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Carbonic anhydrase and matrix metalloproteinase inhibitors. Inhibition of human tumor-associated isozymes IX and cytosolic isozyme I and II with sulfonylated hydroxamates

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Abstract—A series of sulfonylated hydroxamates were synthesized and evaluated as dual inhibitors of both human carbonic anhydrases (hCAs) and matrix metalloproteinases (MMPs), two metalloenzyme families involved in carcinogenesis and tumor invasion processes. The new derivatives were tested on three CA isozymes, the cytosolic isozymes I and II, and the transmembrane, tumor-associated isozyme IX, and also on human gelatinases (MMP-2 and MMP-9). Some of the new derivatives proved to be potent and selective inhibitors of CA II, but only compounds **3b** and **6b**, devoid of the arylsulfonyl moiety, proved to have a better inhibitory activity on hCA IX than on hCA I and II, in the micromolar range.

1. Introduction

The α-carbonic anhydrases (CAs, EC 4.2.1.1) are a family of metalloenzymes involved in the catalysis of an important physiological reaction: the hydration of CO_2 to bicarbonate and a proton ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$). At least 13 enzymatically active isoforms have been discovered in higher vertebrates.[1] CAs are involved in pH regulation, secretion of electrolytes, respiration,[2] and biosynthetic reactions which require CO_2/bicarbonate as substrate such as gluconeogenesis, lipogenesis, ureagenesis, and pyrimidine synthesis among others.[3] Other roles for these enzymes were highlighted, such as calcification and bone resorption.[4] The discovery that CA IX, a transmembrane tumor-associated protein,[5] was prevalently expressed in several human cancer cells and not in their normal counterparts[6] suggests a role for some CA isoforms in oncogenesis.[3] Several studies showed a clearcut relationship between high CA IX levels in tumors and a poor prognosis.[7] CA IX also acts on cell adhesion and differentiation by its N-terminal proteoglycan-related region which is absent in other transmembrane CA isozymes, such as CA XII (which is present in some tumors[3]) and CA XIV (which is not associated with tumors).[8] However, only the catalytic activity of CA IX seems to be involved in oncogenesis, the role of the proteoglycan-like region being largely unknown at this moment.

Tumor cells have a lower extracellular pH (pH_e) than normal cells due to lactic acid produced by glycolysis.[9] An acidic pH_e contributes to increased tumor progression by promoting the action of growth factors,[10] proteases[11] and an increased rate of mutation.[12] Recently CO_2 in addition to lactic acid was demonstrated to be significant source of acidity in tumors,[13a] pointing out the implication of CAs (such as CA IX and XII) in tumor progression.[3] The expression of CA IX is both regulated by the von Hippel-Lindau (VHL) tumor suppressor protein and by hypoxia present in many tumor types.[3,13a] Thus, an inactivation of the VHL factor gene enhances the expression of CA IX,[12c] whereas hypoxia induces the expression of CA IX via a direct transcriptional activation of *CA9* gene by the hypoxia-inducible factor-1 (HIF-1).[12d] Moreover, hypoxia stimulates CA IX to acidify the pH_e (by an yet unknown mechanism

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of action), proving that the expression levels and the catalytic activity of CA IX are dependent on the availability of oxygen within the tumor.[13a] Many CA isoforms also participate to metabolons in which various anion exchangers (AEs) or sodium bicarbonate co-transporters (NBCs) interact physically and functionally with the enzyme.[9–11] Indeed, for allowing the control of their pH and bicarbonate levels, cells express various anion and bicarbonate transport proteins that rapidly and selectively move bicarbonate and/or protons across the plasma membrane (Fig. 1).[13b,c]

CA IX was clearly demonstrated to be involved in the acidification of the pH_e by Svastova et al.[13a] Teicher and collaborators showed earlier that acetazolamide (**AAZ**) decreased the tumor growth in vivo and enhanced the action of some chemotherapeutic agents, such as cisplatin, melphalan, and $PtCl_4$, when used in combination therapy.[14] Several CA IX-selective sulfonamide inhibitors were able to reduce the extracellular acidification of Madin-Darby canine kidney (MDCK)-CA IX cells in hypoxia but not in normoxia.[13a] Furthermore, a decrease of the extracellular pH reduces the cytotoxicity of weakly basic chemotherapeutic drugs such as paclitaxel, mitoxantrone, and topotecan.[15] Taken together, these data suggest the growing interest for specific CA IX/XII inhibitors in cancer therapy. Such compounds may prevent the decrease in pH_e and may be used in combinations with other antitumor drugs to increase the efficacy or the uptake of weakly basic drugs.[9,14] A decrease in pH_e is also known to lead to activation of matrix metalloproteinases (MMPs), zinc endopeptidases critically involved in extracellular matrix breakdown and metastasis.[13b,16]

In the last few years sulfonylated derivatives have been reported as MMP inhibitors (MMPIs).[16] Up-regulation of specific MMPs has been associated to various pathologies including arthritis (MMP-1, -3, -13) and metastatic cancer (MMP-2, -9), and therefore inhibition of these enzymes has been considered an effective therapeutic approach.

Inhibition of both CAs as well as MMPs is correlated to the coordination of the inhibitor molecule to the catalytic metal ion. Thus, CA and MMP inhibitors must contain a zinc-binding function attached to a scaffold interacting with other binding regions of the enzymes.[17a] The most potent MMPIs reported up to now incorporate a hydroxamate moiety which is able to bind bidentately to the catalytic Zn(II) ion of the enzyme. Recently, it has been shown that *N*-hydroxyurea, one of the simplest hydroxamates known, also binds bidentately to the Zn(II) ion within the CA active site.[17b] A large number of sulfonylated amino acid hydroxamates have been developed in the search of potent and selective inhibitors after the discovery of CGS 27023A (Fig. 2),[18] the first broad-spectrum inhibitor of this class entered in clinical development.

In a previous paper,[19] we reported a series of sulfonylated hydroxamates structurally related to CGS 27023A that were also effective as CAIs. The best CA inhibitory activity was seen with compounds of type **A** (Fig. 2) where X was an hydrogen atom, while bulkier X (benzyl, substituted benzyl, etc.) was advantageous for obtaining an increased activity on MMP. These data suggested that it should be possible to develop dual enzyme inhibitors that would inhibit both these metalloenzymes, based on the nature of the X and R substituents in the **A** formula.

Based on these findings, the aim of this work was to further investigate the possibility of inhibiting both CAs and MMPs, two families of enzymes involved in tumor formation, growth, and metastasis, by the same class of

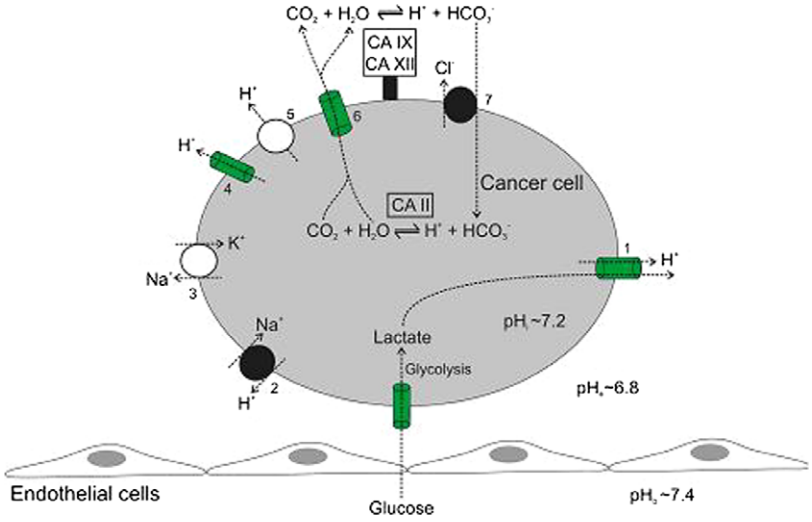
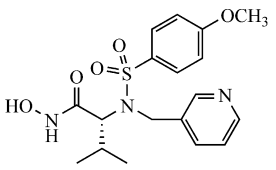
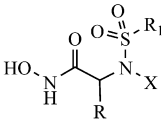


Figure 1. Interaction of cytosolic (CA II) and transmembrane (CA IX and XII) isozymes with other proteins involved in pH homeostasis and anion transport such as (1) the monocarboxylate transporter; (2) the Na^+–H^+-antiporter; (3) the ATP-dependent Na^+–K^+-antiporters; (4) the H^+– ATP-ase; (5) acquaporins; (6) membrane-bound CAs (CA IX, XII or XIV); (7) bicarbonate/chloride anion exchangers (AEs).[13b] The acidic extracellular pH leads to MMP activation. Blocking of both CA and MMP isoforms involved in such processes (CA II, IX, MMP-2, MMP-9, etc.) may thus lead to novel approaches to fight cancer.



