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Carbonic anhydrase inhibitors. Inhibition of human cytosolic isoforms I and II with (reduced) Schiff's bases incorporating sulfonamide, carboxylate and carboxymethyl moieties

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ABSTRACT

A library of Schiff bases was synthesized by condensation of aromatic amines incorporating sulfonamide, carboxylic acid or carboxymethyl functionalities as Zn^{2+}-binding groups, with aromatic aldehydes incorporating *tert*-butyl, hydroxy and/or methoxy groups. The corresponding amines were thereafter obtained by reduction of the imines. These compounds were assayed for the inhibition of two cytosolic human carbonic anhydrase (hCA, EC 4.2.1.1) isoenzymes, hCA I and II. The K_i values of the Schiff bases were in the range of 7.0–21,400 nM against hCA II and of 52–8600 nM against hCA I, respectively. The corresponding amines showed K_i values in the range of 8.6 nM–5.3 μM against hCA II, and of 18.7–251 nM against hCA I, respectively. Unlike the imines, the reduced Schiff bases are stable to hydrolysis and several low-nanomolar inhibitors were detected, most of them incorporating sulfonamide groups. Some carboxylates also showed interesting CA inhibitory properties. Such hydrosoluble derivatives may show pharmacologic applications.

1. Introduction

Carbonic anhydrase CA, the catalyst for the interconversion between carbon dioxide and bicarbonate, is an essential enzyme in bacteria, archaea and eukaryotes. In humans, the CA-catalysed reaction involves four simple chemical entities, water, CO_2, HCO_3^- and H^+ essential in a host of physiological and pathological processes, such as respiration, pH and CO_2 homeostasis, electrolyte secretion lipogenesis.[1–3] Sixteen isozymes have been described so far in mammals. Some of these isoenzymes are cytosolic (CA I, CA II, CA III, CA VII and CA XIII), other are membrane bound (CA IV, CA IX, CA XII and CA XIV), two are mitochondrial (CA VA, CA VB) and one is secreted in saliva and milk (CA VI). CA I and CA II are the two major CA isozymes present at high concentrations in the cytosol in erythrocytes, and CA II is the most active of all CAs.[1–3]

Many of the CAs are therapeutic targets with the potential to be inhibited or activated. CA II and CA I inhibitors are used for the treatment of glaucoma, epilepsy, or as diuretics. For such reasons, the design of new, potent inhibitors, may lead to clinical applications for treating a multitude of diseases.[1–3]

Schiff bases incorporating aromatic/heterocyclic sulfonamide moieties in their molecules have been extensively investigated as CA inhibitors (CAIs).[4,5] Some of these derivatives were the first ones reported to have some selectivity for the inhibition of some human (h) CA isoforms, such as hCA I, II or IV.[4,5] Furthermore, the reversible reaction between an amine and an aldehyde/ketone has been employed to generate Dynamic Combinatorial Libraries-DCLs of sulfonamide CAIs, in which the enzyme acts as a template for the imine reaction formation, 'selected' for the most effective inhibitors.[6]

Here we reported two new libraries of sulfonamide CAIs, that is, imines (Schiff bases obtained by reacting aromatic amines also incorporating zinc-binding functions of the sulfonamide, carboxylate and carboxymethyl type) and the corresponding amines (obtained by the reduction of the Schiff bases). Many of the new derivatives showed a good inhibition against the physiologically

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dominant and relevant isoforms hCA I and hCA II. We also report here some CAIs presenting higher affinity for hCA I as compared to hCA II, which is an unusual trend, since most sulfonamides are better hCA II than hCA I inhibitors.

2. Chemistry

The rationale of this study was to use 'amines' also incorporating potential zinc-binding functions of the sulfonamide, carboxylate and carboxymethyl type, which have been condensed with aldehydes possessing both lipophilic (*tert*-butyl-phenyl) or hydrophilic (hydroxyphenyl) or mixed-type (hydro- and lipophilic, e.g., 2-hydroxy-5-methoxy-phenyl) moieties in their molecule in order to generate a library of Schiff bases (which will be called imines in the following). These imines were then reduced to afford the corresponding secondary amines (which will be called amines in the discussion).

The following amines were included in the study: 4-aminobenzoic acid (**A**), methyl 4-aminobenzoate (**B**), sulfanilamide (**C**), 4-aminomethylbenzene-sulfonamide hydrochloride (**D**), 4-(2-aminoethyl) benzenesulfonamide (**E**), and the following aldehydes: benzaldehyde (**1**), 4-*tert*-butylbenzaldehyde (**2**), 4-hydroxybenandehyde (**3**) and 2-hydroxy-5-methoxybenzaldehyde (**4**). The 20 Schiff bases obtained were then reduced in the presence of an excess of sodium borohydride affording the corresponding 20 amines (Scheme 1).

3. CA inhibition data

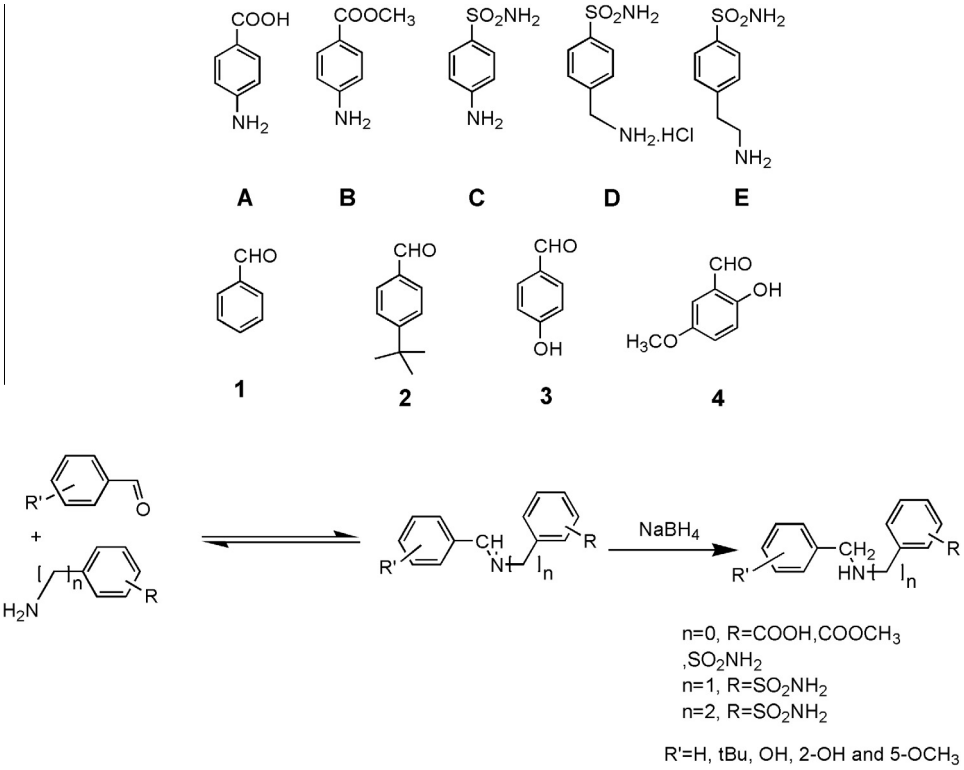
As mentioned in the introductory part, many of the Schiff base sulfonamide CAIs showed not only excellent inhibitory power against many physiologically relevant isoforms, but also interesting selectivity profiles for the inhibition of membrane-associated versus cytosolic CA isoforms.[4–6] Thus, we have investigated the libraries of prepared compounds for the inhibition of the physiologically relevant cytosolic isoforms hCA I and II (Table 1).[7]

