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Carbonic Anhydrase Inhibitors: Inhibition of Cytosolic Isozymes I and II with Sulfamide Derivatives

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Abstract—A novel class of effective CAIs has been identified, starting from a very weak carbonic anhydrase inhibitor (CAI), sulfamide, whose X-ray crystal structure in the adduct with hCA II has recently been reported. A series of *N*,*N*-disubstituted- and *N*-substituted-sulfamides were prepared from the corresponding amines and *N*-(*tert*-butoxycarbonyl)-*N*-[4-(dimethylazaniumylidene)-1,4-dihydropyridin-1-ylsulfonyl]azanide or the unstable *N*-(*tert*-butoxycarbonyl)sulfamoyl chloride. The disubstituted compounds being too bulky, were ineffective as CAIs, whereas mono-substituted derivatives (incorporating aliphatic, cyclic and aromatic moieties) as well as a bis-sulfamide, behaved as micro-nanomolar inhibitors of two cytosolic isozymes, hCA I and hCA II, responsible for critical physiological processes in higher vertebrates. Aryl-sulfamides were more effective than aliphatic derivatives. Low nanomolar inhibitors have been detected, which generally incorporated 4-substituted phenyl moieties in their molecule. This is the first example of CAIs in which low nanomolar inhibitors were generated starting from a very ineffective lead molecule.

Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) catalyze a very simple physiological reaction, the interconversion between carbon dioxide and the bicarbonate ion, and are involved in crucial physiological processes connected with respiration and transport of CO_2/bicarbonate between metabolizing tissues and the lungs, pH and CO_2 homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions (such as gluconeogenesis, lipogenesis and ureagenesis), bone resorption, calcification, tumorigenicity, and many other physiologic or pathologic processes.[1–3] Thus, it is not surprising that many of their isozymes (14 are presently known in higher vertebrates) have been discovered as important targets for inhibitors with clinical applications.[2,3] Almost all of the most potent inhibitors of CAs (such as the clinically used acetazolamide **AAZ**, methazolamide **MZA**, ethoxzolamide **EZA**, dichlorophenamide **DCP**, dorzolamide **DZA** or brinzolamide **BRZ**) contain a terminal sulfonamide as anchoring group to coordinate the catalytic zinc.[1–3] These sulfonamides are widely used clinically, mainly as antiglaucoma agents, but also for the therapy of other diseases, for example increased intracranial pressure, various neurological/neuromuscular pathologies, such as epilepsy, genetic hemiplegic migraine and ataxia, tardive diskinesia, hypokalemic periodic paralysis, essential tremor and Parkinson's disease, and mountain sickness. Accordingly, drugs of this pharmacological class are under constant development.[2,3]

Recently, the X-ray crystallographic structure of sulfamide ($H_2NSO_2NH_2$) bound to the physiologically relevant isozyme hCA II has been reported by this group.[4] It was shown that sulfamide binds to the zinc ion of the enzyme (Fig. 1) via its deprotonated amide group (Zn–N distance of 1.76 Å), and this nitrogen donates an H-bond to Oγ of Thr 199 via its remaining hydrogen.[4] Thus, Oγ acts as an acceptor for the NH group of the inhibitor, but

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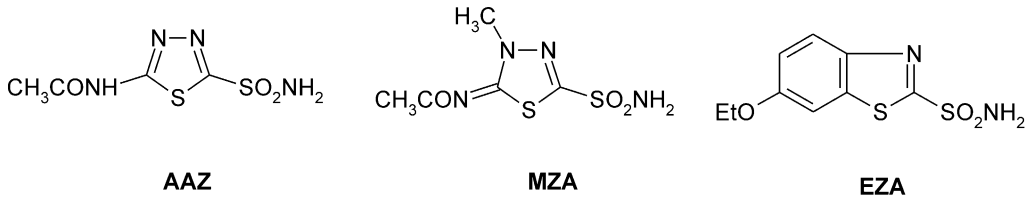
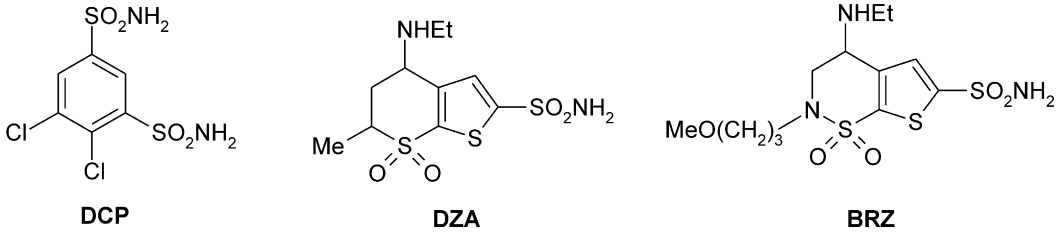




