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RESEARCH ARTICLE

Secondary/tertiary benzenesulfonamides with inhibitory action against the cytosolic human carbonic anhydrase isoforms I and II

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Abstract
Carbonic anhydrase inhibitors of primary sulfonamide type, RSO_2NH_2, have clinical applications as diuretics, antiglaucoma, antiepileptic, antiobesity and antitumor drugs. Here we investigated inhibition of two human cytosolic isozymes, hCA I and II, with a series of secondary/tertiary sulfonamides, incorporating tosyl moieties ($CH_3C_6H_4SO_2NR1R2$). Most compounds inhibited both isoforms in low micromolar range, with inhibition constants between 0.181–6.01 μM against hCA I, and 0.209–0.779 μM against hCA II, respectively. These findings point out that substituted benzenesulfonamides may be used as leads for generating interesting CAIs probably possessing a distinct mechanism of action compared to primary sulfonamides. Indeed, classical RSO_2NH_2 inhibitors bind in deprotonated form to the Zn(II) ion from the CA active site and participate in many other favorable interactions with amino acid residues lining the cavity. The secondary/tertiary sulfonamides cannot bind to the zinc due to steric hindrance and probably are accommodated at the entrance of the active site, in coumarin binding-site.

Keywords: Carbonic anhydrase, benzenesulfonamide, tosyl, inhibitor

Introduction

Carbonic anhydrase (CA, EC 4.2.1.1) inhibitors (CAIs) of the primary sulfonamide type, such as acetazolamide, ethoxzolamide or dichlorphenamide, are clinically used for decades, for various classes of diuretics and systemically acting antiglaucoma agents[1–5]. The CAs are involved in important physiological and pathological functions, such as pH and CO_2 homeostasis, respiration and transport of CO_2/HCO_3^- between metabolizing tissues and the lungs, ion secretion in different tissues/organs and biosynthetic reactions (such as gluconeogenesis, lipogenesis and ureagenesis[1–8]).

Among the sixteen isoenzymes described up to now in mammals, CA I and CA II are present at high concentrations in the cytosol of erythrocytes and the gastrointestinal tract, and CA II has the highest turnover rate of all isoforms[9–14].

Carbonic anhydrase inhibitors (CAIs) or activators have several medical applications, such as in the treatment of glaucoma, as diuretics, in the management of several neurological disorders, including epilepsy, possibly in the treatment of Alzheimer's disease, whereas several agents are in clinical evaluations as antiobesity or antitumor drugs/diagnostic tools[1–7].

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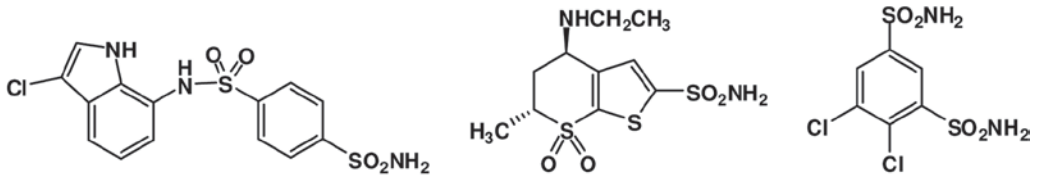

Figure 1. Some clinically used sulfonamides (dorzolamide and dichlorophenamide) or agents in clinical development (indisulam).

A class of derivatives which showed very promising applications among the various CAIs reported by Supuran's group in the last years, were the thioureas obtained from isothiocyanato sulfonamides (such as e.g. 4-isothiocyanatobenzenesulfonamide) and amines, hydrazines or amino acids[15–17]. Such compounds generally showed potent inhibitory activity against the cytosolic isozyme hCA II as well as the transmembrane, tumor-associated isozyme hCA IX, being thus interesting candidates for developing anti-glaucoma/antitumor therapies based on them[15–18]. Many sulfonamide derivatives have been widely used as pro-drugs or drugs, as shown in Figure 1 for some clinically used/investigational primary sulfonamide CAIs. For instance, sulfadiazine is used as an antibiotic, sulfapyridine is mainly used for treatment of bacterial infections, acetazolamide is mainly used as an anti-glaucoma agent[1–5]. However, all potent sulfonamide CAIs investigated till recently were of the primary type, i.e. RSO_2NH_2[1].

Our groups recently investigated the interaction of several CA isozymes with alternative chemotypes, such as salicylic acid derivatives, antioxidant phenolic compounds, organic nitrates, etc[9–13,18]. We would like to extend these earlier investigations to some secondary/tertiary sulfonamide derivatives, as this class of compounds was under investigated till recently[1,3].

In this work we evaluated CA inhibition with tosyl pyrroles and other aromatic benzenesulfonamide derivatives which incorporate tosyl moieties. A series of 18 such compounds was evaluated for their ability to inhibit the dominant cytosolic human (h) isoforms, hCA I and II.

