Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 2567–2573
Bioorganic & Medicinal Chemistry Letters

Carbonic anhydrase inhibitors. Interaction of indapamide and related diuretics with 12 mammalian isozymes and X-ray crystallographic studies for the indapamide–isozyme II adduct☆

Claudia Temperini, Alessandro Cecchi, Andrea Scozzafava and Claudiu T. Supuran*

Università degli Studi di Firenze, Laboratorio di Chimica Bioinorganica, Room 188, Via della Lastruccia 3, I-50019 Sesto Fiorentino (Firenze), Italy

Received 5 March 2008; revised 13 March 2008; accepted 16 March 2008
Available online 20 March 2008

Abstract—Diuretics such as hydrochlorothiazide, hydroflumethiazide, quinethazone, metolazone, chlorthalidone, indapamide, furosemide, and bumetanide containing primary sulfamoyl moieties were reevaluated as inhibitors of 12 human carbonic anhydrases (hCAs, EC 4.2.1.1). These drugs considerably inhibit (low nanomolar range) some CA isozymes involved in critical physiologic processes, among the 16 present in vertebrates, for example, metholazone against CA VII, XII, and XIII, chlorthalidone against CA VB, VII, IX, XII, and XIII, indapamide against CA VII, IX, XII, and XIII, furosemide against CA I, II, and XIV, and bumethanide against CA IX and XII. The X-ray crystal structure of the hCA II–indapamide adduct was also resolved at high resolution.

Carbonic anhydrase (CA, EC 4.2.1.1) inhibitors, such as acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide) **1**, and the structurally related sulfonamides methazolamide **2**, ethoxzolamide **3**, and dichlorophenamide **4**, inhibit non-selectively all the 16 different isoforms of this metalloenzyme characterized so far in vertebrates.[1,2] Acetazolamide played a major role in the development of renal physiology and pharmacology, as well as for the design of many of the presently widely used diuretic agents, such as among others the thiazide and high ceiling diuretics.[1–8] CAs catalyze a very simple physiological reaction, the interconversion between carbon dioxide and the bicarbonate ion, and are thus involved in crucial physiological processes connected with the respiration and transport of CO_2/bicarbonate between metabolizing tissues and 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/pathologic processes.[1–5,7,8] Many CAs isoforms have been shown to be present in various tissues of the kidney,[9,10] such as CA II, IV, VB, IX, XII, and XIV, where these play a crucial function in at least three renal physiological processes: (i) the acid–base balance homeostasis; (ii) the bicarbonate reabsorption processes, and (iii) the renal NH_4^+ output.[2,11,12] Inhibition of both cytosolic (CA II) and membrane-bound (CA IV and CA XIV) enzymes seems to be involved in the diuretic effects of these sulfonamides.[2,9–12]

Sulfonamides **1**–**4** are used for the treatment of edema due to the congestive heart failure, and for drug-induced edema, in addition to their applications as antiglaucoma agents.[1,2,11] The structurally related compound to acetazolamide, benzolamide **5**, has a renal effect on bicarbonate excretion around 10 times as potent as that of acetazolamide, but the compound remained as an orphan drug and has not been developed for clinical use. Using acetazolamide **1** as lead, a large number of other quite successful sulfonamide diuretics were developed in the 60s and 70s, such as benzothiadiazines **6** (hydrochlorothiazide **6a**, hydroflumethiazide **6b**, and the like), quinethazone **7**, metolazone **8**, chlorthalidone **9**, indapamide **10**, furosemide **11**, and bumetanide **12**.[1–3] Some of them are among the most widely clinically used diuretics,[6,11,13] and as these all possess primary SO_2NH_2 moieties, acting as excellent zinc-binding groups for the metal ion present within the CA active site,[1–3] it is to be

Keywords: Carbonic anhydrase; Isozyme; Sulfonamide; Diuretic; Benzothiadiazine; Furosemide; Indapamide; X-ray crystallography.

☆ The X-ray coordinates of the hCA II–indapamide **10** adduct are available in PDB with the ID 3BL1.

* Corresponding author. Tel.: +39 055 457 3005; fax: +39 055 4573385; e-mail: claudiu.supuran@unifi.it

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.03.051

expected that these should also have CA inhibitory properties. However, this issue has been investigated only in the 60s or 70s when these drugs were launched, and when only one CA isozyme (i.e., CA II) was presumed to exist and be responsible for all the physiologic effects of the sulfonamide drugs.[11] Here, we reinvestigate the interaction of some of these clinically used diuretics with all 12 catalytically active mammalian CA isoforms and also report the X-ray crystal structure of one of them (indapamide **10**) with CA II.

