SYNTHESE UND BIOLOGISCHE EVALUIERUNG VON NATURSTOFFDERIVATEN

Dissertation

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Frau Dipl.-LebChem. Sophie Hoenke

Gutachter:

1. Prof. Dr. René Csuk (Martin-Luther-Universität Halle-Wittenberg)
2. Prof. Dr. Dieter Schinzer (Otto-von-Guericke Universität Magdeburg)

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Vorwort

Die vorliegende Arbeit entstand im Zeitraum von August 2018 bis September 2022 am Institut für Chemie im Bereich Organische Chemie der Naturwissenschaftlichen Fakultät II der Martin-Luther-Universität Halle-Wittenberg im Arbeitskreis von Prof. Dr. René Csuk. Diese Dissertation wurde in kumulativer Form angefertigt. Die Forschungsergebnisse sind bereits in internationalen englischsprachigen *‚peer-reviewed*‘ Fachzeitschriften erschienen. Dort sind alle experimentellen Daten, die Ergebnisse und deren Diskussion nachzulesen.

Im Mittelpunkt dieser Dissertation stehen die Einordnung der Forschungsergebnisse, sowie eine übergreifende Diskussion der wissenschaftlichen Veröffentlichungen.

Danksagung

Der geschriebenen Arbeit möchte ich einige Worte des Dankes voranstellen.

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Abkürzungsverzeichnis

A2780	Zelllinie: Ovarienkarzinom
A375	Zelllinie: Melanom
ABC(-Transporter)	*ATP binding cassette* – ATP-bindende-Kassette(-Transporter)
AG	Arbeitsgruppe
(aktivierte) B-Zellen	(aktivierte) B-Lymphozyten
Bax	*Bcl-2-associated X protein* – Bcl-2-assoziiertes X-Protein
Bcl-2	*B-cell lymphoma 2* – B-Zellen-Lymphom 2
BODIPY	Bordipyrromethen
B.Sc.	*Bachelor of Science* – Bachelor der Naturwissenschaften
BSH	BioSolutions Halle GmbH
bzw.	beziehungsweise
Caspase	*cysteinyl-aspartate specific protease* – Cysteinyl-Aspartat-spezifische Protease
CDDO	*2-cyano-3,12-dioxo-oleana-1,9(11)-dien-28-oic acid*
CDK	*cyclin dependent kinase* – cyclinabhängige Kinase
CT	Computertomographie
CTC	*circulating tumor cells* – zirkulierende Tumorzellen
d.h.	das heißt
Dipl.-LebChem.	Diplom-Lebensmittelchemiker
DMSO	Dimethylsulfoxid
DNA	*deoxyribonucleic acid* – Desoxyribonukleinsäure
DOI	*Digital Object Identifier* – Digitaler Objektbezeichner
Dr. rer. nat.	*doctor rerum naturalium* – Doktor der Naturwissenschaften

EC_{50}	mittlere Effektkonzentration
ER	Endoplasmatisches Retikulum
ESI-MS	Elektrosprayionisation-Massenspektrometrie
et al.	*et alii/ et aliae* – und andere
FACS	*fluorescence-activated cell sorter* - Durchflusszytometer
FaDu	Zelllinie: Pharynxkarzinom
^{18}FDG	2-Fluor-2-desoxy-D-glukose, mit ^{18}Fluor markiert
FITC	Fluoresceinisothiocyanat
G_1/G_0-/ G2-Phase	$Gap_{1/0}$-/ Gap_2-Phase – Lücke (zwischen Synthese- oder Zellteilungsphase)
HeLa	Zelllinie: Zervixkarzinom (Henrietta Lacks)
HT29	Zelllinie: Dickdarmkarzinom
IACR	*International Agency for Research on Cancer* - Internationale Agentur für Krebsforschung
IR	Infrarot
ISC	*intersystem crossing*- Intersystem Crossing
Laser	*light amplification by stimulated emission of radiation* – Licht-Verstärkung durch stimulierte Emission von Strahlung
LED	*light-emitting diode* – lichtemittierende Diode
M-Phase	Mitose-Phase
MCF-7	Zelllinie: Brustkarzinom
Me	Methyl
µM	Mikromolar µmol/L (Mikromol pro Liter)
MRT	Magnetresonanztomographie
M.Sc.	*Master of Science* – Master der Naturwissenschaften
NF-κB	nuklearer Transkriptionsfaktor (κ-Ketten-Verstärker der aktivierten B-Zellen)
NIH 3T3	Zelllinie: nicht-maligne Mausfibroblasten
NIR	nahes Infrarot
nm	Nanometer
NMR	*Nuclear Magnetic Resonance* – Kernspinresonanz
p53-Signalweg	Protein-53-Signalweg
P	Publikation
PD	Photodiagnose
PDT	Photodynamische Therapie
PET	Positronen-Emissions-Tomographie

pH	-log(c(H^+)) (negativer dekadischer Logarithmus der Protonenkonzentration)
Prof.	Professor
PS	Photosensibilisator
Rb-Signalweg	Retinoblastom-Protein-Signalweg
RNA	*ribonucleic acid* – Ribonukleinsäure
ROS	Reaktive Sauerstoffspezies
RTK/Ras/PI3K-Signalwege	Rezeptor-Tyrosinkinasen/ Protein *rat sarcoma*/ Phosphoinositid-3-Kinasen-Signalwege
S-Phase	Synthesephase
SRB	Sulforhodamin B
subG_1-Phase	unterhalb der G_1(/G_0)-Phase
TCA	*trichloroacetic acid* – Trichloressigsäure
TRIS-Puffer	Tris(hydroxymethyl)aminomethan-Puffer
UV	Ultraviolett
UV/vis(-Spektroskopie)	UV/*visible* – ultraviolett/sichtbar (Spektroskopie)
v.a.	vor allem
z.B.	zum Beispiel
z.T.	zum Teil

1 Einleitung

„*Wodurch unterscheidet sich der Stoffwechsel wachsenden Gewebes von dem Stoffwechsel ruhenden Gewebes?*“ fragte Otto Warburg schon in den Zwanzigerjahren des letzten Jahrhunderts in Bezug auf Krebs. [1] Warburg befasste sich eingehend mit dieser Krankheit und erkannte, dass sie viele Ursachen hat und auch mit einem veränderten Stoffwechsel einhergeht. [2]

Durch Forschungen wie der Warburgs sowie verbesserten Lebensstandard hat es die Menschheit im letzten Jahrhundert zu einer gesünderen und älter werdenden Gesellschaft gebracht. Fortschritte in den Therapiemethoden, angepasste Impfstoffe und Medikamente ermöglichen es, viele Krankheiten gut zu behandeln oder in vielen Fällen sogar zu heilen. Allerdings gibt es nach wie vor Erkrankungen, für die es noch keinen erfolgreichen Behandlungsansatz gibt. Zu den häufigsten – sogenannten modernen – Krankheiten weltweit mit vielen Todesopfern zählt Krebs. [3,4,5] Abbildung 1 zeigt für das Jahr 2020 die weltweiten Zahlen an Krebsneudiagnosen. Auch in Deutschland gibt es schätzungsweise jährlich fast eine halbe Million Neuerkrankte und 250.000 Sterbefälle mit der Diagnose Krebs. [6] Die vielschichtigen Ursachen und Veränderungen im Tumorgewebe sind Bestandteil der Forschung, um neue und gezieltere Behandlungsmöglichkeiten zu entwickeln.

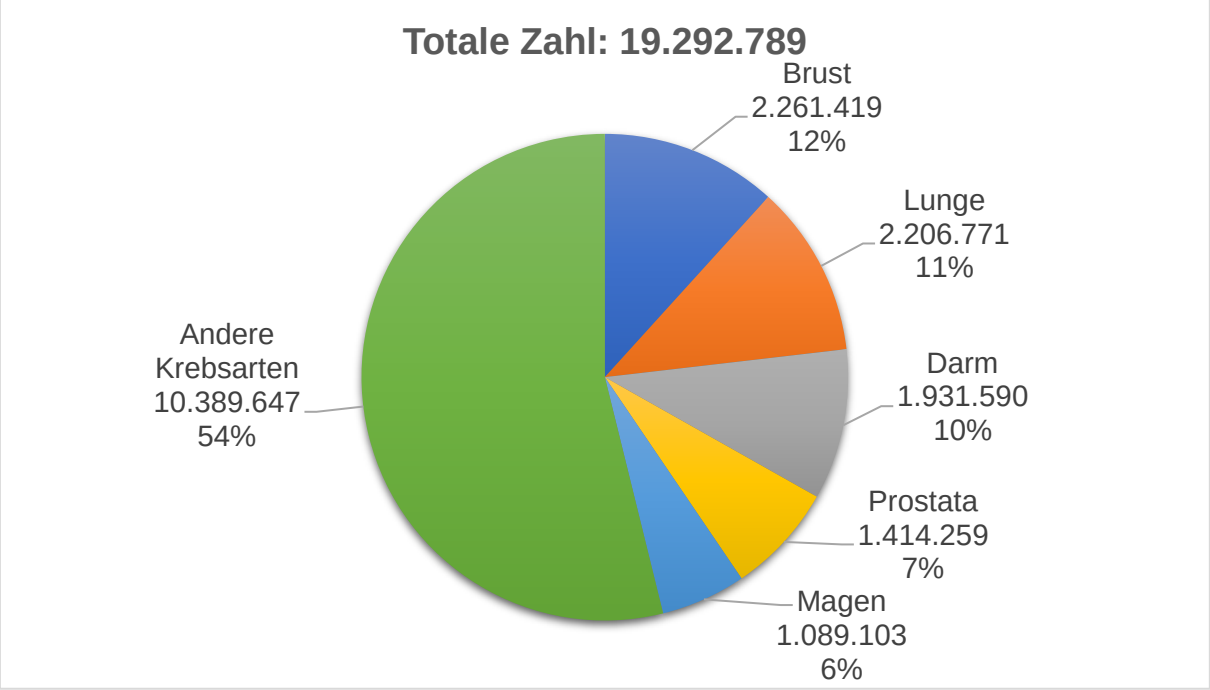


Abbildung 1 Weltweite Krebsfallzahlen im Jahr 2020. [4]

Geschichtliche Berichte belegen, dass Krebs schon vor über 2000 Jahren von Hippokrates beschrieben wurde und von ihm seinen Namen erhielt, weil die Form der von ihm untersuchten Tumore an das gleichnamige Krustentier erinnert. Noch frühere Beschreibungen von Geschwülsten wurden auf Papyri entdeckt, was die lange Geschichte von Krebserkrankungen

beim Menschen belegt. [7] Heute versteht man darunter eine Vielzahl von Erkrankungen von Gewebe, das durch fortlaufende Veränderungen im Genom maligne Tumore ausbildet, was auch als Neoplasie bezeichnet wird. Ein sogenannter gutartiger (benigner) Tumor, der ebenfalls unkontrolliert wächst und umgebendes Gewebe dadurch verdrängt, unterscheidet sich von einem bösartigen (malignen) Tumor insofern, dass letzterer invasives Wachstum aufweist. Krebs entsteht, wenn Zellen sich ungehindert vermehren können, d.h. auf Kosten der Umgebung wachsen, sich teilen und in Normalgewebe der Umgebung oder als Metastasen im gesamten Organismus eindringen. [8–11] Dabei unterscheidet man nach dem Ursprungsgewebe der Entartung zwischen Karzinomen aus Epithelzellen, Sarkomen aus Bindegewebs- oder Muskelzellen sowie weiteren Krebsformen wie z.B. Leukämie oder Lymphome. [10]

1.1 Die Krankheit Krebs

1.1.1 Entstehung

Für die Entstehung von Tumoren sind viele kaskadische Mutationen nötig. Durch schnelleres Wachstum oder weniger Einschränkungen bilden sich Krebszellen und deren Klone. Die Unabhängigkeit von Wachstumsfaktoren und Umgebungssignalen geben den Krebszellen einen selektiven Vorteil. [5] Die Vielzahl der nötigen Mutationen erklärt die Häufung von Krebsneuerkrankungen mit zunehmendem Alter, wie die Zahlen auch für Deutschland belegen: zwei Drittel der Neuerkrankten sind älter als 65 Jahre. [12]

Neben vererbbaren Veranlagungen, die allein keinen Krebs auslösen, dessen Entstehung aber begünstigen, zählen Umwelteinflüsse, wie beispielsweise UV-Strahlung (Ultraviolett), Luftverschmutzung und kanzerogene Stoffe zu den Promotoren von Krebs. Es ist zu beobachten, dass Krebs meist erst nach einem längeren Zeitraum entsteht, was eine weitere Erklärung für die Vielzahl von Veränderungen ist, die aufeinanderfolgen müssen, um zur Krebsbildung zu führen. Persönliche Angewohnheiten können das Krebsrisiko steigern. Belegbar ist ein direkter Zusammenhang zwischen dem Tabak- und Alkoholkonsum und diversen Krebsarten, hauptsächlich im Atemweg und Rachenraum; d.h. ein Verzicht senkt das Risiko deutlich. [5,6,12–14]

Die IARC (*International Agency for Research on Cancer*) hat zum persönlichen Vorbeugen einen Kodex mit wichtigen Hinweisen erarbeitet. [15] Auch Ernährung und Bewegung können über entzündliche Vorgänge einen Einfluss auf das Krebsrisiko haben, da die meisten Mutationen und Veränderungen im Stoffwechsel durch chronische Entzündungen entstehen; diese sind über eine Anpassung der Gewohnheiten größtenteils vermeidbar. [5,6,12,13]

1.1.2 Merkmale

Zu den wichtigsten Merkmalen von Tumoren zählt das unkontrollierte Wachstum von Gewebe durch vermehrte Proliferation bzw. Zellteilung und Unterdrückung von Apoptose, dem gezielten Zelltod (siehe unten). [9] Ausgelöst wird dies durch Mutationen bestimmter Gene: der Proto-Onkogene, die das Wachstum regulieren, und Tumorsuppressorgene. Bei ersteren fördern Mutationen über die Bildung der Onkogene und deren Überaktivierung das Krebsrisiko; bei letzteren können die Mutationen die Genfunktionen hemmen, was entsprechend die Tumorbildung vorantreibt. [10,16]

Die meisten Mutationen betreffen Kontrollpunkte und Signalwege von Zellzyklus und Apoptose sowie den Metabolismus der Zelle. [10,17] Folglich fehlt die Regulation in den Zellen aufgrund der Vermeidung von Wachstumssuppressoren und der Aufrechterhaltung von Wachstumssignalen. [8] Die häufigsten betroffenen Wege sind der Rb-Signalweg (Riboblastom), der p53-Signalweg (Protein53) und der RTK/Ras/PI3K-Signalweg (Rezeptor-Tyrosinkinasen/ Protein *rat sarcoma*/ Phosphoinositid-3-Kinasen), d.h., dass die Regulation für die wichtigsten Kontrollwege der Zellfunktion verändert oder außer Kraft gesetzt werden. Durch Entzündungen wird in den meisten Krebsfällen der NF-κB (nuklearer Transkriptionsfaktor (κ-Ketten-Verstärker der aktivierten B-Zellen)) verändert, was über dessen Aktivierung die Krebsentstehung vorantreibt. [5,11,12]

Für das Wachstum des Tumors und dessen Ausbreitung ist die Nährstoffversorgung wichtig, was die Zellen durch die Bildung neuer Blutgefäße – der Angiogenese – gewährleisten. [10,18] Die erhöhte Glukoseversorgung ist auch für den veränderten Glukosestoffwechsel – den Warburg-Effekt – nötig, der als Energie- und Kohlenstoffquelle für die Biosynthese weiterer Zellbestandteile sorgt. [19,20] Durch weitere Signale an umgebenden Zellen und Blutgefäße können Tumorzellen die Metastasierung steuern und somit invasiv auf den Körper wirken. [17,10]

1.1.2.1 Apoptose

Neben Proliferation spielt auch Apoptose eine wichtige Rolle für eine funktionierende Zelle. [10] Unter Apoptose wird im Allgemeinen eine Art des programmierten Zelltodes verstanden. Die Anfänge der Untersuchungen liegen bei Lockshin *et al.* [21] in den Siebzigerjahren des letzten Jahrhunderts. Mit Apoptose wird eine Form des Zelltodes bezeichneten, der mit Caspaseaktivitäten (*cysteinyl-aspartate specific protease*) und typischen morphologischen Veränderungen wie dem Zellschrumpfen, der Fragmentierung der Zellkerne und der Zellen selbst sowie der Chromatinkondensation einhergeht. [21,22,23] Die ebenfalls von Caspasen eingeleitete Umlagerung von Phosphatidylserin dient der Rezeptorbereitstellung für Makrophagen, die die Zellreste über Phagozytose verwerten sollen. [22,16,24]

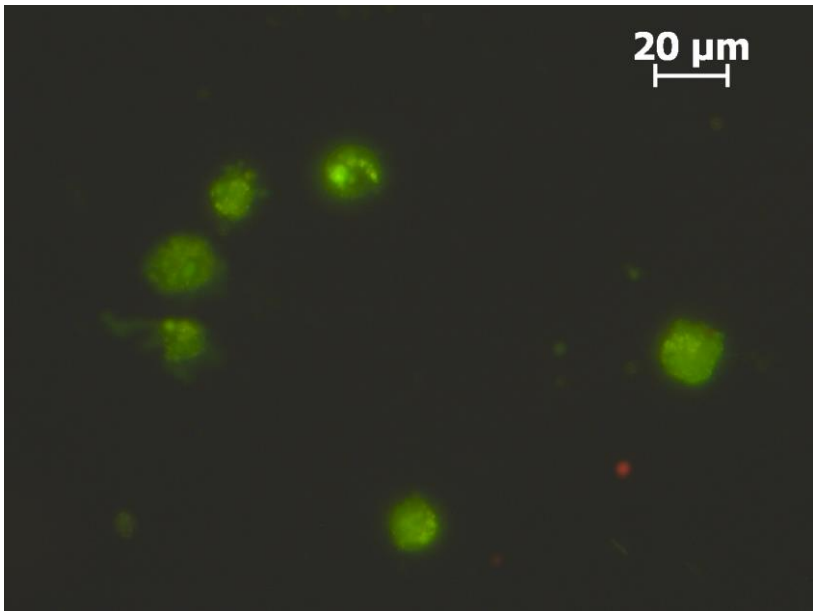


Abbildung 2 Apoptotische Zellen der Zelllinie A2780 mit Acridinorange (grün) und Propidiumiodid (rot) gefärbt unter dem Fluoreszenzmikroskop.

Etwas allgemeiner ist eine neuere Auffassung der Apoptose, die diese als caspasevermittelten Zelltod bezeichnet, denn Caspasen regulieren die Abläufe und sorgen direkt und indirekt für das morphologische Erscheinungsbild der Zellen. Damit ist dies nur eine Form des programmierten Zelltodes, wobei programmiert für genetisch kodiert steht. Denn auch Autophagie und Onkose können dazugezählt werden. Die Autophagie beschreibt den Abbau und die Verwertung von geschädigtem Material durch die Zelle selbst; Onkose führt über Anschwellen der Organellen zu Nekrose. Ein Zelltod durch Nekrose kann auch über den apoptotischen Weg eingeleitet werden, was man sekundäre Apoptose und auch apoptotische oder sekundäre Nekrose nennt. [24,25]

Die bei der Krebsentstehung beteiligten Mutationen können auch die Caspasewege beeinflussen, was zu Veränderungen der Apoptose führt. [26] Im Zusammenhang mit Krebs spielt Apoptose insofern eine Rolle, dass sie meist von den Tumorzellen gehemmt wird, diese also gegen die Apoptosesignalgebung resistent sind, was zur unkontrollierten Proliferation und damit der Neogenese beiträgt. Das Verständnis der Resistenzmechanismen und Apoptosewege soll bei der Therapie eingesetzt werden, damit Tumorzellen gezielt eliminiert werden können. [27]

1.1.3 Diagnose

Für die ärztliche Diagnostik von Krebs werden hauptsächlich radiologische Bildgebungsverfahren, wie CT (Computertomographie) und MRT (Magnetresonanz-tomographie), eingesetzt, die außerdem die Morphologie des Krebses untersuchbar machen. Bei der PET (Positronen-Emissions-Tomographie) kann der Warburg-Effekt, der den höheren

Glukoseverbrauch der Tumorzellen beschreibt, genutzt werden, um diese Zellen im Ganzkörperscan sichtbar zu machen. Diese nehmen auch ^{18}F-markierte FDG (2-Fluor-2-desoxy-glukose) vermehrt auf. [10,12,20,28]

Günstig für die weitere Behandlung ist die frühe Diagnostik – am besten vor der Metastasierung –, um eine erfolgreiche Behandlung zu gewährleisten. Daher werden neue Diagnosemöglichkeiten getestet und verbessert. Blutproben können Krebszellen – sogenannte CTCs (*circulating tumor cells)* – anzeigen, denn bereits vor der Metastasierung werden diese durch den Körper transportiert. Auch Proteine oder Metabolite, die auf die Tumorzellen und ihren Gewebeursprung hinweisen, können im Blut nachgewiesen werden. Immunoassays können mittels Antikörpern Metastasen erkennen und somit bei der Diagnostik helfen. [9,10,12]

1.1.4 Behandlung

Zur etablierten Krebsbehandlung gehören die operative Entfernung von Tumoren sowie die Strahlen- und Chemotherapie. Bei letzterer kommen hauptsächlich Zytostatika zum Einsatz, die den Tumor in Wachstum und Ausbreitung hemmen sollen, indem sie beispielsweise während der Translation eingreifen. Zusätzlich versucht man das Immunsystem zu stärken und es gibt auch Ansätze, Krebs über Immunmodulation direkt zu bekämpfen. [11] Doxorubicin ist ein bekannter Wirkstoff, der durch Interkalation in die DNA (Desoxyribonukleinsäure) und Quervernetzungen mit den Nukleinbasen, die Topoisomerase II inhibiert, die normalerweise die Doppelstrangbrüche herbeiführt. [12,29] Ein Problem in der Krebstherapie, speziell mittels Zytostatika, ist die Belastung für die Normalzellen mit bekannten Nebenwirkungen wie Haarausfall oder generelle Immunschwäche. Am eigentlichen Krebsgeschehen unbeteiligte Zellen werden damit ebenfalls in der Proliferation gehemmt, denn die Selektivität gegen Tumorzellen beruht nur auf ihrer höheren Stoffwechsel- und Wachstumsrate. [11,12]

Weitere Probleme ergeben sich aus den Resistenzen, die Tumorzellen ausbilden können. Zum einen sind Krebszellen durch ihre vielen Mutationen bereits immun bzw. unabhängig von Apoptose- oder Überlebenssignalen oder zeigen deutliche Unterschiede in ihrem Metabolismus. [27] Aber auch während der Behandlung kann es zu neuen Mutationen kommen, die Resistenzen erzeugen, wenn sie z.B. Transport- und Reparaturmechanismen oder das eigentliche Target betreffen. [12,30] Krebszellen können außerdem durch Überexpression von Transportern wie dem ABC-Transporter (*ATP binding cassette)* die Therapeutika aus den Zellen schleusen und die Wirkung damit umgehen. [16] Daher wird nach neuen potentiellen Wirkstoffen geforscht, die Resistenzen wieder umgehen können.

1.1.5 PDT (Photodynamische Therapie) und PD (Photodiagnose)

Für die PDT sind Licht, Sauerstoff und ein Photosensibilisator wichtig. Die Belichtung soll ROS (reaktive Sauerstoffspezies) generieren, v.a. Singulett-Sauerstoff, der als Hauptsubstanz für die zytotoxische Wirkung nachgewiesen ist. Der PS (Photosensibilisator) kann dafür nach Belichtung seinen angeregten Singulett- in den Triplett-Zustand überführen, indem Energie über ISC (*inter system crossing*) übertragen wird. Aus dem Triplettzustand können dann über die photodynamische Reaktion über verschiedene Wege reaktive Spezies generiert werden. Schädigungen an Zellbestandteilen sollen im Tumor den Zelltod herbeiführen, möglichst durch Apoptose; aber auch lokale Entzündungen sind möglich. [31–33]

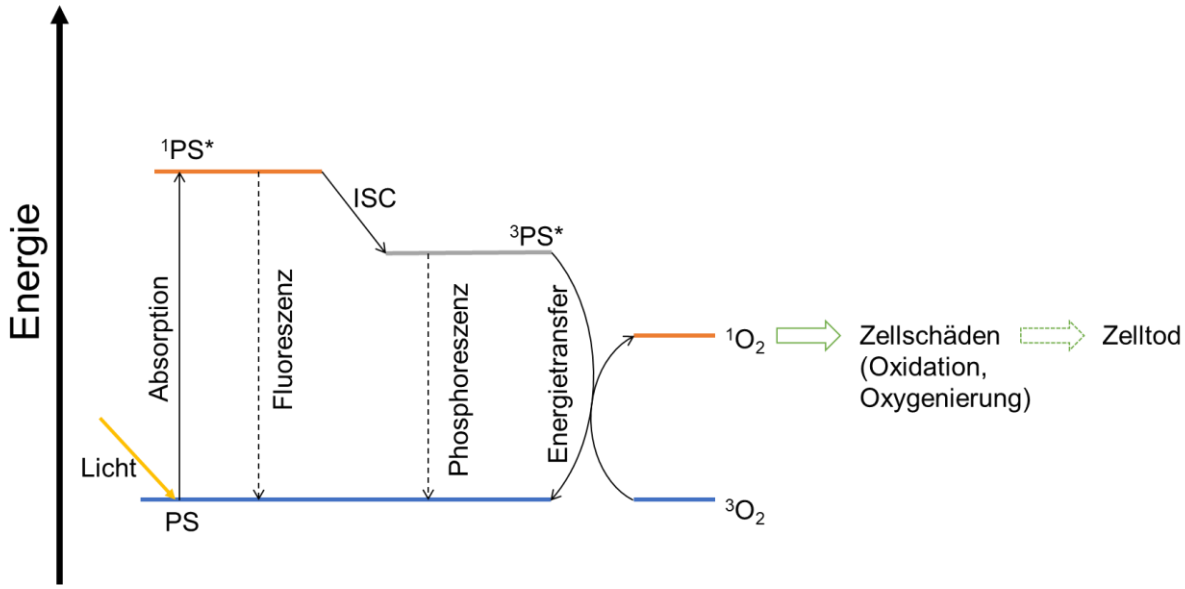


Abbildung 3 Die Wirkung von Photosensibilisatoren (PS) in der PDT an einem vereinfachten Jabłoński-Diagramm. [32–34]

Als Lichtquelle wurden verschiedene Systeme, z.B. Laser (*light amplification by stimulated emission of radiation*) und LEDs (*light-emitting diode*), getestet, wobei es auf gute Dosierbarkeit, monochromatisches Licht, aber auch die Kosten ankommt. Über Fiberglas kann es auch zu inneren Läsionen gelangen. [33] Das therapeutische Fenster liegt bei einer Wellenlänge von 650 nm – 800 nm, da langwelligeres Licht tiefer in Gewebe eindringt, aber über 800 nm nicht mehr genug Energie für die photodynamische Reaktion bzw. Singulettsauerstoffbildung besitzt. Daher sollten die eingesetzten Fluoreszenzfarbstoffe in diesem Bereich angeregt werden sowie photostabil und im Dunklen möglichst atoxisch sein. [31–33]

Die momentan in der Forschung untersuchten Verbindungen können durch ihre direkte Wirkung auf die DNA allerdings auch selbst kanzerogen wirken. Daher wird nach neuen geeigneten Verbindungen gesucht. [31] Ein Problem bei der Behandlung stellt auch hier die Resistenz dar, die beispielsweise durch Ausschleusen der Moleküle oder vermehrte

Antioxidansausschüttung vom Krebs herbeigeführt werden kann. Die Kombination mit weiteren Verbindungen, die dies verhindern, soll die Wirkung der PDT verbessern. [33]
Auch weitere Anpassungen der Struktur sind möglich, sodass die Verbindung pH-abhängig aktiviert wird, was man in der saureren Umgebung von Tumoren nutzen kann, oder bestimmte Strukturen gezielt angegriffen werden. Dies kann beispielsweise über Peptide und Antikörper oder Kohlenhydrate erreicht werden, die für die tumorspezifische Aufnahme sorgen und auf für die Neoplasie nötige oder überexprimierte Moleküle wirken. Außerdem sollten Photosensibilisatoren amphiphil gestaltet werden, damit sie in der Blutbahn transportiert und anschließend von den Krebszellen aufgenommen werden können. [31–33]

Viele der oben genannten Eigenschaften sind auch für Farbstoffe in der Diagnose wichtig: keine Toxizität im Dunklen, wenig Nebenwirkungen wie Photosensibilität, amphiphile Strukturen für gute Bioverfügbarkeit, geeigneter Absorptionsbereich für Gewebe und Photostabilität. [31,35] Außerdem sollten die Stoffe nicht zu lange im Körper verbleiben bzw. nicht in umliegendes Gewebe diffundieren, um Nebenwirkungen zu vermeiden. [36]
Vor allem für die Erkennung von Hauterkrankungen wie Melanomen kann die PD verwendet werden. Bei diesem Verfahren werden tumorspezifische Fluoreszenzmarker eingesetzt, die sich nach ihrer Akkumulation im Tumorgewebe mittels Lichtquelle anregen lassen und dieses gut erkennbar machen. Dies hilft bei der operativen Entfernung und ermöglicht in Kombination mit der PDT neue nicht invasive Verwendungen, wobei eine Tumorselektivität vor der Photosensiblität des umliegenden Gewebes schützt. [31,36,37] Den Einsatz von Stoffen, die gleichzeitig sowohl der Diagnose als auch zur Therapie dienen, nennt man Theranostik. [38]
Zum einen können Antikrebswirkstoffe an Fluoreszenzfarbstoffe gebunden werden; zum anderen sind die in der PDT eingesetzten Stoffe Fluoreszenzmarker und müssen mit einem Tumortarget versehen werden. Zu bereits untersuchten Verbindungen gehören beispielsweise Porphyrin- und Cyaninderivate [29,37,38]; aber auch BODIPYs (Bordipyrromethen) sind in den Fokus der Forschung gerückt. [32,39,40,41]

2 Grundlagen

2.1 Triterpene

Als vielversprechend haben sich Triterpene gezeigt, denn sie sind in der Natur weit verbreitet mit über 20.000 Vertretern, v.a. als sekundäre Pflanzeninhaltstoffe. [41,18,42] Sie werden enzymatisch aus Isopreneinheiten synthetisiert und können nach ihren Aufbaumerkmalen in verschiedene Gruppen eingeteilt werden. Am häufigsten sind tetra- und pentacyclische Triterpene, wobei zu den letzteren die hier am meisten untersuchten Säuren mit Lupan-, Oleanan- oder Ursangrundgerüst gehören. [43,44] Wie die Strukturformeln (Abbildung 4) zeigen, unterscheiden sie sich am fünften Ring: Lupane bestehen am E-Ring aus Cyclopentan mit einem Isopropyl-Rest an C-19; die beiden anderen Grundgerüste bilden am E-Ring ein weiteres Cyclohexan mit zwei Methylgruppen, die beim Oleanan beide an C-20 und beim Ursan jeweils eine an C-19 und C-20 gebunden sind. [43,45]

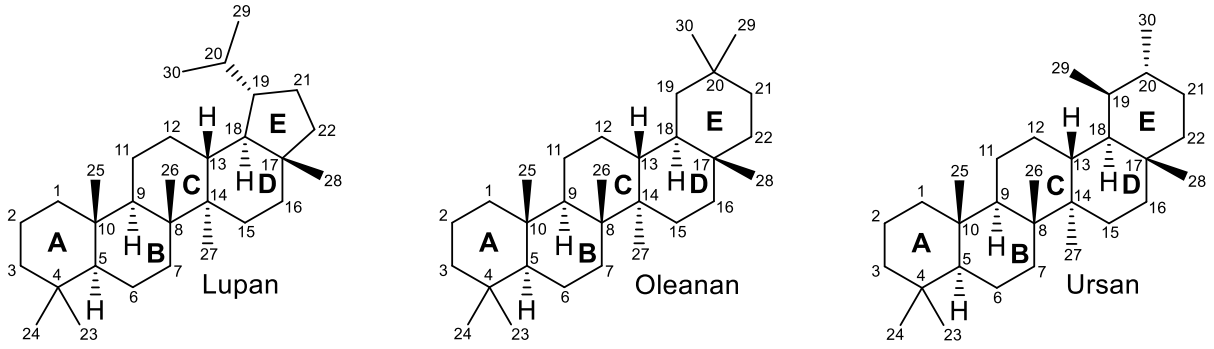


Abbildung 4 Strukturformeln der Triterpengrundgerüste. [45]

In der Natur kommen sie in Pflanzen in äußeren Schichten und Wachsen vor, wie z.B. in Blättern oder Borke. Dabei liegen sie in freier Form oder als Saponine vor, die noch ein bis zwei Zuckerreste aufweisen, was sie amphiphil macht. [18,30,46] Auch durch polare Gruppen am Grundgerüst können Triterpene zu Emulgatoren werden, sodass sie wegen der Grenzflächenaktivität gegen Mikroorganismenmembrane wirken können. Weiteren Schutz bieten sie in den Wachsen gegen mechanische Schäden, da sie diese aufgrund ihrer sterischen Hinderung festigen und Wasser abweisen. [43]

Triterpene wurden bereits in der Vergangenheit gegen viele Erkrankungen verwendet, sodass ihre pharmakologische Wirkung in den letzten Jahren genauer untersucht wurde. Zu ihren Wirkungen zählen antiinflammatorische, antioxidative, analgetische, antivirale, antimikrobielle, immunmodulierende, antiangionetische und antitumorale sowie tumorpräventive Effekte, was sie auch für die Krebsbehandlung interessant macht. [42,43,47] Triterpene gelten normal als nicht toxisch und kommen in Obst und Gewürzen vor, was deren tumorpräventive Eigenschaften erklären könnte, d.h. deren Verzehr kann auch vor einer Krebsentstehung schützen. [5,18]

Gegen diverse Tumorzelllinien konnten insbesondere Triterpensäuren erfolgreich eingesetzt werden, d.h. sie zeigten z.T. auch selektive Zytotoxizität. [48,49] Ein Problem stellt die geringe Löslichkeit und damit geringe Bioverfügbarkeit dar [18,30,50], die durch Derivatisierung verbessert werden soll, wie auch die Zytotoxizität und Selektivität. [51] Zu vielen Vertretern wie der Betulinsäure, Oleanolsäure, Glycyrrhetinsäure und Ursolsäure wurden auch Untersuchungen zu den Wirkmechanismen in Krebszelllinien durchgeführt. [41,48,52]

2.1.1 Forschung zur Wirkung der Triterpene gegen Tumore

Betulinsäure kann nachweislich über den mitochondrialen Weg Apoptose einleiten. [53] Zu dieser Wirkung führen Modulationen von Proteinen der Bcl-2-Familie (*B-cell lymphoma 2*), die proapoptotische Proteine fördern. Auch NF-κB kann durch Betulinsäure aktiviert werden. Zusätzlich werden die Topoisomerasen inhibiert und somit die DNA-Replikation. [18,52] Betulinsäure ist selektiv gegen maligne Zelllinien und wird mit sinkendem pH-Wert wirksamer. [54] Sogar gegen einige resistente Ziellinien ist sie effektiv, da die Apoptoseeinleitung unabhängig von p53 ist. [18,55]

Oleanolsäure kann Apoptose über die Caspasen-9, -3 und -8 einleiten. [18,52] Auch gegen resistente Zelllinien ist sie erfolgreich getestet worden und zeigte zytotoxische Wirkung. Durch die Caspasen wird das Membranpotential der Mitochondrien herabgesetzt und das proapoptotische Bax (*Bcl-2-associated X protein*) vermehrt exprimiert. Weiterhin werden Proteine gehemmt wie Survivin und Bcl-2, was auch den Zelltod vorantreibt. Auch die DNA-Polymerase β wird inhibiert, sodass die DNA-Reparatur behindert ist. Zusätzlich kann Oleanolsäure p53 aktivieren und infolgedessen ebenfalls weitere Apoptosewege einleiten. [52]

Ursolsäure beeinflusst die Bcl-2-Proteine und unterdrückt NF-κB. [18] In Modellversuchen kann sie auch die Ausschüttung von Survivin und Bcl-2 herabsetzen und von Bax erhöhen. Im Zellzyklus bewirkt sie einen S-Phasenarrest (Synthese-Phasenarrest) durch die Inhibierung der DNA-Polymerasen und Topoisomerase II. Außerdem wirkt Ursolsäure gegen einige Krebsarten tumorpräventiv. [52]

Glycyrrhetinsäure kann in den Zelllinienversuchen über Zellzyklusarrest und Caspasenaktivierung apoptotisch wirken und die DNA-Fragmentierung sowie die p53-Ausschüttung vorantreiben. Auch allgemein gegen die Entstehung von Tumoren kann sie z.B. durch UV-Schutz wirken. Die Selektivität gegen maligne Zelllinien ist sehr von Vorteil. [52]

Die Erhöhung der Bioverfügbarkeit durch Einschluss der Triterpensäuren in hydrophile Cyclodextrine verbessert die Aktivität, wie an Ursol- und Oleanolsäure gezeigt wurde. [56] Sie wirken meist auf viele Targets und Zellmechanismen und können dadurch Resistenzen gegen bestimmte Signale umgehen, sodass sie sogar gegen multiresistente Tumore eingesetzt werden können [30,18,57] Auch das Mischen verschiedener Triterpene oder die Zugabe zu

bekannten Wirkstoffen können synergistisch deren Wirkung verbessern. [18,58] Ziele sind dabei das Stoppen der unkontrollierten Proliferation von Krebszellen, durch z.B. Zellzyklusarrest oder Differenzierung der Zellen, oder der gezielte Zelltod. Wegen ihrer gegen Krebs wirkenden Eigenschaften sind die Triterpensäuren eine vielversprechende Lösung und deren Derivate können bereits erste Erfolge zeigen. [18,42,51]

2.2 Farbstoffe

2.2.1 BODIPY

BODIPY steht für Bordipyrromethen und liegt meist als Difluorid vor [59–62], wird aber auch als Pyrromethen-Bordifluorid-Komplex [63] bzw. nach dem Grundgerüst 4-Bora-3a,4a-diaza-*s*-indacen [61] bezeichnet. Diese Farbstoffklasse ist vielseitig einsetzbar, da die Strukturen chemisch gut verändert werden können, um sie an die Zielstruktur anzupassen. Ihre Eignung beruht auf ihrer Stabilität und guten Fluoreszenzeigenschaften, was sie als Biomarker verwenden lässt. [60,61,64] Auch die Zytotoxizität wurde untersucht; verschiedene Derivate zeigen zwar keine Wirkung, werden aber in Zellmembranen, im ER (endoplasmatisches Retikulum) und Lysosomen nachgewiesen. [65] Für iodierte Formen konnte eine zytotoxische Wirkung nach Lichteinwirkung nachgewiesen werden [64]; auch Kupfer-Komplexe zeigen Zytotoxizität gegen Krebszelllinien. [66]

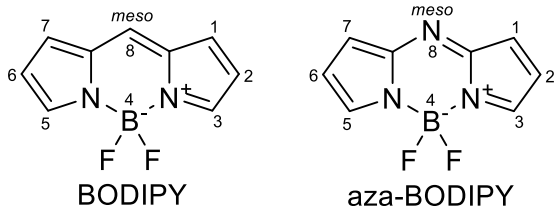


Abbildung 5 Strukturformeln der Grundgerüste von (aza-)BODIPY. [32,61,62]

Erste BODIPY-Derivate wurden und werden für die Theranostik – PD sowie PDT – getestet, um Tumorzellen durch Singulettsauerstoff zu zerstören. Sie müssen geeignete Substituenten aufweisen, um die nötigen Eigenschaften wie bei Anregung eine hohe Anzahl an Molekülen im Triplettzustand zu besitzen. [64] So wurden sie schon gezielt für die PDT und Wirkung in den Lysosomen moduliert und getestet. [67] Eine Wirkung im grünen Lichtspektrum hat Nachteile für den Einsatz, da rotes Licht tiefer ins Gewebe eindringt. Dies kann beispielsweise über Austauschen von Kohlenstoff durch ein Stickstoffatom in *meso*-Position und den damit einhergehenden batochromen Effekt erreicht werden. Diese Strukturen werden als aza-BODIPYs bezeichnet [68]; in den früheren Veröffentlichungen auch als BF_2-chelatisierte Azadipyrromethene. [34,69,70] Derivate stellen einen geeigneten Photosensibilisator dar. [32,35] Auch finden sie weitere Anwendung in der Biologie aufgrund ihrer Eigenschaften wie relativ

einfache Synthese bzw. Anpassung, Photostabilität und Extinktion im roten Wellenlängenbereich bis zum NIR (nahes Infrarot, 650 nm bis 1100 nm). [71] Wasserlösliche Derivate wurden als für die Theranostik geeignet befunden [72]; amphiphile Strukturen haben sich als Verbesserung des BODIPY-Grundgerüstes gezeigt; auch Nanoeinheiten sowie Micellen können die Bioverfügbarkeit und Anreicherung in Tumorzellen verbessern. [73] Nicht-toxische aza-BODIPY-Derivate können zur Markierung lebender Zellen und damit zur Nachverfolgung der Therapie verwendet werden. [34,68,69]
Über eine geringe Zytotoxizität im Dunklen und gute Anregung durch Lichtstimulation kann eine bessere therapeutische Selektivität der BODIPYs erreicht werden.[74]

2.2.2 Rhodamin

Eine weitere Farbstoffklasse, mit der ich mich in meiner Arbeit genauer befasst habe, sind Rhodamine. Bereits Ende des 19. Jahrhunderts wurden deren Synthesen beschrieben und von Ceresole patentiert [75]; im Laufe der Zeit kamen weitere Synthesewege hinzu. [76–78] Rhodamine gehören zu den Xanthenen, die zwei Aminogruppen enthalten und meist aus drei Phenylringen bestehen, wobei zwei durch die Sauerstoffbrücke in *ortho*-Position zum zentralen Kohlenstoffatom an der Drehung gehindert sind. [63,76] Rhodamine sind je nach Substituenten dunkelblaue bis violette Feststoffe [77] oder auch grüne Kristalle oder Pulver [79,80] und in Wasser, DMSO (Dimethylsulfoxid) und Ethanol unter meist grüner bis roter Fluoreszenz löslich. [81,82,83] Sie werden als Farbstoffe für Papier und Tinte sowie als Laser- und Fluoreszenzfarbstoffe eingesetzt. [40,63,76,78]

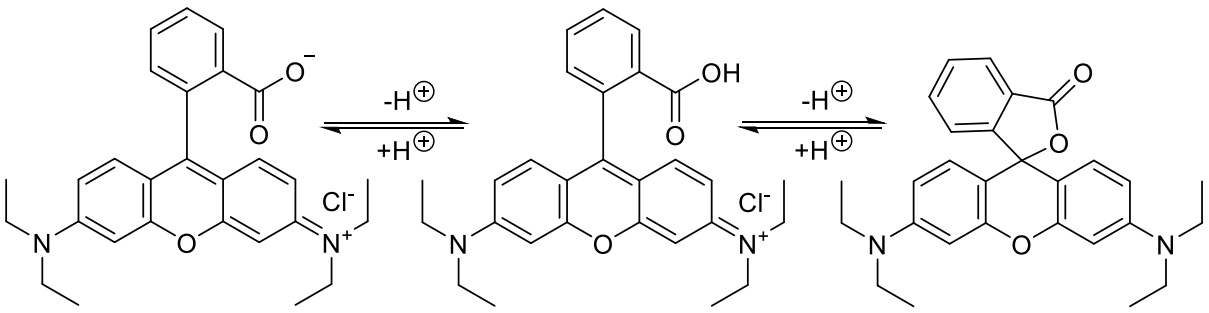


Abbildung 6 Rhodamin B bei verschiedenen pH-Werten. [81,84]

Ein bekanntes Beispiel dieser Klasse ist Rhodamin B, das als Fluoreszenzmarker in Wasser oder in der Histologie zur Färbung der Mitochondrien verwendet wird. [80,83,85] Als Lebensmittelzusatzstoff ist es umstritten, da es in Zellversuchen eine leichte mutagene Wirkung zeigte [86], aber es ist allgemein als nicht toxisch oder krebserregend eingestuft. [79,87] In ungebundener und offenkettiger Form kommt Rhodamin B in Lösungen mit neutralem und physiologischem pH-Wert als Zwitterion vor, wodurch es sich wegen der fehlenden Ladung nicht in Mitochondrien einlagert. [88] Abhängig vom pH-Wert oder Lösungsmittel kann es auch

als Kation im Sauren oder als Spirolacton vorliegen, das nicht fluoresziert – die sogenannte Leukoform. [76,84,89] Die Spirolactonbildung kann durch Veresterung oder Amidierung mit sekundären Aminen verhindert werden, die auch unter physiologischen Bedingungen stabiler sind. [84] Auch photoinduziert kann die Lactonform wieder geöffnet werden [90], was interessante Anwendungsmöglichkeiten der Markierung von Biomolekülen [40,78,89] mit sich bringt, wie die Maskierung von Rhodaminderivaten, um mehrere Färbungen in Zellen bei derselben Wellenlänge möglich zu machen.[91]

2.3 Untersuchungen zum Zelltod

2.3.1 SRB-Assay (Sulforhodamin B-Assay)

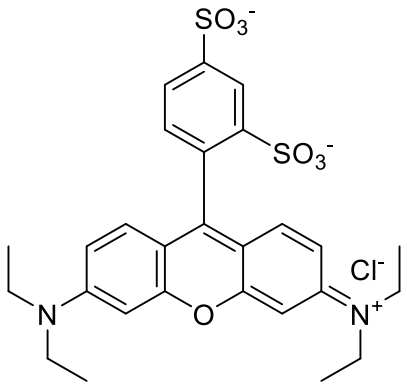


Abbildung 7 Strukturformel von Sulforhodamin B. [76]

Die Durchführung des SRB-Assays wurde in den Publikationen bereits ausführlich beschrieben. An dieser Stelle soll kurz der Hintergrund betrachten werden. Der Assay selbst wurde von Skehan *et al.* entwickelt und optimiert. [92] Der Farbstoff Sulforhodamin B – die Struktur ist in Abbildung 7 dargestellt – kann sich elektrostatisch an Proteine, im Speziellem an deren basische Aminosäurereste, anlagern. Da dieser Vorgang pH-abhängig ist, kann der Farbstoff im Sauren an die Proteine binden und im Basischen wieder in Lösung gebracht werden; im Protokoll werden dafür Essigsäure und TRIS-Puffer (Tris(hydroxymethyl)amino-methan-Puffer) verwendet. [93] Die Bindung erfolgt stöchiometrisch und auch das Ablösen der Proteine erfolgt quantitativ, sodass über die Messung der optischen Dichte der Proteingehalt bestimmt werden kann. [94] Dieser gibt, nachdem die Zellen mit TCA (Trichloressigsäure) fixiert wurden, Aufschluss über die Zellzahl, die dem Proteingehalt proportional ist. Der Assay ist gut in 96-*well* Platten einzusetzen und reproduzierbar, sodass er verlässliche Ergebnisse liefert und für die Berechnung der Konzentration von Substanzen verwendet werden kann, die auf die Krebszellen wirken. Die EC_{50}, die mittlere, auf die Zellen wirkende Effektkonzentration, stellt eine gute Vergleichsgröße für die Substanzwirkung dar. [92] Zur Bestimmung der Selektivität der Substanzen gegen Tumorzellen kann der Selektivitätsindex (SI) aus dem

Quotienten der EC_{50}-Werte für eine nicht-maligne Zelllinie wie die NIH 3T3 (Mausfibroblasten) gegen die jeweilige Krebszelllinie berechnet werden. Dabei gilt: je höher der Wert ist, desto besser ist die Selektivität. [95,96]

2.3.2 Mikroskopie

Mikroskopie kann mit geeigneten Fluoreszenzfarbstoffen eingesetzt werden, um apoptotische Zellen zu erkennen, indem man die für diese typischen morphologischen Unterschiede sichtbar macht. Dies ist beispielsweise mit Acridinorange und Propidiumiodid – auch durch Ethidiumbromid ersetzbar – möglich. Acridinorange lässt lebende Zellen insgesamt grün fluoreszieren und lagert sich dabei an verschiedenen Stellen an. [97,98] Dadurch kann man bei Vorliegen von Apoptose das Zellschrumpfen und die Chromatinkondensation und -fragmentierung erkennen. [97,99] Acridinorange interkaliert dabei in die DNA und färbt diese grün und heller als die Umgebung; es bindet auch an RNA (Ribonucleinsäure), hat dann aber eine schwache, rote Färbung. Bei der Kondensation verschiebt sich die Fluoreszenz ebenfalls ins orange. [100] Auch in sauren Zellkompartimenten wie den Lysosomen kann sich Acridinorange anreichern und diese dann rot färben. [98] Die Zellen färben sich dann im Zuge der Apoptose von grün zu gelb bis orange ein. [97,100]

Durch die Zusätze von Ethidiumbromid oder Propidiumiodid kann man nekrotische Zellen besser unterscheiden, denn diese Farbstoffe dringen in tote Zellen mit geschädigter Membran ein und interkalieren in die DNA, wobei erster orange und letzter rot erscheint. [99–101]

2.3.3 Durchflusszytometrie (FACS)

Unter FACS versteht man einen *fluorescence-activated cell sorter*. [102,103] Die meisten Durchflusszytometer sind nicht mehr auf das Sortieren ausgelegt, sondern auch auf eine Untersuchung der Proben. [102] Dadurch wird die Abkürzung auch allgemein für Durchflusszytometrie verwendet. [104]

Zu den untersuchten Proben zählen neben Zellen auch weitere biologische und nichtbiologische Partikel. Sie werden in einer (Puffer-)Lösung suspendiert und möglichst als einzelne Partikel gemessen. Dafür werden sie in einen Mantelstrom gegeben, um vereinzelt an der Lichtquelle und den Detektoren vorbeigeleitet zu werden. Die Zytometer verwenden unterschiedliche Laser und für die meisten Untersuchungen werden die Proben vorher mit Fluoreszenzmarkern behandelt. Gemessen werden dann die Fluoreszenz und die Streuung des Lichtes. Die Daten geben Aufschluss über die Beschaffenheit der Proben und können dem Assay entsprechend ausgewertet werden. Zu den Anwendungsmöglichkeiten gehören

Analysen der DNA, Apoptose, Expression bestimmter Proteine, Viabilität, des intrazellulären pH-Wertes und des Membranpotentials. [102–104,105]

2.3.3.1 Annexin V

Die Annexin V-Färbung beruht auf dessen Affinität zu Phosphatidylserin. Dieses befindet sich normalerweise an der inneren Zellmembran, wird bei der Apoptose dann durch Flippasen auf die äußere Zellmembran umgelagert. [106–108] Dort dient Phosphatidylserin dem Binden von Makrophagen, die die Zellreste durch Phagozytose wiederverwerten. [107–109] Annexin V kann bei Ca^{2+}-Anwesenheit ebenfalls an das Phosphatidylserin an der äußeren Membran binden. [107] Für die Messbarkeit kann Annexin V an Fluoreszenzfarbstoffe gebunden werden. Dafür wird zum Beispiel FITC (Fluoresceinisothiocyanat) eingesetzt, das grün fluoresziert. Dadurch wird die Annexin V-Bestimmung am Durchflusszytometer durchführbar. [110] Im Falle von Nekrose kann es aufgrund der Membranpermeabilisierung auch zur Annexin-Bindung an Phosphatidylserin im Zellinneren kommen. Nekrotische Zellen werden durch Gegenfärbung mit Propidiumiodid ausgeschlossen. Damit kann man die Untersuchung von Annexin V für die Apoptosebestimmung einsetzen. [108]

2.3.3.2 Zellzyklus

Bei der Proliferation von Krebszellen ist deren Zellteilungsrate erhöht und der Zellzyklus gestört. Damit ist der Zeitraum zwischen zwei Mitosen gemeint, welcher neben der Duplizierung des Genoms und der Synthese weiterer Zellbestandteile auch die Teilung umfasst, sodass zwei identische Tochterzellen entstehen. Im Zellzyklus laufen die G_1-, S-, G_2- und M-Phase (*Gap*$_1$-, Synthese-, *Gap*$_2$- und Mitosephase) nacheinander ab. Gesteuert wird der Prozess über Cycline und CDKs (*cyclin dependent kinase*). [9,10]

Zwischen Zellzyklus und Apoptose besteht ein Zusammenhang, da Zellzyklusregulatoren neben der Proliferation auch für die Apoptose wichtig sind. [111,112] An den Kontrollpunkten zwischen den einzelnen Zellzyklusphasen wird der weitere Prozess überprüft und bei Problemen Apoptose cyclingesteuert eingeleitet. [112]

Neben der Apoptose versucht man auch einen Zellzyklusarrest mit den Derivaten zu erreichen. Dies kann ebenfalls am Durchflusszytometer untersucht werden. Dafür werden die Zellen nach der Aufarbeitung mit Propidiumiodid gefärbt, das sich in der DNA einlagert. Über die Messung des Fluoreszenzsignals kann man auf den DNA-Gehalt in der Probe schließen, der sich im Laufe des Zellzyklus verdoppelt, und die Zellen den einzelnen Phasen zuordnen. Unterhalb der G_1/G_0-Phase sind apoptotische Zellen in der sogenannten subG_1-Phase zu erkennen. [113]

3 Diskussion

3.1 Forschungsstand zu Triterpenderivaten

Nachdem Triterpene mehr in den Fokus der Forschung als potentielle Wirkstoffe gegen unzureichend behandelbare Erkrankungen gerieten, wurde auch an einer Weiterentwicklung durch chemische Modifikationen gearbeitet, was durch eine Vielzahl an Publikationen in den letzten 20 Jahren belegt wird. Im Folgenden wird, ausgehend von dieser Arbeit, gezielt nur auf Derivate der verwendeten Triterpensäuren eingegangen. Betulinsäure wurde beispielsweise bereits auf unterschiedliche Weisen modifiziert; so gibt es mehrere Studien, die sich mit Estern und Amiden befassen, wobei funktionelle Gruppen meist an C-3 oder C-28 angebracht werden. Die Ergebnisse sind uneindeutig, da einige Derivate schlechter gegen die verwendeten Krebszelllinien wirken, andere wiederum besser als die Betulinsäure. Es konnte aber z.T. auch die Selektivität erhöht und in Zelllinien Apoptose nachgewiesen werden. Außerdem zeigte sich, dass Amide bessere Wirkung aufweisen als Ester. [114] Über Konjugation mit Zuckerresten sollte in einem Versuch das strukturell verwandte Betulin besser für die Zellen verfügbar werden, um die Wirkung zu erhöhen; allerdings zeigte sich durch Zuckerfreisetzung sogar eine Verbesserung der Zellfunktion. [115]

Auch zu Oleanolsäure wurden die Derivate in Publikationen so gewählt, dass diese hydrophiler werden, was in den meisten Fällen auch zum Erfolg führte. Zusätzliche Untersuchungen konnten zumeist apoptotische Wirkung feststellen. [116] An Oleanolsäure wurde auch erfolgreich der C-Ring modifiziert, sodass die Aktivität gesteigert und neben dem Zellzyklusarrest in der G_1/G_0-Phase auch Apoptose nachgewiesen wurde. [117] Es gibt bereits klinische Studien zu einem Derivat der Oleanolsäure: CDDO (*2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid*) und dem entsprechenden Methylester (CDDO-Me). Diese Verbindungen zeigten in Mausmodellen vielversprechende Wirkung durch Apoptose und Differenzierung der Krebszellen. [57,118] Dem CDDO-Me ähnlich wurden auch Derivate mit Glycyrrhetinsäure synthetisiert, die vergleichbare Wirkung zeigen. [119] Auch zur Ursolsäure finden sich zahlreiche Veröffentlichungen, die sich mit Modifikationen wie Estern und Amiden an C-3 und C-28 befassen. Sie können in vielen Fällen eine verbesserte Wirkung und Selektivität sowie den Zelltod durch Apoptose nachweisen. Dabei stellte sich Piperazin als geeigneter Spacer heraus. [120]

3.1.1 Arbeitsgruppe Csuk

Im Arbeitskreis von Professor Csuk werden seit einigen Jahren ebenfalls Triterpene derivatisiert und diese in Hinblick auf ihre Zytotoxizität gegenüber Tumorzelllinien und Inhibierung verschiedener Enzyme untersucht. Der Fokus liegt in dieser Arbeit bei antitumoraktiven pentazyklischen Triterpensäurederivaten, im Speziellen bei z.B. Modifikationen von Betulin-, Platan-, Oleanol-, Maslin-, Glycyrrhetin- und Ursolsäure (Strukturen in Abbildung 8). Es gibt viele Studien zu Estern und Amiden der verschiedenen Säuren. Je nach Position und Art des Substituenten sind die Ester bzw. analogen Amide zytotoxischer. [121–123,124,125–128] Es zeigte sich, dass kurze, nicht zu lipophile Aminosäuren die bessere Wahl für Ester an der Hydroxygruppe (C-3) von Glycyrrhetinsäure sind, während die Carboxylgruppe verestert vorliegen sollte. [95,125,126] Bei Maslinsäure konnten Derivate mit einer besseren Zytotoxizität als jene der unsubstituierten Säure synthetisiert werden, bei denen ebenfalls kurze, lipophile Ester die größte Wirkung zeigten und Amide sowie längerkettige Derivate etwas geringere Zytotoxizität aufwiesen. [129] Einige Amide der Oleanolsäure konnten die Zytotoxizität verbessern [121]; weitere Amide mit aromatischen Resten steigerten zudem die Selektivität. [128]

Außerdem wurden an den freien Hydroxygruppen verschiedene Reste getestet: ebenfalls Ester, Ether, Glycoside und Sulfamate liefern zytotoxische Verbindungen. [127,130,131] Aber auch Ring- und weitere Modifikationen wurden untersucht. Beispielsweise wurden die Hydroxygruppen oxidiert, durch Aminogruppen ersetzt oder in ihrer absoluten Konfiguration invertiert. So führte dies in der Regel zu Verbindungen mit höherer Zytotoxizität. [122,132] Zu den Ringmodifikationen, die meist aber keinen großen Einfluss auf die Zytotoxizität hatten, gehörten Ringerweiterungen und -öffnungen [133] sowie weitere Modifikationen an funktionellen Gruppen [134] oder Oxidationen zu Per- und Endoperoxiden [135], letztere Derivate zeigten bessere Zytotoxizität.

Zusätzlich erfolgten Derivatisierungen mit Rhodamin B; dies ergab Konjugate mit sehr guter Zytotoxizität und Selektivität. Diese Verbindungen reicherten sich bevorzugt in den Mitochondrien der Krebszellen an. [136]

Zusammenfassend sind einige Ringveränderungen schlecht für die Aktivität. Ester sind meist besser als Amide, abgesehen von Benzylamiden. Viele Derivate sind zytotoxischer, einige auch selektiver, als die jeweiligen Säuren und bewirken meist Apoptose.

3.2 Ziel der Arbeit

An diese Erfolge knüpft meine Arbeit an. Durch unterschiedliche Modifikationsansätze wird versucht, dem Ziel zytotoxischer und selektiver Substanzen nahe zu kommen. Krebs ist eine komplexe Erkrankung, die am besten über viele Ansätze erforscht und behandelt werden kann. Dafür wurden weitere Amide von pentazyklischen Triterpensäuren untersucht. Neben Rhodamin B wird auch ein aza-BODIPY als fluoreszierender Substituent getestet. Zusätzlich gibt es zu ausgewählten Verbindungen Untersuchungen zu Wirkmechanismen und -orten. Die Ergebnisse der Studien sind als Publikationen erschienen (siehe Anhang) und werden im Folgenden diskutiert und eingeordnet.

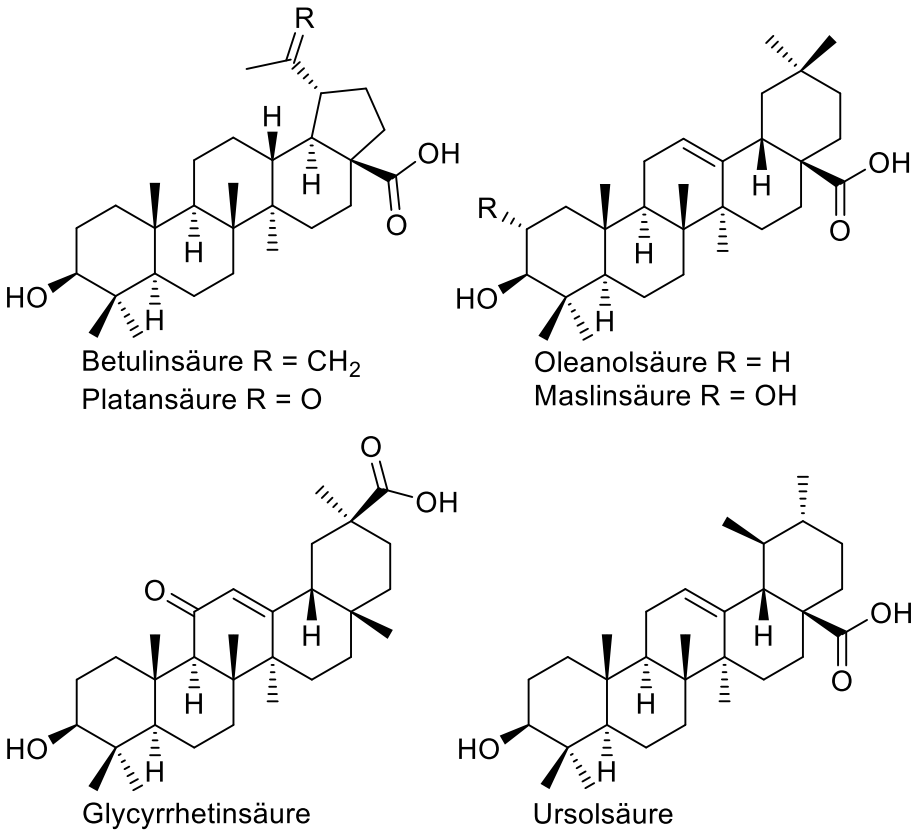


Abbildung 8 Strukturformeln der verwendeten pentazyklischen Triterpensäuren. [45]

3.3 Zusammenfassende Forschungsergebnisse und Ausblick

Da Triterpene weit in der Natur verbreitet sind und viele Wirkungen hervorrufen können, sind diese bereits recht gut erforscht. Für eine mögliche Anwendung in der Krebstherapie gibt es aber auch besonderes Interesse an Triterpensäuren, denn sie können auf viele Wege Apoptose auslösen. Um die Wirkung, Selektivität und Bioverfügbarkeit zu verbessern, wurden bereits Derivate erforscht. In der AG (Arbeitsgruppe) gibt es zu der Thematik auch seit einigen Jahren interessante Forschungsansätze und Synthesen. Dabei werden unterschiedliche Säuren und verschiedene Modifikationen sowie Derivatisierungen vorgenommen. Aus früheren Veröffentlichungen der AG kann man ableiten, dass eine Acetylierung der freien Hydroxygruppen die Wirkung gegen Krebszelllinien verbessert. Außerdem sind bei den Säurederivaten je nach Substituenten die Ester oder Amide aktiver. (siehe 3.1.1)

Ein zur sonstigen Derivatisierung anderer Ansatz stellte die Dimerisierung von Triterpensäuren in **P-1** dar. Am Beispiel von Amiden verschiedener Kettenlänge als Spacer wurden zwei Ursolsäure- bzw. Oleanolsäuremoleküle verknüpft. Die Bildung von Dimeren hat allerdings keine Verbesserung der Zytotoxizität bewirkt. Dennoch sind im Allgemeinen die acetylierten Verbindungen aktiver und Amide stabiler, was in diesem Falle aber auch zu geringerer Verfügbarkeit führen könnte, wenn die aktiven Strukturen nicht am Wirkort abgespalten werden können. Aber – als Spacer eingesetzt – haben sich die stabileren Amide bisher bewährt. Für andere Anwendungen gibt es bisher keine Testung.

Abwandlungen des Grundgerüstes der Triterpensäure am Beispiel des A-Ringes von Maslinsäure führten zur Verbesserung der Wirkung gegen Krebszelllinien im Vergleich zur nicht derivatisierten Maslinsäure, wie in **P-5** erläutert wird. Diese neue Struktur kann in der Zukunft auch für weitere Derivate wie Amide eingesetzt werden.

Für weitere Untersuchungen wurden Amide verschiedener acetylierter Säuren verwendet. Dafür wurden 4-Aminoiso-/5-Aminochinoline an Betulin- und Platansäure gebunden; außerdem 1,4-Diazabicyclo[3.2.2]nonan und 1,3-Diazabicyclo[3.2.2]nonan an selbige sowie Ursol- und Oleanolsäure, um möglichst verschiedene Wirkungen über unterschiedliche Triterpensäuregrundgerüste zu erhalten. Bei den Iso-/chinolinderivaten lagen die EC_{50}-Werte in der Regel im Bereich von 2 µM bis 10 µM; sie zeigten außerdem gute Selektivitätsindices bis 62,5 für A2780 und das Betulinsäure-4-aminoisochinolinderivat (**P2-19**). Die Selektivität für einige Derivate könnte auch noch höher liegen, aber nicht genauer ermittelt werden, weil die Messgrenze in den meisten Fällen bei 30 µM lag und die Werte für NIH 3T3 darüber lagen.

Der Vergleich zwischen den Säuren mit einer Hydroxygruppe an C-3 und zwei Hydroxygruppen an C-2 und C-3 lässt keinen direkten Zusammenhang zwischen diesen Veränderungen am A-Ring und der Zytotoxizität gegen maligne Zelllinien erkennen. Durch die zweite Hydroxygruppe ist der A-Ring zwar hydrophiler, aber die Löslichkeit des Derivates der Dihydroxy-Platansäure ist sogar schlechter, sodass es im Medium ausfällt und nicht als

Wirkstoff geeignet ist. Im Allgemeinen sind von der Platansäure abgeleitete Derivate häufig schlechter löslich als die Analoga anderer Säuren, obwohl durch die hydrophile Ketogruppe an C-29 anderes zu vermuten wäre. [123,131,137,138,139]

Zu den Derivaten **P2-6**, **P2-8** und **P2-19** wurden weitere biologische Untersuchungen durchgeführt, die das Einleiten von Apoptose durch deren Einwirken auf Zellen der Linie A375 bestätigen. Zu sehen war dies am Annexin V-Assay und den mikroskopischen Bildern. Außerdem wurde der Zellzyklus gestoppt, also die Zellen vermehrt in der S-Phase gehalten sowie in die G_1-Phase gedrängt, was ebenfalls am Durchflusszytometer bestätigt wurde.

Die in **P4** beschriebenen Bicyclononane waren im Bereich 2 µM bis 6 µM etwas aktiver, aber zeigten keine Selektivität gegen die NIH 3T3. Außerdem waren die meisten Derivate nicht für weitere Untersuchungen geeignet, da sie sich nicht in DMSO oder im Medium lösten. Lediglich die Platansäurederivate und das Betulinsäurederivat mit 1,3-Diazabicyclo[3.2.2]nonan (**P4-27**, **P4-30** und **P4-31**) waren löslich und erstere an den getesteten Zelllinien im Bereich von 2,3 µM bis 8,2 µM mittelmäßig aktiv, aber mit Werten für die NIH 3T3 von 2,2 µM bzw. 5,0 µM für 1,4-Diazabicyclo[3.2.2]nonan (**P4-27**) bzw. 1,3-Diazabicyclo[3.2.2]nonan (**P4-31**) nicht selektiv gegen maligne Zelllinien, sondern im Gegenteil aktiver gegen die nicht-maligne Zelllinie. Für das Betulinsäurederivat (**P4-30**) war die Zytotoxizität geringer mit Werten zwischen 4,7 µM (A375) und 6,3 µM (FaDu) aber zeigte Selektivität gegenüber der NIH 3T3 mit einem EC_{50}-Wert von 9,3 µM. Damit war Betulinsäure in diesem Fall am besten für die Derivatisierung und weitere Untersuchungen geeignet.

Mit Diaminen (**P4**) wie *cis*- und *trans*-1,4-Diaminocyclohexan sowie Ethylendiamin und Piperazin konnten die Triterpensäuren bereits in ihrer Wirkung gesteigert werden und eignen sich außerdem als Spacer für weitere Funktionalisierung. Insgesamt waren die Werte der Diamine mit EC_{50}-Werten der Krebszelllinien im Bereich von circa 1 µM bis 3 µM besser, aber die Selektivität gegen die NIH 3T3 war mit Werten im selben Bereich nicht gegeben. Diese könnte durch weitere Funktionalisierung wie Rhodaminkopplung gesteigert werden.

Derivate der Oleanol-, Ursol-, Betulin- und Glycyrrhetinsäure – über Piperazin gespacert – mit aza-BODIPY (**P6**) waren unter den getesteten SRB-Bedingungen nicht zytotoxisch, konnten aber durch Färbung am Fluoreszenzmikroskop im ER nachgewiesen werden. Daher erfüllen diese Derivate wichtige Kriterien, um als Biomarker oder auch spezieller für die PD eingesetzt zu werden. Sie sind durch das Triterpensäuregerüst amphiphil, sodass sie von den Zellen gut aufgenommen werden können. Außerdem schädigen sie aufgrund der fehlenden Toxizität nicht das Gewebe. Durch diese Vorteile wäre auch eine Anwendung in der PDT denkbar, denn zusätzlich liegt deren Absorption um 667 nm für eine Belichtung im Gewebe im geeigneten Wellenlängenbereich, d.h. man könnte eine gute Selektivität durch die Lichtstimulation erreichen.

Die bisher besten Ergebnisse in Bezug auf Zytotoxizität und Selektivität lieferten Rhodamin B-Derivate, wie auch der Vergleich mit anderen Farbstoffen zeigte, sodass auch die richtige Wahl des Substituenten entscheidend ist. [140] Rhodamin B selbst zeigte unter den SRB-Bedingungen bis 30 µM keine zytotoxische Wirkung; bereits einige Ester und Amide des Rhodamin B änderten dies, jedoch ohne Selektivität mit Ausnahme des selektiven Derivates **P3-4**. Dafür sind die Derivate der Triterpensäuren geeigneter. Es gibt die Möglichkeit der Bindung als Ester an den Hydroxygruppen des C-3 oder an der Säurefunktion an C-28 über Spaceramine. Die Bindungsstelle des Rhodamins zeigt ebenfalls einen Einfluss auf das zytotoxische Verhalten der Derivate: Rhodamin B an C-3 ist für die untersuchten Säuren weniger aktiv und selektiv. Für die Bindung an C-28 ist Piperazin als sekundäres Amin ein geeigneter Spacer. Denn mit primären Aminen kommt es am Rhodamin B zur Spirolactambildung. Die Verbindungen sind dann nicht aktiv und konnten über verschieden Versuche mit Belichtungen auch nicht aktiviert werden. [141] Die geringe Wirkung wurde mit weiteren Säuren mit Ethylendiamin als Spacer bestätigt. [142] Noch besser als Piperazin ist Homopiperazin geeignet und die entsprechenden Derivate können die zytotoxische Wirkung noch erhöhen.

Die (Homo-)Piperazin- und Rhodamin-Ester-Derivate sind in bisher allen getesteten Fällen über Gegenfärbung in den Mitochondrien nachweisbar und als Mitocan einzustufen; darunter versteht man Substanzen, die in den Mitochondrien gegen Krebs (Cancer) wirken. Dabei können sich die Rhodamine als Kationen in der Mitochondrieninnenmebran, die ein negatives Transmembranpotential aufweist, anreichern und so zur Selektivität in Krebszellen führen. [143] Unter den Steroidderivaten konnte die Verbindung **P3-11** im Annexin V-Assay als apoptotisch, **P3-10** als nekrotisch wirkend nachgewiesen werden. [144] Im Vergleich dazu konnten auch bei weiteren Derivaten durch Untersuchungen am Durchflusszytometer Nekrose oder Apoptose [138,145] und auch eine zytostatische Wirkung durch einen G_1-Phasenarrest [146] nachgewiesen werden.

Oben beschriebene Erkenntnisse können in weiteren Publikationen der AG Csuk bestätigt werden. [139,142,145,147] Unter den Rhodaminderivaten ist als Trend zu erkennen, dass eine größere Anzahl an acetylierten Hydroxygruppen am A-Ring ebenfalls die Zytotoxizität erhöht. Auch Veränderungen der Spacer, weitere Terpene und andere Rhodamine werden in der AG Csuk zurzeit untersucht, zu denen es bereits weitere Veröffentlichungen gibt. [139,142,146,148]

4 Referenzen

[1] O. Warburg, *Naturwissenschaften* **1924**, *12*, 1131.

[2] O. Warburg, *Klin. Wochenschr.* **1925**, *4*, 534.

[3] a) "GLOBOCAN 2020: New Global Cancer Data | UICC", zu finden unter https://www.uicc.org/news/globocan-2020-new-global-cancer-data, **2022**; b) "Cancer", zu finden unter https://www.who.int/news-room/fact-sheets/detail/cancer, **2022**.

[4] "Cancer today", zu finden unter https://gco.iarc.fr/today/home, **2022**.

[5] P. Anand, A. B. Kunnumakara, C. Sundaram, K. B. Harikumar, S. T. Tharakan, O. S. Lai, B. Sung, B. B. Aggarwal, *Pharm. Res.* **2008**, *25*, 2097.

[6] F. Erdmann, C. Spix, A. Katalinic, M. Christ, J. Folkerts, J. Hansmann, K. Kranzhöfer, B. Kunz, K. Manegold, A. Penzkofer et al., *Krebs in Deutschland für 2017/2018,* 13. Aufl., Robert Koch-Institut, Berlin, **2021**.

[7] S. I. Hajdu, *Cancer* **2011**, *117*, 1097.

[8] D. Hanahan, R. A. Weinberg, *Cell* **2000**, *100*, 57.

[9] P. C. Heinrich, G. Löffler, *Biochemie und Pathobiochemie,* 9. Aufl., Springer, Heidelberg, **2011**.

[10] B. Alberts, A. Johnson, J. Lewis, D. Morgan, M. Raff, K. Roberts, P. Walter, *Molecular Biology of the Cell*, Garland Science, New York, **2015**.

[11] W. Clauss, C. Clauss, *Humanbiologie kompakt*, Springer Berlin Heidelberg, Berlin, Heidelberg, **2018**.

[12] W. Hiddemann, C. R. Bartram, *Die Onkologie*. *Teil 1: Epidemiologie - Pathogenese - Grundprinzipien der Therapie; Teil 2: Solide Tumoren,* 2. Aufl., Springer Berlin Heidelberg, Berlin, Heidelberg, **2010**.

[13] V. J. Cogliano, R. Baan, K. Straif, Y. Grosse, B. Lauby-Secretan, F. El Ghissassi, V. Bouvard, L. Benbrahim-Tallaa, N. Guha, C. Freeman et al., *J. Natl. Cancer Inst.* **2011**, *103*, 1827.

[14] O. Warburg in *Über die Katalytischen Wirkungen der Lebendigen Substanz*. *Arbeiten aus dem Kaiser Wilhelm-Institut für Biologie · Berlin-Dahlem* (Hrsg.: O. Warburg), Springer Berlin Heidelberg, Berlin, Heidelberg, **1928**, S. 501–509.

[15] "Europäischer Kodex zur Krebsbekämpfung - Centre international de recherche sur le cancer (OMS). Comission européenne: 12 façons de réduire votre risque de cancer", zu finden unter https://cancer-code-europe.iarc.fr/index.php/de/, **2016**.

[16] R. Brandes, F. Lang, R. F. Schmidt, *Physiologie des Menschen*. *Mit Pathophysiologie*. Mit 850 Farbabbildungen, 32. Aufl., Springer, Berlin, Heidelberg, **2019**.

[17] D. Hanahan, R. A. Weinberg, *Cell* **2011**, *144*, 646.

[18] M. N. Laszczyk, *Planta Med.* **2009**, *75*, 1549.

[19] M. V. Liberti, J. W. Locasale, *Trends Biochem. Sci.* **2016**, *41*, 211.

[20] M. G. Vander Heiden, L. C. Cantley, C. B. Thompson, *Science* **2009**, *324*, 1029.

[21] R. A. Lockshin, J. Beaulaton, *Life Sci.* **1974**, *15*, 1549.

[22] J. Doherty, E. H. Baehrecke, *Nat. Cell Biol.* **2018**, *20*, 1110.

[23] a) J. C. Reed, *Trends Mol. Med.* **2001**, *7*, 314; b) A. H. Wyllie, *Br. Med. Bull.* **1997**, *53*, 451; c) D. Wlodkowic, W. Telford, J. Skommer, Z. Darzynkiewicz, *Methods Cell Biol.* **2011**, *103*, 55.

[24] S. L. Fink, B. T. Cookson, *Infect. Immun.* **2005**, *73*, 1907.

[25] a) M. T. Silva, *FEBS Lett.* **2010**, *584*, 4491; b) L. Wang, J. F. Scabilloni, J. M. Antonini, Y. Rojanasakul, V. Castranova, R. R. Mercer, *Am. J. Physiol. Lung Cell Mol. Physiol.* **2006**, *290*, L695-L702.

[26] M. O. Hengartner, *Nature* **2000**, *407*, 770.

[27] F. H. Igney, P. H. Krammer, *Nat. Rev. Cancer* **2002**, *2*, 277.

[28] L. M. R. Ferreira, *Exp. Mol. Pathol.* **2010**, *89*, 372.

[29] G. Battogtokh, Y. S. Choi, D. S. Kang, S. J. Park, M. S. Shim, K. M. Huh, Y.-Y. Cho, J. Y. Lee, H. S. Lee, H. C. Kang, *Acta Pharm. Sin. B* **2018**, *8*, 862.

[30] X.-J. Yan, L.-H. Gong, F.-Y. Zheng, K.-J. Cheng, Z.-S. Chen, Z. Shi, *Drug Discov. Today* **2014**, *19*, 482.

[31] R. R. Allison, C. H. Sibata, *Photodiagn. Photodyn. Ther.* **2010**, *7*, 61.

[32] S. G. Awuah, Y. You, *RSC Adv.* **2012**, *2*, 11169.

[33] P. Agostinis, K. Berg, K. A. Cengel, T. H. Foster, A. W. Girotti, S. O. Gollnick, S. M. Hahn, M. R. Hamblin, A. Juzeniene, D. Kessel et al., *CA-Cancer J. Clin.* **2011**, *61*, 250.

[34] A. Gorman, J. Killoran, C. O'Shea, T. Kenna, W. M. Gallagher, D. F. O'Shea, *J. Am. Chem. Soc.* **2004**, *126*, 10619.

[35] A. Kamkaew, S. H. Lim, H. B. Lee, L. V. Kiew, L. Y. Chung, K. Burgess, *Chem. Soc. Rev.* **2013**, *42*, 77.

[36] R. R. Allison, C. H. Sibata, *Photodiagn. Photodyn. Ther.* **2008**, *5*, 247.

[37] S. B. Brown, E. A. Brown, I. Walker, *Lancet Oncol.* **2004**, *5*, 497.

[38] M. Ethirajan, Y. Chen, P. Joshi, R. K. Pandey, *Chem. Soc. Rev.* **2011**, *40*, 340.

[39] a) M. Frasconi, Z. Liu, J. Lei, Y. Wu, E. Strekalova, D. Malin, M. W. Ambrogio, X. Chen, Y. Y. Botros, V. L. Cryns et al., *J. Am. Chem. Soc.* **2013**, *135*, 11603; b) V. Pandey, M. K. Raza, M. Sonowal, I. Gupta, *Bioorg. Chem.* **2021**, *106*, 104467; c) A. Turksoy, D. Yildiz, E. U. Akkaya, *Coord. Chem. Rev.* **2019**, *379*, 47.

