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RESEARCH ARTICLE

Kinetic and in silico analysis of thiazolidin-based inhibitors of α-carbonic anhydrase isoenzymes

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Abstract

Carbonic anhydrases (CAs, EC 4.2.1.1) are inhibited by sulfonamides, inorganic anions, phenols, salicylic acid derivatives (acting as drug or prodrugs). A novel class of CA inhibitors (CAIs), interacting with the CA isozymes I and II (cytosolic) in a different manner, is reported here. Kinetic measurements allowed us to identify thiazolidin-based compounds as submicromolar-low micromolar inhibitors of these two CA isozymes. Molecular docking studies of a set of such inhibitors within CA I and II active site allowed us to understand the inhibition mechanism. This new class of inhibitors bind differently compared to other classes of inhibitors known to date: they were found between the phenol-binding site, filling thus the middle of the enzyme cavity.

Keyword: Carbonic anhydrase, thiazolidin, sulfonamide, docking, enzyme inhibition

Introduction

The carbonic anhydrases (CAs, EC. 4.2.1.1) represent a class of ubiquitous zinc-containing enzymes widespread in the all living organisms, which classically participate in the maintenance of pH homeostasis in mammalians, catalyzing the reversible hydration of CO_2 in a two-step reaction to yield HCO_3^- and H^{+1}. At least sixteen CA isozymes have been described up to now in mammals, the most active ones as catalysts for carbon dioxide hydration being CA II[1–3]. CA II is found primarily in red blood cells but also in many other secretory tissues of the kidney, lung, eye, etc[1–5]. CA VI is a secretory isoform that was initially described in the ovine parotid gland, saliva, and normal human serum. Other CA isoforms are found in a variety of tissues where they participate in several important biological processes such as acid-base balance, respiration, carbon dioxide and ion transport, bone resorption, lipogenesis and electrolyte secretion[1–5].

Recently, our groups investigated the interaction of CA I and II isozymes with several types of phenols such as the simple phenol, hydroxy-/methoxysubstituted benzoic acids as well as di-/tri-methoxy benzenes, anti-oxidant bisphenols and several of its substituted derivatives, for example, salicyclates and some of their derivatives[6–12]. In this study, we extend these earlier investigations to thiazolidines, a class of derivatives which have been reported to possess a wide range of biological activities including antibacterial, antitumor, and anti-inflammatory activity[13–16].

In the present study, we have purified human CA I and II from fresh blood and examined the *in vitro* inhibition effects of some thiazolidin derivative compounds mentioned above on these enzymes, using the esterase activity of hCA I and hCA II, with 4-nitrophenyl acetate as substrate.

Materials and methods

CNBr-activated sepharose 4B, protein assay reagents, p-aminobenzene sulfonamide, acetazolamide (AZA), 4-nitrophenylacetate (NPA) and chemicals for

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electrophoresis were purchased from Sigma-Aldrich Co, Munich, Germany. All other chemicals were of analytical grade and obtained from either Sigma or Merck.

Purification of carbonic anhydrase isozymes, protein determination and SDS polyacrylamide gel electrophoresis

Purification of hCA I and hCA II were performed using affinity chromatography as previously described[17]. Protein quantity was determined spectrophotometrically at 595 nm according to the Bradford method during the purification steps, using bovine serum albumin as the standard[18]. SDS polyacrylamide gel electrophoresis was performed after purification of the enzymes. It was carried out in 10% and 3% acrylamide for the running and the stacking gel, respectively, containing 0.1% SDS according to Laemmli procedure[19].

CA inhibition

Carbonic anhydrase activity was assayed by following the change in absorbance at 348 nm of 4-nitrophenylacetate (NPA) to 4-nitrophenylate ion over a period of 3 min at 25°C using a spectrophotometer (Shimadzu UV-VIS) according to the method described by Verpoorte et al.[20]. The enzymatic reaction, in a total volume of 3.0 mL, contained 1.4 mL 0.05 M Tris-SO_4 buffer (pH 7.4), 1 mL 3 mM NPA, 0.5 mL H_2O and 0.1 mL enzyme solution. A reference measurement was obtained by preparing the same cuvette without enzyme solution. The inhibitory effects of compounds 1–6b were examined. All compounds were tested in triplicate at each concentration used. Different inhibitor concentrations were used. Control cuvette activity in the absence of inhibitor was taken as 100%. For each inhibitor an activity%- [inhibitor] graph was drawn. To determine K_I values, three different inhibitor concentrations were tested. In these experiments, NPA was used as substrate at five different concentrations (0.15–0.75 mM). K_I-s were obtained from IC_{50} by the Cheng–Prussoff equation[21,22].

