European Journal of Medicinal Chemistry Reports 12 (2024) 100177

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry Reports

journal homepage: www.editorialmanager.com/ejmcr/default.aspx

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

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ARTICLE INFO

Keywords:
Sulfamates
Carbonic anhydrase II
Inhibitor

ABSTRACT

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

1. Introduction

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

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

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

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

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

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

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https://doi.org/10.1016/j.ejmcr.2024.100177
Received 13 May 2024; Received in revised form 7 June 2024; Accepted 9 June 2024
Available online 11 June 2024

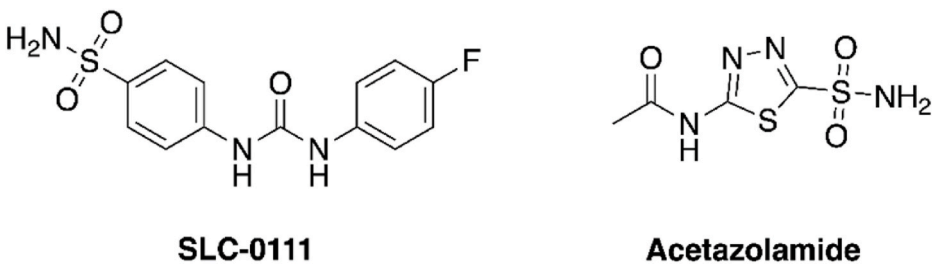
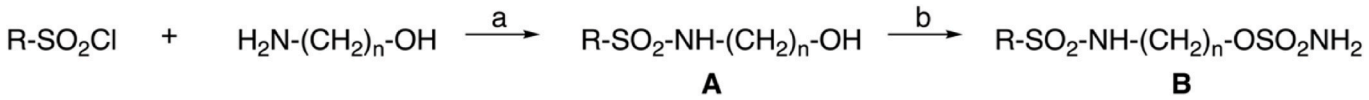
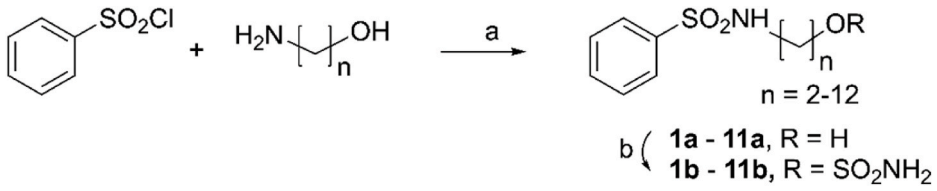


Fig. 1. Structure of established inhibitors SLC-0111 and acetazolamide (standard).