[40] M. S. T. Gonçalves, *Chem. Rev.* **2009**, *109*, 190.

[41] A. Petronelli, G. Pannitteri, U. Testa, *Anti-Cancer Drugs* **2009**, *20*, 880.

[42] A. Bishayee, S. Ahmed, N. Brankov, M. Perloff, *Front. Biosci. (Landmark Ed.)* **2011**, *16*, 980.

[43] C. H. Brieskorn, *Pharm. Unserer Zeit* **1987**, *16*, 161.

[44] a) J. D. Connolly, R. A. Hill, *Nat. Prod. Rep.* **1989**, *6*, 475; b) R. Xu, G. C. Fazio, S. P. T. Matsuda, *Phytochemistry* **2004**, *65*, 261.

[45] E. Breitmaier, *Terpene*. *Aromen, Düfte, Pharmaka, Pheromone*, B. G. Teubner, Stuttgart, Leipzig, **1999**.

[46] a) R. B. Boar, J. Allen, *Phytochemistry* **1973**, *12*, 2571; b) R. Hänsel, J. Hölzl, *Lehrbuch der pharmazeutischen Biologie*. *Ein Lehrbuch für Studenten der Pharmazie im zweiten Ausbildungsabschnitt*, Springer Berlin Heidelberg, Berlin, Heidelberg, **1996**; c) J. Liu, Y. Wang, R. H. Liu, X. He, *Food Funct.* **2016**, *7*, 3244.

[47] a) K. T. Liby, M. M. Yore, M. B. Sporn, *Nat. Rev. Cancer* **2007**, *7*, 357; b) Y. Allouche, G. Beltrán, J. J. Gaforio, M. Uceda, M. D. Mesa, *Food Chem. Toxicol.* **2010**, *48*, 2885; c) J.-L. Ríos, *J. Ethnopharmacol.* **2010**, *128*, 1; d) J. M. R. Patlolla, C. V. Rao, *Current Pharm. Biotechnol.* **2012**, *13*, 147; e) A. T. Nelson, A. M. Camelio, K. R. Claussen, J. Cho, L. Tremmel, J. DiGiovanni, D. Siegel, *Bioorg. Med. Chem.* **2015**, *25*, 4342; f) S. Fulda, *Mol. Nutr. Food Res.* **2009**, *53*, 140.

[48] W. N. Setzer, M. C. Setzer, *Mini-Rev. Med. Chem.* **2003**, *3*, 540.

[49] a) M. Chudzik, I. Korzonek-Szlacheta, W. Król, *Molecules* **2015**, *20*, 1610; b) E. Pisha, H. Chai, I. S. Lee, T. E. Chagwedera, N. R. Farnsworth, G. A. Cordell, C. W. Beecher, H. H. Fong, A. D. Kinghorn, D. M. Brown, *Nat. Med.* **1995**, *1*, 1046.

[50] F. Wu, T. Xu, C. Liu, C. Chen, X. Song, Y. Zheng, G. He, *Sci. World J.* **2013**, *2013*, 913654.

[51] J. Hodon, L. Borkova, J. Pokorny, A. Kazakova, M. Urban, *Eur. J. Med. Chem.* **2019**, *182*, 111653.

[52] J. A. Salvador, A. S. Leal, D. P. Alho, B. M. Gonçalves, A. S. Valdeira, V. I. Mendes, Y. Jing in *Studies in Natural Products Chemistry* (Hrsg.: Atta-ur-Rahman), Elsevier, Amsterdam, **2014**, S. 33–73.

[53] a) S. Fulda, G. Kroemer, *Drug Discov. Today* **2009**, *14*, 885; b) S. Fulda, C. Scaffidi, S. A. Susin, P. H. Krammer, G. Kroemer, M. E. Peter, K. M. Debatin, *J. Biol. Chem.* **1998**, *273*, 33942.

[54] V. Zuco, R. Supino, S. C. Righetti, L. Cleris, E. Marchesi, C. Gambacorti-Passerini, F. Formelli, *Cancer Lett.* **2002**, *175*, 17.

[55] a) S. Fulda, C. Friesen, M. Los, C. Scaffidi, W. Mier, M. Benedict, G. Nuñez, P. H. Krammer, M. E. Peter, K.-M. Debatin, *Cancer Res.* **1997**, *57*, 4956; b) P. Yogeeswari, D. Sriram, *Curr. Med. Chem.* **2005**, *12*, 657.

[56] C. Oprean, M. Mioc, E. Csányi, R. Ambrus, F. Bojin, C. Tatu, M. Cristea, A. Ivan, C. Danciu, C. Dehelean et al., *Biomed. Pharmacother.* **2016**, *83*, 1095.

[57] M. K. Shanmugam, A. H. Nguyen, A. P. Kumar, B. K. H. Tan, G. Sethi, *Cancer Lett.* **2012**, *320*, 158.

[58] a) V. H. Villar, O. Vögler, F. Barceló, M. Gómez-Florit, J. Martínez-Serra, A. Obrador-Hevia, J. Martín-Broto, V. Ruiz-Gutiérrez, R. Alemany, *J. Nutr. Biochem.* **2014**, *25*, 429; b) J. Liu, *J. Ethnopharmacol.* **2005**, *100*, 92.

[59] A. Treibs, F.-H. Kreuzer, *Justus Liebigs Ann. Chem.* **1968**, *718*, 208.

[60] J. Karolin, L. B.-A. Johansson, L. Strandberg, T. Ny, *J. Am. Chem. Soc.* **1994**, *116*, 7801.

[61] G. Ulrich, R. Ziessel, A. Harriman, *Angew. Chem. Int. Ed.* **2008**, *47*, 1184.

[62] S. Çetindere in *Photophysics, Photochemical and Substitution Reactions*. *Recent Advances* (Hrsg.: S. Saha, R. Kumar Kanaparthi, T. V. Soldatovi), IntechOpen, **2021**.

[63] E. Thiel in *Ullmann's encyclopedia of industrial chemistry*, Wiley-VCH, Weinheim, Wiley online library, **2010**.

[64] J. Bañuelos, *Chem. Rec.* **2016**, *16*, 335.

[65] a) L. Jiao, C. Yu, T. Uppal, M. Liu, Y. Li, Y. Zhou, E. Hao, X. Hu, M. G. H. Vicente, *Org. Biomol. Chem.* **2010**, *8*, 2517; b) J. M. Franke, B. K. Raliski, S. C. Boggess, D. V. Natesan, E. T. Koretsky, P. Zhang, R. U. Kulkarni, P. E. Deal, E. W. Miller, *J. Am. Chem. Soc.* **2019**, *141*, 12824.

[66] T. Yogo, Y. Urano, Y. Ishitsuka, F. Maniwa, T. Nagano, *J. Am. Chem. Soc.* **2005**, *127*, 12162.

[67] C. Wang, Y. Qian, *Org. Biomol. Chem.* **2019**, *17*, 8001.

[68] X.-D. Jiang, R. Gao, Y. Yue, G.-T. Sun, W. Zhao, *Org. Biomol. Chem.* **2012**, *10*, 6861.

[69] A. T. Byrne, A. E. O'Connor, M. Hall, J. Murtagh, K. O'Neill, K. M. Curran, K. Mongrain, J. A. Rousseau, R. Lecomte, S. McGee et al., *Br. J. Canc.* **2009**, *101*, 1565.

[70] a) J. Murtagh, D. O. Frimannsson, D. F. O'Shea, *Org. Lett.* **2009**, *11*, 5386; b) J. Killoran, L. Allen, J. F. Gallagher, W. M. Gallagher, D. F. O'Shea, *Chem. Commun.* **2002**, *38*, 1862.

[71] a) Z. Shi, X. Han, W. Hu, H. Bai, B. Peng, L. Ji, Q. Fan, L. Li, W. Huang, *Chem. Soc. Rev.* **2020**, *49*, 7533; b) P. Batat, M. Cantuel, G. Jonusauskas, L. Scarpantonio, A. Palma, D. F. O'Shea, N. D. McClenaghan, *J. Phys. Chem. A* **2011**, *115*, 14034.

[72] G. Kalot, A. Godard, B. Busser, J. Pliquett, M. Broekgaarden, V. Motto-Ros, K. D. Wegner, U. Resch-Genger, U. Köster, F. Denat et al., *Cells* **2020**, *9*.

[73] a) N. Mehraban, H. S. Freeman, *Materials* **2015**, *8*, 4421; b) D. Chen, Z. Zhong, Q. Ma, J. Shao, W. Huang, X. Dong, *ACS Appl. Mater. Interfaces* **2020**, *12*, 26914; c) J. Wang, C. Yu, E. Hao, L. Jiao, *Coord. Chem. Rev.* **2022**, *470*, 214709.

[74] a) S. O. McDonnell, M. J. Hall, L. T. Allen, A. Byrne, W. M. Gallagher, D. F. O'Shea, *J. Am. Chem. Soc.* **2005**, *127*, 16360; b) Y. Sun, X.-A. Yu, J. Yang, L. Gai, J. Tian, X. Sui, H. Lu, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2021**, *246*, 119027.

[75] a) M. Ceresole, US377349A, **1888**; b) M. Ceresole, U.S. Patent 456081 18910714, **1891**; c) M. Ceresole, U.S. Patent 516589 A 18940313, **1894**.

[76] T. Gessner, U. Mayer in *Ullmann's encyclopedia of industrial chemistry*, Wiley-VCH, Weinheim, Wiley online library, **2010**.

[77] S. J. Dwight, S. Levin, *Org. Lett.* **2016**, *18*, 5316.

[78] M. Beija, C. A. M. Afonso, J. M. G. Martinho, *Chem. Soc. Rev.* **2009**, *38*, 2410.

[79] "Rhodamin B", zu finden unter https://gestis.dguv.de/data?name=491216, **2022**.

[80] "Rhodamin B for fluorescence | Sigma-Aldrich", zu finden unter https://www.sigmaaldrich.com/DE/de/product/sigma/83689, **2022**.

[81] A. R. Gear, *J. Biol. Chem.* **1974**, *249*, 3628.

[82] a) "Tetramethylrhodamine, methyl ester (TMRM) (CAS 152842-32-5) (ab275547) | Abcam", zu finden unter https://www.abcam.com/tetramethylrhodamine-methyl-ester-tmrm-ab275547.html, **2022**; b) "Tetramethylrhodamin-methylester-perchlorat for fluorescence, ≥95% (HPCE) | Sigma-Aldrich", zu finden unter https://www.sigmaaldrich.com/DE/de/product/sial/87919, **2022**; c) L. D. Lavis, R. T. Raines, *ACS Chem. Biol.* **2008**, *3*, 142.

[83] Jay A. Brown, MD, MPH, *Haz-Map* **2019**.

[84] E. Birtalan, B. Rudat, D. K. Kölmel, D. Fritz, S. B. L. Vollrath, U. Schepers, S. Bräse, *Biopolymers* **2011**, *96*, 694.

[85] "Rhodamin B", zu finden unter https://www.thermofisher.com/order/catalog/product/A13572.0B, **2022**.

[86] E. R. Nestmann, G. R. Douglas, T. I. Matula, C. E. Grant, D. J. Kowbel, *Cancer Res.* **1979**, *39*, 4412.

[87] a) "List of Classifications – IARC Monographs on the Identification of Carcinogenic Hazards to Humans", zu finden unter https://monographs.iarc.who.int/list-of-classifications, **2022**; b) NOAA Office of Response and Restoration, US GOV, "C.I. FOOD RED 15 | CAMEO Chemicals | NOAA", zu finden unter https://cameochemicals.noaa.gov/chemical/16068, **2022**.

[88] a) L. V. Johnson, M. L. Walsh, L. B. Chen, *Proc. Natl. Acad. Sci. USA* **1980**, *77*, 990; b) Z. Darzynkiewicz, F. Traganos, L. Staiano-Coico, J. Kapuscinski, M. R. Melamed, *Cancer Res.* **1982**, *42*, 799.

[89] S. Brauch, M. Henze, B. Osswald, K. Naumann, L. A. Wessjohann, S. S. van Berkel, B. Westermann, *Org. Biomol. Chem.* **2012**, *10*, 958.

[90] a) K.-H. Knauer, R. Gleiter, *Angew. Chem. Int. Ed.* **1977**, *16*, 113; b) V. N. Belov, M. L. Bossi, J. Fölling, V. P. Boyarskiy, S. W. Hell, *Chem. Eur. J.* **2009**, *15*, 10762.

[91] V. N. Belov, C. A. Wurm, V. P. Boyarskiy, S. Jakobs, S. W. Hell, *Angew. Chem. Int. Ed.* **2010**, *49*, 3520.

[92] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst.* **1990**, *82*, 1107.

[93] W. Voigt in *Methods in Molecular Medicine™*, *Vol. 110* (Hrsg.: R. D. Blumenthal), Humana Press, Totowa, NJ, **2005**, S. 39–48.

[94] V. Vichai, K. Kirtikara, *Nat. Protoc.* **2006**, *1*, 1112.

[95] R. Csuk, S. Schwarz, R. Kluge, D. Ströhl, *Arch. Pharm.* **2011**, *344*, 505.

[96] R. Csuk, S. Schwarz, B. Siewert, R. Kluge, D. Ströhl, *Z. Naturforsch. B* **2012**, *67*, 731.

[97] P. P. Banerjee, A. Bandyopadhyay, P. Mondal, M. K. Mondal, P. Chowdhury, A. Chakraborty, M. Sudarshan, S. Bhattacharya, A. Chattopadhyay, *Nucleus* **2019**, *62*, 243.

[98] Y.-C. Lin, J.-F. Lin, T.-F. Tsai, H.-E. Chen, K.-Y. Chou, S.-C. Yang, Y.-M. Tang, T. I.-S. Hwang, *Sci. Rep.* **2017**, *7*, 14103.

[99] D. Ribble, N. B. Goldstein, D. A. Norris, Y. G. Shellman, *BMC Biotech.* **2005**, *5*, 12.

[100] A. J. McGahon, S. J. Martin, R. P. Bissonnette, A. Mahboubi, Y. Shi, R. J. Mogil, W. K. Nishioka, D. R. Green in *Methods in Cell Biology*, *v.46* (Hrsg.: L. M. Schwartz, B. A. Osborne), Elsevier, **1995**, S. 153–185.

[101] K. Liu, P. Liu, R. Liu, X. Wu, *Med. Sci. Monit. Basic Res.* **2015**, *21*, 15.

[102] T. S. Hawley, R. G. Hawley (Hrsg.) *Methods in Molecular Biology*, *Vol. 263*, Humana Press, Totowa, NJ, **2004**.

[103] M. G. Macey (Hrsg.) *Flow Cytometry*. *Principles and Applications*, Humana Press, Totowa, NJ, **2007**.

[104] M. J. Jaroszeski (Hrsg.) *Methods in Molecular Biology*, *Vol. 91*, Humana Press, Totowa, NJ, **1998**.

[105] a) A. Krishan, H. Krishnamurthy, S. Totey (Hrsg.) *Applications of Flow Cytometry in Stem Cell Research and Tissue Regeneration*, John Wiley & Sons, Hoboken, **2011**; b) H. M. Shapiro, *Practical Flow Cytometry,* 4. Aufl., Wiley-Liss, Hoboken, N.J, **2003**.

[106] M. S. Bretscher, *Nat New Biol.* **1972**, *236*, 11.

[107] M. van Engeland, L. J. Nieland, F. C. Ramaekers, B. Schutte, C. P. Reutelingsperger, *Cytometry* **1998**, *31*, 1.

[108] P. Allen, D. Davies in *Flow Cytometry*. *Principles and Applications* (Hrsg.: M. G. Macey), Humana Press, Totowa, NJ, **2007**, S. 147–163.

[109] V. A. Fadok, D. R. Voelker, P. A. Campbell, J. J. Cohen, D. L. Bratton, P. M. Henson, *J. Immunol.* **1992**, *148*, 2207.

[110] Z. Darzynkiewicz, X. Huang, M. Okafuji, M. A. King in *Methods in Cell Biology*, *v.75* (Hrsg.: Z. Darzynkiewicz, M. Roederer, H. Tanke), Elsevier, Amsterdam, **2004**, S. 307–341.

[111] a) Y. Furuya, P. Lundmo, A. D. Short, D. L. Gill, J. T. Isaacs, *Cancer Res.* **1994**, *54*, 6167; b) W. Meikrantz, R. Schlegel, *J. Cell. Biochem.* **1995**, *58*, 160; c) G. Gil-Gómez, A. Berns, H. J. Brady, *EMBO J.* **1998**, *17*, 7209.

[112] F. Q. B. Alenzi, *Br. J. Biomed. Sci.* **2004**, *61*, 99.

[113] a) P. Rabinovitch, "Introduction to cell cycle analysis", **1994**; b) S. W. Sherwood, R. T. Schimke in *Methods in Cell Biology*, *v.46* (Hrsg.: L. M. Schwartz, B. A. Osborne), Elsevier, **1995**, S. 77–97; c) P. Pozarowski, Z. Darzynkiewicz in *Methods in Molecular Biology*, *v. 281* (Hrsg.: A. H. Schönthal), Human Press, Totowa, N.J, **2004**, S. 301–312; d) L. Gong, Q. Yan, Y. Zhang, X. Fang, B. Liu, X. Guan, *Cancer Commun.* **2019**, *39*, 48.

[114] a) H.-W. Cui, Y. He, J. Wang, W. Gao, T. Liu, M. Qin, X. Wang, C. Gao, Y. Wang, M.-Y. Liu et al., *Eur. J. Med. Chem.* **2015**, *95*, 240; b) B. Eignerova, M. Tichy, J. Krasulova, M. Kvasnica, L. Rarova, R. Christova, M. Urban, B. Bednarczyk-Cwynar, M. Hajduch, J. Sarek, *Eur. J. Med. Chem.* **2017**, *140*, 403.

[115] M. Grymel, G. Pastuch-Gawołek, A. Lalik, M. Zawojak, S. Boczek, M. Krawczyk, K. Erfurt, *Molecules* **2020**, *25*, 6019.

[116] a) J. Hao, J. Liu, X. Wen, H. Sun, *Bioorg. Med. Chem.* **2013**, *23*, 2074; b) M. Medina-O'Donnell, F. Rivas, F. J. Reyes-Zurita, A. Martinez, S. Martin-Fonseca, A. Garcia-Granados, R. M. Ferrer-Martín, J. A. Lupiañez, A. Parra, *Eur. J. Med. Chem.* **2016**, *118*, 64; c) W.-B. Mo, C.-H. Su, J.-Y. Huang, J. Liu, Z.-F. Chen, K.-G. Cheng, *Chem. Cent. J.* **2016**, *10*, 69.

[117] B. Pattnaik, V. Lakshma Nayak, S. Ramakrishna, U. Venkata Mallavadhani, *Bioorg. Chem.* **2016**, *68*, 152.

[118] R. Borella, L. Forti, L. Gibellini, A. de Gaetano, S. de Biasi, M. Nasi, A. Cossarizza, M. Pinti, *Molecules* **2019**, *24*, 4097.

[119] E. B. Logashenko, O. V. Salomatina, A. V. Markov, D. V. Korchagina, N. F. Salakhutdinov, G. A. Tolstikov, V. V. Vlassov, M. A. Zenkova, *ChemBioChem* **2011**, *12*, 784.

[120] a) Y. Meng, Y. Song, Z. Yan, Y. Xia, *Molecules* **2010**, *15*, 4033; b) S.-X. Hua, R.-Z. Huang, M.-Y. Ye, Y.-M. Pan, G.-Y. Yao, Y. Zhang, H.-S. Wang, *Eur. J. Med. Chem.* **2015**, *95*, 435; c) D. Kashyap, H. S. Tuli, A. K. Sharma, *Life Sci.* **2016**, *146*, 201; d) W. Li, H. Zhang, M. Nie, W. Wang, Z. Liu, C. Chen, H. Chen, R. Liu, Z. Baloch, K. Ma, *Oncol. Lett.* **2018**, *15*, 2323; e) J. Zou, J. Lin, C. Li, R. Zhao, L. Fan, J. Yu, J. Shao,

Curr. Cancer Drug Targets **2019**, *19*, 245; f) J.-W. Shao, Y.-C. Dai, J.-P. Xue, J.-C. Wang, F.-P. Lin, Y.-H. Guo, *Eur. J. Med. Chem.* **2011**, *46*, 2652.

[121] L. Heller, A. Knorrscheidt, F. Flemming, J. Wiemann, S. Sommerwerk, I. Z. Pavel, A. Al-Harrasi, R. Csuk, *Bioorg. Chem.* **2016**, *68*, 137.

[122] M. Sahn, A. Grupe, L. Heller, H. Hussain, A. Al-Harrasi, R. Csuk, *Mediterr. J. Chem.* **2018**, *7*, 39.

[123] M. Kahnt, L. Heller, P. Grabandt, A. Al-Harrasi, R. Csuk, *Eur. J. Med. Chem.* **2018**, *143*, 259.

[124] a) R. K. Wolfram, A. Barthel-Niesen, R. Schäfer, L. Heller, A. Al-Harrasi, R. Csuk, *Mediterr. J. Chem.* **2017**, *6*, 180; b) B. Siewert, R. Csuk, *Eur. J. Med. Chem.* **2014**, *74*, 1.

[125] S. Schwarz, R. Csuk, *Bioorg. Med. Chem.* **2010**, *18*, 7458.

[126] R. Csuk, S. Schwarz, B. Siewert, R. Kluge, D. Ströhl, *Arch. Pharm.* **2012**, *345*, 223.

[127] R. Csuk, A. Barthel-Niesen, A. Barthel, R. Schäfer, A. Al-Harrasi, *Eur. J. Med. Chem.* **2015**, *100*, 98.

[128] S. Sommerwerk, L. Heller, J. Kuhfs, R. Csuk, *Eur. J. Med. Chem.* **2016**, *122*, 452.

[129] B. Siewert, E. Pianowski, R. Csuk, *Eur. J. Med. Chem.* **2013**, *70*, 259.

[130] a) R. Csuk, C. Nitsche, R. Sczepek, S. Schwarz, B. Siewert, *Arch. Pharm.* **2013**, *346*, 232; b) S. Schwarz, B. Siewert, N. M. Xavier, A. R. Jesus, A. P. Rauter, R. Csuk, *Eur. J. Med. Chem.* **2014**, *72*, 78.

[131] S. Sommerwerk, L. Heller, R. Csuk, *Arch. Pharm.* **2015**, *348*, 46.

[132] a) R. Csuk, B. Siewert, C. Dressel, R. Schäfer, *Eur. J. Med. Chem.* **2012**, *56*, 237; b) R. Csuk, S. Stark, C. Nitsche, A. Barthel, B. Siewert, *Eur. J. Med. Chem.* **2012**, *53*, 337; c) L. Heller, S. Sommerwerk, F. Tzschöckell, J. Wiemann, S. Schwarz, B. Siewert, A. Al-Harrasi, R. Csuk, *Arch. Pharm.* **2015**, *348*, 889; d) S. Sommerwerk, L. Heller, I. Serbian, R. Csuk, *Tetrahedron* **2015**, *71*, 8528.

[133] R. Csuk, S. Schwarz, B. Siewert, R. Kluge, D. Ströhl, *Eur. J. Med. Chem.* **2011**, *46*, 5356.

[134] a) R. Csuk, S. Schwarz, B. Siewert, R. Kluge, D. Ströhl, *Z. Naturforsch. B* **2011**, *66*, 521; b) R. Csuk, S. Schwarz, R. Kluge, D. Ströhl, *Arch. Pharm.* **2012**, *345*, 28; c) B. Siewert, J. Wiemann, A. Köwitsch, R. Csuk, *Eur. J. Med. Chem.* **2014**, *72*, 84; d) R. Csuk, A. Niesen-Barthel, R. Schäfer, A. Barthel, A. Al-Harrasi, *Eur. J. Med. Chem.* **2015**, *92*, 700.

[135] a) A. Niesen, A. Barthel, R. Kluge, A. Köwitzsch, D. Ströhl, S. Schwarz, R. Csuk, *Arch. Pharm.* **2009**, *342*, 569; b) I. Serbian, R. K. Wolfram, L. Fischer, A. Al-Harrasi, R. Csuk, *Mediterr. J. Chem.* **2018**, *7*, 286; c) R. Csuk, A. Niesen-Barthel, A. Barthel, R. Kluge, D. Ströhl, *Eur. J. Med. Chem.* **2010**, *45*, 3840.

[136] a) S. Sommerwerk, L. Heller, C. Kerzig, A. E. Kramell, R. Csuk, *Eur. J. Med. Chem.* **2017**, *127*, 1; b) M. Kahnt, J. Wiemann, L. Fischer, S. Sommerwerk, R. Csuk, *Eur. J. Med. Chem.* **2018**, *159*, 143; c) R. K. Wolfram, L. Fischer, R. Kluge, D. Ströhl, A. Al-Harrasi, R. Csuk, *Eur. J. Med. Chem.* **2018**, *155*, 869; d) R. K. Wolfram, L. Heller, R. Csuk, *Eur. J. Med. Chem.* **2018**, *152*, 21.

[137] a) M. Kahnt, N. H. L. Fischer, A. Al-Harrasi, R. Csuk, *Molecules* **2018**, *23*; b) T. C. Denner, S. Hoenke, O. Kraft, H.-P. Deigner, A. Al-Harrasi, R. Csuk, *Results Chem.* **2022**, *4*, 100371.

[138] M. Kozubek, S. Hoenke, H.-P. Deigner, R. Csuk, *Results Chem.* **2022**, *4*, 100429.

[139] N. V. Heise, D. Major, S. Hoenke, M. Kozubek, I. Serbian, R. Csuk, *Molecules* **2022**, *27*, 2220.

[140] a) S. Friedrich, I. Serbian, S. Hoenke, R. K. Wolfram, R. Csuk, *Med. Chem. Res.* **2020**, *29*, 926; b) B. Brandes, S. Hoenke, L. Fischer, R. Csuk, *Eur. J. Med. Chem.* **2020**, *185*, 111858; c) O. Kraft, M. Kozubek, S. Hoenke, I. Serbian, D. Major, R. Csuk, *Eur. J. Med. Chem.* **2021**, *209*, 112920.

[141] J. Wiemann, L. Fischer, J. Kessler, D. Ströhl, R. Csuk, *Bioorg. Chem.* **2018**, *81*, 567.

[142] O. Kraft, S. Hoenke, R. Csuk, *Eur. J. Med. Chem. Rep.* **2022**, *5*, 100043.

[143] J. Neuzil, L.-F. Dong, J. Rohlena, J. Truksa, S. J. Ralph, *Mitochondrion* **2013**, *13*, 199.

[144] I. Serbian, S. Hoenke, R. Csuk, *Eur. J. Med. Chem.* **2020**, *199*, 112425.

[145] M. Kozubek, I. Serbian, S. Hoenke, O. Kraft, R. Csuk, *Results Chem.* **2020**, *2*, 100057.

[146] B. Brandes, S. Hoenke, C. Schultz, H.-P. Deigner, R. Csuk, *Steroids* **2022**, *189*, 109148.

[147] N. Heise, S. Hoenke, V. Simon, H.-P. Deigner, A. Al-Harrasi, R. Csuk, *Steroids* **2021**, *172*, 108876.

[148] a) N. V. Heise, S. Hoenke, I. Serbian, R. Csuk, *Eur. J. Med. Chem. Rep.* **2022**, *6*, 100073; b) O. Kraft, A.-K. Hartmann, S. Hoenke, I. Serbian, R. Csuk, *Int. J. Mol. Sci.* **2022**, *23*, 4362; c) M. Kozubek, T. C. Denner, M. Eckert, S. Hoenke, R. Csuk, *Results Chem.* **2023**, *5*, 100708.

5 Anhang

Für die Arbeit wichtige Publikationen

P-1 Synthesis of amide-spacered dimers of ursolic and oleanolic acid

S. Hoenke, I. Wiengarn, I. Serbian, A. Al-Harrasi, R. Csuk, *Mediterr. J. Chem.* **2019**, *9*, 24.

Abstract

Transdermal therapeutic systems can release drug substances slowly and in a controlled manner from a drug depot. To provide a slow-release form of ursolic and oleanolic acid amide-spacered dimers were synthesized from the parent acids and diamines of variable chain lengths. These dimers were assayed in sulforhodamine B (SRB) assays for their cytotoxicity since as a pre-requisite for their use in slow-release forms these substances must not exert any unfavorable side-effects such as cytotoxicity. As a result of long term incubation up to 96 hours, none of these compounds showed any significant cytotoxicity in sulforhodamine B assays.

Keywords: ursolic acid, oleanolic acid, triterpenes, dimers.

DOI: 10.13171/mjc91190811415rc

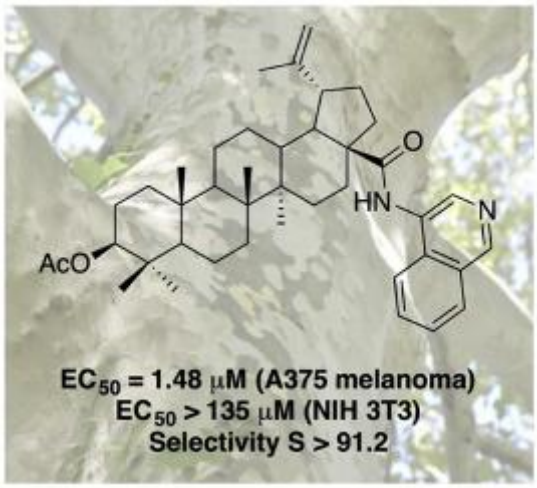
P-2 Betulinic acid derived amides are highly cytotoxic, apoptotic and selective

S. Hoenke, N. V. Heise, M. Kahnt, H.-P. Deigner, R. Csuk, *Eur. J. Med. Chem.* **2020**, *207*, 112815.

Abstract

Betulinic and platanic acid derived amides were prepared and screened for their cytotoxic activity. All of the compounds were shown to be cytotoxic for a panel of human tumor cell lines, and especially apoptotic betulinic acid derived compounds **6**, **8** and **19** showed low EC_{50} values. Of special interest was a 4-isoquinolinyl amide of 3-O-acetyl-betulinic acid (compound **19**), being the most cytotoxic compound of this series and holding EC_{50} values as low as EC_{50} = 1.48 µM (A375 melanoma cells) while being significantly less cytotoxic for non-malignant fibroblasts NIH 3T3 with a selectivity index of > 91.2. This finding parallels previous results obtained for SAA21, a augustic acid derived compound thus making the 4-isoquinolinyl moiety to a privileged scaffold.

Graphical abstract



Keywords: betulinic acid, platanic acid, amides, cytotoxicity, isoquinoline.

DOI: 10.1016/j.ejmech.2020.112815

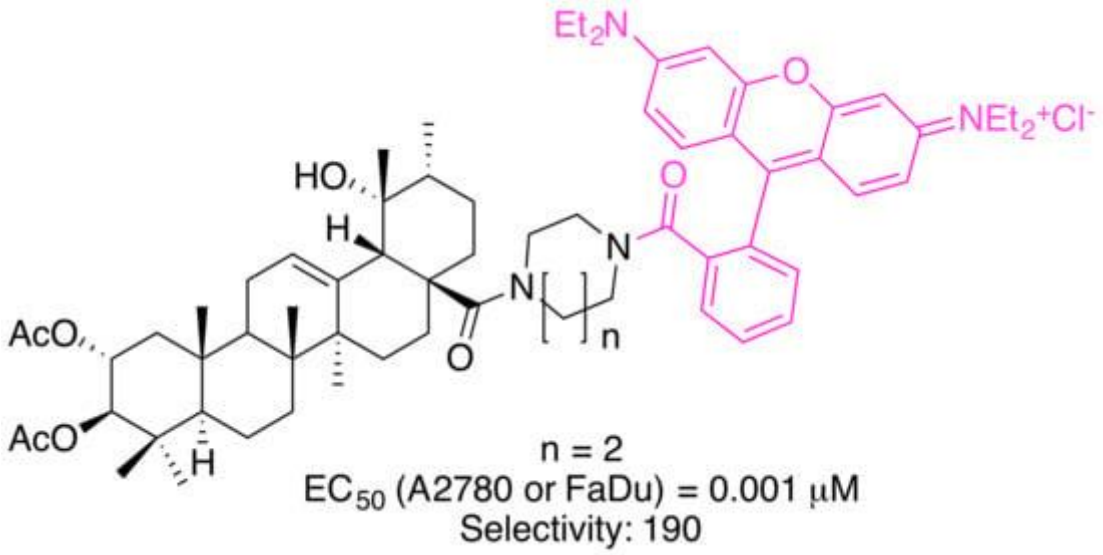
P-3 Mitocanic Di- and Triterpenoid Rhodamine B Conjugates

S. Hoenke, I. Serbian, H.-P. Deigner, R. Csuk, *Molecules* **2020**, *25*, 5443.

Abstract

The combination of the “correct” triterpenoid, the “correct” spacer and rhodamine B (**RhoB**) seems to be decisive for the ability of the conjugate to accumulate in mitochondria. So far, several triterpenoid rhodamine B conjugates have been prepared and screened for their cytotoxic activity. To obtain cytotoxic compounds with EC_{50} values in a low nano-molar range combined with good tumor/non-tumor selectivity, the **RhoB** unit has to be attached via an amine spacer to the terpenoid skeleton. To avoid spirolactamization, secondary amines have to be used. First results indicate that a homopiperazinyl spacer is superior to a piperazinyl spacer. Hybrids derived from maslinic acid or tormentic acid are superior to those from oleanolic, ursolic, glycyrrhetinic or euscaphic acid. Thus, a tormentic acid-derived **RhoB** conjugate **32**, holding a homopiperazinyl spacer can be regarded, at present, as the most promising candidate for further biological studies.

Graphical abstract



Keywords: triterpenoic acid, maslinic acid, tormentic acid, betulinic acid, oleanolic acid, rhodamine B, cytotoxicity.

DOI: 10.3390/molecules25225443

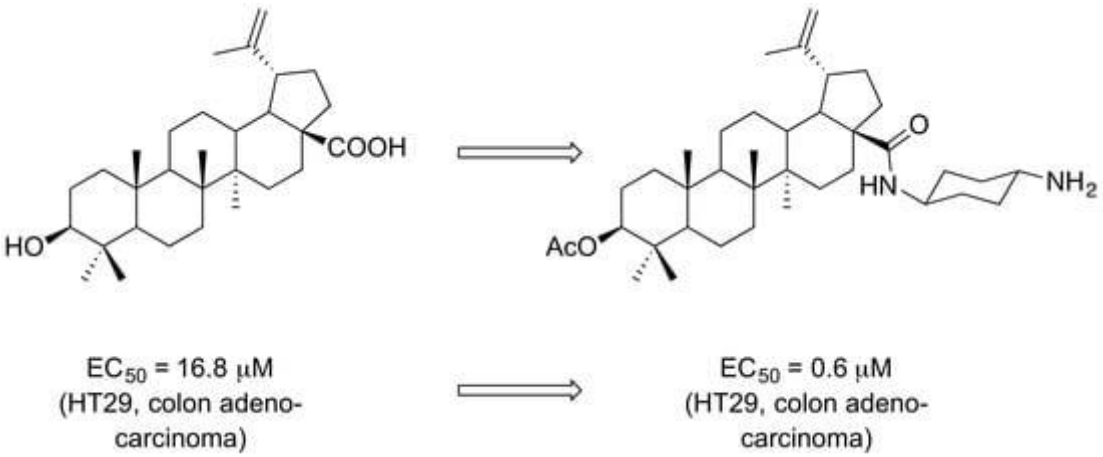
P-4 The Presence of a Cyclohexyldiamine Moiety Confers Cytotoxicity to Pentacyclic Triterpenoids

S. Hoenke, M. A. Christoph, S. Friedrich, N. Heise, B. Brandes, H.-P. Deigner, A. Al-Harrasi, R. Csuk, *Molecules* **2021**, *26*, 2102.

Abstract

Pentacyclic triterpenoids oleanolic acid, ursolic acid, betulinic acid, and platanic acid were acetylated and converted into several amides **9**-**31**; the cytotoxicity of which has been determined in sulforhodamine B assays employing seral human tumor cell lines and nonmalignant fibroblasts. Thereby, a betulinic acid/trans-1,4-cyclohexyldiamine amide showed excellent cytotoxicity (for example, EC_{50} = 0.6 µM for HT29 colon adenocarcinoma cells).

Graphical Abstract



Keywords: oleanolic acid, ursolic acid, betulinic acid, platanic acid, 1,4-cyclohexyldiamines, cytotoxicity.

DOI: 10.3390/molecules26072102

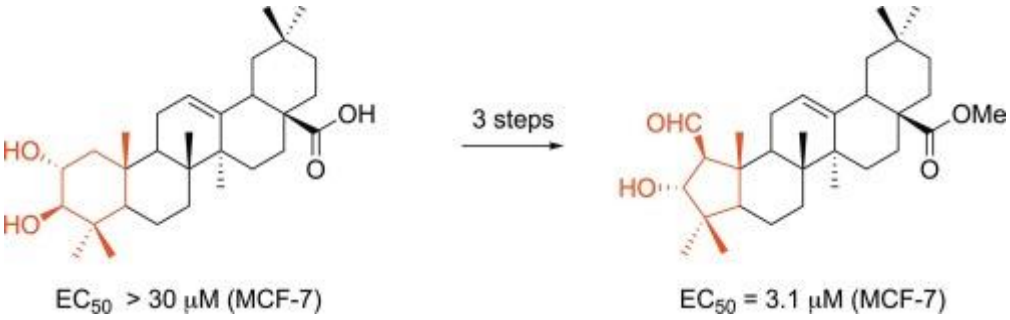
P-5 A Malaprade cleavage, a McMurry ring closure and an intramolecular aldol contraction of maslinic acid's ring A

S. Hoenke, I. Serbian, R. Csuk, *Results Chem.* **2022**, *4*, 100547.

Abstract

A silica gel supported Malaprade cleavage of methyl maslinoate (→3) followed by a titanium-on-graphite supported McMurry coupling was established to access a 2,12-terpenoid. The microwaves supported aldol reaction of dialdehyde **3** gave ring contracted **5**. While parent maslinic acid is of minor cytotoxicity, improved cytotoxicity was observed in SRB assays for **3** while ring contracted compound **5** was most cytotoxic for MCF-7 breast adenocarcinoma cells holding a low EC_{50} = 3.1 µM.

Graphical abstract



Keywords: Maslinic acid, Ring contraction, Aldol reaction, Cytotoxicity, McMurry reaction.

DOI: 10.1016/j.rechem.2022.100547

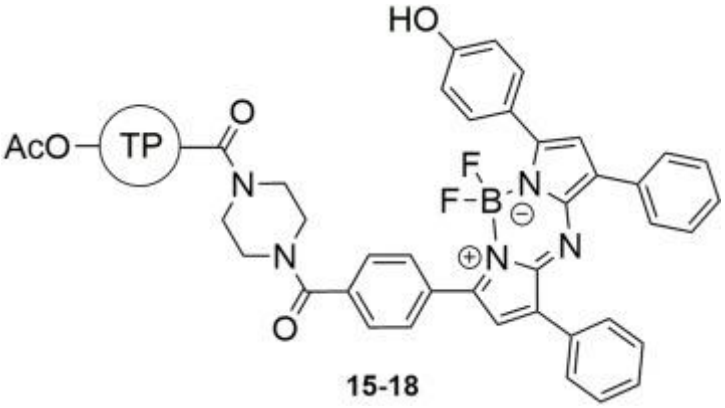
P-6 Non-cytotoxic aza-BODIPY triterpene conjugates to target the endoplasmic reticulum

S. Hoenke, B. Brandes, R. Csuk, *Eur. J. Med. Chem. Rep.* **2022**, 100099.

Abstract

In search of suitable tool to target the endoplasmic reticulum of human tumor cells, a non-cytotoxic aza-BODIPY derivative was designed and accessed in a simple synthesis in a few steps from commercially available starting materials. This aza-BODIPY was conjugated to 3-*O*-acetyl-triterpene carboxylic acids (glycyrrhetinic, ursolic, oleanolic, and betulinic acid) using a piperazinyl spacer. The resulting conjugates exhibited no cytotoxicity but were able to selectively target the ER.

Graphical abstract



Keywords: Triterpenes, aza-BODIPY, Endoplasmic reticulum, Malignant cells

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Lebenslauf

Persönliche Daten

Name	Sophie Hoenke
Geburtsdatum	09.08.1995
Geburtsort	Magdeburg
Staatsangehörigkeit	deutsch

Bildungsweg

Seit 08/2018	Promotionsstudium am Institut für Chemie, Bereich organische Chemie an der Martin-Luther-Universität Halle-Wittenberg unter der Leitung von Professor Csuk
10/2013 bis 05/201	Diplomstudium Lebensmittelchemie an der Martin-Luther-Universität Halle-Wittenberg
08/2005 bis 06/2013	Allgemeine Hochschulreife am Rudolf-Hildebrand-Gymnasium Stendal

Publikationsliste

1. „In search of new cinnamic acid derived flavours and fragrances“
   J. M. Gießel, A. Loesche, **S. Hoenke**, R. Csuk, *Results Chem.* **2019**, *1*, 100010. DOI: 10.1016/j.rechem.2019.100010
2. „Synthesis of amide-spacered dimers of ursolic and oleanolic acid“
   S. Hoenke, I. Wiengarn, I. Serbian, A. Al-Harrasi, R. Csuk, *Mediterr. J. Chem.* **2019**, *9*, 24. DOI: 10.13171/mjc91190811415rc
3. „Synthesis and Cytotoxicity Evaluation of DOTA-Conjugates of Ursolic Acid“
   M. Kahnt, **S. Hoenke**, L. Fischer, A. Al-Harrasi, R. Csuk, *Molecules* **2019**, *24*, 2254. DOI: 10.3390/molecules24122254
4. „The cytotoxicity of oleanane derived aminocarboxamides depends on their aminoalkyl substituents“
   M. Kahnt, A. Loesche, I. Serbian, **S. Hoenke**, L. Fischer, A. Al-Harrasi, R. Csuk, *Steroids* **2019**, *149*, 108422.
   DOI: 10.1016/j.steroids.2019.05.014
5. „Ureidobenzenesulfonamides as efficient inhibitors of carbonic anhydrase II“
   I. Serbian, P. Schwarzenberger, A. Loesche, **S. Hoenke**, A. Al-Harrasi, R. Csuk, *Bioorg. Chem.* **2019**, *91*, 103123.
   DOI: 10.1016/j.bioorg.2019.103123
6. „Unexpected cytotoxicity of a triisopropylsilylated syringaldehyde derived cinnamic acid amide“
   B. A. Weber, R. K. Wolfram, **S. Hoenke**, L. Fischer, A. Al-Harrasi, R. Csuk, *Mediterr. J. Chem.* **2019**, *9*, 45.
   DOI: 10.13171/mjc91190820520rc

7. „A hitherto unreported impurity in Terazosin – elucidation of the structure, synthesis and cytotoxicity“
   B. Brandes, **S. Hoenke**, H.-P. Deigner, R. Csuk, *Mediterr. J. Chem.* **2020**, *10*, 502.
   DOI: 10.13171/mjc10502005171436rc
8. „Design, synthesis and cytotoxicity of BODIPY FL labelled triterpenoids“
   B. Brandes, **S. Hoenke**, L. Fischer, R. Csuk, *Eur. J. Med. Chem.* **2020**, *185*, 111858.
   DOI: 10.1016/j.ejmech.2019.111858
9. „A unified strategy for the synthesis of amorfrutins A and B and evaluation of their cytotoxicity“
   B. Brandes, **S. Hoenke**, M. Türk, B. Weber, H.-P. Deigner, A. Al-Harrasi, R. Csuk, *Mediterr. J. Chem.* **2020**, *11*, 23.
   DOI: 10.13171/mjc10902011171546rc
10. „The presence of a cationic center is not alone decisive for the cytotoxicity of triterpenecarboxylic acid amides“
    B. Brandes, L. Koch, **S. Hoenke**, H.-P. Deigner, R. Csuk, *Steroids* **2020**, *163*, 108713.
    DOI: 10.1016/j.steroids.2020.108713
11. „Synthesis and cytotoxic evaluation of malachite green derived oleanolic and ursolic acid piperazineamides“
    S. Friedrich, I. Serbian, **S. Hoenke**, R. K. Wolfram, R. Csuk, *Med. Chem. Res.* **2020**, *29*, 926.
    DOI: 10.1007/s00044-020-02536-1
12. „Development of indanones and isatins as non-cytotoxic inhibitors of cholinesterases“
    J. M. Gießel, A. Loesche, **S. Hoenke**, I. Serbian, A. Al-Harrasi, R. Csuk, *Mediterr. J. Chem.* **2020**, *10*, 121.
    DOI: 10.13171/mjc10202002161233rc
13. „Betulinic acid derived amides are highly cytotoxic, apoptotic and selective“
    S. Hoenke, N. V. Heise, M. Kahnt, H.-P. Deigner, R. Csuk, *Eur. J. Med. Chem.* **2020**, *207*, 112815.
    DOI: 10.1016/j.ejmech.2020.112815
14. „Mitocanic Di- and Triterpenoid Rhodamine B Conjugates“
    S. Hoenke, I. Serbian, H.-P. Deigner, R. Csuk, *Molecules* **2020**, *25*, 5443.
    DOI: 10.3390/molecules25225443
15. „Synthesis and cytotoxic evaluation of hydroxycinnamic acid rhodamine B conjugates“
    M. Kozubek, I. Serbian, **S. Hoenke**, O. Kraft, R. Csuk, *Results Chem.* **2020**, *2*, 100057.
    DOI: 10.1016/j.rechem.2020.100057
16. „Synthesis of some steroidal mitocans of nanomolar cytotoxicity acting by apoptosis“
    I. Serbian, **S. Hoenke**, R. Csuk, *Eur. J. Med. Chem.* **2020**, *199*, 112425.
    DOI: 10.1016/j.ejmech.2020.112425
17. „Ester and amide derivatives of rhodamine B exert cytotoxic effects on different human tumor cell lines“
    I. Serbian, **S. Hoenke**, O. Kraft, R. Csuk, *Med. Chem. Res.* **2020**, *29*, 1655.
    DOI: 10.1007/s00044-020-02591-8
18. „Evaluation of cholinesterase inhibitory activity and cytotoxicity of synthetic derivatives of di- and triterpene metabolites from *Pinus silvestris* and *Dipterocarpus alatus* resins“
    I. E. Smirnova, O. B. Kazakova, A. Loesche, **S. Hoenke**, R. Csuk, *Med. Chem. Res.* **2020**, *29*, 1478.
    DOI: 10.1007/s00044-020-02566-9

19. „Glycyrrhetinic amides and their cytotoxicity“
    N. Heise, **S. Hoenke**, A. Al-Harrasi, H.-P. Deigner, R. Csuk, *Mediterr. J. Chem.* **2021**, *11*, 255.
    DOI: 10.13171/mjc02110161595Cesuk
20. „Type and position of linkage govern the cytotoxicity of oleanolic acid rhodamine B hybrids“
    N. Heise, **S. Hoenke**, V. Simon, H.-P. Deigner, A. Al-Harrasi, R. Csuk, *Steroids* **2021**, 108876.
    DOI: 10.1016/j.steroids.2021.108876
21. „A simple but unusual rearrangement of an oleanane to a taraxerane-28,14 β -olide“
    N. Heise, B. Siewert, D. Ströhl, **S. Hoenke**, O. Kazakova, R. Csuk, *Steroids* **2021**, *172*, 108853.
    DOI: 10.1016/j.steroids.2021.108853
22. „The Presence of a Cyclohexyldiamine Moiety Confers Cytotoxicity to Pentacyclic Triterpenoids“
    S. Hoenke, M. A. Christoph, S. Friedrich, N. Heise, B. Brandes, H.-P. Deigner, A. Al-Harrasi, R. Csuk, *Molecules* **2021**, *26*, 2102.
    DOI: 10.3390/molecules26072102
23. „Synthesis and *In Silico* Docking of New Pyrazolo[*4,3-e*]pyrido[*1,2-a*]pyrimidine-based Cytotoxic Agents“
    M. Horchani, N. V. Heise, **S. Hoenke**, R. Csuk, A. H. Harrath, H. B. Jannet, A. Romdhane, *Int. J. Mol. Sci.* **2021**, *22*, 10258.
    DOI: 10.3390/ijms221910258
24. „Cytotoxic Potential of a-Azepano- and 3-Amino-3,4-SeCo-Triterpenoids“
    O. Kazakova, I. Smirnova, E. Tret'yakova, R. Csuk, **S. Hoenke**, L. Fischer, *Int. J. Mol. Sci.* **2021**, *22*, 1714.
    DOI: 10.3390/ijms22041714
25. „Synthesis of messagenin and platanic acid chalcone derivatives and their biological potential“
    E. Khusnutdinova, Z. Galimova, A. Lobov, I. Baikova, O. Kazakova, H. N. T. Thu, N. van Tuyen, Y. Gatilov, R. Csuk, I. Serbian **et al.**, *Nat. Prod. Res.* **2021**, 1.
    DOI: 10.1080/14786419.2021.1922904
26. „Apoptotic activity of substituted 3-*O*-acetyl-betulinic acid benzylamides“
    M. Kozubek, L. Höhlich, **S. Hoenke**, H.-P. Deigner, A. Al-Harrasi, R. Csuk, *Eur. J. Med. Chem. Rep.* **2021**, *3*, 100016.
    DOI: 10.1016/j.ejmcr.2021.100016
27. „Cytotoxic triterpenoidesafirinium conjugates target the endoplasmic reticulum“
    O. Kraft, M. Kozubek, **S. Hoenke**, I. Serbian, D. Major, R. Csuk, *Eur. J. Med. Chem.* **2021**, *209*, 112920.
    DOI: 10.1016/j.ejmech.2020.112920
28. „Racemization-free synthesis of *N*α-2-thiophenoyl-phenylalanine-2morpholinoanilide enantiomers and their antimycobacterial activity“
    L. Mann, M. Lang, P. Schulze, J. H. Halz, R. Csuk, **S. Hoenke**, R. W. Seidel, A. Richter, *Amino Acids* **2021**, *53*, 1187.
    DOI: 10.1007/s00726-021-03044-1
29. „Antibacterial and Cytotoxic Activity of Ruthenium-*p*-cymene Complexes with 2-Methylquinolin-8-ol Derivatives“
    A. Nain-Perez, L. C. A. Barbosa, M. H. Araujo, J. P. A. Martins, J. A. Takahashi, G. Oliveira,

R. Diniz, L. Heller, **S. Hoenke**, R. Csuk, *ChemistrySelect* **2021**, *6*, 2942.
DOI: 10.1002/slct.202100733

30. „Structure−Activity Relationship of Anti-*Mycobacterium abscessus* Piperidine-4-carboxamides, a New Class of NBTI DNA Gyrase Inhibitors“
A. Beuchel, D. Robaa, D. A. Negatu, A. Madani, N. Alvarez, M. D. Zimmerman, A. Richter, L. Mann, **S. Hoenke**, R. Csuk et al., *ACS Med. Chem. Lett.* **2022**.
DOI: 10.1021/acsmedchemlett.1c00549
31. „Hydroxyethylamide substituted triterpenoic acids hold good cytotoxicity for human tumor cells“
T. C. Denner, **S. Hoenke**, O. Kraft, H.-P. Deigner, A. Al-Harrasi, R. Csuk, *Results Chem.* **2022**, *4*, 100371.
DOI: 10.1016/j.rechem.2022.100371
32. „An improved partial synthesis of corosolic acid and its conversion to highly cytotoxic mitocans“
N. V. Heise, **S. Hoenke**, I. Serbian, R. Csuk, *Eur. J. Med. Chem. Rep.* **2022**, 100073.
DOI: 10.1016/j.ejmcr.2022.100073
33. „An improved partial synthesis of corosolic acid and its conversion to highly cytotoxic mitocans“
N. V. Heise, D. Major, **S. Hoenke**, M. Kozubek, I. Serbian, R. Csuk, *Molecules* **2022**, *27*, 2220.
DOI: 10.3390/molecules27072220
34. „Betulinic acid and glycyrrhetinic acid derived piperazinyl spacered rhodamine B conjugates are highly cytotoxic and necrotic“
M. Kozubek, **S. Hoenke**, H.-P. Deigner, R. Csuk, *Results Chem.* **2022**, 100429.
DOI: 10.1016/j.rechem.2022.100429
35. „Synthesis and cytotoxicity of betulin and betulinic acid derived 30-oxo-amides“
M. Kozubek, **S. Hoenke**, T. Schmidt, H.-P. Deigner, A. Al-Harrasi, R. Csuk, *Steroids* **2022**, *182*, 109014.
DOI: 10.1016/j.steroids.2022.109014
36. „Platanic acid derived amides are more cytotoxic than their corresponding oximes“
M. Kozubek, **S. Hoenke**, T. Schmidt, D. Ströhl, R. Csuk, *Med. Chem. Res.* **2022**.
DOI: 10.1007/s00044-022-02902-1
37. „Madecassic Acid—A New Scaffold for Highly Cytotoxic Agents“
O. Kraft, A.-K. Hartmann, **S. Hoenke**, I. Serbian, R. Csuk, *Int. J. Mol. Sci.* **2022**, *23*.
DOI: 10.3390/ijms23084362
38. „A tormentic acid-homopiperazine-rhodamine B conjugate of single-digit nanomolar cytotoxicity and high selectivity for several human tumor cell lines“
O. Kraft, **S. Hoenke**, R. Csuk, *Eur. J. Med. Chem. Rep.* **2022**, *5*, 100043.
DOI: 10.1016/j.ejmcr.2022.100043
39. „Synthesis and cytotoxicity of apoptosis-inducing N-heterocyclic triterpene amides“
B. Brandes, **S. Hoenke**, N. Starke, I. Serbian, H.-P. Deigner, A. Al-Harrasi, R. Csuk, *Eur. J. Med. Chem. Rep.* **2022**, 100085.
DOI: 10.1016/j.ejmcr.2022.100085
40. „Synthesis and characterization of steroidal, anellated aminothiophenes by Gewald reaction”
O. Kraft, G. C. Mittag, **S. Hoenke**, N. V. Heise, A. Al-Harrasi, R. Csuk, *Mediterr. J. Chem.* **2022**, *12*, 140.
DOI: 10.13171/mjc02209261446csuk

41. „A Malaprade cleavage, a McMurry ring closure and an intramolecular aldol contraction of maslinic acid's ring A"
    S. Hoenke, I. Serbian, R. Csuk, *Results Chem.* **2022**, *4*, 100547.
    DOI: 10.1016/j.rechem.2022.100547
42. "Selective and low-cost triterpene urea and amide derivatives of high cytotoxicity and selectivity"
    B. Brandes, T. E. Orlamünde, **S. Hoenke**, T. C. Denner, A. Al-Harrasi, R. Csuk, *Results Chem.* **2022**, 100610.
    DOI: 10.1016/j.rechem.2022.100610
43. „Converting bile acids into mitocans"
    B. Brandes, **S. Hoenke**, C. Schultz, H.-P. Deigner, R. Csuk, *Steroids* **2022**, 109148.
    DOI: 10.1016/j.steroids.2022.109148
44. „Non-cytotoxic aza-BODIPY triterpene conjugates to target the endoplasmic reticulum"
    S. Hoenke, B. Brandes, R. Csuk, *Eur. J. Med. Chem. Rep.* **2022**, 100099.
    DOI: 10.1016/j.ejmcr.2022.100099
45. „New amides derived from sclareolide as anticholinesterase agents"
    J. G. Silva, T. F. Borgati, S. M. G. Lopes, N. Heise, **S. Hoenke**, R. Csuk, L. C. A. Barbosa, *Bioorg. Chem.* **2023**, *130*, 106249.
    DOI: 10.1016/j.bioorg.2022.106249
46. „On the influence of the rhodamine substituents onto the cytotoxicity of mitocanic maslinic acid"
    M. Kozubek, T. C. Denner, M. Eckert, **S. Hoenke**, R. Csuk, *Results Chem.* **2023**, *5*, 100708.
    DOI: 10.1016/j.rechem.2022.100708

Erklärung über die Autorenanteile der Publikationen

P-1 Synthesis of amide-spacered dimers of ursolic and oleanolic acid
S. Hoenke, I. Wiengarn, I. Serbian, A. Al-Harrasi, R. Csuk, *Mediterr. J. Chem.* **2019**, *9*, 24.
Die Synthese, Auswertung und weiteren Untersuchungen wurden von Frau B.Sc. I. Wiengarn, Herrn Dr. I. Serbian und mir durchgeführt, wobei ich die biologische Evaluierung geleitet habe. Den Entwurf, die Überarbeitung und fachliche Betreuung haben Profs. Dr. A. Al-Harrasi und Dr. R. Csuk übernommen.

P-2 Betulinic acid derived amides are highly cytotoxic, apoptotic and selective
S. Hoenke, N. V. Heise, M. Kahnt, H.-P. Deigner, R. Csuk, *Eur. J. Med. Chem.* **2020**, *207*, 112815.
Herr M.Sc. N. Heise und Dr. M Kahnt haben die Synthesen übernommen und ich habe die biologische Evaluierung durchgeführt. Der Entwurf stammte von Profs. Dr. H.-P. Deigner und Dr.R. Csuk, der auch die fachliche Beratung und Überarbeitung übernommen hat.

P-3 Mitocanic Di- and Triterpenoid Rhodamine B Conjugates
S. Hoenke, I. Serbian, H.-P. Deigner, R. Csuk, *Molecules* **2020**, *25*, 5443.
Nach einem Konzept von Profs. Dr. H.-P. Deigner und Dr. R. Csuk erfolgten die Untersuchungen und Validierungen durch mich mit Unterstützung von Herrn Dr. I. Serbian.

P-4 The Presence of a Cyclohexyldiamine Moiety Confers Cytotoxicity to Pentacyclic Triterpenoids
S. Hoenke, M. A. Christoph, S. Friedrich, N. Heise, B. Brandes, H.-P. Deigner, A. Al-Harrasi, R. Csuk, *Molecules* **2021**, *26*, 2102.
Durch die Profs. Dr. A. Al-Harrasi, Dr. H.-P. Deigner und Dr. R. Csuk entstand das Konzept für die Untersuchungen, die von den weiter genannten Personen durchgeführt wurden. Die Synthesen waren Teil der Bachelorarbeit von Herrn B.Sc. M. A. Christoph sowie der Masterarbeit von Herrn M.Sc. S. Friedrich. Ich habe außerdem die biologischen Untersuchungen durchgeführt. Die Manuskriptüberarbeitung und weitere Betreuung hat Prof. Dr. R. Csuk vorgenommen.

P-5 A Malaprade cleavage, a McMurry ring closure and an intramolecular aldol contraction of maslinic acid's ring A

S. Hoenke, I. Serbian, R. Csuk, *Results Chem.* **2022**, *4*, 100547.

Nach dem Entwurf von Prof. Dr. R. Csuk haben Dr. I. Serbian und ich die Untersuchungen geführt und beschrieben. Die Überarbeitung hat ebenfalls Prof. Dr. R. Csuk vorgenommen.

P-6 Non-cytotoxic aza-BODIPY triterpene conjugates to target the endoplasmic reticulum

S. Hoenke, B. Brandes, R. Csuk, *Eur. J. Med. Chem. Rep.* **2022**, 100099.

Die Synthese, Auswertung und weiteren Untersuchungen erfolgten durch Herrn M.Sc. B. Brandes und mich. Die fachliche Leitung und Überarbeitung des Manuskripts gingen von Prof. Dr. R. Csuk aus.

Selbständigkeitserklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst, keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.
Die Arbeit wurde bisher an keiner anderen Universität oder Hochschule vorgelegt.

Halle (Saale), den 17.01.2023 Sophie Hoenke

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Synthesis of amide-spacered dimers of ursolic and oleanolic acid

Sophie Hoenke [1], Isabell Wiengarn [1], Immo Serbian [1], Ahmed Al-Harrasi [2] and René Csuk [1,*]

[1] Martin-Luther-University Halle-Wittenberg, Organic Chemistry, Kurt-Mothes-Str. 2, D-06120 Halle (Saale), Germany

[2] University of Nizwa, Chair of Oman's Medicinal Plants and Marine Natural Products, P.O. Box 33, PC 616, Birkat Al-Mauz, Nizwa, Sultanate of Oman

Abstract: Transdermal therapeutic systems can release drug substances slowly and in a controlled manner from a drug depot. To provide a slow-release form of ursolic and oleanolic acid amide-spacered dimers were synthesized from the parent acids and diamines of variable chain lengths. These dimers were assayed in sulforhodamine B (SRB) assays for their cytotoxicity since as a pre-requisite for their use in slow-release forms these substances must not exert any unfavorable side-effects such as cytotoxicity. As a result of long term incubation up to 96 hours, none of these compounds showed any significant cytotoxicity in sulforhodamine B assays.

Keywords: ursolic acid; oleanolic acid; triterpenes; dimers.

1. Introduction

In recent years, the search for biologically active compounds has increasingly turned to secondary natural substances. A newly awakened interest consists in the isolation, synthesis and biological investigation of dimeric compounds. Such dimers consist of two identical monomeric basic structures, which are connected to each other by a suitable linker. Some dimers seem to hold increased biological activity as compared to the corresponding monomers [1-5]. The formation of dimers, trimers, etc… is a common phenomenon in nature to regulate, for example, the activity of proteins [6,] especially of enzymes. The synthesis and biological investigation, however, of dimeric structures derived from triterpenes have been fairly studied [7-10]. The chemistry of triterpenoic dimers started with Dolby's and Debono's dimerization [11] of citronellal. Since then several dimers have been described [7]. Of special interest are 3-amino-oleanolic acid derived dimers holding an adipinic acid-derived spacer [12] showing some activity for the HIV-1 protease; oleanolic acid derivatives [13, 14] were inhibitors of the glycogen phosphorylase.

The number of dimers obtained with an incorporation of the C-28 carboxyl group remains limited probably due to its steric hindrance [15] also found for lupane derived dimers [16]. Only a few dimers holding an amide spacer [13, 14, 16] have been described so far. These compounds were tested as enzyme inhibitors or antivirals but – by and large – no cytotoxic data have been provided for these compounds.

To surpass some problems usually associated with low solubility and to develop an application providing a slow-release of the drug, trans-dermal applications have been suggested and used quite successfully for different kinds of drugs[17]. Thereby, the transdermal application involves the application of ointments, creams or gels to the skin, and the active ingredients of which are absorbed by the skin. This allows them to enter the bloodstream and from there to their actual site of action. Transdermal patches, also known as "transdermal therapeutic systems" (TTS), are a frequently used method of transdermal application. They can release drug substances slowly and in a controlled manner from a drug depot. As a pre-requisite for their use, these substances must not exert any unfavorable side-effects, such as cytotoxicity.

2. Results and Discussion

Oleanolic acid (**OA**) and ursolic acid (**UA**) exert weak cytotoxicity; previous studies showed an increase in cytotoxicity upon their acetylation [18-20]. Also, **OA** and **UA** derived amides holding an additional amino group attached distally to an alkyl group (or a cyclic amine) were shown to be higher cytotoxic than their parent compounds [18-23].

We became interested in the syntheses of dimeric **OA** and **UA** amides linked together by an alkyl chain of variable length. **OA** and **UA** were acetylated and 3-*O*-acetylated **1** [24-26] and **2** [27, 28] were obtained (Scheme 1). The reaction of **1** with oxalyl chloride followed by the addition of 0.5 equivalents of ethylene-diamine gave dimeric **3**.

**Corresponding author: René Csuk*
Email address: rene.csuk@chemie.uni-halle.de
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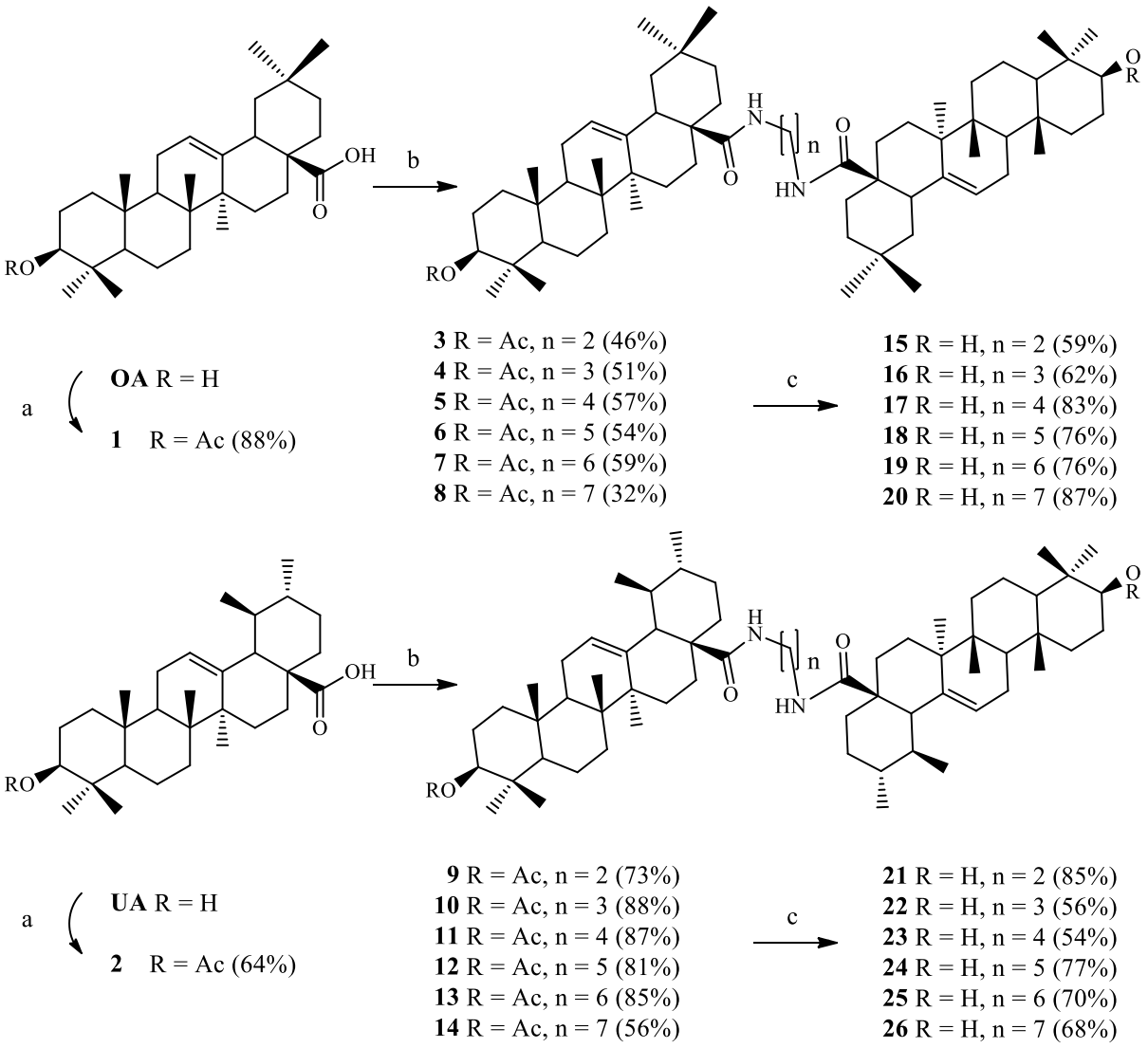
The reaction of **1** with various diamines of variable spacer length furnished **OA** derived dimers **3**-**8**. Similarly, from **2**, **UA** derived dimers **9**-**14** were obtained in 56-88% isolated yield. Deacetylation of **3**-**14** gave dimers **15**-**26**, each holding an unprotected hydroxyl moiety at positions C-3 and C-3', respectively.



Scheme 1. a) Acetic anhydride, pyridine, cat. DMAP, stirring at 25 °C, 1 day; b) i. oxalyl chloriode, triethylamine, dichloromethane, cat. DMF, stirring at 25 °C, 2 h, ii. DCM, 1,n-diamine, stirring at 25 °C, 2 h; c) KOH, MeOH, microwaves, λ = 365 nm, 100 °C, 5 min.

All of the dimers were screened for their cytotoxic activity in sulforhodamine assays (SRB), but none of these compounds showed any significant cytotoxicity (EC_{50} > 30 □M cut-off) when tested with several human tumor cell lines (A375, HT29, SW1736, MCF7, A2780, FaDu, A549) as well with non-malignant mouse fibroblasts (NIH 3T3). No cytotoxic effect was observed even upon applying a prolonged incubation time of 96 hours. Presently, the skin penetration and the stability of these dimers as well as they're *in vivo* degradation is studied in more detail in our laboratories.

3. Conclusion

A slow-release form of ursolic and oleanolic acid, amide-spacered dimers were synthesized from the parent acids and diamines of variable chain lengths. These dimers were assayed in SRB assays; even upon long term incubation up to 96 hours, none of these compounds showed any significant cytotoxicity in sulforhodamine B assays.

4. Experimental

NMR spectra were recorded using the Varian spectrometers Gemini 2000 or Unity 500 (□ given in ppm, *J* in Hz; typical experiments: H-H-COSY, HMBC, HSQC, NOESY; Fig. 1 shows the numbering scheme as well as key HMBC correlations), MS

spectra were taken on a Finnigan MAT LCQ 7000 (electrospray, voltage 4.1 kV, sheath gas nitrogen) instrument. The optical rotations were measured on a Perkin-Elmer polarimeter at 20 °C; TLC was performed on silica gel (Merck 5554, detection with cerium molybdate reagent); melting points are uncorrected (*Leica* hot stage microscope or BÜCHI Melting Point M-565), and elemental analyses were performed on a Foss-Heraeus Vario EL (CHNS) unit. IR spectra were recorded on a Perkin Elmer FT-IR spectrometer Spectrum 1000 or a Perkin-Elmer Spectrum Two (UATR Two Unit). The solvents were dried according to usual procedures. The purity of the compounds was determined by HPLC and found to be >96%. Ursolic (**UA**), oleanolic (**OA**) acid were obtained from different commercial suppliers in bulk quantities. All compounds were obtained as colorless solids.

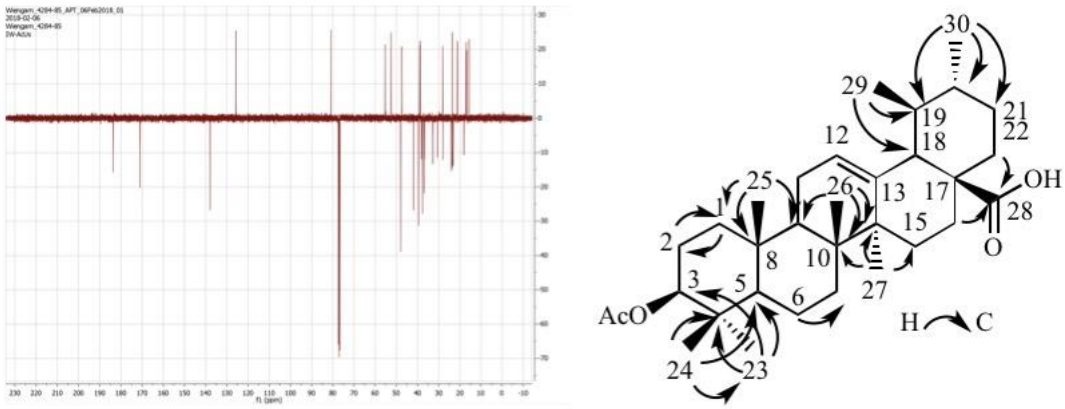


Figure 1. A typical ^{13}C APT-NMR spectrum of **2**, numbering scheme and key HMBC correlations.

(3β) 3-Acetyloxy-olean-12-en-28-oic acid (1)
Acetylation of **OA** (5.0 g, 0.01 mol) with acetic anhydride (3.11 mL, 0.03 mol) in dry pyridine (50 mL) in the presence of a catal. amount of DMAP for 1 day at 25 °C followed by usual aqueous work-up and column chromatography (silica gel, *n*-hexane/ethyl acetate, 8:2) gave **1** (4.4 g, 88; R_F = 0.7 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); m.p.: 263–265 °C (lit.:[29] 260–261°C); $[\alpha]_D$ = +117.7° (*c* 0.37, $CHCl_3$), (lit.: [30] +119° (*c* 0.1, $CHCl_3$); MS (ESI, MeOH): *m/z* = 497.5 (75 %, $[M-H]^-$), 995.2 (100 %, $[2M- H]^-$), 1017.7 (29 %, $[2M-2H+Na]^-$).

(3β) 3-Acetyloxy-urs-12-en-28-oic acid (2)
Following the procedure given for the synthesis of **1**, from **UA** compound **2** (3.5 g, 64%) was obtained; R_F = 0.7 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); m.p.: 242–244°C (lit.: [31] 242.7–244.1 °C); $[\alpha]_D$ = +69.89° (*c* 0.86, $CHCl_3$), (lit.: [31] +71.2° (*c* 1.0, $CHCl_3$); MS (ESI, MeOH): *m/z* = 497.5 (64 %, $[M-H]^-$), 542.9 (30 %, $[M+HCO_2]^-$), 995.1 (68 %, $[2M-H]^-$), 1017.5 (100 %, $[2M-2H+Na]^-$).

General procedure for the synthesis of the dimers
To a solution of **1** or **2** (0.37 mmol) in dry DCM (abs., 30 mL) at 0 °C oxalyl chloride (1.2 mmol), triethylamine (1.2 mmol) and DMF (2 drops) were slowly added, and the mixture was allowed to react at 25 °C for 2 h. The volatiles were removed under reduced pressure, the residue was dissolved in dry DCM (5 mL), and the amine (0.18 mmol) and catalyst amounts of DMAP were added. After stirring at 25°C for 1 h, usual aqueous workup followed by column chromatography gave dimeric **3**–**14**.

General procedure for deacetylation
To a solution of compound **3–14**, (1 equiv) in dry MeOH (5 mL) powdered KOH (20 equiv) was added, and the mixture was stirred at 100 °C (microwave irradiation, □ = 365 nm) for 5 min. Usual aqueous workup followed by column chromatography furnished products **15–26**; respectively.

N^1, N^2-Bis-[(3β)-3-acetyloxy-olean-12-en-28-oyl]-1,2-diaminoethane (3)
Compound **3** (0.17 g, 46%) showed: m.p. 240-242°C; R_F = 0.7 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +49.56° (*c* 0.32, $CHCl_3$);
IR (KBr): ν = 3424*br*, 2948*m*, 2878*m*, 1736*s*, 1638*m*, 1524*m*, 1466*m*, 1368*m*, 1246*m*, 1212*w*, 1186*w*, 1148*w*, 1096*w*, 1028*m*, 1008*m*, 986*m* cm^{-1};
^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.47 (*s*, 2H, 2 x NH), 5.39 (*t*, *J* = 3.5 Hz, 2H, 2 x 12-H), 4.52–4.46 (*m*, 2H, 2 x 3-H), 3.50–3.40 (*m*, 2H, 2 x 33-H), 3.11 (*td*, 2H, 2 x 33-H), 2.54 (*dd*, *J* = 13.1, 4.0 Hz, 2H, 2 x 18-H), 2.04 (*s*, 6H, 2 x 32-H), 2.00 (*dd*, *J* = 13.8 Hz, 4.0 Hz, 2H, 2 x 16-H), 1.96–1.89 (*m*, 2H, 2 x 11-H), 1.72–1.55 (*m*, 18H, 2 x 1-H + 2 x 2-H + 2 x 6-H + 2 x 7-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 19-H), 1.53–1.49 (*m*, 2H, 2 x 15-H), 1.47–1.40 (*m*, 2H, 2 x 22-H), 1.40–1.26 (*dd*, *J* = 12.5, 3.3 Hz, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.25 (*dd*, *J* = 10.1, 2.2 Hz, 2H, 2 x 22-H), 1.19 (*d*, *J* = 3.9 Hz, 4H, 2 x 19-H + 2 x 21-H), 1.15 (*s*, 6H, 2 x 27-H) 1.02 (*dd*, *J* = 10.6, 3.3 Hz, 4H, 2 x 1-H + 2 x 15-H), 0.93 (*s*, 6H, 2 x 25-H), 0.90 (*s*, 6H, 2 x 29-H), 0.89 (*s*, 6H, 2 x 30-H), 0.86 (*s*, 6H,

2 x 23-H), 0.85 (*s*, 6H, 2 x 26-H), 0.82 (*s*, 2H, 2 x 5-H), 0.74 (*s*, 6H, 2 x 24-H);
^{13}C NMR (125 MHz, $CDCl_3$): δ = 179.3 (2 x C-28), 170.9 (2 x C-28), 144.5 (2 x C-13), 122.9 (2 x C-12), 80.8 (2 x C-3), 55.19 (2 x C-5), 47.5 (2 x C-9), 46.6 (2 x C-17), 46.2 (2 x C-19), 41.9 (2 x C-18), 41.9 (2 x C-14), 39.9 (2 x C-33), 39.4 (2 x C-8), 38.1 (2 x C-1), 37.6 (2 x C-4), 36.8 (2 x C-10), 34.1 (2 x C-21), 32.9 (2 x C-29), 32.8 (2 x C-7), 32.2 (2 x C-22), 30.6 (2 x C-20), 28.0 (2 x C-23), 27.3 (2 x C-15), 25.7 (2 x C-27), 23.6 (2 x C-11), 23.5 (2 x C-30), 23.5 (2 x C-2), 23.5 (2 x C-16), 21.2 (2 x C-32), 18.1 (2 x C-6), 16.8 (2 x C-24), 16.6 (2 x C-26), 15.4 (2 x C-25) ppm;
MS (ESI, MeOH): *m/z* = 1021.7 (50 %, $[M+H]^+$), 1043.8 (100 %, $[M+Na]^+$);
analysis calcd for $C_{66}H_{104}N_2O_6$ (1021.54): C 77.60, H 10.26, N 2.74; found: C 77.41, H 10.45, N 2.49.

N^1, N^3-Bis-[(3β)-3-acetyloxy-olean-12-en-28-oyl]-1,3-diaminopropane (4)
Compound **4** (0.18g, 51%) showed: m.p. 204–206°C; R_F = 0.5 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +52.27° (*c* 0.32, $CHCl_3$);
IR (KBr): ν = 3337*br*, 2945*m*, 2863*w*, 1736*m*, 1635*m*, 1526*m*, 1433*m*, 1365*m*, 1244*s*, 1026*m* cm^{-1};
^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.42 (*t*, *J* = 5.9 Hz, 2H, 2 x NH), 5.40 (*t*, *J* = 3.3 Hz, 2H, 2 x 12-H), 4.48 (*dd*, *J* = 10.0, 5.9 Hz, 2H, 2 x 3-H), 3.25 (*dq*, *J* = 12.9, 6.5 Hz, 2H, 2 x 33-H), 3.11 (*dq*, *J* = 12.3, 6.0 Hz, 2H, 2 x 33-H), 2.64 (*dd*, *J* = 13.0, 3.1 Hz, 2H, 2 x 18-H), 2.04 (*s*, 6H, 2 x 32-H), 1.97 (*td*, *J* = 13.6, 3.5 Hz, 2H, 2 x 16-H), 1.92–1.87 (*m*, 2H, 2 x 11-H), 1.78–1.55 (*m*, 20H, 2 x 1-H + 2 x 2-H + 2 x 6-H + 2 x 7-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 19-H + 2 x 34-H), 1.52–1.49 (*m*, 2H, 2 x 15-H), 1.44 (*dd*, *J* = 12.6, 3.5 Hz, 2H, 2 x 22-H), 1.35 (*qd*, *J* = 14.0, 13.0, 3.5 Hz, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.26 (*d*, *J* = 13.1 Hz, 2H, 2 x 22-H), 1.21–1.15 (*m*, 4H, 2 x 19-H + 2 x 21-H), 1.14 (*s*, 6H, 2 x 27-H), 1.06–1.00 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.92 (*s*, 6H, 2 x 25-H), 0.91 (*s*, 6H, 2 x 29-H), 0.90 (*s*, 6H, 2 x 30-H), 0.86 (*s*, 6H, 2 x 23-H), 0.84 (*s*, 6H, 2 x 26-H), 0.82 (*s*, 2H, 2 x 5-H), 0.74 (*s*, 6H, 2 x 24-H) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.5 (2 x C-28), 170.9 (2 x C-28), 144.6 (2 x C-13), 122.6 (2 x C-12), 80.8 (2 x C-3), 55.1 (2 x C-5), 47.4 (2 x C-9), 46.6 (2 x C-17), 46.2 (2 x C-19), 41.95 (2 x C-18), 41.8 (2 x C-14), 39.3 (2 x C-8), 38.1 (2 x C-1), 37.6 (2 x C-4), 36.8 (2 x C-10), 36.0 (2 x C-33), 34.2 (2 x C-21), 33.0 (2 x C-29), 32.9 (2 x C-7), 32.3 (2 x C-22), 30.7 (2 x C-20), 29.7 (C-34), 28.0 (2 x C-23), 27.3 (2 x C-15), 25.7 (2 x C-27), 23.6 (2 x C-11), 23.6 (2 x C-30), 23.5 (2 x C-2), 23.4 (2 x C-16), 21.2 (2 x C-32), 18.1 (2 x C-6), 16.9 (2 x C-24), 16.6 (2 x C-26), 15.4 (2 x C-25) ppm;
MS (ESI, MeOH): *m/z* = 1035.7 (86 %, $[M+H]^+$), 1057.8 (100 % $[M+Na]^+$;
analysis calcd for $C_{67}H_{106}N_2O_6$ (1035.57): C 77.71, H 10.32, N 2.71; found: C 77.50, H 10.46, N 2.42.