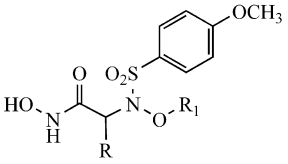
CGS 27023A



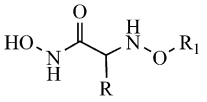
A

R= H, Me, *i*-Pr, *sec*-Bu
R_1= aromatic group
X= H for good CAIs
X= Benzyl, *i*-Pr for good MMPIs

Figure 2.



B



C

1a-d, f-h (R = H)
2d,e (R = *i*-Pr)

3b,c (R = H)
4c (R = Me)
5b,c (R = *n*-Bu)
6b (R = *sec*-Bu)

a: R_1= Et; **b:** R_1= Bn; **c:** R_1= 4-Cl-Bn; **d:** R_1= *i*-Pr; **e:** R_1= *sec*-Bu; **f:** R_1= *n*-Tetradecyl; **g:** R_1= Adamantyl; **h:** R_1= 4-Bn-O-Bn.

Figure 3.

inhibitors, that is, the hydroxamates. As hydroxamates (except *N*-hydroxyurea[17b]) were never investigated until now as CA IX inhibitors but only against isoforms CA I, II, and IV,[19] in this paper, we report the preparation of sulfonylated hydroxamates of type **B** where R_1 is an alkyl group[20] and the arylsulfonyl moiety is a 4-methoxybenzenesulfonyl, like in CGS 27023A, and their interaction with several physiologically relevant CA and MMP isoforms, that is, CA I, II, IX, and MMP-2 and MMP-9. Moreover, we synthesized a series of *N*-alkyloxy amino acid hydroxamates of type **C** (Fig. 3) in order to prove the importance of the sulfonamide moiety for the inhibitory activity on both these classes of enzymes.

2. Chemistry

Synthesis of compounds **1a**–**d**,**f**–**h**, **2d**,**e** is described in Scheme 1. 4-Methoxybenzenesulfonyl chloride was coupled with the appropriate *O*-alkylhydroxylamine hydrochloride **7a**–**h** in the presence of *N*-methylmorpholine to give the corresponding *N*-alkyloxysulfonamides **8a**–**h**. Reaction of **8a**–**h** with *tert*-butylbromoacetate or *tert*-butyl 2-bromo-3-methyl butanoate yielded the corresponding *tert*-butyl esters **9a**–**d**,**f**–**h** and **10d**,**e**. Deprotection of the *tert*-butyl esters with TFA followed by condensation with *O*-(*tert*-butyldimethylsilyl)hydroxylamine in the presence of EDCI gave the *O*-silylated hydroxamates **13a**–**d**,**f**–**h** and **14d**,**e**, which were transformed into the corresponding hydroxamic acids **1a**–**d**, **f**–**h** and **2d**,**e** by acidic cleavage.

Compounds **4c**, **5b**,**c**, and **6b** were synthesized as outlined in Scheme 2. Treatment of *O*-alkylhydroxylamine hydrochloride **7b**,**c** with glyoxylic acid afforded the corresponding *N*-(alkyloxy)iminoacetic acids **15b**,**c**. The addition of alkyllithiums to oximes[21] **15b**,**c** provided the *N*-alkyloxyamino acids **16c**, **17b**,**c**, and **18b** which, after condensation with *O*-(*tert*-butyldimethylsilyl)hydroxylamine in the presence of EDCI followed by acidic deprotection, gave hydroxamic acids **4c**, **5b**,**c**, and **6b**, respectively.

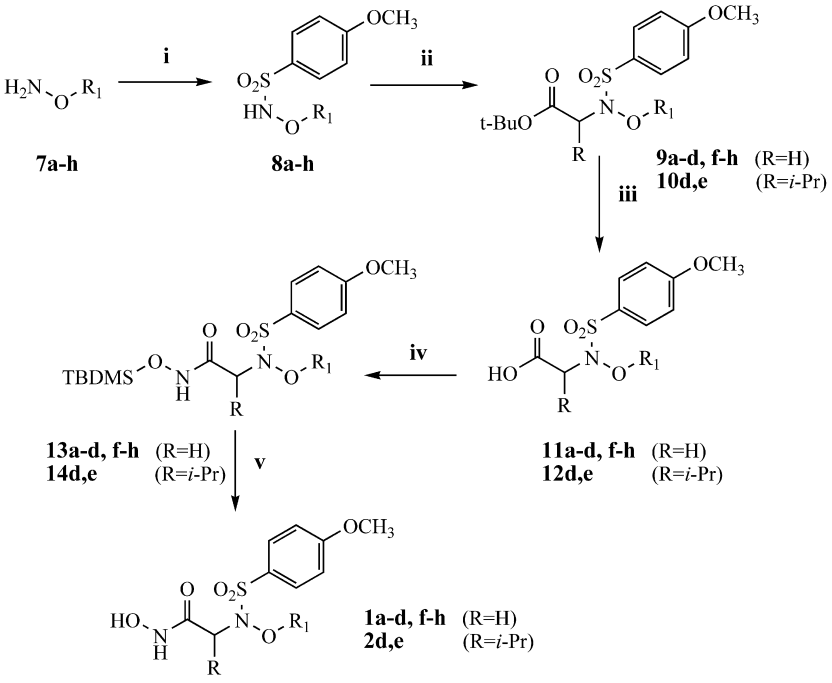
Compounds **3b**,**c** were prepared from **15b**,**c** as described in Scheme 3. Reduction of *N*-(alkyloxy)iminoacetic acids **15b**,**c** with borane-triethylamine complex in hydroalcoholic solution in presence of HCl yielded the corresponding *N*-alkyloxyaminoacetic acids **22b**,**c**. Treatment of **22b**,**c** with *O*-(*tert*-butyldimethylsilyl)hydroxylamine in the presence of EDCI followed by acidic deprotection provided hydroxamic acids **3b**.

3. Results and discussion

Compounds **1**–**6** and standard, clinically used CAIs, such as acetazolamide **AAZ**, methazolamide **MZA**, ethoxozolamide **EZA**, dichlorophenamide **DCP**, and indisulam **IND** (Fig. 4), have been tested for the inhibition of two cytosolic, ubiquitous isozymes of human origin, that is, hCA I and hCA II,[1,2] as well as the human tumor-associated isoform hCA IX.