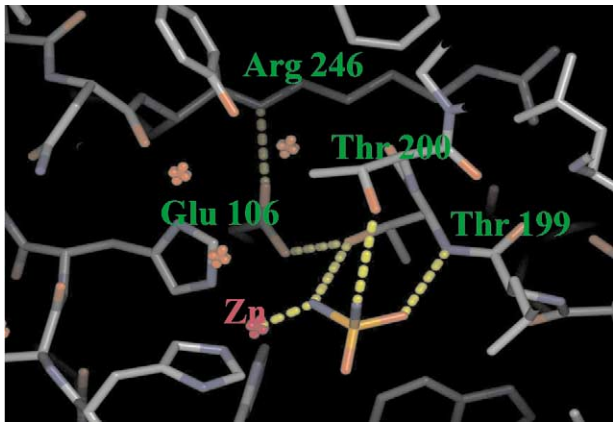
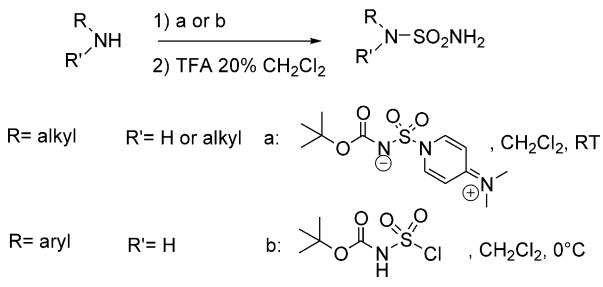


Figure 1. The sulfamide–hCA II adduct: the Zn(II) ion of the enzyme, its three histidine ligands (His 94, 96 and 119 CPK colors) and coordinated sulfamidate are shown, together with active site residues/water molecules (in red) involved in hydrogen bond networks (dotted, in yellow) with the inhibitor molecule: Thr 199 and Thr 200, Glu 106 and Arg 246 (figure generated from the PDB file described in ref. 4).

in turn, its hydroxyl moiety donates an H-bond to one of the terminal carboxylate oxygens of the adjacent Glu106. Via its second oxygen, this carboxylate mediates a hydrogen-bond network as acceptor to the backbone NH of Arg246 and via a water molecule to the hydroxyl oxygen of Tyr7. This extended extra-coordination results in a distorted tetrahedral arrangement around the metal ion, the remaining three ligands of zinc being His94, His96 and His119 (as in the uninhibited enzyme).[4] The second oxygen of the SO_2 group of the inhibitor was shown to be close to the backbone NH of Thr199 (2.81 Å), which accordingly accepts a H-bond from Thr199NH. This oxygen replaces the 'deep water' in the ligand-free enzyme.[4] Finally, the second, uncharged NH_2 group of sulfamide forms a hydrogen bond of 2.90 Å to an adjacent water molecule, which in turn is involved in an H-bond network with three other water molecules present in the binding pocket. In addition, this latter sulfamide nitrogen forms a second weak hydrogen bond to the OH group of Thr 200 (3.20 Å) and a third contact has been found to another water molecule (3.26 Å). In summary, this very simple inhibitor, present as the negatively charged $(NH)SO_2NH_2^-$ ion, shows a large number of favorable contacts in the binding pocket of CA, and may be used as a lead molecule, since sulfamide itself is a weak inhibitor (K_I of 35 μM against hCA I, and of 82 μM against hCA II — for the esterase activity of these enzymes)[4,5] (Fig. 1). From data of Figure 1, it is obvious that the second amino moiety of the inhibitor points towards the exit of the active site, being thus positioned in a very appropriate manner for derivatization. This is the approach we report here, that is derivatization of sulfamide at one of its NH_2 moieties, which led to nanomolar inhibitors of the two CA isozymes most abundant in vertebrates, CA I and CA II.