N^1, N^4-Bis-[(3β)-3-acetyloxy-olean-12-en-28-oyl]-1,4-diaminobutane (5)
Compound **5** (0.21g, 57%) showed: m.p. 180–186°C; R_F = 0.5 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +46.94° (*c* 0.37, $CHCl_3$);
IR (KBr): ν = 3398*br*, 2950*m*, 2874*m*, 1736*s*, 1644*m*, 1522*m*, 1466*m*, 1366*w*, 1246*s*, 1212*w*, 1148*w*, 1048*w*, 1098*m*, 1006*w* cm^{-1};
^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.04 (*s*, 2H, 2 x NH), 5.38 (*t*, *J* = 3.6 Hz, 2H, 2 x 12-H), 4.52–4.47 (*m*, 2H, 2 x 3-H), 3.36 (*dq*, *J* = 13.0, 6.6 Hz, 2H, 2 x 33-H), 3.04 (*dq*, *J* = 11.3, 6.3 Hz, 2H, 2 x 33-H), 2.54 (*dd*, *J* = 13.0, 4.0 Hz, 2H, 2 x 18-H), 2.06 (*s*, 6H, 2 x 32-H), 1.97 (*d*, *J* = 3.6 Hz, 2H 2 x 16-H), 1.92 (*dd*, *J* = 8.8, 3.2 Hz, 2H, 2 x 11-H), 1.81–1.51 (*m*, 22H, 2 x 1-H + 2 x 2-H + 2 x 6-H + 2 x 7-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 19-H + 2 x 34-H), 1.48 (*dt*, *J* = 6.5, 3.4 Hz, 2H, 2 x 15-H), 1.43 (*s*, 2H, 2 x 22-H), 1.41–1.35 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.30–1.25 (*m*, 2H 2 x 22-H), 1.23–1.19 (*m*, 4H, 2 x 19-H + 2 x 21-H), 1.16 (*s*, 6H 2 x 27-H), 1.06 (s, 4H, 2 x 1-H + 2 x 15-H), 0.97 (d, *J* = 6.6 Hz, 6H, 2 x 25-H), 0.95 (*s*, 6H 2 x 29-H), 0.91 (*s*, 6H 2 x 30-H), 0.88 (*s*, 6H, 2 x 23-H), 0.87 (*s*, 6H 2 x 26-H), 0.86–0.82 (*m*, 2H, 2 x 5-H), 0.76 (*s*, 6H 2 x 24-H) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.2 (2 x C-28), 170.9 (2 x C-28), 145.0 (2 x C-13), 122.6 (2 x C-12), 80.8 (2 x C-3), 55.1 (2 x C-5), 47.4 (2 x C-9), 46.7 (2 x C-17), 46.2 (2 x C-19), 42.2 (2 x C-18), 42.0 (2 x C-14), 39.3 (2 x C-8), 38.9 (2 x C-33), 38.1 (2 x C-1), 37.6 (2 x C-4), 36.8 (2 x C-10), 34.1 (2 x C-21), 32.9 (2 x C-29), 32.6 (2 x C-7), 32.3 (2 x C-22), 30.7 (2 x C-20), 28.0 (2 x C-23), 27.2 (2 x C-15), 26.9 (2 x C-34), 25.6 (2 x C-27), 23.7 (2 x C-11), 23.6 (2 x C-30), 23.5 (2 x C-2), 23.4 (2 x C-16), 21.2 (2 x C-32), 18.1 (2 x C-6), 16.9 (2 x C-24), 16.6 (2 x C-26), 15.4 (2 x C-25) ppm;
MS (ESI, MeOH): *m/z* =1049.7 (78 %, $[M+H]^+$), 1071.7 (100 %, $[M+Na]^+$);
analysis calcd for $C_{68}H_{108}N_2O_6$ (1049.60): C 77.81, H 10.37, N 2.67; found: C 77.62, H 10.53, N 2.55.

N^1, N^5-Bis-[(3β)-3-acetyloxy-olean-12-en-28-oyl]-1,5-diaminopentane (6)
Compound **6** (0.21g, 54%) showed: m.p. 175°C; R_F = 0.5 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +47.07° (*c* 0.333, $CHCl_3$);
IR (KBr): ν = 3426*br*, 2946*s*, 2876*m*, 1735*s*, 1641*m*, 1522*m*, 14465*m*, 1366*m*, 1245*s*, 1212*w*, 1148*w*, 1096*w*, 1027*m*, 1006*m*, 986*m*, 970*w* cm^{-1};
^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.91 (*s*, 2H, 2 x NH), 5.37 (*t*, *J* = 3.6 Hz, 2H, 2 x 12-H), 4.49 (*dd*, *J* = 10.3, 5.7 Hz, 2H, 2 x 3-H), 3.34 (*dq*, *J* = 13.8, 7.1 Hz, 2H, 2 x 33-H), 3.03–2.93 (*m*, 2H, 2 x 33-H), 2.50 (*dd*, *J* = 13.2, 4.0 Hz, 2H, 2 x 18-H), 2.05 (*s*, 6H, 2 x 32-H), 1.98 (*d*, *J* = 3.8 Hz, 2H, 2 x 16-H), 1.92 (*dt*, *J* = 8.8, 4.8 Hz, 2H, 2 x 11-H), 1.81–1.51 (*m*, 26H, 2 x 1-H + 2 x 2-H + 2 x 6-H + 2 x 7-H + 2 x 9-H+ 2 x 11-H+

2 x 16-H + 2 x 19-H + 2 x 34-H + 2 x 35-H), 1.48 (*q*, *J* = 7.0, 6.1 Hz, 2H, 2 x 15-H), 1.45–1.40 (*m*, 2H, 2 x 22-H), 1.36 (*ddd*, *J* = 17.6, 8.2, 4.3 Hz, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.33–1.24 (*m*, 2H, 2 x 22-H), 1.22–1.17 (*m*, 4H, 2 x 19-H + 2 x 21-H), 1.16 (*s*, 6H, 2 x 27-H), 1.12–1.00 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.94 (*s*, 6H, 2 x 25-H), 0.90 (*s*, 6H, 2 x 29-H), 0.90 (*s*, 6H, 2 x 30-H), 0.87 (*s*, 6H, 2 x 23-H), 0.86 (*s*, 6H, 2 x 26-H), 0.83 (*m*, 2H, 2 x 5-H), 0.76 (*s*, 6H, 2 x 24-H) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.2 (2 x C-28), 170.9 (2 x C-28), 145.1 (2 x C-13), 122.6 (2 x C-12), 80.7 (2 x C-3), 55.1 (2 x C-5), 47.4 (2 x C-9), 46.7 (2 x C-17), 46.2 (2 x C-19), 42.3 (2 x C-18), 42.0 (2 x C-14), 39.3 (2 x C-8), 39.3 (2 x C-33), 38.1 (2 x C-1), 37.6 (2 x C-4), 36.8 (2 x C-10), 34.1 (2 x C-21), 32.9 (2 x C-29), 32.5 (2 x C-7), 32.3 (2 x C-22), 30.7 (2 x C-20), 29.1 (2 x C-34), 28.0 (2 x C-23), 27.2 (2 x C-27), 25.6 (2 x C-15), 24.6 (C-35), 23.7 (2 x C-11), 23.6 (2 x C-30), 23.5 (2 x C-2), 23.5 (2 x C-16), 21.2 (2 x C-32), 18.1 (2 x C-6), 16.9 (2 x C-24), 16.6 (2 x C-26), 15.4 (2 x C-25) ppm;
MS (ESI, MeOH): *m/z* = 1063.7 (100 %, $[M+H]^+$), 1085.7 (80 %, $[M+Na]^+$);
analysis calcd for $C_{69}H_{110}N_2O_6$ (1063.62): C 77.92, H 10.42, N 2.63; found: C 77.67, H 10.69, N 2.47.

N^1, N^6-Bis-[(3β)-3-acetyloxy-olean-12-en-28-oyl]-1,6-diaminohexane (7)
Compound **7** (0.23g, 54%) showed: m.p. 188–190°C (lit.: [13, 14] 191–192 °C); R_F = 0.4 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +47.07° (*c* 0.333, $CHCl_3$);
IR (KBr): ν = 3427*br*, 2946*s*, 2876*m*, 1736*s*, 1654*m*, 1522*m*, 1466*m*, 1368*m*, 1246*s*, 1212*w*, 1148*w*, 1096*w*, 1028*m*, 1008*m*, 986*m* cm^{-1};
^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.41 (*t*, *J* = 6.0 Hz, 2H, 2 x NH), 5.40 (*t*, *J* = 3.3 Hz, 2H, 2 x 12-H), 4.49 (*dd*, *J* = 9.7, 6.2 Hz, 2H, 2 x 3-H), 3.26 (*dq*, *J* = 12.9, 6.4 Hz, 2H, 2 x 33-H), 3.12 (*dq*, *J* = 12.4, 6.0 Hz, 2H, 2 x 33-H), 2.64 (*dd*, *J* = 13.3, 4.6 Hz, 2H, 2 x 18-H), 2.04 (*s*, 6H, 2 x 32-H), 1.98 (*td*, *J* = 13.7, 3.7 Hz, 2H, 2 x 16-H), 1.93–1.87 (*m*, 2H, 2 x 11-H), 1.79–1.48 (*m*, 30H, 2 x 1-H + 2 x 2-H + 2 x 6-H + 2 x 7-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 19-H + 2 x 34-H + 2 x 35-H), 1.46 (*d*, *J* = 2.7 Hz, 2H, 2 x 15-H), 1.43 (*d*, *J* = 3.0 Hz, 2H, 2 x 22-H), 1.42–1.33 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.29 (*dd*, *J* = 18.6, 7.7 Hz, 2H, 2 x 22-H), 1.20 (*d*, *J* = 2.4 Hz, 4H, 2 x 19-H + 2 x 21-H), 1.16 (*s*, 6H, 2 x 27-H), 1.10–0.99 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.92 (*s*, 6H, 2 x 25-H), 0.91 (*s*, 6H 2 x 29-H), 0.90 (*s*, 6H, 2 x 30-H), 0.86 (*s*, 6H, 2 x 23-H), 0.85 (*s*, 6H, 2 x 26-H), 0.82 (*s*, 2H, 2 x 5-H), 0.74 (*s*, 6H, 2 x 24-H) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.1 (2 x C-28), 170.9 (2 x C-28), 145.1 (2 x C-13), 122.5 (2 x C-12), 80.7 (2 x C-3), 55.1 (2 x C-5), 47.4 (2 x C-9), 46.7 (2 x C-17), 46.2 (2 x C-19), 42.3 (2 x C-18), 42.0 (2 x C-14), 39.3 (2 x C-8), 39.2 (2 x C-33), 38.1 (2 x C-1), 37.6 (2 x C-4), 36.8 (2 x C-10), 34.1 (2 x C-21), 32.9 (2 x C-29), 32.5 (2 x C-7), 32.3 (2 x C-22), 30.7 (2 x C-20), 29.2 (2 x C-34), 28.0 (2 x C-23), 27.2 (2 x C-27), 26.6 (2 x C-15), 25.6 (2 x C-35), 23.7 (2 x C-11), 23.6 (2 x C-30), 23.5 (2 x C-2), 23.4 (2 x C-16), 21.2 (2 x C-32), 18.1 (2 x C-6), 16.9 (2 x C-24), 16.6 (2 x C-26), 15.4 (2 x C-25) ppm;
MS (ESI, MeOH): *m/z* = 558.6 (5 %, $[M+Ca]^{2+}$), 1077.7 (100 %, $[M+H]^+$), 1099.9 (65 %, $[M+Na]^+$);
analysis calcd for $C_{70}H_{112}N_2O_6$ (1077.65): C 78.02, H 10.48, N 2.60; found: C 77.76, H 10.61, N 2.44.

N^1, N^7-Bis-[(3β)-3-acetyloxy-olean-12-en-28-oyl]-1,7-diaminoheptane (8)
Compound **8** (0.12g, 32%) showed: m.p. 166°C; R_F = 0.4 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +47.82° (*c* 0.31, $CHCl_3$);
IR (KBr): ν = 3426*br*, 2944*s*, 2876*m*, 1734*s*, 1640*m*, 1524*m*, 1466*m*, 1370*m*, 1248*s*, 1212*w*, 1148*w*, 1096*w*, 1028*m*, 1008*m*, 986*m* cm^{-1};
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.90 (*t*, *J* = 5.3 Hz, 2H, 2 x NH), 5.36 (*t*, *J* = 3.4 Hz, 2H, 2 x 12-H), 4.49 (*dd*, *J* = 9.6, 6.3 Hz, 2H, 2 x 3-H), 3.34 (*dq*, *J* = 13.6, 7.1 Hz, 2H, 2 x 33-H), 3.03–2.93 (*m*, 2H, 2 x 33-H), 2.49 (*dd*, *J* = 12.8, 3.5 Hz, 2H, 2 x 18-H), 2.04 (*s*, 6H, 2 x 32-H), 1.97 (*dd*, *J* = 13.7, 3.6 Hz, 2H, 2 x 16-H), 1.91 (*dd*, *J* = 8.6, 3.3 Hz, 2H, 2 x 11-H), 1.81–1.50 (*m*, 32H, 2 x 1-H + 2 x 2-H + 2 x 6-H + 2 x 7-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 19-H + 2 x 34-H + 2 x 35-H + 36-H), 1.45 (*dd*, *J* = 12.8, 5.9Hz, 2H, 2 x 15-H), 1.39 (*m*, 2H, 2 x 22-H), 1.35 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.28 (*dd*, *J* = 12.9, 8.0 Hz, 2H, 2 x 22-H), 1.19 (*d*, *J* = 6.3 Hz, 4H, 2 x 19-H + 2 x 21-H), 1.15 (*s*, 6H, 2 x 27-H), 1.04 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.94 (*s*, 6H, 2 x 25-H), 0.90 (*s*, 6H 2 x 29-H), 0.90 (*s*, 6H, 2 x 30-H), 0.87 (*s*, 6H, 2 x 23-H), 0.85 (*s*, 6H, 2 x 26-H), 0.82 (*s*, 2H, 2 x 5-H), 0.76 (*s*, 6H, 2 x 24-H) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.0 (2 x C-28), 170.9 (2 x C-28), 145.1 (2 x C-13), 122.5 (2 x C-12), 80.8 (2 x C-3), 55.1 (2 x C-5), 47.4 (2 x C-9), 46.7 (2 x C-17), 46.2 (2 x C-19), 42.3 (2 x C-18), 42.1 (2 x C-14), 39.3 (2 x C-8), 39.2 (2 x C-33), 38.1 (2 x C-1), 37.6 (2 x C-4), 36.8 (2 x C-10), 34.1 (2 x C-21), 32.9 (2 x C-29), 32.5 (2 x C-7), 32.3 (2 x C-22), 30.7 (2 x C-20), 29.3 (2 x C-34), 28.9 (C-36), 28.0 (2 x C-23), 27.2 (2 x C-27), 27.0 (2 x C-15), 25.6 (2 x C-35), 23.7 (2 x C-11), 23.5 (2 x C-30), 23.5 (2 x C-2), 23.4 (2 x C-16), 21.2 (2 x C-32), 18.1 (2 x C-6),16.9 (2 x C-24), 16.6 (2 x C-26), 15.4 (2 x C-25) ppm;
MS (ESI, MeOH): *m/z* =1092.9 (20 %, $[M+H]^+$), 1114.8 (100 %, $[M+Na]^+$);
analysis calcd for $C_{71}H_{114}N_2O_6$ (1091.68): C 78.11, H 10.53, N 2.57; found: C 77.94, H 10.61, N 2.36.

N^1, N^2-Bis-[(3β)-3-acetyloxy-urs-12-en-28-oyl]-1,2-diaminoethane (9)
Compound **9** (0.28g, 73%) showed: m.p. 218°C; R_F = 0.5 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +43.5° (*c* 0.34, $CHCl_3$);

IR (KBr): ν = 3420*br*, 2948*s*, 2872*m*, 1736*s*, 1646*m*, 1522*m*, 1466*m*, 1456*m*, 1390*m*, 1370*m*, 1246*s*, 1146*w*, 1092*w*, 1028*m*, 1006*w* cm^{-1};
^1H NMR (400 MHz, $CDCl_3$): δ = 6.50 (*m*, 2H, 2 x NH), 5.36 (*t*, *J* = 3.4 Hz, 2H, 2 x 12-H), 4.49 (*dd*, *J* = 9.7, 6.1 Hz, 2H, 2 x 3-H), 3.46 (*d*, *J* = 9.0 Hz, 2H, 2 x 33-H), 3.08 (*d*, *J* = 8.7 Hz, 2H, 2 x 33-H), 2.04 (*s*, 6H, 2 x 32-H), 1.99 (*d*, *J* = 4.1 Hz, 2H, 2 x 16-H), 1.95 (*dd*, *J* = 7.6, 3.4 Hz, 2H, 2 x 11-H), 1.90 (*m*, 2H, 2 x 18-H), 1.89–1.82 (*m*, 2H, 2 x 22-H), 1.75–1.60 (*m*, 12H, 2 x 1-H + 2 x 2-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H), 1.52 (*m*, 6H, 2 x 15-H + 2 x 7-H + 2 x 6-H), 1.47–1.40 (*m*, 6H, 2 x 21-H + 2 x 22-H + (2 x 19-H), 1.41–1.24 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.08 (*s*, 6H, 2 x 27-H), 1.07–1.01 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.95 (*s*, 6H, 2 x 30-H), 0.94 (*s*, 6H, 2 x 25-H), 0.88 (*s*, 6H, 2 x 24-H), 0.86 (*s*, 6H, 2 x 23-H), 0.85 (*s*, 6H, 2 x 26-H), 0.82 (*m*, 2H, 2 x 5-H), 0.75 (*s*, 6H, 2 x 29-H) ppm;
^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.3 (2 x C-28), 170.9 (2 x C-28), 139.3 (2 x C-13), 125.8 (2 x C-12), 80.8 (2 x C-3), 55.2 (2 x C-5), 53.4 (2 x C-18), 47.6 (2 x C-17), 47.4 (2 x C-9), 42.3 (2 x C-14), 39.8 (C-33), 39.7 (2 x C-8), 39.5 (2 x C-19), 39.0 (2 x C-20), 38.2 (2 x C-1), 37.6 (2 x C-4), 36.8 (2 x C-10), 36.7 (2 x C-22), 32.5 (2 x C-7), 30.8 (2 x C-21), 28.0 (2 x C-23), 27.8 (2 x C-15), 24.7 (2 x C-16), 23.5 (2 x C-27), 23.3 (2 x C-2), 21.2 (2 x C-32), 21.1 (2 x C-30), 18.1 (2 x C-6), 17.2 (2 x C-24), 16.8 (2 x C-29), 16.7 (2 x C-26), 15.5 (2 x C-25) ppm;
MS (ESI, MeOH): *m/z* = 530.5 (10 %, $[M+Ca]^{2+}$), 1021.7 (100 %, $[M+H]^+$), 1043.8 (80 %, $[M+Na]^+$);
analysis calcd for $C_{66}H_{104}N_2O_6$ (1021.54): C 77.60, H 10.26, N 2.74; found: C 77.45, H 10.41, N 2.51.

N^1, N^3-Bis-[(3β)-3-acetyloxy-urs-12-en-28-oyl]-1,3-diaminopropane (10)
Compound **10** (0.33g, 88%) showed: m.p. 206°C; R_F = 0.5 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +30.34° (*c* 0.31, $CHCl_3$);
IR (KBr): ν = 3393*br*, 2939*m*, 2860*m*, 1735*s*, 1645*m*, 1517*m*, 1454*m*, 1369*m*, 1246*s*, 1146*w*, 1092*w*, 1028*m*, 1006*w* cm^{-1};
^1H NMR (500 MHz, $CDCl_3$): δ = 6.36 (*t*, *J* = 5.4 Hz, 2H, 2 x NH), 5.35 (*t*, *J* = 3.5 Hz, 2H, 2 x 12-H), 4.49 (*dd*, *J* = 10.5, 5.3 Hz, 2H, 2 x 3-H), 3.24 (*dq*, *J* = 13.1, 6.6 Hz, 2H, 2 x 33-H), 3.09 (*dq*, *J* = 11.8, 5.8 Hz, 2H, 2 x 33-H), 2.04 (*s*, 6H, 2 x 32-H), 1.99 (*d*, *J* = 3.9 Hz, 2H 2 x 16-H), 1.94 (*ddd*, *J* = 17.8, 10.4, 2.7 Hz, 2H, 2 x 11-H), 1.88 (*m*, 2H, 2 x 18-H), 1.87–1.82 (*m*, 2H, 2 x 22-H), 1.76–1.52 (*m*, 14H, 2 x 1-H + 2 x 2-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 34-H), 1.49 (*m*, 6H, 2 x 15-H + 2 x 7-H + 2 x 6-H), 1.48–1.40 (*m*, 6H, 2 x 21-H + 2 x 22-H + (2 x 19-H), 1.37–1.26 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.05 (*s*, 6H, 2 x 27-H), 1.07–1.01 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.95 (*s*, 6H, 2 x 30-H), 0.93 (*s*, 6H, 2 x 25-H), 0.88 (*s*, 6H, 2 x 24-H), 0.87 (*s*, 6H, 2 x 23-H), 0.86 (*s*, 6H, 2 x 26-H), 0.82 (*s*, 2H, 2 x 5-H), 0.76 (*s*, 6H 2 x 29-H) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.5 (2 x C-28), 170.9 (2 x C-28), 139.4 (2 x C-13), 125.6 (2 x C-12), 80.8 (2 x C-3), 55.2, (2 x C-5), 53.5 (2 x C-18), 47.7 (2 x C-17), 47.4 (2 x C-9), 42.3 (2 x C-14), 39.7 (2 x C-8), 39.5 (2 x C-19), 38.9 (2 x C-20), 38.3 (2 x C-1), 37.6 (2 x C-4), 37.4 (2 x C-10), 36.8 (2 x C-22), 36.3 (C-33), 32.7 (2 x C-7), 30.9 (2 x C-21), 29.6 (C-34), 28.0 (2 x C-23), 27.8 (2 x C-15), 24.8 (2 x C-16), 23.4 (2 x C-2), 23.2 (2 x C-27), 21.2 (2 x C-32), 21.1 (2 x C-30), 18.1 (2 x C-6), 17.2 (2 x C-24), 17.0 (2 x C-29), 16.8 (2 x C-26), 15.5 (2 x C-25) ppm;
MS (ESI, MeOH): *m/z* = 1035.7 (100 %, $[M+H]^+$), 1057.8 (70 %, $[M+Na]^+$);
analysis calcd for $C_{67}H_{106}N_2O_6$ (1035.57): C 77.71, H 10.32, N 2.71; found: C 77.58, H 10.46, N 2.53.

N^1, N^4-Bis-[(3β)-3-acetyloxy-urs-12-en-28-oyl]-1,4-diaminobutane (11)
Compound **11** (0.33g, 87%) showed: m.p. 206°C; R_F = 0.4 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +30.2° (*c* 0.32, $CHCl_3$);
IR (KBr): ν = 3445*br*, 2942*m*, 2859*m*, 1735*s*, 1637*m*, 1421*m*, 1325*m*, 1245*s*, 1147*w*, 1045*w*, 1028*m*, 1005*w* cm^{-1};
^1H NMR (500 MHz, $CDCl_3$): δ = 5.99 (*t*, *J* = 5.1 Hz, 2H, 2 x NH), 5.31 (*t*, *J* = 3.9 Hz, 2H, 2 x 12-H), 4.49 (*dd*, *J* = 10.5, 5.4 Hz, 2H, 2 x 3-H), 3.36 (*dd*, *J* = 13.4, 6.5 Hz, 2H, 2 x 33-H), 2.99 (*dq*, *J* = 11.0, 6.1 Hz, 2H, 2 x 33-H), 2.05 (*s*, 6H, 2 x 32-H), 2.00– 1.98 (*m*, 2H, 2 x 16-H), 1.95 (*ddd*, *J* = 13.5, 7.0, 3.5 Hz, 2H, 2 x 11-H), 1.90–1.87 (*m*, 2H, 2 x 18-H), 1.86 (*m*, 2H, 2 x 22-H), 1.76–1.55 (*m*, 16H, 2 x 1-H + 2 x 2-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 34-H), 1.54–1.49 (*m*, 6H, 2 x 15-H + 2 x 7-H + 2 x 6-H), 1.45 (*m*, 6H, 2 x 21-H + 2 x 22-H + (2 x 19-H), 1.41–1.26 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.09 (*s*, 6H, 2 x 27-H), 1.07–1.02 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.95 (*s*, 6H, 2 x 30-H), 0.88 (*s*, 6H, 2 x 24-H), 0.86 (*s*, 6H, 2 x 23-H), 0.85 (*s*, 6H, 2 x 26-H), 0.76 (*s*, 6H 2 x 29-H) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.0 (2 x C-28), 170.9 (2 x C-28), 140.0 (2 x C-13), 125.4 (2 x C-12), 80.8 (2 x C-3), 55.2 (2 x C-5), 53.8 (2 x C-18), 47.7 (2 x C-17), 47.4 (2 x C-9), 42.4 (2 x C-14), 39.7 (2 x C-33), 39.7 (2 x C-8), 39.5 (2 x C-19), 39.0 (2 x C-20), 38.9 (2 x C-1), 37.6 (2 x C-4), 36.8 (2 x C-10), 36.7 (2 x C-22), 32.6 (2 x C-7), 30.8 (2 x C-21), 28.0 (2 x C-23), 27.8 (2 x C-15), 27.0 (2 x C-34), (2 x C-16), 23.5 (2 x C-27), 23.1 (2 x C-2), 21.2 (2 x C-32), 21.1 (2 x C-30), 17.7 (2 x C-6), 17.2 (2 x C-24), 16.9 (2 x C-29), 16.7 (2 x C-26), 15.5 (2 x C-25) ppm;
MS (ESI, MeOH): *m/z* = 1049.7 (100 %, $[M+H]^+$), 545.0 (20 %, $[M+Ca]^{2+}$), 1069.3 (54 %, $[2M+Ca]^{2+}$), 1071.8 (40 %, $[M+Na]^+$);
analysis calcd for $C_{68}H_{108}N_2O_6$ (1049.60): C 77.81, H 10.37, N 2.67; found: C 77.69, H 10.57, N 2.41.

N^1, N^5-Bis-[(3β)-3-acetyloxy-urs-12-en-28-oyl]-1,5-diaminopentane (12)
Compound **12** (0.31g, 81%) showed: m.p. 188°C; R_F = 0.4 (silica gel, toluene/ethyl acetate/formic

acid/heptane, 80:26:5:1); $[\alpha]_D$ = +36.32° (*c* 0.31, $CHCl_3$);
IR (KBr): ν = 3403*br*, 2926*m*, 2871*m*, 1734*s*, 1638*m*, 1514*m*, 1454*m*, 1369*m*, 1243*s*, 1147*w*, 1093*w*, 1027*m*, 1006*w* cm^{-1};
^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.99 (*t*, *J* = 5.1 Hz, 2H, 2 x NH), 5.31 (*t*, *J* = 3.9 Hz, 2H, 2 x 12-H), 4.49 (*dd*, *J* = 10.5, 5.4 Hz, 2H, 2 x 3-H), 3.36 (*dd*, *J* = 13.4, 6.5 Hz, 2H, 2 x 33-H), 2.99 (*dq*, *J* = 11.0, 6.1 Hz, 2H, 2 x 33-H), 2.05 (*s*, 6H, 2 x 32-H), 2.00 –1.98 (*m*, 2H, 2 x 16-H), 1.95 (*ddd*, *J* = 13.5, 7.0, 3.5 Hz, 2H, 2 x 11-H), 1.90–1.87 (*m*, 2H, 2 x 18-H), 1.86 (*m*, 2H, 2 x 22-H), 1.76–1.55 (*m*, 18H, 2 x 1-H + 2 x 2-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 34-H + 35-H), 1.54–1.49 (*m*, 6H, 2 x 15-H + 2 x 7-H + 2 x 6-H), 1.45 (*m*, 6H, 2 x 21-H + 2 x 22-H + 2 x 19-H), 1.41–1.26 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.09 (*s*, 6H, 2 x 27-H), 1.07–1.02 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.95 (*s*, 6H, 2 x 30-H), 0.88 (*s*, 6H, 2 x 24-H), 0.86 (*s*, 6H, 2 x 23-H), 0.85 (*s*, 6H, 2 x 26-H), 0.76 (*s*, 6H 2 x 29-H) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.2 (2 x C-28), 170.9 (2 x C-28), 140.0 (2 x C-13), 125.4 (2 x C-12), 80.7 (2 x C-3), 55.2 (2 x C-5), 53.9 (2 x C-18), 47.6 (2 x C-17), 47.4 (2 x C-9), 42.5 (2 x C-14), 39.7 (2 x C-8), 39.5 (2 x C-19), 39.3 (2 x C-33), 39.1 (2 x C-20), 38.3 (2 x C-1), 37.6 (2 x C-4), 37.2 (2 x C-10), 36.8 (2 x C-22), 32.6 (2 x C-7), 30.8 (2 x C-21), 29.0 (2 x C-34), 28.0 (2 x C-23), 27.8 (2 x C-15), 24.8 (2 x C-16), 24.5 (C-35), 23.4 (2 x C-2), 23.1 (2 x C-27), 21.2 (2 x C-32), 21.1 (2 x C-30), 18.1 (2 x C-6), 17.2 (2 x C-24), 16.9 (2 x C-29), 16.7 (2 x C-26), 15.5 (2 x C-25) ppm;
MS (ESI, MeOH): *m/z* = 1063.7 (100 %, $[M+H]^+$), 1085.8 (90 %, $[M+Na]^+$), 551.5 (10 %, $[M+Ca]^{2+}$);
analysis calcd for $C_{69}H_{110}N_2O_6$ (1063.62): C 77.92, H 10.42, N 2.63; found: C 77.63, H 10.69, N 2.50.

N^1, N^6-Bis-[(3β)-3-acetyloxy-urs-12-en-28-oyl]-1,6-diaminohexane (13)
Compound **13** (0.33g, 85%) showed: m.p. 162°C; R_F = 0.6 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +38.90° (*c* 0.32, $CHCl_3$);
IR (KBr): ν = 3428*br*, 2946*m*, 2872*m*, 1736*s*, 1646*m*, 1522*m*, 1456*m*, 1370*m*, 1246*s*, 1148*w*, 1092*w*, 1028*m*, 1006*w* cm^{-1};
^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.94 (*t*, *J* = 5.5 Hz, 2H, 2 x NH), 5.31–5.29 (*m*, 2H, 2 x 12-H), 4.49 (*dd*, *J* = 10.5, 5.3 Hz, 2H, 2 x 3-H), 3.30 (*dq*, *J* = 13.6, 6.9 Hz, 2H, 2 x 33-H), 3.03–2.95 (*m*, 2H, 2 x 33-H), 2.05 (*s*, 6H, 2 x 32-H), 2.02–1.98 (*m*, 2H, 2 x 16-H), 1.95 (*ddd*, *J* = 14.9, 6.2, 3.3 Hz, 2H, 2 x 11-H), 1.90–1.87 (*m*, 4H, 2 x 18-H + 2 x 22-H), 1.77–1.55 (*m*, 20H, 2 x 1-H + 2 x 2-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 34-H + (2 x 35-H), 1.50 (*m*, 6H, 2 x 15-H + 2 x 7-H + 2 x 6-H), 1.48–1.39 (*m*, 6H, 2 x 21-H + 2 x 22-H + 2 x 19-H), 1.39–1.27 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.09 (*s*, 6H, 2 x 27-H), 1.07–1.01 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.95 (*s*, 6H, 2 x 30-H), 0.88 (*s*, 6H, 2 x 24-H), 0.87 (*s*, 6H, 2 x 23-H), 0.86 (*s*, 6H, 2 x 26-H), 0.82 (*m*, 2H, 2 x 5-H), 0.77 (*s*, 6H 2 x 29-H) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.2 (2 x C-28), 170.9 (2 x C-28), 140.0 (2 x C-13), 125.4 (2 x C-12), 80.8 (2 x C-3), 55.2 (2 x C-5), 53.9 (2 x C-18), 47.7 (2 x C-17), 47.4 (2 x C-9), 42.5 (2 x C-14), 39.7 (2 x C-8), 39.5 (2 x C-19), 39.2 (2 x C-33), 39.1 (2 x C-20), 38.3 (2 x C-1), 37.6 (2 x C-4), 37.2 (2 x C-10), 36.7 (2 x C-22), 32.6 (2 x C-7), 30.8 (2 x C-21), 29.2 (2 x C-34), 28.0 (2 x C-23), 27.8 (2 x C-15), 24.8 (2 x C-16), 24.5 (2 x C-35), 23.4 (2 x C-2), 23.2 (2 x C-27), 21.2 (2 x C-32), 21.1 (2 x C-30), 18.1 (2 x C-6), 17.2 (2 x C-24), 16.9 (2 x C-29), 16.7 (2 x C-26), 15.5 (2 x C-25) ppm;
MS (ESI, MeOH): *m/z* = 1077.7 (100 %, $[M+H]^+$), 1099.8 (72 %, $[M+Na]^+$), 558.5 (5 %, $[M+Ca]^{2+}$);
analysis calcd for $C_{70}H_{112}N_2O_6$ (1077.65): C 78.02, H 10.48, N 2.60; found: C 77.88, H 10.69, N 2.41.

N^1, N^7-Bis-[(3β)-3-acetyloxy-urs-12-en-28-oyl]-1,7-diaminoheptane (14)
Compound **14** (0.23g, 56%) showed: m.p. 176°C; R_F = 0.4 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +36.18° (*c* 0.33, $CHCl_3$);
IR (KBr): ν = 3418*br*, 2925*m*, 2855*m*, 1735*s*, 1655*m*, 1516*m*, 1454*m*, 1369*m*, 1243*s*, 1147*w*, 1092*w*, 1027*m*, 1005*w* cm^{-1};
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.91 (*t*, *J* = 5.4 Hz, 2H, 2 x NH), 5.30 (*m*, 2H, 2 x 12-H), 4.52–4.47 (*m*, 2H, 2 x 3-H), 3.31 (*dq*, *J* = 13.5, 7.0 Hz, 2H, 2 x 33-H), 3.04–2.95 (*m*, 2H, 2 x 33-H), 2.04 (*s*, 6H, 2 x 32-H), 2.00 (*d*, *J* = 4.2 Hz, 2H, 2 x 16-H), 1.99–1.91 (*m*, 2H, 2 x 11-H), 1.89–1.80 (*m*, 4H, 2 x 18-H + 2 x 22-H), 1.77–1.53 (*m*, 22H, 2 x 1-H + 2 x 2-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 34-H + 2 x 35-H + 36-H), 1.50 (*m*, 6H, 2 x 15-H + 2 x 7-H + 2 x 6-H), 1.49–1.41 (*m*, 6H, 2 x 21-H + 2 x 22-H + 2 x 19-H), 1.40–1.25 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.09 (*s*, 6H, 2 x 27-H), 1.05 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.95 (*s*, 6H, 2 x 30-H), 0.88 (*s*, 6H, 2 x 24-H), 0.87 (*s*, 6H, 2 x 23-H), 0.86 (*s*, 6H, 2 x 26-H), 0.81 (*m*, 2H, 2 x 5-H), 0.78 (*s*, 6H 2 x 29-H) ppm;
^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.1 (2 x C-28), 170.9 (2 x C-28), 140.1 (2 x C-13), 125.4 (2 x C-12), 80.8 (2 x C-3), 55.2 (2 x C-5), 54.0 (2 x C-18), 47.6 (2 x C-17), 47.4 (2 x C-9), 42.5 (2 x C-14), 39.7 (2 x C-8), 39.5 (2 x C-19), 39.4 (2 x C-33), 39.1 (2 x C-20), 38.3 (2 x C-1), 37.6 (2 x C-4), 37.1 (2 x C-10), 36.8 (2 x C-22), 32.6 (2 x C-7), 30.8 (2 x C-21), 29.2 (2 x C-34), 28.9 (C-36), 28.0 (2 x C-23), 27.8 (2 x C-15), 27.0 (2 x C-35), 24.8 (2 x C-16), 23.4 (2 x C-2), 23.1 (2 x C-27), 21.2 (2 x C-32) 21.1 (2 x C-30), 18.1(2 x C-6), 17.2 (2 x C-24), 16.9 (2 x C-29), 16.7 (2 x C-26), 15.5 (2 x C-25) ppm;
MS (ESI, MeOH): *m/z* = 1091.7 (100 %, $[M+H]^+$), 1113.0 (92 %, $[M+Na]^+$), 565.9 (5 %, $[M+Ca]^{2+}$);
analysis calcd for $C_{71}H_{114}N_2O_6$ (1091.68): C 78.11, H 10.53, N 2.57; found: C 78.01, H 10.64, N 2.38.

N[1],N[2]-Bis-[(3β)-3-hydroxy-olean-12-en-28-oyl]-1,2-diaminoethane (15)

Compound **15** (0.22 g, 59%) showed: m.p. 239°C; R_F = 0.5 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +51.41° (*c* 0.35, $CHCl_3$);

IR (KBr): ν = 3393*m*, 2927*m*, 2865*w*, 1640*m*, 1518*m*, 1463*m*, 1386*w*, 1364*w*, 1262*w*, 1094*w*, 1029*m*, 996*m* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 6.55 (*s*, 2H, 2 x NH), 5.40 (*t*, *J* = 3.3 Hz, 2H, 2 x 12-H), 3.46 (*d*, *J* = 9.3 Hz, 2H, (2 x 31-H), 3.21 (*dd*, *J* = 11.1, 4.6 Hz, 2H, 2 x 3-H), 3.12 (*d*, *J* = 8.7 Hz, 2H, (2 x 31-H), 2.54 (*dd*, *J* = 13.1, 3.4 Hz, 2H, 2 x 18-H), 2.00 (*dd*, *J* = 13.7, 3.9 Hz, 2H, 2 x 16-H), 1.93 (*dd*, *J* = 11.0, 7.3 Hz, 2H, 2 x 11-H), 1.79–1.53 (*m*, 18H, 2 x 1-H + 2 x 2-H + 2 x 6-H + 2 x 7-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 19-H), 1.52–1.50 (*m*, 2H, 2 x 15-H), 1.49–1.39 (*m*, 2H, 2 x 22-H), 1.39–1.29 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.29–1.22 (*m*, 2H, 2 x 22-H), 1.21–1.17 (*m*, 4H, 2 x 19-H + 2 x 21-H), 1.15 (*s*, 6H, 2 x 27-H), 1.02 (*dd*, *J* = 14.2, 3.7 Hz, 4H, 2 x 1-H + 2 x 15-H), 0.98 (*s*, 6H, 2 x 23-H), 0.90 (*s*, 6H, 2 x 25-H), 0.89 (*s*, 6H, (2 x 30-H), 0.89 (*s*, 6H, 2 x 29-H), 0.77 (*s*, 6H, 2 x 24-H), 0.73 (*s*, 6H, 2 x 26-H), 0.71 (*s*, 2H, 2 x 5-H ppm;

^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.3 (2 x C-28), 144.4 (2 x C-13), 123.1 (2 x C-12), 78.9 (2 x C-3), 55.1 (2 x C-5), 47.5 (2 x C-9), 46.6 (2 x C-17), 46.1 (2 x C-19), 41.9 (2 x C-18), 41.9 (2 x C-14), 40.1 (2 x C-31), 39.3 (2 x C-8), 38.7 (2 x C-1), 38.4 (2 x C-4), 36.9 (2 x C-10), 34.1 (2 x C-21), 32.9 (2 x C-29), 32.7 (2 x C-7), 32.3 (2 x C-22), 30.6 (2 x C-20), 28.0 (2 x C-23), 27.3 (2 x C-15), 27.1 (2 x C-2), 25.8 (2 x C-27), 23.6 (2 x C-11), 23.5 (2 x C-30), 23.5 (2 x C-16), 18.2 (2 x C-6), 16.8 (2 x C-26), 15.5 (2 x C-24), 15.3 (2 x C-25) ppm;

MS (ESI, MeOH): *m/z* = 938.1 (42 %, $[M+H]^+$), 960.2 (100 %, $[M+Na]^+$);

analysis calcd for C62H100N2O4 (937.47): C 79.43, H 10.75, N 2.99; found: C 79.17, H 10.91, N 2.76.

N[1],N[3]-Bis-[(3β)-3-hydroxy-olean-12-en-28-oyl]-1,3-diaminopropane (16)

Compound **16** (0.22 g, 59%) showed: m.p. 218°C; R_F = 0.3 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +50.78° (*c* 0.30, $CHCl_3$);

IR (KBr): ν = 3374*br*, 2928*m*, 2864*w*, 1632*s*, 1527*s*, 1465*m*, 1432*m*, 1380*w*, 1363*w*, 1262*w*, 1094*w*, 1045*m*, 1030*m*, 998*w*, 773*w* cm^{-1};

^1H NMR (500 MHz, $CDCl_3$): δ = 6.47 (*t*, *J* = 5.1 Hz, 2H, 2 x NH), 5.41 (*t*, *J* = 3.5 Hz, 2H, 2 x 12-H), 3.27 (*dq*, *J* = 13.4, 6.8 Hz, 2H, 2 x 31-H), 3.21 (*dd*, *J* = 11.4, 4.3 Hz, 2H, 2 x 3-H), 3.13 (*dq*, *J* = 12.0, 5.9 Hz, 2H, 2 x 31-H), 2.65 (dd, *J* = 13.0, 4.0 Hz, 2H, 2 x 18-H), 2.02–1.93 (*m*, 2H, 2 x 16-H), 1.93–1.87 (*m*, 2H, 2 x 11-H), 1.79–1.50 (*m*, 20H, 2 x 1-H + 2 x 2-H + 2 x 6-H + 2 x 7-H + 2 x 9-H+ 2 x 11-H+ 2 x 15-H + 2 x 16-H + 2 x 19-H + 32-H), 1.44 (*dd*, *J* = 12.2, 3.1 Hz, 2H, 2 x 22-H), 1.41–1.30 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.30–1.24 (*m*, 2H, 2 x 22-H), 1.22–1.17 (*m*, 4H, 2 x 19-H + 2 x 21-H), 1.16 (*s*, 6H, 2 x 27-H), 1.04 (*dt*, *J* = 13.5, 2.9 Hz, 4H, 2 x 1-H + 2 x 15-H), 0.99 (*s*, 6H, 2 x 23-H), 0.92 (*s*, 6H, 2 x 25-H), 0.90 (*s*, 6H, (2 x 30-H), 0.90 (*s*, 6H, 2 x 29-H), 0.78 (*s*, 6H, 2 x 24-H), 0.74 (*s*, 6H, 2 x 26-H), 0.72 (*s*, 2H, 2 x 5-H) ppm;

^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.5 (2 x C-28), 144.5 (2 x C-13), 122.8 (2 x C-12), 78.9 (2 x C-3), 55.1 (2 x C-5), 47.5 (2 x C-9), 46.7 (2 x C-17), 46.3 (2 x C-19), 41.9 (2 x C-18), 41.84 (2 x C-14), 39.3 (2 x C-8), 38.7 (2 x C-1), 38.4 (2 x C-4), 36.9 (2 x C-10), 36.1 (2 x C-31), 34.2 (2 x C-21), 33.01 (2 x C-29), 32.9 (2 x C-7), 32.4 (2 x C-22), 30.7 (2 x C-20), 29.6 (CH2, C32), 28.0 (2 x C-23), 27.3 (2 x C-15), 27.15 (2 x C-2), 25.7 (2 x C-27), 23.6 (2 x C-11), 23.6 (2 x C-30), 23.5 (2 x C-16), 18.2 (2 x C-6), 16.9 (2 x C-26), 15.5 (2 x C-24), 15.3 (2 x C-25) ppm; MS (ESI, MeOH): *m/z* = 952.1 (100 %, $[M+H]^+$), 974.0 (22 %, $[M+Na]^+$);

analysis calcd for $C_{63}H_{102}N_2O_4$ (951.50): C 79.52, H 10.81, N 2.94; found: C 79.35, H 11.03, N 2.77.

N[1],N[4]-Bis-[(3β)-3-hydroxy-olean-12-en-28-oyl]-1,4-diaminobutane (17)

Compound **17** (0.13 g, 83%) showed: m.p. 186°C; R_F = 0.4 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +47.00° (*c* 0.31, $CHCl_3$);

IR (KBr): ν = 3390*br*, 2926*m*, 2864*w*, 1640*m*, 1520*m*, 1463*m*, 1386*w*, 1260*s*, 1092*s*, 1026*s* cm^{-1};

^1H NMR (400 MHz, $CDCl_3$): δ = 6.06 (*t*, *J* = 5.6 Hz, 2H, 2 x NH), 5.37 (*t*, *J* = 3.5 Hz, 2H, 2 x 12-H), 3.35 (*dq*, *J* = 12.9, 6.4 Hz, 2H, 2 x 31-H), 3.21 (*dd*, *J* = 11.1, 4.6 Hz, 2H, 2 x 3-H), 3.04 (*dq*, *J* = 11.6, 6.5 Hz, 2H, (2 x 31-H), 2.53 (*dd*, *J* = 12.9, 4.1 Hz, 2H, 2 x 18-H), 2.01–1.92 (*m*, 2H, 2 x 16-H), 1.95–1.87 (*m*, 2H, 2 x 11-H), 1.81–1.56 (*m*, 22H, 2 x 1-H + 2 x 2-H + 2 x 6-H + 2 x 7-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 19-H + 2 x 32-H), 1.54 (*dt*, *J* = 13.9, 3.4 Hz, 2H, 2 x 15-H), 1.50–1.39 (*m*, 2H, 2 x 22-H), 1.39–1.27 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.27–1.22 (*m*, 2H 2 x 22-H), 1.22–1.17 (*m*, 4H, 2 x 19-H + 2 x 21-H), 1.18–1.14 (*s*, 6H, 2 x 27-H), 1.07–0.99 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.99 (*s*, 6H, 2 x 23-H), 0.91 (*s*, 6H, 2 x 25-H), 0.91 (*s*, 6H, (2 x 30-H), 0.90 (*s*, 6H, 2 x 29-H),0.78 (*s*, 6H, 2 x 24-H), 0.75 (*s*, 6H, 2 x 26-H), 0.71 (*s*, 2H, 2 x 5-H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.2 (2 x C-28), 145.0 (2 x C-13), 122.7 (2 x C-12), 78.9 (2 x C-3), 55.1 (2 x C-5), 47.5 (2 x C-9), 46.7 (2 x C-17), 46.2 (2 x C-19), 42.1 (2 x C-18), 42.0 (2 x C-14), 39.3 (2 x C-8), 39.0 (2 x C-31), 38.7 (2 x C-1), 38.4 (2 x C-4), 36.9 (2 x C-10), 34.1 (2 x C-21), 32.9 (2 x C-29), 32.5 (2 x C-7), 32.3 (2 x C-22), 30.7 (2 x C-20), 28.0 (2 x C-23), 27.3 (2 x C-32), 27.1 (2 x C-2), 26.9 (2 x C-15), 25.7 (2 x C-27), 23.7 (2 x C-11), 23.6 (2 x C-30), 23.5 (2 x C-16), 18.2 (2 x C-6), 16.9 (2 x C-6), 15.5 (2 x C-24), 15.3 (2 x C-25) ppm; MS (ESI, MeOH): *m/z* = 965.3 (92 %, $[M-H]^-$;

analysis calcd for $C_{64}H_{104}N_2O_4$ (965.52): C 79.61, H 10.86, N 2.90; found: C 79.40, H 10.99, N 2.73.

N^1,N^5-Bis-[(3β)-3-hydroxy-olean-12-en-28-oyl]-1,5-diaminopentane (18)

Compound **18** (0.04 g, 76%) showed: m.p. 182°C; R_F = 0.3 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +35.37° (*c* 0.36, $CHCl_3$);

IR (KBr): ν = 3387*br*, 2924*m*, 2854*w*, 1638*m*, 1518*m*, 1455*m*, 1363*m*, 1037*m*, 996*m* cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.00 (*t*, *J* = 5.5 Hz, 2H, 2 x NH), 5.31 (*t*, *J* = 3.5 Hz, 2H, 2 x 12-H), 3.31 (*dq*, *J* = 13.4, 6.8 Hz, 2H, 2 x 31-H), 3.25–3.18 (*dd*, *J* = 11.2, 4.6 Hz, 2H, 2 x 3-H), 3.01 (*dq*, *J* = 12.8, 6.5 Hz, 2H, (2 x 31-H), 2.02–1.98 (*m*, 2H 2 x 16-H), 1.98–1.94 (*m*, 2H, 2 x 11-H), 1.93 (*d*, *J* = 3.3 Hz, 2H, 2 x 18-H), 1.87 (*dt*, *J* = 11.0, 2.9 Hz, 2H, 2 x 22-H), 1.78–1.50 (*m*, 20H, 2 x 1-H + 2 x 2-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 34-H + 2 x 35-H), 1.44 (*m*, 12H, 2 x 15-H + 2 x 7-H + 2 x 6-H + 2 x 21-H + 2 x 22-H + (2 x 19-H), 1.40–1.25 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.10 (*s*, 6H, 2 x 27-H), 1.08–1.00 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.99 (*s*, 6H, (2 x 23-H), 0.95 (*s*, 6H, 2 x 30-H), 0.92 (*s*, 6H, 2 x 25-H), 0.87 (*s*, 6H, 2 x 26-H), 0.78 (*s*, 6H, 2 x 24-H), 0.78 (*s*, 6H, 2 x 29-H), 0.71 (*m*, 2H, 2 x 5-H) ppm;

^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.1 (2 x C-28), 140.0 (2 x C-13), 125.5 (2 x C-12), 78.9 (2 x C-3), 55.1 (2 x C-5), 53.9 (2 x C-18), 47.7 (2 x C-17), 47.5 (2 x C-9), 42.5 (2 x C-14), 39.7 (2 x C-8), 39.5 (2 x C-19), 39.2 (2 x C-31), 39.1 (2 x C-20), 38.7 (2 x C-1), 38.6 (2 x C-4), 37.2 (2 x C-22), 36.9 (2 x C-10), 32.7 (2 x C-7), 30.8 (2 x C-21), 28.1 (2 x C-23), 27.8 (2 x C-15), 27.1 (2 x C-32), 26.4 (2 x C-33), 24.8 (2 x C-2), 24.5 (2 x C-16), 23. (2 x C-27), 23.2 (2 x C-2), 21.2 (2 x C-30), 18.2 (2 x C-6), 17.2 (2 x C-24), 16.9 (2 x C-29), 15.6 (2 x C-26), 15.5 (2 x C-25) ppm;

MS (ESI, MeOH): *m/z* = 994.7 (100 %, $[M+H]^+$), 1017.6 (90 %, $[M+Na]^+$);

analysis calcd for $C_{65}H_{106}N_2O_4$ (979.55): C 79.70, H 10.91, N 2.86; found: C 79.51, H 11.13, N 2.64.

N^1,N^6-Bis-[(3β)-3-hydroxy-urs-12-en-28-oyl]-1,6-diaminohexane (19)

Compound **19** (0.18 g, 76%) showed: m.p. 183°C (lit.: 191–193 °C); R_F = 0.3 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +50.40° (*c* 0.33, $CHCl_3$);

IR (KBr): ν = 3404*br*, 2927*s*, 2863*m*, 1639*s*, 1521*s*, 1463*s*, 1386*s*, 1242*m*, 1030*s*, 996*s* cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.98–5.93 (*m*, 2H, 2 x NH), 5.37 (*t*, *J* = 3.5 Hz, 2H, 2 x 12-H), 3.35 (*dq*, *J* = 13.7, 7.0 Hz, 2H, 2 x 31-H), 3.22 (*dd*, *J* = 11.2, 4.5 Hz, 2H, 2 x 3-H), 3.03–2.93 (*m*, 2H, 2 x 31-H), 2.50 (*dd*, *J* = 12.8, 3.8 Hz, 2H, 2 x 18-H), 1.97 (*dd*, *J* = 13.6, 3.8 Hz, 2H, 2 x 16-H), 1.91 (*m*, 2H, 2 x 11-H), 1.81–1.57 (*m*, 28H, 2 x 1-H + 2 x 2-H + 2 x 6-H + 2 x 7-H + 2 x 9-H + 2 x 11-H+ 2 x 16-H + 2 x 19-H + 2 x 32-H + 2 x 33-H), 1.57–1.51 (*m*, 2H, 2 x 15-H), 1.50–1.40 (*m*, 2H, 2 x 22-H), 1.40–1.27 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.25 (*m*, 2H 2 x 22-H), 1.19 (d, *J* = 11.3 Hz, 4H, 2 x 19-H + 2 x 21-H), 1.16 (*s*, 6H, 2 x 27-H), 1.07–1.00 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.99 (*s*, 6H, 2 x 23-H), 0.91 (*s*, 6H, 2 x 25-H), 0.91 (*s*, 6H, (2 x 30-H), 0.90 (*s*, 6H, 2 x 29-H), 0.78 (*s*, 6H, 2 x 24-H), 0.76 (*s*, 6H, 2 x 26-H), 0.72 (*s*, 2H, 2 x 5-H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.1 (2 x C-28), 145.1 (2 x C-13), 122.6 (2 x C-12), 78.9 (2 x C-3), 55.1 (2 x C-5), 47.5 (2 x C-9), 46.7 (2 x C-17), 46.2 (2 x C-19), 42.3 (2 x C-18), 42.1 (2 x C-14), 39.3 (2 x C-8), 39.2 (2 x C-31), 38.7 (2 x C-1), 38.4 (2 x C-4) 36.9 (2 x C-10), 34.1 (2 x C-21), 32.9 (2 x C-29), 32.4 (2 x C-7), 32.4 (2 x C-22), 30.7 (2 x C-20), 29.3 (2 x C-32), 28.0 (2 x C-23), 27.2 (2 x C-2), 27.1 (2 x C-15) 26.6 (2 x C-33), 25.7 (2 x C-27), 23.7 (2 x C-11), 23.6 (2 x C-30), 23.5 (2 x C-16), 18.2 (2 x C-6), 16.9 (2 x C-6), 15.5 (2 x C-24), 15.3 (2 x C-25) ppm;

MS (ESI, MeOH): *m/z* = 994.6 (90 %, $[M+H]^+$), 1016.6 (100 %, $[M+Na]^{+)}$;

analysis calcd for C66H108N2O4 (993.58): C 79.78, H 10.96, N 2.82; found: C 79.62, H 11.07, N 2.69.

N^1,N^7-Bis-[(3β)-3-hydroxy-olean-12-en-28-oyl]-1,7-diaminoheptane (20)

Compound **20** (0.12 g, 87%) showed: m.p. 183°C; R_F = 0.3 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +45.85° (*c* 0.36, $CHCl_3$);

IR (KBr): ν = 3407*br*, 2927*s*, 2861*m*, 1641*s*, 1519*m*, 1462*s*, 1386*m*, 1363*m*, 1212*m*, 1030*m*, 996*m* cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.93–5.88 (*m*, 2H, 2 x NH), 5.37 (*t*, *J* = 3.5 Hz, 2H, 2 x 12-H), 3.34 (*dq*, *J* = 13.7, 7.1 Hz, 2H, (2 x 31-H), 3.21 (*dd*, *J* = 11.2, 4.5 Hz, 2H, 2 x 3-H), 3.03–2.93 (*m*, 2H, (2 x 31-H), 2.49 (*dd*, *J* = 12.8, 3.6 Hz, 2H, 2 x 18-H), 1.97 (*dd*, *J* = 13.7, 3.7 Hz, 2H, 2 x 16-H), 1.91 (*dd*, *J* = 8.9, 3.3 Hz, 2H, 2 x 11-H), 1.81–1.56 (*m*, 30H, 2 x 1-H + 2 x 2-H + 2 x 6-H + 2 x 7-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 19-H + 2 x 32-H + 2 x 33-H + 2 x 34-H), 1.54 (*dd*, *J* = 9.7, 4.3 Hz, 2H, 2 x 15-H), 1.50–1.40 (*m*, 2H, 2 x 22-H), 1.40–1.27 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.25 (*m*, 2H 2 x 22-H), 1.19 (*d*, *J* = 11.3 Hz, 4H, 2 x 19-H + 2 x 21-H), 1.16 (*s*, 6H, 2 x 27-H), 1.03 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.99 (*s*, 6H, 2 x 23-H), 0.91 (*s*, 6H, 2 x 25-H), 0.91 (*s*, 6H, (2 x 30-H), 0.90 (*s*, 6H, 2 x 29-H), 0.78 (*s*, 6H, 2 x 24-H), 0.76 (*s*, 6H, 2 x 26-H), 0.72 (*s*, 2H, 2 x 5-H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.1 (2 x C-28), 145.1 (2 x C-13), 122.6 (2 x C-12), 78.9 (2 x C-3), 55.1 (2 x C-5), 47.5 (2 x C-9), 46.8 (2 x C-17), 46.2 (2 x C-19), 42.3 (2 x C-18), 42.0 (2 x C-14), 39.4 (2 x C-8), 39.3 (2 x C-31), 38.7 (2 x C-1), 38.4 (2 x C-4), 36.9 (2 x C-10), 34.1 (2 x C-21), 32.9 (2 x C-29), 32.5 (2 x C-7), 32.3 (2 x C-22), 30.7 (2 x C-20), 29.3 (2 x C-32), 28.9 (C-34), 28.0 (2 x C-23), 27.2 (2 x C-2), 27.1 (2 x C-15), 27.0 (2 x C-33), 25.7 (2 x C-27), 23.8 (2 x C-11), 23.5 (2 x C-30), 23.5 (2 x C-16), 18.2 (2 x C-6), 16.9 (2 x C-6), 15.5 (2 x C-24), 15.3 (2 x C-25) ppm;

MS (ESI, MeOH): *m/z* = 1008.8 (14 %, $[M+H]^+$), 1031.7 (43 %, $[M+Na]^+$);

analysis calcd for $C_{67}H_{110}N_2O_4$ (xx): C 79.86, H 11.00, N 2.78; found: C 79.68, H 11.18, N 2.69.

N^1,N^2-Bis-[(3β)-3-hydroxy-urs-12-en-28-oyl]-1,2-diaminoethane (21)

Compound **21** (0.18 g, 85%) showed: m.p. 228°C; R_F = 0.3 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +44.32° (*c* 0.31, $CHCl_3$);

IR (KBr): ν = 3383*br*, 2927*s*, 2870*m*, 1632*s*, 1533*m*, 1455*m*, 1378*w*, 1280*w*, 1089*w*, 1030*m*, 995*m* cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.53 (*m*, 2H, 2 x NH), 5.36 (*t*, *J* = 3.4 Hz, 2H, 2 x 12-H), 3.48 (*d*, *J* = 9.4 Hz, 2H, 2 x 31-H), 3.21 (*dd*, *J* = 11.2, 4.6 Hz, 2H, 2 x 3-H), 3.09 (*d*, *J* = 9.0 Hz, 2H, (2 x 31-H), 2.02 (*d*, *J* = 4.1 Hz, 2H 2 x 16-H), 2.00–1.94 (*m*, 2H, 2 x 11-H), 1.91 (*m*, 2H, 2 x 18-H), 1.89–1.84 (*m*, 2H, 2 x 22-H), 1.76–1.51 (*m*, 12H, 2 x 1-H + 2 x 2-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H), 1.50–1.48 (*m*, 6H, 2 x 15-H + 2 x 7-H + 2 x 6-H), 1.47 (*m*, 6H, 2 x 21-H + 2 x 22-H + 2 x 19-H), 1.40–1.25 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.09 (*s*, 6H, 2 x 27-H), 1.08–1.02 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.99 (*s*, 6H, 2 x 23-H), 0.95 (*s*, 6H, 2 x 30-H), 0.92 (*s*, 6H, 2 x 25-H), 0.87 (*s*, 6H, 2 x 26-H), 0.78 (*s*, 6H, 2 x 24-H), 0.75 (*s*, 6H, 2 x 29-H), 0.72 (*m*, 2H, 2 x 5-H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.1 (2 x C-28), 139.2 (2 x C-13), 125.9 (2 x C-12), 78.9 (2 x C-3), 55.1 (2 x C-5), 53.3 (2 x C-18), 47.6 (2 x C-17), 47.5 (2 x C-9), 42.3 (2 x C-14), 39.7 (2 x C-8), 39.9 (2 x C-31), 39.5 (2 x C-19), 39.0 (2 x C-20), 38.7 (2 x C-1), 38.5 (2 x C-4), 37.4 (2 x C-22), 36.9 (2 x C-10), 32.6 (2 x C-7), 30.8 (2 x C-21), 28.1 (2 x C-23), 27.8 (2 x C-15), 27.1 (2 x C-2), 24.7 (2 x C-16), 23.5 (2 x C-27), 23.3 (2 x C-11), 21.2 (2 x C-30), 18.1 (2 x C-6), 17.2 (2 x C-24), 16.8 (2 x C-29), 16.7 (2 x C-26), 15.5 (2 x C-25) ppm;

MS (ESI, MeOH): *m/z* = 938.5 (60 %, $[M+H]^+$), 960.5 (100 %, $[M+Na]^+$);

analysis calcd for $C_{62}H_{100}N_2O_4$ (937.49): C 79.43, H 10.75, N 2.99; found: C 70.23, H 10.95, N 2.71.

N^1,N^3-Bis-[(3β)-3-hydroxy-urs-12-en-28-oyl]-1,3-diaminopropane (22)

Compound **22** (0.02 g, 56%) showed: m.p. 242°C; R_F = 0.3 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +20.84° (*c* 0.3, $CHCl_3$);

IR (KBr): ν = 3383*br*, 2927*s*, 2870*m*, 1632*m*, 1532*m*, 1454*m*, 1378*m*, 1259*s*, 1088*m*, 1029*m*, 996*w* cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.42 (*m*, 2H, 2 x NH), 5.36 (*t*, *J* = 3.5 Hz, 2H, 2 x 12-H), 3.31–3-22 (*d*, *J* = 9.4 Hz, 2H, (2 x 31-H), 3.20 (*m*, 2H, 2 x 3-H), 3.10 (*dd*, *J* = 13.4, 5.9 Hz, 2H, 2 x 31-H), 2.03 (*d*, *J* = 4.2 Hz, 2H 2 x 16-H), 2.01–1.94 (*m*, 2H, 2 x 11-H), 1.92 (*m*, 2H, 2 x 18-H), 1.90–1.82 (*m*, 2H, 2 x 22-H), 1.79–1.50 (*m*, 14H, 2 x 1-H + 2 x 2-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 34-H), 1.49 (*m*, 6H, 2 x 15-H + 2 x 7-H + 2 x 6-H), 1.48–1.44 (*m*, 6H, 2 x 21-H + 2 x 22-H + 2 x 19-H), 1.43–1.24 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.10 (*s*, 6H, 2 x 27-H), 1.08–1.00 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.99 (*s*, 6H, 2 x 23-H), 0.94 (*s*, 6H, 2 x 30-H), 0.91 (*s*, 6H, 2 x 25-H), 0.87 (*s*, 6H, 2 x 26-H), 0.78 (*s*, 6H, 2 x 24-H), 0.76 (*s*, 6H, 2 x 29-H), 0.71 (*m*, 2H, 2 x 5-H) ppm;

^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.1 (2 x C-28), 139.3 (2 x C-13), 125.7 (2 x C-12), 78.9 (2 x C-3), 55.1 (2 x C-5), 53.5 (2 x C-18), 47.7 (2 x C-17), 47.5 (2 x C-9), 42.4 (2 x C-14), 39.7 (2 x C-8), 39.5 (2 x C-19), 39.0 (2 x C-20), 38.7 (2 x C-1), 38.6 (2 x C-4), 37.4 (2 x C-22), 36.9 (2 x C-10), 36.4 (2 x C-31), 32.8 (2 x C-7), 30.9 (2 x C-21), 28.1 (2 x C-23), 27.8 (2 x C-15), 27.1 (2 x C-2), 27.2 (2 x C-32), 24.7 (2 x C-16), 23.4 (2 x C-27), 23.3 (2 x C-2), 21.2 (2 x C-30), 18.2 (2 x C-6), 17.2 (2 x C-24), 17.0 (2 x C-29), 15.5 (2 x C-26), 15.5 (2 x C-25) ppm; MS (ESI, MeOH): *m/z* = 951.9 (40 %, $[M+H]^+$), 973.8 (8 %, $[M+Na]^+$);

analysis calcd for $C_{63}H_{102}N_2O_4$ (951.49): C 79.52, H 10.81, N 2.94; found: C 79.32, H 11.03, N 2.76.

N^1,N^4-Bis-[(3β)-3-hydroxy-urs-12-en-28-oyl]-1,4-diaminobutane (23)

Compound **23** (0.03 g, 54%) showed: m.p. 245°C; R_F = 0.3 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +33.23° (*c* 0.16, $CHCl_3$);

IR (KBr): ν = 3422*br*, 2926*s*, 2870*m*, 1638*s*, 1517*m*, 1454*s*, 1378*m*, 1243*w*, 1045*m*, 1029*m*, 997*m* cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.97 (*m*, 2H, 2 x NH), 5.31 (*t*, *J* = 3.6 Hz, 2H, 2 x 12-H), 3.40–3.29 (*m*, 2H, 2 x 31-H), 3.22 (*dt*, *J* = 10.8, 5.3 Hz, 2H, 2 x 3-H), 2.99 (*dd*, *J* = 12.2, 5.8 Hz, 2H, 2 x 31-H), 2.00 (*t*, *J* = 2.3 Hz, 2H 2 x 16-H), 1.95 (*m*, 2H, 2 x 11-H), 1.88 (*d*, *J* = 3.2 Hz, 2H, 2 x 18-H), 1.85 (*d*, *J* = 3.1 Hz, 2H, 2 x 22-H), 1.77–1.50 (*m*, 16H, 2 x 1-H + 2 x 2-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 34-H), 1.45 (*m*, 12H, 2 x 15-H + 2 x 7-H + 2 x 6-H + 2 x 21-H + 2 x 22-H + 2 x 19-H), 1.41–1.24 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.10 (*s*, 6H, 2 x 27-H), 1.08–1.00 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.99 (*s*, 6H, 2 x 23-H), 0.95 (*s*, 6H, 2 x 30-H), 0.92 (*s*, 6H, 2 x 25-H), 0.87 (*s*, 6H, 2 x 26-H), 0.78 (*s*, 6H, 2 x 24-H), 0.77 (*s*, 6H, 2 x 29-H), 0.72 (*m*, 2H, 2 x 5-H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.1 (2 x C-28), 140.0 (2 x C-13), 125.5 (2 x C-12), 78.9 (2 x C-3), 55.1 (2 x C-5), 53.8 (2 x C-18), 47.6 (2 x C-17), 47.5 (2 x C-9), 42.5 (2 x C-14), 39.7 (2 x C-8), 39.5 (2 x C-19), 39.1 (2 x C-20), 38.9 (2 x C-31), 38.7 (2 x C-1), 38.6 (2 x C-4), 37.2 (2 x C-22), 36.9 (2 x C-10), 32.7 (2 x C-7), 30.9 (2 x C-21), 28.1 (2 x C-23), 27.8 (2 x C-15), 27.1 (2 x C-32), 26.9 (2 x C-2), 24.7 (2 x C-16), 23.4 (2 x C-27), 23.2 (2 x C-2), 21.2 (2 x C-30), 18.2 (2 x C-6), 17.2 (2 x C-24), 17.0 (2 x C-29), 15.5 (2 x C-26), 15.5 (2 x C-25) ppm; MS (ESI, MeOH): *m/z* = 967.5 (60 %, $[M+H]^+$), 990.5 (100 %, $[M+Na]^+$);

analysis calcd for $C_{64}H_{104}N_2O_4$ (965.52): C 79.61, H 10.86, N 2.90; found: C 79.45, H 11.03, N 2.84.

N^1,N^5-Bis-[(3β)-3-hydroxy-urs-12-en-28-oyl]-1,5-diaminopentane (24)

Compound **24** (0.1 g, 77%) showed: m.p. 158°C; R_F = 0.3 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +42.57° (*c* 0.31, $CHCl_3$);

IR (KBr): ν = 3396*br*, 2924*m*, 2869*w*, 1641*m*, 1520*w*, 1455*w*, 1386*w*, 1260*m*, 1091*s*, 1026*s* cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.92 (*t*, *J* = 5.6 Hz, 2H, 2 x NH), 5.31 (*t*, *J* = 3.2 Hz, 2H, 2 x 12-H), 3.3 (*dq*, *J* = 13.5, 6.9 Hz, 2H, 2 x 31-H), 3.25–3.18 (*m*, 2H, 2 x 3-H), 3.03–2.92 (*m*, 2H, 2 x 31-H), 2.00 (*d*, *J* = 4.2 Hz, 2H 2 x 16-H), 1.98–1.94 (*m*, 2H, 2 x 11-H), 1.83 (*d*, *J* = 2.3 Hz, 2H, 2 x 18-H), 1.91–1.82 (*m*, 2H, 2 x 22-H), 1.78–1.50 (*m*, 18H, 2 x 1-H + 2 x 2-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 34-H + 35-H), 1.49–1.42 (*m*, 12H, 2 x 15-H + 2 x 7-H + 2 x 6-H + 2 x 21-H + 2 x 22-H + (2 x 19-H), 1.41–1.26 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.09 (*s*, 6H, 2 x 27-H), 1.08–1.00 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.99 (*s*, 6H, (2 x 23-H), 0.95 (*s*, 6H, 2 x 30-H), 0.92 (*s*, 6H, 2 x 25-H), 0.87 (*s*, 6H, 2 x 26-H), 0.78 (*s*, 6H, 2 x 24-H), 0.77 (*s*, 6H, 2 x 29-H), 0.71 (*m*, 2H, 2 x 5-H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.1 (2 x C-28), 140.0 (2 x C-13), 125.5 (2 x C-12), 78.9 (2 x C-3), 55.2 (2 x C-5), 53.9 (2 x C-18), 47.6 (2 x C-17), 47.5 (2 x C-9), 42.5 (2 x C-14), 39.7 (2 x C-8), 39.5 (2 x C-19), 39.3 (2 x C-31), 39.1 (2 x C-20), 38.7 (2 x C-1), 38.6 (2 x C-4), 37.2 (2 x C-22), 36.9 (2 x C-10), 32.7 (2 x C-7), 30.8 (2 x C-21), 29.0 (C-33), 28.1 (2 x C-23), 27.8 (2 x C-15), 27.1 (2 x C-32), 24.8 (2 x C-2), 24.5 (2 x C-16), 23.4 (2 x C-27), 23.2 (2 x C-2), 21.2 (2 x C-30), 18.2 (2 x C-6), 17.2 (2 x C-24), 16.9 (2 x C-29), 15.6 (2 x C-26), 15.5 (2 x C-25) ppm;

MS (ESI, MeOH): *m/z* = 980.3 (20 %, $[M+H]^+$), 1002.3 (100 %, $[M+Na]^+$);

analysis calcd for $C_{65}H_{106}N_2O_4$ (979.55): C 79.50, H 10.91, N 2.86; found: C 79.36, H 11.13, N 2.67.

N^1,N^6-Bis-[(3β)-3-hydroxy-urs-12-en-28-oyl]-1,6-diaminohexane (25)

Compound **25** (0.11 g, 70%) showed: m.p. 182°C; R_F = 0.3 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +35.37° (*c* 0.36, $CHCl_3$);

IR (KBr): ν = 3387*br*, 2924*m*, 2854*w*, 1638*m*, 1518*m*, 1455*m*, 1363*m*, 1037*m*, 996*m* cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.00 (*t*, *J* = 5.5 Hz, 2H, 2 x NH), 5.31 (*t*, *J* = 3.5 Hz, 2H, 2 x 12-H), 3.31 (*dq*, *J* = 13.4, 6.8 Hz, 2H, 2 x 31-H), 3.25–3.18 (*dd*, *J* = 11.2, 4.6 Hz, 2H, 2 x 3-H), 3.01 (*dq*, *J* = 12.8, 6.5 Hz, 2H, 2 x 31-H), 2.02–1.98 (*m*, 2H 2 x 16-H), 1.98–1.94 (*m*, 2H, 2 x 11-H), 1.93 (*d*, *J* = 3.3 Hz, 2H, 2 x 18-H), 1.87 (*dt*, *J* = 11.0, 2.9 Hz, 2H, 2 x 22-H), 1.78–1.50 (*m*, 20H, 2 x 1-H + 2 x 2-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 34-H + 2 x 35-H), 1.44 (*m*, 12H, 2 x 15-H + 2 x 7-H + 2 x 6-H + 2 x 21-H + 2 x 22-H + 2 x 19-H), 1.40–1.25 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.10 (*s*, 6H, 2 x 27-H), 1.08–1.00 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.99 (*s*, 6H, 2 x 23-H), 0.95 (*s*, 6H, 2 x 30-H), 0.92 (*s*, 6H, 2 x 25-H), 0.87 (*s*, 6H, 2 x 26-H), 0.78 (*s*, 6H, 2 x 24-H), 0.78 (*s*, 6H, 2 x 29-H), 0.71 (*m*, 2H, 2 x 5-H) ppm;

^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.1 (2 x C-28), 140.0 (2 x C-13), 125.5 (2 x C-12), 78.9 (2 x C-3), 55.1 (2 x C-5), 53.9 (2 x C-18), 47.7 (2 x C-17), 47.5 (2 x C-9), 42.5 (2 x C-14), 39.7 (2 x C-8), 39.5 (2 x C-19), 39.2 (2 x C-31), 39.1 (2 x C-20), 38.7 (2 x C-1), 38.6 (2 x C-4), 37.2 (2 x C-22), 36.9 (2 x C-10), 32.7 (2 x C-7), 30.8 (2 x C-21), 28.1 (2 x C-23), 27.8 (2 x C-15), 27.1 (2 x C-32), 26.4 (2 x C-33), 24.8 (2 x C-2), 24.5 (2 x C-16), 23. (2 x C-27), 23.2 (2 x C-2), 21.2 (2 x C-30), 18.2 (2 x C-6), 17.2 (2 x C-24), 16.9 (2 x C-29), 15.6 (2 x C-26), 15.5 (2 x C-25) ppm;

MS (ESI, MeOH): *m/z* = 994.7 (100 %, $[M+H]^+$), 1017.6 (90 %, $[M+Na]^+$);

analysis calcd for $C_{66}H_{108}N_2O_4$ (993.58): C 79.78, H 10.96, N 2.82; found: C 79.51, H 11.15, N 2.69.

N^1,N^7-Bis-[(3β)-3-hydroxy-urs-12-en-28-oyl]-1,7-diaminoheptane (26)

Compound **26** (0.05 g, 68%) showed: m.p. 188°C; R_F = 0.3 (silica gel, toluene/ethyl acetate/formic acid/heptane, 80:26:5:1); $[\alpha]_D$ = +30.29° (*c* 0.30, $CHCl_3$);

IR (KBr): ν = 3406*br*, 2963*m*, 2925*w*,1641*m*, 1519*w*, 1455*w*, 1377*w*, 1260*w*, 1260*s*, 1088*s*, 1016*s*, 865*w* cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.90 (*t*, *J* = 5.4 Hz, 2H, 2 x NH), 5.30 (*t*, *J* = 3.6 Hz, 2H, 2 x 12-H), 3.31 (*dq*, *J* = 13.6, 7.0 Hz, 2H, 2 x 31-H), 3.22 (*dd*, *J* = 11.2, 4.7 Hz, 2H, 2 x 3-H), 2.99 (*dq*, *J* = 12.7, 7.0 Hz, 2H, 2 x 31-H), 1.99 (*d*, *J* = 3.5 Hz, 2H 2 x 16-H), 1.96 (*m*, 2H, 2 x 11-H), 1.92 (*td*, *J* = 16.7, 3.4 Hz, 2H, 2 x 18-H), 1.89–1.83 (*m*, 2H, 2 x 22-H), 1.78–1.50 (*m*, 20H, 2 x 1-H + 2 x 2-H + 2 x 9-H+ 2 x 11-H+ 2 x 16-H + 2 x 34-H + 2 x 35-H + 36-H), 1.49–1.41 (*m*, 12H, 2 x 15-H + 2 x 7-H + 2 x 6-H + 2 x 21-H + 2 x 22-H + 2 x 19-H), 1.40–1.25 (*m*, 6H, 2 x 6-H + 2 x 7-H + 2 x 21-H), 1.10 (*s*, 6H, 2 x 27-H), 1.08–1.00 (*m*, 4H, 2 x 1-H + 2 x 15-H), 0.99 (*s*, 6H, 2 x 23-H), 0.95 (*s*, 6H, 2 x 30-H), 0.93 (*s*, 6H, 2 x 25-H), 0.87 (*s*, 6H, 2 x 26-H), 0.78 (*s*, 6H, 2 x 24-H), 0.78 (*s*, 6H, 2 x 29-H), 0.71 (*m*, 2H, 2 x 5-H) ppm;

^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.1 (2 x C-28), 140.0 (2 x C-13), 125.5 (2 x C-12), 78.9 (2 x C-3), 55.1 (2 x C-5), 54.0 (2 x C-18), 47.6 (2 x C-17), 47.5 (2 x C-9), 42.5 (2 x C-14), 39.7 (2 x C-8), 39.5 (2 x C-19), 39.4 (2 x C-31), 39.1 (2 x C-20), 38.7 (2 x C-1), 38.6 (2 x C-4), 37.1 (2 x C-22), 36.9 (2 x C-10), 32.7 (2 x C-7), 30.9 (2 x C-21), 29.2 (C-34), 28.9 (2 x C-33), 28.1 (2 x C-23), 27.8 (2 x C-15), 27.1 (2 x C-32), 24.8 (2 x C-2), 24.4 (2 x C-16), 23.4 (2 x C-27), 23.2 (2 x C-2), 21.2 (2 x C-30), 18.2 (2 x C-6), 17.2 (2 x C-24), 16.9 (2 x C-29), 15.6 (2 x C-26), 15.5 (2 x C-25) ppm;

MS (ESI, MeOH): *m/z* = 1008.9 (55 %, $[M+H]^+$), 1030.9 (30 %, $[M+Na]^+$);

analysis calcd for $C_{67}H_{110}N_2O_4$ (1007.60): C 79.86, H 11.00, N 2.78; found: C 79.66, H 11.18, N 2.67.