3.1. Compounds from aldehydes A and B

3.1.1. Imines

(i) The substituted carboxy and carboxymethyl Schiff bases (**1–4)A$_{im}$** and (**1–4)B$_{im}$** show K_i values in the range of 1040–8600 nM and 780–21,400 nM against hCA I and hCA II, respectively (Scheme 3). These data show less effective inhibition comparing to sulfonamide Schiff bases (see later). Some reports described the inhibition of CAs with carboxylate, acetate or salicylic acid.[2,3] It has been shown that the cytosolic hCA I and II were weakly inhibited by all the investigated anions, with inhibition constants in the range of millimolar. The hCA II was crystallized in an acetate-bound complex and the crystallographic data show that the acetate group has replaced the zinc-bound water, forming a tetrahedral coordination, and thus disrupts the hydrogen-bonded solvent network. From the other side, acetate interacts with Thr199 with the methyl group directed away from the hydrophobic patch.[8]

(ii) All these imines expressed very different inhibition straight against hCA I and hCA II. In general the benzyl moiety or organic scaffold lay in the hydrophobic part of the hCAs and involved in many interactions (if the geometry is suitable) and the COOH or CO_2CH_3 bind weakly in the precatalytic complex. As it had been known, the two metalloenzymes hCA I and hCAII present many structure differences based on the disposition, the number and the nature of amino acids (hydrophilic/hydrophobic) inside



Scheme 1. Synthesis of imines and secondary amines as CA inhibitors by reaction of amines **A**–**E** with aldehydes **1**–**4**, followed by reduction of the resulting Schiff bases. Coding of the Schiff's bases is done by considering the aldehyde reagent and the amine one, such as for example the aldehyde, reacting with amine A leads to imine **1A$_{im}$**. When the reduction was also done, the compound (amine) is labeled as **1A$_{am}$**.

Table 1
Inhibition constant of imines (**1**–**4**)(**A**–**E**)$_{im}$ and the corresponding amines (**1**–**4**)(**A**–**E**)$_{am}$ reported in the paper, by a stopped-flow, CO_2 hydrase assay (K_is are mean from 3 different assays)[7]

Inhibitors	K_i (nM) hCA II	K_i (nM) hCA I
1A$_{im}$	21,400	1230
1A$_{am}$	123.2	56.7
2A$_{im}$	10,400	8270
2A$_{am}$	184.5	266
3A$_{im}$	N.D.	N.D.
3A$_{am}$	200.5	116.4
4A$_{im}$	13,300	1290
4A$_{am}$	142.5	189.5
1B$_{im}$	1160	8600
1B$_{am}$	22,171	200.5
2B$_{im}$	780	7460
2B$_{am}$	158.4	251.5
3B $_{im}$	1300	1040
3B$_{am}$	141.5	209.5
4B$_{im}$	11,250	7450
4B$_{am}$	5329	228.3
1C$_{im}$	105	311
1C$_{am}$	21.4	116.6
2C$_{im}$	78	85
2C$_{am}$	32.4	18.75
3C$_{im}$	11.1	80
3C$_{am}$	701.8	29
4C$_{im}$	7.0	52
4C$_{am}$	86.6	23.5
1D$_{im}$	7.1	93
1D$_{am}$	38.5	30.3
2D$_{im}$	11.0	72
2D$_{am}$	24.0	25.5
3D$_{im}$	10.3	76
3D$_{am}$	79.7	59.5
4D$_{im}$	9.4	92
4D$_{am}$	130.5	51.9
1E$_{im}$	11.0	678
1E$_{am}$	160.0	62.4
2E$_{im}$	11.2	665
2E$_{am}$	8.6	50.3
3E$_{im}$	43	105
3E$_{am}$	236	92.1
4E$_{im}$	12.6	70
4E$_{am}$	51.5	56.05
AZA	250	12
MZA	50	14
DCP	1200	38

the enzyme pocket so that they induce large differences in K_i values for the same compound (e.g. K_i of **2B$_{im}$** is 7460 nM against hCA I and only 780 nM againt hCA II). In this case the selectivity ratio is very good, in the range of 10. Furthermore, it may observed that even very small alterations in the structure of these compounds lead to a drastic change in CAII inhibitory capacity, for example the incorporation of an additional substitutent OCH_3 in **4B$_{im}$** structure comparing to **3B$_{im}$** structure drastically decreases the inhibition power from 1300 to 11,250 nM.

3.1.2. Amines

(i) The reduced imines **1A$_{am}$** and its homologue **1B$_{am}$** are the best inhibitors against hCA I from all acid and ester compounds, with K_i = 56.6 and 200.5 nM, respectively. The unsubstituted benzyl scaffold **1** fits well inside the bulky enzyme pocket of hCA I (bulky space due of the presence of His200 and His67 among others).[1,2] The acid **1A$_{am}$** (56.6 for hCA I and 123.2 for hCA II nM) is more potent than the ester **1B$_{am}$** (200.5 for hCA I and 22171 for hCA II nM) probably due to the presence of better binding function –COOH than $-CO_2CH_3$. The monosubstituted *tert*-butyl compounds **2A$_{am}$**–**2B$_{am}$** and 3**A$_{am}$**–**3B$_{am}$** express small differences of K_i values againt hCA I and hCA II (Fig. 1). The selectivity ratio for the inhibition of hCA I over hCA II is very good for **1B$_{am}$** and **4B$_{am}$**, with hCA II/hCA I = 111 and 23, respectively. The X-ray structures of such adducts can afford better understanding of interaction between certain moieties of the inhibitors and amino acid residues within the enzyme active site and can explain why a minor structural difference between two compounds may lead to very different inhibitory effects against various CAs.[2]

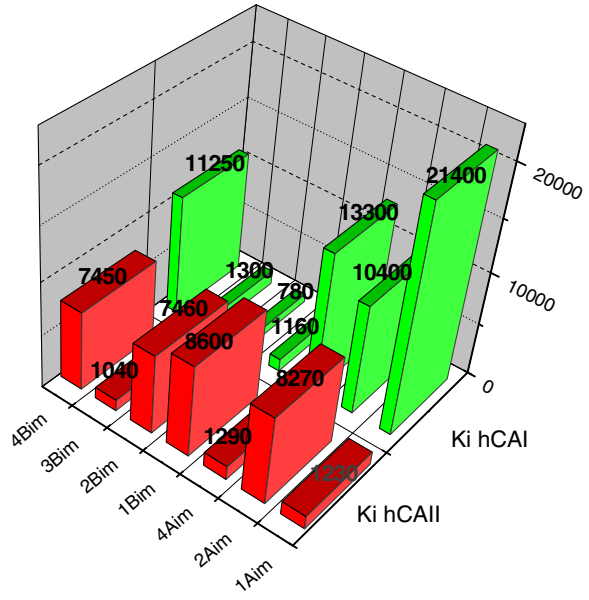


Figure 1. Inhibition constant values against hCA II and hCA I for aromatic Schiff bases with acid and ester substituents (see also Table 1 for the precise K_i values).

(ii) The 8 amines **1A$_{am}$**–**2A$_{am}$**–**3A$_{am}$**–**4A$_{am}$**–**1B$_{am}$**–**2B$_{am}$**–**3B$_{am}$**–**4B$_{am}$** express high inhibition power compared to their corresponding imines, especially for hCA I. It seems that the stereochemically rigid imino compounds would result in poorer binding, decreasing inhibitory properties related to the saturated mobile amino compounds. The difference in inhibition is very important reaching 93 times better for **4Aam**:**4Aim**.This binding is less valid in the case of very selective hCA II. This selectivity is an advantage in order to avoid side effects of drugs based on them.

