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N-Sulfamoylphenyl- and *N*-sulfamoylphenyl-*N*-thiazolyl-β-alanines and their derivatives as inhibitors of human carbonic anhydrases

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ABSTRACT

A series of *N*-substituted and *N*,*N*-disubstituted β-amino acids and their derivatives bearing benzenesulfonamide moiety were designed and synthesized in search of compounds that would be high-affinity and selective inhibitors of human carbonic anhydrases (CA). There are 12 catalytically active human CA isoforms, the cytosolic CA I, CA II, CA III, CA VII, and CA XIII, secreted CA VI, the mitochondrial CA VA and CA VB, membrane-associated CA IV, and transmembrane CA IX, CA XII, and CA XIV. The di-bromo meta-substituted compounds exhibited low nanomolar dissociation constants and over 10-fold selectivity for mitochondrial isozyme CA VB, implicated in diseases of the central nervous system and obesity. These compounds can be used for further development as inhibitors of significant binding affinity and selectivity towards CA VB isozyme.

1. Introduction

Human α-carbonic anhydrases (CA) are metalloenzymes that catalyze the hydration of carbon dioxide into bicarbonate. There are twelve catalytically active human CA isoforms involved in many crucial physiological processes such as intracellular pH regulation, respiration and transport of CO_2/bicarbonate, biosynthetic reactions (e.g. gluconeogenesis, lipogenesis, and ureagenesis) and many other physiological or pathological processes [1]. The enzymes are found in numerous tissues and cellular locations. Five CA isoforms (CA I, CA II, CA III, CA VII, and CA XIII) are cytosolic, CA VI is secreted protein, CA VA and CA VB are mitochondrial ones, CA IV is membrane-associated GPI-anchored enzyme, and three isoforms (CA IX, CA XII, and CA XIV) are the transmembrane proteins. All these isozymes contain catalytically necessary zinc ion in the active site. The enzymes are validated as molecular targets for drugs designed to treat a variety of central nervous system disorders, glaucoma and cancer [2–4].

Recent review on different classes of CA inhibitors [5,6] showed at least five different enzyme inhibition mechanisms of existing CA inhibitors. The most important class of CA inhibitors sulfonamides are zinc binders, which coordinate with Zn^{2+} in tetrahedral or trigonal bipyramidal geometries. Most of all clinically used drugs which target CA isoforms and act as diuretics, antiepileptic/antiobesity and antiglaucoma agents are primary sulfonamides [7]. One of the synthetic sulfonamide passed Phase I clinical trials in 2016 for the treatment of hypoxic, metastatic tumors through the inhibition of CA IX [8]. However, the development of an extremely high affinity and selective inhibitor of CA IX and CA XII proteins (cancer targets) and other important isoforms, e.g. CA VA and CA VB (obesity targets) or CA VII (neuropathic target) remains a great need in target based drug therapy.

All sulfonamide-based inhibitors are characterized by a sulfonamide head group, able to bind the Zn^{2+} ion in a pocket of the CA active site, a ring group and a tail group. Each part can be varied in structure, which modulates the binding affinity and selectivity of the ligand for CA isozymes. Many aromatic sulfonamides have been synthesized and tested as CA inhibitors, mostly developed using two different approaches: the ring approach and the tail approach [5,9,10]. Different aromatic rings have been introduced, including thiophene, furan, thiadiazole, indole, etc. However, benzene moiety bearing compounds were among the most extensive studied compounds with effective inhibition of CAs. The tail groups of various chemical nature attached to benzene ring usually are introduced with the goal to increase the solubility of the ligand [11,12] and to enhance the interaction of the ligand with the active site of the enzyme [13–16].

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Recently, *N*-4-mono-substituted or 4-*N*,*N*-disubstituted derivatives were synthesized using sulfanilamide as a starting material [17], and the inhibition towards CAI, CA II, CAIX and CA XII was tested using stopped-flow assay. The compounds were more potent CA IX and CA XII inhibitors, with inhibition constants exceeding 10 nM. Various acylated, alkylated benzenesulfonamides and hydrazone derivatives were also effective CA II [18], CA IX, and CA XII inhibitors (the inhibition data obtained mostly against four isozymes, namely CA I, CA II, CA IX, and CA XII) [13,18,19]. We have designed *N*-aryl-β-alanine derivatives containing a primary sulfonamide moiety and investigated these compounds as inhibitors of six carbonic anhydrase isoforms: CA I, CA II, CA VI, CA VII, CA XII, and CA XIII [20]. Some of the compounds had low micromolar affinity against CA II.

Tanpure et al. synthesized a series of sulfonamides with dual-tail substitution of acetazolamide, the first clinically used CA inhibitor, where two distinct groups were oriented in opposing directions [21]. The compounds were built to interact with two conserved environments of CA active site, the hydrophobic and hydrophilic side. However, dual-tail compounds with thiadiazole ring were less potent CA I and CA II inhibitors than single-tail derivatives, showing that the tail moieties were not optimally aligned to form close interactions with the hydrophilic and hydrophobic amino acid residues of the CA active site. Compounds with dual tail substitution onto benzenesulfonamides were less investigated [17]. Therefore, in the current work we applied dual tail approach to design and synthesize benzenesulfonamides bearing two distinct tails at *para* position, β-alanine derivatives on one side and thiazole derivatives on another side. The thiazole and β-alanine structural subunits were chosen not accidentally. The *N*-substituted β-alanines and their derivatives are structural units of a number of natural compounds and exhibit a broad spectrum of biological activities, therefore are used as growth regulators, pharmaceuticals, pesticides, and medicinal preparations in agriculture [22]. The thiazole moiety as an attractive scaffold is a component of many natural and synthetic compounds. Thiazoles are easily metabolized by known biochemical reactions, and are non-carcinogenic in nature [23]. Thiazole derivatives have been attracting attention of medicinal chemists due to their wide variety of biological activities, such as antibacterial and antifungal [24–28], anticancer [29–31], antiviral, anti-inflammatory, and antitubercular [32]. The binding affinity toward CAs of the dual tail compounds was compared to the single tail compounds. Furthermore, our results showed that the incorporation of halogen atom (chlorine and bromine) at *meta* position of benzenesulfonamide increases the binding affinity and selectivity towards antiobesity target CA VB.