Chemistry

Sulfamides **1**–**26** have been prepared by an original procedure recently described by Winum et al.,[6] implying the reaction of primary/secondary amines with either *N*-(*tert*-butoxycarbonyl)-*N*-[4-(dimethylazaniumylidene)-1,4-dihydropyridin-1-ylsulfonyl]azanide or the unstable *N*-(*tert*-butoxycarbonyl)sulfamoyl chloride (obtained in situ from chlorosulfonyl isocyanate and *tert*-butanol), followed by removal of the protecting group (Scheme 1). Some of these compounds were previously reported in the literature,[6–16] but they have not been tested as CA



Scheme 1.

inhibitors (CAIs) in earlier studies. A rather large number of substitution patterns (R and R' moieties) have been used in compounds **1**–**26** (aliphatic, aromatic, substituted-aromatic, etc) with both mono- and *N,N*-disubstituted compounds, together with a disulfamide derivative (**26**) prepared, in order to detect best substitution pattern(s) for efficient CA inhibition.[17]

Carbonic anhydrase inhibitory activity

The data of Table 1 show that most of the sulfamides **1**–**26** investigated here act as better inhibitors against isozymes hCA I and hCA II as compared to the lead molecule, sulfamide, which is a weak inhibitor.[4,5] The nature and the number of the groups substituting the sulfamide moiety were the primary factors influencing CA inhibitory properties in this class of derivatives.

Thus, the *N,N*-disubstituted derivatives **5**–**8** are inactive as CAIs (except for **8** against CA II, which behaves as a very weak inhibitors of this isozyme), probably because of their very bulky nature and impossibility to accommodate within the enzyme active site. On the other hand, all the remaining monosubstituted derivatives, as well as the bis-sulfamide **26**, are much more potent CAIs as compared to the lead molecule, being effective against both investigated isozymes. For example, the aliphatic derivatives **1**–**3** and **9**, **10**, as well as benzylsulfamide **4** behave as moderately active CAIs, with inhibition constants in the range of 133–960 nM against hCA I, and 123–890 nM against hCA II, respectively. In this subseries, the bulkiest derivative (the 2-adamantyl-substituted compound **3**) was the most ineffective whereas the benzyl-substituted one (**4**) the most active inhibitor. Very good inhibitors proved to be the aryl-substituted sulfamides **11**–**25**, which showed inhibition constants in the range of 8–62 nM against hCA I, and 7–49 nM against hCA II, respectively. The 4-substituted phenylsulfamides showed better activity as compared to the 3-substituted compound **24**, the naphthyl-substituted derivative **25** or the pentafluorophenyl derivative **23** (which were the most ineffective in this subseries). For the first type of such derivatives, best inhibition has been observed for the 4-trifluoromethylphenyl-, 4-methoxyphenyl-, 4-methylphenyl-, 4-hydroxyphenyl-, phenyl-, and 4-nitrophenyl-substituted sulfamides. A special mention should be done regarding the aliphatic bis-sulfamide **26**, which behaved as an effective CA II inhibitor and a moderate CA I inhibitor, with inhibition constants of 27 nM against hCA II and 149 nM against hCA I, respectively. Thus, many of the new CAIs reported here possess potencies comparable or better than those of the clinically used compounds acetazolamide, methazolamide, ethoxzolamide, dichloro-

Table 1. Inhibition data for derivatives **1**–**26** investigated in the present paper and standard sulfonamide CA inhibitors: RR′N-SO_2NH_2 (**1**–**25**); NH_2SO_2-NH-RR′NH-SO_2NH_2 (**26**)