Molecular docking

Glide/induced fit docking (IFD) module of Schrodinger molecular modeling package has been used for docking

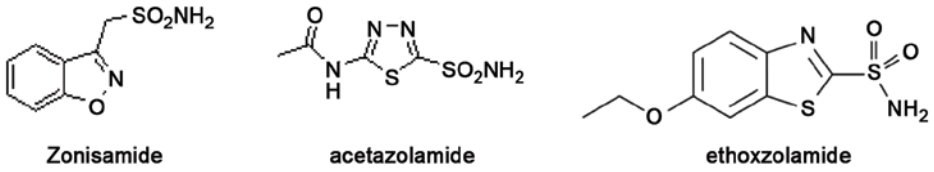

Figure 1. Chemical structures of commonly used medical sulfonamides.

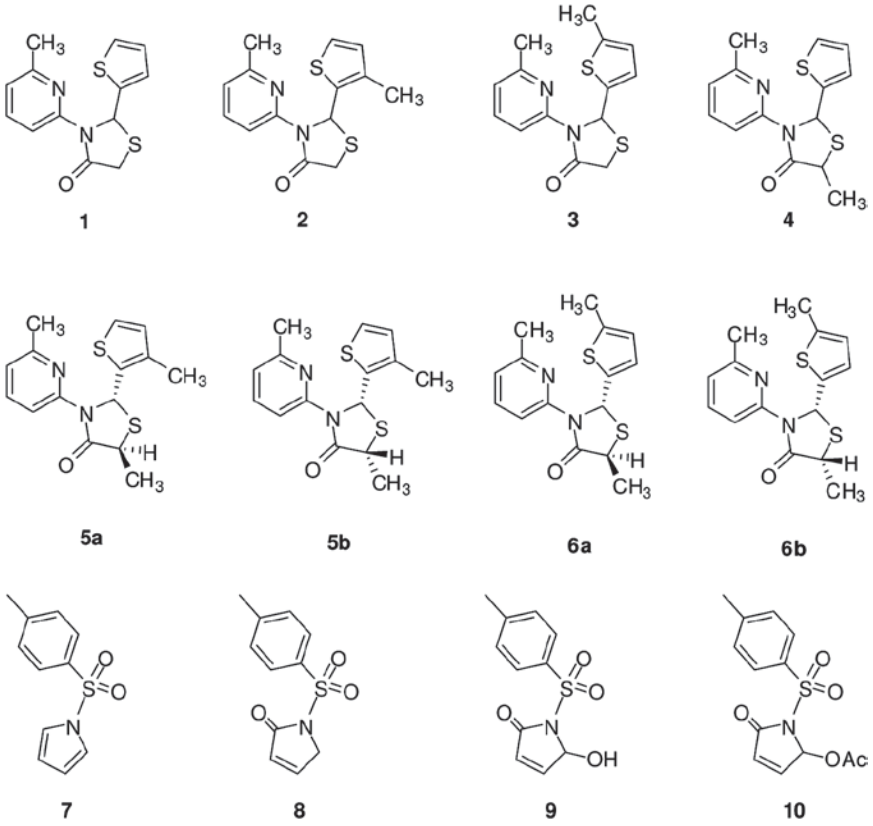

Figure 2. Chemical structures of tested compounds.

