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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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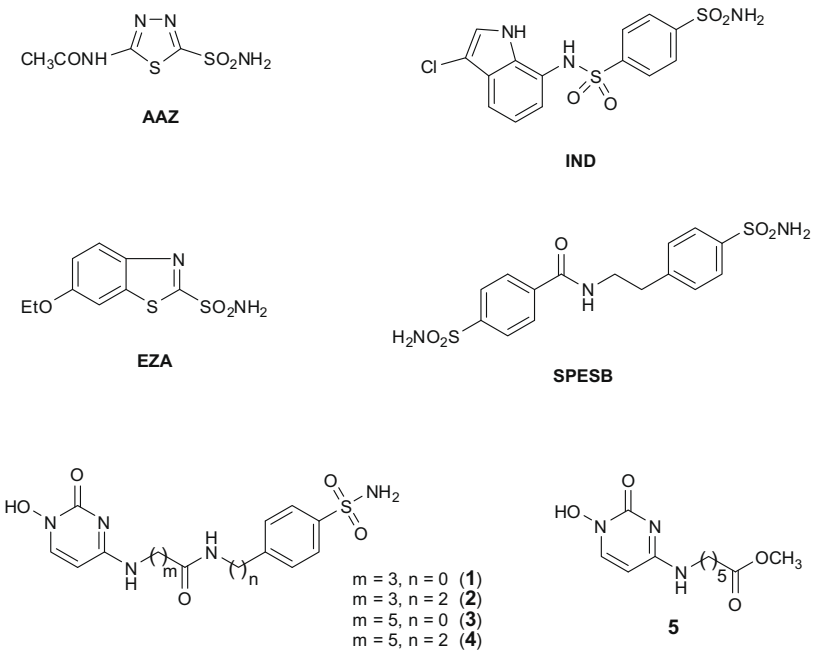


Figure 1. Reference CA inhibitors (AAZ, EZA, IND, and SPESB), and the new compounds (**1**–**5**).

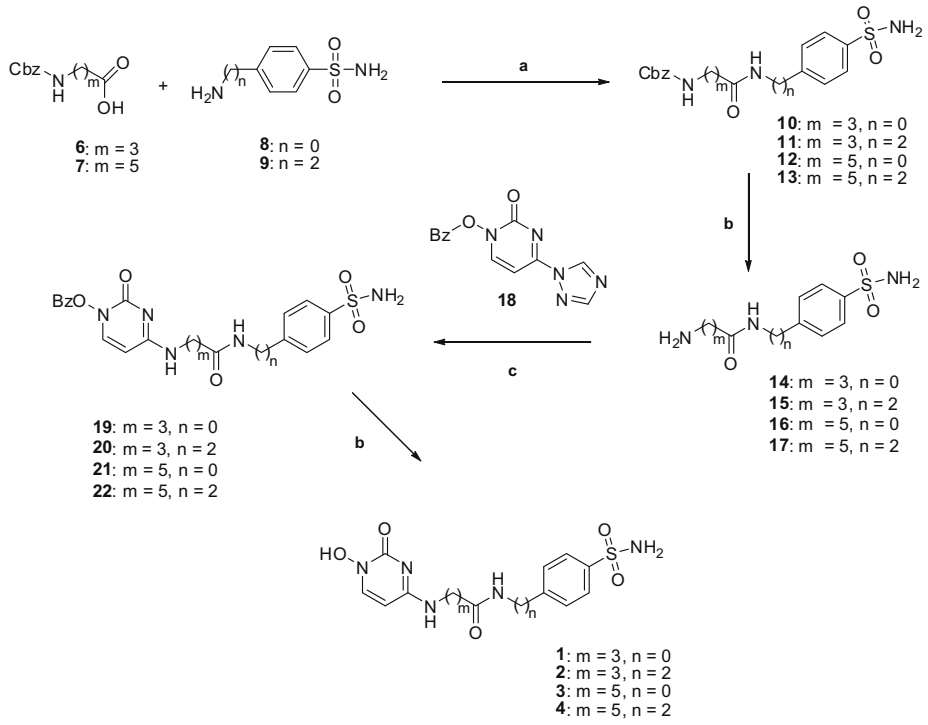
The target compounds (**1**–**4**) were synthesized by a multi-step procedure, as depicted in Scheme 1.[13] In the first step, *N*-protected amino acids **6**–**7** were coupled[14] with the commercially available sulfonamides **8**–**9** using 1-hydroxybenzotriazole hydrate (HOBt) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide 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



Scheme 1. Reagents and conditions: (a) HOBt, EDC·HCl, CH_2Cl_2, DMF, −10 °C to rt; (b) 10% Pd/C, H_2, MeOH, rt; (c) THF, reflux.