3.2. Compounds obtained from aldehydes C, D, E

3.2.1. Imines

(i) The 3 aldehydes **C**, **D** and **E** possess 0, 1 or 2 CH_2 groups respectively, in *para* to the sulfonamide group. It has been demonstrated that an additional CH_2 moiety may lead to important change in inhibition constant value:[9–11] for example the K_i value increase from 72 nM (**2D$_{im}$**) to 665 nM (**2E$_{im}$**) by adding one CH_2 group. All these derivatives showed higher hCA II affinity (7–105 nM) than hCA I (52–678 nM) (Fig. 2 and Chart 1). This result is expected because sulfonamides are known to be better inhibitors for hCA I than hCA II.

(ii) Very good inhibitor properties have been detected: the best compounds are **4C$_{im}$** and **1D$_{im}$** (7 and 7.1 nM) againt hCA II and seven other potent hCA II inhibitors (such as **3C$_{im}$**,

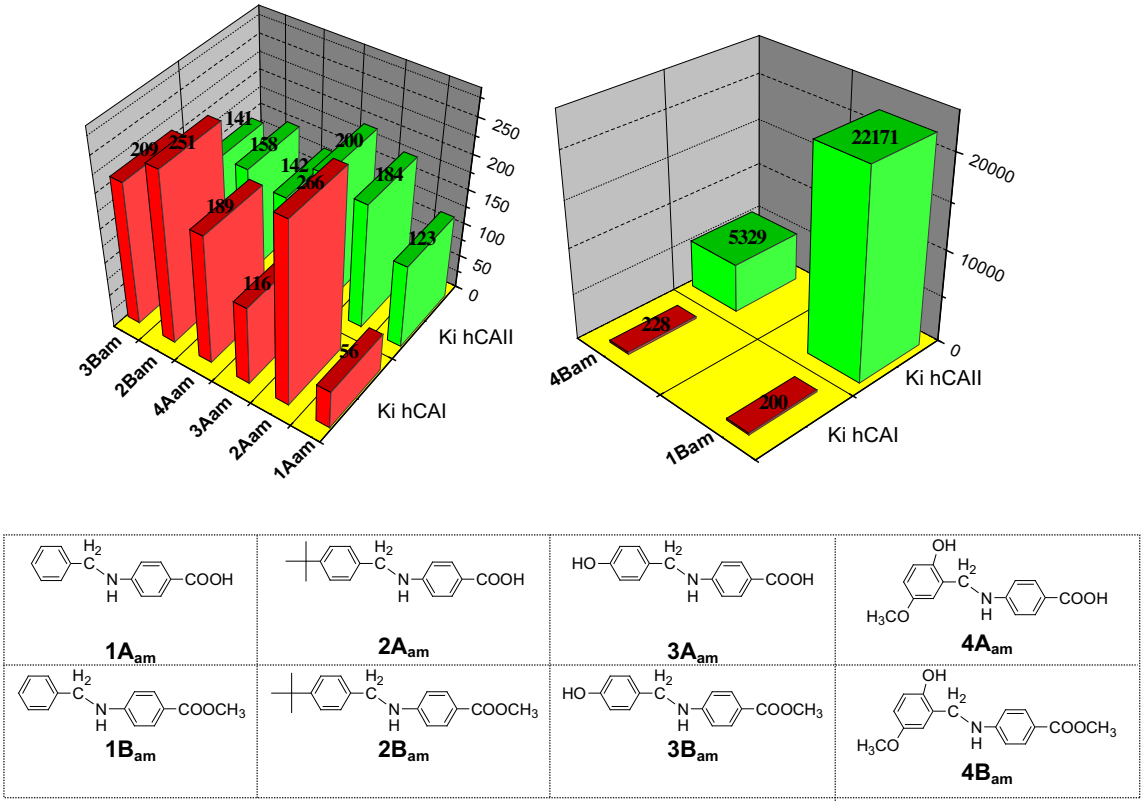


Figure 2. Inhibition constant values against hCA II and hCA I for aromatic amines with carboxylic acid and ester substituents.

1E$_{im}$, **2E$_{im}$**, **4E$_{im}$**, **2D$_{im}$**, **3D$_{im}$**, **4D$_{im}$** with K_i ranging between 9.4 and 12.6 nM. Imine **4C$_{im}$** (52 nM) was the best inhibitor against hCA I. These data clearly indicate that these compounds act as efficient CA inhibitors with potencies comparable to those of reference sulfonamide drugs, or clinically used CA inhibitors as diuretics, antiglaucoma agents, antiepileptics, eg: **AZA**, **MZA**, **DCP** (Table 1).[12]

(iii) All sulfonamides with a benzylamino group **D** (n = 1) express better inhibition properties than compound with phenylethylamino group **E** (n = 2) or aniline group **C** (n = 0), following this order **1D$_{im}$** > **1C$_{im}$** > **1E$_{im}$**, **2D$_{im}$** ~ **2E$_{im}$** > **2C$_{im}$**, **3D$_{im}$** ~ **3C$_{im}$** > **3E$_{im}$**. The phenethylamine group **E** seems to be sterically long and did not bind tightly to the active site of the enzyme. The extended linker would result in poorer binding because of a higher entropic loss upon binding, especially in hCA I. With the compounds originating from aldehyde **4**, the K_i values of **4C$_{im}$**, **4E$_{im}$** and **4D$_{im}$** are close and are the lowest because the hydroxy and methoxy groups participate in H-bonds, strongly stabilizing the enzyme–inhibitor adduct. There are in fact several amino acid residues situated in the middle of the active site cavity of hCA II (such as Asn62, His64, Gln92, Th200) responsible of this kind of interaction.[13,14] Finally due to the good inhibition properties of compounds related to **4**, there is no notable difference for this kind of potent inhibitors **4C$_{im}$** ~ **4D$_{im}$** ~ **4E$_{im}$**.

(iv) Comparing K_i for all compounds derived from **C** or **E**, the order of K_i values for such compounds are similar except for **3E$_{im}$** (against hCA II): **1C$_{im}$** 311–105 > **2C$_{im}$** 85–78 > **3C$_{im}$** 80–11.1 > **4C$_{im}$** 52–7 nM (against hCA II and hCA I respectively) and **1E$_{im}$** 678 > **2E$_{im}$** 665 > **3E$_{im}$** 105 > **4E$_{im}$** 70 nM (only against hCA I) and **1E$_{im}$** 11 > **2E$_{im}$** 11.2 > **4E$_{im}$** 12.6 > **3E$_{im}$** 43 nM (against hCA II). Based on this result, the substituted benzyl *tert*-butyl **2** seem to be more active than the non-substituted phenyl **1** and the substituted phenyl **4** express better activity by the presence of OH and OCH_3 attached to the benzyl group (Fig. 3 and Scheme 2).

3.2.2. Amine

(i) The imines and the corresponding amines are more effective hCA I and hCA II inhibitors as compared to the parent sulfonamide amines **C**, **D** and **E** (Table 2).[15] The data of reduced sulfonamide imines are very different from their corresponding imines. Certainly the presence of $-CH_2-NH-$ groups (in the case of amines) instead of $-CH{=}N-$ groups (in the case of imines) induces flexibility and different interactions with amino acid residues of the isozymes. The best inhibitor againt hCA I is **2C$_{am}$** with K_i = 18.75 nM followed by **4C$_{am}$** with K_i = 23.5 nM, and **2E$_{am}$** against hCA II with K_i = 8.6 nM.

(ii) Compounds with *tert*-butylbenzyl group, **2** possess good activity against hCA I and hCA II with K_i in the range of 8.6–50.3 nM and are slightly more potent than compound from non-substituted benzyl **1**: **2C** 18.75 > **1C** 116.65 nM (except against hCA II), **2E** 665–11.2 > **1E** 678–11, **2D** 25.4–24 > **1D** 30.3–38.5 nM (hCA I and hCA II, respectively). Certainly the bulky *tert*-butyl fills the pocket space and stabilizes this hCAs.