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References

1- M. Vrettou, A.A. Gray, A.R.E. Brewer, A.G.M. Barrett, Strategies for the synthesis of C-2 symmetric natural products- a review, Tetrahedron **2007**, 63, 1487-1536.
2- G. Berube, Natural and synthetic biologically active dimeric molecules: Anticancer agents, anti-HIV agents, steroid derivatives and opioid antagonists, Curr. Med. Chem. **2006**, 13, 131-154.
3- G. Berube, D. Rabouina, V. Perron, B. N'Zemba, R.C. Gaudreault, S. Parenta, E.E. Asselin, Synthesis of unique 17 beta-estradiol homo-dimers, estrogen receptors binding affinity evaluation and cytocidal activity on breast, intestinal and skin cancer cell lines, Steroids **2006**, 71, 911-921.
4- T. Wezeman, K.S. Masters, S. Bräse, Double Trouble-The Art of Synthesis of Chiral Dimeric Natural Products, Angew. Chem. Int. Ed. **2014**, 53, 4524-4526.
5- J. L. Jeffrey, R. Sarpong, An approach to the synthesis of dimeric resveratrol natural products via a palladium-catalysed domino reaction, Tetrahedron Lett. **2009**, 50, 1969-1972.
6- N. J. Marianayagam, M. Sunde, J.M. Matthews, The power of two: protein dimerization in biology, Trends Biochem. Sci. **2004**, 29, 618-625.
7- B. Bednarczyk-Cwynar, A. Gunther, Advances in Chemistry and Pharmacology of Triterpenoid Synthetic Dimers, Curr. Med. Chem. **2017**, 24, 2205-2240.
8- O. B. Kazakova, G.V. Giniyatullina, G.A. Tolstikov, V.E. Kataev, R.Z. Musin, Synthesis and modification of triterpenoids with two lupan backbones, Russ. J. Bioorg. Chem. **2009**, 35, 645-650.
9- G. R. Pettit, N. Melody, F. Hempenstall, J.C. Chapuis, T.L. Groy, L. Williams, Antineoplastic Agents. 595. Structural Modifications of Betulin and the X-ray Crystal Structure of an Unusual Betulin Amine Dimer, J. Nat. Prod. **2014**, 77, 863-872.
10- J. Hu, R.F. Li, J.R. Lu, Y. Ju, Synthesis and anion recognition of a novel oleanolic acid-based cyclic dimer, Tetrahedron Lett. **2011**, 52, 4211-4214.
11- L. J. Dolby, M. Debono, Studies of Terpene Chemistry .1. Acid-Catalyzed Dimerization of Citronellal, J. Org. Chem. **1964**, 29, 2306-2310.
12- C. M. Ma, N. Nakamura, M. Hattori, Chemical modification of oleanene type triterpenes and their inhibitory activity against HIV-1 protease dimerization, Chem. Pharm. Bull. **2000**, 48, 1681-1688.
13- K. G. Cheng, J. Liu, H.B. Sun, J. Xie, Synthesis of Oleanolic Acid Dimers as Inhibitors of Glycogen Phosphorylase, Chem. Biodivers. **2010**, 7, 690-697.
14- K. G. Cheng, C.H. Su, L.D. Yang, J. Liu, Z.F. Chen, Synthesis of oleanolic acid dimers linked at C-28 and evaluation of anti-tumor activity, Eur. J. Med. Chem. **2015**, 89, 480-489.
15- J. R. Lu, J.D. Wu, Y. Ju, Tuning the aggregation mode to induce different chiralities in organogels of mono- and bis-triterpenoid derivatives and the preparation of gold nanoparticles for use as a template, New. J. Chem. **2014**, 38, 6050-6056.
16- T. Honda, E.M. Padegimas, E. David, C. Sundararajan, K.T. Liby, C. Williams, M.B. Sporn, M. Visnick, 2-Cyano-3,10-dioxooleana-1,9(11)-dien-28-oic acid anhydride. A novel and highly potent anti-inflammatory and cytoprotective agent, Bioorg. Med. Chem. Lett. **2010**, 20, 2275-2278.
17- M. R. Prausnitz, R. Langer, Transdermal drug delivery, Nat. Biotechnol. **2008**, 26, 1261-1268.
18- M. Kahnt, L. Fischer, A. Al-Harrasi, R. Csuk, Ethylenediamine Derived Carboxamides of Betulinic and Ursolic Acid as Potential Cytotoxic Agents, Molecules **2018**, 23, 2558; doi:10.3390/molecules23102558.
19- M. Kahnt, L. Heller, P. Grabandt, A. Al-Harrasi, R. Csuk, Platanic acid: A new scaffold for the synthesis of cytotoxic agents, Eur. J. Med. Chem. **2018**, 143, 259-265.
20- M. Kahnt, J. Wiemann, L. Fischer, S. Sommerwerk, R. Csuk, Transformation of asiatic acid into a mitocanic, bimodal-acting rhodamine B conjugate of nanomolar cytotoxicity, Eur. J. Med. Chem. **2018**, 159, 143-148.
21- L. Heller, A. Knorrscheidt, F. Flemming, J. Wiemann, S. Sommerwerk, I.Z. Pavel, A. Al-Harrasi, R. Csuk, Synthesis and proapoptotic activity of oleanolic acid derived amides, Bioorg. Chem. **2016**, 68, 137-151.
22- S. Sommerwerk, L. Heller, C. Kerzig, A.E. Kramell, R. Csuk, Rhodamine B conjugates of triterpenoic acids are cytotoxic mitocans even at nanomolar concentrations, Eur. J. Med. Chem. **2017**, 127, 1-9.
23- S. Sommerwerk, L. Heller, J. Kuhfs, R. Csuk, Selective killing of cancer cells with triterpenoic acid amides - The substantial role of an aromatic moiety alignment, Eur. J. Med. Chem. **2016**, 122, 452-464.
24- A. Loesche, M. Kahnt, I. Serbian, W. Brandt, R. Csuk, Triterpene-based carboxamides act as good inhibitors of butyrylcholinesterase, Molecules **2019**, 24, 948/941-948/916.

25- R. K. Wolfram, L. Fischer, R. Kluge, D. Stroehl, A. Al-Harrasi, R. Csuk, Homopiperazine-rhodamine B adducts of triterpenoic acids are strong mitocans, Eur. J. Med. Chem. **2018**, 155, 869-879.
26- R. K. Wolfram, L. Heller, R. Csuk, Targeting mitochondria: Esters of rhodamine B with triterpenoids are mitocanic triggers of apoptosis, Eur. J. Med. Chem. **2018**, 152, 21-30.
27- W. Jiang, R.-Z. Huang, J. Zhang, T. Guo, M.-T. Zhang, X.-C. Huang, B. Zhang, Z.-X. Liao, J. Sun, H.-S. Wang, Discovery of antitumor ursolic acid long-chain diamine derivatives as potent inhibitors of NF-κB, Bioorg. Chem. **2018**, 79, 265-276.
28- L.-H. Zhang, Z.-H. Zhang, M.-Y. Li, Z.-Y. Wei, X.-J. Jin, H.-R. Piao, Synthesis and evaluation of the HIF-1α inhibitory activities of novel ursolic acid tetrazole derivatives, Bioorg. Med. Chem. Lett. **2019**, 29, 1440-1445.
29- L. Ruzicka, K. Hofmann, Polyterpenes and polyterpenoids. C. Transpositions in the rings A and E of oleanolic acid. Carbon skeleton of pentacyclic triterpenes, Helv. Chim. Acta **1936**, 19, 114-128.
30- S. C. B. Gnoatto, L. Dalla Vechia, C.L. Lencina, A. Dassonville-Klimpt, S. Da Nascimento, D. Mossalayi, J. Guillon, G. Gosmann, P. Sonnet, Synthesis and preliminary evaluation of new ursolic and oleanolic acids derivatives as antileishmanial agents, J. Enzyme Inhib. Med. Chem. **2008**, 23, 604-610.
31- M. Miyazawa, Y. Okuno, K. Imanishi, Suppression of the SOS-Inducing Activity of Mutagenic Heterocyclic Amine, Trp-P-1, by Triterpenoid from Uncaria sinensis in the Salmonella typhimurium TA1535/pSK1002 Umu Test, J. Agric. Food Chem. **2005**, 53, 2312-2315.

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Research paper

Betulinic acid derived amides are highly cytotoxic, apoptotic and selective

Sophie Hoenke [a, 1], Niels V. Heise [a, 1], Michael Kahnt [a], Hans-Peter Deigner [b], René Csuk [a, *]

[a] Martin-Luther-University Halle-Wittenberg, Organic Chemistry, Kurt-Mothes-Str. 2, D-06120, Halle (Saale), Germany
[b] Furtwangen University, Medical and Life Sciences Faculty, Jakob-Kienzle Str. 17, D-78054, Villingen-Schwenningen, Germany

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ABSTRACT

Betulinic and platanic acid derived amides were prepared and screened for their cytotoxic activity. All of the compounds were shown to be cytotoxic for a panel of human tumor cell lines, and especially apoptotic betulinic acid derived compounds **6**, **8** and **19** showed low EC_{50} values. Of special interest was a 4-isoquinolinyl amide of 3-*O*-acetyl-betulinic acid (compound **19**), being the most cytotoxic compound of this series and holding EC_{50} values as low as EC_{50} = 1.48 μM (A375 melanoma cells) while being significantly less cytotoxic for non-malignant fibroblasts NIH 3T3 with a selectivity index of >91.2. This finding parallels previous results obtained for **SAA21**, a augustic acid derived compound thus making the 4-isoquinolinyl moiety to a privileged scaffold.

1. Introduction

Despite all efforts, diseases and deaths from cancer still represent the second most frequent type of death worldwide. In high-income countries, however, deaths from cancer are even more common than those resulting from a cardiovascular disease [1] (see Scheme 1)

The number of patients with cancer has risen steadily in recent years, and a forecast for the year 2040 predicts that the number of patients as well as the number of deaths by cancer will double [2]. The number of newly approved drugs increases continuously; thus in 2019 alone for Germany 25 new drugs (excluding biosimilars) have been brought to market with ten of them to treat cancer patients [3].

The development of new cytotoxic active ingredients continues to be focused on natural substances or on drugs derived from natural substances. Thus, nowadays 50% of all approved drugs can be traced back to a natural product [4]. A special role thereby is taken by terpenes and terpenoids in as much as they represent the largest family of natural compounds that have been investigated for their therapeutic effects [4,5].

More recently, triterpenes in particular have again become a focus of research, and especially triterpene carboxylic acids derived from, for example betulinic acid, maslinic acid, oleanolic acid or ursolic acid (Fig. 1).

Recent investigations have shown that the presence of an intact carboxyl function is necessary to achieve good cytotoxicity. For example, derivatives derived from betulinic acid are usually significantly more active than those derived from betulin [6–8]. The potential of those compounds containing an additional cationic residue, e.g. a quaternary ammonium salt [9,10], a phosphonium moiety [11–13], a BODIPY residue [14,15], a malachite green [16] or – of special interest - a rhodamine B residue [17–20] being connected to the basic skeleton of the triterpene with or without a suitable amine spacer, should be particularly emphasized. Interestingly, however, in the past, some triterpenoid amides also showed good and - above all - quite selective cytotoxic effects. **EM2** [21–25] - a benzylamide of diacetylated maslinic acid (Fig. 2) - is cytotoxic in the low μM range [26] (e.g. EC_{50} against A2780 ovarian cancer cells = 0.5 μM) but this compound also showed good selectivity towards cancer cells (e.g. EC_{50} against non-malignant primary human fibroblasts WW030272 = 156 μM). Furthermore, small structural differences obviously influence both cytotoxicity and selectivity. For example, **SAA9** derived from augustic acid was quite cytotoxic (EC_{50} 2.2 μM, A2780 cells), but also quite unselective ($S = [EC_{50}(A2780)]/[EC_{50}(NIH\ 3T3)] = 0.4$), whereas the analogous

* Corresponding author.
E-mail address: rene.csuk@chemie.uni-halle.de (R. Csuk).
[1] These authors contributed equally.

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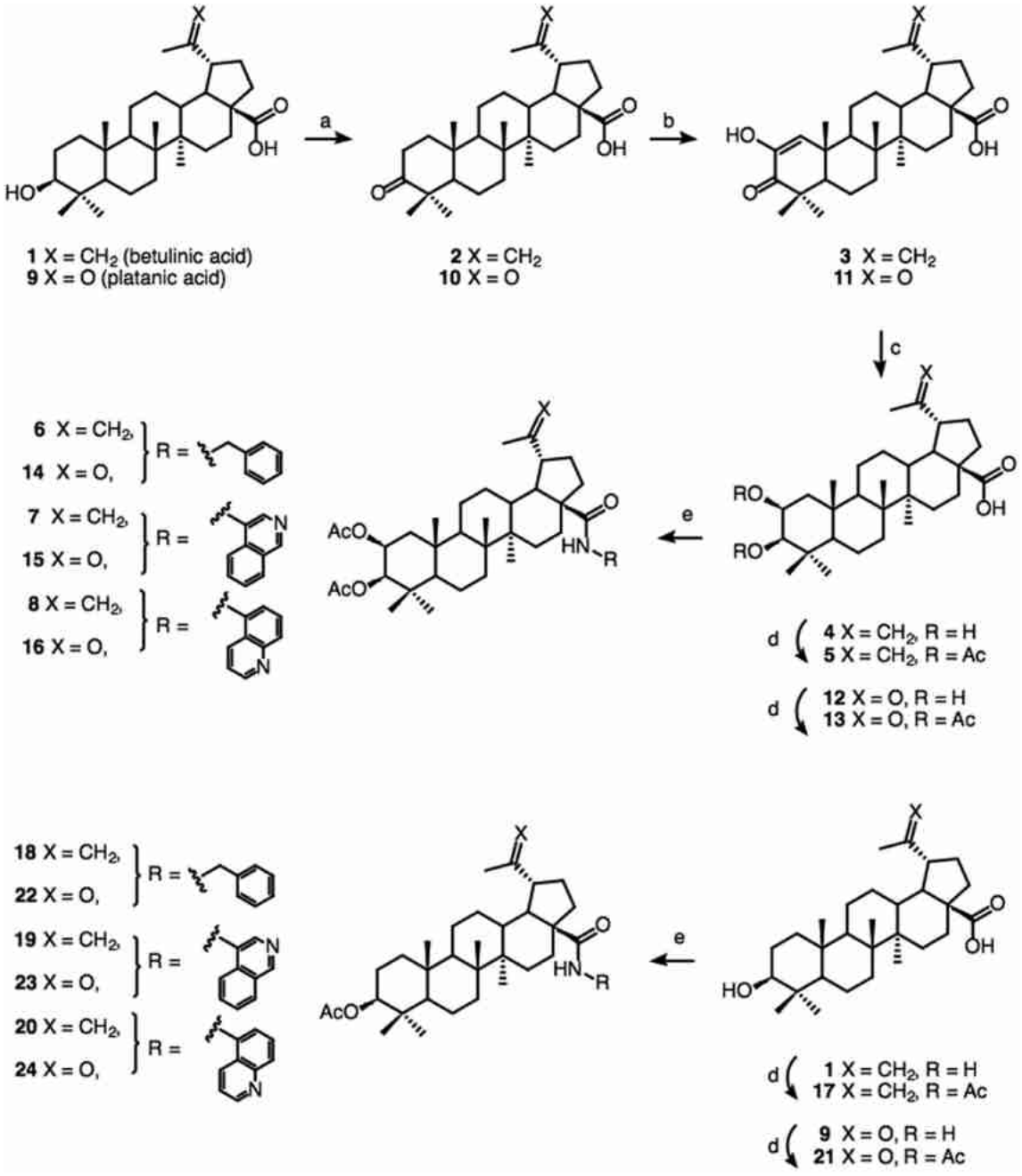


Scheme 1. Reactions and conditions: a) Jones oxidation, 76% for 2, 98% for 10; b) ^{t}BuOK, ^{t}BuOH, THF, 3 h, 50 °C, air, 97% for **3**, 85% for **11**; c) $NaBH_4$, MeOH, 23 °C, 2 h, 55% (for **4**), 74% (for **12**); d) Ac_2O, NEt_3, DMAP (cat.), DCM, 23 °C, 1 d, 70% (for **5**), 48% (for **13**); e) $(COCl)_2$, DCM, 23 °C, 1 h, then benzylamine or 4-amino-isoquinoline or 5-aminoquinoline, DCM, 23 °C, 1 h, 62% (for **6**), 55% (for **7**), 42% (for **8**), 84% (for **14**), 49% (for **15**), 82% (for **16**), 73% (for **18**), 60% (for **19**), 64% (for **20**), 76% (for **22**), 80% (for **23**), 57% (for **24**).

SAA21 derivative showed high cytotoxicity (EC_{50} = 1.2 μM, A2780) but also high selectivity (S = [EC_{50} (A2780)]/[EC_{50} (NIH 3T3)] = > 50) [27].

In the past, many derivatives obtained from betulinic acid proved to be more cytotoxic than those derived from 2,3-*O*-diacylated triterpene carboxylic acids [7,8]. To get a better insight into structure/activity relationships, we decided to synthesize derivatives holding a betulinic acid (or derived from it, platanic acid) backbone but with an extra hydroxyl group at C-2. We also wanted to investigate whether the influence of the orientation of the quinolinyl substituent, as already observed for augustic acid derivatives, was also reflected in these derivatives.

2. Results and discussion

2.1. Chemistry

The synthesis of the compounds was straightforward. Silica gel supported Jones oxidation of betulinic acid (**1**) gave betulonic acid (**2**) in 76% isolated yield. Its treatment with ^{t}BuOK/air in ^{t}BuOH/THF gave **3** whose reduction with $NaBH_4$ furnished (2β, 3β) configurated **4**. Compound **4** was acetylated to yield diacetylated **5**. Compound **5** served as a valuable starting material for the synthesis of the amides. Thereby **5** was activated with oxalyl chloride followed by the addition of either benzylamine, 4-aminoisoquinoline or 5-

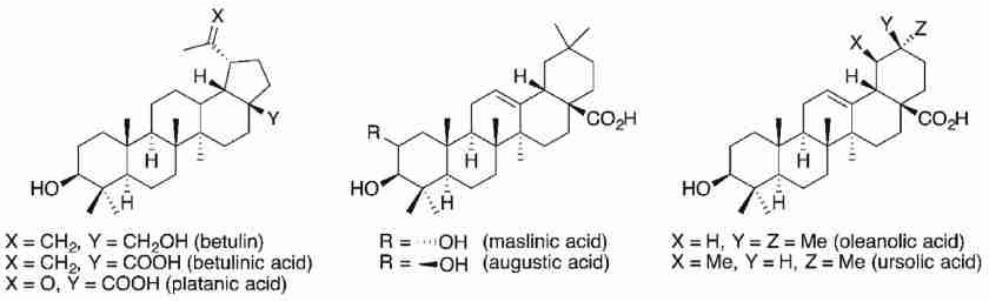


Fig. 1. Structure of betulin, betulinic acid, platanic acid, maslinic acid, augustic acid, oleanolic acid and ursolic acid.

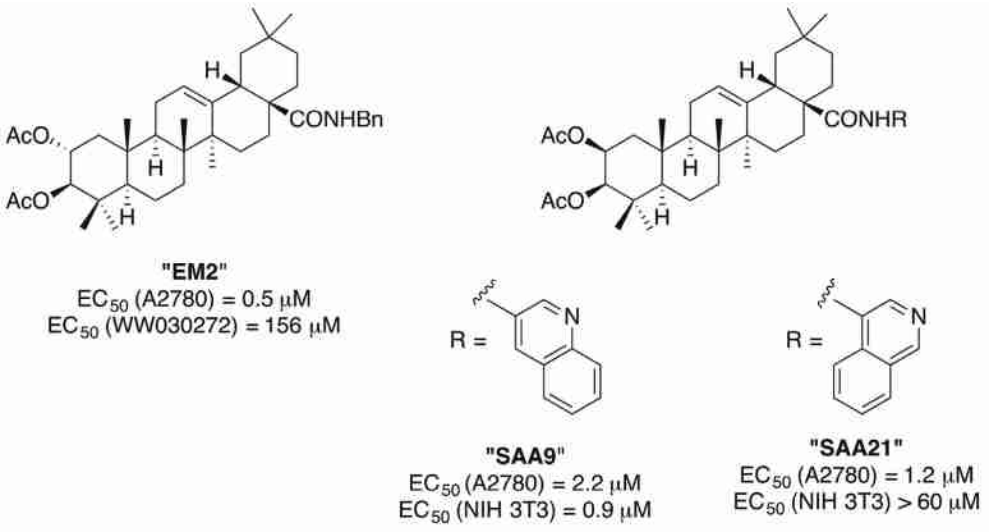


Fig. 2. Structure (and representative EC_{50} values) for maslinic acid derived **EM2** and augustic acid derived compounds **SAA9** and **SAA21**.

aminoquinoline to yield amides **6**–**8**, respectively. In a similar way, platanic acid (**9**) was oxidized, and 2-oxo compound **10** was obtained, whose oxidation (→ **11**), reduction (→**12**) followed by acetylation gave **13**. As described above, from **13** amides **14**–**16** were prepared.

For comparison and to have an insight onto the influence of the extra hydroxyl group in these molecules (as compared to the starting materials), **1** and **9** were acetylated, and the acetates **17** and **21** were obtained. Reaction with oxalyl chloride followed by the addition of either benzylamine, 4-aminoisoquinoline or 5-aminoquinoline furnished amides **18**–**20** and **22**–**24**, respectively.

2.2. Biology

The compounds were screened for their cytotoxic activity using photometric sulforhodamine assays (SRB) [28–30], the results of which are compiled in Table 1 and Fig. 3.

The results from the SRB assays revealed for many compounds cytotoxic activity. While the parent compounds **1** and **9** were not cytotoxic within the borders of the assays (cut-off 30 µM), betulonic acid (**2**) was more cytotoxic than parent **1**. Low cytotoxicity was also observed for **3**–**5**, **9**–**13** and compound **21**. Cytotoxicity was significantly increased as soon as an amide was formed irrespective whether the terpenoid skeleton was derived from **1** or **9**. For all amides **6**–**8**, **14**,**15**, **18**–**18**, **23**, and **24** EC_{50} values in the low µM range were found with betulinic acid derived **19** (a 4-isoquinolinyl derivative) being the most cytotoxic compound of this series. These results parallel previous findings for augustic acid derived **SAA21**, and is also found for analogs **8**, **14** and **24**.

Compound **19**, the most cytotoxic compound of this study also held the highest tumor/non-tumor cell selectivity, especially for A375 melanoma cells (S = 91.2, Table 2 and Fig. 4), A2780 ovarian cancer cells (S = 61.6) and the cell line FaDu (hypopharyngeal carcinoma, S = 59.0). These results seem to indicate that the amide substituent is more important than the substitution pattern at ring E.

For compounds **6**, **8** and **19**, further investigations were carried out to get a deeper insight into their mode of being cytotoxic; thereby, A375 melanoma cell were used. After having treated the cells with the compounds for 1 day, an Annexin V assay (Fig. 5) showed that fewer cells were vital as compared to the control.

Having been treated for 2 days (Fig. 6), an even more pronounced effect was observed. The number of apoptotic cells was increased (circa 44). Approximately 20% of the cells were late-apoptotic, but only 1.5% showed a necrotic cell death. As far as **8** is concerned, about two thirds of the cells were still vital (67.4%). The number of apoptotic cells, however, was significantly increased (18.9%) as compared to necrotic (1.3%) or late apoptotic/secondary necrotic (12.4%) cells. In summary, these compounds mainly act by apoptosis. Thereby, **6** showed only a slight increase of cells being necrotic and late apoptotic and secondary necrotic range (0.7 and 7.9%, respectively), while the largest increase was observed for

Table 1
Cytotoxicity of selected compounds; SRB assay EC_{50} values [μM] after 72 h of treatment; averaged from three independent experiments performed each in triplicate; confidence interval CI = 95%. Human cancer cell lines: A375 (melanoma), HT29 (colorectal carcinoma), MCF-7 (breast adenocarcinoma), A2780 (ovarian carcinoma), FaDu (hypopharyngeal carcinoma), NIH 3T3 (non-malignant fibroblasts); n.s. not soluble under the conditions of the assay; n.d. not determined. Doxorubicin (**DR**) was used as a positive control.

	A375	HT29	MCF7	A2780	FaDu	NIH 3T3
1	>30	>30	>30	>30	>30	>30
2	14.3 ± 1.7	18.5 ± 1.9	12.0 ± 1.5	4.7 ± 0.6	17.4 ± 2.1	19.4 ± 1.2
3	13.76 ± 1.4	26.07 ± 2.8	11.96 ± 1.1	10.49 ± 1.2	14.05 ± 1.1	12.85 ± 0.9
4	11.2 ± 1.8	17.68 ± 2.2	15.8 ± 2.1	12.28 ± 2.2	16.54 ± 2.5	17.28 ± 2.3
5	15.81 ± 1.0	17.40 ± 1.2	11.25 ± 0.8	8.68 ± 1.2	13.5 ± 0.9	14.11 ± 0.9
6	3.14 ± 0.6	20.72 ± 6.1	11.3 ± 2.1	4.42 ± 1.0	5.4 ± 1.0	>70
7	3.34 ± 0.2	>15	7.33 ± 1.0	4.76 ± 0.6	4.02 ± 0.3	>30
8	2.9 ± 0.1	>30	6.26 ± 0.8	3.24 ± 0.5	3.49 ± 0.4	>120
9	>30	>30	>30	>30	>30	>30
10	>30	>30	>30	>30	>30	>30
11	>30	>30	24.8 ± 1.7	20.6 ± 1.7	>30	>30
12	>30	>30	>30	>30	>30	>30
13	22.54 ± 5.7	>30	>30	>30	>30	9.57 ± 3.5
14	2.9 ± 0.5	7.22 ± 4.3	5.2 ± 2.1	2.36 ± 1.6	5.57 ± 4.4	>30
15	4.88 ± 0.6	18.43 ± 4.9	6.96 ± 2.0	6.15 ± 0.8	6.41 ± 0.8	>30
16	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
17	19.2 ± 1.7	21.3 ± 2.0	11.0 ± 0.5	18.3 ± 0.5	7.2 ± 1.2	>30
18	4.05 ± 0.2	>30	26.8 ± 6.8	6.29 ± 0.8	9.59 ± 0.9	>30
19	1.48 ± 0.1	>30	4.65 ± 0.5	2.16 ± 0.2	2.26 ± 0.2	>135
20	3.05 ± 0.1	>30	12.12 ± 1.8	5.08 ± 0.3	5.18 ± 0.3	>30
21	>30	>30	>30	>30	>30	>30
22	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
23	5.66 ± 0.4	>30	9.34 ± 1.3	7.84 ± 0.6	7.67 ± 0.2	>30
24	4.60 ± 0.3	>30	7.06 ± 1.5	5.4 ± 0.9	6.42 ± 0.5	>30
DR	n.d.	>30	1.1 ± 0.3	0.01 ± 0.01	n.d.	1.3 ± 0.6

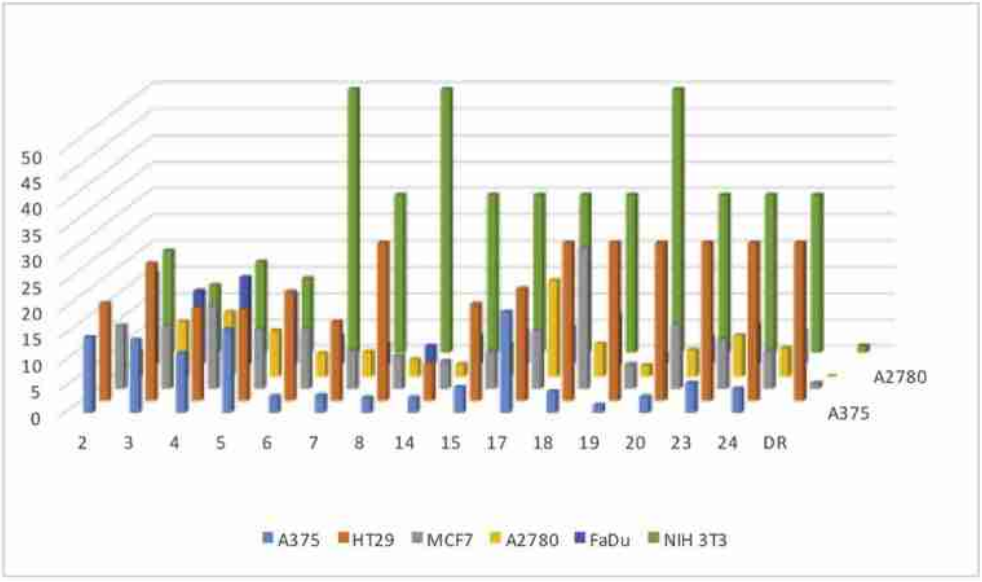


Fig. 3. Cytotoxicity of selected compounds; SRB assay EC_{50} values [μM] after 72 h of treatment.

apoptotic cells (9.5%). Compound **8** also gave an increased number of apoptotic cells, but here more were found being in the late phase. A similar behavior was observed upon treatment of the cells with **19** (8.6% apoptosis and 9.6%% late apoptosis).

Visual inspection (Fig. 7) of the cells supported the findings from the FACS investigations (vide infra). Thus, after a 24 h incubation, cell division had already been reduced/stopped. The number of apoptotic cells was slightly increased resulting in a small number of cells showing morphological differences/characteristics.

After an incubation for 48h, however, all compounds caused dramatic changes in the number and morphology of the cells due to apoptosis. For example, the cells were shrunken, strangulation occurred and also blebbing was observed.

Changes in the cell cycle (Fig. 8 and Fig. 9) were visible after 24 h of incubation. Compound **6** no longer showed a G2/M peak (0.0%), and the number of cells being in the S phase was significantly (30.3%), while more cells were in the G1 phase (63.8%). In addition, approximately 6% of the cells were in the SubG1 phase. For compound **8**, an increase of cells in the subG1 phase could be noted (3.5%). Compound **19** showed a similar behavior as **6**. Hereby, 61.1% of the cells were in G1 and 36.6% in S phase. Visual inspection of the cells supported the findings from the FACS investigations (vide

Table 2
Selectivity S = EC_{50} (NIH 3T3)/EC_{50} (tumor cell line) for the individual compounds.

	A375	HT29	MCF7	A2780	FaDu
3	0.9	0.5	1.1	1.2	0.9
4	1.5	1.0	1.1	1.4	1.0
5	0.9	0.8	1.3	1.6	1.0
6	22.3	3.4	6.2	15.8	13.0
7	9.0	2.0	4.1	6.3	7.5
8	41.4	4.0	19.2	37.0	34.4
13	0.4	0.3	0.3	0.3	0.3
14	10.3	4.2	5.8	12.7	5.4
15	6.1	1.6	4.3	4.9	4.7
17	1.6	1.4	2.7	1.6	4.2
18	7.4	1.0	1.1	4.8	3.1
19	91.2	4.5	29.0	62.5	59.7
20	9.8	1.0	2.5	5.9	5.8
23	5.3	1.0	3.2	3.8	3.9
24	6.5	1.0	4.3	5.6	4.7

infra). Thus, after a 24 h incubation, cell division had already been reduced/stopped. The number of apoptotic cells was slightly increased resulting in a small number of cells showing morphological differences/characteristics.

3. Conclusion

A small series of amides derived from betulinic acid (**1**), platanic acid (**9**) and (2β, 3β)-dihydroxy-20(29)-en-28-oic acid (**4**) as well as (2β, 3β)-20-oxo-30-norlupan-28-28-oic acid (**12**) was prepared and screened for their cytotoxic activity. All of the compounds were shown to be cytotoxic for a panel of human tumor cell lines. Especially betulinic acid derived compounds **6**, **8** and **19** held excellent cytotoxicity. They act mainly by apoptosis. Compound **19**, a 4-isoquinolinyl amide of 3-*O*-acetyl-betulinic acid, was the most cytotoxic compound holding EC_{50} values as low as EC_{50} = 1.48 μM (A375 melanoma cells) while being significantly less cytotoxic for non-malignant fibroblasts NIH 3T3 with a selectivity index of >91.2. This finding parallels previous results obtained for **SAA21**, an augustic acid derived compound.

4. Experimental

NMR spectra were recorded using the Varian spectrometers Gemini 2000 or Unity 500 (δ given in ppm, J in Hz; typical experiments: H—H—COSY, HMBC, HSQC, NOESY), MS spectra were taken on a Finnigan MAT LCQ 7000 (electrospray, voltage 4.1 kV, sheath

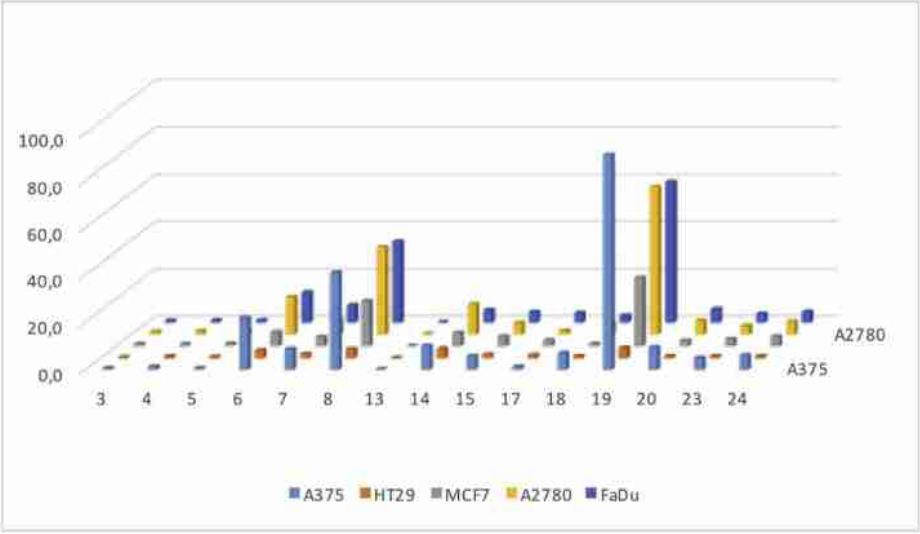
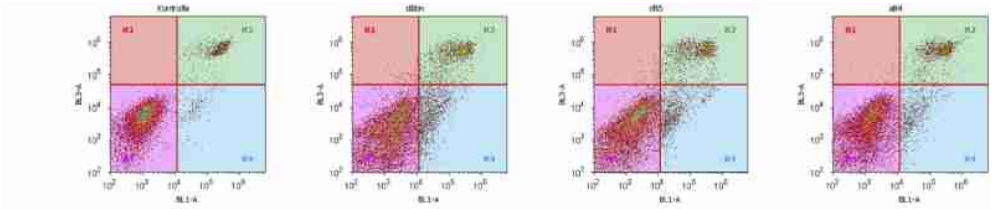
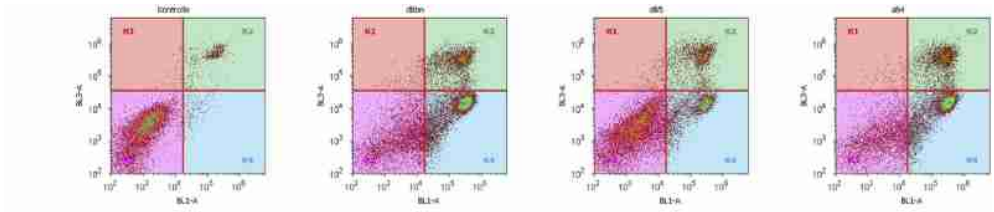


Fig. 4. Selectivity S = EC_{50} (NIH 3T3)/EC_{50} (tumor cell line) of selected compounds.



	control	compound **6**	compound **8**	Compound **19**
R1	0.331	0.667	0.749	0.351
R2	7.352	7.930	10.295	9.649
R3	90.123	81.857	80.405	83.505
R4	2.194	9.546	6.495	8.551

Fig. 5. Annexin V-FITC/PI assay: treatment of A375 cells with **6**, **8** and **19** (6.2 μM/4.8 μM/3.0 μM) for 24 h. Examples of density plots determined by flow cytometry (Attune® Cytometric Software v. 1.2.5). R1: necrotic, R2: secondary necrotic/late stage apoptotic, R3: vital, R4: apoptotic.



	control	compound **6**	compound **8**	Compound **19**
R1	0.547	1.449	1.342	1.115
R2	5.108	20.078	12.388	21.382
R3	92.723	34.287	67.409	33.194
R4	1.622	44.187	18.861	44.309

Fig. 6. Annexin V-FITC/PI assay: treatment of A375 cells with **6**, **8** and **19** (6.2 μM/4.8 μM/3.0 μM) for 48 h. Examples of density plots determined by flow cytometry (Attune® Cytometric Software v. 1.2.5). R1: necrotic, R2: secondary necrotic/late stage apoptotic.

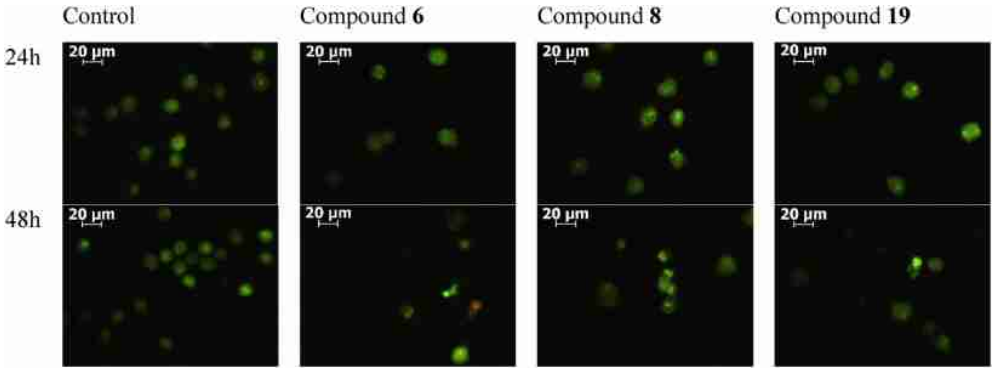
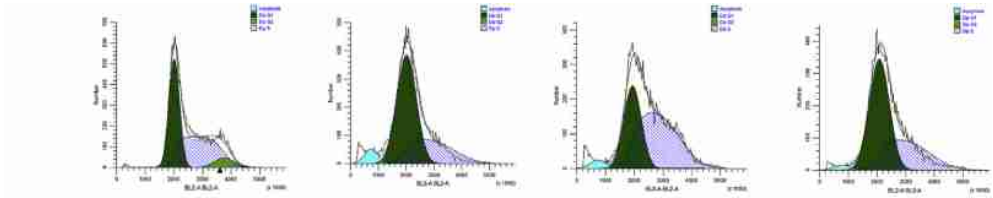


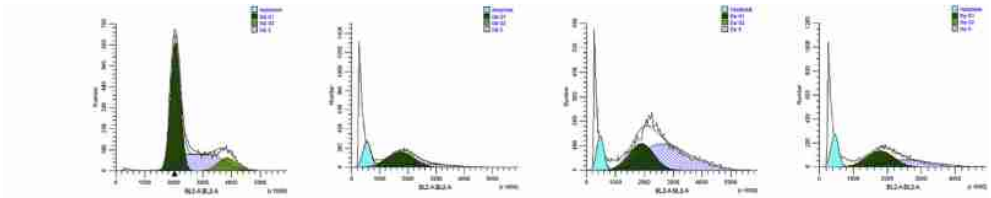
Fig. 7. Microscopic evaluation of the A375 cells treated with compounds **6**, **8**, **19**.



	control	compound **6**	compound **8**	Compound **19**
subG1	0.46%	5.98%	3.53%	2.38%
G1	44.59%	63.76%	37.51%	61.06%
S	47.46%	30.27%	58.53%	36.56%
G2/M	7.48%	0.00%	0.43%	0.00%

Fig. 8. Cell Cycle investigation. Examples for histogram determined by flow cytometry after 24 h, green G1 G2/M, striped S phase, blue subG1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

gas nitrogen) instrument. The optical rotations were measured either on a JASCO P-2000 or a Perkin-Elmer polarimeter at 20 °C; TLC was performed on silica gel (Merck 5554, detection with cerium molybdate reagent); melting points are uncorrected (*Leica* hot stage microscope), and elemental analyses were performed on a Foss-Heraeus Vario EL (CHNS) unit. IR spectra were recorded on a Perkin Elmer FT-IR spectrometer Spectrum 1000 or a Perkin-Elmer Spectrum Two (UATR Two Unit). UV-VIS spectra were taken on a Perkin-Elmer Lambda 14 spectrometer or on a Perkin-Elmer Lambda 750 S (UV/VIS/NIR) spectrometer. The solvents were dried according to usual procedures. The purity of the compounds was determined by HPLC and found to be >96%. Column chromatography was performed on a Büchi Reveleris Prep purification system using Chromabond Flash cartridges (SiOH, 40.63 μm) from Macherey-Nagel or Reveleris high resolution cartridges from Büchi. Betulinic acid and platanic acid were obtained from Betulinines (Stříbrná Skalice, Czech Republic) in bulk quantities and used as received. A description of the biological screening, additional



	control	compound **6**	compound **8**	Compound **19**
subG1	0.57%	25.69%	11.49%	23.32%
G1	57.30%	52.38%	31.39%	41.85%
S	31.30%	21.92%	57.06%	34.84%
G2/M	10.83%	0.00%	0.00%	0.00%

Fig. 9. Cell Cycle investigation. Examples for histogram determined by flow cytometry after 48 h, green G1 G2/M, striped S phase, blue subG1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

spectroscopic data and depictions of the ^{1}H and ^{13}C NMR spectra are provided in the supplementary materials file.

4.1. General procedure for acetylation (GP A)

One equivalent of each triterpene carboxylic acid (compounds **1**, **4**, **9**, **12**) was dissolved in dry dichloromethane (DCM, 100 mL), and acetic anhydride (3 eq. per hydroxyl group), triethylamine (3 eq.) and catalytic amounts of DMAP were added. The reaction mixture was stirred at 23 °C for 1 day. A methanolic solution of ammonia (satd., 5 mL) was added, and the reaction mixture was stirred for another 30 min at 23 °C. For workup, aq. hydrochloric acid (10%, 20 mL) was added, the aqueous phase was extracted with DCM (3 x 10 mL), the combined organic phases were washed with brine (20 mL) and dried (Na_2SO_4). The volatiles were removed under diminished pressure, and the crude product (**5**, **13**, **17**, **2**1) was purified by column chromatography.

4.2. General procedure for the synthesis of benzyl amides (GP B)

The corresponding acetylated compound (**5**, **13**, **17** and **21**) was dissolved in dry DCM (10 mL) and oxalyl chloride (4 eq.) and catalytic quantities of DMF were added. The reaction mixture was stirred at 23 °C for 1 h. The volatiles were removed under diminished pressure, the residue was dissolved in dry DCM (10 mL), and the corresponding amine (3 eq.) was added. The reaction mixture was stirred at 23 °C for 1 h. Usual aqueous workup followed column chromatography gave compounds **6**, **14**, **18** and **22**, respectively.

4.3. General procedure for the synthesis of quinolinyl amides (GP C)

The corresponding acetylated compound (**5**, **13**, **17** and **21**) was dissolved in dry DCM (10 mL), and activated with oxalyl chloride (4 eq.) and catalytic quantities of DMF as described above. The volatiles were removed under reduced pressure, the residue was dissolved in dry DCM (4 mL), and dry pyridine (8 mL), and the respective amine (3 eq.) and catalytic quantities of DMAP and triethylamine were added. The reaction mixture was stirred with the help of microwave irradiation (Anton Parr, Graz/Austria, Monowave) at 120 °C for 2 h. Usual aqueous workup followed by column chromatographic purification gave compounds **7**, **8**, **15**, **16**, **19**, **20**, **23** and **24**, respectively.

4.4. Syntheses

4.4.1. 3-Oxolup-20(29)-en-28-oic acid (betulonic acid, 2)

Oxidation of betulinic acid (**1**, 10.0 g, 21.9 mmol) with silica gel supported Jones reagent [31] [from acetone (500 mL), CrO_3 (2.51 g, 25.1 mmol), H_2SO_4 (2.5 mL), H_2O (10 mL), silica gel (100 mL)] at 0 °C for 1 h followed by quenching the reaction by the addition of MeOH (5 mL), removal of the solvents and Soxhlet-extraction of the residue with diethyl ether (600 mL, 3.5 h) and column chromatography (SiO_2, hexanes/EtOAc, EtOAc: 3% → 15%) gave **2** (6.23 g, 76%) as a colorless solid; m.p. 246–248 °C (lit.: [32] 245–247 °C); $[\alpha]_D$ = +37.4° (*c* 0.283, $CHCl_3$) [lit.: [33] $[\alpha]_D$ = +33.8° (*c* 0.33 $CHCl_3$)]; R_F = 0.42 (SiO_2, hexanes/EtOAc, 7:1).

4.4.2. 2-Hydroxy-3-oxolupa-1,20(29)-dien-28-oic acid (3) [173106-22-4]

To a solution of **2** (8.55 g, 18.8 mmol) in ^{t}BuOH (500 mL) and THF (70 mL), ^{t}BuOK (30.0 g, 0.267 mol) [34] was added, and the mixture was stirred for 3 h at 50 °C while air was bubbled through. The solvents were removed under diminished pressure, and the residue was dissolved in EtOAc (100 mL). Usual aqueous workup followed by column chromatography (SiO_2, hexanes/EtOAc, EtOAc: 5% → 20%) gave **3** (7.41 g, 97%) as a colorless solid; m.p. 204–205 °C (lit.: [35] 204–205 °C); $[\alpha]_D$ = +40.5° (*c* 0.265, $CHCl_3$) [lit.: [35] $[\alpha]_D$ = +13° (*c* 0.75, $CHCl_3$)]; R_F = 0.68 (SiO_2, hexanes/EtOAc, 3:1).

4.4.3. 2β,3β-Dihydroxylup-20(29)-en-28-oic acid (4)

To a solution of **3** (8.525 g, 18.2 mmol) in THF (200 mL) and MeOH (40 mL), $NaBH_4$ (1.0 g, 26.4 mmol) [31] was added, and the mixture was stirred for 2 days at 23 °C. Usual aqueous workup followed by column chromatography (SiO_2, hexanes/EtOAc, EtOAc: 10% → 30%) gave **4** (4.03 g, 55%) as a colorless solid; m.p. 270–273 °C (decomp.) (lit.: [36] 273–276 °C); $[\alpha]_D$ = +32.4° (c 0.21, $CHCl_3$) [lit.: [36] $[\alpha]_D$ = +31.1 (*c* 0.262, pyridine)]; R_F = 0.54 (SiO_2, hexanes/EtOAc, 1:1).

4.4.4. 2β,3β-Diacetyloxy-lup-20(29)-en-28-oic acid (5)

Acetylation of **4** (2.38 g, 5.0 mmol) according to GPA followed by chromatography (SiO_2, hexanes/EtOAc, EtOAc: 3% → 15%) gave **5** (1.97 g, 70%) as a colorless solid; m.p. 260–265 °C (decomp.) (lit.: [36] 246–248 °C); $[\alpha]_D$ = +34.6° (*c* 0.199, $CHCl_3$); R_F = 0.63 (SiO_2, hexanes/EtOAc, 7:1); IR (ATR): ν = 2944*m*, 1742*s*, 1366*m*, 1230*s*, 1189*m*, 1031 m cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.32 (*m*, 1H, 2-H), 4.74 (*s*, 1H, 29-H_a), 4.66–4.56 (*m*, 2H, 3-H, 29-H_b), 3.00 (*m*, 1H, 19-H), 2.32–2.09 (*m*, 2H, 16-H_a, 13-H), 2.03 (*s*, 3H, 34-H), 2.02 (*s*, 3H, 32-H), 2.07–1.92 (*m*, 1H, 1-H_a), 1.96 (*m*, 2H, 21-H_a, 22-H_a), 1.72 (*d*,

J = 1.6 Hz, 2H, 12-H_a, 6-H_a), 1.69 (*s*, 3H, 30-H), 1.65−1.14 (*m*, 13H, 18-H, 6-H_b, 22-H_b, 7-H_b, 16-H_b, 21-H_b, 15-H_a, 11-H, 9-H, 1-H_b, 15-H_b), 1.11 (*s*, 3H, 26-H), 1.02 (*s*, 3H, 25-H), 0.99 (*m*, 1H, 12-H_b), 0.97 (*s*, 3H, 27-H), 0.96 (*s*, 3H, 24-H), 0.92 (*m*, 1H, 5-H), 0.88 (*s*, 3H, 23-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 181.38 (C-28), 170.90 (C-33), 170.41 (C-31), 150.39 (C-20), 109.97 (C-29), 78.11 (C-3), 69.79 (C-2), 56.49 (C-17), 55.39 (C-5), 51.09 (C-9), 49.41 (C-18), 47.08 (C-19), 42.76 (C-1), 42.35 (C-14), 40.97 (C-8), 38.53 (C-13), 37.56 (C-4), 37.20 (C-10), 37.16 (C-22), 34.31 (C-7), 32.32 (C-16), 30.72 (C-21), 29.71 (C-15), 29.14 (C-23), 25.54 (C-12), 21.41 (C-34), 21.14 (C-11), 21.02 (C-32), 19.51 (C-30), 18.15 (C-6), 17.64 (C-25), 16.89 (C-26), 16.35 (C-24), 14.76 (C-27) ppm; MS (ESI, MeOH): *m/z* 437 (8%, $[M + H\text{-}2HOAc]^+$), 497 (10%, $[M + H\text{-}HOAc]^+$), 1135 (100%, $[2M + Na]^+$); analysis calcd for $C_{34}H_{52}O_6$ (556.77): C 73.34, H 9.41; found: C 73.11, H 9.63.

4.4.5. Benzyl 2β,3β-Diacetyloxy-lup-20(29)-en-28-amide (6)

Reaction of **5** (250 mg, 0.45 mmol) with benzyl amine (0.3 mL, 2.8 mmol) according to GPB followed by chromatography (SiO_2, EtOAc/hexanes, EtOAc 8% → 20%) gave **6** (180 mg, 62%) as a colorless solid; m.p. 138−141 °C; $[\alpha]_D$ = +39.0° (*c* 0.222, $CHCl_3$); R_F = 0.64 (SiO_2, toluene/EtOAc/heptane/HCOOH, 80:26:10:5); IR (ATR): ν = 2943*w*, 1741*s*, 1620*w*, 1365*m*, 1231*s*, 1188*m*, 1029*m*, 698 m cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38−7.24 (*m*, 5H, 37-H, 38-H, 39-H, 40-H, 41-H), 5.88 (*t*, J = 5.7 Hz, 1H, NH) 5.36−5.26 (*m*, 1H, 2-H_a), 4.74 (*d*, J = 2.0 Hz, 1H, 29-H_a), 4.60 (*m*, 2H, 29-H_b, 3-H), 4.48 (*dd*, J = 15.1, 5.8 Hz, 1H, 35-H_a), 4.37 (*dd*, J = 14.6, 5.6 Hz, 1H, 35-H_b), 3.16 (*td*, J = 11.0, 4.1 Hz, 1H, 19-H), 2.55−2.44 (*td*, 1H, 13-H), 2.03 (*s*, 3H, 32-H), 2.01 (*s*, 3H, 34-H), 2.06−1.87 (*m*, 3H, 1-H, 16-H_a, 21-H_a), 1.68 (*s*, 3H, 30-H), 1.81−1.21 (*m*, 16H, 22-H_a, 12-H, 16-H_b, 18-H, 6-H, 22-H_b, 21-H_b, 7-H, 11-H, 1-H_b, 9-H), 1.12 (*s*, 1H, 15-H), 1.10 (*s*, 3H, 26-H), 1.02 (*s*, 3H, 25-H), 0.95 (*s*, 3H, 27-H), 0.93 (*s*, 3H, 24-H), 0.90 (*m*, 1H, 5-H), 0.88 (*s*, 3H, 23-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 176.01 (C-28), 170.88 (C-33), 170.40 (C-31), 150.97 (C-20), 139.32 (C-36), 128.80 (C-38, C-40), 127.93 (C-37, C-41), 127.47 (C-39), 109.61 (C-29), 78.13 (C-3), 69.76 (C-2), 55.78 (C-17), 55.42 (C-5), 51.27 (C-9), 50.32 (C-18), 46.83 (C-19), 43.94 (C-8), 43.44 (C-35), 42.82 (C-4), 42.38 (C-1), 41.05 (C-10), 38.56 (C-22), 37.77 (C-13), 34.43 (C-7), 33.94 (C-16), 31.02 (C-21), 29.46 (C-15), 29.11 (C-23), 25.72 (C-12), 21.41 (C-32), 21.25 (C-11), 21.02 (C-34), 19.63 (C-30), 18.18 (C-6), 17.67 (C-25), 16.92 (C-26), 16.42 (C-24), 14.69 (C-27) ppm; MS (ESI, MeOH): *m/z* 646 (100%, $[M+H]^+$), 668 (76%, $[M+Na]^+$), 1291 (52%, $[2M + H]^+$), 1313 (84%, $[2M + Na]^+$); analysis calcd for $C_{41}H_{59}NO_4$ (645.93): C 76.24, H 9.21, N 2.17; found: C 76.02, H 9.37, N 2.30.

4.4.6. Isoquinolin-4-yl 2β,3β-diacetyloxy-lup-20(29)-en-28-amide (7)

According to GPC from **5** (150 mg, 0.27 mmol) and 4-aminoisoquinoline (150 mg, 1.0 mmol) followed by chromatography (SiO_2, hexanes/EtOAc, EtOAc: 20% → 55%) **7** (76 mg, 42%) was obtained as an off-white solid; m.p. 186−190 °C (decomp.); $[\alpha]_D$ = −3.2° (*c* 0.216, $CHCl_3$); R_F = 0.56 (SiO_2, $CHCl_3$/MeOH, 95:5); UV−Vis ($CHCl_3$): λ_{max} (log ε) = 282 nm (4.20), 323 nm (4.24); IR (ATR): ν = 2942*m*, 1741*s*, 1367*m*, 1251*s*, 1187*m*, 1030*m*, 577*w* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.12 (*s*, 1H, 38-H), 8.75 (*s*, 1H, 36-H), 8.01 (*d*, J = 8.2 Hz, 1H, 40-H), 7.83−7.70 (*m*, 2H, 42-H, 41-H), 7.68−7.59 (*m*, 1H, 43-H), 5.32 (*td*, 1H, 2-H), 4.75 (*s*, 1H, 29-H_a), 4.66−4.56 (*m*, 2H, 29-H_b, 3-H), 3.19 (*s*, 1H, 19-H), 2.59 (*td*, 1H, 13-H), 2.18−1.92 (*m*, 3H, 1-H_a, 12-H_a, 21-H_a), 2.02 (*s*, 3H, 32-H), 2.02 (*s*, 3H, 34-H), 1.72 (*s*, 3H, 30-H), 1.91−1.19 (*m*, 17H, 12-H_b, 18-H, 1-H_b, 21-H_b, 22-H, 16-H, 7-H, 11-H, 15-H, 9-H, 17-H), 1.10 (*s*, 3H, 26-H), 1.03 (*s*, 3H, 27-H), 1.02 (*m*, 6H, 24-H, 25-H), 0.98−0.91 (*m*, 1H, 5-H), 0.88 (*s*, 3H, 23-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 175.20 (C-28), 170.68 (C-33), 170.19 (C-31), 150.33 (C-20), 149.89 (C-38), 138.61 (C-36), 131.07 (C-35), 130.56 (C-41), 128.75 (C-39), 128.11 (C-40), 127.31 (C-43), 124.76 (C-44), 120.64 (C-42), 109.65 (C-39), 77.88 (C-3), 69.49 (C-2), 56.68 (C-17), 55.57 (C-5), 51.05 (C-9), 50.19 (C-18), 46.43 (C-19), 42.71 (C-8), 42.17 (C-4), 40.90 (C-10), 38.44 (C-1), 37.58 (C-13), 37.32 (C-22), 36.94 (C-14), 34.22 (C-7), 34.08 (C-16), 30.82 (C-21), 29.61 (C-15), 28.88 (C-23), 25.49 (C-12), 21.17 (C-32), 21.02 (C-11), 20.79 (C-34), 19.45 (C-30), 17.92 (C-6), 17.43 (C-25), 16.69 (C-26), 16.28 (C-24), 14.54 (C-27) ppm; MS (ESI, MeOH): *m/z* 683 (92%, $[M+H]^+$), 705 (44%, $[M+Na]^+$), 1365 (100%, $[2M + H]^+$), 1388 (36%, $[2M + Na]^+$); analysis calcd for $C_{43}H_{58}N_2O_5$ (682.95): C 75.62, H 8.56, N 4.10; found: C 75.48, H 8.76, N 4.24.

4.4.7. Quinolin-5-yl 2β,3β-diacetyloxy-lup-20(29)-en-28-amide (8)

According to GPC from **5** (250 mg, 0.45 mmol) and 5-aminoquinoline (250 mg, 1.7 mmol) followed by chromatography (SiO_2, hexanes/EtOAc, EtOAc: 20% → 55%) **8** (130 mg, 42%) was obtained as an off-white solid; m.p. 249−252 °C (decomp.); $[\alpha]_D$ = +2.9° (*c* 0.162, $CHCl_3$); R_F = 0.53 (SiO_2, $CHCl_3$/MeOH, 95:5); UV−Vis ($CHCl_3$): λ_{max} (log ε) = 305 nm (4.47), 317 nm (4.45); IR (ATR): ν = 2942*m*, 1741*s*, 1474*m*, 1366*m*, 1232*s*, 1187*m*, 1030*m*, 798*m*, 730 m cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.84 (*d*, J = 3.1 Hz, 1H, 41-H), 8.29 (*d*, J = 8.4 Hz, 1H, 36-H), 8.04−7.94 (*m*, 1H, 37-H), 7.77−7.66 (*m*, 2H, 38-H, 43-H), 7.46 (*dd*, J = 8.6, 4.4 Hz, 1H, 42-H), 5.31 (*m*, 1H, 2-H), 4.74 (*s*, 1H, 29-H_a), 4.65−4.58 (*m*, 2H, 29-H_b, 3-H), 3.16 (*td*, 1H, 19-H), 2.58 (*td*, 1H, 13-H), 2.32 (*m*, 1H, 16-H_a), 2.02 (*s*, 3H, 32-H), 2.02 (*s*, 3H, 34-H), 2.20−1.95 (*m*, 3H, 1-H_a, 21-Ha, 22-H_a), 1.72 (*s*, 3H, 30-H), 1.88−1.21 (*m*, 16H, 16-H_b, 12-H, 15-H_a, 22-H_b, 18-H, 6-H, 21-H_b, 7-H, 11-H, 15-H_b, 9-H, 1-H_b), 1.10 (*s*, 3H, 26-H), 1.03 (*s*, 3H, 27-H), 1.01 (*m*, 6H, 24-H, 25-H), 0.93 (*m*, 1H, 5-H), 0.88 (*s*, 3H, 23-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 175.66 (C-28), 170.91 (C-33), 170.41 (C-31), 150.54 (C-20), 148.81 (C-41), 148.39 (C-39), 133.54 (C-44), 132.84 (C-36), 130.38 (C-43), 125.85 (C-37), 124.09 (C-35), 123.17 (C-38), 120.89 (C-42), 109.92 (C-29), 78.10 (C-3), 69.71 (C-2), 56.92 (C-17), 55.44 (C-5), 51.27 (C-9), 50.43 (C-18), 46.68 (C-19), 42.93 (C-8), 42.40 (C-1), 41.14 (C-10), 38.62 (C-22), 37.83 (C-13), 37.55 (C-14), 37.17 (C-4), 34.46 (C-7), 34.15 (C-16), 31.05 (C-21), 29.86 (C-15), 29.10 (C-23), 25.71 (C-12), 21.40 (C-32), 21.27 (C-11), 21.02 (C-34), 19.66 (C-30), 18.15 (C-6), 17.66 (C-25), 16.92 (C-26), 16.54 (C-24), 14.78 (C-27) ppm; MS (ESI, MeOH): *m/z* 683 (100%, $[M+H]^+$), 705 (22%, $[M+Na]^+$), 1365 (44%, $[2M + H]^+$), 1387 (58%, $[2M + Na]^+$); analysis calcd for $C_{43}H_{58}N_2O_5$ (682.95): C 75.62, H 8.56, N 4.10; found: C 75.41, H 8.74, N 4.29.

4.4.8. 3,20-Dioxo-30-norlupan-28-oic acid (10)

Silica gel assisted Jones oxidation [31] of platanic acid (**9**, 5.0 g, 10.9 mmol) followed by column chromatography (SiO_2, hexanes/EtOAc, EtOAc: 10% → 30%) gave **10** (4.90 g, 98%) as a colorless solid; m.p. 230 °C (decomp.) (lit.: [37] 232−233 °C); $[\alpha]_D$ = +4.2° (*c* 0.308, $CHCl_3$) (lit.: [37] $[\alpha]_D$ = −11° (*c* 1.0, C_6H_6)]; R_F = 0.64 (SiO_2, hexanes/EtOAc, 1:1).

4.4.9. 2-Hydroxy-3,20-dioxo-30-norlupan-1-en −28-oic acid (11)

Following the procedure [34] given for the synthesis of **3**, from **10** (4.846 g, 10.6 mmol) **11** (4.30 g, 85%) was obtained and directly used in the next step; an analytical sample showed m.p. 225−228 °C (lit.: [29] 224−228 °C); $[\alpha]_D$ = +9.2° (*c* 0.71, MeOH) [lit.: [29] $[\alpha]_D$ = +9.0° (*c* 0.66, MeOH); MS (ESI, MeOH): *m/z* 472 (14%, $[M+H]^+$), 493 (100%, $[M+Na]^+$), 509 (32%, $[M+K]^+$).

4.4.10. 2β,3β-dihydroxy-20-oxo-30-norlupan-28-oic acid (12)

To a solution of **11** (4.9 g, 10.4 mmol) in THF (100 mL) and MeOH (20 mL), $NaBH_4$ (0.5 g, 13.2 mmol) [31] was added in several portions, and the mixture was stirred for 1 d at 23 °C. Usual aqueous work-up followed by chromatography (SiO_2, $CHCl_3$/acetone, acetone: 5% → 20%) gave **12** (3.7 g, 74%) as a colorless solid; m.p. 265−269 °C (lit.: [29] 266−270 °C) $[\alpha]_D$ = −11.2° (*c* 0.130, $CHCl_3$)

(lit.: $[\alpha]_D = -6.2°$ (*c* 0.215, $CHCl_3$)]; $R_F = 0.31$ (SiO_2, hexanes/EtOAc, 1:1).

4.4.11. 2β,3β-Diacetyloxy-20-oxo-30-norlupan-28-oic acid (13)

According to GPA from **12** (1.75 g, 7.4 mmol) followed by chromatography (SiO_2, hexanes/EtOAc, EtOAc: 5% → 50%), **13** (993 mg, 48%) was obtained as a colorless solid; m.p. 150 °C; $[\alpha]_D = +6.9°$ (*c* 0.146, $CHCl_3$); $R_F = 0.33$ (SiO_2, hexanes/EtOAc, 3:1); IR (ATR): ν = 2944*w*, 1740*m*, 1365*m*, 1231*s*, 1189*w*, 1134*w*, 1031*m*, 946*w*, 752*m*, 667*w*, 605*w*, 510*w* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.31 (*td*, 2H, 2-H), 4.60 (*d*, *J* = 3.9 Hz, 1H, 3-H), 3.24 (*td*, *J* = 11.3, 4.7 Hz, 2H, 19-H), 2.29 (*m*, 1H, 16-H_a), 2.18 (*s*, 3H, 29-H), 2.15−2.05 (*m*, 1H, 18-H), 2.02 (*s*, 3H, 33-H), 2.01 (*s*, 3H, 31-H), 2.06−1.92 (*m*, 3H, 1-H_a, 13-H, 22-H_a), 1.67−1.15 (*m*, 13H, 22-H_b, 6-H, 15-H_a, 21-H, 16-H_b, 7-H, 11-H, 1-H_b, 15-H_b), 1.10 (*s*, 3H, 25-H), 1.08 (*s*, 2H, 12-H), 1.01 (*s*, 3H, 24-H), 1.00 (*s*, 3H, 27-H), 0.95 (*m*, 1H, 5-H), 0.93 (*s*, 3H, 26-H), 0.88 (*s*, 3H, 23-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 212.12 (C-20), 181.60 (C-28), 170.84 (C-32), 170.37 (C-30), 78.02 (C-3), 69.79 (C-2), 56.34 (C-17), 55.32 (C-5), 51.35 (C-19), 50.90 (C-9), 49.26 (C-18), 42.55 (C-14), 42.27 (C-1), 40.85 (C-8), 37.62 (C-13), 37.55 (C-4), 37.15 (C-10), 36.84 (C-22), 34.18 (C-7), 31.59 (C-16), 30.21 (C-29), 29.71 (C-15), 29.12 (C-23), 28.41 (C-21), 27.19 (C-12), 21.39 (C-33), 21.13 (C-11), 21.00 (C-31), 18.10 (C-6), 17.61 (C-24), 16.87 (C-25), 16.29 (C-26), 14.79 (C-27) ppm; MS (ESI, MeOH): *m*/*z* 439 (28%, $[M + H\text{-}HOAc]^+$), 499 (32%, $[M + H\text{-}2HOAc]^+$), 576 (100%, $[M + NH_4]^+$), 581 (48%, $[M+Na]^+$); analysis calcd for $C_{33}H_{50}O_7$ (558.76): C 70.94, H 9.02; found: C 70.73, H 9.24.

4.4.12. Benzyl 2β,3β-diacetyloxy-20-oxo-30-norlupan-28-amide (14)

According to GPB from **13** (12.41 g, 2.22 mmol) and benzylamine (0.49 mL, 4.5 mmol) followed by chromatography (SiO_2, hexanes/EtOAc, 3:2), **14** (1.21 g, 84%) was obtained as a colorless solid; m.p. 150−154 °C; $[\alpha]_D = +21.5°$ (*c* 0.355, $CHCl_3$); $R_F = 0.49$ (SiO_2, hexanes/EtOAc, 3:1); UV−Vis ($CHCl_3$): λ_{max} (log ε) = 259 nm (3.22); IR (ATR): ν = 2943*m*, 1741*s*, 1660*m*, 1516*m*, 1231*s*, 1188*m*, 1030*m*, 699*m*, 603*w* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.36−7.23 (*m*, 5H, 36-H, 37-H, 38-H, 39-H, 40-H), 5.97 (*t*, *J* = 5.7 Hz, 1H, NH) 5.30 (*td*, *J* = 3.6 Hz, 1H, 2-H), 4.59 (*d*, *J* = 3.9 Hz, 1H, 3-H), 4.51−4.44 (*m*, 1H, 34-H_a), 4.38−4.30 (*m*, 1H, 34-H_b), 3.46 (*td*, *J* = 11.2, 4.1 Hz, 1H, 19-H), 2.27 (*td*, *J* = 12.1, 4.1 Hz, 1H, 13-H), 2.16 (*s*, 3H, 29-H), 2.12−2.03 (*m*, 2H, 18-H, 21-H_a), 2.02 (*s*, 3H, 33-H), 2.01 (*s*, 3H, 31-H), 1.98−1.93 (*m*, 1H, 1-H_b), 1.93−1.85 (*m*, 1H, 16-H_a), 1.81−1.73 (*m*, 1H, 22-H_a), 1.71−1.19 (*m*, 12H, 16-H_b, 22-H_b, 6-H, 21-H_b, 15-H_a, 7-H, 11-H, 1-H_b, 9-H), 1.14 (*m*, 1H, 15-H_b), 1.09 (s, 3H, 25-H), 1.13−1.05 (*m*, 2H, 12-H), 1.01 (*s*, 3H, 24-H), 0.97 (*s*, 3H, 27-H), 0.95−0.92 (*m*, 1H, 5-H), 0.89 (*s*, 3H, 26-H), 0.87 (*s*, 3H, 23-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 212.76 (C-20), 175.70 (C-28), 170.65 (C-32), 170.19 (C-30), 138.96 (C-35), 128.68 (C-38, C-37), 127.73 (C-40, C-36), 127.39 (C-38), 77.89 (C-3), 69.60 (C-2), 55.48 (C-17), 55.22 (C-5), 50.97 (C-19), 50.93 (C-9), 49.92 (C-18), 43.30 (C-34), 42.42 (C-14), 42.17 (C-1), 40.79 (C-8), 37.95 (C-22), 37.39 (C-4), 36.99 (C-10), 36.68 (C-13), 34.15 (C-7), 32.98 (C-16), 30.30 (C-29), 29.33 (C-15), 28.94 (C-23), 28.54 (C-21), 27.13 (C-12), 21.24 (C-33), 21.06 (C-11), 20.84 (C-31), 17.97 (C-6), 17.50 (C-24), 16.73 (C-25), 16.19 (C-26), 14.60 (C-27) ppm; MS (ESI, MeOH): *m*/*z* 648 (72%, $[M+H]^+$), 994 (52%, $[3M+2Na]^{2+}$), 1295 (34%, $[2M + H]^+$), 1317 (72%, $[2M + Na]^+$); analysis calcd for $C_{40}H_{57}NO_6$ (647.90): C 74.15, H 8.87, N 2.16; found: C 73.97, H 9.03, N 2.31.

4.4.13. Isoquinolin-4-yl 2β,3β -diacetyloxy-20-oxo-30-norlupan-28-amide (15)

According to GPC from **13** (137 mg, 0.24 mmol) and 4-aminoisoquinoline (150 mg, 1.0 mmol) **15** (83 mg, 49%) was obtained as an off-white solid; m.p. 205−208 °C (decomp.); $[\alpha]_D = -14.2°$ (*c* 0.258, $CHCl_3$); $R_F = 0.20$ (SiO_2, $CHCl_3$/MeOH, 95:5); UV−Vis ($CHCl_3$): λ_{max} (log ε) = 228 nm (3.78); IR (ATR): ν = 2943*w*, 1741*m*, 1666*w*, 1588*w*, 1449*w*, 1396*m*, 1368*m*, 1253*s*, 1188*m*, 1032*m*, 947*w*, 873*w*, 780*w*, 750*w*, 653*w*, 622*w*, 576*w*, 497*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 9.13 (*s*, 1H, 37-H), 8.71 (*s*, 1H, 35-H), 8.02 (*d*, *J* = 8.2 Hz, 1H, 39-H), 7.82−7.72 (*m*, 2H, 42-H, 40-H), 7.65 (*m*, 1H, 41-H), 5.31 (*m*, 1H, 2-H), 4.60 (*d*, *J* = 3.9 Hz, 1H, 3-H), 3.45 (*td*, *J* = 11.3, 5.7 Hz, 1H, 19-H), 2.41−2.32 (*m*, 1H, 13-H), 2.29−2.20 (*m*, 2H, 16-H_a, 18-H), 2.18 (*m*, 1H, 21-H_a), 2.17 (*s*, 3H, 29-H), 2.09 (*m*, 1H, 22-H_a), 2.02 (*s*, 3H, 33-H), 2.01 (*s*, 3H, 31-H), 1.97 (*m*, 1H, 1-H_b), 1.88 (*m*, 1H, 16-H_b), 1.74 (*m*, 2H, 15-H, 22-H_b), 1.62−1.21 (*m*, 10H, 21-H_b, 6-H, 7-H, 11-H, 15-H_b, 9-H, 1-H_b), 1.09 (*s*, 3H, 25-H), 1.07 (*m*, 2H, 12-H), 1.06 (*s*, 3H, 27-H), 1.02 (*s*, 3H, 24-H), 1.00 (*s*, 3H, 25-H), 0.97−0.91 (*m*, 1H, 5-H), 0.88 (*s*, 3H, 23-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 212.60 (C-20), 175.39 (C-28), 170.86 (C-32), 170.39 (C-30), 150.25 (C-37), 138.78 (C-35), 131.38 (C-34), 130.94 (C-40), 128.97 (C-38), 128.44 (C-39), 128.28 (C-43), 127.66 (C-41), 120.82 (C-42), 78.04 (C-3), 69.73 (C-2), 56.77 (C-17), 55.40 (C-5), 51.11 (C-19), 50.86 (C-9), 50.11 (C-18), 42.72 (C-14), 42.34 (C-1), 41.03 (C-8), 38.18 (C-22), 37.55 (C-4), 37.16 (C-10), 36.89 (C-13), 34.34 (C-7), 33.53 (C-16), 30.51 (C-29), 29.91 (C-15), 29.10 (C-23), 28.66 (C-21), 27.29 (C-12), 21.39 (C-33), 21.22 (C-11), 21.01 (C-31), 18.12 (C-6), 17.65 (C-24), 16.89 (C-25), 16.44 (C-26), 14.84 (C-27) ppm; MS (ESI, MeOH): *m*/*z* 685 (100%, $[M+H]^+$); analysis calcd for $C_{42}H_{56}N_2O_6$ (684.92): C 73.65, H 8.24, N 4.09; found: C 73.40, H 8.42, N 4.17.

4.4.14. Quinolin-5-yl 2β,3β -diacetyloxy-20-oxo-30-norlupan-28-amide (16)

According to GPC fron **13** (244 mg, 0.44 mmol) and 5-aminoquinoline (25 mg, 1.8 mmol) followed by chromatography (SiO_2, hexanes/EtOAc, EtOAc: 10% → 60%) **16** (244 mg, 82%) was isolated as an off-white solid; m.p. 290−292 °C (decomp.); $[\alpha]_D = -28.6°$ (*c* 0.213, $CHCl_3$); $R_F = 0.26$ (SiO_2, $CHCl_3$:MeOH, 95:5); UV−Vis ($CHCl_3$): λ_{max} (log ε) = 304 nm (4.34), 316 nm (4.31); IR (ATR): ν = 2939*w*, 1741*m*, 1364*m*, 1249*s*, 1027*w*, 809 s cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.93 (*d*, *J* = 2.8 Hz, 1H, 40-H), 8.17 (*d*, *J* = 8.5 Hz, 1H, 42-H), 8.02 (*dd*, *J* = 6.9, 2.5 Hz, 1H, 41-H), 7.78−7.68 (*m*, 2H, 37-H, 35-H), 7.45 (*dd*, *J* = 8.6, 4.2 Hz, 1H, 36-H), 5.34−5.28 (*m*, 1H, 2-H), 4.60 (*d*, *J* = 3.9 Hz, 1H, 3-H), 3.51−3.41 (*m*, 1H, 19-H), 2.41−2.31 (*m*, 1H, 13–H), 2.23 (*m*, 3H, 16-H_a, 18-H, 21-H_a), 2.17 (*s*, 3H, 29-H), 2.14−2.04 (*m*, 1H, 22-H_a), 2.02 (*s*, 3H, 33-H), 2.01 (*s*, 3H, 31-H), 1.97 (*s*, 1H, 1-H_a), 1.89 (*m*, 1H, 16-H_b), 1.80−1.67 (*m*, 1H, 15-H_a, 22-H_b), 1.64−1.22 (*m*, 10H, 21-H_b, 6-H, 7-H, 11-H, 15-H_b, 9-H, 1-H_b), 1.10 (*s*, 3H, 25-H), 1.13−1.03 (*m*, 2H, 12-H), 1.06 (*s*, 3H, 27-H), 1.02 (*s*, 3H, 24-H), 1.00 (*s*, 3H, 26-H), 0.97−0.91 (*m*, 1H, 5-H), 0.88 (*s*, 3H, 23-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 212.51 (C-20), 175.35 (C-28), 170.84 (C-32), 170.36 (C-30), 150.19 (C-40), 147.96 (C-38), 132.88 (C-34), 130.79 (C-42), 129.57 (C-35), 127.46 (C-41), 123.84 (C-43), 122.61 (C-37), 121.14 (C-36), 78.02 (C-3), 69.72 (C-2), 56.75 (C-17), 55.41 (C-5), 51.11 (C-19), 50.91 (C-9), 50.13 (C-18), 42.73 (C-14), 42.35 (C-1), 41.05 (C-8), 38.18 (C-22), 37.56 (C-4), 37.16 (C-10), 36.94 (C-13), 34.35 (C-7), 33.53 (C-16), 30.49 (C-29), 29.92 (C-15), 29.10 (C-23), 28.67 (C-21), 27.28 (C-12), 21.40 (C-33), 21.24 (C-11), 21.01 (C-31), 18.12 (C-6), 17.66 (C-24), 16.89 (C-25), 16.47 (C-26), 14.85 (C-27) ppm; MS (ESI, MeOH): *m*/*z* 685 (100%, $[M+H]^+$), 707 (8%, $[M+Na]^+$), 1370 (18%, $[2M + H]^+$), 1391 (10%, $[2M + Na]^+$); analysis calcd for $C_{42}H_{56}N_2O_6$ (684.92): C 73.65, H 8.24, N 4.09; found: C 73.47, H 8.39, N 4.20.

4.4.15. 3β-Acetyloxy-lup-20(29)-en-28-oic acid (17) [10376-50-8]

According to GPA from **1** (0.5 g, 1.10 mmol) followed by crystallization from EtOH, **17** (489 mg, 89%) was obtained as a colorless solid; m.p. 275−278 °C (lit.: [35] 277−278 °C); $[\alpha]_D = +20.6°$ (*c* 0.350, $CHCl_3$) [lit.: [35] $[\alpha]_D = +22°$ (*c* 0.49, $CHCl_3$)]; $R_F = 0.58$ (SiO_2, hexanes/EtOAc, 4:1).

4.4.16. Benzyl 3β-acetyloxy-lup-20(29)-en-28-amide (18)

According to GPB from **17** (407 mg, 0.82 mmol) and benzylamine (0.3 mL, 2.8 mmol) followed by chromatography (SiO_2, hexanes/EtOAc, 9:1), **18** (350 mg, 73%) was obtained as a colorless solid; m.p. 135 °C, 210 °C (decomp.) (lit.: [30,38] 124–127 °C); $[\alpha]_D = 20.9°$ (*c* 0.348, $CHCl_3$) [lit.: [30,38] $[\alpha]_D = +23.2°$ (*c* 0.35, $CHCl_3$)]; $R_F = 0.65$ (SiO_2, hexanes/EtOAc, 7:1).