Inhibitor No.	R	R′	K_I (nM)	
			hCA I[a]	hCA II[a]
Acetazolamide	—	—	900	12
Methazolamide	—	—	780	14
Ethoxzolamide	—	—	25	8
Dichlorophenamide	—	—	1200	38
Dorzolamide	—	—	> 50,000	9
Brinzolamide	—	—	—	3
Sulfamide ($H_2NSO_2NH_2$)	—	—	35,000	82,000
1	*n*-Bu	H	173	148
2	Cyclohexyl	H	164	450
3	2-Adamantyl	H	960	890
4	$PhCH_2$	H	133	123
5	*i*-Bu	*i*-Bu	> 100,000	> 100,000
6	*i*-Pr	*i*-Pr	> 100,000	> 100,000
7	Cyclohexyl	Cyclohexyl	> 100,000	> 100,000
8	$PhCH_2$	$PhCH_2$	> 100,000	647
9	$-(CH_2)_5$	—	155	148
10	$-(CH_2)_6$	—	163	131
11	Ph	H	13	12
12	4-Me-C_6H_4	H	15	13
13	4-CF_3-C_6H_4	H	8	7
14	4-Cl-C_6H_4	H	19	15
15	4-Br-C_6H_4	H	23	21
16	4-I-C_6H_4	H	18	17
17	4-MeO-C_6H_4	H	14	11
18	4-HO-C_6H_4	H	16	12
19	4-O_2N-C_6H_4	H	18	13
20	4-EtO_2C-C_6H_4	H	26	19
21	4-NC-C_6H_4	H	20	16
22	4-Me_2N-C_6H_4	H	17	21
23	C_6F_5	H	34	32
24	3-Benzoyl-C_6H_4	H	62	49
25	2-Naphthyl	H	39	36
26	$(CH_2)_2$–SS–$(CH_2)_2$		149	27

[a]Human (cloned) isozymes, by the esterase method.[18]

phenamide and so on (Table 1). Isozyme hCA II was more prone to inhibition by these sulfamides as compared to isozyme hCA I, but the differences between them are rather small, especially if compared to the heterocyclic/aromatic sulfonamides used clinically.[19] It should also be mentioned that the lead molecule itself had a higher affinity for isozyme I as compared to isozyme II, behavior which unfortunately has not been maintained in the derivatized sulfamides investigated here. In fact, very few compounds with selectivity for CA I over CA II have been reported up to now, and sulfamide was one of them.

Conclusions

We report here a novel class of effective CAIs, starting from a very weak CAI, sulfamide, whose X-ray crystal structure in the adduct with hCA II has recently been reported. *N,N*-disubstituted sulfamides were too bulky, and ineffective as CAIs, whereas mono-substituted derivatives (incorporating aliphatic, cyclic and aromatic moieties) as well as a bis-sulfamide, behaved as micro-nanomolar inhibitors of two isozymes, hCA I and hCA II, responsible for critical physiological processes in higher vertebrates. Aryl-sulfamides were more effective than aliphatic derivatives. In the first group of such compounds, low nanomolar inhibitors have been obtained, which generally incorporated 4-substituted phenyl moieties in their molecule. This is the first example of CAIs in which low nanomolar inhibitors were generated starting from a very ineffective lead molecule.

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17. An example of synthesis is illustrated below: compound **13**: to a cold solution of 4-aminobenzotrifluoride (1 equiv) and triethylamine (1.5 equiv) in methylene chloride was added a solution of *N*-(*tert*butoxycarbonyl)sulfamoyl chloride in methylene chloride (prepared ab initio by adding chlorosulfonylisocyanate (1 equiv) on *tert*-butanol (1 equiv) at 0 °C). The mixture was stirred 1 h at room temperature and then concentrated in vacuo. The residue was treated with a mixture of ether- pentane and filtered. The precipitate was reacted with a solution of 20% TFA in methylene chloride until complete disapearance of starting material on TLC. Then the reaction was concentrated, and the expected compound was precipitated in a mixture ether-pentane and filtered. Yield: 90%. Colorless crystals, mp: 144–146 °C; ^{1}H NMR (200 MHz, DMSO-d_6): 10 (1H, s, NH), 7.6 (2H, d, $J = 8.5$ Hz, Ar–H), 7.35 (2H, d, $J = 8.5$ Hz, Ar–H), 7.3 (2H, s, NH_2); MS ESI +30 eV: 263 $[M + Na]^+$.
18. A stopped flow variant of the Poker and Stone spectrophotometric method (Pocker, Y.; Stone, J. T. *Biochemistry* **1967**, *6*, 668) has been employed, using an SX.18MV-R Applied Photophysics stopped flow instrument.
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