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Carbonic anhydrase inhibitory activity of sulfonamides and carboxylic acids incorporating cyclic imide scaffolds

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ABSTRACT

A series of sulfonamides incorporating cyclic imide moieties were investigated as inhibitors of several human α-carbonic anhydrase (hCA, EC 4.2.1.1) isoforms. Several carboxylic acids possessing the same scaffolds as the sulfonamides were also included in the study, since the sulfonamidate and the carboxylate are among the frequently used zinc-binding groups (ZBGs) for obtaining zinc enzymes inhibitors. The cytosolic isoform hCA I was moderately inhibited by most of the 30 investigated derivatives; many low nanomolar hCA II inhibitors were detected, whereas some of these compounds were low nanomolar/subnanomolar inhibitors of the transmembrane, tumor-associated isoforms hCA IX and XII. In this series of compounds the SO_2NH^- and the COO^- ZBGs showed similar efficacy for obtaining potent inhibitors, although some carboxylates had isoform-selective inhibition profiles for the transmembrane CAs.

The primary sulfonamides constitute the most investigated class of inhibitors of the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1),[1–4] with several such derivatives in clinical use for decades. This class of pharmacological agents led to drugs with a variety of uses, among which diuretics,[5] antiglaucoma drugs,[3b,6] antiepileptics,[7] antiobesity[8] and antitumor agents/diagnostic tools for hypoxic tumors,[2,3a,9] among others. This is mainly due to the fact that in vertebrates, humans included, a large number of different isoforms are present, which are involved in a variety of different functions, mainly related to the acid-base equilibrium and chemosensing (as CO_2, bicarbonate and protons are the substrates/reaction products of the physiologic reaction catalyzed by these enzymes) but also in some biosynthetic pathways.[1–3] In humans, 15 α-CA isoforms were described, hCA I–hCA VA, hCA VB, ChA VI–hCA XIV, with 12 of them being catalytically active and three (hCA VIII, X and XI) devoid of activity but still playing significant functions in tumorigenesis and other physiologic or pathologic processes.[10] In other organisms all over the phylogenetic tree, five other genetic families encoding CAs (apart the α-class) were reported, the β-, γ-, δ-, ζ- and η-CAs, making these enzymes among the ubiquitous biocatalysts in nature.[11–14]

Although there are many other classes of CA inhibitors (CAIs) apart the sulfonamides and their isosters, such as the dithiocarbamates and xanthates, the coumarins and their derivatives, the polyamines, etc.,[1–4] sulfonamides still attract much interest due to their pharmacological properties, facility of preparation, stability and ease of administration.[15] In the case of the CAIs incorporating this functionality, for a long period only nonselective inhibitors were described.[15] However by using structure-based drug design strategies, mainly guided by the X-ray crystal structural data of many adducts of sulfonamides with various CA isoforms, in the last years interesting advances have been obtained, with many classes of isoforms-selective sulfonamide CAIs described.[16,17] Compounds such as **A**, incorporating phthalimide moieties,[17a] or the corresponding derivatives with tetrachloro- or tetrabromo-phthalimide tails **B** and **C**,[17b,c] were shown to possess potent inhibitory action against CA isoforms of relevant physyiologic functions (such as CA II, IV, VII, IX and XII). The similar *ortho*-benzenedisulfonimide derivatives **D**, designed by considering the phthalimides **A** as lead compounds,[17a] were also shown recently to possess interesting inhibitory profiles against the tumor-associated isoforms CA IX and XII. All these derivatives were much more isoform-selective compared to classical, first generation CAIs such as acetazolamide **AAZ**.

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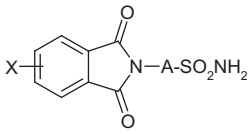
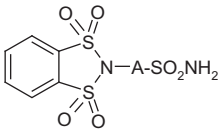
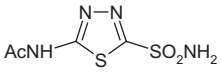


A: X = H, A= aryl, hetaryl
B: X = Cl_4, A= aryl, hetaryl
C: X = Br_4, A= aryl, hetaryl



D: A= aryl, hetaryl



AAZ

Considering these interesting inhibition profiles of compounds incorporating cyclic imide or cyclic disulfonimide moieties, here we investigate sulfonamides and carboxylates possessing the same substitution pattern, but a rather variable cyclic imide scaffold (Fig. 1). Indeed, the compounds shown in Figure 1 were reported earlier[18] and investigated as cyclooxygenase-2 (COX-2) inhibitors by one of our groups. They were obtained by the classical condensation of sulfanilamide/homosulfanilamide/4-aminobenzoic acid with cyclic anhydrides in refluxing acetic acid.[18]

Here we report the inhibitory properties of these 30 cyclic imides[19] possessing sulfamoyl or carboxyl ZBGs against four physiologically relevant CA isoforms, the cytosolic hCA I and II, as well as the transmembrane, tumor-associated hCA IX and XII. One should mention that although the COO^- moiety is a good ZBGs for the design of many zinc enzyme inhibitors, the carboxylates were less investigated as CAIs, except for some recent studies from one of our groups.[11b,20,21]