4.4.17. Isoquinolin-4-yl 3β-acetyloxy-lup-20(29)-en-28-amide (19)

According to GPC from **17** (300 mg, 0.60 mmol) and 4-aminoisoquinoline (300 mg, 2.1 mmol) followed by chromatography (SiO_2, hexanes/EtOAc, EtOAc: 15% → 60%), **19** (214 mg, 56%) was obtained as an off-white solid; m.p. 235 °C (decomp.); $[\alpha]_D = -10.7°$ (*c* 0.124, $CHCl_3$); $R_F = 0.5$ (SiO_2, $CHCl_3$/MeOH, 95:5); UV–Vis ($CHCl_3$): λ_{max} (log ε) = 229 nm (3.87); IR (ATR): ν = 2943*m*, 2870*w*, 1731*m*, 1656*w*, 1585*w*, 1516*w*, 1468*m*, 1411*w*, 1391*m*, 1373*m*, 1244*s*, 1182*w*, 1135*w*, 1106*w*, 1026*m*, 979*m*, 884*m*, 797*w*, 778*w*, 749*s*, 559*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 9.08 (*s*, 1H, 36-H), 8.69 (*s*, 1H, 34-H), 7.99 (*d*, *J* = 8.2 Hz, 1H, 38-H), 7.82–7.70 (*m*, 2H, 40-H, 39-H), 7.63 (*t*, *J* = 7.1 Hz, 1H, 41-H), 4.74 (*s*, 1H, 29-H_a), 4.62 (*s*, 1H, 19-H_b), 4.50–4.42 (*m*, 1H, 3-H), 3.23–3.14 (*m*, 1H, 19-H), 2.58 (*m*, 1H, 13-H), 2.33–2.24 (*m*, 1H, 16-H_a), 2.12–2.05 (*m*, 2H, 22-H_a, 21-H_a), 2.03 (*s*, 3H, 32-H), 1.71 (*s*, 3H, 30-H), 1.86–1.17 (*m*, 16H, 16-H_b, 12-H_a, 15-H_a, 1-H_a, 18-H, 22-H_b, 2-H, 21-H_b, 11-H_a, 7-H, 6-H_b, 15-H_b, 9-H, 11-H_b), 1.03 (*s*, 3H, 27-H), 1.00 (*s*, 3H, 25-H), 0.97 (*m*, 2H, 12-H_b, 1-H_b), 0.84 (*m*, 6H, 23-H, 25-H), 0.83 (*s*, 3H, 24-H), 0.79 (*m*, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 175.58 (C-28), 171.18 (C-31), 150.68 (C-20), 149.77 (C-36), 138.49 (C-34), 131.49 (C-33), 130.90 (C-39), 128.88 (C-37), 128.68 (C-42), 128.35 (C-38), 127.62 (C-41), 121.15 (C-40), 109.80 (C-29), 81.08 (C-3), 56.93 (C-17), 55.65 (C-5), 50.76 (C-9), 50.45 (C-18), 46.66 (C-19), 42.77 (C-14), 41.03 (C-8), 38.64 (C-1), 38.59 (C-4), 37.95 (C-10), 37.89 (C-13), 37.29 (C-22), 34.56 (C-7), 34.21 (C-16), 31.06 (C-21), 30.00 (C-15), 28.08 (C-23), 25.79 (C-12), 23.84 (C-2), 21.45 (C-32), 21.12 (C-11), 19.68 (C-30), 18.34 (C-6), 16.62 (C-24), 16.38 (C-25, C-26), 14.83 (C-27) ppm; MS (ESI, MeOH): *m*/*z* 626 (76%, $[M+H]^+$), 1249 (100%, $[2M + H]^+$); analysis calcd for $C_{41}H_{56}N_2O_3$ (624.91): C 78.80, H 9.03, N 4.48; found: C 78.61, H 9.18, N 4.61.

4.4.18. Quinolin-5-yl 3β-acetoxy-lup-20(29)-en-28-amide (20)

According to GPC from **17** (200 mg, 0.4 mmol) and 5-aminoquinoline (200 mg, 1.4 mmol) followed by chromatography (SiO_2: hexanes/EtOAc, EtOAc: 25% → 50%), **20** (160 mg, 64%) was obtained as an off-white solid; m.p. 277–280 °C (decomp.); $[\alpha]_D = +21.2°$ (*c* 0.261, $CHCl_3$); $R_F = 0.54$ (SiO_2, $CHCl_3$/MeOH, 95:5); UV–Vis ($CHCl_3$): λ_{max} (log ε) = 236 nm (3.93); IR (ATR): ν = 2943*m*, 1730*m*, 1649*w*, 1594*w*, 1477*m*, 1392*w*, 1367*m*, 1244*s*, 1181*w*, 1028*m*, 979*m*, 883*m*, 797*s*, 755*m*, 651*w* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.91–8.86 (*m*, 1H, 39-H), 8.15 (*d*, *J* = 8.5 Hz, 1H, 41-H), 7.97 (*dd*, *J* = 6.8, 2.6 Hz, 1H, 34-H), 7.71–7.63 (*m*, 2H, 36-H, 35-H), 7.40 (*dd*, *J* = 8.5, 4.2 Hz, 1H, 40-H), 4.74 (*s*, 1H, 29-H_a), 4.62 (*s*, 1H, 29-H_b), 4.50–4.41 (*m*, 1H, 3-H), 3.19 (*td*, *J* = 10.9, 4.7 Hz, 1H, 19-H), 2.57 (*td*, *J* = 12.6, 3.4 Hz, 1H, 13-H), 2.30–2.20 (*m*, 1H, 16-H_a), 2.11–1.99 (*m*, 2H, 21-H_a, 22-H_a), 2.03 (*s*, 3H, 32-H), 1.71 (*s*, 3H, 30-H), 1.89–1.15 (*m*, 17H, 16-H_b, 12-H_a, 15-H_a, 9-H, 1-H_a, 22-H_b, 2-H, 6-H_a, 21-H_b, 11-H_a, 7-H, 6-H_b, 15-H_b, 18-H, 11-H_b), 1.03 (*s*, 3H, 27-H), 1.11–0.91 (*m*, 2H, 12-H_b, 1-H_b), 0.99 (*s*, 3H, 24-H), 0.84 (*s*, 3H. 23-H), 0.83 (*s*, 3H, 25-H), 0.82 (*s*, 3H, 26-H), 0.79 (*m*, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 175.51 (C-28), 171.17 (C-31), 150.66 (C-20), 150.13 (C-39), 148.40 (C-37), 133.14 (C-42), 130.88 (C-41), 129.47 (C-35), 127.29 (C-34), 123.89 (C-33), 122.56 (C-36), 121.00 (C-40), 109.81 (C-29), 81.06 (C-3), 56.88 (C-17), 55.64 (C-5), 50.75 (C-18), 50.46 (C-9), 46.71 (C-19), 42.76 (C-14), 41.03 (C-8), 38.64 (C-4), 38.64 (C-1), 37.94 (C-22), 37.91 (C-13), 37.28 (C-10), 34.56 (C-7), 34.19 (C-16), 31.06 (C-21), 29.99 (C-15), 28.07 (C-23), 25.77 (C-12), 23.84 (C-2), 21.45 (C-32), 21.12 (C-11), 19.66 (C-30), 18.32 (C-6), 16.62 (C-24), 16.38 (C-25, C-26), 14.83 (C-27) ppm; MS (ESI, MeOH): *m*/*z* 625 (100%, $[M+H]^+$), 1249 (44%, $[2M + H]^+$); $C_{41}H_{56}N_2O_3$ (624.91): C 78.80, H 9.03, N 4.48; found: C 78.53, H 9.23, N 4.57.

4.4.19. 3β-Acetyloxy-20-oxo-30-norlupan-28-oic acid (21)

According to GPA from **9** (15.02 g, 32.8 mmol) followed by crystallization from EtOH, **21** (13.8 g, 84%) was obtained as a colorless solid; m.p. 268–270 °C (decomp.) (lit.: [39] 252–255 °C); $[\alpha]_D = -9.1°$ (*c* 0.34, $CHCl_3$) [lit.: [39] $[\alpha]_D = -9.5°$ (*c* 0.80, $CHCl_3$)]; $R_F = 0.50$ (SiO_2, toluene/EtOAc/heptane/HCOOH, 80:26:10:5).

4.4.20. Benzyl 3β-acetyloxy-20-oxo-30-norlupan-28-amide (22)

According to GPB from **21** (250 mg, 0.5 mmol) and benzylamine (0.2 mL, 1.9 mmol) followed by chromatography (SiO_2, hexanes/EtOAc, EtOAc: 5% → 30%) **22** (244 mg, 76%) [40] was obtained as a colorless solid; m.p. 290 °C (decomp.); $[\alpha]_D = +0.5°$ (*c* 0.159, $CHCl_3$); $R_F = 0.35$ (SiO_2, hexanes/EtOAc, 3:1); IR (ATR): ν = 2943*m*, 1733*m*, 1654*m*, 1519*m*, 1453*w*, 1367*m*, 1244*s*, 1027*m*, 979*m*, 753*m*, 698*m*, 609*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.37–7.27 (*m*, 5H, 35-H, 37-H, 38-H, 34-H, 36-H), 5.95 (*t*, *J* = 5.7 Hz, 1H, NH), 4.48 (*m*, 2H, 3-H, 32-H_a), 4.37–4.27 (*m*, 1H, 32-H_b), 3.48 (*m*, 1H, 19-H), 2.26 (*m*, 1H, 13-H), 2.17 (*s*, 3H, 29-H), 2.13–2.04 (*m*, 1H, 18-H), 2.03 (*s*, 3H, 31-H), 1.88 (*m*, 1H, 16-H_a), 1.77 (*m*, 1H, 21-H_a), 1.68–1.20 (*m*, 17H, 1-H_a, 2-H, 6-H, 7-H, 9-H, 11-H, 12-H, 15-H, 16-H_b, 21-H_b, 22-H_b), 1.19–1.02 (*m*, 1H, 1-H_b), 0.98 (*s*, 3H, 27-H), 0.88 (*s*, 3H, 26-H), 0.84–0.80 (*m*, 9H, 23-H, 26-H, 27-H), 0.78 (*m*, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 213.01 (C-20), 175.88 (C-28), 171.09 (C-30), 139.12 (C-33), 128.84 (C-35, C-37), 127.89 (C-34, C-38), 127.55 (C-36), 81.01 (C-3), 55.66 (C-17), 55.55 (C-5), 51.17 (C-19), 50.57 (C-9), 50.19 (C-18), 43.46 (C-32), 42.42 (C-14), 40.84 (C-8), 38.52 (C-1), 38.11 (C-4), 37.94 (C-22), 37.27 (C-10), 36.94 (C-13), 34.41 (C-7), 33.13 (C-16), 30.47 (C-29), 29.62 (C-15), 28.72 (C-21), 28.08 (C-23), 27.39 (C-12), 23.81 (C-2), 21.44 (C-31), 21.09 (C-11), 18.32 (C-6), 16.63 (C-24), 16.36 (C-25), 16.22 (C-26), 14.82 (C-27) ppm; MS (ESI, MeOH): *m*/*z* 590 (100%, $[M+H]^+$), 1179 (66%, $[2M + H]^+$), 1201 (54%, $[2M + Na]^+$); analysis calcd for $C_{38}H_{55}NO_4$ (589.86): C 77.38, H 9.40, N 2.37; found: C 77.19, H 9.65, N 2.50.

4.4.21. Isoquinolin-4-yl 3β-acetyloxy-20-oxo-30-norlupan-28-amide (23)

According to GPC from **21** (250 mg, 0.5 mmol) and 4-aminoisoquinoline (250 mg, 1.7 m mol) followed by chromatography SiO_2, hexanes/EtOAc, EtOAc: 20% → 80%) **23** (228 mg, 72%) was obtained as an off-white solid; m.p. 235 °C (decomp.); $[\alpha]_D = -20.3°$ (*c* 0.211, $CHCl_3$); $R_F = 0.26$ (SiO_2, $CHCl_3$/MeOH, 95:5); UV–Vis ($CHCl_3$): λ_{max} (log ε) = 228 nm (3.74); IR (ATR): ν = 2943*m*, 1710*m*, 1585*w*, 1516*w*, 1467*m*, 1368*m*, 1245*s*, 1183*w*, 1024*m*, 979*m*, 893*w*, 749*s*, 574*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 9.12 (*s*, 1H, 35-H), 8.72 (*s*, 1H, 33-H), 8.02 (*d*, *J* = 8.1 Hz, 1H, 37-H), 7.78 (*m*, 2H, 38-H, 39-H), 7.65 (*t*, *J* = 7.3 Hz, 1H, 40-H), 4.47 (*dd*, *J* = 10.9, 5.3 Hz, 1H, 3-H), 3.50–3.42 (*m*, 1H, 19-H), 2.36 (*m*, 1H, 13-H), 2.30–2.20 (*m*, 2H, 16-H_a, 18-H), 2.17 (*s*, 3H, 29-H), 2.15–2.06 (*m*, 1H, 22-H_a), 2.03 (*s*, 3H, 31-H), 1.92–1.82 (*m*, 1H, 16-H_b), 1.78–1.14 (*m*, 15H, 22-H_b, 15-H_a, 1-H_a, 2-H, 21-H, 6-H_a, 11-H_a, 7-H, 15-H_b, 6-H_b, 9-H, 11-H_b), 1.08 (*s*, 2H, 12-H), 1.06 (*s*, 3H, 27-H), 0.99 (*s*, 3H, 25-H), 0.96 (*s*, 1H, 1-H_b), 0.83 (*d*, *J* = 7.1 Hz, 9H, 23-H, 24-H, 26-H), 0.80 (*m*, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 212.90 (C-20), 175.69 (C-28), 171.38 (C-30), 150.31 (C-35), 138.85 (C-33), 131.70 (C-32), 131.26 (C-38), 128.70 (C-37), 127.98 (C-40), 121.22 (C-39), 81.25 (C-3), 57.06 (C-17), 55.83 (C-5), 51.14 (C-19), 50.86 (C-9), 50.47 (C-18), 42.82 (C-14), 41.19 (C-8), 38.80 (C-1), 38.43 (C-4), 38.21 (C-22), 37.54 (C-10), 37.24 (C-13), 34.71 (C-7), 33.76 (C-16), 30.77 (C-29), 30.32 (C-15), 28.93 (C-21), 28.33 (C-23), 27.65 (C-12), 24.07 (C-2), 21.70 (C-31), 21.36 (C-11), 18.57 (C-6), 16.88 (C-24), 16.62 (C-25), 16.57 (C-26), 15.15 (C-

27) ppm; MS (ESI, MeOH): *m/z* 627 (100%, $[M+H]^+$), 1253 (58%, $[2M + H]^+$); analysis calcd for $C_{40}H_{54}N_2O_4$ (626.88): C 76.64, H 8.68, N 4.47; found: C 76.51, H 8.96, N 4.62.

4.4.22. Quinolin-5-yl 3β -acetyloxy-20-oxo-30-norlupan-28-amide (24)

According to GPC from **21** (250 mg, 0.5 mmol) and 5-aminoquinoline (250 mg, 1.7 mmol) followed by chromatography (SiO_2, hexanes/EtOAc, EtOAc: 25% → 80%) **24** (177 mg, 57%) was obtained as an off-white solid; m.p. 277–280 °C (decomp.); $[\alpha]_D = -35.9°$ (*c* 0.106, $CHCl_3$); $R_F = 0.36$ (SiO_2, $CHCl_3$/MeOH, 95:5); UV–Vis ($CHCl_3$): λ_{max} (log ε) = 235 nm (3.95); IR (ATR): ν = 2944*m*, 1732*m*, 1711*m*, 1595*w*, 1515*w*, 1476*m*, 1367*m*, 1244*s*, 1182*w*, 1133*w*, 1024*m*, 979*m*, 798*s*, 752*s*, 665*w*, 610*w*, 496*w* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.90 (*d*, *J* = 3.0 Hz, 1H, 38-H), 8.21 (*d*, *J* = 8.4 Hz, 1H, 40-H), 8.03 (*d*, *J* = 7.7 Hz, 1H, 33-H), 7.71 (*m*, 2H, 35-H, 34-H), 7.45 (*dd*, *J* = 8.5, 4.3 Hz, 1H, 39-H), 4.51–4.42 (*m*, 1H, 3-H), 3.47 (*m*, 1H, 19-H), 2.36 (*s*, 1H, 13-H), 2.25 (*m*, 2H, 16-H_a, 18-H), 2.17 (*s*, 3H, 29-H), 2.14–2.05 (*m*, 2H, 21-H_a, 22-H_a), 2.03 (*s*, 3H, 31-H), 1.88 (*m*, 1H, 16-H_b), 1.80–1.16 (*m*, 14H, 22-H_b, 15-H_a, 1-H_a, 2-H, 6-H_a, 11-H_a, 7-H, 15-H_b, 6-H_b, 9-H, 11-H_b, 21-H_b), 1.09 (*s*, 2H, 12-H), 1.07 (*s*, 3H, 27-H), 0.99 (*s*, 3H 25-H), 0.97 (*s*, 1H, 1-H_b), 0.83 (*m*, 9H, 23-H, 24-H, 26-H), 0.79 (*s*, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 212.59 (C-20), 175.45 (C-28), 171.12 (C-30), 149.77 (C-38), 133.06 (C-36), 131.51 (C-40), 129.82 (C-34), 127.05 (C-33), 123.97 (C-32), 122.86 (C-35), 121.06 (C-39), 107.47 (C-41), 80.97 (C-3), 56.79 (C-17), 55.57 (C-5), 50.94 (C-19), 50.59 (C-9), 50.24 (C-18), 42.57 (C-14), 40.95 (C-8), 38.55 (C-1), 38.17 (C-4), 37.95 (C-22), 37.29 (C-10), 37.04 (C-13), 34.46 (C-7), 33.48 (C-16), 30.49 (C-29), 30.07 (C-15), 28.69 (C-21), 28.08 (C-23), 27.38 (C-12), 23.81 (C-2), 21.45 (C-31), 21.12 (C-11), 18.31 (C-6), 16.63 (C-24), 16.37 (C-25), 16.35 (C-26), 14.91 (C-27) ppm; MS (ESI, MeOH): *m/z* 628 (100%, $[M+H]^+$), 1253 (24%, $[2M + H]^+$); analysis calcd for $C_{40}H_{54}N_2O_4$ (626.88): C 76.64, H 8.68, N 4.47; found: C 76.46, H 8.87, N 4.65.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2020.112815.

References

[1] G.R. Dagenais, D.P. Leong, S. Rangarajan, F. Lanas, P. Lopez-Jaramillo, R. Gupta, R. Diaz, A. Avezum, G.B.F. Oliveira, A. Wielgosz, S.R. Parambath, P. Mony, K.F. Alhabib, A. Temizhan, N. Ismail, J. Chifamba, K. Yeates, R. Khatib, O. Rahman, K. Zatonska, K. Kazmi, L. Wei, J. Zhu, A. Rosengren, K. Vijayakumar, M. Kaur, V. Mohan, A. Yusufali, R. Kelishadi, K.K. Teo, P. Joseph, S. Yusuf, Variations in common diseases, hospital admissions, and deaths in middle-aged adults in 21 countries from five continents (PURE): a prospective cohort study, Lancet 395 (2020) 785–794.
[2] J. Ferlay, M. Colombet, I. Soerjomataram, C. Mathers, D.M. Parkin, M. Pineros, A. Znaor, F. Bray, Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods, Int. J. Canc. 144 (2019) 1941–1953.
[3] NN, accessed 2020-04-17, https://www.vfa.de/de/presse/pressemitteilungen/pm-036-2019-bilanz-2019-neue-medikamente-bringen-fortschritt-fuer-patienten.html last.
[4] D.J. Newman, G.M. Cragg, Natural products as sources of new drugs from 1981 to 2014, J. Nat. Prod. 79 (2016) 629–661.
[5] J. Gershenzon, N. Dudareva, The function of terpene natural products in the natural world, Nat. Chem. Biol. 3 (2007) 408–414.
[6] J.A.R. Salvador, Pentacyclic Triterpenes as Promising Agents in Cancer, Nova Science Publishers, Inc., New York, 2010.
[7] R. Csuk, in: G.G.E. Chen, P.B.S.E. Lai (Eds.), Targeting Cancer by Betulin and Betulinic Acid in "Novel Apoptotic Regulators in Carcinogenesis", Springer, Dordrecht, 2012, pp. 267–287.
[8] R. Csuk, Betulinic acid and its derivatives: a patent review (2008-2013), Expert Opin. Ther. Pat. 24 (2014) 913–923.
[9] D. Biedermann, B. Eigenrova, M. Hajduch, J. Sarek, Synthesis and Evaluation of Biological Activity of the Quaternary Ammonium Salts of Lupane-, Oleanane-, and Ursane-type Acids, Synthesis, 2010, pp. 3839–3848.
[10] V.E. Kataev, I.Y. Strobykina, L.Y. Zakharova, Quaternary ammonium derivatives of natural terpenoids. Synthesis and properties, Russ. Chem. Bull. 63 (2014) 1884–1900.
[11] A.Y. Spivak, D.A. Nedopekina, R.R. Khalitova, R.R. Gubaidullin, V.N. Odinokov, Y.P. Bel'skii, N.V. Bel'skaya, V.A. Khazanov, Triphenylphosphonium cations of betulinic acid derivatives: synthesis and antitumor activity, Med. Chem. Res. 26 (2017) 518–531.
[12] A.Y. Spivak, D.A. Nedopekina, E.R. Shakurova, R.R. Khalitova, R.R. Gubaidullin, V.N. Odinokov, U.M. Dzhemilev, Y.P. Bel'skii, N.V. Bel'skaya, S.A. Stankevich, E.V. Korotkaya, V.A. Khazanov, Synthesis of lupane triterpenoids with triphenylphosphonium substituents and studies of their antitumor activity, Russ. Chem. Bull. 62 (2013) 188–198.
[13] J. Zielonka, J. Joseph, A. Sikora, M. Hardy, O. Ouari, J. Vasquez-Vivar, G. Cheng, M. Lopez, B. Kalyanaraman, Mitochondria-targeted triphenylphosphonium-based compounds: syntheses, mechanisms of action, and therapeutic and diagnostic applications, Chem. Rev. 117 (2017) 10043–10120.
[14] B. Brandes, S. Hoenke, L. Fischer, R. Csuk, Design, synthesis and cytotoxicity of BODIPY FL labelled triterpenoids, Eur. J. Med. Chem. 185 (2020) 111858.
[15] S. Krajčovičová, J. Stankova, P. Dzubak, M. Hajduch, M. Soural, M. Urban, Synthetic approach for rapid preparation of BODIPY conjugates and their use in imaging of cellular drug uptake and distribution, Chem. A Eur J. 24 (2018).
[16] S. Friedrich, I. Serbian, S. Hoenke, R.K. Wolfram, R. Csuk, Synthesis and cytotoxic evaluation of malachite green derived oleanolic and ursolic acid piperazineamides, Med. Chem. Res. 29 (2020) 926–933.
[17] M. Kahnt, J. Wiemann, L. Fischer, S. Sommerwerk, R. Csuk, Transformation of asiatic acid into a mitocanic, bimodal-acting rhodamine B conjugate of nanomolar cytotoxicity, Eur. J. Med. Chem. 159 (2018) 143–148.
[18] S. Sommerwerk, L. Heller, C. Kerzig, A.E. Kramell, R. Csuk, Rhodamine B conjugates of triterpenoic acids are cytotoxic mitocans even at nanomolar concentrations, Eur. J. Med. Chem. 127 (2017) 1–9.
[19] R.K. Wolfram, L. Fischer, R. Kluge, D. Ströhl, A. Al-Harrasi, R. Csuk, Homopiperazine-rhodamine B adducts of triterpenoic acids are strong mitocans, Eur. J. Med. Chem. 155 (2018) 869–879.
[20] R.K. Wolfram, L. Heller, R. Csuk, Targeting mitochondria: esters of rhodamine B with triterpenoids are mitocanic triggers of apoptosis, Eur. J. Med. Chem. 152 (2018) 21–30.
[21] I.Z. Pavel, R. Csuk, C. Danciu, S. Avram, F. Baderca, A. Cioca, E.A. Moaca, C.V. Mihali, I. Pinzaru, D.M. Muntean, C.A. Dehelean, Assessment of the anti-angiogenic and anti-inflammatory properties of a maslinic acid derivative and its potentiation using zinc chloride, Int. J. Mol. Sci. 20 (2019).
[22] I.Z. Pavel, C. Danciu, C. Oprean, C.A. Dehelean, D. Muntean, R. Csuk, D.M. Muntean, In vitro evaluation of the antimicrobial ability and cytotoxicity on two melanoma cell lines of a benzylamide derivative of maslinic acid, Anal. Cell Pathol. (2016), 2787623.
[23] I.Z. Pavel, C.A. Dehelean, L. Farczadi, D.M. Muntean, L. Vlase, C. Danciu, R. Csuk, F. Birsasteanu, D.M. Muntean, Assessment of a maslinic acid derivative and its metabolite in rat blood by liquid chromatography coupled with mass spectrometry, Rev. Chim.-Bucharest 68 (2017) 1089–1094.
[24] I.Z. Pavel, O.M. Duicu, I. Scurtu, D. Coricovac, C. Dehelean, R. Csuk, D.M. Muntean, Assessment of the effects of a maslinic acid derivative in isolated mouse liver mitochondria, Eur. J. Clin. Invest. 45 (2015), 29-29.
[25] I.Z. Pavel, A.E. Parvu, C.A. Dehelean, L. Vlase, R. Csuk, D.M. Muntean, Assessment of the antioxidant effect of a maslinic acid derivative in an experimental model of acute inflammation, FARMACIA 65 (2017) 390–395.
[26] B. Siewert, E. Pianowski, A. Obernauer, R. Csuk, Towards cytotoxic and selective derivatives of maslinic acid, Bioorg. Med. Chem. 22 (2014) 594–615.
[27] S. Sommerwerk, L. Heller, J. Kuhfs, R. Csuk, Selective killing of cancer cells with triterpenoic acid amides - the substantial role of an aromatic moiety alignment, Eur. J. Med. Chem. 122 (2016) 452–464.
[28] M. Kahnt, L. Heller, P. Grabandt, A. Al-Harrasi, R. Csuk, Platanic acid: a new scaffold for the synthesis of cytotoxic agents, Eur. J. Med. Chem. 143 (2018) 259–265.
[29] M. Kahnt, L. Heller, A. Al-Harrasi, R. Schäfer, R. Kluge, C. Wagner, C. Otgonbayar, R. Csuk, Platanic acid-derived methyl 20-amino-30-norlupan-

28-oates are potent cytotoxic agents acting by apoptosis, Med. Chem. Res. 27 (2018) 1757–1769.
[30] J. Wiemann, L. Heller, V. Perl, R. Kluge, D. Ströhl, R. Csuk, Betulinic acid derived hydroxamates and betulin derived carbamates are interesting scaffolds for the synthesis of novel cytotoxic compounds, Eur. J. Med. Chem. 106 (2015) 194–210.
[31] S. Sommerwerk, R. Csuk, Convenient and chromatography-free partial syntheses of maslinic acid and augustic acid, Tetrahedron Lett. 55 (2014) 5156–5158.
[32] A. Barthel, S. Stark, R. Csuk, Oxidative transformations of betulinol, Tetrahedron 64 (2008) 9225–9229.
[33] Y. Fukuda, K. Sakai, S. Matsunaga, H. Tokuda, R. Tanaka, Cancer chemopreventive effect of orally administrated lupane-type triterpenoid on ultraviolet light B induced photocarcinogenesis of hairless mouse, Canc. Lett. 240 (2006) 94–101.
[34] Y. Wei, C.-M. Ma, M. Hattori, Synthesis and evaluation of A-seco type triterpenoids for anti-HIV-1protease activity, Eur. J. Med. Chem. 44 (2009) 4112–4120.
[35] M. Urban, J. Sarek, J. Klinot, G. Korinkova, M. Hajduch, Synthesis of A-seco derivatives of betulinic acid with cytotoxic activity, J. Nat. Prod. 67 (2004) 1100–1105.
[36] J. Hao, X. Zhang, P. Zhang, J. Liu, L. Zhang, H. Sun, Efficient access to isomeric 2,3-dihydroxy lupanes: first synthesis of alphitolic acid, Tetrahedron 65 (2009) 7975–7984.
[37] N.G. Komissarova, N.G. Belenkova, O.V. Shitikova, L.V. Spirikhin, M.S. Yunusov, Oxidative decarboxylation of triterpene C-28 acids of lupane series, Nat. Prod. Commun. 3 (2008) 1569–1574.
[38] R.K. Wolfram, L. Heller, R. Csuk, Targeting mitochondria: esters of rhodamine B with triterpenoids are mitocanic triggers of apoptosis, Eur. J. Med. Chem. 152 (2018) 21–30.
[39] A. Vystrcil, M. Budesinsky, Triterpenes. XVI. Unusual epimerization of the C-19 acetyl group in 20-oxo-30-norlupane derivatives, Collect. Czech Chem. Commun. 35 (1970) 295–311.
[40] T. Durst, Z. Merali, J.T. Arnason, E.P. Sanchez-Vindas, L.J. Poveda Alvarez, Anxiolytic Marcgraviaceae Compositions Containing Betulinic Acid, Betulinic Acid Derivatives, and Methods of Preparation and Use, 2002, p. CA2446748C.

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Review

Mitocanic Di- and Triterpenoid Rhodamine B Conjugates

Sophie Hoenke [1], Immo Serbian [1], Hans-Peter Deigner [2] and René Csuk [1,*]

[1] Organic Chemistry, Martin-Luther University Halle-Wittenberg, Kurt-Mothes Street 2, D-06120 Halle, Germany; sophie.hoenke@chemie.uni-halle.de (S.H.); immo.serbian@chemie.uni-halle.de (I.S.)

[2] Medical and Life Science Faculty, Institute of Precision Medicine, Furtwangen University, Jakob–Kienzle–Street 17, D-78054 Villigen–Schwenningen, Germany; dei@hs-furtwangen.de

* Correspondence: rene.csuk@chemie.uni-halle.de; Tel.: +49-345-5525660

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Abstract: The combination of the "correct" triterpenoid, the "correct" spacer and rhodamine B (**RhoB**) seems to be decisive for the ability of the conjugate to accumulate in mitochondria. So far, several triterpenoid rhodamine B conjugates have been prepared and screened for their cytotoxic activity. To obtain cytotoxic compounds with EC_{50} values in a low nano-molar range combined with good tumor/non-tumor selectivity, the Rho B unit has to be attached via an amine spacer to the terpenoid skeleton. To avoid spirolactamization, secondary amines have to be used. First results indicate that a homopiperazinyl spacer is superior to a piperazinyl spacer. Hybrids derived from maslinic acid or tormentic acid are superior to those from oleanolic, ursolic, glycyrrhetinic or euscaphic acid. Thus, a tormentic acid-derived **RhoB** conjugate **32**, holding a homopiperazinyl spacer can be regarded, at present, as the most promising candidate for further biological studies.

Keywords: triterpenoic acid; maslinic acid; tormentic acid; betulinic acid; oleanolic acid; rhodamine B; cytotoxicity

1. Introduction

Cancer remains one of the leading causes of death worldwide, and the incidence is increasing. Cancer is the second-leading cause of death globally, accounting for 9.6 million deaths in 2018 [1]. It is expected that in 2030, 21 million people worldwide will suffer from cancer [2]. Tremendous progress, however, has been made in the treatment of individual cancers [3–6]. This is due, on one hand, to improved early detection and prophylaxis and, on the other, to the development of highly efficient drugs in a wide range of different substance classes. Thus, the probability of premature death from cancer per year decreased from 8.3% in the year 2000 to 6.9% in 2015. It is expected to be as low as 5.3% in 2030, saving approximately 1.1 million lives per year [7].

Both proteins and small molecules have proven their worth in therapy and are in use in the clinic. However, low-molecular-weight drugs often bear the stigma of reduced selectivity in which the cytotoxic drug not only targets cancerous cells, but it also damages healthy tissue. This increases serious side effects and stress symptoms in patients, including nausea, heart or brain damage, impairments to the central nervous system and damage to cells of the inner ear; losses of fertility, hearing and hair have also been noted [8].

These serious side effects and impairments limit the use and acceptance of a drug, as they reduce patient compliance due to a significantly reduced quality of life. This not only endangers the chances of successful therapy, but it often also leads to discontinuation of the therapy [9,10].

Since the problems of reconversion of cancer cells into normal cells ("reprogramming", for example, of terminally differentiated cancer cells into cancer cells of benign phenotypes) [11] have only remotely

been solved today, cancer cells have to be removed either by surgery or destroyed by radiation or chemotherapy. Cell death by chemotherapy can be induced in many different ways [12–17], but the mitochondria play a major role in the life or death of a cell. Thus, agents that target mitochondria and induce a controlled cell death, so called "mitocans", have received increased attention in recent years [17–38]. This seems even more significant inasmuch as cancer cells are closely linked to dysregulated apoptosis of the cells; as a consequence, drug resistance of the cancer cells can develop [39].

Mitocans (as well as other cytotoxic agents) are often able to induce apoptosis; however, the death of a cell, irrespective of whether this cell is malignant, is not random at all. Triggering of controlled cell death is always preferable to an unselective rupture of membranes following the application of extreme but locally applied heat, freeze–thaw cycles or steep osmotic gradients. Controlled cell death can be triggered on a cellular level from nuclear, reticular, cytoskeletal, lysosomal, membrane or, most important, mitochondrial origins [16].

Usually, the cells in a living organism closely cooperate, and cells are constantly in an equilibrium between life and death. Triggering programmed cell death routines removes damaged, infected and out-of-control cells from the organism. The problem arises from the latter cells, since most cancer cells do not respond to extrinsic apoptotic triggers. Thus, mitochondria present a target of emerging interest for cancer therapy as they can trigger apoptosis through an intrinsic pathway. Apoptosis usually starts with loss of mitochondrial membrane potential, followed by the release of cytochrome c and activation of caspase 3 [40]. Furthermore, permeabilization of the outer mitochondrial membrane and the release of cytochrome c are required in many cell death stimuli [41]. This release of cytochrome c can be regarded as a "point of no return" finally leading to the death of the cell. This highlights the importance of mitochondrial compartmentation and explains the devastating effect following permeabilization of the mitochondrial membrane [42–44]. In addition to membrane permeabilization, the opening of the mitochondrial permeability transition pore is also considered an important event resulting in mitochondrial depolarization and the release of apoptotic factors [29].

In recent years, triterpenes have repeatedly and increasingly entered the focus of scientific interest. Extensive studies on their apoptotic and cytotoxic properties have been performed. A major concern in dealing with cancer is the MDR (multiple drug resistance) phenotype [39]. These cancer cells overexpress ATP-dependent transporters that eject toxic compounds from the cell before they cause harm to the cell. Some triterpenes are known inhibitors of the efflux pump MDR1, but they are also known to downregulate the transcription factor NF-κB. For cancer, it is widely accepted [45] that NF-κB promotes tumor migration and tumor proliferation.

2. Results

Mitochondrial membranes of malignant cells hold an increased membrane potential compared to non–malignant cells [46]. This effect fosters the accumulation of cationic molecules [17,47,48], hence inducing high selectivity for mitocans holding a (more or less) lipophilic cation such as a rhodamine scaffold. The same effect applies for triphenylphosphonium cations [49–57] and to a small extent for quaternary ammonium ions [58–60], zwitterionic *N*-oxides [60,61] and triterpenes substituted with BODIPYs [62–66] or a safirinium moiety [67]. However, the presence of a cationic center is not alone decisive for achieving high cytotoxic effects [60].

Rhodamine B (**RhoB**) seems to be a privileged scaffold. This fluorescent dye, also known as rhodamine 610, C.I. Pigment violet 1, basic violet 10, and C.I. 45170 [68], was invented in 1888 ("Tetraethyl-rhodamine") by M. Cérésole [69,70], and since then it has been widely used in biology, biotechnology and as a biosensor [71,72]. **RhoB** exists in an equilibrium [73–77] between an "open" positively charged form A (Figure 1) that is fluorescent and a "closed", non–fluorescent form B. Under acidic conditions, pink-colored A dominates, while colorless B dominates under basic conditions. Further, in less polar organic solvents, the zwitterionic form C undergoes a rapid reversible conversion to B [78–81].

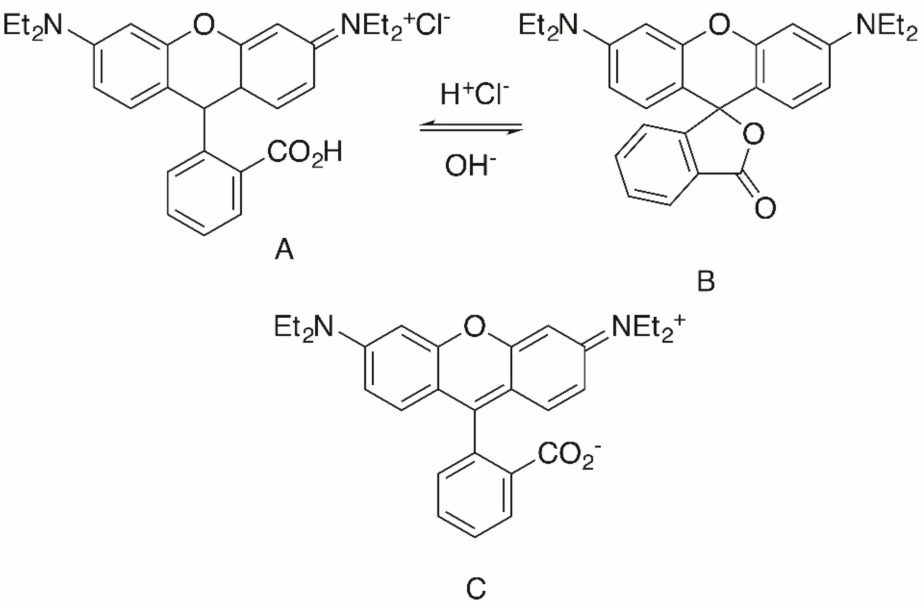

Figure 1. Structure of Rhodamine B (**Rho**B) in its "open" form **A**, "closed" lactone form **B** and the zwitterion **C**.

RhoB is suspected to be carcinogenic [82–85]. The LD_{50} value for orally administered RhoB in rats is >500 mg/kg, and an older report classified **RhoB** (as well as Rho6G) as possibly carcinogenic in rats [85]. **RhoB**, however, seems not to be mutagenic in Chinese hamster ovary cells [86], but it presents a genotoxic hazard for mammalian organisms [87]. As far as the **RhoB**–triterpene conjugates are concerned, two types of compounds have been accessed so far: triterpenes with a **RhoB** moiety directly attached to the skeleton of the triterpene, and compounds wherein these two units are separated by a suitable spacer.

To date, hybrid molecules have been prepared from oleanolic acid (**OA**, Figure 2), ursolic acid (**UA**), glycyrrhetinic acid (**GA**), betulinic acid (**BA**), maslinic acid (**MA**), augustic acid (**AU**), 11-keto-β-boswellic acid (**KBA**), asiatic acid (**AA**), tormentic acid (**TA**) and euscaphic acid (**EA**).

By means of suitable double-staining experiments, it could be shown that these hybrids are actually effective as mitocans [88], and preliminary molecular modeling studies suggest these compounds might target the mitochondrial NADH dehydrogenase and mitochondrial succinate dehydrogenase [89]. Both enzymes are part of the mitochondrial electron transport chain; this also suggests an increased production of reactive oxygen species (ROS). An increased production of ROS would lead to an oxidative damage of the cell and trigger apoptosis through an intrinsic pathway. Therefore, the integrity of the **RhoB** basic structure seems to be of crucial importance. It has been shown that derivatives from the triphenylmethane dye malachite green still exhibit increased cytotoxicity as compared to their parent compounds [90]. The cytotoxicity, however, of these hybrids was much lower than those observed for the **RhoB** derivatives (vide infra).

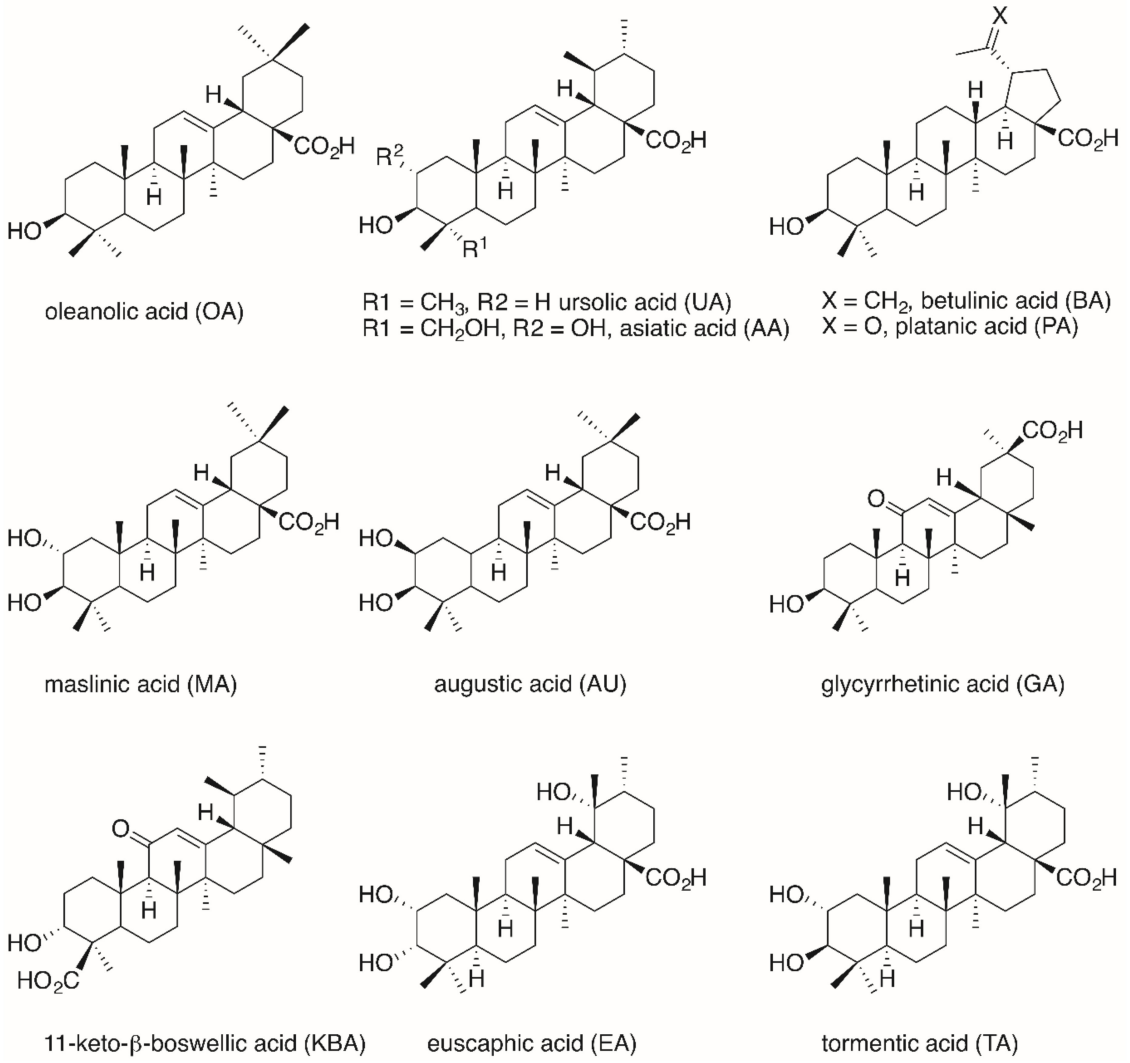

Figure 2. Structure of some important pentacyclic triterpenoic acids.

The triterpenoid skeleton is equally important. Here, too, it was shown that "simple" RhoB conjugates **1**–**9** (Figure 3) also had lower cytotoxicity than the corresponding triterpenoid analogs, but their tumor cell/non-tumor cell selectivity was also diminished (Table 1) [91].

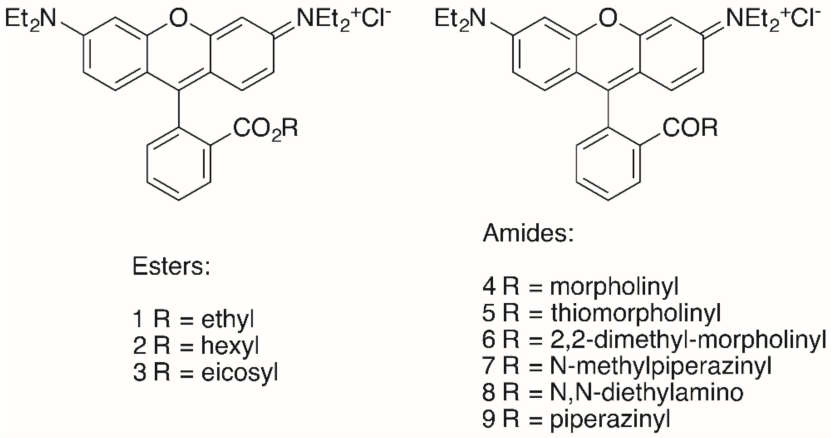

Figure 3. Structures of "simple" **RhoB** conjugates **1**–**9**.

Table 1. Cytotoxicity of selected "simple" **RhoB** conjugates.

Compound	A375	HT29	MCF-7	A2780	FaDu	NIH 3T3
RhoB	>30	>30	>30	>30	>30	>30
1	0.38	0.41	0.23	0.21	0.30	0.96
2	0.19	0.19	0.14	0.17	0.15	0.32
3	>30	>30	>30	>30	>30	>30
4	7.09	5.46	1.54	1.66	4.53	>30
5	1.79	1.54	0.44	0.52	1.12	5.09
6	3.05	1.74	0.49	0.70	1.52	7.92
7	16.05	17.34	3.74	3.62	11.78	>30
8	1.03	0.54	0.32	0.27	0.64	3.27
9	>30	>30	17.80	26.40	>30	>30

EC_{50} in μM from SRB assays; cut-off 30 μM.

Of special interest seems the morpholinyl derivative **4** inasmuch as this compound held the highest selectivity of this series with respect to MCF-7 carcinoma cells (S = ($EC_{50,\ NIH\ 3T3}$ / $EC_{50,\ MCF\text{-}7}$) > 19.5) and A2780 ovarian cancer cells (S = ($EC_{50,\ NIH\ 3T3}$ / $EC_{50,\ A27807}$) > 18.1) [90].

The highest cytotoxicity was observed for the hexyl ester **2** (EC_{50} = 0.15–0.19 μM) for the different tumor cell lines. Interestingly, an eicosyl ester **3** with a lipophilicity similar to that of triterpenoids did not show even moderate cytotoxicity [90], while hydroxycinnamic acid rhodamine B conjugates displayed good cytotoxicity in the low μM range [92].

The importance of the presence of a triterpenoid backbone is also evident from studies concerning RhoB steroid conjugates (Figure 4) [93]. In these studies, the reaction of the steroids cholesterol, testosterone, prednisone and abiraterone with an activated **RhoB** chloride furnished ester conjugates holding low EC_{50} values (SRB assays with several human tumor cell lines, Table 2). Thus, a testosterone conjugate **10** held EC_{50} = 60 nm for MCF-7 cells, but acted by necrosis (20%, A2780 cells). A prednisone conjugate **11** was less cytotoxic (0.2 μM for MCF-7 cells) but acted in A2780 cells mainly by apoptosis (48%) and late apoptosis (14%). In addition, this compound showed a higher selectivity for the A2780 tumor cells (S = 73) than for NIH 3T3 fibroblasts. For comparison, an abiratone conjugate **12** was less cytotoxic and also less selective [93].

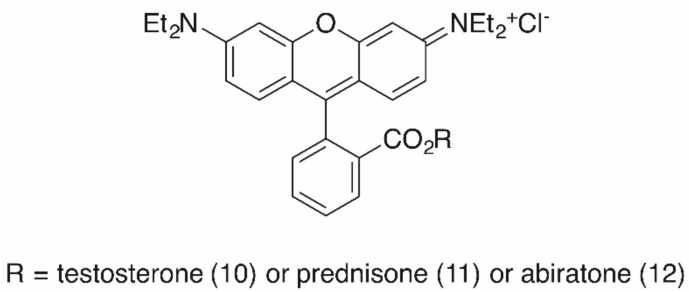

Figure 4. RhoB steroid conjugates from the esterification of RhoB with testosterone (→ **10**), prednisone (→ **11**) or abiratone (→ **12**), respectively.

Table 2. Cytotoxicity of selected steroidal **RhoB** conjugates.

Compound	A375	HT29	MCF-7	A2780	FaDu	NIH 3T3
10	0.16	0.12	0.06	0.08	0.26	0.25
11	0.11	0.64	0.21	0.31	0.40	1.81
12	0.22	0.21	0.23	0.13	0.24	0.37

EC_{50} in μM from SRB assays.

A closer look at the cell cycle by FACS (with A2780 cells) showed a decrease of the G1 and G2/M peak with an increase of cells in the S phase. For cells treated with **11,** the S phase peak and the

subG1/apoptosis peak increased significantly. However, for all compounds the selectivity between tumor cells and non-malignant fibroblasts NIH 3T3 was small and never exceeded 7.3 (**11**, for MCF-7 cells) [93].

A similar behavior was observed for dehydroabietylamine (DHAA) derivatives **13**–**16** (Figure 5, Table 3). These products were easily obtained from dehydroabietylamine by the microwave-assisted multicomponent Ugi reaction using paraformaldehyde, an isocyanide and **RhoB** with yields between 47 and 50% [94].

Table 3. Cytotoxicity of selected **DHAA**-derived **RhoB** conjugates.

Compound	A375	HT29	MCF-7	A2780	FaDu	NIH 3T3
13	3.2	0.18	0.10	0.37	0.23	0.28
14	0.23	0.32	0.16	0.57	0.35	0.41
15	0.20	0.28	0.12	0.66	0.32	0.44
16a/16b	>30	>30	>30	>30	>30	>30

EC_{50} in μM from SRB assays.

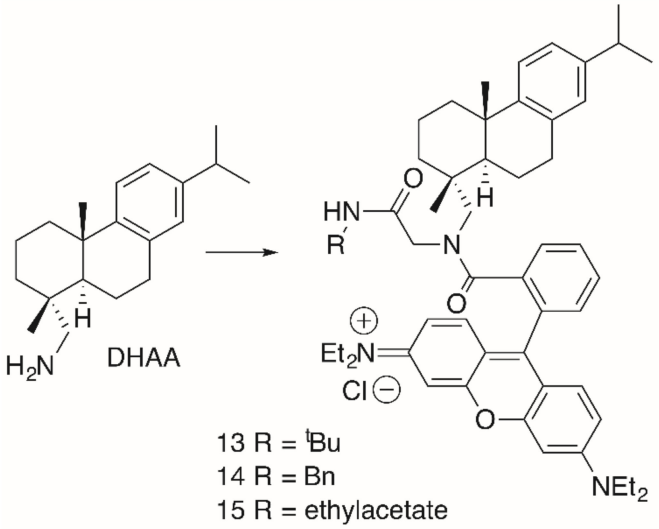

Figure 5. Dehydroabiethylamine (**DHAA**)-derived **RhoB** conjugates **13**–**15** obtained by Ugi-multi component reactions.

Although the cytotoxicity of these compounds was good, their pharmacological potential was restricted by low selectivity values. Interestingly enough, products **16a**/**16b** (Figure 6), having been obtained from a simple Schotten–Baumann reaction with **DHAA** and **RhoB,** were not cytotoxic at all [94]. As mentioned above, **RhoB** conjugates derived from primary amines are able to form intramolecular non-fluorescent spirolactams (here **16a**). From a photo-induced ring opening reaction, **16b** was obtained from **16a** very quickly within 10 s of irradiation either with visible light or with UV light (λ = 254 or 366 nm). This equilibrium is also strongly influenced by changes in temperature, and at room temperature **16a** dominates the equilibrium [94].

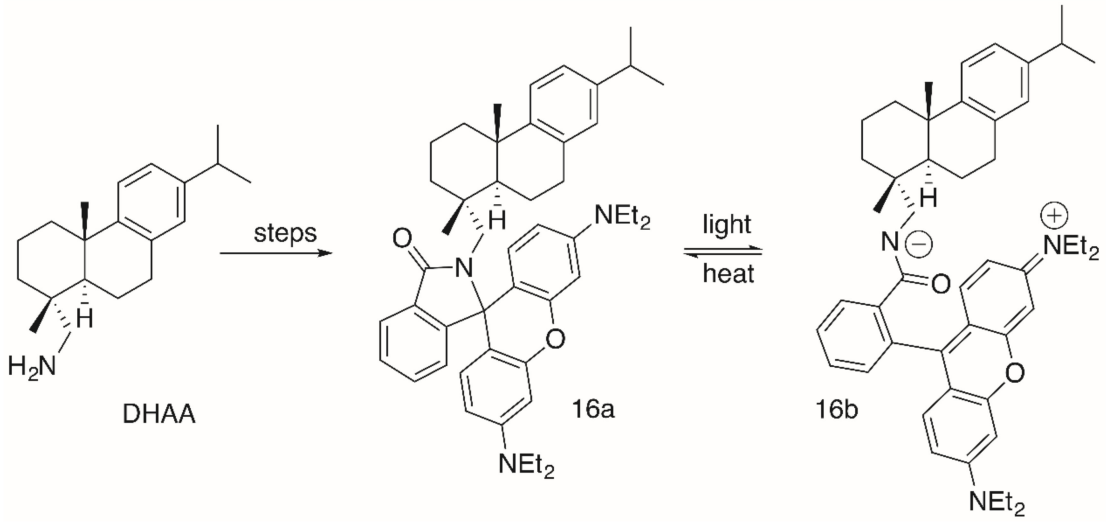

Figure 6. Synthesis of **DHAA**-derived **RhoB** conjugates **16a**/**16b** and their equilibrium.

As far as the triterpene **RhoB** conjugates are concerned, the **RhoB** moiety can be attached to the triterpenoid scaffold either directly (e.g., in form of a triterpene **RhoB** ester) or with the aid of a suitable spacer. Pentacyclic triterpenoic acids (Figure 2) holding an **RhoB** moiety without an extra spacer have been prepared by esterification of **UA**, **OA**, **GA** and **BA** with **RhoB**, respectively, (Figure 7) [95].

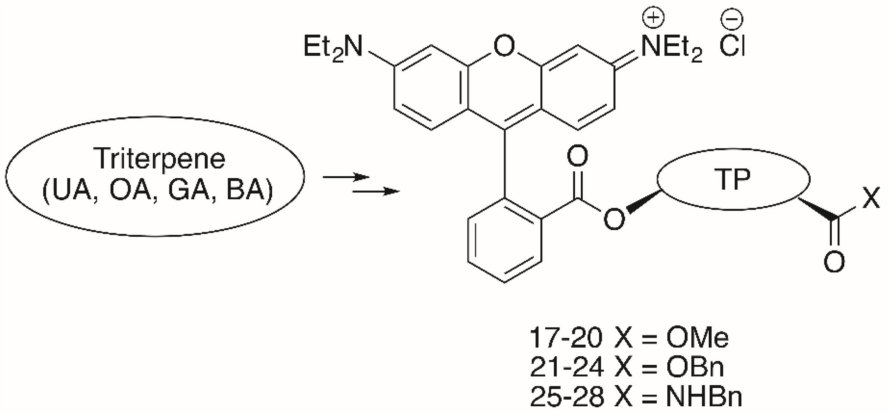

Figure 7. Un-spacered **UA-**, **OA-, GA-** and **BA**-derived esters **17**–**24** and amides **25**–**28**.

All of these compounds had EC_{50} values between 0.02 and 15.8 μM (Table 4); thereby, the cytotoxicity of benzyl esters **21**–**24** was lower than the cytotoxicity of the methyl esters **17**–**20**, while the benzyl amides **25**–**28** were the most cytotoxic compounds of this series. The presence of a benzyl ester group as in **21**–**24** seems to be disadvantageous, while the opposite is true for the benzyl amides **25**–**28**. Compound **27** was the most cytotoxic compound (EC_{50} = 0.02–0.08 μM), but it was not selective for human tumor cells. Extra staining experiments showed this compound to be accumulated in the mitochondria of A2780 cells and to act mainly by apoptosis [95].

Table 4. Cytotoxicity of un-spacered esters **UA-**, **OA-**, **GA-** and **BA**-derived esters **17**–**24** and amides **25**–**28**.

Compound	TP	R	FaDu	A2780	HT29	MCF-7	SW1736	NIH 3T3
17	UA	OMe	1.96	1.75	1.85	1.83	1.72	1.84
18	OA	OMe	1.99	1.14	2.75	2.31	1.76	2.63
19	GA	OMe	0.19	0.08	0.15	0.18	0.15	0.20
20	BA	OMe	1.29	0.42	0.61	0.81	0.74	1.77
21	UA	OBn	15.79	10.10	11.41	13.75	12.66	15.42
22	OA	OBn	9.12	3.35	8.90	9.40	9.05	11.25
23	GA	OBn	1.54	0.90	1.42	1.47	1.13	1.28
24	BA	OBn	7.59	3.36	5.33	5.05	6.43	8.04
25	UA	NBn	0.44	0.34	0.45	0.30	0.24	0.37
26	OA	NBn	0.50	0.32	0.46	0.36	0.27	0.40
27	GA	NBn	0.06	0.02	0.06	0.04	0.04	0.08
28	BA	NBn	0.54	0.31	0.53	0.47	0.45	0.54

EC_{50} in μM from SRB assays.

Noteworthy in this context is the higher cytotoxicity of the glycyrrhetinic acid derivatives as compared to analogs derived from **OA**, **UA** or **BA**. Extensions in the design of these compounds led to the synthesis of triterpene conjugates with further modifications in the backbone (→ tormentic acid (**TA**) and euscaphic acid (**EA**)) as well as to changes in the ring size of the heterocyclic spacer between the backbone of the triterpene and the **RhoB** moiety (Figure 8).

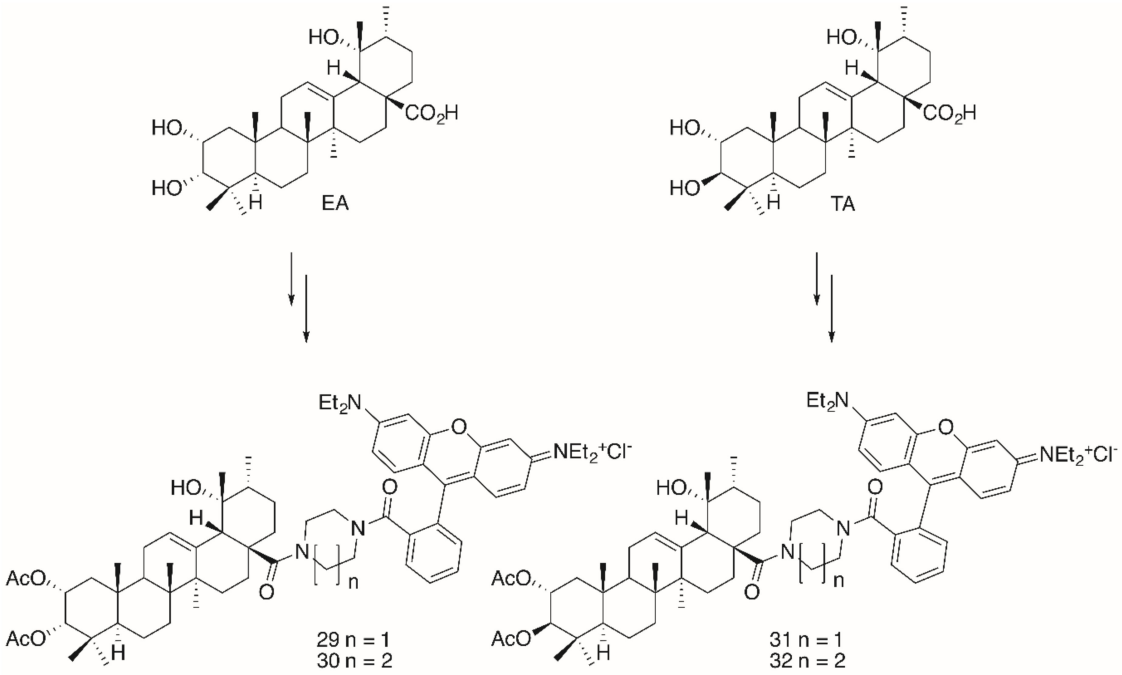

Figure 8. Synthesis of euscaphic (**EA**)- or tormentic acid (**TA**)-derived **RhoB** conjugates **29**–**32**.

The significantly higher cytotoxicity (Table 5) of **TA-**derived **32** seems particularly noteworthy when comparing the different spacers: Thereby, the presence of a homopiperazinyl spacer [96] (as in **32**) proved to be clearly superior to the piperazinyl moiety (as in **31**). A similar trend was also noted for **EA-**derived compounds **29** and **30**. On the other hand, **TA-**derived compounds were more cytotoxic than the corresponding **EA** derivatives. Interestingly, the absolute configuration at C–2 and C–3 in **TA** corresponds exactly to the configuration found in maslinic acid (**MA**). Several **MA** derivatives (for example [97,98], a diacetylated benzylamide **EM2**, Figure 9) were of higher cytotoxicity and better selectivity than their corresponding **OA** or **UA** derivatives.

Table 5. Cytotoxicity of euscaphic (**EA**)- or tormentic acid (**TA**)-derived **RhoB** conjugates **29**–**32**, asiatic acid (**AA**)-derived **33** and maslinic acid (**MA**)-derived amide **EM2**.

Compound	A375	HT29	MCF-7	A2780	FaDu	NIH 3T3
29	0.19	0,19	0.094	0.066	0.074	0.21
30	0.012	0.012	0.022	0.004	0.004	0.164
31	0.14	0.16	0.0084	0.037	0.041	0.25
32	0.06	0.005	0.008	0.001	0.001	0.19
33	n.d.	0.017	0.012	0.008	n.d.	0.178
EM2	n.d.	4.70	7.70	0.50	n.d.	33.8

EC_{50} in μM from SRB assays.

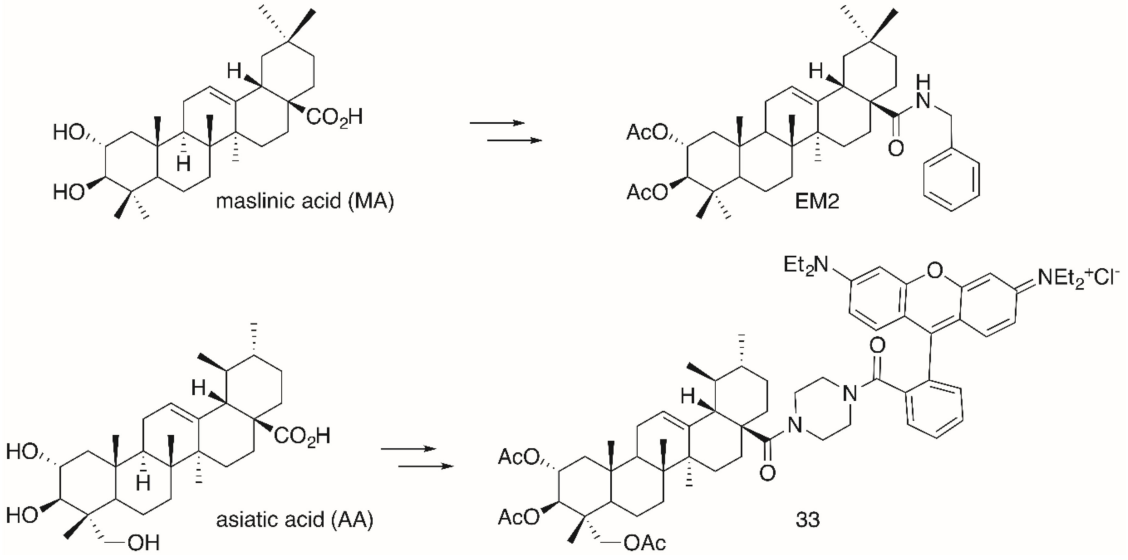

Figure 9. Synthesis of maslinic acid (**MA**)-derived **EM2** holding the same absolute configuration of hydroxyl groups in ring A as asiatic acid (**AA**)-derived conjugate **33**.

The same configuration is found in asiatic acid (**AA**). Again, its acetylated piperazinyl-rhodamine B conjugate **33** was most cytotoxic to many human tumor cell lines, being accumulated in the mitochondria, and it also acted as a mitocan [99]. However, for this compound an unusual non-linear rate of growth was detected for some human tumor cell lines (e.g., colorectal carcinoma HT29 and melanoma 518A2). In a bimodal manner at two different concentrations the tumor cells were killed, a phenomenon that might be due to an accelerated recovery of the mitochondrial membrane potential or due to a modulation of the mitochondrial permeability pores. However, at present a concentration-triggered activation of a metabolizing enzyme cannot completely be ruled out [99].

A graphical comparison of all derivatives (using the target line A2780 as an example) is given in Figure 10 including a comparison of tumor cell/non-tumor cell selectivity (A2780 vs. NIH 3T3) of all compounds.

From Figure 10 the high potential of compound **32** (selectivity for A2780 or FaDu cells, ca. 190) becomes clearly visible, making this compound an interesting drug for advanced testing and biological screening.

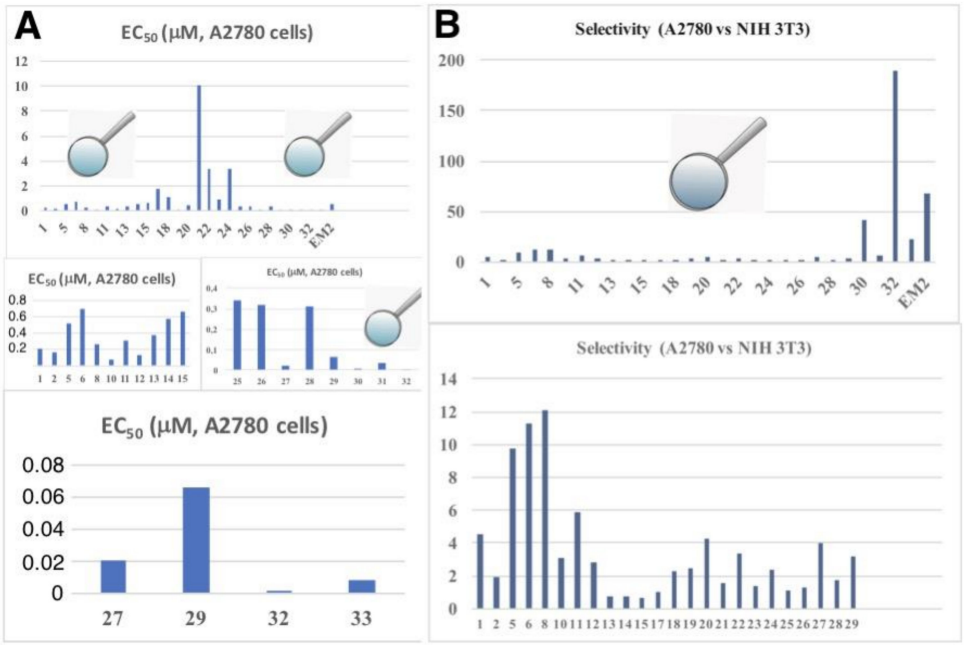

Figure 10. Graphical representation of the cytotoxicity of all compounds (EC_{50} in µM) for the cell line A2780 (**A**) combined with a comparison of tumor cell/non-tumor cell selectivity (A2780 vs. NIH 3T3, selectivity = ($EC_{50,\ NIH3T3}/EC_{50,\ A2780}$)) of all compounds (**B**).

3. Conclusions

OA-derived **RhoB** conjugates appear to be superior to analog **UA**-derived compounds in the majority of cases with respect to their cytotoxicity. Although **AKBA**-derived derivatives have good cytotoxicity properties, they were found to be less cytotoxic compared to other triterpene carboxylic acid derivatives, but they often showed better tumor cell/non-tumor cell selectivity. So far, the best cytotoxicity properties have been found for **MA-**, **EA-** and **TA-**derived derivatives. These allowed the transition to compounds of nano-molar activity, while many other triterpene carboxylic acid derivatives were cytotoxic only on a micro-molar concentration range. **MA-** derived derivatives seem to be approximately equivalent to **EA-**derived compounds. They are currently only surpassed in many tumor cell lines only by the analogous derivatives from **TA**. From results available so far, it can be concluded that compounds holding a homopiperazinyl spacer are superior to those with a piperazinyl spacer. This underlines the importance of the spacer for obtaining good cytotoxicity properties. Replacement of the secondary amide derived spacer by a primary amine like ethylenediamine has invariably led to **RhoB** conjugates of insignificant cytotoxicity (EC_{50} > 30 µM) due to the formation of a spirolactam holding no positive charge in the RhoB part.

However, the presence of a distal cation is not sufficient to obtain compounds with excellent cytotoxicity, as has been shown for several quaternary ammonium compounds or compounds where the **RhoB** part has been replaced by, for example, malachite green, a BODIPY residue or a safirinium group. In addition, the latter compounds do not act as mitocans, since their primary target is the endoplasmic reticulum.

A statement on the extent to which the replacement of the **RhoB** group with another rhodamine has a positive effect on biological activity cannot be made at present. The cytotoxic properties of these compounds, other spacers and other triterpene carboxylic acids are currently the subject of further investigation. The combination of the "correct" parent structure, the "correct" spacer and the "correct" **RhoB** seems to be decisive for the ability of the conjugate to accumulate in mitochondria. So far, a tormentic acid acid-derived **RhoB** conjugate **32** holding a homopiperazinyl spacer can be regarded as the most promising candidate for further biological studies. At present, no extended investigations have been carried out on the precise mode of action of these molecules.

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References

1. Available online: https://www.who.int/health-topics/cancer-tab=tab_1 (accessed on 20 October 2020).
2. Bray, F.; Jemal, A.; Grey, N.; Ferlay, J.; Forman, D. Global cancer transitions according to the Human Development Index (2008–2030): A population-based study. *Lancet Oncol.* **2012**, *13*, 790–801. [CrossRef]
3. Siegel, R.L.; Mph, K.D.M.; Sauer, A.G.; Fedewa, S.A.; Butterly, L.F.; Anderson, J.C.; Cercek, A.; Smith, R.A.; Jemal, A. Colorectal cancer statistics, 2020. *CA: A Cancer J. Clin.* **2020**, *70*, 145–164. [CrossRef] [PubMed]
4. Markham, M.J.; Wachter, K.; Agarwal, N.; Bertagnolli, M.M.; Chang, S.M.; Dale, W.; Diefenbach, C.S.M.; Rodriguez-Galindo, C.; George, D.J.; Gilligan, T.D.; et al. Clinical Cancer Advances 2020: Annual Report on Progress Against Cancer From the American Society of Clinical Oncology. *J. Clin. Oncol.* **2020**, *38*, 1081. [CrossRef] [PubMed]
5. Oostveen, M.; Pritchard-Jones, K. Pharmacotherapeutic Management of Wilms Tumor: An Update. *Pediatr. Drugs* **2019**, *21*, 1–13. [CrossRef]
6. Di Paolo, A.; Arrigoni, E.; Luci, G.; Cucchiara, F.; Danesi, R.; Galimberti, S. Precision Medicine in Lymphoma by Innovative Instrumental Platforms. *Front. Oncol.* **2019**, *9*, 1417. [CrossRef]
7. Available online: https://www.paho.org/hq/index.php?option=com_docman&view=download&category_slug=4-cancer-country-profiles-2020&alias=51561-global-cancer-profile-2020&Itemid=270&lang=fr (accessed on 20 October 2020).
8. Green, D.R. Cancer and Apoptosis: Who Is Built to Last? *Cancer Cell* **2017**, *31*, 2–4. [CrossRef]
9. Pretta, A.; Trevisi, E.; Bregni, G.; Deleporte, A.; Hendlisz, A.; Sclafani, F. Treatment compliance in early-stage anal cancer. *Ann. Oncol.* **2020**, *31*, 1282–1284. [CrossRef]
10. Muzumder, S.; Srikantia, N.; Vashishta, G.D.; Udayashankar, A.H.; Raj, J.M.; Sebastian, M.G.J.; Kainthaje, P.B. Compliance, toxicity and efficacy in weekly versus 3-weekly cisplatin concurrent chemoradiation in locally advanced head and neck cancer. *J. Radiother. Pr.* **2018**, *18*, 21–25. [CrossRef]
11. Gong, L.; Yan, Q.; Zhang, Y.; Fang, X.; Liu, B.; Guan, X.-Y. Cancer cell reprogramming: A promising therapy converting malignancy to benignity. *Cancer Commun.* **2019**, *39*, 1–13. [CrossRef]
12. Fuchs, Y.; Steller, H. Live to die another way: Modes of programmed cell death and the signals emanating from dying cells. *Nat. Rev. Mol. Cell Biol.* **2015**, *16*, 329–344. [CrossRef]
13. Wang, C.X.; Youle, R.J. The Role of Mitochondria in Apoptosis. *Annu. Rev. Genet.* **2009**, *43*, 95–118. [CrossRef] [PubMed]
14. Johnstone, R.W.; Ruefli, A.A.; Lowe, S.W. Apoptosis: A link between cancer genetics and chemotherapy. *Cell* **2002**, *108*, 153–164. [CrossRef]
15. Fiers, W.; Beyaert, R.; Declercq, W.; Vandenabeele, P. More than one way to die: Apoptosis, necrosis and reactive oxygen damage. *Oncogene* **1999**, *18*, 7719–7730. [CrossRef] [PubMed]
16. Galluzzi, L.; Pedro, J.M.B.-S.; Kroemer, G. Organelle-specific initiation of cell death. *Nat. Cell Biol.* **2014**, *16*, 728–736. [CrossRef] [PubMed]
17. Bing, Y.; Dong, L.; Neuzil, J. Mitochondria: An intriguing target for killing tumour-initiating cells. *Mitochondrion* **2016**, *26*, 86–93. [CrossRef]
18. Pei, J.; Panina, S.B.; Kirienko, N.V. An Automated Differential Nuclear Staining Assay for Accurate Determination of Mitocan Cytotoxicity. *J. Vis. Exp.* **2020**, *159*, e61295. [CrossRef]
19. Panina, S.B.; Pei, J.; Baran, N.; Konopleva, M.; Kirienko, N.V. Utilizing Synergistic Potential of Mitochondria-Targeting Drugs for Leukemia Therapy. *Front. Oncol.* **2020**, *10*, 435. [CrossRef]
20. Ralph, S.J.; Neuzil, J. Mitochondria as Targets for Cancer Therapy. In *Mitochondria and Cancer*; Costello, L., Singh, K., Eds.; Springer: New York, NY, USA, 2009; pp. 211–249.