Table 1
Inhibitory activities of the target compounds (**1–4**) towards human CA I, II, and IX, compared with the model compound **5** and the reference inhibitors

Compound[a]			K_I (nM)			Selectiv.
	m	n	CA I	CA II	CA IX	CA IX/II
1	3	0	23	31	3.4	9.2
2	3	2	22	28	3.5	8.1
3	5	0	27	13	8.5	1.5
4	5	2	22	25	4.1	6.2
5			4.3×10^4	2.7×10^5	>1000	
AAZ			250	12	25	0.48
EZA			25	8	34	0.25
IND			31	15	24	0.62
SPESB			40	5	18	0.28

[a] See Figure 1.

involve the anionic O-atom. Thus, for the BSA/HPM conjugates the interaction with Zn(II) at the active site of the CAs should occur through the BSA moiety. However, further favorable interactions between the HPM group and other regions of these enzyme active site should occur, in order to explain the high inhibitory activities evidenced by these new compounds towards the tested enzymes. Moreover, since the mechanism of the new CA inhibitors seems to be determined by the sulfonamide moieties, they are most probably reversible noncompetitive inhibitors as well.[2,22]

The interaction of these conjugates with MMPs was not studied herein because, based on preliminary studies with a series of simple HPM model compounds with a set of MMP isoforms (MMP-2, -7, -9, -14), the inhibitory activities were found to be in the micromolar range (see Table S1 of Supplementary data).[11] For the present set of compounds, no further structural modifications were made for enhancing their binding to the MMPs, thus identical order of magnitude is expected for their MMP inhibitory activities.

Molecular modeling studies were performed aimed at getting some rationalization of the inhibitory activities observed for our compounds. The models were docked into the crystal structures of CA II and CA IX (respectively, entries 1G54 and 3IAI of the RCSB Protein Data Bank),[23] using the GOLD software,[24] following a previously validated method.[25] The docking results showed a major binding feature, common to all ligands, which is due to the good accommodation of the benzenesulfonyl moiety into the active site of the CAs, and the coordination of catalytic zinc ion by the anionic N-atom of the sulfonamide. Two H-bonds are also formed, one between its NH group and the hydroxyl group of Thr198 (numeration according to the hCA II structure of UniProtKB database, entry P00918),[26] and another one between its sulfonyl oxygen and the backbone NH moiety of the same residue. In Figure 2 are displayed some of the docking results for CA II, which analysis clearly evidences that all these compounds bind in very similar manners. The benzene ring of the BSA moiety interacts at the hydrophobic surface of the enzyme, namely with the residues Leu197, Val142 and Val121. The rest of the molecule is extended outwards the active site, but, unexpectedly, it has the spacer bent over, which allows the HPM moiety to interact with the hydrophilic part of the cavity. The HPM moiety of the different ligands may form at least one H-bond, namely through the carbonyl O-atom or the OH groups. For **1** and **3**, this H-bond is established between the HPM-carbonyl and the hydroxyl group of Thr199, while for **2** and **4** that H-bonding involves the HPM-hydroxyl and the side-chain carbonyl group of Asn67. In the case of **3**, an additional H-bond (which is not seen for the other compounds) is established between the HPM-NH group and the side-chain carbonyl of Asn67. This extra H-bond may explain the highest inhibitory activity towards CA II, observed for compound **3**. In addition, all the compounds, but **2**, form H-bonds between the carbonyl oxygen atom of the spacer chains (between the HPM and the sulfonamide groups) and the NH_2 group of Gln92.

Concerning the docking with CA IX, the results showed that the binding conformations of the ligands are quite similar to those into CA II, namely the BSA moiety forming the same interactions with the enzyme as mentioned for the CA II, and the HPM group binding