Materials and methods

Chemistry

Compounds **1–19** investigated here were reported earlier[3,12].

Enzymology: CO2 hydration assay method

An applied photophysics stopped flow instrument has been used for assaying the CA-catalyzed CO_2 hydration activity[19]. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity, in triplicate measurements. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were done thereafter with distilled-deionized water. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex[5,19,20]. The curve-fitting algorithm allowed us to obtain the IC_{50} values, working at the lowest concentration of substrate of 1.7 mM, from which K_I values were calculated by using the Cheng–Prusoff equation[21,22].

Results and discussion

Diverse chemotypes of the primary sulfonamide one may show interest for the inhibition of the CAs, a superfamily of widely spread enzymes in organisms all over the tree of life[1]. In a previous work[3] we have investigated some non-primary sulfonamides, such as derivatives **1–11**, for their inhibition against some CA isoforms, by an esterase method with *para*-nitrophenyl acetate (NPA) as susbtrate. As NPA is not the physiologic CA substrate, and many isoforms show weak esterase activity, we re-evaluate here these compounds by monitoring their inhibition against the dominant isoforms hCA I and II, with a stopped-flow CO_2 hydrase method[19].

Recently, our groups also investigated the interactions of some methoxy-benzenesulfonamide derivatives and some of their substituted derivatives with different isozymes, such as hCA I, II and IX[22], evidencing several low micromolar/submicromolar inhibitors as well as the possibility to design isozyme selective CAIs. Indeed, the inhibition profile of various isozymes with this class of agents is very variable, with inhibition constants ranging from the millimolar to the submicromolar range for some methoxy-benzenesulfonamide or hydroxamate derivatives[22]. All these data provide information on the fact that many non-primary sulfonamides may show significant CA inhibitory activity, and that the mechanism of action of such derivatives is different from that of the RSO_2NH_2derivatives[3,22–29]. It appeared thus of interest to extend the previous studies[3], including in this research some benzenesulfonamides with clinical applications, such as sulfapyridine **18,** sulfadiazine **19**, as well as some

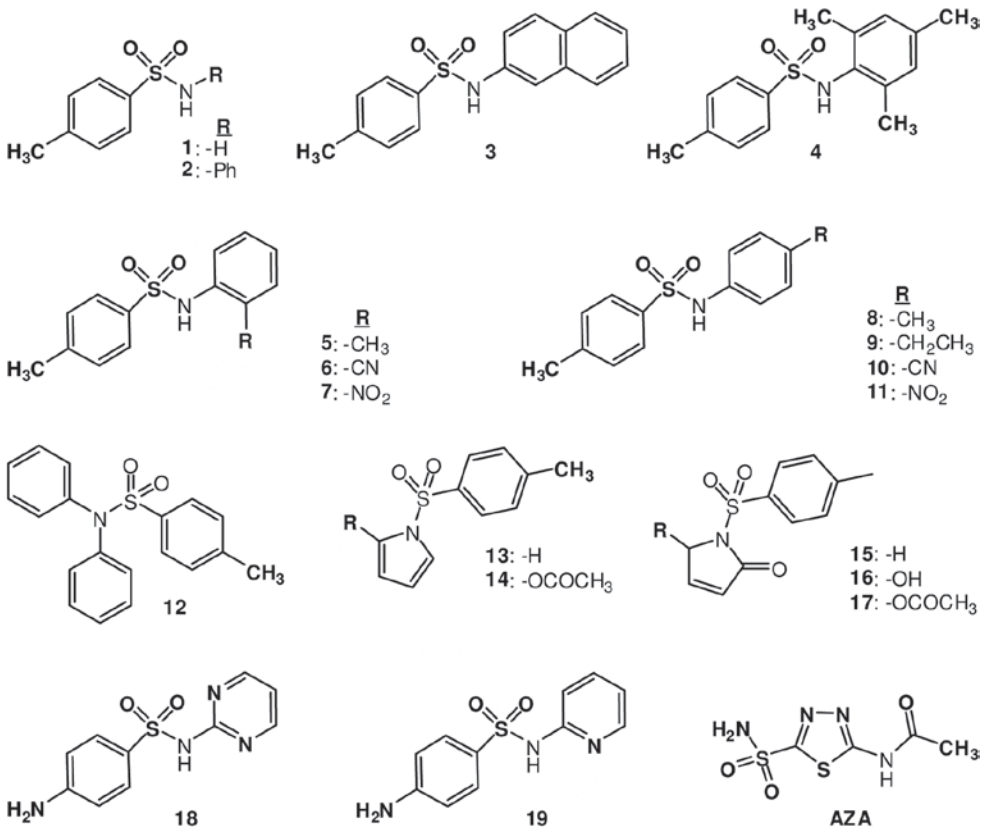

Figure 2. Structures of tested compounds and the standard drug acetazolamide (AZA).

of their substituted derivatives incorporating amino, cyano, methyl and nitro moieties as substituents at one of the aromatic ring in various different positions (Figure 2).

