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Research paper

2-Benzylpiperazine: A new scaffold for potent human carbonic anhydrase inhibitors. Synthesis, enzyme inhibition, enantioselectivity, computational and crystallographic studies and in vivo activity for a new class of intraocular pressure lowering agents

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ABSTRACT

Two series of 2-benzylpiperazines have been prepared and tested for the inhibition of physiologically relevant isoforms of human carbonic anhydrases (hCA, EC 4.2.1.1). The new compounds carry on one nitrogen atom of the piperazine ring a sulfamoylbenzamide group as zinc-binding moiety, and different alkyl/acyl/sulfonyl groups on the other nitrogen. Regio- and stero-isomers are described. The majority of these compounds showed Ki values in the low-medium nanomolar range against hCA I, II and IV, but not IX. In many instances interaction with the enzyme was enantioselective. The binding mode has been studied by means of X-ray crystallography and molecular modelling. Two compounds, evaluated in rabbit models of glaucoma, were able to significantly reduce intraocular pressure, making them interesting candidates for further studies.

1. Introduction

Carbonic Anhydrase (CA, EC 4.2.1.1) is a very efficient enzyme, which catalyzes the hydration of carbon dioxide to produce bicarbonate and a proton. This process is crucial for most organisms, and, as a consequence, during the evolution of life seven genetically different families of this enzyme (classified as α- θ-CAs) evolved [1]. α-CA are present in vertebrate, and in mammals 16 different α-CA isoforms were isolated and characterized so far, varying for tissue distribution and cellular localization (membrane, cytosol, mitochondria) [2]. Some isoforms have been studied in detail also by means of X-ray crystallography, obtaining pictures of the active site, where a zinc ion plays a pivotal role in the catalytic reaction. Moreover, other structural features of the active site have been highlighted, such as the presence of two different areas, one lined by hydrophobic residues and the other one by hydrophilic amino-acids, offering the opportunity to modulate ligand design in different ways [3].

CA inhibitors are in clinical use for more than sixty years as diuretics and antiglaucoma drugs [4]. Glaucoma is a multifactorial ocular disease characterized by optic nerve degeneration generally related to high intraocular pressure, which can lead to blindness [5]. CA inhibitors such as dorzolamide and brinzolamide are

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effective in reducing intra-ocular pressure (IOP) after topical administration; however, since these drugs display several side effect, novel therapeutic agents are needed [6]. The isoform mainly involved in aqueous humor secretion are CA II and CA IV [7]. Oral and topical CA inhibitors are also used in other ocular diseases, among which cystoid macular oedema [8].

Classical inhibitors contain a benzene or heterocyclic sulfonamide moiety, which coordinates the zinc ion. This pharmacophoric group has been inserted into a variety of structures obtaining compounds endowed with high potency and in some instances isoform selectivity [3]. Due to the high number of CA isoforms, potency and selectivity are crucial properties for therapeutic applications, especially those suggested in recent times, such as anticonvulsant, antiobesity, anticancer, analgesic, and anti-infective drugs [9–13].

The piperazine ring is a widely used scaffold for drug discovery, and piperazine derivatives are known to produce a wide range of pharmacological activities [14]. Several piperazines have been tested also on CA, showing a broad spectrum of potency; some examples are shown in Chart 1. Compounds **I** and **II** are mainly N-arylpiperazines, carrying an arylsulfonamide moiety as zinc binding group (ZBG), linked to the second piperazine N-atom directly (**II**) or through an urea moiety (**I**). Both series of molecules were tested on hCA I, II, IX and XII. Compounds with general formula I showed good potency (K_i in the low nanomolar) especially on hCA II, and in few instances also on hCA IX (Ar = phenyl or 4-Cl-phenyl) or hCA XII (Ar = 3-cyano-2-pyridinyl, 4-trifluoromethyl-2-pyrimidinyl) [15]. Compounds with general formula II were weaker inhibitors (K_i in the high nanomolar), the less sensitive enzyme being hCA I; only few compounds showed K_i values below 50 nM on hCA XII (R = benzyl, 2-pyrimidinyl) or on hCA IX (R = benzyl) [16]. Other examples of piperazine-based CA inhibitors can be found in the literature [17,18], but to our knowledge, no C-substituted piperazine has been tested so far on hCA.

Aiming to find new potent and selective CA inhibitors, we designed and prepared two series of compounds (A and B, Chart 1), characterized by the presence of a piperazine ring carrying a sulfamoylbenzamide moiety as ZBG on one N atom, and different alkyl/acyl/sulfonyl groups on the other one. The 6-membered ring has been further decorated with a lipophilic substituent (e.g. a benzyl group), which could interact with the lipophilic area of the active site, while the amide linkers or the chargeable amine functionality could be engaged with the hydrophilic domain. In our design these interactions could introduce high potency and hopefully also some isoform selectivity. Thus, compounds **1**–**16** were prepared and tested as CA inhibitors on four different isoforms (hCA I, hCA II, hCA IV and hCA IX), together with the serendipitously discovered **17**, which could comply with both A and B general formulas. The presence in these molecules of a stereogenic centre prompted us to synthesize both the *R* and *S* enantiomers, allowing us to perform a study of enantioselectivity, in order to understand the features associated with effective enzyme inhibitory properties.