(iii) Compounds with 2-hydroxy-5-methoxybenzyl group **4** are more potent than compounds from 4-hydroxybenzyl **3** except for **4D** against hCA II. It is obvious that the disubstituted aromatic moiety **4** assures more H-bond interaction and tighter bonding with amino acid residues of the hydrophobic pocket.

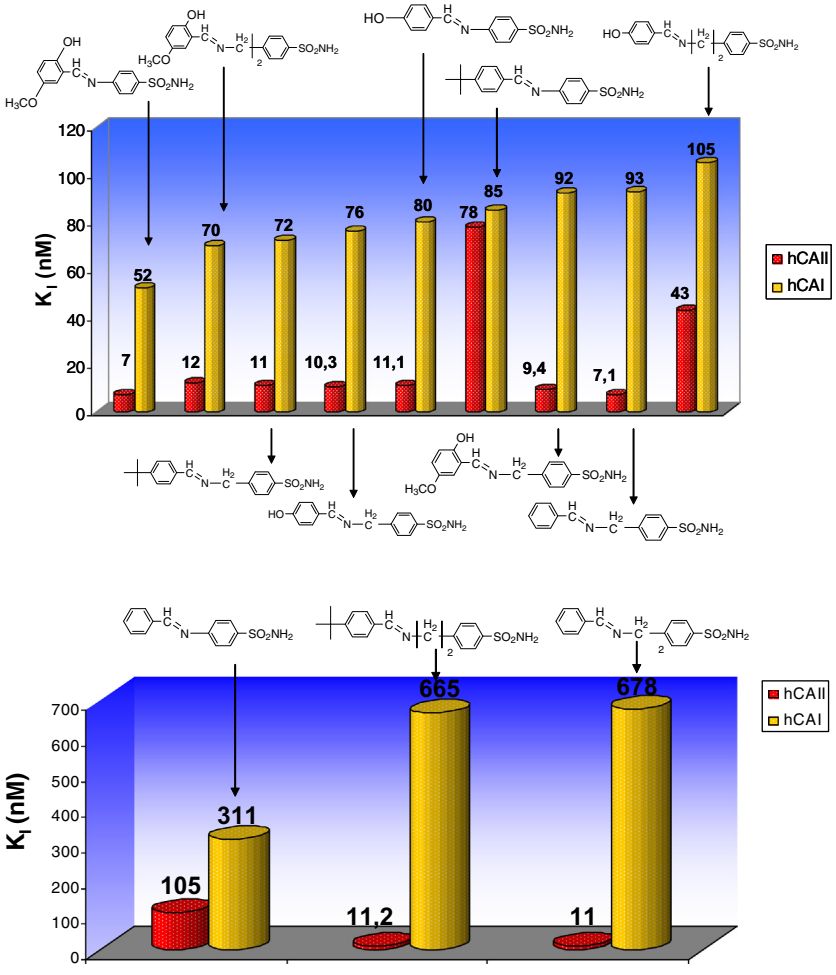
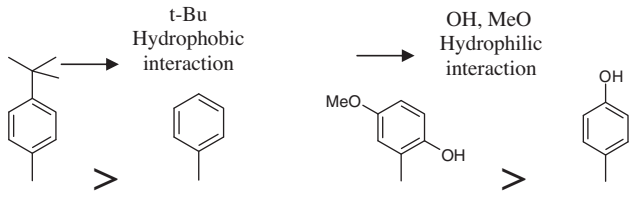


Figure 3. K_i (nM) for sulfonamide inhibitors againt hCA I and hCA II.



Scheme 2. Interaction sites of incorporated substituents.

Table 2
K_i values of sulfonamide amines **C**–**E**

Sulfonamide mines	K_i (nM)	
	hCA I	hCA II
C	28,000	300
D	25,000	170
E	21,000	160

4. Conclusions

Two libraries of CA inhibitors of (20 imines and 20 amines) have been synthesised by condensation between five amines incorporating carboxylic acid, carboxymethyl or sulfonamide groups, with fouraromatic aldehydes substitued by *tert*-butyl, hydroxy or hydroxy and methoxy groups, followed by reduction of the imino to the amino group.

(i) Moderate inhibition constants in the range of micromolar were assayed for compounds with carboxy, **A** or methoxy, **B** substituents. The flexible amines express higher inhibition compared to their more rigid imine (for hCA I). High selectivity of 10^2 is expressed for hCA II over hCA.
(ii) Low nanomolar K_i values of sulfonamide imines and amines (with **C**, **D**, **E**) reaching 7 and 8.6 nM respectively.
(iii) Better inhibition power was determined against hCA II versus hCA I for sulfonamide imines.
(iv) We discovered new amine and imine derivatives with inhibitory properties against hCA I and hCA II and good water solubility. These compounds are potential candidates in ophtalmologic studies l in which eye drops containing CAIs are useful for the treatment of glaucoma and ocular hypertension.

5. Experimental

5.1. Chemistry

All reagents were obtained from Aldrich and used without further purification. All organic solutions were routinely dried by

using sodium sulfate. ^{1}H NMR spectra were recorded on an ARX 300 MHz Bruker spectrometer in $CDCl_3$ with the use of the residual solvent $CHCl_3$ peak as reference. Elemental analyses were performed at Service d'Analyse de l'Université Montpellier II.

5.1.1. General synthesis of imine compounds

1 mmol of amine **A**–**E** and 1.1 mmol of aldehyde **1**–**4** were introduced in 50 ml of $CHCl_3$:MeOH (5:1, v/v). The solution was stirred at reflux for 18 h. The solvent was then evaporated and the pure imine compounds were obtained after precipitated by ether.

5.1.2. Imines

5.1.2.1. $1A_{im}$: 4-(Benzylideneimino) benzoic acid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.251–7.279 (d, 2H, Ar-H from benzoic acid moiety), 7.491–7.585 (m, 3H, Ar-H), 7.92 (dd, 2H, Ar-H), 8.168–8.191 (d, 2H, Ar-H from benzoic acid), 8.472 (s, 1H, CH=N). Elem. anal. calcd for $C_{14}H_{11}NO_2$: C, 74.65; H, 4.92; N, 6.22; O, 14.21; found C, 74.36; H, 5.11; N, 6.09.

5.1.2.2. $2A_{im}$: 4-(4-*tert*-Butylbenzylideneimino) benzoic acid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.191 (s, 9H, tBu), 7.042–7.07 (d, 2H, Ar-H from benzoic acid), 7.349–7.377 (d, 2H, Ar-H), 7.656–7.684 (d, 2H, Ar-H), 7.894–7.922 (d, 2H, Ar-H from benzoic acid), 8.282 (s, 1H, CH=N). Elem. anal. calcd for $C_{18}H_{19}NO_2$: C, 76.84; H, 6.81; N, 4.98; O, 11.37; found C, 75.90; H, 6.60; N, 4.75.

5.1.2.3. $3A_{im}$: 4-(4-Hydroxybenzylideneimino) benzoic acid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.880–6.852 (d, 2H, Ar-H), 7.147–7.120 (d, 2H, Ar-H from benzoic acid), 7.727–7.700 (d, 2H, Ar-H), 7.981–7.953 (d, 2H, Ar-H from benzoic acid), 8.316 (s, 1H, CH=N). Elem. anal calcd for $C_{14}H_{11}NO_3$: C, 69.70; H, 4.60; N, 5.81; O, 19.90; found C, 69.76; H, 4.96; N, 5.80.

