Bioorganic & Medicinal Chemistry Letters 18 (2008) 3938–3941

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Carbonic anhydrase inhibitors: Thioxolone versus sulfonamides for obtaining isozyme-selective inhibitors?

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ARTICLE INFO

Article history:
Received 3 May 2008
Revised 6 June 2008
Accepted 6 June 2008
Available online 12 June 2008

Keywords:
Carbonic anhydrase
Isozymes I–XV
Sulfonamide
Thioxolone
Isozyme-selective inhibitor
Acetazolamide

ABSTRACT

Inhibition of 13 mammalian isoforms of the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1), CA I–XV, with thioxolone (6-hydroxy-1,3-benzoxathiol-2-one) and two sulfonamides was investigated. Thioxolone was inefficient for generating isozyme-selective inhibitors, since except for CA I which is inhibited in the nanomolar range (K_I of 91 nM), the remaining 12 isoforms were inhibited with a very flat profile (K_Is in the range of only 4.93–9.04 μM). In contrast to thioxolone, 3,5-dichloro-4-hydroxybenzenesulfonamide as well as the clinically used heterocyclic sulfonamide acetazolamide showed K_Is in the range of 58 nM–78.6 μM and 2.5 nM–200 μM, respectively, against the 13 investigated mammalian CAs. The sulfonamide zinc-binding group is thus superior to the thiol one for generating CA inhibitors with a varied and sometimes isozyme-selective inhibition profile against the mammalian enzymes.

Inhibitors or activators of the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1) have several medical applications, such as among others 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.[1,2] This variety of medical applications is undoubtedly due to the large number of isoforms presently known in mammals (16 isozymes in non-primates and 15 in primates, all belonging to the α-CA gene family, have been described to date)[1,2] and to their specific physiologic functions.[1–4] The reverse of the medal is constituted by the fact that it is relatively difficult to design agents (inhibitors or activators) with specificity or selectivity for any of these isoforms,[1–3] and as a consequence, many pharmacological agents belonging to the class of the CA inhibitors (CAIs) or CA activators (CAAs) act as promiscuous inhibitors/activators of most isozymes with physiological/pathological relevance, and as drugs they show undesired side effects. The search of isozyme-selective or -specific inhibitors and activators is thus a goal pursued by many groups.[1,2,6–10]

The major classes of CAIs are constituted by the unsubstituted sulfonamides and their bioisosteres (i.e., the sulfamates, sulfamides, and related compounds)[1–5,9–12] and by the metal-complexing anions,[1,2] which bind to the Zn(II) ion of these enzymes either by substituting the non-protein zinc ligand to generate a tetrahedral adduct or by addition to the metal coordination sphere, generating trigonal-bipyramidal species, and are non-competitive inhibitors with CO_2 as substrate (Fig. 1A and B).[1–5] This is in net contrast to the binding mode of phenols, a third class of CAIs,[8] which does not substitute the non-protein zinc ligand, but interact with it by means of the two hydrogen bonds as shown in Figure 1C.[8a] Such a particular binding mode offers the possibility to design CAIs possessing a different inhibition mechanism as compared to the classical sulfonamide or sulfamate inhibitors,[2–4] as we recently showed by investigating a series of phenols as inhibitors of the 12 catalytically active CA isoforms.[8b] On the same research line, Vu et al.[7] showed that a simple coumarine derivative is an effective bovine CA II inhibitor, but its binding mode has not been detailed up to now, whereas Tripp's group, in an elegant study,[6] showed thioxolone (6-hydroxy-1,3-benzoxathiol-2-one) **1** to be a CAI with characteristics of a prodrug, since the compound is rapidly hydrolyzed by human (h) hCA II to the corresponding 4-mercapto-benzene-1,3-diol **2**, which acts as a medium potency hCA II inhibitor (an IC_{50} of 1.77 μM has been reported for thioxolone by an esterase assay method with 4-nitrophenyl acetate as substrate,[6a] and a K_I of 314 μM was then reported by the same group by an ^{18}O-exchange activity measurement assay).[6b] The binding mode of the actual CAI, that is, thiophenol **2**, was then determined by means of X-ray crystallography,[6b] being shown that the SH moiety of **2** acts as zinc-binding group, being coordinated to the Zn(II) ion within the hCA II active site, and also participating to a hydrogen bond with the OH moiety of Thr199, an amino acid residue involved in the binding of most CAIs to the enzyme (Fig. 1D).[1–6]