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



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

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

2. Results and discussion

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

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

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

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

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

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

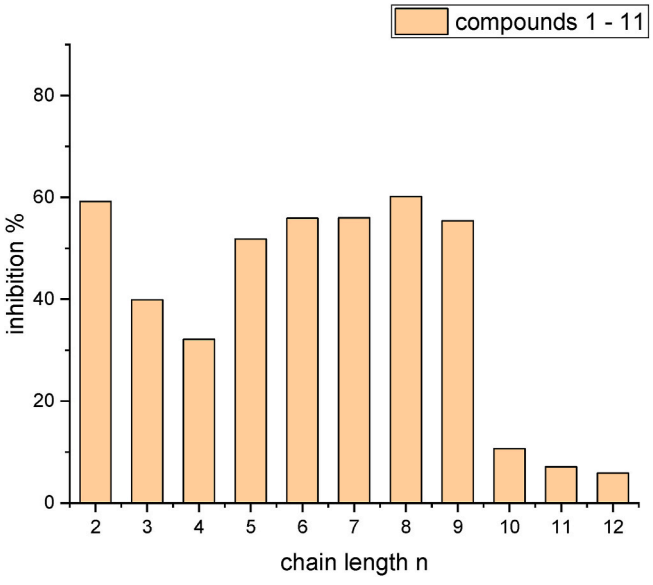
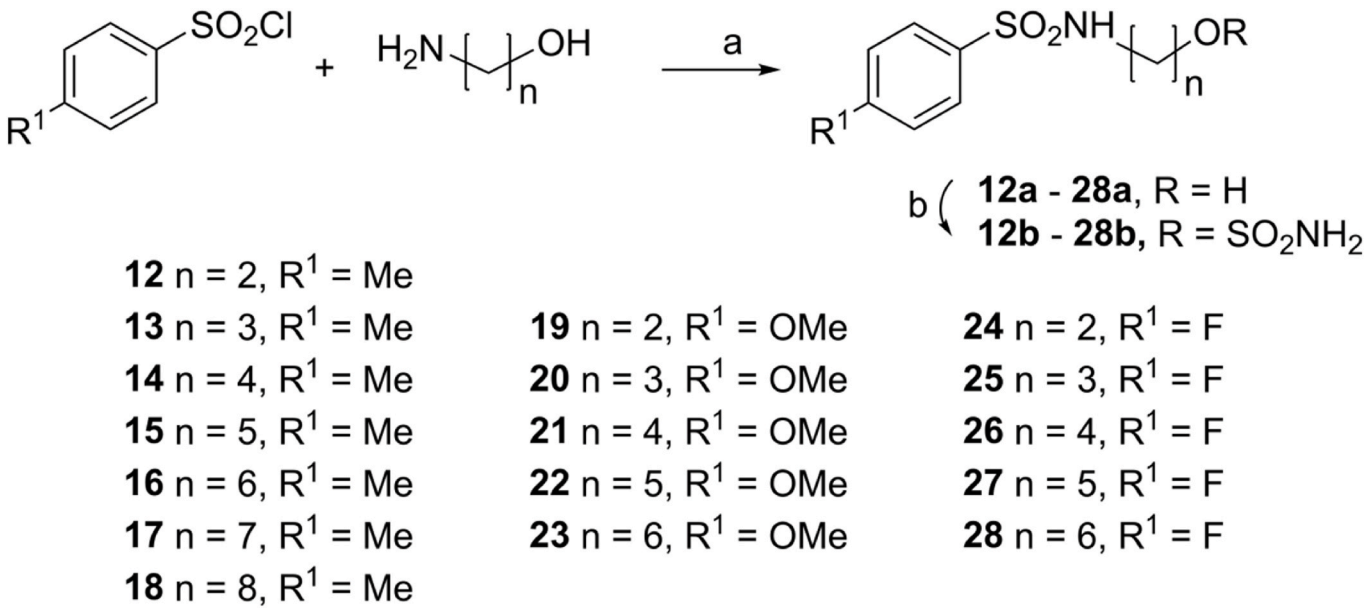


Fig. 2. Inhibition (in %) of unsubstituted compounds **1b**–**11b**.



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

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

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

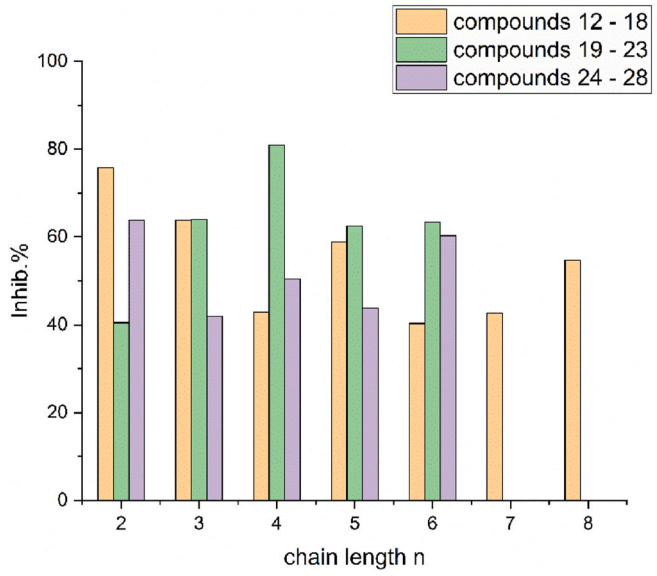


Fig. 3. Inhibition (in %) of the second series **12b–28b**.

