8.83, N 3.97.

4.2.146. 6-[(4-Cyclohexylphenyl)sulfonamido]hexyl Sulfamate (**72b**)

Applying GPB: from **72a** (200 mg, 0.6 mmol): **72b** (186 mg, 75%); white solid; R_f = 0.63 ($CHCl_3$/EtOAc, 2:3); m.p. = 86–88 °C; UV–Vis: 229 nm (3.81); IR: ν = 3370*w*, 3280*m*, 2929*w*, 2854*w*, 1558*w*, 1456*w*, 1356*s*, 1173*vs*, 1156*s*, 1094*w*, 995*s*, 922*s*, 828*w*, 770*vs*, 700*w*, 595*m*, 571*m*, 550*vs*, 493*m* cm^{-1}; ^{1}H NMR:δ = 7.71–7.66 (*m*, 2H, 2-H, 2′-H), 7.48–7.41 (*m*, 3H, NH, 3-H, 3′-H), 7.37 (*s*, 2H, NH_2), 3.97 (*t*, *J* = 6.5 Hz, 2H, 14-H), 2.71 (*q*, *J* = 6.6 Hz, 2H, 9-H), 2.59 (*ddd*, *J* = 11.5, 8.4, 2.8 Hz, 1H, 5-H), 1.84–1.75 (*m*, 4H, 6-H_a, 6′-H_a, 7-H_a, 7′-H_a), 1.75–1.66 (*m*, 1H, 8-H_a), 1.55 (*p*, *J* = 6.7 Hz, 2H, 13-H), 1.48–1.30 (*m*, 6H, 6-H_b, 6′-H_b, 7-H_b, 7′-H_b, 10-H), 1.29–1.17 (*m*, 5H, 8-H_b, 11-H, 12-H); ^{13}C NMR:δ = 152.3 (C-1), 138.2 (C-4), 127.5 (C-2), 126.7 (C-3), 69.1 (C-14), 43.7 (C-9), 42.5 (C-5), 33.7 (C-6), 29.0 (C-13), 28.3 (C-10), 26.3 (C-7), 25.6 (C-8), 24.7 (C-11) ppm; MS: *m*/*z* = 441.1 (100%, [M + Na]$^+$), 419.1 (95%, [*M* + H]$^+$); anal. calcd. for $C_{18}H_{30}N_2S_2O_5$ (418.57): C 51.65, H 7.22, N 6.69; found: C 51.24, H 7.60, N 6.33.

4.2.147. 4-Cyclohexyl-N-(7-hydroxyheptyl)benzene Sulfonamide (**73a**)

Applying GPA: from 4-cyclohexylbenzenesulfonyl chloride (500 mg, 1.93 mmol) and 7-amino-heptanol (380 mg, 2.90 mmol): **73a** (592 mg, 87%); white solid; R_f = 0.47 ($CHCl_3$/EtOAc, 2:3); m.p. = 67–68 °C; UV–Vis: 229 nm (4.15); IR: ν = 3272*m*, 2923*m*, 2852*m*, 1599*w*, 1474*w*, 1463*w*, 1449*w*, 1429*m*, 1409*w*, 1318*s*, 1281*w*, 1186*w*, 1156*vs*, 1093*m*, 1058*m*, 1034*m*, 999*w*, 894*w*, 867*w*, 847*w*, 823*m*, 780*w*, 707*s*, 665*m*, 632*m*, 598*s*, 570*vs*, 543*w*, 529*m*, 506*w* cm^{-1}; ^{1}H NMR:δ = 7.71–7.66 (*m*, 2H, 2-H, 2′-H), 7.46–7.40 (*m*, 3H, NH, 3H-, 3′-H), 4.29 (*t*, *J* = 5.1 Hz, 1H, OH), 3.35 (*td*, *J* = 6.7, 5.1 Hz, 2H, 15-H), 2.70 (*t*, *J* = 7.1 Hz, 2H, 9-H), 2.63–2.54 (*m*, 1H, 5-H), 1.84–1.75 (*m*, 4H, 6-H_a, 6′-H_a, 7-H_a, 7′-H_a), 1.74–1.66 (*m*, 1H, 8-H_a), 1.48–1.26 (*m*, 8H, 6-H_b, 6′-H_b, 7-H_b, 7′-H_b, 10-H, 14-H), 1.26–1.11 (*m*, 7H, 8-H_b, 11-H, 12-H, 13-H) ppm; ^{13}C NMR:δ = 152.0 (C-1), 138.2 (C-4), 127.3 (C-2), 126.5 (C-3), 60.7 (C-15), 43.5 (C-5), 42.5 (C-9), 33.5 (C-6), 32.4 (C-14), 28.9 (C-10), 28.4 (C-11), 26.2 (C-7), 26.0 (C-13), 25.4 (C-8), 25.3 (C-12) ppm; MS: *m*/*z* = 375.8 (100%, [M + Na]$^+$); anal. calcd. for $C_{19}H_{31}NSO_3$ (353.52): C 64.55, H 8.84, N 3.96; found: C 64.12, H 9.12, N 3.64.

4.2.148. 7-[(4-Cyclohexylphenyl)sulfonamido]heptyl Sulfamate (**73b**)

Applying GPB: from **73a** (200 mg, 0.57 mmol): **73b** (201 mg, 82%); white solid; R_f = 0.61 ($CHCl_3$/EtOAc, 2:3); m.p. = 102–103 °C; UV–Vis: 229 nm (4.18); IR: ν = 3345*m*, 3273*m*, 3244*w*, 2928*m*, 2857*w*, 1598*w*, 1562*vw*, 1474*w*, 1452*w*, 1430*w*, 1411*vw*, 1394*w*, 1375*s*, 1309*s*, 1281*w*, 1177*s*, 1153*vs*, 1107*w*, 1093*m*, 1077*vw*, 1062*m*, 1049*w*, 1006*w*, 997*m*, 972*s*, 923*m*,

899*m*, 867*vw*, 835*w*, 813*s*, 781*w*, 731*m*, 708*s*, 668*m*, 598*m*, 590*m*, 568*s*, 550*s*, 532*m*, 515*m*, 507*w* cm^{-1}; ^1H NMR:δ = 7.71–7.66 (*m*, 2H, 2-H, 2′-H), 7.47–7.40 (*m*, 3H, 3-H, 3′-H, NH), 7.37 (*s*, 2H, NH_2), 3.98 (*t*, *J* = 6.5 Hz, 2H, 15-H), 2.71 (*q*, *J* = 6.6 Hz, 2H, 9-H), 2.63–2.54 (*m*, 1H, 5-H), 1.84–1.75 (*m*, 4H, 6-H_a, 6′-H_a, 7-H_a, 7′-H_a), 1.74–1.67 (*m*, 1H, 8-H_a), 1.63–1.53 (*m*, 2H, 14-H), 1.48–1.30 (*m*, 6H, 6-H_b, 6′-H_b, 7-H_b, 7-H_b, 10-H), 1.29–1.15 (*m*, 7H, 8-H_b, 11-H, 12-H, 13-H) ppm; ^{13}C NMR:δ = 152.1 (C-1), 138.1 (C-4), 127.4 (C-2), 126.6 (C-3), 69.0 (C-15), 43.6 (C-9), 42.5 (C-5), 33.6 (C-6), 28.9 (C-14), 28.2 (C-11), 28.0 (C-10), 26.2 (C-7), 25.9 (C-13), 25.5 (C-8), 24.9 (C-12) ppm; MS: *m*/*z* = 455.8 (100%, $[M + Na]^+$); anal. calcd. for $C_{19}H_{32}N_2S_2O_5$ (432.59): C 52.75, H 7.46, N 6.48; found: C 52.45, H 7.88, N 6.13.

4.2.149. 4-Cyclohexyl-N-(8-hydroxyoctyl)benzene Sulfonamide (**74a**)

Applying GPA: from 4-cyclohexylbenzenesulfonyl chloride (500 mg, 1.93 mmol) and 8-amino-octanol (421 mg, 2.90 mmol): **74a** (618 mg, 87%); white solid; R_f = 0.45 ($CHCl_3$/EtOAc, 2:3); UV–Vis: 229 nm (4.16); IR: ν = 3277*m*, 2924*s*, 2851*m*, 1599*w*, 1495*vw*, 1478*w*, 1466*w*, 1449*w*, 1427*m*, 1409*w*, 1318*s*, 1280*w*, 1185*w*, 1157*vs*, 1133*w*, 1094*m*, 1052*s*, 1016*w*, 997*w*, 982*w*, 901*m*, 883*w*, 866*w*, 849*w*, 823*m*, 780*w*, 755*m*, 733*m*, 706*s*, 651*m*, 632*m*, 598*s*, 570*vs*, 531*m*, 507*m*, 470*w* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.71–7.67 (*m*, 2H, 2-H, 2′-H), 7.46–7.42 (*m*, 2H, 3-H, 3′-H), 7.42–7.41 (*m*, 1H, NH), 4.29 (*t*, *J* = 5.2 Hz, 1H, OH), 3.36 (*td*, *J* = 6.6, 5.1 Hz, 2H, 16-H), 2.70 (*q*, *J* = 6.6 Hz, 2H, 9-H), 4.31–4.28 (*m*, 1H, 5-H), 1.83–1.75 (*m*, 4H, 6-H_a, 6′-H_a, 7-H_a, 7′-H_a), 1.74–1.67 (*m*, 1H, 8-H_a), 1.47–1.28 (*m*, 8H, 6-H_b, 6′-H_b, 7-H_b, 7′-H_b, 10-H, 15-H), 1.27–1.10 (*m*, 9H, 8-H_b, 11-H, 12-H, 13-H, 14-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 152.0 (C-4), 138.2 (C-1), 127.3 (C-2), 126.5 (C-2), 60.7 (C-16), 43.5 (C-5), 42.5 (C-9), 33.5 (C-6), 32.5 (C-15), 28.9 (C-10), 28.8 (C-14), 28.6 (C-11), 26.2 (C-7), 25.9 (C-13), 25.4 (C-8), 25.4 (C-12) ppm; MS: *m*/*z* = 390.3 (100%, $[M + Na]^+$); anal. calcd. for $C_{20}H_{33}NSO_3$ (367.55): C 65.36, H 9.05, N 3.81; found: C 65.06, H 9.37, N 3.56.

4.2.150. 8-[(4-Cyclohexylphenyl)sulfonamido]octyl Sulfamate (**74b**)

Applying GPB: from **74a** (200 mg, 0.54 mmol): **74b** (226 mg, 93%); white solid; R_f = 0.68 ($CHCl_3$/EtOAc, 2:3); m.p. = 91–93 °C; UV–Vis: 229 nm (4.11); IR: ν = 3367*w*, 3299*w*, 3274*w*, 2953*w*, 2922*m*, 2851*m*, 1598*w*, 1544*w*, 1475*w*, 1463*w*, 1453*w*, 1409*m*, 1396*w*, 1372*s*, 1316*s*, 1183*m*, 1157*s*, 1149*s*, 1106*w*, 1093*m*, 1069*w*, 1057*m*, 1039*m*, 1016*w*, 1004*m*, 996*m*, 981*s*, 944*w*, 935*m*, 887*m*, 857*w*, 827*m*, 813*s*, 782*w*, 763*w*, 730*m*, 712*s*, 659*m*, 632*w*, 589*m*, 575*s*, 555*vs*, 528*s*, 507*m*, 481*w* cm^{-1}; ^1H NMR:δ = 7.71–7.66 (*m*, 2H, 2-H, 2′-H), 7.47–7.40 (*m*, 3H, 3-H, 3′-H, NH), 7.37 (*s*, 2H, NH_2), 3.99 (*t*, *J* = 6.5 Hz, 2H, 16-H), 2.74–2.67 (*m*, 2H, 9-H), 2.64–2.55 (*m*, 1H, 5-H), 1.83–1.74 (*m*, 4H, 6-H_a, 6′-H_a, 7-H_a, 7′-H_a), 1.74–1.67 (*m*, 1H, 8-H_a), 1.64–1.55 (*m*, 2H, 15-H), 1.49–1.12 (*m*, 15H, 6-H_b, 6′-H_b, 7-H_b, 7′-H_b, 8-H_b, 10-H, 11-H, 12-H, 13-H, 14-H) ppm; ^{13}C NMR:δ = 152.0 (C-1), 138.2 (C-4), 127.4 (C-2), 126.5 (C-3), 69.0 (C-16), 43.6 (C-5), 42.5 (C-9), 33.6 (C-6), 28.9 (C-15), 28.4 (C-10), 28.3 (C-13), 28.3 (C-12), 26.2 (C-7), 25.9 (C-11), 25.4 (C-8), 25.0 (C-14) ppm; MS: *m*/*z* = 469.9 (100%, $[M + Na]^+$); anal. calcd. for $C_{20}H_{34}N_2S_2O_5$ (446.62): C 53.79, H 7.67, N 6.27; found: C 53.35, H 7.96, N 5.98.

4.2.151. 4-Cyclohexyl-N-(9-hydroxynonyl)benzene Sulfonamide (**75a**)

Applying GPA: from 4-cyclohexylbenzenesulfonyl chloride (500 mg, 1.93 mmol) and 9-amino-nonanol (462 mg, 2.90 mmol): **75a** (723 mg, 98%); white solid; R_f = 0.84 ($CHCl_3$/EtOAc, 2:3); m.p. = 55–57 °C; UV–Vis: 229 nm (4.11); IR: ν = 3279*m*, 2922*s*, 2851*m*, 1599*w*, 1475*w*, 1467*w*, 1448*w*, 1426*m*, 1409*w*, 1318*s*, 1281*w*, 1269*w*, 1186*w*, 1157*vs*, 1095*m*, 1054*m*, 1036*m*, 1016*w*, 998*w*, 974*w*, 902*w*, 883*w*, 851*vw*, 822*m*, 780*w*, 733*m*, 706*s*, 654*m*, 632*m*, 599*s*, 569*vs*, 529*m*, 508*w*, 472*vw*, 460*vw* cm^{-1}; ^1H NMR:δ = 7.70–7.66 (*m*, 2H, 2-H, 2′-H), 7.46–7.40 (*m*, 3H, NH, 3-H, 3′-H), 4.30 (*td*, *J* = 5.1, 1.1 Hz, 1H, OH), 3.36 (*td*, *J* = 6.5, 5.1 Hz, 2H, 17-H), 2.71 (*q*, *J* = 6.7 Hz, 2H, 9-H), 2.63–2.55 (*m*, 1H, 5-H), 1.83–1.75 (*m*, 4H, 6-H_a, 6′-H_a, 7-H_a, 7′-H_a), 1.74–1.67 (*m*, 1H, 8-H_a), 1.48–1.27 (*m*, 6H, 7-H_b, 7′-H_b, 8-H_b, 10-H, 16-H), 1.26–1.11 (*m*, 11H, 6-H_b, 6′-H_b, 11-H, 12-H, 13-H, 14-H, 15-H) ppm; ^{13}C NMR:δ = 152.0 (C-4), 138.2 (C-1), 127.3 (C-2), 126.5 (C-3), 60.7 (C-17), 43.6 (C-5), 42.5 (C-9), 33.6 (C-6), 32.5 (C-16), 28.9 (C-10, C-14), 28.8 (C-12), 28.5 (C-13), 26.2 (C-7), 26.0 (C-11), 25.5 (C-8), 25.4

(C-15) ppm; MS: *m*/*z* = 457.1 (100%, [M + Na]$^+$); anal. calcd. for $C_{21}H_{35}NSO_3$ (381.58): C 54.76, H 7.88, N 6.08; found: C 54.31, H 8.06, N 5.76.

4.2.152. 9-[(4-Cyclohexylphenyl)sulfonamido]nonyl Sulfamate (**75b**)

Applying GPB: from **75a** (200 mg, 0.52 mmol): **75b** (190 mg, 79%); white solid; R_f = 0.87 ($CHCl_3$/EtOAc, 2:3); m.p. = 88–89 °C; UV–Vis: 230 nm (4.04); IR: ν = 3379*w*, 3278*m*, 2962*w*, 2924*m*, 2855*m*, 1600*w*, 1542*w*, 1477*w*, 1461*vw*, 1451*w*, 1424*m*, 1399*w*, 1376*s*, 1326*w*, 1310*s*, 1281*w*, 1214*vw*, 1186*s*, 1154*vs*, 1118*w*, 1094*m*, 1078*vw*, 1060*m*, 1034*m*, 993*w*, 967*s*, 907*s*, 885*m*, 866*w*, 819*vs*, 781*w*, 734*w*, 704*s*, 652*m*, 631*m*, 598*m*, 587*m*, 573*s*, 555*vs*, 534*m*, 524*m*, 505*m*, 483*m*, 454*vw* cm^{-1}; ^{1}H NMR:δ = 7.68–7.63 (*m*, 2H, 2-H, 2′-H), 7.44–7.38 (*m*, 3H, NH, 3-H, 3′-H), 7.34 (*s*, 2H, NH_2), 3.97 (*t*, *J* = 6.5 Hz, 2H, 17-H), 2.68 (*q*, *J* = 6.7 Hz, 2H, 9-H), 2.61–2.52 (*m*, 1H, 5-H), 1.82–1.72 (*m*, 4H, 6-H_a, 6′-H_a, 7-H_a, 7′-H_a), 1.72–1.63 (*m*, 1H, 8-H_a), 1.62–1.54 (*m*, 2H, 16-H), 1.46–1.08 (*m*, 17H, 6-H_b, 6′-H_b, 7-H_b, 7′-H_b, 8-H_b, 10-H, 11-H, 12-H, 13-H, 14-H, 15-H) ppm; ^{13}C NMR:δ = 152.0 (C-4), 138.2 (C-1), 127.4 (C-2), 126.5 (C-3), 69.0 (C-17), 43.6 (C-5), 42.5 (C-9), 33.6 (C-6), 28.9 (C-16), 28.7 (C-10), 28.4 (C-12), 28.4 (C-14), 28.3 (C-13), 26.2 (C-7), 25.9 (C-11), 25.4 (C-8), 25.0 (C-15) ppm; MS: *m*/*z* = 457.1 (100%, [M + Na]$^+$); anal. calcd. for $C_{21}H_{36}N_2S_2O_5$ (460.65): C 54.76, H 7.88, N 6.08; found: C 54.35, H 8.01, N 5.74.

4.2.153. 4-Cyclohexyl-N-(10-hydroxydecyl)benzene Sulfonamide (**76a**)

Applying GPA: from 4-cyclohexylbenzenesulfonyl chloride (450 mg, 1.74 mmol) and 10-amino-decanol (452 mg, 2.61 mmol): **76a** (625 mg, 91%); white solid; R_f = 0.82 ($CHCl_3$/EtOAc, 2:3); m.p. = 71–72 °C; UV–Vis: 229 nm (4.17); IR: ν = 3494*w*, 3130*w*, 2915*s*, 2877*w*, 2849*s*, 1598*w*, 1476*w*, 1466*w*, 1448*m*, 1440*m*, 1408*w*, 1397*w*, 1346*w*, 1310*s*, 1288*w*, 1267*w*, 1188*w*, 1150*vs*, 1114*m*, 1095*m*, 1074*s*, 1043*w*, 1031*w*, 1020*m*, 999*w*, 973*w*, 914*m*, 876*w*, 865*vw*, 840*w*, 825*m*, 781*w*, 734*w*, 720*m*, 701*s*, 606*vs*, 575*s*, 523*m*, 505*w*, 468*m* cm^{-1}; ^{1}H NMR:δ = 7.70–7.66 (*m*, 2H, 2-H, 2′-H), 7.46–7.39 (*m*, 3H, NH, 3-H, 3′-H), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.36 (*td*, *J* = 6.6, 5.2 Hz, 2H, 18-H), 2.74–2.67 (*m*, 2H, 9-H), 2.64–2.54 (*m*, 1H, 5-H), 1.84–1.75 (*m*, 4H, 6-H_a, 6′-H_a, 7-H_a, 7′-H_a), 1.74–1.67 (*m*, 1H, 8-H_a), 1.47–1.27 (*m*, 8H, 6-H_b, 6′-H_b, 7-H_b, 7′-H_b, 10-H, 17-H), 1.27–1.09 (*m*, 13H, 8-H_b, 11-H, 12-H, 13-H, 14-H, 15-H, 16-H) ppm; ′^{13}C NMR:δ = 152.0 (C-4), 138.2 (C-1), 127.3 (C-2), 126.6 (C-3), 60.7 (C-18), 43.6 (C-5), 42.5 (C-9), 33.6 (C-6), 32.5 (C-17), 29.0 (C-10), 28.9 (C-12), 28.9 (C-15), 28.8 (C-13), 28.5 (C-14), 26.2 (C-7), 26.0 (C-11), 25.5 (C-16), 25.4 (C-8) ppm; MS: *m*/*z* = 457.1 (100%, [M + Na]$^+$); anal. calcd. for $C_{22}H_{37}NSO_3$ (395.60): C 66.79, H 9.43, N 3.54; found: C 66.25, H 9.81, N 3.16.

4.2.154. 10-[(4-Cyclohexylphenyl)sulfonamido]decyl Sulfamate (**76b**)

Applying GPB: from **76a** (180 mg, 0.46 mmol): **76b** (161 mg, 75%); white solid; R_f = 0.9 ($CHCl_3$/EtOAc, 2:3); m.p. = 100–101 °C; UV–Vis: 229 nm (4.33); IR: ν = 3369*w*, 3298*w*, 3275*w*, 2953*w*, 2919*m*, 2849*m*, 1598*w*, 1543*w*, 1475*w*, 1464*w*, 1454*w*, 1409*w*, 1396*w*, 1373*s*, 1317*s*, 1183*m*, 1157*s*, 1150*s*, 1093*m*, 1060*w*, 1052*m*, 1037*w*, 1021*m*, 1001*m*, 978*m*, 937*m*, 915*w*, 882*m*, 845*w*, 827*m*, 815*s*, 798*m*, 782*w*, 763*w*, 731*m*, 713*s*, 661*m*, 632*w*, 591*m*, 575*s*, 555*vs*, 530*m*, 504*w*, 476*w*, 471*w*, 417*w* cm^{-1}; ^{1}H NMR:δ = 7.71–7.66 (*m*, 2H, 2-H, 2′-H), 7.46–7.40 (*m*, 3H, NH, 3-H, 3′-H), 7.37 (*s*, 2H, NH_2), 4.00 (*t*, *J* = 6.5 Hz, 2H, 18-H), 2.71 (*q*, *J* = 6.7 Hz, 2H, 9-H), 2.63–2.54 (*m*, 1H, 5-H), 1.83–1.75 (*m*, 4H, 6-H_a, 6′-H_a, 7-H_a, 7′-H_a), 1.74–1.67 (*m*, 1H, 8-H_a), 1.61 (*p*, *J* = 6.5 Hz, 2H, 17-H), 1.48–1.37 (*m*, 3H, 8-H_b, 10-H), 1.36–1.12 (*m*, 16H, 6-H_b, 6′-H_b, 7-H_b, 7′-H_b, 11-H, 12-H, 13-H, 14-H, 15-H, 16-H) ppm; ^{13}C NMR:δ = 152.0 (C-4), 138.2 (C-1), 127.4 (C-2), 126.5 (C-3), 69.0 (C-18), 43.6 (C-5), 42.5 (C-9), 33.6 (C-6), 28.9 (C-10), 28.8 (C-13), 28.8 (C-17), 28.5 (C-12), 28.5 (C-15), 28.3 (C-14), 26.2 (C-7), 26.0 (C-11), 25.4 (C-8), 25.1 (C-16) ppm; MS: *m*/*z* = 457.1 (100%, [M + Na]$^+$); anal. calcd. for $C_{22}H_{38}N_2S_2O_5$ (474.68): C 55.67, H 8.07, N 5.90; found: C 55.25, H 8.41, N 5.65.

4.2.155. 4-(Adamantan-1-yl)-N-(8-hydroxyoctyl)benzene Sulfonamide (**77a**)

Applying GPA: from 4-(adamantan-1-yl)benzenesulfonyl chloride (300 mg, 0.96 mmol) and 8-amino-octanol (212 mg, 1.45 mmol): **77a** (363 mg, 90%); white solid; R_f = 0.70 ($CHCl_3$/EtOAc, 2:3); m.p. = 101–102 °C; UV–Vis: 231 nm (4.15); IR: ν = 3463*w*, 3147*w*, 2917*s*, 2903*m*, 2849*m*, 1477*w*, 1465*w*, 1447*w*, 1399*w*, 1381*w*, 1367*w*, 1360*w*, 1345*w*, 1316*s*, 1304*m*, 1291*m*, 1158*s*, 1095*m*, 1085*m*, 1058*m*, 1029*m*, 1014*w*, 975*w*, 957*w*, 951*w*, 939*m*, 846*w*, 832*w*, 806*m*, 774*w*, 742*m*, 721*w*, 683*m*, 674*m*, 599*s*, 579*vs*, 534*m*, 483*m* cm^{-1}; ^1H NMR:δ = 7.73–7.69 (*m*, 2H, 2-H, 2′-H), 7.58–7.54 (*m*, 2H, 3-H, 3′-H), 7.44 (*t*, *J* = 5.8 Hz, 1H, NH), 4.29 (*td*, *J* = 5.1, 1.0 Hz, 1H, OH), 3.35 (*td*, *J* = 6.6, 5.2 Hz, 2H, 22-H), 2.74–2.66 (*m*, 2H, 15-H), 2.10–2.03 (*m*, 3H, 7-H, 9-H, 11-H), 1.88 (*d*, *J* = 3.0 Hz, 6H, 6-H, 12-H, 13-H), 1.79–1.69 (*m*, 6H, 8-H, 10-H, 14-H), 1.42–1.27 (*m*, 4H, 16-H, 21-H), 1.26–1.10 (*m*, 8H, 17-H, 18-H, 19-H, 20-H) ppm; ^{13}C NMR:δ = 155.2 (C-4), 137.8 (C-1), 126.4 (C-2), 125.5 (C-3), 60.7 (C-22), 42.5 (C-15), 42.2 (C-12, C-6, C-13), 36.2 (C-5), 36.0 (C-10, C-8, C-14), 32.5 (C-21), 28.9 (C-16), 28.8 (C-18), 28.6 (C-19), 28.2 (C-7, C-9, C-11), 25.9 (C-17), 25.4 (C-20) ppm; MS: *m*/*z* = 457.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{24}H_{37}NSO_3$ (419.62): C 68.70, H 8.89, N 3.34; found: C 68.47, H 9.19, N 2.96.

4.2.156. 8-[(4-(Adamantan-1-yl)phenyl)sulfonamido]octyl Sulfamate (**77b**)

Applying GPB: from **77a** (190 mg, 0.42 mmol): **77b** (147 mg, 66%); white solid; R_f = 0.87 ($CHCl_3$/EtOAc, 2:3); m.p. = 85–86 °C; UV–Vis: 231 nm (4.20); IR: ν = 3360*w*, 3254*m*, 2929*m*, 2899*s*, 2847*m*, 1568*w*, 1448*m*, 1398*w*, 1361*m*, 1344*w*, 1316*vs*, 1293*m*, 1183*m*, 1145*vs*, 1111*w*, 1092*s*, 1076*m*, 1065*m*, 1044*w*, 1031*w*, 1013*m*, 999*w*, 982*w*, 976*w*, 964*m*, 953*m*, 933*s*, 906*m*, 898*m*, 877*m*, 861*w*, 837*w*, 795*s*, 773*w*, 756*m*, 744*s*, 730*w*, 721*m*, 694*s*, 665*w*, 633*w*, 595*s*, 575*vs*, 551*vs*, 537*m*, 526*m*, 505*w*, 480*m*, 473*m* cm^{-1}; ^1H NMR:δ = 7.73–7.68 (*m*, 2H, 2-H, 2′-H), 7.59–7.54 (*m*, 2H, 3-H, 3′-H), 7.45 (*t*, *J* = 5.9 Hz, 1H, NH), 7.37 (*s*, 2H, NH_2), 3.99 (*t*, *J* = 6.5 Hz, 2H, 22-H), 2.71 (*q*, *J* = 6.7 Hz, 2H, 15-H), 2.07 (*p*, *J* = 3.1 Hz, 3H, 7-H, 9-H, 11-H), 1.88 (*d*, *J* = 2.9 Hz, 6H, 6-H, 12-H, 13-H), 1.79–1.69 (*m*, 6H, 8-H, 10-H, 14-H), 1.64–1.55 (*m*, 2H, 21-H), 1.37–1.25 (*m*, 2H, 16-H), 1.23–1.13 (*m*, 8H, 17-H, 18-H, 19-H, 20-H) ppm; ^{13}C NMR:δ = 155.2 (C-4), 137.8 (C-1), 126.4 (C-2), 125.5 (C-3), 69.0 (C-22), 42.5 (C-15), 42.2 (C-6, C-12, C-13), 36.2 (C-5), 36.0 (C-8, C-10, C-14), 28.9 (C-21), 28.4 (C-19), 28.3 (C-16), 28.3 (C-18), 28.2 (C-7, C-9, C-11), 25.9 (C-17), 25.0 (C-20) ppm; MS: *m*/*z* = 457.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{24}H_{38}N_2S_2O_5$ (498.70): C 57.80, H 7.68, N 5.62; found: C 57.64, H 7.43, N 5.45.

4.2.157. 4-(Adamantan-1-yl)-N-(9-hydroxynonyl)benzene Sulfonamide (**78a**)

Applying GPA: from 4-(adamantan-1-yl)benzenesulfonyl chloride (150 mg, 0.48 mmol) and 9-amino-nonanol (115 mg, 0.72 mmol): **78a** (201 mg, 96%); white solid; R_f = 0.72 ($CHCl_3$/EtOAc, 2:3); m.p. = 84–85 °C; UV–Vis: 231 nm (4.28); IR: ν = 3503*vw*, 3281*w*, 2903*s*, 2849*m*, 1597*w*, 1496*vw*, 1449*m*, 1400*w*, 1319*s*, 1293*m*, 1157*vs*, 1094*m*, 1031*m*, 1014*w*, 976*w*, 839*w*, 806*m*, 752*m*, 741*m*, 674*s*, 596*vs*, 576*s*, 475*w* cm^{-1}; ^1H NMR:δ = 7.71 (*d*, *J* = 8.6 Hz, 2H, 2-H, 2′-H), 7.56 (*d*, *J* = 8.6 Hz, 2H, 3-H, 3′-H), 7.44 (*t*, *J* = 5.8 Hz, 1H, NH), 4.29 (*t*, *J* = 5.1 Hz, 1H, OH), 3.40–3.33 (*m*, 2H, 23-H), 2.71 (*q*, *J* = 6.8 Hz, 2H, 15-H), 2.07 (*s*, 3H, 7-H, 9-H, 11-H), 1.88 (*d*, *J* = 2.7 Hz, 6H, 6-H, 12-H, 13-H), 1.79–1.69 (*m*, 6H, 8-H, 10-H, 14-H), 1.43–1.35 (*m*, 2H, 22-H), 1.34–1.27 (*m*, 2H, 16-H), 1.27–1.10 (*m*, 10H, 17-H, 18-H, 19-H, 20-H, 21-H) ppm; ^{13}C NMR:δ = 155.2 (C-4), 137.9 (C-1), 126.4 (C-2), 125.5 (C-3), 60.7 (C-23), 42.5 (C-15), 42.2 (C-6, C-12, C-13), 36.2 (C-5), 36.0 (C-8, C-10, C-14), 32.5 (C-22), 28.9 (C-16, C-18), 28.9 (C-20), 28.5 (C-19), 28.2 (C-7, C-9, C-11), 26.0 (C-17), 25.5 (C-21) ppm; MS: *m*/*z* = 457.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{25}H_{39}NSO_3$ (433.65): C 69.24, H 9.07, N 3.23; found: C 68.97, H 9.33, N 2.95.

4.2.158. 9-[(4-(Adamantan-1-yl)phenyl)sulfonamido]nonyl Sulfamate (**78b**)

Applying GPB: from **78a** (110 mg, 0.25 mmol): **78b** (110 mg, 84%); white solid; R_f = 0.92 ($CHCl_3$/EtOAc, 2:3); m.p. = 94–96 °C; UV–Vis: 231 nm (4.19); IR: ν = 3347*w*, 3269*w*, 2922*m*, 2903*m*, 2850*m*, 1429*w*, 1401*w*, 1370*m*, 1317*s*, 1297*m*, 1176*m*, 1156*vs*, 1117*w*, 1103*w*, 1093*w*, 1076*w*, 1059*w*, 1033*w*, 1013*w*, 963*m*, 926*m*, 879*w*, 848*w*, 839*w*, 823*m*, 805*m*, 775*w*, 747*m*, 729*w*, 697*m*, 648*w*, 598*s*, 572*s*, 564*s*, 534*w*, 522*w*, 513*w*, 484*w* cm^{-1}; ^1H NMR:δ = 7.71 (*d*, *J* = 8.2 Hz,

2H, 2-H, 2′-H), 7.57 (*d*, *J* = 8.2 Hz, 2H, 3-H, 3′-H), 7.44 (*t*, *J* = 5.9 Hz, 1H, NH), 7.37 (*s*, 2H, NH_2), 3.99 (*t*, *J* = 6.5 Hz, 2H, 23-H), 2.71 (*q*, *J* = 6.7 Hz, 2H, 15-H), 2.07 (*s*, 3H, 7-H, 9-H, 11-H), 1.88 (*d*, *J* = 2.9 Hz, 6H, 6-H, 12-H, 13-H), 1.74 (*s*, 6H, 8-H, 10-H, 14-H), 1.60 (*p*, *J* = 6.7 Hz, 2H, 22-H), 1.37–1.27 (*m*, 4H, 16-H, 21-H), 1.27–1.11 (*m*, 8H, 17-H, 18-H, 19-H, 20-H) ppm; ^{13}C NMR:δ = 155.7 (C-4), 138.3 (C-1), 126.8 (C-2), 126.0 (C-3), 69.4 (C-23), 42.9 (C-15), 42.7 (C-6, C-12, C-13), 36.6 (C-5), 36.5 (C-8, C-10, C-14), 29.4 (C-22), 29.2 (C-16), 28.9 (C-18), 28.9 (C-20), 28.8 (C-19), 26.4 (C-17), 25.5 (C-21) ppm; MS: *m*/*z* = 457.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{25}H_{40}N_2S_2O_5$ (512.72): C 58.56, H 7.86, N 5.46; found: C 58.27, H 8.05, N 5.16.

4.2.159. 4-(Adamantan-1-yl)-N-(10-hydroxydecyl)benzene Sulfonamide (**79a**)

Applying GPA: from 4-(adamantan-1-yl)benzenesulfonyl chloride (300 mg, 0.97 mmol) and 10-amino-decanol (251 mg, 1.45 mmol): **79a** (377 mg, 87%); white solid; R_f = 0.74 ($CHCl_3$/EtOAc, 2:3); m.p. = 76–79 °C; UV–Vis: 231 nm (4.20); IR: ν = 3496*vw*, 3282*w*, 2903*s*, 2849*s*, 1597*w*, 1449*m*, 1400*w*, 1369*w*, 1320*s*, 1293*m*, 1157*vs*, 1094*m*, 1056*m*, 1031*m*, 1014*w*, 976*w*, 881*vw*, 839*w*, 806*m*, 740*m*, 721*w*, 674*s*, 596*vs*, 577*s*, 495*w*, 487*w*, 476*w*, 451*vw* cm^{-1}; ^{1}H NMR:δ = 7.73–7.68 (*m*, 2H, 2-H, 2′-H), 7.59–7.53 (*m*, 2H, 3-H, 3′-H), 7.44 (*t*, *J* = 5.8 Hz, 1H, NH), 4.30 (*t*, *J* = 5.1 Hz, 1H, OH), 3.36 (*td*, *J* = 6.5, 5.2 Hz, 2H, 24-H), 2.71 (*q*, *J* = 6.6 Hz, 2H, 15-H), 2.09–2.03 (*m*, 3H, 7-H, 9-H, 11-H), 1.88 (*d*, *J* = 3.0 Hz, 6H, 6-H, 12-H, 13-H), 1.73 (*d*, *J* = 4.4 Hz, 6H, 8-H, 10-H, 14-H), 1.43–1.34 (*m*, 2H, 23-H), 1.34–1.26 (*m*, 2H, 16-H), 1.26–1.09 (*m*, 12H, 17-H, 18-H, 19-H, 20-H, 21-H, 22-H) ppm; ^{13}C NMR:δ = 155.2 (C-4), 137.9 (C-1), 126.4 (C-2), 125.5 (C-3), 60.7 (C-24), 42.5 (C-15), 42.2 (C-6, C-12, C-13), 36.2 (C-5), 36.0 (C-8, C-10, C-14), 32.5 (C-23), 29.0 (C-16), 28.9 (C-19), 28.9 (C-21), 28.8 (C-20), 28.5 (C-18), 28.2 (C-7, C-9, C-11), 26.0 (C-17), 25.5 (C-22) ppm; MS: *m*/*z* = 457.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{26}H_{41}NSO_3$ (447.68): C 69.76, H 9.23, N 3.13; found: C 69.41, H 9.55, N 2.94.

4.2.160. 10-[(4-(Adamantan-1-yl)phenyl)sulfonamido]decyl Sulfamate (**79b**)

Applying GPB: from **79a** (170 mg, 0.38 mmol): **79b** (92 mg, 46%); white solid; R_f = 0.85 ($CHCl_3$/EtOAc, 2:3); m.p. = 87–89 °C; UV–Vis: 231 nm (4.18); IR: ν = 3291*w*, 2919*m*, 2903*m*, 2850*m*, 1597*vw*, 1567*vw*, 1563*vw*, 1471*w*, 1450*w*, 1426*w*, 1400*w*, 1366*m*, 1322*s*, 1294*m*, 1179*m*, 1158*vs*, 1119*w*, 1103*w*, 1093*m*, 1064*w*, 1051*w*, 1031*w*, 1014*w*, 954*m*, 936*m*, 880*w*, 831*m*, 805*m*, 775*w*, 762*w*, 747*m*, 731*w*, 722*w*, 682*m*, 628*w*, 597*s*, 572*s*, 561*s*, 523*w*, 474*w* cm^{-1}; ^{1}H NMR:δ = 7.74–7.69 (*m*, 2H, 2-H, 2′-H), 7.59–7.54 (*m*, 2H, 3-H, 3′-H), 7.44 (*t*, *J* = 5.8 Hz, 1H, NH), 7.37 (*s*, 2H, NH_2), 4.00 (*t*, *J* = 6.5 Hz, 2H, 24-H), 2.71 (*q*, *J* = 6.6 Hz, 2H, 15-H), 2.07 (*p*, *J* = 3.1 Hz, 3H, 7-H, 9-H, 11-H), 1.88 (*d*, *J* = 2.9 Hz, 6H, 6-H, 12-H, 13-H), 1.80–1.68 (*m*, 6H, 8-H, 10-H, 14-H), 1.61 (*p*, *J* = 6.6 Hz, 2H, 23-H), 1.36–1.27 (*m*, 4H, 16-H, 22-H), 1.27–1.11 (*m*,10H, 17-H, 18-H, 19-H, 20-H, 21-H) ppm; ^{13}C NMR:δ = 155.6 (C-4), 138.3 (C-1), 126.8 (C-2), 126.0 (C-3), 69.4 (C-24), 42.9 (C-15), 42.7 (C-6, C-12, C-13), 36.6 (C-5), 36.5 (C-8, C-10, C-14), 29.4 (C-16), 29.3 (C-19), 29.3 (C-23), 29.0 (C-18), 28.9 (C-21), 28.8 (C-20), 28.6 (C-7, C-9, C-11), 26.4 (C-17), 25.5 (C-22) ppm; MS: *m*/*z* = 457.1 (100%, $[M + Na]^+$); anal. calcd. for $C_{26}H_{42}N_2S_2O_5$ (526.75): C 59.29, H 8.04, N 5.32; found: C 58.97, H 8.31, N 5.03.

4.3. Molecular Modeling

For the molecular docking studies, MOE 2020 software (2020 0901) was employed. The enzyme structure, obtained from the PDB Database (PDB ID: 3HS4), was prepared using the QuickPrep tool (version 2020). Ligands were deprotonated to align with the crystal structure of the co-crystallized ligand. Docking utilized a pharmacophore model focusing on the interaction between the sulfonamide group and the zinc ion. Ligands were initially positioned with the Triangle Matcher algorithm, producing 30 poses, which were scored with the London dG scoring function. The top five poses underwent refinement with a rigid receptor model and were rescored using the GBVI/WSA dG method. The best pose was assessed by comparing it to the expected logical structure and interactions. The docking protocol was validated by successfully redocking the co-crystallized ligand, acetazolamide.

4.4. Enzymatic Assay

The enzymatic assays have been performed with carbonic anhydrase II (*b*CAII, ≥3000 W-A units/mg from bovine erythrocytes) from Sigma (Taufkirchen, Germany) using a BMG Labtech Spectrostar Omega apparatus (BMG Labtech, Ortenberg, Germany) measuring λ = 415 nm. Conditions have been described previously [72]. K_i and K_i' values were determined from Lineweaver–Burk, Dixon and Cornish Bowden plots, respectively.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules29133015/s1, page 1 to page 53: ^{1}H and ^{13}C NMR spectra of all compounds; page 54 to page 58: representative HRMS spectra; page 59 to page 64: 2D and 3D depiction (docking calculations).

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Arylsulfonamido-alkyl-sulfamates act as inhibitors of bovine carbonic anhydrase II

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ABSTRACT

A small library of arylsulfonamido-alkyl sulfamates was prepared by a two-step synthesis from readily available starting materials. The compounds were tested for their ability to inhibit bovine carbonic anhydrase II. Several of them were found as good competitive inhibitors holding K_i values as low as $K_i = 0.9$ μM (compound **47b**). The activity was influenced by the substitution pattern of the arylsulfonamide moiety as well as the length of the spacer to the distal sulfamate group. Molecular docking studies were used to substantiate these findings. For the aryl-substituted analogues, the increase in inhibitory activity for compounds with a shorter spacer can be explained by stabilization via aromatic π-interactions. For the cyclopropyl or methylsulfonyl substituted analogues, their inhibitory activity can be attributed to their reduced steric hindrance. These results provide a basis for designing effective CA II inhibitors.

1. Introduction

The search for efficient inhibitors of carbonic anhydrases (CAs) has become increasingly important in recent years. CAs are found in all kinds of organisms and their function is not limited to pH regulation. Especially in the last decade, a variety of other functions of these enzymes have been reported. CAs are involved in many physiological processes, including respiration and transport of CO_2 and bicarbonate, calcification and bone resorption, but also in biosynthetic reactions, including gluconeogenesis, adipogenesis and ureagenesis. It is therefore not surprising that *h*CAs are considered therapeutic targets for diseases such as neuropathic pain (CA II, VII), oxidative stress (CA III), inflammatory processes (CA IV, IX and XII), epilepsy (II, VII, XIV), hypoxic tumors (IX, XII), obesity (VA/VB), edema (I, II) and glaucoma (II, IV, XII) [1–14].

We have investigated the potential of sulfamates in the past [15–19]. Compounds of this class have previously been studied as antiviral agents; they have also been used as antibiotic compounds in clinical trials and suggested for the treatment of hormone-dependent breast and prostate tumors [11]. Furthermore, they have also been proposed for the treatment of obesity [9], hyperlipidemia and atherosclerosis [11]. In our own studies, pentacyclic triterpenoids in particular played an important role, but also ureido sulfamates [20,21]. We were able to show that the sulfamates of pentacyclic triterpene-carboxylic acids in particular exhibited cytotoxic activity even in the (sub)-nanomolar concentration range, and that ureido-sulfamates proved to be selective CA inhibitors, some of which exceeded the effect of the gold standard SLC-0111 (Fig. 1) [20,21]; the latter compound is currently undergoing extensive clinical trials for the treatment of pancreatic and breast cancer.

Analyzing fungal extracts, activity-guided screenings for possible CA II inhibitors revealed that compound **A** showed no activity against CA II, but the readily accessible sulfamate of structure **B**, however, did. Initial molecular modeling calculations suggested that the inhibitory effect of structurally analogous compounds should depend on the type and position of a substituent on the aromatic ring, but also on the chain length of the spacer and possible substituents acting as side chains of the spacer; the latter aspect has recently been sufficiently verified and confirmed [16].

Since inhibitors of CA II are of great importance not only for the therapy of glaucoma but also for the alleviation of some side effects of modern antibody-based therapy of Alzheimer's disease (e.g. brain swelling, edema), this aspect should be investigated in more detail. (Scheme 1).

The latter compounds are easily accessible from the former by simple reaction with sulfamoyl chloride. The compounds thus obtained were tested for their inhibitory activity using *b*CAII.

Human and bovine carbonic anhydrase II are enzymes with a high degree of structural similarity, allowing them to efficiently catalyze the

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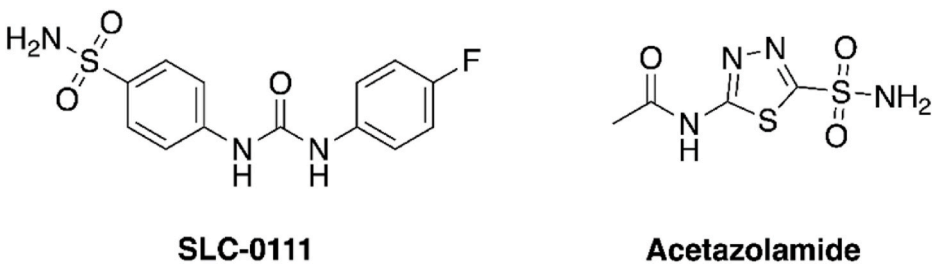
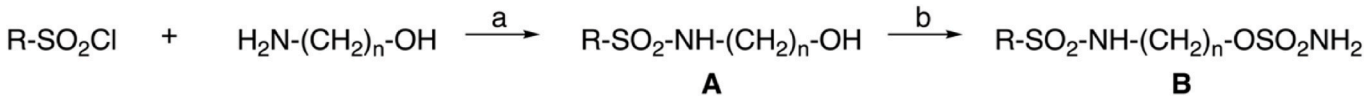
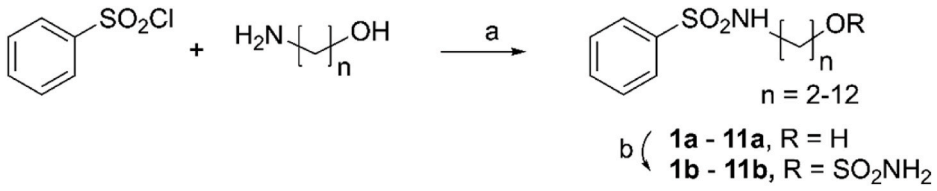


Fig. 1. Structure of established inhibitors SLC-0111 and acetazolamide (standard).



Scheme 1. Synthesis of sulfamates (structure **B**) from sulfonyl chlorides and amino-alcohols. Reactions and conditions: a) DCM, NEt_3, 20 °C, 3–24 h; b) DCM, NEt_3, sulfamoyl chloride, 0 °C → 20 °C, 3–24 h.



Scheme 2. Synthesis of target compounds **1b**–**11b** differing in the length of the alkyl spacer; a) DCM, NEt_3, 20 °C, 3–24 h; b) DCM, NEt_3, sulfamoyl chloride, 0 °C → 20 °C, 3–24 h.

reversible conversion of carbon dioxide to bicarbonate and a proton. Both human and bovine CA II hold a similar three-dimensional structure. This structure includes an active site where a zinc ion is coordinated by three histidine residues (His94, His96, and His119). The presence of these conserved histidine residues is crucial as they facilitate the enzyme's catalytic function by stabilizing the transition state and activating water molecules necessary for the reaction. Despite these significant similarities, there are differences between human and bovine CA II, particularly in their N-terminal amino acid sequences. The variations in this region between human and bovine CA II may lead to differences in the proton transfer pathway of the catalytic cycle [22–24].

2. Results and discussion

Several series of compounds were synthesized to systematically characterize the influence of different structural elements on biological activity. In a first series of compounds, the influence of the chain length of the spacer between sulfonamide and sulfamate was investigated. The reaction of phenylsulfonyl chloride with 1,ω-aminoalcohols gave the intermediates **1a**-**11a**, whose further reaction led to the sulfamates **1b**–**11b** (Scheme 2).

Compounds **1a**-**11a** and **1b**–**11b** were screened for their inhibitory activity employing *b*CAII; the results of these assys are compiled in Table 1. Thereby, no activity was established for **1a**-**11a**.

In this group it is noticeable that the most active compounds either have a very short alkyl spacer (**1**, n = 2, with I = 59.2 %), or a maximum of inhibitory activity is reached with a chain length of the spacer of n = 8 (compound **7**, I = 60.2 %). A significant decrease in activity is associated with compounds with even longer spacers (Fig. 2).

To determine the influence of electron donating or electron pushing substituents, series 2 was synthesized (Scheme 3); these compounds have in common that they contain a corresponding substituent (Me, OMe or F) in the *para* position.

Table 1
Inhibition (I in %) of *b*CA II by compounds **1b**–**11b** (at 1 μM) concentration of the inhibitor); acetazolamide (**AAZ**) was used as a positive standard. All experiments were performed in triplicate with three technical replicas.

Cmp.	Inhibition [%]	Cmp.	Inhibition [%]
AAZ	99.2 ± 0.2	**6b**	56.0 ± 0.8
1a-11a	<5	**7b**	60.2 ± 0.7
1b	59.2 ± 0.8	**8b**	55.4 ± 0.7
2b	39.9 ± 0.4	**9b**	10.7 ± 0.4
3b	32.2 ± 0.3	**10b**	7.2 ± 0.9
4b	51.8 ± 0.7	**11b**	5.9 ± 0.9
5b	56.0 ± 0.5		

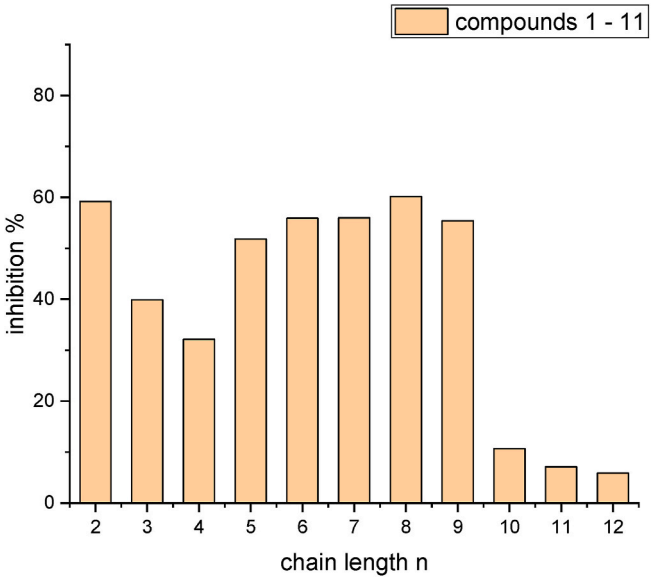
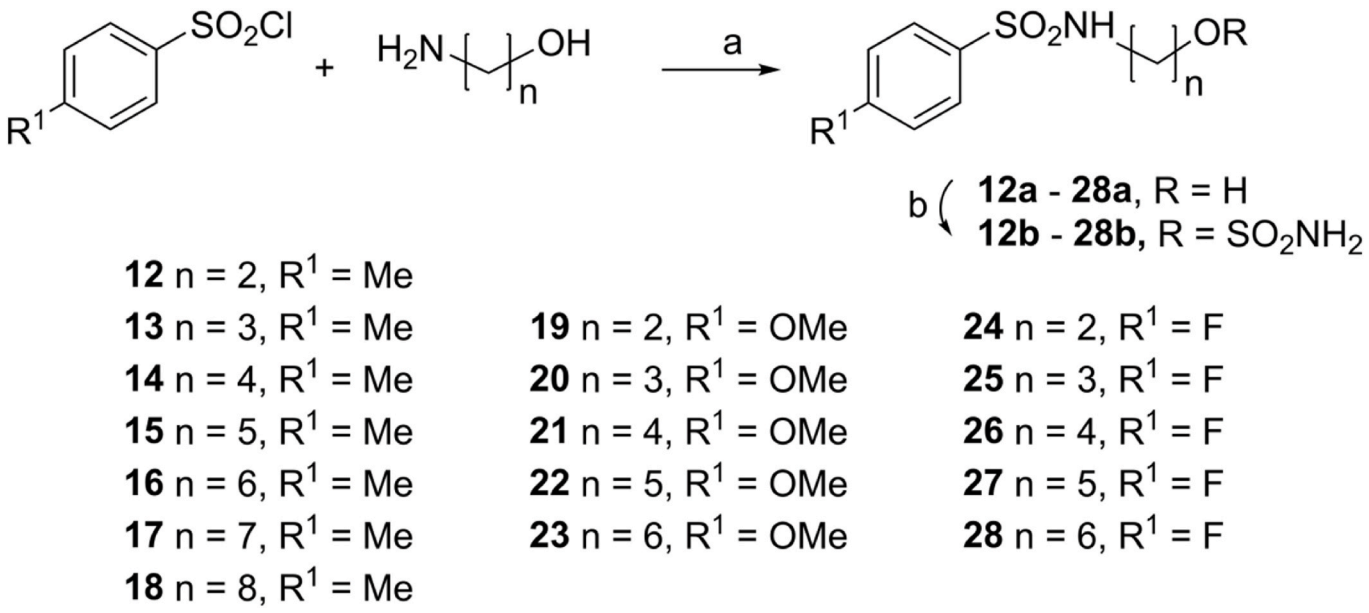


Fig. 2. Inhibition (in %) of unsubstituted compounds **1b**–**11b**.



Scheme 3. Structure of compounds of the second series with electron pushing substituents; a) DCM, NEt_3, 20 °C, 3–24 h; b) DCM, NEt_3, sulfamoyl chloride, 0 °C → 20 °C, 3–24 h.

Table 2
Inhibition (I in %) of *b*CA II by compounds **12b–28b** (at 1 μM) concentration of the inhibitor); acetazolamide (**AAZ**) was used as a positive standard. All experiments were performed in triplicate with three technical replicas.

Cmp.	Inhibition [%]	Cmp.	Inhibition [%]
AAZ	99.2 ± 0.2	**20b**	64.0 ± 3.4
12a-28a	<5	**21b**	80.9 ± 3.2
12b	75.8 ± 0.6	**22b**	62.5 ± 2.5
13b	63.8 ± 0.8	**23b**	63.4 ± 1.1
14b	42.9 ± 0.1	**24b**	63.8 ± 1.4
15b	58.9 ± 0.2	**25b**	41.9 ± 0.7
16b	40.3 ± 0.7	**26b**	50.5 ± 0.2
17b	42.8 ± 0.5	**27b**	43.8 ± 0.9
18b	54.7 ± 0.6	**28b**	60.3 ± 0.4
19b	40.5 ± 0.7		

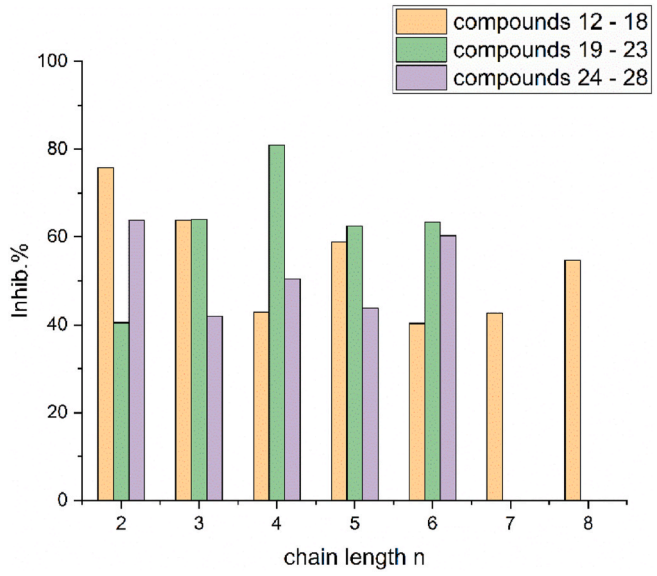


Fig. 3. Inhibition (in %) of the second series **12b–28b**.

Initial molecular modeling calculations had shown that a substituent in the *para* position should result in a better fit of the molecule in the active center of CA II than would have been the case with a substituent in the *ortho* or *meta* position. The results of the assays are summarized in Table 2 and Fig. 3.

In a third series, the (un)-substituted phenylsulfonamide moiety of both series 1 and 2 was replaced by a phenylvinylsulfonamide moiety to yield via **29a-35a** finally **29b–35b** (Scheme 4). Reaction of the amino alcohols with pyridine-3-sulfonyl chloride (yielding **36a** and **37a**, respectively) followed by reaction with sulfamoyl chloride gave the compounds **36b** and **37b**. For comparison, in this series, benzoyl chloride was also reacted with ethanolamine to give **38a**, whose reaction with sulfamoyl chloride gave **38b**. The results of the enzymatic tests are summarized in Table 3 and Fig. 4.

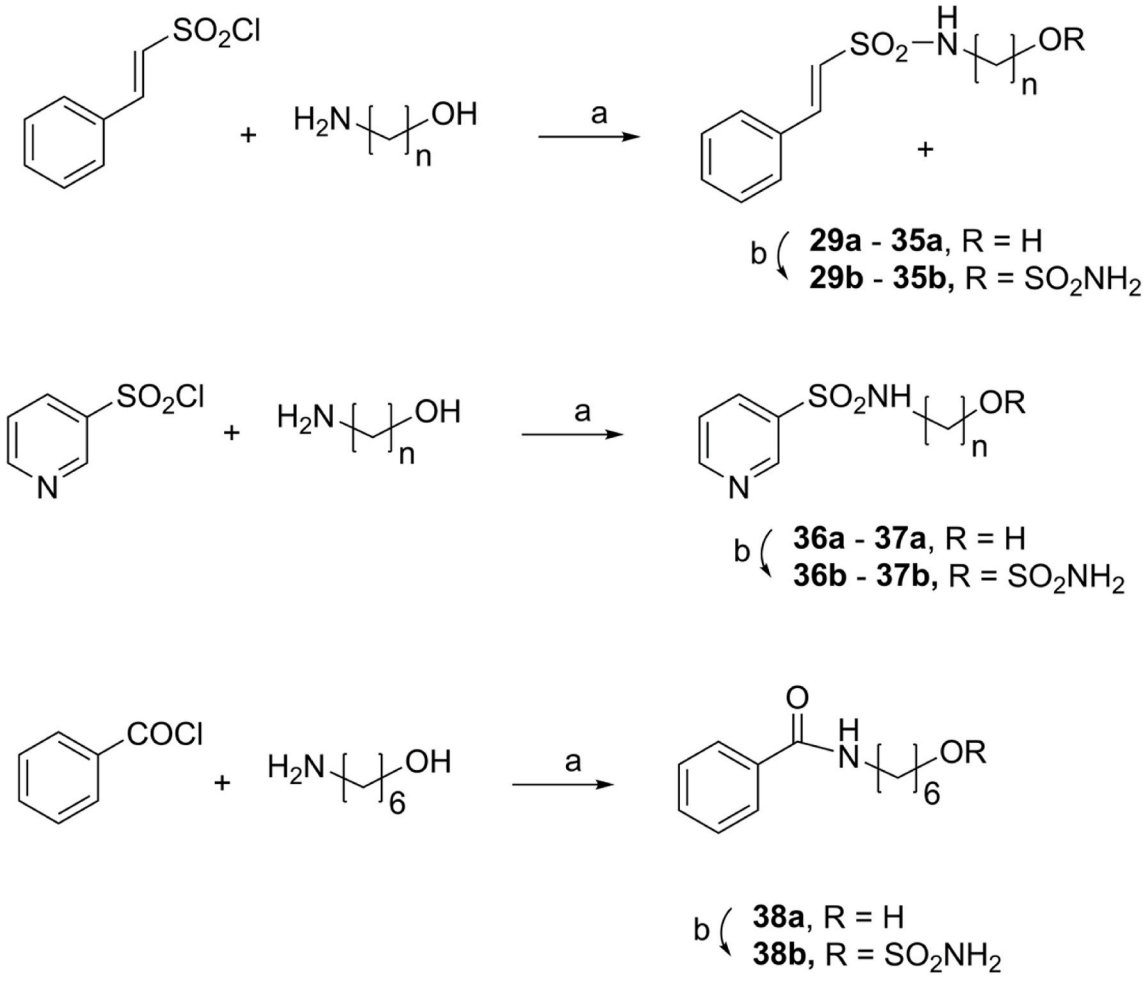
Compounds **29b–35b** show decreased activity with longer chains, suggesting steric hindrance or altered molecular interactions. Conversely, compounds **36b** and **37b** display enhanced activity with elongated chains. Notably, compound **38b** exhibits activity similar to compound **33b** despite similar chain lengths.

Finally, to clarify the question of whether an aromatic moiety is necessary at all, cyclopropylsulfonyl chloride or methylsulfonyl chloride (Scheme 5) was used as the starting material in a 4th series. The reaction with the amino alcohols gave the compounds **39a-47a**, which were converted to the compounds **39b–47b**. The reaction of **46a** with methylsulfonyl chloride gave **48** as a by-product. The results of the assays are summarized in Table 4 and Fig. 5.

