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Carbonic Anhydrase Inhibitors: Aliphatic N-phosphorylated Sulfamates—A Novel Zinc-anchoring Group Leading to Nanomolar Inhibitors

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A small library of phosphorylated sulfamates (*N*-(*O*-alkylsulfamoyl)-phosphoramidic acids) incorporating long aliphatic chains (C8–C16) has been synthesized and investigated for their interaction with two physiologically relevant carbonic anhydrase (CA) isozymes. These compounds behaved as very potent inhibitors of both isozymes, with inhibition constants in the range of 8.2–16.1 nM against isozyme hCA I, and 5.3–11.9 nM against isozyme hCA II. Activity was optimal for the *n*-octyl derivative (similarly with that of the corresponding unsubstituted sulfamates) and gradually decreased for the longer chain derivatives. Some of these compounds are much more effective CA inhibitors as compared to the clinically used derivatives acetazolamide, sulfanilamide or topiramate, which are used as standards for the enzymatic determinations. The phosphorylated sulfamate moiety represents a novel zinc-binding group for the design of effective CA inhibitors.

Keywords: Carbonic anhydrase; Isozyme I, II; Sulfamate; N-phosphorylated sulfamate

INTRODUCTION

Inhibition of the metallo-enzyme carbonic anhydrase (CA, EC 4.2.1.1) by sulfonamides was discovered in 1940 by Mann and Keilin,[1] and led to the report by Krebs[2] that only the unsubstituted aromatic sulfonamides of type $ArSO_2NH_2$ act as strong CA inhibitors (CAIs), and that the potency of such compounds is drastically reduced by *N*-substitution of the sulfonamide moiety. This constituted the beginning of extensive structure–activity correlations which led to some valuable drugs during a short period of time, but also led to the fact that for more than 40 years only this class of sulfonamides was investigated for its interaction with these enzymes.[3–6]

Thus, up to recently, only sulfonamides of type $ArSO_2NH_2$ (where Ar is an aromatic or heterocyclic moiety) were known to possess high affinity for CAs. Some recent data indicate that potent CAIs may be designed from many other types of compounds.[3–6] Figure 1 shows the most general structure of a CAI complexed to the enzyme active site. Such a compound must possess: (i) a zinc binding group (ZBG) by which it interacts with the metal ion of the enzyme and the residues Thr 199 and Glu 106 in its neighborhood; (ii) an organic scaffold—usually an aromatic or heterocyclic moiety, which may be present or absent in new generation CAIs; (iii) a tail attached to the scaffold, which usually was absent in the first and second generation of sulfonamide CAIs, but which is extremely important (and generally present) for the last generation of such derivatives.[3–10] All these structural elements interact both with the hydrophobic as well as the hydrophylic halves of the active site, whereas the ZBG interacts with Thr 199 and Glu 106 as shown in the Figure.[7–13] Thus, sulfonamides constitute just a particular case for this type of general interaction.

Indeed, in recent years, an entire range of new ZBGs have been reported, mostly by our groups,[7–13]

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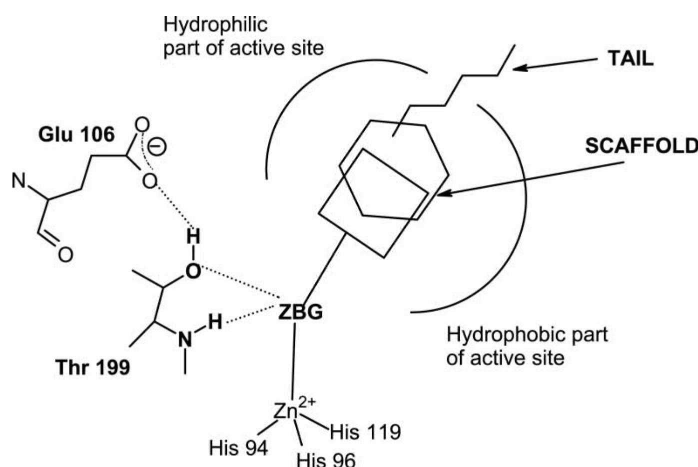

FIGURE 1 The general structure of a CAI complexed to the enzyme active site: ZBG = zinc binding group; the organic scaffold may be present or absent; the tail too. These structural elements interact both with the hydrophobic as well as the hydrophylic halves of the active site, whereas ZBG interacts with Thr 199 and Glu 106.[7–10]

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as shown in Figure 2. These new ZBGs include in addition to the classical sulfonamide function: sulfamates, sulfamides, substituted sulfonamide, Schiff's bases, urea and hydroxyurea, as well as hydroxamates.