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

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

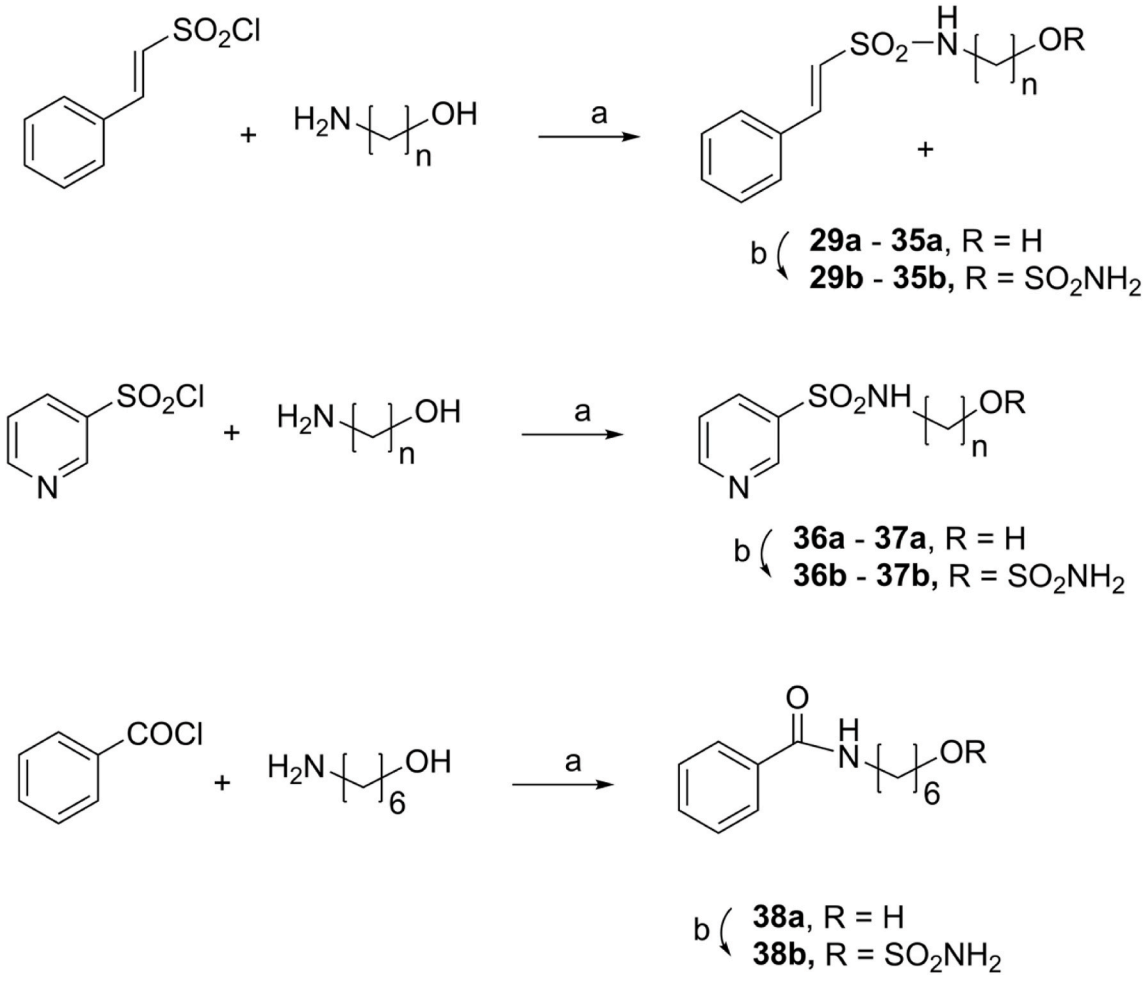
Compounds **29b–35b** show decreased activity with longer chains, suggesting steric hindrance or altered molecular interactions. Conversely, compounds **36b** and **37b** display enhanced activity with elongated chains. Notably, compound **38b** exhibits activity similar to compound **33b** despite similar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