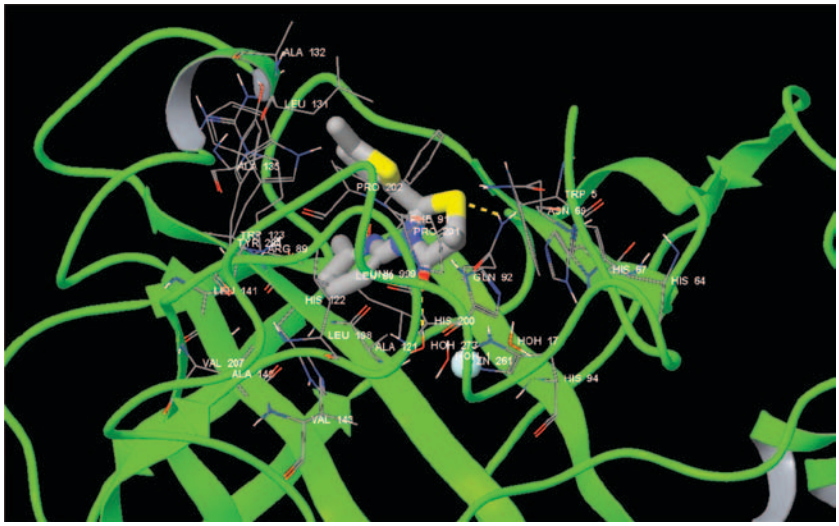

Figure 3. Compound 6b at hCA-I. (See colour version of this figure online at www.informahealthcare.com/enz)

Table 1. hCA I and II inhibition data (K_I) with compounds 1–10, ZNA and AZA, by an esterase assay with 4-nitrophenylacetate as substrate.

Compound	hCA I (μM)	hCA II (μM)
1	6.8	1.34
2	47.5	2.48
3	5.38	1.36
4	5.51	1.32
5a	27.5	2.29
5b	29.4	3.59
6a	5.58	0.93
6b	4.43	0.81
7[a]	42.4	37.5
8[a]	37.3	23.1
9[a]	14.6	0.51
10[a]	25.7	0.73
ZNA[b]	14.8	1.07
AZA	36.2	0.37

Mean from at least three determinations. Errors in the range of 1–3% of the reported value (data not shown).
[a]From ref.[6].
[b]From ref.[10].

studies (Figures 2 and 3). Extra precision (XP) option is used[23–25].

Results and discussion

There are many studies in the literature on the interactions of different compounds and CAs. The interaction of 1-tosyl pyrrol-2-one derivatives, phenolic and metoxy/bromo phenolic compounds with two human isozymes, CA I–II, has recently been investigated[5,6,26], evidencing several inhibitors. Indeed, the inhibition profile of various isozymes with this class of agents is very variable, with inhibition constants ranging from the millimolar to the sub-micromolar level for many simple pyrrol-2-one-containing molecules[5,6].

We report here a study on the inhibitory effects of thiazolidin-based compounds of type 1–6b on the esterase activity of hCA I and II. The sulfonamide CAI AZA has been used as controls in our experiments, and for comparison reasons (Figure 1). Data of Table 1 show the following regarding inhibition of hCA I and II with compounds 1–6b, by an esterase assay, with 4-NPA as substrate:

Table 2. Molecular docking binding scores of compounds 1–6b and ZNA within the hCA I and II active site.

Molecule	Binding score at hCA-I (kcal/mol)	Binding score at hCA-II (kcal/mol)
1	−6.34	−8.45
2	−5.26	−8.18
3	−6.27	−8.42
4	−6.40	−8.66
5a	−5.90	−8.72
5b	−5.71	−7.87
6a	−6.39	−8.48
6b	−6.60	−9.09
ZNA	−7.10	−10.89

(i) Against the slow cytosolic isozyme hCA I, compounds 7 and 2 behave as weak inhibitors, with K_I values of 42.4 μM and 47.5 μM, respectively. Thus, compound 2 was an ineffective hCA I inhibitor. A second group of derivatives compounds 5a, 5b, 8-10 and ZNA showed better inhibitory activity as compared to the previously mentioned compounds, with K_I values of 14.6–37.3 μM, (Table 1). It is also interesting to note that thiazolidin-4-one derivatives 1, 3, 6a and 6b were better hCA I inhibitors compared to the other compounds investigated here. AZA and ZNA are also medium inhibitors with this assay and substrate against hCA I (K_I-s of 14.8 and 36.2 μM, respectively). Kinetic investigations (Lineweaver Burk plots, data not shown) indicate that similarly to phenolic compounds, sulfonamides and inorganic anions[17,26–35], all the investigated compounds act as non-competitive inhibitors with 4-NPA as substrate, i.e. they bind in different regions of the active site