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0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.06.024

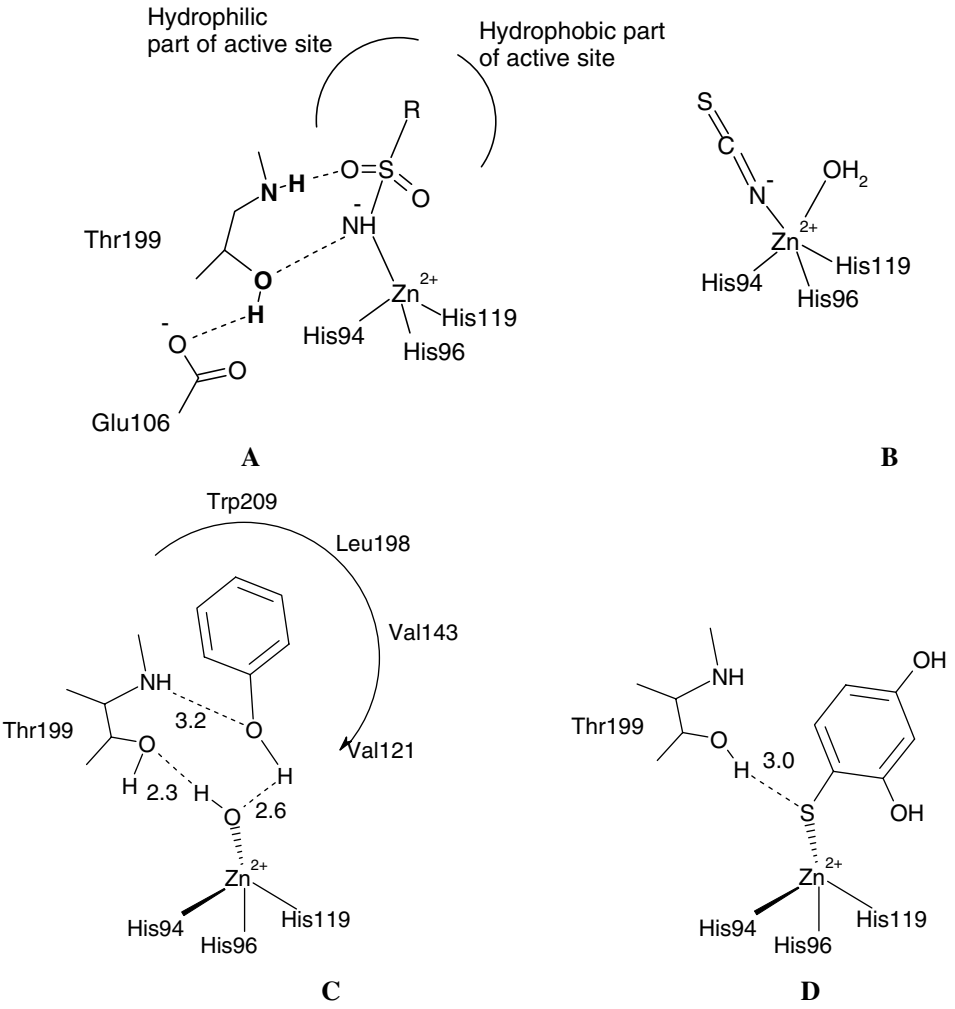
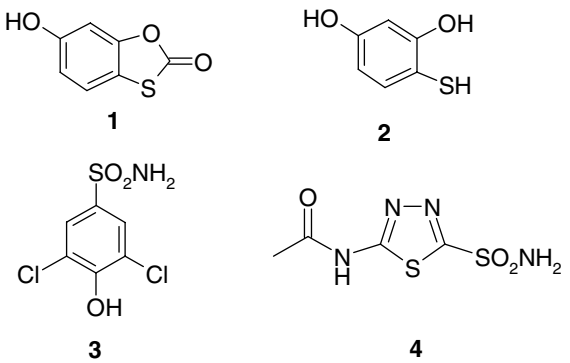


Figure 1. Schematic representation for the interactions of four classes of CAIs with the enzyme active site: (A) sulfonamides (and their isosteres, sulfamate, and sulfamide) inhibitors, (B) inorganic anion inhibitors (thiocyanate as an example), (C) phenol(s), (D) thiophenols (4-mercapto-benzene-1,3-diol). The interactions in which phenol and 4-mercapto-benzene-1,3-diol participate when bound to the hCA II active site are shown in detail (figures represent distances in Å; hydrogen bonds are represented as dashed lines).