21. Ralph, S.J.; Rodríguez-Enríquez, S.; Neuzil, J.; Moreno-Sánchez, R. Bioenergetic pathways in tumor mitochondria as targets for cancer therapy and the importance of the ROS-induced apoptotic trigger. *Mol. Asp. Med.* **2010**, *31*, 29–59. [CrossRef]
22. Ralph, S.J.; Rodríguez-Enríquez, S.; Neuzil, J.; Saavedra, E.; Moreno-Sánchez, R. The causes of cancer revisited: "Mitochondrial malignancy" and ROS-induced oncogenic transformation – Why mitochondria are targets for cancer therapy. *Mol. Asp. Med.* **2010**, *31*, 145–170. [CrossRef] [PubMed]
23. Chiu, H.Y.; Tay, E.X.Y.; Ong, D.S.T.; Taneja, R. Mitochondrial Dysfunction at the Center of Cancer Therapy. *Antioxidants Redox Signal.* **2020**, *32*, 309–330. [CrossRef]
24. Singh, Y.; Viswanadham, K.K.D.R.; Pawar, V.K.; Meher, J.; Jajoriya, A.K.; Omer, A.; Jaiswal, S.; Dewangan, J.; Bora, H.K.; Singh, P.; et al. Induction of Mitochondrial Cell Death and Reversal of Anticancer Drug Resistance via Nanocarriers Composed of a Triphenylphosphonium Derivative of Tocopheryl Polyethylene Glycol Succinate. *Mol. Pharm.* **2019**, *16*, 3744–3759. [CrossRef] [PubMed]
25. Nguyen, C.; Pandey, S. Exploiting Mitochondrial Vulnerabilities to Trigger Apoptosis Selectively in Cancer Cells. *Cancers* **2019**, *11*, 916. [CrossRef] [PubMed]
26. Olivas-Aguirre, M.; Pottosin, I.; Dobrovinskaya, O. Mitochondria as emerging targets for therapies against T cell acute lymphoblastic leukemia. *J. Leukoc. Biol.* **2019**, *105*, 935–946. [CrossRef]
27. Sassi, N.; Biasutto, L.; Mattarei, A.; Carraro, M.; Giorgio, V.; Citta, A.; Bernardi, P.; Garbisa, S.; Szabò, I.; Paradisi, C.; et al. Cytotoxicity of a mitochondriotropic quercetin derivative: Mechanisms. *Biochim. et Biophys. Acta (BBA) - Gen. Subj.* **2012**, *1817*, 1095–1106. [CrossRef] [PubMed]
28. Sassi, N.; Mattarei, A.; Azzolini, M.; Bernardi, P.; Szabò, I.; Paradisi, C.; Zoratti, M.; Biasutto, L. Mitochondria-targeted resveratrol derivatives act as cytotoxic pro-oxidants. *Curr. Pharm. Des.* **2014**, *20*, 172–179. [CrossRef]
29. Chen, G.; Wang, F.; Trachootham, D.; Huang, P. Preferential killing of cancer cells with mitochondrial dysfunction by natural compounds. *Mitochondrion* **2010**, *10*, 614–625. [CrossRef]
30. Guzman-Villanueva, D.; Weissig, V. Mitochondria-Targeted Agents: Mitochondriotropics, Mitochondriotoxics, and Mitocans. *Muscarinic Receptors* **2016**, *240*, 423–438. [CrossRef]
31. Rohlena, J.; Dong, L.-F.; Neuzil, J. Targeting the mitochondrial electron transport chain complexes for the induction of apoptosis and cancer treatment. *Curr. Pharm. Biotechnol.* **2013**, *14*, 377–389. [CrossRef]
32. Rohlena, J.; Dong, L.; Ralph, S.J.; Neuzil, J. Anticancer Drugs Targeting the Mitochondrial Electron Transport Chain. *Antioxidants Redox Signal.* **2011**, *15*, 2951–2974. [CrossRef]
33. Kornblihtt, L.I.; Carreras, M.C.A.; Blanco, G. Targeting Mitophagy in Combined Therapies of Haematological Malignancies. *Autophagy Curr. Trends Cell. Physiol. Pathol.* **2016**, *19*, 411–431. [CrossRef]
34. Neuzil, J.; Dong, L.-F.; Rohlena, J.; Truksa, J.; Ralph, S.J. Classification of mitocans, anti-cancer drugs acting on mitochondria. *Mitochondrion* **2013**, *13*, 199–208. [CrossRef] [PubMed]
35. Neuzil, J.; Dyason, J.C.; Freeman, R.; Dong, L.; Prochazka, L.; Wang, X.-F.; Scheffler, I.; Ralph, S.J. Mitocans as anti-cancer agents targeting mitochondria: Lessons from studies with vitamin E analogues, inhibitors of complex II. *J. Bioenerg. Biomembr.* **2007**, *39*, 65–72. [CrossRef] [PubMed]
36. Neuzil, J.; Wang, X.-F.; Dong, L.-F.; Low, P.; Ralph, S.J. Molecular mechanism of 'mitocan'-induced apoptosis in cancer cells epitomizes the multiple roles of reactive oxygen species and Bcl-2 family proteins. *FEBS Lett.* **2006**, *580*, 5125–5129. [CrossRef] [PubMed]
37. Morrison, B.J.; Andera, L.; Reynolds, B.A.; Ralph, S.J.; Neuzil, J. Future use of mitocans against tumour-initiating cells? *Mol. Nutr. Food Res.* **2009**, *53*, 147–153. [CrossRef] [PubMed]
38. Hahn, T.; Polanczyk, M.J.; Borodovsky, A.; Ramanathapuram, L.V.; Akporiaye, E.T.; Ralph, S.J. Use of anti-cancer drugs, mitocans, to enhance the immune responses against tumors. *Curr. Pharm. Biotechnol.* **2013**, *14*, 357–376. [CrossRef] [PubMed]
39. Amaral, L.; Spengler, G.; Molnar, J. Identification of Important Compounds Isolated from Natural Sources that Have Activity Against Multidrug-resistant Cancer Cell Lines: Effects on Proliferation, Apoptotic Mechanism and the Efflux Pump Responsible for Multi-resistance Phenotype. *Anticancer. Res.* **2016**, *36*, 5665–5672. [CrossRef]
40. Abdelmageed, N.; Morad, S.A.; Elghoneimy, A.A.; Syrovets, T.; Simmet, T.; El-Zorba, H.; El-Banna, H.A.; Cabot, M.; Abdel-Aziz, M.I. Oleanolic acid methyl ester, a novel cytotoxic mitocan, induces cell cycle arrest and ROS-Mediated cell death in castration-resistant prostate cancer PC-3 cells. *Biomed. Pharmacother.* **2017**, *96*, 417–425. [CrossRef]

41. Bhola, P.D.; Letai, A. Mitochondria—Judges and Executioners of Cell Death Sentences. *Mol. Cell* **2016**, *61*, 695–704. [CrossRef]
42. Jacotot, E.E.; Ferri, K.K.; El Hamel, C.C.; Brenner, C.C.; Druillennec, S.S.; Hoebeke, J.; Rustin, P.P.; Métivier, D.D.; Lenoir, C.C.; Geuskens, M.; et al. Control of Mitochondrial Membrane Permeabilization by Adenine Nucleotide Translocator Interacting with HIV-1 Viral Protein R and Bcl-2. *J. Exp. Med.* **2001**, *193*, 509–520. [CrossRef]
43. Zamzami, N.; Kroemer, G. The mitochondrion in apoptosis: How Pandora's box opens. *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 67–71. [CrossRef]
44. Zamzami, N.; Maisse, C.; Métivier, D.; Kroemer, G. Chapter 8 Measurement of membrane permeability and permeability transition of mitochondria. *Echinoderms Part B* **2001**, *65*, 147–158. [CrossRef]
45. Yang, H.; Dou, Q.P. Targeting apoptosis pathway with natural terpenoids: Implications for treatment of breast and prostate cancer. *Curr. Drug Targets* **2010**, *11*, 733–744. [CrossRef] [PubMed]
46. Fulda, S.; Kroemer, G. Mitochondria as Therapeutic Targets for the Treatment of Malignant Disease. *Antioxidants Redox Signal.* **2011**, *15*, 2937–2949. [CrossRef] [PubMed]
47. Serafim, T.L.; Carvalho, F.S.; Pereira, G.; Greene, A.; Perkins, E.; Holy, J.; Krasutsky, D.A.; Kolomitsyna, O.N.; Krasutsky, P.A.; Oliveira, P.J. Lupane triterpenoids as mitocans against breast cancer cells. *Eur. J. Clin. Invest.* **2013**, *43*, 48–49.
48. Serafim, T.L.; Carvalho, F.S.; Bernardo, T.C.; Pereira, G.C.; Perkins, E.; Holy, J.; Krasutsky, D.A.; Kolomitsyna, O.N.; Krasutsky, P.A.; Oliveira, P.J. New derivatives of lupine triterpenoids disturb breast cancer mitochondria and induce cell death. *Bioorg. Med. Chem.* **2014**, *22*, 6270–6287. [CrossRef] [PubMed]
49. Grymel, M.; Zawojak, M.; Adamek, J. Triphenylphosphonium Analogues of Betulin and Betulinic Acid with Biological Activity: A Comprehensive Review. *J. Nat. Prod.* **2019**, *82*, 1719–1730. [CrossRef]
50. Kolevzon, N.; Kuflik, U.; Shmuel, M.; Benhamron, S.; Ringel, I.; Yavin, E. Multiple Triphenylphosphonium Cations as a Platform for the Delivery of a Pro-Apoptotic Peptide. *Pharm. Res.* **2011**, *28*, 2780–2789. [CrossRef]
51. Spivak, A.Y.; Nedopekina, D.A.; Khalitova, R.R.; Gubaidullin, R.; Odinokov, V.N.; Bel'Skii, Y.P.; Bel'Skaya, N.V.; Khazanov, V.A. Triphenylphosphonium cations of betulinic acid derivatives: Synthesis and antitumor activity. *Med. Chem. Res.* **2017**, *26*, 518–531. [CrossRef]
52. Akhmetova, V.R.; Nedopekina, D.A.; Shakurova, E.R.; Khalitova, R.R.; Gubaidullin, R.R.; Odinokov, V.N.; Dzhemilev, U.M.; Bel'Skii, Y.P.; Bel'Skaya, N.V.; Stankevich, S.A.; et al. Synthesis of lupane triterpenoids with triphenylphosphonium substituents and studies of their antitumor activity. *Russ. Chem. Bull.* **2013**, *62*, 188–198. [CrossRef]
53. Huang, H.; Wu, H.; Huang, Y.; Zhang, S.; Lam, Y.-W.; Ao, N. Antitumor activity and antitumor mechanism of triphenylphosphonium chitosan against liver carcinoma. *J. Mater. Res.* **2018**, *33*, 2586–2597. [CrossRef]
54. Strobykina, I.Y.; Andreeva, O.V.; Belenok, M.G.; Semenova, M.N.; Semenov, V.V.; Chuprov-Netochin, R.N.; Sapunova, A.S.; Voloshina, A.D.; Dobrynin, A.B.; Semenov, V.E.; et al. Triphenylphosphonium conjugates of 1,2,3-triazolyl nucleoside analogues. Synthesis and cytotoxicity evaluation. *Med. Chem. Res.* **2020**, *29*, 1–15. [CrossRef]
55. Jara, J.A.; Castro-Castillo, V.; Saavedra-Olavarría, J.; Peredo, L.; Pavanni, M.; Jaña, F.; Letelier, M.E.; Parra, E.; Becker, M.I.; Morello, A.; et al. Antiproliferative and Uncoupling Effects of Delocalized, Lipophilic, Cationic Gallic Acid Derivatives on Cancer Cell Lines. Validation in Vivo in Singenic Mice. *J. Med. Chem.* **2014**, *57*, 2440–2454. [CrossRef] [PubMed]
56. Kafkova, A.; Trnka, J. Mitochondria-targeted compounds in the treatment of cancer. *Neoplasma* **2020**, *67*, 450–460. [CrossRef] [PubMed]
57. Ye, Y.; Zhang, T.; Yuan, H.; Li, D.; Lou, H.; Fan, P. Mitochondria-Targeted Lupane Triterpenoid Derivatives and Their Selective Apoptosis-Inducing Anticancer Mechanisms. *J. Med. Chem.* **2017**, *60*, 6353–6363. [CrossRef]
58. Sarek, J.; Biedermann, D.; Eignerova, B.; Hajduch, M. Synthesis and Evaluation of Biological Activity of the Quaternary Ammonium Salts of Lupane-, Oleanane-, and Ursane-Type Acids. *Synthesis* **2010**, *22*, 3839–3848. [CrossRef]
59. Kataev, V.E.; Strobykina, I.Y.; Zakharova, L.Y. Quaternary ammonium derivatives of natural terpenoids. Synthesis and properties. *Russ. Chem. Bull.* **2014**, *63*, 1884–1900. [CrossRef]
60. Brandes, B.; Koch, L.; Hoenke, S.; Deigner, H.-P.; Csuk, R. The presence of a cationic center is not alone decisive for the cytotoxicity of triterpene carboxylic acid amides. *Steroids* **2020**, *163*, 108713. [CrossRef]

61. Perreault, M.; Maltais, R.; Dutour, R.; Poirier, D. Explorative study on the anticancer activity, selectivity and metabolic stability of related analogs of aminosteroid RM-133. *Steroids* **2016**, *115*, 105–113. [CrossRef]
62. Brandes, B.; Hoenke, S.; Fischer, L.; Csuk, R. Design, synthesis and cytotoxicity of BODIPY FL labelled triterpenoids. *Eur. J. Med. Chem.* **2020**, *185*, 111858. [CrossRef]
63. Barut, B.; Çoban, Ö.; Özgür, Y.C.; Baş, H.; Sari, S.; Biyiklioglu, Z.; Demirbaş, Ü.; Özel, A. Synthesis, DNA interaction, in vitro/in silico topoisomerase II inhibition and photodynamic therapy activities of two cationic BODIPY derivatives. *Dye. Pigment.* **2020**, *174*, 108072. [CrossRef]
64. Barut, B.; Özgür, Y.C.; Sari, S.; Çoban, Ö.; Keleş, T.; Biyiklioglu, Z.; Abudayyak, M.; Demirbaş, Ü.; Özel, A. Novel water soluble BODIPY compounds: Synthesis, photochemical, DNA interaction, topoisomerases inhibition and photodynamic activity properties. *Eur. J. Med. Chem.* **2019**, *183*, 11685. [CrossRef] [PubMed]
65. Taki, S.; Ardestani, M.S. Novel nanosized AS1411-chitosan-BODIPY conjugate for molecular fluorescent imaging. *Int. J. Nanomed.* **2019**, *14*, 3543–3555. [CrossRef] [PubMed]
66. Li, M.; Li, X.; Cao, Z.; Wu, Y.; Chen, J.-A.; Gao, J.; Wang, Z.; Guo, W.; Gu, X. Mitochondria-targeting BODIPY-loaded micelles as novel class of photosensitizer for photodynamic therapy. *Eur. J. Med. Chem.* **2018**, *157*, 599–609. [CrossRef] [PubMed]
67. Kraft, O.; Kozubek, M.; Hoenke, S.; Serbian, I.; Major, D.; Csuk, R. Cytotoxic triterpenoid–safirinium conjugates target the endoplasmic reticulum. *Eur. J. Med. Chem.* **2020**, 112920. [CrossRef] [PubMed]
68. Cooksey, C. Quirks of dye nomenclature. 5. Rhodamines. *Biotech. Histochem.* **2015**, *91*, 1–6. [CrossRef]
69. Ceresole, M. Rhodamin Dye. U.S. Patent 516589 A 18940313, 13 March 1894.
70. Ceresole, M. Red Dyestuff. U.S. Patent 456081 18910714, 14 July 1891.
71. Kim, H.N.; Lee, M.H.; Kim, H.J.; Kim, J.S.; Yoon, J. A new trend in rhodamine-based chemosensors: Application of spirolactam ring-opening to sensing ions. *Chem. Soc. Rev.* **2008**, *37*, 1465–1472. [CrossRef]
72. Wang, D.; Wang, Z.; Li, Y.; Song, Y.; Song, Y.; Zhang, M.; Yu, H. A single rhodamine spirolactam probe for localization and pH monitoring of mitochondrion/lysosome in living cells. *New J. Chem.* **2018**, *42*, 11102–11108. [CrossRef]
73. Adamovich, L.P.; Mel'nik, V.V.; McHedlov-Petrosyan, N.O. Rhodamine B equilibriums in water-salt solutions. *Zh. Fiz. Khim.* **1979**, *53*, 356–359.
74. Hinckley, D.A.; Seybold, P.G. Thermodynamics of the rhodamine B lactone zwitterion equilibrium: An undergraduate laboratory experiment. *J. Chem. Educ.* **1987**, *64*, 362. [CrossRef]
75. Ramart-Lucas, P. Structure of rhodamine from its absorption spectrum. *Compt. Rend.* **1938**, *207*, 1416–1418.
76. Ramette, R.W.; Blackburn, T.R. Specific effects of cations on rhodamine B equilibriums. *J. Phys. Chem.* **1958**, *62*, 1601–1603. [CrossRef]
77. Ramette, R.W.; Sandell, E.B. Rhodamine B equilibriums. *J. Am. Chem. Soc.* **1956**, *78*, 4872–4878. [CrossRef]
78. Hinckley, D.A.; Seybold, P.G.; Borris, D.P. Solvatochromism and thermochromism of rhodamine solutions. *Spectrochim. Acta Part A Mol. Spectrosc.* **1986**, *42*, 747–754. [CrossRef]
79. Beija, M.; Afonso, C.A.M.; Martinho, J.M.G. Synthesis and applications of Rhodamine derivatives as fluorescent probes. *Chem. Soc. Rev.* **2009**, *38*, 2410–2433. [CrossRef]
80. Arbeloa, F.L.; Estevez, M.J.T. Photophysics of rhodamines: Molecular structure and solvent effects. *J. Phys. Chem.* **1991**, *95*, 2203–2208. [CrossRef]
81. Arbeloa, T.L.; Estévez, M.T.; López-Arbeloa, F.; Aguirresacona, I.U.; Arbeloa, I.L. Luminescence properties of rhodamines in water/ethanol mixtures. *J. Lumin* **1991**, *48*, 400–404. [CrossRef]
82. Milvy, P.; Kay, K. Mutagenicity of 19 major graphic arts and printing dyes. *J. Toxicol. Environ. Heal. Part A* **1978**, *4*, 31–36. [CrossRef]
83. Smart, P.L. A review of the toxicity of twelve fluorescent dyes used for water tracing. *NSS Bull.* **1984**, *46*, 21–33.
84. Umeda, M. Experimental production of sarcoma in rats by injection of rhodamine B. I. *Gann* **1952**, *43*, 120–122.
85. Umeda, M. Experimental study of xanthene dyes as carcinogenic agents. *Gann* **1956**, *47*, 51–78.
86. Nestmann, E.R.; Douglas, G.R.I.; Matula, T.E.; Grant, C.; Kowbel, D.J. Mutagenic activity of rhodamine dyes and their impurities as detected by mutation induction in Salmonella and DNA damage in Chinese hamster ovary cells. *Cancer Res.* **1979**, *39*, 4412–4417. [PubMed]
87. Elliott, G.S.; Mason, R.W.; Edwards, I.R. Studies on the pharmacokinetics and mutagenic potential of rhodamine b. *J. Toxicol. Clin. Toxicol.* **1990**, *28*, 45–59. [CrossRef] [PubMed]

88. Sommerwerk, S.; Heller, L.; Kerzig, C.; Kramell, A.E.; Csuk, R. Rhodamine B conjugates of triterpenoic acids are cytotoxic mitocans even at nanomolar concentrations. *Eur. J. Med. Chem.* **2017**, *127*, 1–9. [CrossRef] [PubMed]
89. Macasoi, I.; Mioc, M.; Vaduva, D.B.; Ghiulai, R.; Mioc, A.; Soica, C.; Muntean, D.; Dumitrascu, V. In silico Evaluation of the Antiproliferative Mithocondrial Targeted Mechanism of Action of Some Pentacyclic Triterpene Derivatives. *Rev. Chim.* **2019**, *69*, 3361–3363. [CrossRef]
90. Friedrich, S.; Serbian, I.; Hoenke, S.; Wolfram, R.K.; Csuk, R. Synthesis and cytotoxic evaluation of malachite green derived oleanolic and ursolic acid piperazineamides. *Med. Chem. Res.* **2020**, *29*, 926–933. [CrossRef]
91. Serbian, I.; Hoenke, S.; Kraft, O.; Csuk, R. Ester and amide derivatives of rhodamine B exert cytotoxic effects on different human tumor cell lines. *Med. Chem. Res.* **2020**, *29*, 1655–1661. [CrossRef]
92. Kozubek, M.; Serbian, I.; Hoenke, S.; Kraft, O.; Csuk, R. Synthesis and cytotoxic evaluation of hydroxycinnamic acid rhodamine B conjugates. *Results Chem.* **2020**, *2*, 100057. [CrossRef]
93. Serbian, I.; Hoenke, S.; Csuk, R. Synthesis of some steroidal mitocans of nanomolar cytotoxicity acting by apoptosis. *Eur. J. Med. Chem.* **2020**, *199*, 112425. [CrossRef]
94. Wiemann, J.; Fischer, L.; Kessler, J.; Ströhl, D.; Csuk, R. Ugi Multicomponent-reaction: Syntheses of cytotoxic dehydroabietylamine derivatives. *Bioorganic Chem.* **2018**, *81*, 567–576. [CrossRef]
95. Wolfram, R.K.; Heller, L.; Csuk, R. Targeting mitochondria: Esters of rhodamine B with triterpenoids are mitocanic triggers of apoptosis. *Eur. J. Med. Chem.* **2018**, *152*, 21–30. [CrossRef]
96. Wolfram, R.K.; Fischer, L.; Kluge, R.; Ströhl, D.; Al-Harrasi, A.; Csuk, R. Homopiperazine-rhodamine B adducts of triterpenoic acids are strong mitocans. *Eur. J. Med. Chem.* **2018**, *155*, 869–879. [CrossRef]
97. Siewert, B.; Pianowski, E.; Obernauer, A.; Csuk, R. Towards cytotoxic and selective derivatives of maslinic acid. *Bioorg. Med. Chem.* **2014**, *22*, 594–615. [CrossRef]
98. Sommerwerk, S.; Heller, L.; Kuhfs, J.; Csuk, R. Urea derivates of ursolic, oleanolic and maslinic acid induce apoptosis and are selective cytotoxic for several human tumor cell lines. *Eur. J. Med. Chem.* **2016**, *119*, 1–16. [CrossRef]
99. Kahnt, M.; Wiemann, J.; Fischer, L.; Sommerwerk, S.; Csuk, R. Transformation of asiatic acid into a mitocanic, bimodal-acting rhodamine B conjugate of nanomolar cytotoxicity. *Eur. J. Med. Chem.* **2018**, *159*, 143–148. [CrossRef] [PubMed]

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The Presence of a Cyclohexyldiamine Moiety Confers Cytotoxicity to Pentacyclic Triterpenoids

Sophie Hoenke [1], Martin A. Christoph [1], Sander Friedrich [1], Niels Heise [1], Benjamin Brandes [1], Hans-Peter Deigner [2], Ahmed Al-Harrasi [3], and René Csuk [1,*]

[1] Organic Chemistry, Martin–Luther University Halle–Wittenberg, Kurt–Mothes, Str. 2, D-06120 Halle (Saale), Germany; sophie.hoenke@chemie.uni-halle.de (S.H.); martin.christoph@student.uni-halle.de (M.A.C.); sander.friedrich@student.uni-halle.de (S.F.); niels.heise@student.uni-halle.de (N.H.); Benjamin.brandes@chemie.uni-halle.de (B.B.)
[2] Institute of Precision Medicine, Medical and Life Science Faculty, Furtwangen University, Jakob–Kienzle–Str. 17, D-78054 Villigen–Schwenningen, Germany; dei@hs-furtwangen.de
[3] Chair of Oman's Medicinal Plants and Marine Natural Products, University of Nizwa, P.O. Box 33 Birkat Al-Mauz, PC 616 Nizwa, Oman; aharrasi@unizwa.edu.om
* Correspondence: rene.csuk@chemie.uni-halle.de; Tel.: +49-345-5525660

Abstract: Pentacyclic triterpenoids oleanolic acid, ursolic acid, betulinic acid, and platanic acid were acetylated and converted into several amides **9**-**31**; the cytotoxicity of which has been determined in sulforhodamine B assays employing seral human tumor cell lines and nonmalignant fibroblasts. Thereby, a betulinic acid/*trans*-1,4-cyclohexyldiamine amide showed excellent cytotoxicity (for example, EC_{50} = 0.6 μM for HT29 colon adenocarcinoma cells).

Keywords: oleanolic acid; ursolic acid; betulinic acid; platanic acid; 1,4-cyclohexyldiamines; cytotoxicity

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1. Introduction

Pentacyclic triterpenes represent an important class of secondary natural products [1–10]. Their research began very early in the history of chemistry: for example, J. T. Lowitz' betulin was the first to be extracted from plant material in pure form and described in its physicochemical properties as early as 1788 [11]. Great progress in their discovery, isolation, and especially in the elucidation of their complex structures was made at the beginning and in the middle of the 20th century. In this period, the first partial syntheses also took place. Their pharmaceutical/medical potential, however, was only recognized much later. The observation of E. Pisha in 1995 describing, for the first time, the cytotoxic effect of betulinic acid on melanoma cells was groundbreaking in this respect [12]. Since this observation, the research of this class of natural substances has intensified tremendously.

While unsubstituted triterpene carboxylic acids, e.g., oleanolic acid (**OA**) [13–15], ursolic acid (**UA**) [6], betulinic acid (**BA**) [16], and platanic acid [17–20] (**PA**) (Figure 1) have a relatively low cytotoxicity, the 3-*O*-acetylated amides of these compounds have especially become the focus of scientific interest in recent years. For example, triterpenoic benzyl amides (such as EM2) [21,22] and (homo)-piperazinyl-amides [23,24], as well as the rhodamine B conjugates [24] of the latter, have cytotoxic effects on numerous human tumor cell lines even in nanomolar concentrations [25]. However, some of these mitocanic compounds are quite cytotoxic to nonmalignant cells, and selective cytotoxicity can only be achieved by a several well-defined conjugates [25].

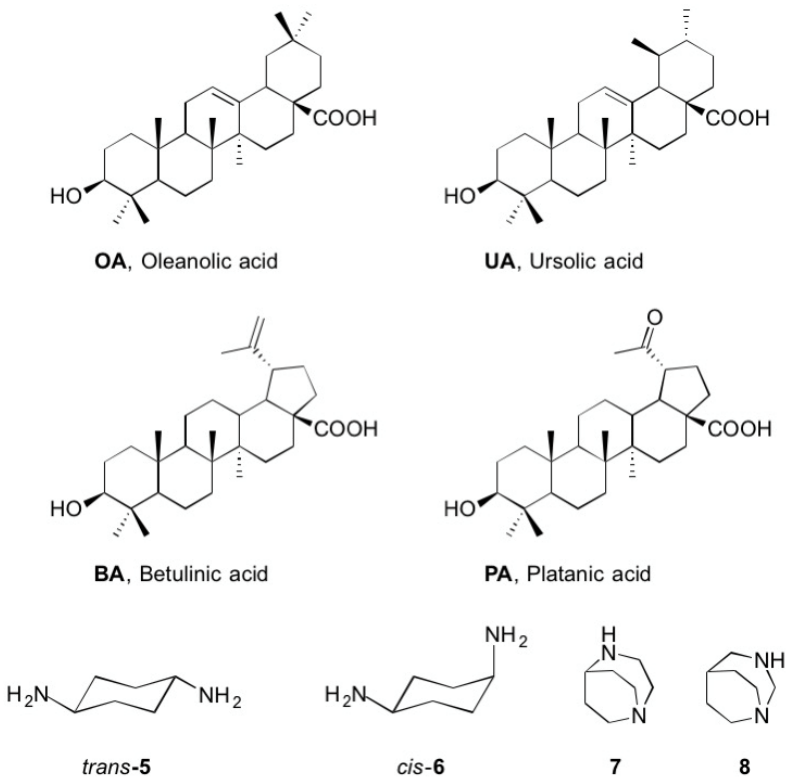


Figure 1. Structures of triterpenoic acids **OA**, **UA**, **BA**, and **PA** as well as of trans- and cis-1,4-cylohexyldiamines **5** and **6**, as well as bicyclic diamines **7** and **8**, respectively.

To get a deeper insight in the importance and role of a diamine-derived spacer, two different types of diamines (Figure 1) were selected. Both types have never been used in the context of triterpenes. On the one hand we selected 1,4-cyclohexyldiamines **5** and **6**, and on the other hand 1,4- (or 1,3)-diazabicyclo[3.2.2]nonanes **7** and **8**, respectively.

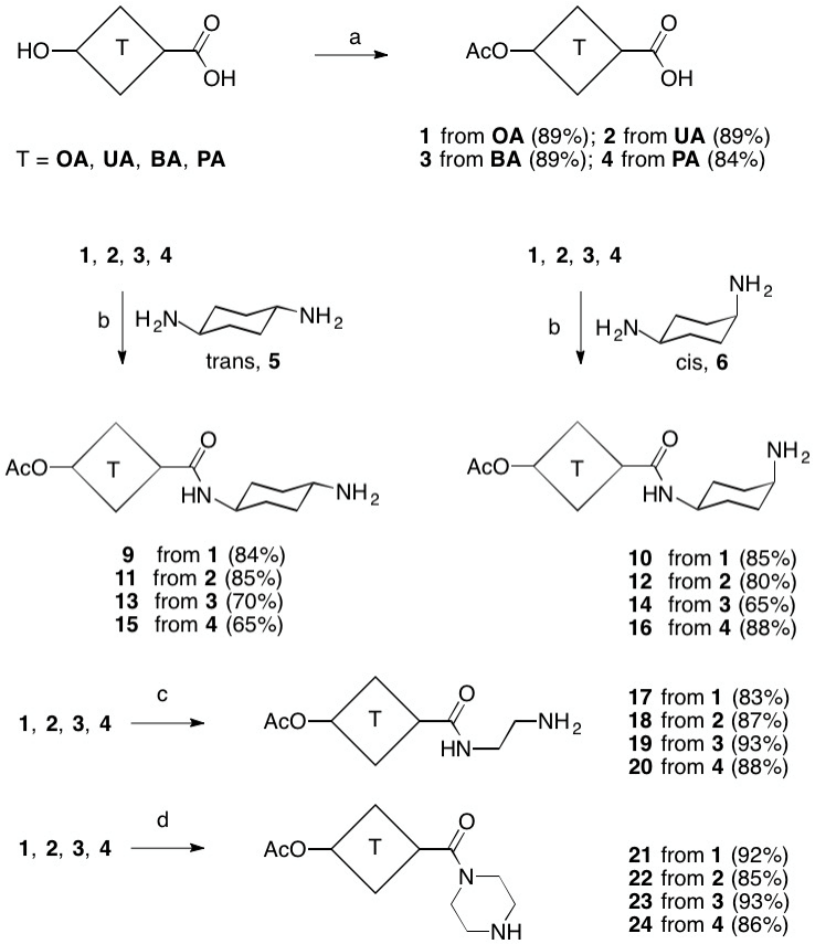
Triterpenoic amides holding both a piperazinyl or homopiperazinyl moiety and an extra rhodamine B moiety were moderately to highly cytotoxic to a variety of human tumor cell lines [25]. A somewhat differentiated picture, however, was found for compounds holding an ethylenediamine moiety instead [24]. Several of these compounds were cytotoxic while other analogs were not [17,18,21,26]. To evaluate the influence of a moiety being more rigid than ethylenediamine but as bulky as piperazine, we came across 1,4-cyclohexyldiamines **5** and **6**. The skeleton shows some similarity to piperazine concerning its flexibility and the conformation of the ring but also holds two primary amino groups, thus resembling ethylenediamine.

Since the rigidity of the diamine attached to the essential carboxyl group of the triterpenoid backbone might influence the cytotoxicity of the compounds, 1,4-diazabicyclo[3.2.2] nonane (**7**) [27] and 1,3-diazabicyclo[3.2.2]nonane (**8**) [28] were selected as amine components, too. Due to their structural similarity to a (homo)-piperazinyl spacer aside from holding an additional chain between the nitrogen, this would result in an increased rigidity of the molecule.

2. Results

The 1,4-dicyclohexyldiamine scaffold exists in two different stereoisomers holding either a *cis*- or a *trans*-configuration of the amino groups. Additionally, both compounds are *meso*-compounds. For the synthesis of the target compounds, triterpenoic acids oleanolic acid (**OA**), ursolic acid (**UA**), betulinic acid (**BA**), and platanic acid (**PA**) (Figure 1) were acetylated yielding well-known 3-*O*-acetyl compounds **1**–**4**, respectively.

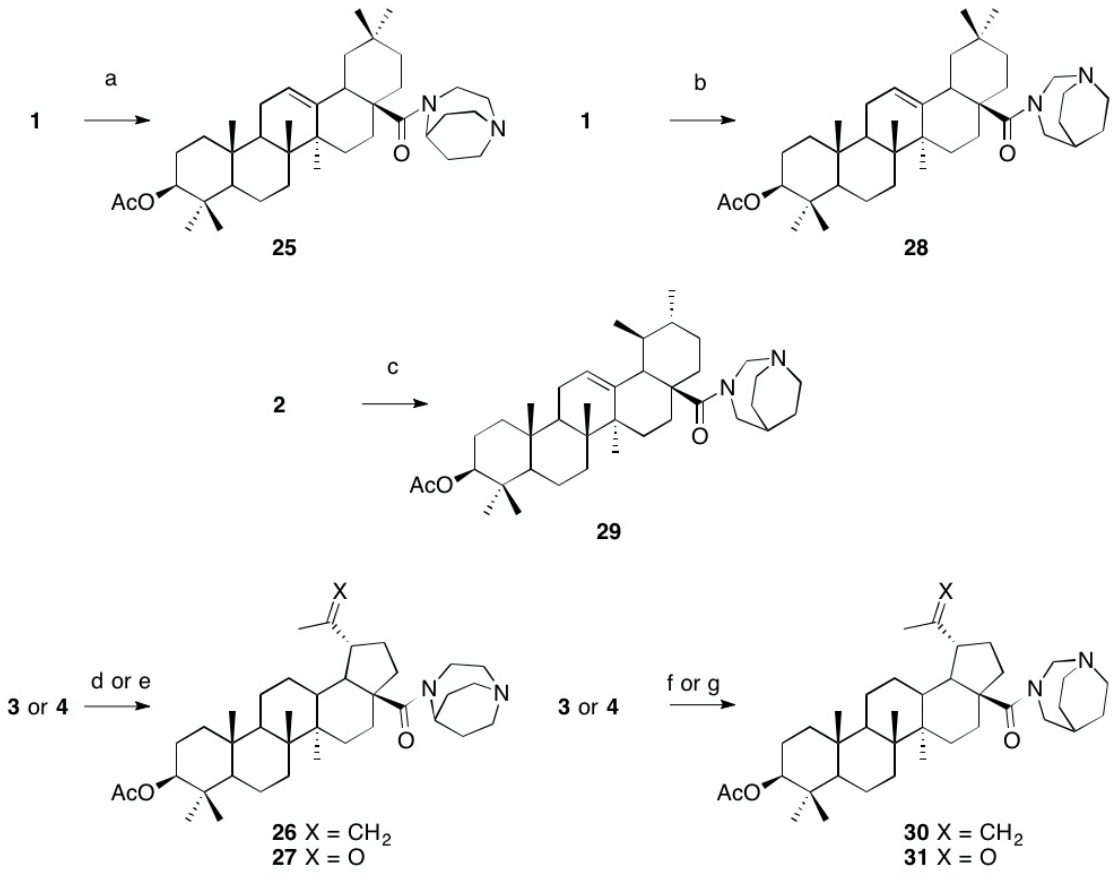
After having been activated with oxalyl chloride *trans*-1,4-cyclohexyldiamine (**5**) or *cis*-1,4-dicyclohexyldiamine (**6**) was added, and final target compounds **9**–**16** were obtained in yields between 65–85% (Scheme 1).



Scheme 1. Reactions and conditions: (**a**) Ac_2O, DCM, TEA, DMAP (cat.), 20 °C, 1d; (**b**) DCM, DMF (cat.), $(COCl)_2$, then **5** or **6**, TEA, DMAP (cat.), 23 °C, 1d; (**c**) DCM, DMF (cat.), $(COCl)_2$, then H_2N-$(CH_2)_2$-NH_2, TEA, DMAP (cat.), 23 °C, 1d; and (**d**) DCM, DMF (cat.), $(COCl)_2$, then piperazine, TEA, DMAP (cat.), 23 °C, 1d.

The compounds are characterized, as exemplified for **OA** derived *trans*-**9** and *cis*-**10** in their ^{13}C NMR spectra holding a signal for C-28 (amide) at $\delta = 179.8$ ppm. The synthesis of the two amines **7** and **8** started from commercially available quinuclidin-3-one hydrochloride as previously reported [27,28]. Acetates **1**–**4** were activated with oxalyl chloride followed by the addition of either ethylenediamine (→ products **17**–**20**, Scheme 1), piperazine (→ products **21**–**24**, Scheme 1), **7** (→ products **25**–**27**, Scheme 2), or **8** (→ products **28**–**31**, Scheme 2).

For cytotoxicity screening, parent triterpenoic acid, acetates **1**–**4** and products **9**–**31** were subjected to sulforhodamine B assays (SRB) employing several human tumor cell lines as well as nonmalignant fibroblasts (NIH 3T3). The results from these assays are summarized in Tables 1–3.



Scheme 2. Reactions and conditions: (**a**) DCM, DMF (cat.), $(COCl)_2$, then **7**, TEA, DMAP (cat.), 23 °C, 1d, 73%; (**b**) DCM, DMF (cat.), $(COCl)_2$, then **8**, TEA, DMAP (cat.), 23 °C, 1d, 73%; (**c**) DCM, DMF (cat.), $(COCl)_2$, then **8**, TEA, DMAP (cat.), 23 °C, 1d, 73%; (**d** or **e**) from **3**: DCM, DMF (cat.), $(COCl)_2$, then **7**, TEA, DMAP (cat.), 23 °C, 1d, 74%, from **4**: 99%; (**f**) or (**g**) from **3** and **8**: 95%; from **4** and **8**: 73%.

Table 1. Cytotoxicity of parent compounds **OA**, **UA**, **BA**, **PA**, acetates **1–4**, starting materials **5–8** as well as products **9–16** (EC_{50}-values in μM from SRB-assays) after 72 h of treatment, the values are averaged from three independent experiments performed each in triplicate, confidence interval CI = 95%; mean ± standard mean error); n.d. not determined; n.s. not soluble; betulinic acid (**BA**) and doxorubicin (**DX**) were used as positive controls. Cell lines: malignant: A375 (melanoma), HT29 (colon adenocarcinoma), MCF-7 (breast adenocarcinoma), A2780 (ovarian carcinoma), FaDu (hypopharyngeal carcinoma); nonmalignant: NIH 3T3 (fibroblasts).

Compound	A375	HT29	MCF-7	A2780	FaDu	NIH 3T3
OA	>30	>30	>30	>30	>30	>30
UA	15.4 ± 1.0	12.4 ± 1.1	14.7 ± 0.4	17.3 ± 0.9	18.2 ± 1.7	16.3 ± 1.4
BA	17.7 ± 0.4	16.8 ± 2.0	12.3 ± 1.1	9.4 ± 1.1	13.7 ± 0.9	19.3 ± 1.1
PA	>30	>30	>30	>30	>30	>30
1	13.1 ± 1.1	20.5 ± 1.7	12.9 ± 1.9	9.4 ± 0.5	11.8 ± 0.9	17.5 ± 1.5
2	11.4 ± 1.4	17.3 ± 1.5	12.1 ± 1.2	8.3 ± 0.9	10.7 ± 0.8	16.4 ± 1.7
3	19.2 ± 1.7	21.3 ± 2.0	11.0 ± 0.5	18.3 ± 0.5	7.2 ± 1.2	>30
4–8	>30	>30	>30	>30	>30	>30
9	1.8 ± 0.3	1.7 ± 0.2	2.0 ± 0.3	2.1 ± 0.2	2.0 ± 0.1	1.8 ± 0.4
10	2.4 ± 0.2	2.9 ± 0.1	2.8 ± 0.6	2.7 ± 0.2	2.9 ± 0.2	2.2 ± 0.2
11	1.9 ± 0.2	2.6 ± 0.1	2.5 ± 0.4	2.6 ± 0.3	2.6 ± 0.1	1.9 ± 0.3
12	1.9 ± 0.3	2.4 ± 0.1	2.4 ± 0.4	2.3 ± 0.2	2.5 ± 0.1	1.9 ± 0.2
13	0.9 ± 0.1	0.6 ± 0.1	1.3 ± 0.4	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
14	1.3 ± 0.2	0.8 ± 0.1	1.2 ± 0.4	1.2 ± 0.35	1.1 ± 0.2	1.1 ± 0.1

15	0.9 ± 0.1	2.1 ± 0.1	2.8 ± 0.4	1.8 ± 0.2	1.5 ± 0.1	0.5 ± 0.1
16	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
DX	n.d.	0.9 ± 0.2	1.1 ± 0.3	0.02 ± 0.01	1.7 ± 0.3	0.06 ± 0.03

Table 2. (EC_{50} in μM from SRB) of ethylenediamine amides **17**–**20** and piperazine derived amides **21**–**24**. The values are averaged from three independent experiments performed each in triplicate, confidence interval CI = 95%; mean ± standard mean error).

Compound	HT29	MCF-7	A2780	NIH 3T3
17	2.0 ± 0.2	1.7 ± 0.2	3.1 ± 0.1	2.1 ± 0.1
18	1.8 ± 0.1	2.0 ± 0.1	2.3 ± 0.1	2.6 ± 0.3
19	1.0 ± 0.3	1.3 ± 0.1	1.4 ± 0.2	1.4 ± 0.1
20	3.3 ± 1.2	3.1 ± 0.1	3.2 ± 0.2	2.1 ± 0.1
21	1.3 ± 0.1	1.7 ± 0.2	1.7 ± 0.1	1.7 ± 0.1
22	1.9 ± 0.3	2.0 ± 0.1	2.1 ± 0.1	2.1 ± 0.1
23	1.0 ± 0.1	1.4 ± 0.1	1.9 ± 0.1	0.9 ± 0.1
24	2.4 ± 0.3	2.8 ± 0.1	3.1 ± 0.1	0.7 ± 0.1

Table 3. (EC_{50} in μM from SRB) of diazabicyclononanes derived amides **25**–**31**. The values are averaged from three independent experiments performed each in triplicate, confidence interval CI = 95%; mean ± standard mean error); n.s. not soluble.

Compound	A375	HT29	MCF-7	A2780	FaDu	NIH 3T3
25, 26	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
27	2.3 ± 0.2	5.2 ± 0.2	4.2 ± 0.8	3.9 ± 0.4	2.7 ± 0.4	2.2 ± 0.2
28, 29	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
30	4.7 ± 0.2	4.8 ± 0.3	6.0 ± 0.9	5.5 ± 0.4	6.3 ± 0.5	9.3 ± 0.7
31	6.0 ± 0.5	8.2 ± 0.3	6.3 ± 0.5	6.4 ± 0.3	6.2 ± 0.6	5.0 ± 0.4

As can be seen from Tables 1–3, there is, by and large, no significant difference in the cytotoxicity of the individual compounds; the cytotoxic activity is independent of the used spacer. Betulinic acid (**BA**)-derived derivatives are slightly more cytotoxic than the other derivatives. The selectivity between malignant cells and the nonmalignant fibroblast cell line NIH 3T3 is practically not affected by the choice of different spacers. The better cytotoxicity of the 3-*O*-acetylated pentacyclic triterpenoids as compared to their analogues holding an unprotected hydroxyl moiety at position C-3 might be explained by a better bioavailability of the former. The better cytotoxicity of derivatives derived from **BA** as compared to those from **PA**, however, cannot be explained but seems to be quite general inasmuch as the parent compound **BA** is more cytotoxic and PA, and the same also holds true for the corresponding 3-*O*-acetates. Comparison of the cytotoxicity of compounds **15** and **16** would have been interesting but failed because of the insolubility of **16** under the conditions of the assay. This might also have been caused by the presence of a weak hydrogen bond between the axially arranged amino group in **16** with the amide group. This assumption is also confirmed by the fact that **16** has a higher melting point than **15**, and it can be assumed that solvation leads to an additional bias against the axial position.

All compounds show only low selectivity. This finding distinguishes these compounds from substituted benzylamides (such as EM2) [21] but also from derivatives with an additional rhodamine B [24,25] moiety being present.

3. Conclusions

Pentacyclic triterpenoids oleanolic acid, ursolic acid, betulinic acid, and platanic acid were acetylated. These acetates were treated with oxalyl chloride followed by the addition of ethylenediamine or several monocyclic and bicyclic diamines to provide amides **9**–**31**, whose cytotoxicity has been determined in sulforhodamine B assays employing seral human

tumor cell lines and nonmalignant fibroblasts. Thereby, a betulinic acid/trans 1,4-cyclohexyldiamine amide showed excellent cytotoxicity (for example, EC_{50} = 0.6 μM for HT29 colon adenocarcinoma cells), but the selectivity tumor cell/nontumor cell could not be improved. This finding distinguishes these compounds from previously investigated substituted benzylamides but also from piperazinyl-rhodamine B conjugates.

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 an Advion expression^L 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: N_2). Thin-layer chromatography was performed on precoated 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). 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. The triterpenoic acids were bought from "Betulinines" (Stříbrná Skalice, Czech Republic) and used as received.

4.1. Cell Lines and Culture Conditions

Following human cancer cell lines A375 (malignant melanoma), HT29 (colon adenocarcinoma), MCF-7 (breast cancer), A2780 (ovarian carcinoma), FaDu (pharynx carcinoma), and nonmalignant mouse fibroblasts NIH 3T3 were used. All cell lines were obtained from the Department of Oncology (Martin Luther University Halle-Wittenberg). Cultures were maintained as monolayers in RPMI 1640 medium with L-glutamine (Capricorn Scientific GmbH, Ebsdorfergrund, Germany) supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich GmbH, Steinheim, Germany) and 1% penicillin/streptomycin (Capricorn Scientific GmbH, Ebsdorfergrund, Germany) at 37 °C in a humidified atmosphere with 5% CO_2.

4.2. Cytotoxicity Assay (SRB Assay)

For the evaluation of the cytotoxicity of the compounds the sulforhodamine-B (Kiton-Red S, ABCR GmbH, Karlsruhe, Germany), a microculture colorimetric assay was used as previously reported. The EC_{50} values were averaged from three independent experiments performed each in triplicate calculated from semilogarithmic dose-response curves applying a nonlinear 4P Hills-slope equation. In short, cells were seeded into 96 well plates at day 0 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 nontoxic to the cells. After 72 h treatment, the supernatant media from the 96 well plates were discarded, and 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.

4.3. General Procedure for the Synthesis of Acetates **1**–**4** *(GPA)*

To a solution of the triterpenoic acid (**OA**, **UA**, **BA**, **PA**, 1 eq.) in dry DCM, acetic anhydride (3 eq.), triethylamine (3 eq.) and DMAP (cat.) were added, and stirring at 20 °C was continued for 1 day. Usual aqueous workup followed by recrystallization from ethanol furnished products **1**–**4**.

4.4. General Procedure for the Synthesis of Amides **9–31** *(GPB)*

To the solution of the acetylated triterpenoic acid (**1–4**, 1 eq.) in dry DCM, a drop of dry DMF and oxalyl chloride (4 eq.) were added at 0 °C. Stirring at 25 °C was continued until the evolution of gases had ceased. The volatiles were removed under reduced pressure. The corresponding amine (3 eq.) was dissolved in dry DCM (20 mL), and a solution of TEA (4.2 eq.), DMAP (cat.) in dry DCM (10 mL) was added. To this mixture, the reaction mixture (dissolved in dry DCM) from above was slowly added at 0 °C, and stirring at 23 °C was continued for 1 day. The usual aqueous workup followed by liquid column chromatography ($CHCl_3$/MeOH) gave the products **9–31**, respectively.

3β-Acetyloxy-olean-12-en-28-oic acid (**1**). Following GPA, compound **1** (4.89 g, 89%) was obtained as a colorless solid; R_f = 0.54 (hexanes/ethyl acetate, 3:1); m.p.: 259–263 °C (lit.: [29] 266–268 °C); $[\alpha]_D^{20}$= +74.1° (c 0.43, $CHCl_3$) [lit.: [29] $[\alpha]_D^{20}$= +74.0° (c 1, $CHCl_3$)]; MS (ESI, MeOH): m/z 499.1 ($[M + H]^+$, 9), 521.3 (38%, $[M + Na]^+$), 1019.4 (100%, $[2M + Na]^+$).

3β-Acetoxy-urs-12-en-28-oic acid (**2**). Following GPA, compound **2** (4.89 g, 89%) was obtained as a colorless solid; R_f = 0.71 (toluene/ethyl acetate/heptane/formic acid, 80:26:10:5); m.p.: 287–290 °C (lit.:[30] 289–290 °C); $[\alpha]_D^{20}$ = +68.9° (c 0.315, $CHCl_3$) [lit.:[31] $[\alpha]_D^{20}$ = +72.3° (c 0.5, $CHCl_3$)]; MS (ESI, MeOH): m/z 499.0 ($[M + H]^+$, 74), 516.3 (36%, $[M + NH_4]^+$), 521.5 (34%, $[M + Na]^+$);

3β-Acetoxy-lup-20(29)-en-28-oic acid (**3**). Following GPA, compound **3** (4.90 g, 89%) was obtained as a colorless solid; R_f = 0.58 (hexanes/ethyl acetate, 4:1); m.p.: 281–283 °C (lit.:[32] 280–282 °C); $[\alpha]_D^{20}$ = +25.6° (c 0.35, $CHCl_3$) [lit.:[33] $[\alpha]_D^{20}$ = +26.4° (c 0.54, $CHCl_3$)]; MS (ESI, MeOH): m/z 487.1 (28%, $[M- H]^-$) 995.3 (100%, $[2M - H]^-$), 1018.2 (28%, $[2M - 2H + Na]^-$).

3β-Acetoxy-20-oxo-30-norlupan-28-oic acid (**4**). Following GPA, compound **4** (13.8 g, 84%) was obtained as a colorless solid; R_f = 0.50 (toluene/ethyl acetate/heptane/formic acid, 80:26:10:5); m.p.: 268–270 °C (decomp.), (lit.:[34] 252–255 °C); $[\alpha]_D^{20}$ = −9.1° (c 0.34, $CHCl_3$) [lit.:[34] $[\alpha]_D^{20}$ = −9.5° (c 0.8, $CHCl_3$)]; MS (ESI, MeOH): m/z 999.3 (100%, $[2M - H]^-$).

Trans-cyclohexyl-1,4-diamine (5) and cis-cyclohexyl-1,4-diamine (**6**). These compounds were commercially obtained from Merck and used as received.

1,4-Diazabicyclo[3.2.2]nonane (7) and 1,3-diazabicyclo[3.2.2]nonane (**8**). These compounds were prepared from quinuclidin-3-one hydrochloride as previously reported [27,28].

(3β)-28-[(trans-4-Aminocyclohexyl)amino]-28-oxoolean-12-en-3-yl acetate (**9**). Following GPB, compound **9** (1.01 g, 84%) was obtained as a colorless solid; R_f = 0.66 ($CHCl_3$/MeOH, 8:2); m.p.: 203–205 °C (decomp.); $[\alpha]_D^{20}$ = +23.6° (c 0.35, MeOH); IR (ATR):ν = 3423 w, 2945 m, 1703 m, 1618 m, 1430 s, 1332 s, 1036 s, 817 s. 749 s cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ = 6.64 (m, 1H, NH), 5.43 (t, J = 3.6 Hz, 1H, 12-H), 4.48 (dd, J = 11.2, 4.9 Hz, 1H, 3-H), 3.89 (s, 1H, 33-H), 3.35–3.32 (m, 1H, 36-H), 2.85–2.79 (m, 1H, 18-H), 2.20–2.07 (m, 1H, 16-H_a), 2.05 (s, 3H, 32-H), 1.99–1.28 (m, 25H, 34-H, 35-H, 37-H, 38-H, 1-H_b, 22-H, 2-H, 15-H_a, 6-H, 11-H, 7-H, 1-H_a, 21-H, 9-H, 19-H_b), 1.23 (s, 3H, 27-H), 1.22–1.02 (m, 3H, 15-H_b, 16-H_b, 19-H_a), 1.00 (s, 3H, 25-H), 0.98 (s, 3H, 29-H), 0.94 (s, 3H, 30-H), 0.91 (s, 3H, 24-H), 0.91 (s, 3H, 23-H), 0.89 (m, 1H, 5-H), 0.84 (s, 3H, 26-H) ppm; ^{13}C NMR (126 MHz, CD_3OD): δ = 179.8 (C-28), 145.5 (C-13), 123.9 (C-12), 82.4 (C-3), 56.6 (C-5), 49.9 (C-9), 49.5 (C-33), 47.7 (C-19), 47.5 (C-17), 46.3 (C-36), 43.8 (C-18), 43.1 (C-14), 40.7 (C-8), 39.3 (C-1), 38.7 (C-4), 38.1 (C-10), 35.1 (C-21), 34.2 (C-34, C-38), 33.8 (C-35, C-37), 33.5 (C-30), 31.6 (C-20), 28.5 (C-15), 28.5 (C-23), 28.1 (C-7), 27.8 (C-22), 26.3 (C-27), 24.5 (C-2), 24.5 (C-11), 24.0 (C-16), 23.9 (C-29), 21.1 (C-32), 19.3 (C-6), 18.1 (C-26), 17.1 (C-24), 15.9 (C-25) ppm; ESI, MeOH): m/z 595.4 (100%, $[M + H]^+$), 1189.3 (5%, $[2M + H]^+$); analysis calcd for $C_{38}H_{62}N_3O_4$ (594.91): C 76.72, H 10.50, N 4.71; found: C 76.49, H 10.71, N 4.55. Please see Supplementary Materials.

(3β)-28-[(cis-4-Aminocyclohexyl)amino]-28-oxoolean-12-en-3-yl acetate (**10**). Following GPB, compound **10** (1.02 g, 85%) was obtained as a colorless solid; R_f = 0.673 ($CHCl_3$/MeOH, 8:2); m.p.: 197–200 °C (decomposition); $[\alpha]_D^{20}$ = +3.8° (c 0.14, $CHCl_3$); IR (ATR): ν = 3406 w,

2944 m, 1704 m, 1621 m, 1524 s, 1428 s, 1313 s, 1099 m, 1028 s, 821 s, 763 m cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ = 6.61–6.55 (m, 1H, NH), 5.37 (t, 1H, 12-H), 4.42 (d, 1H, 3-H), 3.60–3.52 (m, 1H, 33-H), 3.29–3.24 (m, 1H, 36-H), 2.10–1.93 (m, 1H, 18-H), 1.99 (s, 3H, 32-H), 1.93–1.85 (m, 8H, 34-H, 35-H, 37-H, 38-H), 1.85–1.21 (m, 14H, 1-H_b, 22-H_a, 2-H, 16-H_a, 15-H_b, 6-H, 11-H_b, 7-H_b, 1-H_a, 21-H_b, 9-H, 19-H_b), 1.16 (s, 3H, 27-H), 1.16–0.97 (m, 5H, 15-H_a,16-H_b,19-H_a, 21-H_a, 22-H_b), 0.94 (s, 3H, 26-H), 0.91 (s, 3H, 24-H), 0.88 (s, 3H, 25-H), 0.85 (s, 3H, 29-H), 0.84 (s, 3H, 30-H), 0.83–0.81 (m, 1H, 5-H), 0.78 (s, 3H, 23-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 179.8 (C-28), 172.8 (C-31), 145.4 (C-13), 123.9 (C-12), 82.4 (C-3), 56.6 (C-5), 49.8 (C-9), 49.5 (C-33), 47.6 (C-19), 46.2 (C-17), 43.7 (C-18), 43.1 (C-14), 42.8 (C-36), 40.6 (C-21), 39.2 (C-8), 38.6 (C-4), 38.0 (C-1), 37.0 (C-10), 34.2 (C-7), 33.8 (C-34, C-38), 33.5 (C-29, C-30), 31.5 (C-35, C-37), 28.0 (C-20), 27.7 (C-22), 27.3 (C-15), 27.2 (C-16), 26.3 (C-27), 24.5 (C-2), 21.1 (C-32), 19.2 (C-6), 18.3 (C-23), 18.1 (C-26), 17.1 (C-24), 15.9 (C-25) ppm; MS (ESI, MeOH): m/z 595.4 (100%, $[M + H]^+$), 1190.4 (8%, $[2M + H]^+$); analysis calcd for $C_{38}H_{62}N_3O_4$ (594.91): C 76.72, H 10.50, N 4.71; found: C 76.59, H 10.75, N 4.46.

(3β)-28-[(trans-4-Aminocyclohexyl)amino]-28-oxoursan-12-en-3-yl acetate (**11**). Following GPB, compound **11** (1.02 g, 85%) was obtained as a colorless solid; R_f = 0.66 ($CHCl_3$/MeOH, 8:2); m.p.: 189–193°C (decomp.); $[\alpha]_D^{20}$ = +34.4° (c 0.31, MeOH); IR (ATR): ν = 3420 w, 2928 m, 1734 m, 1621 m, 1523 m,1314 s, 1244 s, 1096 s, 1027 s, 985 m, 902 m, 822 s cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ = 6.90 (m, 1H, NH), 5.28 (t, J = 3.7 Hz, 1H, 12-H), 4.42 (m, 1H, 3-H), 3.64–3.51 (m, 1H, 33-H), 3.10–2.98 (m, 1H, 36-H), 2.16–2.12 (m, 1H, 18-H), 2.07–1.97 (m, 3H, 16-H_b, 35-H_a, 37-H_a), 1.99 (s, 3H, 30), 1.94–1.87 (m, 3H, 11, 21-H_a), 1.84–1.72 (m, 3H, 15-H_b, 34-H_b, 38-H_b), 1.69–1.26 (m, 17H, 1-H_b, 2-H, 6-H, 7-H, 9-H, 16-H_a, 19-H, 22-H, 34-H_a, 35-H_b, 37-H_b, 38-H_a), 1.10 (s, 3H, 27-H), 1.07–0.99 (m, 3H, 1-H_a, 15-H_a, 20-H), 0.95 (s, 3H, 25-H), 0.93 (s, 3H, 32-H), 0.87 (s, 3H, 26-H), 0.85 (s, 3H, 24-H), 0.84 (s, 3H, 23-H), 0.82–0.80 (m, 1H, 5-H), 0.79 (s, 3H, 29-H) ppm; ^{13}C NMR (126 MHz, CD_3OD): δ = 179.5 (C-28), 172.8 (C-31), 139.9 (C-13), 126.8 (C-12), 82.4 (C-3), 56.7 (C-5), 53.9 (C-18), 50.6 (C-36), 49.9 (C-9), 48.8 (C-17), 48.7 (C-33), 43.4 (C-14), 40.8 (C-19), 40.2 (C-20), 39.4 (C-1), 38.7 (C-8), 38.0 (C-4), 34.2 (C-10), 31.9 (C-7), 31.1 (C-21), 30.7 (C-38, C-34), 30.5 (C-37, C-35), 28.9 (C-15), 28.6 (C-23), 24.9 (C-16), 24.5 (C-2), 24.4 (C-11), 24.1 (C-27), 21.6 (C-32), 21.1 (C-30), 19.3 (C-6), 18.2 (C-29), 17.2 (C-24), 16.0 (C-25) ppm; MS (ESI, MeOH): m/z 595.4 (100%, $[M + H]^+$), 1211.6 (4%, $[2M + Na]^+$); analysis calcd for $C_{38}H_{62}N_3O_4$ (594.91): C 76.72, H 10.50, N 4.71; found: C 76.60, H 10.83, N 4.52.

(3β)-28-[(Cis-4-Aminocyclohexyl)Amino]-28-Oxoursan-12-En-3-Yl Acetate (**12**). Following GPB, compound **12** (0.96 g, 80%) was obtained as a colorless solid; R_f = 0.67 ($CHCl_3$/MeOH, 8:2); m.p.: 186–190 °C (decomp.); $[\alpha]_D^{20}$ = +26.7° (c 0.10, $CHCl_3$); IR (ATR): ν = 3416 m, 2929 m, 1625 m, 1520 m, 1326 s, 1245 s, 1028 s, 823 m cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ = 6.52 (m, 1H, NH), 5.36 (t, J = 3.6 Hz, 1H, 12-H), 4.42 (dd, J = 11.0, 5.0 Hz, 1H, 3-H), 3.61–3.51 (m, 1H, 33-H), 3.28–3.26 (m, 1H, 36-H), 2.12–2.05 (m, 1H, 18-H), 1.99 (s, 3H, 30-H), 2.06–1.96 (m, 3H, 16-H_b, 35-H_a, 37-H_a), 1.96–1.89 (m, 3H, 11-H, 21-H_a), 1.89–1.75 (m, 3H, 15-H_b, 34-H_b, 38-H_b), 1.74–1.27 (m, 16H, 34-H_a, 35-H_b, 37-H_b,38-H_a, 1-H_b, 22-H, 2-H, 16-H, 6-H, 9-H, 7-H), 1.12 (s, 3H, 27-H), 1.08–1.00 (m, 3H, 1-H_a, 15-H_a, 20-H), 0.95 (s, 3H, 25-H), 0.94 (s, 3H, 32-H), 0.98 (m, 3H, 26-H), 0.85 (s, 3H, 24-H), 0.84 (s, 3H, 23-H), 0.83–0.81 (m, 1H, 5-H), 0.79 (s, 3H, 29-H) ppm; ^{13}C NMR (126 MHz, CD_3OD): δ = 179.7 (C-28), 172.8 (C-31), 140.4 (C-13), 127.0 (C-12), 82.3 (C-3), 56.6 (C-5), 54.6 (C-18), 49.8 (C-9), 49.6 (C-36), 49.0 (C-17), 48.8 (C-33), 43.5 (C-14), 40.9 (C-19), 40.0 (C-20), 39.4 (C-1), 38.7 (C-8), 38.0 (C-4), 34.1 (C-22), 31.9 (C-7), 29.0 (C-15), 28.6 (C-23), 28.0 (C-21), 27.1 (C-34, C-38), 27.0 (C-35, C-37), 25.2 (C-16), 24.5 (C-2), 24.4 (C-11), 23.9 (C-27), 21.5 (C-32), 21.1 (C-30), 19.2 (C-6), 18.1 (C-29), 17.6 (C-26), 17.2 (C-24), 16.1 (C-25) ppm; MS (ESI, MeOH): m/z 595.4 (100%, $[M + H]^+$), 1189.4 (10%, $[2M + H]^+$); analysis calcd for $C_{38}H_{62}N_3O_4$ (594.91): C 76.72, H 10.50, N 4.71; found: C 76.54, H 10.69, N 4.48.

(3β)-28-[(trans-4-Aminocyclohexyl)amino]-28-oxolup-20(29)-en-3-yl acetate (**13**). Following GPB, compound **13** (0.42 g, 70%) was obtained as a colorless solid; R_f = 0.595 ($CHCl_3$/MeOH, 8:2); m.p.: 205–212 °C (decomp.); $[\alpha]_D^{20}$ = +0.1° (c 0.17, MeOH); IR

(ATR):ν = 2940 m, 1731 m, 1637 m, 1513 m, 1369 m, 1244 s, 1026 m, 978 m, 882 m, 751 s cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 4.71 (s, 1H, 29-H_a), 4.57 (s, 1H, 29-H_b), 4.49–4.41 (m, 1H, 3-H), 3.96–3.80 (m, 1H, 36-H), 3.76–3.63 (m, 1H, 33-H), 3.22–3.03 (m, 1H, 19-H), 2.40 (td, J = 12.3, 3.6 Hz, 1H, 13), 2.02 (s, 3H, 32-H), 1.98–1.02 (m, 28H, 37-H, 35-H, 38-H, 34-H, 1-H_a, 22-H_a, 12-H_a, 2-H, 18-H, 16-H, 15-H_a, 6-H, 11-H, 7-H, 1-H_b, 21-H_a, 9-H, 15-H_b), 1.66 (s, 3H, 30-H), 1.01–0.96 (m, 2H, 1-H_b, 12-H_b), 0.94 (s, 3H, 27-H), 0.92 (s, 3H, 26-H), 0.82 (d, J = 1.5 Hz, 6H, 23-H, 24-H), 0.81 (s, 3H, 25-H), 0.78–0.73 (m, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 175.6 (C-28), 171.1 (C-31), 151.1 (C-20), 109.5 (C-29), 81.1 (C-3), 55.6 (C-17), 55.6 (C-5), 50.7 (C-9), 50.3 (C-18), 50.1 (C-33), 47.4 (C-19), 47.0 (C-36), 42.6 (C-14), 40.9 (C-8), 39.2 (C-13), 38.5 (C-22), 38.5 (C-1), 37.9 (C-10), 37.2 (C-4), 34.4 (C-7), 33.9 (C-16), 33.8 (C-34, C-38), 31.5 (C-35, C-37), 31.0 (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), 19.6 (C-30), 18.3 (C-6), 16.6 (C-24), 16.4 (C-25), 16.3 (C-26), 14.7 (C-27) ppm; MS (ESI, MeOH/$CHCl_3$, 4:1): m/z 593.3 (100%, $[M - H]^-$), 629.3 (80%, $[M + Cl]^-$); analysis calcd for $C_{38}H_{62}N_3O_4$ (594.91): C 76.72, H 10.50, N 4.71; found: C 76.47, H 10.89, N 4.43.

(3β)-28-[(Cis-4-Aminocyclohexyl)Amino]-28-Oxolup-20(29)-En-3-Yl Acetate (**14**). Following GPB, compound **14** (0.39 g, 65%) was obtained as a colorless solid; R_f = 0.634 ($CHCl_3$/MeOH, 8:2); m.p.: 230–235 °C (decomp.); $[\alpha]_D^{20}$ = +9.7° (c 0.19, MeOH); IR (ATR):ν = 2940 s, 1731 m, 1620 m, 1505 m, 1368 m, 1244 s, 1027 m, 978 m, 751 s cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 4.73 (s, 1H, 29-H_a), 4.59 (s, 1H, 29-H_b), 4.46 (dd, J = 10.3, 5.7 Hz, 1H, 3-H), 4.00 (s, 1H, 36-H), 3.47 (s, 1H, 33-H), 3.10 (td, J = 11.0, 3.9 Hz, 1H, 19-H), 2.46 (dd, J = 11.7, 2.0 Hz, 1H, 13-H), 2.03 (s, 3H, 32-H), 2.02–1.05 (m, 28H, 37-H, 35-H, 38-H, 34-H, 1-H_a, 22-H_a, 12-H_a, 2-H, 18-H, 16-H, 15-H_a, 6-H, 11-H, 7-H, 1-H_b, 21-H_a, 9-H, 15-H_b), 1.67 (s, 3H, 30-H), 1.03–0.96 (m, 2H, 1-H_a, 12-H_b), 0.95 (s, 3H, 27-H), 0.92 (s, 3H, 26-H), 0.84 (s, 6H, 23-H, 24-H), 0.83 (s, 3H, 25-H), 0.80–0.75 (m, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 175.6 (C-28), 171.0 (C-31), 150.8 (C-20),109.5 (C-29), 80.9 (C-3), 55.6 (C-17), 55.4 (C-5), 50.5 (C-9), 50.0 (C-18), 48.0 (C-33), 46.7 (C-19), 44.3 (C-36), 42.4 (C-14), 40.8 (C-8), 38.5 (C-22), 38.4 (C-1), 37.8 (C-10), 37.7 (C-13), 37.1 (C-4), 34.4 (C-7), 33.8 (C-16), 30.9 (C-21), 29.7 (C-34, C-38), 29.4 (C-15), 27.9 (C-23), 26.9 (C-35, C-37), 25.6 (C-12), 23.7 (C-2), 21.3 (C-32), 21.0 (C-11), 19.5 (C-30), 18.0 (C-6), 16.5 (C-24), 16.3 (C-26), 16.2 (C-25), 14.6 (C-27) ppm; MS (ESI, MeOH/$CHCl_3$, 4:1): m/z 595.5 (100%, $[M + H]^+$); analysis calcd for $C_{38}H_{62}N_3O_4$ (594.91): C 76.72, H 10.50, N 4.71; found: C 76.55, H 10.83, N 4.61.

(3β)-28-[(trans-4-Aminocyclohexyl)amino]-20,28-dioxo-30-norlupan-3-yl acetate (**15**). Following GPB, compound **15** (0.385 g, 65%) was obtained as a colorless solid; R_f = 0,595 ($CHCl_3$/MeOH, 8:2); m.p.: 251–255 °C (decomp.); $[\alpha]_D^{20}$ = −17.0° (c 0.15, MeOH); IR (ATR):ν = 3384 w, 2941 s, 1710 m, 1633 m, 1516 m, 1368 m, 1025 m, 751 s cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 4.49–4.41 (m, 1H, 3-H), 3.84–3.71 (m, 1H, 35-H), 3.46–3.27 (m, 1H, 19-H), 3.25–2.97 (m, 1H, 32-H), 2.33–2.18 (m, 1H, 13-H), 2.15 (s, 3H, 29-H), 2.07 (d, J = 15.8 Hz, 2H, 18-H, 21-H_a), 2.03 (s, 3H, 31-H), 1.94–1.81 (m, 1H, 16-H_a), 1.77–1.04 (m, 27-H, 36-H, 34-H, 37-H, 33-H, 22-H, 12-H, 2-H, 1-H_a, 16-H_b, 21-H_b, 15-H_a, 6-H, 11-H, 7-H, 9-H, 15-H_b), 0.98 (s, 3H, 27-H), 0.96–0.92 (m, 1H, 1-H_b), 0.90 (s, 3H, 26-H), 0.83–0.82 (m, 6H, 24-H, 25-H), 0.81 (s, 3H, 23-H), 0.80–0.76 (m, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 212.8 (C-20), 175.8 (C-28), 171.1 (C-30), 80.9 (C-3), 55.5 (C-5), 55.4 (C-17) 51.3 (C-19), 50.5 (C-9), 50.3 (C-33), 50.2 (C-18), 47.0 (C-36), 42.4 (C-14), 40.9 (C-8), 38.5 (C-1), 38.2 (C-22), 37.9 (C-10), 37.3 (C-4), 37.0 (C-13), 34.4 (C-7), 33.1 (C-16), 30.9 (C-33, C-37), 30.3 (C-29), 29.7 (C-36, C-34), 29.6 (C-15), 28.7 (C-21), 28.1 (C-23), 27.3 (C-12), 23.8 (C-2), 21.4 (C-31), 21.1 (C-11), 18.3 (C-6), 16.6 (C-24), 16.3 (C-25), 16.3 (C-26), 14.8 (C-27) ppm; MS (ESI, MeOH/$CHCl_3$ 4:1): m/z 597.4 (100%, $[M + 2H]^+$); analysis calcd for $C_{37}H_{60}N_2O_4$ (596.88): C 74.45, H 10.13, N 4.69; found: C 74.19, H 10.32, N 4.42.

(3β)-28-[(cis-4-Aminocyclohexyl)amino]-20,28-dioxo-30-norlupan-3-yl acetate (**16**). Following GPB, compound **16** (465 mg, 78%) was obtained as a colorless solid; R_f = 0.65 ($CHCl_3$/MeOH, 8:2); m.p.: 257–260 °C (decomp.); $[\alpha]_D^{20}$ = −9.1° (c 0.14, $CHCl_3$); IR

(ATR):ν = 2936 m, 1729 m, 1600 s, 1517 m, 1369 m, 1245 s, 988 s, 804 m, 7451 m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.58–4.26 (m, 1H, 3-H), 4.00–3.83 (m, 1H, 35-H), 3.43 (dt, J = 11.4, 6.0 Hz, 1H, 19-H), 3.11–2.85 (m, 1H, 32-H), 2.21 (dt, J = 11.8, 4.5 Hz, 1H, 13-H), 2.14 (s, 3H, 29-H), 2.09–2.03 (m, 2H, 18-H, 21-H_a), 2.01 (s, 3H, 31-H), 1.95–1.85 (m, 1H, 16-H_b), 1.77–1.01 (m, 27-H, 36-H, 34-H, 37-H, 33-H, 22-H, 12-H, 2-H, 1-H_b, 16-H_b, 15-H, 21-H_b, 6-H, 11-H, 7-H, 9-H), 0.97 (s, 3H, 27-H), 0.88 (s, 3H, 26-H), 0.87 (s, 1H, 1-H_a), 0.82 (s, 3H, 25-H), 0.81 (s, 3H, 24-H), 0.80 (s, 3H, 23-H), 0.79–0.73 (m, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 213.0 (C-20), 175.3 (C-28), 171.0 (C-30), 81.0 (C-3), 55.5 (C-17), 55.4 (C-5), 51.3 (C-19), 50.5 (C-9), 50.2 (C-18), 47.6 (C-32), 45.1 (C-35), 42.3 (C-14), 40.8 (C-8), 38.5 (C-1), 38.2 (C-22), 37.9 (C-10), 37.2 (C-4), 36.9 (C-13), 34.3 (C-7), 33.2 (C-16), 30.8 (C-37, C-33), 30.4 (C-29), 29.6 (C-15), 28.7 (C-21), 28.0 (C-23), 27.9 (C-36, C-34), 27.6 (C-12), 23.8 (C-2), 21.4 (C-31), 21.1 (C-11), 18.3 (C-6), 16.6 (C-24), 16.3 (C-25), 14.8 (C-27) ppm; MS (ESI, MeOH/$CHCl_3$, 4:1): m/z 597.2 (95%, $[M - H]^-$), 631.3 (100%, $[M + Cl]^-$); analysis calcd for $C_{37}H_{60}N_2O_4$ (596.88): C 74.45, H 10.13, N 4.69; found: C 74.23, H 10.39, N 4.37.

(3β)-28-[(2-Aminoethyl)amino]-28-oxoolean-12-en-3-yl acetate (**17**). This compound (0.69 g, 83%) was obtained from **1** following GPB as a colorless solid;[35,36] m.p. 211–214 °C (lit.:[36] 212–215 °C); $[\alpha]_D^{20}$ = +38.3° (c 0.4, $CHCl_3$) [lit.:[36] $[\alpha]_D^{20}$ = +37.8° (c 0.35, $CHCl_3$); MS (ESI, MeOH): m/z 541.2 (100%, $[M + H]^+$).

(3β)-28-[(2-Aminoethyl)amino]-28-oxours-12-en-3-yl acetate (**18**). This compound (0.81 g, 87%) was obtained from **2** following GPB as a colorless solid;[37–40] m.p. 202–205 °C (lit.:[37] 140–142 °C); $[\alpha]_D^{20}$ = +39.0° (c 0.2, $CHCl_3$) [lit.:[18] $[\alpha]_D^{20}$ = +39.4° (c 0.555, $CHCl_3$); MS (ESI, MeOH): m/z 541.3 (100%, $[M + H]^+$).

(3β)-28-[(2-Aminoethyl)amino]-28-oxolup-20(29)-en-3-yl acetate (**19**). This compound (0.86 g, 93%) was obtained from **3** following GPB as a colorless solid;[41] m.p. 150–153 °C (lit.:[18] 152–154 °C); $[\alpha]_D^{20}$ = +8.1° (c 0.25, $CHCl_3$) [lit.:[18] $[\alpha]_D^{20}$ = +8.4° (c 0.33, $CHCl_3$); MS (ESI, MeOH): m/z 541.2 (100%, $[M + H]^+$).

(3β)-28-[(2-Aminoethyl)amino]-20,28-dioxo-30-norlupan-3-yl acetate (**20**). This compound (0.80 g, 88%) was obtained from **4** following GPB as a colorless solid;[42] m.p. 231–234 °C (lit.:[19] 230–234 °C); $[\alpha]_D^{20}$ = −8.5° (c 0.20, $CHCl_3$) [lit.:[19] $[\alpha]_D^{20}$ = −8.5° (c 0.16, $CHCl_3$); MS (ESI, MeOH): m/z 543.1 (100%, $[M + H]^+$).

(3β)-28-Oxo-piperazin-1-yl-olean-12-en-3-yl acetate (**21**). This compound (0.91 g, 92%) was obtained from **1** following GPB as a colorless solid; [43–46] m.p. 170–175 °C (lit.: [24] 170–176 °C); MS (ESI, MeOH): m/z 567.4 (50%, $[M + H]^+$).

(3β)-28-Oxo-piperazin-1-yl-ursan-12-en-3-yl acetate (**22**). This compound (0.85 g, 85%) was obtained from **2** following GPB as a colorless solid; [47,48] m.p. 157–160 °C (lit.: [24] 158–161 °C); MS (ESI, MeOH): m/z 567.3 (60%, $[M + H]^+$).

(3β)-28-Oxo-piperazin-1-yl-lup-20(29)-en-3-yl acetate (**23**). This compound (0.90 g, 93%) was obtained from **3** following GPB as a colorless solid; [47,48] m.p. 177–180 °C (lit.: [24] 177–181 °C); MS (ESI, MeOH): m/z 567.3 (38%, $[M + H]^+$).

(3β)-20,28-Dioxo-piperazin-1-yl-30-norlupan-3-yl acetate (**24**). This compound (0.82 g, 86%) was obtained from **4** following GPB as a colorless solid; [47,48] m.p. 115–123 °C (lit.: [24] 115–125 °C); MS (ESI, MeOH): m/z 569.2 (25%, $[M + H]^+$).

(3β)28-(1,4-Diazabicyclo[3.2.2]nonyl-4-yl)-28-oxoolean-12-en-3-yl acetate (**25**). Following GPB from **1** (626 mg, 1.26 mmol) and **7** (500 mg, 2.51 mmol), **25** (462 mg, 73%) was obtained as colorless solid; m.p. 271–274 °C; R_f = 0.7 ($CHCl_3$/MeOH, 9:1); $[\alpha]_D$ = +20.5° (c 0.15, $CHCl_3$); IR (ATR): ν = 2943 br, 1732 m, 1621 m, 1463 w, 1393 m, 1363 m, 1243 s, 1174 m, 1140 m, 1115 w, 1026 m, 1005 m, 749 m cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.15–5.99 (m, 2H, 34-H), 5.27–5.24 (m, 1H, 12-H), 4.76 (m, 1H, 39-H), 4.51–4.44 (m, 1H, 3-H), 4.38–3.99 (m, 5H, 35-H_a + 37-H_2 + 40-H_2), 3.75 (t, J = 12.0 Hz, 1H, 35-H_b) , 3.04 (d, J = 13.6 Hz, 1H, 18-H), 2.38–2.25 (m, 2H, 38-H_2), 2.24–2.11 (m, 2H, 41-H_2), 2.03 (s, 3H, 32-H_3), 2.00–1.81 (m, 3H, 11-H_2 + 16-H_a), 1.61 (m, 7H, 1-H_a + 6-H_a + 9-H + 15-H_a + 19-H_a + 22-H_2), 1.48–1.16 (m, 10H,

1-H_b + 2-H_2 + 6-H_b + 7-H_2 + 16-H_b + 19-H_b + 21-H_2), 1.13 (s, 3H, 26-H_3), 0.92 (s, 3H, 23-H_3), 0.91 (s, 3H, 25-H_3), 0.90 (s, 3H, 30-H_3), 0.86 (s, 3H, 29-H_3), 0.84 (s, 3H, 24H_3), 0.81 (s, 1H, 5-H), 0.67 (s, 3H, 27-H_3) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 174.9 (C-28), 171.0 (C-31), 144.1 (C-13), 122.0 (C-12), 80.9 (C-3), 72.4 (C-34), 55.4 (C-35), 55.3 (C-5), 47.9 (C-37), 47.6 (C-10), 46.1 (C-17), 45.2 (C-39), 43.7 (C-18), 41.9 (C-14), 40.9 (C-40), 39.1 (C8), 38.1 (C-22), 37.7 (C-20), 37.0 (C-4), 33.8 (C-1), 33.5 (C-21), 32.9 (C-23), 32.8 (C-16), 32.5 (C-7), 28.0 (C-29), 27.9 (C-15), 25.8 (C-26), 24.0 (C-38), 23.5 (C-41), 23.3 (C-19), 22.6 (C-2), 22.5 (C-11), 21.3 (C-32), 18.2 (C-6), 17.0 (C-25), 16.6 (C-24), 15.4 (C-27), 14.1 (C-30), 8.6 (C-9) ppm; MS (ESI, MeOH): m/z = 607.5 (100%, $[M + H]^+$), 608.5 (40%, $[M + 2H]^+$); analysis calcd for $C_{39}H_{62}N_2O_3$ (606.94): C 77.18, H 10.30, N 4.62; found: C 76.84, H 10.58, N 4.45.

(3β)28-(1,4-Diazabicyclo[3.2.2]non-4-yl)-28-oxolup-20(29)en-3-yl acetate (**26**). Following GPB from **3** (250 mg, 0.50 mmol) and **7** (249 mg, 1.25 mmol), **26** (228 mg, 74%) was obtained as a colorless solid; m.p. 242–246 °C; R_f = 0.4 (DCM/MeOH, 9:1); $[\alpha]_D$ = −0.9° (c 0.17, $CHCl_3$); IR (ATR): ν = 2941 m, 1731 m, 1624 m, 1475 m, 1398 m, 1385 m, 1242 s, 1117 m, 1028 m, 978 m, 749 m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.72 (d, J = 2.3 Hz, 1H, 29-H_a), 4.62 (dq, J = 4.6, 2.5 Hz, 1H, 39-H), 4.58 (dt, J = 2.4, 1.4 Hz, 1H, 29-H_b), 4.49–4.45 (m, 1H, 3-H), 3.79–3.64 (m, 2H, 34-H_2), 3.16–2.88 (m, 7H, 13-H + 19-H_2 + 35-H + 37-H_2 + 40-H_2), 2.12 (dt, J = 13.5, 3.5 Hz, 1H, 41-H_a), 2.04 (s, 3H. 32-H_3), 2.02–1.89 (m, 5H, 1-H_a + 16-H_a + 21-H_a + 22-H_a + 41-H_b), 1.89–1.45 (m, 14H, 2-H_2 + 7-H_2 + 12-H_2 + 15-H_a + 18-H_2 + 30-H_3 + 38-H_2), 1.44–1.06 (m, 7H, 6-H_2 + 9-H + 11-H_2 + 16-H_b + 22-H_b), 0.96 (s, 3H, 27-H_3), 0.94 (s, 3H, 25-H_3), 0.92–0.89 (m, 3H, 1-H_b + 15-H_b + 21-H_b), 0.86 (s, 3H, 23-H_3), 0.85 (s, 3H, 26-H_3), 0.84 (s, 3H, 24-H_3), 0.79 (dd, J = 8.7, 3.3 Hz, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 173.3 (C-28), 171.0 (C-31), 151.4 (C-20), 109.1 (C-29), 81.0 (C-3), 55.6 (C-5), 55.2 (C-17), 53.0 (C-18), 50.8 (C-9), 47.4 (C-39), 46.8 (C-37), 46.3 (C-35), 45.7 (C-19), 45.2 (C-34), 41.9 (C-14), 40.7 (C-8), 38.4 (C-1), 37.8 (C-4), 37.2 (C-10), 36.9 (C-13), 36.2 (C-22), 34.3 (C-7), 32.5 (C-40), 31.5 (C-16), 29.9 (C-21), 27.9 (C-23), 26.6 (C-15), 26.5 (C41 + C38), 25.6 (C-12), 23.7 (C-2), 21.3 (C-32), 21.2 (C-11), 19.7 (C-30), 18.2 (C-6), 16.5 (C-24), 16.3 (C-25), 16.1 (C-26), 14.7 (C-27) ppm; MS (ESI, MeOH): m/z = 607.6 (100%, $[M + H]^+$), 608.6 (45%, $[M + 2H]^+$), 1214.2 (5%, $[2M + 2H]^+$); analysis calcd for $C_{39}H_{62}N_2O_3$ (606.94): C 77.18, H 10.30, N 4.62; found: C 76.97, H 10.51, N 4.44.

(3β)28-(1,4-Diazabicyclo[3.2.2]non-4-yl)-20,28-dioxo-30-norlupan-3-yl acetate (**27**). Following GPB from **4** (250 mg, 0.49 mmol) and **7** (238 mg, 1.19 mmol), **27** (270 mg, 99%) was obtained as a colorless solid; m.p. 253 °C (decomp.); R_f = 0.3 (DCM/MeOH/, 9:1); $[\alpha]_D$ = −8.7° (c 0.21, $CHCl_3$); IR (ATR): ν = 2940 br, 1731 m, 1622 m, 1367 m, 1243 s, 1026 m, 978 m, 772 s cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.60 (s, 1H, 39-H), 4.46 (dd, J = 10.5, 5.5 Hz, 1H, 3-H), 3.71 (m, 2H, 34-H_2), 3.25 (td, J = 11.3, 3.5 Hz, 1H, 19-H), 3.18–2.88 (m, 6H, 35-H_2 + 37-H_2 + 40-H_2), 2.79 (td, J = 12.0, 3.8 Hz, 1H, 13-H), 2.16 (s, 3H, 29-H_3), 2.15–2.05 (m, 2H, 18-H + 21-H_a), 2.03 (s, 3H, 32-H_3), 2.01–1.80 (m, 2H, 16-H_a + 21-H_b), 1.79–1.55 (m, 6H, 15-H_a + 16-H_b + 38-H_2 + 41-H_2), 1.54–1.11 (m, 14H, 1-H_a + 2-H_a + 6-H_2 + 7-H_2 + 9-H + 11-H_2 + 12-H_2 + 15-H_b + 22-H_2), 1.05 (dd, J = 13.4, 3.5 Hz, 1H, 2-H_b), 0.98 (s, 3H, 27-H_3), 0.96 (s, 1H, 1-H_b), 0.93 (s, 3H, 26-H_3), 0.83 (m, 6H, 23-H_3 + 25-H_3), 0.82 (s, 3H, 24-H_3), 0.81–0.76 (m, 1H, 5-H) ppm; ^{13}C NMR (126 MHz $CDCl_3$): δ = 213.1 (C-20), 173.3 (C-28), 171.0 (C-31), 80.9 (C-3), 55.5 (C-5), 55.1 (C-17), 52.9 (C-18), 50.7 (C-9), 50.1 (C-19), 47.4 (C-39), 46.8 (C-35), 46.2 (C-37), 46.2 (C-40), 44.9 (C-34), 41.8 (C-14), 40.6 (C-8), 38.4 (C-1), 37.8 (C-4), 37.1 (C-10), 37.1 (C-22), 35.9 (C-13), 35.7 (C-16), 34.2 (C-7), 32.0 (C-38), 30.3 (C-29), 29.9 (C-15), 28.9 (C-21), 27.9 (C-24), 27.5 (C-41), 27.3 (C-12), 23.7 (C-2), 21.3 (C-32), 21.2 (C-11), 18.1 (C-6), 16.5 (C-23), 16.2 (C-25), 16.0 (C-26), 14.7 (C-27) ppm; MS (ESI, MeOH): m/z = 609.5 (25%, $[M + H]^+$); analysis calcd for $C_{38}H_{60}N_2O_4$ (608.91): C 74.96, H 9.93, N 4.60; found: C 74.72, H 10.13, N 4.48.

