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Design, solid-phase synthesis, and biological evaluation of novel 1,5-diarylpyrrole-3-carboxamides as carbonic anhydrase IX inhibitors

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ABSTRACT

Following previous studies we herein report the synthesis and the pharmacological evaluation of a new class of human carbonic anhydrase (hCA) inhibitors, 1,5-diarylpyrrole-3-carboxamides prepared by a solid-phase strategy involving a PS(HOBt) resin. A molecular modeling study was conducted in order to simulate the binding mode of this new family of enzyme inhibitors within the active site of hCA IX. This study revealed that the 3-position of the pyrrole was opened to the solvent, so we introduced an amino side-chain, protonated at physiological pH both to enhance the aqueous solubility and to decrease the cell membrane penetration. This strategy consisted of preparing membrane-impermeant inhibitors that may selectively target the tumor-associated hCA IX. Physico-chemical characterizations including aqueous solubility and lipophilic parameters are described. Pharmacological studies revealed high hCA IX inhibitory potency in the nanomolar range. Some compounds are selective for hCA IX displaying hCA I/hCA IX and hCA II/hCA IX ratios higher than 20 and 5, respectively.

1. Introduction

The carbonic anhydrases (CA) are zinc enzymes that catalyze the reversible hydration of carbon dioxide into a bicarbonate anion and a proton ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$).[1] To date, 16 different α-CA isozymes were identified in humans and are distinguished by their localization and their catalytic activity. Many of these isozymes are involved in numerous physiological processes and pathological disorders including edema, glaucoma, obesity, epilepsy.[1] Throughout the last three decades, expression of different carbonic anhydrase isoforms was investigated in various types of cancer cell.[2,3] Functional implication of catalytically active carbonic anhydrases in cancer was revealed after the identification of two transmembrane CAs: CA IX, which is almost exclusively associated with tumors and CA XII, which is expressed in some tumor types. As CA XII is also found in normal cells, it is a less attractive target than CA IX in the cancer therapy. CA IX expression is strongly upregulated by hypoxia, which is a key feature of many tumors.[4–6] The overall consequence of CA IX overexpression in tumors is a pH imbalance leading to the acidification of the extracellular space of hypoxic tumors (pHe ≃ 6), in contrast to normal tissue (pHe ≃ 7.4).[7–9] This low pHe is associated with tumorigenic transformation, chromosomal rearrangements, extracellular matrix breakdown, migration, and invasion.[1–3,6] Acidic pHe is also related to a chemoresistance by a reduced uptake of weakly basic anticancer drugs. The tumor-associated CA IX is thus clearly an attractive target in cancer therapy.[10]

Recently,[11] one of our groups reported that celecoxib **1** (Fig. 1), a COX-2 sulfonamide containing inhibitor, was a strong inhibitor of hCA IX (K_i = 16 nM) as well as hCA II (K_i = 21 nM). The crystal structure of the hCA II-celecoxib adduct demonstrated that this coxib binds with its sulfonamide toward the zinc ion resulting in a tetrahedral coordination, as observed for the numerous sulfonamides acting as CA inhibitors.[12] In a recent work,[13] we described benzylanilinosulfonamides **2** as very potent hCA IX inhibitors (K_i = 1.8–27 nM). Although very promising as hCA IX inhibitors, these compounds revealed to be poorly selective when compared to the ubiquitous hCA II (K_i = 17–49 nM), and they are characterized by two chiral centers which are certainly not advantageous for further drug development.

With respect to these results, we designed novel constrained derivatives **3a–3e** based on the benzylanilinosulfonamide scaffold, and including a central pyrrole ring, isoster of the pyrazole present in celecoxib (Fig. 1).

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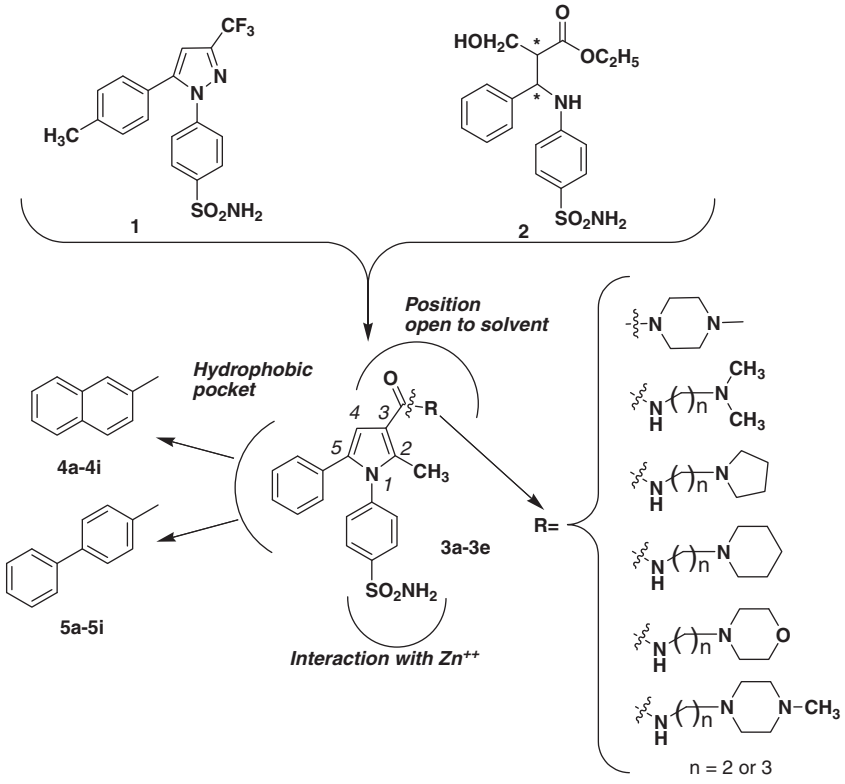


Figure 1. Design of 4-(5-aryl-2-methyl-pyrrol-1-yl)-benzenesulfonamides from the coxib scaffold and from the benzylanilinosulfonamides described as hCA IX inhibitors.

As the 3D-coordinates of the catalytic domain of hCA IX were available,[14] the binding conformation of this novel 1-(4-aminosulfonylphenyl)-2-methyl-5-phenyl-1*H*-pyrrole template was obtained by docking in the active site of hCA IX (Fig. 2). Interestingly, this study revealed that the phenyl ring in the 5-position of the pyrrole partly fills a hydrophobic pocket bordered by residues Pro-202, Leu-198, Val-131, and Val-121 in CA IX. In order to better accommodate this lipophilic pocket, we suggested the introduction of bulkier hydrophobic moieties such as a 2-naphthyl (**4a–4i**) or a 4-biphenylyl (**5a–5i**) in this position.

The study also revealed that the 3-position of the pyrrole was opened to the solvent, and thus adequately oriented for the introduction of an additional residue. For this position, we selected an amino side-chain, protonated at physiological pH, to decrease the cell membrane penetration, thus preventing the inhibition of cytosolic hCAs I and II which are ubiquitous. On the contrary, the active site of hCA IX is located on the external side of the cell membrane. This strategy consisting of preparing membrane-impermeant inhibitors that may selectively target the tumor-associated hCA IX was already applied.[15] Moreover, the presence of an amino side-chain in position 3 could improve the drug solubility.

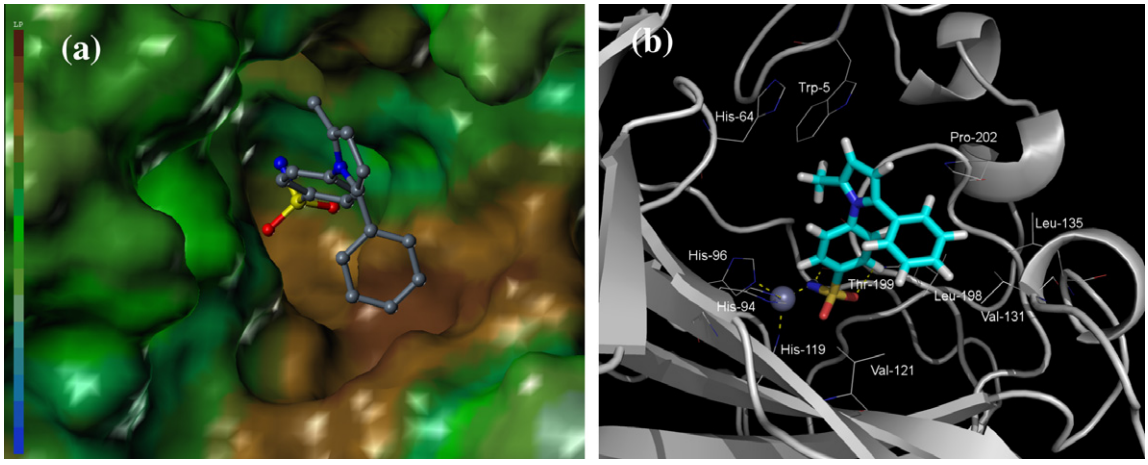


Figure 2. View of the 1-(4-aminosulfonylphenyl)-2-methyl-5-phenyl-1*H*-pyrrole template inside the hCA IX active site with (a) the lipophilic potential mapped onto the Connoly surface (brown: high, green: medium, and blue: low lipophilicity) and (b) secondary structure and main active site residues. The zinc ion is represented by a sphere. Pictures made using (a) Molcad and (b) Pymol, respectively.

