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RESEARCH ARTICLE

Investigation of arenesulfonyl-2-imidazolidinones as potent carbonic anhydrase inhibitors

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Abstract

A series of arenesulfonyl-2-imidazolidinones incorporating methyl, isopropyl, methoxy, halogen and phenyl moieties were prepared and tested as possible inhibitors of two members of the pH regulatory enzyme family, carbonic anhydrase (CA; EC 4.2.1.1). The inhibitory potencies of the compounds against human isoforms hCA I and hCA II were analyzed by an esterase assay with 4-nitrophenyl acetate as substrate, and the inhibition constants (K_I) were calculated. Most compounds investigated here exhibited micromolar inhibition constants against the two isoenzymes. K_I values were in the range of 10.2–40.6 μM for hCA I and of 13.1–31.4 μM for hCA II, respectively. Most of the imidazolidinones showed interesting CA inhibitory efficacy, some of them having comparable affinity (for hCA I) as the clinically used sulfonamide acetazolamide (AZA), but their efficacy against hCA II was much lower compared to AZA.

Keywords

Carbonic anhydrase, glaucoma, imidazolidinone, inhibitor

History

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Introduction

The role of metalloenzyme carbonic anhydrase in various physiological processes has been recognized for a long period, being shown that the deregulated expression and/or abnormal performance of the 16 presently known isozymes may have important pathological consequences[1–4]. There are several human diseases whose pathopysiological characteristics involve disbalance in the conversion between carbon dioxide and bicarbonate (the two substrates of CA enzymes), resulting in perturbed ion transport, shift in pH and abnormal fluid secretion[1–4]. Thus, it seems plausible that modulation of CA activity to normal levels either by inhibition or activation offers interesting therapeutic options[1,2]. The first use and clinical testing of CA inhibitors (CAIs) dates back several decades ago, to a period before the recognition of the diversity of isoforms within the CA family and their differential distribution in various human tissues and organs[1–4]. Traditionally, the use of inhibitors was based on histochemical, biochemical and functional evidences for the presence of active CAs, potentially contributing to disease, and consequences of the treatment were evaluated mainly symptomatically[1–4]. Due to their favorable outcomes, sulfonamides have become widely accepted drugs in the treatment of several CA-based diseases, especially as antiglaucoma agents, diuretics and antiulcer agents among others[2–4]. However, systemic and topically administered CA inhibitors regularly showed serious side effects[3–9]. It is now clearly known that these undesired effects are due to the existence of at least 12 different active CA isoforms (together with the three inactive isozymes, the CA-related proteins, CARPs VIII, X and XI)[3–10], that are indiscriminately inhibited irrespective of whether they play a real role in disease or are just coexpressed in the same tissue and elsewhere in the body. In addition, certain drugs directed primarily against different CA-unrelated targets may also inhibit the activity of CAs[1–5]. Thus, it is critically important to thoroughly characterize the affinity of different isozymes for different CAIs, and also to better understand the structure activity relationships[1–10].

Our groups recently investigated the interaction of CA I and II isozymes with several types of phenols, pyrrole derivatized sulfonamides, dopaminergic bromophenolic compounds, antioxidant bisphenols and several of its substituted derivatives, e.g. salicyclates and some of their derivatives[8,9]. Here, we extend these earlier investigations to series of arenesulfonyl-2-imidazolidinone-based compounds. Diarenesulfonylurea and thiourea functionalities have attracted reasonable attention[11–15], especially after the discovery of sulofenur[16]. It is generally assumed that the strong cytotoxicity and, as a consequence, the antitumor properties of the diarenesulfonylurea is due to the

