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New hydroxypyrimidinone-containing sulfonamides as carbonic anhydrase inhibitors also acting as MMP inhibitors

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ABSTRACT

A set of benzenesulfonamide (BSA) derivatives bearing a hydroxypyrimidinone (HPM) moiety were synthesized and investigated for their inhibitory activity against several carbonic anhydrase (CA, EC 4.2.1.1) isozymes. They all revealed to be very potent inhibitors (nanomolar order) of the cytosolic CA I and II isozymes, but especially of the transmembrane, tumor-associated CA IX isozyme, a beneficial feature for a potential antitumor effect of these compounds. Further structure optimization aimed at improving the specificity of CA inhibition and enhancing their matrix metalloproteinase (MMP) inhibitory activity may also lead to new compounds with an attractive dual mechanism of action as antitumor agents.

Several isoforms of carbonic anhydrases (CAs, EC 4.2.1.1) are considered important targets for the design of inhibitors with clinical applications, namely for anti-glaucoma, anti-epileptic, and obesity treatments, or even in oncology for tumor control and imageology.[1–4] Among them, the transmembrane tumor-associated isozyme IX and the cytosolic isozymes I and II have been the object of major interest as druggable targets. Examples of potent CA inhibitors are acetazolamide (AAZ) and ethoxzolamide (EZA), compounds in clinical use, or indisulam (IND), in phase II clinical trials as anticancer agent (see Fig. 1). All of these CA inhibitors contain a terminal primary sulfonamide (RSO_2NH_2, where R is generally an aromatic/heteroaromatic moiety), as the zinc-binding group (ZBG).[3] The wide range of pharmaceutical applications of this class of compounds justifies its continuing research to overpass side-effects or even to get further improvements in terms of bioavailability, specificity, or eventual adjuvant effects.[5,6]

The conjugation of hydroxamic (HA) and benzenesulfonamide (BSA) moieties in the same molecular entity, for dual inhibition of matrix metalloproteinases (MMPs) and CAs, has been recently reported and its potential pharmacologic interest has been outlined, since some members of MMP family are also known to be involved in carcinogenesis and tumor progression processes.[7] Although the HA moiety (CONHOH) has been recognized as one of the strongest ZBGs for the MMP inhibition (MMPi), there are some drawbacks associated to its metabolic lability.[1b] Thus, there is a recent search trend for effective MMP inhibitors with nonhydroxamic ZBGs, including a variety of heterocycles, such as hydroxypyrone/-pyridinones[8,9] and pyrimidinetrione compounds.[10]

We have recently reported a small set of simple hydroxypyrimidinones (1-hydroxy-2(1*H*)-pyrimidinones) (HPM), a kind of endocyclic secondary HA, which proved to be more potent MMPi (micromolar range)[11] than simple primary HA (millimolar range).[12] That feature, together with the remarkable inertness of these compounds to the in vivo metabolization, make HPM as potential pharmacophore targets as alternatives to HAs for MMPi. Thus, we have decided to explore new molecular entities, incorporating hydroxypyrimidinone (HPM) and BSA moieties, and to study their biological potential. In this Letter, we report the synthesis of a small series of such novel bifunctional derivatives (Fig. 1, compounds **1**–**4**), as well as their inhibitory properties against the cytosolic and tumor-associated carbonic anhydrase isozymes I, II and IX. All these compounds contain the two key functions, BSA and HPM, separated by different linkers, that mainly differ on the chain-size (5–9 atom chain) and positioning of the amide linkage between both functional groups. The rationalization of their CA inhibitory profiles is aided by comparison with the reference inhibitors and a model compound, **5**, as well as by modeling studies.

Abbreviations: BSA, benzenesulfonamide; HPM, hydroxypyrimidinone; CA, carbonic anhydrase; MMP, matrix metalloproteinase; ZBG, zinc-binding group; HA, hydroxamic acid.

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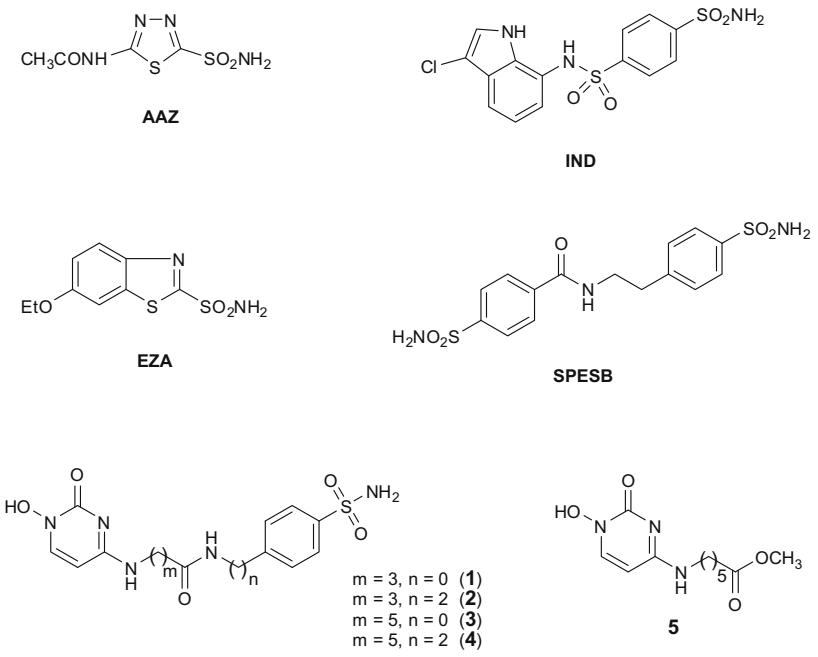

Figure 1. Reference CA inhibitors (AAZ, EZA, IND, and SPESB), and the new compounds (**1**–**5**).