2. Results and discussion

2.1. Chemistry

The synthetic pathway to obtain the carboxylic acids **9a–9c** is outlined in Scheme 1. Reaction of ethyl acetoacetate with sodium hydride in THF, followed by addition of suitable α-bromomethylketones **6a–6c** gave the 1,4-diketones **7a**–**7c** with a good yield (61–84%).[16] The pyrrole cyclization step was performed following the usual Paal–Knoor cyclization between the 1,4-diketones **7a**–**7c** and 4-aminobenzenesulfonamide in the presence of a catalytic amount of *p*-toluenesulfonic acid in refluxing EtOH, to afford 1-[4-(aminosulfonyl)phenyl]-5-aryl-2-methyl-1*H*-pyrrol-3-carboxylic ethyl esters **8a–8c** (yield: 73–83%). The hydrolysis of the ethyl esters **8a–8c** with sodium hydroxide in refluxing EtOH yielded the corresponding carboxylic acids **9a–9c** (yield: 78–94%).

To introduce a large chemical diversity among the 1-[4-(aminosulfonyl)phenyl]-5-aryl-2-methyl-1*H*-pyrrol-3-carboxamides, a solid-phase strategy involving a PS(HOBt) resin as coupling agent was developed on a Quest 205® synthesizer (Scheme 2). The choice of the activating agent is crucial, because we have already demonstrated that the sulfonamide moiety can react with HBTU (*O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate) during the amidification reaction, leading to the formation of stable tetramethylsulfonylguanidines.[17] Another procedure for loading PS-HOBt with carboxylic acids uses PyBroP as coupling reagent.[18] In our case, the use of diisopropylcarbodiimide (DIC) and 4-dimethylaminopyridine (DMAP) was preferred: indeed, it requires fewer equivalents of carboxylic acid because a double loading of the resin is not required differently from PyBroP.[19] This method is a two steps procedure: (a) formation of resin-bound active esters of PS-HOBt using the coupling process with DIC/DMAP ('catch'); (b) amide formation is effected by mixing the PS-HOBt ester resin (previously purified by washing sequences) with a mix of amine (in default) and DIEA in dichloromethane ('release'). The best conditions for coupling were 8 h at room temperature.

The obtained amides were easily purified by flash chromatography or single crystallization. This pathway afforded the phenyl **3a–3e**, the 2-naphthyl **4a–4i** and the 4-biphenylyl **5a–5i** derivatives with moderate to good yields (30–86%) (Table 1).

2.2. Carbonic anhydrase inhibition

The inhibitory potency (K_i, nM) of all the derivatives was evaluated against the tumor-associated hCA IX and against the ubiquitous and physiologically relevant hCA I and hCA II (Table 1). The isozyme selectivity towards hCA IX was expressed as the hCA I/hCA IX and hCA II/hCA IX K_i ratios.

From the inhibition data reported in Table 1, we observed that all the compounds exhibit a weak inhibitory activity against hCA I. The K_i values of the R1-phenyl derivatives **3a**–**3e** were found between 781 and 2151 nM as observed for the R1-naphthyl molecules **4a**–**4i** (427–10,000 nM). The 4-biphenylyl derivatives **5a**–**5i** were also weak hCA I inhibitors with K_i values higher than 1736 nM. This weak inhibitory potency may be explained by the fact that the hCA I active site is more restricted due to the presence of a His200 residue.[1,20,21]

All the synthesized molecules were found much more potent against hCA II (K_i: 9–620 nM) than against hCA I. The phenyl **3a**–**3e** and the 4-biphenylyl **5a**–**5i** derivatives were moderate hCA II inhibitors with K_i values ranging from 127 to 620 nM. The best hCA II inhibitors ($K_i \leqslant 70$ nM) belonged to the R1-naphthyl series (**4a**–**4e** and **4h**).

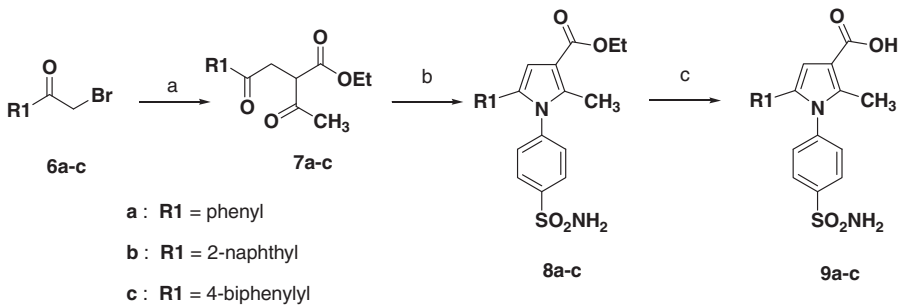
Against the hCA IX isozyme, the newly synthesized molecules showed a powerful inhibitory potency (K_i: 10–242 nM). Among the R1-phenyl (K_i: 89–242 nM) and the R1-biphenylyl (K_i: 68–207 nM) derivatives, the nature of the R2-side-chain poorly influenced the hCA IX inhibitory potency, differently from the R1-naphthyl compounds (K_i: 10–147 nM). Indeed, the most potent hCA IX inhibitors are the 2-naphthyl compounds **4a**, **4b**, **4d**, and **4h** (K_i: 10–22 nM) whereas **4c**, **4e**–**4g**, and **4i** are less active on hCA IX (K_i: 125–147 nM).

Docking of **4a**, the most potent hCA IX inhibitor identified, inside the hCA IX binding cleft (Fig. 3) revealed a binding orientation very similar to the one initially reported for the 1-(4-aminosulfonylphenyl)-2-methyl-5-phenyl-1*H*-pyrrole template (Fig. 2). In this orientation, **4a** is found to be deeply inserted in the hCA IX cavity, with its naphthyl group perfectly fitting the hydrophobic pocket. This might explain, in part, the generally weaker inhibitory potency of the phenyl (**3a–3e**) or the biphenylyl (**5a–5i**) that are, respectively, too small or too bulky to adequately fit this pocket. As expected, the amino side-chain was found in a position open to the solvent with the carboxamido function H-bonded to the Trp-5 NH.

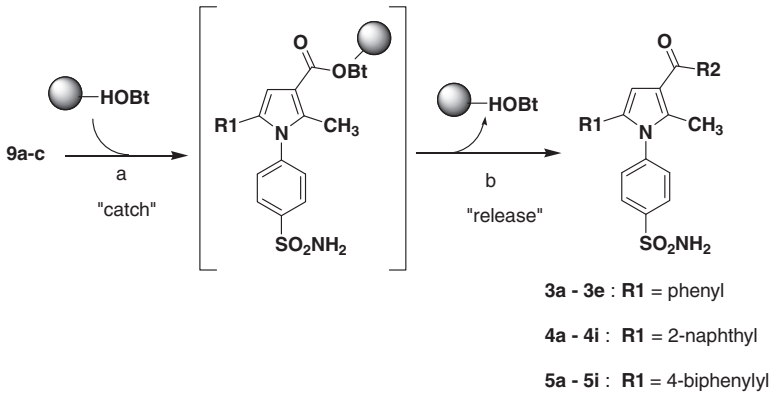
As far as hCA IX selectivity is concerned, all the molecules were much more potent on hCA IX than hCA I and had a moderate to high hCA I/hCA IX K_i ratio ranging from 4 to 246. Regarding the hCA II/hCA IX selectivity all the molecules, except the R1-naphthyl compounds **4c**–**4e**, were moderately selective inhibitors of hCA IX (hCA II/hCA IX K_i ratio $\geqslant 1$). The R1-naphthyl **4a** and the R1-biphenylyl derivatives **5a–5c**, **5i** were the most selective hCA IX inhibitors characterized by hCA I/hCA IX and hCA II/hCA IX ratios higher than 20 and 5, respectively. Among these five selective hCA IX inhibitors, **4a** (K_i: 10 nM) was 7- to 11-fold more potent on hCA IX than the other inhibitors.

2.3. Lipophilicity and solubility

In drug development, lipophilicity and solubility are important physico-chemical parameters which strongly influence the bioavailability. The lipophilicity was theoretically determined with ACD software,[22] and expressed as the logarithm of the distribution



Scheme 1. Reagents and conditions: (a) (i) NaH, ethyl acetoacetate, anhydrous THF, 0 °C, 30 min; (ii) α-bromomethylketone **6a–6c**, 0 °C, 2 h then rt, 8 h; (b) 4-aminobenzenesulfonamide, *p*-toluenesulfonic acid, EtOH, reflux, 24 h; (c) NaOH, EtOH, reflux, 24 h.