An interesting, and rather uninvestigated ZBG is constituted by the phosphorylated-sulfonamide function, with several compounds of the type $Ar—SO_2NH—PO_3H_2$ (where Ar = Ph, 4-halogeno-C_6H_4—; 4-Me-C_6H_4— and 4-H_2N-C_6H_4—, etc) recently shown by our group[14] to act as good inhibitors of isozymes CA I and II (K_I values in the range of 8–280 nM against isozyme CA II, and 42–450 nM against isozyme CA I).[14] Ultimately, this group investigated a large number of sulfamates as CAIs, detecting several sub-nanomolar inhibitors of several physiologically relevant isozymes such as the cytosolic CA I and II, or the trans-membrane, tumor associated isozyme CA IX.[8,12,15,16]. Thus, it appeared of interest to synthesize and test for their CA inhibitory activity sulfamates possessing a phosphoryl moiety substituting the nitrogen atom, of the type $R—O—SO_2NH—PO_3H_2$. Here we present the first CA inhibition data for this type of compound, which proved to be very effective inhibitors of isozymes I and II.

MATERIALS AND METHODS

Compounds **1–4** used in the activity measurements were prepared as described earlier.[17] The sulfonamides **5**, **6** and the sulfamate **7** used as standards were commercially available (Sigma-Aldrich or Johnson & Johnson).

CA Assays

An SX.18MV-R Applied Photophysics stopped-flow instrument was used for assaying the CO_2 hydration

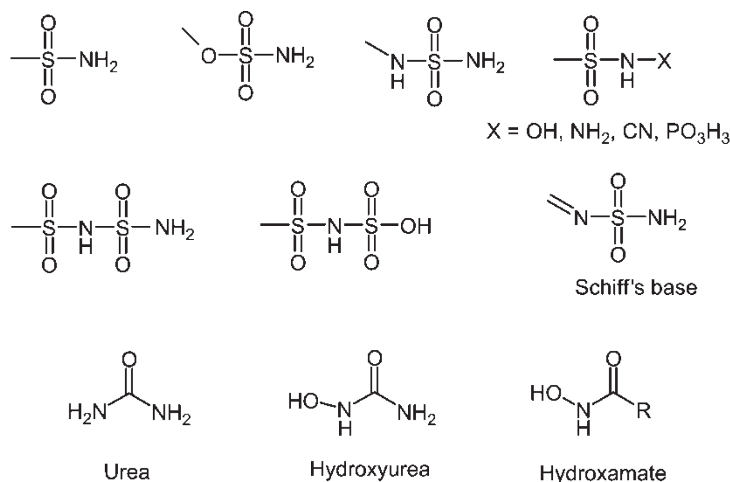

FIGURE 2 Zinc binding groups present in CAIs: sulfonamides, sulfamates, sulfamides, substituted sulfonamides, Schiff's bases, urea, hydroxyurea and hydroxamates among others.

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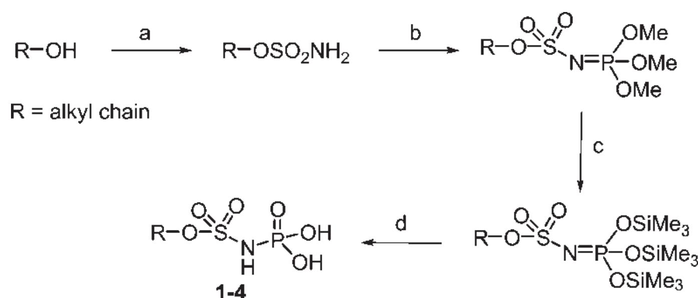

SCHEME 1 Synthesis of *N*-(*O*-alkylsulfamoyl)phosphoramidic acids **1–4**. Reagents and conditions: (a) sulfamoyl chloride, DMA; (b) $PO(OMe)_3$, DIAD, THF; (c) Me_3SiBr; (d) H_2O, pH = 7, 7days.

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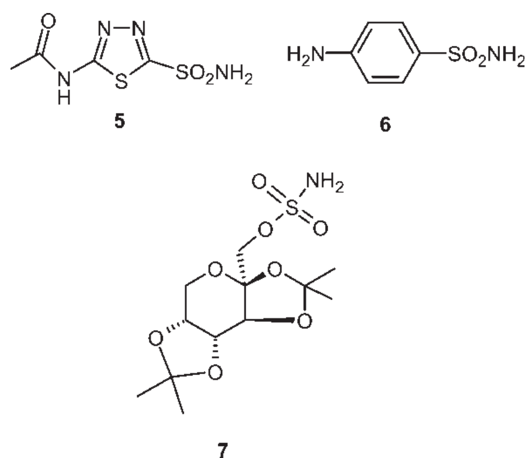
activity of CA isozymes.[18] Phenol red (at a concentration of 0.2 mM) was used as indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.5) as buffer, 0.1 M Na_2SO_4 (for maintaining constant ionic strength), and following the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. Saturated CO_2 solutions in water at 20°C were used as substrate. Stock solutions of inhibitors were prepared at a concentration of 1–3 mM (in DMSO–water 1:1, v/v) and dilutions up to 0.1 nM made with the assay buffer mentioned above. The enzyme concentrations were 0.1 μM for hCA II and 1 μM for hCA I, and inhibition constants were calculated as described in Ref. 18