The data listed in Table 1 show that similar to the classical CAIs **1**–**5**, the clinically used sulfonamide diuretics **6**–**12** also act as inhibitors of all 12 investigated CA isozymes, with an inhibition profile different from that of inhibitors investigated earlier, such as **1**–**5**. The following should be noted from these inhibition data. (i) Hydrochlorothiazide **6a** acts as a medium potency inhibitor of isoforms hCA I, II, VB, IX, and XII, with inhibition constants in the range of 290–603 nM, the compound being a weaker inhibitor of isoforms hCA VA, VI, VII, XIII, and XIV (K_Is in the range of 3.655–5.010 μM) and an exceedingly weak one against hCA III (K_I of 0.79 mM). (ii) Hydroflumethiazide **6b** shows an inhibition profile distinct from that of the closely structurally related **6a**, being a rather efficient inhibitor of the following isoforms hCA II, VB, VII, IX, XII, and XIV, with inhibition constants in the range of 305–435 nM. This sulfonamide is a weaker inhibitor of hCA I, IV, and VI (K_Is in the range of 2.84–8.25 μM) and shows a very weak inhibition against isozymes hCA III, VA, and XIII (K_Is of 10.2–870 μM). Thus, even small structural changes in the benzothiadiazine scaffold, such as the substitution of the chlorine atom in *ortho* to the sulfamoyl moiety by a trifluoromethyl group, such as in the pair **6a/6b**, have dramatic consequences for the CA inhibitory properties of the two compounds (Table 1). (iii) Quinethazone **7** is the only diuretic among compounds **1**–**12** investigated here which is not approved for clinical use in Europe (but it is approved in USA),[6,11b] and this derivative was not available to be investigated here. The literature data[6] show it to be a very weak hCA I and a modest hCA II inhibitor, with inhibition constants in the range of 1.26–35 μM (Table 1). (iv) Metolazone **8** shows very weak hCA I and III inhibitory properties (K_Is in the range of 54–610 μM), being a low micromolar inhibitor of hCA II, VI, and XIV, with inhibition constants in the range of 1.714–5.432 μM. However, the drug is a medium potency inhibitor of isozymes hCA IV, VA, VB, and IX (K_Is in the range of 216–750 nM) and a very efficient one against hCA VII, hCA XII, and mCA XIII (K_Is in the range of 2.1–15 nM). (v) Chlorthalidone **9** also shows a very interesting inhibition profile, acting as a weak hCA III inhibitor (with a K_I of 11 μM, this compound is one of the most effective hCA III inhibitors ever detected among all known sulfonamides except trifluoromethanesulfonamide which has a K_I of 0.9 μM),[3b,15] and a rather weak hCA VI and hCA XIV inhibitor (K_Is in the range of 1.347–4.95 μM). Chlorthalidone is a moderate hCA VA inhibitor (K_I of 917 nM) and an effective, or very effective inhibitor of the other mammalian CA isozymes. Thus, the ubiquitous hCA I and II, as well as hCA IV, show inhibition constants in the range of 138–348 nM, but isoforms VB, VII, IX, XII, and XIII are inhibited in the low nanomolar range (K_Is in the range of 2.8–23 nM). (vi) Indapamide (the clinically used R-enantiomer) **10** acts as an inefficient CA I and III inhibitor (K_Is in the range of 51.9 to >200 μM), is a rather weak inhibitor of isoforms CA II, VA, VI, and XIV (K_Is in the range of 890–4950 nM) but shows significant inhibitory activity against CA IV and VB (K_Is in the range of 213–274 nM) and excellent inhibition of CA VII, IX, XII, and XIII, with inhibition constants in the low nanomolar range (K_Is in of 0.23–36 nM). These data are indeed remarkable, also considering the wide use of the drug as diuretic and its beneficial effects in patients with type 2 diabetes mellitus, as recently reported in an important clinical trial.[13] A special mention should be done regarding CA VII (K_I of 0.23 nM) since this isoform is present only within the brain, unlike other cytosolic CAs.[1,15b] In this organ, CA VII is involved in epileptogenesis among others, being one of the targets of the anticonvulsant sulfonamides and sulfamates.[1,15b] There are no literature data regarding a possible anticonvulsant effect of this compound, but the present data strongly suggest one, and experiments are warranted to test this activity which may lead to novel applications for the drug or to the design of novel classes of CA VII-selective inhibitors.[1,15b] (vii) Furosemide **11** acts as a very weak hCA III inhibitor (K_I of 3200 μM), but it shows moderate inhibitory activity against many isoforms, such as CA IV, VA, VB, VI, VII, IX, XII, and XIII, with K_Is in the range of 261–564 nM. The compound is, on the other hand, a much better inhibitor of CA I, II, and XIV, with K_Is in the range of 52–65 nM. (viii) Bumethanide is again an extremely weak hCA III inhibitor (K_I of 3400 μM), similarly to furosemide with which it is structurally related. However, bumethanide is also a weak inhibitor of hCA I, II, VI, and XIII (K_Is in the range of 2570–6980 nM), probably due to the quite bulky phenoxy moiety in *ortho* to the sulfamoyl zinc-binding group. The compound shows better inhibitory activity against isoforms CA IV, VA, VB, and XIV (K_Is in the range of 159–700 nM) but very good inhibition of the tumor-associated isoforms CA IX and XII (K_Is in the range of 21.1–25.8 nM, that is, the same order of magnitude as acetazolamide **1**, methazolamide **2**, or ethoxzolamide **3**) and CA VII (K_I of 62 nM).

But what is the relevance of this study for the drug design of CAIs with diverse pharmacological applications? Up to now, these widely used drugs were considered to be inactive as CAIs, due to the fact that these were launched in a period when only CA II was well known (and considered as responsible of all physiologic effects of CAIs). It may indeed be observed that in contrast to the classical CAIs of type **1**–**5** (generally low nanomolar CA II inhibitors), all compounds **6**–**12** (except furosemide **11**) are much weaker inhibitors of this isozyme, usually in the micromolar range. Indeed, only furosemide **11** is a good CA II inhibitor among these diuretics, with a K_I of 65 nM, whereas all others show K_Is in the range of 138–6980 nM (Table 1). Again with the exception of furosemide **11**, diuretics **6**–**12** have low affinity