Scheme 2. Reagents and conditions: (a) PS-HOBt(HL), DIC, DMAP, CH_2Cl_2/DMF (80/20), rt, 3 h. (b) R-NH_2, or *N*-methylpiperazine, DIEA, CH_2Cl_2, rt, 8 h.

coefficient (log *D*) calculated at physiological pH (7.4) (Table 1). The log *D*7.4 of all the synthesized pyrroles was ranging from 0.64 to 3.52. The replacement of the R1-phenyl by a 2-naphthyl increased the log *D*7.4 value (Δ log *D*7.4 = +1.12 to +1.19; compare **3a**–**4a**; **3b**–**4b**; **3c**–**4c**; **3d**–**4e**; **3e**–**4h**). A higher log *D* increase (Δ log *D*7.4 = +1.42) was observed when the R1-phenyl is replaced by 4-biphenylyl moiety (compare **3a**–**5a**; **3b**–**5b**; **3c**–**5c**; **3d**–**5e**; **3e**–**5h**). Surprisingly, when the *N,N*-dimethylaminoethylamino side-chain was replaced by the more lipophilic *N,N*-dimethylaminopropylamino moiety, the log *D*7.4 strongly dropped (Δ log *D*7.4 = −1.1; compare: **3a**–**3b**; **4a**–**4b**; **5a**–**5b**) whatever the nature of R1. This result was attributed to the increase of the calculated pK_a value (from 8.37 to 9.41, ACD software[22]) of the *N,N*-dimethylamino function. Indeed, the insertion of a supplementary methylene group reduced the impact of the carboxamido function on the dimethylamino moiety. So, this pK_a increase enhanced the cationic form of the molecule at pH 7.4, and reduced its log *D*7.4, as well as its solubility. Indeed, the theoretical solubility at pH 7.4 of the *N,N*-dimethylaminoethylamino derivatives was 13-fold lower than their *N,N*-dimethylaminopropylamino counterparts (compare: **3a**–**3b**; **4a**–**4b**; **5a**–**5b**).

The ACD program calculated the drug solubility at pH 7.4 (Table 1).[22] For an identical R2 side-chain, the theoretical solubility at pH 7.4 decreased with the following sequence: phenyl > 2-naphthyl > 4-biphenylyl (compare **3a**–**4a**–**5a**; **3b**–**4b**–**5b**; **3c**–**4c**–**5c**; **3d**–**4e**–**5e**; **3e**–**4h**–**5h**). The R2-*N,N*-dimethylaminopropylamino compounds (**3b**, **4b**, **5b**) were more soluble than their *N,N*-dimethylaminoethylamino counterparts (**3a**, **4a**, **5a**), as expected by their log *D*7.4

3. Conclusion

Combining the structure of celecoxib **1** and benzylanilinosulfonamides **2**, we designed a series of novel 4-(5-aryl-2-methyl-pyrrol-1-yl)-benzenesulfonamides with the aim to obtain selective hCA IX inhibitors. The R1-aryl which interacts with the hydrophobic pocket was a phenyl, a 2-naphthyl or a 4-biphenylyl, while a carboxamidoalkylamino R2-side-chain substituted the pyrrole at the position 3. At physiological pH (7.4), this R2-residue is positively charged, particularly in the case of the *N,N*-dimethylaminopropylamino moiety (calculated pK_a = 9.42 for **3b**, **4b**, and **5b**). This positive charge is reinforced when the extracellular space of tumor cells is acidified by the overexpression of hCA IX and proton transporters. Even a log *D*7.4 >1.5–2.0 is an optimal value for membrane penetration,[23] we assumed that this cationic R2-residue hinders the crossing of the cell membrane, preventing to reach cytosolic CAs, particularly ubiquitous CA I and CA II; this poor cell penetration increased the hCA IX selectivity. The R2-naphthyl derivatives **4a** and **4b** were the most potent hCA IX inhibitors with a K_i value of 10 and 11 nM, respectively (Table 1). They are selective regarding their hCA I inhibitory potency (K_i ratio: 78 and 39, respectively). As far as the hCA II/h CA IX selectivity is concerned, the derivative **4a** is moderately selective for hCA IX (K_i ratio = 5.5), while **4b** is non-selective (K_i ratio = 1.5). Overall **4a** seems to emerge as a lead for further drug development. However, their capacity to cross the cell membrane needs to be assessed on in vitro models prior to selecting either.

4. Experimental section

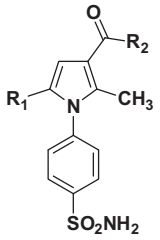
4.1. Chemistry

All commercial reagents and solvents were used without further purification. Tetrahydrofuran (THF) was distilled from sodium/benzophenone prior to use. All reactions were monitored by analytical thin-layer chromatography (TLC) on 0.25 mm precoated Kieselgel 60F_{254} plates (Merck); compounds were visualized by UV (254 and 366 nm) and/or with iodine. Flash chromatography (FC) was performed with silica gel Kieselgel Si 60 0.040–0.063 mm (Merck). Melting points (Mp) were determined with a Büchi 535 capillary melting point apparatus and remain uncorrected. The structures of each compound were supported by IR (neat, FT-Brücker Vector 22 instrument) and by ^{1}H NMR at 300 MHz on a Brucker DPX-300 spectrometer. Chemical shifts (δ) are reported in ppm downfield from tetramethylsilane (TMS), *J* values are in hertz, and the splitting patterns are designed as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br s, broad singlet. The purity of compounds was checked using LC–MS system Thermo Electron Surveyor MSQ. The mass spectra were operated in the atmospheric pressure chemical ionization mode (APCI+). Elemental analyses (C, H, N) were performed on a ThermoFinnigan Flash EA 112 elemental analyser.

4.1.1. Ethyl 2-acetyl-4-oxo-4-phenylbutanoate (7a)

To a suspension of sodium hydride (16.5 mmol, 0.66 g) in tetrahydrofuran (30 mL) cooled at 0 °C was added dropwise a solution of ethyl acetoacetate (15 mmol, 1.89 mL) in THF (5 mL). The reaction mixture was stirred 30 min at 0 °C before adding a solution of 2-bromo-1-phenyl-ethan-1-one **6a** (16.5 mmol, 3.28 g) in THF (20 mL). The solution was stirred 2 h at 0 °C, then 8 h at rt. The reaction was quenched with 1 N HCl solution (30 mL) and extracted with diethylether (3 × 50 mL). The organic layers were combined, dried over $MgSO_4$, filtered and the solvent eliminated under reduced pressure. The residue was purified by FC (heptane/EtOAc; 85:15) to give **7a** as a yellow oil (yield: 84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.02–7.94 (m, 2H), 7.62–7.55 (m, 1H),

Table 1
Inhibitory potency (*K*i, nM), isozyme selectivity ratio data for the R2-phenyl-(**3a**–**3e**), the R2-2-naphthyl (**4a**–**4i**), and the R2-4-biphenylylpyrazoles (**5a**–**5i**) towards hCA I, hCA II, and hCA IX, and calculated log *D*7.4 and solubility



Compd	R1	R2	K_i[a] (nM)			Ratio[b]		Calculated[c]	
			hCA I	hCA II	hCA IX	hCA I/hCA II	hCA II/hCA IX	log *D*7.4	Solubility (μM)
3a	Phenyl	NH-$(CH_2)_2$-N(CH3)2	1691	391	242	7	1.6	1.76	127
3b	Phenyl	NH-$(CH_2)_3$-N(CH3)2	1460	127	131	11	1	0.64	1680
3c	Phenyl	N(CH2CH2)2N–	2151	516	135	16	3.8	1.21	114
3d	Phenyl	NH-$(CH_2)_2$-N(CH2CH2)2CH2	781	286	89	9	3	2.10	101
3e	Phenyl	NH-$(CH_2)_2$-N(CH2CH2)2O	2131	620	200	11	3	1.75	38
4a	2-Naphthyl	NH-$(CH_2)_2$-N(CH3)2	781	55	10	78	5.5	2.88	15
4b	2-Naphthyl	NH-$(CH_2)_3$-N(CH3)2	427	16	11	39	1.5	1.82	201
4c	2-Naphthyl	N(CH2CH2)2N–	>10,000	10	133	>75	0.07	2.40	13
4d	2-Naphthyl	NH-$(CH_2)_2$-N(CH2CH2)2CH2	5940	10	30	198	0.3	2.22	79
4e	2-Naphthyl	NH-$(CH_2)_2$-N(CH2CH2)2CH2	479	9	125	4	0.1	3.29	13
4f	2-Naphthyl	NH-$(CH_2)_2$-N(CH2CH2)2N–	2489	196	126	20	1.6	2.72	9
4g	2-Naphthyl	NH-$(CH_2)_3$-N(CH2CH2)2N–	1751	243	147	12	1.7	2.71	12
4h	2-Naphthyl	NH-$(CH_2)_2$-N(CH2CH2)2O	5412	70	22	246	3.2	2.94	5
4i	2-Naphthyl	NH-$(CH_2)_3$-N(CH2CH2)2O	1435	256	137	11	1.9	2.93	6
5a	4-Biphenylyl	NH-$(CH_2)_2$-N(CH3)2	1736	475	83	21	5.7	3.18	11
5b	4-Biphenylyl	NH-$(CH_2)_3$-N(CH3)2	2169	553	111	20	5	2.04	150
5c	4-Biphenylyl	N(CH2CH2)2N–	7085	538	68	104	8	2.63	10
5d	4-Biphenylyl	NH-$(CH_2)_2$-N(CH2CH2)2CH2	6799	604	153	44	4	2.45	61
5e	4-Biphenylyl	NH-$(CH_2)_2$-N(CH2CH2)2CH2	5853	247	167	35	1.5	3.52	10
5f	4-Biphenylyl	NH-$(CH_2)_2$-N(CH2CH2)2N–	4424	518	207	21	2.5	2.95	7
5g	4-Biphenylyl	NH-$(CH_2)_3$-N(CH2CH2)2N–	5542	205	198	28	1	2.94	9
5h	4-Biphenylyl	NH-$(CH_2)_2$-N(CH2CH2)2O	6135	352	187	33	2	3.17	4
5i	4-Biphenylyl	NH-$(CH_2)_3$-N(CH2CH2)2O	3924	574	101	39	5.7	3.16	5

[a] Errors in the range of 3–5% of the reported values from three different assays. Acetazolamide was chosen as the standard reference drug: K_i for hCA I, II, and IX are 250, 12, and 25 nM, respectively).
[b] The K_i ratios are indicative of isozyme selectivity: a weakly selective inhibitor is characterized by a low ratio value.
[c] Calculated at pH 7.4 by ACD Software.