5.1.2.4. $4A_{im}$: 4-(2-Hydroxy-5-methoxybenzylideneimino)benzoic acid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.772 (s, 3H, OCH_3), 6.891–6.900 (d, 1H, Ar-H), 6.910–6.940 (d, 1H, Ar-H), 6.973–6.982, 7.004–7.012 (dd, 1H, Ar-H), 7.252–7.279 (d, 2H, Ar-H from benzoic acid), 8.055–8.082 (d, 2H, Ar-H from benzoic acid), 8.569 (s, 1H, CH=N). Elem. anal. calcd for $C_{15}H_{13}NO_4$: C, 66.41; H, 4.83; N, 5.16; O, 23.59; found C, 66.30; H 5.2; N 4.93.

5.1.2.5. $1B_{im}$: Methyl 4-(benzylideneimino)benzoate. ^{1}H NMR (300 MHz, 9:1 $CDCl_3$:CD_3OD) δ: 3.930 (s, 3H, $COOCH_3$), 7.198–7.227 (d, 2H, Ar-H from methyl benzoate), 7.5 (m, 3H, Ar-H), 7.901–7.928 (d, 2H, Ar-H), 8.066–8.095 (d, 2H, Ar-H from methyl benzoate), 8.441 (s, 1H, CH=N). Elem. Anal calcd for $C_{15}H_{13}NO_2$: C, 75.30; H, 5.48; N, 5.85; O, 13.37; found C, 75.25; H, 5.69; N, 5.61.

5.1.2.6. $2B_{im}$: Methyl 4-(4-*tert*-butylbenzylideneimino)benzoate. ^{1}H NMR (300 MHz, 9:1 $CDCl_3$/CD_3OD) δ: 1.363 (s, 9H, tBu), 3.889 (s, 3H, $COOCH_3$), 7.244–7.271 (d, 2H, Ar-H from methyl benzoate), 7.564–7.592, 7.859–7.886 (d, 2H, Ar-H), 8.025–8.053 (d, 2H, Ar-H from methyl benzoate), 8.497 (s, 1H, CH=N). Elem. anal calcd for $C_{19}H_{21}NO_2$: C, 77.26; H, 7.17; N, 4.74; O, 10.83; found C, 77.07; H, 7.38; N, 4.43.

5.1.2.7. $3B_{im}$: Methyl 4-(4-hydroxybenzylideneimino)benzoate. ^{1}H NMR (300 MHz, 9:1 $CDCl_3$:CD_3OD) δ: 3.946 (s, 3H, $COOCH_3$), 6.940–6.968 (d, 2H, Ar-H), 7.196–7.224 (d, 2H, Ar-H from methyl benzoate), 7.828–7.857 (d, 2H, Ar-H), 8.070–8.098 (d, 2H, Ar-H from methyl benzoate), 8.377 (s, 1H, CH=N). Elem. anal calcd for $C_{15}H_{13}NO_3$: C, 70.58; H, 5.13; N, 5.49; O, 18.80; found C, 69.81; H, 5.01; N, 5.31.

5.1.2.8. $4B_{im}$: Methyl 4-(2-hydroxy-5-methoxybenzylideneimino)benzoate. ^{1}H NMR (300 MHz, 9:1 $CDCl_3$:CD_3OD) δ: 3.725 (s, 3H, OCH_3), 3.846 (s, 3H, $COOCH_3$), 6.816–6.925 (m, 3H, Ar-H); 7.240–7.268, 7.984–8.012 (d, 2H, Ar-H from methyl benzoate), 8.953 (s, 1H, CH=N). Elem. anal calcd for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91; O, 22.43; found C, 67.12; H, 5.29; N, 4.7.

5.1.2.9. $1C_{im}$: 4-(Benzylidene-imino)-benzenesulfonamide. ^{1}H NMR (300 MHz, 9:1 $CDCl_3$:CD_3OD) δ: 7.128–7.155 (d, 2H, Ar-H from benzenesulfonamide) 7.38 (m, 3H, Ar-H), 7.79 (m, 4H, 2 Ar-H and 2 Ar-H from benzenesulfonamide), 8.319 (s, 1H, CH=N). Elem. anal calcd for $C_{13}H_{12}N_2O_2S$: C, 59.98; H, 4.65; N, 10.76; O, 12.29; S, 12.32; found C, 59.95; H, 4.91; N, 10.38; S, 12.14.

5.1.2.10. $2C_{im}$: 4-[(4-*tert*-Butyl-benzylidene)-imino]-benzenesulfonamide. ^{1}H NMR (300 MHz, 9:1 $CDCl_3$: CD_3OD) δ: 1.189 (s, 9H, tBu). 7.079–7.108 (d, 2H Ar-H from benzenesulfonamide), 7.346–7.374, 7.650–7.678 (d, 2H, Ar-H), 7.741–7.770 (d, 2H, Ar-H from benzenesulfonamide), 8.255 (s, 1H, CH=N). Elem. anal. calcd for $C_{17}H_{20}N_2O_2S$: C, 64.53; H, 6.37; N, 8.85; O, 10.11; S, 10.13; found C, 64.63; H, 6.47; N, 8.55; S, 9.70.

5.1.2.11. $3C_{im}$: 4-[(4-Hydroxy-benzylidene)-imino]-benzenesulfonamide. ^{1}H NMR (300 MHz, 9:1 $CDCl_3$:CD_3OD) δ: 6.538–6.567 (d, 2H, Ar-H), 6.895–6.924 (d, 2H, Ar-H from benzene sulfonamide), 7.402–7.431 (d, 2H, Ar-H), 7.543–7.571 (d, 2H, Ar-H from benzene sulfonamide), 8.017 (s, 1H, CH=N). Elem. anal. calcd for $C_{13}H_{12}N_2O_3S$: C, 56.51; H, 4.38; N, 10.14; O, 17.37; S, 11.60; found C, 56.31; H, 4.46; N, 10.05; S, 11.75.

5.1.2.12. $4C_{im}$: 4-[(2-Hydroxy-5-methoxy-benzylidene)-imino]-benzenesulfonamide. ^{1}H NMR (300 MHz, 9:1 $CDCl_3$:CD_3OD) δ: 3.684 (s, 3H, OCH_3), 6.918–7.049 (m, 3H, H-Ar), 7.352–7.381, 7.936–7.965 (d, 2H, Ar-H from benzenesulfonamide), 8.642 (s, 1H, CH=N). Elem. anal calcd for $C_{14}H_{14}N_2O_4S$: C, 54.89; H, 4.61; N, 9.14; O, 20.89; S, 10.47; found C, 55.00; H, 4.87; N, 8.71; S, 10.22.

5.1.2.13. $1D_{im}$: 4-[(Benzylidene-imino)-methyl]-benzenesulfonamide. ^{1}H NMR (300 MHz, 9 $CDCl_3$:1 CD_3OD) δ: 5 (s, 2H, CH_2), 7.066–7.095 (d, 2H, Ar-H from benzenesulfonamide), 7.275–7.375 (m, 3H, Ar-H), 7.692–7.760 (m, 4H, 2Ar-H from benzenesulfonamide and 2Ar-H), 8.25 (s, 1H, CH=N). Elem. anal calcd for $C_{14}H_{14}N_2O_2S$: C, 61.29; H, 5.14; N, 10.21; O, 11.66; S, 11.69; found C, 61.08; H, 5.17; N, 9.87; S, 11.38.