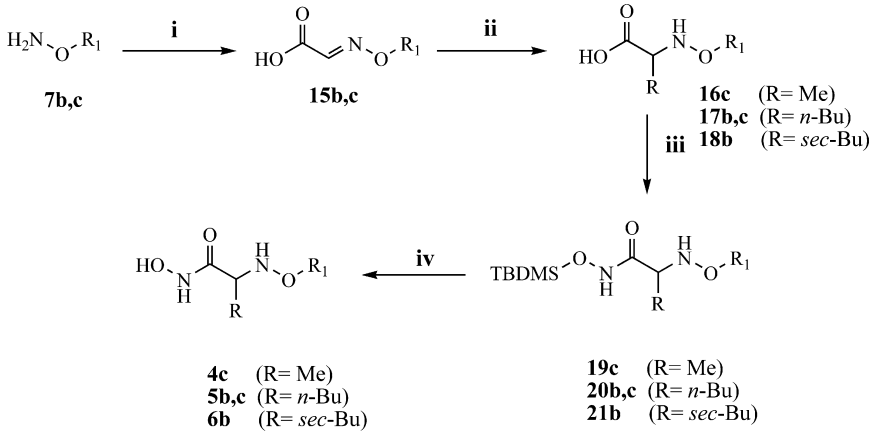
Compounds **1**–**6** have also been tested in vitro on MMP-2 (human gelatinase A) and MMP-9 (human gelatinase B), using CGS 27023A as reference drug. The two gelatinases play a significant role in some key functions of tumor cells, facilitating metastatic tumor dispersion and angiogenesis, resistance to apoptosis, and activation of EGF receptors.[22] Inhibition data against these enzymes are shown in Tables 1 and 2.

The following SAR may be observed for compounds **1a**–**d**,**f**–**h**, **2d**,**e** (type **B**) presented in Table 1: (i) sulfonylated hydroxamates reported here generally act as better inhibitors against the cytosolic isozymes I and II than against the tumor-associated isozyme hCA IX, being particularly active on hCA II, with K_I values in the range of 91–97 nM; (ii) these derivatives are also much more effective on hCA I and II than the reference drug CGS 27023A, some of them having K_I values in the same range as the clinically used derivatives; (iii) the introduction of the R substituent in the α-position to the hydroxamate moiety does not seem to affect the



a: R_1= Et; **b:** R_1= Bn; **c:** R_1= 4-Cl-Bn; **d:** R_1= *i*-Pr; **e:** R_1= *sec*-Bu; **f:** R_1= *n*-Tetradecyl; **g:** R_1= Adamantyl; **h:** R_1= 4-Bn-O-Bn.

Scheme 1. Reagents: (i) $ClSO_2PhOCH_3$, *N*-methylmorpholine, THF; (ii) $BrCHRCO_2{}^tBu$, Cs_2CO_3, Bu_4NHSO_4, DMF; (iii) TFA, CH_2Cl_2; (iv) $TBDSiONH_2$, EDCI, CH_2Cl_2; (v) TFA, CH_2Cl_2.



b: R_1= Bn; **c:** R_1= 4-Cl-Bn

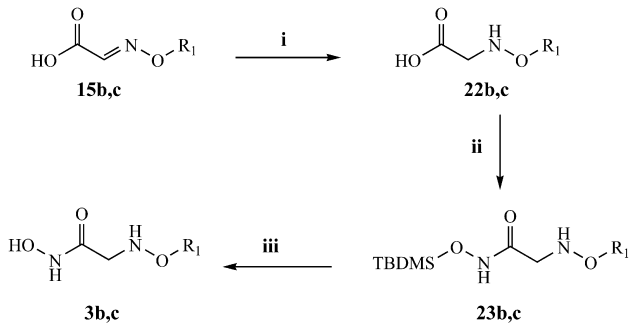
Scheme 2. Reagents: (i) $HCOCO_2H$, AcONa, MeOH/H_2O 1:1; (ii) RLi, THF; (iii) $TBDMSiONH_2$, EDCI, CH_2Cl_2; (iv) TFA, CH_2Cl_2.

biological activity (e.g., **1d** compared to **2d**). (iv) The most interesting compounds from the point of view of CA-binding affinity of this series are **1d** and **2d**, which show a 100-fold selectivity for hCA II over the other isozymes. They both bear an *i*-Pr group in the R_1 position, so this substituent seems to be the best among those considered to achieve this kind of selectivity profile.

(v) These derivatives display a low micromolar inhibitory activity on gelatinases, being particularly effective on MMP-9; (vi) the α-substitution leads to an increased activity on both gelatinases, with **2d** and **2e** showing the best affinities; (vii) they are all less active on gelatinases than CGS 27023A.

The most potent compound of this series is **2d** which has an *i*-Pr group in R_1 and R substituents. This compound displays good inhibitory activity against both hCA II (K_I = 94 nM) and MMP-9 (IC_{50} = 370 nM).

In Table 2 are reported the biological results for compounds **3b**,**c**, **4c**, **5b**,**c**, and **6b** (type **C**): (i) *N*-alkyloxy amino acid hydroxamates presented here generally act as less effective inhibitors of hCA I and II than the



b: R_1= Bn; **c:** R_1= 4-Cl-Bn

Scheme 3. Reagents: (i) BH_3–TEA, HCl 1 N, EtOH; (ii) TBDMSiONH$_2$, EDCI, CH_2Cl_2; (iii) TFA/H_2O.

previous series of derivatives; (ii) the most interesting compounds are **3b** and **6b**, which are selective for hCA IX over hCA I, II, showing also the best activities on hCA IX over both series of derivatives (K_I = 6.7 and 7.1 μM, respectively, on this CA isoform); (iii) like observed for the previous series, the α-substitution (R) does not affect the inhibitory activity on hCAs, generally. Compound **6b** seems to be the only case in which it is possible to see an influence of the R substituent. In-fact, the introduction of a *sec*-butyl group in R maintains a similar selectivity for hCA IX over hCA I with respect to its R un-substituted analogue **3b** but is able to increase about 2.5 times the selectivity for hCA IX over hCA II. Also the R_1 substituent seems to influence hCA affinity: in fact 4-chloro-benzyl-derivatives clearly appear to be more active than benzyl analogues on hCA I.

(iv) Against gelatinases these compounds are less active than sulfonamide derivatives; (v) they present an inverse activity profile with respect to sulfonamides, becoming more effective on MMP-2 than on MMP-9, also if in the micromolar range; (vi) the α-substituted compounds have an increased activity on both gelatinases (e.g., **5b** compared to **3b**) except when R becomes a too bulky group, as *sec*-butyl in **6b**. (vii) *O*-benzyl derivatives prove to be more active on gelatinases than 4-chloro-benzyl analogues (e.g., **3b** compared to **3c** and **5b** compared to **5c**), thus the best inhibitor of this series on gelatinases is **5b** (IC_{50} = 33.7 μM on MMP-2).

Considering these results, it should be concluded that the lack of the arylsulfonyl moiety causes a 500- to 1000-fold decrease in hCA I, II inhibitory activity (e.g., **3b** compared to **1b**) without affecting activity on hCA IX, and a 90- to 300-fold reduction of gelatinase inhibition together with an inversion of selectivity pattern between MMP-2 and MMP-9.

4. Conclusions

In conclusion, the present paper reports the design and synthesis of a series of sulfonylated hydroxamates as dual inhibitors of both, hCAs and MMPs, families of enzymes involved in carcinogenesis and tumor invasion processes. The new derivatives have been tested on three CA isozymes, the cytosolic isozymes I and II, and the transmembrane, tumor-associated isozyme IX, and also on human gelatinases (MMP-2 and MMP-9).

All reported derivatives, similarly to the clinically used sulfonamides, act as better CA II than CA IX inhibitors (Table 1), with compounds **1d** and **2d** being selective inhibitors of CA II over CA I and IX, in the nanomolar range. Compound **2d** was also the most active on MMP-2, as the best MMP inhibitory activity was seen for α-substituted derivatives. Only compounds **3b** and **6b**, devoid of the arylsulfonyl moiety, proved to have the best inhibitory activity on hCA IX than on hCA I and II, in the micromolar range.

Thus, although these compounds are not as effective as the clinically used sulfonamides on the single enzymes, their peculiar selectivity profile and the ability to inhibit both CAs and MMPs make these derivatives interesting for more detailed SAR studies.