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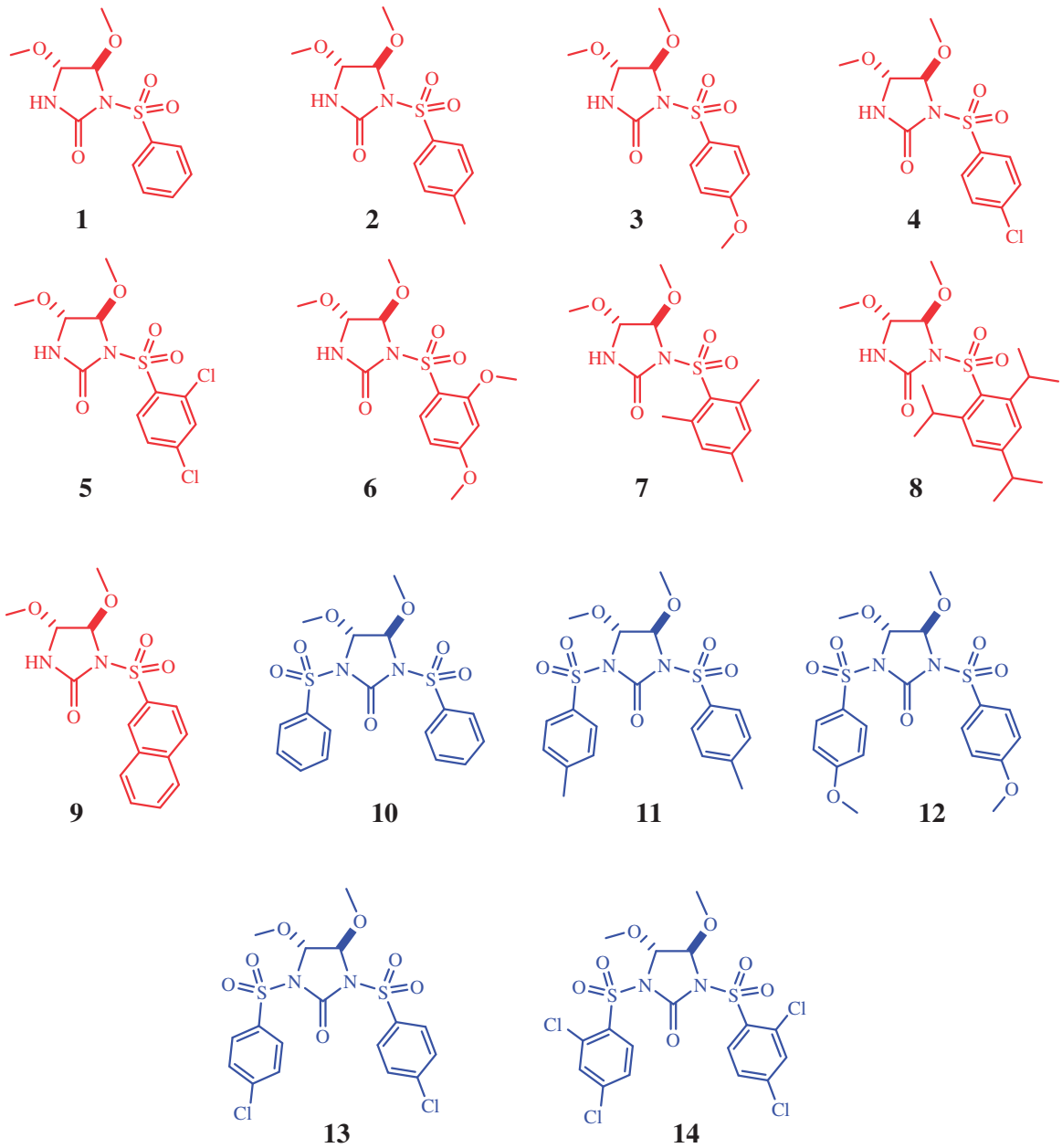

Figure 1. Structure of the tested compounds.

uncoupling of mitochondria[11,12], however, other mechanisms, such as inhibition of the mitochondrial isozyme V of carbonic anhydrase (CAs) have also been hypothesized, since hydrolysis of the cytotoxic agent leading to the formation of unsubstituted sulfonamides as the principal products has been reported both *in vivo* and *in vitro*[17].

It is well known that aromatic/heterocyclic sulfonamides (formed after such a hydrolytic process) act as very potent inhibitors of CAs[18,19], and that these enzymes are involved in a multitude of crucial physiologic processes[20]. However, clinical trials of sulofenur have yielded unsatisfactory results because of its high protein binding and limited dosing caused by the appearance of anemia, and methemoglobinemia, a side effect that is likely caused by its aniline-related metabolites[21,22]. Trying to overcome the serious side effects of sulofenur, several cyclic diarenesulfonylurea such as 4-phenyl-2-imidazolidinone[23–26], arylidenehydantoin have been synthesized and screened for antitumor activity against various human solid tumors[27,28] (Figure 1).

In the present study, we have purified human CA I, II (hCA I, hCA II) isoenzymes and examined the *in vitro* inhibition effects of some arenesulfonyl-2-imidazolidinones mentioned above on these enzymes.