In contrast to the series with an aromatic moiety this series increases the inhibitory activity with longer chain lengths. Both series of compounds with the cyclopropyl – and methyl residue indicate the importance of longer alkyl chains for increased activity.

In contrast to the series featuring an aromatic moiety, the current series demonstrates a notable increase in inhibitory activity with longer chain lengths. This observation suggests a distinct structural dependence on alkyl chain elongation for heightened efficacy. Both series of compounds, characterized by the presence of cyclopropyl and methyl residues, underscore the important role of longer alkyl chains in augmenting activity (Fig. 6).

Nevertheless, Fig. 7 highlights a contrasting trend where the highest enzymatic activity is observed in compounds possessing shorter chain lengths along with a methyl group in *para*-position.



Scheme 4. Synthesis and structure of compounds of the third series; a) DCM, NEt_3, 20 °C, 3–24 h; b) DCM, NEt_3, sulfamoyl chloride, 0 °C → 20 °C, 3–24 h.

Table 3
Inhibition (I in %) of *b*CA II by compounds **29b**–**38b** (at 1 μM) concentration of the inhibitor); acetazolamide (**AAZ**) was used as a positive standard. All experiments were performed in triplicate with three technical replicas.

Cmp.	Inhibition [%]	Cmp.	Inhibition [%]
AAZ	99.2 ± 0.2	**33b**	51.4 ± 3.4
29a-38a	<5	**34b**	45.9 ± 1.3
29b	73.5 ± 0.7	**35b**	33.5 ± 0.4
30b	56.2 ± 1.6	**36b**	34.5 ± 1.4
31b	54.0 ± 1.1	**37b**	61.1 ± 0.5
32b	51.4 ± 1.6	**38b**	48.4 ± 0.6

Tabelle 4
Inhibition (I in %) of *b*CA II by compounds **39b**–**47b** (at 1 μM) concentration of the inhibitor); acetazolamide (**AAZ**) was used as a positive standard. All experiments were performed in triplicate with three technical replicas.

Cmp.	Inhibition [%]	Cmp.	Inhibition [%]
AAZ	99.1 ± 0.4	**43**	47.9 ± 0.1
39a-47a	<5	**44**	45.2 ± 1.8
39	23.7 ± 0.1	**45**	55.4 ± 2.6
40	14.7 ± 0.3	**46**	25.6 ± 1.6
41	41.2 ± 0.3	**47**	62.7 ± 1.8
42	39.4 ± 0.9	**48**	<5

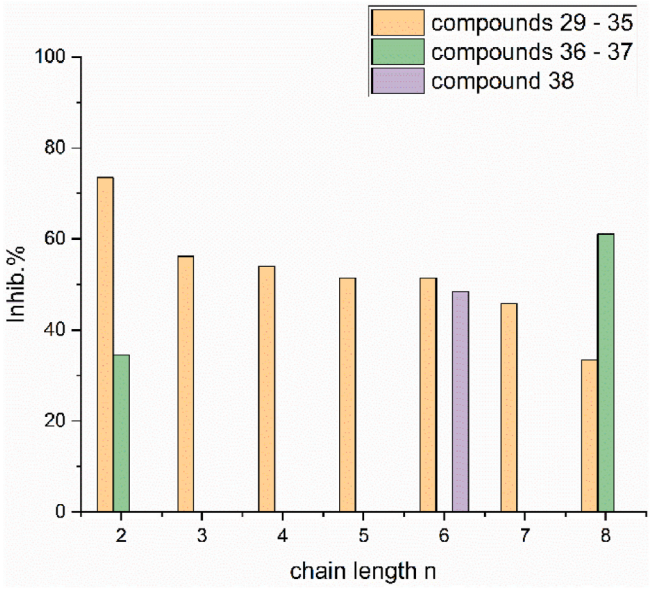


Fig. 4. Inhibition (in %) of the third series **29b**–**38b**.

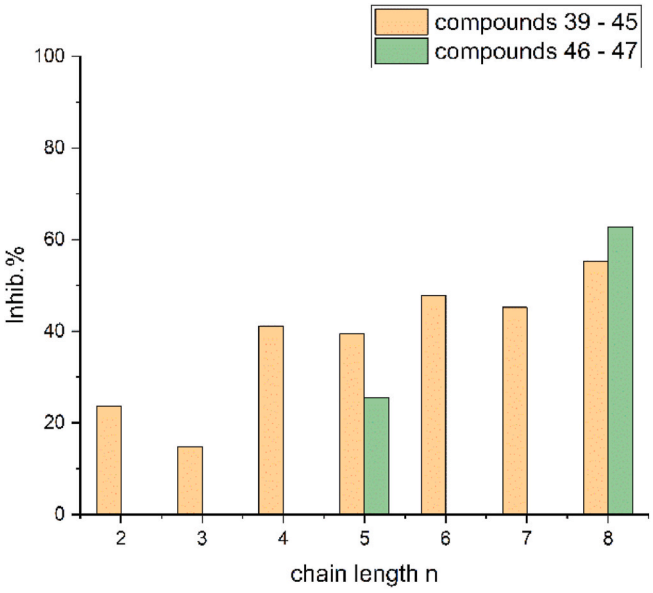
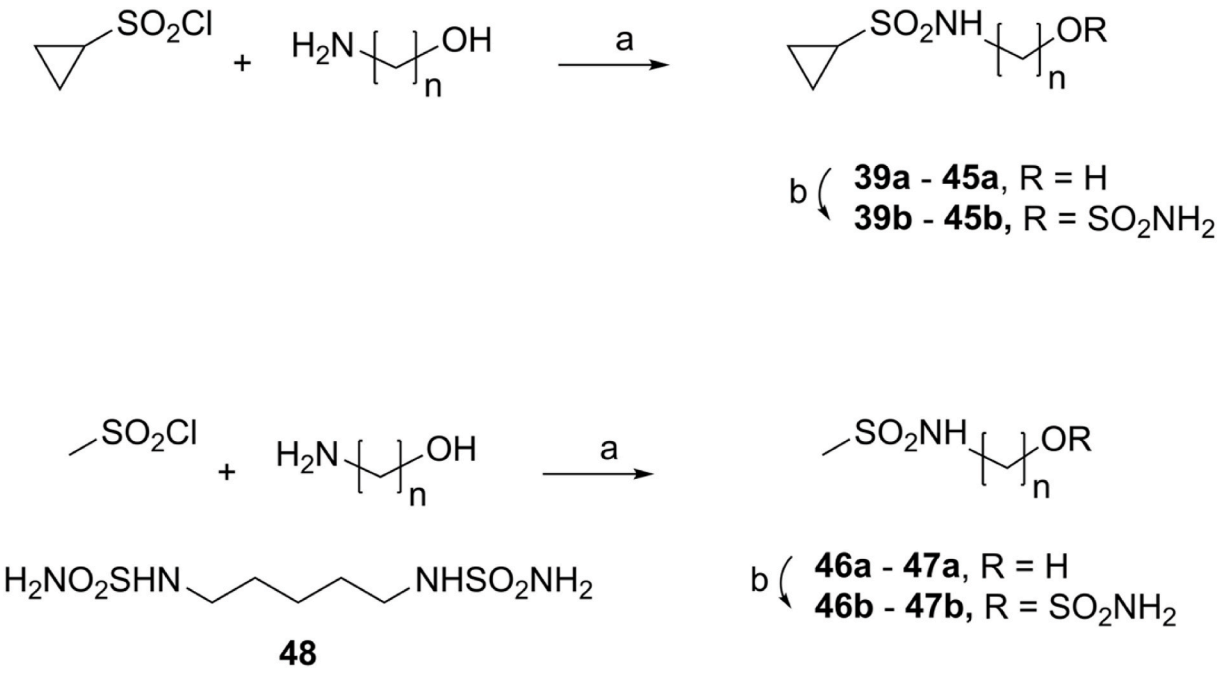


Fig. 5. Inhibition (in %) of the 4th series **39b**–**47b**.

Other electron pushing substituents exhibit a comparable effect, but with slightly less activity (Fig. 8).

Extra measurements were performed for compounds **21b**, **24b**, **29b** and **47b** to determine their respective inhibition constants K_i. Dixon plots are depicted in Fig. 9 together with the respective inhibition constants. All of these compounds proved to be competitive inhibitors.



Scheme 5. Structure of compounds of the 4th series; a) DCM, NEt_3, 20 °C, 3–24 h; b) DCM, NEt_3, sulfamoyl chloride, 0 °C → 20 °C, 3–24 h.

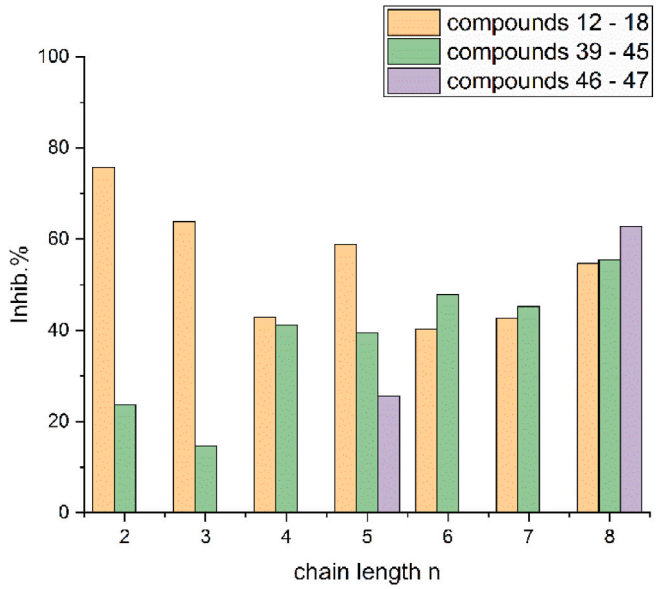


Fig. 6. Inhibition (in %) of the 4th series **39b**–**47b** without an aromatic moiety vs compounds **12b**–**18b**.

To rationalize these results on a molecular basis, some molecular modeling calculations were performed. For the molecular docking studies, the Molecular Operating Environment (MOE 2020) software was utilized. The enzyme structure was obtained from the PDB Database (PDB ID: 3HS4) and prepared using the QuickPrep tool. Ligands were deprotonated to match the crystal structure of the co-crystallized ligand. Docking was performed with a pharmacophore model that included the interaction of the sulfamate group with the zinc ion. Initial placement of the ligands was carried out using the Triangle Matcher algorithm, generating 30 poses that were scored using the London dG scoring function. The top five poses were then refined with a rigid receptor model and rescored using the GBVI/WSA dG scoring method. The best pose was evaluated through comparison with the expected logical structure and interactions. Successful redocking of the co-crystallized ligand, acetazolamide, validated the docking protocol.

The calculations indicate that the increased enzymatic activity of compound **29b** compared to compound **35b** can be explained inasmuch as that for compounds holding shorter alkyl chains, there is enhanced stabilization via aromatic-π interactions with His94, in addition to favorable side chain donor interactions facilitated by the double bond with Gln92. Furthermore, the increase in inhibition observed with longer chain lengths of the cyclopropyl and methylsulfonyl species may be attributed to the reduced steric hindrance due to the relatively compact size of the sulfonamide moiety. Fig. 10 depicts the results from these calculations (results for compounds **7b**, **12b**, **21b**, **28b**, **36b**, **37b**, **38b**, **39b**, **45b** and **46b** can be found in the Supplementary Materials File).

3. Conclusion

This sudy presents the successful synthesis of a diverse library of arylsulfonamido-alkylsulfamates and their evaluation as inhibitors of carbonic anhydrase II (CA II). Among the synthesized compounds, several exhibited potent competitive inhibition, notably compound **47b**, with a K_i value of 0.9 μM. The inhibitory activities were significantly influenced by the structural features of the arylsulfonamide moiety and the spacer length connecting it to the sulfamate group. Shorter spacers in aryl-substituted analogues enhanced inhibitory activity, potentially due to favorable aromatic π-interactions within the active site of CA II. In contrast, cyclopropyl- and methylsulfonyl-substituted analogues showed high inhibitory activity attributed to minimized steric hindrance. These findings provide valuable insights into the design of effective CA II inhibitors, highlighting the importance of both the substituent pattern and spacer length in optimizing inhibitor efficacy. Further research could focus on fine-tuning these structural parameters to develop more potent and selective CA II inhibitors for therapeutic applications.

4. Experimental

4.1. General

Starting materials were obtained from local vendors; solvents were dried under usual conditions; equipment and assays were used as previously described [15–21].

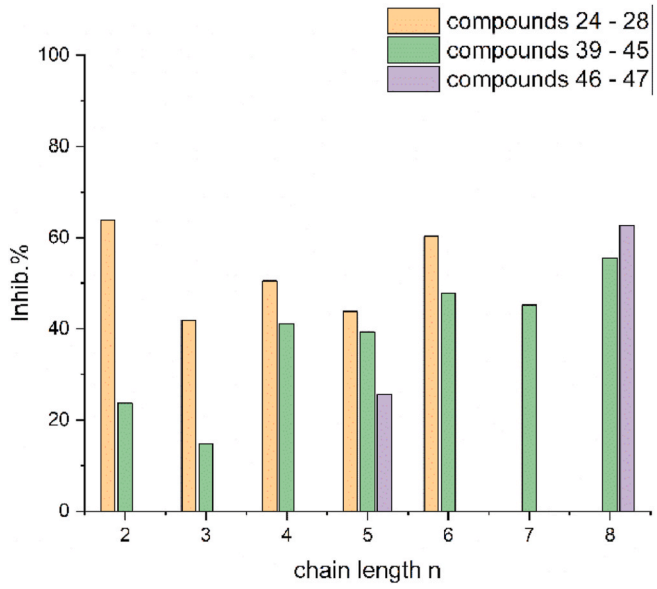


Fig. 7. Inhibition (in %) of the 4th series **39b**–**47b** without an aromatic moiety vs compounds **24b**–**28b** with fluorine moiety.

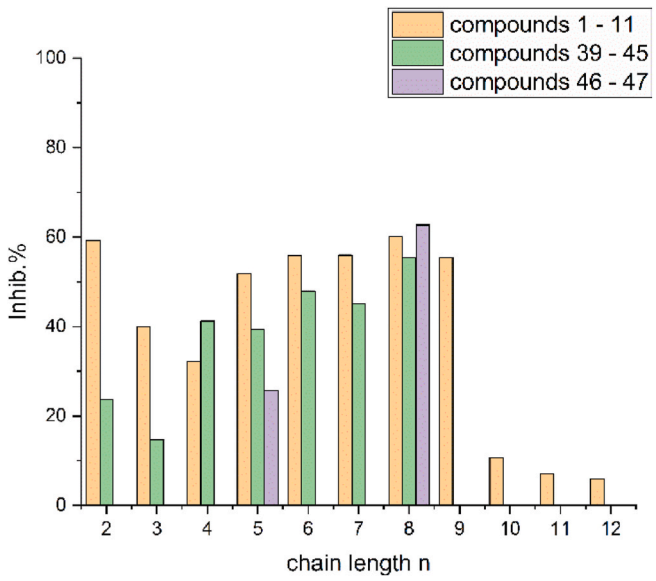
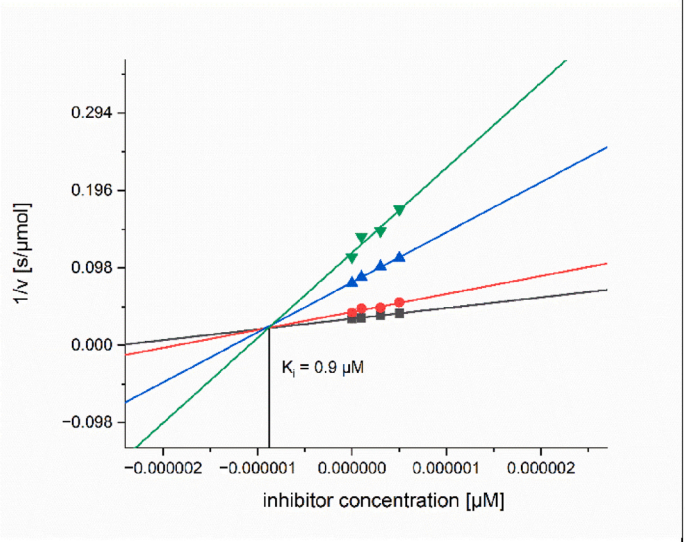
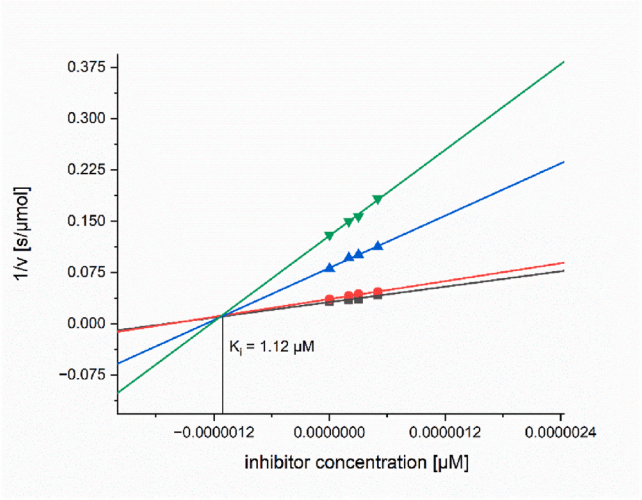


Fig. 8. Inhibition (in %) of the 4th series **39b**–**47b** without an aromatic moiety vs compounds **1b**–**11b**.

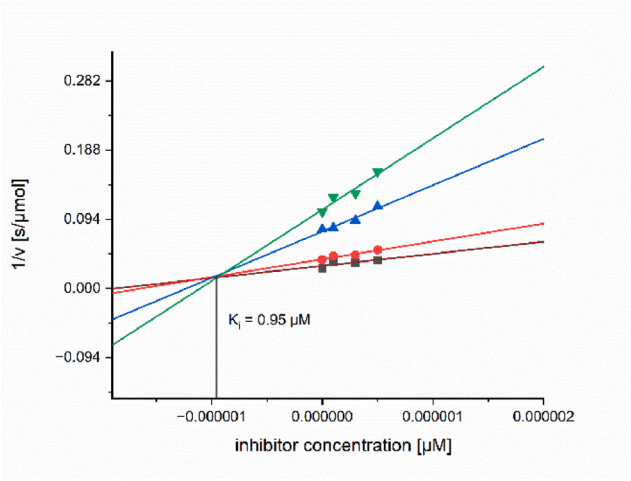
21b (inhibitor concentration used: 0.1, 0.3, 0.5 μM); $K_i = 0.90 \pm 0.03$ μM



24b (inhibitor concentration used: 0.2, 0.3, 0.5 μM); $K_i = 1.12 \pm 0.02$ μM



29b (inhibitor concentration used: 0.1, 0.3, 0.5 μM); $K_i = 0.95 \pm 0.04$ μM



47b (inhibitor concentration used: 0.1, 0.2, 0.3 μM); $K_i = 0.59 \pm 0.02$ μM

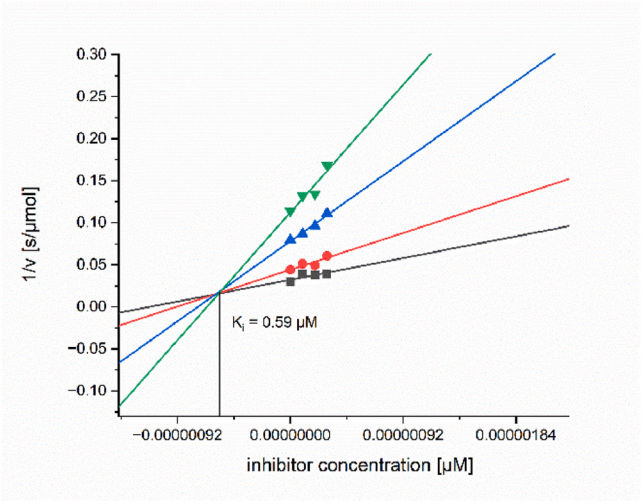


Fig. 9. Dixon plots for **21b**, **24b**, **29b** and **47b**.

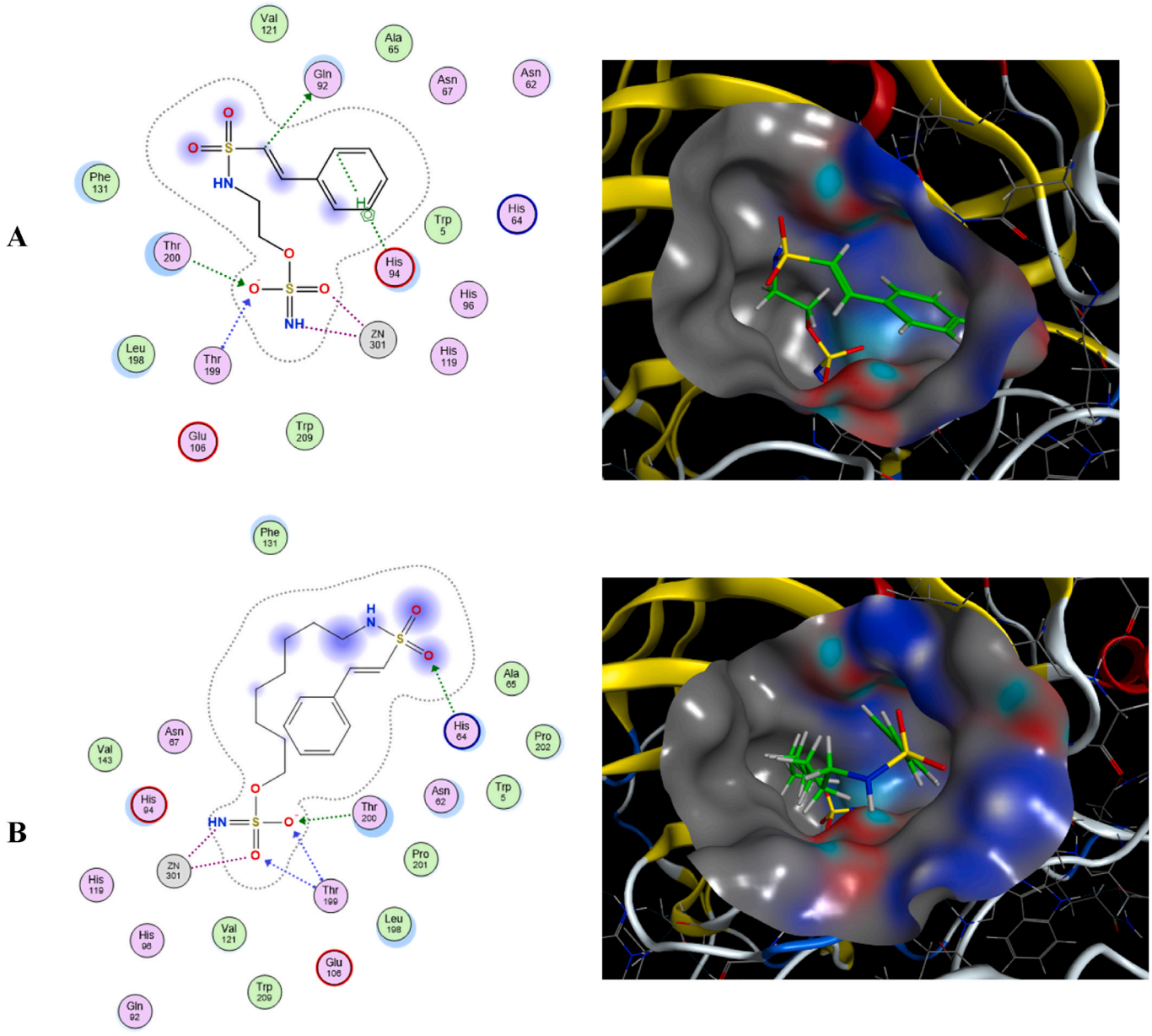


Fig. 10. Calculated 2D and 3D representations showing the interactions of **29b** (A) and **35b** (B) within the active site of CA II.

*4.2. Synthesis of the sulfonamides (general procedure A, GPA) **1a–47a***

To a solution containing the corresponding amino-alcohol (1.5 equiv.) in dry dichloromethane (DCM, 12 mL), dry triethylamine (2 equiv., TEA) and the corresponding sulfonyl chloride (1 equiv.) were added at 22 °C. The reaction mixture was stirred at 22 °C for 3 h. The volatiles were removed under diminished pressure, and the residue was subjected to column chromatography (SiO_2) to yield **1a–47a**. For long-chain amino-alcohols (n = 8–12), the solvent composition was modified, and a 1: 1 mixture of DCM and acetonitrile was used instead.

*4.3. Synthesis of the sulfamates (general procedure B, GPB) **1b–47b***

To a solution of **1a–47a** (1 equiv.) in dry DCM (6 mL), TEA (3 equiv.) was added, followed by the slow addition of sulfamoyl chloride (3 equivalents). The reaction mixture was stirred at 22 °C until TLC showed completion of the reaction. The volatiles were removed under reduced pressure, and the residue was subjected to column chromatography (SiO_2) to afford **1b–47b**.

4.4. Characterization of the compounds

Experimental details, full characterization (m.p., R_f, IR, UV/vis, MS, micro-analysis) and a depiction of all NMR spectra (^1H, ^{13}C) can be found in the Supplementary Materials file.

CRediT authorship contribution statement

Toni C. Denner: Writing – review & editing, Writing – original draft, Investigation. **Niels V. Heise:** Writing – review & editing, Writing – original draft, Investigation. **René Csuk:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

We like to thank Dr. D. Ströhl, Y. Schiller and S. Ludwig for the NMR spectra. MS, UV/vis and IR spectra were recorded by M. Schneider who also performed the micro-analyses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmcr.2024.100177.

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Stereochemistry matters: Inhibition of carbonic anhydrase II by amino acid derived sulfamates depends on their absolute configuration

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ABSTRACT

Aminoalcohols were converted into the corresponding enantiomeric phenylsulfonamide sulfamates. These compounds proved to be inhibitors of carbonic anhydrase II. Interestingly, a sulfamate derived from (*S*)-tryptophan was no inhibitor at all while its (*R*) configurated enantiomer was an excellent inhibitor of carbonic anhydrase II (CA II). A rationale can be deduced from molecular modeling studies. The sulfamates derived from (*R*) or (*S*) proline held very low inhibition constants for this enzyme as $K_i = 0.77$ μM and 0.70 μM, respectively.

1. Introduction

The enzyme carbonic anhydrase II (CA II) has recently become the focus of increasing scientific interest [1–11] because inhibitors of this enzyme have been shown to be synergistic in the treatment of glioblastoma, which is otherwise very difficult to treat [12]. Spacered aryl-substituted type **A** sulfonamides (Fig. 1) have been shown [13] to be effective inhibitors of CA II. CA II has been detected not only in glioblastoma but also in astrocytoma, oligodendroglioma and medulloblastoma [14,15]. The presence of CA II has also been documented in oligoastrocytoma [16]. Glioblastoma is considered to be the most common and lethal type of brain tumor, with an average survival time of about 15 months after diagnosis [17,18].

In glioblastoma, there is a correlation between malignancy and CA II expression [15,16]. A high level of expression usually results in a shorter remaining lifespan. Therefore, it was of primary interest to synthesize first-in-class type **B** compounds and determine their CA II inhibitory properties.

2. Results and discussion

The synthesis of model compound **1b** can be carried out in a number of ways and, on the basis of preliminary tests, we decided to use the very simple synthesis shown in Scheme 1, which not only promised high yields but also provided sufficient degrees of freedom to make in future as many modifications as possible [19]. Analogue syntheses were described very early in the scientific literature and proved to be very robust and reliable.

The reaction of benzenesulfonyl chloride with ethanolamine afforded the sulfonamide **1a**, known from the literature [20–22], in 95 % yield, whose reaction with sulfamoyl chloride afforded the model compound **1b** in 82 % isolated yield.

Compound **1b** proved to be an inhibitor of CA II in preliminary tests (vide infra; concentration of the inhibitor: 10 μM); accompanying molecular modeling calculations suggested that substituents in the α-position should have an influence on the extent of inhibition. The use of readily available amino alcohols derived from α-aminocarboxylic acids would also allow conclusions to be drawn about the possible influence of the absolute configuration of the final products obtained.

The reaction of benzenesulfonyl chloride with the enantiomerically pure amino alcohols (both enantiomers of alaninol, leucinol, prolinol, valinol, phenylalaninol, methioninol, tryptophanol and (*S*, *S*)-isoleucinol) gave the products **2a** – **16a**, whose reaction with sulfamoyl chloride afforded the final products **2b**–**16b**.

In their ^{1}H NMR spectra, the aminoalkylbenzenesulfonamides **2a**-**16a** are characterized by the signal for NH at $\delta = 7.3$–7.6 ppm and that for OH at $\delta = 4.4$–4.8 ppm. In the ^{13}C NMR spectra the CH_2–OH group is found in the range $\delta = 60$–65 ppm, as expected. In the ATR-IR spectra the *C*–N valence vibration is found at $\nu = 1306$–1331 cm^{-1}, the SO_2 valence vibration between $\nu = 1151$–1165 cm^{-1} and the *C*–O valence vibration at about $\nu = 1050$ cm^{-1}. As expected, the UV-VIS-Spectra show the max absorption for the benzenesulfonyl residue at $\lambda = 221$ nm.

Biological testing of the compounds was performed in 96-well microtiter plates and yielded the inhibition data summarized in Table 1; Fig. 2 depicts the inhibition percentages. Acetazolamide (**AAZ**) was included as a positive control [23–26].

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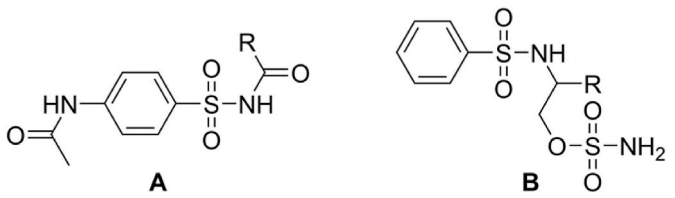


Fig. 1. Structures known CA II inhibitors of type **A**, and of presumptive inhibitors type **B**.

For the enantiomeric sulfamates derived from Ala, Leu, Phe, Pro and Val, approximately equal inhibition-% were determined; at a concentration of 10 μM, inhibition-% between 50 and 98 % was achieved; the product **12b** derived from (*S*, *S*)-isoleucine did not prove to be a very good inhibitor at all.

Interestingly, the product **16b** derived from (*S*)-Trp did not show any inhibitory activity, whereas the product **15b** derived from (*R*)-Trp proved to be one of the most potent inhibitors and 97.9 % inhibition was achieved. In comparison, 99.3 % inhibition was measured for the standard **AAZ** at the same concentration.

Extra measurements were performed to determine their respective inhibition constants K_i. Representative Dixon plots for compounds **6b** and **7b** are depicted in Fig. 3. For **15b** (derived from (*R*)-Trp) a Dixon and a Cornish-Bowden plot are depicted in Fig. 4.

For these compounds K_i values as low as 0.77 and 0.70 μM were observed, respectively.

Molecular modeling calculations were carried out to gain a better understanding. As exemplified for **6**–**9** (Fig. 5; high resolution picture can be found in the supplementary materials file) no significant differences in their respective mode of binding were observed for these compounds.

This result is in excellent agreement with the results from the bioassays. As far as compounds **15b** and **16b** are concerned, it was found that the amino group of the sulfamate of compounds **15 b**/**16b** binds to the Zn^{2+}, with **16b** (Fig. 6) forming an additional bond with the oxygen. Another bond occurs with Thr199 of the enzyme.

In **15b** (see c), a sidechain donor interaction is expected from the amino group of the BSC residue to Thr200, with Thr200 thereby acting as both an acceptor and a sidechain donor, forming a bond with an oxygen of the sulfamate residue. In addition, an aromatic H interaction with Phe131 and the aromatic indole ring system is possible, which is not expected for **16b**. From the measured inhibition percentage, it could be concluded that the interactions of **16b** with CA II is possibly too weak and its binding in the active site is not sufficiently stabilized so that no enzyme inhibitory activity is detected, whereas in the (*R*) configuration the additional aromatic H interactions and the side chain donor/acceptor interactions ensure good binding in the enzyme's pocket so that high inhibition percentages was measured.

Table 1
Results from the inhibition assays for CA II (concentration of inhibitor 10 μM); experiments were performed in triplicate with each 3 replicas. Acetazolamide (**AAZ**) was used as a positive control. Compounds **1a** – **16a** showed no inhibition at all.

Compound	[%] inhibition	compound	[%] inhibition
AAZ	99.3 ± 0.1	**1b**	64.8 ± 0.4
2b	85.3 ± 0.2	**3b**	69.4 ± 1.7
4b	89.1 ± 0.6	**5b**	74.5 ± 0.3
6b	98.3 ± 0.1	**7b**	97.5 ± 0.2
8b	50.9 ± 1.5	**9b**	72.3 ± 0.5
10b	81.2 ± 2.2	**11b**	69.6 ± 0.8
12b	69.1 ± 1.0	**13b**	<5
14b	<5	**15b**	97.9 ± 0.2
16b	<5		

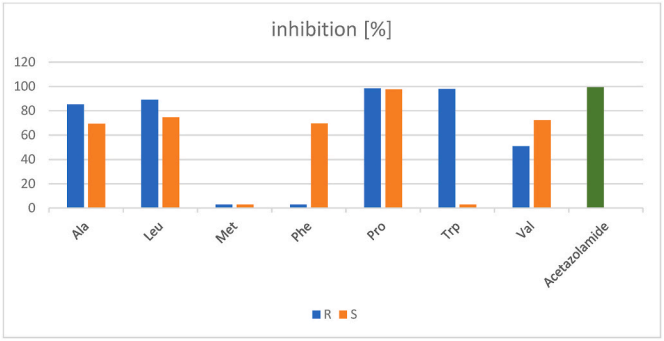
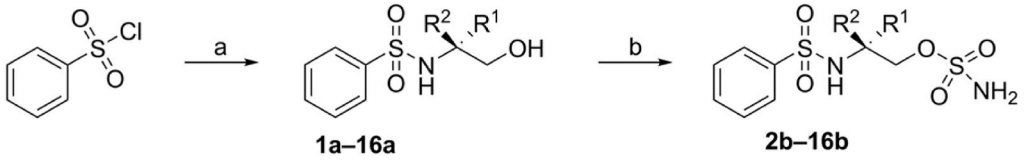


Fig. 2. Inhibition of CA II by amino acid derived sulfamates; acetazolamide was used as a positive control.

3. Conclusion

As a result of this first series of compounds, it has been shown that apparently amino acid derived (*R*)-configured compounds are better inhibitors of CA II than their (*S*)-configured enantiomers. It was also shown that the presence of relatively large, bulky residue in α-position can induce higher inhibitory activity, which can be explained by additional stabilizing interactions in the active site of the enzyme. These compounds therefore proved suitable for further derivatization to produce even better inhibitors. The absolute configuration of tryptophan derivatives, as exemplified by compounds **15b** and **16b**, significantly



1 $R^1 = R^2 = H$
2 $R^1 = CH_3$, $R^2 = H$ (from (*R*)-Ala)
3 $R^1 = H$, $R^2 = CH_3$ (from (*S*)-Ala)
4 $R^1 = CH_2\text{-}CH(CH_3)_2$, $R^2 = H$ (from (*R*)-Leu)
5 $R^1 = H$, $R^2 = \text{-}CH_2\text{-}CH(CH_3)_2$ (from (*S*)-Leu)
6 $R^1 = CH_2$-pyrrolidin-2-yl, $R^2 = H$ (from (*R*)-Pro)
7 $R^1 = H$, $R^2 = CH_2$-pyrrolidin-2-yl (from (*S*)-Pro)
8 $R^1 = CH(CH_3)_2$, $R^2 = H$ (from (*R*)-Val)
9 $R^1 = H$, $R^2 = CH(CH_3)_2$ (from (*S*)-Val)
10 $R^1 = CH_2$-Phe, $R^2 = H$ (from (*R*)-Phe)
11 $R^1 = H$, $R^2 = CH_2$-Phe (from (*S*)-Phe)
12 $R^1 = CH(CH_3)\text{-}CH_2\text{-}CH_3$ (from (*S*)-Isoleu), R = H
13 $R^1 = CH_2\text{-}CH_2\text{-}S\text{-}CH_3$, $R^2 = H$ (from (*R*)-Met)
14 $R^1 = H$, $R^2 = CH_2\text{-}CH_2\text{-}S\text{-}CH_3$ (from (*S*)-Met)
15 $R^1 = CH_2$-(1H-indol-3-yl), $R^2 = H$ (from (*R*)-Trp)
16 $R^1 = H$, $R^2 = CH_2$-(1H-indol-3-yl) from (*S*)-Trp)

Scheme 1. Reactions and conditions: a) DCM, NEt_3, 20 °C 3–24 h; b) DCM, NEt_3, sulfamoyl chloride, 0 °C → 20 °C, 3–24 h.

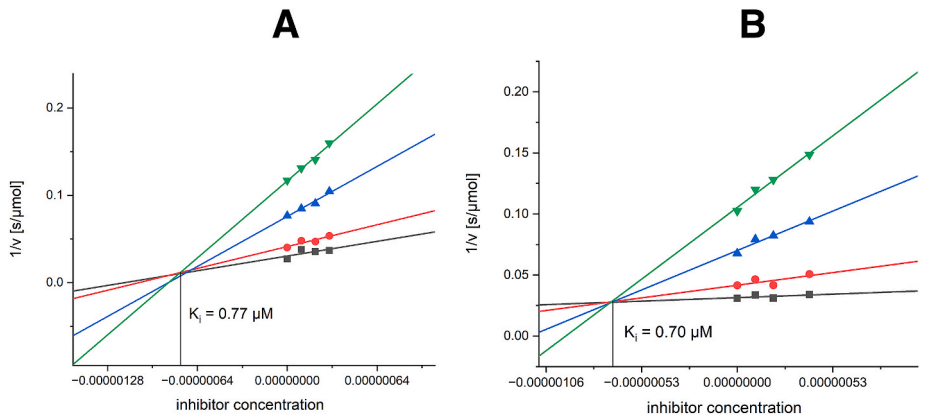


Fig. 3. Dixon plots for compounds **6b** (A, (*R*)-Pro derived, concentration of inhibitor: 0.1, 0.2, 0.3 µM) and **7b** (B, (*S*)-Pro-derived, concentration of the inhibitor: 0.1, 0.2, 0.4 µM).

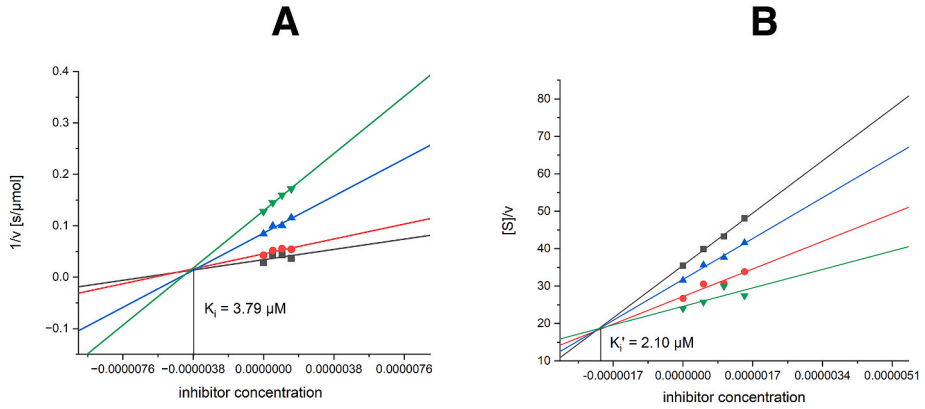


Fig. 4. Dixon (**A**) and Cornish-Bowden plot (**B**) for (*R*)-Trp derived compound **15b** (concentration of the inhibitor: 0.5, 1, 1.5 µM).

influences their interactions with CA II. Molecular modeling studies shed light on the binding modes of these enantiomers with CA II. Compound **15b** likely forms strong interactions within the active site, such as aromatic hydrogen interactions and side chain donor/acceptor interactions, contributing to its potent inhibitory activity. In contrast, compound **16b** exhibits significantly weaker interactions, which are obviously insufficient to stabilize the binding ofvthe molecule to the enzyme. The active site of the CA II enzyme likely has stereochemical preferences for certain ligands. Compound **15b**, with its specific spatial arrangement and functional groups, fits snugly into the active site, allowing for optimal binding interactions. This facilitates effective inhibition of the enzyme. Conversely, compound **16b**, holding a different absolute configuration, fails to achieve effective binding orientations within the active site, thus resulting in the absence of inhibitory activity. In summary, the stereochemistry of tryptophan derivatives dictates their interactions with CA II, impacting their inhibitory activity. Compound **15b**, with its (*R*)-configuration, demonstrated potent inhibition due to favorable interactions and high binding affinity, while compound **16b**, holding a (*S*)-configuration, exhibits weaker interactions and lack of inhibitory activity, underscoring the importance of absolute configuration in enzyme inhibition.

4. Experimental

4.1. General

Equipment and conditions of the assay were previously described [23–26]. Sulfamoyl chloride (SC), benzenesulfonyl chloride (BSC) and both (*R*)- and (*S*)- amino-alcohols (enantiomerically pure) were obtained from Sigma-Aldrich GmbH (Taufkirchen, Germany) and used as received.

4.2. Syntheses

4.2.1. Synthesis of the sulfonamides (General Procedure A, GPA) *1a–16a*

To a solution containing the corresponding amino-alcohol (1.5 equiv.) in dry dichloromethane (DCM, 12 mL), dry triethylamine (2 equiv., TEA) and BSC (1 equiv.) were added at 22 °C. The reaction mixture was stirred at 22 °C for 3 h. The volatiles were removed under diminished pressure, and the residue was subjected to column chromatography (SiO_2) to yield **1a–16a**.

4.2.2. Synthesis of the sulfamates (General procedure B, GPB) *1b–16b*

To a solution of **1a–16a** (1 equiv.) in dry DCM (10 mL), TEA (4 equiv.) was added, followed by the slow addition of SC (4 equivalents). The reaction mixture was stirred at 22 °C for 3–24 h. The volatiles were removed under reduced pressure, and the residue was subjected to column chromatography (SiO_2) to afford **1b–16b**.

4.3. CA II assay

Carbonic anhydrase II (bCAII, ≥3000 W-A units/mg from bovine erythrocytes) as well as 4-nitrophenyl acetate (4-NA) were purchased from Sigma-Aldrich GmbH (Taufkirchen, Germany).

BMG Labtech Spectrostar Omega (BMG Labtech, Ortenberg, Germany) working in the slow kinetics mode (measuring the absorbance at a wavelength of λ = 415 nm with center scanning) was used for the enzymatic studies. In short: A mixture of 4-NA solution (125 µL, 6 mM in

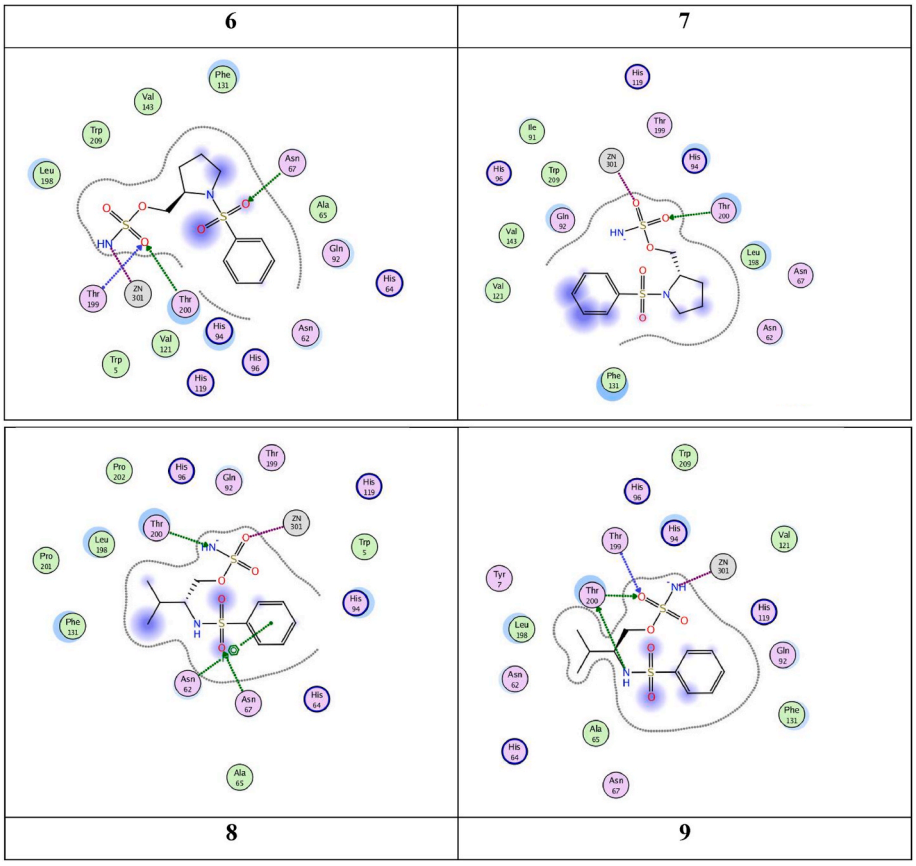


Fig. 5. 2D representation of binding and location of compounds **6**–**9** in the active site of CA II.

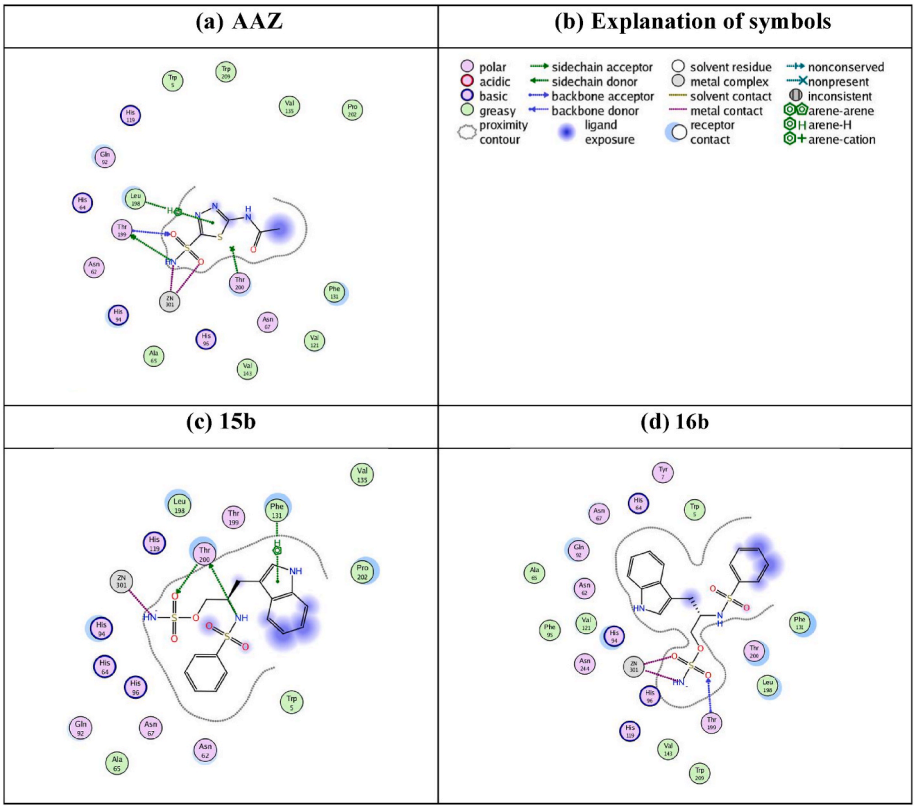


Fig. 6. 2D representation of selected interactions of compounds reference compound acetazolamide (a), and compounds **15b** (c) and **16b** (d).

50 mM Tris-HCl buffer, pH 8), enzyme solution (25 μL, 0.3 mg/mL) and compounds solutions (25 μL, 3 different concentrations and water as a blank) was incubated at 37 °C for 20 min. The substrate (25 μL, [4-NA] = 0.75 mM, 0.50 mM, 0.25 mM, 0.15 mM) was added to start the enzymatic reaction. The absorbance data was recorded under a controlled temperature of 37 °C for 30 min at 1 min intervals at λ = 415 nm. The relative inhibition was determined as the quotient of the slopes (compound divided by blank) of the linear ranges.

4.4. Individual syntheses

4.4.1. N-(2-Hydroxyethyl)benzene sulfonamide (**1a**)

Following GPA from BSC (500 mg, 2.83 mmol), TEA (0.78 mL, 5.66 mmol) and 2-amino-ethanol (259 mg, 4.25 mmol) **1a** (542 mg, 95 %) was obtained as a white, waxy solid; R_f = 0.07 (SiO_2, hexanes/ethyl acetate, 6:4); m. p. = 78–79 °C (lit. [19]: 79–80 °C); UV–Vis (MeOH): λ_{max} (log ε) = 220 nm (3.95); IR (ATR): ν = 3500*br*, 3274*br*, 2939*w*, 2883*w*, 1479*w*, 1447 *m*, 1309*s*, 1093 *m*, 1055 *m*, 999*w*, 949 *m*, 881*w*, 804*w*, 753 *m*, 718 *m*, 688 *m*, 582 *m*, 567 *m*, 450*w* cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 7.82–7.78 (m, 2H, 2-H, 2′-H), 7.66–7.56 (m, 4H, 3-H, 3′-H, 4-H, NH), 4.67 (t, J = 5.4 Hz, 1H, OH), 3.40–3.35 (m, 2H, 6-H), 2.79 (t, J = 6.4 Hz, 2H, 5-H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 140.6 (C-1), 132.3 (C-4), 129.2 (C-3), 126.4 (C-2), 59.9 (C-6), 45.1 (C-5) ppm; MS (ESI, MeOH): m/z = 200.0 (60 %, $[M - H]^-$); analyss calcd. for $C_8H_{11}NSO_3$ (201.24): C 47.75, H 5.51, N 6.96; found: C 47.50, H 5.76, N 6.69.

4.4.2. (R)-N-(1-Hydroxypropane-2-yl)-benzenesulfonamide (**2a**)

Following GPA from (*R*)-alaninol (350 mg, 4.66 mmol), TEA (1 mL, 7.78 mmol) and BSC (650 mg, 3.89 mmol) **2a** (772 mg, 77 %) was obtained as a colorless, viscous oil; R_f = 0.55 (SiO_2, hexanes/ethyl acetate, 3:7); $[\alpha]_D^{20}$ = +18.94° (*c* 0.200, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (4.03); IR (ATR) ν = 3498*w*, 3272*w*, 3067*vw*, 2976*w*, 2938*w*, 2880*w*, 1447 *m*, 1429 *m*, 1307*s*, 1292 *m*, 1232*w*, 1156*vs*, 1088*s*, 1047*s*, 971*s*, 890 *m*, 755*s*, 719*s*, 689*vs*, 639 *m*, 581*vs*, 569*vs*, 479*s*, 427 *m*, 422 *m*, 415 m cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 7.85–7.79 (m, 2H, 5-H, 9-H), 7.65–7.56 (m, 3H, 6-H, 7-H, 8-H), 7.50 (d, J = 5.0 Hz, 1H, NH), 4.63 (s, 1H, OH), 3.33–3.25 (m, 1H, 2-H), 3.14–3.05 (m, 2H, 1-H), 0.87 (d, J = 6.3 Hz, 3H, 3-H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 141.9 (C-4), 132.1 (C-7), 129.1 (C-6, C-8), 126.3 (C-5, C-9), 65.0 (C-1), 51.0 (C-2), 17.6 (C-3) ppm; MS (ESI, MeOH): m/z = 238.2 (78 %, $[M+Na]^+$), 254.2 (9 %, $[M+K]^+$), 260.2 (16 %, $[M+2Na–H]^+$); analysis calcd. for $C_9H_{13}NO_3S$ (215.27): C 50.22, H 6.09, N 6.51; found: C 50.01, H 6.31, N 6.37.

4.4.3. (S)–N-(1-Hydroxypropane-2-yl)-benzenesulfonamide (**3a**)

Following GPA from (*S*)-alaninol (260 mg, 3.45 mmol), TEA (0.9 mL, 5.76 mmol) and BSC (500 mg, 2.88 mmol) **3a** (520 mg, 70 %) was obtained as a colorless, viscous oil [27,28]; R_f = 0.56 (SiO_2, hexanes/ethyl acetate, 3:7); $[\alpha]_D^{20}$ = −19.65° (*c* 0.276, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (4.02); IR (ATR) ν = 3495*w*, 3272 *m*, 3068*vw*, 2976*w*, 2938*w*, 2880*w*, 1636*vw*, 1586*vw*, 1447 *m*, 1307*s*, 1292 *m*, 1233*w*, 1156*vs*, 1088*s*, 1047*s*, 971*s*, 927*w*, 890 *m*, 755*s*, 719*s*, 688*vs*, 639 *m*, 581*vs*, 569*vs*, 480*s*, 426 *m*, 421 *m*, 413 m cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 7.85–7.79 (m, 2H, 5-H, 9-H), 7.65–7.55 (m, 3H, 6-H, 7-H, 8-H), 7.50 (d, J = 4.9 Hz, 1H, NH), 4.66 (t, J = 5.4 Hz, 1H, OH), 3.32–3.25 (m, 1H, 2-H), 3.14–3.06 (m, 2H, 1-H), 0.87 (d, J = 6.4 Hz, 3H, 3-H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 141.9 (C-4), 132.1 (C-7), 129.1 (C-6, C-8), 126.3 (C-5, C-9), 65.0 (C-1), 50.9 (C-2), 17.5 (C-3) ppm; MS (ESI, MeOH): m/z = 238.2 (78 %, $[M+Na]^+$), 254.2 (9 %, $[M+K]^+$), 260.2 (17 %, $[M+2Na–H]^+$); analysis calcd. for $C_9H_{13}NO_3S$ (215.27): C 50.22, H 6.09, N 6.51; found: C 49.97, H 6.23, N 6.42.

4.4.4. (R)-N-(1-Hydroxy-4-methylpentane-2-yl)-benzenesulfonamide (**4a**)

Following GPA from (*R*)-leucinol (408 mg, 3.48 mmol), TEA (0.78 mL, 5.66 mmol) and BSC (500 mg, 2.83 mmol) **4a** (572 mg, 64 %) was obtained as a white solid; R_f = 0.72 (SiO_2, hexanes/ethyl acetate, 3:7); m. p. = 104.5–106.5 °C; $[\alpha]_D^{20}$ = +33.92° (*c* 0.176, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (4.04); IR (ATR) ν = 3508*w*, 3172*w*, 2957 *m*, 2932*w*, 2870*w*, 1467*w*, 1448 *m*, 1433 *m*, 1387*w*, 1367*w*, 1341*w*, 1309*s*, 1289*w*, 1203*vw*, 1165*vs*, 1125*w*, 1091*s*, 1073*w*, 1062*w*, 1032*vs*, 1020 *m*, 999*w*, 975*w*, 962 *m*, 941 *m*, 922*w*, 888*w*, 827*w*, 806*w*, 758*s*, 721*s*, 690*s*, 672*w*, 599*s*, 580*s*, 558*vs*, 497 *m*, 473 *m*, 461 *m*, 415 m cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 7.84–7.76 (m, 2H, 8-H, 12-H), 7.67–7.53 (m, 3H, 9-H, 10-H, 11-H), 7.46 (d, J = 7.3 Hz, 1H, NH), 4.59 (t, J = 5.3 Hz, 1H, OH), 3.29–3.17 (m, 1H, 1-H_a), 3.13–2.96 (m, 2H, 1-H_b, 2-H), 1.49–1.37 (m, 1H, 4-H), 1.34–1.24 (m, 1H, 3-H_a), 1.34–1.24 (m, 1H, 3-H_b), 0.73 (d, J = 6.6 Hz, 3H, 5-H), 0.54 (d, J = 6.5 Hz, 3H, 6-H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 142.0 (C-7), 132.1 (C-10), 129.0 (C-9, C-11), 126.3 (C-8, C-12), 64.1 (C-1), 53.2 (C-2), 40.5 (C-3), 23.6 (C-4), 23.2 (C-5), 21.4 (C-6) ppm; MS (ESI, MeOH): m/z = 280.3 (100 %, $[M+Na]^+$); analysis calcd. for $C_{12}H_{19}NO_3S$ (257.35): C 56.01, H 7.44, N 5.44; found: C 55.81, H 7.63, N 5.20.

4.4.5. (S)–N-(1-Hydroxy-4-methylpentane-2-yl)-benzenesulfonamide (**5a**)

Following GPA from (*S*)-leucinol (406 mg, 3.46 mmol), TEA (0.78 mL, 5.66 mmol) and BSC (500 mg, 2.83 mmol) **5a** (420 mg, 47 %) was obtained as a white solid [28]; R_f = 0.71 (SiO_2, hexanes/ethyl acetate, 3:7); m. p. = 104.5–106.5 °C; $[\alpha]_D^{20}$ = −35.19° (*c* 0.118, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (4.04); IR (ATR) ν = 3506*w*, 3171*w*, 3065*vw*, 2956*w*, 2932*w*, 2869*w*, 1467*w*, 1448*w*, 1433*w*, 1387*w*, 1367*w*, 1341*vw*, 1309 *m*, 1289*w*, 1202*vw*, 1165*vs*, 1125*w*, 1091*s*, 1073*w*, 1062*w*, 1032*vs*, 1019 *m*, 998*w*, 974*w*, 962 *m*, 941 *m*, 922*w*, 888*w*, 827*w*, 806*w*, 758*s*, 721*s*, 690*s*, 672*w*, 599*s*, 580*s*, 558*vs*, 497 *m*, 473 *m*, 460 *m*, 429*w*, 416 m cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 7.86–7.75 (m, 2H, 8-H, 12-H), 7.66–7.53 (m, 3H, 9-H, 10-H, 11-H), 7.46 (*d*, J = 7,3 Hz, 1H, NH), 4.59 (t, J = 5.4 Hz, 1H, OH), 3.28–3.20 (m, 1H, 1-H_a), 3.12–2.98 (m, 2H, 1-H_b, 2-H), 1.51–1.36 (m, 1H, 4-H), 1.35–1.22 (m, 1H, 3-H_a), 1.15–1.02 (m, 1H, 3-H_b), 0.73 (d, J = 6.6 Hz, 3H, 5-H), 0.54 (d, J = 6.5 Hz, 3H, 6-H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 142.0 (C-7), 132.1 (C-10), 129.0 (C-9, C-11), 126.3 (C-8, C-12), 64.0 (C-1), 53.0 (C-2), 40.4 (C-3), 23.6 (C-4), 23.2 (C-5), 21.4 (C-6) ppm; MS (ESI, MeOH): m/z = 279.9 (100 %, $[M+Na]^+$); analysis calcd. for $C_{12}H_{19}NO_3S$ (257.35): C 56.01, H 7.44, N 5.44; found: C 55.73, H 7.68, N 5.21.

4.4.6. (R)-[(1-Phenylsulfonyl)pyrrolidin-2-yl]-methanol (**6a**)

Following GPA from (*R*)-prolinol (349 mg, 3.45 mmol), TEA (0.78 mL, 5.66 mmol) and BSC (500 mg, 2.83 mmol) **6a** (511 mg, 61 %) was obtained as a colorless, viscous oil; R_f = 0.62 (SiO_2, hexanes/ethyl acetate, 3:7); $[\alpha]_D^{20}$ = +83.85° (*c* 0.309, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 222 nm (4.02); IR (ATR) ν = 3511*w*, 3065*vw*, 2953*w*, 2877*w*, 1585*vw*, 1477*w*, 1446 *m*, 1331*s*, 1310 *m*, 1291*w*, 1251*w*, 1198 *m*, 1155*vs*, 1091*s*, 1073 *m*, 1043*s*, 1014 *m*, 989 *m*, 928*w*, 900*w*, 875*w*, 820*w*, 757 *m*, 736 *m*, 718*s*, 692*s*, 613*s*, 591*vs*, 571*vs*, 535 *m*, 439*w*, 406*w* cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 7.86–7.79 (m, 2H, 7-H, 11-H), 7.75–7.59 (m, 3H, 8-H, 9-H, 10-H), 4.82 (t, J = 5.6 Hz, 1H, OH), 3.62–3.48 (m, 2H, 1-H), 3.40–3.23 (m, 2H, 5-H), 3.12–2.98 (m, 1H, 2-H), 1.87–1.65 (m, 2H, 3-H), 1.49–1.23 (m, 2H, 4-H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 136.8 (C-6), 133.0 (C-9), 129.4 (C-8, C-10), 127.2 (C-7, C-11), 63.8 (C-1), 61.0 (C-2), 49.0 (C-5), 27.6 (C-3), 23.3 (C-4) ppm; MS (ESI, MeOH): m/z = 264.2 (100 %, $[M+Na]^+$), 504.5 (35 %, $[2\ M\ +\ Na]^+$); analysis calcd. for $C_{11}H_{15}NO_3S$ (241.31): C 54.75, H 6.27, N 5.80; found: C 54.55, H 6.63, N 5.61.