2. Chemistry

Compounds reported here were synthesized following the procedures shown in Schemes 1–3. The enantiomers were prepared starting from the suitable (*S*) or (*R*) synthone using the same methods, with the exception of compounds (*S*)-**11** and (*R*)-**11**, which were prepared in different ways.

The synthesis of compounds with general formula A started from (*S*) or (*R*)-1,3 dibenzylpiperazine **18** (Scheme 1), obtained as reported by Gerdes [19]. Treatment of (*S*) or (*R*)-**18** with 4-

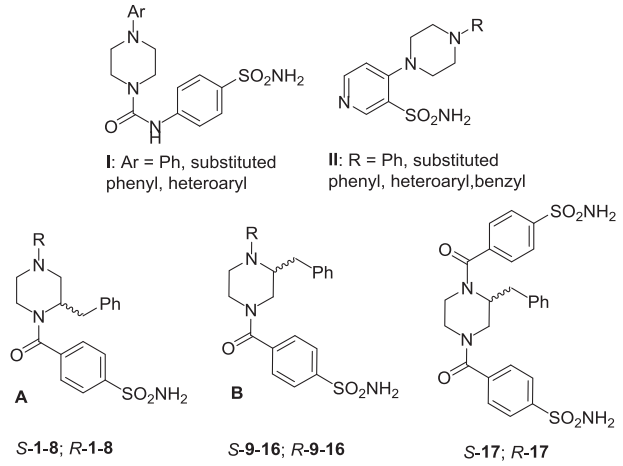
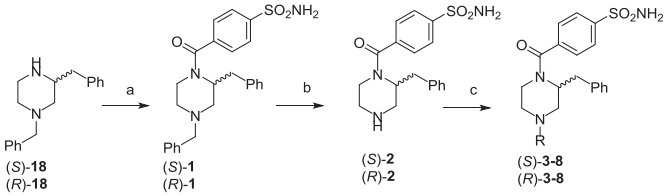


Chart 1. Structure of piperazine CA inhibitors reported in the literature (I, II) and synthesized in this paper (1-17).

sulfamoylbenzoyl chloride in acetonitrile gave amides (*S*) and (*R*)-**1** which underwent catalytic hydrogenation, leading to secondary amines (*S*)- and (*R*)-**2**. Compounds (*S*) and (*R*)-**3**-**8** were then obtained treating **2** with the suitable reagent in acetonitrile. Triethylamine was added when this reaction was performed with acid chlorides (benzoyl chloride for **5**, methanesulfonyl chloride for **7**, phenylsulfonyl chloride for **8**). The base was not necessary when preparing compounds **4** and **6**, for which acetic anhydride or 2,5-dioxopyrrolidin-1-yl 2-phenylacetate [20], respectively, were used, nor for the synthesis of **3**, for which only 0.5 eq of methyl iodide were used.

(*S*)-**18** was used also in the synthesis of (*S*)-**11** (Scheme 2). It was treated with formic acid/formaldehyde to give the *N*-methyl derivative (*S*)-**19**, then its catalytic hydrogenation led to the secondary amine (*S*)-**20** [21]. Reaction with 4-sulfamoylbenzoyl chloride gave the desired *N*-methyl derivative (*S*)-**11.**

The synthesis of the other compounds with the general formula B started from (*S*) or (*R*)-2-benzyl piperazine **21** (Scheme 3), obtained as reported by Levy [22]. Treatment of (*S*) and (*R*)-**21** with 4-sulfamoylbenzoyl chloride at room temperature afforded almost exclusively the double addition products (*S*) and (*R*)-**17.** (*S*) and (*R*)-**9** were obtained by reaction of (*S*) and (*R*)-**21** at 0 °C with 0.7 eq of 2,5-dioxopyrrolidin-1-yl 4-sulfamoylbenzoate [23], in order to avoid the formation of the double addition product **17** [24], and transformed into the final compounds (*S*) or (*R*)-**12**-**16** as done for their regio-isomers (compounds **4**–**8** A series). The N-benzyl derivatives (*S*) and (*R*)-**10** were obtained by reaction of (S) or (R)-**9** with benzyl bromide using $NaHCO_3$ as base, while for compound (*R*)-**11** methyl iodide (0.5 eq) was used without addition of a base.



Reagents: (a) 4-sulfamoylbenzoyl chloride, TEA, acetonitrile; (b) MeOH/HCl, H_2/Pd/C; (c) R-Z, acetonitrile, base (when necessary). For the meaning of R-Z and base see Table 3.

Scheme 1. Synthesis of compounds **1**-**8**.