Heterocyclic[13] and aliphatic[14] thiols have in fact been evaluated as CAIs by our group earlier, being shown that many such derivatives are potent inhibitors of several physiologically relevant isoforms, such as CA I, II, IV, and IX. However, no X-ray crystallographic characterization of such adducts has been performed yet, the aromatic thiophenol **2** being the first thiol compound for which the X-ray crystal structure in complex with hCA II has been reported by Tripps group.[6b] In the same interesting work,[6b] in which only hCA II inhibition with compound **1** has been investigated, it has been stated that the thioxolones as a class of prodrug CAIs may show advantage over the sulfonamides in obtaining isozyme-selective inhibitors. Here, we test this hypothesis of Barrese et al.,[6b] and investigate the inhibition of the 13 catalytically active mammalian isozymes (CA I–XV) with thioxolone **1** as compared to a simple, structurally similar to **1** sulfonamide, that is, compound **3**, as well as the classical, clinically used sulfonamide inhibitor acetazolamide **4**.[15,16] Sulfonamide **3** has been chosen as a comparison inhibitor to **1**, as it contains the benzenesulfonamide scaffold substituted with three groups, one of which is an OH moiety in *para* to the zinc-binding group (ZBG), also present in the hydrolysis product of compounds **1** and **2**. Thus, the common features of **2** and **3** is that they both possess a ZBG (of thiol and sulfonamide type, respectively) and a *para*-OH phenol group with respect to the ZBG. The difference consists in the substitution pattern of the remaining moieties, as **2** contains an additional OH moiety in *ortho* to the ZBG, whereas **3** has two chlorine atoms in *meta* to the ZBG. However, the two scaffolds are simple and similar enough to make a comparison between their inhibitory activity relevant enough to resolve the issue whether thioxolones may offer advantages over sulfonamides for the design of isozyme-selective CAIs. Acetazolamide **4** on the other hand has been included in the study as it is the best characterized, reference CAI.[1,2]

Table 1
Inhibition of mammalian isozymes CA I–XV (h, human; m, murine isoform) with thioxolone **1**, and sulfonamides **3** (aromatic) and **4** (heterocyclic), by a stopped-flow, CO_2 hydration assay method[16]

Isozyme[a]	K_I[c] (μM)		
	1	**3**	**4**
hCA I	0.091 ± 0.002	7.50 ± 0.34	0.250 ± 0.013
hCA II	6.82 ± 0.31[d]	0.058 ± 0.003	0.012 ± 0.0006
hCA III	6.56 ± 0.26	78.6 ± 3.4	200 ± 11
hCA IV	8.41 ± 0.40	0.072 ± 0.002	0.074 ± 0.003
hCA VA	7.06 ± 0.35	0.62 ± 0.03	0.063 ± 0.002
hCA VB	9.04 ± 0.21	0.61 ± 0.01	0.054 ± 0.003
hCA VI	4.93 ± 0.23	6.17 ± 0.27	0.011 ± 0.0005
hCA VII	5.73 ± 0.18	0.072 ± 0.004	0.0025 ± 0.0001
hCA IX[b]	6.67 ± 0.30	0.081 ± 0.003	0.025 ± 0.001
hCA XII[b]	7.79 ± 0.39	0.076 ± 0.004	0.0057 ± 0.0002
mCA XIII	8.23 ± 0.25	6.53 ± 0.25	0.017 ± 0.0006
hCA XIV	8.59 ± 0.44	8.12 ± 0.43	0.041 ± 0.002
mCA XV	7.87 ± 0.29	0.78 ± 0.03	0.072 ± 0.004

[a] h, human; m, murine isozyme.
[b] Catalytic domain.
[c] Means ± standard error from three different assays.
[d] In Ref. 6a an IC_{50} of 1.77 μM is reported by an esterase assay method with 4-nitrophenyl acetate as substrate, and in Ref. 6b; a K_I of 314 μM is reported by the same group by an ^{18}O-exchange activity measurement assay.