5. Experimental

5.1. General synthetic methods and materials

All commercially available starting materials and solvents were of reagent grade. Solutions containing products were dried over anhydrous sodium sulfate (Na_2SO_4). Melting points were determined on a Kofler

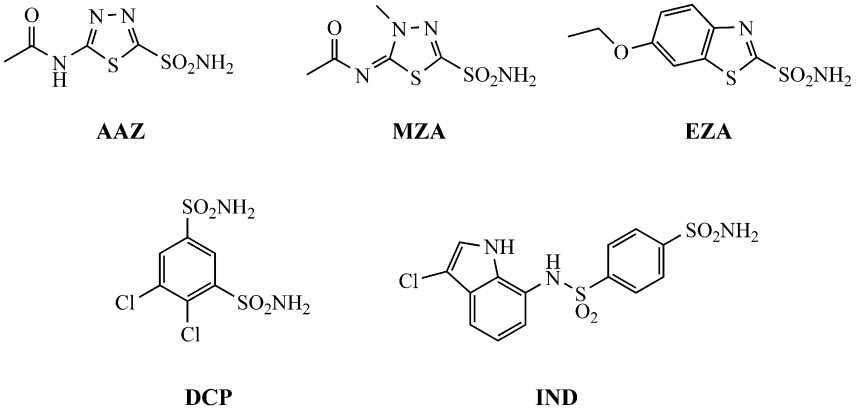
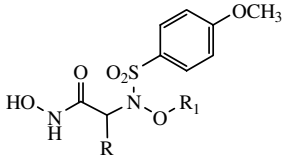


Figure 4.

Table 1. Inhibition data of sulfonylated hydroxamates **1a**–**d**,**f**–**h**, **2d**,**e** against hCA I, II, IX, and MMP-2, 9 compared to standard CA inhibitors and to CGS 27023A

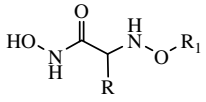


B: 1a-d, f-h
2d,e

Compound			IC_{50} (μM)*				
	R	R_1	hCA I[a]	hCA II[a]	hCA IX[b]	MMP-2	MMP-9
AAZ			0.31	0.012	0.025		
MZA			0.78	0.014	0.027		
EZA			0.025	0.008	0.034		
DCP			1.20	0.038	0.050		
IND			0.031	0.015	0.024		
CGS 27023A			281	8.6	7.8	0.025	0.0048
1a	H	Et	8.91	8.7	9.0	9.6	2.2
1b	H	Bn	0.88	0.096	7.5	1.06	0.87
1c	H	4-Cl–Bn	156	4.2	9.3	1.5	0.33
1d	H	*i*-Pr	4.1	0.091	9.5	4.9	3.4
1f	H	*n*-tetradecyl	0.89	0.097	7.4	30.0	33
1g	H	Adamantyl	0.90	0.096	7.8	1.98	2.0
1h	H	4-Bn-*O*-Bn	5.9	0.095	8.7	2.1	1.83
2d	*i*-Pr	*i*-Pr	9.0	0.094	9.3	0.66	0.370
2e	*i*-Pr	*sec*-Bu	9.7	9.6	9.2	0.73	0.286

CA inhibition values are expressed as K_I (μM).
[a] Human (cloned) isozymes, by the CO_2 hydration method.
[b] Catalytic domain of human, cloned isozyme,[23] by the CO_2 hydration method.[24]
* Errors in the range of 5–10% of the reported value (from three different assays).

Table 2. Inhibition data of hydroxamates **3**–**6** against hCA I, II, IX, and MMP-2, 9 compared to standard CA inhibitors and to CGS 27023A



C: 3b,c, 4c
5b,c, 6b

Compound			IC_{50} (μM)*				
	R	R_1	hCA I[a]	hCA II[a]	hCA IX[b]	MMP-2	MMP-9
AAZ			0.31	0.012	0.025		
MZA			0.78	0.014	0.027		
EZA			0.025	0.008	0.034		
DCP			1.20	0.038	0.050		
IND			0.031	0.015	0.024		
CGS 27023A			281	8.6	7.8	0.025	0.0048
3b	H	Bn	490	94	6.7	94	256
3c	H	4-Cl–Bn	10.0	4.8	8.8	137	261
4c	Me	4-Cl-Bn	9.8	4.7	9.2	68	141
5b	*n*-Bu	Bn	520	7.8	7.6	33.7	81.5
5c	*n*-Bu	4-Cl–Bn	10.2	6.1	6.9	81.8	125
6b	*sec*-Bu	Bn	435	267	7.1	151	352

CA inhibition values are expressed as K_I (μM).
[a] Human (cloned) isozymes, by the CO_2 hydration method.
[b] Catalytic domain of human, cloned isozyme,[23] by the CO_2 hydration method.[24]
* Errors in the range of 5–10% of the reported value (from three different assays).

hot-stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Gemini 200 (200 MHz) using $CDCl_3$ or DMSO-d_6 as solvent unless otherwise indicated. The chemical shift values are reported in ppm (δ) and coupling constants (J) in Hertz (Hz). Mass spectra were recorded on a GC/MS Trace

GCQ Plus Thermo Quest Finnigan spectrometer using a direct injection probe and an electron beam energy of 40 eV. Reactions were routinely monitored by thin-layer chromatography (TLC) on 0.25 mm silica gel plates (Merck 60 F_{254}) and hydroxamic acids were visualized with $FeCl_3$ aqueous solution. Flash chromatography was carried out through glass columns containing silica gel 60 (Merck 230–400 mesh). Elemental analyses were carried out by our analytical laboratory and were consistent with theoretical values to within ±0.4%.

The clinically used sulfonamide CA inhibitors (CAIs) acetazolamide **AAZ**, methazolamide **MZA**, ethoxozolamide **EZA**, dichlorophenamide **DCP**, and indisulam **IND**, employed as standard inhibitors in the enzyme assays, are commercially available from Sigma–Aldrich or have been prepared as previously described.[25]

5.1.1. General procedure for the preparation of *N*-alkyloxysulfonamides (8a–h). A solution of 4-methoxybenzenesulfonyl chloride (7.0 mmol) in anhydrous THF (18.0 mL) was added dropwise to a stirred and cooled (0 °C) solution of the appropriate *O*-alkyl hydroxylamine hydrochloride **7a**–**h** (7.0 mmol) and *N*-methylmorpholine (1.5 mL, 14 mmol) in anhydrous THF (18.0 mL). After 30 min under these conditions, the reaction mixture was stirred for 3 days at room temperature. The resulting mixture was diluted with H_2O (100 mL) and extracted with EtOAc (100 mL) giving, after work-up, crude residues which were purified by flash chromatography on silica gel to yield **8a**–**h** as pure solids.

5.1.1.1. *N*-Ethyloxy-4-methoxybenzenesulfonamide (8a). The title compound was prepared as previously described.[20]

5.1.1.2. *N*-Benzyloxy-4-methoxybenzenesulfonamide (8b). The title compound was prepared from *O*-benzylhydroxylamine hydrochloride and 4-methoxybenzenesulfonyl chloride following the general procedure. Compound **8b** was used without further purification: white solid (74% yield); mp 105–107 °C; ^{1}H NMR ($CDCl_3$) δ: 3.87 (s, 3H), 4.96 (s, 2H), 6.87 (s, 1H), 6.96–7.00 (m, 2H), 7.43 (m, 5H), 7.84–7.88 (m, 2H).

5.1.1.3. *N*-4-Chlorobenzyloxy-4-methoxybenzenesulfonamide (8c). The title compound was prepared from *O*-(4-chlorobenzyl)hydroxylamine hydrochloride and 4-methoxybenzenesulfonyl chloride following the general procedure. Compound **8c** was used without further purification: white solid (76% yield); mp 144–146 °C; ^{1}H NMR ($CDCl_3$) δ: 3.87 (s, 3H), 4.92 (s, 2H), 6.85 (s, 1H), 6.96–7.01 (m, 2H), 7.24–7.30 (m, 4H), 7.82–7.86 (m, 2H).