4.4.7. (S)-((1-Phenylsulfonyl)pyrrolidin-2-yl)-methanol (**7a**)

Following GPA from (*S*)-prolinol (351 mg, 3.47 mmol), TEA (0.78 mL, 5.66 mmol) and BSC (500 mg, 2.83 mmol) **7a** (393 mg, 47 %) was obtained as a colorless, viscous oil [29–31]; R_f = 0.62 (SiO_2, hexanes/ethyl acetate, 3:7); $[\alpha]_D^{20}$ = −84.06° (*c* 0.248, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 222 nm (4.03); IR (ATR) ν = 3510*w*, 3064*vw*,

2953*w*, 2877*w*, 1638*vw*, 1585*vw*, 1477*w*, 1446 *m*, 1331*s*, 1310 *m*, 1291*w*, 1251*w*, 1198 *m*, 1155*vs*, 1091*s*, 1073 *m*, 1043*s*, 1014 *m*, 989 *m*, 928*w*, 900*w*, 875*w*, 820*w*, 757 *m*, 736 *m*, 718*s*, 691*s*, 613*s*, 591*vs*, 571*vs*, 535 *m*, 480 *m*, 440 *m*, 419*w*, 404*w* cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 7.88–7.78 (m, 2H, 7-H, 11-H), 7.77–7.56 (m, 3H, 8-H, 9-H, 10-H), 4.82 (t, J = 5.6 Hz, 1H, OH), 3.62–3.48 (m, 2H, 1-H), 3.39–3.23 (m, 2H, 5-H), 3.11–2.98 (m, 1H, 2-H), 1.83–1.68 (m, 2H, 3-H), 1.49–1.30 (m, 2H, 4-H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 136.8 (C-6), 133.0 (C-9), 129.4 (C-8, C-10), 127.2 (C-7, C-11), 63.7 (C-1), 61.0 (C-2), 49.0 (C-5), 27.6 (C-3), 23.3 (C-4) ppm; MS (ESI, MeOH): m/z = 264.2 (100 %, $[M+Na]^+$), 504.5 (8 %, $[2\ M\ +\ Na]^+$); analysis calcd. for $C_{11}H_{15}NO_3S$ (241.31): C 54.75, H 6.27, N 5.80; found: C 54.46, H 6.57, N 5.63.

4.4.8. (*R*)-*N*-(1-Hydroxy-3-methylbutane-2-yl)benzenesulfonamide (**8a**)

Following GPA from (*R*)-valinol (353 mg, 3.42 mmol), TEA (0.78 mL, 5.66 mmol) and BSC (500 mg, 2.83 mmol) **8a** (560 mg, 67 %) was obtained as a white solid; R_f = 0.71 (SiO_2, hexanes/ethyl acetate, 3:7); m. p. = 76.0–78.0 °C; $[\alpha]_D^{20}$ = +2.79° (*c* 0.258, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (4.09); IR (ATR) ν = 3499*w*, 3284*w*, 3067*vw*, 2963*w*, 2877*w*, 2741*vw*, 1724*vw*, 1639*vw*, 1578*vw*, 1465*w*, 1447 *m*, 1391*w*, 1370*w*, 1319*s*, 1310*s*, 1292 *m*, 1156*vs*, 1093*s*, 1072*s*, 1039*s*, 1000 *m*, 988 *m*, 965 *m*, 951 *m*, 925*w*, 908 *m*, 861*w*, 815*w*, 755*s*, 720*s*, 689*s*, 654 *m*, 595*vs*, 563*vs*, 523*s*, 469 *m*, 460 *m*, 440 *m*, 419 m cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 7.91–7.74 (m, 2H, 7-H, 11-H), 7.65–7.50 (m, 3H, 8-H, 9-H, 10-H), 7.32 (d, J = 8.4 Hz, 1H, NH), 4.47 (t, J = 5.4 Hz, 1H, OH), 3.26–3.07 (m, 2H, 1-H), 3.02–2.87 (m, 1H, 2-H), 1.93–1.76 (m, 1H, 3-H), 0.72 (*dd*, J = 9.8, 6.9 Hz, 6H, 4-H, 5-H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 142.1 (C-6), 132.0 (C-9), 128.9 (C-8, C-10), 126.4 (C-7, C-11), 61.0 (C-1), 60.0 (C-2), 27.5 (C-3), 19.3 (C-4), 16.9 (C-5) ppm; MS (ESI, MeOH): m/z = 266.3 (100 %, $[M+Na]^+$), 288.3 (9 %, $[M+2Na–H]^+$); analysis calcd. for $C_{11}H_{17}NO_3S$ (243.3): C 54.30, H 7.04, N 5.76; found: C 54.03, H 7.32, N 5.50.

4.4.9. (*S*)–*N*-(1-Hydroxy-3-methylbutane-2-yl)benzenesulfonamide (**9a**)

Following GPA from (*S*)-valinol (361 mg, 3.49 mmol), TEA (0.78 mL, 5.66 mmol) and BSC (500 mg, 2.83 mmol) **9a** (420 mg, 47 %) was obtained as a white solid [28]; R_f = 0.68 (SiO_2, hexanes/ethyl acetate, 3:7); m. p. = 76.0–78.0 °C; $[\alpha]_D^{20}$ = −2.95° (*c* 0.151, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (4.08); IR (ATR) ν = 3465 *m*, 3199*w*, 3067*vw*, 2960*w*, 2926*w*, 2903*w*, 2880*w*, 1474 *m*, 1466 *m*, 1448*w*, 1389*w*, 1370*w*, 1357*w*, 1315 *m*, 1304*s*, 1288 *m*, 1220*w*, 1180*w*, 1165*s*, 1141*w*, 1118*w*, 1097*s*, 1074 *m*, 1035*vs*, 1007*w*, 998*w*, 955*vw*, 910 *m*, 847*w*, 773*w*, 754*s*, 720*s*, 687*s*, 673*w*, 600*vs*, 570*vs*, 538 *m*, 518*s*, 483 *m*, 463 *m*, 428*w*, 412*w* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.83–7.79 (m, 2H, 7-H, 11-H), 7.63–7.53 (m, 3H, 8-H, 9-H, 10-H), 7.32 (d, J = 8.5 Hz, 1H, NH), 4.48 (s, 1H, OH), 3.22–3.10 (m, 2H, 1-H), 2.98–2.90 (m, 1H, 2-H), 1.89–1.77 (m, 1H, 3-H), 0.72 (*dd*, J = 12.3, 6.9 Hz, 6H, 4-H, 5-H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 142.1 (C-6), 132.0 (C-9), 128.9 (C-8, C-10), 126.4 (C-7, C-11), 61.0 (C-1), 60.0 (C-2), 27.5 (C-3), 19.3 (C-4), 16.9 (C-5) ppm; MS (ESI, MeOH): m/z = 266.3 (100 %, $[M+Na]^+$), 288.3 (7 %, $[M+2Na–H]^+$); analysis calcd. for $C_{11}H_{17}NO_3S$ (243.3): C 54.30, H 7.04, N 5.76; found: C 54.11, H 7.27, N 5.48.

4.4.10. (*R*)-*N*-(1-Hydroxy-3-phenylpropane-2-yl)benzenesulfonamide (**10a**)

Following GPA from (*R*)-phenylalaninol (522 mg, 3.46 mmol), TEA (0.78 mL, 5.66 mmol) and BSC (500 mg, 2.83 mmol) **10a** (882 mg, 87 %) was obtained as a white solid; R_f = 0.46 (SiO_2, hexanes/ethyl acetate, 1:1); m. p. = 87.0–89.0 °C; $[\alpha]_D^{20}$ = +51.93° (*c* 0.09, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (3.83); IR (ATR) ν = 3517 *m*, 3296 *m*, 3091*vw*, 3067*vw*, 3055*vw*, 3040*vw*, 3022*vw*, 2940*w*, 2927*w*, 2884*vw*, 1606*vw*, 1585*vw*, 1498*w*, 1491*w*, 1484*w*, 1459*w*, 1449 *m*, 1424 *m*, 1381*w*, 1346*w*, 1323*s*, 1310*s*, 1304*s*, 1270*w*, 1224 *m*, 1200*w*, 1155*vs*, 1096 *m*, 1080*s*, 1067*s*, 1032*s*, 1003 *m*, 980*s*, 925 *m*, 902*w*, 887 *m*, 847 *m*, 818*w*, 755 *m*, 747*vs*, 722*s*, 705*s*, 686*s*, 660*s*, 595*vs*, 567*vs*, 545*vs*, 510*vs*, 448*s*, 431 *m*, 412 m cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.67–7.60 (m, 3H, 11-H, 15-H, NH), 7.56–7.51 (m, 1H, 13-H), 7.48–7.41 (m, 2H, 12-H, 14-H), 7.20–7.10 (m, 3H, 6-H, 7-H, 8-H), 7.08–7.02 (m, 2H, 5-H, 9-H), 4.75 (t, J = 5.4 Hz, 1H, OH), 3.32–3.24 (m, 2H, 1-H), 3.23–3.15 (m, 1H, 2-H), 2.82 (*dd*, J = 13.6, 5.8 Hz, 1H, 3-H_a), 2.49–2.42 (m, 1H, 3-H_b) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 141.7 (C-10), 138.3 (C-4), 131.9 (C-13), 129.1 (C-5, C-9), 128.9 (C-12, C-14), 128.0 (C-6, C-8), 126.1 (C-11, C-15), 126.0 (C-7), 62.7 (C-1), 57.0 (C-2), 37.0 (C-3) ppm; MS (ESI, MeOH): m/z = 314.3 (100 %, $[M+Na]^+$); analysis calcd. for $C_{15}H_{17}NO_3S$ (291.37): C 61.83, H 5.88, N 4.81; found: C 61.64, H 6.02, N 4.61.

4.4.11. (*S*)–*N*-(1-Hydroxy-3-phenylpropane-2-yl)benzenesulfonamide (**11a**)

Following GPA from (*S*)-phenylalaninol (523 mg, 3.46 mmol), TEA (0.78 mL, 5.66 mmol) and BSC (500 mg, 2.83 mmol) **11a** (608 mg, 60 %) was obtained as a white solid; R_f = 0.72 (SiO_2, hexanes/ethyl acetate, 3:7); m. p. = 87.2–89.2 °C (lit. [28]: 64–66 °C; $[\alpha]_D^{20}$ = +49.96° (*c* 0.162, MeOH) (lit. [28]: $[\alpha]_D^{20}$ = +18.7° (*c* 0.005 $CHCl_3$)); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (4.02); IR (ATR) ν = 3516 *m*, 3296 *m*, 3092*vw*, 3067*vw*, 3055*vw*, 3040*vw*, 3022*vw*, 2939*w*, 2927*w*, 2884*w*, 1606*vw*, 1498*w*, 1491*w*, 1484*w*, 1458*w*, 1449 *m*, 1424 *m*, 1381*w*, 1346*w*, 1323*s*, 1310 *m*, 1304 *m*, 1270*w*, 1224*w*, 1200*w*, 1181*vw*, 1155*vs*, 1096 *m*, 1080*s*, 1067*s*, 1032*s*, 1003*w*, 981*s*, 926 *m*, 902*w*, 887 *m*, 847 *m*, 818*w*, 755 *m*, 747*vs*, 722*s*, 705*s*, 686*s*, 661*s*, 596*vs*, 568*vs*, 545*vs*, 525 *m*, 510*vs*, 495*s*, 448*s*, 425 *m*, 415 m cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.66–7.61 (m, 3H, 11-H, 15-H, NH), 7.56–7.51 (m, 1H, 13-H), 7.47–7.42 (m, 2H, 12-H, 14-H), 7.19–7.10 (m, 3H, 6-H, 7-H, 8-H), 7.07–7.03 (m, 2H, 5-H, 9-H), 4.75 (t, J = 5.4 Hz, 1H, OH), 3.30–3.23 (m, 2H, 1-H), 3.21–3.15 (m, 1H, 2-H), 2.82 (*dd*, J = 13.6, 5.8 Hz, 1H, 3-H_a), 2.46 (*dd*, J = 13.6, 7.0 Hz, 1H, 3-H_b) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 141.7 (C-10), 138.3 (C-4), 131.9 (C-13), 129.1 (C-5, C-9), 128.9 (C-12, C-14), 128.0 (C-6, C-8), 126.1 (C-11, C-15), 126.0 (C-7), 62.7 (C-1), 57.0 (C-2), 37.0 (C-3) ppm; MS (ESI, MeOH): m/z = 314.4 (100 %, $[M+Na]^+$), 336.4 (7 %, $[M+2Na–H]^+$); analysis calcd. for $C_{15}H_{17}NO_3S$ (291.37): C 61.83, H 5.88, N 4.81; found: C 61.50, H 6.03, N 4.62.

4.4.12. *N*-((2*S*,3*S*)-1-Hydroxy-3-methylpentane-2-yl)benzenesulfonamide (**12a**)

Following GPA from (*S*)-isoleucinol (399 mg, 3.40 mmol), TEA (0.78 mL, 5.66 mmol) and BSC (500 mg, 2.83 mmol) **12a** (585 mg, 67 %) was obtained as a colorless, waxy solid [28]; R_f = 0.73 (SiO_2, hexanes/ethyl acetate, 3:7); m. p. = 61–63 °C (lit. [28]: 58–60 °C; $[\alpha]_D^{20}$ = −0.14° (*c* 0.166, MeOH) (lit. [28]: $[\alpha]_D^{20}$ = +25.0° (c 0.006 $CHCl_3$)); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (4.04); IR (ATR) ν = 3497*w*, 3287 *m*, 3063*vw*, 2963*w*, 2932*w*, 2875*w*, 1723*vw*, 1481*w*, 1463*w*, 1448 *m*, 1430 *m*, 1379*w*, 1321*s*, 1286 *m*, 1246*w*, 1157*vs*, 1093 *m*, 1072*s*, 1027 *m*, 1002 *m*, 993 *m*, 978 *m*, 967 *m*, 941 *m*, 893 *m*, 872*w*, 825*w*, 805*w*, 752*s*, 721*s*, 689*s*, 647 *m*, 585*vs*, 560*vs*, 533*s*, 487 *m*, 463 *m*, 439 *m*, 428 *m*, 420 m cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 7.85–7.78 (m, 2H, 8-H, 12-H), 7.64–7.52 (m, 3H, 9- H, 10-H, 11-H), 7.34 (d, J = 8.3 Hz, 1H, NH), 4.44 (t, J = 5.0 Hz, 1H, OH), 3.28–3.12 (m, 2H, 1-H), 3.04–2.95 (m, 1H, 2-H), 1.59–1.45 (m, 1H, 3-H), 1.41–1.28 (m, 1H, 4-H), 1.04–0.82 (m, 1H, 4-H), 0.77–0.68 (m, 6H, 5-H, 6-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 142.1 (C-7), 132.0 (C-10), 128.9 (C-9, C-11), 126.4 (C-8, C-12), 60.6 (C-1), 59.3 (C-2), 35.1 (C-3), 23.8 (C-4), 15.2 (C-6), 11.5 (C-5) ppm; MS (ESI, MeOH): m/z = 280.4 (100 %, $[M+Na]^+$); analysis calcd. for $C_{12}H_{19}NO_3S$ (257.35): C 56.01, H 7.44, N 5.44; found: C 55.76, H 7.73, N 5.08.

4.4.13. (*R*)-*N*-(1-Hydroxy-4-(methylthio)butane-2-yl)benzenesulfonamide (**13a**)

Following GPA from (*R*)-methioninol (414 mg, 3.06 mmol), TEA (0.71 mL, 5.10 mmol) and BSC (450 mg, 2.55 mmol) **13a** (662 mg, 94 %)

was obtained as a colorless, viscous oil; $R_f = 0.61$ (SiO_2, hexanes/ethyl acetate, 3:7); $[\alpha]_D^{20} = +20.73°$ (*c* 0.107, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 222 nm (4.05); IR (ATR) ν = 3490*w*, 3276*w*, 3067*vw*, 2918*w*, 1478*w*, 1447 *m*, 1428 *m*, 1318 *m*, 1308*s,* 1292 *m*, 1152*vs,* 1091*s,* 1072 *m*, 1051 *m*, 1000*w*, 979 *m*, 857*w*, 755 *m*, 719*s,* 689*s,* 671*w*, 647*w*, 582*s,* 559*s,* 427*vw* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.83–7.79 (m, 2H, 7-H, 11-H), 7.64–7.59 (m, 1H, 9- H), 7.59–7.55 (m, 3H, NH, 8-H, 10-H), 4.70 (t, *J* = 5.4 Hz, 1H, OH), 3.32–3.26 (m, 1H, 1- H_a), 3.19–3.12 (m, 2H, 1-H_b, 2-H), 2.28 (*ddd*, *J* = 12.9, 9.2, 5.3 Hz, 1H, 4-H_a), 2.10 (*ddd*, *J* = 12.9, 9.1, 6.6 Hz, 1H, 4-H_b), 1.86 (s*,* 3H, 5-H), 1.74 (*dddd*, *J* = 13.6, 8.9, 6.5, 4.1 Hz, 1H, 3-H_a), 1.43 (*dtd*, *J* = 13.9, 8.6, 5.2 Hz, 1H, 3-H_b) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 141.9 (C-6), 132.2 (C-9), 129.0 (C-8, C-10), 126.3 (C-7, C-11), 63.4 (C-1), 54.2 (C-2), 30.5 (C-3), 29.4 (C-4), 14.3 (C-5) ppm; MS (ESI, MeOH): *m*/z = 297.9 (100 %, $[M+Na]^+$); analysis calcd. for $C_{11}H_{17}NO_3S_2$ (275.38): C, 47.98; H, 6.22; N, 5.09; found: C 47.73, H 6.48, N 5.98.

4.4.14. (S)–N-(1-Hydroxy-4-(methylthio)butane-2-yl)benzenesulfonamide (14a)

Following GPA from (*S*)-methioninol (414 mg, 3.06 mmol), TEA (0.71 mL, 5.10 mmol) and BSC (450 mg, 2.55 mmol) **14a** (635 mg, 91 %) was obtained as a colorless, viscous oil; $R_f = 0.63$ (SiO_2, hexanes/ethyl acetate, 3:7); $[\alpha]_D^{20} = -21.82°$ (*c* 0.113, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 222 nm (3.93); IR (ATR) ν = 3495*w*, 3274*w*, 2918*w*, 1478*w*, 1447 *m*, 1428 *m*, 1318 *m*, 1308*s,* 1292 *m*, 1152*vs,* 1091*s,* 1073 *m*, 1051 *m*, 1000*w*, 978 *m*, 857*w*, 755 *m*, 719*s,* 689*s,* 647*w*, 582*s,* 560*s,* 444*w* cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 7.83–7.78 (m, 2H, 7-H, 11-H), 7.65–7.54 (m, 4H, NH, 8-H, 9-H, 10-H), 4.70 (t, *J* = 5.2 Hz, 1H, OH), 3.31–3.24 (m, 1H, 1-H_a), 3.21–3.09 (m, 2H, 1-H_b, 2-H), 2.28 (*ddd*, *J* = 12.9, 9.2, 5.3 Hz, 1H, 4-H_a), 2.10 (*ddd*, *J* = 13.0, 9.1, 6.6 Hz, 1H, 4-H_b), 1.87 (s*,* 3H, 5-H), 1.74 (*dddd*, *J* = 13.7, 9.2, 6.6, 4.1 Hz, 1H, 3-H_a), 1.42 (*ddt*, *J* = 13.9, 8.6, 5.3 Hz, 1H, 3-H_b) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 141.9 (C-6), 132.2 (C-9), 129.1 (C-8, C-10), 126.3 (C-7, C-11), 63.4 (C-1), 54.2 (C-2), 30.5 (C-3), 29.4 (C-4), 14.3 (C-5) ppm; MS (ESI, MeOH): *m*/z = 297.9 (100 %, $[M+Na]^+$); analysis calcd. for $C_{11}H_{17}NO_3S_2$ (275.38): C 47.98, H 6.22, N 5.09; found: C 47.75, H 5.94, N 4.82.

4.4.15. (R)-N-(1-Hydroxy-3-(1h-indol-3-yl)propane-2-yl) benzenesulfonamide (15a)

Following GPA from (*R*)-tryptophanol (453 mg, 2.38 mmol), TEA (0.55 mL, 3.96 mmol) and BSC (350 mg, 1.98 mmol) **15a** (501 mg, 77 %) was obtained as an off-white solid; $R_f = 0.85$ (SiO_2, hexanes/ethyl acetate, 3:7); m. p. = 100.0–102.0 °C; $[\alpha]_D^{20} = +69.68°$ (*c* 0.028, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (4.52); IR (ATR) ν = 3404 *m*, 3297*w*, 2925*w*, 1457 *m*, 1447 *m*, 1421 *m*, 1307*s,* 1253*w*, 1232*w*, 1179*w*, 1153*vs,* 1090*s,* 1031*s,* 1011 *m*, 1001*w*, 971 *m*, 879*w*, 840*w*, 741*vs,* 719*s,* 686*s,* 591*s,* 569*s,* 555*s,* 546*vs,* 503 *m*, 498 *m*, 490 *m*, 476*w*, 459 *m*, 441*w*, 424 m cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 10.72–10.67 (m, 1H, NH_{indole}), 7.71–7.66 (m, 2H, 13-H, 17-H), 7.57 (d, *J* = 7.1 Hz, 1H, NH), 7.53–7.46 (m, 1H, 15-H), 7.44–7.38 (m, 2H, 14-H, 16-H), 7.32–7.24 (m, 2H, 7-H, 10-H), 7.05–6.98 (m, 2H, 5-H, 8-H), 6.94–6.87 (m, 1H, 9- H), 4.67 (*t*, *J* = 5.4 Hz, 1-H, OH), 3.32–3.18 (m, 3H, 1-H, 2-H), 2.91 (*dd*, *J* = 14.4, 6.9 Hz, 1H, 3-H_a), 2.62 (*dd*, *J* = 14.3, 5.8 Hz, 1H, 3-H_b) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 141.7 (C-12), 136.1 (C-6), 131.8 (C-15), 128.7 (C-14, C-16), 127.2 (C-11), 126.2 (C-13, C-17), 123.6 (C-5), 120.7 (C-8), 118.1 (C-9, C-10), 111.3 (C-7), 110.4 (C-4), 62.5 (C-1), 56.0 (C-2), 27.0 (C-3) ppm; MS (ESI, MeOH): *m*/z = 352.9 (16 %, $[M+Na]^+$); analysis calcd. for $C_{17}H_{18}N_2O_3S$ (330.40): C 61.80, H 5.49, N 8.48; found: C 61.55, H 5.71, N 8.29.

4.4.16. (S)–N-(1-Hydroxy-3-(1h-indol-3-yl)propane-2-yl) benzenesulfonamide (16a)

Following GPA from (*S*)-tryptophanol (516 mg, 2.71 mmol), TEA (0.63 mL, 4.52 mmol) and BSC (400 mg, 2.26 mmol) **16a** (589 mg, 79 %) was obtained as a white solid; $R_f = 0.85$ (SiO_2, hexanes/ethyl acetate, 3:7); m. p. = 100.0–101.0 °C; $[\alpha]_D^{20} = -67.23°$ (*c* 0.175, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (4.57); IR (ATR) ν = 3476*w*, 3418*w*, 3346*w*, 3145*w*, 3056*w*, 2919*w*, 2885*w*, 1479*w*, 1458 *m*, 1446 *m*, 1417 *m*, 1381*w*, 1354*w*, 1346*w*, 1325 *m*, 1311 *m*, 1304 *m*, 1231*w*, 1289 *m*, 1254*w*, 1170 *m*, 1158*s,* 1149*s,* 1093*s,* 1054 *m*, 1039*s,* 1026*s,* 999*w*, 962 *m*, 926*w*, 888 *m*, 840*w*, 812*w*, 770*w*, 757*w*, 745 *m*, 736*vs,* 719*s,* 687*vs,* 641*w*, 596*s,* 586*s,* 568*s,* 548*vs,* 462*w*, 428 s cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 10.72–10.67 (m, 1H, NH_{indole}), 7.70–7.65 (m, 2H, 13-H, 17-H), 7.56 (d, *J* = 7.0 Hz, 1H, NH), 7.53–7.47 (m, 1H, 15-H), 7.44–7.38 (m, 2H, 14-H, 16-H), 7.30–7.26 (m, 2H, 7-H, 10-H), 7.05–6.99 (m, 2H, 5-H, 8-H), 6.93–6.87 (m, 1H, 9- H), 4.66 (*t*, *J* = 5.4 Hz, 1-H, OH), 3.32–3.18 (m, 3H, 1-H, 2-H), 2.91 (*dd*, *J* = 14.4, 6.9 Hz, 1H, 3-H_a), 2.61 (*dd*, *J* = 14.3, 5.8 Hz, 1H, 3-H_b) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 141.7 (C-12), 136.1 (C-6), 131.8 (C-15), 128.7 (C-14, C-16), 127.2 (C-11), 126.2 (C-13, C-17), 123.6 (C-5), 120.7 (C-8), 118.1 (C-9, C-10), 111.3 (C-7), 110.4 (C-4), 62.5 (C-1), 56.0 (C-2), 27.0 (C-3) ppm; MS (ESI, MeOH): *m*/z = 352.9 (7 %, $[M+Na]^+$); analysis calcd. for $C_{17}H_{18}N_2O_3S$ (330.40): C 61.80, H 5.49, N 8.48; found: C 61.62, H 5.72, N 8.21.

4.4.17. 2-(Phenylsulfonamido]ethyl sulfamate (1b)

Following GPB from **1a** (175 mg, 0.87 mmol), **1b** (202 mg, 82 %) was obtained as a white, waxy solid; $R_f = 0.38$ (SiO_2, chloroform/ethyl acetate, 4:6); m. p. = 71–73 °C; UV–Vis (MeOH): λ_{max} (log ε) = 221 nm (3.92); IR (ATR): ν = 3342 *m*, 3263 *m*, 3258 *m*, 1447*w*, 1379*s*, 1356 *m*, 1313*s*, 1178*s*, 1153*s*, 1112*w*, 1093 *m*, 1074*w*, 1053 *m*, 1003 *m*, 952*s*, 914 *m*, 899 *m*, 884 *m*, 835*s*, 754 *m*, 722*s*, 689*s*, 675 *m*, 623 *m*, 597 *m*, 585*s*, 562*s*, 544*vs*, 508 *m*, 499 *m*, 474*w*, 442*w* cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 7.95–7.90 (m*,* 1H, NH), 7.83–7.78 (m*,* 2H, 2-H), 7.69–7.57 (m*,* 3H, 3-H, 4-H), 7.50 (s, 2H, NH_2), 4.00 (t*,* *J* = 5.7 Hz, 2H, 5-H), 3.05 (q*,* *J* = 5.6 Hz, 2H, 6-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 140.2 (C-1), 132.5 (C-4), 129.3 (C-3), 126.4 (C-2), 67.5 (C-6), 41.5 (C-5) ppm; MS (ESI, MeOH): *m/z* = 303.1 (100 %, $[M+Na]^+$); anal. calcd. for $C_8H_{12}N_2S_2O_5$ (280.31): C 34.28, H 4.32, N 9.99; found: C 33.01, H 4.52, N 9.86.

4.4.18. (R)-2-(Phenylsulfonamido)propyl sulfamate (2b)

Following GPB from **2a** (300 mg, 1.39 mmol), TEA (0.77 mL, 5.56 mmol) and SC (645 mg, 5.56 mmol) **2b** (187 mg, 46 %) was obtained as a white solid; $R_f = 0.72$ (SiO_2, $CHCl_3$/ethyl acetate, 4:6); m. p. = 107.9–109.9 °C; $[\alpha]_D^{20} = +46.65°$ (*c* 0.104, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (3.79); IR (ATR) ν = 3399*w*, 3288 *m*, 3122*vw*, 3069*vw*, 3001*vw*, 2953*vw*, 1572*w*, 1481*vw*, 1447*w*, 1429*w*, 1382 *m*, 1366 *m*, 1327*s,* 1295 *m*, 1260*w*, 1168*s,* 1151*s,* 1125 *m*, 1090 *m*, 1057*w*, 1024*w*, 1005 *m*, 961*s,* 937*s,* 900 *m*, 840 *m*, 834*s,* 752 *m*, 720*s,* 684*s,* 650 *m*, 619*s,* 612*s,* 565*s,* 549*vs,* 496 *m*, 475*s,* cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 7.89 (d, *J* = 7.6 Hz, 1H, NH), 7.85–7.80 (m, 2H, 5-H, 9-H), 7.68–7.56 (m, 3H, 6-H, 7-H, 8-H), 7.49 (s*,* 2H, NH_2), 3.90 (*dd*, *J* = 9.7, 4.9 Hz, 1H, 1-H), 3.78 (*dd*, *J* = 9.7, 6.3 Hz, 1H, 1-H), 3.48–3.39 (m, 1H, 2-H), 0.90 (d, *J* = 6.8 Hz, 3H, 3-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 141.4 (C-4), 132.4 (C-7), 129.2 (C-6, C-8), 126.3 (C-5, C-9), 71.5 (C-1), 47.8 (C-2), 17.1 (C-3) ppm; MS (ESI, MeOH): *m*/z = 316.9 (100 %, $[M+Na]^+$); analysis calcd. for $C_9H_{14}N_2O_5S_2$ (294.34): C 36.73, H 4.79, N 9.52; found: C 36.39, H 4.95, N 9.33.

4.4.19. (S)-2-(Phenylsulfonamido)propyl sulfamate (3b)

Following GPB from **3a** (200 mg, 0.93 mmol), TEA (0.52 mL, 3.72 mmol) and SC (430 mg, 3.72 mmol) **3b** was obtained as a white solid (184 mg, 67 %); $R_f = 0.71$ (SiO_2, $CHCl_3$/ethyl acetate, 4:6); m. p. = 107.7–109.7 °C; $[\alpha]_D^{20} = -47.83°$ (*c* 0.133, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (3.78); IR (ATR) ν = 3399*w*, 3287 *m*, 3122*vw*, 3069*vw*, 3001*vw*, 2953*vw*, 2849*vw*, 1573 *m*, 1481*vw*, 1447*w*, 1429*w*, 1382 *m*, 1366 *m*, 1327*s,* 1295 *m*, 1260*w*, 1168*s,* 1151*s,* 1125 *m*, 1090 *m*, 1057*w*, 1024*w*, 1005 *m*, 960*s,* 937*s,* 900 *m*, 834*s,* 752 *m*, 720*s,* 684*s,* 649 *m*,

618*s,* 612*s,* 565*s,* 548*vs,* 496 *m*, 475*s,* 403 m cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.89 (d, J = 7.6 Hz, 1H, NH), 7.86–7.80 (m, 2H, 5-H, 9-H), 7.67–7.56 (m, 3H, 6-H, 7-H, 8-H), 7.49 (s, 2H, NH_2), 3.90 (*dd*, J = 9.7, 4.9 Hz, 1H, 1- H), 3.78 (*dd*, J = 9.7, 6.3 Hz, 1H, 1-H), 3.48–3.38 (m, 1H, 2-H), 0.90 (d, J = 6.8 Hz, 3H, 3-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 141.4 (C-4), 132.4 (C-7), 129.2 (C-6, C-8), 126.3 (C-5, C-9), 71.5 (C-1), 47.8 (C-2), 17.1 (C-3) ppm; MS (ESI, MeOH): *m/*z = 316.9 (100 %, $[M+Na]^+$), 338.9 (5 %, $[M+2Na–H]^+$); analysis calcd. for $C_9H_{14}N_2O_5S_2$ (294.34): C 36.73, H 4.79, N 9.52; found: C 36.44, H 4.87, N 9.39.

4.4.20. (R)-4-Methyl-2-(phenylsulfonamido)pentyl sulfamate (**4b**)

Following GPB from **4a** (202 mg, 0.79 mmol), TEA (0.43 mL, 3.11 mmol) and SC (361 mg, 3.12 mmol) **4b** (185 mg, 70 %) was obtained as a colorless, viscous oil; R_f = 0.71 (SiO_2, $CHCl_3$/ethyl acetate, 4:6); $[\alpha]_D^{20}$ = +83.25° (*c* 0.055, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (3.81); IR (ATR) ν = 3277 *m*, 3104*vw*, 3070*vw*, 2959*w*, 2871*w*, 1558*w*, 1468*w*, 1448 *m*, 1427*w*, 1365*s,* 1324*s,* 1309*s,* 1230*w*, 1178*s,* 1153*vs,* 1091*s,* 1073 *m*, 1026*w*, 986*s,* 921*s,* 804*s,* 755*s,* 719*s,* 689*s,* 635 *m*, 593*vs,* 567*vs,* 550*vs,* 490*s,* 419 *m*, 407 m cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.87 (d, J = 7.4 Hz, 1H, NH), 7.84–7.80 (m, 2H, 8-H, 12-H), 7.66–7.56 (m, 3H, 9-H, 10-H, 11-H), 7.48 (s, 2H, NH_2), 3.91 (*dd*, J = 9.6, 4.0 Hz, 1H, 1-H_a), 3.81 (*dd*, J = 9.6, 6.2 Hz, 1H, 1-H_b), 1.40–1.28 (m, 1H, 4-H), 1.25–1.12 (m, 3H, 2-H, 3-H), 0.70 (d, J = 6.6 Hz, 3H, 5-H), 0.46 (d, J = 6.5 Hz, 3H, 6-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 141.2 (C-7), 132.4 (C-10), 129.1 (C-9, C-11), 126.4 (C-8, C-12), 70.9 (C-1), 50.1 (C-2), 39.9 (C-3), 23.4 (C-4), 22.9 (C-5), 21.1 (C-6) ppm; MS (ESI, MeOH): *m/*z = 358.9 (100 %, $[M+Na]^+$), 374.9 (7 %, $[M+K]^+$), 380.9 (13 %, $[M+2Na–H]^+$). analysis calcd. for $C_{12}H_{20}N_2O_5S_2$ (336.42): C 42.84, H 5.99, N 8.33; found: C 42.64, H 6.27, N 8.05.

4.4.21. (S)-4-Methyl-2-(phenylsulfonamido)pentyl sulfamate (**5b**)

Following GPB from **5a** (200 mg, 0.78 mmol), TEA (0.43 mL, 3.11 mmol) and SC (365 mg, 3.15 mmol) **5b** (170 mg, 65 %) was obtained as a colorless, viscous oil; R_f = 0.72 (SiO_2, $CHCl_3$/ethyl acetate, 4:6); $[\alpha]_D^{20}$ = −81.61° (*c* 0.036, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (3.81); IR (ATR) ν = 3277 *m*, 3103*vw*, 3070*vw*, 2959*w*, 2871*w*, 1622*vw*, 1559*w*, 1468*w*, 1448 *m*, 1427*w*, 1365*s,* 1324*s,* 1309*s,* 1230*w*, 1178*s,* 1153*vs,* 1091*s,* 1073 *m*, 1026*w*, 985*s,* 921*s,* 804*s,* 755*s,* 719*s,* 689*s,* 635 *m*, 592*vs,* 567*vs,* 550*vs,* 489*s,* 416 *m*, 409 *m*, 403 m cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.87 (d, J = 7.4 Hz, 1H, NH), 7.84–7.80 (m, 2H, 8-H, 12-H), 7.66–7.56 (m, 3H, 9-H, 10-H, 11-H), 7.48 (s, 2H, NH_2), 3.90 (*dd*, J = 9.6, 4.0 Hz, 1H, 1-H_a), 3.81 (*dd*, J = 9.6, 6.2 Hz, 1H, 1-H_b), 1.38–1.31 (m, 1H, 4-H) 1.24–1.15 (m, 3H, 2-H, 3-H), 0.70 (d, J = 6.6 Hz, 3H, 5-H), 0.47 (d, J = 6.5 Hz, 3H, 6-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 141.2 (C-7), 132.4 (C-10), 129.1 (C-9, C-11), 126.4 (C-8, C-12), 70.9 (C-1), 50.1 (C-2), 39.9 (C-3), 23.4 (C-4), 22.9 (C-5), 21.1 (C-6) ppm; MS (ESI, MeOH): *m/*z = 358.9 (100 %, $[M+Na]^+$), 374.9 (7 %, $[M+K]^+$), 380.9 (13 %, $[M+2Na–H]^+$); analysis calcd. for $C_{12}H_{20}N_2O_5S_2$ (336.42): C 42.84, H 5.99, N 8.33; found: C 42.56, H 6.21, N 8.04.

4.4.22. (R)-(1-(Phenylsulfonyl)pyrrolidin-2-yl)methyl sulfamate (**6b**)

Following GPB from **6a** (200 mg, 0.83 mmol), TEA (0.47 mL, 3.32 mmol) and SC (390 mg, 3.37 mmol) **6b** (207 mg, 78 %) was obtained as a colorless, viscous oil); R_f = 0.67(SiO_2, $CHCl_3$/ethyl acetate, 4:6); $[\alpha]_D^{20}$ = +97.66° (c 0.116, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 222 nm (3.81); IR (ATR) ν = 3336 *m*, 3253 *m*, 3121*vw*, 2983*w*, 2918*vw*, 2849*vw*, 2687*vw*, 1756*vw*, 1698*vw*, 1583*vw*, 1572*w*, 1478*vw*, 1461*w*, 1447 *m*, 1380 *m*, 1363*s,* 1320*s,* 1313*s,* 1293 *m*, 1252*w*, 1242*w*, 1204 *m*, 1178*s,* 1153*vs,* 1115 *m*, 1089*s,* 1049 *m*, 1023*w*, 1002 *m*, 987*s,* 970*s,* 933 *m*, 913*s,* 872 *m*, 837*s,* 764*s,* 717*s,* 691*s,* 664 *m*, 596*s,* 571*vs,* 542*vs,* 501 *m*, 489 *m*, 471 *m*, 420 *m*, 407 m cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.87–7.84 (m, 2H, 7-H, 11-H), 7.75–7.71 (m, 1H, 9- H), 7.65–7.62 (m, 2H, 8-H, 10-H), 7.58 (s, 2H, NH_2), 4.16 (*dd*, J = 9.7, 3.6 Hz, 1H, 1-H_a), 4.00 (*dd*, J = 9.7, 7.4 Hz, 1H, 1-H_b), 3.85–3.78 (m, 1H, 2-H), 3.34–3.29 (m, 1H, 5-H_a), 3.10–3.04 (m, 1H, 5-H_b), 1.81–1.72 (m, 2H, 3-H), 1.59–1.49 (m, 1H, 4-H_a), 1.47–1.40 (m, 1H, 4-H_b) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 136.2 (C-6), 133.3 (C-9), 129.6 (C-8, C-10), 127.4 (C-7, C-11), 70.3 (C-1), 57.7 (C-2), 49.2 (C-5), 28.0 (C-3), 23.4 (C-4) ppm; MS (ESI, MeOH): *m/*z = 169.9 (13 %, $[M + H + NH_4]^{2+}$), 343.0 (100 %, $[M+Na]^+$); analysis calcd. for $C_{11}H_{16}N_2O_5S_2$ (320.38): C 41.24, H 5.03, N 8.74; found: C 40.97, H 5.36, N 8.51.

4.4.23. (S)-(1-(Phenylsulfonyl)pyrrolidin-2-yl)methyl sulfamate (**7b**)

Following GPB from **7a** (190 mg, 0.79 mmol), TEA (0.46 mL, 3.31 mmol) and SC (390 mg, 3.37 mmol) **7b** (130 mg, 52 %) was obtained as a colorless, viscous oil; R_f = 0.65 (SiO_2, $CHCl_3$/ethyl acetate, 4:6); $[\alpha]_D^{20}$ = −98.42° (*c* 0.043, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 222 nm (3.82); IR (ATR) ν = 3367*w*, 3273*w*, 3104*vw*, 2960*vw*, 2881*vw*, 1756*vw*, 1561*w*, 1447 *m*, 1367*s,* 1331*s,* 1312 *m*, 1292*w*, 1247*w*, 1177*s,* 1155*vs,* 1092*s,* 1075 *m*, 1046 *m*, 987*s,* 925*s,* 823 *m*, 779 *m*, 755*s,* 738 *m*, 718*s,* 691*s,* 657 *m*, 615*s,* 597*s,* 571*vs,* 550*vs,* 497*s,* 408 m cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.87–7.84 (m, 2H, 7-H, 11-H), 7.76–7.71 (m, 1H, 9-H), 7.67–7.62 (m, 2H, 8-H, 10-H), 7.58 (s, 2H, NH_2), 4.16 (*dd*, J = 9.7, 3.6 Hz, 1H, 1-H_a), 4.00 (*dd*, J = 9.7, 7.4 Hz, 1H, 1-H_b), 3.85–3.79 (m, 1H, 2-H), 3.34–3.30 (m, 1H, 5-H_a), 3.11–3.03 (m, 1H, 5-H_b), 1.81–1.71 (m, 2H, 3-H), 1.57–1.50 (m, 1H, 4-H_a), 1.47–1.40 (m, 1H, 4-H_b) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 136.2 (C-6), 133.3 (C-9), 129.6 (C-8, C-10), 127.4 (C-7, C-11), 70.3 (C-1), 57.7 (C-2), 49.2 (C-5), 28.0 (C-3), 23.4 (C-4) ppm; MS (ESI, MeOH): *m/*z = 170.0 (13 %, $[M + H + NH_4]^{2+}$), 342.9 (100 %, $[M+Na]^+$); analysis calcd. for $C_{11}H_{16}N_2O_5S_2$ (320.38): C 41.24, H 5.03, N 8.74; found: C 40.93, H 5.41, N 8.52.

4.4.24. (R)-3-Methyl-2-(phenylsulfonamido)butyl sulfamate (**8b**)

Following GPB from **8a** (201 mg, 0.83 mmol), TEA (0.45 mL, 3.29 mmol) and SC (384 mg, 3.32 mmol) **8b** (212 mg, 80 %) was obtained as a colorless, viscous oil; R_f = 0.63 (SiO_2, $CHCl_3$/ethyl acetate, 4:6); $[\alpha]_D^{20}$ = −36.07° (*c* 0.028, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (3.85); IR (ATR) ν = 3610*vw*, 3280 *m*, 3106*vw*, 2967*w*, 2878*vw*, 1816*vw*, 1708*vw*, 1558*w*, 1464*w*, 1448 *m*, 1365*s,* 1322*s,* 1178*s,* 1158*vs,* 1091*s,* 1044 *m*, 979*s,* 954*s,* 925*s,* 795 *m*, 755*s,* 720*s,* 688*s,* 654 *m*, 591*s,* 549*vs,* 527*s,* 467 *m*, 447 *m*, 434 *m*, 424 *m*, 410 *m*, 402 m cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.85–7.77 (m, 3H, NH, 7-H, 11-H), 7.65–7.54 (m, 3H, 8-H, 9-H, 10-H), 7.47 (s, 2H, NH_2), 3.94–3.79 (m, 2H, 1-H), 3.25–3.13 (m, 1H, 2-H), 1.83–1.65 (m, 1H, 3-H), 0.73 (d, J = 6.8 Hz, 3H, 4-H), 0.67 (d, J = 6.9 Hz, 3H, 5-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 141.4 (C-6), 132.3 (C-9), 129.1 (C-8, C-10), 126.4 (C-7, C-11), 68.5 (C-1), 57.2 (C-2), 28.1 (C-3), 18.7 (C-4), 17.4 (C-5) ppm; MS (ESI, MeOH): *m/*z = 344.9 (100 %, $[M+Na]^+$), 360.8 (6 %, $[M+K]^+$); analysis calcd. for $C_{11}H_{18}N_2O_5S_2$ (322.39): C 40.98, H 5.63, N 8.69; found: C 40.63, H 5.87, N 8.35.

4.4.25. (S)-3-Methyl-2-(phenylsulfonamido)butyl sulfamate (**9b**)

Following GPB from **9a** (200 mg, 0.82 mmol), TEA (0.45 mL, 3.29 mmol) and SC (380 mg, 3.30 mmol) **9b** (200 mg, 75 %) was obtained as a colorless, viscous oil; R_f = 0.61 (SiO_2, $CHCl_3$/ethyl acetate, 4:6); $[\alpha]_D^{20}$ = +34.93° (*c* 0.041, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (3.84); IR (ATR) ν = 3281 *m*, 3105*vw*, 2967*w*, 2878*vw*, 1558*w*, 1464*w*, 1448 *m*, 1365*s,* 1322*s,* 1178*s,* 1158*vs,* 1091*s,* 1044 *m*, 979*s,* 954*s,* 925*s,* 842 *m*, 795 *m*, 754*s,* 720*s,* 688*s,* 669 *m*, 653 *m*, 591*s,* 550*vs,* 527*s,* 408 m cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.85–7.77 (m, 3H, NH, 7-H, 11-H), 7.66–7.53 (m, 3H, 8-H, 9-H, 10-H), 7.47 (s, 2H, NH_2), 3.93–3.81 (m, 2H, 1-H), 3.24–3.13 (m, 1H, 2-H), 1.79–1.69 (m, 1H, 3-H), 0.73 (d, J = 6.8 Hz, 3H, 4-H), 0.67 (d, J = 6.9 Hz, 3H, 5-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 141.4 (C-6), 132.3 (C-9), 129.1 (C-8, C-10), 126.4 (C-7, C-11), 68.5 (C-1), 57.2 (C-2), 28.1 (C-3), 18.7 (C-4), 17.4 (C-5) ppm; MS (ESI, MeOH): *m/*z = 344.9 (100 %, $[M+Na]^+$), 366.9 (3 %, $[M+2Na–H]^+$); analysis calcd. for $C_{11}H_{18}N_2O_5S_2$ (322.39): C 40.98, H 5.63, N 8.69; found: C 40.73, H 5.88, N 8.27.

*4.4.26. (R)-3-Phenyl-2-(phenylsulfonamido)propyl sulfamate (**10b**)*

Following GPB from **10a** (197 mg, 0.77 mmol), TEA (0.38 mL, 2.77 mmol) and SC (320 mg, 2.77 mmol) **10b** (225 mg, 78 %) was obtained as a colorless, viscous oil; $R_f = 0.71$ (SiO_2, $CHCl_3$/ethyl acetate, 4:6); $[\alpha]_D^{20} = +65.75°$ (*c* 0.056, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 216 nm (3.42); IR (ATR) ν = 3612*vw*, 3538*vw*, 3277*w*, 3065*w*, 3030*vw*, 2956*vw*, 1603*vw*, 1585*w*, 1557*w*, 1497*w*, 1479*w*, 1448 *m*, 1365*s,* 1322 *m*, 1309 *m*, 1178*s,* 1154*vs,* 1091*s,* 1001 *m*, 988*s,* 932*s,* 812 *m*, 747*s,* 719*s,* 700*s,* 687*s,* 629 *m*, 595*s,* 566*vs,* 551*vs,* 508*s,* 503*s,* 479*s,* 428*s,* 418 s cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.07 (d, *J* = 8.0 Hz, 1H, NH), 7.61–7.57 (m, 2H, 11-H, 15-H), 7.56–7.51 (m, 3H, 12-H, 13-H, 14-H), 7.46–7.40 (m, 2H, 5-H, 9-H), 7.17–7.12 (m, 3H, 6-H, 7-H, 8-H), 7.05–7.00 (m, 2H, NH_2), 3.96–3.85 (m, 2H, 1-H), 3.59–3.53 (m, 1H, 2-H), 2.76 (*dd*, *J* = 13.8, 6.1 Hz, 1H, 3-H_a), 2.49–2.44 (m, 1H, 3-H_b) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 141.1 (C-10), 137.1 (C-4), 132.1 (C-13), 129.0 (C-5, C-9), 129.0 (C-12, C-14), 128.2 (C-6, C-8), 126.3 (C-11, C-15), 126.1 (C-7), 70.0 (C-1), 53.9 (C-2), 36.7 (C-3) ppm; MS (ESI, MeOH): *m*/z = 392.8 (100 %, $[M+Na]^+$); analysis calcd. for $C_{15}H_{18}N_2O_5S_2$ (370.44): C 48.64, H 4.90, N 7.56; found: C 48.41, H 5.12, N 7.39.

*4.4.27. (S)-3-Phenyl-2-(phenylsulfonamido)propyl sulfamate (**11b**)*

Following GPB from **11a** (200 mg, 0.78 mmol), TEA (0.38 mL, 2.77 mmol) and SC (323 mg, 2.79 mmol) **11b** (166 mg, 58 %) was obtained as a colorless, viscous oil; $R_f = 0.71$ (SiO_2, $CHCl_3$/ethyl acetate, 4:6); $[\alpha]_D^{20} = -66.17°$ (*c* 0.012, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 216 nm (3.43); IR (ATR) ν = 3277 *m*, 3065*vw*, 3030*vw*, 2926*vw*, 2857*vw*, 1603*vw*, 1585*vw*, 1556*w*, 1497*w*, 1480*w*, 1448 *m*, 1365*s,* 1322 *m*, 1310 *m*, 1178*s,* 1154*vs,* 1090*s,* 1049*w*, 1001 *m*, 988*s,* 929*s,* 850 *m*, 811*s,* 747*s,* 719*s,* 700*s,* 687*s,* 629 *m*, 594*s,* 566*vs,* 551*vs,* 508*s,* 478*s,* 441*s,* 421*s,* 413*s,* 406 s cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.07 (d, *J* = 8.0 Hz, 1H, NH), 7.60–7.50 (m, 5H, 12-H, 13-H, 14-H, NH_2), 7.46–7.39 (m, 2H, 11-H, 15-H), 7.18–7.10 (m, 3H, 6-H, 7-H, 8-H), 7.06–6.99 (m, 2H, 5-H, 9-H), 3.93 (*dd*, *J* = 9.7, 4.3 Hz, 1H, 1-H_a), 3.88 (*dd*, *J* = 9.7, 5.4 Hz, 1H, 1-H_b), 3.61–3.52 (m, 1H, 2-H), 2.75 (*dd*, *J* = 13.8, 6.1 Hz, 1H, 3-H_a), 2.48–2.45 (m, 1H, 3-H_b) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 141.1 (C-10), 137.1 (C-4), 132.1 (C-13), 129.0 (C-5, C-9), 129.0 (C-12, C-14), 128.2 (C-6, C-8), 126.3 (C-11, C-15), 126.1 (C-7), 70.0 (C-1), 53.9 (C-2), 36.7 (C-3) ppm; MS (ESI, MeOH): *m*/z = 392.8 (100 %, $[M+Na]^+$), 408.8 (14 %, $[M+K]^+$), 762.7 (3 %, $[2\ M\ +\ Na]^+$); analysis calcd. for $C_{15}H_{18}N_2O_5S_2$ (370.44): C 48.64, H 4.90, N 7.56; found: C 48.37, H 5.13, N 7.46.

*4.4.28. (2S,3S)-3-methyl-2-(phenylsulfonamido)pentyl sulfamate (**12b**)*

Following GPB from **12a** (204 mg, 0.79 mmol), TEA (0.44 mL, 3.18 mmol) and SC (367 mg, 3.17 mmol) **12b** (130 mg, 52 %) was obtained as a colorless, viscous oil; $R_f = 0.70$ (SiO_2, $CHCl_3$/ethyl acetate, 4:6); $[\alpha]_D^{20} = -41.48°$ (*c* 0.021, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (3.81); IR (ATR) ν = 3604*vw*, 3537*vw*, 3280*w*, 3106*vw*, 2967*w*, 2879*w*, 1624*vw*, 1559*w*, 1448 *m*, 1365*s,* 1323 *m*, 1310 *m*, 1178*s,* 1157*vs,* 1090*s,* 1024 *m*, 978*s,* 923*s,* 831 *m*, 808 *m*, 755*s,*720*s,* 689*s,* 588*s,* 549*vs,* 475*s,* 462*s,* 442*s,* 427 *m*, 418 *m*, 408 m cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.85 (d, *J* = 8.1 Hz, 1H, NH), 7.83–7.79 (m, 2H, 8-H, 12-H), 7.65–7.59 (m, 1H, 10-H), 7.59–7.54 (m, 2H, 9-H, 11-H), 7.46 (s, 2H, NH_2), 3.93 (*dd*, *J* = 10.0, 5.4 Hz, 1H, 1-H_a), 3.87 (*dd*, *J* = 10.0, 4.9 Hz, 1H, 1-H_b), 3.25 (*dq*, *J* = 7.8, 5.3 Hz, 1H, 2-H), 1.48–1.42 (m, 1H, 4-H_a), 1.31–1.23 (m, 1H, 4-H_b), 0.91–0.83 (m, 1H, 3- H), 0.73 (d, *J* = 6.9 Hz, 3H, 5-H), 0.65 (t, *J* = 7.4 Hz, 3H, 6-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 141.6 (C-7), 132.3 (C-10), 129.1 (C-9, C-11), 126.4 (C-8, C-12), 68.4 (C-1), 56.1 (C-2), 35.3 (C-3), 24.0 (C-4), 14.6 (C-6), 11.1 (C-5) ppm; MS (ESI, MeOH): *m*/z = 359.0 (100 %, $[M+Na]^+$); analysis calcd. for $C_{12}H_{20}N_2O_5S_2$ (336.42): C 42.84, H 5.99, N 8.33; found: C 42.66, H 5.25, N 42.31.

*4.4.29. (R)-4-(Methylthio)-2-(phenylsulfonamido)butyl sulfamate (**13b**)*

Following GPB from **13a** (300 mg, 1.10 mmol), TEA (0.61 mL, 4.38 mmol) and SC (506 mg, 4.38 mmol) **13b** (176 mg, 46 %) was obtained as a colorless, viscous oil; $R_f = 0.53$ (SiO_2, $CHCl_3$/ethyl acetate, 4:6); m. p. = 62.5–65.3 °C; $[\alpha]_D^{20} = +43.50°$ (c 0.01, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (4.07); IR (ATR) ν = 3247*w*, 3097*w*, 3024*w*, 2891*w*, 1447 *m*, 1325 *m*, 1235 *m*, 1153*vs,* 1092*s,* 1072 *m*, 1039*s,* 997 *m*, 916*w*, 885*w*, 755*s,* 723*s,* 689*s,* 623*w*, 580*s,* 548*vs,* 484*w* cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.88–7.84 (m, 2H, 7-H, 11-H), 7.85–7.80 (m, 1H, NH), 7.73–7.68 (m, 1H, 9-H), 7.67–7.55 (m, 4H, 8-H, 10-H, NH_2), 4.25–4.17 (m, 1H, 2-H), 3.55–3.48 (m, 1H, 4-H_a), 3.43–3.36 (m, 1H, 1-H_a), 3.36–3.27 (m, 2H, 1-H_b, 4-H_b), 2.82 (s, 3H, 5-H), 2.37–2.24 (m, 1H, 3-H_a), 2.13–2.05 (m, 1H, 3-H_b) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 140.2 (C-6), 132.9 (C-9), 129.5 (C-8, C-10), 126.5 (C-7, C-11), 55.1 (C-2), 47.8 (C-1), 41.1 (C-4), 33.0 (C-3), 25.7 (C-5) ppm; MS (ESI, MeOH): *m*/z = 297.8 (100 %, $[M+Na]^+$); analysis calcd. for $C_{11}H_{18}N_2O_5S_3$ (354.45): C 37.27, H 5.12, N 7.90; found: C 36.97, H 5.38, N 7.72.

*4.4.30. (S)-4-(Methylthio)-2-(phenylsulfonamido)butyl sulfamate (**14b**)*

Following GPB from **14a** (300 mg, 1.10 mmol), TEA (0.61 mL, 4.38 mmol) and SC (506 mg, 4.38 mmol) **14b** (206 mg, 53 %) was obtained as a white solid; $R_f = 0.58$ (SiO_2, $CHCl_3$/ethyl acetate, 4:6); m. p. = 63.0–65.0 °C; $[\alpha]_D^{20} = -43.84°$ (*c* 0.025, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (4.04); IR (ATR) ν = 3248*w*, 3100*w*, 3024*w*, 2886*w*, 1446 *m*, 1324 *m*, 1312 *m*, 1234 *m*, 1153*vs,* 1092*s,* 1072 *m*, 1039*s,* 997 *m*, 886*w*, 755*s,* 721*s,* 689*s,* 623*w*, 580*s,* 549*vs* cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.88–7.84 (m, 2H, 7-H, 11-H), 7.82–7.79 (m, 1H, NH), 7.73–7.68 (m, 1H, 9-H), 7.67–7.56 (m, 4H, 8-H, 10-H, NH_2), 4.23–4.17 (m, 1H, 2-H), 3.55–3.47 (m, 1H, 4-H_a), 3.42–3.35 (m, 1H, 1-H_a), 3.33–3.26 (m, 2H, 1-H_b, 4-H_b), 2.81 (s, 3H, 5-H), 2.34–2.25 (m, 1H, 3-H_a), 2.14–2.04 (m, 1H, 3-H_b) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 140.3 (C-6), 132.9 (C-9), 129.5 (C-8, C-10), 126.5 (C-7, C-11), 55.1 (C-2), 47.8 (C-1), 41.1 (C-4), 33.0 (C-3), 25.7 (C-5) ppm; MS (ESI, MeOH): *m*/z = 297.9 (100 %, $[M+Na]^+$); analysis calcd. for $C_{11}H_{18}N_2O_5S_3$ (354.45): C 37.27, H 5.12, N 7.90; found: C 36.97, H 5.36, N 7.67.

*4.4.31. (R)-3-(1H-Indol-3-yl)-2-(phenylsulfonamido)propyl sulfamate (**15b**)*

Following GPB from **15a** (190 mg, 0.56 mmol), TEA (0.31 mL, 2.24 mmol) and SC (259 mg, 2.24 mmol) **15b** (116 mg, 49 %) was obtained as a white solid; $R_f = 0.78$ (SiO_2, $CHCl_3$/ethyl acetate, 4:6); m. p. = 69.0–72.0 °C; $[\alpha]_D^{20} = +65.31°$ (*c* 0.038, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (4.56); IR (ATR) ν = 3401*w*, 3280*w*, 1457*w*, 1447 *m*, 1423*w*, 1359 *m*, 1320 *m*, 1309 *m*, 1179*s,* 1154*vs,* 1089*s,* 1033*w*, 1025*w*, 1001 *m*, 986 *m*, 928 *m*, 817 *m*, 742*s,* 719 *m*, 686*s,* 592*s,* 579 *m*, 567*s,* 550*s,* 515 *m*, 506 *m*, 499 *m*, 490 *m*, 481*w*, 470 *m*, 458 *m*, 424 *m*, 413*w* cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.76 (s, 1H, NH_{indole}), 8.04 (d, *J* = 7.5 Hz, 1H, NH), 7.70–7.64 (m, 2H, 13-H, 17-H), 7.54–7.46 (m, 3H, NH_2, 15-H), 7.45–7.38 (m, 2H, 14-H, 16-H), 7.31–7.26 (m, 1H, 10-H), 7.11–6.99 (m, 3H, 5-H, 7-H, 8-H), 6.89 (*ddt*, *J* = 8.0, 7.0, 1.0 Hz, 1H, 9-H), 3.92 (*dq*, *J* = 9.7, 4.6 Hz, 2H, 1-H), 3.59–3.50 (m, 1H, 2-H), 2.88 (*dd*, *J* = 14.3, 7.8 Hz, 1H, 3-H_a), 2.63 (*dd*, *J* = 14.2, 6.8 Hz, 1H, 3-H_b) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 141.0 (C-12), 136.1 (C-6), 132.1 (C-15), 128.8 (C-14, C-16), 126.8 (C-11), 126.2 (C-13, C-17), 124.0 (C-5), 120.8 (C-8), 118.3 (C-9), 117.7 (C-10), 111.4 (C-7), 109.1 (C-4), 69.7 (C-1), 52.8 (C-2), 26.8 (C-3) ppm; MS (ESI, MeOH): *m*/z = 431.8 (100 %, $[M+Na]^+$); analysis calcd. for $C_{17}H_{19}N_3O_5S_2$ (409.48): C 49.87, H 4.68, N 10.26; found: C 49.65, H 4.97, N 10.01.

