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Carbonic anhydrase inhibitors: Design, synthesis, kinetic, docking and molecular dynamics analysis of novel glycine and phenylalanine sulfonamide derivatives

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ABSTRACT

The inhibition of two human cytosolic carbonic anhydrase isozymes I and II, with some novel glycine and phenylalanine sulfonamide derivatives were investigated. Newly synthesized compounds **G1–4** and **P1–4** showed effective inhibition profiles with K_I values in the range of 14.66–315 μM for hCA I and of 18.31–143.8 μM against hCA II, respectively. In order to investigate the binding mechanisms of these inhibitors, in silico docking studies were applied. Atomistic molecular dynamic simulations were performed for docking poses which utilize to illustrate the inhibition mechanism of used inhibitors into active site of CAII. These sulfonamide containing compounds generally were competitive inhibitors with 4-nitrophenylacetate as substrate. Some investigated compounds here showed effective hCA II inhibitory effects, in the same range as the clinically used sulfonamide, sulfanilamide or mafenide and might be used as leads for generating enzyme inhibitors possibly targeting other CA isoforms which have not been yet assayed for their interactions with such agents.

1. Introduction

Carbonic anhydrase (EC 4.2.1.1, CA) is a family of metalloenzymes that catalyze the rapid conversion of CO_2 to HCO_3^- and H^+, and involved in the biochemical process.[1] 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, ureagenesis, gluconeogenesis, lipogenesis and electrolyte secretion.[1–4] Many CA isozymes involved in these processes are important therapeutic targets with the potential to be inhibited/activated for the treatment of a range of disorders such as edema, glaucoma, obesity, cancer, epilepsy and osteoporosis.[1,4]

Our groups recently investigated the interaction of some mammalian CA isozymes with several types of sulfonamide derivatives, such as sulfanilamide and a series of chromone containing sulfonamides, benzenesulfonamides, for example, and some of their derivatives.[5] Here we extend these earlier investigations to series of sulfonamides, some of which are widely used as prodrug or as drugs. Sulfonamides possess many types of biological activities, and representatives of this class of pharmacological agents are widely used in clinic as antibacterial, hypoglycemic, diuretic, anti-hypertensive and antiviral drugs among others.[1,4–6] Recently, a host of structurally novel sulfonamide derivatives have been reported to show substantial antitumor activity in vitro and/or in vivo.[6–8]

In the present study we have purified CA I and II (hCA I and hCA II) from human erythrocytes and examined the in vitro inhibition effects of above mentioned aminoacid derivatives on these enzymes, using the esterase activity of hCA I and II, with 4-nitrophenyl acetate as substrate. Molecular modeling studies also

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applied for better understanding of molecular mechanisms of used compounds.

2. Results and discussion

2.1. Chemistry

The rationale of investigating sulfonamide derivatives as CA inhibitors (CAIs) is due to the fact that the simple benzenesulfonamide ($PhSO_2NH_2$) has been shown to be competitive inhibitor with both CO_2 and 4-nitrophenyl acetate as substrate for CA isoforms.[1,5a] Sulfonamide type inhibitors bind to CAs, with coordination to the Zn(II) ion from the enzyme active site by substituting the fourth, non-protein ligand, a water molecule or hydroxide ion, such as for example acetazolamide (**AZA**), a clinically used compound since 1954.[8,9]

The X-ray crystal structure has been extensively used for understanding the inhibition mechanism of CAIs. For example, for the adduct of hCA II with sulfamide,[8a,9] it has been observed that the compound binds to CA by anchoring its NH moiety to the zinc ion of the enzyme active site, through a hydrogen bond, as well as through a second hydrogen bond to the NH amide of Thr199, an amino acid conserved in all α-CAs and critically important for the catalytic cycle of these enzymes.[6–9] Only recently, our groups investigated the interactions of some methanesulfonates, chromone containing sulfonamides, benzenesulfonamides, salicylic acid derivatives, some pesticides, some natural product polyphenols and phenolic acids with all mammalian isozymes, CA I–XV,[8–11] evidencing some low micromolar/submicromolar inhibitors as well as the possibility to design isozyme selective CAIs. Indeed, the inhibition profile of various isozymes with this class of agents is very variable, with inhibition constants ranging from the millimolar to the submicromolar range for many simple sulfonamides (Fig. 1).[5a,10]