7.50–7.43 (m, 2H), 4.27–4.18 (m, 3H), 3.74 (dd, *J* = 18.4 Hz, 8.2 Hz, 1H), 3.53 (dd, *J* = 18.4 Hz, 5.5 Hz, 1H), 2.44 (s, 3H), 1.29 (t, *J* = 7.2 Hz, 3H). IR (cm^{-1}: neat): 1740, 1716, 1640. LC–MS ($APCI^+$) *m*/*z*: 249 $(M+H)^+$.

4.1.2. Ethyl 2-acetyl-4-(2-naphthyl)-4-oxobutanoate (7b)

The title compound was prepared from 2-bromo-1-(2-naphthyl)-ethan-1-one **6b** and ethyl acetoacetate according to the same procedure than that described for ethyl 2-acetyl-4-oxo-4-phenylbutanoate **7a**. The crude product was purified by FC (toluene/EtOAc; 9:1) and crystallized from diisopropyl ether to afford **7b** as white crystals (yield: 68%). Mp: 64–66 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.49 (s, 1H), 8.05–7.82 (m, 4H), 7.68–7.50 (m, 2H), 4.34–4.20 (m, 3H), 3.85 (dd, *J* = 18.4 Hz, 8.1 Hz, 1H), 3.65 (dd, *J* = 18.4 Hz, 5.9 Hz, 1H), 2.47 (s, 3H), 1.29 (t, *J* = 7 Hz, 3H). IR (cm^{-1}: neat): 1736, 1710, 1680. LC–MS ($APCI^+$) *m*/*z*: 299 $(M+H)^+$.

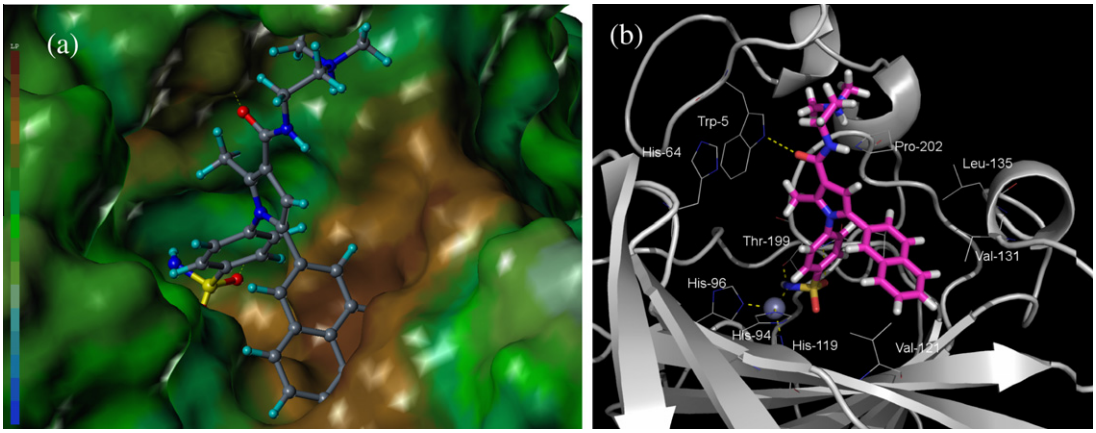


Figure 3. View of 4a inside the hCA IX active site with (a) the lipophilic potential mapped onto the Connoly surface (brown: high, green: medium, and blue: low lipophilicity) and (b) the secondary structure and main active site residues. The zinc ion is represented by a sphere. Pictures made using (a) Molcad and (b) Pymol, respectively.

4.1.3. Ethyl 2-acetyl-4-(4-biphenylyl)-4-oxobutanoate (7c)

The title compound was prepared from 2-bromo-1-(4-biphenylyl)-ethan-1-one **6c** and ethyl acetoacetate according to the same procedure than that described for ethyl 2-acetyl-4-oxo-4-phenylbutanoate **7a**. The crude product was purified by FC (heptan/EtOAc; 8:2) and crystallized from diisopropyl ether to afford **7c** as white crystals (yield: 61%). Mp: 87–89 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.08 (d, J = 8.8 Hz, 2H), 7.71 (d, J = 8.8 Hz, 2H), 7.67–7.62 (m, 2H), 7.53–7.39 (m, 3H), 4.29–4.22 (m, 3H), 3.77 (dd, J = 18.4 Hz, 8.3 Hz, 1H), 3.57 (dd, J = 18.4 Hz, 5.5 Hz, 1H), 2.48 (s, 3H), 1.32 (t, J = 7 Hz, 3H). IR (cm^{-1}: neat): 1737, 1713, 1672. LC–MS (APCI$^+$) m/z: 325 (M+H)$^+$.

4.1.4. Ethyl 1-[4-(aminosulfonyl)phenyl]-2-methyl-5-phenyl-1*H*-pyrrole-3-carboxylate (8a)

A solution of ethyl 2-acetyl-4-oxo-4-phenylbutanoate **7a** (10 mmol, 2.48 g), 4-aminobenzenesulfonamide (10 mmol, 1.77 g) and 4-toluenesulfonic acid (0.01 mmol, 1.9 mg), in absolute EtOH (100 mL) was refluxed for 24 h. After cooling to rt the solvent was evaporated under reduced pressure and the crude product was purified by FC (cyclohexane/EtOAc; 6:4) and crystallized from toluene to give the title compound **8a** as yellow crystals (yield: 83%). Mp: 192–194 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.88 (d, J = 8.6 Hz, 2H), 7.58–7.46 (m, 4H), 7.26–7.14 (m, 3H), 7.07–7.04 (m, 2H), 6.67 (s, 1H), 4.23 (q, J = 7.0 Hz, 2H), 2.33 (s, 3H), 1.29 (t, J = 7.0 Hz, 3H). IR (cm^{-1}: neat): 1666, 1342, 1230, 1168. LC–MS (APCI$^+$) m/z: 385 (M+H)$^+$.

4.1.5. Ethyl 1-[4-(aminosulfonyl)phenyl]-2-methyl-5-(2-naphthyl)-1*H*-pyrrole-3-carboxylate (8b)

The title compound was prepared from ethyl 2-acetyl-4-(2-naphthyl)-4-oxobutanoate **7b** and 4-aminobenzenesulfonamide according to the same procedure than that described for **8a**. The crude product was purified by crystallization (EtOH) to afford **8b** as yellow crystals (yield: 73%). Mp: 195–197 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.88 (d, J = 8.6 Hz, 2H), 7.84–7.78 (m, 1H), 7.77–7.67 (m, 3H), 7.57–7.51 (m, 4H), 7.49–7.43 (m, 2H), 7.11 (dd, J = 8.4 Hz, 1.7 Hz, 1H), 6.86 (s, 1H), 4.26 (q, J = 6.9 Hz, 2H), 2.38 (s, 3H), 1.31 (t, J = 6.9 Hz, 3H). IR (cm^{-1}: neat): 1667, 1337, 1231, 1170. LC–MS (APCI$^+$) m/z: 435 (M+H)$^+$.

4.1.6. Ethyl 1-[4-(aminosulfonyl)phenyl]-2-methyl-5-(4-biphenylyl)-1*H*-pyrrole-3-carboxylate (8c)

The title compound was prepared from ethyl 2-acetyl-4-(4-biphenylyl)-4-oxobutanoate **7c** and 4-aminobenzenesulfonamide according to the same procedure than that described for **8a**. The crude product was purified by FC (cyclohexane/EtOAc; 6:4) and crystallized from CH_3CN to afford **8c** as white crystals (yield: 80%). Mp: 137–139 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.92 (d, J = 8.7 Hz, 2H), 7.66–7.59 (m, 2H), 7.57–7.51 (m, 6H), 7.46–7.39 (m, 2H), 7.36–7.30 (m, 1H), 7.14 (d, J = 8.2 Hz, 2H), 6.79 (s, 1H), 4.24 (q, J = 7 Hz, 2H), 2.34 (s, 3H), 1.30 (t, J = 7 Hz, 3H). IR (cm^{-1}: neat): 1674, 1340, 1236, 1162. LC–MS (APCI$^+$) m/z: 461 (M+H)$^+$.