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

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

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

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



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

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

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

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

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

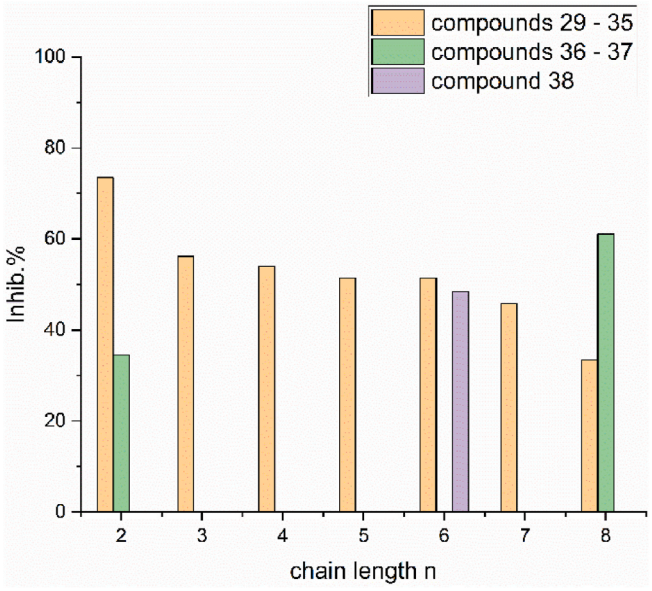


Fig. 4. Inhibition (in %) of the third series **29b**–**38b**.