The target compounds (**1**–**4**) were synthesized by a multi-step procedure, as depicted in Scheme 1.[13] In the first step, *N*-protected amino acids **6**–**7** were coupled[14] with the commercially available sulfonamides **8**–**9** using 1-hydroxybenzotriazole hydrate (HOBt) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl) as coupling reagents to generate compounds **10**–**13**. Then, the benzyloxycarbonyl (Cbz) protecting group was removed by catalytic hydrogenation to afford intermediates **14**–**17**. In the next step these compounds were reacted with 1-(benzyloxy)-4-(1′,2′,4′-triazol-1′-yl)-2-(1*H*)-pyrimidinone **18**, a hydroxypyrimidinone derivative previously described,[11,15] to afford the *O*-protected hydroxypyrimidinone-sulfonamide ligands **19**–**22**. Finally, removal of the benzyl protecting group by catalytic hydrogenation afforded the final compounds **1**–**4**.

The model compound **5** was synthesized by the same procedure, starting with the reaction of the hydroxypyrimidinone derivative **18** with 6-aminohexanoic acid methyl ester hydrochloride[16] and potassium carbonate to obtain the *O*-protected hydroxypyrimidinone, 4-[5-(methyloxycarbonyl)-pentylamino]-1-(benzyloxy)-2(1*H*)-pyrimidinone **23**, which was then deprotected by catalytic hydrogenation to afford ligand **5.**

The new HPM–BSA conjugates (**1**–**4**) were tested for their inhibitory activities towards a set of physico-pathologically relevant human CAs (the ubiquitous cytosolic CA I and II, and the tumor-associated isoform CA IX), as previously reported.[7] All these hybrid compounds presented high inhibitory activities (Table 1), with K_I in low nanomolar range (3.4–31 nM), although acting more efficiently against CA IX, a beneficial feature for antitumor effects, mainly for hypoxic tumors that overexpress this isozyme. Regarding CA I, although this enzyme is usually one of the least inhibited by the sulfonamides,[3,17] in our case, the inhibitory activities for CA I and II are quite similar. As compared with the reference inhibitors (i.e., AAZ, IND, EZA, and *N*-(4-sulfamoylphenylethyl)-4-sulfamoylbenzamide (SPESB), with K_I values ranging from 250 to 25 nM), in general, our compounds revealed higher activities, although with similar values. Regarding CA II, **3** was the most active compound (K_I = 13 nM), while **1** was the less active (K_I = 31 nM). For this enzyme, the variation in inhibitory activities was higher than for CA I. This fact suggests that, probably, CA II has more specific interactions or hindrances with these inhibitors than CA I, allowing a higher differentiation on the stability of their protein–ligand complexes. As compared with the reference inhibitors, our compounds showed in general slightly lower activities against CA II.

Concerning the inhibition of CA IX, all our compounds displayed very strong activities, with K_I values ranging from 3.4 to 8.5 nM. These values are below the ones observed for the reference inhibitors, including the one with closest structural similarity, SPESB (K_I value of 18 nM), or even IND, the inhibitor in phase II clinical trials against cancer (K_I value of 24 nM). The CA IX/II selectivities are determinant features for the interest of these new compounds, and, unlike the reference compounds, they demonstrated to be more selective for inhibiting the cancer-associated isozyme CA IX than the ubiquitous CA II. These selectivities ranged from 1.5 (for compound **3**) to 9.2 (for **1**), thus quite convenient for an eventual cancer therapy.

Since the studied compounds include two functional groups that potentially may bind the zinc (the HPM and BSA), we have also studied a model compound, **5** (containing only the HPM). Thus, by testing such compound without the BSA (the preferred ZBG in the case of the CAs), we envisage to get some insight on eventual competition between the HPM and the BSA moieties for the binding to the catalytic zinc. The low inhibitory activities observed for **5** (with K_I 43 and 270 μM for CA I and II, respectively, and higher than 1 μM for CA IX) indicate that this chelating group hardly interacts with the metal ion in the active site of the CAs, thus being unable to block their activity. The inhibitory activity against CA II is comparable but slightly lower than that found for acetohydroxamic acid (K_I = 47 μM),[18] most probably because the monodentate coordination to the Zn(II) ion by the primary hydroxamate moiety (deprotonated at the nitrogen atom) is hampered in the present case, because the HPM is a secondary HA. Although the inhibition activity found for this HPM derivative is also in the same range of that found for phenolic inhibitors, a different metal-binding type should also be involved. In fact, the phenol–(metal–enzyme) interaction involves the OH moiety hydrogen-bonded to the zinc-bound water/hydroxide ion,[19] but the hydroxylic proton is quite more acidic in HPM than in phenol (p*K*a ca. 7 and 10, respectively),[20,21] thus suggesting that the HPM–(metal–enzyme) interaction should