*4.4.32. (S)-3-(1H-Indol-3-yl)-2-(phenylsulfonamido)propyl sulfamate (**16b**)*

Following GPB from **16a** (300 mg, 0.91 mmol), TEA (0.50 mL, 3.64 mmol) and SC (421 mg, 3.64 mmol) **16b** (146 mg, 39 %) was obtained as a white solid; $R_f = 0.77$ (SiO_2, $CHCl_3$/ethyl acetate, 4:6); m. p. = 69.0–72.0 °C; $[\alpha]_D^{20} = -66.24°$ (*c* 0.021, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 221 nm (4.58); IR (ATR) ν = 3400*w*, 3276*w*, 1457*w*, 1447 *m*, 1423*w*, 1360 *m*, 1320 *m*, 1309 *m*, 1178*s,* 1155*vs,* 1089*s,* 1001 *m*, 987 *m*,

929 *m*, 817 *m*, 768*w*, 742*s*, 719 *m*, 687 *m*, 627*w*, 593*s*, 567*s*, 550*s*, 512 *m*, 504 *m*, 468*w*, 459*w*, 424 m cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 10.79–10.73 (m, 1H, NH_{indole}), 8.04 (d, J = 7.5 Hz, 1H, NH), 7.70–7.64 (m, 2H, 13-H, 17-H), 7.58–7.46 (m, 3H, NH_2, 15-H), 7.44–7.38 (m, 2H, 14-H, 16-H), 7.30–7.25 (m, 1H, 10-H), 7.10–6.99 (m, 3H, 5-H, 7-H, 8-H), 6.88 (*ddd*, J = 8.0, 7.0, 1.0 Hz, 1H, 9-H), 3.92 (*qd*, J = 9.7, 4.6 Hz, 2H, 1-H), 3.59–3.49 (m, 1H, 2-H), 2.87 (*dd*, J = 14.4, 7.8 Hz, 1H, 3-H_a), 2.63 (*dd*, J = 14.4, 6.6 Hz, 1H, 3-H_b) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 141.0 (C-12), 136.1 (C-6), 132.1 (C-15), 128.8 (C-14, C-16), 126.8 (C-11), 126.2 (C-13, C-17), 124.0 (C-5), 120.8 (C-8), 118.3 (C-9), 117.7 (C-10), 111.4 (C-7), 109.0 (C-4), 69.7 (C-1), 52.8 (C-2), 26.8 (C-3) ppm; MS (ESI, MeOH): *m/z* = 431.7 (100 %, $[M+Na]^+$); analysis calcd. for $C_{17}H_{19}N_3O_5S_2$ (409.48): C 49.87, H 4.68, N 10.26; found: C 49.61, H 4.87, N 9.97.

CRediT authorship contribution statement

Toni C. Denner: Writing – review & editing, Writing – original draft, Investigation. **Elsa L. Klett:** Writing – review & editing, Writing – original draft, Investigation. **Niels V. Heise:** Writing – review & editing, Writing – original draft, Investigation. **René Csuk:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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Ureidobenzenesulfonamides as Selective Carbonic Anhydrase I, IX, and XII Inhibitors

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Abstract: Sulfonamides remain an important class of drugs, especially because of their inhibitory effects on carbonic anhydrases. Herein, we have synthesized several sulfonamides and tested them for their inhibitory activity against carbonic anhydrases hCA I, hCA II, hCA IX, and hCA XII, respectively. Thereby, biphenyl- and benzylphenyl-substituted sulfonamides showed high selectivity against hCA IX and hCA XII; these enzymes are common targets in the treatment of hypoxic cancers, and noteworthy inhibitory activity was observed for several compounds toward hCA I that might be of interest for future applications to treat cerebral edema. Compound **3** (4-[3-(2-benzylphenyl)ureido]benzenesulfonamide) held an exceptionally low K_i value of 1.0 nM for hCA XII.

Keywords: carbonic anhydrase; inhibitor; ureidobenzenesulfonamides

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1. Introduction

The development of sulfonamides began as early as 1908 [1], when the antibacterial effect of "Prontosil" (4-(2,4-diaminophenyl)diazinyl)-benzene sulfonamide [2] was first successfully used to treat bacterial sepsis in humans [3]. While today, sulfonamides have—more or less—lost their importance as antibacterial drugs due to the development of other drug classes, a new era began with the observation that representatives of this substance class are excellent inhibitors of the enzyme carbonic anhydrase [4].

Carbonic anhydrases (CAs; EC 4.2.1.1) are essential for life, as they balance acid and base equilibria in tissues and blood through the conversion of carbon dioxide and water into bicarbonate and protons. The importance of CAs can be seen from their high turn-over numbers [5], which are even faster than those measured for the enzyme acetylcholinesterase (AChE), being necessary for synaptic transmission and hence belonging to the fastest catalyzing enzymes. Furthermore, it has been shown that an isoform, carbonic anhydrase IX, is overexpressed in many types of cancer, thereby leading to an acidosis of the surrounding tissue and, consequently, promoting tumor growth, invasion, and proliferation [6]. In addition, changes in the tumor microenvironment that are induced by hypoxia promote aggressive and resistant cancer phenotypes [7], thus resulting in a poor prognosis in cancer patients [8]. Especially in recent years, the development of carbonic anhydrase inhibitors (CAIs) is of major interest [9,10], as CAIs may be supportive in anticancer therapy [11]. Especially, targeting hCA IX and XII seems to be of major interest, as these enzymes are overexpressed in hypoxic tumors including breast, cervix, and lung carcinomas [12–17].

These metalloenzymes play a role in numerous physiological and pathological processes. Two out of the fifteen human CA isoforms, namely, hCA IX and XII, are heavily expressed in hypoxic tumors due to the activation of HIF-1/2 (the transcription factor

hypoxia inducible factor). CA XII is moderately present, while CA IX is almost nonexistent in normal tissues. This renders them highly promising prospects for developing innovative anticancer drugs. Thus, HIF-1 is associated with tumorigenesis and other physiological or pathological processes, along with biochemical mechanisms that are responsible for these phenomena. Indeed, in hypoxia and particularly in hypoxic tumors, numerous proteins that are implicated in metabolism, pH regulations, angiogenesis, and immunological response are overexpressed due to the HIF signal cascade, rendering these tumor cells rather distinct from their normal counterparts.

However, the application of sulfonamide-based inhibitors acting on all different isoforms of CA may result in severe side effects like paresthesias and others, and especially in patients with pre-existing renal, pulmonary, and hepatic diseases, the most important concerns are acidosis, respiratory failure, and encephalopathy [15]. Consequently, isoform selectivity is of the utmost importance in order to avoid or minimize undesired side effects.

Several sulfonamides have already been synthesized and extensively investigated for their potential as inhibitors of CAs, as they appear to be very promising agents for the treatment of hypoxic tumors, especially for inhibiting the CA isoforms IX and XII since they are in vivo under the control of HIF-1, the transcription factor hypoxia-inducible factor. In particular, SLC-0111 (Figure 1), a sulfonamide inhibitor in clinical trials, seems to be very promising [18–20]. This compound is used in Phase 1b/II for combination treatment together with gemcitabine of metastatic pancreatic ductal adenocarcinoma.

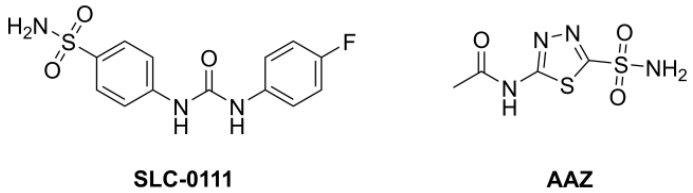


Figure 1. Structure of **SLC-0111** and of acetalzolamide (**AAZ**).

Thus, HIF-1 is associated with tumorigenesis and other physiological or pathological processes, along with biochemical mechanisms that are responsible for these phenomena. Indeed, in hypoxia and particularly in hypoxic tumors, numerous proteins that are implicated in metabolism, pH regulations, angiogenesis, and immunological response are overexpressed due to the HIF signal cascade, rendering these tumor cells rather distinct from their normal counterparts. In response to hypoxia, tumor cells also experience transcriptional reprogramming involving increased expression of hCA IX, which maintains the acid–base balance. An overexpression of CA IX has been demonstrated to be associated with a negative prognosis in multiple cancers.

Furthermore, **SLC-0111** administration negated the hypoxia-triggered overexpression of CA IX and reduced cell viability, and **SLC-0111** also hindered cell migration in a wound healing experiment. In addition, transcriptomic profiles exhibited contrasting responses to **SLC-0111** administration between normoxic and hypoxic conditions, despite eliciting upregulation of various tumor suppressor genes in both scenarios.

SLC-0111 holds an ureido moiety that is found in several compounds used in a broad variety of biomedical applications. Recently, some ureidobenzenesulfonamides have been reported as efficient inhibitors of carbonic anhydrase II [21]. The inhibition of this enzyme is essential in order to reduce intraocular pressure in patients suffering from glaucoma; it is expected that in 2040, the number of persons suffering from glaucoma will increase to 111.8 million [22]. However, the treatment of primary open-angle glaucoma, a multifactorial optic neuropathy that is linked to progressive retinal ganglion cell death and visual field loss, is one of the primary uses of CA II inhibitors. Elevated intraocular pressure is the primary risk factor for primary open-angle glaucoma, although there are numerous other related variables. Because of the unsatisfactory side effect profile of oral carbonic anhydrase inhibitors, topical CAIs took a long time to develop but have been shown to be a valuable adjunct to the treatment of primary open-angle glaucoma. They work by preventing the

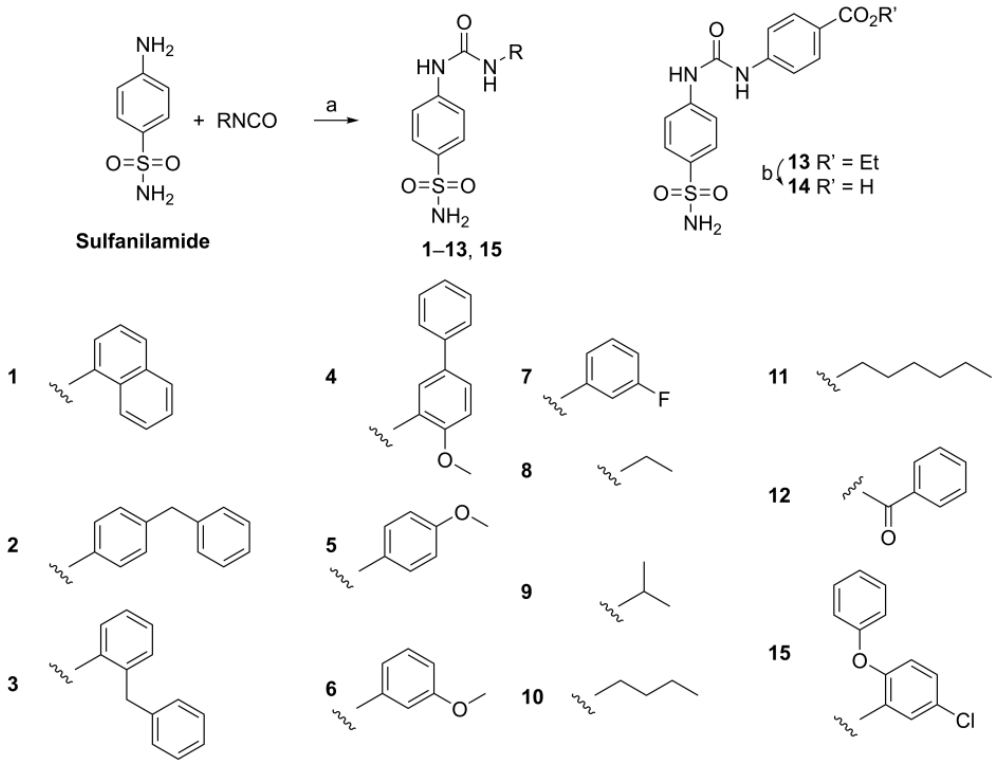
ciliary epithelium's CA II from functioning. As a result, fewer bicarbonate ions are formed, which decreases intraocular pressure and fluid transfer.

Furthermore, out of all the organs studied, the mammalian central nervous system (CNS) possesses the greatest number of CA isoforms (at least 9). Isoforms I, VB, VII, VIII, X, XI, XII, and XIV are also found, with hCA II being the most prevalent. Carbonic anhydrase inhibitors have been used therapeutically in a number of brain pathologies because of the broad expression range of CA isoforms in the brain. In epilepsy and idiopathic intracranial hypertension, where acetazolamide (**AAZ**, Figure 1) is one of the medications now in clinical use, inhibition via CAIs has been shown to be clinically beneficial. Moreover, migraine, neuropathic pain, diabetes-induced blood–brain barrier failure, and amyloid β-induced mitochondrial dysfunction that is characteristic of Alzheimer's disease are possible therapeutic uses for CAIs targeting CNS isoforms.

Consequently, the treatment of cerebral edema became a subject of scientific interest again, since swelling of the brain is a known side effect of some new drugs (e.g., lecanemab [23] and donanemab [24,25]) that were recently approved for the therapy of Alzheimer's disease. Treatment of cerebral edema might include the intravenous injection of a CA inhibitor, especially of an inhibitor acting on hCA I [26,27].

2. Results

Starting from a readily available starting material, compounds **1**–**15** were synthesized (Scheme 1) as previously described via the reaction of sulfanilamide with isocyanates [21]. This approach is especially suited for accessing the target compounds when the isocyanate is readily available. Recently, an alternative one-pot procedure has been published [28].



Scheme 1. Synthesis of compounds **1**–**15**: (a) MeCN, 12 h, 23 °C: **1** naphthalen-1-yl, **2** 4-benzylphenyl, **3** 2-benzylphenyl, **4** 4-methoxy-(1,1′-biphenyl)-3-yl, **5** 4-methoxyphenyl, **6** 3-methoxyphenyl, **7** 3-fluorophenyl, **8** ethyl, **9** isopropyl, **10** butyl, **11** hexyl, **12** benzoyl, **13** ethyl-4-benzoate, **15** 5-chloro-2-phenoxyphenyl; (b) KOH, MeOH, Δ, 4 h, 98%.

Furthermore, the ureido moieties incorporated into benzenesulfonamides holding an extra ureido moiety can be regarded as an interesting class of CA inhibitors (CAIs), since

the presence of a urea functionality in the zinc-binding group is among the most promising trends in the design of CAIs.

In this study, we were especially interested in the investigation of benzylphenyl and biphenyl derivates, since previous docking studies indicated high binding affinities, which parallels previous results from the literature [21].

Compounds **1–15** were obtained in good yields and subjected to screening through stop–flow experiments employing enzymes hCA I, hCVA II, hCA IX, and hCA XII, respectively. Acetazolamide (**AAZ**, Figure 1) was used as a positive standard. The results from these assays are summarized in Table 1; data for **SLC-0111** were taken from the literature for comparison [29].

Table 1. K_i values (in nM): Inhibition of human carbonic anhydrase I, II, IX, XII of compounds **1–15** with the standard inhibitor acetazolamide (**AAZ**) and **SLC-0111** (positive control) and calculated selectivity of hCA IX and hCA XII against hCA I and hCA II, respectively. Selectivity was calculated as the ratio of the respective K_i values.

Cmp	hCA I	hCA II	hCA IX	S(I/IX)	S(II/IX)	hCA XII	S(I/XII)	S(II/XII)
1	242.8	33.5	42.9	5.66	0.78	15.5	15.66	2.16
2	785.2	356.2	15.2	51.66	23.43	6.4	122.69	55.66
3	78.3	61.5	8.2	9.55	7.50	1	78.30	61.50
4	6650	5034	188.6	35.26	26.69	32.9	202.13	153.01
5	94.4	56.5	149.7	0.63	0.38	79.6	1.19	0.71
6	84.5	56.8	210.4	0.40	0.27	90.4	0.93	0.63
7	263.3	53.6	165.3	1.59	0.32	6.7	39.30	8.00
8	871.6	595.2	134.3	6.49	4.43	86.5	10.08	6.88
9	447.7	98.6	183.7	2.44	0.54	45.9	9.75	2.15
10	86.2	84.9	84.9	1.02	1.00	2.9	29.72	29.28
11	693.9	497.1	57.7	12.03	8.62	6.8	102.04	73.10
12	948.5	209.2	207.7	4.57	1.01	63.9	14.84	3.27
13	773.3	63.5	216.7	3.57	0.29	60.7	12.74	1.05
14	368.1	84.4	174.9	2.10	0.48	53.4	6.89	1.58
15	591.1	300.4	161.4	3.66	1.86	14.2	41.63	21.15
SLC-0111	5080	960	45.1	112.6	21.3	4.5	1128.9	213.3
AAZ	250	12.1	25.8	9.69	0.47	5.7	43.86	2.12

As a result of these assays, most of the compounds showed inhibition for all isoenzymes.

However, it must be noted that compounds **2** and **3** exerted promising K_i values for hCA XII (K_i = 6.4 and 1.0 nm, respectively), with 3 being also an excellent inhibitor for hCA IX (K_i = 8.2 nm). A graphic comparison of all compounds is depicted in Figure 2.

Compound **3** exerted significantly lower K_i values for hCA I (compared with standard **AAZ**) and proved to be a better inhibitor of hCA IX and hCA XII than **AAZ** (Figure 3A). A comparison of the K_i values for compound **3** together with its isoform selectivity is depicted in Figure 3B.

Compound **4** is not a good inhibitor of both hCA I and hCA II, with K_i values > 5 μM. However, with K_i values of 188 nM and 32.9 nM for hCA IX and hCA XII, respectively, compound **4** holds a noteworthy selectivity, especially for hCA XII, with selectivity factors of 153 to 202, respectively, when compared with hCA I and hCA II. A comparison of the selective index is depicted in Figure 4.

While the biphenyl motif proved to be a very weak binder to hCA I and hCAII, the phenylbenzyl substituted ureas were good inhibitors of all tested isoenzymes and exhibited extraordinarily low K_i values for hCA IX and hCA XII (15 nM and 6.4 nM for compound **2**). Compound **3** even showed lower K_i values than **AZZ** with 8.2 nM against hCA IX and 1 nM against hCA XII. The low K_i values for compounds **2** and **3** also resulted in good selectivity factors of 23 to 122 for compound **2**. Compound **3**, however, held no pronounced selectivity for hCA IX but high selectivity of 78 and 62 for hCA XII.

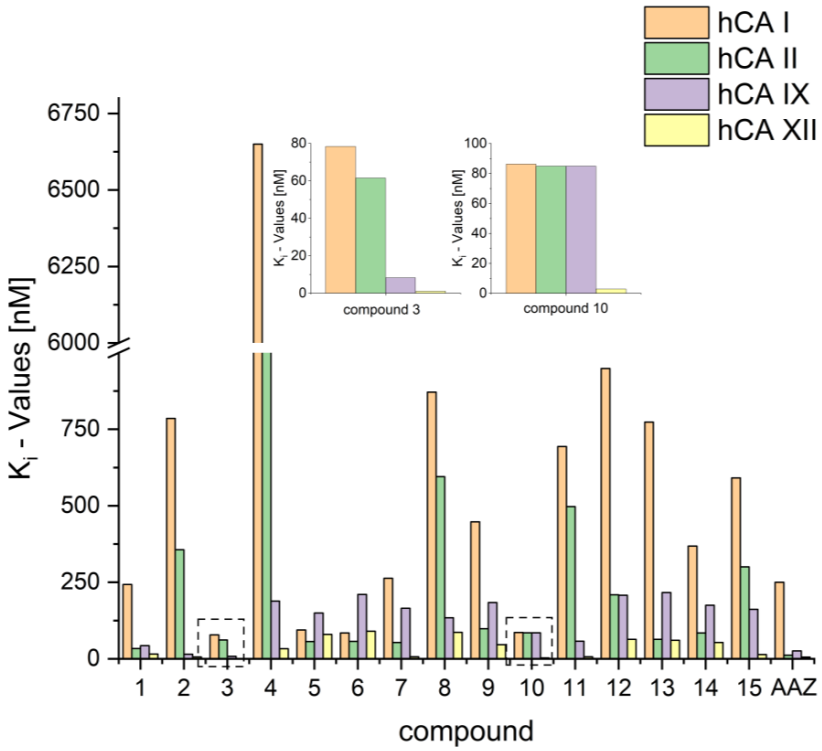


Figure 2. Depiction of K_i values for compounds **1–15** and **AAZ**.

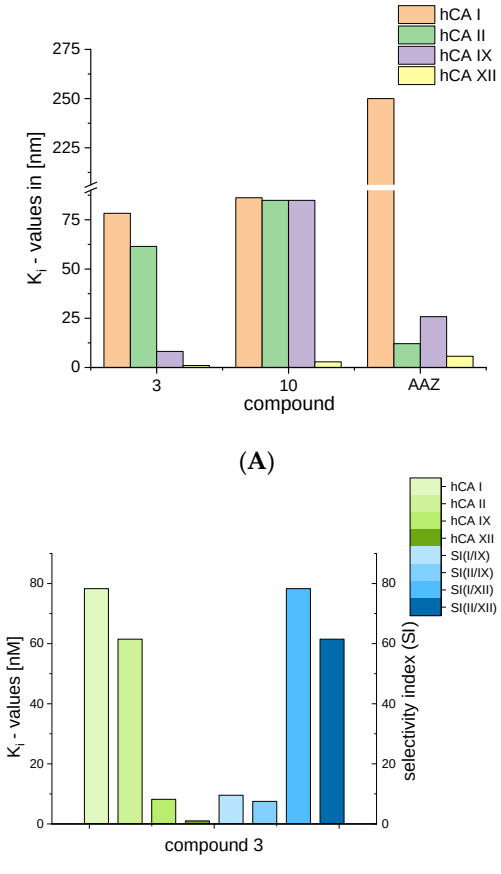


Figure 3. (**A**): Comparison of K_i values for compounds **3** and **10** and standard acetazolamide (**AAZ**); (**B**): comparison of K_i values for compound **3** with respect to isoforms of hCA.

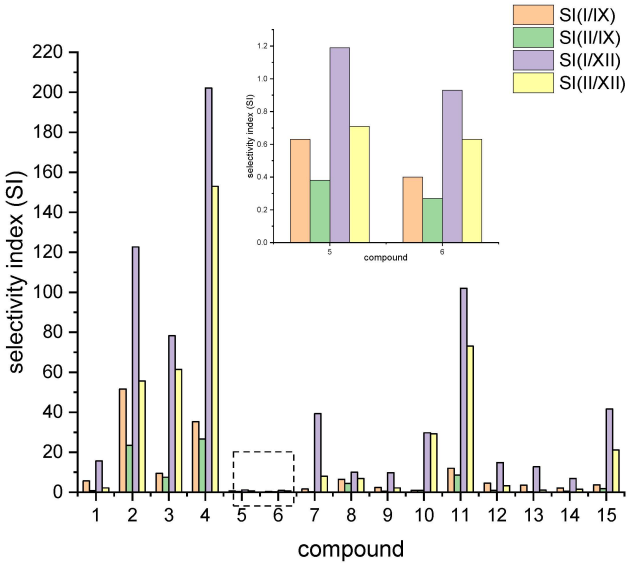


Figure 4. Comparison of selectivity index for compounds **1**–**15** with respect to the different isoforms.

The compound **SLC-0111** (Figure 1, K_i = 454 nM for hCA XII), which is currently used in clinical studies, was the best inhibitor of this study; compound **3**, regarding hCA XII (K_i = 1.0 nM), showed similar ADME profiles (Figure 5; calculated online www.swissadme.ch; accessed on 1 July 2023); this makes this compound an interesting candidate for further biological studies. The predicted ADME data for all compounds are compiled in the Supplementary Materials file.

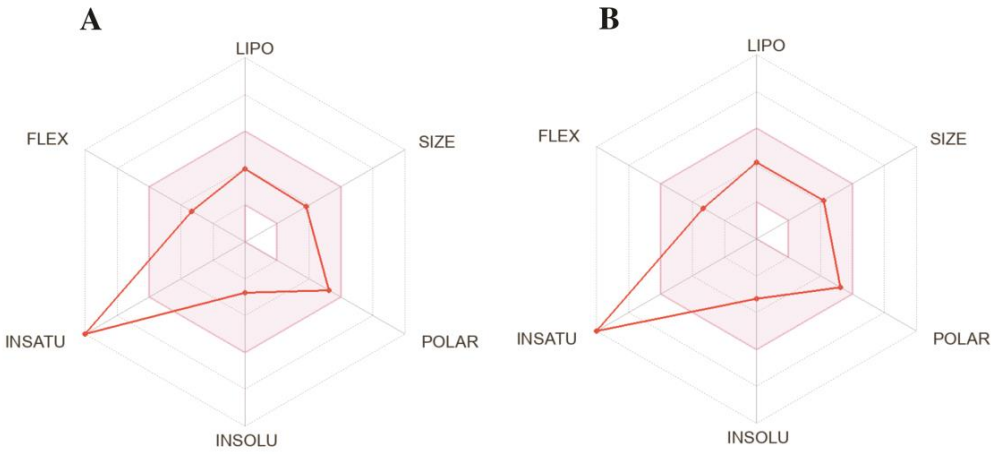


Figure 5. Predicted ADME properties of clinically tested SLC-0111 (**A**) and compound **3** (**B**).

As mentioned above, treatment of cerebral edema usually includes the application of a CA inhibitor. It might be assumed that a selective hCA I inhibitor might be beneficial for the therapy of this serious and life-threatening disease. Upon close examination of our results, a striking inhibitory disparity between compounds **8** and **10** became evident, approaching nearly 10-fold (with K_i values of 871.6 and 86.2 nM, respectively). Intriguingly, these compounds displayed minimal scaffolding differences, underscoring the pivotal role of subtle alkyl chain structural variances in dictating their divergent inhibitory activities against the targeted isoform. To unravel the molecular basis of these differences, X-ray diffraction experiments against hCA I were called for in order to obtain an insight into the ligand–protein interactions at the atomic level and focus on the tail interaction that underpinned the observed divergent potencies.

Regarding the complex between **8** and hCA I, after the initial rounds of refinement, the calculated Fo-Fc map showed, inside the active site, a clear electron density that was

compatible with the sulfonamide moiety (Figure 6B), which interacts directly with the zinc atom in the active sites. In addition, the sulfonamide group formed the characteristic hydrogen bond with residue Thr199, stabilizing the complex, which is typical of this class of inhibitors (Figure 6A).

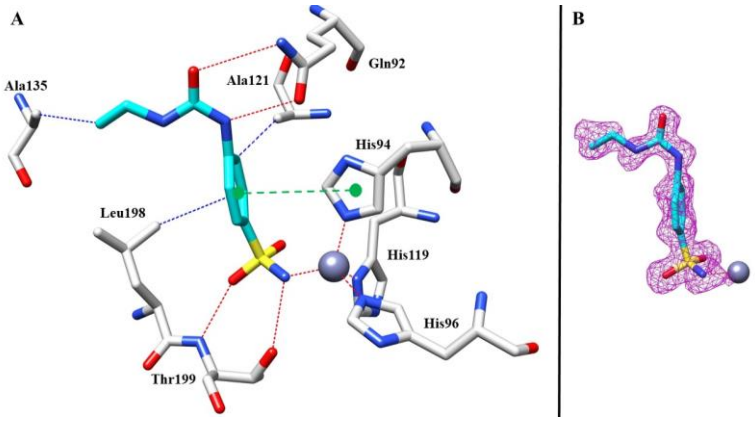


Figure 6. (**A**) Compound **8** inside the active site of hCA I. Hydrophobic (blue), hydrophilic (red), and π-stacking (green) interactions are labeled. (**B**) Electron density 2Fo-Fc map of **8** bound to zinc in hCA I active site; contoured at the 1.0 σ level.

Other hydrogen bonds were observed for the ureido moiety with a side chain of Gln92, and the benzene ring formed a π-stacking interaction with the aromatic ring of His94. Finally, we observed hydrophobic connections between Leu198 and Ala121 with the benzenesulfonamide moiety and between Ala135 and the end of the ethyl tail.

Moving on the second complex investigated, 10 with hCA I, we also found a clear density for the sulfonamide moiety inside the active site of the protein (Figure 7B). The benzenesulfonamide scaffold showed the same binding mode as mentioned above, with the hydrogen bonds between the sulfonamide group and Thr199 stabilizing the binding of the inhibitor, similar to the previous complex with hCA I (Figure 7A).

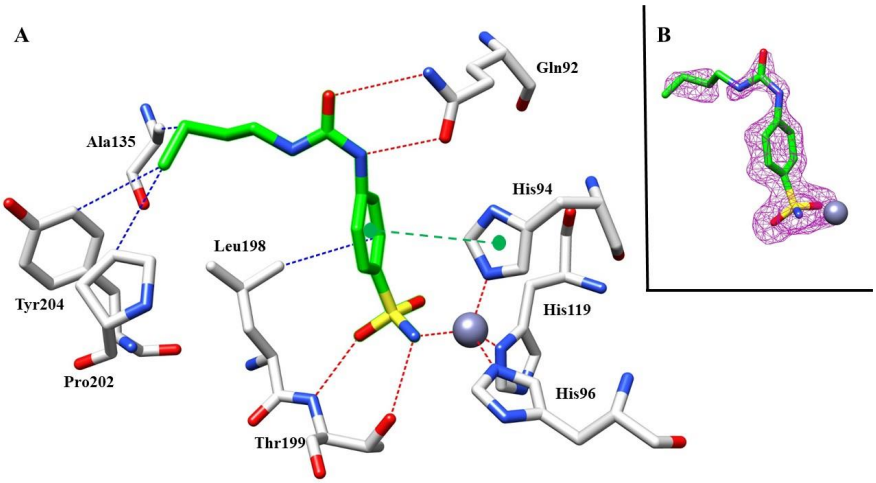


Figure 7. (**A**) Compound **10** inside the active site of hCA I. Hydrophobic (blue), hydrophilic (red), and π-stacking (green) interactions are labeled. (**B**) Electron density 2Fo-Fc map of **10** bound to zinc in hCA I active site; contoured at the 1.0 σ level.

Although a structural comparison of the two inhibitors with hCA I showed similar features, such as the same sulfonamide moiety interacting with the catalytic zinc ion and hydrogen bonds with ureido group and π-stacking interactions, the longer butyl chain of **10** compared with the ethyl one of **8** allows for more hydrophobic interactions with the

side chains of Pro202 and Tyr204. This simple modification could explain the different inhibition potency against hCA I of nearly 10-fold between **8** and **10**.

3. Experimental Procedure

Reagents were bought from commercial suppliers and used without further purification. The solvents were dried according to usual procedures. TLC was performed on silica gel (Macherey-Nagel (Macherey Nagel GmbH, Düren, Germany), detection with UV absorption). Melting points are uncorrected (Büchi M-565). NMR spectra were recorded using VARIAN spectrometers (Varian GmbH, Darmstadt, Germany) at 27 °C (δ given in ppm; *J* in Hz, typical experiments for assignments: ^{13}C APT, HMBC, HSQC). ASAP-MS spectra were taken on an Advion expression CMS-L (Advion Interchim, Ithaca, NY, USA) with an ASAP/APCI Ion source (capillary voltage 150 V, capillary temperature 220 °C, and voltage of the ion source: 15 V; APCI source temperature 300 °C with 5 µA). IR spectra were recorded on a Perkin-Elmer Spectrum Two (UATR Two Unit; Perkin-Elmer GmbH, Rodgau, Germany. CA inhibition assays were performed as previously described.

3.1. General Procedure for the Synthesis of Compounds **1**–**13** *(GP)*

A solution of sulfanilamide (2.5 mmol, 431 mg) and the corresponding isocyanate (1 eq.) in dry acetonitrile (10 mL) was stirred for 12 h at 23 °C, and the precipitate was filtered off and subsequently washed with acetonitrile, ethanol, and diethyl ether (5 mL each), and dried in vacuo to afford **1**–**13** each as a colorless crystalline solid.

3.1.1. 4-[3-(Naphthalen-1-yl)ureido]benzenesulfonamide (**1**)

Following GP, **1** was obtained in 72% yield; R_f = 0.54 (silica gel, $CHCl_3$/DMF/MeOH, 16:1:1); m.p.: 252–254 °C (lit.: [21] 254 °C); MS (APCI): *m*/*z* (%) = 342.1 ([M+H]$^+$, 88).

3.1.2. 4-[3-(4-Benzylphenyl)ureido]benzenesulfonamide (**2**)

Following GP, **2** was obtained in 48% yield; R_f = 0.50 (silica gel, $CHCl_3$/DMF/MeOH, 16:1:1); m.p.: 230–232 °C (lit: [21] 231 °C); MS (APCI): *m*/*z* (%) = 382.1 ([M+H]$^+$, 40).

3.1.3. 4-[3-(2-Benzylphenyl)ureido])benzenesulfonamide (**3**)

Following GP, **3** was obtained in 45% yield; R_f = 0.59 (silica gel, $CHCl_3$/DMF/MeOH, 16:1:1); m.p.: 219–221 °C (lit.: [21] 220 °C); MS (APCI): *m*/*z* (%) = 382.1 ([M+H]$^+$, 31).

3.1.4. 4-{3-[(4-Methoxy-(1,1′-biphenyl)-3-yl]ureido}benzenesulfonamide (**4**)

Following GP, **4** was obtained in 42% yield; R_f = 0.53 (silica gel, $CHCl_3$/DMF/MeOH, 16:1:1); m.p.: 271–272 °C (lit.: [21] 270 °C); MS (APCI): *m*/*z* (%) = 398.2 ([M+H]$^+$, 25).

3.1.5. 4-[3-(4-Methoxyphenyl)ureido])benzenesulfonamide (**5**)

Following GP, **5** was obtained in 65% yield; R_f = 0.45 (silica gel, $CHCl_3$/DMF/MeOH, 16:1:1); m.p.: 227–229 °C (lit.: [21] 229 °C); MS (APCI): *m*/*z* (%) = 322.4 ([M+H]$^+$, 18).

3.1.6. 4-[3-(3-Methoxyphenyl)ureido]benzenesulfonamide (**6**)

Following GP, **6** was obtained in 53% yield; R_f = 0.45 (silica gel, $CHCl_3$/DMF/MeOH, 16:1:1); m.p.: 238–239 °C (lit.: [21] 238 °C); MS (APCI): *m*/*z* (%) = 322.1 ([M+H]$^+$, 17).

3.1.7. 4-[3-(3-Fluorophenyl)ureido]benzenesulfonamide (**7**)

Following GP, **7** was obtained in 70% yield; R_f = 0.42 (silica gel, $CHCl_3$/DMF/MeOH, 16:1:1); m.p.: 240–241 °C (lit.: [21] 240 °C); MS (APCI): *m*/*z* (%) = 310.3 ([M+H]$^+$, 97).

3.1.8. 4-(3-Ethylureido)benzenesulfonamide (**8**)

Following GP, **8** was obtained in 68% yield; R_f = 0.37 (silica gel, $CHCl_3$/DMF/MeOH, 16:1:1); m.p.: 229–231 °C (lit.: [21] 232 °C); MS (APCI): *m*/*z* (%) = 244.0 ([M+H]$^+$, 35).

3.1.9. 4-(3-Isopropylureido)benzenesulfonamide (**9**)

Following GP, **9** was obtained in 54% yield; R_f: = 0.65 (silica gel, $CHCl_3$/DMF/MeOH, 16:1:1); m.p.: 221–223 °C (lit.: 223 °C); MS (APCI): *m*/*z* (%) = 256.3 ([M+H]$^+$, 96).

3.1.10. 4-(3-Butylureido)benzenesulfonamide (**10**)

Following GP, **10** was obtained in 68% yield; R_f = 0.43 (silica gel, $CHCl_3$/DMF/MeOH, 16:1:1); m.p.: 194–196 °C (lit.: [21] 195 °C); MS (APCI): *m*/*z* (%) = 272.3 ([M+H]$^+$, 25).

3.1.11. 4-(3-Hexylureido)benzenesulfonamide (**11**)

Following GP, **11** was obtained in 71% yield; R_f = 0.49 (silica gel, $CHCl_3$/DMF/MeOH, 16:1:1); m.p.: 202–203 °C (lit.: [21] 201 °C); MS (APCI): *m*/*z* (%) = 300.3 ([M+H$^+$], 35).

3.1.12. N-[(4-Sulfamoylphenyl)carbamoyl]benzamide (**12**)

Following GP, **12** was obtained in 51% yield; R_f = 0.71 (silica gel, $CHCl_3$/DMF/MeOH, 16:1:1); m.p.: 262–264 °C (lit.: [21] 263 °C); MS (APCI): *m*/*z* (%) = 320.5 ([M+H]$^+$, 11).

3.1.13. Ethyl 4-[3-(4-sulfamoylphenyl)ureido]benzoate (**13**)

Following GP, **13** was obtained in 91% yield; R_f = 0.35 (silica gel, $CHCl_3$/DMF/MeOH, 16:1:1); m.p.: 261–263 °C (lit.: [21] 263 °C); MS (APCI): *m*/*z* (%) = 364.3 ([M+H]$^+$, 23).

3.1.14. 4-[3-(4-Sulfamoylphenyl)ureido]benzoic acid (**14**)

To a suspension of **13** (200 mg, 0.55 mmol) in MeOH (10 mL), finely grounded KOH (310 mg, 5.5 mmol) was added, and the mixture was heated under reflux for 4 h. The precipitate was filtered off, washed with MeOH, and dried to afford **14** (196 mg, 98%) as a colorless solid; R_f = 0.10 (silica gel, $CHCl_3$/DMF/MeOH, 16:1:1); m.p. 310–313 °C (lit.: [21] 277–303 °C); MS (APCI): *m*/*z* (%) = 336.4 ([M+H]$^+$, 97).

3.1.15. 4-[3-(5-Chloro-2-phenoxyphenyl)ureido]benzenesulfonamide (**15**)

To a solution of sulfanilamide (206 mg, 1.2 mmol) in acetonitrile (10 mL), 5-chloro-2-phenoxyphenylisocyanate (295 mg, 1.2 mmol) was added, and the mixture was stirred at 21 °C for 1 day. The volatiles were removed under reduced pressure, and the residue subjected to column chromatography (silica gel, hexane/ethyl acetate, 2:1) to afford **15** (361 mg, 72%) as a colorless solid; R_f = 0.40 (silica gel, hexanes/ethyl acetate, 2:1); m.p.: 209 °C (lit: [21] 290 °C); MS (APCI): *m*/*z* (%) = 418.3 ([M+H]$^+$, 21%).

3.2. Crystallization and X-ray Data Collection

Crystal of hCA I was obtained using the hanging drop vapor diffusion method using a 24-well Linbro plate. Then, 2 µL of 10 mg/mL solution of hCA I in Tris-HCl 20 mM pH 9.0 was mixed with 2 µL of a solution of 28–31% PEG4000, 0.2 M sodium acetate, and 0.1 M Tris pH 8.5–9.0 and was equilibrated against the same solution at 296 K. The complex was prepared by soaking the hCA I native crystals in the mother liquor solution containing the inhibitor at a concentration of 10 mM for two days. All crystals were flash-frozen at 100 K using a solution obtained by adding 15% (*v*/*v*) glycerol to the mother liquor solution as cryoprotectant. Data on crystal of the complex were collected using synchrotron radiation at the XRD2 beamline at Elettra Synchrotron (Trieste, Italy) with a wavelength of 1.0 Å and a DECTRIS Pilatus 6M detector. Data were integrated and scaled using the XDS program [30]. Data processing statistics are shown in Supporting Information.

3.3. Structure Determination

The crystal structure of hCA I (PDB accession code: 1JV0) without solvent molecules and other heteroatoms was used to obtain initial phases using Refmac5 [31]. Then, 5% of the unique reflections were selected randomly and excluded from the refinement data set for the purpose of R_{free} calculations. The initial |Fo − Fc| difference electron density maps unambiguously showed the inhibitor molecules. Refinements proceeded using

normal protocols of positional, isotropic atomic displacement parameters alternating with manual building of the models using COOT [32]. The quality of the final models was assessed with COOT and RAMPAGE [33]. Crystal parameters and refinement data are summarized in Supporting Information. Atomic coordinates were deposited in the Protein Data Bank (PDB accession code: 8CDX, 8CDZ). Graphical representations were generated with Chimera [34].

4. Conclusions

In conclusion, the synthesized ureidobenzenesulfonamides exhibited remarkably favorable K_i values within the enzyme assay. Notably, the ureidobenzenesulfonamides featuring biphenyl (**4**) and benzylphenyl (**2** and **3**) substitutions demonstrated exceptional selectivity toward tumor-associated hCA IX and hCA XII. Compound **3** showed a low K_i for the latter enzyme of 1 nM.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules28237782/s1.

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Isosteviol – A new scaffold for the synthesis of carbonic anhydrase II inhibitors

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ABSTRACT

The diterpene isosteviol can easily be synthesized via hydrolysis from stevioside, a renewable resource adding particular utility to its potential for drug synthesis. Of late, there has been an increase in scientific studies focusing on inhibitors of the enzyme carbonic anhydrase II, CA II, since this enzyme is not used just in glaucoma treatment but also in mitigating the side effects of Alzheimer's disease antibody therapy. The presence of a sulfamate or a sulfonamide group is a key structural feature in many CA II inhibitors. Thus, isosteviol was transformed into sulfamates, either spacered or unspacered, to scrutinize their ability to perform as CA II inhibitors. Three particular derivatives were discovered to be effective inhibitors. As a competitive inhibitor with a K_i = 2.59 μM, a 16-*O*-acetyl-isosteviol compound holding a 5-amino-1,3,4-thiadiazol-2-yl-amino substituent attached to a succinoyl ester nearly entirely inhibited CA II.

1. Introduction

Interest in the development of inhibitors of carbonic anhydrases (CAs) has increased in recent years. This newly awakened interest can be explained by several different facts. On the one hand, CAs are involved in a variety of physiological processes, including respiration, transportation of carbon dioxide and bicarbonate, pH and carbon dioxide homeostasis, gluconeogenesis, adipogenesis, ureagenesis as well as calcification [1–10]. As a result, the use of CAs for therapeutic purposes has led to the need to develop isoenzyme-specific inhibitors in order to minimize the type and number of possible interactions and undesirable side effects. In addition to an increased interest in inhibitors for the isoenzymes CA IX and CA XII, which are of particular importance in the treatment of hypoxic tumors, inhibitors of CA II, in particular, have recently gained in importance [11–18]. This can be explained on the one hand by their successful use in the treatment of eye diseases (e.g., glaucoma) [19–25] and on the other hand by the observation that CA inhibitors (CAIs) can also be applied to reduce oedema, in particular macular and cerebral oedema [26–31]. An abnormal level of hCA II has also been associated with neuropathic pain or epilepsy. Especially for the treatment of cerebral oedema it seems significant that the active substances lecanemab, aducanumab and donanemab recently used to treat Alzheimer's disease (AD) might lead to brain oedema and swelling in a significant number of patients as side effects, which can also result in death if not appropriately treated [32–37]. As the number of people suffering from AD or who will suffer from it in the next few years is very large worldwide and still increasing [38] (55 million in 2020, expected in 2030 78 million and 139 million in 2050), and the number of people who will be treated with these novel drugs will also increase, the development of CA II inhibitors seems of the highest interest.

Over the years, many different structures have been considered. What many structures, however, have in common is that they hold a sulfamate group as the effective structural element. For example, methazolamide [39,40] is used to treat glaucoma and acute mountain disease, ethoxzolamide [41,42] found applications to treat duodenal ulcers, and indisulam [43–45] is applied in the therapy of melanoma and leucemia. In this context, acetazolamide (**AAZ**) [46–49], a relatively non-selective CAI, and **SLC-0111** [50–57] (Scheme 1), a particularly successful CAI in the treatment of cancer, are particularly worth mentioning. Previous studies have also shown that the backbone to which the sulfonamide or the sulfamate group is attached is of particular importance, too, especially concerning a cell/organ targeting of the compounds. Thereby, our own earlier studies were mainly focused on pentacyclic triterpenes, some of which proved to be particularly good inhibitors of CA IX and CA II [58–64]. However, a tacit prerequisite in the development of novel CAIs is also good = low-cost accessibility of the molecular target. This is often not the case with pentacyclic triterpenes, and individual representatives of this substance class (e.g.,

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betulinic acid, platanic acid) hold also poor solubility properties, which makes future therapeutic application much more difficult.

A relatively new basic structure is provided by the diterpene isosteviol, which can be obtained in very good yields by acid hydrolysis from the sweetener stevioside (Scheme 1); the latter is available in large quantities and at low cost. In contrast to many triterpenoidic scaffolds isosteviol is not cytotoxic. Stevioside is also to be regarded as a renewable raw material as it can usually be obtained by extraction from the easily cultivated plant *Stevia rebaudiana* Bertoni (Asteraceae) which is also known as sugar leaf.

2. Results and discussion

Stevioside (Scheme 1) was converted into isosteviol (**1**) by acid hydrolysis as previously reported. Extensive NMR spectroscopic investigations also allowed an unambiguous assignment of all signals in the ^{1}H and ^{13}C NMR spectra [65,66]. Thereby, the use of a 2D ^{13}C INADEQUATE experiment proved very helpful in the unambiguous assignment of all carbons especially of the methyl groups. The NMR data for **1** have been compiled in Table. 1.

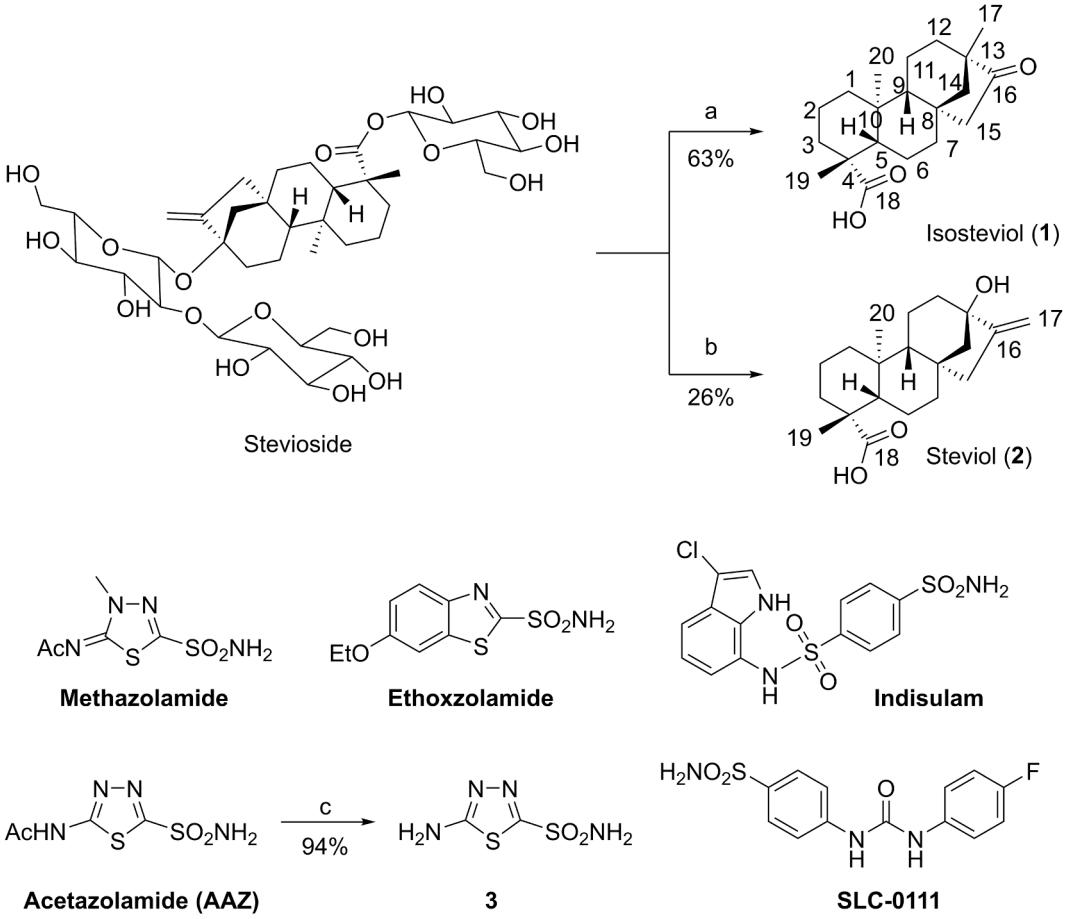
These data served in turn as the basis for the assignment of the signals in the corresponding derivatives of this investigation. The reagents required for subsequent reactions, sulfamoyl chloride was bought from local vendors and 5-amino-1,3,4-thiadiazole-2-sulfonamide (**3**), was obtained following a known synthesis (Scheme 1) from acetazolamide (**AAZ**).

Reduction of isosteviol (**1**) with sodium borohydride in dry methanol (Scheme 2) gave "dihydro-isosteviol" (**4**) [67–69] in very good yields.

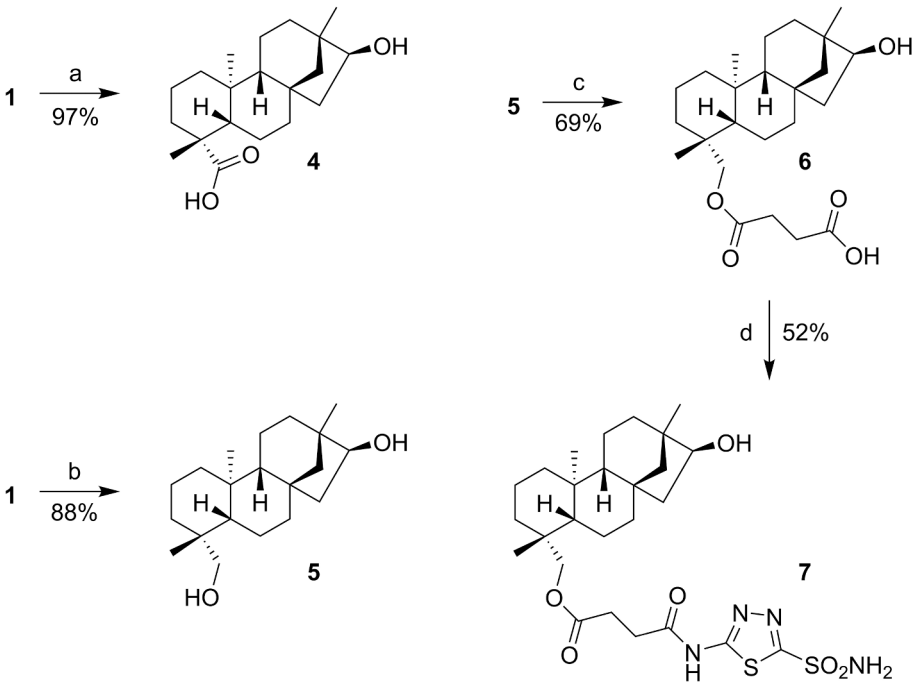
Table 1
Complete assignments of ^{1}H (500 MHz) and ^{13}C NMR (126 MHz) spectra for isosteviol (1) in $CDCl_3$; 21 °C; numbering as depicted in Scheme 1.

Atom	δ (^{13}C NMR) (ppm)	δ (^{1}H NMR) (ppm)	$^{3}J_{H,H}$ (Hz)
1	39.6	1.71; 0.90	13.3, 4.3
2	18.7	1.81; 1.41	
3	37.5	2.15, 1.01	13.1, 4.0; 13.6, 4.2
4	43.5	–	
5	56.9	1.14	12.1, 2.3
6	21.4	1.86; 1.73	
7	41.3	1.64; 1.48	13.3, 3.1; 13.6, 4.0
8	39.6	–	
9	54.6	1.16	
10	38.0	–	
11	20.2	1.67; 1.19	
12	37.2	1.59, 1.35	
13	48.6	–	
14	54.1	1.53; 1.40	11.6, 2.7; 11.4, 3.7
15	48.3	2.62; 1.80	18.6, 3.7; 18.5
16	222.7	–	
17	19.7	0.96	
18	183.9	–	
19	28.8	1.23	
20	13.2	0.77	

This reduction is highly selective and only traces of the corresponding diastereomeric alcohol could be detected by HPTLC-ESI-MS. The high selectivity of this reaction is in good agreement with previous reports, and can be explained by the fact that the borohydride anion attacks from the least sterically hindered side thus leading to a product holding a



Scheme 1. Synthesis of isosteviol (1) and steviol (2) from stevioside: a) MeOH, conc. HCl, reflux 2 h, 20 °C overnight, 63 %; b) H_2O; $NaIO_4$, KOH, 24 h, 21 °C, 26 %; c) synthesis of 3 from acetalzolamide (AAZ): conc. aq. HCl, reflux, 3 h, 94 %; structure of clinically used CA inhibitors methazolamide, ethoxzolamide, indisulam and SCC-0111.



Scheme 2. Reactions and conditions: a) $NaBH_4$, EtOH, 0 °C, 1 h, 97 %; b) $LiAlH_4$, THF, reflux, 3 h, 88 %; c) pyridine, DMAP (cat.), succinic anhydride, microwaves, 200 °C, 3 h, 69 %; d) THF, NMM, ethyl chloroformate, 20 °C, 15 min, then 3, reflux, 48 h, 52 %.

gauche orientation of the hydroxyl group attached to C-16 and the methyl group C-17. The structure of this product has also previously been confirmed by x-ray diffraction [67,68].

Reduction of **1** with lithium aluminum hydride gave diol **5** whose microwave assisted reaction with succinic anhydride in pyridine in the presence of cat. amounts of DMAP yielded 69 % of **6**. The position of the succinoyl residue was determined from 2D-NMR spectra; the preferential acylation at the C-18 position can be explained by the higher reactivity of the primary hydroxyl group compared to the secondary hydroxyl group at C-18. Reaction of **6** with ethyl chloroformate, 4-methyl-morpholine in dry THF followed by further reaction with **3** for 48 h under reflux led to the corresponding product **7**. The ^{1}H and ^{13}C NMR spectra of **7** show the typical signals for the diterpenoid backbone, the signals for the spacer (^{1}H NMR: CH_2: δ = 2.77–2.84 and δ = 2.71–2.64 ppm), and the thiadiazol moiety (^{13}C NMR: δ = 164.3 and 161.0 ppm). In the ESI-MS a m/z = 429.4 corresponds to a quasi-molecule ion $[M + Na]^+$, thereby additionally confirming the structure as do the results from the micro-analysis.

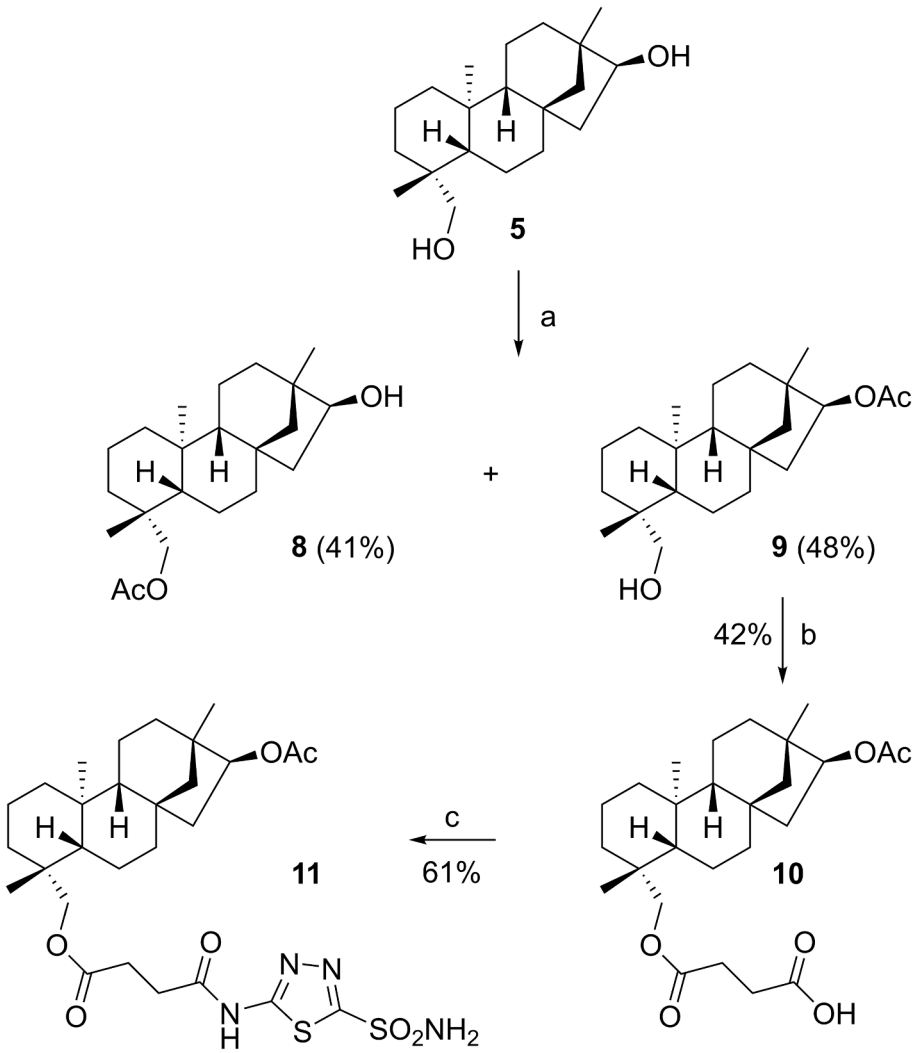
Acetylation of **5** (Scheme 3) gave a mixture of acetates **8** and **9** that were separated by chromatography. Similarly to the synthesis of **6**, compound **9** was converted into succinoyl-spacered **10** whose reaction with **3** afforded thiadiazol derivative **11** in 61 % isolated yield.

To determine the influence of the spacer and of the thiadiazole fragment, sulfamates were also prepared for comparison purposes by direct reaction (Scheme 4) of the alcohol with sulfamoyl chloride. Reaction of the mono-acetates **8** and **9** with sulfamoyl chloride gave the compounds **12** and **13** being acetylated/sulfamoylated at different positions, respectively. The target compound **15** (Scheme 4) was obtained from **4** under the same conditions. A double sulfamoylation reaction was carried out on **5**, and **14** was obtained in 83 % yield. For comparison, steviol (**2**) was sulfamoylated, and sulfamate **16** was obtained.

The compounds were screened for their ability to inhibit CA II. Thereby, acetazolamide (AAZ) – a sulfonamide type inhibitor with high affinity for CA II under physiological conditions, was used as a positive standard. The results are summarized in Table 2 and Fig. 1, which show that **11**, **12** and **15** are the best inhibitors of this enzyme, while **14** and **16** showed little activity. Compounds **12** and **13** differ only in the position of their acetate and sulfamate groups, but **12** is a better inhibitor than **13**; a sulfamate at position C-16 seems to be superior to a localization at C-18, and **15** also retained a good inhibitory activity. However, the presence of a second sulfamate group (as in **14**) led to an almost complete loss of activity. The same was true for the comparison between steviol-derived **16** (with almost no activity) and isosteviol-derived **15** (94.6 % inhibition of CA II). This difference underlines the significant impact of structural variations between the two scaffolds.

The moderate inhibitory activity of **7** suggests that the presence of an unprotected hydroxyl group may reflect greater flexibility or less favorable interactions with the active site of the enzyme, resulting in only moderate inhibition of the enzyme. The remarkable increase in inhibitory activity observed for **11** highlights the potent effect of protecting the hydroxyl group as an acetate. This modification is likely to enhance binding interactions, possibly through hydrogen bonding or hydrophobic interactions, resulting in almost complete inhibition. Compounds **11** and **12** both retain an acetyl group, highlighting the significant effect of the presence of an acetyl moiety. This suggests that an acetyl group increases binding affinity, probably through some additional interactions with the enzyme. In contrast, compound **14**, which has sulfamate groups at both positions C-16 and C-18, showed only minimal inhibition (<5%). A probable reason for this could be that the simultaneous presence of sulfamate groups at both positions could lead to steric hindrance or unfavorable interactions. Compound **12**, displaying a notable 94.5 % inhibition, reveals again an enhancing effect between acetyl group at position C-18, and the sulfamate group at position C-16. Compound **13**, holding an acetyl group at position C-16 and a sulfamate moiety at C-18 exhibited inhibition of 73.5 %, thus emphasizing the nuanced impact of individual modifications at distinct positions. Compound **12**, with a remarkable inhibition of 94.5 %, again shows a potentiating effect between the acetyl group at position C-18 and the sulfamate group at position C-16.

Some molecular modeling calculations were performed, and Fig. 1 shows the main interactions of compounds **11**, **12** and **15** in 2D representation; for **11** a 3D representation is shown, too. Thereby, the protein structure was prepared and validated using the standard MOE tool. To validate the docking's accuracy, the co-crystallized ligand was redocked



Scheme 3. Reactions and conditions: a) DCM, Ac_2O, DMAP (cat.), 20 °C, 1 day: 10: (41 %), 11 (48 %); b) pyridine, DMAP (cat.), succinic anhydride, microwaves, 200 °C, 3 h, 42 %; c) THF, NMM, ethyl chloroformate, 20 °C, 15 min, then 3, reflux, 48 h, 61 %.

into the enzyme binding site and compared with the crystallographic pose. Additionally, experimental results and known binding mechanisms of comparable inhibitors were utilized to verify the docking parameters.

For the most active compounds, i.e., **11**, **12** and **15**, extra kinetic measurements were performed showing all of them as competitive inhibitors for CA II. Table 3 summarizes these results, and the corresponding Dixon-plots are depicted in Fig. 2.

3. Conclusion

The diterpene isosteviol can be obtained by hydrolysis from stevioside which is a renewable resource and therefore of special interest for synthesis. Inhibitors of the enzyme carbonic anhydrase II are currently in the focus of increasing scientific research because, in addition to their use in the treatment of glaucoma, their use to reduce the side effects of antibody therapies for Alzheimer's disease has recently been discussed. The central structural feature of many CA II inhibitors is the presence of a sulfonamide or a sulfamate group. Isosteviol was therefore converted into (un)-spacered sulfamates, and their ability to act as CA II inhibitors was investigated. Three derivatives, in particular, were found to be effective inhibitors. A conjugate of isosteviol carrying a 5-aminosulfonyl-1,3,4-thiadiazol-2-yl-amino substituent acetylated at C-16 of the isosteviol skeleton and spacered with a succinoyl ester inhibited CA II almost completely as a competitive inhibitor showing a $K_i = 2.59$ μM.