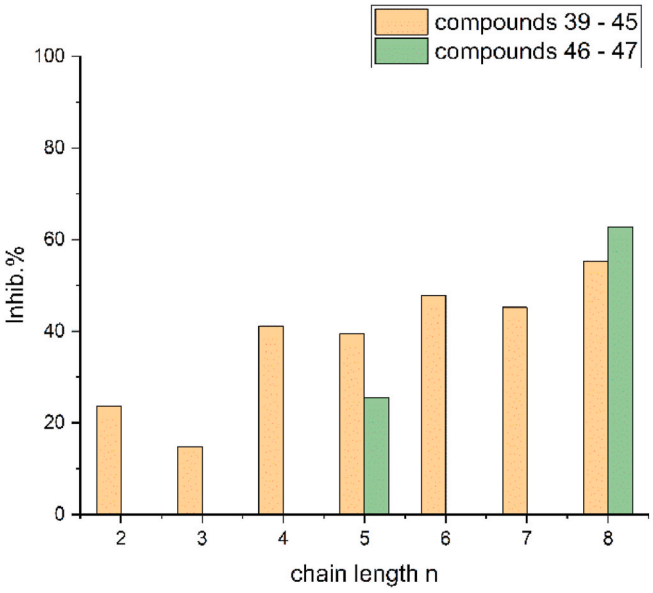
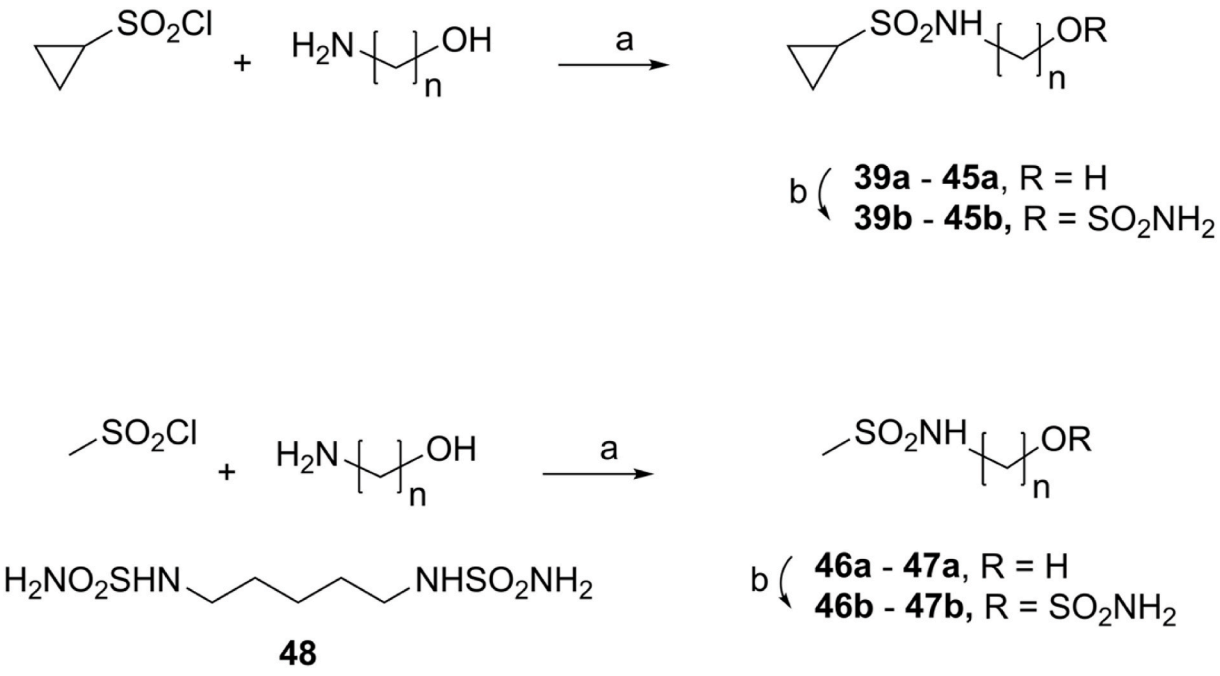


Fig. 5. Inhibition (in %) of the 4th series **39b**–**47b**.

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

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



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

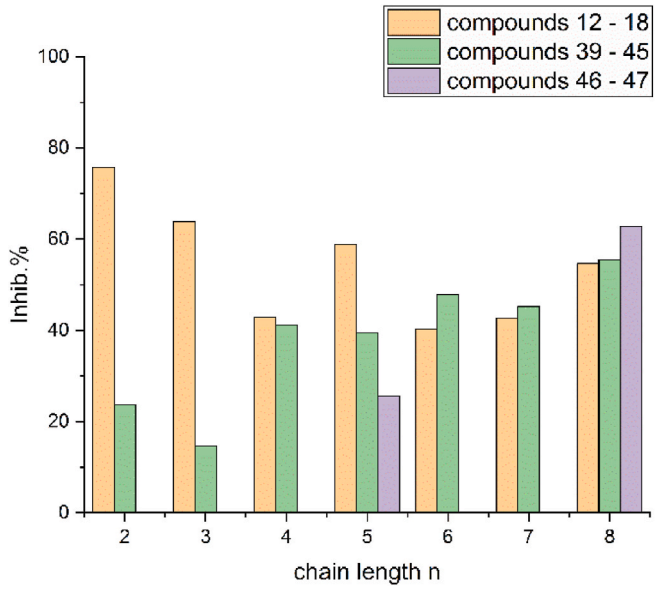


Fig. 6. Inhibition (in %) of the 4th series **39b**–**47b** without an aromatic moiety vs compounds **12b**–**18b**.