RESULTS AND DISCUSSION

The different *N*-(*O*-alkylsulfamoyl)phosphoramidic acids **1–4** were prepared as previously described in a four step synthesis starting from the corresponding alcohol (*n*-octanol, *n*-dodecanol, *n*-tetradecanol, and *n*-hexadecanol, respectively), via a phospha-λ^5-azene intermediate (Scheme 1).[17] Reaction of the alcohol with sulfamoyl chloride in *N*,*N*-dimethylacetamide led to the corresponding sulfamate in high yield. The sulfamate was then reacted with trimethylphosphite in the presence of diisopropylazodicarboxylate (DIAD) through a redox reaction to afford the phospha-λ^5-azene which was silylated with pure trimethylbromosilane. Hydrolysis of the silylated phospha-λ^5-azene with water gave quantitatively the *N*-(*O*-alkylsulfamoyl)phosphoramidic acids.[17]



Inhibition data for the phosphorylated sulfamates **1–4**, the standard sulfonamides acetazolamide **5** and sulfanilamide **6**, the clinically used sulfamate topiramate **7**, as well as the corresponding aliphatic (unsubstituted) sulfamates **8–11**, previously investigated by us,[15,16] against the physiologically relevant isozymes CA I and CA II, are shown in Table I.

TABLE I CA inhibition data with compounds **1–4** and standard inhibitors (derivatives **5–7**) against human isozymes hCA I and hCA II

Compound	K_I (nM)*	
	hCA I [a]	hCA II [a]
1 n-C_8H_{17}—O—SO_2NH—PO_3H_2	8.2	5.3
2 n-$C_{12}H_{25}$—O—SO_2NH—PO_3H_2	10.5	9.9
3 n-$C_{14}H_{29}$—O—SO_2NH—PO_3H_2	14.6	11.9
4 n-$C_{16}H_{33}$—O—SO_2NH—PO_3H_2	16.1	11.2
5 acetazolamide	900	12
6 sulfanilamide	28,000	300
7 topiramate	250	5
8[b] n-C_8H_{17}—O—SO_2NH_2	3.5	2.7
9[c] n-$C_{12}H_{25}$—O—SO_2NH_2	270	10
10[c] n-$C_{14}H_{29}$—O—SO_2NH_2	150	87
11[c] n-$C_{16}H_{33}$—O—SO_2NH_2	58,000	97

*Errors in the range of 5–10% of the reported value (from 3 different assays). [a] Human (cloned) isozymes. [b] From ref. 15. [c] From ref. 16.

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The following features of CA inhibition with these compounds may be stressed: (i) the phosphorylated sulfamates **1–4** represent a novel, very potent class of CAIs. Indeed, against isozyme hCA I, these compounds showed inhibition constants in the range 8.2–16.1 nM, being much more effective inhibitors than the standard compounds **5–7**, or the corresponding aliphatic sulfamates, except for octylsulfamate **8**, which is 2.3 times more effective an inhibitor as compared to the corresponding phosphorylated derivative **1**. Against isozyme hCA II, the situation is rather similar, with derivatives **1–4** showing inhibition constants in the low nanomolar range (5.3–11.9 nM), of the same order of magnitude as those of the clinically used derivatives acetazolamide **5** and topiramate **7**, or of the two aliphatic sulfamates **8** and **9**. Sulfanilamide and the long chain sulfamates **10** and **11** are on the other hand less effective hCA II inhibitors as compared to the previously mentioned compounds, with inhibition constants in the range of 87–300 nM; (ii) the length of the aliphatic chain is critically important for the CA inhibitory properties of the phosphorylated sulfamates **1–4** (as it is for the corresponding unsubstituted sulfamates **8–11**).[15,16] Indeed, optimum activity was observed for the 8-carbon atoms chain compound **1** (or **8**), with a gradual decrease in activity with increasing length of the aliphatic chain, from C8 to C16. However, this decrease was not so dramatic for derivatives **1–4** as compared to the corresponding unsubstituted sulfamates **8–11**. Remarkable are the differences in hCA I inhibitory properties of the compounds **4** and **11**, possessing the same aliphatic chain: whereas the sulfamate **11** is a weak CA I inhibitor, the corresponding phosphorylated compound **4** (although being the most inefficient CAI in the small series of phosphorylated sulfamates investigated here) behaves as a quite potent hCA I inhibitor, being among others much more effective than acetazolamide or topiramate; (iii) no important differences in susceptibility to inhibition by this class of compounds between the two investigated isozymes was observed, which is rather remarkable since sulfonamide CAIs show a net preference for CA II over CA I.

In conclusion, we found that the phosphorylated sulfamate zinc binding group was very efficient for the design of low nanomolar CA inhibitors. Aliphatic compounds incorporating C8–C16 chains lead to inhibitors with affinities of 8–16 nM against hCA I, and 5–12 nM against hCA II.

Acknowledgements

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