Data of Table 1 allow the following insights regarding inhibition of the 13 catalytically active mammalian isozymes CA I–XV with thioxolone **1** and sulfonamides **3** and **4**: (i) thioxolone showed a behavior of efficient hCA I inhibitor, with a K_I of 91 nM, being a much weaker inhibitor of isozymes II–XV, for which it showed K_Is in the range of 4.93–9.04 μM. The most striking feature of these data is the very flat inhibition profile of all isoforms except hCA I, with a variation of around only 4 μM units among very different isoforms such as the cytosolic ones CA II, III, VII, and XIII, the mitochondrial ones CA VA and VB, the membrane-anchored ones CA IV and XV, the secreted one CA VI, as well as the transmembrane ones CA IX, XII, and XIV; (ii) the aromatic sulfonamide **3** behaved as a very inefficient CA III inhibitor (K_I of 78.6 μM), was a more efficient, low micromolar inhibitor for isoforms hCA I, hCA VI, mCA XIII, and hCA XIV (K_Is in the range of 6.17–8.12 μM), and showed submicromolar inhibition constants against hCA VA, hCA VB, and mCA XV (K_Is in the range of 0.61–0.78 μM). On the other hand, **3** was a nanomolar inhibitor of isozymes hCA II, hCA IV, hCA VII, hCA IX, and hCA XII, with inhibition constants in the range of 58–81 nM (Table 1). It is already obvious that the inhibition profile of the 13 CA isozymes with sulfonamide **3** is much more varied as compared to that of thioxolone **1**, since the variation of K_Is among the various isozymes is in the range of 58 nM (for the best inhibited one, CA II) to 78.6 μM, for the least inhibited isoform, that is, CA III; (iii) a rather similar situation as the one described for compound **3**, is observed comparing the inhibition data or the 13 CA isozymes with the clinically used sulfonamide acetazolamide **4** (only that the heterocyclic sulfonamide **4** is generally a much more potent inhibitor as compared to the aromatic one **3** against most CA isoforms, a feature already observed earlier when comparing heterocyclic over aromatic sulfonamide CAIs).[1–3] Thus, acetazolamide is a weak hCA III inhibitor (K_I of 200 μM) and a submicromolar hCA I inhibitor (K_I of 0.250 μM), being a low nanomolar inhibitor for all other isoforms, with K_Is in the range of 2.5–74 nM (Table 1). It is obvious from these data, as already mentioned above, that acetazolamide is a promiscuous CAI, which leads to many undesired side effects of this drug when used for the treatment of glaucoma[22] or gastrointestinal ulcers.[23] However, it is also clear that sulfonamides have no real competitors in terms of potency and variation of inhibitory properties among the various isozymes, as compared to other classes of CAIs, such as the phenols[8] or the thiophenols (including the prodrugs of thioxolone type).[6] It should also be mentioned that there are examples of sulfonamides[7,24] and sulfamides[25] showing a high degree of selectivity for inhibiting some physiologically relevant isoforms, such as CA IX and XII among others.

In conclusion, we prove here that thioxolone as a prodrug is inefficient for generating isozyme-selective CAIs, since except for CA I which is inhibited in the nanomolar range (K_I of 91 nM), the remaining 12 catalytically active mammalian CA isoforms (CA II–XV) were inhibited with a very flat profile (K_Is in the range of only 4.93–9.04 μM). In contrast to thioxolone, an aromatic simple sulfonamide (3,5-dichloro-4-hydroxybenzenesulfonamide) as well as the clinically used heterocyclic sulfonamide acetazolamide showed K_Is in the range of 58 nM–78.6 μM, and 2.5 nM–200 μM, respectively, against the 13 investigated mammalian CA isoforms.

Acknowledgments

This research was financed in part by two grants of the 6th Framework Programme of the European Union (EUROXY and DeZnIT Projects). Thanks are addressed to Prof. S. Parkkila and Dr. M. Hilva (Tampere University, Finland) for the gift of mCA XV used in this work.

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