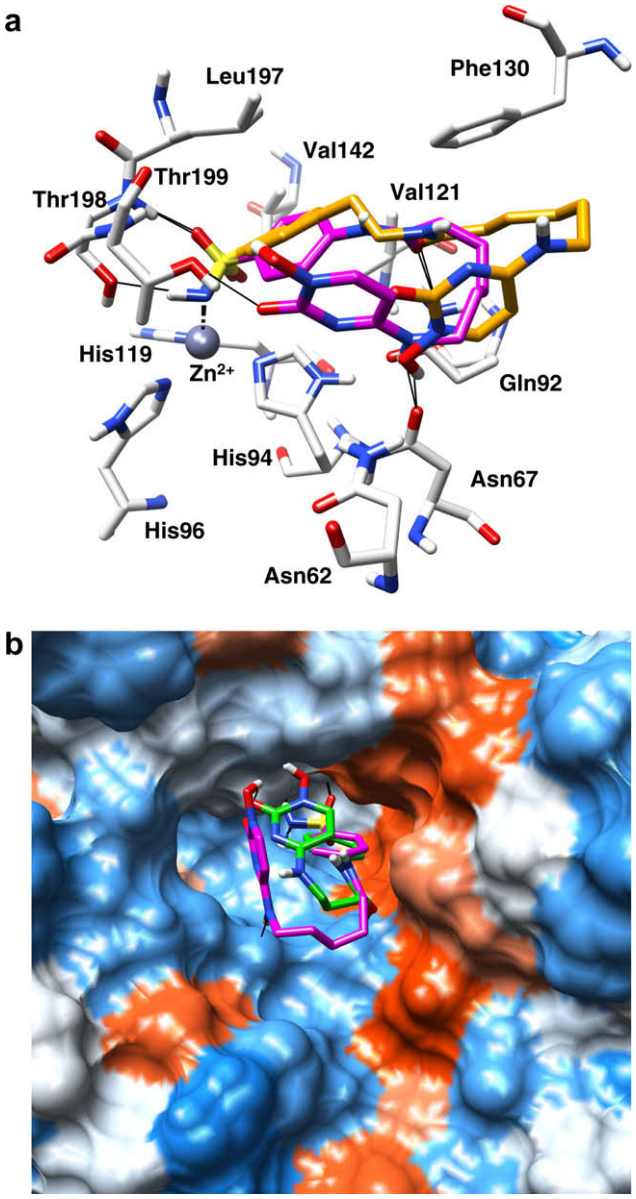


Figure 2. Docking of compounds into the CA II active site: **3** (magenta) superimposed with **4** (orange) (a), and **1** (green) superimposed with **3** (magenta), with the protein surface displayed (b). Red surfaces represent the hydrophobic and blue the hydrophilic regions of the protein; black dashed lines represent the ligand coordination to the metal (gray sphere), and the full black lines represent the H-bonds formed with the protein.

with the hydrophilic part of the protein (see Fig. 3). All the ligands form an H-bond with the side-chain NH_2 group of Gln224, through the carbonyl group of the amide spacer (hCA IX numeration, entry Q16790 of UniProtKB database). In all cases, the linker chain seems to establish van der Waals interactions with some residues forming the hydrophobic part of the active site, namely Val253, Val262 and Leu272. **1** and **2** are able to form two additional H-bonds with the protein, through the HPM moiety. In the case of **1**, these bonds are formed between the OH groups of the HPM and Thr333, and between its carbonyl group and the NH_2 of Gln224 (Fig. 3). As for compound **2**, these H-bonds are formed with Gln203, between the OH of the HPM with the side-chain carbonyl group, and the carbonyl O-atom of the HPM with the NH_2 group, respectively. Concerning compounds **3** and **4**, these form only one extra H-bond with the enzyme, through the hydroxyl group of the HPM moiety; while for **3** this interaction is with the OH group of Thr333, for **4** it

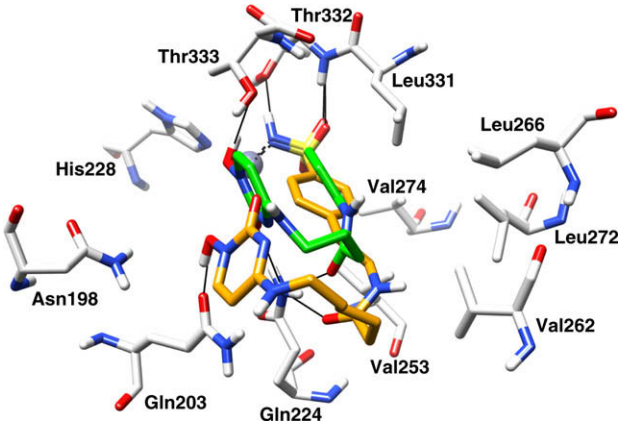


Figure 3. Docking of compounds **1** (green) and **4** (orange) into the CA IX active site. Black dashed lines represent the ligand coordination to the zinc (gray sphere), and the full black lines represent the H-bonds formed with the protein.

is with the side-chain carbonyl O-atom of Gln203. Globally, the docking results seem to have elucidated the high stability of the complexes formed, and consequently give support the high inhibitory activities observed for these compounds. Inhibitor **1** displayed the highest number of interactions with the CA IX, thus resulting in the highest activity observed among this series.