(3β)28-(1,3-Diazabicyclo[3.2.2]nonyl-3-yl)-28-oxoolean-12-en-3-yl acetate (**28**). Following GPB from **1** (375 mg, 0.75 mmol) and **8** (300 mg, 1.52 mmol), **28** (462 mg, 73%) was obtained as an off-white solid; m.p. 130 °C (decomp.); R_f = 0.3 ($CHCl_3$/MeOH, 98:2); $[\alpha]_D$ = +10.5° (c 0.16, $CHCl_3$); IR (ATR): ν = 3221 brw, 2942 brm, 1731 m, 1610 m, 1530 m, 1446 s, 1366 m,

1244 s, 1026 s, 655 s cm^{-1}; ^1H NMR (400 MHz, MeOH-d_4): δ = 5.83 (ddt, J = 9.9, 3.7, 1.7 Hz, 1H, 34-H_a), 5.72–5.66 (m, 1H, 34-H_b), 5.36 (t, J = 3.7 Hz, 1H, 12-H), 4.45 (dd, J = 10.9, 5.0 Hz, 1H, 3-H), 3.46 (d, J = 6.5 Hz, 1H, 39-H_a), 3.27 (d, J = 6.7 Hz, 1H, 39-H_b), 3.21 (dt, J = 8.9, 2.8 Hz, 1H, 18-H), 2.84 (dq, J = 15.0, 5.9 Hz, 2H, 36-H_2), 2.73 (m, 3H, 18-H + 40-H_2), 2.28 (q, J = 2.5 Hz, 2H, 37-H_2), 2.14–2.05 (m, 2H, 9-H + 11-H_a), 2.02 (s, 3H, 32-H_3), 1.97–1.87 (m, 4H, 2-H_2 + 11-H_b + 15-H_a) 1.84–1.73 (m, 2H, 6-H_a + 19-H_a), 1.69–1.51 (m, 7H, 16-H_a + 21-H_2 + 38-H_2 + 41-H_2), 1.51–1.20 (m, 7H, 1-H_2 + 6-H_b + 7-H_2 + 22-H_2), 1.18 (s, 3H, 26-H_3), 1.16–0.99 (m, 3H, 15-H_b + 16-H_b + 19-H_b), 0.97 (s, 3H, 25-H_3), 0.94 (s, 3H, 30-H_3), 0.91 (s, 3H, 23-H_3), 0.88 (s, 3H, 29-H_3), 0.87 (s, 3H, 24-H_3), 0.85 (s, 1H, 5-H), 0.79 (s, 3H, 27-H_3) ppm; ^{13}C NMR (101 MHz, MeOH-d_4): δ = 179.3 (C-28), 171.5 (C-31), 143.7 (C-13), 124.9 (C-34), 123.2 (C-34),122.7 (C-12), 81.1 (C-3), 56.1 (C-40), 55.3 (C-5), 49.6 (C36), 47.1 (C-38), 46.3 (C-17), 46.2 (C-19), 41.5 (C-14), 41.3 (C-18), 39.3 (C-8), 37.9 (C-1), 37.3 (C-20), 36.7 (C-4), 35.7 (C-39), 34.1 (C-21), 33.7 (C-10), 32.8 (C-16), 32.5 (C-22), 32.1(C-23), 30.2(C-7), 27.2 (C-29) , 27.1 (C-15), 25.0 (C-26), 24.6 (C-41),23.4 (C-38) 23.2 (C-37), 23.1 (C-11), 22.6 (C-30), 22.2 (C-2), 19.7 (C-32), 17.9 (C-6), 16.5 (C-27), 15.7 (C-24), 14.5 (C-25) ppm; MS (ESI, MeOH): m/z = 607.4 (100%, $[M + H]^+$), 608.4 (60%, $[M + 2H]^+$); analysis calcd for $C_{39}H_{62}N_2O_3$ (606.94): C 77.18, H 10.30, N 4.62; found: C 76.93, H 10.56, N 4.49.

(3β)28-(1,3-Diazabicyclo[3.2.2]nonyl)-3-yl)-28-oxours-12-en-3-yl acetate (**29**). Following GPB from **2** (375 mg, 0.75 mmol) and **8** (404 mg, 2.03 mmol), **29** (332 mg, 73%) was obtained as a colorless solid; m.p. 135–139 °C (decomp.); R_f = 0.35 (DCM/MeOH, 96:4); $[\alpha]_D$ = +28.6° (c 0.14, $CHCl_3$); IR (ATR): ν = 2924 br, 1733 s, 1651 m, 1455 m, 1369 m, 1243 s, 1141 w, 1026 m, 653 m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.77 (m, 1H, 34-H_a), 5.67 (m, 1H, 34-H_b), 5.27 (t, J = 3.7 Hz, 1H, 12-H), 4.60–4.44 (dd, J = 10.0, 6.0 Hz, 1H, 3-H), 3.41–3.19 (m, 2H, 39-H_2), 3.07 –2.82 (m, 2H, 36-H_2), 2.64 (dt, J = 10.8, 5.2 Hz, 1H, 40-H_a), 2.53 (dt, J = 9.0, 5.5 Hz, 2H, 18-H, 40-H_b), 2.29–2.10 (m, 3H, 11-H_2 + 16-H_a), 2.06 (s, 3H, 32-H_3), 2.02–1.83 (m, 6H, 19-H + 1-H_a + 2-H_2 + 7-H_2), 1.80–1.70 (m, 3H, 15-H_a + 37-H_2), 1.64 (m, 4H, 6-H_a + 21-H_a + 22-H_2), 1.59–1.25 (m, 10H, 6-H_b + 9-H + 16-H_b + 20-H_2 + 21-H_b + 38-H_2 + 41-H_2), 1.09 (s, 3H, 27-H_3), 1.08–0.95 (m, 2H, 1-H_b + 15-H_b), 0.96 (s, 3H, 25-H_3), 0.94 (s, 3H, 30-H_3) 0.89 (s, 3H, 29-H_3), 0.88 (s, 3H, 24-H_3), 0.87 (s, 3H, 23-H_3), 0.85–0.81 (m, 1H, 5-H), 0.79 (s, 3H, 26-H_3) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 178.1 (C-28), 171.0 (C-31), 139.2 (C-13), 125.8(C-12), 125.2 (C-34), 125.1 (C-34), 80.9 (C-3), 56.7 (C-18), 55.2(C-5), 53.9 (C-19), 52.3 (C-36), 50.3 (C-40), 47.8 (C-17), 47.5 (C-9), 42.4 (C-14), 39.8 (C-38), 39.6 (C-8), 39.1 (C-20), 38.3 (C-1), 37.7 (C-4), 37.3 (C-37), 36.9 (C-10), 36.2 (C-7), 36.2 (C-39), 32.7(C-22), 31.0 (C-21), 28.1 (C-24), 27.9 (C-16), 26.3 (C-11), 24.9 (C-41), 23.5 (C-2), 23.5 (C-15), 23.2 (C-27), 21.3 (C-32), 21.2 (C-25), 18.2 (C-6), 17.3 (C-29), 17.0 (C-26), 16.7 (C-23), 15.6 (C-30) ppm; MS (ESI, MeOH): m/z = 607.3 (100%, $[M + H]^+$), 608.3 (65%; $[M + 2H]^+$; m/z = 605.3 (100%, $[M - H]^-$); analysis calcd for $C_{39}H_{62}N_2O_3$ (606.94): C 77.18, H 10.30, N 4.62; found: C 76.87, H 10.57, N 4.43.

(3β)28-(1,3-Diazabicyclo[3.2.2]non-3-yl)-28-oxolup-20(29)-en-3-yl acetate (**30**). Following GPB from **3** (200 mg, 0.40 mmol) and **8** (238 mg, 1.19 mmol), **30** (125 mg, 95%) was obtained as an amorphous colorless solid; R_f = 0.30 (DCM/MeOH, 98:2); $[\alpha]_D$ = +5.5° (c 0.17, $CHCl_3$); IR (ATR): ν = 2942 m, 1732 m, 1638 m, 1450 m, 1368 m, 1243 s, 1027 m, 978 m, 881 m, 772 m, 653 m cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.75 (m, 1H, 34-H_a), 5.66 (m, 1H, 34-H_b), 4.72 (d, J = 2.4 Hz, 1H, 29-H_a), 4.59 (dt, J = 2.5, 1.4 Hz, 1H, 29-H_b), 4.51–4.42 (m, 1H, 3-H), 3.37 (qt, J = 13.9, 6.8 Hz, 2H, 39-H_2), 3.11–2.92 (m, 3H, 19-H + 36-H_a + 40-H_a), 2.65–2.52 (m, 4H, 36-H_b + 40-H_b), 2.37 (td, J = 12.4, 3.6 Hz, 1H, 9-H), 2.17 (tp, J = 5.7, 2.9, 2.3 Hz, 2H, 13-H + 18-H), 2.03 (s, 3H, 32-H_3), 2.00–1.72 (m, 4H, 1-H_a + 12-H_a + 21-H_a + 22-H_a), 1.68 (m, 4H, 21-H_b + 30-H_3), 1.66–1.56 (m, 6H, 2-H_2 + 6-H_a + 11-H_a + 15-H_a + 22-H_b), 1.55–0.97 (m, 14H, 1-H_b + 6-H_b + 7-H_2 + 11-H_b + 12-H_b + 15-H_b + 16-H_2 + 37-H_2 + 38-H + 41-H_2), 0.95 (s, 3H, 27-H_3), 0.93 (s, 3H, 26-H_3), 0.84 (s, 3H, 23-H_3), 0.83 (s, 3H, 25-H_3), 0.82 (s, 3H, 24-H_3), 0.80–0.74 (m, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 177.7 (C-28), 171.0 (C-31), 151.0 (C-20), 118.9 (C-34), 109.4 (C-29), 80.9 (C-3), 55.9 (C36), 55.4 (C-5), 50.5 (C-9), 50.3(C-40), 50.1 (C-18), 49.2 (C-17), 46.8 (C-19), 42.4 (C-14), 40.8 (C-8), 38.4 (C-13),

38.1 (C-1), 37.8 (C-4), 37.7 (C-38), 37.1 (C-10), 34.3 (C-22), 34.2 (C-7), 33.0 (C-16), 30.9 (C-21), 29.5 (C-15), 27.9 (C-23), 25.6 (C-12), 25.4 (C-41) ,23.7 (C-2), 21.8 (C-37), 21.3 (C-32), 21.0 (C-11), 19.4 (C-30), 18.2 (C-6), 16.5 (C-24), 16.2 (C-25), 16.2 (C-26), 14.6 (C-27) ppm; MS (ESI, MeOH): m/z = 607.3 (100%, $[M + H]^+$, 1241.9 (5%, $[2M + 3H_2O + 2H]^{2+}$); analysis calcd for $C_{39}H_{62}N_2O_3$ (606.94): C 77.18, H 10.30, N 4.62; found: C 76.81, H 10.53, N 4.41.

(3β)28-(1,4-Diazabicyclo[3.2.2]non-4-yl)-20,28-dioxo-30-norlupan-3-yl-acetate (**31**). Following GPB from **4** (250 mg, 0.39 mmol) and **8** (238 mg, 1.19 mmol), **31** (178 mg, 73%) was obtained as a colorless solid; m.p. 130–132 °C; R_f = 0.40 (DCM/MeOH, 95:5); $[\alpha]_D$ = −7.0° (c 0.15, $CHCl_3$); IR (ATR): ν = 2942 brm, 1732 m, 1656 m, 1517 m, 1449 m, 1363 m, 1244 m, 1195 m, 1026 m, 979 m, 772 m, 654 m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.89–5.64 (m, 2H, 34-H_2), 4.45 (dd, J = 11.0, 5.2 Hz, 1H, 3-H), 3.52 (m, 2H, 39-H_2), 3.37 (m, 2H, 36-H_2), 3.33–3.18 (m, 1H, 19-H), 2.87 (dt, J = 26.8, 6.1 Hz, 2H, 40-H_2), 2.42–2.30 (m, 2H, 37-H_2), 2.29–2.17 (m, 1H, 13-H), 2.14 (s, 3H, 29-H_3), 2.12–2.04 (m, 2H, 18-H + 38-H), 2.02 (s, 3H, 32-H_3), 2.00–1.81 (m, 1H, 21-H_a), 1.67–1.11 (m, 18H, 1-H_a + 2-H_a + 6-H_2 + 7-H_2 + 9-H + 11-H_2 + 12-H_2 + 15-H_2 + 16-H_a + 22-H_2 + 41-H_2), 1.05 (m, 3H, 2-H_b + 16-H_b + 21-H_b), 0.97 (s, 3H, 27-H_3), 0.94–0.91 (m, 1H, 1-H_b), 0.89 (s, 3H, 26-H_3), 0.84–0.80 (m, 9H, 23-H_3 + 24-H_3 + 25-H_3), 0.79–0.73 (m, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 212.7 (C-20), 176.8 (C-28), 170.9 (C-31), 125.6 (C-34), 80.8 (C-3), 56.2 (C36), 55.7 (C-17), 55.4 (C-5), 51.3 (C-4), 51.2 (C-19), 50.4 (C-9), 50.0 (C-18), 49.4 (C-40), 42.2 (C-14), 40.7 (C-8), 38.3 (C-1), 37.8 (C-38), 37.8 (C-22), 37.1 (C-10), 36.8 (C-13), 35.0 (C-39), 34.2 (C-7), 32.6 (C-16), 30.1 (C-29), 29.5 (C-15), 28.6 (C-21), 27.9 (C-24), 27.9 (C-25), 27.7 (C-12), 27.2 (C-41), 23.8 (C-2), 23.6 (C-37), 21.3 (C-32), 20.9 (C-11), 18.2 (C-6), 16.5 (C-23), 16.2 (C-26), 14.6 (C-27) ppm; MS (ESI, MeOH): m/z = 609.2 (100%, $[M + H]^+$), 610.2 (50%; $[M + 2H]^+$); analysis calcd for $C_{38}H_{60}N_2O_4$ (608.91): C 74.96, H 9.93, N 4.60; found: C 74.76, H 10.14, N 4.41.

Supplementary Materials: The Supplementary Materials are available online at www.mdpi.com/1420-3049/26/7/2102/s1—representative ^1H-, ^{13}C NMR as well as IR (ATR) spectra.

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References

1. Al-Harrasi, A.; Khan, A.L.; Rehman, N.U.; Csuk, R. Biosynthetic diversity in triterpene cyclization within the Boswellia genus. *Phytochemistry* **2021**, *184*, 112660.
2. Almahli, H. Pentacyclic triterpene acids: Use, mode of action, biological activity and synthesis. *J. Chem. Biol. Phys. Sci.* **2020**, *10*, 294–315.
3. Ayeleso, T.B.; Matumba, M.G.; Mukwevho, E. Oleanolic acid and its derivatives: Biological activities and therapeutic potential in chronic diseases. *Molecules* **2017**, *22*, 1915.
4. Ghiulai, R.; Rosca, O.J.; Antal, D.S.; Mioc, M.; Mioc, A.; Racoviceanu, R.; Macasoi, I.; Olariu, T.; Dehelean, C.; Cretu, O.M.; et al. Tetracyclic and pentacyclic triterpenes with high therapeutic efficiency in wound healing approaches. *Molecules* **2020**, *25*, 5557.

5. Hordyjewska, A.; Ostapiuk, A.; Horecka, A.; Kurzepa, J. Betulin and betulinic acid: Triterpenoids derivatives with a powerful biological potential. *Phytochem. Rev.* **2019**, *18*, 929–951.
6. Khwaza, V.; Oyedeji, O.O.; Aderibigbe, B.A. Ursolic acid-based derivatives as potential anti-cancer agents: An update. *Int. J. Mol. Sci.* **2020**, *21*, 5920.
7. Rios, J.L.; Manez, S. New Pharmacological Opportunities for Betulinic Acid. *Planta Med.* **2018**, *84*, 8–19.
8. Salvador, J.A.R.; Leal, A.S.; Valdeira, A.S.; Goncalves, B.M.F.; Alho, D.P.S.; Figueiredo, S.A.C.; Silvestre, S.M.; Mendes, V.I.S. Oleanane-, ursane-, and quinone methide friedelane-type triterpenoid derivatives: Recent advances in cancer treatment. *Eur. J. Med. Chem.* **2017**, *142*, 95–130.
9. Sharma, N.; Palia, P.; Chaudhary, A.; Shalini; Verma, K.; Kumar, I. A review on pharmacological activities of lupeol and its triterpene derivatives. *J. Drug Deliv. Ther.* **2020**, *10*, 325–332.
10. Sun, Q.; He, M.; Zhang, M.; Zeng, S.; Chen, L.; Zhou, L.; Xu, H. Ursolic acid: A systematic review of its pharmacology, toxicity and rethink on its pharmacokinetics based on PK-PD model. *Fitoterapia* **2020**, *147*, 104735.
11. Lowitz, J.T. Über eine neue fast benzoesäureartige Substanz der Birken. *Crell's Ann. Chem.* **1788**, *2*, 312–317.
12. Pisha, E.; Chai, H.; Lee, I.S.; Chagwedera, T.E.; Farnsworth, N.R.; Cordell, G.A.; Beecher, C.W.W.; Fong, H.H.S.; Kinghorn, A.D.; Brown, D.M.; et al. Discovery of Betulinic Acid as a Selective Inhibitor of Human-Melanoma That Functions by Induction of Apoptosis. *Nat. Med.* **1995**, *1*, 1046–1051.
13. Feng, J.-H.; Chen, W.; Zhao, Y.; Ju, X.-L. Anti-tumor activity of oleanolic, ursolic and glycyrrhetinic acid. *Open Nat. Prod. J.* **2009**, *2*, 48–52.
14. Paduch, R.; Kandefer-Szerszen, M. Antitumor and Antiviral Activity of Pentacyclic Triterpenes. *Mini-Rev. Org. Chem.* **2014**, *11*, 262–268.
15. Paszel-Jaworska, A.; Romaniuk, A.; Rybczynska, M. Molecular Mechanisms of Biological Activity of Oleanolic Acid—A Source of Inspiration for A New Drugs Design. *Mini-Rev. Org. Chem.* **2014**, *11*, 330–342.
16. Csuk, R. Betulinic acid and its derivatives: A patent review (2008–2013). *Expert Opin. Ther. Pat.* **2014**, *24*, 913–923.
17. Hoenke, S.; Heise, N.V.; Kahnt, M.; Deigner, H.-P.; Csuk, R. Betulinic acid derived amides are highly cytotoxic, apoptotic and selective. *Eur. J. Med. Chem.* **2020**, *207*, 112815.
18. Kahnt, M.; Fischer, L.; Al-Harrasi, A.; Csuk, R. Ethylenediamine derived carboxamides of betulinic and ursolic acid as potential cytotoxic agents. *Molecules* **2018**, *23*, 2558.
19. Kahnt, M.; Heller, L.; Al-Harrasi, A.; Schaefer, R.; Kluge, R.; Wagner, C.; Otgonbayar, C.; Csuk, R. Platanic acid-derived methyl 20-amino-30-norlupan-28-oates are potent cytotoxic agents acting by apoptosis. *Med. Chem. Res.* **2018**, *27*, 1757–1769.
20. Kahnt, M.; Heller, L.; Grabandt, P.; Al-Harrasi, A.; Csuk, R. Platanic acid: A new scaffold for the synthesis of cytotoxic agents. *Eur. J. Med. Chem.* **2018**, *143*, 259–265.
21. Siewert, B.; Pianowski, E.; Csuk, R. Esters and amides of maslinic acid trigger apoptosis in human tumor cells and alter their mode of action with respect to the substitution pattern at C-28. *Eur. J. Med. Chem.* **2013**, *70*, 259–272.
22. Siewert, B.; Pianowski, E.; Obernauer, A.; Csuk, R. Towards cytotoxic and selective derivatives of maslinic acid. *Bioorg. Med. Chem.* **2014**, *22*, 594–615.
23. Wolfram, R.K.; Fischer, L.; Kluge, R.; Stroehl, D.; Al-Harrasi, A.; Csuk, R. Homopiperazine-rhodamine B adducts of triterpenoic acids are strong mitocans. *Eur. J. Med. Chem.* **2018**, *155*, 869–879.
24. Sommerwerk, S.; Heller, L.; Kerzig, C.; Kramell, A.E.; Csuk, R. Rhodamine B conjugates of triterpenoic acids are cytotoxic mitocans even at nanomolar concentrations. *Eur. J. Med. Chem.* **2017**, *127*, 1–9.
25. Hoenke, S.; Serbian, I.; Deigner, H.-P.; Csuk, R. Mitocanic Di- and triterpenoid rhodamine B conjugates. *Molecules* **2020**, *25*, 5443.
26. Kahnt, M.; Loesche, A.; Serbian, I.; Hoenke, S.; Fischer, L.; Al-Harrasi, A.; Csuk, R. The cytotoxicity of oleanane derived aminocarboxamides depends on their aminoalkyl substituents. *Steroids* **2019**, *149*, 108422.
27. De Costa, B.R.; He, X.; Linders, J.T.M.; Dominguez, C.; Gu, Z.Q.; Williams, W.; Bowen, W. Synthesis and evaluation of conformationally restricted N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(1-pyrrolidinyl)ethylamines at σ receptors. 2. Piperazines, bicyclic amines, bridged bicyclic amines, and miscellaneous compounds. *J. Med. Chem.* **1993**, *36*, 2311–2320.
28. Mikhlina, E.E.; Rubtsov, M.V. Reaction of 3-quinuclidone with hydrazoic acid. *Zh. Obshch. Khim.* **1963**, *33*, 2167–2172.
29. Takemoto, T.; Kometani, K. Triterpene glycosides (mubenins) from the seeds of Stauntonia hexaphylla. *Justus Liebigs Ann. Chem.* **1965**, *685*, 237–246.
30. Ewen, E.S.; Spring, F.S. Triterpene resinols and related acids. XIV. The oxidation of acetylursolic acid. *J. Chem. Soc.* **1943**, 523–525.
31. Taketa, A.T.C.; Breitmaier, E.; Schenkel, E.P. Triterpenes and triterpenoidal glycosides from the fruits of Ilex paraguariensis (Mate). *J. Braz. Chem. Soc.* **2004**, *15*, 205–211.
32. Petrenko, N.I.; Elantseva, N.V.; Petukhova, V.Z.; Shakirov, M.M.; Shul'ts, E.E.; Tolstikov, G.A. Synthesis of Betulonic Acid Derivatives Containing Amino-Acid Fragments. *Chem. Nat. Compd.* **2002**, *38*, 331–339.
33. Thibeault, D.; Gauthier, C.; Legault, J.; Bouchard, J.; Dufour, P.; Pichette, A. Synthesis and structure-activity relationship study of cytotoxic germanicane- and lupane-type 3β-O-monodesmosidic saponins starting from betulin. *Bioorg. Med. Chem.* **2007**, *15*, 6144–6157.
34. Vystrcil, A.; Budesinsky, M. Triterpenes. XVI. Unusual epimerization of the C-19 acetyl group in 20-oxo-30-norlupane derivatives. *Collect. Czech. Chem. Commun.* **1970**, *35*, 295–311.

35. Brandes, B.; Hoenke, S.; Fischer, L.; Csuk, R. Design, synthesis and cytotoxicity of BODIPY FL labelled triterpenoids. *Eur. J. Med. Chem.* **2020**, *185*, 111858.
36. Loesche, A.; Kahnt, M.; Serbian, I.; Brandt, W.; Csuk, R. Triterpene-based carboxamides act as good inhibitors of butyrylcholinesterase. *Molecules* **2019**, *24*, 948.
37. Bai, K.-K.; Yu, Z.; Chen, F.-L.; Li, F.; Li, W.-Y.; Guo, Y.-H. Synthesis and evaluation of ursolic acid derivatives as potent cytotoxic agents. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2488–2493.
38. Becker, C.S.; Chukanov, N.V.; Grigor'ev, I.A. New Amino-Bisphosphonate Building Blocks in the Synthesis of Bisphosphonic Derivatives Based on Lead Compounds. *Phosphorus Sulfur Silicon Relat. Elem.* **2015**, *190*, 1201–1212.
39. Kahnt, M.; Hoenke, S.; Fischer, L.; Al-Harrasi, A.; Csuk, R. Synthesis and cytotoxicity evaluation of DOTA-conjugates of ursolic acid. *Molecules* **2019,** 24, 2254.
40. Wang, J.; Jiang, Z.; Xiang, L.; Li, Y.; Ou, M.; Yang, X.; Shao, J.; Lu, Y.; Lin, L.; Chen, J.; et al. Synergism of ursolic acid derivative US597 with 2-deoxy-D-glucose to preferentially induce tumor cell death by dual-targeting of apoptosis and glycolysis. *Sci. Rep.* **2014**, *4*, 5006/1–5006/12.
41. Pettit, G.R.; Melody, N. Betulastatin Compounds for Cancer Cell Growth Inhibition. International Patent Application No WO2019094709A1, 16 May 2019.
42. Heller, L.; Kahnt, M.; Loesche, A.; Grabandt, P.; Schwarz, S.; Brandt, W.; Csuk, R. Amino derivatives of platanic acid act as selective and potent inhibitors of butyrylcholinesterase. *Eur. J. Med. Chem.* **2017**, *126*, 652–668.
43. Brandes, B.; Koch, L.; Hoenke, S.; Deigner, H.-P.; Csuk, R. The presence of a cationic center is not alone decisive for the cytotoxicity of triterpene carboxylic acid amides. *Steroids* **2020**, *163*, 108713.
44. Friedrich, S.; Serbian, I.; Hoenke, S.; Wolfram, R.K.; Csuk, R. Synthesis and cytotoxic evaluation of malachite green derived oleanolic and ursolic acid piperazineamides. *Med. Chem. Res.* **2020**, *29*, 926–933.
45. Kahnt, M.; Wiemann, J.; Fischer, L.; Sommerwerk, S.; Csuk, R. Transformation of asiatic acid into a mitocanic, bimodal-acting rhodamine B conjugate of nanomolar cytotoxicity. *Eur. J. Med. Chem.* **2018**, *159*, 143–148.
46. Nie, W.; Luo, J.-G.; Wang, X.-B.; Yin, H.; Sun, H.-B.; Yao, H.-Q.; Kong, L.-Y. Synthesis of new α-glucosidase inhibitors based on oleanolic acid incorporating cinnamic amides. *Chem. Pharm. Bull.* **2011**, *59*, 1051–1056.
47. Hua, S.-X.; Huang, R.-Z.; Ye, M.-Y.; Pan, Y.-M.; Yao, G.-Y.; Zhang, Y.; Wang, H.-S. Design, synthesis and in vitro evaluation of novel ursolic acid derivatives as potential anticancer agents. *Eur. J. Med. Chem.* **2015**, *95*, 435–452.
48. Yang, X.; Li, Y.; Jiang, W.; Ou, M.; Chen, Y.; Xu, Y.; Wu, Q.; Zheng, Q.; Wu, F.; Wang, L.; et al. Synthesis and Biological Evaluation of Novel Ursolic acid Derivatives as Potential Anticancer Prodrugs. *Chem. Biol. Drug Des.* **2015**, *86*, 1397–1404.

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A Malaprade cleavage, a McMurry ring closure and an intramolecular aldol contraction of maslinic acid's ring A

Sophie Hoenke, Immo Serbian, René Csuk *

Martin-Luther University Halle-Wittenberg, Organic Chemistry, Kurt-Mothes-Str.2, D-06120 Halle (Saale), Germany

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ABSTRACT

A silica gel supported Malaprade cleavage of methyl maslinoate (→**3**) followed by a titanium-on-graphite supported McMurry coupling was established to access a 2,12-terpenoid. The microwaves supported aldol reaction of dialdehyde **3** gave ring contracted **5**. While parent maslinic acid is of minor cytotoxicity, improved cytotoxicity was observed in SRB assays for **3** while ring contracted compound **5** was most cytotoxic for MCF-7 breast adenocarcinoma cells holding a low $EC_{50} = 3.1$ μM.

Introduction

In the search for cytotoxic compounds from pentacyclic triterpenes [1–12], we were previously able to show that endoperoxides of the general structure **D** (Fig. 1) exhibit a strong cytotoxic effect albeit their tumor/non-tumor cell selectivity was slightly diminished. [13,14] A key step in the preparation of these compounds was an oxidation in allylic position [13–15], but thereby a mandatory prerequisite is the presence of a double bond between positions C-2 and C-3. These compounds have been made accessible in several steps from the esters of the corresponding triterpene carboxylic acids **A** with an elimination reaction as the key-step to afford the double bond. [16–27] In search of a shorter synthesis that also exhibited higher atomic economy, we came across the titanium-mediated McMurry reaction. [28–31] For the realization of this synthesis strategy, maslinic acid (**MA**, Scheme 1) [32–40] represents an excellent starting material. This triterpene carboxylic acid can be obtained from the waste products of olive oil production. [41–45] This olive pomace is available in enormous quantities but **MA** can also be obtained from commercially available olives, which can be purchased in large amounts at relatively low cost in any supermarket. As an alternative a partial synthesis starting from oleanolic acid has been devised. [46].

Results and discussion

In countries that do not produce olive oil themselves, it is difficult to get hold of the press residues from the oil production; transport costs are also high, as these residues have to be delivered as refrigerated transport in order to avoid fermentation processes of the pomace. As previously shown, there is no huge difference in the amount of **MA** that can be extracted from these press residues or from the olives. We therefore decided to use olives as a starting material. Thus, maslinic acid was extracted from pitted olives and obtained in a yield of 0.33 %. Its esterification with methyl iodide in the presence of potassium carbonate gave the corresponding ester **1**. Maslinic acid and its ester **1** [47–51] are stable, and they can be stored at 5 °C for months without any significant decomposition.

Cleavage of vicinal diols has been accomplished by a variety of different methods, [52] but, interestingly enough, there are only two reports in literature applying this reaction to dihydroxylated terpenes. While lead tetraacetate led to a mixture of a dialdehyde and an anellated 2,7-oxepandiol, [53] a sodium periodate oxidation of a triterpene derived amide was reported to give an almost quantitative yield of the corresponding dialdehyde. [54].

We found the method of a silica gel-assisted Malaprade cleavage particularly valuable. [54] These reaction conditions applied onto **MA** or its methyl ester **1** proceeded well within a few minutes and afforded the dialdehydes **2** and **3** in good yields. These compounds are characterized in their ^{13}C NMR spectra by the presence of two signals at $\delta =$ 206.9 (C-3) and 202.7 (C-2) ppm, respectively.

Based on our previous experiences with reductive carbonyl coupling reactions, the McMurry reaction was carried out using the titanium-graphite surface compound (prepared from C_8K and $TiCl_3$). [55–57] While the McMurry reaction of **2** gave an inseparable mixture of many

* Corresponding author.
E-mail address: rene.csuk@chemie.uni-halle.de (R. Csuk).

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compounds, the reaction of **3** went very smoothly, and after a short reaction time of 4 h, **4** was obtained in 89 % isolated yield. Thus, this synthesis route represents a drastic shortcut of previous synthesis strategies to terpenoid 2,12-dienes, allowing a more efficient access to this class of compounds. This represents one of the few examples of a successful McMurry coupling in the field of terpenoid natural products [58,59] and furthermore, represents the first application of the highly active Ti on graphite surface compound in this class of compounds.

During the work-up of the McMurry reaction, we observed the formation of two major impurities. HPTLC-MS analysis revealed that one of these impurities held a $m/z = 485.2$, and for the other a quasi-molecule ion $m/z = 467.1$ was detected with the latter obviously originating from the former. Initial structural studies suggested that this might be the product of an intramolecular aldol reaction of the dialdehyde **3**; the second product would result from de-hydration of the initial product.

For confirmation, a microwave assisted reaction of dialdehyde **3** with ethylamine at 70 °C for 6 h was performed that led to the formation of **5** in an aldol reaction with a contracted ring A compared to the original starting material. Close inspection of the reaction by HPTLC showed the presence of minute amounts of another product holding also a m/z 485.2 probably a stereoisomer of **5**; this product could not be isolated in sufficient amounts for characterization. Under forced conditions (12 h, 70 °C), **6** was formed from **3**. Compound **6** was also detected (HPTLC-MS) in the reaction mixture resulting from the treatment of **5** with ion exchange resin (IR120 H^+) in refluxing toluene in the presence of $MgSO_4$.

A putative sequence for the formation of **5** would start by a deprotonation of 1-H leading to an enolate that attacks the carbonyl group at C-3 followed by a protonation leading finally to compound **5**. Elimination of water results in the formation of **6**.

Application of the Zimmerman-Traxler model allows a prediction of the configuration at the two newly created stereogenic centers; as a result, the formyl group and the hydroxyl group at C-1 have to hold a *trans*-configuration. Their absolute configuration can be deduced from NMR data (depicted in Fig. 2). Thus, the vicinal coupling between H-1 and H-3 was 8.6 Hz; this is very close to the expected value of $^3J = 8.7$ Hz and typical for a *trans* orientation; for *cis*-oriented protons a $^3J \sim 5$ Hz would be expected. Furthermore, a 2D-NOESY spectrum showed an interaction between the aldehydic hydrogen and the methyl group C-15. As a consequence, a (*1S*, *3R*) configuration was deduced for compound **5** from these data.

As far as we know, this aldol reaction represents only the third ever application of an intramolecular aldol reaction in the field of triterpenes. [60,61].

As previously shown, many derivatives of pentacyclic triterpenoic acids exhibited pronounced cytotoxicity for a series of human tumor cell lines. Hence, we became interest in the cytotoxic activity of our products. Sulforhodamine assays (SRB) were performed the results of which are summarized in Table 1.

While parent **MA** holds only low cytotoxicity, a significantly higher cytotoxicity was observed for the dialdehyde **3** ranging between $EC_{50} = 4.4$ μM for MCF-7 and 12.3 μM for HT29 cells. The tumor cell/non-tumor cell selectivity also increased for the most cell lines, as shown by the comparison of human breast adenocarcinoma MCF-7 with the non-malignant fibroblasts NIH 3 T3. **MA** is more cytotoxic to NIH 3 T3 with $EC_{50} = 21.1$ μM compared with MCF-7 with EC_{50} over 30 μM, while the EC_{50} of **3** for MCF-7 is half the value of NIH 3 T3 with $EC_{50} = 4.4$ μM and 9.3 μm, respectively. A further increase in cytotoxicity was observed for **5** being of good cytotoxicity for MCF-7 cells ($EC_{50} = 3.1$ μM); for the other cell lines EC_{50} in the low single-digit range between 3.7 μM – 5.2 μM were observed. This compound however, held no significant tumor cell/non-tumor cell selectivity inasmuch as for NIH 3 T3 an $EC_{50} = 3.8$ μM was determined.

Conclusion

In search for an efficient route to 2,12-dieno-triterpenoids we developed a short sequence of reactions starting with a silica gel supported Malaprade cleavage of methyl maslinoate (→**3**) followed by a graphite supported McMurry coupling. This represents one of the few examples of a successful McMurry coupling in the field of terpenoid natural products. Furthermore, this is the first application of the highly active Ti on graphite surface compound in this class of compounds. Dialdehyde **3** proved to be a starting material for a microwave supported aldol reaction to afford ring contracted *abeo*-**5**. Cytotoxicity screenings showed parent maslinic acid of minor cytotoxicity but improved cytotoxicity was observed in SRB assays for **3** while ring contracted compound **5** was most cytotoxic for MCF-7 breast adenocarcinoma cells

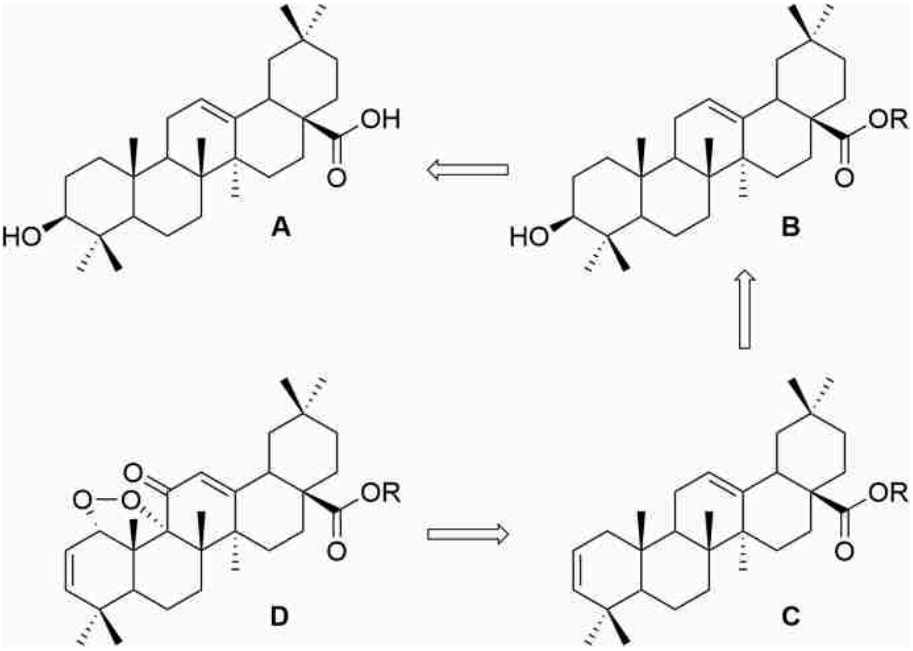


Fig. 1. Synthesis of highly cytotoxic triterpenoic endoperoxides **D** from parent triterpenoic acids A via protection (**A** → **B**), elimination (**B** → **C**) and allylic oxidation (**C** → **D**).

holding a low EC_{50} = 3.1 μM.

Experimental

Equipment, materials and the conditions of the SRB assay have previously been described. [45,49,50] ^{1}H, ^{13}C NMR, MS and IR spectra of known compounds correspond to previously published data.

Preparation of the periodate-on-silica

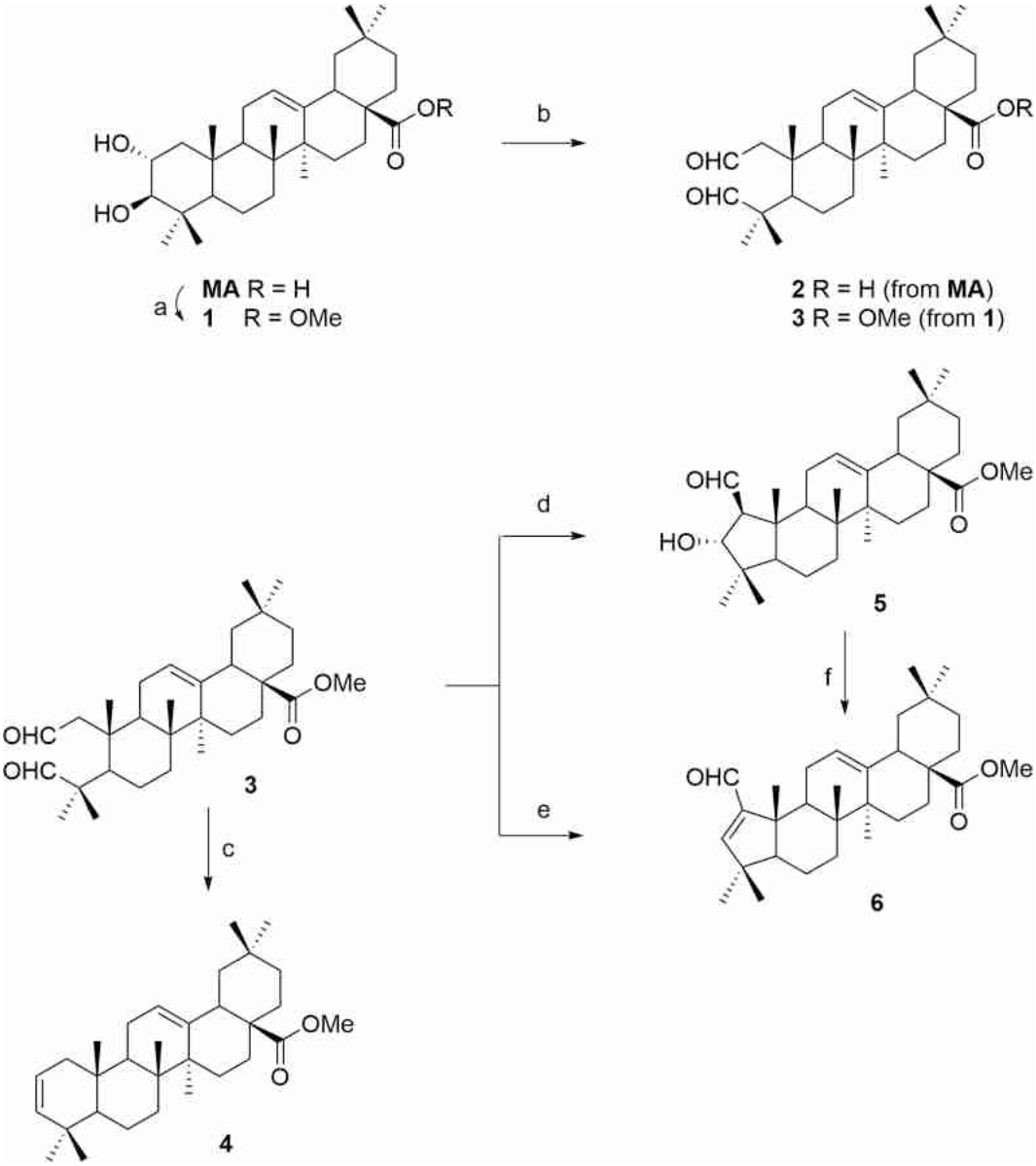
To a solution of sodium metaperiodate (5.04 g, 24.0 mmol) in hot water (85 °C, 10 mL) was added silica gel (20 g) under vigorous stirring. The powder was stirred (ca 200 rpm) for another 30 min and used directly for the reactions. [54].

*Maslinic acid (2α, 3β-Dihydroxyolean-12-en-28-oic acid, **MA**)*

Pitted olives (super market, 5 kg) were crushed and dried at 21 °C for 1 week. Dried olives (ca 1 kg) were mixed and ground with silica gel (1:0.7 ratio) and extracted with methanol (2.0 L) for 1 week. This extraction procedure was performed 3 times. The combined methanol phases were concentrated, and the residue was washed with hexane, dried, and mixed with silica gel (1:1 ratio) and ground. This mixture was extracted for 24 h with ether using a Soxhlet extractor, and the crude product from this extraction was purified by chromatography (silica gel, hexanes/ethyl acetate, 6:4). Maslinic acid (3.55 g, 0.33 %) was obtained as colorless crystals; m.p. 267–270 °C (lit.: [62] m.p. 266–270 °C); $[\alpha]_D$ = +57.6° (*c* 0.24, $CHCl_3$) [lit.: [63] $[\alpha]_D$ = +60.0° (*c* 0.1, $CHCl_3$)]; MS (ESI, MeOH): m/z = 471.5 ($[M-H]^-$, 45 %).

*Methyl (2α,3β)-2,3-dihydroxyolean-12-en-28-oate (**1**)*

Reaction of **MA** (2.0 g, 4.2 mmol) with iodomethane (3.0 mL, 4.6 mmol) in dry DMF (25 mL) in the presence of dry K_2CO_3 (2.80 g, 20.3 mmol) at 21 °C for 12 h followed by usual aq. work-up and chromatography (silica gel, hexanes/ethyl acetate, 8:2) gave **1** (1.65 g, 81 %) as



Scheme 1. Synthesis of **1**–**6**; reactions and conditions: a) MeI, K_2CO_3, DMF, 21 °C, 12 h, 81 %; b) $NaIO_4$ on silica, 90 °C, 5 min, microwaves, 98 % (of **2** from **MA**) and 91 % (of **3** from **1**); c) Ti on graphite, THF, reflux, 12 h, 89 %; d) $EtNH_2$, $MgSO_4$, THF, 70 °C, microwaves, 4 h, 61 %; e) $EtNH_2$, $MgSO_4$, THF, 70 °C, microwaves, 12 h, 10 %; f) IR120 (H^+), $MgSO_4$, toluene, reflux, product **6** was detected by HPTLC-MS only (not isolated).

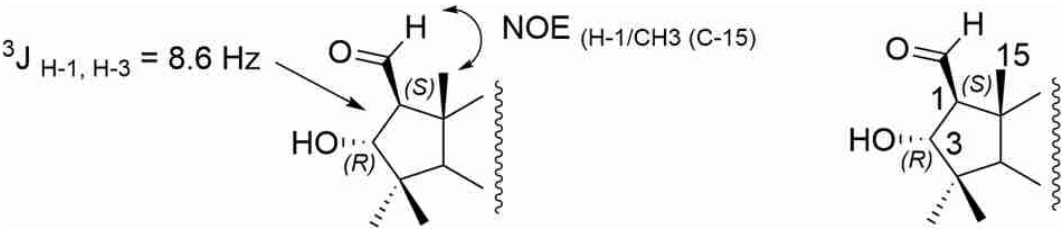


Fig. 2. NMR spectroscopic data of compound **5** to establish the absolute configuration at the newly created stereogenic centers.

Table 1
Cytotoxicity of **MA** and selected compounds (in μM from SRB assays, 92 h of incubation, all experiments were performed in triplicate with three technical replicas each; n.d. not determined); doxorubicin (**DOX**) and staurosporine (**STA**) have been used as positive controls; cell lines: human malignant: 518A2 (melanoma), FaDu (pharynx carcinoma), HT29 (colon adenocarcinoma), MCF-7 (breast adenocarcinoma), A549 (alveolar adenocarcinoma), SW1736 (thyroid anaplastic carcinoma), A2780 (ovarian carcinoma); non-malignant: NIH 3 T3 (murine fibroblasts).

	518A2	FaDu	HT29	MCF-7	A549	SW1736	A2780	NIH 3 T3
MA	13.7 ± 1.0	19.6 ± 0.8	28.8 ± 0.5	> 30	23.5 ± 0.5	17.8 ± 1.1	19.5 ± 0.8	21.1 ± 0.2
1	15.6 ± 1.1	18.3 ± 1.4	12.8 ± 0.9	16.3 ± 1.0	19.7 ± 0.5	16.8 ± 1.2	17.3 ± 1.5	21.4 ± 1.9
2	11.4 ± 0.9	13.2 ± 0.7	11.5 ± 0.6	7.3 ± 1.0	6.3 ± 0.9	10.4 ± 1.7	6.3 ± 1.5	10.9 ± 1.2
3	9.2 ± 0.8	11.0 ± 1.1	12.3 ± 0.7	4.4 ± 0.5	5.8 ± 0.4	10.5 ± 1.0	5.1 ± 0.9	9.3 ± 1.0
5	4.1 ± 0.3	4.7 ± 0.2	4.6 ± 0.3	3.1 ± 0.7	4.3 ± 0.3	5.2 ± 0.5	3.7 ± 0.1	3.8 ± 0.5
DOX	n.d.	n.d.	0.9 ± 0.01	1.1 ± 0.01	n.d.	n.d.	0.1 ± 0.01	0.01 ± 0.001
STA	n.d.	n.d.	0.9 ± 0.01	1.1 ± 0.3	n.d.	n.d.	0.01 ± 0.01	0.45 ± 0.04

a colorless solid; m.p. 229–231 °C (lit.: [49] 229–231 °C); $[\alpha]_D = +60.6°$ (*c* 0.21, $CHCl_3$) [lit.: [49] $[\alpha]_D = +60.9°$ (*c* 6.5, $CHCl_3$)]; MS (ESI, MeOH): m/z = 488.5 ($[M + H]^+$, 100 %).

*2,3-Dioxo-2,3-seco-olean-12-en-28-oic acid (**2**)*

A mixture of **MA** (200 mg, 0.42 mmol) and the silica assisted periodate reagent (800 mg) in dry DCM (40 mL) was heated for 5 min at 90 °C (microwave assisted). The mixture was allowed to cool to 21 °C followed by usual aq. work-up and flash chromatographic (silica gel, hexanes/ethyl acetate, 3:2) to yield **2** (194 mg, 98 %) as a colorless solid; m.p. 166–170 °C; $[\alpha]_D = +102.0°$ (*c* 0.54, $CHCl_3$); R_F = 0.65 (silica gel, hexanes/ethyl acetate, 3:2); IR (KBr): ν = 3416 m, 2950 s, 1718 s, 1694 s, 1464w, 1387w, 1366 m, 1305 m, 1273 m, 1034 m, 646 m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 9.81 (dd, *J* = 1.9, 1.9 Hz, 1H, 2-CHO), 9.59 (s, 1H, 3-CHO), 5.25 (dd, *J* = 3.4, 3.4 Hz, 1H, 12-H), 2.80 (dd, *J* = 13.7, 4.1 Hz, 1H, 18-H), 2.53 and 2.28 (dd, *J* = 17.6, 2.1 Hz, 2H, 1-$H_{a,b}$), 2.21 (dd, *J* = 10.4, 7.2 Hz, 1H, 9-H), 2.10 (m, 1H, 5-H), 1.95 (m, 1H, 16-H_a), 1.90 (m, 2H, 11-$H_{a,b}$), 1.74 (m, 1H, 22-H_a), 1.63 (m, 1H, 15-H_a), 1.59 (m, 1H, 16-H_b), 1.57 (m, 1H, 19-H_a), 1.53 (m, 1H, 22-H_a), 1.49 (m, 2H, 6-$H_{a,b}$), 1.49 (m, 1H, 7-H_a), 1.30 (m, 1H, 7-H_b), 1.30 (m, 1H, 21-H_a), 1.20 (m, 1H, 21-H_b), 1.12 (m, 1H, 19-H_b), 1.10 (s, 3H, 23-H), 1.10 (s, 3H, 27-H), 1.09 (m, 1H, 15-H_b), 1.07 (s, 3H, 24-H), 0.97 (s, 3H, 26-H), 0.90 (s, 3H, 29-H), 0.87 (s, 3H, 30-H), 0.74 (s, 3H, 25-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.9 (CHO, C-3), 202.7 (CHO, C-2), 185.5 (C-28), 143.6 (C-13), 122.1 (C-12), 52.1 (C-1), 51.0 (C-4), 49.2 (C-5), 46.7 (C-17), 45.8 (C-19), 43.4 (C-10), 42.2 (C-14), 41.3 (C-9), 41.0 (C-18), 39.5 (C-8), 33.9 (C-21), 33.1 (C-30), 32.5 (C-22), 32.0 (C-7), 30.8 (C-20), 27.8 (C-15), 25.5 (C-27), 24.1 (C-23), 23.9 (C-11), 23.6 (C-29), 22.9 (C-16), 20.4 (C-6), 19.9 (C-24), 19.5 (C-26), 17.1 (C-25) ppm; MS (ESI, MeOH): m/z = 469.7 ($[M-H]^-$, 100 %), 939.3 ([2 M−H]-, 87 %); analysis calcd for $C_{30}H_{46}O_4$ (470.69): C 76.55, H 9.85; found: C 76.32, H 9.97.

*Methyl 2,3-dioxo-2,3-seco-olean-12-en-28-oate (**3**)*

Following the procedure given for **2**, from **1** (100 mg, 0.2 mmol) and periodate reagent (0.4 g) followed by chromatography (silica gel, hexanes/ethyl acetate, 6:4) **3** (90 mg, 91 %) was obtained as a white solid; m.p. 110–112 °C; $[\alpha]_D = +91.5°$ (*c* 0.54, $CHCl_3$); R_F = 0.79 (silica gel, hexanes/ethyl acetate, 6:4); IR (KBr): ν = 3446br, 2049 s, 1725 s, 1462 s, 1386 m, 1366 m, 1303w, 1261 s, 1234 m, 1196 m,1164 s, 1103 m, 1058 m, 1035 s, 954 m, 818 m, 788 m, 756 s, 650 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 9.81 (dd, *J* = 2.2, 2.2 Hz, 1H, 2-CHO), 9.60 (s, 1H, 3-CHO), 5.27 (dd, *J* = 3.6, 3.6 Hz, 1H, 12-H), 3.60 (s, 3H, OMe), 2.85 (dd, *J* = 13.7, 4.7 Hz, 1H, 18-H), 2.53 (dd, *J* = 17.6, 2.3 Hz, 1-H_a), 2.27 (dd, *J* = 17.6, 2.3 Hz, 1H, 1-H_b), 2.20 (dd, *J* = 10.9, 6.8 Hz, 1H, 9-H), 2.09 (m, 1H, 5-H), 1.95 (m, 1H- 16-H_a), 1.90 (m, 1H, 16-H_b), 1.66 (m, 1H, 7-H_a), 1.62 (m, 1H, 11-H_a), 1.60 (m, 1H, 19-H_a), 1.59 (m, 1H, 11-H_b), 1.54 (m, 1H, 15-H_b), 1.50 (m, 1H, 6-H_a), 1.50 (m, 1H, 7-H_b), 1.49 (m, 1H, 6-H_b), 1.30 (m, 2H, 22-$H_{a,b}$), 1.30 (m, 1H, 21-H_a), 1.18 (m, 1H, 21-H_b), 1.12 (m, 1H, 19-H_b), 1.11 (s, 3H, 29-H), 1.10 (s, 3H, 27-H), 1.09 (m, 1H, 15-H_a), 1.08 (s, 3H, 24-H), 0.97 (s, 3H, 26-H), 0.90 (s, 3H, 23-H), 0.87 (s, 3H, 30-H), 0.74 (s, 3H, 25-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.9 (CHO, C-3), 202.7 (CHO, C-2), 178.3 (C-28), 143.9 (C-13), 121.9 (C-12), 52.2 (C-1), 51.6 (C-31), 51.0 (C-4), 49.3 (C-5), 46.8 (C-17), 45.8 (C-19), 42.2 (C-14), 41.5 (C-18), 41.4 (C-9), 40.1 (C-8), 39.5 (C-10), 34.0 (C-21), 33.2 (C-30), 32.4 (C-7), 32.0 (C-22), 30.8 (C-20), 27.8 (C-15), 25.5 (C-27), 24.1 (C-29), 24.0 (C-16), 23.7 (C-23), 23.1 (C-11), 20.5 (C-6), 20.0 (C-24), 19.5 (C-26), 16.9 (C-25) ppm; MS (ESI, MeOH): m/z = 485.4 [M + H]+, 100 %); analysis calcd for $C_{31}H_{48}O_4$ (484.72): C 76.82, H 9.98; found: C 76.61, H 10.13.

*Methyl oleana-2,12-dien-28-oate (**4**)*

Natural Graphite (3.0 g, 250 mmol) was degased for 15 min at 150 °C; under vigorous stirring, potassium (1.2 g, 31.0 mmol) was added at this temperature in several portions. The black graphite turned bronze colored upon this reaction. Stirring at 150 °C was continued for another 30 min. $TiCl_3$ (1.58 g, 10.2 mmol) was added in one portion with stirring to the bronze-colored C_8K (31 mmol) in dry THF (30 mL) at 21 °C under argon; the mixture was heated under reflux for 1.5 h. A solution of **3** (2.42 g, 5.0 mmol) in dry THF was added, and the suspension was heated under reflux overnight. For work-up, the solids were filtered off (short path of silica gel), the filtrate was concentrated under reduced pressure, and the residue subjected to chromatography (silica gel, hexanes/ethyl acetate, 6:1) to yield **4** (1.05 g, 89 %) as a colorless solid; m.p. 179–181 °C (lit.: [64] m.p. 182–185 °C), $[\alpha]_D = +144.4°$ (*c* 0.55, $CHCl_3$) [lit.: [64] $[\alpha]_D = +142°$ (*c* 0.50, $CHCl_3$); MS (ESI, MeOH): m/z = 237.4 ($[M + H]^+$, 100 %).

Methyl (1SR, 3R) 3(2 → 1)-abeo-3-hydroxy-2-oxoolean-12-en-28-oate ***(5)***

A mixture of dialdehyde **3** (100 mg, 0.21 mmol), ethylamine in THF (20 mL, 25 %) and $MgSO_4$ (25 mg) was heated at 70 °C (microwave assisted) for4h. Usual aqueous work-up followed by chromatography (silica gel, hexanes/ethyl acetate, 7:3) gave **5** (62 mg, 61 %) as a colorless solid; m.p. 181–184 °C; $[\alpha]_D = +24.4°$ (*c* 0.68, $CHCl_3$); R_F = 0.35 (silica gel, hexanes/ethyl acetate, 7:3); IR (KBr): ν = 3484br, 2953 s, 2918 s, 2856 s, 1727 s, 1648 m, 1464 s, 1386 s, 1364 m, 1266 s, 1206 m, 1178 m, 1056 s, 1040 s, 824 m, 790 m, 752 m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 9.94 (d, *J* = 2.7 Hz, 2-H), 5.25 (dd, *J* = 3.4, 3.4 Hz, 1H, 12-H), 4.34 (d, *J* = 8.6 Hz, 1H, 3-H), 3.61 (s, 3H, 31-H), 2.84 (dd, *J* = 13.8, 4.3 Hz, 1H, 18-H), 2.22 (dd, *J* = 8.6, 2.6 Hz, 1H, 1-H), 2.12–1.90 (m, 3H, 11-H_a + 16-H_a + 9-H), 1.90–1.80 (m, 1H, 11-H_b), 1.74–1.56 (m, 4H, 22-H_a + 16-H_b + 15-H_a + 19-H_a), 1.56–1.36 (m, 4H, 22-H_b + 7-H_a + 6-$H_{a,b}$), 1.37–1.22 (m, 2H, 7-H_b + 21-H_a), 1.22–1.02 (m, 3H, 21-H_b + 19-H_b + 15-H_b), 1.16 (s, 3H, 27-H), 1.00 (s, 3H, 25-H), 0.98 (s, 3H, 24-H), 0.94 (s, 3H, 23-H), 0.91 (s, 3H, 29-H), 0.89)s, 3H, 30-H), 0.71 (s, 3H, 26-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 205.4 (C-2), 178.3 (C-28), 144.6 (C-13), 121.6 (C-12), 80.9 (C-3), 73.6 (C-1), 62.3 (C-5), 51.7 (C-31), 47.2 (C-9), 46.8 (C-4), 46.5 (C-17), 45.9 (C-19), 42.0 (C-14), 41.5 (C-18), 40.6 (C-8), 40.2 (C-10), 34.0 (C-21), 33.2 (C-30), 32.8 (C-7), 32.4 (C-22), 30.8 (C-20), 28.0 (C-15), 26.8 (C-11), 26.3 (C-27), 25.6 (C-23), 25.4 (C-24), 23.8 (C-29), 23.1 (C-16), 17.7 (C-26), 14.2 (C-25) ppm; MS (ESI, MeOH): *m/z* = 485.2 ($[M + H]^+$, 74 %), 502.7 ($[M + NH_4]^+$, 90 %), 507.3 ($[M + Na]^+$, 100 %); analysis calcd for $C_{31}H_{48}O_4$ (484.72): C 76.82, H 9.98, found: C 76.57, H 10.19.

Methyl 3(2 → 1)-abeo-2-oxoolean-1,12-dien-28-oate ***(6))***

Following the procedure given for **5**, from **3** (100 mg, 0.21), ethylamine in THF (20 mL) and $MgSO_4$ (25 mg) but applying a reaction time of 12 h, **6** (10 mg, 10 %) was obtained as an amorphous solid; $[\alpha]_D = +32.4°$ (*c* 0.05, $CHCl_3$); R_F = 0.54 (silica gel, hexanes/ethyl acetate, 7:3); ^1H NMR (500 MHz, $CDCl_3$): δ = 9.68 (s, 1H, CHO), 6.56 (s, 1H, 3-H), 5.31 (dd, *J* = 3.5, 3.5 Hz, 12-H), 3.63 (s, 3H, OMe), 2.85 (dd, *J* = 13.7, 4.0 Hz, 18-H), 2.42–2.21 (m, 2H, 11-$H_{a,b}$), 2.10 (dd, *J* = 10.8, 6.7 Hz, 9-H), 2.03–1.91 (m, 1H, 16-H_a), 1.76–1.45 (m, 9H, 22Ha + 15-H_a, 16-H_b + 19Ha + 22-H_b + 5-H + 7-H_a + 6-$H_{a,b}$), 1.44–0.99 (m, 5H, 21-H_a + 7-H_b + 21^-H_b, + 19-H_b + 15-H_b), 1.20 (s, 3H, 26-H), 1.15 (s, 3H, 30-H), 1.14 (s, 3H, 29-H), 1.02 (s, 3H, 24-H), 0.92 (s, 3H, 27-H), 0.89 (s, 3H, 23-H), 0.81 (s, 3H, 25-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 191.2 (C-2), 178.5 (C-28), 163.5 (C-3), 157.1 (C-1), 143.4 (C-13), 123.4 (C-12), 62.9 (C-5), 51.7 (C-31), 51.1 (C-10), 46.9 (C-19), 45.8 (C-17), 44.6 (C-9), 44.1 (C-8), 42.2 (C-4), 41.6 (C-18), 41.2 (C-14), 34.0 (C-21), 33.8 (C-7), 33.3 (C-30), 32.5 (C-22), 30.8 (C-20), 28.7 (C-23), 28.1 (C-15), 27.4 (C-11), 26.6 (C-27), 23.8 (C-29), 23.2 (C-16), 20.9 (C-24), 18.7 (C-25), 18.5 (C-26), 17.0 (C-6) ppm; MS (ESI, MeOH): *m/z* = 449.3 ($[M + H\text{-}H_2O]^+$, 100 %), 467.1 ($[M + H]^+$, 90 %), 489.2 ($[M + Na]^+$, 40 %); analysis calcd for $C_{31}H_{46}O_3$ (466.71): C 79.78, H 9.94; found: C 76.55, H 10.17.

CRediT authorship contribution statement

Sophie Hoenke: Investigation. **Immo Serbian:** Investigation. **René Csuk:** Conceptualization, Writing – original draft, Writing – review & editing.

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

Data will be made available on request.

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References

[1] D.R. Barreto Vianna, J. Gotardi, S.C. Baggio Gnoatto, D.A. Pilger, Natural and Semisynthetic Pentacyclic Triterpenes for Chronic Myeloid Leukemia Therapy: Reality, Challenges and Perspectives, ChemMedChem 16 (2021) 1835–1860.
[2] R. Csuk, Betulinic acid and its derivatives: a patent review (2008–2013), Expert Opin. Ther. Pat. 24 (8) (2014) 913–923.
[3] S. Fulda, Betulinic acid: a natural product with anticancer activity, Mol. Nutr. Food Res. 53 (2009) 140–146.
[4] S. Fulda, G. Kroemer, Targeting mitochondrial apoptosis by betulinic acid in human cancers, Drug Discovery Today 14 (2009) 885–890.
[5] M.H. Ghante, P.G. Jamkhande, Role of Pentacyclic Triterpenoids in Chemoprevention and Anticancer Treatment: An Overview on Targets and Underling Mechanisms, J Pharmacopuncture 22 (2019) 55–67.
[6] J. Hodon, L. Borkova, J. Pokorny, A. Kazakova, M. Urban, Design and synthesis of pentacyclic triterpene conjugates and their use in medicinal research, Eur. J. Med. Chem. 182 (2019), 111653.
[7] H. Hussain, I. Ali, D. Wang, F.L. Hakkim, B. Westermann, I. Ahmed, A.M. Ashour, A. Khan, A. Hussain, I.R. Green, S.T.A. Shah, Glycyrrhetinic acid: a promising scaffold for the discovery of anticancer agents, Expert Opin. Drug Discovery 16 (12) (2021) 1497–1516.
[8] S.M. Kamble, S.N. Goyal, C.R. Patil, Multifunctional pentacyclic triterpenoids as adjuvants in cancer chemotherapy: a review, RSC Adv. 4 (63) (2014) 33370–33382.
[9] A. Kaps, P. Gwiazdon, E. Chodurek, Nanoformulations for delivery of pentacyclic triterpenoids in anticancer therapies, Molecules 26 (2021) 1764.
[10] S.K. Król, M. Kiełbus, A. Rivero-Müller, A. Stepulak, Comprehensive review on betulin as a potent anticancer agent, BioMed Res. Int. 2015 (2015) 1–11.
[11] M. Olech, W. Ziemichod, N. Nowacka-Jechalke, The Occurrence and Biological Activity of Tormentic Acid-A Review, Molecules 26 (2021) 3797.
[12] J.A.R. Salvador, V.M. Moreira, Semisynthetic pentacyclic triterpenoids in the cancer setting: ursolic acid and its derivatives, in: J.A.R. Salvador (Ed.), Pentacyclic Triterpenes as Promiusing Agents in Cancer, Nova Science Publishers Inc, New York, 2010, pp. 249–276.
[13] R. Csuk, A. Niesen-Barthel, A. Barthel, R. Kluge, D. Ströhl, Synthesis of an antitumor active endoperoxide from 11-keto-beta-boswellic acid, Eur. J. Med. Chem. 45 (2010) 3840–3843.
[14] A. Niesen, A. Barthel, R. Kluge, A. Kowitzsch, D. Ströhl, S. Schwarz, R. Csuk, Antitumoractive Endoperoxides from Triterpenes, Arch. Pharm. 342 (2009) 569–576.
[15] A. Chung, M.R. Miner, K.J. Richert, C.J. Rieder, K.A. Woerpel, Formation of an Endoperoxide upon Chromium-Catalyzed Allylic Oxidation of a Triterpene by Oxygen, J. Org. Chem. 80 (2015) 266–273.
[16] A.S.R. Anjaneyulu, M.N. Rao, L.R. Row, A. Sree, Sapogenins of Acacia concinna DC-IV. Wagner-Meerwein rearrangements in acacic acid lactone and some selected triterpenes, Tetrahedron 35 (1979) 519–525.
[17] R.B. Boar, D.W. Hawkins, J.F. McGhie, D.H.R. Barton, Nickel boride as a desulfurizing agent. Improved route to triterpene 2-enes, J. Chem. Soc., Perkin Trans. 1 (1973) 654–657.
[18] C. Djerassi, D.B. Thomas, A.L. Livingston, C.R. Thompson, X.X.X.I. Terpenoids, The structure and stereochemistry of medicagenic acid, J. Am. Chem. Soc. 79 (1957) 5292–5297.
[19] G. Drefahl, S. Huneck, The reduction products of various triterpene oximes and triterpenic acid amides, Chem. Ber. 93 (1960) 1967–1975.
[20] A. García-Granados, J. Dueñas, J.N. Moliz, A. Parra, F.L. Pérez, J.A. Dobado, J. Molina, Semi-synthesis of triterpene A-ring derivatives from oleanolic and maslinic acids. Theoretical and experimental 13C chemical shifts, J. Chem. Res., Synop. 2000 (2) (2000) 56–57.
[21] A. Garcia-Granados, P.E. Lopez, E. Melguizo, J.N. Moliz, A. Parra, Y. Simeo, J. A. Dobado, Epoxides, Cyclic Sulfites, and Sulfate from Natural Pentacyclic Triterpenoids: Theoretical Calculations and Chemical Transformations, J. Org. Chem. 68 (2003) 4833–4844.
[22] X. Liu, X. Gao, X. Jin, C. Zhao, Z. Feng, Y. Sui, L. Zhao, X. Yan, Synthesis, characterization and cytotoxic activity of novel oleanolic acid and ursolic acid derivatives, Youji Huaxue 38 (2018) 3227–3235.
[23] A. Parra, P.E. Lopez, A. Garcia-Granados, Different pathways for the deoxygenation of the A-ring of natural triterpene compounds, Nat. Prod. Res. 24 (2010) 177–196.
[24] G. Snatzke, H.W. Fehlhaber, Simple method for the localization of isolated double bonds in steroids and triterpenes, Justus Liebigs Ann. Chem. 663 (1963) 123–135.

[25] R. Tschesche, E. Henckel, G. Snatzke, Triterpenes., XIV. Constitution of bredemolic and crategolic acids, Justus Liebigs Ann. Chem. 676 (1964) 175–187.
[26] L. Zaprutko, Triterpenoids., Part 8. Structure elucidation of new CrO3 and tert-butyl chromate oxidation products of methyl oleanolate and some of its derivatives, Pol. J. Chem. 68 (1994) 1753–1765.
[27] L. Zaprutko, Triterpenoids., Part 9. Structure elucidation of a new sodium dichromate oxidation product of methyl oleanolate and some of its derivatives, Pol. J. Chem. 69 (1995) 1003–1012.
[28] A. Bongso, R. Roswanda, Y.M. Syah, Recent advances of carbonyl olefination via McMurry coupling reaction, RSC Adv. 12 (2022) 15885–15909.
[29] M. Ephritikhine, A new look at the McMurry reaction, Chem. Commun. (Cambridge) (1998) 2549–2554.
[30] R. Lindner, A. Kuehnle, On-Surface Reactions, ChemPhysChem 16 (2015) 1582–1592.
[31] T. Takeda, A. Tsubouchi, The McMurry coupling and related reactions, Org. React. (Hoboken, NJ, U. S.), 82 (2013) 1-470.
[32] N. Choudhary, N. Singh, A.P. Singh, A.P. Singh, Medicinal uses of maslinic acid: a review, J. Drug Delivery Ther. 11 (2021) 237–240.
[33] J. Deng, H. Wang, X. Mu, X. He, F. Zhao, Q. Meng, Advances in Research on the Preparation and Biological Activity of Maslinic Acid, Mini-Rev. Med. Chem. 21 (2021) 79–89.
[34] Z. Jing, W. Rui, R. Li, Y. Hao, H. Fang, Review of the Biological Activity of Maslinic Acid, Curr. Drug Targets 22 (2021) 1496–1506.
[35] X. Lin, U. Ozbey, U.Y. Sabitaliyevich, R. Attar, B. Ozcelik, Y. Zhang, M. Guo, M. Liu, S.S. Alhewairini, A.A. Farooqi, Maslinic acid as an effective anticancer agent, Cell. Mol. Biol. (Noisy-le-grand) 64 (2018) 87–91.
[36] G. Lozano-Mena, M. Sanchez-Gonzalez, M.E. Juan, J.M. Planas, Maslinic acid, a natural phytoalexin-type triterpene from olives - a promising nutraceutical? Molecules 19 (2014) 11538.
[37] X.-P. Qian, X.-H. Zhang, L.-N. Sun, W.-F. Xing, Y. Wang, S.-Y. Sun, M.-Y. Ma, Z.-P. Cheng, Z.-D. Wu, C. Xing, B.-N. Chen, Y.-Q. Wang, Corosolic acid and its structural analogs: A systematic review of their biological activities and underlying mechanism of action, Phytomedicine 91 (2021), 153696.
[38] C. Sanchez-Quesada, A. Lopez-Biedma, F. Warleta, M. Campos, G. Beltran, J. J. Gaforio, Bioactive Properties of the Main Triterpenes Found in Olives, Virgin Olive Oil, and Leaves of Olea europaea, J. Agric. Food Chem. 61 (2013) 12173–12182.
[39] H. Sharma, P. Kumar, R.R. Deshmukh, A. Bishayee, S. Kumar, Pentacyclic triterpenes: New tools to fight metabolic syndrome, Phytomedicine 50 (2018) 166–177.
[40] L. Yu, X. Xie, X. Cao, J. Chen, G. Chen, Y. Chen, G. Li, J. Qin, F. Peng, C. Peng, The Anticancer Potential of Maslinic Acid and Its Derivatives: A Review, Drug Des Devel Ther 15 (2021) 3863–3879.
[41] K. Chouaib, F. Hichri, A. Nguir, M. Daami-Remadi, N. Elie, D. Touboul, H. Ben Jannet, M.A. Hamza, Semi-synthesis of new antimicrobial esters from the natural oleanolic and maslinic acids, Food Chem. 183 (2015) 8–17.
[42] N. Blanco-Cabra, K. Vega-Granados, L. Moya-Andérico, M. Vukomanovic, A. Parra, L. Álvarez de Cienfuegos, E. Torrents, Novel oleanolic and maslinic acid derivatives as a promising treatment against bacterial biofilm in nosocomial infections: An in vitro and in vivo study, ACS Infect. Dis. 5 (9) (2019) 1581–1589.
[43] I. Fernandez-Pastor, A. Fernandez-Hernandez, S. Perez-Criado, F. Rivas, A. Martinez, A. Garcia-Granados, A. Parra, Microwave-assisted extraction versus Soxhlet extraction to determine triterpene acids in olive skins, J. Sep. Sci. 40 (2017) 1209–1217.
[44] A. Garcia-Granados, A. Martinez, J.N. Moliz, A. Parra, F. Rivas, 2-α, 3-β-dihydroxyolean-12-3n-28-oic acid (maslinic acid), Molecules 3 (1998) M88.
[45] B. Siewert, R. Csuk, Membrane damaging activity of a maslinic acid analog, Eur. J. Med. Chem. 74 (2014) 1–6.
[46] S. Sommerwerk, L. Heller, I. Serbian, R. Csuk, Straightforward partial synthesis of four diastereomeric 2,3-dihydroxy-olean-12-en-28-oic acids from oleanolic acid, Tetrahedron 71 (45) (2015) 8528–8534.
[47] S. Alam, F. Khan, 3D-QSAR studies on Maslinic acid analogs for Anticancer activity against Breast Cancer cell line MCF-7, Sci. Rep. 7 (2017) 1–13.
[48] S. Schwarz, S. Sommerwerk, S.D. Lucas, L. Heller, R. Csuk, Sulfamates of methyl triterpenoates are effective and competitive inhibitors of carbonic anhydrase II, Eur. J. Med. Chem. 86 (2014) 95–102.
[49] B. Siewert, E. Pianowski, R. Csuk, Esters and amides of maslinic acid trigger apoptosis in human tumor cells and alter their mode of action with respect to the substitution pattern at C-28, Eur. J. Med. Chem. 70 (2013) 259–272.
[50] B. Siewert, J. Wiemann, A. Koewitsch, R. Csuk, The chemical and biological potential of C ring modified triterpenoids, Eur. J. Med. Chem. 72 (2014) 84–101.
[51] H. Tan, A. Ashour, Y. Katakura, K. Shimizu, A structure-activity relationship study on antiosteoclastogenesis effect of triterpenoids from the leaves of loquat (Eriobotrya japonica), Phytomedicine 22 (2015) 498–503.
[52] A.S. Perlin, Glycol-cleavage oxidation, Adv. Carbohydr. Chem. Biochem. 60 (2006) 183–250.
[53] N. Aimi, K. Kawada, H. Murakami, Y. Fukumasu, S. Sakai, Partial Synthesis of Dihydroisothysanolactone, and C-13-Nmr Study of Thysanolactone, Heterocycles 23 (1985) 1863–1867.
[54] S. Sommerwerk, L. Heller, J. Kuhfs, R. Csuk, Urea derivates of ursolic, oleanolic and maslinic acid induce apoptosis and are selective cytotoxic for several human tumor cell lines, Eur. J. Med. Chem. 119 (2016) 1–16.
[55] R. Csuk, A. Fürstner, H. Weidmann, A simple and efficient new synthesis of vicinal diols by reductive coupling of carbonyl compounds, J. Chem. Soc., Chem. Commun. (24) (1986) 1802–1803.
[56] A. Fürstner, R. Csuk, C. Rohrer, H. Weidmann, Magnesium- and titanium-induced reductive coupling of carbonyl compounds. Efficient syntheses of pinacols and alkenes, J. Chem. Soc., Perkin Trans. 1 (1988) 1729–1734.
[57] A. Fürstner, H. Weidmann, Highly reactive titanium on graphite. A universal reducing agent for the coupling of carbonyl compounds to alkenes, Synthesis (1987) 1071–1075.
[58] W.G. Dauben, K.L. Lorenz, D.W. Dean, G. Shapiro, I. Farkas, Synthesis of the trinervitane ring system, Tetrahedron Lett. 39 (1998) 7079–7082.
[59] M.P. Mermet-Mouttet, K. Gabriel, D. Heissler, A synthesis of the ring system of terpestacin, Tetrahedron Lett. 40 (1999) 843–846.
[60] K. Kuczynska, J. Jazwinski, Z. Pakulski, P. Cmoch, R. Luboradzki, Oxidative Functionalization of Trinor-18 alpha-olean-17(22)-ene Derivatives. Annulation of the E-Ring by an Intramolecular Aldol Reaction, J. Org. Chem. 86 (2021) 7636–7647.
[61] J.W. Apsimon, R.F. Lawuyi, Approaches to the Synthesis of Triterpenes. 9. Diethylaluminum Ethoxide as a Reagent for the Intramolecular Aldol Condensation, Synthetic Commun. 17 (1987) 1773–1776.
[62] X. Wen, H. Sun, J. Liu, G. Wu, L. Zhang, X. Wu, P. Ni, Pentacyclic triterpenes. Part 1: The first examples of naturally occurring pentacyclic triterpenes as a new class of inhibitors of glycogen phosphorylases, Bioorg. Med. Chem. Lett. 15 (2005) 4944–4948.
[63] M.S. Zheng, Y.-K. Lee, Y. Li, K. Hwangbo, C.-S. Lee, J.-R. Kim, S.-K.-S. Lee, H.-W. Chang, J.-K. Son, Inhibition of DNA topoisomerases I and II and cytotoxicity of compounds from Ulmus davidiana var. japonica, Arch. Pharmacal Res. 33 (2010) 1307–1315.
[64] C.H. Brieskorn, W. Krause, Further triterpenes from Melissa officinalis L, Arch. Pharm. (Weinheim) 307 (1974) 603–612.

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Non-cytotoxic aza-BODIPY triterpene conjugates to target the endoplasmic reticulum

Sophie Hoenke, Benjamin Brandes, René Csuk *

Martin-Luther University Halle-Wittenberg, Organic Chemistry, Kurt-Mothes-Str. 2, D-06120, Halle (Saale), Germany

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ABSTRACT

In search of suitable tool to target the endoplasmic reticulum of human tumor cells, a non-cytotoxic aza-BODIPY derivative was designed and accessed in a simple synthesis in a few steps from commercially available starting materials. This aza-BODIPY was conjugated to 3-*O*-acetyl-triterpene carboxylic acids (glycyrrhetinic, ursolic, oleanolic, and betulinic acid) using a piperazinyl spacer. The resulting conjugates exhibited no cytotoxicity but were able to selectively target the ER.

1. Introduction

Visualization of the structures of (living) cells is most important to understand metabolic processes in detail. Thereby, the endoplasmic reticulum (ER) plays a major role in the survival of mammalian cells but also in their maintenance and homeostasis. Since in ER vital processes such as protein assembling, protein folding and post-translational modifications take place, investigations concerning the ER have gained increased attention. ER stress is known to lead to dysregulation. This dysregulation is considered to play an important role in non-communicative diseases such as diabetes and cancer but also in neurodegenerative diseases and strokes [1].

