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Benzenesulfonamides incorporating bulky aromatic/heterocyclic tails with potent carbonic anhydrase inhibitory activity

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ABSTRACT

Three series of sulfonamides incorporating long, bulky tails were obtained by applying synthetic strategies in which substituted anthranilic acids, quinazolines and aromatic sulfonamides have been used as starting materials. They incorporate long, bulky diamide-, 4-oxoquinazoline-3-yl- or quinazoline-4-yl moieties in their molecules, and were investigated for the inhibition of four physiologically relevant carbonic anhydrase (CA, EC 4.2.1.1) isoforms, the cytosolic human (h) hCA I and II, as well as the transmembrane hCA IX and XII. Most of the new sulfonamides showed excellent inhibitory effects against the four isoforms, with K_Is of 7.6–322 nM against hCA I, of 0.06–85.4 nM against hCA II; of 6.7–152 nM against hCA IX and of 0.49–237 nM against hCA XII; respectively. However no relevant isoform-selective behavior has been observed for any of them, although hCA II and XII, isoforms involved in glaucoma-genesis were the most inhibited ones. The structure–activity relationship for inhibiting the four CAs with these derivatives is discussed in detail.

1. Introduction

The sulfonamides continue to be one of the important families of inhibitors of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1), with many representatives in clinical use as diuretics, antiglaucoma, antiepileptic, antiobesity drugs, or agents useful for the treatment of some neurological disorders, such as idiopathic intracranial hypertension.[1–10] Recently, a sulfonamide CA inhibitor (CAI) developed by one of our groups by applying the tail approach, SLC-0111, entered Phase I clinical trials for the treatment of patients with advanced solid, metastatic tumors overexpressing CA IX/XII.[1–4]

The tail approach[1] reported by one of these groups more than 15 years ago allowed for the facile preparation of a large number of potent and isoform-selective classes of CA inhibitors (CAIs).[1–4] Indeed, an initial drug design strategy based on appending tails of different size, shape or nature to pharmacophores incorporating sulfonamides as zinc-binding group (ZBG),[4–10] as opposed to the ring approach[2] which explored various aromatic/heterocyclic ring systems on which the SO_2NH_2 moiety was bound, afforded CA inhibitors (CAIs) possessing both high affinity and desired pharmacologic properties.[4–10] This approach, based on an 'extension' of the aromatic/heterocyclic scaffolds through the anchoring tails has been thereafter explored for sulfamates, sulfamides, and dithiocarbamates (as alternative ZBGs to the sulfonamide) but also to CAIs incorporating scaffolds belonging to the aliphatic or glycosidic chemical species.[10–12] The advantage of the tail approach over other drug design strategies was thereafter explained at the molecular level, after the report of many X-ray crystallographic structures of adducts of various CA isoforms with such inhibitors. These studies demonstrated that the active site of most CA isoenzymes is a rather large conical cavity in which the Zn(II) ion is positioned at its bottom. The lining of the active site builds two adjacent very diverse halves, one entirely hydrophilic, the opposing one completely hydrophobic,[12] with the highest variability of amino acid residues between the different isoforms on the edge/entrance of the active site. This is exactly the region in which the tails of the inhibitors are usually accomodated,[12–16] explaining why these specific interactions between the inhibitor tail and amino acid residues at the entrance of the active site may lead to

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compounds showing selectivity for inhibiting isoforms with pharmacological applications.[1–4] In this way many sulfonamides/sulfamates/sulfamides/dithiocarbamates with excellent CA IX/XII over CA I and II selectivity were reported,[16] whereas the coumarins, which bind exclusively in this outward region of the active site, showed the highest isoform selectivity among all CAI classes reported to date.[17–24]

Here we report a new series of sulfonamide CAIs obtained by applying the tail approach. Our interest was to obtain compounds with even longer tails than the ones we reported earlier, in order to investigate whether prolonging the scaffold towards the outside part of the enzyme active cavity may have interesting consequences for the inhibition profiles of such sulfonamides against Ca isoforms with applications in pharmacology, such as CA I and II (cytosolic isoforms) as well as CA IX and XII (trans-membrane, tumor-associated isoforms).

2. Results and discussion

2.1. Chemistry

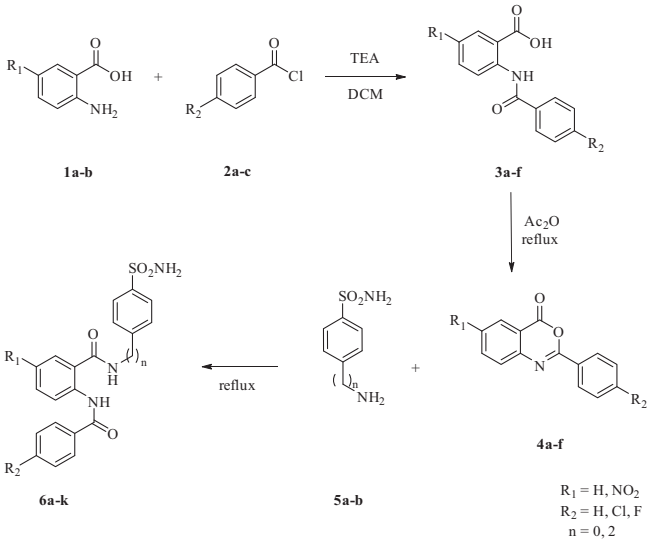
Aromatic benzene sulfonamides (sulfanilamide and 4-aminoethylbenzenesulfonamide) have been used in the present drug design as main scaffolds as they led to CA IX/XII-selective CAIs in several tail-approach studies.[15,24] Furthermore, the amino moiety present in these two compounds has a good reactivity and it is easily derivatizable by reaction with a variety of derivatives.