In conclusion, a set of new bifunctional compounds containing HPM and BSA moieties have been synthesized and evaluated for their CA inhibitory activities. These compounds demonstrated to inhibit CA I, II and IX with low nanomolar activity, and showed preference for inhibiting the tumor-associated isozyme CA IX, over the ubiquitous cytosolic CA I and II. The high CA inhibitory activities evidenced for all these compounds are supported by docking modelling studies which showed that, besides the expected strong binding between BSA moiety and catalytic zinc ion at the active site of the CAs, there is an extra interaction between the HPM moiety and the hydrophilic part of these enzymes. Considering the excellent CA inhibitory activities observed against this set of physiologically relevant CAs, these HPM-containing sulfonamides may be useful to further design of new CA inhibitors. These might be endowed with the extra-favorable properties associated with the HPM (ranging from the MMPi activity, metal chelator, and bioavailability), and thus reveal interesting for novel applications in therapy, with eventual dual-drug activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.109.

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13. Experimental procedures and spectral data for the synthesized compounds. The full characterization of all compounds can be found in Supplementary data.

    General procedure for the synthesis of compounds **10–13**: A solution of EDC·HCl (1 mmol) in dichloromethane (3.5 mL) was added to a mixture of the *N*-Cbz-amino acid (**6** or **7**) (1 mmol), sulfonamide (**8** or **9**) (1 mmol) and HOBt (1 mmol) in DMF (1.8 mL) at -10 °C. The mixture was stirred under a nitrogen atmosphere at room temperature for 24 h. After this period, the solvent was removed under reduced pressure and the residue obtained was diluted in water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with 0.5 M citric acid and brine, dried over magnesium sulfate and filtered. The filtrate was evaporated in vacuum to dryness, followed by recrystallization of the residual solid from MeOH/diethyl ether to afford the pure products **10–13**.

    3-(4-Sulfamoyl-phenylcarbamoyl)-propylcarbamic acid benzyl ester (**10**): white crystals, 53% yield, mp = 172–174 °C. FTIR (KBr) ν_{max} 3333, 3363, 2936, 2958, 1694, 1651, 1588, 1531, 1507, 1402, 1345, 1160, 898 cm^{-1}. ^1H NMR (CD_3OD) δ 1.86 (2H, quin, J = 6.9 Hz, $CH_2CH_2CH_2$), 2.42 (2H, t, J = 7.5 Hz, $CH_2C(O)$), 3.19 (2H, t, J = 6.6 Hz, $NHCH_2$), 5.04 (2H, s, CH_2O), 7.72 (2H, d, J = 8.7 Hz, $CHCS(O)_2$), 7.81 (2H, d, J = 9 Hz, HNC*CH*), 7.31 (5H, s, Ar*H*CH_2) ppm. ^{13}C NMR (CD_3OD) δ 26.9 ($CH_2CH_2CH_2$), 35.2 ($CH_2C(O)$), 41.3 ($NHCH_2CH_2CH_2$), 67.5 (CH_2O), 120.4 (2C, HNC*C*H), 128.2 (2C, H*C*CS(O)$_2$), 139.6 (*C*S(O)$_2$), 143.6 (HN*C*CH), 127.7, 128.4, 129.5, 138.5 (6C, Ar*C*CH_2O), 166.9 (O*C*(O)), 174.2 (*C*(O)NHC) ppm.

    General procedure for the synthesis of compounds **14–17**: A suspension of Pd/C 10% (20 mg) in dry MeOH (10 mL) was pre-hydrogenated with H_2 (1 atm) for 30 min. To this suspension, a solution of *N*-Cbz protected compound **10–13** (0.5 mmol) in dry MeOH (10 mL) was added and the mixture stirred under H_2 (1 atm) for 3 h at room temperature. The catalyst was removed by filtration and the solvent evaporated under reduced pressure to afford products **14–17** as white solids which were used in the following steps without further purification.

    N-(4-Sulfamoyl-phenyl)-4-aminobutanamide (**14**): Amorphous white solid, 91% yield. FTIR (KBr) ν_{max} 3362, 3261, 2958, 1668, 1592, 1541, 1400, 1315, 1158, 839, 748 cm^{-1}. ^1H NMR (CD_3OD) δ 1.82 (2H, quin, J = 6.9 Hz, $CH_2CH_2CH_2$), 2.42 (2H, t, J = 7.2 Hz, $CH_2C(O)$), 2.71 (2H, t, J = 6.9 Hz, NH_2CH_2), 7.69 (2H, d, J = 8.7 Hz, $CHCS(O)_2$), 7.78 (2H, d, J = 8.7 Hz, HNC*C*H) ppm. ^{13}C NMR (CD_3OD) δ 28.7 ($CH_2CH_2CH_2$), 35.3 ($CH_2C(O)$), 41.7 (NH_2CH_2), 120.4 (2C, HNC*C*H), 128.2 (2C, HC*C*S(O)$_2$), 139.6 (*C*S(O)$_2$), 143.5 (HN*C*), 174.2 (*C*(O)NH) ppm.