2. Results and discussion

2.1. Chemistry

The halogen-substituted 3-((4-sulfamoylphenyl)amino)propanoic acids **2** and **3** were synthesized by treating β-alanine **1** with HCl or HBr in the presence of hydrogen peroxide (Scheme 1). 3-((2-Bromo-4-sulfamoylphenyl)amino)propanoic acid (**4**) was obtained from acid **1** and *N*-bromosuccinimide in DMF. Esters **5–7** were synthesized by esterification of acids **2–4** with an excess (10 times) of methanol under reflux in the presence of sulfuric acid as a catalyst. Reaction of esters **5–7** with hydrazine hydrate in propan-2-ol under reflux yielded hydrazides **8–10**, whose reactions with corresponding aldehydes in propan-2-ol at reflux temperature yielded hydrazones **11–16**. Hydrazone **17** was synthesized from acid hydrazide (synthesis reported in [20]) and 4-fluorobenzaldehyde.

Hydrazones can form four isomers owing to the presence of amide and azomethine groups in their structure. The geometrical isomers (*cis/trans*) originate from the azomethine group and rotamer (*E*/*Z*) formation is due to the restricted rotation of the amide group. As shown by the NMR spectra, hydrazones **11–17** exist as a mixture of *E*/*Z* isomers in DMSO-d_6 solutions. In all ^{1}H NMR spectra for **11–17**, resonances for the CO—NH and N=CH group protons are present in double sets and the signal intensity ratio is 0.65:0.35. The *Z* isomer predominates because of a hindered rotation around the CO—NH bond [33,34]. However, no formation of geometrical isomers was observed.

Benzimidazole **18** was synthesized from acid **1** according to *Phillips* method by refluxing with *o*-phenylenediamine in dilute hydrochloric acid. The presence of CNH proton singlet at 12.27 ppm and the increased number of aromatic proton peaks in the ^{1}H NMR spectrum for **18** indicate the formation of benzimidazole moiety.

The *N*,*N*-disubstituted β-alanines bearing thiazole and dihydrothiazole rings in their structure were synthesized. Thioureido acids are convenient precursors for the synthesis of such compounds. The precursor for a series of compounds, 3-(1-(4-sulfamoylphenyl)thioureido)propanoic acid (**19**), was synthesized from acid **1** via 2-thiodihydrouracil [20], which was immediately cleaved in alkaline medium (10% aqueous NaOH solution) to form a corresponding sodium salt, the solution was acidified with dilute hydrochloric acid to pH 5 to yield acid **19**. In the ^{13}C NMR spectrum for **19**, the carbon resonance at 181.89 ppm confirms the presence of the C=S group in the molecule.

Thiazole ring can be formed by *Hantzsch* reaction. Thus, reaction of thioureido acid **19** and monochloroacetic acid provided thiazolone **20**, which subsequently was functionalized (Scheme 2). Bromination of **20** was carried out with bromine in acetic acid at 60 °C in the presence of sodium acetate to give compound **21**. In the ^{1}H NMR spectrum for **20**, the singlet attributable to the SCH_2 group in dihydrothiazolone ring is at 3.99 ppm, whereas, it is absent in the ^{1}H NMR spectrum for **21**. Methylene group in thiazolone ring undergoes facile condensation reaction with aldehydes. Reactions of **20** with corresponding aldehydes in methanol in the presence of TEA provided compounds **22–26**. In their ^{1}H NMR spectra, the proton singlet attributed to the C=CH group in the thiazolone moiety has been observed in the aromatic region. Ester **27** was obtained by the esterification reaction of acid **20** with methanol in the presence of sulfuric acid.

Reactions of thioureido acid **19** with 2,3-dichloronaphthoquinone in acetic acid and α-haloketones in acetone provided thiazoles **28** and **29–34**, respectively. ^{1}H and ^{13}C NMR spectra of these compounds display additional resonances in the aromatic region indicating the formation of new benzene rings. For the structure-activity relationship, compound **31** was modified by introducing bromine into thiazole moiety, yielding derivative **35**. Similarly, the reactions with carboxylic group subsequently provided corresponding ester **36**, hydrazide **37**, hydrazones **38–41**, and pyrazole derivative **42** (Scheme 3). In the ^{1}H NMR spectrum for **42**, the proton singlet attributable to the CH group in the pyrazole ring is at 6.14 ppm and proton singlets of the CH_3 groups are observed at 2.15 ppm and 2.37 ppm.

With the aim to evaluate the influence of the position of SO_2NH_2 group in benzene ring on CAs affinities, compounds **44–47** were synthesized (Scheme 4). These compounds dissolve better in common organic solvents than *p*-substituted derivatives.

2.2. Binding affinities to CAs

The main goal of the present study was to evaluate the effects of *N*-*para*-mono-substitution, *N*-*meta*-mono-substitution and *N*,*N*-disubstitution of benzenesulfonamides to the binding affinity of