Several synthetic strategies have been thus employed for prolonging the scaffold of these aromatic sulfonamides in order to obtain long-tailed CAIs, as shown in Schemes 1–3.

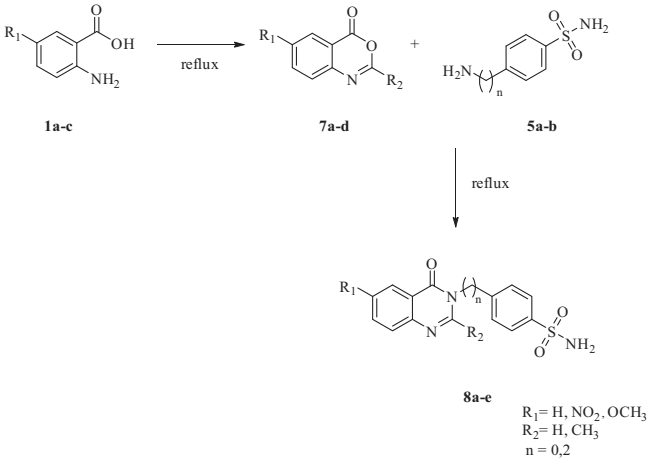
The anthranilic acid derivatives **1** were chosen for the first approach, due to the fact that the amino moiety in *ortho* to the carboxylic acid has a good reactivity with acyl halides, can participate in cyclization reactions and in addition, diamides with the geometry shown in Scheme 1 were not investigated earlier. Thus, 2-amino-5-substituted benzoic acids **1a**,**b** were reacted with 4-substituted-benzoyl chlorides **2a–c** leading to intermediates **3a–f** which were thereafter cyclized in the presence of acetic anhydrides to the key intermediates **4a–f**, which by reaction with sulfanilamide (**5a**) or 4-aminoethylbenzenesulfonamide (**5b**) led to a first group of long-tailed sulfonamides **6a–k** incorporating diamide functionalities (Scheme 1).

For the second approach, again anthranilic acid derivatives **1** were considered, but this time in such a way as to obtain cyclic compounds in which the amino and carboxylic moieties of the starting material are involved. Again such derivatives were not investigated earlier as CAIs. Condensation of 2-amino-5-substituted benzoic acids **1a–c** with acetic anhydride or triethylorthoformate afforded the cyclic intermediates **7a–d** which were subsequently reacted with the two sulfonamides mentioned above, **5a**,**b**, leading to the second group of sulfonamides reported here **8a–e**, incorporating 4-oxoquinazolin-3(4*H*)-yl tails in their molecules (Scheme 2).

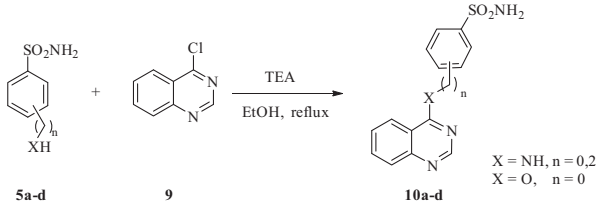
The last synthetic approach is shown in Scheme 3. In this case we used as model reaction the one between cyanuryl chloride and amino-sulfonamides,[15b] by which we have reported a large series of highly isoform-selective CAIs. Indeed, the chlorine atoms from cyanuryl chloride and the chlorine from **9** have a good reactivity with various nucleophiles, leading thus to chemical diversity by a facile synthetic approach. Thus, sulfanilamide, 4-aminoethyl-benzenesulfonamide or 4-hydroxybenzenesulfonamide were treated with 4-chloroquinazoline **9**, leading to the 4-substituted quinazolines **10a–d** (Scheme 3). Compounds **10a** and **10b** are also commercially available from Aurora Screening library, but we prepared them by the procedure illustrated above.



Scheme 1. Preparation of 2-benzamido-*N*-(4-sulfamoylphenyl)benzamides **6a–k**.



Scheme 2. Preparation of 4-(4-oxoquinazolin-3(4*H*)-yl)benzenesulfonamides **8a–e**.



Scheme 3. Preparation of 4-substituted quinazolines **10a–d**.

The sulfonamides investigated here as CAIs were characterized by physico-chemical and spectroscopic methods which confirmed their structures (see Section 4).

2.2. Carbonic anhydrase inhibition

The sulfonamides **6a–6k**, **8a–8e** and **10–10d** reported here were investigated for their enzyme inhibitory action against four physiologically relevant CA isoforms, the human (h) hCA I, II, IX and XII (Table 1). Acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide) was used as standard drug in the assay.[25]