5.1.1.4. *N*-Isopropoxy-4-methoxybenzenesulfonamide (8d). The title compound was prepared as previously described.[20]

5.1.1.5. *N*-*sec*-Butyloxy-4-methoxybenzenesulfonamide (8e). The title compound was prepared from *O*-*sec*-butylhydroxylamine hydrochloride and 4-methoxybenzenesulfonyl chloride following the general procedure. The crude product was purified by flash chromatography on silica gel (*n*-hexane/EtOAc, 7:2) to give **8e** (62% yield) as white solid. R_f = 0.14; ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 7.5 Hz, 3H), 1.16 (d, J = 6.2 Hz, 3H), 1.40–1.58 (m, 2H), 3.88 (s, 3H), 4.03 (m, 1H), 6.7 (s, 1H), 6.98–7.02 (m, 2H), 7.83–7.88 (m, 2H).

5.1.1.6. *N*-*n*-Tetradecyloxy-4-methoxybenzenesulfonamide (8f). The title compound was prepared from *O*-*n*-tetradecylhydroxylamine hydrochloride and 4-methoxybenzenesulfonyl chloride following the general procedure. Compound **8f** was used without further purification: white solid (92% yield); ^{1}H NMR ($CDCl_3$) δ: 0.83 (t, J = 6.0 Hz, 3H), 1.25 (m, 24H), 3.88 (s, 3H), 3.96 (t, J = 6.7 Hz, 2H), 6.83 (s, 1H), 6.98–7.02 (m, 2H), 7.83–7.88 (m, 2H).

5.1.1.7. *N*-Adamantyloxy-4-methoxybenzenesulfonamide (8g). The title compound was prepared from *O*-adamantylhydroxylamine hydrochloride and 4-methoxybenzenesulfonyl chloride following the general procedure. The crude product was purified by flash chromatography on silica gel (hexane/EtOAc, 4:1) to give **8g** (48% yield) as white solid. R_f = 0.22; ^{1}H NMR ($CDCl_3$) δ: 1.56 (m, 6H), 1.76 (d, 6H), 2.15 (m, 3H), 3.88 (s, 3H), 6.36 (s, 1H), 6.97–7.01 (m, 2H), 7.82–7.87 (m, 2H).

5.1.1.8. *N*-Benzyloxy-benzyloxy-4-methoxybenzenesulfonamide (8h). The title compound was prepared as previously described.[20]

5.1.2. General procedure for the preparation of *tert*-butyl esters (9a–d,f–h, 10d,e). A solution of the appropriate sulfonamide **8a**–**h** (1 mmol) in anhydrous DMF (3.0 mL) was treated with *tert*-butylbromoacetate or *tert*-butyl 2-bromo-3-methyl butanoate (1.2 mmol), cesium carbonate (1 mmol), and tetrabutylammonium hydrogensulfate (1 mmol). The reaction mixture was stirred for 3 days at room temperature, diluted with H_2O (20.0 mL) and extracted with EtOAc (3× 20.0 mL) giving, after work-up, crude residues which were purified by flash chromatography on silica gel to yield **9a**–**d**,**f**–**h**, and **10d**,**e** as pure solids.

5.1.2.1. *tert*-Butyl {ethyloxy[(4-methoxyphenyl)sulfonyl]amino}acetate (9a). The title compound was prepared as previously described.[20]

5.1.2.2. *tert*-Butyl {benzyloxy[(4-methoxyphenyl)sulfonyl]amino}acetate (9b). The title compound was prepared from **8b** and *tert*-butylbromoacetate following the general procedure. Compound **9b** was used without further purification: yellow gel (92% yield). ^{1}H NMR ($CDCl_3$) δ: 1.47 (s, 9H), 3.63 (s, 2H), 3.84 (s, 3H), 5.32 (s, 2H), 6.94–6.98 (m, 2H), 7.31–7.36 (m, 5H), 7.76–7.80 (m, 2H).

5.1.2.3. *tert*-Butyl {4-Chlorobenzyloxy[(4-methoxyphenyl)sulfonyl]amino}acetate (9c). The title compound was prepared from **8c** and *tert*-butylbromoacetate following the general procedure. Compound **9c** was used without

further purification: yellow oil (80% yield); ^{1}H NMR ($CDCl_3$) δ: 1.46 (s, 9H), 3.61 (s, 2H), 3.85 (s, 3H), 5.28 (s, 2H), 6.95–7.00 (m, 2H), 7.28–7.30 (m, 4H), 7.74–7.78 (m, 2H).

5.1.2.4. *tert*-Butyl {isopropoxy[(4-methoxyphenyl)sulfonyl]amino}acetate (9d). The title compound was prepared as previously described.[20]

5.1.2.5. *tert*-Butyl {*n*-tetradecyloxy[(4-methoxyphenyl)sulfonyl]amino}acetate (9f). The title compound was prepared from **8f** and *tert*-butylbromoacetate following the general procedure. Compound **9f** was used without further purification: yellow gel (76% yield); ^{1}H NMR ($CDCl_3$) δ: 0.87 (t, J = 6.6 Hz, 3H), 1.24 (m, 24H), 1.45 (s, 9H), 3.58 (s, 2H), 3.88 (s, 3H), 4.26 (t, 2H), 6.99–7.04 (m, 2H), 7.77–7.83 (m, 2H).

5.1.2.6. *tert*-Butyl {adamantyloxy[(4-methoxyphenyl)sulfonyl]amino}acetate (9g). The title compound was prepared from **8g** and *tert*-butylbromoacetate following the general procedure. The crude product was purified by flash chromatography on silica gel (*n*-hexane/EtOAc 4:1) to give **9g** (49% yield) as a yellow solid. R_f = 0.32; ^{1}H NMR ($CDCl_3$) δ: 1.49 (s, 9H), 1.62 (m, 6H), 1.90 (d, 6H), 2.18 (m, 3H), 3.75 (s, 2H), 3.88 (s, 3H), 6.98–7.02 (m, 2H), 7.79–7.83 (m, 2H).

5.1.2.7. *tert*-Butyl {benzyloxybenzyloxy[(4-methoxyphenyl)sulfonyl]amino}acetate (9h). The title compound was prepared as previously described.[20]

5.1.2.8. *tert*-Butyl 2-{isopropoxy[(4-methoxyphenyl)sulfonyl]amino}-3-methylbutanoate (10d). The title compound was prepared from **8d** and racemic *tert*-butyl 2-bromo-3-methyl butanoate following the general procedure. The crude product was purified by flash chromatography on silica gel (*n*-hexane/EtOAc 5:1) to give **10d** (25% yield) as a yellow oil. R_f = 0.27; ^{1}H NMR ($CDCl_3$) δ: 0.88 (d, J = 6.4 Hz, 6H), 1.16 (brs, 9H), 1.22 (d, J = 6.2 Hz, 3H), 1.24 (d, J= 6.2 Hz, 3H), 2.20 (m, 1H), 3.69 (d, J = 10.6 Hz, 1H), 3.87 (s, 3H), 4.41 (septet, J = 6.4 Hz, 1H), 6.95–7.01 (m, 2H), 7.82–7.87 (m, 2H).