5.1.2.14. $2D_{im}$: 4-{[(4-*tert*-Butyl-benzylidene)-imino]-methyl}-benzenesulfonamide. ^{1}H NMR (300 MHz, 9 $CDCl_3$:1 CH_3OD) δ: 1.177 (s, 9H, tBu), 3.162 (s, 2H, CH_2), 7.073–7.102 (d, 2H, Ar-H from benzenesulfonamide), 7.337–7.365, 7.639–7.667 (d, 2H, Ar-H), 7.732–7.760 (d, 2H, Ar-H from benzenesulfonamide), 8.248 (s, 1H, CH=N). Elem. anal calcd for $C_{18}H_{22}N_2O_2S$: C, 66.26; H, 8.34; N, 7.73; O, 8.83; S, 8.85; found C, 66.53; H, 8.84; N, 7.96.

5.1.2.15. $3D_{im}$: 4-{[(4-Hydroxy-benzylidene)-imino]-methyl}-benzenesulfonamide. ^{1}H NMR (300 MHz, 9 $CDCl_3$: 1 CD_3OD) δ: 4.77 (s, 2H, CH_2), 6.584–6.613 (d, 2H, Ar-H), 6.714–6.742 (d, 2H, Ar-H from benzenesulfonamide), 7.157–7.185 (d, 2H, Ar-H), 7.524–7.554 (d, 2H, Ar-H from benzenesulfonamide), 8.266 (s, 1H, CH=N). Elem. anal calcd for $C_{14}H_{14}N_2O_3S$: C, 57.92; H, 4.86; N, 9.65; O, 16.53; S, 11.04; found C, 56.26; H, 4.98; N, 8.81; S, 11.40.

5.1.2.16. $4D_{im}$: 4-{[(2-Hydroxy-5-methoxy-benzylidene)-imino]-methyl}-benzenesulfonamide. ^{1}H NMR (300 MHz, 9 $CDCl_3$: 1 CD_3OD) δ: 3.821 (s, 3H, OCH_3), 6.914–6.923 (d, 1H, Ar-H), 6.977–7.007 (d, 1H, Ar-H), 7.043–7.053, 7.073–7.083 (dd, 1H,

Ar-H), 7.358–7.386, 7.980–8.008 (d, 2H, Ar-H from benzenesulfonamide), 8.586 (s, 1H, CH=N). Elem. anal calcd for $C_{15}H_{16}N_2O_4S$: C, 56.24; H, 5.03; N, 8.74; O, 19.98; S, 10.01; found C, 55.16; H, 5.14; N, 8.07; S, 9.59.

5.1.2.17. $1E_{im}$: 4-[2-(Benzylidene-imino)-ethyl]-benzenesulfonamide. ^{1}H NMR (300 MHz, CD_3CN) δ: 3.059–3.105 (t, 2H, CH_2), 3.858–3.905 (td, 2H, CH_2), 5.636 (s, 2H, SO_2NH_2), 7.448–7.475 (m, 5H, 2 Ar-H from benzenesulfonamide and 3 Ar-H), 7.700–7.732 (m, 2H, Ar-H), 7.780–7.808 (d, 2H, Ar-H from benzenesulfonamide), 8.249 (s, 1H, CH=N). Elem. anal calcd for $C_{15}H_{16}N_2O_2S$: C, 62.48; H, 5.59; N, 9.71; O, 11.10; S, 11.12; found C, 62.23; H, 6.02; N, 9.41; S, 9.15.

5.1.2.18. $2E_{im}$: 4-{2-[(4-*tert*-Butyl-benzylidene)-imino]-ethyl}-benzenesulfonamide. ^{1}H NMR (300 MHz, CD_3CN) δ: 1.338 (s, 9H, tBu), 3.049–3.095 (t, 2H, CH_2), 3.840–3.889 (td, 2H, CH_2), 5.625 (s, 2H, SO_2NH_2), 7.441–7.469 (d, 2H, Ar-H from benzenesulfonamide), 7.476–7.505, 7.623–7.651 (d, 2H, Ar-H), 7.775–7.802 (d, 2H, Ar-H from benzenesulfonamide), 8.206 (s, 1H, CH=N).[1] Elem. anal calcd for $C_{19}H_{24}N_2O_2S$: C, 66.25; H, 7.02; N, 8.13; O, 9.29; S, 9.31; found C, 64.44; H, 7.52; N, 7.55; S, 10.34.

5.1.2.19. $3E_{im}$: 4-{2-[(4-Hydroxy-benzylidene)-imino]-ethyl}-benzenesulfonamide. ^{1}H NMR (300 MHz, 9 $CDCl_3$: 1 CH_3OH) δ: 3.251–3.298 (t, 2H, CH_2), 4.022–4.069 (t, 2H, CH_2), 5.06 (s, 1H, OH), 7.029–7.057, 7.598–7.625 (d, 2H, Ar-H from benzenesulfonamide), 7.749–7.777, 7.994–8.022 (d, 2H, Ar-H), 8.318 (s, CH=N). Elem.anal. calcd for $C_{15}H_{16}N_2O_3S$: C, 59.19; H, 5.30; N, 9.20; O, 15.77; S, 10.54; found C, 58.50; H, 5.46; N, 9.75; S, 9.93.

5.1.2.20. $4E_{im}$: 4-{2-[(2-Hydroxy-5-methoxy-benzylidene)-imino]-ethyl}-benzenesulfonamide. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.051–3.096 (t, 2H, CH_2), 3.739 (t, 3H, O-CH_3), 3.849–3.895 (t, 2H, CH_2), 6.739–6.748, 6.795–6.825 (d, 1H, Ar-H), 6.867–6.877, 6.897–6.907 (dd, 1H, Ar-H), 7.352–7.381, 7.786–7.813 (d, 1H, Ar-H from benzenesulfonamide), 8.238 (s, 1H, CH=N). Elem. anal calcd for $C_{16}H_{18}N_2O_4S$: C, 57.47; H, 5.43; N, 8.38; O, 19.14; S, 959; found C, 57.26; H, 5.13; N, 8.65.

5.1.3. General synthesis of amine compounds

One mole of imine was dissolved in 30 ml of MeOH. 10 equivalent of $NaBH_4$ was added during one hour. The mixture is left under stirring for 3 more hours. The mixture was evaporated than dissolved in acid solution and the amine is extracted in ether. Some amines were purified in flash column to eliminate all salt residues. We may note that all the amino compounds reported bellow are chromatographically pure following a study reported previously: see Ref.[6b]

5.1.4. Amines

5.1.4.1. $1A_{am}$: 4-(Benzylideneamino) benzoic acid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.434 (s, 2H, CH_2-Ar), 6.652–6.681 (d, 2H, Ar-H from benzoic acid), 7.372–7.385 (m, 5H, Ar-H), 7.927–7.955 (d, 2H, Ar-H).

5.1.4.2. $2A_{am}$: 4-(4-*tert*-Butylbenzylideneamino)benzoic acid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.234 (s, 9H, tBu), 4.239 (s, 2H, CH_2), 6.472–6.501 (d, 2H, Ar-H from benzoic acid), 7.221–7.293 (m, 4H, Ar-H), 7.652–7.681 (d, 2H, Ar-H from benzoic acid).

5.1.4.3. $3A_{am}$: 4-(4-Hydroxybenzylideneamino)benzoic acid. ^{1}H NMR (300 MHz, CD_3OD) δ: 6.592–6.621 (d, 2H, Ar-H from benzoic acid), 6.746–6.773, 7.170–7.197 (d, 2H, Ar-H), 7.751–7.774 (d, 2H, Ar-H from benzoic acid).

5.1.4.4. $4A_{am}$: 4-(2-Hydroxy-5-methoxybenzylideneamino)benzoic acid. ^{1}H NMR (300 MHz, CD_3OD) δ: 3.633 (s, 3H, OCH_3), 4.323–4.339 (d, 2H, CH_2), 6.593–6.650 (m, 3H, Ar-H), 6.711–6.794 (m, 2H, Ar-H from benzoic acid), 7.740–7.767 (d, 2H, Ar-H from benzoic acid).