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

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

3. Conclusion

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

4. Experimental

4.1. General

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

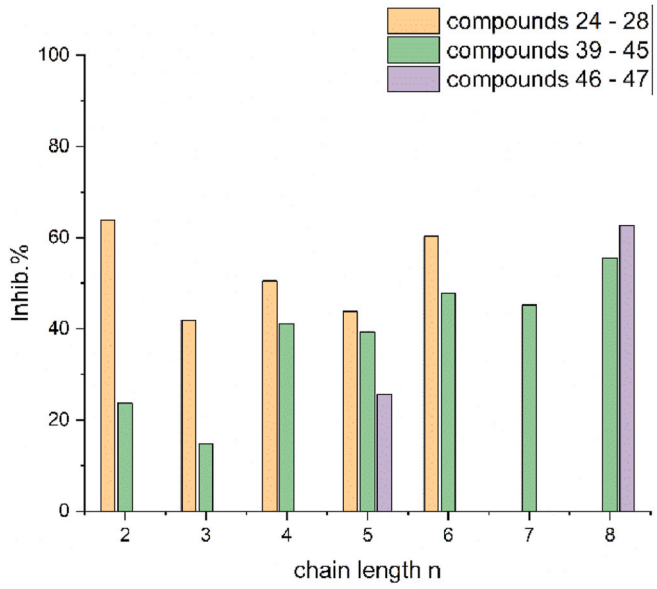


Fig. 7. Inhibition (in %) of the 4th series **39b**–**47b** without an aromatic moiety vs compounds **24b**–**28b** with fluorine moiety.

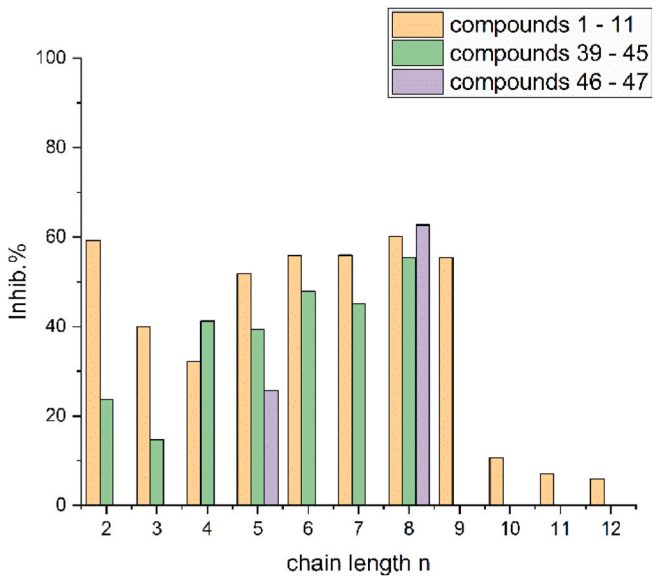
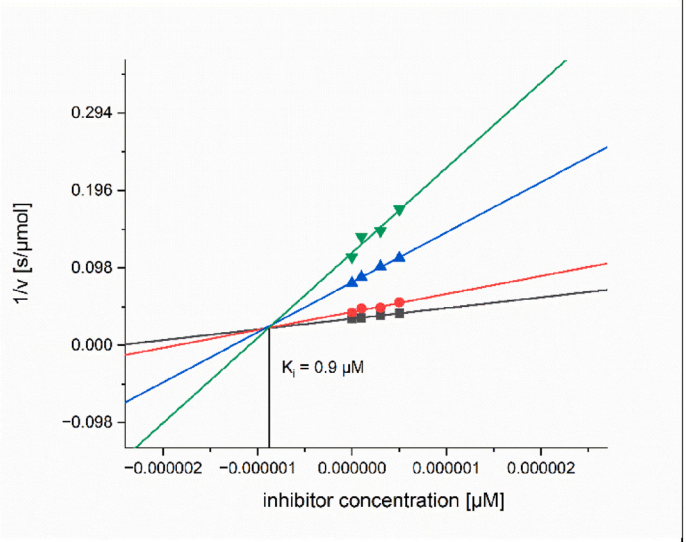
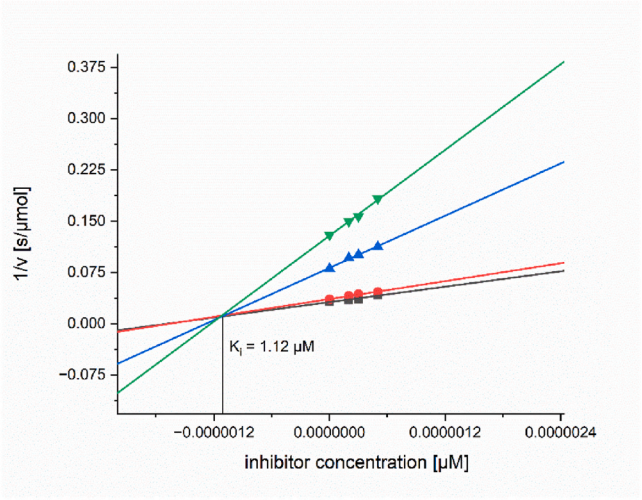