4. Experimental

4.1. General

General information is provided in the supplementary material file.

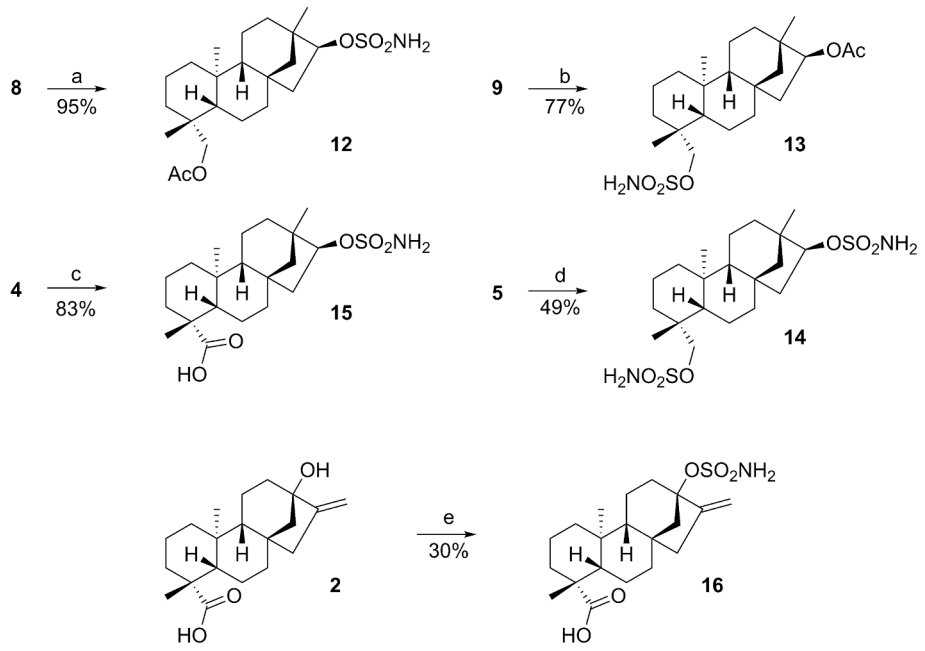
4.2. Syntheses

4.2.1. (4α, 8β, 13β) 13-Methyl-16-oxo-17-norkauran-18-oic acid (1, isosteviol)

Hydrolysis of stevioside (86.0 g, 0.10 mol) in MeOH (500 mL) with aq. HCl (33 %, 90 mL) under reflux for 2 h followed by stirring at 21 °C continued overnight, addition of water (1200 mL), filtration, drying and re-crystallization of the filter cake from EtOH (300 mL) gave **1** (21.6 g, 63 %) as a colorless solid; $R_f = 0.71$ (SiO_2, $CHCl_3$/MeOH 9:1); m.p. = 229–231 °C [lit.: [70] 228–230 °C]; $[\alpha]_D^{20} = -84.02°$ ($c = 0.15$, $CHCl_3$) [lit.: [71] $[\alpha]_D^{20} = -79.3°$ (EtOH)]; MS (ESI, MeOH/$CHCl_3$, 4:1): *m/z* (%) = 317.0 (100 %, $[M-H]^-$).

4.2.2. (4α) 13-Hydroxy-kaur-16-en-18-oic acid (2, steviol)

Reaction of stevioside (100.0 g, 0.12 mol) in dist. water (8.0 L) with $NaIO_4$ (160.0 g, 0.75 mol) as previously described followed by



Scheme 4. Reactions and conditions: a) DMA, sulfamoyl chloride, 1 day, 21 °C, 95 %; b) DMA, sulfamoyl chloride, 1 day, 21 °C, 77 %; c) DMA, sulfamoyl chloride, 1 day, 21 °C, 49 %; d) DMA, sulfamoyl chloride, 1 day, 21 °C, 83 %; e) DMA, sulfamoyl chloride, 1 day, 21 °C, 30 %.

Table 2
Inhibition of CA II; experiments were performed in triplicate; concentration of the inhibitor 10 μM; AAZ was used as a positive standard.

Compound	Inhibition [%]
7	24.2 ± 1.1
11	99.9 ± 0.1
12	94.5 ± 0.8
13	73.5 ± 1.4
14	< 5
15	94.6 ± 0.2
16	< 5
AAZ	99.9 ± 0.1

crystallization from MeOH gave **2** (10.5 g, 26 %) as a colorless solid; R_f = 0.47 (SiO_2, $CHCl_3$/MeOH, 9:1); m.p. = 205 °C [lit.: [71] 212–213 °C]; $[\alpha]_D^{20} = -62.88°$ ($c = 0.09$, $CHCl_3$) [lit.: [72] $[\alpha]_D^{20} = -55.2°$ ($CHCl_3$); MS (ESI, MeOH): *m/z* (%) = 317.2 (100 %, $[M-H]^-$).

*4.2.3. 5-Amino-1,3,4-thiadiazole-2-sulfonamide (**3**)*

Hydrolysis of acetazolamide (**AAZ**, 9.0 g, 40.7 mmol) in conc. HCl (60 mL) for 3 h as previously described gave **3** (6.9 g, 94 %) as a white solid; m.p. 195 °C decomp. (lit.: [73] 215–216 °C); R_F = 0.3 (SiO_2, $CHCl_3$/MeOH, 9:1); MS (ESI, MeOH): *m/z* = 179.0 (100 %, $[M-H]^-$).

*4.2.4. (4α, 8β, 13β, 16β) 16-Hydroxystachan-18-oic acid (**4**)*

A solution of isosteviol **1** (0.5 g, 1.6 mmol) and sodium borohydride (0.09 g, 2.4 mmol) in dry ethanol (20 mL) was stirred at 0 °C for 1 h. The volatiles were removed under reduced pressure, and the residue was extracted with $CHCl_3$ and H_2O. The combined organic layers were washed with brine, and dried ($MgSO_4$) to afford **4** (0.49 g, 97 %) as a colorless solid; R_f = 0.58 (SiO_2, $CHCl_3$/MeOH, 9:1); m.p. 185–186 °C (lit.: [70] 169 °C); $[\alpha]_D^{20} = -59.33°$ ($c = 0.098$, MeOH); IR (ATR): *ν* = 3472 *m*, 2947 *m*, 2894 *m*, 2836 m, 1701 *m*, 1649 *m*, 1470*w*, 1451*w*, 1386*w*, 1371*w*, 1324*w*, 1290*w*, 1265*w*, 1235*w*, 1187*w*, 1153*w*, 1120*w*, 1070*w*, 1055*w*, 1025*w*, 997*w*, 974*w*, 908*w*, 851*w*, 786*w*, 769*w*, 761*w*, 736*w*, 703*w*, 619*w*, 579*w*, 533*w* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): *δ* = 3.86 (*dd*, *J* = 10.7, 4.6 Hz, 1H, 16-H), 2.12 (*d*, *J* = 13.4 Hz, 1H, 3-H), 1.93–1.67 (*m*, 6H, 2-H_a, 15-H_a, 6-H_a, 12-H_a, 1-H_a, 15-H_b), 1.65–1.47 (*m*, 4H, 11-H, 6-H_b, 7-H_a), 1.45–1.33 (*m*, 1H, 2-H_b), 1.33–1.24 (*m*, 2H, 7-H_b, 14-H_a), 1.22 (*s*, 4H, 12-H_b, 19-H), 1.09–0.94 (*m*, 4H, 3-H_b, 14-H_b, 5-H, 9-H), 0.90 (*s*, 3H, 17-H), 0.88–0.86 (*m*, 1H, 1-H_b), 0.83 (*s*, 3H, 20-H); ^{13}C NMR (101 MHz, $CDCl_3$): *δ* = 183.6 (C-18), 80.6 (C-16), 57.2 (C-5), 56.0 (C-9), 55.4 (C-14), 43.7 (C-4), 42.8 (C-15), 42.3 (C-13), 42.1 (C-8), 41.8 (C-7), 39.9 (C-1), 38.4 (C-10), 37.9 (C-3), 33.9 (C-12), 29.1 (C-19), 25.0 (C-17), 21.9 (C-6), 20.6 (C-11), 19.0 (C-2), 13.3 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 318.9 (90 %, $[M-H]^-$); analysis calcd for $C_{20}H_{32}O_3$ (320.47): C 74.96, H 10.07; found: 74.71, H 10.28.

*4.2.5. (4α, 8β, 13β, 16β) Stachane-16,18-diol (**5**)*

To a solution of **1** (5.0 g, 0.016 mol) in dry THF (50 mL), $LiAlH_4$ (5.0 g, 0.13 mol) was slowly added at ambient temperature followed by heating under reflux for 3 h. Usual aq. work-up followed by chromatography (silica gel, $CHCl_3$/MeOH, 95:5) gave **5** (4.2 g, 88 %) as a white solid; R_f = 0.45 (SiO_2, $CHCl_3$/MeOH, 9:1); m.p. 156–157 °C; $[\alpha]_D^{20} = -25.08°$ (c = 0.167, MeOH); IR (ATR): *ν* = 3305*br*, 2929 *m*, 2891*w*, 2867*w*, 2838 *m*, 1480*w*, 1454*w*, 1446*w*, 1385*w*, 1371*w*, 1327*w*, 1210*w*, 1157*w*, 1123*w*, 1083*w*, 1062 *m*, 1025 *m*, 999*w*, 970*w*, 958*w*, 930*w*, 840*w*, 759*w*, 704*w*, 626*w*, 535*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): *δ* = 3.84 (*dd*, *J* = 10.9, 4.5 Hz, 1H, 16-H), 3.75 (*d*, *J* = 11.0 Hz, 1H, 18-H_a), 3.41 (*d*, *J* = 11.1 Hz, 1H, 18-H_b), 1.86–1.71 (*m*, 3H, 15-H_a, 3-H_a, 12-H_a), 1.71–1.63 (*m*, 2H, 15-H_a, 1-H_a), 1.61–1.45 (*m*, 5H, 6-H_a, 11-H, 2-H_a, 7-H_a), 1.42–1.15 (*m*, 5H, 2-H_b, 7-H_b, 14-H_a, 12-H_b, 6-H_b), 1.05–0.99 (*m*, 2H, 14-H_b, 9-H), 0.99–0.96 (*m*, 1H, 5-H), 0.95 (*s*, 3H, 19-H), 0.94–0.92 (*m*, 1H, 3-H_b), 0.90 (*s*, 3H, 17-H), 0.88 (*s*, 3H, 20-H), 0.86–0.80 (*m*, 1H, 1-H_b) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): *δ* = 80.8 (C-16), 65.8 (C-18), 57.1 (C-5), 56.8 (C-9), 55.6 (C-14), 43.1 (C-15), 42.2 (C-13), 42.1 (C-7, C-8), 39.7 (C-1), 38.6 (C-4), 37.8 (C-10), 35.7 (C-3), 33.8 (C-12), 27.3 (C-19), 25.1 (C-17), 20.4 (C-6), 20.4 (C-11), 18.2 (C-2), 15.6 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 345.5 (50 %, $[M+K]^+$); analysis calcd for $C_{20}H_{34}O_2$ (306.49): C 78.38, H 11.18; found: C 78.11, H 11.34.

*4.2.6. (4α, 8β, 13β, 16β) 4-[(16-Hydroxy-stachan-18-oyl)oxy]-4-oxobutanoic acid (**6**)*

To a solution of **5** (0.6 g, 1.96 mmol) in dry pyridine (10 mL), DMAP (cat.) and succinic anhydride (0.55 g, 5.5 mmol) were added. The reaction mixture was stirred at 200 °C (microwave assisted; Anton-Paar

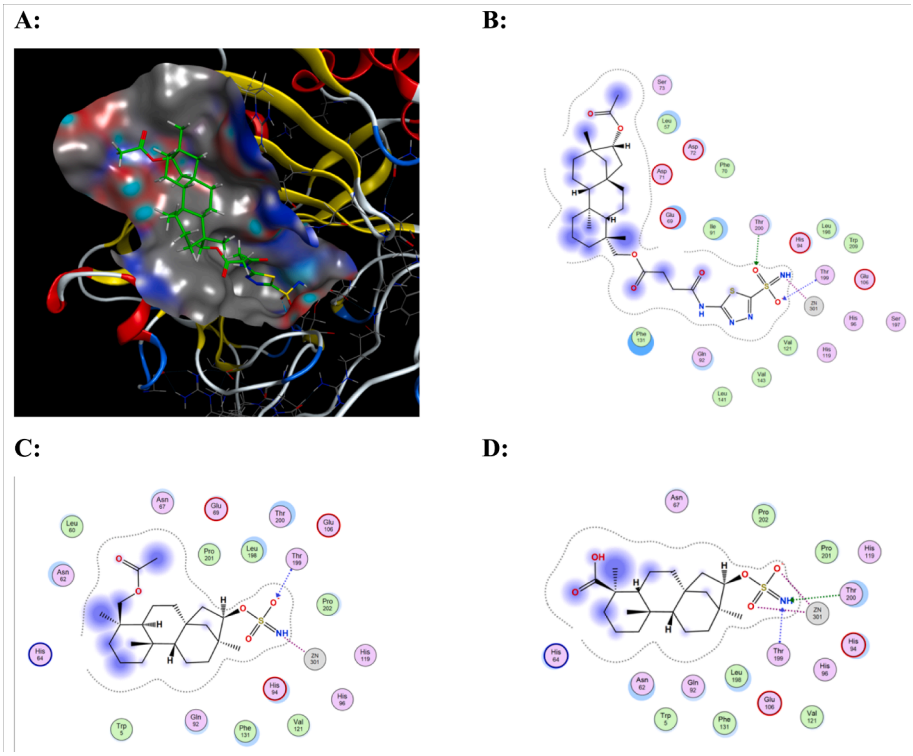


Fig. 1. Calculated preferred localization and orientation of compounds 11, 12 and 15 and CA II: A: 3D modeling results for 11; B: 2D modeling result for 11; C: 2D modeling result for 12; D: 2D modeling result for 15.

Table 3
Determination of inhibition-% and K_i values (in μM) for compounds 11, 12 and 15.

Compound	11	12	15
K_i [μM]	2.59 ± 0.12	3.60 ± 0.17	4.10 ± 0.28
Inhibition-%	99.9 ± 0.1	94.5 ± 0.8	94.6 ± 0.2

GmbH, Monowave) for 3 h. Usual aqueous work up and chromatography (SiO_2, hexanes/ethyl acetate, 7:3) gave **6** (0.552 g, 69 %) as a colorless solid; R_f = 0.23 (SiO_2, hexanes/ethyl acetate, 5:5); m.p. 149–150 °C; $[\alpha]_D^{20}$ = –20.13° (*c* = 0.141, MeOH); IR (ATR): *ν* = 2930*w*, 2841*w*, 1706 *m*, 1455*w*, 1394*w*, 1373*w*, 1320*w*, 1275*w*, 1189*w*, 1165*w*, 1069*w*, 1001*w*, 970*w*, 834*w*, 759*w*, 637*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): *δ* = 4.27 (*d*, *J* = 11.0 Hz, 1H, 18-H_a), 3.89 (*d*, *J* = 11.0 Hz, 1H, 18-H_b), 3.85 (*dd*, *J* = 10.8, 4.6 Hz, 1H, 16-H), 2.70–2.58 (*m*, 4H, 22-H, 23-H), 1.86–1.78 (*m*, 1H, 15-H_a), 1.79–1.71 (*m*, 1H, 12-H_a), 1.71–1.63 (*m*, 3H, 3-H_a, 1-H_a, 15-H_b), 1.62–1.46 (*m*, 5H, 6-H_a, 11-H, 2-H_a, 7-H_a), 1.42–1.15 (*m*, 5H, 2-H_b, 7-H_b, 14-H_a, 6-H_b, 12-H_b), 1.06–0.99 (*m*, 2H, 14-H_b, 9-H), 0.99–0.94 (*m*, 2H, 3-H_b, 5-H), 0.93 (*s*, 3H, 19-H), 0.90 (*s*, 6H, 17-H, 20-H), 0.84 (*td*, *J* = 13.1, 4.1 Hz, 1H, 1-H_b) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): *δ* = 177.4 (C-24), 172.5 (C-21), 80.8 (C-16), 67.9 (C-18), 57.0 (C-5), 56.7 (C-9), 55.6 (C-14), 43.0 (C-15), 42.2 (C-8, C-13), 42.0 (C-7), 39.6 (C-1), 37.7 (C-10), 37.1 (C-4), 36.4 (C-3), 33.8 (C-12), 29.2 (C-22), 29.1 (C-23), 27.7 (C-19), 25.1 (C-17), 20.4 (C-6), 20.4 (C-11), 18.1 (C-2), 15.5 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 429.4 (95 %, $[M + Na]^+$); analysis calcd for $C_{24}H_{38}O_5$ (406.56): C 70.90, H 9.42; found: C 70.68, H 9.63.

4.2.7. (4α, 8β, 13β, 16β) 16-Hydroxystan-18-yl 4-{[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]amino}-4-oxobutanoate (7)

Compound **6** (0.32 g, 0.79 mmol) was dissolved in dry THF (50 mL),

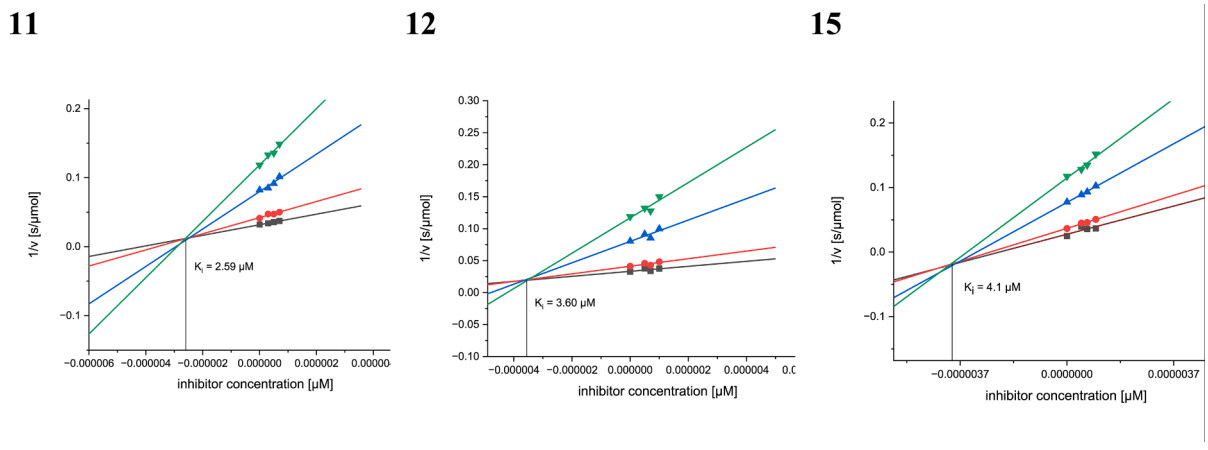


Fig. 2. Dixon plots for compounds 11, 12 and 15.

4-methylmorpholine (0.16 g, 1.58 mmol) and ethyl chloroformate (0.171 g, 1.58 mmol) were added. The reaction mixture was stirred at 20 °C for 15 min. Compound **3** (0.215 g, 1.2 mmol) was added, and the mixture was heated under reflux for another 48 h. The solvent was removed, the residue dissolved in $CHCl_3$, washed with aq. NaOH (2M), water and brine and dried ($MgSO_4$). Chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) gave **7** (0.232 g, 52 %) as a white solid; $R_f = 0.44$ (SiO_2, $CHCl_3$/MeOH, 9:1); m.p. 126–127 °C; $[\alpha]_D^{20} = -12.16°$ ($c = 0.06$, MeOH); UV–Vis (MeOH): λ_{max} (log ε) = 265 nm (3.81); IR (ATR): ν = 3502*br*, 3286*br*, 2919 *m*, 2850 *m*, 1685 *m*, 1518 *m*, 1451*w*, 1419*w*, 1345 *m*, 1325 *m*, 1291*w*, 1221*w*, 1170 *m*, 1156 *m*, 1088*w*, 1051*w*, 1000*w*, 973*w*, 952*w*, 926*w*, 797*w*, 756*w*, 627*w*, 587*w*, 510*w* cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 8.31 (*s*, 3H, NH, NH_2), 4.46 (*d*, J = 4.1 Hz, 1H, OH), 4.24 (*d*, J = 10.9 Hz, 1H, 18-H_a), 3.74 (*d*, J = 11.0 Hz, 1H, 18-H_b), 3.65 (*dt*, J = 9.5, 4.1 Hz, 1H, 16-H), 2.84–2.77 (*m*, 2H, 23-H), 2.71–2.64 (*m*, 2H, 22-H), 1.72–1.64 (*m*, 2H, 15-H_a, 12-H_a), 1.63–1.33 (*m*, 8H, 6-H_a, 1-H_a, 3-H_a, 11-H_a, 15-H_b, 2-H_a, 11-H_b, 7-H_a), 1.33–1.22 (*m*, 2H, 2-H_b, 7-H_b), 1.22–1.10 (*m*, 2H, 14-H_a, 6-H_b), 1.10–0.99 (*m*, 1H, 12-H_b), 0.99–0.87 (*m*, 4H, 3-H_b, 14-H_b, 5-H, 9-H), 0.85 (*s*, 3H, 19-H), 0.83 (*s*, 3H, 20-H), 0.81 (*s*, 3H, 17-H), 0.80–0.71 (*m*, 1H, 1-H_b) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 171.8 (C-21), 171.2 (C-24), 164.3 (C-26), 161.0 (C-25), 78.4 (C-16), 66.4 (C-18), 62.8, 56.2 (C-5), 56.2 (C-9), 55.1 (C-14), 42.7 (C-15), 41.6 (C-8), 41.6 (C-13), 41.5 (C-7), 38.9 (C-1), 37.1 (C-10), 36.7 (C-4), 35.6 (C-3), 33.6 (C-12), 29.9 (C-23), 28.3 (C-22), 27.2 (C-19), 25.0 (C-17), 19.7 (C-11), 19.6 (C-6), 17.5 (C-2), 15.1 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 567.4 (95 %, $[M-H]^-$); analysis calcd for $C_{26}H_{40}N_4O_6S_2$ (568.75): C 54.91, H 7.09, N 9.85; found: C 54.77, H 7.30, N 9.63.

*4.2.8. (4α, 8β, 13β, 16β) 16-Hydroxystachan-18-yl acetate (**8**) and (4α, 8β, 13β, 16β) 18-hydroxystach-16-yl acetate (**9**)*

To a solution of **5** (2.0 g, 6.6 mmol) in dry DCM, acetic anhydride (2 g, 40 mmol), triethylamine (4.0 g, 19.8 mmol), and DMAP (cat.) were added, and the mixture was stirred at 20 °C for 1 day. Usual aqueous work-up followed by column chromatography (SiO_2, hexanes/ethyl acetate, 8:2) gave **8** (0.931 g, 41 %) and **9** (1.08 g, 48 %) each as a white solid.

Data for **8**: R_f = 0.89 (SiO_2, hexanes/ethyl acetate, 6:4); m.p. 149–150 °C; $[\alpha]_D^{20} = -26.13°$ ($c = 0.169$, MeOH); IR (ATR): ν = 3526 *m*, 3499*w*, 2963*w*, 2932 *m*, 2903 *m*, 2840 *m*, 1720 *s*, 1481*w*, 1444*w*, 1393 *m*, 1371 *m*, 1319*w*, 1259 *s*, 1209*w*, 1125*w*, 1089*w*, 1052*w*, 1046 *m*, 1031 *m*, 1001*w*, 982 *m*, 913*w*, 855*w*, 773*w*, 738*w*, 704*w*, 626*w*, 609*w*, 511*br*, 465*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 4.23 (*d*, J = 11.0 Hz, 1H, 18-H_a), 3.84 (*d*, J = 10.9 Hz, 2H, 16-H, 18-H_b), 2.03 (*s*, 3H, 22-H), 1.83 (*ddd*, J = 14.2, 4.4, 3.1 Hz, 1H, 15-H_a), 1.79–1.71 (*m*, 1H, 12-H_a), 1.72–1.64 (*m*, 3H, 3-H_a, 1-Ha, 15-H_b), 1.62–1.53 (*m*, 3H, 6-H_a, 11-H), 1.53–1.46 (*m*, 2H, 2-H_a, 7-H_a), 1.41–1.34 (*m*, 2H, 2-H_b, 7-H_b), 1.29 (*m*, 1H, 14-H_a), 1.25–1.17 (*m*, 2H, 6-H_b, 12-H_b), 1.06–0.98 (*m*, 2H, 14-H_b, 9-H), 0.99–0.94 (*m*, 2H, 3-H_b, 5-H), 0.93 (*s*, 3H, 19-H), 0.91 (*s*, 3H, 20-H), 0.90 (*s*, 3H, 17-H), 0.84 (*td*, J = 13.3, 4.0 Hz, 1H, 1-H_b) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 171.5 (C-21), 80.7 (C-16), 67.3 (C-18), 57.0 (C-5), 56.8 (C-9), 55.6 (C-14), 43.1 (C-15), 42.2 (C-13), 42.2 (C-8), 42.0 (C-7), 39.6 (C-1), 37.7 (C-10), 37.1 (C-4), 36.3 (C-3), 33.8 (C-12), 27.7 (C-19), 25.1 (C-17), 21.1 (C-22), 20.4 (C-11), 20.3 (C-6), 18.1 (C-2), 15.6 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 371.5 (45 %, $[M + Na]^+$); analysis calcd for $C_{22}H_{36}O_3$ (348.53): C 75.82, H 10.41; found: C 75.61, H 10.55.

Data for **9**: R_f = 0.85 (SiO_2, hexanes/ethyl acetate, 6:4); m.p. 163–165 °C; $[\alpha]_D^{20} = -40.49°$ ($c = 0.118$, MeOH); IR (ATR): ν = 3529 *m*, 2994*w*, 2928 *m*, 2871*w*, 2845 *m*, 1712 *s*, 1467*w*, 1451*w*, 1437*w*, 1386 *m*, 1370 *m*, 1258 *s*, 1207*w*, 1153*w*, 1119*w*, 1057 *m*, 1034 *s*, 974 *m*, 909*w*, 851*w*, 762*w*, 714*w*, 631*w*, 608*w*, 502*br* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 4.72 (*dd*, J = 8.7, 6.4 Hz, 1H, 16-H), 3.74 (*d*, J = 10.9 Hz, 1H, 18-H_a), 3.39 (*d*, J = 10.1 Hz, 1H, 18-H_b), 2.06 (*s*, 3H, 22-H), 1.83–1.74 (*m*, 4H, 15-H, 3-H_a, 12-H_a), 1.70–1.62 (*m*, 1H, 1-H_a), 1.61–1.46 (*m*, 5H, 6-H_a, 11-H, 2-H_a, 7-H_a), 1.42–1.29 (*m*, 3H, 2-H_b, 7-H_b, 14-H_a), 1.29–1.12 (*m*, 2H, 12-H_b, 6-H_b), 1.08–1.01 (*m*, 2H, 14-H_b, 9-H), 0.99–0.96 (*m*, 1H, 5-H), 0.95 (*s*, 3H, 19-H), 0.94–0.91 (*m*, 1H, 3-H_b), 0.90 (*s*, 3H, 17-H), 0.88–0.86 (*m*, 1H, 1-H_b), 0.85 (*s*, 3H, 20-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 171.5 (C-21), 81.8 (C-16), 65.7 (C-18), 57.1 (C-5), 56.7 (C-9), 55.2 (C-14), 42.4 (C-8), 41.9 (C-13), 41.6 (C-7), 40.9 (C-15), 39.7 (C-1), 38.6 (C-4), 37.8 (C-10), 35.6 (C-3), 34.7 (C-12), 27.2 (C-19), 25.1 (C-17), 21.3 (C-22), 20.3 (C-11), 20.2 (C-6), 18.1 (C-2), 15.6 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 371.3 (40 %, $[M + Na]^+$); analysis calcd for $C_{22}H_{36}O_3$ (348.53): C 75.82, H 10.41; found: C 77.59, H 10.62.

*4.2.9. 4-{[(4α, 8β, 13β, 16β) 16-(Acetyloxy)stachan-18-yl]oxy}-4-oxobutanoic acid (**10**)*

To a solution of **9** (0.542 g, 1.55 mmol) in dry pyridine (10 mL), DMAP (cat.) and succinic anhydride (0.311 g, 3.11 mmol) were added. The reaction mixture was stirred (microwave, as above) at 200 °C for 3 h. Usual aqueous work up and chromatography (SiO_2, hexanes/ethyl acetate, 7:3) gave **10** (0.292 g, 42 %) as a colorless solid; R_f = 0.14 (SiO_2, hexanes/ethyl acetate, 7:3); m.p. 100–102 °C; $[\alpha]_D^{20} = -26.6°$ ($c = 0.114$, MeOH); IR (ATR): ν = 2927 *m*, 2848 *m*, 1733 *s*, 1712 *s*, 1455 *m*, 1439 *m*, 1372 *m*, 1241 *s*, 1159 *s*, 1057 *m*, 1035 *m*, 995*w*, 973*w*, 938*w*, 839*w*, 754 *m*, 629*w*, 605 *m*, 550*br*, 484*w*, 456*w* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 4.76–4.69 (*m*, 1H, 16-H), 4.28 (*d*, J = 11.0 Hz, 1H, 18-H_a), 3.86 (*d*, J = 10.5 Hz, 1H, 18-H_b), 2.71–2.57 (*m*, 4H, 24-H, 25-H), 2.06 (*s*, 3H, 22-H), 1.82–1.72 (*m*, 2H, 15-H, 12-H_a), 1.71–1.61 (*m*, 2H, 1-H_a, 3-H_a), 1.61–1.45 (*m*, 5H, 11-H, 6-H_a, 2-H_a, 7-H_a), 1.42–1.29 (*m*, 3H, 2-H_b, 7-H_b, 14-H_a), 1.29–1.18 (*m*, 2H, 6-H_b, 12-H_b), 1.10–0.94 (*m*, 4H, 14-H_b, 9-H, 5-H, 3-H_b), 0.93–0.91 (*m*, 3H, 19-H), 0.89 (*s*, 3H, 17-H), 0.87 (*s*, 3H, 20-H), 0.85–0.79 (*m*, 1H, 1-H_b); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 177.8 (C-26), 172.4 (C-23), 171.6 (C-21), 81.7 (C-16), 67.8 (C-18), 57.0 (C-5), 56.6 (C-9), 55.2 (C-14), 42.4 (C-8), 41.7 (C-13), 41.6 (C-7), 40.8 (C-15), 39.5 (C-1), 37.7 (C-4), 37.1 (C-10), 36.4 (C-3), 34.6 (C-12), 29.1 (C-25), 29.1 (C-24), 27.7 (C-19), 25.0 (C-17), 21.3 (C-22), 20.3 (C-11), 20.2 (C-6), 18.0 (C-2), 15.5 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 447.6 (85 %, $[M-H]^-$); analysis calcd for $C_{26}H_{40}O_6$ (448.60): C 69.61, H 8.99; found: C 69.45, H 9.14.

*4.2.10. (4α, 8β, 13β, 16β) 18-(Acetyloxy)-stachan-16-yl 4-{[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]amino}-4-oxobutanoate (**11**)*

Compound **10** (0.371 g, 0.83 mmol) was dissolved in dry THF (50 mL), 4-methylmorpholine (0.167 g, 1.65 mmol) and ethyl chloroformate (0.179 g, 1.65 mmol.) were added. The reaction mixture was stirred at 20 °C for 15 min. Compound **3** (0.18 g, 1 mmol) was added, and the mixture was heated under reflux for another 48 h. The solvent was removed, the residue dissolved in $CHCl_3$, washed with aq. NaOH (2M), water and brine and dried ($MgSO_4$). Chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) gave **11** (0.31 g, 61 %) as a white solid; R_f = 0.62 (SiO_2, $CHCl_3$/MeOH, 9:1); m.p. 135–136 °C; $[\alpha]_D^{20} = -27.17°$ ($c = 0.069$, MeOH); UV–Vis (MeOH): λ_{max} (log ε) = 265 nm (3.96); IR (ATR): ν = 3253*br*, 2927*br*, 2847*w*, 1705 *m*, 1527 *m*, 1432*br*, 1370 *m*, 1243 *m*, 1173 *m*, 1086*w*, 1057*w*, 1034*w*, 973*w*, 912*w*, 754*w*, 655*w*, 605 *m*, 508*w* cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 8.31 (*s*, 2H, NH_2), 4.66 (*t*, J = 7.5 Hz, 1H, 16-H), 4.23 (*d*, J = 11.0 Hz, 1H, 18-H_a), 3.74 (*d*, J = 11.0 Hz, 1H, 18-H_b), 2.83–2.75 (*m*, 2H, 25-H), 2.72–2.63 (*m*, 2H, 24-H), 2.01 (*s*, 3H, 22-H), 1.74–1.64 (*m*, 3H, 15-H, 12-H_a), 1.63–1.40 (*m*, 6H, 1-H_a, 3-H_a, 11-H, 6-H_a, 7-H_a), 1.37–1.10 (*m*, 5H, 7-H_b, 14-H_a, 2-H_b, 12-H_b, 6-H_b), 1.09–0.98 (*m*, 2H, 14-H_b, 9-H), 0.97–0.88 (*m*, 2H, 5-H, 3-H_b), 0.86 (*s*, 3H, 19-H), 0.85 (*s*, 3H, 17-H), 0.81 (*s*, 3H, 20-H), 0.78–0.76 (*m*, 1H, 1-H_b) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 171.8 (C-23), 171.5 (C-26), 170.5 (C-21), 164.0 (C-28), 161.5 (C-27), 80.6 (C-16), 66.3 (C-18), 56.0 (C-5), 55.7 (C-9), 54.3 (C-14), 41.7 (C-8), 41.0 (C-13), 40.9 (C-7), 40.2 (C-15), 38.8 (C-1), 35.6 (C-3), 34.0 (C-12), 30.1 (C-25), 28.4 (C-24), 27.2 (C-19), 24.7 (C-17), 20.9 (C-22), 19.6 (C-11), 19.6 (C-6), 17.4 (C-2), 15.0 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 609.9 (95 %,

[M−H]$^-$); analysis calcd for $C_{28}H_{42}N_4O_7S_2$ (610.79): C 55.06, H 6.93, N 9.17; found: C 54.81, H 7.20, N 9.34.

4.2.11. (4α, 8β, 13β, 16β) 18-[(Aminosulfonyl)oxy]stachan-16-yl acetate (12)

Compound **8** (0.14 g, 0.4 mmol) was suspended in dry dimethylacetamide (DMA, 5 mL) and stirred with sulfamoyl chloride (0.116 g, 1.0 mmol) for 1 day. The reaction mixture was quenched with MeOH (10 mL). The solvent evaporated under reduced pressure; column chromatography (SiO_2, $CHCl_3$/ethyl acetate, 9:1) gave **12** (0.163 g, 95 %) as a colorless solid; R_f = 0.9 (SiO_2, $CHCl_3$/ethyl acetate, 4:6); m.p. 171–173 °C; $[\alpha]_D^{20}$ = –34.40° (*c* = 0.121, MeOH); IR (ATR): *ν* = 3344 *m*, 3239*w*, 2964*w*, 2946*w*, 2918 *m*, 2883*w*, 2842 *m*, 1700 *s*, 1557*w*, 1462 *m*, 1442*w*, 1373 *s*, 1298*w*, 1271*w*, 1247*w*, 1221*w*, 1180 *s*, 1126*w*, 1030 *m*, 981 *m*, 953 *m*, 915 *m*, 884*w*, 861 *s*, 817 *m*, 788 *m*, 735*w*, 712*w*, 611*w*, 590*w*, 562*w*, 544 *m*, 518*w*, 479*w*, 456*w*, 431*w*, 423 *m* cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): *δ* = 7.34 (*s*, 2H, NH_2), 4.41 (*dd*, *J* = 10.7, 3.8 Hz, 1H, 16-H), 4.22 (*d*, *J* = 11.0 Hz, 1H, 18-H_a), 3.77 (*d*, *J* = 11.0 Hz, 1H, 18-H_b), 2.07 (*dt*, *J* = 14.6, 3.2 Hz, 1H, 15-H_a), 1.99 (*s*, 3H, 22-H), 1.76–1.54 (*m*, 5H, 15-H_b, 1-H_a, 3-H_a, 12-H_a, 11-H_a), 1.53–1.42 (*m*, 4H, 2-H_a, 11-H_b, 6-H_a, 7-H_a), 1.39–1.26 (*m*, 2H, 7-H_b, 2-H_b), 1.25–1.14 (*m*, 3H, 14-H_a, 6-H_b, 12-H_b), 1.11–1.00 (*m*, 2H, 14-H_b, 9-H), 1.01–0.94 (*m*, 2H, 3-H_b, 5-H), 0.92 (*s*, 3H, 17-H), 0.90 (*s*, 3H, 19-H), 0.86 (*s*, 3H, 20-H), 0.85–0.78 (*m*, 1H, 1-H_b) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): *δ* = 170.4 (C-21), 86.4 (C-16), 66.0 (C-18), 56.0 (C-5), 55.6 (C-9), 53.8 (C-14), 41.7 (C-8), 41.6 (C-13), 41.0 (C-7), 39.9 (C-15), 38.8 (C-1), 37.0 (C-10), 36.6 (C-4), 35.7 (C-3), 33.7 (C-12), 27.3 (C-19), 24.1 (C-17), 20.7 (C-24), 19.7 (C-11), 19.6 (C-6), 17.5 (C-2), 15.0 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 426.7 (95 %, [M−H]$^-$); analysis calcd for $C_{22}H_{37}NO_5S$ (427.60): C 61.8, H 8.72, N 3.28; found: C 61.56, H 7.96, N 2.99.

4.2.12. (4α, 8β, 13β, 16β) 16-[(Aminosulfonyl)oxy]stachan-18-yl acetate (13)

Compound **9** (0.2 g, 0.58 mmol) was suspended in dry dimethylacetamide (DMA, 5 mL) and stirred with sulfamoyl chloride (0.134 g, 1.16 mmol) for 1 day. The reaction mixture was quenched with MeOH (10 mL). The solvent was evaporated under reduced pressure and the column chromatography (SiO_2, $CHCl_3$/ethyl acetate, 9:1) gave **13** (0.19 g, 77 %) as a colorless solid; R_f = 0.93 (SiO_2, $CHCl_3$/ethyl acetate, 4:6); m.p. 175–177 °C; $[\alpha]_D^{20}$ = −37.88° (*c* = 0.103, MeOH); IR (ATR): *ν* = 3272*br*, 3115*br*, 2928 *m*, 2846 *m*, 1715 *m*, 1630*w*, 1563*w*, 1455*w*, 1371 *m*, 1260 *m*, 1182 *m*, 1122*w*, 1057*w*, 1035*w*, 969 *m*, 924 *m*, 836 *m*, 754*w*, 715*w*, 676*w*, 611*w*, 552*w*, 503*w*, 483*w* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* = 4.75–4.69 (*m*, 3H, NH_2, 16-H), 4.35 (*d*, *J* = 9.3 Hz, 1H, 18-H_a), 3.95 (*d*, *J* = 9.3 Hz, 1H, 18-H_b), 2.06 (*s*, 3H, 22-H), 1.83–1.75 (*m*, 4H, 3-H_a, 12-H_a, 15-H), 1.72–1.64 (*m*, 1H, 1-H_a), 1.62–1.47 (*m*, 5H, 11-H, 6-H_a, 2-H_a, 7-H_a), 1.46–1.31 (*m*, 3H, 2-H_b, 7-H_b, 14-H_a), 1.30–1.14 (*m*, 2H, 12-H_b, 6-H_b), 1.10–1.02 (*m*, 4H, 14-H_b, 9-H, 5-H, 3-H_b), 1.01 (*s*, 3H, 19-H), 0.90 (*s*, 3H, 17-H), 0.88 (*s*, 3H, 20-H), 0.86–0.82 (*m*, 1H, 1-H_b) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): *δ* = 171.6 (C-21), 81.7 (C-16), 74.7 (C-18), 56.9 (C-5), 56.6 (C-9), 55.1 (C-14), 42.3 (C-8), 41.6 (C-7, C-13), 40.9 (C-15), 39.3 (C-1), 37.7 (C-10), 37.3 (C-4), 35.8 (C-3), 34.6 (C-12), 27.4 (C-19), 25.0 (C-17), 21.3 (C-22), 20.3 (C-11), 20.2 (C-6), 17.9 (C-2), 15.6 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 426.7 (95 %, [M−H]$^-$); analysis calcd for $C_{22}H_{37}NO_5S$ (427.6): C 61.80, H 8.72, N 3.28; found: C 61.56, H 8.97, N 2.99.

4.2.13. (4α, 8β, 13β, 16β) Stachane-16,18-diyl disulfamate (14)

Compound **5** (0.27 g, 0.88 mmol) was suspended in dry dimethylacetamide (DMA, 5 mL) and stirred with sulfamoyl chloride (0.254 g, 2.2 mmol) for 1 day. The reaction mixture was quenched with MeOH (10 mL). The solvent was evaporated under reduced pressure and column chromatography (SiO_2, $CHCl_3$/ethyl acetate, 9:1) gave **14** (0.2 g, 49 %) as a colorless solid; R_f = 0.26 (SiO_2, $CHCl_3$/MeOH, 9:1); m.p. 167–169 °C; $[\alpha]_D^{20}$ = −30.97° (*c* = 0.128, MeOH); IR (ATR): *ν* = 3399*w*, 3377*br*, 3280 *m*, 2987*w*, 2940 *m*, 2893 *m*, 2845 *m*, 1533*w*, 1456*w*, 1343 *s*, 1183 *s*, 1169 *s*, 1049*w*, 997 *s*, 941 *s*, 914 *s*, 863 *m*, 840 *m*, 798 *m*, 759*w*, 595 *m*, 551 *m*, 488*w*, 473*w* cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): *δ* = 7.36 (*s*, 4H, NH_2), 4.41 (*dd*, *J* = 10.8, 3.8 Hz, 1H, 16-H), 4.11 (*d*, *J* = 9.6 Hz, 1H, 18-H_a), 3.76 (*d*, *J* = 9.6 Hz, 1H, 18-H_b), 2.08 (*dt*, *J* = 14.7, 2.9 Hz, 1H, 15-H_a), 1.77–1.60 (*m*, 4H, 15-H_b, 3-H_a, 12-H_a, 1-H_a), 1.60–1.42 (*m*, 5H, 6-H_a,11-H, 2-H_a, 7-H_a), 1.41–1.11 (*m*, 5H, 2-H_b, 7-H_b, 14-H_a, 12-H_b, 6-H_b), 1.11–0.96 (*m*, 4H, 14-H_b, 9-H, 5-H, 3-H_b), 0.93 (*s*, 6H, 17-H, 19-H), 0.87 (*s*, 3H, 20-H), 0.86–0.79 (*m*, 1H, 1-H_b) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): *δ* = 86.4 (C-16), 71.7 (C-18), 55.9 (C-5), 55.5 (C-9), 53.7 (C-14), 41.7 (C-13), 41.6 (C-8), 41.0 (C-7), 39.8 (C-15), 38.7 (C-1), 37.0 (C-10), 36.6 (C-4), 35.4 (C-3), 33.7 (C-12), 27.1 (C-19), 24.1 (C-17), 19.7 (C-11), 19.6 (C-6), 17.4 (C-2), 15.0 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 463.2 (95 %, [M−H]$^-$) 927.3 (60 %, [2M−H]$^-$); analysis calcd for $C_{20}H_{36}N_2O_6S_2$ (464.64): C 51.70, H 7.81, N 6.03; found: C 51.52, H 8.02, N 5.83.

4.2.14. (4α, 8β, 13β, 16β) 16-[(Aminosulfonyl)oxy]stachan-18-oic acid (15)

Compound **4** (0.27 g, 0.84 mmol) was suspended in dry dimethylacetamide (DMA, 5 mL) and stirred with sulfamoyl chloride (0.196 g, 1.7 mmol) for 1 day. The reaction mixture was quenched with MeOH (10 mL). The solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography (SiO_2, $CHCl_3$/ethyl acetate, 8:2) to yield **15** (0.28 g, 83 %) as a colorless solid; R_f = 0.43 (SiO_2, $CHCl_3$/MeOH, 9:1); m.p. 155–157 °C; $[\alpha]_D^{20}$ = –60.39° (*c* = 0.149, MeOH); IR (ATR): *ν* = 3345*br*, 3255 *m*, 2932 *m*, 2845*w*, 1692 *m*, 1607 *m*, 1574*w*, 1455 *m*, 1362*w*, 1339*br*, 1264*w*, 1249*w*, 1226 *m*, 1174 *s*, 1159 *m*, 1020*w*, 967*w*, 943*w*, 928*w*, 880*w*, 868*w*, 854*w*, 822*w*, 786*w*, 766*w*, 740*w*, 713*w*, 666*w*, 646*w*, 615*w*, 584*w*, 567*w*, 546*w*, 525*w*, 434*w* cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): *δ* = 11.96 (*s*, 1H, OH), 7.34 (*s*, 2H, NH_2), 4.40 (*dd*, *J* = 10.7, 4.0 Hz, 1H, 16-H), 2.10–1.97 (*m*, 2H, 15-H_a, 3-H_a), 1.82–1.61 (*m*, 5H, 2-H_a, 15-H_b, 6-H_a, 12-H_a, 1-H_a), 1.60–1.41 (*m*, 4H, 6-H_b, 11-H, 7-H_a), 1.37–1.15 (*m*, 4H, 2-H_b, 7-H_b, 14-H_a, 12-H_b), 1.09 (*s*, 3H, 19-H), 1.07–0.96 (*m*, 3H, 14-H_b, 5-H, 9-H), 0.92 (*s*, 4H, 3-H_b, 17-H), 0.85 (*td*, *J* = 13.4, 4.1 Hz, 1H, 1-H_b), 0.77 (*s*, 3H, 20-H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): *δ* = 178.6 (C-18), 86.4 (C-16), 56.0 (C-5), 54.9 (C-9), 53.6 (C-14), 42.8 (C-4), 41.8 (C-13), 41.6 (C-8), 40.9 (C-7), 39.8 (C-15), 39.4 (C-1), 37.6 (C-10), 37.6 (C-3), 33.7 (C-12), 28.6 (C-19), 24.1 (C-17), 21.4 (C-6), 19.7 (C-11), 18.6 (C-2), 13.0 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 398.2 (60 %, [M−H]$^-$) 797.3 (90 %, [2M−H]$^-$); analysis calcd for $C_{20}H_{33}NO_5S$ (399.55): C 60.12, H 8.33, N 3.51; found: C 59.96, H 8.55, N 3.33.

4.2.15. (4α) 13-(Aminosulfonyl)oxy-kaur-16-en-18-oic acid (16)

Compound **2** (0.27 g, 0.85 mol) was suspended in dry dimethylacetamide (DMA, 5 mL) and stirred with sulfamoyl chloride (0.196 g, 1.7 mmol) for 1 day. The reaction mixture was quenched with MeOH (10 mL). The solvent was evaporated under reduced pressure, and column chromatography (SiO_2, $CHCl_3$/ethyl acetate, 7:3) gave **16** (0.1 g, 30 %) as a colorless solid; R_f = 0.54 (SiO_2, $CHCl_3$/MeOH, 9:1); m.p. 121–122 °C; $[\alpha]_D^{20}$ = –33.49° (*c* = 0.067, MeOH); IR (ATR): *ν* = 3380*br*, 3276*br*, 2927*br*, 2849*w*, 1690 *s*, 1556*w*, 1463*w*, 1448*w*, 1363*br*, 1262*w*, 1176 *s*, 1028 *m*, 1000 *m*, 958 *m*, 926 *s*, 904 *m*, 852 *m*, 843 *m*, 799*w*, 755 *m*, 671*w*, 616*w*, 589*w*, 550*w*, 491*w* cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): *δ* = 12.01 (*s*, 1H, COOH), 7.39 (*s*, 2H, NH_2), 4.96 (*brs*, 1H, 17-H_a), 4.87 (*brs*, 1H, 17-H_b), 2.54–2.51 (*m*, 1H, 14-H_a), 2.25–2.14 (*m*, 1H, 3-H_a), 2.11–1.99 (*m*, 3H, 15-H, 12-H_a), 1.82–1.70 (*m*, 6H, 2-H_a, 6-H, 1-H_a, 11-H_a, 14-H_b), 1.63–1.47 (*m*, 3H, 12-H_b, 11-H_b, 7-H_a), 1.46–1.29 (*m*, 2H, 7-H_b, 2-H_b), 1.11 (*s*, 3H, 19-H), 1.06–1.02 (*m*, 1H, 5-H), 1.02–0.92 (*m*, 2H, 9-H, 3-H_b), 0.88 (*s*, 3H, 20-H), 0.79 (*td*, *J* = 13.3, 3.1 Hz, 1H, 1-H_b) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): *δ* = 178.6 (C-18), 151.1 (C-16), 104.6 (C-17), 91.2 (C-13), 55.7 (C-5), 52.5 (C-9), 45.9 (C-15), 42.8 (C-4), 42.2 (C-14), 42.2 (C-8), 40.6 (C-7), 40.1 (C-1), 38.8

(C-10), 37.5 (C-12), 37.4 (C-3), 28.5 (C-19), 21.5 (C-6), 20.0 (C-11), 18.8 (C-2), 15.2 (C-20) ppm; MS (ESI, MeOH): *m/z* (%) = 396.3 (60 %, $[M-H]^-$, 793.3 (95 %, $[2M-H]^-$); analysis calcd for $C_{20}H_{31}NO_5S$ (397.53): C 60.43, H 7.86, N 3.52; found: C 60.19, H 8.01, N 3.36.

CRediT authorship contribution statement

Toni C. Denner: Writing – review & editing, Writing – original draft, Investigation. **Niels V. Heise:** Writing – review & editing, Writing – original draft, Investigation. **René Csuk:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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Lupane acetates in small molecule drug hybrids: Probing their inhibitory activity for carbonic anhydrase II

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ABSTRACT

Earlier studies had shown the potential of modified pentacyclic triterpenes as possible inhibitors of carbonic anhydrase II (CA II). In an extension of our earlier studies, betulin, betulinic acid and, for comparison purposes, glycyrrhetinic acid, ursolic acid and oleanolic acid were therefore converted into the respective acetates and linked to either taurinamide or de-acetylated acetazolamide via a variable linker. In particular, the derivatives **8** and **18** derived from betulinic acid or betulin and provided with a long spacer were found to be strong competitive inhibitors of CA II, thereby holding K_i = 1.27 and 0.20 μM, respectively.

1. Introduction

The development of inhibitors for carbonic anhydrases is an area of increasing scientific interest. Whereas many years ago these enzymes were thought to be responsible only for the fixation of carbon dioxide to bicarbonate, and therefore only important for the regulation of intracellular and extracellular pH, today many different isoenzymes are known to be involved in an almost incalculable number of biological processes. For this reason, the development of new inhibitors seems particularly important [1–5].

In the past, CA II inhibitors have only played a role in the treatment of glaucoma [6–11]. This should not be underestimated, as it is estimated that around 76 million people worldwide suffered from this eye disease in the year 2020, and in 2040 around 111.8 million persons will be affected [12]; furthermore, glaucoma is the leading cause of global irreversible blindness [13]. CA II inhibitors have recently become particularly important because CA II has also been implicated in epilepsy [14–18] (in addition to CA VII and CA IV), neuropathic pain [19–21] (in addition to CA VII) and edema [22–24] (in addition to CA I). Edema and brain swelling have also been reported as adverse effects in patients with Alzheimer's disease treated with lecanemab, donanemab or aducanumab – three anti-amyloid monoclonal antibodies [25–28].

During our studies on pentacyclic triterpenes some years ago, we became aware of the CA II inhibitory activity of some derivatives and decided to investigate this aspect in more detail [29–33].

Based on these initial findings, the pentacyclic triterpene betulinic acid (**BA**, Scheme 1) in particular offered itself as a readily available starting material. Classical sulfonamides have been known as inhibitors of CA II since the 1950s and 1960s. In the context of a small molecule drug conjugate (SMDC) concept [34–37], conjugates between the triterpene and a suitable sulfonamide therefore appeared to be of interest. We therefore chose structural elements derived from the known inhibitors, acetazolamide [38–40] or taurinamide [41,42].

2. Results and discussion

Betulinic acid (**BA**, **1**, Scheme 1) was acetylated as previously described, and acetate **2** was obtained in 96% yield. Reaction of **2** with oxalyl chloride followed by adding either taurinamide or deacetylated acetazolamide (**10**) yielded conjugates **3** and **4** in 69% and 32% yield, respectively.

To investigate the influence of a linker onto the activity of the compounds, acetate **2** was converted *in situ* with oxalyl chloride into the corresponding acid chloride; its subsequent reaction with 2-(2-aminoethoxy)-ethan-1-ol gave 96% of **5** whose reaction with succinic anhydride furnished **6**. The latter compound was again activated *in situ* with oxalyl chloride followed by the reaction with taurinamide or **10** to furnish conjugates **7** and **8**, respectively. In a similar way, from the reaction of **5** with sulfamoyl chloride conjugate **9** was obtained.

Acetylation of betulin (**BN**, **11**, Scheme 2) afforded diacetate **12** in 93% yield whose selective de-acetylation with catal. amounts of calcium hydride gave 84% of 3-*O*-acetyl-betulin (**13a**); 28-*O*-acetyl-betulin (**13b**) was prepared according to literature [43,44]. The former compound was reacted with sulfamoyl chloride to yield 95% of sulfamate

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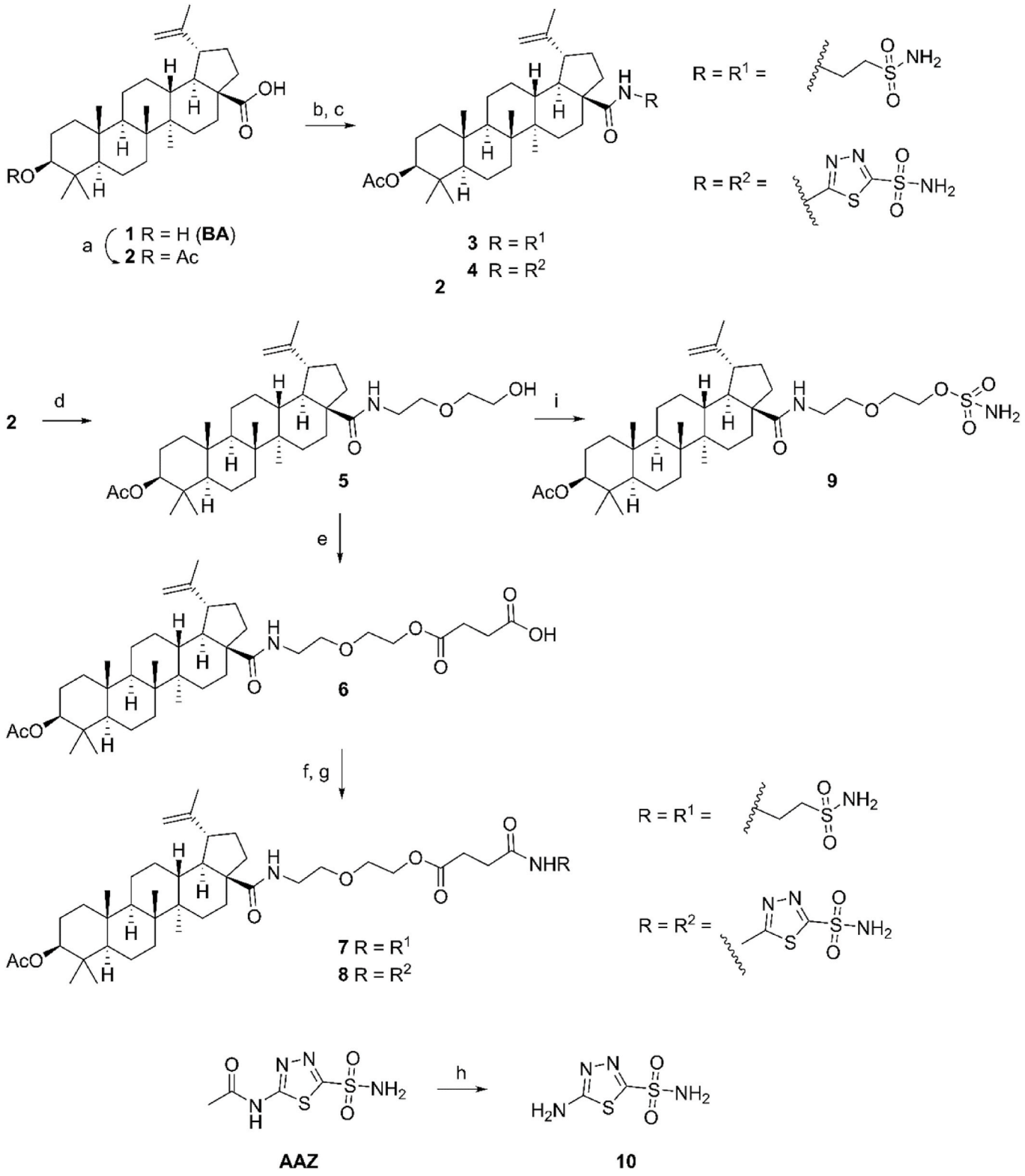


Scheme 1. Reactions and conditions: a) Ac_2O, NEt_3, DMAP (cat.), 20 °C, 12 h, 96%; b) $(COCl)_2$, DMF (cat.), DCM, 20 °C, 30 min, then taurinamide, NEt_3, DCM, 20 °C, 12 h → 3 (69%); c) $(COCl)_2$, DMF (cat.), DCM, 20 °C, 30 min, then **10**, NEt_3, DCM, microwave-assisted, 120 °C, 2 h → 4 (32%); d) $(COCl)_2$, DMF (cat.), DCM, 20 °C, 30 min, then H_2N–$(CH_2)_2$–*O*–$(CH_2)_2$–OH, NEt_3, DCM, 12 h, 20 °C, 96%; e) pyridine, DMAP (cat.), succinic anhydride, Δ, 3.5 h, 84%; f) $(COCl)_2$, DMF (cat.), DCM, 20 °C, 30 min, then taurinamide, NEt_3, DCM, 20 °C, 12 h → 7 (68%); g) $(COCl)_2$, DMF (cat.), DCM, 20 °C, 30 min, then **10**, NEt_3, DCM, 20 °C, 12 h → 8 (80%); h) conc. aq. HCl, Δ, 3 h, 96%; i) DMA, sulfamoyl chloride, 20 °C, 24 h, 83%.

14. Interestingly, while the reaction of **13a** with sulfamoyl chloride in dry DMA exclusively furnished **14**, from the reaction of **13a** with sodium hydride/sulfamoyl chloride a mixture of 28-sulfamate **14** and 3-sulfamate **15** was obtained. The formation of **15** can be explained by in intramolecular migration of the acetyl group from position C-3 to C-28 followed by sulfamoylation. The structure of **15** was confirmed beyond doubt by MS and especially NMR studies. In addition, the MS spectra showed a quasi-molecule ion $[M-H]^- m/z = 562.2$, and an independent synthesis starting from **13b** led to the same product.

Reaction of **13a** with succinic anhydride yielded 81% of spacered **16** whose reaction with taurinamide or **10** - as described above - yielded conjugates **17** and **18**, respectively.