5.1.4.5. $1B_{am}$: Methyl 4-(benzylideneamino)benzoate. ^{1}H NMR (300 MHz, CD_3OD) δ: 3.231 (s, 3H, OCH_3), 3.771 (s, 2H, CH_2), 5.962–6.002 (t, 2H, Ar-H), 6.734 (m, 5H, Ar-H), 7.261–7.274 (d, 2H, Ar-H).

5.1.4.6. $2B_{am}$: Methyl 4-(4-*tert*-butylbenzylideneamino)benzoate. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.321 (s, 9H, tBu), 3.845 (s, 2H, OCH_3), 4.334–4.352 (d, 2H, CH_2), 6.578–6.607 (d, 2H, Ar-H from methyl-benzoate), 7.259–7.294, 7.374–7.396 (d, 2H, Ar-H), 7.849–7.878 (d, 2H, Ar-H from methyl-benzoate). ^{13}C (75 Hz, $CDCl_3$) δ: 18.2, 31, 34.5, 47.2, 51.4, 58.3, 111.8, 118.5, 125.7, 127, 131.7, 135, 150.6, 151.8, 167.2.

5.1.4.7. $3B_{am}$: Methyl 4-(4-hydroxybenzylideneamino)benzoate. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.845 (s, 2H, OCH_3), 6.578–6.607 (d, 2H, Ar-H from methyl-benzoate), 7.259–7.274, 7.374–7.396 (d, 2H, Ar-H), 7.849–7.878 (d, 2H, Ar-H from methyl-benzoate).

5.1.4.8. $4B_{am}$: Methyl 4-(2-hydroxy-5-methoxybenzylideneamino)benzoate. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.59 (s, 3H, CH_3–O–Ar), 3.730 (s, 3H, $COOCH_3$), 4.220 (s, 2H, CH_2N)6.589–6.657 (m, 3H, 3H from methyl-benzoate and 1H from Ar-H), 6.719–6.760 (m, 2H, Ar-H), 7.651–7.678 (d, 2H, Ar-H from methyl-benzoate).

5.1.4.9. $1C_{am}$: 4-(Benzylidene-amino)-benzenesulfonamide. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.410 (s, 2H, CH_2), 6.698 (s, 2H, Ar-H), 7.058 (s, 1H, Ar-H), 7.313–7.552 (m, 6H, Ar-H).

5.1.4.10. $2C_{am}$: 4-[(4-*tert*-Butyl-benzylidene)-amino]-benzenesulfonamide. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.262 (s, 9H, tB), 4.275–4.294 (d, 2H, CH_2), 6.621–6.645 (d, 2H, Ar-H from benzenesulfonamide), 6.889 (s, 2H, SO_2NH_2), 6.932–6.970 (t, 1H, NH), 7.251–7.274, 7.335–7.363 (dd, 2H, Ar-H), 7.465–7.494 (d, 2H, Ar-H from benzenesulfonamide).

5.1.4.11. $3C_{am}$: 4-[(4-Hydroxy-benzylidene)-amino]-benzenesulfonamide. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.760–6.789 (d, 2H, Ar-H from benzenesulfonamide), 7.032–7.061 (d, 2H), 7.124–7.153 (d, 2H), 7.718–7.748 (d, 2H, Ar-H from benzenesulfonamide).

5.1.4.12. $4C_{am}$: 4-[(2-Hydroxy-5-methoxy-benzylidene)-amino]-benzenesulfonamide. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.581 (s, 3H, CH_3), 4.208 (s, 2H, CH_2), 6.519–6.579 (m, 3H, 2 Ar-H from benzenesulfonamide and 1 Ar-H), 6.665–6.694 (m, 2H, Ar-H), 7.470–7.499 (d, 2H, Ar-H from benzenesulfonamide).

5.1.4.13. $1D_{am}$: 4-[(Benzylidene-amino)-methyl]-benzenesulfonamide. ^{1}H NMR (300 MHz, CD_3OD/$CDCl_3$) δ: 3.734–3.797 (d, 4H, CH_2), 7.305 (m, 5H, Ar-H), 7.414–7.438, 7.8–7.85 (d, 2H, Ar-H from benzenesulfonamide). ^{13}C (75 Hz, $CDCl_3$) δ: 5, 56, 57, 130, 131, 132.1, 132.2, 132.3, 145.2, 146, 148.

5.1.4.14. $2D_{am}$: 4-{[(4-*tert*-Butyl-benzylidene)-amino]-methyl}-benzenesulfonamide. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.246 (s, 9H, tBu), 3.641–3.744 (d, 4H, CH_2), 7.175–7.202 (d, 2H, Ar-H), 7.294–7.316, 7.434–7.462 (d, 2H, Ar-H from benzenesulfonamide), 7.790–7.818 (d, 2H, Ar-H).

5.1.4.15. $3D_{am}$: 4-{[(4-Hydroxy-benzylidene)-amino]-methyl}-benzenesulfonamide. ^{1}H NMR (300 MHz, CD_3OD) δ: 3.574, 3.721 (s, 2H, CH_2), 6.637–6.665 (d, 2H, Ar-H), 7.051–7.078 (d, 2H, Ar-H from benzenesulfonamide), 7.417–7.444 (d, 2H, Ar-H), 7.782–7.809 (d, 2H, Ar-H from benzenesulfonamide).

5.1.4.16. $4D_{am}$: 4-{[(2-Hydroxy-5-methoxy-benzylidene)-amino]-methyl}-benzenesulfonamide. ^{1}H NMR (300 MHz, CD_3OD) δ: 3.580 (s, 3H, CH_3),, 3.721 (s, 2H, CH_2), 6.637–6.665 (d, 2H, Ar-H), 7.051–7.078 (d, 2H, Ar-H from benzenesulfonamide), 7.417–7.444 (d, 2H, Ar-H), 7.782–7.809 (d, 2H, Ar-H from benzenesulfonamide).

5.1.4.17. $1E_{am}$: 4-[2-(Benzylidene-amino)-ethyl]-benzenesulfonamide. ^{1}H NMR (300 MHz, D_2O) δ: 2.109, 3.701 (t, 2H, CH_2), 4.219 (s, 2H, CH_2N), 7.152 (m, 7H, Ar-H), 7.731–7.759 (2H, Ar-H from benzenesulfonamide).

5.1.4.18. $2E_{am}$: 4-{2-[(4-*tert*-Butyl-benzylidene)-amino]-ethyl}-benzenesulfonamide. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.132 (s, 9H, tBu), 2.171 (s, 4H, CH_2), 3.574 (s, 2H, CH_2N), 7.000–7.028 (d, 2H, Ar-H from benzenesulfonamide), 7.136–7.191 (td, 4H, Ar-H), 7.636–7.664 (d, 2H, Ar-H from benzenesulfonamide).

5.1.4.19. $3E_{am}$: 4-{2-[(4-Hydroxy-benzylidene)-amino]-ethyl}-benzenesulfonamide. ^{1}H NMR (300 MHz, CD_3OD) δ: 3.035, 4.044 (t, 2H, CH_2), 6.739–6.768 (d, 2H, Ar-H from benzenesulfonamide), 7.230–7.259, 7.359–7.387 (d, 2H, Ar-H), 7.767–7.795 (t, 2H, Ar-H from benzenesulfonamide).