The rationale for testing compounds **1a**–**10c** (Fig. 1) as CAIs was the following: (i) we wanted to compare the efficacy of the carboxylate versus the sulfonamidate moiety as ZBGs for their inhibitory power against various isoforms, such as hCA I, II, IX and XII, in compounds incorporating the same scaffold (i.e., succinimide-substituted benzenesulfonamide and benzoic acids; phthalimide-substituted benzenesulfonamide and benzoic acids, etc.); (ii) we were interested to delineate the structure–activity relationship (SAR) for the inhibition of these isoforms with compounds incorporating cyclic imides with diversely substituted scaffolds. Thus, in addition to the monocyclic succinimides **1a**–**1c**, derivatives of tetrahydrophthalimide (**2b**–**2c**), phthalimide (**3a**–**3c**) as well as phthalimides substituted with various moieties at the benzene nucleus (such as methyl-, *tert*-butyl-, dichloro-, tetrachloro- and nitro-groups) of types **4a**–**8c** were also included in the study. Furthermore, derivatives incorporating the heterocyclic pyrazine-2,3-dicarboximide (**9a**–**9c**) or the bulkier naphthalene-1,8-dicarboximide (**10a**–**10c**) moieties were also included in the study, in order to explore as much chemical space as possible. The following SAR was observed for the inhibition of the four CA isoforms mentioned above with derivatives **1a**–**10c** (Table 1):

(i) The slow cytosolic isoform hCA I (usually considered an off-target isoform when CAIs for antiglaucoma or anticancer activity are envisaged)[15] was well inhibited by many of the investigated compounds, such as **1b**, **2a**–**5b**, **6a**–**6c** and **7c**, which showed K_Is ranging between 21.2 and 35.7 nM, being thus much more inhibitory than the standard drug acetazolamide **AAZ** (K_I of 250 nM). It may be observed that both sulfonamides and carboxylates belong to this category of effective hCA I inhibitors, with some cases in which the nature of the ZBG seems to have been of little importance for the biological activity. For example the sulfonamide/carboxylate pairs incorporating the same scaffolds (tetrahydrophthalimide, phthalimide and 5-methyl-phthalimide) **2b/2c**, **3b/3c** and **4b/4c**, show very similar inhibition constants against this isoform, suggesting a quite similar binding mode to the enzyme. However other such pairs differ considerably in their activity. For example the succinimide derivatives (**1b** and **1c**), the tetrachlorophthalimides (**7b** and **7c**) or the heterocyclic imides **9b** and **9c**, show very different hCA I inhibition profiles: for the first and last pairs, the carboxylic acids are weaker inhibitors compared to the corresponding sulfonamide, whereas for the second one, the carboxylic acid **7c** is 11.7-times a better inhibitor compared to the corresponding sulfonamide **7b**. For the two different sulfonamides, generally the sulfanilamide derivatives were slightly more inhibitory compared to the corresponding homosulfanilamides, but the differences of activity were rather small (Table 1). Ten of the investigated compounds (**1a**, **1c**, **5c**, **7a**, **7b**, **8a**, **8b**, **9a**, **9b**, and **10a**) showed weaker hCA I inhibitory activity (K_Is in the range of 126–489 nM) whereas compounds **8c**, **9c**, **10b** and **10c** were very weak inhibitors, with no significant inhibition observed up to concentrations of 1 μM (Table 1). Except **10b** they are carboxylates and incorporate 5-nitrophthalimide, pyrazine-2,3-dicarboximide or naphthalene-1,10-dicarboximide moieties.

(ii) The physiologically dominant hCA II (drug target for antiglaucoma agents) was efficiently inhibited by many of the sulfonamides investigated here (but not by the carboxylates, which were either not inhibitory at all up to 1 μM—for example, **1c**, **4c**, **5c**–**9c**—or were very inefficient inhibitors—for example, **2c** and **3c** had K_Is in the range of 166–190 nM). Thus, compounds **1b**, **2a**, **2b**, **3a**, **3b**, **4b**, **5a**, **5b**, **6a**, **6b**, **7b**, **8b**, **9b** and **10a** had K_Is lower or in the same range as the clinically used drug **AAZ**, more precisely of 2.2–11.3 nM (Table 1). It may be noted that diverse cyclic imide scaffolds lead to highly efficient hCA II inhibitors (succinimides, tetrahydrophthalimide, phthalimide and substituted phthalimides with Me, tert-Bu, dichloro- , tetrachloro and nitro groups on the benzene ring, but also some of the imides derived from pyrazine–2,3-dicarboxylic acid or naphthalene-1,8-dicarboxylic acid). Generally the sulfanilamide derivatives were better hCA II inhibitors than the homosulfanilamides. The scaffold leading to the best inhibition was just the unsubstituted phthalimide (compounds **3a** and **3b**) which in fact have been reported earlier[17a] (and tested as CAIs by using the esterase activity of the enzyme; the conclusions of the earlier work[17a] are thus confirmed here). The introduction of diverse substituents on the benzene ring of the substituted phthalimides thus leads to a decrease of the inhibitory power. Several of the sulfonamides investigated here (**1a**, **2c**, **3c**, **4a**, **7a**, **8a**, **9a and 10b**) were medium potency hCA II inhibitors, with K_Is in the range of 26.2–238 nM.

(iii) The hCA IX inhibition profile with the compounds investigated here was quite interesting. For reasons difficult to explain, two sulfonamides (**8a** and **8b**) showed very weak, micromolar inhibitory activity against this transmembrane isoform. They incorporate the 5-nitrophthalimide moiety in their molecules. It should be noted that the remaining sulfonamides and many of the investigated carboxylic acids had excellent hCA IX inhibitory activity, with K_Is in the low nanomolar range, of 2.4–29.3 nM (being thus much more effective or equipotent to **AAZ**, a drug shown to possess also antitumor effects in hypoxic tumors).[9] The only carboxylates showing weaker inhibitory activity (K_Is in the range of 350–506 nM) were **1c**, **4c** and **5c**, which incorporate maleimide, 5-methyl-phthalimide and 5-tert-butyl- phthalimide moieties in their molecules. Interestingly, for the other scaffolds investigated here, the sulfonamides and carboxylates showed very