5.1.2.9. *tert*-Butyl 2-{*sec*-butyloxy[(4-methoxyphenyl)sulfonyl]amino}-3-methylbutanoate (10e). The title compound was prepared from **8e** and racemic *tert*-butyl 2-bromo-3-methyl butanoate following the general procedure. The crude product was purified by flash chromatography on silica gel (*n*-hexane/EtOAc 8:1) to give **10e** (31% yield) as a dense oil. R_f = 0.3; ^{1}H NMR ($CDCl_3$) δ: 0.84–0.96 (m, 9H), 1.17–1.26 (m, 12H), 1.35–1.56 (m, 1H), 1.67–1.80 (m, 1H), 2.20 (m, 1H), 3.68 (d, J = 10.6 Hz, 1H), 3.87 (s, 3H), 4.23 (m, 1H), 6.95–7.01 (m, 2H), 7.82–7.88 (m, 2H).

5.1.3. General procedure for the preparation of carboxylic acids (11a–d,f–h, 12d,e). TFA (4.4 mL, 57 mmol) was added dropwise to a stirred and cooled (0 °C) solution of the appropriate *tert*-butyl ester **9a–d,f–h**, **10d,e** (1 mmol) in freshly distilled CH_2Cl_2 (4.4 mL). The solution was stirred for 5 h at 0 °C and the solvent was removed in vacuo to give **11a–d,f–h**, **12d,e** as solids which were purified by crystallization.

5.1.3.1. {Ethyloxy[(4-methoxyphenyl)sulfonyl]amino}-acetic acid (11a). The title compound was prepared as previously described.[20]

5.1.3.2. {Benzyloxy[(4-methoxyphenyl)sulfonyl]amino}acetic acid (11b). The title compound was prepared from **9b** following the general procedure. Compound **11b** was used without further purification: yellow solid (99% yield); ^{1}H NMR ($CDCl_3$) δ: 3.80 (s, 2H), 3.86 (s, 3H), 4.84 (br s, 1H), 5.28 (s, 2H), 6.97–7.01 (m, 2H), 7.31–7.36 (m, 5H), 7.76–7.80 (m, 2H).

5.1.3.3. {4-Chlorobenzyloxy[(4-methoxyphenyl)sulfonyl]amino}acetic acid (11c). The title compound was prepared from **9c** following the general procedure. Compound **11c** was used without further purification: yellow oil (98% yield); ^{1}H NMR ($CDCl_3$) δ: 3.78 (s, 2H); 3.86 (s, 3H); 5.23 (s, 2H); 6.96–7.02 (m, 2H); 7.30 (m, 4H); 7.75–7.81 (m, 2H).

5.1.3.4. {Isopropoxy[(4-methoxyphenyl)sulfonyl]amino}acetic acid (11d). The title compound was prepared as previously described.[20]

5.1.3.5. {*n*-Tetradecyloxy[(4-methoxyphenyl)sulfonyl]amino}acetic acid (11f). The title compound was prepared from **9f** following the general procedure. Compound **11f** was used without further purification: yellow solid (92% yield); ^{1}H NMR ($CDCl_3$) δ: 0.87 (*t*, J = 6.6 Hz, 3H), 1.25 (m, 24H), 3.55 (br s, 1H), 3.75 (s, 2H), 3.89 (s, 3H), 4.24 (t, J = 7.1 Hz, 2H), 7.01–7.05 (m, 2H), 7.78–7.83 (m, 2H).

5.1.3.6. {Adamantyloxy[(4-methoxyphenyl)sulfonyl]amino}acetic acid (11g). The title compound was prepared from **9g** following the general procedure. Compound **11g** was used without further purification: yellow solid (99% yield); mp 190–192 °C; ^{1}H NMR ($CDCl_3$) δ: 1.63 (m, 6H), 1.88 (d, 6H), 2.20 (m, 3H), 3.80 (d, 2H), 3.89 (s, 3H), 7.01–7.05 (m, 2H), 7.79–7.83 (m, 2H).

5.1.3.7. {Benzyloxybenzyloxy[(4-methoxyphenyl)sulfonyl]amino}acetic acid (11h). The title compound was prepared as previously described.[20]

5.1.3.8. 2-{Isopropoxy[(4-methoxyphenyl)sulfonyl]amino}-3-methylbutanoic acid (12d). The title compound was prepared from **10d** following the general procedure. Compound **12d** was used without further purification: white solid (87% yield); ^{1}H NMR ($CDCl_3$) δ: 0.92 (d, J = 6.6 Hz, 6H), 1.21–1.30 (m, 6H), 2.15 (m, 1H), 3.69 (d, J = 10.6 Hz, 1H), 3.87 (s, 3H), 4.43 (septet, J = 6.4 Hz, 1H), 6.94–6.99 (m, 2H), 7.79–7.84 (m, 2H), 9.19 (br s, 1H).

5.1.3.9. 2-{*sec*-Butyloxy[(4-methoxyphenyl)sulfonyl]amino}-3-methylbutanoic acid (12e). The title compound was prepared from **10e** following the general procedure. Compound **12e** was used without further purification: white solid (100% yield); ^{1}H NMR ($CDCl_3$) δ: 0.84–0.95 (m,

6H), 1.09 (m, 3H), 1.22–1.29 (m, 3H), 1.39–1.59 (dq, 1H), 1.67–1.84 (dq, 1H), 2.12 (m, 1H), 3.81 (d, $J = 10.6$ Hz, 1H), 3.87 (s, 3H), 4.30 (m, 1H), 6.02 (br s, 1H), 6.95–6.99 (m, 2H), 7.80–7.84 (m, 2H).

5.1.4. General procedure for the preparation of *O*-TBDMS acid hydroxyamides (13a–d,f–h, 14d,e). 1-[3-(dimethylamino)propyl]-3-ethyl carbodiimide hydrochloride (EDCI) was added portionwise (1 mmol) to a stirred and cooled (0 °C) solution of the carboxylic acid **11a**–**d**,**f**–**h**, **12d**,**e** (1 mmol) and *O*-(*tert*-butyldimethylsilyl)hydroxylamine (1 mmol) in freshly distilled CH_2Cl_2 (18 mL). After stirring at rt for 20 h, the mixture was washed with water (20 mL) and the organic phase was dried and evaporated in vacuo. The crude residue was purified by flash chromatography on silica gel to yield pure **13a**–**d**,**f**–**h**, **14d**,**e** as oils.

5.1.4.1. 2-{Ethyloxy[(4-methoxyphenyl)sulfonyl]amino}acetic acid (*tert*-butyldimethylsilyl)hy-droxyamide (13a). The title compound was prepared as previously described.[20]

5.1.4.2. 2-{Benzyloxy[(4-methoxyphenyl)sulfonyl]amino}acetic acid (*tert*-butyldimethylsilyl)hy-droxyamide (13b). The title compound was prepared from **11b** following the general procedure. The crude product was purified by flash chromatography on silica gel (*n*-hexane/EtOAc 1:1) to give **13b** (13% yield) as a yellow solid; ^{1}H NMR ($CDCl_3$) δ: 0.16 (s, 6H), 0.95 (s, 9H), 3.58 (s, 2H), 3.86 (s, 3H), 4.96 (s, 2H), 6.96–7.01 (m, 2H), 7.34–7.38 (m, 5H), 7.77–7.82 (m, 2H).

5.1.4.3. 2-{4-Chlorobenzyloxy[(4-methoxyphenyl)sulfonyl]amino}acetic acid (*tert*-butyldimethylsilyl)hydroxyamide (13c). The title compound was prepared from **11c** following the general procedure. Compound **13c** was used without further purification: yellow oil (81% yield); ^{1}H NMR ($CDCl_3$) δ: 0.096 (s, 6H); 0.91 (s, 9H); 3.60 (s, 2H); 3.87 (s, 3H); 5.29 (s, 2H); 6.96–6.97 (m, 2H); 7.30–7.33 (m, 4H); 7.74–7.79 (m, 2H).