    General procedure for the synthesis of compounds **19–23**: A solution of amide **14–17** (0.58 mmol) and 1-(benzyloxy)-4-(1′,2′,4′-triazol-1′-yl)-2-(1*H*)-pyrimidinone (0.50 mmol) **18** in dry THF (10 mL) was stirred for 24-48 h at reflux temperature, under N_2. Then, the solvent was evaporated under reduced pressure and the resulting residue was purified by CC on silica gel, using a mixture of methanol/dichloromethane (0.1:1) as eluent to obtain the product, which was then recrystallized from methanol/diethyl ether mixtures to afford pure compound **19–22**. Compound **23** was synthesized by the same procedure but using 6-aminohexanoic acid methyl ester hydrochloride instead of an amide and adding 1 equiv of potassium carbonate to neutralize the hydrochloride.

    4-{3-[(4-Sulfamoyl-phenyl)carbamoyl]propylamino}-1-(benzyloxy)-2(1H)-pyrimidinone (**19**): light yellow crystals, 42 % yield, mp 255-257 °C. FTIR (KBr) ν_{max} 3378, 3261, 2958, 2879, 1699, 1633, 1590, 1536, 1504, 1472, 1402, 1320, 1151, 854, 748, 790 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.80 (2H, quin, J = 6.9 Hz, $CH_2CH_2CH_2$), 2.39 (2H, t, J = 7.2 Hz, $CH_2C(O)$), 3.25-3.30 (2H, m, $NHCH_2$), 5.05 (2H, s, CH_2O), 5.51 (1H, d, J = 9 Hz, NHC=C*H*), 7.57 (1H, d, J = 9 Hz, NC*H*=CH), 7.38-7.43 (4H, m, $CHCS(O)_2$ and (O)CHNCC*H*), 7.74 (5H, s, Ar*H*CH_2) ppm. ^{13}C NMR (DMSO-d6) δ 24.5 ($CH_2CH_2CH_2$), 33.9 ($CH_2C(O)$), 39.2 ($NHCH_2$), 77.3 (CH_2O), 93.2 (NCH=*C*H), 118.6 (2C, (O)CHNC*C*H), 126.7 (2C, H*C*CS(O)$_2$), 127.1, 128.0, 129.7, 142.2 (6C, Ar*C*CH_2O), 134.4 (*C*S(O)$_2$), 138.1 ((O)CHN*C*), 142.9 (N*C*H=CH), 152.9 (N*C*(O)N), 162.4 (N=*C*NH), 171.5 (*C*(O)NH) ppm.

    General procedure for the synthesis of compounds **1–5**: Compounds **1–5** were obtained by deprotection of **19–23**, using for removal of the Bz protecting group the procedure already described for the Cbz group.

    4-{3-[(4-Sulfamoyl-phenyl)carbamoyl]propylamino}-1-hydroxy-2(1H)-pyrimidinone (**1**): white solid, 89% yield, mp 140-142 °C. FTIR (KBr) ν_{max} 3272, 2923, 2874, 1636, 1593, 1533, 1403, 1327, 1157, 837, 764 cm^{-1}. ^1H NMR (CD_3OD) δ 1.91 (2H, quin, J = 6.9 Hz, $CH_2CH_2CH_2$), 2.43 (2H, t, J = 7.2 Hz, $CH_2C(O)$), 3.38 (2H, t, J = 6.6 Hz, $NHCH_2$), 5.66 (1H, d, J = 7.2 Hz, NCH=C*H*), 7.56 (1H, d, J = 7.2 Hz, NC*H*=CH), 7.74 (4H, q, J = 11.4 Hz, $CHCS(O)_2$ and (O)CHNCC*H*) ppm. ^{13}C NMR (CD_3OD) δ 26.3 ($CH_2CH_2CH_2$), 35.4 ($CH_2C(O)$), 41.2 ($NHCH_2$), 95.4 (NCH=*C*H), 143.7 (NCH=CH), 120.5 (2C, (O)CHNC*C*H), 128.1 (2C, *C*HCS(O)$_2$), 139.5 (*C*S(O)$_2$), 143.6 (N*C*(O)N), 164.3 (N=*C*NH), 174.2 (*C*(O)NH) ppm. HRMS (ESI) calcd for $C_{14}H_{17}N_5O_5S$+H 368, 10286, found 368, 10349.
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