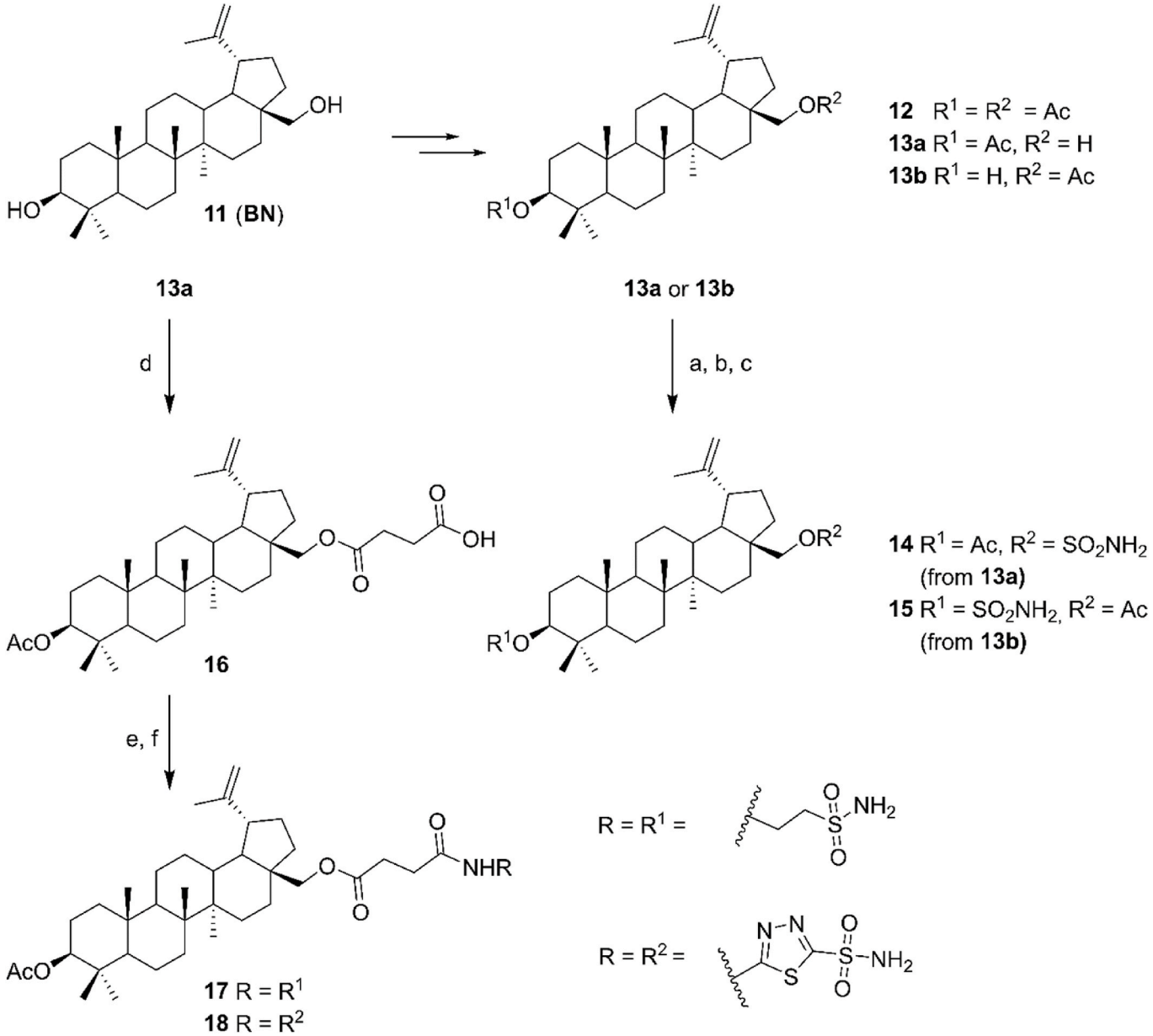
To test the influence of the triterpenoid backbone on the biological activity, glycyrrhetinic acid (**GA**, **19**, Scheme 3), ursolic acid (**UA**, **20**) and oleanolic acid (**OA**, **21**) were also included in the investigations.

These pentacyclic triterpenoic acids were converted into acetates **22**–**24**; their reaction with succinic anhydride afforded **25**–**27** which upon activation with oxalyl chloride and reaction with taurinamide yielded final compounds **28**–**30**, respectively.

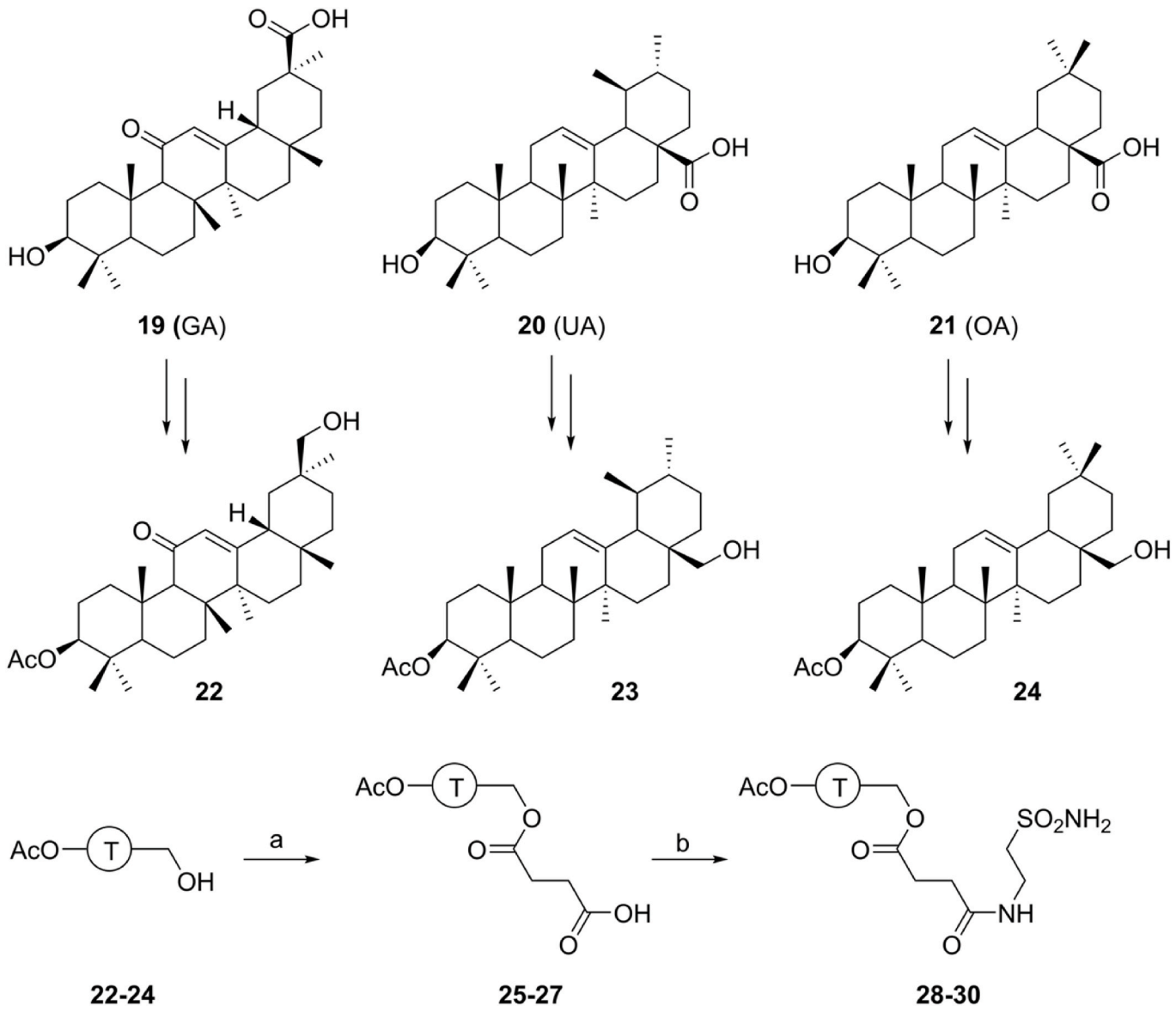
The products were subjected to enzymatic assays employing CA II; the results from these assays are compiled in Table 1.

Most compounds proved to be weak inhibitors for CA II except for **8** and **18** which were excellent inhibitors. Therefore, extra kinetic studies were performed for these compounds as well as for **9** and **14**. These compounds were shown to be competitive inhibitors, and their respective K_i values are compiled in Table 2. A Dixon plot for **8** is depicted in Fig. 1.

In this series of compounds, those with a lupane scaffold appear to be better inhibitors than those holding an ursane or an oleanane scaffold. Compounds with a longer spacer between the sulfamate group and the triterpene are better inhibitors than those with only a short-chained spacer. One reason for this could be that in compounds with a longer spacer, the triterpene does not have to penetrate the active site of the enzyme; from the structure of the active site no polar interactions with



Scheme 2. Reactions and conditions: a) DMA, sulfamoyl chloride, 24 h, 20 °C, 95% (from **13a**); b) **13a**, DCM, sulfamoyl chloride, 24 h, 20 °C, → 40% **14** and 44% **15**; c) DMA, sulfamoyl chloride, 24 h, 20 °C, 90% (from **13b**); d) according to lit. [29]; 80%; e) ethyl chloroformate, 4-methylmorpholine, THF, 20 °C 15 min, then taurinamide, Δ, 24h, → **17** (80%); f) ethyl chloroformate, 4-methylmorpholine, THF, 20 °C 15 min, then **10**, Δ, 24h, → **18** (85%).



Scheme 3. Reactions and conditions: a) according to lit. [29], b) THF, ethyl chloroformate, 4-methylmorpholine 20 °C, then taurinamide, reflux, 24 h, → **28** (68% from **25**), → **29** (81% from **26**), → 30 (70% from **27**).

Table 1
Inhibition in %) of *b*CA II by compounds **3,4, 7–9, 14, 15, 17, 18, 28–30** (at 1 μM concentration of the inhibitor); acetazolamide (**AAZ**) was used as a positive standard. All experiments were performed in triplicate with three technical replicas. Under the conditions of the assay, **AAZ** showed an inhibition of 98%.

Comp	Inhibition [%]	Comp	Inhibition [%]
3	<5	**15**	58.9 ± 3.9
4	22.5 ± 0.6	**17**	38.1 ± 0.1
7	11.1 ± 2.7	**18**	93.0 ± 0.1
8	98.4 ± 0.7	**28**	12.7 ± 0.4
9	<5	**29**	<5
14	76.4 ± 5.2	**30**	<5

the triterpene can be expected anyway. Molecular modelling calculations support this assumption. A 2D representation of **8** and CA II is depicted in Fig. 2.

In a direct comparison between **17**, **28**, **29** and **30**, compound **17** holds unambiguously superior inhibitory properties as compared to the other compounds of this investigation.

Table 2
Detailed investigation of the most active inhibitors (from Table 1); K_i are given in μM.

Comp	K_i	Comp	K_i
8	1.27 ± 0.04	**15**	2.77 ± 0.03
14	1.40 ± 0.08	**18**	0.20 ± 0.03

3. Conclusion

Previous studies had shown the potential of modified pentacyclic triterpenes as possible inhibitors of carbonic anhydrase II. Betulinic, betulinic, glycyrrhetinic, ursolic and oleanolic acids appear to be of particular interest - also due to their good commercial availability. Therefore, in an extension of our previous studies, these triterpenes were converted to the corresponding acetates and coupled via a variable linker to either taurinamide or de-acetylated acetazolamide, the latter compound being a well-known and excellent inhibitor of carbonic anhydrases. As a result, in particular, derivatives **8** and **18**, derived from betulinic acid or betulin and provided with a long spacer, were found to

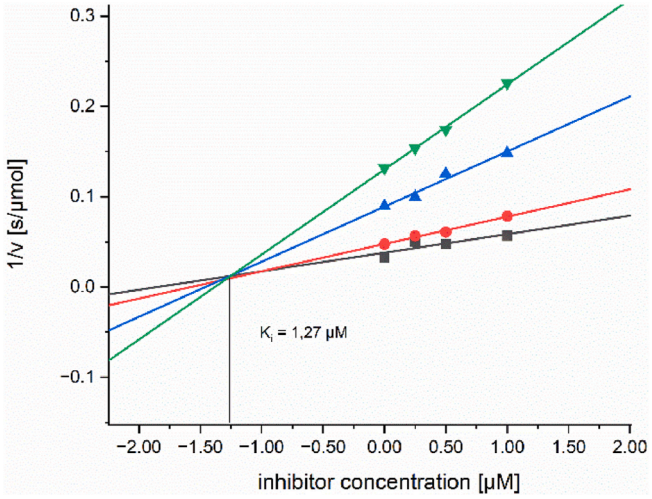


Fig. 1. Dixon plot for **8**; a K_i value of 1.27 µM was determined (concentration of the inhibitor: green: 0.75 µM, blue: 0.5 µM; red: 0.25 µM; black: 0.15 mM). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

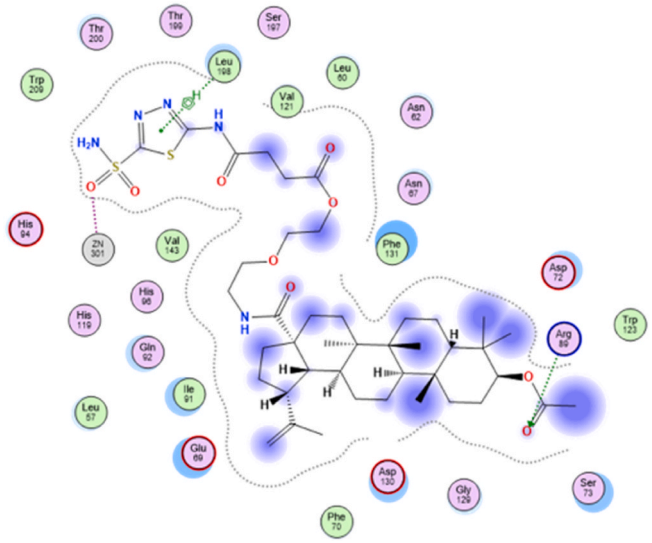


Fig. 2. Depiction of the interactions of CA II with compound **8**.

be strong competitive inhibitors of CA II holding K_i values of $K_i = 1.27 \pm 0.04$ µM (for **8**) and $K_i = 0.129 \pm 0.02$ µM (for **18**), respectively.

4. Experimental

4.1. General

Starting materials were obtained from local vendors; solvents were dried under usual conditions; equipment and assays were used as previously described [29,30,32]. Triterpenes were obtained from Betulinines (Stříbrná Skalice, CZ) and used as received.

4.2. Syntheses

4.2.1. (3β) Acetyloxy-lup-20(29)-en-28-oic acid (2)

Acetylation of betulinic acid (**1**, 10.0 g, 21.8 mmol) with acetic anhydride (250 mL, 3.25 mol) was performed [45] as previously described; recrystallization from ethanol gave **2** (8.4 g, 96%) as a colourless solid; R_f = 0.65 (hexanes/ethyl acetate; 3:1); m.p. 277–278 °C [lit. [45]: 277–278 °C]; $[\alpha]_D^{20} = +18.9°$ ($c = 0.013$, $CHCl_3$) [lit. [45]: $[\alpha]_D^{20} = +22.0°$ ($c = 0.049$, $CHCl_3$)]; ESI-MS (MeOH): m/z = 497.3 ($[M - H]^-$), 995.2 ($[2M - H]^-$), 1017.5 ($[2M\text{-}2H + Na]^-$).

4.2.2. (3β) 28-{[2-(Aminosulfonyl)ethyl]amino}-28-oxolup-20(29)-en-3-yl acetate (3)

To a solution of **2** (300 mg, 0.60 mmol) in dry DCM (10 mL), oxalyl chloride (0.2 mL, 2.4 mmol) and cat. amounts of DMF were added, and the reaction mixture was stirred for 30 min at 20 °C followed by an evaporation of the volatiles. A solution of taurinamide (150 mg, 1.2 mmol) and triethylamine (0.3 mL, 2.0 mmol) in dry DCM (20 mL) was slowly added, and the mixture was stirred overnight at 20 °C. Usual aqueous work-up followed by column chromatography (SiO_2, chloroform MeOH, 95:5) gave **3** (250 mg, 69%) as a s colourless solid; R_f = 0.45 (hexanes/ethyl acetate; 7:3); m.p. 137–140 °C; $[\alpha]_D^{20} = +1.3°$ (c = 0.083, MeOH); IR (ATR): ν = 2941 *m*, 2869*w*, 1733 *m*, 1633 *m*, 1520 *m*, 1449*w*, 1375 *m*, 1327 *m*, 1245*s*, 1190*w*, 1136*s*, 1024 *m*, 979 *m*, 880*w*, 796*w*, 753 *m*, 569*w*, 489 m cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.41 (t, J = 6.1 Hz, 1H, NH), 5.19 (s, 2H, NH_2), 4.72 (d, J = 1.5 Hz, 1H, 29-H_b), 4.59 (d, J = 1.5 Hz, 1H, 29-H_a), 4.50–4.42 (m, 1H, 3-H), 3.84–3.70 (m, 2H, 33-H), 3.37–3.21 (m, 2H, 34-H), 3.06 (*dt*, J = 11.3, 5.8 Hz, 1H, 19-H), 2.40 (*dt*, J = 12.4, 3.6 Hz, 1H, 13-H), 2.03 (s, 3H, 32-H), 1.99–1.85 (m, 2H, 7-H_a, 16-H_a), 1.82–1.73 (m, 1H, 22-H_b), 1.67 (s, 3H, 30-H), 1.73–1.59 (m, 2H, 1-H_a, 12-H_a), 1.61–1.52 (m, 3H, 2, 18-H), 1.52–1.29 (m, 8H, 6-H, 7-H_b, 11-H_a, 15-H_a, 16-H_b, 21-H_a, 22-H_a), 1.29–1.19 (m, 3H, 9, 11-H_b, 21-H_b), 1.14 (*dt*, J = 13.7, 3.2 Hz, 1H, 15-H_b), 1.02–0.96 (m, 2H, 1-H_b, 12-H_b), 0.95 (s, 3H, 27-H), 0.92 (s, 3H, 26-H), 0.83 (s, 3H, 24-H), 0.83 (s, 3H, 23-H), 0.82 (s, 3H, 25-H), 0.80–0.74 (m, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 177.3 (C-28), 171.2 (C-31), 150.8 (C-20), 109.7 (C-29), 81.1 (C-3), 55.9 (C-17), 55.6 (C-5), 55.0 (C-34), 50.7 (C-9), 50.2 (C-18), 46.9 (C-19), 42.6 (C-14), 40.9 (C-8), 38.5 (C-1), 38.3 (C-22), 37.9 (C-10), 37.9 (C-13), 37.3 (C-4), 34.5 (C-33), 33.6 (C-7), 30.9 (C-16), 29.8 (C-21), 29.5 (C-15), 28.1 (C-23), 25.7 (C-12), 23.8 (C-2), 21.4 (C-32), 21.1 (C-11), 19.5 (C-30), 18.3 (C-6), 16.6 (C-25), 16.3 (C-26), 16.3 (C-24), 14.7 (C-27) ppm; MS (ESI, MeOH/$CHCl_3$, 4:1): m/z = 605.2 ($[M+H]^+$); analysis calcd for $C_{34}H_{56}N_2SO_5$ (604.88): C 67.51, H 9.33, N 4.63; found: C 67.39, H 9.55, N 4.42.

4.2.3. (3β) 28-{[5-(Aminosulfonyl)1,3,4-thiadiazol-2-yl]amino}-28-oxolup-20(29)-en-3-yl acetate (4)

From the reaction of **2** (500 mg, 1.0 mmol) in dry DCM (20 mL), with oxalyl chloride (0.4 mL, 4.8 mmol) and cat. amounts of DMF as described above, followed by adding **10** (230 mg, 1.3 mmol) and triethylamine (0.3 mL, 2 mmol) in dry DCM (20 mL) and subsequent stirring in a (microwave apparatus, Monowave, Anton Paar GmbH) at 120 °C for 2h, followed by usual aqueous work-up and column chromatography (SiO_2, $CHCl_3$/MeOH, 95:5) **4** (208 mg, 32%) was obtained as a s colourless solid; m.p. 162–165 °C; $[\alpha]_D^{20} = +13.2°$ (c = 0.082, MeOH); R_F = 0.35 (SiO_2, $CHCl_3$/MeOH, 9:1); UV–Vis (MeOH): λ_{max} (log ε) = 262 nm (3.62); IR (ATR): ν = 3263*br*, 2941*br*, 2671*w*, 1733*w*, 1710 *m*, 1693 *m*, 1643*w*, 1510*s*, 1467 *m*, 1455*w*, 1366*s*, 1316*w*, 1291*w*, 1265 *m*, 1245*s*, 1174*s*, 1153*w*, 1129 *m*, 1070 *m*, 1026 *m*, 979 *m*, 944*w*, 915*w*, 889 *m*, 796*w*, 749*w*, 700*w*, 661 *m*, 616*s*, 602*s*, 560*w*, 509 *m*, 489*w*, 454*w* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.63 (s, 1H, NH), 6.69 (s, 2H, NH_2), 4.77–4.74 (m, 1H, 29′-H_a), 4.64–4.61 (m, 1H, 29-H_b), 4.51–4.41 (m, 1H, 3-H), 3.01 (*dt*, J = 10.9, 4.6 Hz, 1H, 19-H), 2.54–2.44 (m, 1H, 13-H), 2.27 (d, J = 13.9 Hz, 1H, 7-H_a), 2.04 (s, 3H, 32-H), 1.84–1.56 (m, 9H, 16-H_a, 12-H_a, 7-H_b, 30-H, 1-H_a, 18-H, 2-H), 1.53–1.13 (m, 12H; 6-H, 11-H_a, 16-H_b, 22-H, 9-H, 11-H_b, 21-H, 15-H), 1.08–1.01 (m, 2H, 1-H_b, 12-H_b), 0.98 (s, 3H, 27-H), 0.88 (s, 3H, 26-H), 0.84 (s, 3H, 24-H), 0.83 (s, 3H, 23-H), 0.82 (s, 3H, 25-H), 0.81–0.75 (m, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 175.4 (C-28), 171.2 (C-31), 164.2 (C-34), 163.0 (C-33), 149.9 (C-20), 110.3 (C-29), 81.1 (C-3), 57.1 (C-17), 55.6 (C-5), 50.7 (C-9), 50.1 (C-18), 46.4 (C-19), 42.6 (C-14), 40.9 (C-8), 38.6 (C-1), 37.9 (C-10), 37.7 (C-13), 37.3 (C-4), 34.4 (C-22), 32.5 (C-7), 30.5 (C-

16), 29.8 (C-21), 29.7 (C-15), 28.1 (C-23), 25.6 (C-12), 23.8 (C-2), 21.5 (C-32), 21.1 (C-11), 19.5 (C-30), 18.3 (C-6), 16.6 (C-25), 16.4 (C-26), 16.3 (C-24), 14.8 (C-27) ppm; MS (ESI, MeOH/$CHCl_3$, 4:1): *m/z* = 659.5 ([M − H]$^-$); analysis calcd for $C_{34}H_{52}N_4O_5S_2$ (660.93): C 61.79, H 7.93, N 8.48; found: C 61.50, H 9.17, N 8.23.

4.2.4. (3β) 28-{[2-(2-Hydroxyethoxy)ethyl]amino}-28-oxolup-20(29)-en-3-yl acetate (5)

As described above from the reaction of **2** (1.0 g, 2 mmol) in dry DCM (20 mL) with oxalyl chloride (1.0 mL, 5.0 mmol) and cat. amounts of DMF, followed by adding 2-(2-aminoethoxy)-ethan-1-ol (0.6 mL, 5.0 mmol) and triethylamine (0.6 mL, 4 mmol) in dry DCM (20 mL), stirring for 12 h at 20 °C and usual work-up and chromatography (SiO_2, $CHCl_3$/MeOH, 95:5) **5** (1.12 g mg, 96%) was obtained as a colourless solid; R_f = 0.38 (DCM/MeOH, 10:0.1); m.p. 205–207 °C; $[\alpha]_D^{20}$ = +13.0° (*c* = 0.170, MeOH); IR (ATR): *ν* = 3313*w*, 2932 *m*, 2866*w*, 1733*s*, 1639 *m*, 1545 *m*, 1467*w*, 1444*w*, 1368 *m*, 1248*vs*, 1130 *m*, 1070*w*, 1026 *m*, 976*w*, 898*w*, 878*w*, 656*w*, 542*w* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* = 6.02 (t, *J* = 5.2 Hz, 1H, NH), 4.72 (d, *J* = 1.4 Hz, 1H, 29-H_b), 4.58 (d, *J* = 1.4 Hz, 1H, 29-H_a), 4.49–4.41 (m, 1H, 3-H), 3.73 (s, 2H, 36-H), 3.60–3.52 (m, 4H, 34-H, 35-H), 3.53–3.44 (m, 1H, 33-H_b), 3.44–3.34 (m, 1H, 33-H_a), 3.10 (*dt*, *J* = 11.0, 3.9 Hz, 1H, 19-H), 2.42 (*dt*, *J* = 12.8, 3.4 Hz, 1H, 13-H), 2.21 (s, 1H, OH), 2.02 (s, 3H), 2.00–1.86 (m, 2H, 16-H_a, 21-H_b), 1.78–1.67 (m, 3H, 1-H_b, 12-H_b, 22-H_b), 1.67 (s, 3H, 30-H), 1.64–1.52 (m, 4H, *2-H,* 18-H, 21-H_a), 1.51–1.44 (m, 2H, 6-H_a, 15-H_a), 1.44–1.28 (m, 6H, 6-H_b, *7-H,* 11-H_a, 16-H_b, 22-H_a), 1.26 (s, 1H, 9-H), 1.22 (*dt*, *J* = 12.2, 4.0 Hz, 1H, 11-H_b), 1.16–1.09 (m, 1H, 15-H_b), 1.05–0.97 (m, 2H, 1-H_a, 12-H_a), 0.95 (s, 3H, 27-H), 0.92 (s, 3H, 26-H), 0.84–0.82 (m, 6H, 23, 24-H), 0.81 (s, 3H, 25-H), 0.79–0.75 (m, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): *δ* = 176.5 (C-28), 171.1 (C-31), 151.0 (C-20), 109.5 (C-29), 81.1 (C-3), 72.3 (C-35), 70.3 (C-34), 61.9 (C-36), 55.9 (C-17), 55.6 (C-5), 50.7 (C-9), 50.2 (C-18), 47.0 (C-19), 42.6 (C-14), 40.9 (C-8), 39.1 (C-33), 38.5 (C-1), 38.5 (C-22), 37.9 (C-4), 37.9 (C-13), 37.3 (C-10), 34.5 (C-7), 33.8 (C-21), 31.0 (C-16), 29.5 (C-15), 28.1 (C-23), 25.7 (C-12), 23.8 (C-2), 21.4 (C-32), 21.1 (C-11), 19.6 (C-30), 18.3 (C-6), 16.6 (C-25), 16.3 (C-24, 26), 14.7 (C-2) ppm; ESI-MS (MeOH): = 584.5 ([M − H]$^-$); 620.5 ([M+Cl]$^-$); analysis calcd for $C_{36}H_{59}NO_5$ (585.86): C 73.80, H 10.15, N 2.39; found: C 73.62, H 10.39, N 2.17.

4.2.5. 4-[(2-2-{[(3β)-(Acetyloxy)-28-oxolup-20(29)en-28-yl]amino} ethoxy)ethoxy]-4-oxo-butanoic acid (6)

Compound **5** (500 mg, 0.85 mmol) was acylated in dry pyridine [DMAP (catalytic amounts), with succinic anhydride (430 mg, 4.3 mmol)] under reflux for 3.5 h; usual aqueous work-up followed by column chromatography (SiO_2, $CHCl_3$/MeOH, 95:5) gave **6** (490 mg, 84%) obtained as a colourless solid; R_f = 0.48 (DCM/MeOH, 10:0.5); m.p. 78–82 °C; $[\alpha]_D^{20}$ = +16.1° (*c* = 0.118, MeOH); IR (ATR): *ν* = 2942 *m*, 2869*w*, 1733*s*, 1636*w*, 520*w*, 1466*w*, 1374 *m*, 1245*s*, 1161 *m*, 1130 *m*, 1028 *m*, 979 *m*, 881*w*, 545*w* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* = 6.06 (t, *J* = 5.5 Hz, 1H, NH), 4.72 (d, *J* = 1.6 Hz, 1H, 29-H_b), 4.58 (d, *J* = 1.4 Hz, 1H, 29-H_a), 4.49–4.43 (m, 1H, 3-H), 4.26–4.23 (m, 2H, 36-H), 3.66–3.62 (m, 2H, 35-H), 3.55–3.50 (m, 2H, 34-H), 3.49–3.44 (m, 1H, 33-H_a), 3.43–3.37 (m, 1H, 33-H_b), 3.09 (*dt*, *J* = 11.0, 4.0 Hz, 1H, 19-H), 2.70–2.62 (m, *J* = 3.8 Hz, 4H, 38-H, 39-H), 2.46–2.38 (m, 1H, 13-H), 2.03 (s, 3H, 32-H), 2.00–1.86 (m, 2H, 16-H_a, 21-H_b), 1.78–1.69 (m, 2H, 1-H_b, 12-H_a), 1.67 (s, 3H, 30-H), 1.65–1.52 (m, 4H, *2-H,* 18-H, 22-H_a), 1.52–1.44 (m, 3H, 6-H_a, 15-H_a, 21-H_a), 1.44–1.29 (m, 6H, 6-H_b, 7-H, 11-H_a, 16-H_b, 22-H_b), 1.26 (d, *J* = 7.7 Hz, 2H, 9, 11-H_b), 1.16–1.10 (m, 1H, 15-H_b), 1.00 (s, 2H, 1-H_a, 12-H_b), 0.95 (s, 3H, 27-H), 0.92 (s, 3H, 26-H), 0.83 (s, 3H, 25-H), 0.83 (s, 3H, 23-H), 0.82 (s, 3H, 24-H), 0.79–0.76 (m, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): *δ* = 176.8 (C-28), 176.4 (C-40), 172.2 (C-37), 171.2 (C-31), 151.0 (C-20), 109.6 (C-29), 81.1 (C-3), 70.1 (C-34), 69.0 (C-35), 63.9 (C-36), 55.9 (C-17), 55.6 (C-5), 50.7 (C-9), 50.2 (C-18), 46.9 (C-19), 42.6 (C-14), 40.9 (C-8), 39.1 (C-33), 38.5 (C-1), 38.5 (C-22), 37.9 (C-4), 37.9 (C-13), 37.3 (C-10), 34.5 (C-7), 33.8 (C-21), 31.0 (C-16), 29.5 (C-15), 29.1 (C-39), 29.0 (C-38), 28.1 (C-23), 25.7 (C-12), 23.8 (C-2), 21.4 (C-32), 21.1 (C-11), 19.6 (C-30), 18.3 (C-6), 16.6 (C-25), 16.3 (C-26), 16.3 (C-24), 14.7 (C-27) ppm; ESI-MS (MeOH): *m/z* = 684.6 ([M − H]$^-$); analysis calcd for $C_{40}H_{63}NO_8$ (685.93): C 70.04, H 9.26, N 2.04; found: C 69.86, H 9.33, N 1.85.

4.2.6. 2-(2-{[(3β)-3-(Acetyloxy)-28-oxolup-20(29)en-28-ylamino} ethoxy)ethyl 4-{[2-(aminosulfonyl)ethyl]amino}-4-oxobutanoate (7)

From the reaction of **6** (250 mg, 0.36 mmol) (10 mL) with oxalyl chloride (0.1 mL, 1.2 mmol) and cat. amounts of DMF in dry DCM in dry DCM for 30 min, followed by the reaction with taurinamide (75 mg, 0.6 mmol) and triethylamine (0.1 mL, 0.7 mmol) in dry DCM (20 mL) at 21C for 12 h, usual aqueous work-up and column chromatography (SiO_2, $CHCl_3$/MeOH, 95:5) **7** (190 mg, 68%) was obtained as a colourless, viscous solid; R_f = 0.20 ($CHCl_3$/MeOH, 9:1); m.p. 44–48 °C; $[\alpha]_D^{20}$ = +6.3° (*c* = 0.131, MeOH); IR (ATR): *ν* = 3327*w*, 3208*w*, 2944 *m*, 2870*w*, 1722*w*, 1688*s*, 1658 *m*, 1532 *m*, 1450*w*, 1374*w*, 1330 *m*, 1264*s*, 1141*s*, 1040*s*, 979*w*, 901*w*, 751*s*, 601*w*, 501 s cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* = 6.84 (t, *J* = 5.8 Hz, 1H, NH_a), 6.22 (t, *J* = 5.5 Hz, 1H, NH_b), 5.62–5.56 (m, 2H, NH_2), 4.71 (s, 1H, 29-H_b), 4.58 (s, 1H, 29-H_a), 4.48–4.42 (m, 1H, 3-H), 4.24–4.19 (m, 2H, 36-H), 3.79–3.71 (m, 2H, 41-H), 3.71–3.62 (m, 2H, 35-H), 3.57–3.51 (m, 2H, 34-H), 3.48–3.37 (m, 2H, 33-H), 3.37–3.29 (m, 2H, 42), 3.07 (*dt*, *J* = 10.7, 10.2, 3.4 Hz, 1H, 19-H), 2.67 (t, *J* = 6.4 Hz, 2H, 38-H), 2.51 (t, *J* = 6.4 Hz, 2H, 39-H), 2.47–2.36 (m, 1H, 13-H), 2.03 (s, 3H, 32), 2.01–1.84 (m, 2H, 16-H_a, 21-H_b), 1.80–1.72 (m, 1H, 1-H_b), 1.70–1.65 (m, 2H, 12-H_b, 22-H_b), 1.67 (s, 3H, 30-H), 1.64–1.46 (m, 5H, *2-H,* 6-H_a, 18, 21-H_a), 1.45–1.29 (m, 8H, 6-H_b, 7-H, 11-H, 15-H_a, 16-H_b, 22-H_a), 1.29–1.18 (m, 1H, 9-H), 1.18–1.09 (m, 1H, 15-H_b), 1.06–0.96 (m, 2H, 1-H_a, 12-H_a), 0.95 (s, 3H, 27-H), 0.92 (s, 3H, 26-H), 0.84–0.82 (m, 6H, 23, 24-H), 0.82 (s, 3H, 25-H), 0.79–0.73 (m, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): *δ* = 176.9 (C-28), 173.3 (C-37), 172.7 (C-40), 171.2 (C-31), 151.0 (C-20), 109.6 (C-29), 81.1 (C-3), 70.2 (C-34), 69.0 (C-35), 64.0 (C-36), 55.6 (C-5), 54.8 (C-42), 54.2 (C-17), 50.6 (C-9), 50.2 (C-18), 47.0 (C-19), 42.6 (C-14), 40.9 (C-8), 39.1 (C-33), 38.5 (C-1), 38.5 (C-22), 37.9 (C-4), 37.9 (C-13), 37.3 (C-10), 35.0 (C-41), 34.5 (C-7), 33.7 (C-21), 31.0 (C-39), 30.7 (C-16), 29.5 (C-38), 29.4 (C-15), 28.1 (C-23), 25.7 (C-12), 23.8 (C-2), 21.4 (C-32), 21.1 (C-11), 19.5 (C-30), 18.3 (C-6), 16.6 (C-25), 16.3 (C-26), 14.7 (C-24), 14.7 (C-27) ppm; ESI-MS (MeOH/$CHCl_3$, 4:1): *m/z* = 814.7 ([M+Na]$^+$); analysis calcd for $C_{42}H_{69}N_3O_9S$ (792.08): C 63.69, H 8.78, N 5.31; found: C 63.41, H 8.93, N 5.02.

4.2.7. 2-(2-{[(3β)-3-(Acetyloxy)-28-oxolup-20(29)en-28-yl]amino} ethoxy)ethyl 4-{[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]amino}-4-oxo-butanoate (8)

From the reaction of **6** (250 mg, 0.36 mmol) with oxalyl chloride (0.1 mL, 1.2 mmol) and cat. amounts of DMF as described above, followed by a reaction with **10** (100.0 mg, 0.5 mmol) and triethylamine (0.1 mL, 0.7 mmol) for 12 h at 20 °C and usual work-up and column chromatography (SiO_2, $CHCl_3$/MeOH, 95:5) **8** (246 mg, 80%) was obtained as a s colourless solid; R_f = 0.10 ($CHCl_3$/MeOH, 95:5); m.p. 92–94 °C; $[\alpha]_D^{20}$ = +9.5° (*c* = 0.082, MeOH); UV–Vis (MeOH): λ_{max} (log *ε*) = 264 nm (3.77); IR (ATR): *ν* = 2943 *m*, 2870*w*, 1731 *m*, 1713 *m*, 1638*w*, 1525 *m*, 1449*w*, 1365 *m*, 1248*s*, 1174*s*, 1132 *m*, 1028*w*, 979*w*, 913*w*, 754 *m*, 732*w*, 654*w*, 603 *m*, 509 m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): *δ* = 12.19 (s, 1H, NH_b), 6.75 (s, 2H, NH_2), 6.19 (t, *J* = 5.5 Hz, 1H, NH_a), 4.71 (s, 1H, 29-H_b), 4.58 (s, 1H, 29-H_a), 4.46 (*dd*, *J* = 10.3, 5.8 Hz, 1H, 3-H), 4.25 (dd, *J* = 3.3 Hz, 2H, 36-H), 3.67 (t, *J* = 4.3 Hz, 2H, 35-H), 3.55 (t, *J* = 5.1 Hz, 2H, 34-H), 3.42 (*qq*, *J* = 13.8, 5.2 Hz, 2H, 33-H), 3.06 (*dt*, *J* = 10.9, 4.3 Hz, 1H, 19-H), 2.97 (t, *J* = 6.0 Hz, 2H, 38-H), 2.81 (t, *J* = 6.4 Hz, 2H, 39-H), 2.40 (*dt*, *J* = 12.6, 3.2 Hz, 1H, 13-H), 2.03 (s, 3H, 32), 1.99 (*dt*, *J* = 12.3, 2.5 Hz, 1H, 21-H_b), 1.94–1.88 (m, 1H, 16-H_a), 1.80–1.73 (m, 1H, 1-H_b), 1.71–1.62 (m, 2H, 12-H_b, 22-H_b), 1.67 (s, 3H, 30-H), 1.63–1.52 (m, 4H, *2-H,* 18, 21-H_a), 1.52–1.28 (m, 8H, *6-*H, *7-*H,

11-H_a, 15-H_b, 16-H_b, 22-H_a), 1.28–1.23 (m, 1H, 9-H), 1.21 (*dt*, $J = 12.4$, 3.9 Hz, 1H, 11-H_b), 1.13 (d, $J = 13.2$ Hz, 1H, 15-H_a), 1.04–0.96 (m, 2H, 1-H_a, 12-H_a), 0.95 (s, 3H, 27-H), 0.91 (s, 3H, 26-H), 0.84–0.81 (m, 6H, 23, 24-H), 0.81 (s, 3H, 25-H), 0.77 (d, $J = 9.7$ Hz, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 177.1$ (C-28), 172.7 (C-31), 171.2, 170.9 (C-40), 164.6 (C-42), 162.7 (C-41), 150.9 (C-20), 109.7 (C-29), 81.1 (C-3), 70.2 (C-34), 68.9 (C-35), 64.3 (C-36), 55.9 (C-17), 55.6 (C-5), 50.6 (C-9), 50.2 (C-18), 47.0 (C-19), 42.6 (C-8), 40.9 (C-14), 39.2 (C-33), 38.5 (C-1, C-22), 38.0 (C-13), 37.9 (C-4), 37.3 (C-10), 34.5 (C-7), 33.8 (C-21), 31.0 (C-38), 30.9 (C-16), 29.5 (C-15), 28.9 (C-39), 28.1 (C-23), 25.7 (C-12), 23.8 (C-2), 21.5 (C-32), 21.1 (C-11), 19.5 (C-30), 18.3 (C-6), 16.6 (C-25), 16.3 (C-26), 16.3 (C-24), 14.8 (C-27)) ppm; ESI-MS (MeOH/$CHCl_3$, 4:1): m/z = 846.9 ([M − H]$^-$); analysis calcd for $C_{42}H_{65}N_5O_9S_2$ (848.12): C 59.48, H 7.73, N 8.26; found: C 59.26, H 8.01, N 7.96.

4.2.8. (3β)-28-[(2-{2-[(Aminosulfonyl)oxy]ethoxy}ethyl)amino]-28-oxolup-20(29)-en-3-yl acetate (9)

From the reaction of **5** (120 mg, 0.2 mmol) with sulfamoyl chloride (2 equ.) in dry DMA (2 mL) at 21 °C for 1 day followed by usual aqueous work-up and column chromatography (SiO_2, $CHCl_3$/MeOH, 95:5) **9** (113 mg, 83%) was obtained as a colourless solid; R_f = 0.23 (SiO_2, $CHCl_3$/MeOH, 95:5); m.p. 114–117 °C; IR (ATR): ν = 3330*br*, 3259*br*, 3072*w*, 2942 *m*, 2869*w*, 1733*w*, 1713 *m*, 1638 *m*, 1518 *m*, 1465*w*, 1451 *m*, 1368*s*, 1316*w*, 1246*s*, 1181*s*, 1130 *m*, 1025*s*, 979 *m*, 923*s*, 885*w*, 775 *m*, 656*w*, 598*w*, 551*s*, 513*w*, 488*w*, 466*w* cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): $\delta = 7.58$ (s, 1H, NH), 7.45 (s, 2H, NH_2), 4.68–4.64 (m, 1H, 29-H_a), 4.55–4.51 (m, 1H, 29-H_b), 4.39–4.33 (m, 1H, 3-H), 4.10 (s, 2H, 34-H), 3.62 (s, 2H, 35-H), 3.41 (s, 2H, 36-H), 3.29–3.09 (m, 2H, 33-H), 3.04–2.98 (m, 1H, 19-H), 2.58–2.52 (m, 1H, 13-H), 2.12 (d, $J = 11.5$ Hz, 1H, 16-H_a), 1.99 (s, 3H, 32-H), 1.79–1.65 (m, 2H, 21-H_a, 22-H_a), 1.65–1.55 (m, 6H, 2-H_a, 12-H_a, 1-H_a, 30-H), 1.53–1.20 (m, 13H, 2-H_b, 18-H, 6-H, 16-H_b, 11-H_a, 22-H_b, 15-H_a, 7-H, 9-H, 21-H_b, 11-H_b), 1.19–0.99 (m, 3H; 15-H_b, 1-H_b, 12-H_b), 0.92 (s, 3H, 27-H), 0.85 (s, 3H, 24-H), 0.80 (s, 3H, 26-H), 0.79 (s, 7H, 5-H, 23-H, 25-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 175.7 (C-28), 170.1 (C-31), 150.9 (C-20), 109.2 (C-29), 79.9 (C-3), 69.1 (C-36), 68.0 (C-34), 68.0 (C-35), 54.9 (C-17), 54.7 (C-5), 49.8 (C-9), 49.6 (C-18), 46.2 (C-19), 41.9 (C-14), 40.3 (C-8), 38.1 (C-33), 37.8 (C-1), 37.6 (C-22), 37.3 (C-4), 36.7 (C-13), 36.6 (C-10), 33.8 (C-7), 32.3 (C-16), 30.3 (C-21), 28.8 (C-15), 27.7 (C-23), 25.2 (C-12), 23.4 (C-2), 20.9 (C-32), 20.6 (C-11), 19.0 (C-30), 17.7 (C-6), 16.4 (C-25), 15.9 (C-26), 15.8 (C-24), 14.3 (C-27) ppm; ESI-MS (MeOH): m/z = 665.3 ([M+H]$^+$), 687.9 ([M+Na]$^+$; analysis calcd for $C_{36}H_{60}N_2O_7S$ (664.94) C 65.03, H 9.10, N 4.21; found: C 64.79, H 4.39, N 3.97.

4.2.9. 5-Amino-1,3,4-thiadiazole-2-sulfonamide (10)

A solution of acetazolamide (10.0 g, 45.2 mmol) was heated under reflux in conc. HCl (60 mL) for 3 h [46]. After neutralization with aq. NaOH, saturation with NaCl and extraction with THF removal of the organic solvent gave **10** as a white solid (7.8 g, 96%); m.p. 197 °C decomp. (lit. [46]: 195 °C); R_f = 0.32 (SiO_2, $CHCl_3$/MeOH, 9:1); UV–Vis (MeOH): λ_{max} (log ε) = 278 nm (3.80) IR (ATR): ν = 3428*w*, 3321 *m*, 2639*w*, 1601*s*, 1490*s*, 1448 *m*, 1338*s*, 1172 *m*, 1139 *m*, 939 *m*, 647*s*, 581 s cm^{-1}; 1H NMR (500 MHz, DMSO-d_6): $\delta = 8.04$ (s, 2H, NH_2), 7.84 (s, 2H, NH_2) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 171.7, 157.9 ppm; ESI-MS (MeOH): m/z = 179.0 ([M − H]$^-$).

4.2.10. 3,28-Di-O-acetyl-betulin (12)

Compound **12** was prepared from betulin (**11**,10.0 g, 32 mmol) by acetylation with acetic anhydride as previously [47] described, and recrystallization from ethanol gave **12** (11.8 g, 93%) as a white solid; R_f = 0.70 (SiO_2, hexanes/ethyl acetate, 8:2); m.p. 220–222 °C (lit. [47]: 216–218 °C); $[\alpha]_D^{20}$ = +17.5° (*c* = 0.032, $CHCl_3$), [lit. [47]: $[\alpha]_D^{20}$ = +19.7° ($CHCl_3$)]; ESI-MS (MeOH): m/z = 467.5 ([M + H-HOAc]$^+$).

4.2.11. 3-O-acetyl-betulin (13a)

Compound **13a** was prepared from **12** by selective deacetylation with cat. amounts of CaH_2 as previously [47] described; R_f = 0.40 (SiO_2, hexanes/ethyl acetate, 8:2); m.p. 258 °C (lit. [47]: 258–260 °C); $[\alpha]_D^{20}$ = +26.2° (*c* = 0.013, $CHCl_3$), [lit. [47]: $[\alpha]_D^{20}$ = +25.7° ($CHCl_3$)]; ESI-MS (MeOH): m/z = 992.0 ([2 M + Na]$^+$).

4.2.12. 28-O-acetyl-betulin (13b)

Compound **13b** was prepared from **11** as previously [43,44] reported; R_f = 0.25 (SiO_2, hexanes/ethyl acetate, 9:1); m.p. 206–208 °C (lit. [43]: 205–208 °C); $[\alpha]_D^{20}$ = +8.8° (*c* = 1.1, $CHCl_3$), [lit.: [44]$[\alpha]_D^{20}$ = +8.5° (*c* = 1.58, $CHCl_3$)]; ESI-MS (MeOH): m/z = 992.3 ([2 M + Na]$^+$).

4.2.13. (3β)-28-[(Aminosulfonyl)oxy]lup-20(29)-en-3-yl acetate (14)

Method A: To a solution of **13a** (200 mg, 0.41 mmol) in dry DMA (2.0 mL) at 0 °C, sulfamoyl chloride (2 eq.) was added, and the reaction mixture was stirred at 20 °C for 1 day. Usual aqueous work up followed by column chromatography (SiO_2, $CHCl_3$/MeOH, 95:5) gave **14** (220 mg, 95%) as a colourless solid; m.p. 130–134 °C; $[\alpha]_D^{20}$ = +10.4° (*c* = 0.163, MeOH); R_f = 0.35 (SiO_2, hexanes/ethyl acetate, 8:2); IR (ATR): ν = 2944 *m*, 2872*w*, 1711 *m*, 1456*w*, 1367*s*, 1265*s*, 1255*s*, 1181*s*, 1028*w*, 972*s*, 917 *m*, 902 *m*, 574*s*, 606*w*, 549 *m*, 500*w* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.83$ (s, 2H, NH_2), 4.71–4.66 (m, 1H, 29-H_a), 4.63–4.57 (m, 1H, 29-H_b), 4.46 (*dd*, $J = 10.4$, 5.6 Hz, 1H, 3-H), 4.39 (d, $J = 8.2$ Hz, 1H, 28-H_a), 3.94 (d, $J = 9.4$ Hz, 1H, 28-H_b), 2.39 (*dt*, $J = 10.7$, 5.9 Hz, 1H, 19-H), 2.04 (s, 3H, 32), 2.01–1.85 (m, 3H, 16-H_a, 21-H_a, 22-H_a), 1.74 (*dt*, $J = 13.7$, 4.2 Hz, 1H, 15-H_b), 1.68 (s, 3H, 30-H), 1.67–1.58 (m, 5H, 1-H_b, *2-H*, 12-H_b, 13-H, 18-H), 1.55–1.35 (m, 6H, 6-H_a, *7-H*, 11-H_a, 21-H_b), 1.33–1.24 (m, 3H, 9-H, 16-H_b), 1.20 (*dd*, $J = 12.6$, 4.3 Hz, 1H, 11-H_b), 1.06 (s, 4H, 1-H_a, 12-H_a, 15-H_a, 22-H_b), 1.03 (s, 3H, 24-H), 0.97 (s, 3H, 27-H), 0.85–0.84 (m, 6H, 23, 25-H), 0.83 (s, 3H, 26-H), 0.79 (d, J = 9.5 Hz, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 171.3 (C-31), 149.8 (C-20), 110.3 (C-29), 81.1 (C-3), 70.5 (C-28), 55.5 (C-5), 50.4 (C-9), 48.9 (C-18), 47.9 (C-19), 46.8 (C-17), 42.8 (C-14), 41.0 (C-8), 38.5 (C-1), 37.9 (C-13), 37.2 (C-4, C-10), 34.3, 34.2 (C-7, C-22), 29.5 (C-16), 29.3 (C-21), 28.1 (C-23), 27.0 (C-15), 25.2 (C-12), 23.8 (C-2), 21.5 (C-32), 20.9 (C-11), 19.2 (C-30), 18.3 (C-6), 16.6 (C-26), 16.3 (C-25), 16.1 (C-24), 14.9 (C-27) ppm; ESI-MS (MeOH/$CHCl_3$, 4:1): m/z = 562.3 ([M − H]$^-$); analysis calcd for $C_{32}H_{53}NO_5S$ (563.83): C 68.17, H 9.48, N 2.48; found: C 67.83, H 9.63, 2.03.

Method B: To a solution of **13a** (200 mg, 0.41 mmol) in dry DCM (20 mL), sodium hydride (4 eq.) and sulfamoyl chloride (2 eq.) were added at 0 °C, and the reaction mixture was stirred at 20 °C for 1 day. Usual aqueous work up followed by column chromatography (SiO_2, $CHCl_3$/MeOH, 95:5) gave **14** (90 mg, 40%) as a colourless solid; data as above.

4.2.14. (3β)-3-[(Aminosulfonyl)oxy]lup-20(29)-en-28-yl acetate (15)

Compound **13b** (200 mg, 0.41 mmol) was dissolved in dry DCM (20 mL), and at 0 °C, sodium hydride (4 eq.) and sulfamoyl chloride (2 eq.) were added; the reaction mixture was stirred at 20 °C for 1 day. Usual aqueous work up followed by column chromatography (SiO_2, $CHCl_3$/MeOH, 95:5) gave **15** (100 mg, 44%) as a colourless solid; m.p. 110–112 °C; $[\alpha]_D^{20}$ = +10.5° (*c* = 0.111, MeOH); R_f = 0.3 (SiO_2, hexanes/ethyl acetate, 8:2); IR (ATR): ν = 2944 *m*, 2871*w*, 1738 *m*, 1717 *m*, 1455*w*, 1364*s*, 1236*s*, 1180*s*, 1033 *m*, 933*s*, 909*vs*, 883*s*, 838 *m*, 753 *m*, 586*w*, 548 *m*, 513*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 4.81$ (s, 2H, NH_2), 4.68 (s, 1H, 29-H_a), 4.59 (s, 1H, 29-H_b), 4.24 (d, $J = 11.2$ Hz, 1H, 28-H_a), 4.21 (*dd*, $J = 12.1$, 4.7 Hz, 1H, 3-H), 3.84 (d, $J = 11.0$ Hz, 1H, 28-H_b), 2.44 (*dt*, $J = 11.1$, 5.7 Hz, 1H, 19-H), 2.06 (s, 3H, 32), 2.03 (*dd*, J = 13.8, 3.9 Hz, 1H, 2-H_b), 1.99–1.93 (m, 1H, 21-H_b), 1.88–1.81 (m, 2H, 2-H_a, 16-H_b), 1.80–1.76 (m, 1H, 22-H_a), 1.75–1.72 (m, 1H, 1-H_a), 1.73–1.62 (m, 3H, 12-H_b, 13, 15-H_b), 1.68 (s, 4H, 30-H), 1.60 (d, J = 11.7 Hz, 1H, 18-H), 1.55–1.49 (m, 1H, 6-H_a), 1.46–1.35 (m, 5H, 6-H_b, *7-H*, 11-H_a, 21-H_a), 1.29–1.17 (m, 3H, 9-H, 11-H_b, 16-H_a), 1.11–1.04 (m,

2H, 12-H_a, 15-H_a, 22-H_b), 1.03 (s, 3H, 26-H), 1.01 (s, 3H, 23-H), 1.00–0.93 (m, 1H, 1-H_b), 0.96 (s, 3H, 27-H), 0.85 (s, 3H, 24-H), 0.84 (s, 3H, 25-H), 0.77 (d, J = 9.0 Hz, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 171.8 (C-31), 150.2 (C-20), 110.0 (C-29), 92.0 (C-3), 62.9 (C-28), 55.8 (C-5), 50.4 (C-9), 48.9 (C-18), 47.8 (C-19), 46.4 (C-17), 42.8 (C-8), 41.0 (C-14), 38.9 (C-1), 38.7 (C-4), 37.7 (C-13), 37.1 (C-10), 34.7 (C-7), 34.2 (C-22), 29.9 (C-16), 29.7 (C-21), 28.2 (C-23), 27.2 (C-12), 25.3 (C-15), 24.6 (C-2), 21.2 (C-32), 21.0 (C-11), 19.3 (C-30), 18.4 (C-6), 16.3 (C-25), 16.2 (C-26), 16.2 (C-24), 14.9 (C-27) ppm; ESI-MS (MeOH/$CHCl_3$, 4:1): m/z = 562.2 ([M − H]$^-$); analysis calcd for $C_{32}H_{53}NO_5S$ (563.83): C 68.17, H 9.48, N 2.48; found: C 67.93, H 9.61, N 2.25.

4.2.15. 4-{[(3β)-3-(Acetyloxy)lup-20(29)-en-28-yl]oxy}-4-oxobutanoic acid (16)

Compound **16** was prepared from **13** and succinic anhydride as previously [29] described; m.p. 123–124 °C [lit. [29]: 122–125 °C]; $[\alpha]_D^{20}$ = +8.4° (c = 0.023, MeOH) [lit. [29]: $[\alpha]_D^{20}$ = +12.1° (c = 0.198, MeOH)]; ESI-MS (MeOH): m/z = 583.6 ([M − H]$^-$).

4.2.16. (3β)-3-(Acetyloxy)lup-20(29)-en-28-yl-4-2-(aminosulfonyl) ethylamino-4-oxo butanoate (17)

To a solution of **16** (1.00 g, 1.7 mmol) in dry THF (50 mL), 4-methylmorpholine (2 eq.) and ethyl chloroformate (2 eq.) were added. The reaction mixture was stirred at 20 °C for 15 min. Taurinamide (1.2 eq.) was added, and the mixture was heated under reflux for 24 h. The solvent was removed, the residue dissolved in $CHCl_3$, washed with aq. NaOH (2 M), water and brine and dried ($MgSO_4$). Chromatography (hexanes/ethyl acetate, 3:7) gave **17** (950 mg, 80%) as a white solid; m.p. 123–126 °C; $[\alpha]_D^{20}$ = +6.7° (c = 0.096, MeOH); R_f = 0.28 (SiO_2, hexanes/ethyl acetate, 3:7); IR (ATR): ν = 3355*w*, 2943 *m*, 2872*w*, 1731*s*, 1660 *m*, 1544*w*, 1452*w*, 1391*w*, 1367 *m*, 1333 *m*, 1244*s*, 1191*w*, 1146*s*, 1107*w*, 1027 *m*, 979 *m*, 900*w*, 883 *m*, 609*w*, 547*w*, 498 m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.69 (s, 1H, NH), 5.79–4.90 (m, 2H, NH_2), 4.68 (s, 1H, 29-H_a), 4.58 (s, 1H, 29-H_b), 4.46 (*dd*, J = 10.4, 5.8 Hz, 1H, 3-H), 4.26 (d, J = 10.8 Hz, 1H, 28-H_a), 3.86 (d, J = 10.7 Hz, 1H, 28-H_b), 3.83–3.73 (m, 2H, 37-H), 3.42–3.24 (m, 2H, 38-H), 2.79–2.64 (m, 2H, 35-H), 2.59–2.46 (m, 2H, 34-H), 2.46–2.34 (m, 1H, 19-H), 2.03 (s, 3H, 32-H), 1.98–1.88 (m, 1H, 16-Ha), 1.85–1.78 (m, 1H, 16-Hb), 1.77–1.72 (m, 1H, 22-H_a), 1.67 (s, 3H, 30-H), 1.70–1.54 (m, 7H, 1-H_a, 2-H, 12-H_a, 13-H, 15-H_a, 18-H), 1.53–1.45 (m, 1H, 6-H_a), 1.44–1.34 (m, 5H, 6-H_b, 7-H, 11-H_a, 21-H_a), 1.32–1.13 (m, 3H, 9-H, 11-H_b, 21-H_b), 1.10–0.98 (m, 4H, 1-H_b, 12-H_b, 15-H_b, 22-H_b), 1.02 (s, 3H, 26-H), 0.96 (s, 3H, 27-H), 0.85–0.83 (m, 6H, 23-H, 24-H), 0.83 (s, 3H, 25-H), 0.78 (d, J = 9.3 Hz, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 173.8 (C-33), 172.8 (C-31), 171.2 (C-36), 150.1 (C-20), 110.1 (C-29), 81.1 (C-3), 63.5 (C-28), 55.5 (C-5), 54.5 (C-38), 50.4 (C-9), 48.9 (C-18), 47.8 (C-19), 46.6 (C-17), 42.8 (C-14), 41.0 (C-8), 38.5 (C-1), 37.9 (C-4), 37.7 (C-13), 37.2 (C-10), 35.1 (C-22), 34.7 (C-7), 34.3 (C-37), 31.1 (C-34), 29.9 (C-35), 29.7 (C-16), 29.6 (C-21), 28.1 (C-24), 27.2 (C-15), 25.3 (C-12), 23.8 (C-2), 21.4 (C-32), 20.9 (C-11), 19.2 (C-30), 18.3 (C-6), 16.6 (C-25), 16.3 (C-26), 16.2 (C-23), 14.9 (C-27) ppm; ESI-MS (MeOH): m/z = 714.0 ([M+Na]$^+$); analysis calcd for $C_{38}H_{62}N_2O_7S$ (690.97): C 66.05, H 9.04, N 4.05; found: C 65.87, H 9.32, N 3.86.

4.2.17. (3β)-3-(Acetyloxy)lup-20(29)-en-28-yl 4-{[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]amino}-4-oxobutanoate (18)

Compound **18** was prepared as described for **17** starting from **10**; yield: 620 mg (85%); m.p. 162–164 °C [lit. [29]: 161–164 °C]; R_f = 0.55 (SiO_2, hexanes/ethyl acetate, 7:3); ESI-MS (MeOH): m/z = 745.7 ([M − H]$^-$).

4.2.18. (3β, 20β) 3-Acetyloxy-29-hydroxyolean-12-en-11-one (22)

Compound **22** (2.26 g, 85%) was prepared as previously [29] reported from **19** (2.6 g, 5.52 mmol) and obtained as a colourless solid; m.p. 265–267 °C (lit. [29]: 264–266 °C); R_f = 0.45 (silica gel, $CHCl_3$/E-t_2O/hexanes/HCOOH, 25:25:43:7); $[\alpha]_D^{20}$ = +87.9° (c = 0.06, $CHCl_3$), [lit. [29]: $[\alpha]_D^{20}$ = +91.6° (c = 0.129, $CHCl_3$)]; ESI-MS (MeOH/$CHCl_3$, 4:1): m/z = 497.9 ([M − H]$^-$).

4.2.19. (3β)-28-Hydroxyursan-12-en-3-yl acetate (23)

Compound **23** (3.58 g, 65%) was prepared as previously [48] reported from **20** (6.0 g, 11.4 mmol); m.p. 260–263 °C (lit. [48]: 258–261 °C); R_f = 0.50 (silica gel, hexanes/ethyl acetate, 8:2); $[\alpha]_D^{20}$ = +67.8° (c = 0.11, $CHCl_3$) [lit. [49]: $[\alpha]_D^{20}$ = +70.5° (c = 0.145, $CHCl_3$)]; ESI-MS (MeOH): m/z = 485.4 ([M + H]$^+$).

4.2.20. (3β)-28-Hydroxyolean-12-en-3-yl acetate (24)

Compound **24** (3.75 g, 68%) was prepared as previously [50,51] reported from **21** (6.0 g, 11.4 mmol); m.p. 236–238 °C (lit. [50]: 233–234 °C); R_f = 0.55 (silica gel, hexanes/ethyl acetate, 8:2); $[\alpha]_D^{20}$ = +65.1° (c = 0.12, $CHCl_3$) [lit. [51]: $[\alpha]_D^{20}$ = +71° (c = 0.70, $CHCl_3$)]; ESI-MS (MeOH): m/z = 485.3 ([M + H]$^+$).

4.2.21. 4-{[(3β,20β)3-(Acetyloxy)-11-oxoolean-12-en-30-yl]oxy}-4-oxobutanoic acid (25)

Compound **25** (630 mg g, 96%) was prepared as previously [29] reported from **22** (450 mg, 0.55 mmol); m.p. 110–113 °C (lit. [29]: m.p. 109–111 °C); R_f = 0.45 (silica gel, hexanes/ethyl acetate, 1:1); $[\alpha]_D^{20}$ = +109.2° (c = 0.07, $CHCl_3$) (lit. [29]: $[\alpha]_D^{20}$ = +110.5° (c = 0.118, $CHCl_3$)); MS (ESI, MeOH/$CHCl_3$, 4:1) m/z = 600.0 ([M + H]$^+$).