Fig. 8. Inhibition (in %) of the 4th series **39b**–**47b** without an aromatic moiety vs compounds **1b**–**11b**.

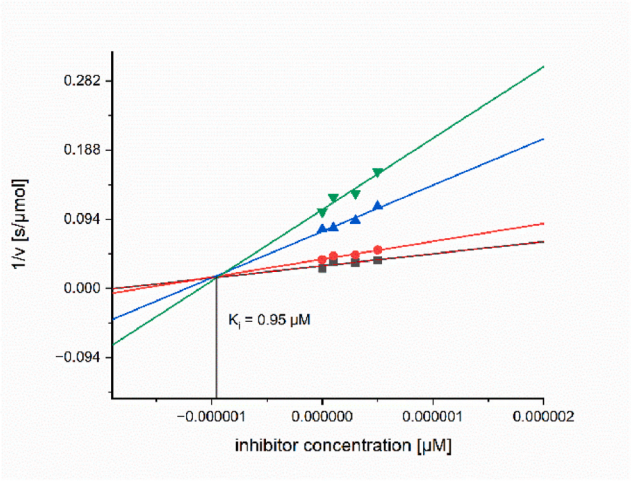
21b (inhibitor concentration used: 0.1, 0.3, 0.5 μM); $K_i = 0.90 \pm 0.03$ μM



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



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



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

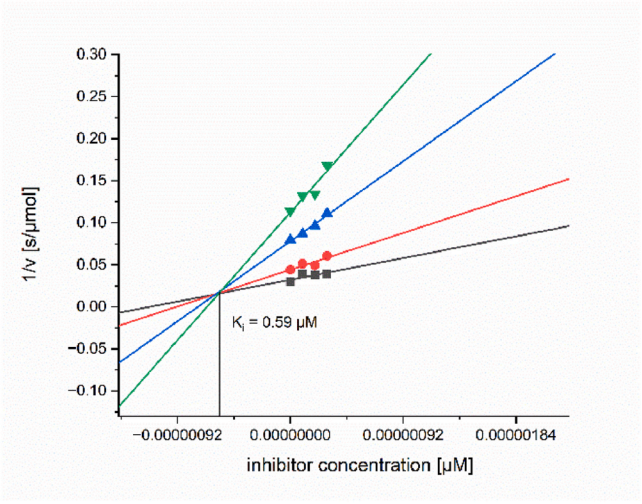


Fig. 9. Dixon plots for **21b**, **24b**, **29b** and **47b**.