Materials and methods

CNBr-activated Sepharose 4B, protein assay reagents, p-aminobenzene sulfonamide L-tyrosine, 4-nitrophenylacetate and chemicals for electrophoresis were purchased from Sigma–Aldrich Co. All other chemicals were of analytical grade and obtained from Sigma-Aldrich (Bornem, Belgium).

General method for the synthesis of compounds

A solution containing 0.001 mol 6-(un)substituted 3-formylchromone in 5–7 mL ethanol was stirred with heating until dissolved. Then catalytic amount of p-toluenesulfonic acid (p-TsOH) was added followed by the addition of 0.001 mol of 3- and 4-aminobenzene sulfonamide in equal volumes of

ethanol. Reaction mixture was refluxed for 3.5 h and kept overnight. Solid product was obtained by filtration and purified by recrystallization from a mixture of hot ethanol and acetone (1:1).

Kinetic studies

Purification of hCA I and hCA II were previously described[8,9]. The activity assay was carried out according to Verpoorte et al.[29].

Protein determination

Protein quantity was determined spectrophotometrically at 595 nm according to the Bradford method during the purification steps, using bovine serum albumin as the standard[30].

SDS polyacrylamide gel electrophoresis

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[31].

Results and discussion

Our groups recently investigated the interactions of sulfonamides and some of their substituted derivatives with all mammalian CA enzymes[17–24], demonstrating some low micromolar/submicromolar inhibitors as well as the possibility to design isozyme selective CAIs. The inhibition profile of various CAs with this class of agents is very variable, with inhibition constants ranging from the millimolar to the submicromolar range[10]. Thus, it seemed reasonable to us to extend the previous studies[32], including in this investigation a series of arenesulfonyl-2-imidazolidinone compounds.

The purification of the two CA isozymes was performed with a simple one step method by a Sepharose-4B-aniline-sulfanilamide affinity column chromatoghrapy[9]. hCA I was purified, 108.6-fold with a specific activity of 963.12 EU mg^{-1} and overall yield of 56%, hCA II was purified, 903.2-fold with a specific activity of 6790 EU mg^{-1} and overall yield of 66%. Inhibitory effects of compounds **1–14** on enzyme activities were tested under *in vitro* conditions. The sulfonamide CAI acetazolamide **AZA** has been used as a negative control in our experiments, and for comparison reasons. Data of Table 1 show the following regarding inhibition of hCA I and II with compounds **1–14** and **AZA** (as standard):

(i) Against the slow cytosolic isoform hCA I, the arenesulfonyl-2-imidazolidinone derivatives investigated here showed moderate to effective inhibitory properties. Thus, derivatives **1–14** exhibited weaker inhibition of this isoform, with K_I in the range of 37.1–41.3 μM, with a comparable potency as the reference compound AZA (K_I: 36.2 μM) (Table 1). The compounds **1–7** were more effective inhibitors against hCA I, with K_I in the range of 10.2–23.4 μM, being more effective than AZA. However, compounds **4** and **5** acted as the most effective hCA I inhibitor (K_I-s: 2.14–2.59 μM) (Table 1). These results demonstrate the contribution of the halogen atoms to the inhibition efficacy.

(ii) Against the ubiquitous and dominant rapid cytosolic isozyme hCA II, compounds **1–14**, acted as weak inhibitors (K_I in the range of 12.9–31.4 μM) compared to the clinically used sulfonamide **AZA** (Table 1). Similar to the trend in hCA I, compounds **1–7** were more effective inhibitors than others with K_I in the range of 12.9–19.1 μM. However, remaining seven derivatives **8–14** as weaker hCA II inhibitors (K_I-s: 21.1–31.4 μM), with much lower K_I values than that of the reference compound AZA (Table 1). Findings of our study indicates that arenesulfonyl-2-imidazolidinone derivatives show good inhibitory activity on hCA I with comparable or lower K_I values than the clinically used sulfonamide AZA, but this trend was not seen for the rapid enzyme hCA II.

Table 1. hCA I and II inhibition data with compounds **1–10**, by an esterase assay with 4-nitrophenylacetate as substrate[9].

Compound	hCA I (μM)	hCA II (μM)
1	16.2	13.1
2	15.3	12.9
3	11.6	13.2
4	10.2	17.6
5	11.3	19.1
6	13.9	18.2
7	21.4	16.7
8	37.1	22.2
9	39.3	31.4
10	40.2	23.3
11	40.6	24.6
12	42.2	21.1
13	40.5	21.6
14	41.3	27.4
AZA	36.2*	0.37*

*From Ref. 9.

Declaration of interest

The authors report no declarations of interest. The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGP-VPP-163.

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