4.2.22. 4-{[(3β)-3-(Acetyloxy)urs-12-en-28-yl]oxy}-4-oxobutanoic acid (26)

Compound **26** (0.84 g g, 78%) was prepared as previously [29] reported from **23** (0.9 g, 1.8 mmol); m.p. 110–113 °C (lit. [29]: m.p. 112–114 °C); R_f = 0.50 (silica gel, hexanes/ethyl acetate, 7:3); $[\alpha]_D^{20}$ = +45.7° (c = 0.11, $CHCl_3$) [lit. [29]: $[\alpha]_D^{20}$ = +42.7° (c = 0.131, $CHCl_3$)]; MS (ESI, MeOH/$CHCl_3$, 4:1): m/z = 583.9 ([M − H]$^-$).

4.2.23. 4-{[(3β)-3-(Acetyloxy)-olean-12-en-28-yl]oxy}-4-oxobutanoic acid (27)

Compound **27** (0.9 g, 84%) was prepared as previously [29] reported from **24** (0.9 g, 1.8 mmol); m.p. 124–127 °C (lit. [29]: 124–126 °C); R_f = 0.45 (silica gel, hexanes/ethyl acetate, 7:3); $[\alpha]_D^{20}$ = +45.2° (c = 0.04, $CHCl_3$) [lit. [29]: $[\alpha]_D^{20}$ = +49.6° (c = 0.126, $CHCl_3$)]; MS (ESI, MeOH/$CHCl_3$, 4:1): m/z = 583.9 ([M − H]$^-$).

4.2.24. (3β) 3-(Acetyloxy)-11-oxoolean-12-en-28-yl-4-(2-sulfamoylethyl)-4-oxobutanoate (28)

To a solution of **25** (30 mg, 0.05 mmol) in dry THF (5 mL), 4-methylmorpholine (2 eq.) and ethyl chloroformate (2 eq.) were added. The reaction mixture was stirred at 20 °C for 15 min. Taurinamide (1,2 eq.) was added, and the mixture was heated under reflux for 24 h. The solvent was removed, the residue dissolved in $CHCl_3$, washed with aq. NaOH (2 M), water and brine and dried ($MgSO_4$). Chromatography (hexanes/ethyl acetate, 3:7) gave **28** (24 mg, 68%) as a white solid; m.p. 108–110 °C; $[\alpha]_D^{20}$ = +47.1° (c 0.199, MeOH); R_f = 0.29 (SiO_2, hexanes/ethyl acetate, 3:7); IR (ATR): ν = 3346*w*, 2929 *m*, 2874*w*, 1728*s*, 1657*s*, 1544*w*, 1456*w*, 1388*w*, 1366*w*, 1332 *m*, 1244*s*, 1207*w*, 1191*w*, 1144*s*, 1092*w*, 1028 *m*, 985 *m*, 902*w*, 752*w*, 562*w*, 496*w* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.92–6.57 (m, 1H, NH), 5.60 (s, 1H, 12-H), 5.88–4.98 (m, 2H, NH_2), 4.50 (*dt*, J = 11.4, 5.5 Hz, 1H, 3-H), 4.34–4.21 (m, 1H, 30-H_a), 3.91–3.70 (*m*, 3 zH, 30-H_b, 37-H), 3.43–3.21 (m, 2H, 38-H), 2.81 (d, J = 16.5 Hz, 1H, 19-H_a), 2.71 (s, 2H, 35-H), 2.63–2.48 (m, 2H, 34-H), 2.48–2.40 (m, 1H, 1-H_a), 2.40–2.34 (m, 1H, 9-H), 2.24–2.12 (m, 1H, 18-H), 2.04 (s, 3H, 32-H), 1.92–1.00 (m, 16H, 15-H_a, 16-H_a, 15-H_b, 2-H, 6-H_a, 7-H_a, 6-H_b, 22-H_a, 7-H_b, 22-H_b, 16-H_b, 21-H, 1-H_b, 19-H_b), 1.36 (s,

3H, 27-H), 1.17 (s, 3H, 25-H), 1.14 (s, 3H, 26-H), 1.12 (s, 3H, 24-H), 1.02 (s, 3H, 29-H), 0.87 (m, 6H, 23-H, 28-H), 0.82–0.79 (m, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 209.86 (C-11), 173.30 (C-33), 173.22 (C-36), 172.51 (C-31), 170.99 (C-13), 128.03 (C-12), 80.53 (C-3), 74.47 (C-30), 63.46 (C-9), 54.99 (C-5), 54.36 (C-38), 46.74 (C-18), 44.27 (C-8), 43.41 (C-14), 38.84 (C-19), 38.21 (C-1), 37.84 (C-4), 36.99 (C-10), 36.74 (C-22), 35.91 (C-20), 34.44 (C-37), 32.66 (C-7), 32.22 (C-17), 31.02 (C-34), 30.72 (C-35), 29.40 (C-21), 28.53 (C-28), 28.02 (C-23), 26.40 (C-15), 25.77 (C-16), 24.01 (C-29), 23.54 (C-2), 23.31 (C-27), 21.29 (C-32), 19.47 (C-6), 18.78 (C-26), 18.74 (C-24), 16.66 (C-25) ppm; ESI-MS (MeOH): *m/z* = 727.3 ([M+Na]$^+$), 705.2 ([M+H]$^+$); analysis calcd for $C_{38}H_{60}N_2O_8S$ (704.96): C 64.74, H 8.58, N 3.97; found: C 64.45, H 8.76, N 3.55.

4.2.25. (3β) 3-(Acetyloxy)urs-12-en-28-yl-4-(2-sulfamoylethyl)-4-oxobutanoate (29)

Reaction of **26** (100 mg, 0,17 mmol) in dry THF (10 mL) with 4-methylmorpholine (2 eq.), ethyl chloroformate (2 eq.) and taurinamide (1.2 eq.) under reflux for 24 h followed by chromatography (hexanes/ethyl acetate, 3:7) gave **29** (95 mg, 81%) as a white solid; m.p. 117–120 °C; $[\alpha]_D^{20}$ = +27.6° (*c* = 0.094, MeOH); R_f = 0.34 (SiO_2, hexanes/ethyl acetate, 3:7); IR (ATR): ν = 3347*w*, 2947 *m*, 2925 *m*, 2670*w*, 1732 *m*, 1658 *m*, 1544*w*, 1456*w*, 1389*w*, 1369*w*, 1332 *m*, 1244*s*, 1176*w*, 1145*s*, 1095*w*, 1025 *m*, 1005 *m*, 985*w*, 902*w*, 753*w*, 664*w*, 607*w*, 496*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.73–6.50 (m, 1H, NH), 5.56–4.84 (m, 2H, NH_2), 5.17–5.11 (m, 1H, 12-H), 4.56–4.44 (m, 1H, 3-H), 4.07 (d, *J* = 10.8 Hz, 1H, 28-H_a), 3.82–3.69 (m, 2H, 37-H), 3.62 (d, *J* = 10.8 Hz, 1H, 28-H_b), 3.39–3.26 (m, 2H, 38-H), 2.76–2.60 (m, 2H, 35-H), 2.57–2.39 (m, 2H, 34-H), 2.04 (s, 3H, 32-H), 1.99–1.85 (m, 3H, 11-H, 16-H_a), 1.77–1.69 (m, 1H, 15-H_a), 1.69–1.59 (m, 3H, 1-H_a, 2-H), 1.59–1.50 (m, 4H, 6-H_a, 7-H_a, 9-H, 22-H_a), 1.49–1.37 (m, 4H, 6-H_b, 18-H, 19-H, 21-Ha), 1.37–1.27 (m, 3H, 7-H_b, 22-H_b), 1.25–1.16 (m, 2H, 16-H_b, 21-H_b), 1.09 (s, 3H, 27-H), 1.12–1.04 (m, 2H, 1-H_b, 15-H_b), 0.97 (s, 6H, 2-H 5, 30-H), 0.93 (s, 3H, 29-H), 0.87 (s, 3H, 23-H), 0.86 (s, 3H, 24-H), 0.85–0.83 (m, 1H, 5-H), 0.81 (s, 3H, 26-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 173.6 (C-33), 172.8 (C-36), 171.2 (C-31), 138.3 (C-13), 125.7 (C-12), 81.1 (C-3), 71.9 (C-28), 55.4 (C-5), 54.5 (C-38), 54.4 (C-18), 47.7 (C-9), 42.1 (C-14), 40.1 (C-8), 39.5 (C-20), 39.3 (C-19), 38.6 (C-1), 37.8 (C-4), 37.1 (C-10), 36.9 (C-17), 35.8 (C-22), 35.1 (C-37), 32.8 (C-7), 31.2 (C-34), 30.6 (C-21), 29.5 (C-35), 28.2 (C-23), 26.2 (C-15), 23.7 (C-2), 23.5 (C-16), 23.5 (C-11), 23.5 (C-27), 21.4 (C-29), 21.4 (C-32), 18.3 (C-6), 17.4 (C-26), 16.9 (C-24, C-30), 15.9 (C-25) ppm; ESI-MS (MeOH): *m/z* = 714.2 (100%, [M+Na]$^+$); analysis calcd for $C_{38}H_{62}N_2O_7S$ (690.98): C 66.05, H 9.04, N 4.05; found: C 65.83, H 9.29, N 3.76.

4.2.26. (3β) 3-(Acetyloxy)olean-12-en-28-yl-4-(2-sulfamoylethyl)-4-oxobutanoate (30)

Reaction of **27** (35 mg, 0,06 mmol) in dry THF (5 mL) with 4-methylmorpholine (2 eq.), ethyl chloroformate (2 eq.) and taurinamide (1.2 eq.) under reflux for 24 h as described above followed by chromatography (hexanes/ethyl acetate, 3:7) gave **30** (29 mg, 70%) as a white solid; m.p. 116–118 °C; $[\alpha]_D^{20}$ = +48.4° (c 0.124, MeOH); R_f = 0.35 (SiO_2, hexanes/ethyl acetate, 3:7); IR (ATR): ν = 3355*w*, 2946 *m*, 2926 *m*, 2664*w*, 1732 *m*, 1660*w*, 1544*w*, 1463*w*, 1386*w*, 1364*w*, 1332 *m*, 1245*s*, 1199*w*, 1145*s*, 1095*w*, 1027 *m*, 1004 *m*, 986 *m*, 967*w*, 904*w*, 754*w*, 731*w*, 660*w*, 495*w* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.58 (s, 1H, NH), 5.23–5.17 (m, 1H, 12-H), 5.71–4.74 (m, 2H, NH_2), 4.53–4.43 (m, 1H, 3-H), 4.04 (d, *J* = 10.8 Hz, 1H, 28-H_a), 3.88–3.74 (m, 2H, 37-H), 3.69 (d, *J* = 10.8 Hz, 1H, 28-H_b), 3.43–3.22 (m, 2H, 38-H), 2.80–2.63 (m, 2H, 35-H), 2.57–2.38 (m, 2H, 34-H), 2.04 (s, 3H, 32-H), 2.02–1.98 (m, 1H, 18-H), 1.97–1.81 (m, 3H, 11-H, 16-H_a), 1.78–1.67 (m, 1H, 19-H_a), 1.66–1.58 (m, 4H, 1-H_a, 2-H, 15-H_a), 1.57–1.40 (m, 4H, 6-H_a, 7-H_a, 9-H, 22-H_a), 1.39–1.22 (m, 4H, 6-H_b, 7-H_b, 22-H_b, 21-H_a), 1.20–1.11 (m, 2H, 16-H_b, 21-H_b), 1.15 (s, 3H, 27-H), 1.11–0.98 (m, 3H, 1-H_b, 15-H_b, 19-H_b), 0.95 (s, 3H, 25-H), 0.93 (s, 3H, 26-H), 0.89 (s, 3H, 29-H), 0.87 (s, 6H, 23-H, 30-H), 0.86 (s, 3H, 24-H), 0.84–0.79 (m, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 173.6 (C-33), 172.8 (C-31), 171.2 (C-36), 143.7 (C-13), 123.0 (C-12), 81.1 (C-3), 71.4 (C-28), 55.4 (C-5), 54.5 (C-38), 47.6 (C-9), 46.3 (C-19), 42.7 (C-18), 41.8 (C-14), 39.9 (C-8), 38.4 (C-1), 37.9 (C-4), 37.0 (C-10), 36.0 (C-17), 35.1 (C-37), 34.1 (C-21), 33.3 (C-29), 32.6 (C-7), 31.6 (C-22), 31.2 (C-20), 31.0 (C-34), 29.5 (C-35), 28.2 (C-23), 26.1 (C-27), 25.7 (C-15), 23.7 (C-30), 23.7 (C-2, C-11), 22.3 (C-16), 21.4 (C-32), 18.4 (C-6), 16.9 (C-26), 16.8 (C-24), 15.7 (C-25) ppm; ESI-MS (ESI, MeOH): *m/z* = 714.2 ([M+Na]$^+$); analysis calcd for $C_{38}H_{62}N_2O_7S$ (690.98): C 66.05, H 9.04, N 4.05; found: C 65.71, H 9.33, N 3.72.

4.3. CA II assay

Carbonic anhydrase II (bCAII, ≥3000 W-A units/mg from bovine erythrocytes) as well as 4-nitrophenyl acetate (4-NA) were purchased from Sigma. BMG Labtech Spectrostar Omega working in the slow kinetics mode and measuring the absorbance at λ = 415 nm applying center scanning for the enzymatic studies. In short: A mixture of 4-NA solution (125 μL, 6 mM in 50 mM Tris-HCl buffer, pH 8), enzyme solution (25 μL, 0.3 mg/mL) and compounds solutions (25 μL, 3 different concentrations and water as a blank) was incubated at 37 °C for 20 min. The substrate (25 μL, [4-NA] = 0.75 mM, 0.50 mM, 0.25 mM, 0.15 mM) was added to start the enzymatic reaction. The absorbance data was recorded under a controlled temperature of 37 °C for 30 min at 1 min intervals at λ = 415 nm. The relative inhibition was determined as the quotient of the slopes (compound divided by blank) of the linear ranges.

CRediT authorship contribution statement

Toni-Christopher Denner: Writing – review & editing, Writing – original draft, Investigation. **Niels V. Heise:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Julian Zacharias:** Writing – review & editing, Writing – original draft, Investigation. **René Csuk:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmcr.2024.100139.

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An asiatic acid derived trisulfamate acts as a nanomolar inhibitor of human carbonic anhydrase VA

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ABSTRACT

This investigation delves into the inhibitory capabilities of a specific set of triterpenoic acids on diverse isoforms of human carbonic anhydrase (*h*CA). Oleanolic acid (**1**), maslinic acid (**2**), betulinic acid (**3**), platanic acid (**4**), and asiatic acid (**5**) were chosen as representative triterpenoids for evaluation. The synthesis involved acetylation of parent triterpenoic acids **1**–**5**, followed by sequential reactions with oxalyl chloride and benzylamine, deacetylation of the amides, and subsequent treatment with sodium hydride and sulfamoyl chloride, leading to the formation of final compounds **21**–**25**.

Inhibition assays against *h*CAs I, II, VA, and IX demonstrated noteworthy outcomes. A derivative of betulinic acid, compound **23**, exhibited a K_i value of 88.1 nM for *h*CA VA, and a derivative of asiatic acid, compound **25**, displayed an even lower K_i value of 36.2 nM for the same isoform. Notably, the latter compound displayed enhanced inhibitory activity against *h*CA VA when compared to the benchmark compound acetazolamide (**AAZ**), which had a K_i value of 63.0 nM. Thus, this compound surpasses the inhibitory potency and isoform selectivity of the standard compound acetazolamide (**AAZ**). In conclusion, the research offers insights into the inhibitory potential of selected triterpenoic acids across diverse *h*CA isoforms, emphasizing the pivotal role of structural attributes in determining isoform-specific inhibitory activity. The identification of compound **25** as a robust and selective *h*CA VA inhibitor prompts further exploration of its therapeutic applications.

1. Introduction

The development of inhibitors of the enzyme class of carbonic anhydrases (CAs) has gained importance for many years [1–10]. Although first described in 1933 by Meldrum and Roughton [11,12] for the efficient catalysis of the reversible hydration of CO_2 in blood to bicarbonate and protons, eight genetically distinct CA families are known today, and CAs are involved in many physiological processes. These include respiration, gluconeogenesis, adipogenesis and numerous other biosynthetic reactions. Therefore, especially human CAs (*h*CAs) are the subject of numerous investigations as therapeutic targets for a wide variety of diseases, including the therapy of edema, glaucoma, epilepsy, obesity, inflammatory diseases, neuropathic pain, Alzheimer's disease, oxidative stress and especially of hypoxic tumors [1–10].

The connection between cancer and individual CA isoforms, especially *h*CA IX and *h*CA XII, has been known for many years [13–19]. Both *h*CAs are under control of the hypoxia inducible factor [20–24]. For individual inhibitors of *h*CA XI and *h*CA XII, it has now been demonstrated that they reduce the number of cancer stem cells, inhibit the growth of primary tumors and also slow down metastasis. In addition, they also interfere with an iron-dependent cell death mechanism (ferroptosis) [25–27].

Links between lifestyle combined with inappropriate food and cancer are now considered to be well established, and it is therefore not surprising that obesity is among the top global health problems; enzyme isoforms CAs VA and VB have been suggested as targets to develop anti-obesity drugs [28–32].

Some time ago, we were able to demonstrate the quite high cytotoxicity of pentacyclic triterpenes for human tumor cell lines with simultaneously significantly lower cytotoxicity for non-malignant fibroblasts. In addition, some compounds were found to be inhibitors of CA II [33–40]. It was therefore obvious to extend the investigations of this substance class, especially regarding compounds exhibiting isoform selectivity.

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The number of publications dealing with triterpene derivatives as inhibitors of CAs is limited. Thereby, methyl triterpenoates were shown [35] to be good inhibitors of CA II, and several derivatives of betulin and betulinic acid inhibited CA IX [36,41]. Recently, we were able to show that succinyl spacered acetazolamide hybrids of acetylated triterpenoids such as betulin, oleanolic acid, ursolic acid and glycyrrhetinic acid were inhibitors of CA II [34]. Thereby, it was revealed that small structural differences govern the inhibition activity of these conjugates [34]. The latter finding called for a more intensive study of sulfamated triterpenoids and their ability to inhibit isoforms of CAs.

2. Results and discussion

Oleanolic acid (**1**), maslinic acid (**2**), betulinic acid (**3**), platanic acid (**4**) and asiatic acid (**5**) (Scheme 1) were chosen as a small library of representative triterpenoic acids. Compounds **1** and **2** are representatives of triterpenes holding an oleanane skeleton, **3** and **4** are lupanes and compound **5** is a trihydroxylated triterpenoid of the ursane type.

For the synthesis of the target compounds, parent triterpenoic acids **1**–**5** were acetylated, and acetates **6**–**10** were obtained. Their treatment with oxalyl chloride followed by the reaction with benzylamine yielded benzyl amides **11**–**15**, which upon de-acetylation afforded **16**–**20**. Their treatment with sodium hydride in dry THF followed by the addition of sulfamoyl chloride yielded final products **21**–**25**, respectively.

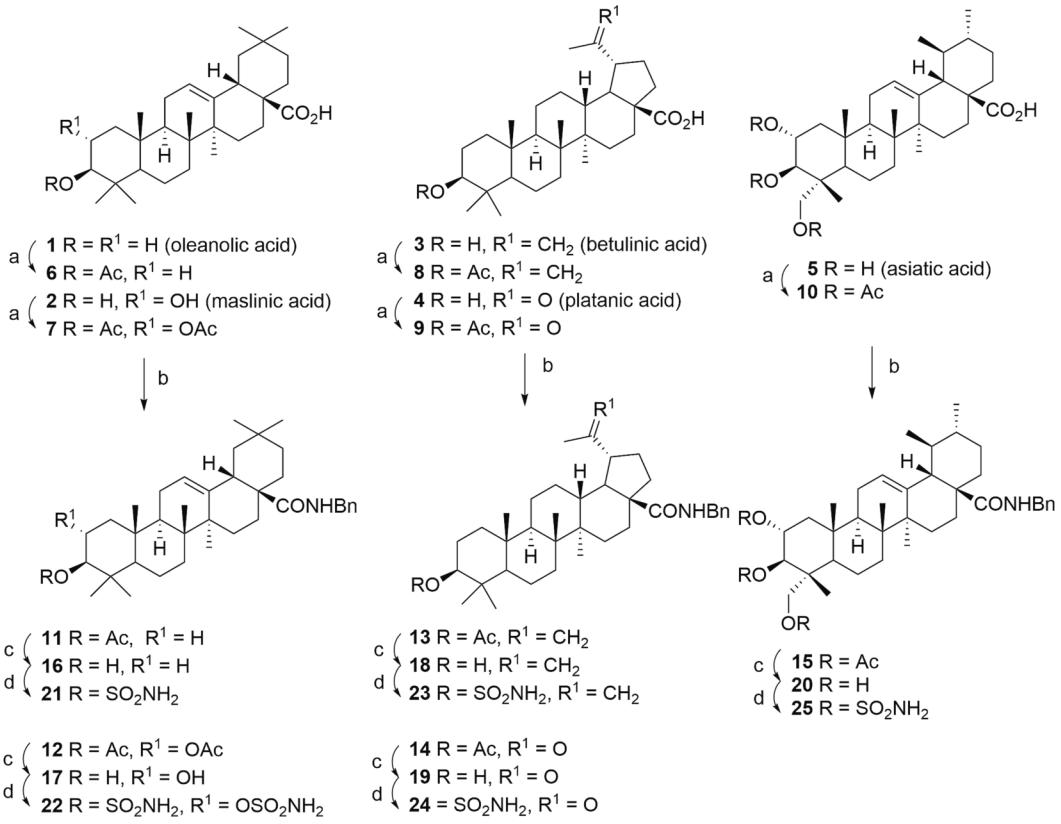
Compounds **21**–**25** were screened for their ability to inhibit *h*CAs I, II, VA, and IX, respectively. The results from these assays are summarized in Table 1 and depicted in Fig. 1.

While none of these compounds was an excellent inhibitor for *h*CA I and only a fair inhibitor of *h*CA II, especially betulinic acid derived **23** showed a K_i value of 88.1 nM for *h*CA VA, and an even lower $K_i = 36.2$ nM was measured for asiatic acid derived compound **25**. Thus, this compound was a better inhibitor for this enzyme than standard acetazolamide (**AAZ**) holding a $K_i = 63.0$ nM for *h*CA VA. The SI values as depicted in Fig. 2 for compounds **21**—**25** indicate that the former exhibits a similar selectivity towards the different isoenzymes as standard **AAZ**. In contrast, compound **23** held the lowest selectivity towards *h*CA IX. The most significant difference, however, in their respective SI values were observed for **21** and **22**, although both compounds share an oleanane skeleton, but differ in their number of hydroxyl groups. It might be assumed that the number of sulfamate groups holds a significant impact on the selectivity index for *h*CA I vs *h*CA IX.

Only oleanolic acid derived compound **21** was a moderate inhibitor of *h*CA IX with a $K_i = 95.2$ nM. Due to its high selectivity towards *h*CA I (Fig. 2), **21** might be an interesting starting point for the development of drugs to deal with brain edema. This seems of special interest inasmuch as a potential association between neuronal cell swelling induced by plaques and the symptomatic manifestations of Alzheimer's disease have been discovered. Concerning Alzheimer's disease ramifications

Table 1
Inhibition of carbonic anhydrase isoforms *h*CA I, *h*CA II, *h*CA VA and hCA IX; values were obtained as means from three different assays by a stopped flow technique as described in the Supplementary Materials File; errors were in the range of ± 5–10 % of the reported values. Acetazolamide (**AAZ**) was used as a positive control. K_i values are reported in nM.

Compound	*h*CA I	*h*CA II	*h*CA VA	*h*CA IX
21	925.0	390.4	457.0	95.2
22	3781	507.7	362.3	1923
23	892.8	564.4	88.1	995.3
24	2494	428.9	206.1	1122
25	5230	297.5	36.2	1126
AAZ	250.0	12.1	63.0	25.8



Scheme 1. Synthesis of sulfamated triterpenoic acid derivatives **21**–**25**; reactions and conditions: a) Ac_2O, Et_3N, DMAP (cat.), DCM, 24 h, 20 °C: **6** (90 %), **7** (91 %), **8** (90 %), **9** (86 %), **10** (87 %); b) $(COCl)_2$, DCM, DMF (cat.), 0 °C → room temperature 3 h, then Bn-NH_2, room temperature, 1 h: **11** (93 %), **12** (89 %), **13** (93 %), **14** (85 %), **15** (93 %); c) KOH in MeOH, 20 °C: **16** (92 %), **17** (90 %), **18** (92 %), **19** (87 %), **20** (84 %); d) NaH, THF then $ClSO_2NH_2$, room temperature, 1 week: **21** (86 %), **22** (84 %), **23** (87 %), **24** (86 %), **25** (89 %).

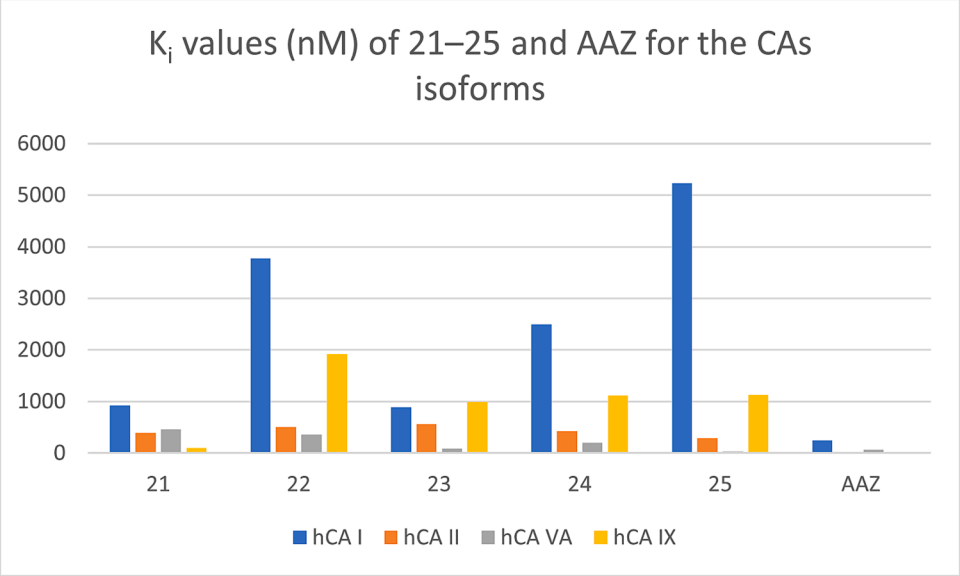


Fig. 1. Inhibition of *h*CAs I, II, VA and IX by triterpenoid sulfamates **21**–**25** and acetalzolamide (**AAZ**) for comparison; K_i values are reported in nM.

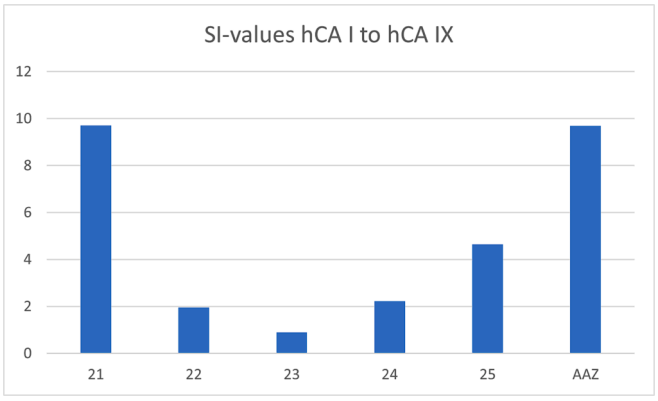


Fig. 2. Selectivity index (SI) (ratio K_i (*h*CA I) vs K_i (*h*CA IX)) of **21**–**25** and of standard **AAZ** showing **21** of holding a selectivity for *h*CA IX similarly to that of **AAZ**.

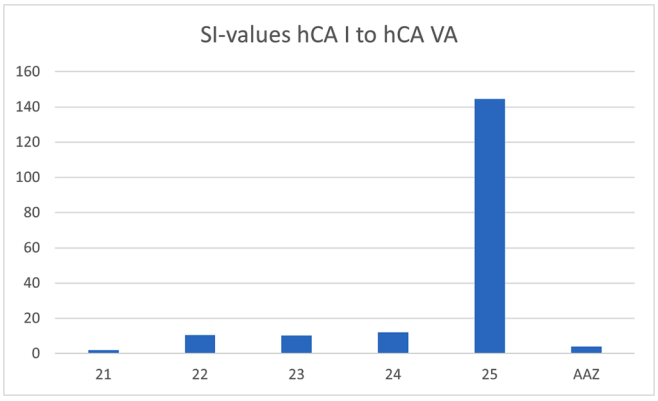


Fig. 3. Selectivity index (SI) (ratio K_i (*h*CA I) vs K_i (*h*CA VA)) of **21**–**25** and of standard **AAZ** showing **25** of holding a high selectivity of 144.5 especially for *h*CA VA.

subsequently to lecanemab or donanemab treatment [42–44], namely brain edema and hemorrhage, it was observed that these complications affected a substantial proportion of participants - up to approximately one-third - in the clinical trial cohort [42]. Furthermore, an activation of microglial cells within regions of cerebral edema is evident in brain swelling patients. This observation propounds the proposition that these microglia might play a contributory role in Alzheimer's disease pathogenesis subsequently to therapeutic interventions. It should be noted that microglial activation is also discernible in Alzheimer's disease models and is currently acknowledged as one of the primary pathological hallmarks of this disease [45]. Unspecific CA inhibitors (pan-CA inhibitors) are presently applied to reduce brain swelling and brain edema [46,47], and the development of more specific inhibitors is called for. Compound **21** might be a good candidate for further development.

The results from the CA screening also show that there is a strong dependence of isoform-specific inhibitory activity and the skeleton of the triterpenoid scaffold. Some more calculated selectivity values (calculated from the ratio of the K_i-values) are depicted in Figs. 3 and 4. As a result, asiatic acid derived compound **25** was the best inhibitor for *h*CA VA holding a K_i = 36.2 nM and a high selectivity towards this enzyme (as compared to *h*CA I) of 144.5.

Hence, compound **25** is a better inhibitor for this enzyme holding also a higher selectivity for this isoform than standard compound acetazolamide (**AAZ**). Comparison of isoform selectivity concerning *h*CA II vs *h*CA VA shows (Fig. 4) compounds **23** and **25** to be more selective towards *h*CA VA than standard **AAZ**.

Fig. 4 shows a comparison for the isoform selectivity towards *h*CA II and *h*CA VA. It is evident that compounds **23** and **25** exhibit stronger selectivity towards *h*CA VA than *h*CA II. This selectivity is even greater than that of **AAZ**. In this context, compounds **21** and **22** (both holding an oleanane skeleton) showed less selectivity towards *h*CA VA. Compounds **23** and **24** (both lupanes) demonstrated more pronounced differences in their selectivity. Compound **23**, derived from betulinic acid, displayed a threefold higher selectivity towards *h*CA VA as compared to the platanic acid-derived compound **24**. Trihydroxylated compound **25** (ursane type) exhibited the highest selectivity among all compounds of this study.

Parent triterpenes are well known for their poor solubility in water. While parent parent triterpenoic acids are almost insoluble (solubility between 0.01 and 0.02 μg/mL) in water, compound **25** showed an about 20-fold improved solubility. The excellent properties of this compound call for additional biological testing. Due to legal restrictions on the implementation of animal experiments to determine in vivo toxicity, toxicity was estimated using the ProTox II (https://tox-new.charite.de/

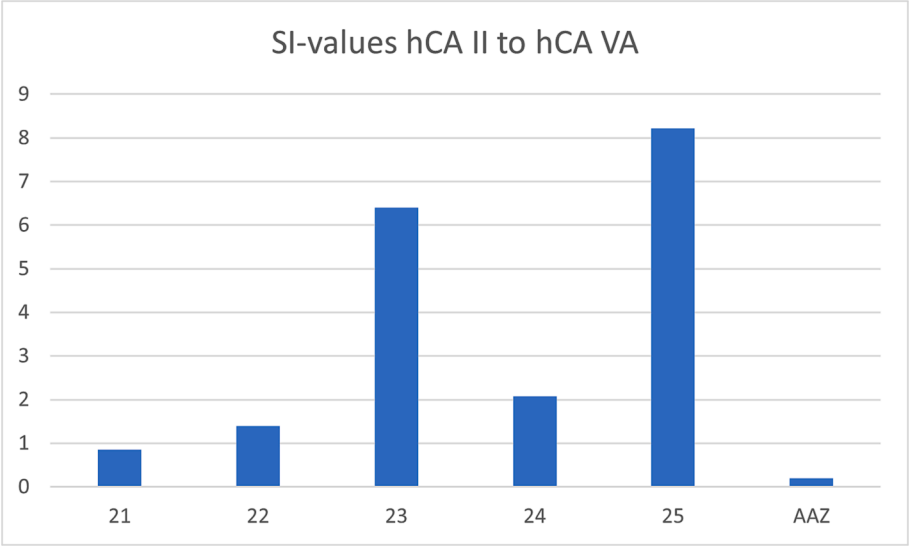


Fig. 4. Selectivity index (SI) (ratio K_i (*h*CA II) vs K_i (*h*CA VA)) of **21**–**25** and of standard **AAZ** showing **23** and **25** to be significantly more selective for *h*CA VA than to *h*CA II and performing better than **AAZ**.

protox_II) as an alternative. This resulted in an estimated calculated toxicity of $LD_{50} > 3$ g/kg. Future studies will also include investigations concerning the cytotoxicity of these compounds.

3. Conclusion

The present study investigates the inhibitory potential of a selected group of triterpenoic acids on various human carbonic anhydrase (*h*CA) isoforms. Oleanolic acid (**1**), maslinic acid (**2**), betulinic acid (**3**), platanic acid (**4**), and asiatic acid (**5**) were chosen as representative triterpenoids for analysis. To synthesize the target compounds, parent compounds underwent acetylation, followed by reaction with oxalyl chloride and benzylamine, de-acetylation of the amides followed by their reaction with sodium hydride and sulfamoyl chloride to yield final sulfamoylated benzyl amides **21**–**25**, respectively.

The inhibitory potential of these compounds was assessed against *h*CAs I, II, VA, and IX. A betulinic acid-derived compound **23** displayed a K_i value of 88.1 nM for *h*CA VA, while asiatic acid-derived compound **25** demonstrated an even lower K_i value of 36.2 nM for the same isoform. Notably, compound **25** exhibited superior inhibitory activity against *h*CA VA compared to the standard compound acetazolamide (**AAZ**), which displayed a K_i value of 63.0 nM. The study's findings reveal a strong correlation between isoform-specific inhibitory activity and the triterpenoid scaffold's structural framework. Selectivity values calculated from the K_i ratios illustrate this relationship, with asiatic acid-derived compound **25** emerging as the most potent *h*CA VA inhibitor, displaying both a low K_i value of 36.2 nM but also a selectivity of 144.5 compared to *h*CA I. In conclusion, this study sheds light on the inhibitory potential of a selected group of triterpenoic acids against various *h*CA isoforms. The results underscore the importance of structural characteristics in determining isoform-specific inhibitory activity. The identification of asiatic acid-derived compound **25** as a potent and selective inhibitor for *h*CA VA warrants further exploration for its potential therapeutic applications especially for the treatment of obesity.

4. Experimental

4.1. General

Starting materials were obtained from local vendors [oleanolic acid, betulinic acid and platanic acid were obtained from betulinines (Stříbrná Skalice, Czech Republic), asiatic acid and maslinic acid were bought from Merck (Darmstadt, Germany)]; solvents were dried under usual conditions; equipment was used as previously described. Details of the biological testing, analytical data for **21**–**24** and representative NMR spectra (^{1}H and ^{13}C) are provided in the Supplementary File. The physical data (m.p., $[\alpha]_D^{20}$, IR, UV/Vis, ^{1}H NMR, ^{13}C NMR, MS) of known compounds **6**–**24** described in this manuscript was in full agreement with data described in the literature.

4.2. Acetylation of the triterpenoic acids (General procedure A, GPA)

Acetylation of **1**–**5** (1 equiv.) was performed in dry DCM with acetic anhydride (3 equiv.), Et_3N (3 equiv.) and DMAP (catal. amount) for 24 h at ambient temperatures. Usual aqueous work-up followed by recrystallization from EtOH yielded products **6**–**10**.

4.3. Synthesis of the benzylamides 11–15 (General procedure B, GPB)

The acetylated triterpenoic acid **6**–**10** (1 equiv.) was dissolved in dry DCM, oxalyl chloride (4.0 equiv.) and dry DMF (cat. amount) were added at 0 °C, stirring at room temperature was continued for 3 h, the volatiles were removed, and benzyl amine (2 equiv.) was added. Stirring at room temperature was continued for another hour followed by usual workup and chromatography to afford **11**–**15**.

4.4. Deacetylation of 11–15 (General procedure C, GPC)

To a solution of methanolic KOH (1.2 equiv.) a solution of **11**–**15** was slowly added, and stirring at 20 °C was continued until TLC showed completeness of the reaction. The product was precipitated with cold aq. HCl (3.5 %), collected, dried, and purified by chromatography to afford **16**–**20**.

4.5. Synthesis of the sulfamates 21–25 (General procedure D, GPD)

To a solution of **16**–**20** (0.5 mmol) in dry THF (25 mL), NaH (freshly washed with dry *n*-hexane, 0.80 mmol) was added at 5 °C, and the mixture was stirred until the evolution of gases had ceased. A solution of sulfamoyl chloride (1.0 mmol) in dry THF (2 mL) was added, and stirring at room temperature was continued for 1 week. The volatiles were removed under diminished pressure, the residue was parted between water (100 mL) and diethyl ether (100 mL); usual workup of the organic layer followed by chromatography furnished **21**–**25** each as a white

solid.

4.6. Syntheses

4.6.1. 3-O-Acetyl-oleanolic acid (6)

From **1** by GPA, compound **6** was obtained in 90 % yield; white solid; m.p. 260–262 °C (lit.: [48] m.p. 267–268 °C); $[\alpha]_D^{20}$ = +69.4° (*c* 0.30, $CHCl_3$) [lit.: [49] $[\alpha]_D^{20}$ = +74.0° (*c* 1.0, $CHCl_3$)]; MS (ESI, MeOH): *m/z* = 499.2 (55 %, $[M + H]^+$).

4.6.2. 2,3-Di-O-acetyl-maslinic acid (7)

From **2** by GPA, compound **7** was obtained in 91 % yield; white solid; m.p. 224–226 °C (lit.: [50] 170–173 °C); $[\alpha]_D^{20}$ = +30.0° (*c* 0.45, $CHCl_3$) [lit.: [50] $[\alpha]_D^{20}$ = +30.0° (*c* 0.83, $CHCl_3$)]; MS (ESI, MeOH): *m/z* = 557.5 (52 %, $[M + H]^+$).

4.6.3. 3-O-Acetyl-betulinic acid (8)

From **3** by GPA, compound **8** was obtained in 90 % yield; white solid; m.p. 280–283 °C (lit.: [51] 277–278 °C); $[\alpha]_D^{20}$ = +20.0° (*c* 0.25, $CHCl_3$) [lit.: [51] $[\alpha]_D^{20}$ = +22.0° (*c* 0.49, $CHCl_3$)]; MS (ESI, MeOH): *m/z* = 497.5 (26 %, $[M-H]^-$).

4.6.4. 3-O-Acetyl-platanic acid (9)

From **4** by GPA, compound **9** was obtained in 86 % yield; white solid; m.p. 266–268 °C (lit.: [52] 256–259 °C); $[\alpha]_D^{20}$ = –9.4° (*c* 0.4, $CHCl_3$) [lit.: [52] $[\alpha]_D^{20}$ = –9.1° (*c* 0.34, $CHCl_3$)]; MS (ESI, MeOH): *m/z* = 999.5 (100 %, $[2\ M-H]^-$).

4.6.5. 2,3,23-Tri-O-acetyl-asiatic acid (10)

From **5** by GPA, compound **10** was obtained in 87 % yield; white solid; m.p. 162–164 °C (lit.: [53] 159–161 °C); $[\alpha]_D^{20}$ = +37.2° (*c* 0.4, $CHCl_3$) [lit.: [53] $[\alpha]_D^{20}$ = +35.96° (*c* 0.34, $CHCl_3$)]; MS (ESI, MeOH): *m/z* = 615.3 (17 %, $[M + H]^+$).

4.6.6. 3β-Acetyloxy-N-benzyl-olean-12-en-28 amide (11)

From **6** by GPB, compound **11** was obtained in 93 % yield; white solid; m.p. 257–260 °C (lit.: [50] 254–259 °C); $[\alpha]_D^{20}$ = +30.1° (*c* 0.5, $CHCl_3$) [lit.: [50] $[\alpha]_D^{20}$ = +29° (*c* 0.56, $CHCl_3$)]; MS (ESI, MeOH): *m/z* = 588.7 (60 %, $[M + H]^+$).

4.6.7. (2α,3β)-Bis(acetyloxy)-N-benzyl-olean-12-en-28-amide (12)

From **7** by GPB, compound **12** was obtained in 89 % yield; white solid; m.p. 144–146 °C (lit.: [50] 143–145 °C); $[\alpha]_D^{20}$ = –6.9° (*c* 0.4, $CHCl_3$) [lit.: [50] $[\alpha]_D^{20}$ = –7.0° (*c* 0.32, $CHCl_3$); MS (ESI, MeOH): *m/z* = 646.5 (100 %, $[M + H]^+$).

4.6.8. 3β-Acetyloxy-N-benzyl-lup-20(29)en-28-amide (13)

From **8** by GPB, compound **13** was obtained in 93 % yield; white solid; m.p. 124–126 °C (lit.: [54] 124–127 °C); $[\alpha]_D^{20}$ = +24.1° (*c* 0.45, $CHCl_3$) [lit.: [54] $[\alpha]_D^{20}$ = +23.2° (*c* 0.35, $CHCl_3$); MS (ESI, MeOH): *m/z* = 588.4 (52 %, $[M + H]^+$).

4.6.9. 3β-Acetyloxy-N-benzyl-30-oxo-30-norlupan-28-amide (14)

From **9** by GPB, compound **14** was obtained in 85 % yield; white solid; m.p. 288–290 °C (lit.: [55] 290 °C (decomp.); $[\alpha]_D^{20}$ = +1.7° (*c* 0.25, $CHCl_3$) (lit.: [55] $[\alpha]_D^{20}$ = + 0.5° (*c* 0.159, $CHCl_3$); MS (ESI, MeOH): *m/z* = 590.1 (100 %, $[M + H]^+$).

4.6.10. (2α,3β,4α) 2,3,23-Tris(acetyloxy)-N-benzyl-urs-12-en-28-amide (15)

From **10** by GPB, compound **15** was obtained in 93 % yield; white solid; m.p. 137–139 °C (lit.: [53] 136–138 °C); $[\alpha]_D^{20}$ = +0.75° (*c* 0.40, $CHCl_3$) [lit.: [53] $[\alpha]_D^{20}$ = +0.68° (*c* 0.345, $CHCl_3$); ESI (MS, MeOH): *m/z* = 704.5 (100 %, $[M + H]^+$).

4.6.11. 3β-Hydroxy-N-benzyl-olean-12-en-28-amide (16)

From **11** by GPC, compound **16** was obtained in 92 % yield; white solid; m.p. 247–249 °C (lit: [50] 247–249 °C); $[\alpha]_D^{20}$ = +31.3° (*c* 0.70, CHCl3) [lit.: [50] $[\alpha]_D^{20}$ = +30.4° (*c* 0.56, $CHCl_3$); MS (ESI, MeOH): *m/z* = 546.7 (40 %, $[M + H]^+$).

4.6.12. (2α, 3β)-Dihydroxy-N-benzyl-olean-12-en-28-amide (17)

From **12** by GPC, compound **17** was obtained in 90 % yield; white solid; m.p. 149–151 °C (lit.: [50] 148–151 °C); $[\alpha]_D^{20}$ = +32.1° (*c* 0.50, $CHCl_3$) [lit.: [50] $[\alpha]_D^{20}$ = +29° (*c* 0.29, $CHCl_3$); MS (ESI, MeOH): *m/z* = 563.3 (17 %, $[M + H]^+$).

4.6.13. 3β-Hydroxy-N-benzyl-lup-20(29)en-28-amide (18)

From **13** by GPC, compound **18** was obtained in 92 % yield; white solid; m.p. 247–248 °C (lit.: [54] 246–248 °C); $[\alpha]_D^{20}$ = +15.1° (*c* 0.45, $CHCl_3$) [lit.: [54] $[\alpha]_D^{20}$ = +14.3° (*c* 0.31, $CHCl_3$); MS (ESI, MeOH): *m/z* = 546.4 (100 %, $[M + H]^+$).

4.6.14. 3β-Hydroxy-20-oxo-N-benzyl-30-norlupan-28-amide (19)

From **14** by GPC, compound **19** was obtained in 87 % yield; white solid; m.p. 267–268 °C (lit.: [41] 266–268 °C); $[\alpha]_D^{20}$ = –6.2° (*c* 0.25, $CHCl_3$) [lit.: [41] $[\alpha]_D^{20}$ = –6.1° (*c* 0.3, $CHCl_3$); MS (ESI, MeOH): *m/z* = 548.2 (100 %, $[M + H]^+$).

4.6.15. (2α, 3β, 4α)-2,3,23-Trihydroxy-N-benzyl-urs-12-en-28-amide (20)

From **15** by GPC, compound **20** was obtained in 84 % yield; white solid; m.p. 160–163–°C (lit: [56] 155–159 °C); $[\alpha]_D^{20}$ = +29.5° (*c* 0.45, $CHCl_3$) [lit.: [56] $[\alpha]_D^{20}$ = +27.26° (*c* 0.31, $CHCl_3$); MS (ESI, MeOH): *m/z* = 578.2 (100 %, $[M + H]^+$).

4.6.16. (3β)-[(Aminosulfonyl)oxy]-N-benzyl-olean-12-en-28-amide (21)

From **16** by GPD, compound **21** was obtained in 86 % yield; white, amorphous solid; $[\alpha]_D^{20}$ = +27.9° (*c* 0.25, $CHCl_3$) [lit.: [41] $[\alpha]_D^{20}$ = +28.1° (*c* 0.385, $CHCl_3$); MS (ESI, MeOH): *m/z* = 625.2 (100 %, $[M + H]^+$).

4.6.17. (2α, 3β)-Bis[(aminosulfonyl)oxy]-N-benzyl-olean-12-en-amide (22)

From **17** by GPD, compound **22** was obtained in 84 % yield; white, amorphous solid; $[\alpha]_D^{20}$ = +7.5° (*c* 0.25, $CHCl_3$) [lit.: [41] $[\alpha]_D^{20}$ = +7.3° (*c* 0.3, $CHCl_3$); MS (ESI, MeOH): *m/z* = 720.2 (100 %, $[M + H]^+$).

4.6.18. (3β)-[(Aminosulfonyl)oxy]-N-benzyl-lup-20(29)-en-28-amide (23)

From **18** by GPD, compound **23** was obtained in 87 % yield; white, amorphous solid; $[\alpha]_D^{20}$ = +18.4° (*c* 0.5, $CHCl_3$) [lit.: [41] $[\alpha]_D^{20}$ = +17.2° (*c* 0.345, $CHCl_3$); MS (ESI, MeOH): *m/z* = 625.4 (100 %, $[M + H]^+$).

4.6.19. (3β)-[(Aminosulfonyl)oxy]-N-benzyl-20-oxo-30-norlupan-28-amide (24)

From **19** by GPD, compound **24** was obtained in 86 % yield; white, amorphous solid; $[\alpha]_D^{20}$ = –3.1° (*c* 0.25, $CHCl_3$) [lit.: [41] $[\alpha]_D^{20}$ = –2.9° (*c* 0.34, $CHCl_3$); MS (ESI, MeOH): *m/z* = 627.5 (35 %, $[M + H]^+$).

4.6.20. (2α, 3β, 4α)-2,3,23-Tris[(aminosulfonyl)oxy]-N-benzyl-urs-12-en-28-amide (25)

From **20** by GPD, compound **25** was obtained in 89 % yield; white solid; m.p. 198–199 °C $[\alpha]_D^{20}$ = +8.59 (c 0.136, MeOH); R_f = 0.26 (SiO_2, $CHCl_3$/MeOH, 9:1); UV–Vis ($CHCl_3$): λ_{max} (log ε) = 259 nm (3.25); IR (ATR): ν = 3361*w*, 3278*w*, 3062*w*, 2923*w*, 2870*w*, 1627*w*, 1557*w*,

1525*w*, 1497*w*, 1455*w*, 1360 *s*, 1176*vs*, 1012*w*, 971 *m*, 960 *s*, 924 *s*, 877*w*, 828 *s*, 754*w*, 743*w*, 723*w*, 699*w*, 666*w*, 651*w*, 641*w*, 601*w*, 580 *m*, 555 *m*, 537 *m*, 497*w*, 482*w*, 460*w* cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 7.73$ (t, $J = 6.0$ Hz, 1H, NH), 7.48 – 7.10 (*m*, 11*H*, NH_2, 33-H, 34-H, 35-H, 36-H, 37-H), 5.21 (t, $J = 3.6$ Hz, 1H, 12-H), 4.67 (ddd, $J = 11.8$, 9.8, 4.7 Hz, 1H, 2-H), 4.37 (d, $J = 9.9$ Hz, 1H, 3-H), 4.20 (qd, $J = 15.0$, 5.8 Hz, 2H, 31-H), 3.90 – 3.80 (*m*, 2H, 24-H), 2.32 (dd, $J = 12.5$, 4.5 Hz, 1H, 1-H_a), 2.23 (d, $J = 10.7$ Hz, 1H, 18-H), 2.00 – 1.62 (*m*, 4H, 16-H_a, 11-H_a, 15-H_a, 16-H_b), 1.61 – 1.20 (*m*, 11*H*, 22-H, 9-H, 7-H_a, 6-H, 21-H_a, 19-H, 21-H_b, 5-H, 11-H_b), 1.20 – 1.06 (*m*, 2H, 7-H_b, 1-H_b), 1.05 (s, 3H, 27), 0.97 (s, 4H, 26, 20-H), 0.92 (s, 4H, 30, 15-H_b), 0.84 (d, $J = 6.4$ Hz, 3H, 29), 0.81 (s, 3H, 23), 0.52 (s, 3H, 25) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): $\delta = 176.1$ (C-28), 140.0 (C-32), 138.6 (C-13), 128.0 (C-34, C-36), 127.3 (C-33, C-37), 126.4 (C-35), 124.0 (C-12), 82.6 (C-3), 76.2 (C-2), 69.2 (C-24), 51.9 (C-18), 46.6 (C-5, C-9), 46.5 (C-17), 44.1 (C-1), 42.8 (C-4), 42.2 (C-31), 41.6 (C-14), 39.0 (C-8, C-10), 38.7 (C-19), 38.4 (C-20), 37.0 (C-22), 31.8 (C-7), 30.4 (C-21), 27.2 (C-15), 23.5 (C-16), 23.2 (C-27), 23.0 (C-11), 21.1 (C-30), 17.2 (C-6), 17.1 (C-29), 16.7 (C-25), 16.2 (C-26), 13.5 (C-23) ppm; MS (ESI, MeOH): $m/z = 812.9$ (100 %, $[M-H]^-$); anal. calcd. for $C_{37}H_{58}N_4S_3O_{10}$ (815.07): C 54.52, H 7.17, N 6.87; found: C 54.27, H 7.39, N 6.55.

CRediT authorship contribution statement

Toni C. Denner: Writing – review & editing, Writing – original draft, Investigation. **Niels V. Heise:** Writing – review & editing, Writing – original draft, Investigation. **Immo Serbian:** Writing – review & editing, Writing – original draft, Investigation. **Andrea Angeli:** Writing – review & editing, Writing – original draft, Investigation. **Claudiu T. Supuran:** Writing – review & editing, Writing – original draft, Validation, Supervision. **René Csuk:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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Small Structural Differences Govern the Carbonic Anhydrase II Inhibition Activity of Cytotoxic Triterpene Acetazolamide Conjugates

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Abstract: Acetylated triterpenoids betulin, oleanolic acid, ursolic acid, and glycyrrhetinic acid were converted into their succinyl-spacered acetazolamide conjugates. These conjugates were screened for their inhibitory activity onto carbonic anhydrase II and their cytotoxicity employing several human tumor cell lines and non-malignant fibroblasts. As a result, the best inhibitors were derived from betulin and glycyrrhetinic acid while those derived from ursolic or oleanolic acid were significantly weaker inhibitors but also of diminished cytotoxicity. A betulin-derived conjugate held a K_i = 0.129 μM and an EC_{50} = 8.5 μM for human A375 melanoma cells.

Keywords: triterpenoic acid; carbonic anhydrase II; acetazolamide conjugate; cytotoxicity

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1. Introduction

The ubiquitous metalloenzymes carbonic anhydrases (CAs) [1–6] are present in bacteria [7,8] and fungi [9–12], plants and animals. Inhibitors of these enzymes have been clinically exploited for decades, and the discovery of multiple human isoforms [13–18] has led to many new applications and the development of new therapeutic principles, among them antiglaucoma [19–21] and antitumor drugs but also antiepileptic [22–26] and antiobesity drugs [27–29] as well as agents for the management of Alzheimer's disease [30,31], neuropathic pain, cerebral ischemia, and some forms of arthritis [32–34]. Furthermore, the development of inhibitors for bacterial carbonic anhydrases is thought as a new concept to develop antibacterial drugs [35–42]. In addition, drug conjugates were investigated for their ability to treat a variety of disorders in a multitargeting approach [43–47]. The most investigated compound, however, is SLC-0111 (U-104, Figure 1) [48–53] for the management of advanced, metastatic solid tumors; this compound is now in Phase Ib/II clinical trials [54].

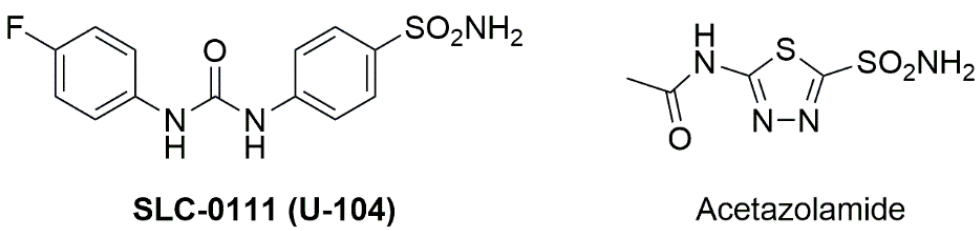


Figure 1. Structure of well-established CA inhibitors SLC-0111 and acetazolamide.

For many years, especially CA IX and CA XII were in the focus of scientific interest to combat cancer. Recently, CA II in the endothelium of glial tumors became a potential target for therapy [55–64]. Furthermore, the CA II inhibitor acetazolamide was suggested as a chemosensitizer for treating temozolomide resistant gliomas [65–69]. In addition, CA

II was found among up-regulated genes and as a candidate gene correlated with glioma malignancy and patient survival. [70]

The association of CA II with several pathological diseases prompted us to synthesize novel CA II inhibitors. Recently, it was revealed that methyl sulfamates, [71] derived from methyl triterpenoates such as oleanolic acid, ursolic acid, and glycyrrhetinic acid or betulinic acid, are effective and competitive inhibitors of CA II and several cycloartane derivatives [72] held significant activity for CA II, too. Euphol [73] was also shown to induce autophagy and to sensitize temozolomide cytotoxicity in glioblastoma cells. Several 1H-1,2,3-triazoles derived from pentacyclic triterpenoid 3-*O*-acetyl-(11-keto)-boswellic acids [74] were inhibitors CA II. Furthermore, hybrids of bile acids [75–78] have been shown to act as inhibitors of CAs.

Consequently, we became interested in the design, synthesis and CA II screening of conjugates derived from pentacyclic triterpenes such as betulin, oleanolic acid, ursolic acid, and glycyrrhetinic acid (Figure 2) with well-known inhibitor acetazolamide.

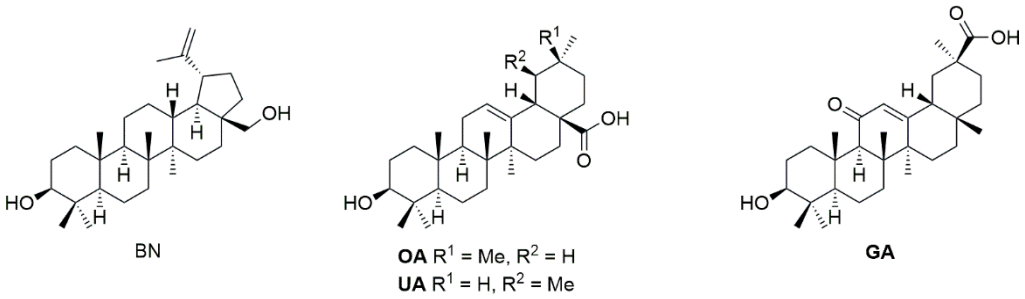


Figure 2. Structures of starting materials betulin (BN), oleanolic acid (OA), ursolic acid (UA), and glycyrrhetinic acid (GA).

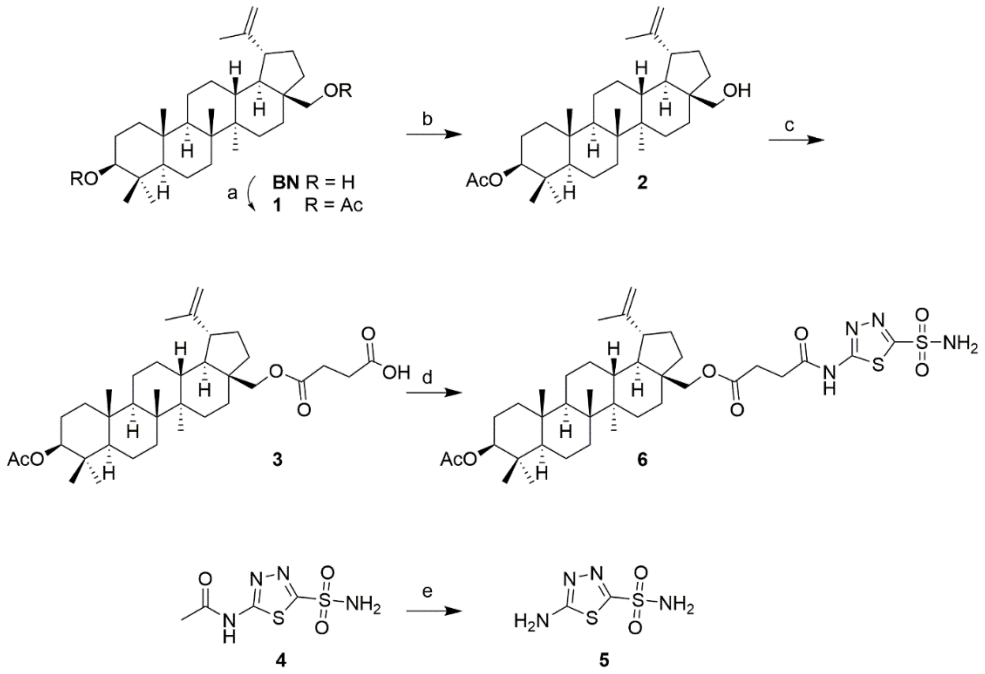
2. Results

Based on our previous results [71], triterpenoids (Figure 2) betulin (**BN**), oleanolic acid (**OA**), ursolic acid (**UA**), and glycyrrhetinic acid (**GA**) appeared to us as suitable and representative starting materials. **BN** was converted (Scheme 1) to the diacetate **1** according to known procedures, the selective mono-deacylation of which with CaH_2 in a methanol/water mixture gave the 3-*O*-acetate **2**. Reaction of **2** with succinic anhydride in pyridine in the presence of DMAP (cat.) furnished the succinyl derivative **3**.