Tosyl pyrrole and benzenesulfonamides were synthesized by tosylation of pyrrole, aniline, *o*-methylaniline, *p*-methylaniline, trimethylaniline, diphenylamine, β-naphtylamine, *p*-ethylaniline, *o*-cyano-aniline, *p*-cyano-aniline, *o*-nitroaniline, *p*-nitroaniline[20]. Inhibitory effects of compounds **1–19** and standard inhibitors on the enzyme activity were tested under *in vitro* conditions; K_I values were calculated by using the Cheng–Prusoff equation[21] and are given in Table 1.

Data of Table 1 show the following regarding inhibition of hCA I and II with compounds **1–19**:

1. Against the slow cytosolic isozyme hCA I, compounds **2–12** and **14–17** behave as efficient inhibitors, with K_I values in the range of 0.181–0.869 μM similar to the clinically used sulfonamide AZA (K_I: 0.250 μM). Isoform hCA I was weakly inhibited by compound **13** which has a very different K_I value (K_I: 6.01 μM) from the structurally related derivative **14** (K_I: 0.339 μM). Dorzolamide and dichlorophenamide were ineffective inhibitors for hCA I isoenzyme in this assay. The most powerful agent was compound **3**, with K_I values in the range of 0.181 μM. Derivatives **1**, **18** and **19** were ineffective inhibitors for the isoenzyme, with inhibition constants in the range of 22.12–28.38 μM, similar to dorzolamide, a clinically used antiglaucoma agent[1].

Table 1. hCA I and CA II inhibition data of the tested and reference compounds.

	K_I (μM)	
Compound	hCA I	hCA II
1	22.120[a]	0.715[a]
2	0.419	0.395
3	0.181	0.485
4	0.612	0.290
5	0.386	0.615
6	0.674	0.602
7	0.869	0.381
8	0.501	0.354
9	0.753	0.533
10	0.519	0.734
11	0.482	0.699
12	0.377	0.459
13	6.010	0.779
14	0.339	0.395
15	0.406	0.534
16	0.584	0.639
17	0.316	0.392
18	26.19[a]	0.341[a]
19	28.38[a]	0.276[a]
AAZ	0.250	0.012
Indisulam	0.31[b]	0.015[b]
Dorzolamide	50[b]	0.009[b]
Dichlorophenamide	1.20[b]	0.038[b]

*Mean from at least three determinations. Errors in the range of 1–2 % of the reported value (data not shown), [a]From ref. 3, [b]From ref. 1.

2. Against the rapid cytosolic isozyme hCA II compounds 1–19 investigated here showed a moderate-weak inhibition, with K_I values in the range of 0.276–0.779 μM (Table 1). Compound **13** was the weakest hCA II inhibitor, with a K_I value of 0.779 μM, although it is not the bulkiest one among the investigated secondary/tertiary sulfonamides in the investigated series. It should be noted that the three clinically used/investigational compounds (AZA, indisulam, dorzolamide, dichlorophenamide, Figure 1) showed much more potent hCA II inhibitory activity compared to the secondary/tertiary sulfonamides investigated here, with K_I-s in the range of 0.009–0.038 μM (Table 1). These findings clearly illustrate that a small variation in the structure of a sulfonamide CAI may have drastic consequences for the enzyme inhibitory activity and selectivity profile against various isozymes of such derivatives. Thus, all the secondary/tertiary sulfonamide derivatives **1–19** investigated here showed moderate hCA II inhibitory activity (Table 1).

Conclusions

We show here that compounds belonging to the secondary/tertiary sulfonamide class, incorporatring tosyl moeiteis, are inhibitors of hCA I and II in the submicromolar – micromolar range. These findings point out that substituted benzenesulfonamides may be used as leads for generating interesting CAIs probably possessing a distinct mechanism of action compared to the primary sulfonamides. Indeed, the classical RSO_2NH_2 inhibitors bind in deprotonated for to the Zn(II) ion from the CA active site and participate in many other favorable interactions with amino acid residues lining the cavity. The secondary/tertiary sulfonamides cannot bind to the zinc due to steric hindrance and probably are accommodated at the entrance of the active site, in the coumarin binding-site[29–31]. Indeed, coumarins and lactones/thiolactones were recently shown to be mechanism-based inhibitors, which bind in hydrolyzed state at the entrance of the active site cavity, without interference with the catalytic metal ion[29–32]. As the entrance of the active site cavity is the most variable region of the various CA isoforms with therapeutic applications[1], this may lead to CAIs with a favourable inhibition profile and enhanced selectivity for the target over the offtarget isoforms.

Declaration of interest

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