4.1.7. 1-[4-(Aminosulfonyl)phenyl]-2-methyl-5-phenyl-1*H*-pyrrole-3-carboxylic acid (9a)

A solution of the ester **8a** (10 mmol, 3.84 g) and sodium hydroxyde (50 mmol, 2 g) in 250 mL of aqueous ethyl alcohol (95%) was refluxed for 24 h. After cooling the reaction mixture was concentrated under reduced pressure. The resulting crude residue was diluted into water (150 mL) and the pH adjusted to 2 with HCl 1 N. The mixture was stirred for 1 h at rt. The white precipitate was extracted with EtOAc (3 × 400 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and the solvent evaporated under reduced pressure. The residual solid was crystallized in CH_3CN to afford title compound **9a** as white crystals (yield: 78%). Mp: 223–226 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.06 (br s, 1H), 7.88 (d, J = 8.2 Hz, 2H), 7.56–7.43 (m, 4H), 7.27–7.12 (m, 3H), 7.08–7.01 (m, 2H), 6.68 (s, 1H), 2.32 (s, 3H). IR (cm^{-1}: neat): 1655, 1331, 1253, 1161. LC–MS (APCI$^+$) m/z: 357 (M+H)$^+$.

4.1.8. 1-[4-(Aminosulfonyl)phenyl]-2-methyl-5-(2-naphthyl)-1*H*-pyrrole-3-carboxylic acid (9b)

The title compound was prepared from carboxylic ethyl ester **8b** according to the same procedure than that described for **9a**. The crude product was crystallized from cyclohexane to afford **9b** as white crystals (yield: 85%). Mp: 235–236 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.1 (br s, 1H), 7.87 (d, J = 8.6 Hz, 2H), 7.83–7.78 (m, 1H), 7.73–7.67 (m, 2H), 7.64 (s, 1H), 7.55–7.49 (m, 4H), 7.47–7.42 (m, 2H), 7.12 (dd, J = 8.5 Hz, 1.8 Hz, 1H), 6.83 (s, 1H), 2.36 (s, 3H). IR (cm^{-1}: neat): 1649, 1339, 1256, 1166. LC–MS (APCI$^+$) m/z: 407 (M+H)$^+$.

4.1.9. 1-[4-(Aminosulfonyl)phenyl]-2-methyl-5-(4-biphenylyl)-1*H*-pyrrole-3-carboxylic acid (9c)

The title compound **9c** was prepared in 94% yield from carboxylic ethyl ester **8c** according to the same procedure than that described for **9a** to afford **9c** (yield: 94%) without further purification. Mp: 246–248 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.09 (br s, 1H), 7.91 (d, J = 8.5 Hz, 2H), 7.64–7.59 (m, 2H), 7.56–7.50 (m, 6H), 7.46–7.39 (m, 2H), 7.36–7.30 (m, 1H), 7.13 (d, J = 8.2 Hz, 2H), 6.76 (s, 1H), 2.34 (s, 3H). IR (cm^{-1}: neat): 1651, 1334, 1247, 1171. LC–MS (APCI$^+$) m/z: 433 (M+H)$^+$.

4.1.10. *N*-[2-(Dimethylamino)ethyl]-1-[4-(aminosulfonyl)phenyl]-2-methyl-5-phenyl-1*H*-pyrrole-3-carboxamide (3a)

DMAP (0.59 mmol, 0.072 g) dissolved in 10 mL of CH_2Cl_2 was added to PS-HOBt(HL) resin (0.98 mmol, 1 g) and stirred for 5 min at rt. Then a solution of the carboxylic acid **9a** (1.47 mmol, 0.52 g) dissolved in a mixture of CH_2Cl_2/DMF (80:20) was added and stirred for 10 min. Finally, *N*,*N*′-diisopropylcarbodiimide (4.41 mmol, 0.68 mL) dissolved in 1 mL of CH_2Cl_2 was added to the mixture and stirred at rt for 3 h. The resin was collected by filtration and washed successively with DMF (3 × 20 mL), CH_2Cl_2 (3 × 20 mL), DMF (3 × 20 mL), and THF (3 × 20 mL), to afford the resin-bound-HOBt active ester. A solution of *N*,*N*-dimethylethylenediamine (0.78 mmol, 0.087 mL) and *N*,*N*-diisopropylethylamine (0.98 mmol, 0.16 mL) in CH_2Cl_2 (20 mL) was added to the resin-bound-HOBt active ester, and stirred at 25 °C for 8 h. At the end of the reaction, DMF (5 mL) was added and the resin collected by filtration and washed with a mixture of CH_2Cl_2/DMF (80/20) (3 × 20 mL). The filtrate was then concentrated under reduced pressure. The crude product was purified by FC (CH_2Cl_2/MeOH; 6:4) and crystallized from EtOH to afford **3a** as white crystals (yield: 30%). Mp: 248–250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.87 (d, J = 8.8 Hz, 2H), 7.79 (t, J = 5.7 Hz, 1H), 7.51 (s, 2H), 7.43 (d, J = 8.8 Hz, 2H), 7.26–7.12 (m, 3H), 7.05–7.01 (m, 2H), 6.84 (s, 1H), 3.35–3.26 (m, 2H), 3.37 (t, J = 6.9 Hz, 2H), 2.32 (s, 3H), 2.17 (s, 6H). IR (cm^{-1}: neat): 3069, 1629, 1569, 1536, 1339, 1164. LC–MS (APCI$^+$) m/z: 427 (M+H)$^+$. Anal. Calcd for $C_{22}H_{26}N_4O_3S$: C, 61.95; H, 6.14; N, 13.14. Found: C, 64.65; H, 6.04; N, 12.98.

4.1.11. *N*-(Dimethylaminopropyl)-1-[4-(aminosulfonyl)phenyl]-2-methyl-5-phenyl-1*H*-pyrrole-3-carboxamide (3b)

The title compound was prepared from the carboxylic ethyl ester **9a** and *N*,*N*-dimethyl-1,3-propanediamine according to the same procedure than that described for **3a**. The crude product was purified by FC (CH_2Cl_2/MeOH; 6:4) to afford **3b** as a white solid (yield: 44%). Mp: 253–255 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.92 (t, J = 5.5 Hz, 1H), 7.86 (d, J = 8.5 Hz, 2H), 7.51 (s, 2H), 7.44 (d, J = 8.5 Hz, 2H), 7.25–7.12 (m, 3H), 7.06–7.01 (m, 2H), 6.83 (s, 1H), 3.27–3.19 (m, 2H), 2.32 (s, 3H), 2.25 (t, J = 7.2 Hz, 2H), 2.13 (s, 6H), 1.68–1.57 (m, 2H). IR (cm^{-1}: neat): 1622, 1570, 1543, 1322, 1156. LC–MS (APCI$^+$) m/z: 441 (M+H)$^+$. Anal. Calcd for $C_{23}H_{28}N_4O_3S$: C, 62.70; H, 6.41; N, 12.72. Found: C, 62.55; H, 6.34; N, 12.62.

4.1.12. 4-{2-Methyl-3-[(4-methylpiperazino)carbonyl]-5-phenyl-1*H*-pyrrolyl-1}-benzenesulfonamide (3c)

The title compound was prepared from the carboxylic ethyl ester **9a** and 1-methylpiperazine according to the same procedure than that described for **3a**. The crude product was purified by FC (CH_2Cl_2/MeOH; 9:1) to afford **3c** as a white solid (yield: 77%). Mp >260 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.86 (d, J = 8.8 Hz, 2H), 7.51 (s, 2H), 7.47 (d, J = 8.8 Hz, 2H), 7.25–7.12 (m, 3H), 7.08–7.02 (m, 2H), 6.44 (s, 1H), 3.66–3.57 (m, 4H), 2.36–2.31 (m, 4H), 2.20 (s, 3H), 2.10 (s, 3H). IR (cm^{-1}: neat): 1600, 1336, 1255, 1160. LC–MS (APCI$^+$) m/z: 439 (M+H)$^+$. Anal. Calcd for $C_{23}H_{26}N_4O_3S$: C, 62.99; H, 5.98; N, 12.78. Found: C, 63.04; H, 6.10; N, 12.54.

4.1.13. *N*-(Piperidino-1-ethyl)-1-[4-(aminosulfonyl)phenyl]-2-methyl-5-phenyl-1*H*-pyrrole-3-carboxamide (3d)

The title compound was prepared from the carboxylic ethyl ester **9a** and *N*-(2-aminoethyl)piperidine according to the same procedure than that described for **3a**. The crude product was purified by FC (CH_2Cl_2/MeOH; 8:2) and crystallized from EtOH to afford **3d** as a white solid (yield: 45%). Mp: 206–208 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.87 (d, J = 8.5 Hz, 2H), 7.78 (t, J = 5.5 Hz, 1H), 7.51 (s, 2H), 7.44 (d, J = 8.5 Hz, 2H), 7.26–7.12 (m, 3H), 7.06–6.99 (m, 2H), 6.83 (s, 1H), 3.38–3.27 (m, 2H), 2.48–2.32 (m, 6H), 2.28 (s, 3H), 1.51–1.37 (m, 6H). IR (cm^{-1}: neat): 1625, 1569, 1536, 1310, 1161. LC–MS (APCI$^+$) m/z: 467 (M+H)$^+$. Anal. Calcd for $C_{25}H_{30}N_4O_3S$: C, 64.35; H, 6.48; N, 12.01. Found: C, 64.45; H, 6.44; N, 12.12.