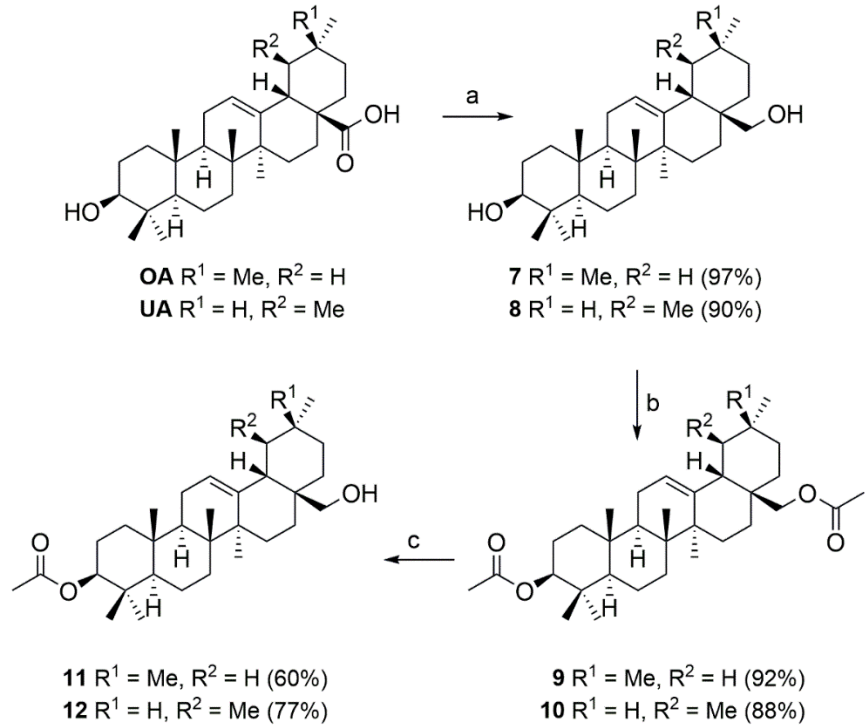
Commercial acetazolamide (**4**) was deacetylated with conc. HCl under reflux and compound **5** was obtained. The reaction of **3** with **5** proved somewhat difficult in the beginning, as both an in situ generation of the corresponding carboxylic acid chloride and its coupling with **5** failed, as was the use of coupling reagents such as EDC, DCC, PyBOP, or T3P. However, a good yield of **6** was obtained by first reacting **3** with ethyl chloroformate in the presence of 4-methyl-morpholine in THF to yield a mixed anhydride in situ, whose reaction with **5** then gave 88% of **6**.

Compound **6** shows the basic carbon skeleton unchanged from the starting material **BN**, and it is further characterized by the presence of the acetyl group at position C-3 (^{1}H NMR δ = 1.99 ppm, and in ^{13}C NMR δ = 171.9 and 21.0 ppm). The succinyl spacer is characterized by the two CH_2 groups (in ^{13}C NMR at δ = 30.0 and 30.9 ppm). The heterocycle shows in its ^{13}C NMR spectrum the characteristic signals at δ = 161.0 and 164.3 ppm; the sulfamate group held in the ^{1}H NMR spectrum the signal for the NH_2 group at δ = 8.30 ppm.

For the preparation of the corresponding analogous compounds derived from **OA**, **UA**, or **GA**, the commercially relatively inexpensive triterpene carboxylic acids **OA**, **UA** first had to be reduced using $LiAlH_4$ (Scheme 2). This allowed the corresponding diols **7** and **8** to be obtained in good yields.



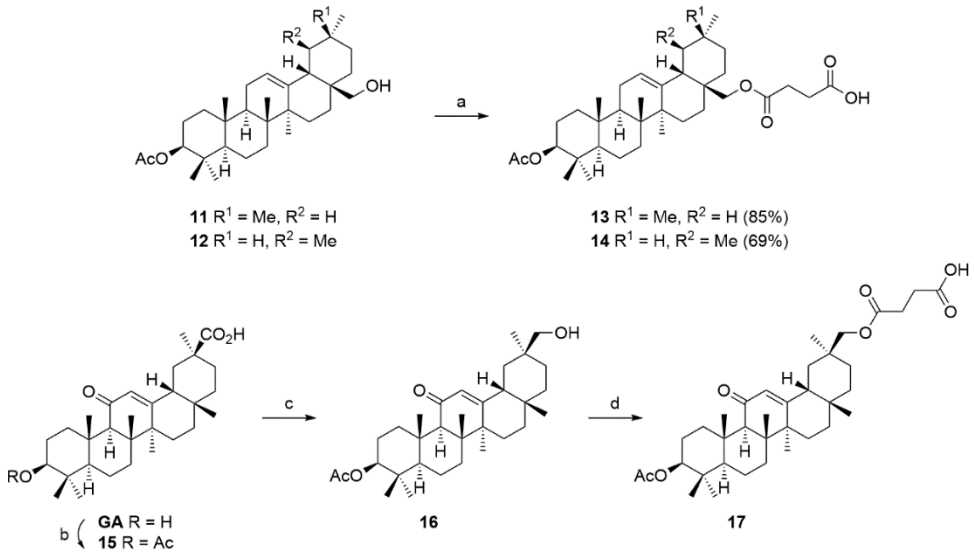
Scheme 1. Reactions and conditions: (**a**) Ac_2O, TEA, DMAP (cat.), DCM, 20 °C, 12 h, 90%; (**b**) CaH_2, MeOH/THF, 20 °C, 12 h, 83%; (**c**) pyridine, DMAP, succinic anhydride, reflux, 15 h, 71%; (**d**) THF, 4-methyl-morpholine, ethyl chloroformate, 20 °C, 15 min, then **5**, reflux, 48 h, 88%; (**e**) conc. HCl, reflux, 3 h, 94%.



Scheme 2. Reactions and conditions: (**a**) $LiAlH_4$, THF, reflux, 2 h; (**b**) Ac_2O, pyridine, 20 °C, 15 h; (**c**) Al(*i*PrO)$_3$, ^{i}PrOH, reflux, 4 h.

Compounds **7** and **8** were converted into the corresponding diacetates **9** and **10**, respectively, whose selective de-acetylation gave compounds **11** and **12**. Analogous conditions as described above could now be carried out for the subsequent reactions to yield the target compounds.

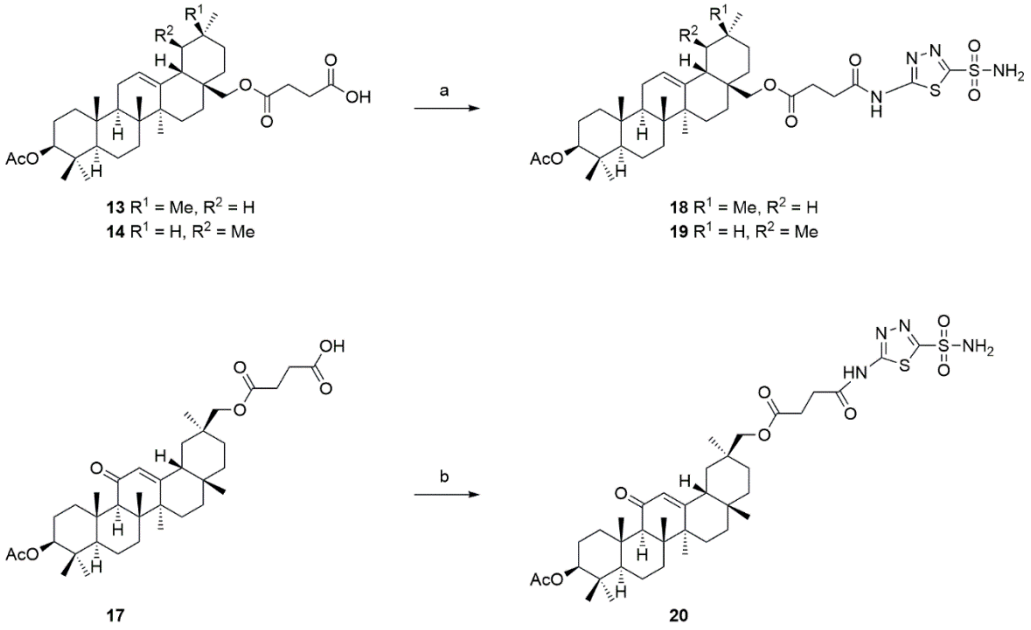
Thus, the mono-acetates **11** and **12** were converted (Scheme 3) to the succinyl derivatives **13** and **14**.



Scheme 3. Reactions and conditions: (**a**) Pyridine, DMAP, succinic anhydride, reflux, 24 h; (**b**) Ac_2O, pyridine, 20 °C, 15 h; (**c**) ethyl chloroformate, TEA, THF, −12 °C, 15 min, then sodium borohydride in water, 15 min; (**d**) pyridine, DMAP, succinic anhydride, reflux, 24 h.

Since the reduction of **GA** by $LiAlH_4$ failed to give good yields, **GA** was first converted into acetate **15**, the reaction of which with ethyl chloroformate/TEA gave an un-isolated mixed anhydride, the reduction of which with $NaBH_4$ at room temperature afforded compound **16** in good yields within a few minutes. Its reaction with succinyl anhydride yielded **17**.

The coupling of **13**, **14,** and **17** with **5** (Scheme 4) gave the products **18**–**20**, respectively.



Scheme 4. Reactions and conditions: (**a**,**b**) THF, 4-methyl-morpholine, ethyl chloroformate, 20 °C, 15 min, then **5**, reflux, 48 h.

Screening of compounds **6**, **18**–**20** for their activity was performed with CA II as previously described; the results from the assays are compiled in Table 1. Acetazolamide (**4**) was used as a positive control.

Table 1. Inhibition percentage of conjugates (at 10 μM concentration) and of standard acetazolamide (**4**).

Compound	Inhibition [%]
4	89.9 ± 0.6
6	93.0 ± 0.1
18	49.4 ± 0.1
19	70.8 ± 0.2
20	96.8 ± 0.2

These assays showed glycyrrhetinic acid-derived conjugate **20** as the best inhibitor for this enzyme followed by betulin-derived **6**. These compounds were even better inhibitors than gold standard acetazolamide (**4**). Oleanolic and ursolic-derived conjugates showed a diminished ability to inhibit CA II. Parent compounds, i.e., betulin, betulinic acid, ursolic acid, oleanolic acid, and glycyrrhetinic acid did not inhibit the enzyme under the conditions of the assay at all. Compounds **2**, **3**, **7**–**17** showed inhibition rates less than 10%.

For compounds with the highest inhibition percentage, i.e., **6** and **19** and **20**, some extra measurements were performed to determine their respective inhibition constants K_i values. The results from these experiments are summarized in Table 2; Figure 3 shows the Dixon plot for compound **6**; this compound acts as a competitive inhibitor for the enzyme and holds a rather low K_i = 0.129 μM.

Table 2. K_i values (in μM) for conjugates **6**, **19,** and **20**.

Compound	K_i (in μM)
6	0.129 ± 0.02
19	0.91 ± 0.17
20	5.22 ± 0.57

Initial molecular modelling calculations were performed to get some insights in the mode of action of the conjugates. These calculations, however, did not provide any reasonable explanation for the different ability of the conjugates to inhibit the enzyme. While it seems plausible that the acetazolamide moiety interacts with the active site of the enzyme in a manner like parent acetazolamide, it cannot be excluded; however, that the conjugates also act as non-zinc binding inhibitors, thus paralleling previous findings for structurally similar pentacyclic triterpenoid arjunolic acid [79].

Previously especially CA IX was extensively studied in the process of tumorigenesis, [15,80] and several derivatives of pentacyclic triterpenoids have been revealed as inhibitors of this isoform, too [81]. The selectivity of the triterpenoid investigated so far toward individual isoforms of CA, however, was not particularly pronounced.

Compounds **6** and **18**–**20** were screened for their cytotoxic activity in sulforhodamine B assays (SRB), employing several human tumor cell lines. The results from these assays are summarized in Table 3. Expression of CA II and its involvement cancer has previously been established for A375 [82], HT29 [83] as well as for MCF-7 cells [84]. Cell line A2780 and non-malignant fibroblasts (NIH 3T3) were employed for comparison.

As a result, the highest cytotoxicity was established for botulin-derived **6** followed by glycyrrhetinic acid-derived **20**. This parallels the finding for the inhibition rates for CA II established for these compounds. A significantly lower cytotoxicity was determined for oleanolic or ursolic acid-derived compounds **18** and **19**, respectively. The malignant/non-malignant cell selectivity, however, was low for all compounds. No cytotoxicity (EC_{50} > 30 μM; cut-off of the assay) was found for parent triterpenoic acids.

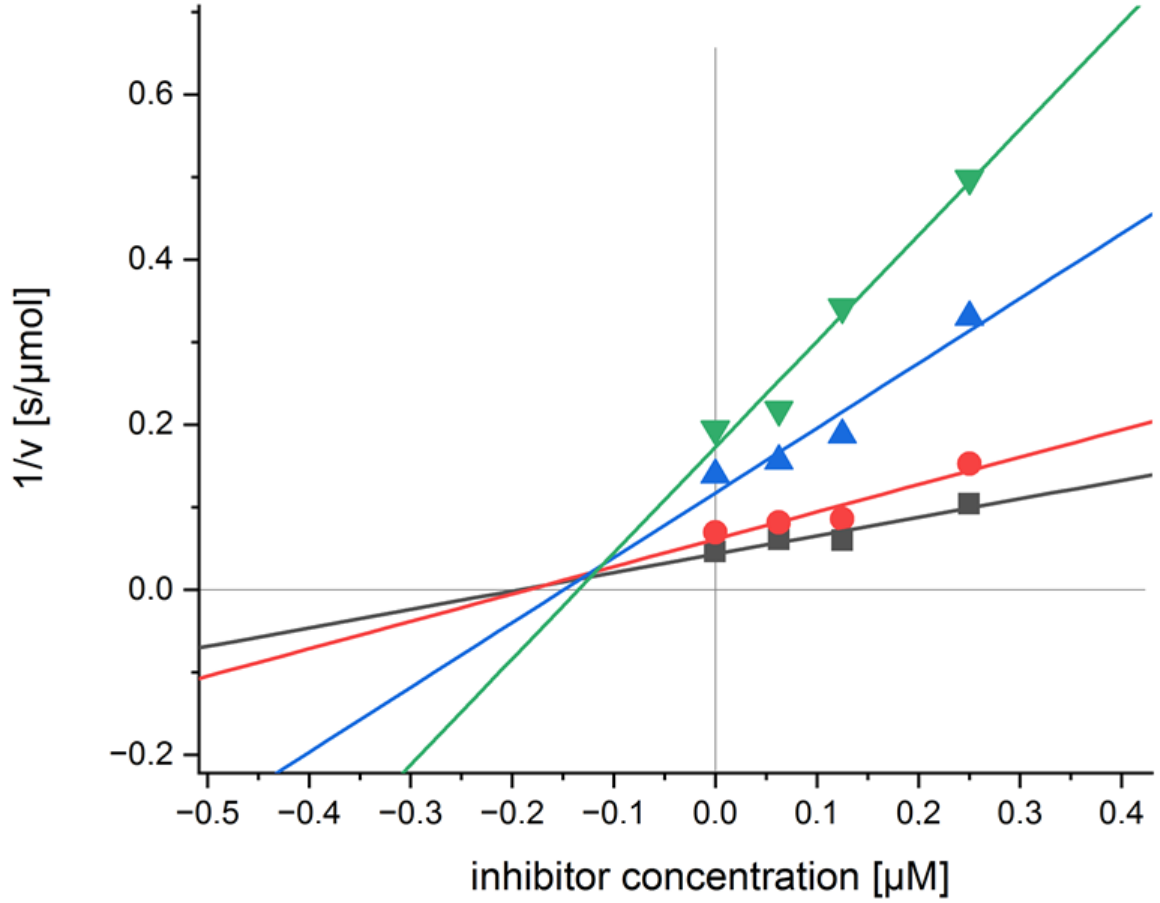


Figure 3. Dixon plot for compound **6** and CA II.

Table 3. Cytotoxicity of acetazolamide (**4**) and conjugates **6**, **18-20** assessed from SRB-assays (EC_{50} values [µM] after 72 h of treatment). Human cancer cell lines: A375 (epithelial melanoma), HT29 (colorectal adenocarcinoma), MCF-7 (breast adenocarcinoma), A2780 (ovarian carcinoma); non-malignant: NIH 3T3 (fibroblasts); n.d. not determined; positive control: doxorubicin (**DX**).

	A375	**HT29**	**MCF-7**	**A2780**	**NIH 3T3**
4	>30	>30	>30	>30	>30
6	8.5 ± 0.7	10.2 ± 1.3	8.9 ± 0.7	9.3 ± 1.2	9.5 ± 1.0
18	10.1 ± 0.8	14.2 ± 1.4	10.6 ± 1.4	11.8 ± 1.4	14.0 ± 1.5
19	13.7 ± 1.1	15.0 ± 0.6	12.4 ± 0.8	12.5 ± 1.7	14.8 ± 1.5
20	9.2 ± 0.5	13.0 ± 1.3	10.5 ± 1.2	9.8 ± 0.8	11.9 ± 1.7
DX	n.d.	0.25 ± 0.02	0.1 ± 0.01	0.1 ± 0.01	0.01 ± 0.001

3. Conclusions

Pentacyclic triterpenoids betulin, oleanolic acid, ursolic acid, and glycyrrhetinic acid were acetylated at position C-3 and converted into their succinyl-spacered acetazolamide conjugates. Their screening for their inhibitory activity onto carbonic anhydrase II and screening for their cytotoxicity in SRB assays employing several human tumor cell lines and non-malignant fibroblasts showed the conjugates derived from betulin and glycyrrhetinic acid to be the best inhibitors while those derived from ursolic or oleanolic acid were significantly weaker inhibitors but also of diminished cytotoxicity. A botulin-derived conjugate held a K_i = 0.129 µM and an EC_{50} = 8.5 µM for human A375 melanoma cells.

4. Experimental

NMR spectra were recorded using the Varian spectrometers (Darmstadt, Germany) DD2 and VNMRS (400 and 500 MHz, respectively). MS spectra were taken on a Advion expressionL CMS mass spectrometer (Ithaca, USA; positive ion polarity mode, solvent: methanol, solvent flow: 0.2 mL/min, spray voltage: 5.17 kV, source voltage: 77 V, APCI corona discharge: 4.2 µA, capillary temperature: 250 °C, capillary voltage: 180 V, sheath gas: N2). Thin-layer chromatography was performed on pre-coated silica gel plates supplied by Macherey-Nagel (Düren, Germany). IR spectra were recorded on a Spectrum 1000 FT-IR-spectrometer from Perkin Elmer (Rodgau, Germany). The UV/Vis-spectra were recorded on a Lambda 14 spectrometer from Perkin Elmer (Rodgau, Germany); optical rotations were measured using a JASCO-P2000 instrument (JASCO Germany GmbH, Pfungstadt, Germany) The melting points were determined using the Leica hot stage microscope Galen III (Leica Biosystems, Nussloch, Germany) and are uncorrected. The solvents were dried according to usual procedures. Microanalyses were performed with an Elementar Vario EL (CHNS) instrument (Elementar Analysensysteme GmbH, Elementar-Straße 1, D-63505 Langenselbold, Germany). All dry solvents were distilled over respective drying agents except for DMF which was distilled and stored under argon and molecular sieve. Reactions using air- or moisture-sensitive reagents were carried out under argon atmosphere in dried glassware. Triethylamine was stored over potassium hydroxide. Biological assays were performed as previously reported employing cell lines obtained from the Department of Oncology [Martin-Luther-University Halle Wittenberg; they were bought from ATCC: malignant: A 375, HT29, MCF7, and A2780; non-malignant: NIH 3T3]. Oleanolic and ursolic acid were obtained from Betulinines (Strbrna Skalice, Czech Republic) and used as received. Glycyrrhetinic acid was bought from Orgentis Chemicals GmbH (Gatersleben).

For the SRB assay: cells were seeded into 96-well plates on day zero at appropriate cell densities to prevent confluence of the cells during the period of the experiment. After 24 h, the cells were treated with different concentrations (1, 3, 7, 12, 20, and 30 µM), but the final concentration of DMSO/DMF never exceeded 0.5%, which was non-toxic to the cells. After 72 h of treatment, the supernatant media from the 96-well plates were discarded, then the cells were fixed with 10% trichloroacetic acid and allowed to rest at 4 °C. After 24 h of fixation, the cells were washed in a strip washer and then dyed with SRB solution (200 µL, 10 mM) for 20 min. Then the plates were washed four times with 1% acetic acid to remove the excess of the dye and allowed to air-dry overnight. Tris base solution (200 µL, 10 mM) was added to each well. The absorbance was measured with a 96-well plate reader from Tecan Spectra.

For the CA II assay: Carbonic anhydrase II (bCA II, ≥3000 W-A units/mg from bovine erythrocytes) as well as 4-nitrophenyl acetate (4-NA) were purchased from Sigma.

A 96-well microplate spectrometer BMG Labtech Spectrostar Omega working in the slow kinetics mode and measuring the absorbance at a distinct wavelength of λ = 415 nm with center scanning was used for the enzymatic studies. In short: A mixture of 4-NA solution (125 µL, 6 mM in 50 mM Tris-HCl buffer, pH 8), enzyme solution (25 µL, 0.3 mg/mL), and compounds solutions (25 µL, 3 different concentrations and water as a blank) was incubated at 37 °C for 20 min. The substrate (25 µL, [4-NA] = 0.75 mM, 0.50 mM, 0.25 mM, 0.15 mM) was added to start the enzymatic reaction. The absorbance data was recorded under a controlled temperature of 37 °C for 30 min at 1 min intervals at λ = 415 nm. The relative inhibition was determined as the quotient of the slopes (compound divided by blank) of the linear ranges.

4.1. Di-O-Acetyl-betulin

Compound 1 was prepared from BN (15.0 g, 34 mmol) by acetylation with acetic anhydride as previously described; re-crystallization from ethanol gave 1 (15.8 g, 90%) as a white solid; R_F = 0.73 (silica gel, hexanes/ethyl acetate, 8:2); m.p.: 221 °C (lit.: [85] 216–218 °C); $[\alpha]_D$ = +16.6° (*c* = 0.061, MeOH), [lit.: [85] $[\alpha]_D$ = +19.7° ($CHCl_3$)]; MS (ESI, MeOH): *m*/*z* = 467.5 (100%, $[M + H\text{-}HOAc]^+$).

4.2. 3-O-Acetyl-betulin

Selective deacetylation of 1 (8.0 g, 15.2 mmol) with cat. amounts of CaH_2 in MeOH/THF (100 mL, 1:1 *v:v*) for 12 h at 20 °C followed by usual aqueous workup and chromatography (silica gel, hexanes/ethyl acetate, 8:2) gave 2 (6.1 g, 83%) as a colorless solid; R_F = 0.40 (silica gel, hexanes/ethyl acetate, 8:2); m.p.: 256–259 °C (lit.: [85] 258–260 °C); $[\alpha]_D$ = +28.8° (*c* = 0.039, $CHCl_3$), [lit.: [85] $[\alpha]_D$ = +25.7° ($CHCl_3$)]; MS (ESI, MeOH): *m*/*z* = 992,0 (100%, $[2M+Na]^+$).

4.3. 4-{[(3β)-3-(Acetyloxy)lup-20(29)-en-28-yl]oxy}-4-oxobutanoic Acid

To a solution of 2 (4.0 g, 8.2 mmol) in dry pyridine (50 mL), DMAP (cat.) and succinic anhydride (1.64 g, 16.4 mmol) were added. The reaction mixture was stirred under reflux for 15 h. Usual aqueous work up and chromatography (silica gel, hexanes/ethyl acetate, 7:3) gave 3 [86–91] (3.4 g, 71%) as a white solid; m.p. 189–191 °C (lit.: [86–88] 190–191 °C); $[\alpha]_D$ = +12.1° (*c* = 0.198, MeOH); R_F = 0.15 (silica gel, hexanes/ethyl acetate, 8:2); IR (ATR): ν = 2943*m*, 2871*w*, 1732*s*, 1713*s*, 1455*w*, 1361*w*, 1366*m*, 1244*s*, 1159*m*, 1027*w*, 979*m*, 883*w*, 754*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 4.68 (s, 1H, 29-H_a), 4.58 (s, 1H, 29-H_b), 4.46 (dd, J = 10.6, 5.6 Hz, 1H, 3-H_a), 4.30 (d, J = 11.0 Hz, 1H, 28-H_a), 3.88 (d, J = 11.1 Hz, 1H, 28-H_b), 2.75–2.54 (m, 4H, 34-H, 35-H), 2.42 (td, J = 11.0, 5.8 Hz, 1H, 19-H), 2.03 (s, 3H, 32-H), 2.01–1.88 (m, 1H, 21-H_a), 1.85–1.79 (m, 1H, 16-H_a), 1.75 (dd, J = 12.5, 7.9 Hz, 1H, 22-H_a), 1.68 (s, 3H, 30-H), 1.72–1.54 (m, 7H, 1-H_a, 13-H, 15-H_a, 12-H_a, 2-H, 9-H), 1.50 (s, 1H, 6-H_a), 1.44–1.35 (m, 5H, 6-H_b, 11-H_a, 21-H_b, 7-H), 1.34–1.14 (m, 3H, 16-H_b, 18-H, 11-H_b), 1.02 (s, 3H, 23-H), 1.12–0.90 (m, 4H, 22-H_b, 12-H_b, 15-H_b, 1-H_b), 0.96 (s, 3H, 27-H), 0.84 (s, 3H, 24-H), 0.84 (s, 3H, 26-H), 0.83 (s, 3H, 25-H), 0.78 (m, 1H, 5-H). ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 177.8 (C-36), 172.6 (C-33), 171.3 (C-31), 150.2 (C-20), 110.0 (C-29), 81.1 (C-3), 63.3 (C-28), 55.5 (C-5), 50.4 (C-18), 48.9 (C-9), 47.9 (C-19), 46.6 (C-17), 42.8 (C-14), 41.0 (C-8), 38.5 (C-1), 37.9 (C-4), 37.7 (C-13), 37.2 (C-10), 34.2 (C-22), 29.9 (C-16), 29.1 (C-35), 28.1 (C-24), 27.2 (C-15), 25.3 (C-12), 23.8 (C-2), 21.4 (C-32), 20.9 (C-11), 19.3 (C-30), 18.3 (C-6), 16.6 (C-25), 16.2 (C-23), 14.9 (C-27) ppm; MS (ESI, MeOH): *m*/*z* = 583.6 (100%, $[M-H]^-$); analysis calcd for $C_{36}H_{56}O_6$ (584.83): C 73.93, H 9.65; found: C 73.67, H 9.88.

4.4. 5-Amino-1,3,4-thiadiazole-2-sulfonamide

A solution of acetazolamide (4, 9.0 g, 40.7 mmol) in conc. HCl (60 mL) was heated under reflux for 3 h. After neutralization with NaOH, saturation with NaCl and extraction with THF (4 × 100 mL) followed by removal of the organic solvent, 5 (6.9 g, 94 %) was obtained as a white solid; m.p. 195 °C decomp. (lit.: [92] 215.5–216); R_F = 0.3 (silica gel, $CHCl_3$/MeOH, 9:1); UV-Vis (MeOH): λ_{max} (log ε) = 278 nm (3.80) IR (ATR): ν = 3427*w*, 3321*m*, 3173*w*, 2870*w*, 2636*w*, 1601*s*, 1496*s*, 1448*m*, 1338*s*, 1172*m*, 1139*m*, 1098*w*, 1058*w*, 941*m*, 647*s*, 581*s*, 484*w* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 8.04 (s, 2H, NH_2), 7.84 (s, 2H, NH_2) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 171.7, 157.9. ppm; MS (ESI, MeOH): *m*/*z* = 179.0 (100%, $[M-H]^-$).

4.5. (3β)-3-(Acetyloxy)lup-20(29)-en-28-yl 4-{[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]amino}-4-oxobutanoate

Compound 3 (500 mg, 0.85 mmol) was dissolved in dry THF (50 mL), 4-methylmorpholine (172 mg, 1.7 mmol) and ethyl chloroformate (185 mg, 1.7 mmol.) were added. The reaction mixture was stirred at 20 °C for 15 min. Compound 5 (184 mg, 1.02 mmol) was added, and the mixture was heated under reflux for another 48 h. The solvent was removed, the residue dissolved in $CHCl_3$, washed with 2 M NaOH, water and brine and dried ($MgSO_4$). Chromatography (silica gel, $CHCl_3$/MeOH, 9:1) gave 4 (560 mg, 88%) as a white solid; m.p. 161–164°C; R_F = 0.55 (silica gel, hexanes/ethyl acetate, 7:3); UV-Vis ($CHCl_3$): λ_{max} (log ε) = 264 nm (3.92) IR (ATR): ν = 2944*m*, 1733*m*, 1701*m*, 1531*w*, 1371*m*, 1245*s*, 1173*s*, 1018*w*, 979*m*, 882*w*, 609*m*, 504*w* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 8.30 (s, 2H, NH_2), 4.69 (s, 1H, 29-H_a), 4.55 (s, 1H, 29-H_b), 4.36 (dd, *J* = 11.4, 4.7 Hz, 1H, 3-H), 4.23 (d, *J* = 10.9 Hz, 1H, 28-H_a), 3.78 (d, *J* = 11.1 Hz, 1H, 28-H_b), 2.87–2.78 (m, 2H, 35-H), 2.76–2.65 (m, 2H, 34-H),

2.43 (s, 1H, 19-H), 1.99 (s, 3H, 32-H), 1.85 (m, 1H, 21-H_a), 1.63 (s, 3H, 30-H), 1.76–1.43 (m, 9H, 16-H_a, 22-H_a, 12-H_a, 13-H, 1-H_a, 9-H, 15-H_a, 2-H), 1.42–1.12 (m, 9H, 6-H, 11-H_a, 21-H_b, 7-H, 18-H, 16-H_b, 11-H_b), 0.95 (s, 3H, 23-H), 0.93 (s, 3H, 27-H), 1.09–0.73 (m, 5H, 22-H_b, 12-H_b, 1H_b, 15-H_b, 5-H), 0.79 (s, 9H, 24-H, 25-H, 26-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 171.9 (C-31), 171.3 (C-33), 170.1 (C-36), 164.3 (C-38), 161.0 (C-37), 149.8 (C-20), 110.0 (C-29), 79.9 (C-3), 61.9 (C-28), 54.6 (C-5), 49.5 (C-18), 48.1 (C-9), 47.0 (C-19), 46.0 (C-17), 42.2 (C-14), 40.4 (C-8), 37.4 (C-4), 37.0 (C-13), 36.6 (10), 34.2, 34.0 (22), 33.5 (7), 30.9 (34), 30.0 (35), 29.1 (16), 28.9, 28.5 (21), 27.6 (24), 26.6 (C-15), (C-12), 23.4 (C-2), 21.0 (C-32), 20.3 (C-11), 18.7 (C-30), 17.7 (C-6), 16.4 (C-25), 15.8 (C-26), 15.5 (C-23), 14.5 (C-27) ppm; MS (ESI, MeOH): *m/z* = 745.7 (100%, $[M-H]^-$); analysis calcd for $C_{38}H_{58}N_4O_7S_2$ (747.03): C 61.10, H 7.83, N 7.50; found: C 60.85, H 8.03, N 7.33.

4.6. (3β) Olean-12-en3-3,28-diol (Erythrodiol)

To a solution of OA (5.0 g, 10.7 mmol, 1.00 eq.) in dry THF (150 mL), $LiAlH_4$ (2.0 g, 53.6 mmol, 5.00 eq.,) was slowly added. Stirring under reflux was continued for another 2 h. After cooling to 20 °C, the reaction was quenched (slow addition of 20 mL MeOH), and aq. HCl (6 M, 50 mL) was added. The reaction mixture was extracted with ethyl acetate (3 × 75 mL); the combined organic phases were washed with aq. NaOH (1 M, 2 × 50 mL), brine (50 mL) and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residue was subjected to chromatography (silica gel, chloroform/hexanes/ethyl acetate, 10:8:2) to yield 7 (4.61 g, 97%) as a colorless solid; m.p. 218–219 °C (lit.: [93] 217–219 °C); R_F = 0.17 (silica gel, chloroform/hexanes/ethyl acetate, 10:8:2); $[\alpha]_D$ = +72.1° (*c* = 0.113, MeOH) (lit.: [94] $[\alpha]_D$ = +75.0° (*c* = 0.325, $CHCl_3$)).

4.7. (3β) Urs-12-ene-3,28-diol (Uvaol, 8)

Following the procedure given for the synthesis of 7, from UA (5.00 g, 10.7 mmol), dry THF (150 mL) and $LiAlH_4$ (2.0 g, 53.6 mmol) followed by chromatography (silica gel, (chloroform/hexanes/ethyl acetate, 10:8:2) 8 (4.36 g, 90%) was obtained as a colorless solid; m.p. 227–229 °C (lit.: [93] 225–227 °C); R_F = 0.18 (silica gel, chloroform/hexanes/ethyl acetate, 10:8:2); $[\alpha]_D$ = +60.5° (*c* = 0.109, MeOH); (lit.: [95] $[\alpha]_D$ = +62.6° (*c* = 0.62, $CHCl_3$)).

4.8. (3β)-Olean-12-ene-3,28-diyl Diacetate

Acetylation of 7 (4.00 g, 9.04 mmol) in dry pyridine (16 mL) with acetic anhydride (2.6 mL, 27.1 mmol) for 15 h at 20 °C followed by usual aqueous work up and chromatography (silica gel, (hexanes/ethyl acetate, 9:1) gave 9 (4.37 g, 92%) as a colorless solid; m.p. 184-186 °C (lit.: [96] 184-186 °C); R_F = 0.43 (silica gel, hexanes/ethyl acetate, 9:1); $[\alpha]_D$ = +62.2° (*c* = 0.124, $CHCl_3$); (lit.: [97] $[\alpha]_D$ = +56.0° (*c* = 1.0$CHCl_3$)).

4.9. (3β)-Urs-12-ene-3,28-diyl Diacetate

Acetylation of 8 (3.48 g, 7.86 mmol) as described above for the synthesis of 9 gave 10 (3.66 g, 88%) as a colorless solid; m.p. 151-153 °C (lit.: [98] 150–151 °C); R_F = 0.41 (silica gel, hexanes/ethyl acetate, 9:1); $[\alpha]_D$ = +51.4° (*c* = 0.115, $CHCl_3$).

4.10. (3β)-28-Hydroxyolean-12-en-3-yl Acetate

A solution of 9 (3.1 g, 5.89 mmol) and aluminum isopropoxide (12.3 g, 58.8 mmol) in isopropanol (150 mL) was heated under reflux for 4 h. Usual aqu. work-up followed by chromatography (silica gel, hexanes/ethyl acetate, 8:2) gave 11 (1.71 g, 60%) as a colorless solid; m.p. 234–236 °C (lit.: [99] 233–234 °C); R_F = 0.55 (silica gel, hexanes/ethyl acetate, 8:2); $[\alpha]_D$ = +67.4° (*c* = 0.122, $CHCl_3$) (lit.: [100] $[\alpha]_D$ = +71° (*c* = 0.70, $CHCl_3$)).

4.11. (3β)-28-Hydroxyolean-12-en-3-yl Acetate

As described above for the synthesis of 11, from 10 (2.7 g, 5.13 mmol) and aluminum propoxide (10.7 g, 51.3 mmol) in isopropanol (150 mL) followed by chromatography (silica gel, hexanes/ethyl acetate, 8:2) 12 (1.92 g, 77%) was obtained as a colorless solid;

m.p. 265–267 °C (lit.: [101] 258–261 °C); R_F = 0.51 (silica gel, hexanes/ethyl acetate, 8:2); $[\alpha]_D$ = +63.1° (*c* = 0.139, $CHCl_3$) (lit.: [102] $[\alpha]_D$ = +70.5° (*c* = 0.145, $CHCl_3$)).

4.12. 4-{[(3β)-3-(Acetyloxy)-olean-12-en-28-yl]oxy}-4-oxobutanoic Acid

To a solution of 11 (0.45 g, 0.928 mmol) in dry pyridine (15 mL) succinic anhydride (0.188 g, 1.86 mmol) and cat. DMAP were added, and the mixture was stirred for 1 day under reflux. Usual aq. work-up followed by chromatography (silica gel, hexanes/ethyl acetate (1% HCOOH), 8:2 → 7:3) gave 13 (0.460 g, 85%) as a colorless solid; m.p. 124–126 °C; R_F = 0.47 (silica gel, hexanes/ethyl acetate, 7:3); $[\alpha]_D$ = +49.6° (*c* = 0.126, $CHCl_3$); IR (ATR): ν = 2946*m*, 2864 *w*, 1734*s*, 1712*s*, 1463*w*, 1432*w*, 1387*m*, 1364*m*, 1244*s*, 1160*s*, 1095*w*, 1027*m*, 1004*m*, 986*m*, 967*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.01 (brs, 1H, COO-H), 5.19 (t, *J* = 3.6 Hz, 1H, 12-H), 4.55–4.44 (m, 1H, 3-H), 4.07 (d, *J* = 11.0 Hz, 1H, 28-H_a), 3.72 (d, *J* = 11.0 Hz, 1H, 28-H_b), 2.71–2.59 (m, 4H, 34-H + 35-H), 2.04 (s, 3H, 32-H), 2.01–1.99 (m, 1H, 18-H), 1.97–1.78 (m, 3H, 16-H_a + 11-H), 1.77–1.46 (m, 9H, 19-H_a + 15-H_a + 2-H + 1-H_a + 9-H + 6-H_a + 7-H_a + 22-H_a), 1.45–1.21 (m, 4H, 6-H_b + 22-H_b + 7-H_b + 21-H_a), 1.19–1.10 (m, 5H, 16-H_b + 21-H_b + 27-H), 1.12–0.95 (m, 3H, 19-H_b + 1-H_b + 15-H_b), 0.94 (s, 3H, 25-H), 0.93 (s, 3H, 26-H), 0.88 (s, 3H, 30-H), 0.86 (s, 6H, 29-H + 24-H), 0.85 (s, 3H, 23-H), 0.84–0.80 (m, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 177.9 (C-36), 172.2 (C-33), 171.3 (C-31), 143.7 (C-13), 123.0 (C-12), 81.1 (C-3), 71.3 (C-28), 55.4 (C-5), 47.6 (C-9), 46.3 (C-19), 42.7 (C-18), 41.8 (C-14), 39.9 (C-8), 38.4 (C-1), 37.8 (C-4), 36.9 (C-10), 36.0 (C-17), 34.1 (C-21), 33.3 (C-30), 32.6 (C-7), 31.6 (C-22), 31.0 (C-20), 29.1 (C-34), 29.1 (C-35), 28.2 (C-24), 26.1 (C-27), 25.7 (C-15), 23.7 (C-29), 23.7 (C-11), 23.7 (C-2), 22.3 (C-16), 21.4 (C-32), 18.4 (C-6), 16.8 (C-26), 16.8 (C-23), 15.7 (C-25) ppm; MS (ESI, MeOH/$CHCl_3$, 4:1): *m/z* = 583.9 (100%, $[M-H]^-$); analysis calcd for $C_{36}H_{56}O_6$ (584.84): C 73.93, H 9.65; found: C 73.71, H 9.86.

4.13. 4-{[(3β)-3-(Acetyloxy)urs-12-en-28-yl]oxy}-4-oxobutanoic Acid

Following the procedure given for 11, from 12 (1.35 g, 2.79 mmol), 14 (1.12 g, 69%) was obtained as a colorless solid; m.p. 112–114 °C; R_F = 0.50 (silica gel, hexanes/ethyl acetate, 7:3); $[\alpha]_D$ = +42.7° (*c* = 0.131, $CHCl_3$); IR (ATR): ν = 2948*m*, 2925*m*, 1734*s*, 1712*s*, 1456*m*, 1432*w*, 1388*m*, 1370*m*, 1269*m*, 1244*s*, 1158*s*, 1095*w*, 1025*m*, 1006*m*, 985*m*, 967*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.01 (*brs*, 1H, COO-H), 5.13 (dd, *J* = 3.7 Hz, 1H, 12-H), 4.53–4.44 (m, 1H, 3-H), 4.10 (d, *J* = 11.0 Hz, 1H, 28-H_a), 3.64 (d, *J* = 11.0 Hz, 1H, 28-H_b), 2.70–2.60 (m, 4H, 34-H + 35-H), 2.04 (s, 3H, 32-H), 1.98–1.88 (m, 2H, 16-H_a + 11-H), 1.76 – 1.68 (m, 1H, 15-H_a), 1.67–1.59 (m, 2H, 1-H_a + 2-H), 1.59–1.48 (m, 4H, 22-H_a + 7-H_a + 9-H + 6-H_a), 1.47–1.28 (m, 6H, 21-H_a + 18-H + 6-H_b + 19-H + 7-H_b + 22-H_b), 1.27–1.13 (m, 2H, 21-H_b + 16-H_b), 1.08 (s, 3H, 27-H), 1.08–1.06 (m, 1H, 1-H_b), 0.99–0.96 (m, 1H, 15-H_b), 0.97 (s, 3H, 26-H), 0.96 (s, 3H, 25-H), 0.93 (d, *J* = 5.8 Hz, 3H, 29-H), 0.91–0.88 (m, 1H, 20-H), 0.86 (s, 3H, 24-H), 0.86 (s, 3H, 23-H), 0.85–0.83 (m, 1H, 5-H), 0.80 (d, *J* = 5.3 Hz, 3H, 30-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 178.0 (C-36), 172.2 (C-33), 171.3 (C-31), 138.3 (C-13), 125.7 (C-12), 81.1 (C-3), 71.8 (C-28), 55.4 (C-5), 54.4 (C-18), 47.7 (C-9), 42.1 (C-14), 40.1 (C-8), 39.5 (C-20), 39.3 (C-19), 38.6 (C-1), 37.8 (C-17), 37.1 (C-4), 36.9 (C-10), 35.8 (C-22), 32.8 (C-7), 30.6 (C-21), 29.2 (C-34), 29.2 (C-35), 28.2 (C-24), 26.1 (C-15), 23.7 (C-2), 23.5 (C-11), 23.5 (C-16), 23.5 (C-27), 21.4 (C-32), 21.4 (C-29), 18.3 (C-6), 17.4 (C-30), 16.9 (C-26), 16.8 (C-23), 15.9 (C-25) ppm; MS (ESI, MeOH/$CHCl_3$, 4:1): *m/z* = 607.9 (100%, $[M + Na]^+$); analysis calcd for $C_{36}H_{56}O_6$ (584.84): C 73.93, H 9.65; found: C 73.68, H 9.91.

4.14. (3β, 20β)-3-Acetyloxy-11-oxoolean-12-en-29-oic Acid

Acetylation of GA (2.50 g, 5.31 mmol) as described above followed by chromatography (silica gel, hexanes/ethyl acetate, 8:2) gave 15 (2.15 g, 79%) as a colorless solid; m.p. 305–307 °C (lit.: [96] 316–318 °C); R_F = 0.41 (silica gel, hexanes/ethyl acetate, 9:1); $[\alpha]_D$ = +163.3° (*c* = 0.142, $CHCl_3$); (lit.: [103] $[\alpha]_D$ = +165.1° (*c* = 0.7, $CHCl_3$)).

4.15. (3β, 20β) 3-Acetyloxy-29-hydroxyolean-12-en-11-one

To a solution of 15 (1.3 g, 2.76 mmol) and triethylamine (1.1 mL, 7.60 mmol) in dry THF (15 mL), at −12 °C, ethyl chloroformate (1.1 mL, 11.1 mmol) was added, and the mixture was stirred for 15 min. The precipitate was filtered off, and the filtrate was slowly added to a freshly prepared solution of sodium borohydride (0.522 g, 13.8 mol) in water (2.5 mL). Stirring at room temperature was continued for another 15 min followed by usual aq. work-up and chromatography (silica gel, $CHCl_3/Et_2O$/hexanes/HCOOH, 25:25:43:7) to yield 16 (1.09 g, 82%) as a colorless solid; m.p. 264–266 °C; R_F = 0.46 (silica gel, $CHCl_3/Et_2O$/hexanes/HCOOH, 25:25:43:7); $[\alpha]_D$ = +91.6° (*c* = 0.129, $CHCl_3$); UV-Vis ($CHCl_3$): λ_{max} (log ε) = 249 nm (4.07); IR (ATR): ν = 3569*w*, 3550 *w*, 2925*m*, 2862*w*, 1725*s*, 1695*m*, 1651*s*, 1626*m*, 1465*w*, 1455*m*,1386*m*, 1366*m*, 1325*w*, 1279*w*, 1246*s*, 1209*m*, 1173*s*, 1143*m*, 1095*w*, 1048*m*, 1025*s*, 1001*m*, 985*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.58 (s, 1H, 12-H), 4.50 (dd, *J* = 11.8, 4.7 Hz, 1H, 3-H), 4.13 (d, *J* = 11.0 Hz, 1H, 30-H_a), 4.03 (d, *J* = 11.0 Hz, 1H, 30-H_b (30)), 2.78 (dt, *J* = 13.6, 3.6 Hz, 1H, 1-H_a), 2.35 (s, 1H, 9-H), 2.13–2.06 (m, 2H, 18-H + 16-H_a), 2.04 (s, 3H, 32-H), 1.86–1.76 (m, 1H, 15-H_a), 1.74–1.53 (m, 7H, 2-H_a + 22-H_a + 7-H_a + 19-H_a + 2-H_b + 21-H_a + 6-H_a) 1.50–1.36 (m, 3H, 6-H_b + 7-H_b + 22-H_b + 19-H_b), 1.36 (s, 3H, 27-H), 1.21– 1.16 (m, 1H, 15-H_b), 1.15 (s, 3H, 25-H), 1.12 (s, 3H, 26-H), 1.07–0.98 (m, 2H, 1-H_b + 16-H_b), 0.95 (s, 3H, 29-H), 0.87 (s, 6H, 28-H + 23-H), 0.86 (s, 3H, 24-H), 0.81–0.77 (m, 1H, 5-H), 0.80 (d, *J* = 5.7 Hz, 3H, 30-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 200.4 (C-11), 171.4 (C-31), 169.8 (C-13), 128.5 (C-12), 80.9 (C-3), 67.0 (C-30), 61.8 (C-9), 55.2 (C-5), 47.0 (C-18), 45.6 (C-8), 43.5 (C-14), 40.2 (C-19), 38.9 (C-1), 38.2 (C-4), 37.1 (C-10), 36.0 (C-22), 34.3 (C-20), 32.8 (C-7), 32.4 (C-17), 30.2 (C-21), 28.6 (C-28), 28.2 (C-24), 27.9 (C-29), 26.7 (C-16), 26.5 (C-15), 23.7 (C-2), 23.5 (C-27), 21.4 (C-32), 18.8 (C-26), 17.5 (C-6), 16.8 (C-23), 16.5 (C-25) ppm; MS (ESI, MeOH/$CHCl_3$, 4:1): *m/z* = 497.9 (100%, $[M-H]^-$); analysis calcd for $C_{32}H_{50}O_4$ (498.74): C 77.06, H 10.10; found: C 76.81, H 10.35.

4.16. 4-{[(3β,20β)3-(Acetyloxy)-11-oxoolean-12-en-30-yl]oxy}-4-oxobutanoic Acid

Following the procedure described above, from 16 (0.270 g, 0.541 mmol) 17 (0.315 g, 97%) was obtained as a colorless solid; m.p. 109–111 °C; R_F = 0.44 (silica gel, hexanes/ethyl acetate, 1:1); $[\alpha]_D$ = +110.5° (*c* = 0.118, $CHCl_3$); UV-Vis ($CHCl_3$): λ_{max} (log ε) = 254 nm (4.05); IR (ATR): ν = 2949*m*, 2928*m*, 2871*w*, 1730*s*, 1657*m*, 1465*w*, 1456*w*, 1388*m*, 1365*m*, 1322*w*, 1244*s*, 1209*m*, 1158*m*, 1091*w*, 1049*w*, 1028*m*, 1001*m*, 986 *m* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.01 (*brs*, 1H, COO-H), 5.64 (s, 1H, 12-H), 4.70 (d, *J* = 10.9 Hz, 1H, 30-H_a), 4.51 (dd, *J* = 11.5, 4.9 Hz, 1H, 3-H), 3.46 (d, *J* = 10.9 Hz, 1H, 30-H_b), 2.85 (dt, *J* = 13.6, 3.5 Hz, 1H, 1-H_a), 2.79–2.71 (m, 2H, 34-H), 2.58–2.49 (m, 2H, 35-H), 2.37 (s, 1H, 9-H), 2.36–2.25 (m, 1H, 18-H), 2.12–2.06 (m, 1H, 16-H_a), 2.04 (s, 3H, 32-H), 1.87–1.75 (m, 1H, 15-H_a), 1.74–1.36 (m, 9H, 2-H + 7-H_a + 6-H_a + 19-H_a + 6-H_b + 22-H_a + 7-H_b + 21-H_a), 1.35 (s, 3H, 27-H), 1.33–1.14 (m, 4H, 21-H_b + 22-H_b + 19-H_b + 16-H_b), 1.12 (s, 6H, 25-H + 26-H), 1.10– 0.98 (m, 2H, 1-H_b + 15-H_b), 0.95 (s, 3H, 29-H), 0.87 (s, 9H, 28-H + 23-H + 24-H), 0.83–0.77 (m, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 202.2 (C-11), 174.8 (C-36), 172.9 (C-33), 171.8 (C-13), 171.1 (C-31), 128.1 (C-12), 80.7 (C-3), 67.7 (C-30), 61.9 (C-9), 55.2 (C-5), 46.7 (C-18), 45.5 (C-8), 43.4 (C-14), 39.2 (C-19), 39.0 (C-1), 38.3 (C-4), 37.4 (C-10), 36.1 (C-22), 34.9 (C-20), 32.7 (C-7), 32.3 (C-17), 31.3 (C-21), 29.5 (C-35), 28.9 (C-34), 28.9 (C-28), 28.2 (C-24), 28.2 (C-29), 26.6 (C-16), 26.6 (C-15), 23.7 (C-2), 23.3 (C-27), 21.4 (C-32), 19.0 (C-26), 17.5 (C-6), 16.8 (C-23), 16.7 (C-25) ppm; MS (ESI, MeOH/$CHCl_3$, 4:1) *m/z* = 600.0 (96%, $[M + H]^+$); analysis calcd for $C_{36}H_{54}O_7$ (598.82): C 72.21, H 9.09; found: C 71.97, H 9.32.

4.17. (3β) 3-(Acetyloxy)olean-12-en-28-yl-4-{[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]amino}-4-oxobutanoate

To a solution of 13 (0.250 g, 0.427 mmol) in dry THF (20 mL) at −15 °C, 4-methylmorpholine (0.1 mL, 0641 mmol) and ethyl chloroformate (0.05 mL, 0.513 mmol) were added, and the mixture was stirred for 10 min at this temperature. Then 5-amino-1,3,4-thiadiazole-2-sulfonamide 5 (0.092 g, 0.513 mmol) was added, and the mixture was heated under reflux for 4 h. The solvents were removed under diminished pressure, and the residue was

subjected to chromatography (silica gel, ($CHCl_3$/MeOH, 95:5) to yield 18 (0.166 g, 52%) as a colorless solid; m.p. 198–200 °C; R_F = 0.36 (silica gel, $CHCl_3$/MeOH, 9:1); $[\alpha]_D$ = +19.2° (*c* = 0.122, $CHCl_3$); UV-Vis ($CHCl_3$): λ_{max} (log ε) = 262 nm (4.45); IR (ATR): ν = 3332*w*, 3234*w*, 2947*m*, 2864*m*, 1734*m*, 1704*s*, 1545*m*, 1536*m*, 1463*w*, 1432*w*, 1422*w*, 1371*s*, 1329*m*, 1245*s*, 1216*m*, 1173*s*, 1092*m*, 1027*m*, 1004*m*, 985*m*, 967*m*, 755*m*, 651*m*, 637*m*, 606 *s*, 504*m* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 13.07 (*brs*, 1H, CON-H), 8.30 (d, *J* = 6.2 Hz, 2H, SN-H), 5.15 (t, *J* = 3.7 Hz, 1H, 12-H), 4.38 (dd, *J* = 11.7, 4.5 Hz, 1H, 3-H), 3.96 (d, *J* = 10.9 Hz, 1H, 28-H_a), 3.60 (d, *J* = 10.9 Hz, 1H, 28-H_b), 2.89–2.76 (m, 2H, 34-H), 2.72–2.65 (m, 2H, 35-H), 2.02–1.97 (m, 1H, 18-H), 1.99 (s, 3H, 32-H), 1.86 (*td*, *J* = 13.9, 4.6 Hz, 1H, 16-H_a), 1.82–1.75 (m, 2H, 11-H), 1.70 (dd, *J* = 13.4, 13.4 Hz, 1H, 19-H_a), 1.62–1.13 (m, 11H, 15-H_a + 2-H + 1-H_a + 9-H + 6-H_a + 21-H_a + 22-H_a, 6-H_b + 22-H_b + 7-H_b), 1.11 (s, 3H, 27-H), 1.09–0.93 (m, 4H, 16-H_b + 7-H_b + 19-H_b + 1-H_b), 0.88 (*s*, 3H, 25-H), 0.85 (d, 3H, 30-H), 0.84 (d, 3H, 29-H), 0.83 (s, 3H, 24-H), 0.83–0.80 (m, 7H, 26-H + 23-H + 5-H); ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 171.6 (C-33), 171.2 (C-36), 170.1 (C-31), 164.3 (C-38), 161.0 (C-37), 143.4 (C-13), 122.2 (C-12), 79.9 (C-3), 70.0 (C-28), 54.5 (C-5), 46.7 (C-9), 45.7 (C-19), 41.8 (C-18), 41.1 (C-14), 39.2 (C-8), 37.7 (C-1), 37.3 (C-4), 36.3 (C-10), 35.4 (C-17), 33.4 (C-7), 32.9 (C-30), 31.8 (C-21), 31.0 (C-22), 30.5 (C-20), 30.0 (C-34), 28.4 (C-35), 27.7 (C-24), 25.7 (C-27), 25.1 (C-15), 23.4 (C-29), 23.2 (C-2), 23.0 (C-11), 21.5 (C-16), 20.9 (C-32), 17.7 (C-6), 16.6 (C-26), 16.2 (C-23), 15.2 (C-25) ppm; MS (ESI, MeOH/$CHCl_3$, 4:1): *m/z* = 770.1 (100%, $[M + Na]^+$); analysis calcd for $C_{38}H_{58}S_2N_4$ (747.02): C 61.10, H 7.83, N 7.50; found: C 60.81, H 8.03, N 7.39.

4.18. (3β)-3-(Acetyloxy)urs-12-en-28-yl-4-{[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]amino} 4-oxobutanoate

Following the procedure given above for the synthesis of 18, from 14 (0.250 g, 0.427 mmol) 21 (0.217 g, 68%) was obtained as a colorless solid; m.p. 190–192 °C; R_F = 0.33 (silica gel, $CHCl_3$/MeOH, 9:1); $[\alpha]_D$ = +27.4° (*c* = 0.129, $CHCl_3$); UV-Vis ($CHCl_3$): λ_{max} (log ε) = 263 nm (4.98); IR (ATR): ν = 3244*w*, 2948*m*, 2925*m*, 2871*m*, 1733*m*, 1705*s*, 1531*m*, 1457*w*, 1431*w*; 1414*w*, 1367*s*, 1245*s*, 1174*s*, 1094*m*, 1027*m*, 1006*m*, 985*m*, 967*m*, 655*m*, 603*s*, 508*m* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 13.07 (*brs*, 1H, CON-H), 8.30 (s, *J* = 2H, SN-H), 5.10 (t, *J* = 3.2 Hz, 1H, H-12), 4.38 (dd, *J* = 11.5, 4.7 Hz, 1H, H-3), 3.94 (d, *J* = 10.8 Hz, 1H, 28-H_a), 3.54 (d, *J* = 10.9 Hz, 1H, 28-H_b), 2.84–2.78 (m, 2H, 34-H), 2.71–2.65 (m, 2H, 35-H), 1.99 (s, 3H, 32-H), 1.92–1.80 (m, 3H, 16-H_a + 11-H), 1.65–1.53 (m, 3H, 15-H_a + 2-H_a + 1-H_a), 1.53–1.42 (m, 5H, 2-H_a + 9-H + 7-H_a + 6-H_a + 22-H_a), 1.40–1.06 (m, 7H, 18-H + 6-H_b + 21-H_a + 22-H_b + 7-H_b + 21-H_b + 16-H_b), 1.04 (s, 3H, 27-H), 1.01–0.96 (m, 1H, 1-H_b), 0.90 (s, 3H, 23-H), 0.87 (d, *J* = 6.7, 3H, 29-H), 0.86 (s, 3H, 26-H), 0.85–0.83 (m, 2H, 15-H_b + 1-H_b), 0.83 (s, 6H, 24-H + 25-H), 0.83–0.80 (m, 1H, 20-H), 0.87 (d, *J* = 5.3 Hz, 3H, 30-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 171.4 (C-33), 171.2 (C-36), 170.1 (C-31), 164.3 (C-38), 161.0 (C-37), 138.0 (C-13), 124.8 (C-12), 79.9 (C-3), 70.4 (C-28), 54.5 (C-5), 53.4 (C-18), 46.8 (C-9), 41.4 (C-14), 39.4 (C-8), 38.7 (C-20), 38.5 (C-19), 37.9 (C-1), 37.3 (C-4), 36.4 (C-17), 36.3 (C-10), 35.1 (C-2), 32.1 (C-7), 30.0 (C-34), 29.9 (C-21), 28.4 (C-35), 27.7 (C-24), 25.5 (C-15), 23.3 (C-2), 23.0 (C-27), 22.9 (C-11), 22.8 (C-16), 21.0 (C-29), 20.9 (C-32), 17.1 (C-6), 17.1 (C-30), 16.6 (C-23), 16.3 (C-26), 15.2 (C-25) ppm; MS (ESI, MeOH/$CHCl_3$, 4:1): *m/z* = 770.1 (100%, $[M + Na]^+$); analysis calcd for $C_{38}H_{58}S_2N_4O_7$ (747.02): C 61.10, H 7.83, N 7.50; found: C 60.89, H 8.07, N 7.36.

4.19. (3β, 20β) 3-(Acetyloxy)-11-oxoolean-12-en-30-yl-4-{[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl] amino}-4-oxobutanoate

Following the procedure given above for the synthesis of 18, from 17 (0.250 g, 0.417 mmol) 20 (0.215 g, 68%) was obtained as a colorless solid; m.p. 179–181 °C; R_F = 0.41 (silica gel, $CHCl_3$/MeOH, 9:1); $[\alpha]_D$ = +83.5° (*c* = 0.114, $CHCl_3$); UV-Vis ($CHCl_3$): λ_{max} (log ε) = 256 nm (4.25); IR (ATR): ν = 3252*w*, 2950*m*, 2872w, 1727*m*, 1709*m*, 1643*m*, 1528*m*, 1466*w*, 1455*w*, 1365*s*, 1323*m*, 1247*s*, 1215*m*, 1173*s*, 1088*w*, 1049*w*, 1028*m*, 1001*m*, 985*m*, 754*s*, 667*m*, 654*m*, 604*s*, 509*m* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 13.08 (*brs*, 1H, CON-H), 8.30 (s, *J* = 2H, SN-H), 5.48 (s, 1H, 12-H), 4.42 (dd, *J* = 11.8, 4.5 Hz, 1H, 3-H), 4.05 (d, *J* = 11.0 Hz, 1H, 30-H_a), 3.92 (d, *J* = 11.0 Hz, 1H, 30-H_b), 2.82 (dd, *J* = 7.5, 5.5 Hz 2H, 35-H),

2.71 (dd, J = 7.6, 5.5 Hz, 2H, 34-H), 2.61 (dt, J = 13.4, 3.6 Hz, 1H, 1-H_a), 2.38 (s, 1H, 9-H), 2.16–2.04 (m, 2H, 18-H + 16-H_a), 2.00 (s, 3H, 32-H), 1.79–1.35 (m, 7H, 16-H_b + 7-H_a + 19-H_a + 2-H_a + 6-H_a + 2-H_b + 6-H_b). 1.34 (s, 3H, 27-H), 1.34–1.31 (m, 3H, 7-H_b + 21-H_b + 22-H_b), 1.24–1.06 (m, 4H, 19-H_b + 22-H_b + 15-H_b + 1-H_b), 1.06 (*s*, 1H, 25-H) 1.04 (s, 1H, 26-H)), 0.86– 0.98 (m, 2H, 15-H_b + 5-H), 0.85 (s, 3H, 29-H), 0.82 (s, 6H, 23-H + 24-H), 0.80 (s, 3H, 28-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 198.9 (C-11), 171.8 (C-36), 171.1 (C-33), 170.1 (C-31), 170.0 (C-13), 127.4 (C-12), 79.7 (C-3), 67.0 (C-30), 60.8 (C-9), 53.7 (C-5), 46.0 (C-18), 44.9 (C-8), 43.0 (C-14), 39.7 (C-19), 37.8 (C-1), 37.5 (C-4), 36.5 (C-10), 35.4 (C-22), 34.0 (C-20), 31.9 (C-7), 31.8 (C-17), 29.9 (C-35), 29.3 (C-21), 28.3 (C-34), 28.1 (C-28), 27.7 (C-24), 27.2 (C-29), 25.9 (C-16), 25.9 (C-15), 23.2 (C-2), 23.1 (C-27), 20.9 (C-32), 18.3 (C-26), 16.9 (C-6), 16.6 (C-23), 16.1 (C-25) ppm; MS (ESI, MeOH/$CHCl_3$, 4:1): m/z = 784.0 (100%, $[M + Na]^+$; analysis calcd for $C_{38}H_{56}S_2N_4O_8$ (761.00): C 59.97, H 7.42, N 7.36; found: C 59.71, H 7.71, N 7.09.

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