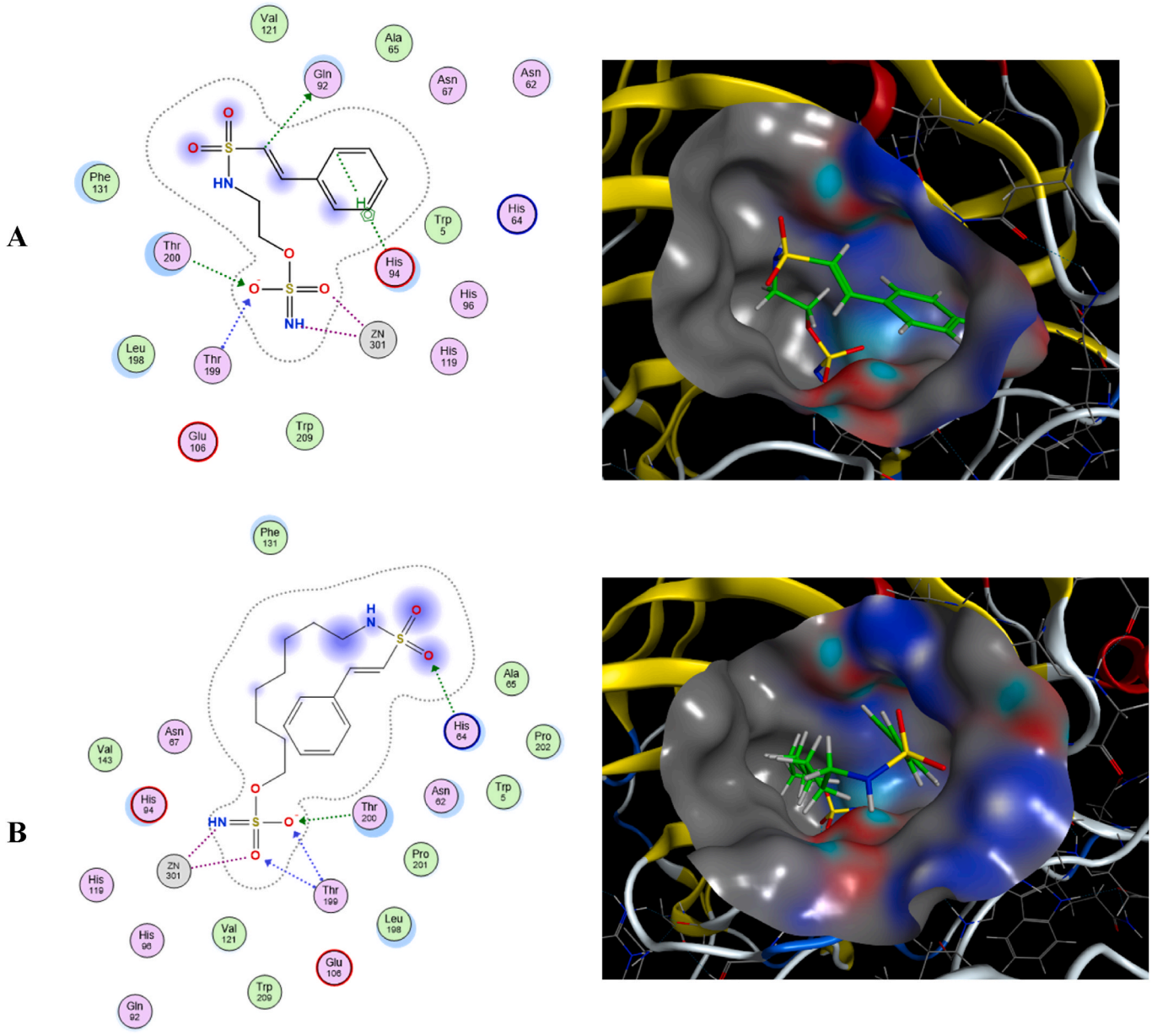


Fig. 10. Calculated 2D and 3D representations showing the interactions of **29b** (A) and **35b** (B) within the active site of CA II.

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

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

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

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

4.4. Characterization of the compounds

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

CRediT authorship contribution statement

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

Declaration of competing interest

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

Data availability

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

Acknowledgments

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

Appendix A. Supplementary data

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

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