5.1.4.20. $4E_{am}$: 4-{2-[(2-Hydroxy-5-methoxy-benzylidene)-amino]-ethyl}-benzenesulfonamide. ^{1}H NMR (300 MHz, D_2O) δ: 3.082, 3.291 (t, 2H, CH_2), 3.708 (s, 3H, OCH_3), 4.163 (s, 2H, CH_2N), 6.795 (m, 3H, Ar-H), 7.391–7.419, 7.779–7.807 (d, 2H, Ar-H from benzenesulfonamide).

5.2. CA inhibition assay

An SX.18MV-R Applied Photophysics (Oxford, UK) stopped-flow instrument has been used to assay the catalytic/inhibition of various CA isozymes as reported by Khalifah.[7] Phenol Red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.4) as buffer, 0.1 M Na_2SO_4 or $NaClO_4$ (for maintaining constant the ionic strength; these anions are not inhibitory in the used concentration), following the CA-catalyzed CO_2 hydration reaction for a period of 5–10 s. Saturated CO_2 solutions in water at 25 °C were used as substrate. Stock solutions of inhibitors were prepared at a concentration of 10 mM (in DMSO–water 1:1, v/v) and dilutions up to 1 nM done with the assay buffer mentioned above. At least 7 different inhibitor concentrations have been used for measuring the inhibition constant. Inhibitor and enzyme solutions were preincubated together for 10 min at room temperature prior to 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 Letter are the mean of such results. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and represent the mean from at least three different determinations.

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References and notes

1. (a) Supuran, C. T. *Nat. Rev. Drug Disc.* **2008**, *7*, 168; (b) De Simone, G.; Alterio, V.; Supuran, C. T. *Expert Opin. Drug Disc.* **2013**, *8*, 793; (c) Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 759; (d) Aggarwal, M.; Kondeti, B.; McKenna, R. *Expert Opin. Ther. Pat.* **2013**, *23*, 717; (e) Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3467; (f) Dubois, L.; Lieuwes, N. G.; Maresca, A.; Thiry, A.; Supuran, C. T.; Scozzafava, A.; Wouters, B. G.; Lambin, P. *Radiother. Oncol.* **2009**, *92*, 423.
2. (a) Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C. T.; De Simone, G. *Chem. Rev.* **2012**, *112*, 4421; (b) Supuran, C. T. *Expert Opin. Ther. Pat.* **2013**, *23*, 677; (c) Supuran, C. T. *Bioorg. Med. Chem.* **2013**, *21*, 1377; (d) Pinard, M. A.; Boone, C. D.; Rife, B. D.; Supuran, C. T.; McKenna, R. *Bioorg. Med. Chem.* **2013**, *21*, 7210.
3. (a) D'Ambrosio, K.; Vitale, R.-M.; Dogne, J.-M.; Masereel, B.; Innocenti, A.; Scozzafava, A.; Simone, G.; Supuran, C. T. *J. Med. Chem.* **2008**, *51*, 3230; (b) Innocenti, A.; Vullo, D.; Scozzafava, A.; Casey, J. R.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 573; (c) Bayram, E.; Senturk, M.; Kufrevioglu, I.; Supuran, C. T. *Bioorg. Med. Chem.* **2008**, *16*, 9101; (d) Winum, J.-Y.; Innocenti, A.; Nasr, J.; Montero, J.-L.; Scozzafava, A.; Vullo, D.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2353.
4. (a) Supuran, C. T.; Nicolae, A.; Popescu, A. *Eur. J. Med. Chem.* **1996**, *31*, 431; (b) Supuran, C. T.; Popescu, A.; Ilisiu, M.; Costandache, A.; Banciu, M. D. *Eur. J. Med. Chem.* **1996**, *31*, 439; (c) Puccetti, L.; Fasolis, G.; Vullo, D.; Chohan, Z. H.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3096; (d) Luca, C.; Barboiu, M.; Supuran, C. T. *Revue Roum. Chim.* **1991**, *36*, 1169.
5. (a) Supuran, C. T.; Scozzafava, A.; Popescu, A.; Bobes-Tureac, R.; Banciu, A.; Creanga, A.; Bobes-Tureac, G.; Banciu, M. D. *Eur. J. Med. Chem.* **1997**, *32*, 445; (b) Supuran, C. T.; Clare, B. W. *Eur. J. Med. Chem.* **1998**, *33*, 489; (c) Popescu, A.; Simion, A.; Scozzafava, A.; Briganti, F.; Supuran, C. T. *J. Enzyme Inhib.* **1999**, *14*, 407; (d) Scozzafava, A.; Banciu, M. D.; Popescu, A.; Supuran, C. T. *J. Enzyme Inhib.* **2000**, *15*, 533.
6. (a) Nasr, G.; Petit, E.; Supuran, C. T.; Winum, J. Y.; Barboiu, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6014; (b) Nasr, G.; Petit, E.; Vullo, D.; Winum, J. Y.; Supuran, C. T.; Barboiu, M. *J. Med. Chem.* **2009**, *52*, 4853.
7. Khalifah, R. G. *J. Biol. Chem.* **1971**, *246*, 2561.
8. Mazumdar, P. A.; Kumaran, D.; Swaminathan, S.; Dasa, A. K. *Acta Crystallogr.* **2008**, *F64*, 163.
9. (a) Casini, A.; Antel, J.; Abbate, F.; Scozzafava, A.; David, S.; Waldeck, H.; Schäfer, S.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 841; (b) Borras, J.; Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. *Bioorg. Med. Chem.* **1999**, *7*, 2397.
10. (a) Winum, J. Y.; Temperini, C.; El Cheikh, K.; Innocenti, A.; Vullo, D.; Ciattini, S.; Montero, J. L.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2006**, *49*, 7024; (b) Supuran, C. T.; Vullo, D.; Manole, G.; Casini, A.; Scozzafava, A. *Curr. Med. Chem. Cardiovasc. Hematol. Agents* **2004**, *2*, 49; (c) Mari, F.; Bertol, E.; Fineschi, V.; Karch, S. B. *J. R. Soc. Med.* **2004**, *97*, 397.
11. (a) Temperini, C.; Innocenti, A.; Guerri, A.; Scozzafava, A.; Rusconi, S.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2210; (b) Wilkinson, B. L.; Bornaghi, L. F.; Houston, T. A.; Innocenti, A.; Supuran, C. T.; Poulsen, S. A. *J. Med. Chem.* **2006**, *49*, 6539; (c) Singh, S.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 666.
12. (a) Abbate, F.; Coetzee, A.; Casini, A.; Ciattini, S.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 337; (b) Briganti, F.; Pierattelli, R.; Scozzafava, A.; Supuran, C. T. *Eur. J. Med. Chem.* **1996**, *31*, 1001.
13. (a) Güzel, Ö.; Temperini, C.; Innocenti, A.; Scozzafava, A.; Salman, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 152; (b) Supuran, C. T.; Mincione, F.; Scozzafava, A.; Briganti, F.; Mincione, G.; Ilies, M. A. *Eur. J. Med. Chem.* **1998**, *33*, 247.
14. (a) Abbate, F.; Casini, A.; Owa, T.; Scozzafava, A.; Supuran, C. T *Bioorg. Med. Chem. Lett.* **2004**, *14*, 217; (b) Fabrizi, F.; Mincione, F.; Somma, T.; Scozzafava, G.; Galassi, F.; Masini, E.; Impagnatiello, F.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 138; (c) Bertol, E.; Mari, F.; Milia, M. G.; Politi, L.; Furlanetto, S.; Karch, S. B. *J. Pharm. Biomed. Anal.* **2011**, *55*, 1186.
15. (a) Vullo, D.; Franchi, M.; Gallori, E.; Antel, J.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2004**, *47*, 1272; (b) Alp, C.; Özsoy, S.; Alp, N. A.; Erdem, D.; Gültekin, M. S.; Küfrevioğlu, Ö. İ.; Şentürk, M.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 818.