Visualization of organelles and their cellular processes can be done most preferentially by fluorescence microscopy, and small fluorescent molecules are often used to fluorescently label the organelles [2]. One of the prerequisites for successful investigations is a superior target selectivity of these molecular probes. Several BODIPYs such as ER-Tracker ™ Green and ER-Tracker ™ red (Fig. 1), both holding a glibenclamide moiety, are most often used; they are commercially available [1]. Several BODIPY-conjugates targeting different organelles or metabolic processes have been reported recently, among them conjugates with platinum drugs [3–11] or curcumin [12], paclitaxel [13–15], PEG conjugates [16–18], chlorambucil [19], capsaicin [20], isoxazoles [21], and mertansine [22]. All of these were intended to act as cytotoxic agents. One of the major aims of this study was to develop a molecular fluorescent probe-based bio-imaging and bio-sensing tool for non-invasive monitoring of biological processes in real time to provide dynamic information about for biological analysis. This has been accomplished by nanodots [23] as well as by fluorescence probes [24–28].

Recently, two studies dealt with BODIPY-triterpene conjugates [29, 30]. These compounds were also of moderate cytotoxicity but held improved tumor cell selectivity as compared to other conjugates. Furthermore, it was shown that triterpenoic acid amides holding a distal rhodamine (B or 101) unit are of superior cytotoxicity thereby acting as a mitocanic agent [31] while triterpene-safirinium conjugates [32] target the ER. Their cytotoxicity was significantly diminished as compared to the rhodamine conjugates [25]. BODYPY FL labelled triterpenoids were shown to hold good cytotoxicity for MCF-7 human breast adenocarcinoma cells but not to other cell lines [30]. From these observations and keeping in mind that the cytotoxicity of triterpene amide conjugates is strongly governed by the distal substituent we assumed that triterpene aza-BODIPY conjugates might be of low cytotoxicity but should provide a good selectivity for the ER.

Our previous studies also showed that the “linker” between the fluorophore and the triterpenoic skeleton should be a piperazinyl moiety [31]. Hence, we set out to access these compounds by synthesizing a piperazinyl substituted BODIPY first followed by coupling this molecule to 3-*O*-acetyl substituted triterpenoic acids [30], such as glycyrrhetinic acid (**GA**), ursolic (**UA**), oleanolic acid (**OA**) and betulinic acid (**BA**).

2. Results and discussion

The synthesis (Scheme 1) of the BODIPYs started from commercially available 4-hydroxy-acetophenone (**1**) whose aldol condensation with benzaldehyde gave 93% of (*E*)-configurated chalcone **2** [30]. The configuration of the double bond was determined from the magnitude of

* Corresponding author.
E-mail address: rene.csuk@chemie.uni-halle.de (R. Csuk).

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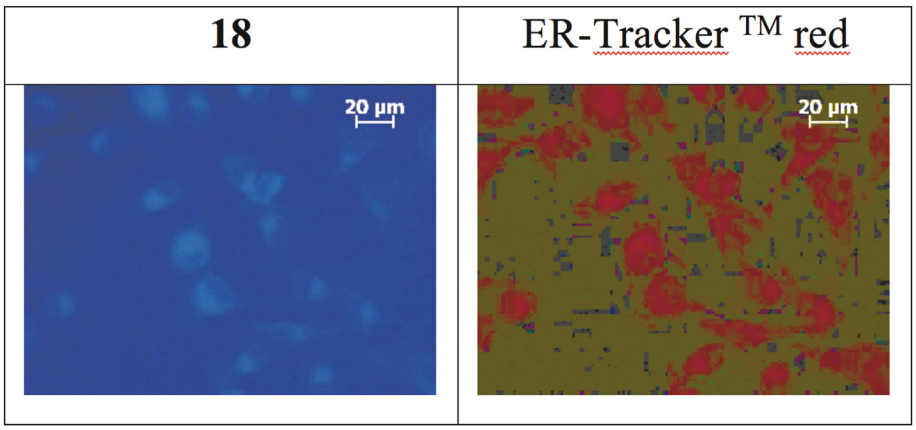
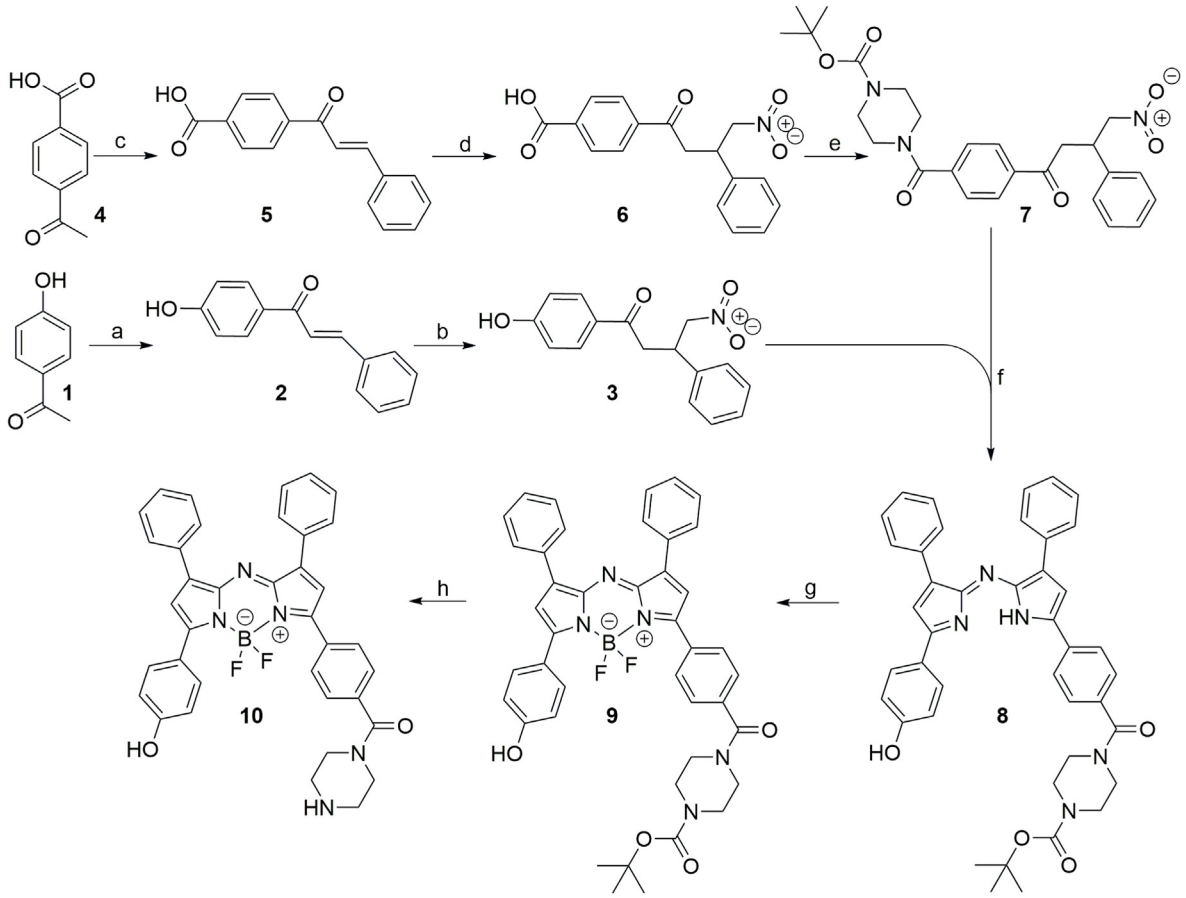


Fig. 1. Extended investigation of **18** and A375 cells: Fluorescence microscopic images (scale bar 20 µm), and ER-Tracker ™ red (staining of the endoplasmic reticulum); double staining experiment. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



Scheme 1. Synthesis of aza-BODIPY (**10**); a) NaOH, EtOH, H_2O, room temperature, 30 min → benzaldehyde, room temperature, 93%; b) CH_3NO_2, KOH, MeOH, room temperature → reflux, 6 h, 64%; c) KOH, H_2O, EtOH, room temperature → benzaldehyde, room temperature, overnight., 90%; d) CH_3NO_2, KOH, MeOH, room temperature → reflux, 6 h, 98%; e) cat. DMF, oxalyl chloride, DCM, 0 °C → room temperature, 1.5 h → TEA, Boc-piperazine, 2 h, room temperature, 72%; f) $NH_4CH_3CO_2$, n-BuOH, reflux, 8 h, 54%; g) $BF_3{\cdot}Et_2O$, DIPEA, room temperature, overnight, 20%; h) HCl in 1,4-dioxane, MeOH, room temperature, 91%.

the ^3J coupling constant of the olefinic hydrogen substituents of 15.5 Hz being typical for an (*E*) configuration. Reaction of **2** with nitromethane furnished **3** [34] in 64% isolated yield. The presence of a nitro substituent in **3** and **6** [29] (vide infra) is characterized by the presence of characteristic signals in their IR spectra at $\tilde{\nu} = 1554$ and 1375 cm^{-1} (for **3**) and $\tilde{\nu} = 1549$ and 1378 cm^{-1} (for **6**). In this divergent approach, 4-acetyl-benzoic acid (**4**) was coupled with benzaldehyde to furnish **5** [35,36] whose reaction with nitromethane yielded **6**. Reaction of **6** with oxalyl chloride in the presence of DMF (cat.) followed by the reaction with Boc-piperazine furnished **7** in 72% yield. Coupling of **3** and **7** in refluxing *n*-BuOH in the presence of ammonium acetate gave **8** whose cyclisation to BODIPY **9** was accomplished with boron trifluoride diethyl etherate and diisopropylethylamine (DIPEA) overnight. Deprotection of **9** gave BODIPY **10** in 91% yield. This compound is characterized in its ^{19}F NMR by a signal multiplet at $\delta = -130.85$ to -132.06 ppm; in the ^{11}B NMR spectrum a signal at $\delta = 1.01$ ppm as a virt. triplet holding a $J_{B,F} = 31.6$

Hz is found; the chemical shift and the magnitude of the coupling constant is typical for this type of compounds [37].

The triterpenoic acids **GA**, **UA**, **OA** and **BA** (Scheme 2) were acetylated as previously described [24] resulting in well-known acetates **11**–**14**. Reaction of the acetates with oxalyl chloride/DMF (cat) followed by adding BODIPY **10** furnished the conjugates **15**–**18** in 47–70% isolated yields (Scheme 3). As exemplified for oleanolic acid derived **17**, the conjugate showed a ^{11}B NMR chemical shift of $\delta = 0.97$ ppm (t, $J = 31.4$ Hz) and a ^{19}F NMR $\delta = -129.19$ to -133.50 ppm, together with the typical signals of the triterpenoid skeleton and the piperazinyl residue hence proving the structure of the conjugate.

To assess the cytotoxicity of the compounds, SRB assays were performed employing several human tumor cell lines, such as A375 (melanoma), HT29 (colon adenocarcinoma), MCF7 (breast adenocarcinoma), A2780 (ovarian carcinoma), HeLa (cervical adenocarcinoma) as well as non-malignant cell lines NIH 3T3 (murine fibroblasts) and HEK293 (human embryonic kidney). The results from these assays are summarized in Table 1. Most of the precursors of the final compounds **15**–**18** were found to be not cytotoxic at all (cut-off of the assay 30 μM). Compounds **2** and **5** held some cytotoxicity for all cell lines. This is in excellent agreement with previously reported data on the cytotoxicity of chalcones [38–41]. Thereby, the presence of an α,β-unsaturated ketone seems crucial for the cytotoxic activity since neither **3**, **6** nor **7** showed a cytotoxic effect to the cells. Piperazinyl-substituted **10** was cytotoxic for all cell lines but without any selectivity.

Upon coupling with the acetylated triterpenoic acids **11**–**14**, **10** lost all cytotoxicity; consequently, target compounds **15**–**18** were not cytotoxic at all within the cut-off of the assay (30 μM). This represents a significant improvement in properties compared to BODIPY FL substituted triterpenes, as the latter compounds held some residual cytotoxicity for some cell lines thus limiting their application in living systems [30].

Fluorescence microscopy allowed to determine the localization of the compounds as exemplified for **18**. Visual inspection of the cells showed compound **18** not to be in the nucleus of the cells while double staining of the cells with **18** and rhodamine 123 excluded its localization in the mitochondria. Staining with ER tracker ™ red, however, revealed that **18** ends up in the ER of the cells (Fig. 1).

It cannot ruled out, however, that the probe affects the biological activity of the cells inasmuch as it is known that even unsubstituted ursolic acid participates in metabolic re-wiring and epigenetic re-programming in human prostate cancer [42]. However, no significant changes in the morphology of the cells have been observed upon incubation for 2 days. Triterpenes, as exemplified for asiatic acid, might enter the endoplasmic reticulum, too, but this process triggered in PPC-1 prostate cancer cells a rapid caspase-dependent cell death within several hours of treatment [43]. Furthermore, it cannot completely excluded that triterpenoic probes might exert an indirect effect onto the cell metabolism by binding on chaperones as previously exemplified for triterpenoid celastrol [44]. Additional experiments showed that also **8** is able to enter the cells and to accumulate in the ER; as previously reported, the same holds true for several safirinium triterpene conjugates [32]. However, no significant changes in the morphology of the cells were observed.

3. Conclusion

The labeling and bio-visualization of living cells has come into the focus of scientific interest, and many theranostic drugs have been developed so far. In search of a suitable tool to target the endoplasmic reticulum (ER) of human tumor cells, we designed a non-cytotoxic aza-BODIPY **10** that was accessed in a simple synthesis in few steps from commercially available starting materials. This aza-BODIPY was conjugated to 3-*O*-acetyl-triterpene carboxylic acids (glycyrrhetinic, ursolic, oleanolic, and betulinic acid) using a piperazinyl spacer between the triterpenoid skeleton and the aza-BODIPY scaffold. The resulting conjugates exhibited no cytotoxicity (as shown by SRB assays using a cut-off of 30 μM) but they were able to selectively target the ER (as shown by fluorescence microscopy and staining experiments). Due to the absence of any cytotoxic effects, these novel triterpene aza-BODIPY conjugates are equally suitable for the investigation of cancer cells as well as of non-malignant cells.

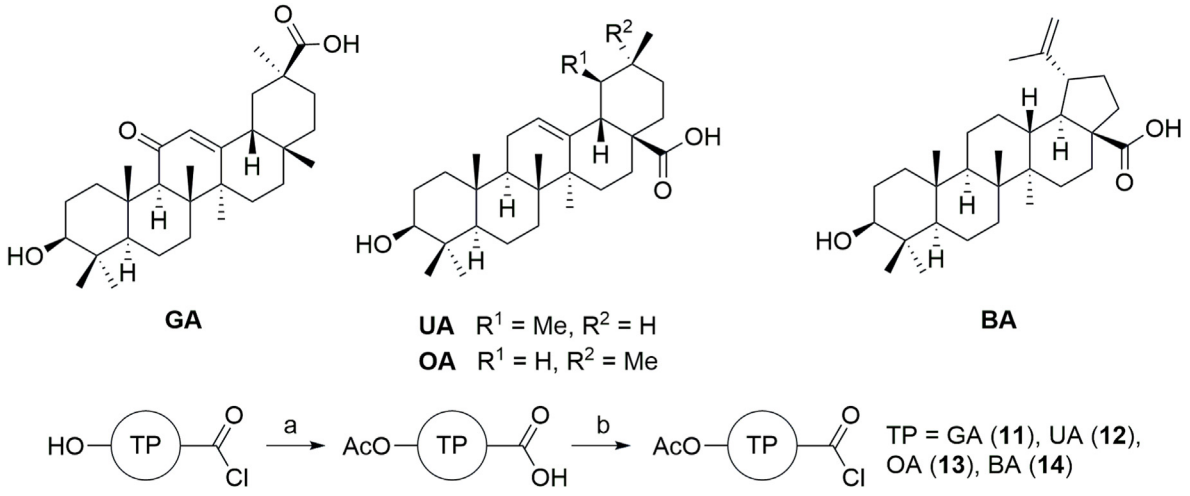
4. Experimental part

4.1. General

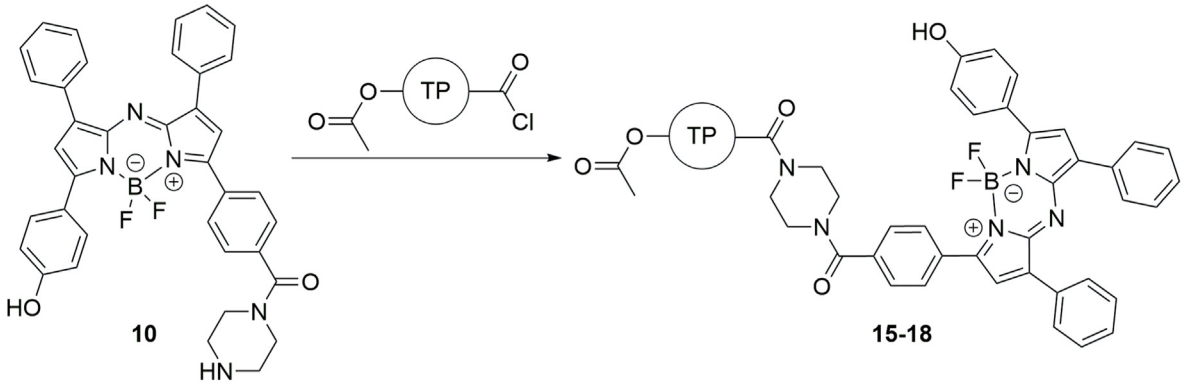
4-Hydroxyacetophenone (**1**) and 4-acetyl-benzoic acid (**4**) were obtained from local suppliers; Ursolic, betulinic and oleanolic acid were obtained from Betulinines (Stribrna Skalice, Czech Republic) and glycyrrhetinic acid was bought from Orgentis GmbH (Neugatersleben, Germany) and used as received. Equipment was used as previously described [24].

4.2. Cell lines and culture conditions

The biological studies were performed employing the human cancer cell lines A375 (malignant melanoma), HT29 (colon adenocarcinoma),



Scheme 2. Structure of parent triterpenoic acids **GA**, **UA**, **OA** and **BA**, their conversion into 3-*O*-acetates and their activation yielding the corresponding acid chlorides.



Scheme 3. Synthesis of aza-BODIPY conjugates **15**–**18**: TEA, DCM, room temperature, overnight; yields: **15** (70%), **16** (63%), **17** (47%), **18** (50%).

Table 1
Cytotoxicity of compounds: starting materials **2**, **3**, **5**–**8**, aza-BODIPY derivatives **9** and **10** and corresponding triterpenoid derivatives **15**–**18** (EC_{50}-values in μM from SRB-assays) after 72 h of treatment; the values are averaged from three independent experiments performed each in triplicate, confidence interval CI = 95%; mean ± standard mean error; malignant cell lines: A375 (melanoma), HT29 (colon adenocarcinoma), MCF-7 (breast adenocarcinoma), A2780 (ovarian carcinoma), HeLa (cervical adenocarcinoma); non-malignant: NIH 3T3 (murine fibroblast), HEK293 (human embryonic kidney) were tested.

	A375	HT29	MCF7	A2780	HeLa	NIH 3T3	HEK293
2	5.44 ± 0.3	9.06 ± 0.8	7.61 ± 0.8	9.53 ± 0.9	8.38 ± 0.2	21.4 ± 2.1	7.66 ± 0.1
3	>30	>30	>30	>30	>30	>30	>30
5	14.4 ± 1.0	20.92 ± 0.6	13.8 ± 1.2	19.1 ± 2.0	21.4 ± 1.2	>30	17.03 ± 0.7
6–**9**	>30	>30	>30	>30	>30	>30	>30
10	7.22 ± 0.4	6.19 ± 0.3	6.88 ± 0.8	10.56 ± 0.7	6.23 ± 0.3	6.38 ± 0.9	10.59 ± 0.3
15–**18**	>30	>30	>30	>30	>30	>30	>30

MCF-7 (breast cancer), A2780 (ovarian carcinoma), non-malignant murine fibroblasts NIH 3T3 and non-malignant human embryonic kidney cells (HEK293). All cell lines were obtained from the Department of Oncology (Martin-Luther-University Halle Wittenberg). Cultures were maintained as monolayers in RPMI 1640 medium with L-glutamine (Capricorn Scientific GmbH, Ebsdorfergrund, Germany) supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich GmbH, Steinheim, Germany) and penicillin/streptomycin (Capricorn Scientific GmbH, Ebsdorfergrund, Germany) at 37 °C in a humidified atmosphere with 5% CO_2.

4.3. Cytotoxicity assay (SRB assay)

For the evaluation of the cytotoxicity of the compounds a sulforhodamine-B (Kiton-Red S, ABCR) micro-culture colorimetric assay was used as previously reported. The EC_{50} values were averaged from three independent experiments performed each in triplicate calculated from semi-logarithmic dose-response curves applying a non-linear 4P Hills-slope equation.

4.4. Microscopic investigation

For the microscopic studies, the cell line A375 was used. On the first day, 10^5 cells were seeded in a Petri dish (3.5 cm diameter, 22 mm cover slip inserted) and incubated under normal conditions for 24 h. The medium was replaced with new medium loaded with **18** (1 μL of an 8 mM solution in 2 mL medium) and incubated for another 24 h. Then the medium was replaced with 1 mL medium loaded with 1 μL Rh123 and 100 μL ER-Tracker ™ red (ER staining Kit - Red Fluorescence, abcam, Milton), respectively, and incubated for 30 min each. The medium was discarded, the cover slip was washed with PBS (with Ca^{2+} and Mg^{2+}) and put on a slide holding 20 μL PBS. The cells were studied using a fluorescence microscope.

4.5. General procedure (GP) for the amidation with acetylated triterpene carboxylic acids chlorides

The acetylated triterpene carboxylic acids **11**–**14** were reacted with oxalyl chloride/DMF as previously reported. They (1 eq.) were dissolved in dry DCM (1 mL), and this solution was added to a solution of **10** (1.2 eq.) and triethylamine (1.2 eq.) under stirring and argon atmosphere. After stirring overnight, the reaction was quenched with MeOH, and the solvents were removed under reduced pressure. The crude product was purified by gradient column chromatography (SiO_2, $CHCl_3$ → $CHCl_3$/MeOH/NH_4OH, 98:1.8:0.2).

*4.6. (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one (**2**) [38239-52-0]*

4-Hydroxyacetophenone (**1**, 10.0 g, 73.4 mmol) was dissolved in ethanol (50 mL) and water (150 mL) was added. Under stirring an aqueous solution of sodium hydroxide (50%, 7.4 mL) was added. Stirring was continued for 0.5 h followed by the dropwise addition of benzaldehyde (8.51 mL, 84.2 mmol). After complete consumption of **1** (as checked by TLC), the reaction was acidified with aqueous HCl (10% vol). The precipitate was filtered off, washed with water, recrystallized from ethanol, and dried to obtain **2** (15.3 g, 93%) as an off-white solid; m.p. 177.1 °C (lit.: [33]: 178 °C); R_F = 0.27 (toluene/ethyl acetate/heptane/HCOOH, 80:26:10:5); IR (ATR): $\tilde{\nu}$ = 3125*w*, 1645 *m*, 1602*s*, 1590*s*, 1577 *m*, 1565*s*, 1514 *m*, 1495 *m*, 1441 *m*, 1383*w*, 1339*s*, 1303*w*, 1284*s*, 1219*vs*, 1175*s*. 1165*s*, 1043 *m*, 1028*w*, 999*w*, 980 *m*, 893*w*, 866*w*, 834 m, 823 *m*, 786 *w*, 764*s*, 734 *m*, 691*s*, 674*s*, 638*w*, 620 *m*, 560*s*, 538 *m*, 504 *m*, 482 *m*, 444*w*, 418*w* cm^{-1}; UV/vis ($CHCl_3$): λ_{max} (log ε) = 314 (4.39) nm; ^1H NMR (400 MHz, DMSO-d_6): δ = 10.39 (s, 1H, 1-OH), 8.11–8.03 (m, 2H, 3-H+3′-H), 7.88 (dd, J = 15.5, 2.5 Hz, 1H, 6-H), 7.85–7.81 (m, 2H, 10-H+10′-H), 7.67 (d, J = 15.5 Hz, 1H, 7-H), 7.48–7.37 (m, 3H, 9-H, 9′-H), 6.93–6.86 (m, 2H, 2-H+2′-H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 187.6 (C-5), 162.7 (C-1), 143.1 (C-7), 135.3 (C-8), 131.6 (C-3+C-3′), 130.7 (C-11), 129.6 (C-4), 129.3 (C-9+C-9′), 129.1 (C-10+C-10′), 122.6 (C-6), 115.8 (C-2+C-2′) ppm; MS (ESI, MeOH/$CHCl_3$, 4:1): m/z = 223.1 (100%, [M − H]$^-$).

*4.7. 1-(4-Hydroxyphenyl)-4-nitro-3-phenylbutan-1-one (**3**) [1093881-86-7]*

Compound **2** (15.2 g, 67.8 mmol) was dissolved in methanol (500 mL) and nitromethane (72.8 mL, 1.4 mol). Potassium hydroxide (5.0 g, 88.1 mmol) was added in several portions, and the reaction mixture was

heated under reflux for 6 h. The reaction was cooled to room temperature, and the solvent was removed under reduced pressure. The oily residue was acidified with aqueous HCl (1 M, 50 mL) and diluted with water (200 mL). The mixture was extracted with ethyl acetate (200 mL), dried ($MgSO_4$), and the organic solvent was removed under reduced pressure. The residue was purified by column chromatography (SiO_2, hexane/acetone, 4:1) and crystallized from hexanes/acetone (4:1, 200 mL) at 0 °C to obtain **3** (12.4 g, 64%) as a beige solid; m.p. 121.1 °C (lit.: [34]: 112–113 °C); R_F = 0.55 (hexanes/acetone, 2:1); IR (ATR): $\tilde{\nu}$ = 3211*w*, 1646*s*, 1600 *m*, 1585*s*, 1554*vs*, 1515*w*, 1496*w*, 1455 *m*, 1441 *m*, 1430 *m*, 1418 *m*, 1375*s*, 1324*w*, 1288*s*, 1278 *m*, 1230*s*, 1212 *m*, 1169*vs*, 1119*w*, 1078*w*, 1032*w*, 1008 *m*, 1000 *m*, 942*vw*, 921*w*, 891*w*, 838*s*, 819*w*, 798 *m*, 767 *m*, 738 *m*, 720 *m*, 703*s*, 635 *m*, 607*w*, 596 *m*, 543 *m*, 525 *m*, 516 *m*, 492*w*, 446*vw*, 408*w* cm^{-1}; UV/vis ($CHCl_3$): λ_{max} (log ε) = 269 (4.21) nm; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.36 (s, 1H, 1-OH), 7.86–7.78 (m, 2H, 3-H+3′-H), 7.40–7.32 (m, 2H, 10-H+10′-H), 7.33–7.25 (m, 2H, 11-H+11′-H), 7.25–7.17 (m, 1H, 12-H), 6.83 (d, J = 8.7 Hz, 2H, 2-H+2′-H), 4.96 (dd, J = 12.9, 5.7 Hz, 8-H_a), 4.85 (dd, J = 12.9, 9.6 Hz, 1H, 8-H_b), 4.02 (ddd, J = 9.6, 6.8 Hz, 1H, 7-H), 3.41 (ddd, J = 17.6, 7.0 Hz, 3H, 6-H_2) ppm; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 195.4 (C-5), 162.2 (C-1), 140.2 (C-9), 130.5 (C-3+C-3′), 128.4 (C-11+C-11′), 128.1 (C-4), 127.8 (C-10+C-10′), 127.1 (C-12), 115.2 (C-2+C-2′), 79.7 (C-8), 40.6 (C-6), 39.4 (C-7) ppm; MS (ESI, $CHCl_3$/MeOH, 4:1): m/z = 284.0 (30%, $[M - H]^-$).

4.8. 4-Cinnamoylbenzoic acid (5) [20118-35-8]

To a suspension of 4-acetylbenzoic acid (**4**, 10.0 g, 61 mmol) in ethanol (200 mL) and water (50 mL) was added dropwise aqueous potassium solution (6 M, 32 mL) under stirring at room temperature. Benzaldehyde (6.5 g, 61 mmol) was added dropwise, and the reaction mixture was allowed to stir overnight [35]. The white suspension was acidified with aqueous HCl (1 M), the solid filtered off, washed with water (3 × 100 mL), air-dried, and recrystallized from ethanol to yield **5** (13.8 g, 90%) as a white solid; m.p. 237.1 °C (lit.: [36]: 234 °C); R_F = 0.37 (toluene/ethyl acetate/heptane/HCOOH, 80:26:10:5); IR (ATR): $\tilde{\nu}$ = 2663*w*, 2546*w*, 1685 *m*, 1655 *m*, 1595 *m*, 1574 *m*, 1506*w*, 1497*w*, 1448*w*, 1426 *m*, 1406*w*, 1336 *m*, 1322 *m*, 1283*s*, 1216 *m*, 1206 *m*, 1183*w*, 1128 *m*, 1033 *m*, 1014 *m*, 996 *m*, 978 *m*, 969 *m*, 944 *m*, 854 *m*, 849 *m*, 778 *m*, 749*vs*, 709*w*, 686*s*, 662 *m*, 572 *m*, 558 *m*, 519 *m*, 488 *m*, 482 *m*, 447*w* cm^{-1}; UV/vis (MeOH): λ_{max} (log ε) = 313 (4.05), 265 (3.88) nm; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.31 (s, 1H, 1-OH), 8.23 (dd, J = 8.3, 1.6 Hz, 2H, 3-H+3′H), 8.11–8.06 (m, 5H, 4-H+4′-H), 7.91 (d, J = 1.3 Hz, 1H, 7-H), 7.89 (ddd, J = 5.7, 2.8, 1.5 Hz, 2H, 10-H+10′-H), 7.77 (d, J = 15.7 Hz, 1H, 8-H), 7.49–7.44 (m, 3H, 11-H+11′-H+12-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 189.0 (C-6), 166.6 (C-1), 144.8 (C-8), 140.7 (C-5), 134.5 (C-9), 134.4 (C-2), 130.8 (C-12), 129.6 (C-4+C-4′), 129.0 (C-10+C-10′), 128.9 (C-11+C-11′), 128.6 (C-3+C-3′), 122.0 (C-7) ppm; MS (ESI, $CHCl_3$/MeOH, 4:1): m/z = 250.9 (100%, $[M - H]^-$), 386.8 (40%, $[M+Cl]^-$).

4.9. 4-(4-Nitro-3-phenylbutanoyl)benzoic acid (6) [1815579-07-7]

To a suspension of **5** (7.1 g, 28.1 mmol) in methanol (500 mL) and nitromethane (30.2 mL, 0.3 mol), potassium hydroxide (2.07 g, 36.9 mmol) was added in several portions. The reaction was stirred and heated under reflux for 6 h, and the solvent was removed under reduced pressure. The residue was treated with aqueous HCl solutions (0.1 M, 200 mL; 1 M, 10 mL) and ethyl acetate (200 mL). The organic phase was separated, dried ($MgSO_4$), and the solvent was removed under reduced pressure to yield **6** (8.65 g, 98%) as a beige solid; m.p. 175.1 °C; R_F = 0.31 (toluene/ethyl acetate/heptane/HCOOH, 80:26:10:5); IR (ATR): $\tilde{\nu}$ = 3101*w*, 3062*w*, 3032*w*, 2897*w*, 2666*w*, 2550*w*, 1696*s*, 1682*vs*, 1571*w*, 1549*vs*, 1506*w*, 1454*w*, 1424 *m*, 1406 *m*, 1378 *m*, 1367*w*, 1322 *m*, 1291*s*, 1220 *m*, 1202 *m*, 1128*w*, 1119*w*, 992*w*, 976*w*, 971*w*, 930*w*, 853 *m*, 806*w*, 769 *m*, 757 *m*, 700*s*, 683*w*, 643*w*, 557 *m*, 509*w* cm^{-1}; UV/vis (MeOH): λ_{max} (log ε) = 294 (3.22), 253 (4.22) nm; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.29 (s, 1H, 1-OH), 8.06–7.96 (m, 4H, 3-H+3′-H+4-H+4′-H), 7.38–7.33 (m, 2H, 11-H+11′-H), 7.32–7.25 (m, 2H, 12-H+12′-H), 7.24–7.18 (m, 1H, 13-H), 4.97 (dd, J = 13.0, 5.7 Hz, 1H, 9-H_a), 4.84 (dd, J = 12.9, 9.6 Hz, 1H, 9-H_b), 4.03 (dq, J = 9.6, 6.9 Hz, 1H, 8-H), 3.67–3.50 (m, 2H, 7-H_2) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 197.7 (C-6), 167.0 (C-1), 140.4 (C-10), 139.9 (C-5), 135.1 (C-2), 130.0 (C-4+C-4′), 128.9 (C-12+C-12′), 128.6 (C-3+C-3), 128.2 (C-11+C-11′), 127.7 (C-13), 80.1 (C-9), 42.0 (C-7), 39.6 (C-8) ppm; MS (ESI, $CHCl_3$/MeOH, 4:1): m/z = 312.0 (100%, $[M - H]^-$; analysis calcd for $C_{17}H_{15}NO_5$ (313.31): C 65.17, H 4.83, N 4.47; found: C 64.79, H 5.03, N 4.20.

4.10. tert-Butyl 4-[4-(4-nitro-3-phenylbutanoyl)benzoyl]piperazine carboxylate (7)

Compound **6** (6.0 g, 19.2 mmol) was suspended in dry DCM (50 mL) under argon atmosphere. The suspension was cooled to 0 °C, and under stirring cat. amounts of dry DMF and oxalyl chloride (3.2 mL, 38.3 mmol) were added. The solution was stirred for 1.5 h at 21 °C; the solvent was removed under reduced pressure. The obtained acid chloride was redissolved in dry THF (2 × 100 mL), and the solvent was removed multiple times to remove excessive oxalyl chloride. The acid chloride was taken up in dry DCM (5 mL) and transferred dropwise to a solution of N-Boc-piperazine (3.9 g, 21.1 mmol), and triethylamine (0.9 mL, 21.1 mmol) in dry DCM (10 mL). After stirring for 2 h, the solvent was removed under reduced pressure, and the beige solid was purified by column chromatography (SiO_2, hexanes/ethyl acetate, 2:1) to yield **7** (6.64 g, 72%) as a white solid; m.p. 63.2 °C; R_F = 0.36 (hexanes/ethyl acetate, 2:1); IR (ATR): $\tilde{\nu}$ = 2976*w*, 2864*w*, 1685*s*, 1631*s*, 1549*s*, 1505*w*, 1456 *m*, 1416*s*, 1378 *m*, 1365*s*, 1286 *m*, 1262*s*, 1247*vs*, 1231*s*, 1207 *m*, 1155*vs*, 1119*s*, 1075*w*, 1006*s*, 997*s*, 893*w*, 842 *m*, 759*s*, 700*s*, 551 m cm^{-1}; UV/vis ($CHCl_3$): λ_{max} (log ε) = 255 (4.12) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.98–7.93 (m, 2H, 4-H+4′-H), 7.50–7.43 (m, 2H, 3-H+3′-H), 7.35–7.30 (m, 2H, 12-H+12′-H), 7.29–7.24 (m, 3H, 11-H+11′-H+13-H), 4.85–4.78 (m, 1H, 7-H_a), 4.73–4.66 (m, 1H, 7-H_b), 4.21 (p, J = 7.1 Hz, 1H, 8-H), 3.74 (s, 2H, 14-H_a+15-H_a), 3.61–3.25 (m, 8H, 9-H_2+14-H_b+14′-H_2+15-H_2+15′-H_2), 1.46 (s, 9H, 18-H_3+18′-H_3+18″-H_3) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 196.3 (C-6), 169.3 (C-1), 154.6 (C-16), 140.4 (C-2), 139.0 (C-10), 137.4 (C-5), 129.2 (C-12+C-12′), 128.5 (C-4+C-4′), 128.1(C-13), 127.6 (C-11+C-11′), 127.5(C-3+C-3′), 80.6 (C-17), 79.6 (C-7), 47.1 (C-14+C-14′), 42.0 (C-15+C-15′), 41.8 (C-9), 39.4 (C-8), 28.5 (C-18+C-18'+C-18″) ppm; MS (ESI, $CHCl_3$/MeOH, 4:1): m/z = 504.2 (100%, $[M+Na]^+$); analysis calcd for $C_{26}H_{31}N_3O_6$ (481.55): C 64.85, H 6.49, N 8.73; found: C 64.69, H 6.71, N 8.55.

4.11. tert-butyl (Z)-4-(4-{2-([5-(4-hydroxyphenyl)-3-phenyl-1H-pyrrol-2-yl]imino}-3-phenyl-2H-pyrrol-5-yl)benzoyl)piperazine-1-carboxylate (8)

Compounds **3** (1.86 g, 6.5 mmol), **7** (3.14 g, 6.5 mmol), and ammonium acetate (17.6 g, 228 mmol) were dissolved in *n*-butanol (50 mL), and the mixture was stirred under reflux for 8 h. The solvent was removed under reduced pressure, and the blue solid was washed with water, filtered off and dissolved in DCM (100 mL). The organic phase was washed with small amounts of water until pH neutral. After drying ($MgSO_4$), the compound was purified by column chromatography (SiO_2, $CHCl_3$/ethyl acetate, 9:2) to afford **8** (3.68 g, 54%) as a blue solid; m.p. 223.6 °C; R_F = 0.41 ($CHCl_3$/MeOH/NH_4OH, 98:1.8:0.2); IR (ATR): $\tilde{\nu}$ = 1690*s*, 1600*s*, 1587 *m*, 1562 *m*, 1465*s*, 1419*s*, 1291 *m*, 1277 *m*, 1263*s*, 1235*vs*, 1219 *m*, 1163*vs*, 1122*s*, 1005*vs*, 848*s*, 824*s*, 768*s*, 758*vs*, 694*s*, 550 m cm^{-1}; UV/vis ($CHCl_3$): λ_{max} (log ε) = 602 (4.31), 317 (4.53) nm; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.70 (s, 1H, 5-NH), 11.54 (t, J = 2.8 Hz, 1H, 1-OH), 8.13–8.10 (m, 2H, 22-H+22′-H), 8.10–8.04 (m, 2H, 15-H+15′-H), 7.80 (s, 1H, 19-H), 7.75–7.70 (m, 2H, 2-H+2′-H), 7.63–7.58 (m, 3H, 10-H+10′-H+13-H), 7.48–7.43 (m, 2H, 14-H+14′-H), 7.43–7.40 (m, 2H, 23-H+23′-H), 7.40–7.37 (m, 2H, 3-H+3′-H), 7.35–7.28 (m, 2H, 11-H+11′-H), 7.15–7.09 (m, 1H, 12-H), 7.05–7.03 (m, 1H, 6-H),

3.64–3.30 (m, 8H, 26-H_2+26′-H_2+27-H_2+27′-H_2), 1.40 (s, 9H, 30 + 30′-H_3+30″-H_3) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 169.6 (C-25), 169.2 (C-1), 167.0 (C-20), 158.0 (C-17), 154.3 (C-28), 146.7 (C-21), 142.0 (C-18), 136.0 (C-9), 134.4 (C-24), 133.3 (C-16), 132.3 (C-7), 131.9 (C-4), 131.1 (C-22+C-22′), 129.3 (C-15+C-15′), 129.0 (C-11+C-11′), 128.7 (C-13+C-14+C-14′), 128.3 (C-23+C-23′), 125.6 (C-5), 125.5 (C-8), 125.4 (C-12), 124.9 (C-10+C-10′), 123.5 (C-2+C-2′), 122.1 (C-19), 117.9 (C-3+C-3′), 104.7 (C-6), 79.6 (C-29), 47.4 (C-26+C-26′), 44.1 (C-27+C-27′), 28.5 (C-30+C-30'+C-30″) ppm; MS (ESI, $CHCl_3$/MeOH, 4:1): m/z = 676.3 (70%, [M − H]$^-$); analysis calcd for $C_{42}H_{39}N_5O_4$ (677.81): C 74.43, H 5.80, N 10.33; found: C 74.17, H 6.03, N 10.03.

4.12. 3-(4-Hydroxyphenyl)-1,7-diphenyl-5-{4-[4-(tert-butoxycarbonyl) piperazine-1-carbonyl] phenyl}-4,4-difluoro-4-bora-3a,4a,8-triaza-s-indacene (9)

To an ice-cold solution of **8** (2.45 g, 3.6 mmol) in dry DCM (200 mL), boron trifluoride diethyl etherate (4.58 mL, 36 mmol) and DIPEA (6.15 mL, 36 mmol) were added dropwise under stirring. The green solution was stirred overnight and quenched with an aqueous solution of $NaHCO_3$ (20 mL, satd.). The mixture was filtered through a pad of Celite, which was rinsed with ethyl acetate until no green color was observed. The organic phase was separated, washed with water (20 mL), brine (20 mL), and dried over $MgSO_4$. Once the solvent was removed under reduced pressure, the green solid was purified by column chromatography (SiO_2, $CHCl_3$ → $CHCl_3$:MeOH:NH_4OH, 9:1.8:0.2) to obtain unchanged starting material **8** (490 mg, 20%), product **9** (1144 mg, 44%) and deprotected **10** (522 mg, 23%); m.p. 256 °C; R_F = 0.28 ($CHCl_3$/ethyl acetate, 4:1); IR (ATR): $\tilde{\nu}$ = 3397*w*, 3007*w*, 2977*w*, 2927*w*, 2868*w*, 2257*vw*, 1686 *m*, 1616 *m*, 1604 *m*, 1538 *m*, 1506*s*, 1475*s*, 1461*s*, 1444*s*, 1421*s*, 1389 *m*, 1365 *m*, 1323 *m*, 1285*s*, 1247*s*, 1233*s*, 1177*s*, 1121*s*, 1100*s*, 1071*s*, 1051 *m*, 1033*s*, 1021*vs*, 1006*vs*, 863 *m*, 820*s*, 761*s*, 744 *m*, 689*s*, 619*s*, 540 m cm^{-1}; UV/vis ($CHCl_3$): λ_{max} (log ε) = 669 (4.04), 467 (3.20), 320 (3.63) nm; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.29 (s, 1H, 1-OH), 8.24–8.16 (m, 4H, 3-H+3′-H+11-H+11′-H), 8.14–8.08 (m, 4H, 14-H+14′-H+22-H+22′-H), 7.80 (s, 1H, 6-H), 7.58–7.47 (m, 7H, 10-H+10′-H+12-H+15-H+15′-H+23-H+23′-H), 7.46–7.40 (m, 2H, 13-H+19-H), 6.99–6.96 (m, 2H, 2-H+2′-H), 3.48–3.23 (m, 8H, 26-H_2+26′-H_2+27-H_2+27′-H_2), 1.40 (s, 9H, 30-H_3+30′-H_3+30-H″-H_3) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 169.0 (C-25), 163.0 (C-1), 162.4 (C-5), 154.3 (C-28), 153.2 (C-16), 146.9 (C-8), 144.8 (C-9), 143.5 (C-17), 140.3 (C-20), 137.3 (C-24), 133.8 (C-3+C-3′), 133.2 (C-21), 132.7 (C-18), 131.7 (C-7), 130.6 (C-12), 129.8 (C-11+C-11′), 129.6 (C-22+C-22′), 129.5 (C-13), 129.4 (C-14+C-14′), 129.2 (C-15+C-15′), 129.1 (C-10+C-10′), 127.7 (C-23+C-23′), 122.3 (C-6), 121.2 (C-4), 118.9 (C-19), 116.7 (C-2+C-2′), 79.7 (C-29), 45.8 (C-26+C-26′), 41.0 (C-27+C-27′), 28.5 (C-30+C-30'+C-30″) ppm; MS (ESI, MeOH): m/z = 724.3 (100%, [M − H]$^-$); analysis calcd for $C_{42}H_{38}BF_2N_5O_4$ (725.59): C 69.52, H 5.28, N 9.65; found: C 69.36, H 5.47, N 9.38.

4.13. 3-(4-Hydroxyphenyl)-1,7-diphenyl-5-(4-(piperazine-1-carbonyl) phenyl)-4,4-difluoro-4-bora-3a,4a,8-triaza-s-indacene (10)

Compound **9** (720 mg, 1 mmol) was dissolved in methanol (15 mL) and HCl solution in dioxane (4 M, 10 mL, 1.1 mmol) was added under stirring. After consumption of the starting material (as checked by TLC), the solvent was removed under reduced pressure, and the solid residue was purified by column chromatograhpy (SiO_2, $CHCl_3$/MeOH/NH_4OH, 90:9:1) to afford **10** (570 mg, 91%) as a green solid; m.p. 200 °C; R_F = 0.17 ($CHCl_3$/MeOH/NH_4OH, 90:9:1); IR (ATR): $\tilde{\nu}$ = 1633*w*, 1596 *m*, 1539 *m*, 1512 *m*, 1504 *m*, 1415*s*, 1364 *m*, 1319 *m*, 1280*s*, 1232*s*, 1178 *m*, 1126*s*, 1094*s*, 1068*s*, 1035*s*, 1021*vs*, 983*s*, 962*s*, 939*s*, 865 *m*, 822*s*, 760*vs*, 744*s*, 714 *m*, 686*vs*, 617*s*, 566*s*, 552*s*, 530*s*, 462*s*, 403 s cm^{-1}; UV/vis ($CHCl_3$): λ_{max} (log ε) = 669 (4.91), 467 (4.07), 319 (4.50) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.17–7.95 (m, 7H, 3-H+3′-H+11-H+11′-H+12-H+14-H-14′-H), 7.56–7.32 (m, 9H, 10-H+10′-H+13-H+15-H+15′-H+22-H+22′-H+23-H+23′-H), 7.16 (s, 1H, 19-H), 6.94–6.79 (m, 3H, 2-H+2′-H+6-H), 3.94–3.08 (m, 8H, 26-H_2+26′-H_2+27-H_2+27′-H_2) ppm; ^{11}B NMR (160 MHz, $CDCl_3$): δ = 1.01 (t, J = 31.6 Hz) ppm; ^{19}F NMR (470 MHz, $CDCl_3$): δ = −130.85–132.06 (m) ppm; MS (ESI, MeOH): m/z = 624.3 (100%, [M − H]$^-$); analysis calcd for $C_{37}H_{30}BF_2N_5O_2$ (625.49): C 71.05, H 4.83, N 11.20; found: C 70.81, H 5.06, N 10.97.

4.14. 3-O-acetyl-glcycyrrhetinic acid (11), 3-O-acetyl-ursolic acid (12), 3-O-acetyl-oleanolic acid (13), 3-O-acetyl-betulinic acid (14)

These compounds were prepared from the parent triterpenoic acids by acetylation as previously described [30].

4.15. 3-(4-Hydroxyphenyl)-1,7-diphenyl-5-{4-[N-(3β-acetyloxy-11-oxo-olean-12-en-28-oyl] piperazine-1-carbonyl)phenyl}-4,4-difluoro-4-bora-3a,4a,8-triaza-s-indacene (15)

Following the GP from **11** (38 mg, 74 μm mol), **15** (58 mg, 70%) was obtained as a green solid; m.p. 287 °C; R_F = 0.15 ($CHCl_3$/MeOH/NH_4OH, 98:1.8:0.2); IR (ATR): $\tilde{\nu}$ = 2947*w*, 2928*w*, 2865*w*, 1728*w*, 1634 *m*, 1603 *m*, 1538 *m*, 1509 *m*, 1483 *m*, 1473 *m*, 1445 *m*, 1417 *m*. 1387 *m*, 1365 *m*, 1320 *m*, 1280 *m*, 1241*s*, 1178*s*, 1126*s*, 1095*s*, 1070*s*, 1036*vs*, 1022*vs*, 1002*vs*, 985*s*, 827 *m*, 761*s*, 746*s*, 688*s*, 673 *m*, 617 *m*, 407 m cm^{-1}; UV/vis ($CHCl_3$): λ_{max} (log ε) = 669 (4.88), 469 (4.05), 320 (4.43) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.08–7.98 (m, 7H, 46-H+46′-H+47-H+53-H+53′-H+58-H+58′-H), 7.57–7.33 (m, 9H, 37-H+37′-H+38-H+38′-H+45-H+45′-H+57-H+57′-H+59-H), 7.12 (s, 1H, 50-H), 6.97–6.88 (m, 3H, 41-H+54-H+54′-H), 5.68 (s, 1H, 12-H), 4.49 (dd, J = 11.7, 4.7 Hz, 1H, 3-H), 3.93–3.41 (m, 8H, 33-H_2+33′-H_2+34-H_2+34′-H_2), 2.79 (d, J = 13.4 Hz, 1H, 1-H_a), 2.37 (s, 1H, 9-H), 2.23 (d, J = 13.1 Hz, 1H, 18-H), 2.07 (s, 3H, 32-H_3), 2.13–2.01 (m, 2H, 16-H_a+19-H_a), 2.01–1.94 (m, 1H, 21-H_a), 1.88–1.78 (m, 1H, 15-H_a), 1.78–1.38 (m, 10H, 2-H_2+6-H_2+7-H_2+19-H_b+21-H_a+22-H_2), 1.36 (s, 3H, 27-H_3), 1.23 (s, 3H, 29-H_3), 1.22–1.15 (m, 1H, 15-H_b), 1.17 (s, 3H, 25-H_3), 1.12 (s, 3H, 26-H_3), 1.10–0.99 (m, 2H, 1-H_b+16-H_b), 0.91–0.87 (m, 6H, 23-H_3+24-H_3), 0.82 (s, 3H, 28-H_3), 0.85–0.74 (m, 1H, 5-H) ppm; ^{11}B NMR (160 MHz, $CDCl_3$): δ = 0.99 (t, J = 31.0 Hz) ppm; ^{19}F NMR (376 MHz, $CDCl_3$): δ = −130.15–132.95 (m) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 200.2 (C-11), 174.5 (C-30), 171.6 (C-31), 170.3 (C-35), 169.8 (C-13), 161.9 (C-51), 160.7 (C-55), 154.1 (40), 146.9 (C-49), 145.2 (C-56), 141.5 (C-42), 135.8 (C-36), 134.1 (C-39), 132.7 (C-47), 132.6 (C-43), 131.8 (C-44), 129.8 (C-59), 129.7 (C-48), 129.4 (C-58+C-58'+C-53+C-53′), 129.2 (C-46+C-46′), 129.0 (C-37+C-37′), 128.6 (C-57+C-57′), 128.5 (C-45+C-45′), 128.3 (C-12), 127.3 (C-38+C-38′), 122.6 (C-52), 120.2 (C-50), 117.9 (C-41), 116.3 (C-51+C-51′), 81.2 (C-3), 61.8 (C-9), 55.0 (C-5), 48.5 (C-18), 47.7 (C-34+C-34′), 45.4 (C-8), 43.9 (C-20), 43.5 (C-21), 43.4 (C-14), 42.5 (C-33+C-33′), 38.8 (C-1), 38.0 (C-4), 37.6 (C-22), 36.9 (C-10), 33.5 (C-19), 32.8 (C-7), 31.9 (C-17), 28.4 (C-28), 28.0 (C-24), 27.1 (C-29), 26.7 (C-16), 26.4 (C-15), 23.5 (C-2), 23.1 (C-27), 21.4 (C-32), 18.7 (C-26), 17.4 (C-6), 16.7 (C-23), 16.4 (C-25) ppm; MS (ESI, $CHCl_3$/MeOH, 4:1): m/z = 1119.3 (100%, [M]$^-$); analysis calcd for $C_{69}H_{76}BF_2N_5O_6$ (1120.20): C 73.98, H 6.84, N 6.25; found: C 73.60, H 7.02, N 6.02.

4.16. 3-(4-Hydroxyphenyl)-1,7-diphenyl-5-{4-[N-(3β-acetyloxy-ursan-12-en-28-oyl] piperazine-1-carbonyl)phenyl)}-4,4-difluoro-4-bora-3a,4a,8-triaza-s-indacene (16)

Following the GP from **12** (30 mg, 58 μm mol), **13** (41 mg, 63%) was obtained as a green solid; m.p. 234 °C; R_F = 0.27 ($CHCl_3$/MeOH/NH_4OH, 98:1.8:0.2); IR (ATR): $\tilde{\nu}$ = 2923*w*, 2870*w*, 1730*w*, 1634 *m*, 1602 *m*, 1540 *m*, 1511 *m*, 1501 *m*, 1483 *m*, 1475*s*, 1444*s*, 1419 *m*, 1388*s*, 1369 *m*, 1320 *m*, 1281 m, 1237*s*, 1178*s*, 1126*s*, 1095*s*, 1071*s*, 1036*vs*, 1022*vs*, 1003*s*, 985*s*, 968 *m*, 944 *m*, 826 *m*, 771*s*, 746 *m*, 688*s*, 663 *m*, 618 *m*, 579 *m*, 411 m cm^{-1}; UV/vis ($CHCl_3$): λ_{max} (log ε) = 666 (4.89), 471 (4.05), 318 (4.43) nm; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.12–7.96 (m, 7H, 46-H+46′-

H+47-H+53-H+53′-H+58-H+58′-H), 7.58–7.31 (m, 9H, 37-H+37′-H+38-H+38′H+45-H+45′-H+57-H+57′-H+59-H), 7.12 (s, 1H, 50-H), 6.98–6.86 (m, 3H, 41-H+54-H+54′-H), 5.20 (s, 1H, 12-H), 4.48 (q, J = 7.8, 6.3 Hz, 1H, 3-H), 3.97–3.18 (m, 8H, 33-H_2+33′-H_2+34-H_2+34′-H_2), 2.46–2.09 (m, 1H, 18-H), 2.03 (s, 3H, 32-H_3), 1.95–1.84 (m, 2H, 11-H_2), 1.83–1.17 (m, 15H, 1-H_a+2-H_2+6-H_2+7-H_2+9-H+16-H_2+19-H+21-H_2+22-H_2), 1.07–0.97 (m, 7H, 1-H_b+15-H_2+20-H+27-H_3), 0.96–0.89 (m, 6H, 25-H_3+30-H_3), 0.89–0.84 (m, 3H, 29-H_3), 0.84–0.75 (m, 7H, 5-H+23-H_3+24-H_3), 0.71 (d, J = 3.9 Hz, 3H, 26-H_3) ppm; ^{11}B NMR (128 MHz, $CDCl_3$): δ = 0.99 (t, J = 31.5 Hz) ppm; ^{19}F NMR (376 MHz, $CDCl_3$): δ = −129.13–134.44 (m) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 175.9 (C-28), 171.1 (C-31), 170.3 (C-35), 162.1 (C-51), 161.0 (C-55), 153.6 (C-40), 146.9 (C-49), 145.3 (C-56), 141.4 (C-42), 138.5 (C-13), 135.7 (C-36), 134.3 (C-39), 132.7 (C-47), 132.6 (C-43), 131.7 (C-44), 129.8 (C-59), 129.6 (C-48), 129.4 (C-53+C-53′), 129.4 (C-58+C-58′), 129.1 (C-46+C-46′), 129.0 (C-37+C-37′), 128.6 (C-45+C-45′), 128.6 (C-57+C-57′), 127.4 (C-38+C-38′), 125.4 (C-12), 122.3 (C-52), 120.3 (C-50), 117.6 (C-41), 116.4 (C-54+C-54′), 81.0 (C-3), 55.3 (C-5+C-18), 48.7 (C-17), 47.5 (C-9), 45.3 (C-33+C-33'+C-34+C-34′), 42.1 (C-14), 39.4 (C-8+C-19), 38.7 (C-20), 38.2 (C-1), 37.6 (C-4), 36.9 (C-10), 34.4 (C-22), 33.0 (C-7), 30.4 (C-21), 28.1 (C-15), 28.0 (C-24), 23.5 (C-16+C-27), 23.3 (C-11), 21.3 (C-32), 21.2 (C-30), 18.2 (C-2+C-6), 17.4 (C-29), 16.9 (C-26), 16.7 (C-23), 15.5 (C-25) ppm; MS (ESI, $CHCl_3$/MeOH, 4:1): m/z = 1106.3 (40%, $[M]^-$); analysis calcd for $C_{69}H_{78}BF_2N_5O_5$ (1106.22): C 74.92, H 7.11, N 6.33; found: C 74.68, H 7.40, N 6.01.

*4.17. 3-(4-Hydroxyphenyl)-1,7-diphenyl-5-{4-[N-(3β-acetyloxy-olean-12-en-28-oyl] piperazine-1-carbonyl)phenyl}-4,4-difluoro-4-bora-3a,4a,8-triaza-s-indacene (**17**)*

Following the GP from **13** (30 mg, 58 μm mol), **15** (30 mg, 47%) was obtained as a green solid; m.p. 283 °C; R_F = 0.23 ($CHCl_3$/MeOH/NH_4OH, 98:1.8:0.2); IR (ATR): $\tilde{\nu}$ = 2942*w.* 2927*w,* 2866*w,* 1729*w,* 1629 *m,* 1602 *m,* 1539 *m,* 1510 *m,* 1501 *m,* 1483 *m,* 1473*s,* 1444*s,* 1418 *m,* 1387*s,* 1366 *m,* 1320 *m,* 1279*s,* 1237*s,* 1178*s,* 1124*s,* 1094*vs,* 1070*s,* 1036*s,* 1021*vs,* 1001*vs,* 985*s,* 968*s,* 943 *m,* 916 *m,* 826 *m,* 760*s,* 746*s,* 714 *m,* 687*s,* 665 *m,* 617 *m,* 586 *m,* 574 *m,* 531 *m,* 408 m cm^{-1}; UV/vis ($CHCl_3$): λ_{max} (log ε) = 667 (4.93), 471 (4.09), 319 (4.49) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.13–7.94 (m, 7H, 46-H+46′-H+47-H+53-H+53′-H+58-H+58′-H), 7.51–7.30 (m, 9H, 37-H+37′-H+38-H+38′-H+45-H+45′-H+57-H+57′-H+59-H), 7.09 (s, 1H, 50-H), 6.96 (d, J = 8.2 Hz, 2H, 54-H+54′-H), 6.93 (s, 1H, 41-H), 5.24 (t, J = 3.7 Hz, 1H, 12-H), 4.46 (t, J = 7.9 Hz, 1H, 3-H), 3.87–3.27 (m, 8H, 33-H_2+33′-H_2+34-H_2+34′-H_2), 3.10–2.99 (m, 1H, 18-H), 2.16–1.98 (m, 1H, 16-H_a), 2.03 (s, 3H, 32-H_3), 1.96–1.77 (m, 2H, 11-H_2), 1.74–1.42 (m, 10H, 1-H_a+2-H_2+6-H_a+9-H+15-H_a+16-H_b+19-H_a+22-H_2), 1.42–1.12 (m, 6H, 6-H_b+7-H_2+19-H_b+21-H_2), 1.09 (s, 3H, 27-H_3), 1.07–0.96 (m, 2H, 1-H_b+15-H_b), 0.90 (s, 3H, 29-H_3), 0.89 (s, 3H, 25-H_3), 0.87 (s, 3H, 30-H_3), 0.82 (s, 3H, 24-H_3), 0.80 (s, 3H, 23-H_3), 0.80–0.76 (m, 1H, 5-H), 0.70 (s, 3H, 26-H_3) ppm; ^{11}B NMR (128 MHz, $CDCl_3$): δ = 0.97 (t, J = 31.4 Hz) ppm; ^{19}F NMR (470 MHz, $CDCl_3$): δ = −129.19–-133.50 (m) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 175.6 (C-28), 171.1 (C-31), 170.2 (C-35), 162.2 (C-55), 161.4 (C-51), 153.3 (C-40), 146.9 (C-49), 145.3 (C-56), 144.4 (C-13), 143.9 (C-42), 135.8 (C-36), 134.2 (C-39), 132.7 (C-47), 132.6 (C-43), 131.6 (C-44), 129.8 (C-59), 129.6 (C-48), 129.4 (C-53+C-53′), 129.4 (C-58+C-58′), 129.1 (C-46+C-46′), 129.0 (C-37+C-37′), 128.6 (C-45+C-45′), 128.6 (C-57+C-57′), 127.3 (C-38+C-38′), 122.1 (C-12), 121.7 (C-52), 120.3 (C-50), 117.5 (C-41), 116.5 (C-54+C-54′), 81.0 (C-3), 55.4 (C-5), 47.6 (C-9), 47.6 (C-17), 46.3 (C-19), 45.3 (C-34+C-34′), 43.6 (C-18), 42.4 (C-33+C-33′), 41.8 (C-8), 39.1 (C-14), 38.1 (C-1), 37.7 (C-4), 36.9 (C-10), 33.9 (C-21), 33.0 (C-30), 32.8 (C-7), 30.3 (C-20), 30.0 (C-22), 28.0 (C-24), 27.8 (C-15), 25.9 (C-27), 24.0 (C-29), 23.5 (C-2), 23.4 (C-11), 22.8 (C-16), 21.3 (C-32), 18.2 (C-6), 16.9 (C-26), 16.6 (C-23), 15.4 (C-25) ppm; MS (ESI, $CHCl_3$/MeOH, 4:1): m/z = 1105.4 (100%, $[M]^-$); analysis calcd for $C_{69}H_{78}BF_2N_5O_5$ (1106.22): C 74.92, H 7.11, N 6.33; found: C 74.77, H 7.34, N 6.02.

*4.18. 3-(4-Hydroxyphenyl)-1,7-diphenyl-5-{4-[N-(3β-acetyloxy-lup-20(29)en-28-oyl] piperazine-1-carbonyl)phenyl}-4,4-difluoro-4-bora-3a,4a,8-triaza-s-indacene (**18**)*

Following the GP from **14** (50 mg, 58 μm mol), **17** (55 mg, 50%) was obtained as a green solid; m.p. 274 °C; R_F = 0.25 ($CHCl_3$/MeOH/NH_4OH, 98:1.8:0.2); IR (ATR): $\tilde{\nu}$ = 2941 *m,* 2867*w,* 1729*w,* 1635 *m,* 1602 *m,* 1537 *m,* 1511 *m,* 1505 *m,* 1502 *m,* 1483 *m,* 1474*s,* 1445*s,* 1418 *m,* 1388*s,* 1372 *m,* 1320 *m,* 1281*s,* 1262 *m,* 1238*s,* 1179*s,* 1125*s,* 1095*s,* 1070*s,* 1036*vs,* 1022*vs,* 1002*vs,* 979*s,* 944 *m,* 916 *m,* 899 *m,* 865 *m,* 841 *m,* 826 *m,* 771*s,* 763*s,* 746*s,* 713 *m,* 688*s,* 617 *m,* 587 *m,* 577 *m,* 530 *m,* 409 m cm^{-1}; UV/vis ($CHCl_3$): λ_{max} (log ε) = 667 (4.89), 472 (4.05), 318 (4.47) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.08–8.01 (m, 7H, 46-H+46′-H+47-H+53-H+53-H+58-H+58′-H), 7.55–7.36 (m, 9H, 37-H+37′-H+38-H+38′-H+45-H+45′-H+57-H+57′-H+59-H), 7.10 (s, 1H, 50-H), 6.95 (s, 1H, 41-H), 6.89 (d, J = 8.3 Hz, 2H, 54-H+54′-H), 4.74–4.67 (m, 1H, 29-H_a), 4.58 (d, J = 2.0 Hz, 1H, 29-H_b), 4.49–4.40 (m, 1H, 3-H), 3.86–3.32 (m, 8H, 33-H_2+33′-H_2+34-H_2+34′-H_2), 3.01–2.90 (m, 1H, 19-H), 2.86–2.75 (m, 1H, 13-H), 2.03 (s, 3H, 32-H_3), 2.06–1.96 (m, 1H, 16-H_a), 1.96–1.77 (m, 2H, 21-H_a+22-H_a), 1.66 (s, 3H, 30-H_3), 1.72–1.49 (m, 6H, 1-H_a+2-H_2+9-H+12-H_a+16-H_b), 1.48–1.19 (m, 10H, 6-H_2+7-H_2+11-H_2+15-H_a+21-H_b+22-H_b+18), 1.12 (d, J = 13.2 Hz, 1H, 15-H_b), 0.99–0.83 (m, 2H, 1-H_b+12-H_b), 0.92 (s, 3H, 27-H_3), 0.89 (s, 3H, 26-H_3), 0.81 (s, 3H, 25-H_3), 0.80 (s, 3H, 24-H_3), 0.78 (s, 3H, 23-H_3), 0.74 (d, J = 8.5 Hz, 1H, 5-H) ppm; ^{11}B NMR (160 MHz, $CDCl_3$): δ = 0.99 (t, J = 31.5 Hz) ppm; ^{19}F NMR (470 MHz, $CDCl_3$): δ = −130.07–-132.33 (m) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 174.1 (C-28), 171.1 (C-31), 170.4 (C-35), 161.9 (C-51), 160.5 (C-55), 154.0 (C-40), 151.0 (C-20), 146.8 (C-49), 145.3 (C-56), 141.7 (C-42), 135.8 (C-36), 134.2 (C-39), 132.6 (C-47), 132.5 (C-43), 131.8 (C-44), 129.8 (C-59), 129.6 (C-48), 129.4 (C-53+C-53′), 129.4 (C-58+C-58′), 129.2 (C-46+C-46′), 129.1 (C-37+C-37′), 128.6 (C-45+C-45′), 128.6 (C-57+C-57′), 127.3 (C-38+C-38′), 122.7 (C-52), 120.1 (C-50), 117.8 (C-41), 116.3 (C-54-H + C-54′), 109.4 (C-29), 81.0 (C-3), 55.5 (C-5), 54.7 (C-17), 52.6 (C-9), 50.7 (C-18), 47.9 (C-34+C-34′), 45.6 (C-19), 42.5 (C-33+C-33′), 41.9 (C-14), 40.7 (C-8), 38.4 (C-1), 37.8 (C-4), 37.1 (C-10), 36.9 (C-13), 36.0 (C-22), 34.3 (C-7), 32.6 (C-16), 31.3 (C-21), 29.8 (C-15), 27.9 (C-24), 25.6 (C-12), 23.7 (C-2), 21.3 (C-32), 21.1 (C-11), 19.6 (C-30), 18.2 (C-6), 16.4 (C-23), 16.2 (C-25), 16.0 (C-26), 14.6 (C-27) ppm; MS (ESI, $CHCl_3$/MeOH, 4:1): m/z = 1006.5 (30%, $[M]^-$); analysis calcd for $C_{69}H_{78}BF_2N_5O_5$ (1106.22): C 74.92, H 7.11, N 6.33; found: C 74.61, H 7.38, N 6.51.

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

Data will be made available on request.

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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.2022.100099.

References

[1] F. de Jong, J. Pokorny, B. Manshian, B. Daelemans, J. Vandaele, J.B. Startek, S. Soenen, M. Van der Auweraer, W. Dehaen, S. Rocha, G. Silveira-Dorta, Development and characterization of BODIPY-derived tracers for fluorescent labeling of the endoplasmic reticulum, Dyes Pigments 176 (2020), 108200.

[2] E. Antina, N. Bumagina, Y. Marfin, G. Guseva, L. Nikitina, D. Sbytov, F. Telegin, BODIPY conjugates as functional compounds for medical diagnostics and treatment, Molecules 27 (2022).

[3] F. Qi, H. Yuan, Y.C. Chen, Y. Guo, S.R. Zhang, Z.P. Liu, W.J. He, Z.J. Guo, BODIPY-based monofunctional Pt (II) complexes for specific photocytotoxicity against cancer cells, J. Inorg. Biochem. 218 (2021).

[4] H.Z. Yao, S. Chen, Z.Q. Deng, M.K. Tse, Y.D. Matsuda, G.Y. Zhu, BODI-Pt, a green-light-activatable and carboplatin-based platinum(IV) anticancer prodrug with enhanced activation and cytotoxicity, Inorg. Chem. 59 (2020) 11823–11833.

[5] V. Ramu, S. Gautam, P. Kondaiah, A.R. Chakravarty, Diplatinum(II) catecholate of photoactive boron-dipyrromethene for lysosome-targeted photodynamic therapy in red light, Inorg. Chem. 58 (2019) 9067–9075.

[6] V. Ramu, P. Kundu, P. Kondaiah, A.R. Chakravarty, Maloplatin-B, a cisplatin-based BODIPY-tagged mito-specific "Chemo-PDT" agent active in red light, Inorg. Chem. 60 (2021) 6410–6420.

[7] V. Ramu, P. Kundu, A. Upadhyay, P. Kondaiah, A.R. Chakravarty, Lysosome specific platinum(II) catecholates with photoactive BODIPY for imaging and photodynamic therapy in near-IR light, Eur. J. Inorg. Chem. 2021 (2021) 831–839.

[8] M.K. Raza, S. Gautam, A. Garai, K. Mitra, P. Kondaiah, A.R. Chakravarty, Monofunctional BODIPY-appended imidazoplatin for cellular imaging and mitochondria-targeted photocytotoxicity, Inorg. Chem. 56 (2017) 11019–11029.

[9] M.K. Raza, S. Gautam, P. Howlader, A. Bhattacharyya, P. Kondaiah, A.R. Chakravarty, Pyriplatin-boron-dipyrromethene conjugates for imaging and mitochondria-targeted photodynamic therapy, Inorg. Chem. 57 (2018) 14374–14385.

[10] E. Kitteringham, D. Wu, S. Cheung, B. Twamley, D.F. O'Shea, D.M. Griffith, Development of a novel carboplatin like cytoplasmic trackable near infrared fluorophore conjugate via strain-promoted azide alkyne cycloaddition, J. Inorg. Biochem. 182 (2018) 150–157.

[11] J. Zhou, Y.Z. Zhang, G.C. Yu, M.R. Crawley, C.R.P. Fulong, A.E. Friedman, S. Sengupta, J.F. Sun, Q. Li, F.H. Huang, T.R. Cook, Highly emissive self-assembled BODIPY-platinum supramolecular triangles, J. Am. Chem. Soc. 140 (2018) 7730–7736.

[12] U. Bhattacharyya, B. Kumar, A. Garai, A. Bhattacharyya, A. Kumar, S. Banerjee, P. Kondaiah, A.R. Chakravarty, Curcumin "drug" stabilized in oxidovanadium(IV)-BODIPY conjugates for mitochondria-targeted photocytotoxicity, Inorg. Chem. 56 (2017) 12457–12468.

[13] T.T. Sun, W.H. Lin, W. Zhang, Z.G. Xie, Self-Assembly of amphiphilic drug-dye conjugates into nanoparticles for imaging and chemotherapy, Chem. Asian J. 11 (2016) 3174–3177.

[14] C.S. Wijesooriya, J.A. Peterson, P. Shrestha, E.J. Gehrmann, A.H. Winter, E.A. Smith, A photoactivatable BODIPY probe for localization-based super-resolution cellular imaging, Angew. Chem., Int. Ed. 57 (2018) 12685–12689.

[15] T. Zhang, W. Zhang, M. Zheng, Z.G. Xie, Near-infrared BODIPY-paclitaxel conjugates assembling organic nanoparticles for chemotherapy and bioimaging, J. Colloid Interface Sci. 514 (2018) 584–591.

[16] I.W. Badon, J. Lee, T.P. Vales, B.K. Cho, H.J. Kim, Synthesis and photophysical characterization of highly water-soluble PEGylated BODIPY derivatives for cellular imaging, J. Photochem. Photobiol., A 377 (2019) 214–219.

[17] Z. Ruan, Y.Y. Zhao, P. Yuan, L. Liu, Y.C. Wang, L.F. Yan, PEG conjugated BODIPY-Br-2 as macrophotosensitizer for efficient imaging-guided photodynamic therapy, J. Mater. Chem. B 6 (2018) 753–762.

[18] S.T. Wang, J.T. Li, Z. Ye, J.L. Li, A.H. Wang, J. Hu, S. Bai, J. Yin, Self-assembly of photosensitive and chemotherapeutic drugs for combined photodynamic-chemo cancer therapy with real-time tracing property, Colloid. Surface. 574 (2019) 44–51.

[19] Y. Jang, T.I. Kim, H. Kim, Y. Choi, Y. Kim, Photoactivatable BODIPY platform: light-triggered anticancer drug release and fluorescence monitoring, ACS Appl. Bio Mater. 2 (2019) 2567–2572.

[20] A. Sampedro, A. Ramos-Torres, C. Schwoppe, C. Muck-Lichtenfeld, I. Helmers, A. Bort, I. Diaz-Laviada, G. Fernandez, Hierarchical self-assembly of BODIPY dyes as a tool to improve the antitumor activity of capsaicin in prostate cancer, Angew. Chem., Int. Ed. 57 (2018) 17235–17239.

[21] D.N. Tomilin, L.N. Sobenina, K.B. Petrushenko, I.A. Ushakov, B.A. Trofimov, Design of novel meso-CF3-BODIPY dyes with isoxazole substituents, Dyes Pigments 152 (2018) 14–18.

[22] R. Tiwari, P.S. Shinde, S. Sreedharan, A.K. Dey, K.A. Vallis, S.B. Mhaske, S.K. Pramanik, A. Das, Photoactivatable prodrug for simultaneous release of mertansine and CO along with a BODIPY derivative as a luminescent marker in mitochondria: a proof of concept for NIR image-guided cancer therapy, Chem. Sci. 12 (2020) 2667–2673.

[23] Y.H. Li, J. Zeng, Z. Wang, T.Y. Wang, S.Y. Wu, X.Y. Zhu, X. Zhang, B.H. Shan, C.Z. Gao, S.H. Wang, F.A.-O. Wu, Sulfur-Doped Organosilica Nanodots as a Universal Sensor for Ultrafast Live/Dead Cell Discrimination, Biosensors 12 (2022) 1000.

[24] [1], E.M. Nolan, S.J. Lippard, Small-molecule fluorescent sensors for investigating zinc metalloneurochemistry, Acc. Chem. Res. 42 (2009) 193–203.

[25] J. Yao, M. Yang, Y. Duan, Chemistry, biology, and medicine of fluorescent nanomaterials and related systems: new insights into biosensing, bioimaging, genomics, diagnostics, and therapy, Chem. Rev. 114 (2014) 6130–6178.

[26] T. Myochin, K. Hanaoka, T. Komatsu, T. Terai, T. Nagano, Design strategy for a near-infrared fluorescence probe for matrix metalloproteinase utilizing highly cell permeable boron dipyrromethene, J. Am. Chem. Soc. 134 (2012) 13730–13737.

[27] X. Wu, Z. Li, X.-X. Chen, J.S. Fossey, T.D. James, Y.-B. Jiang, Selective sensing of saccharides using simple boronic acids and their aggregates, Chem. Soc. Rev. 42 (2013) 8032–8048.

[28] A.N. Butkevich, G. Lukinavičius, E. D'Este, S.W. Hell, Cell-permeant large Stokes shift dyes for transfection-free multicolor nanoscopy, J. Am. Chem. Soc. 139 (2017) 12378–12381.

[29] D. Kodr, J. Stankova, M. Rumlova, P. Dzubak, J. Rehulka, T. Zimmermann, I. Krizova, S. Gurska, M. Hajduch, P.B. Drasar, M. Jurasek, Betulinic acid decorated with polar groups and blue emitting BODIPY dye: synthesis, cytotoxicity, cell-cycle analysis and anti-HIV profiling, Biomedicines 9 (2021) 1104.

[30] B. Brandes, S. Hoenke, L. Fischer, R. Csuk, Design, synthesis and cytotoxicity of BODIPY FL labelled triterpenoids, Eur. J. Med. Chem. 185 (2020), 111858.

[31] S. Hoenke, I. Serbian, H.P. Deigner, R. Csuk, Mitocanic di- and triterpenoid rhodamine B conjugates, Molecules 25 (2020) 5443.

[32] O. Kraft, M. Kozubek, S. Hoenke, I. Serbian, D. Major, R. Csuk, Cytotoxic triterpenoid-safirinium conjugates target the endoplasmic reticulum, Eur. J. Med. Chem. 209 (2021), 112920.

[33] V.N. Ingle, S.T. Kharche, U.G. Upadhyay, Glucosylation of 4 '-hydroxychalcones using glucosyl donor, Indian J. Chem. B 44 (2005) 801–805.

[34] J. Murtagh, D.O. Frimannsson, D.F. O'Shea, Azide conjugatable and pH responsive near-infrared fluorescent imaging probes, Org. Lett. 11 (2009) 5386–5389.

[35] M. Strobl, T. Rappitsch, S.M. Borisov, T. Mayr, I. Klimant, NIR-emitting aza-BODIPY dyes - new building blocks for broad-range optical pH sensors, Analyst 140 (2015) 7150–7153.

[36] P. Pfeiffer, K. Kollbach, E. Haack, Halochromic compounds of polyketones, Justus Liebigs Ann. Chem. 460 (1928) 138–156.

[37] M. Farfan-Paredes, O. Gonzalez-Antonio, D.E. Tahuilan-Anguiano, J. Peon, A. Ariza, P.G. Lacroix, R. Santillan, N. Farfan, Physicochemical and computational insight of F-19 NMR and emission properties of meso-(o-aryl)-BODIPYs, New J. Chem. 44 (2020) 19459–19471.

[38] S. Grigoropoulou, D. Manou, A.I. Antoniou, A. Tsirogianni, C. Siciliano, A.D. Theocharis, C.M. Athanassopoulos, Synthesis and antiproliferative activity of novel dehydroabietic acid-chalcone hybrids, Molecules 27 (2022) 3623.

[39] H.H.H. Mohammed, A.A. Abd El-Hafeez, K. Ebeid, A.I. Mekkawy, M.A.S. Abourehab, E.I. Wafa, S.O. Alhaj-Suliman, A.K. Salem, P. Ghosh, G.E.A. Abuo-Rahma, A.M. Hayallah, S.H. Abbas, New 1,2,3-triazole linked ciprofloxacin-chalcones induce DNA damage by inhibiting human topoisomerase I& II and tubulin polymerization, J. Enzym. Inhib. Med. Chem. 37 (2022) 1346–1363.

[40] N.A. Rehuman, J.M. Oh, M.A. Abdelgawad, E.A.M. Beshr, M.A.S. Abourehab, N. Gambacorta, O. Nicolotti, R.K. Jat, H. Kim, B. Mathew, Development of halogenated-chalcones bearing with dimethoxy phenyl head as monoamine oxidase-B inhibitors, Pharmaceuticals 15 (2022) 1152.

[41] E.A. Veliz, A. Kaplina, S.D. Hettiarachchi, A.L. Yoham, C. Matta, S. Safar, M. Sankaran, E.L. Abadi, E.K. Cilingir, F.A. Vallejo, W.M. Walters, S. Vanni, R.M. Leblanc, R.M. Graham, Chalcones as anti-glioblastoma stem cell agent alone or as nanoparticle formulation using carbon dots as nanocarrier, Pharmaceutics 14 (2022) 1465.

[42] S. Li, R.A.-O. Wu, L. Wang, H.C. Dina Kuo, D. Sargsyan, X. Zheng, Y. Wang, X. Su, A.A.-O. Kong, Triterpenoid ursolic acid drives metabolic rewiring and epigenetic reprogramming in treatment/prevention of human prostate cancer, Mol. Carcinogen 61 (2022) 111–121.

[43] D.M. Gurfinkel, S. Chow, R. Hurren, M. Gronda, C. Henderson, C. Berube, D.W. Hedley, A.D. Schimmer, Disruption of the endoplasmic reticulum and increases in cytoplasmic calcium are early events in cell death induced by the natural triterpenoid Asiatic acid, Apoptosis 11 (2006) 1463–1471.

[44] D. Luo, N. Fan, X. Zhang, F.Y. Ngo, J. Zhao, W. Zhao, M. Huang, D. Li, Y. Wang, J. Rong, Covalent inhibition of endoplasmic reticulum chaperone GRP78 disconnects the transduction of ER stress signals to inflammation and lipid accumulation in diet-induced obese mice, Elife 11 (2022), e72182.