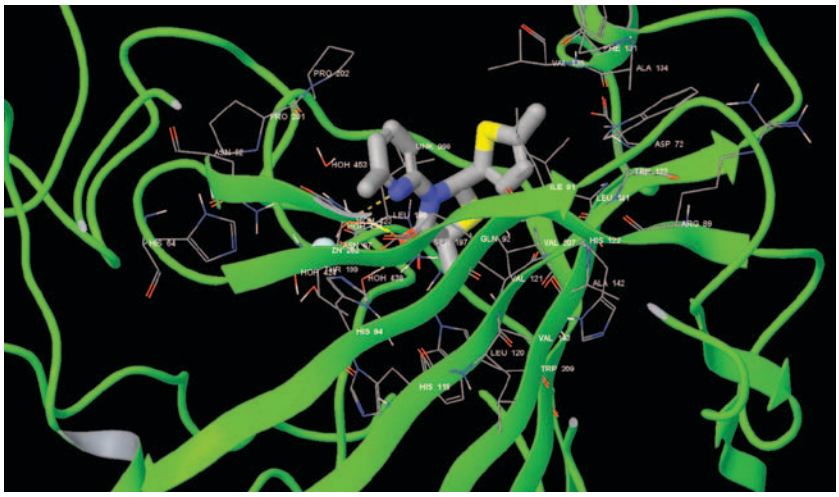

Figure 4. Compound 6b at hCA-II. (See colour version of this figure online at www.informahealthcare.com/enz)

cavity as compared to the substrate. However, the binding site of 4-NPA itself is unknown, but it is presumed to be in the same region as that of CO_2, the physiological substrate of this enzyme[31].

(ii) All compounds had better inhibitory activity against the rapid cytosolic isozyme hCA II (Table 1). Compounds 7 and 8 were weak hCA II inhibitors, with K_I-s of 37.5 and 23.1 μM, respectively (Table 1). The best hCA II inhibitor in this series of derivatives was the bulky 6b (Figure 4), which with a K_I of 0.81 μM, is similar inhibitor ZNA and AZA, a clinically used sulfonamide. It must be stressed that K_I-s measured with the esterase method are most of the time in the micromolar range because hCA I and II are weak esterases[26–34].

Although various carbonic anhydrase inhibitors have been identified up to now, it is critically important to explore further classes of potent CAIs in order to detect compounds with a different inhibition profile to find novel applications for the inhibitors of these widespread enzymes[35–40].

In silico studies

In this study, to better understand the binding mechanisms of studied molecules, fully flexible docking methodology for both receptor residues at the active site and docked ligands was used. Docking studies are performed using Glide XP-IFD algorithm, which was implemented with the Prime module under Schrodinger molecular modeling package[5,12,23–25]. The compounds 1–6b and ZNA were docked at the binding site of the targets (hCA I and hCA II). Glide/IFD docking scores of docked inhibitors at hCA I and II targets and corresponding binding interactions were tabulated in Table 2.

Conclusions

Compound 6b, ((2S,5R)-5-methyl-3-(6-methylpyridin-2-yl)-2-(5-methylthiophen-2-yl)thiazolidin-4-one) influences the activity of hCA I and II isozymes due to the functional groups although similar structures showed weaker action. Compound 6a ((2S,5S)-5-methyl-3-(6-methylpyridin-2-yl)-2-(5-methylthiophen-2-yl)thiazolidin-4-one) shows relatively lower action although it has the same structure except for the configuration of one methyl group. Thus, the nature of the substituents strongly influences the inhibitory potency of these molecules. Our findings indicate thus another class of possible CAIs of interest, in addition to the well-known sulfonamides/sulfamates, the phenols/bromophenol/diphenols bearing bulky ortho moieties in their molecules. Some of the compounds investigated here showed effective CA inhibitory activity, in the low-micromolar range, by the esterase method which usually gives K_I-s an order of magnitude higher as compared to the CO_2 hydratase assay[31]. Probably the inhibition mechanism of these compounds is distinct of the sulfonamides with RSO_2NH_2 groups and similar to that of the 1-tosyl pyrrol-2-one derivatives binding to a distinct part of the active site than that where sulfonamides bind. These findings point out that substituted thiazolidin-4-one derivatives may be used as leads for generating potent CAIs eventually targeting other isoforms which have not been assayed yet for their interactions with such agents.

Declaration of interest

The authors report no conflicts of interest.

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