4.1.14. *N*-(Morpholino-1-ethyl)-1-[4-(aminosulfonyl)phenyl]-2-methyl-5-phenyl-1*H*-pyrrole-3-carboxamide (3e)

The title compound was prepared from the carboxylic ethyl ester **9a** and *N*-(2-aminoethyl)morpholine according to the same procedure than that described for **3a**. The crude product was purified by FC (CH_2Cl_2/MeOH; 95:5) and crystallized from EtOH to afford **3e** as white crystals (yield: 57%). Mp: 254–256 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.87 (d, J = 8.7 Hz, 2H), 7.81 (t, J = 5.9 Hz, 1H), 7.51 (s, 2H), 7.44 (d, J = 8.7 Hz, 2H), 7.26–7.13 (m, 3H), 7.06–7.01 (m, 2H), 6.83 (s, 1H), 3.62–3.56 (m, 4H), 3.39–3.30 (m, 2H), 2.47–2.38 (m, 6H), 2.32 (s, 3H). IR (cm^{-1}: neat): 1625, 1568, 1538, 1312, 1158. LC–MS (APCI$^+$) m/z: 469 (M+H)$^+$. Anal. Calcd for $C_{24}H_{28}N_4O_4S$: C, 61.52; H, 6.02; N, 11.96. Found: C, 61.72; H, 6.23; N, 11.57.

4.1.15. *N*-[2-(Dimethylamino)ethyl]-1-[4-(aminosulfonyl)phenyl]-2-methyl-5-(2-naphthyl)-1*H*-pyrrole-3-carboxamide (4a)

The title compound was prepared from the carboxylic acid **9b** and *N*,*N*-dimethylethylenediamine according to the same procedure than that described for **3a**. The crude product was purified by FC (CH_2Cl_2/MeOH; 8:2) to afford **4a** as a white solid (yield: 82%). Mp: 226–227 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.87 (d, J = 8.8 Hz, 2H), 7.82–7.78 (m, 2H), 7.73–7.68 (m, 2H), 7.59 (d, J = 1.8 Hz, 1H), 7.52–7.42 (m, 6H), 7.11 (dd, J = 8.6 Hz, 1.8 Hz, 1H), 7.00 (s, 1H), 3.38–3.29 (m, 2H), 2.42 (t, J = 6.9 Hz, 2H), 2.37 (s, 3H), 2.21 (s, 6H). IR (cm^{-1}: neat): 2923, 1626, 1568, 1536, 1332, 1280, 1162. LC–MS (APCI$^+$) m/z: 477 (M+H)$^+$. Anal. Calcd for $C_{26}H_{28}N_4O_3S$: C, 65.52; H, 5.92; N, 11.76. Found: C, 65.46; H, 6.03; N, 11.86.

4.1.16. *N*-(Dimethylaminopropyl)-1-[4-(aminosulfonyl)phenyl]-2-methyl-5-(2-naphthyl)-1*H*-pyrrole-3-carboxamide (4b)

The title compound was prepared from the carboxylic acid **9b** and *N*,*N*-dimethyl-1,3-propanediamine according to the same procedure than that described for **3a**. The crude product was crystallized from EtOH to afford **4b** as white crystals (yield: 52%). Mp: 228–230 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.96 (t, J = 5.7 Hz, 1H), 7.91–7.84 (m, 2H), 7.82–7.78 (m, 1H), 7.74–7.67 (m, 2H), 7.59 (s, 1H), 7.54–7.42 (m, 6H), 7.11 (dd, J = 8.5 Hz, 1.7 Hz, 1H), 6.99 (s, 1H), 3.28–3.22 (m, 2H), 2.37 (s, 3H), 2.30–2.23 (m, 2H), 2.14 (s, 6H), 1.67–1.58 (m, 2H). IR (cm^{-1}: neat): 1627, 1571, 1539, 1323, 1281, 1158. LC–MS (APCI$^+$) m/z: 491(M+H)$^+$. Anal. Calcd for $C_{27}H_{30}N_4O_3S$: C, 66.10; H, 6.16; N, 11.42. Found: C, 66.32; H, 6.04; N, 11.22.

4.1.17. 4-{2-Methyl-3-[(4-methylpiperazino)carbonyl]-5-(2-naphthyl)-1*H*-pyrrolyl-1}-benzenesulfonamide (4c)

The title compound was prepared from the carboxylic acid **9b** and 1-methylpiperazine according to the same procedure than that described for **3a**. The crude product was recrystallized from a mixture of CH_3CN/EtOH (5:5) to afford **4c** as white crystals (yield: 52%). Mp >260 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.91–7.78 (m, 3H), 7.75–7.67 (m, 2H), 7.63 (s, 1H), 7.57–7.32 (m, 6H), 7.18–7.07 (m, 1H), 6.58 (s, 1H), 3.72–3.54 (m, 4H), 2.42–2.29 (m, 4H), 2.21 (s, 3H), 2.14 (s, 3H). IR (cm^{-1}: neat): 3357, 1607, 1333, 1254, 1158. LC–MS (APCI$^+$) m/z: 489 (M+H)$^+$. Anal. Calcd for $C_{27}H_{28}N_4O_3S$: C, 66.37; H, 5.78; N, 11.47. Found: C, 66.42; H, 6.10; N, 11.22.

4.1.18. *N*-[2-(Pyrrolidinyl-1-ethyl)]-1-[4-(aminosulfonyl)phenyl]-2-methyl-5-(2-naphthyl)-1*H*-pyrrole-3-carboxamide (4d)

The title compound was prepared from the carboxylic acid **9b** and *N*-(2-aminoethyl)pyrrolidine according to the same procedure

than that described for **3a**. The crude product was purified by FC (CH_2Cl_2/MeOH; 8:2) to afford **4d** as a white solid (yield: 60%). Mp: 148–152 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.96 (t, J = 5.9 Hz, 1H), 7.87 (d, J = 8.8 Hz, 2H), 7.82–7.78 (m, 1H), 7.75–7.68 (m, 2H), 7.60 (s, 1H), 7.54–7.42 (m, 6H), 7.12 (dd, J = 8.6 Hz, 1.9 Hz, 1H), 7.00 (s, 1H), 3.45–3.3 (m, 2H), 2.68–2.53 (m, 6H), 2.38 (s, 3H), 1.78–1.67 (m, 4H). IR (cm^{-1}: neat): 1626, 1569, 1539, 1336, 1271, 1163. LC–MS (APCI$^+$) m/z: 503 (M+H)$^+$. Anal. Calcd for $C_{28}H_{30}N_4O_3S$: C, 66.91; H, 6.02; N, 11.15. Found: C, 67.01; H, 6.14; N, 11.21.

4.1.19. *N*-(Piperidino-1-ethyl)-1-[4-(aminosulfonyl)phenyl]-2-methyl-5-(2-naphthyl)-1*H*-pyrrole-3-carboxamide (4e)

The title compound was prepared from the carboxylic acid **9b** and *N*-(2-aminoethyl)piperidine according to the same procedure than that described for **3a**. The crude product was purified by FC (CH_2Cl_2/MeOH; 8:2) to afford **4e** as a white solid (yield: 86%). Mp: 245–247 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.87 (d, J = 8.8 Hz, 2H), 7.83–7.79 (m, 2H), 7.74–7.69 (m, 2H), 7.59 (d, J = 1.9 Hz, 1H), 7.52–7.42 (m, 6H), 7.10 (dd, J = 8.6 Hz, 1.9 Hz, 1H), 6.99 (s, 1H), 3.39–3.30 (m, 2H), 2.50–2.42 (m, 6H), 2.37 (s, 3H), 1.52–1.47 (m, 6H). IR (cm^{-1}: neat): 2926, 1624, 1570, 1538, 1325, 1281, 1161. LC–MS (APCI$^+$) m/z: 517 (M+H)$^+$. Anal. Calcd for $C_{29}H_{32}N_4O_3S$: C, 67.42; H, 6.24; N, 10.84. Found: C, 67.31; H, 6.31; N, 10.22.

4.1.20. *N*-[2-(4-Methylpiperazino)ethyl]-1-[4-(aminosulfonyl)-phenyl]-2-methyl-5-(2-naphthyl)-1*H*-pyrrole-3-carboxamide (4f)

The title compound was prepared from the carboxylic acid **9b** and 2-(4-methylpiperazino)ethylamine according to the same procedure than that described for **3a**. The crude product was purified by FC (CH_2Cl_2/MeOH; 8:2) and crystallized from EtOH to afford **4f** as white crystals (yield: 39%). Mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.92–7.76 (m, 4H), 7.74–7.65 (m, 2H), 7.58 (d, J = 1.2 Hz, 1H), 7.53–7.39 (m, 6H), 7.11 (dd, J = 8.4, 1.2 Hz, 1H), 6.98 (s, 1H), 3.37–3.28 (m, 2H), 2.47–2.38 (m, 2H), 2.36–2.27 (m, 11H), 2.13 (s, 3H). IR (cm^{-1}: neat): 1628, 1570, 1538, 1335, 1268, 1163. LC–MS (APCI$^+$) m/z: 532 (M+H)$^+$. Anal. Calcd for $C_{39}H_{33}N_5O_3S$: C, 65.51; H, 6.26; N, 13.17. Found: C, 65.41; H, 6.31; N, 13.52.