5.1.4.4. 2-{Isopropoxy[(4-methoxyphenyl)sulfonyl]amino}acetic acid (*tert*-butyldimethylsilyl)hy-droxyamide (13d). The title compound was prepared as previously described.[20]

5.1.4.5. 2-{*n*-Tetradecyloxy[(4-methoxyphenyl)sulfonyl]amino}acetic acid (*tert*-butyldimethylsilyl)hy-droxyamide (13f). The title compound was prepared from **11f** following the general procedure. The crude product was purified by flash chromatography on silica gel (*n*-hexane/EtOAc 3:1) to give **13f** (4% yield) as a yellow solid; ^{1}H NMR ($CDCl_3$) δ: 0.20 (s, 6H), 0.87(t, $J = 6.6$ Hz, 3H), 0.97 (s, 9H), 1.25 (m, 24H), 3.62 (s, 2H), 3.89 (s, 3H), 4.16 (t, $J = 7.1$ Hz, 2H), 7.01–7.05 (m, 2H), 7.77–7.81 (m, 2H), 8.5 (br s, 1H).

5.1.4.6. 2-{Adamantyloxy[(4-methoxyphenyl)sulfonyl]amino}acetic acid (*tert*-butyldimethylsilyl)hy-droxyamide (13g). The title compound was prepared from **11g** following the general procedure. Compound **13g** was used without further purification: yellow solid (75% yield); ^{1}H NMR ($CDCl_3$) δ: 0.23 (s, 6H), 0.99 (s, 9H), 1.62 (m, 6H), 1.85 (d, 6H), 2.20 (m, 3H), 3.80 (d, 2H), 3.89 (s, 3H), 7.01–7.05 (m, 2H), 7.78–7.83 (m, 2H), 8.77 (br s, 1H).

5.1.4.7. 2-{Benzyloxybenzyloxy[(4-methoxyphenyl)sulfonyl]amino}acetic acid (*tert*-butyldimethylsilyl)hydroxyamide (13h). The title compound was prepared as previously described.[20]

5.1.4.8. *N*-(*tert*-Butyldimethylsilyloxy)-2-[*N*-isopropoxy(4-methoxyphenyl)sulfonamido]-3-methylbutanamide (14d). The title compound was prepared from **12d** following the general procedure. Compound **14d** was used without further purification: yellow solid (87% yield); ^{1}H NMR ($CDCl_3$) δ: 0.10 (s, 6H), 0.89–0.92 (m, 15H), 1.19–1.29 (m, 6H), 2.15 (m, 1H), 3.88 (s, 3H), 4.32 (septet, $J = 6.4$ Hz, 1H), 6.98–7.02 (m, 2H), 7.80–7.85 (m, 2H), 9.19 (br s, 1H).

5.1.4.9. *N*-(*tert*-Butyldimethylsilyloxy)-2-[*N*-*sec*-butyloxy(4-methoxyphenyl)sulfonamido]-3-methylbutanamide (14e). The title compound was prepared from **12e** following the general procedure. The crude product was purified by flash chromatography on silica gel (*n*-hexane/EtOAc 4:1) to give **14e** (57% yield) as a yellow solid; ^{1}H NMR ($CDCl_3$) δ: 0.09 (s, 6H) 0.84–0.91 (m, 18H), 1.17–1.24 (m, 3H), 1.34–1.52 (dq, 1H), 1.65–1.83 (dq, 1H), 2.12 (m, 1H), 3.71 (d, $J = 10.6$ Hz, 1H), 3.87 (s, 3H), 4.16 (m, H), 6.98–7.02 (m, 2H), 7.80–7.85 (m, 2H), 8.15 (br s, 1H).

5.1.5. General procedure for the preparation of acid hydroxyamides (1a–d,f–h, 2d,e). TFA (4.4 mL, 57 mmol) was added dropwise to a stirred solution of **13a**–**d**,**f**–**h**, **14d**,**e** (1 mmol) in freshly distilled CH_2Cl_2 (4.4 mL) cooled to 0 °C. The solution was stirred for 5 h at 0 °C and the solvent was removed in vacuo to give a solid. The crude products were recrystallized from Et_2O and *n*-hexane to yield **1a**–**d**,**f**–**h**, **2d**,**e** as pure solids.

5.1.5.1. 2-{Ethyloxy[(4-methoxyphenyl)sulfonyl]amino}-*N*-hydroxyacetamide (1a). The title compound was prepared as previously described.[20]

5.1.5.2. 2-{Benzyloxy[(4-methoxyphenyl)sulfonyl]amino}-*N*-hydroxyacetamide (1b). The title compound was prepared from **13b** following the general procedure. The crude product was purified by preparative thin-layer chromatography ($CHCl_3$/MeOH 9:1) to give **1b** as white solid (4% yield); mp 80–81 °C; ^{1}H NMR ($CDCl_3$) δ: 3.62 (s, 2H), 3.87 (s, 3H), 5.14 (s, 2H), 6.97–7.02 (m, 2H), 7.38 (m, 5H), 7.77–7.80 (m, 2H). EI-MS, *m*/*z*: 51 (38.17), 91 (100), 107 (25.01). Anal. Calcd for $C_{16}H_{18}N_2O_6S$: C, 52.45; H, 4.95; N, 7.65. Found: C, 52.50; H, 5.05, N 7.55.

5.1.5.3. 2-{4-Chlorobenzyloxy[(4-methoxyphenyl)sulfonyl]amino}-*N*-hydroxyacetamide (1c). The title compound was prepared from **13c** following the general procedure. The crude product was purified by flash chromatography on silica gel ($CHCl_3$/MeOH 9:1);

R_f = 0.45. Recrystallization from Et_2O gave **1c** as white solid (9% yield); mp 148–150 °C; ^1H NMR (DMSO-d_6) δ: 3.45 (s, 2H), 3.85 (s, 3H), 5.00 (s, 2H), 7.15–7.20 (m, 2H), 7.33–7.46 (m, 4H), 7.74–7.79 (m, 2H), 9.10 (br s, 1H), 10.70 (br s, 1H). EI-MS, *m*/*z*: 77 (100), 107 (54.53), 125 (60.47), 171 (39.16). Anal. Calcd for $C_{16}H_{17}ClN_2O_6S$: C, 47.94; H, 4.27; N, 6.99. Found: C, 47.99; H, 4.32; N, 6.89.

5.1.5.4. 2-{Isopropoxy[(4-methoxyphenyl)sulfonyl]amino}-*N*-hydroxyacetamide (1d). The title compound was prepared as previously described.[20]

5.1.5.5. 2-{*n*-Tetradecyloxy[(4-methoxyphenyl)sulfonyl]amino}-*N*-hydroxyacetamide (1f). The title compound was prepared from **13f** following the general procedure. Compound **1f** was used without further purification: yellow solid (67% yield); ^1H NMR ($CDCl_3$) δ: 0.87 (t, J = 6.6 Hz, 3H), 1.25 (m, 24H), 3.71 (s, 2H), 3.89 (s, 3H), 4.12 (t, J = 7.1 Hz, 2H), 7.01–7.05 (m, 2H), 7.78–7.83 (m, 2H). EI-MS, *m*/*z*: 43 (100), 107 (19.28), 149 (64.83), 171 (42.86). Anal. Calcd for $C_{23}H_{40}N_2O_6S$: C, 58.45; H, 8.53; N, 5.93. Found: C, 58.60; H, 8.60; N, 5.88.

5.1.5.6. 2-{Adamantyloxy[(4-methoxyphenyl)sulfonyl]amino}-*N*-hydroxyacetamide (1g). The title compound was prepared from **13g** following the general procedure. Recrystallization from Et_2O gave **1g** as white solid (62% yield); mp 190–192 °C; ^1H NMR (DMSO-d_6) δ: 1.55 (m, 6H), 1.81 (m, 6H), 2.11 (m, 3H), 3.79 (d, 2H), 3.87 (s, 3H), 7.15–7.20 (m, 2H), 7.76–7.79 (m, 2H), 10.60 (s, 1H). EI-MS *m*/*z*: 135 (100), 171 (8.85), 239 (1.75). Anal. Calcd for $C_{19}H_{26}N_2O_6S$: C, 55.59; H, 6.38; N, 6.82. Found: C, 55.68; H, 6.47; N, 6.77.