2.2. CA purification, assay and inhibition with some amino acid derivatives

The purification of the two CA isozymes used here was performed with a simple one step method by a Sepharose-4B-aniline-sulfanilamide affinity column chromatoghrapy.[12] Inhibitory effects of **G1–4** and **P1–4** compounds on enzyme activities were tested under in vitro conditions; K_I values were calculated from by using the Cheng–Prusoff equation and are given in Table 1.[13]

We report here the first study on the inhibitory effects of amino acid derivatives **G1–4** and **P1–4** on the esterase activity of hCA I and II:

(i) Against the slow cytosolic isozyme hCA I, **G3** and **P2** behave as good inhibitors, with K_I values in the range of 29.62 and 63.7 μM. Thus, the natural compound derivatives of the groups in hydrophobic benzoic moiety strongly influences hCA I inhibitory activity. It is also interesting to note that the **P3** was much better hCA I inhibitors as compared to the corresponding **G3** and **P2** from which they were prepared. Kinetic investigations indicate that similarly to sulfonamides and inorganic anions,[4,5,8] all the investigated compounds act as competitive inhibitors with 4-NPA as substrate, that is, they bind in different regions of the active site 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.[14]

(ii) A better inhibitory activity has been observed with **G3** and **4** investigated here for the inhibition of the rapid cytosolic isozyme hCA II (Table 1). Structure–activity relationship (SAR) is thus quite sharp for this small series of these compounds: the –COOH **G1** are ineffective leads, with carboxylic acid moieties is already a submicromolar hCA II inhibitor. The

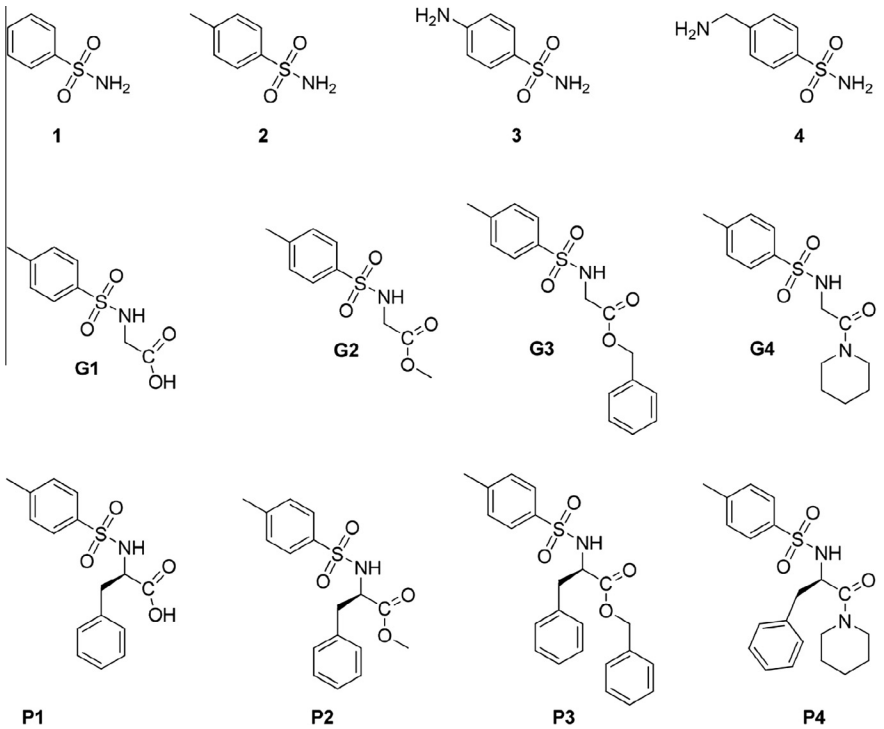

Figure 1. Chemical structures of synthesized and used compounds for this study.