4.1.21. *N*-[3-(4-Methylpiperazino)propyl]-1-[4-(aminosulfonyl)-phenyl]-2-methyl-5-(2-naphthyl)-1*H*-pyrrole-3-carboxamide (4g)

The title compound was prepared from the carboxylic acid **9b** and 3-(4-methylpiperazino)propylamine according to the same procedure than that described for **3a**. The crude product was purified by FC (CH_2Cl_2/MeOH; 7:3) and crystallized from EtOH to afford **4g** as white crystals (yield: 43%). Mp: 239–241 °C. ^{1}H NMR (DMSO-d_6) δ: 7.94 (t, J = 5.4 Hz, 1H), 7.87 (d, J = 8.4 Hz, 2H), 7.83–7.78 (m, 1H), 7.74–7.66 (m, 2H), 7.58 (s, 1H), 7.52–7.41 (m, 6H), 7.10 (dd, J = 8.4, 1.4 Hz, 1H), 6.98 (s, 1H), 3.28–3.21 (m, 2H), 2.52–2.43 (m, 2H), 2.40–2.26 (m, 11H), 2.11 (s, 3H), 1.73–1.62 (m, 2H). IR (cm^{-1}: neat): 1601, 1570, 1534, 1330, 1283, 1166. LC–MS (APCI$^+$) m/z: 546 (M+H)$^+$. Anal. Calcd for $C_{30}H_{35}N_5O_3S$: C, 66.03; H, 6.46; N, 12.83. Found: C, 65.98; H, 6.56; N, 12.42.

4.1.22. *N*-(2-Morpholinoethyl)-1-[4-(aminosulfonyl)phenyl]-2-methyl-5-(2-naphthyl)-1*H*-pyrrole-3-carboxamide (4h)

The title compound was prepared from the carboxylic acid **9b** and *N*-(2-aminoethyl)morpholine according to the same procedure than that described for **3a**. The crude product was crystallized from EtOH to afford **4h** as white crystals (yield: 86%). Mp: 248–250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.95–7.86 (m, 3H), 7.83–7.78 (m, 1H), 7.73–7.65 (m, 2H), 7.58 (s, 1H), 7.54–7.42 (m, 6H), 7.12 (dd, J = 8.6 Hz, 1.9 Hz, 1H), 6.99 (s, 1H), 3.64–3.56 (m, 4H), 3.42–3.31 (m, 2H), 2.48–2.42 (m, 6H), 2.37 (s, 3H). IR (cm^{-1}: neat): 1635, 1570, 1538, 1327, 1271, 1158. LC–MS (APCI$^+$) m/z: 519 (M+H)$^+$. Anal. Calcd for $C_{28}H_{30}N_4O_4S$: C, 64.85; H, 5.83; N, 10.80. Found: C, 64.52; H, 5.99; N, 10.62.

4.1.23. *N*-(3-Morpholinopropyl)-1-[4-(aminosulfonyl)phenyl]-2-methyl-5-(2-naphthyl)-1*H*-pyrrole-3-carboxamide (4i)

The title compound was prepared from the carboxylic acid **9b** and *N*-(3-morpholinopropyl)amine according to the same procedure than that described for **3a**. The crude product was purified by FC (CH_2Cl_2/MeOH; 8:2) and crystallized from EtOH to afford **4i** as white crystals (yield: 43%). Mp: 240–242 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.94 (t, J = 5.5 Hz, 1H), 7.86 (d, J = 8.7 Hz, 2H), 7.83–7.78 (m, 1H), 7.74–7.66 (m, 2H), 7.57 (d, J = 1.6 Hz, 1H), 7.51–7.41 (m, 6H), 7.11 (dd, J = 8.6, 1.6 Hz, 1H), 6.98 (s, 1H), 3.62–3.55 (m, 4H), 3.29–3.22 (m, 2H), 2.39–2.28 (m, 9H), 1.73–1.62 (m, 2H). IR (cm^{-1}: neat): 1624, 1568, 1542, 1332, 1271, 1158. LC–MS (APCI$^+$) m/z: 533 (M+H)$^+$. Anal. Calcd for $C_{29}H_{32}N_4O_4S$: C, 65.39; H, 6.06; N, 10.52. Found: C, 65.21; H, 6.24; N, 10.32.

4.1.24. *N*3-[2-(Dimethylamino)ethyl]-1-[4-(aminosulfonyl)phenyl]-2-methyl-5-(4-biphenylyl)-1*H*-3 pyrrolecarboxamide (5a)

The title compound was prepared from carboxylic acid **3c** and *N*,*N*-dimethylethylenediamine according to the same procedure than that described for **3a**. The crude product was recrystallized from EtOH to afford **5a** as white crystals (yield: 44%). Mp: 245–247 °C. ^{1}H NMR (DMSO-d_6) δ: 7.90 (d, J = 8.4 Hz, 2H), 7.79 (t, J = 5.8 Hz, 1H), 7.65–7.59 (m, 2H), 7.54 (d, J = 8.4 Hz, 2H), 7.51–7.38 (m, 6H), 7.36–7.29 (m, 1H), 7.11 (d, J = 8.4 Hz, 2H), 6.93 (s, 1H), 3.36–3.28 (m, 2H), 2.38 (t, J = 6.8 Hz, 2H), 2.33 (s, 3H), 2.18 (s, 6H). IR (cm^{-1}: neat): 1634, 1560, 1334, 1171. LC–MS (APCI$^+$) m/z: 503 (M+H)$^+$. Anal. Calcd for $C_{28}H_{30}N_4O_3S$: C, 66.91; H, 6.02; N, 11.15. Found: C, 66.75; H, 5.96; N, 11.22.

4.1.25. *N*3-[3-(Dimethylamino)propyl]-1-[4-(aminosulfonyl)phenyl]-2-methyl-5-(2-naphthyl)-1*H*-3-pyrrolecarboxamide (5b)

The title compound was prepared from carboxylic acid **3c** and *N*,*N*-dimethyl-1,3-propanediamine according to the same procedure than that described for **3a**. The crude product was purified by FC (CH_2Cl_2/MeOH; 7:3) and recrystallized from EtOH to afford **5b** as white crystals (yield: 53%). Mp: 216–218 °C. ^{1}H NMR (DMSO-d_6) δ: 8.15 (t, J = 5.3 Hz, 1H), 7.90 (d, J = 7.9 Hz, 2H), 7.68–7.28 (m, 11H), 7.10 (d, J = 7.9 Hz, 2H), 6.97 (s, 1H), 3.35–3.27 (m, 2H), 3.09–2.97 (m, 2H), 2.72 (s, 6H), 2.34 (s, 3H), 1.94–1.81 (m, 2H). IR (cm^{-1}: neat): 1629, 1554, 1329, 1163. LC–MS (APCI$^+$) m/z: 517 (M+H)$^+$. Anal. Calcd for $C_{29}H_{32}N_4O_3S$: C, 67.42; H, 6.24; N, 10.84. Found: C, 67.21; H, 6.27; N, 10.96.

4.1.26. 4-[2-Methyl-3-[(4-methylpiperazino)carbonyl]-5-(4-biphenylyl)-1*H*-1-pyrrolyl]-1-benzenesulfonamide (5c)

The title compound was prepared from carboxylic acid **3c** and 1-methylpiperazine according to the same procedure than that described for **3a**. The crude product was purified by FC (CH_2Cl_2/MeOH; 95:5) and recrystallized from EtOH to afford **5c** as white crystals (yield: 39%). Mp >250 °C. ^{1}H NMR (DMSO-d_6) δ: 7.89 (d, J = 8.5 Hz, 2H), 7.61 (d, J = 7.3 Hz, 2H), 7.56–7.47 (m, 6H), 7.45–7.39 (m, 2H), 7.34–7.29 (m, 1H), 7.13 (d, J = 8.2 Hz, 2H), 6.51 (s, 1H), 3.67–3.56 (m, 4H), 2.38–2.29 (m, 4H), 2.21 (s, 3H), 2.11 (s, 3H). IR (cm^{-1}: neat): 1628, 1569, 1340, 1236, 1162. LC–MS (APCI$^+$) m/z: 515 (M+H)$^+$. Anal. Calcd for $C_{29}H_{30}N_4O_3S$: C, 67.68; H, 5.88; N, 10.89. Found: C, 67.51; H, 5.94; N, 11.01.

4.1.27. *N*3-(2-Tetrahydro-1*H*-1-pyrrolylethyl)-1-[4-(aminosulfonyl)phenyl]-2-methyl-5-(4-biphenylyl)-1*H*-3-pyrrolecarboxamide (5d)

The title compound was prepared from carboxylic acid **3c** and *N*-(2-aminoethyl)piperidine according to the same procedure than

that described for **3a**. The crude product was purified by FC (CH_2Cl_2/MeOH; 8:2) and recrystallized from EtOH to afford **5d** as white crystals (yield: 49%). Mp >250 °C. ^{1}H NMR (DMSO-d_6) δ: 8.32 (t, J = 5.4 Hz, 1H), 7.91 (d, J = 8.4 Hz, 2H), 7.65–7.59 (m, 2H), 7.55 (d, J = 8.4 Hz, 2H), 7.52–7.38 (m, 6H), 7.36–7.29 (m, 1H), 7.11 (d, J = 8.4 Hz, 2H), 7.06 (s, 1H), 3.65–3.54 (m, 4H), 3.31–3.26 (m, 2H), 3.10–2.94 (m, 2H), 2.35 (s, 3H), 2.05–1.82 (m, 4H). IR (cm^{-1}: neat): 1636, 1555, 1326, 1163. LC–MS (APCI$^+$) m/z: 529 (M+H)$^+$. Anal. Calcd for $C_{30}H_{32}N_4O_3S$: C, 68.16; H, 6.10; N, 10.60. Found: C, 68.32; H, 5.96; N, 10.56.

4.1.28. *N*3-(2-Piperidinoethyl)-1-[4-(aminosulfonyl)phenyl]-2-methyl-5-(4-biphenylyl)-1*H*-3-pyrrolecarboxamide (5e)

The title compound was prepared from carboxylic acid **3c** and *N*-(2-aminoethyl)morpholine according to the same procedure than that described for **3a**. The crude product was purified by FC (CH_2Cl_2/MeOH; 9:1) and recrystallized from EtOH to afford **5e** as white crystals (yield: 62%). Mp: 148–150 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.38 (t, J = 4.9 Hz, 1H), 7.91 (d, J = 8.2 Hz, 2H), 7.62 (d, J = 8.2, 2H), 7.56–7.30 (m, 9H), 7.12–7.06 (m, 3H), 3.67–3.58 (m, 2H), 3.21–3.13 (m, 2H), 2.55–2.40 (m, 6H), 2.35 (s, 3H), 1.84–1.73 (m, 4H). IR (cm^{-1}: neat): 1629, 1556, 1330, 1285, 1162. LC–MS (APCI$^+$) m/z: 543 (M+H)$^+$. Anal. Calcd for $C_{31}H_{34}N_4O_3S$: C, 68.61; H, 6.31; N, 10.32. Found: C, 68.51; H, 6.24; N, 10.12.

4.1.29. *N*3-[2-(4-Methylpiperazino)ethyl]-1-[4-(aminosulfonyl)-phenyl]-2-methyl-5-(4-biphenylyl)-1*H*-3-pyrrolecarboxamide (5f)

The title compound was prepared from carboxylic acid **3c** and 2-(4-methylpiperazino)ethylamine according to the same procedure than that described for **3a**. The crude product was purified by FC (CH_2Cl_2/MeOH; 8:2) and recrystallized from EtOH to afford **5f** as white crystals (yield: 43%). Mp: 235–237 °C. ^{1}H NMR (DMSO-d_6) δ: 7.90 (d, J = 8.4 Hz, 2H), 7.79 (t, J = 5.5 Hz, 1H), 7.65–7.59 (m, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.51–7.46 (m, 4H), 7.44–7.38 (m, 2H), 7.36–7.29 (m, 1H), 7.10 (d, J = 8.4 Hz, 2H), 6.90 (s, 1H), 3.36–3.29 (m, 2H), 2.47–2.39 (m, 7H), 2.35–2.28 (m, 6H), 2.14 (s, 3H). IR (cm^{-1}: neat): 1638, 1554, 1329, 1236, 1171. LC–MS (APCI$^+$) m/z: 558 (M+H)$^+$. Anal. Calcd for $C_{31}H_{35}N_5O_3S$: C, 66.76; H, 6.33; N, 12.56. Found: C, 66.71; H, 5.96; N, 12.51.

4.1.30. *N*3-[3-(4-Methylpiperazino)propyl]-1-[4-(aminosulfonyl) phenyl]-2-methyl-5-(4-biphenylyl)-1*H*-3-pyrrolecarboxamide (5g)

The title compound was prepared from carboxylic acid **3c** and 3-(4-methylpiperazino)propylamine according to the same procedure than that described for **3a**. The crude product was purified by recrystallization from EtOH to afford **5g** as white crystals (yield: 63%). Mp >250 °C. ^{1}H NMR (DMSO-d_6) δ: 7.97–7.84 (m, 3H), 7.68–7.58 (m, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.49–7.46 (m, 4H), 7.44–7.39 (m, 2H), 7.35–7.29 (m, 1H), 7.11 (d, J = 8.9 Hz, 2H), 6.91 (s, 1H), 3.27–3.21 (m, 2H), 3.36–3.30 (m, 11H), 2.55–2.42 (m, 2H), 2.12 (s, 3H), 1.69–1.60 (m, 2H). IR (cm^{-1}: neat): 1632, 1555, 1330, 1169. LC–MS (APCI$^+$) m/z: 572 (M+H)$^+$. Anal. Calcd for $C_{32}H_{37}N_5O_3S$: C, 67.22; H, 6.52; N, 12.25. Found: C, 67.14; H, 6.61; N, 12.06.

4.1.31. *N*3-(2-Morpholinoethyl)-1-[4-(aminosulfonyl)phenyl]-2-methyl-5-(4-biphenylyl)-1*H*-3-pyrrolecarboxamide (5h)

The title compound was prepared from carboxylic acid **3c** and *N*-(2-aminoethyl)morpholine according to the same procedure than that described for **3a**. The crude product was purified by FC (CH_2Cl_2/MeOH; 95:5) and recrystallized from EtOH to afford **5h** as white crystals (yield: 57%). Mp >250 °C. ^{1}H NMR (DMSO-d_6) δ: 7.90 (d, J = 8.5 Hz, 2H), 7.82 (t, J = 5.6 Hz, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.57–7.29 (m, 9H), 7.10 (d, J = 8.5 Hz, 2H), 6.91 (s, 1H), 3.63–3.55 (m, 4H), 3.40–3.31 (m, 2H), 2.47–2.38 (m, 6H), 2.33 (s, 3H). IR (cm^{-1}: neat): 1622, 1552, 1339, 1236, 1164. LC–MS (APCI$^+$) m/z: 545 (M+H)$^+$. Anal. Calcd for $C_{30}H_{32}N_4O_4S$: C, 66.16; H, 5.92; N, 10.29. Found: C, 66.45; H, 6.04; N, 10.22.

4.1.32. *N*3-(3-Morpholinopropyl)-1-[4-(aminosulfonyl)phenyl]-2-methyl-5-(4-biphenylyl)-1*H*-3-pyrrolecarboxamide (5i)

The title compound was prepared from carboxylic acid **3c** and *N*-(3-morpholinopropyl)amine according to the same procedure than that described for **3a**. The crude product was recrystallized from EtOH to afford **5i** as white crystals (yield: 36%). Mp >250 °C. ^{1}H NMR (DMSO-d_6) δ: 7.93–7.87 (m, 3H), 7.65–7.59 (m, 2H), 7.54 (d, J = 8.4 Hz, 2H), 7.51–7.46 (m, 4H), 7.45–7.38 (m, 2H), 7.36–7.29 (m, 1H), 7.10 (d, J = 8.4 Hz, 2H), 6.92 (s, 1H), 3.61–3.55 (m, 4H), 3.29–3.21 (m, 2H), 2.39–2.28 (m, 9H), 1.73–1.61 (m, 2H). IR (cm^{-1}: neat): 1629, 1552, 1329, 1165. LC–MS (APCI$^+$) m/z: 559 (M+H)$^+$. Anal. Calcd for $C_{31}H_{34}N_4O_4S$: C, 66.64; H, 6.13; N, 10.03. Found: C, 66.73; H, 6.21; N, 10.23.

4.2. Enzymatic evaluation

The CA catalyzed CO_2 hydration activity was followed by an SX.18MV-R Applied Photophysics (Oxford, UK) stopped-flow instrument. Phenol red (at a concentration of 0.2 mM) was used as indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.5) as buffer, 0.1 M Na_2SO_4 (for maintaining constant the ionic strength). Saturated CO_2 solutions in water at 25 °C were used as substrate. Stock solutions of inhibitor were prepared at a concentration of 10 mM (in DMSO/water 1:1, v/v) and dilutions up to 0.01 nM done with the assay buffer mentioned above. At least seven different inhibitor concentrations were used for measuring the inhibition constant. Inhibitor and enzyme solutions were preincubated together for 10 min at room temperature prior assay, in order to allow for the formation of the E–I complex. Triplicate experiments were done for each inhibitor concentration and the values reported throughout the paper are the mean of such results. K_is were obtained from Lineweaver–Burk plots, as reported earlier.[24]

4.3. Molecular modeling and calculations

The compounds were built using the *SKETCH* module, as implemented in *SYBYL* (version 8.0),[25] and their geometry was optimized using the *MINIMIZE* module. The minimization process uses the *POWELL* method with the *TRIPOS* force field (dielectric constant 1*r*) to reach a final convergence of 0.01 kcal mol^{-1}. Docking simulation was then performed into hCA IX (RCSB Protein Data Bank 3IAI)[14] with the automated *GOLD* program.[26] The active site was defined including all residues in a volume of 10 Å around acetazolamide taken as reference.

The theoretical lipophilicity, expressed as log *D*7.4 calculated at pH 7.4, and solubility, calculated at the same pH were evaluated by means of the ACD/Structure Designer program v12.0.[22]

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