5.1.5.7. 2-{Benzyloxybenzyloxy[(4-methoxyphenyl)sulfonyl]amino}-*N*-hydroxyacetamide (1h). The title compound was prepared as previously described.[20]

5.1.5.8. *N*-Hydroxy-2-[*N*-isopropoxy(4-methoxyphenyl)sulfonamido]-3-methylbutanamide (2d). The title compound was prepared from **14d** following the general procedure. Recrystallization from Et_2O gave **2d** as white solid (50% yield); mp 175–176 °C; ^1H NMR ($CDCl_3$) δ: 0.91 (d, J = 6.6 Hz, 6H), 1.26 (d, J = 6.4 Hz, 3H), 1.30 (d, J = 6.4 Hz, 3H), 2.04 (m, 1H), 3.88 (s, 3H), 4.47 (septet, J = 6.4 Hz, 1H), 6.97–7.03 (m, 2H), 7.78–7.82 (m, 2H), 8.60 (br s, 1H). EI-MS, *m*/*z*: 43 (65.60), 155 (100), 171 (91.26), 361 (M + 1, 2.06). Anal. Calcd for $C_{15}H_{24}N_2O_6S$: C, 49.99; H, 6.71; N, 7.77. Found: C, 50.10; H, 6.85; N, 7.65.

5.1.5.9. *N*-Hydroxy-2-[*N*-*sec*-butyloxy(4-methoxyphenyl)sulfonamido]-3-methylbutanamide (2e). The title compound was prepared from **14e** following the general procedure. Recrystallization from Et_2O gave **2e** as yellow solid (80% yield); ^1H NMR ($CDCl_3$) δ: 0.87–0.97 (m, 6H),1.23–1.29 (m, 3H), 1.40–1.66 (m, 3H), 1.68–1.85 (m, H), 2.00 (m, 1H), 3.68 (d, 1H), 3.88 (s, 3H), 4.28 (m, 1H), 6.97–7.02 (m, 2H), 7.78–7.83 (m, 2H), 8,70 (br s, 1H). Anal. Calcd for $C_{16}H_{26}O_6N_2S$: C, 51.32; H, 7.00; N, 7.48. Found: C, 51.45; H, 7.10; N, 7.35.

5.1.6. General procedure for the preparation of *N*-(alkyloxy)iminoacetic acids (15b,c). Sodium acetate (9.76 g, 12 mmol) was added to a solution of glyoxylic acid hydrate (5.47 g, 60 mmol) and the appropriate *O*-alkyl hydroxylamine hydrochloride **7b**,**c** (60 mmol) in 90 mL of H_2O/MeOH 1:1 and the reaction mixture was stirred overnight at room temperature. The MeOH was evaporated and the residue was extracted with EtOAc. The organic phase was then extracted with saturated $NaHCO_3$ solution. The aqueous phase was acidified to pH 3 at 0 °C with HCl 10% and extracted with EtOAc. The organic extracts were dried and evaporated to give **15b**,**c** as solids.

5.1.6.1. *N*-(Benzyloxy)iminoacetic acid (15b). The title compound was prepared from **7b** following the general procedure. Compound **15b** was used without further purification: white solid (84% yield); mp 78–80 °C; ^1H NMR ($CDCl_3$) δ: 5.33 (s, 2H), 6.62 (br s, 1H), 7.39 (s, 5H), 7.56 (s, 1H).

5.1.6.2. *N*-(4-Chlorobenzyloxy)iminoacetic acid (15c). The title compound was prepared from **7c** following the general procedure. Compound **15c** was used without further purification: white solid (89% yield); mp 120–122 °C; ^1H NMR ($CDCl_3$) δ: 5.27 (s, 2H), 5.63 (br s, 1H), 7.22–7.90 (m, 4H), 7.54 (s, 1H).

5.1.7. General procedure for the preparation of *N*-alkyloxyamino acids (16c, 17b,c, 18b). The appropriate *N*-(alkyloxy)iminoacetic acid **15b**,**c** (8.38 mmol) was dissolved in dry THF (40 mL) under nitrogen and cooled to −40 °C. A solution of methyl, *n*-butyl or *sec*-butyllithium (16.76 mmol) was added and the mixture was stirred at −40 °C for 1 h. The reaction was quenched with saturated NH_4Cl, acidified with HCl 10% to pH 3, and extracted with EtOAc. The organic extracts were combined, washed with brine, dried over Na_2SO_4, filtered, and evaporated to provide **16c**, **17b**,**c**, **18b** as solids.

5.1.7.1. 2-(4-Chlorobenzyloxyamino)propanoic acid (16c). The title compound was prepared from **15c** and methyllithium following the general procedure. Compound **16c** was used without further purification: white solid (38% yield); mp 102–104°C; ^1H NMR ($CDCl_3$) δ: 1.26 (d, J = 7.1 Hz, 3H), 3.72 (q, J = 7.1 Hz, 1H), 3.73 (br s, 2H), 4.68 (s, 2H), 7.20–7.40 (m, 4H). EI-MS, *m*/*z*: 45 (39.19), 77 (19.44), 89 (12.63), 125 (100), 127 (30). Anal. Calcd for C_{10} $H_{12}ClNO_3$: C, 52.30; H, 5.27; N, 6.10. Found: C, 52.32; H, 5.27; N, 6.11.

5.1.7.2. 2-(Benzyloxyamino)hexanoic acid (17b). The title compound was prepared from **15b** and *n*-butyllithium following the general procedure. Recrystallization from $CHCl_3$/*n*-hexane gave **17b** as white solid (25% yield); mp 135–137 °C; ^1H NMR ($CDCl_3$) δ: 0.78–1.00 (m, 3H), 1.20–1.70 (m, 6H), 3.61 (t, J = 6.6 Hz, 1H), 4.18 (br s, 2H), 4.72 (s, 2H), 7.34 (s, 5H). EI-MS, *m*/*z*: 45 (6.81), 65 (5.84), 86 (1.81), 91 (100), 107 (3.20), 108 (2.41). Anal. Calcd for C_{13} H_{19} NO_3: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.82; H, 8.15; N 5.87.

5.1.7.3. 2-(4-Chlorobenzyloxyamino)hexanoic acid (17c). The title compound was prepared from **15c** and *n*-butyllithium following the general procedure. Recrystallization from $CHCl_3$/*n*-hexane gave **17c** as white solid (27% yield); mp 141–143 °C; ^{1}H NMR ($CDCl_3$) δ: 0.70–0.95 (m, 3H), 1.20–1.40 (m, 4H), 1.42–1.65 (m, 2H), 3.30 (br s, 2H), 3.59 (m, 1H), 4.67 (s, 2H), 7.25–7.40 (m, 4H). EI-MS, *m*/*z*: 45 (16.54), 57 (3.27), 72 (2.51), 77 (17.21), 86 (3.43),125 (100). Anal. Calcd for $C_{13}H_{18}Cl\,NO_3$: C, 57.46; H, 6.68; N, 5.15. Found: C, 57.48; H, 6.73; N, 5.15.

5.1.7.4. 2-(Benzyloxyamino)-3-methylpentanoic acid (18b). The title compound was prepared from **15b** and *sec*-butyllithium following the general procedure. Compound **18b** was used without further purification: oil (25% yield); ^{1}H NMR ($CDCl_3$) δ: 0.81–1.70 (m, 9H), 3.42–3.60 (m, 1H), 4.69 (s, 2H), 5.71 (br s, 2H), 7.20–7.42 (m, 5H).

5.1.8. General procedure for the preparation of *O*-TBDMS acid hydroxyamides (19c, 20b,c, 21b). 1-[3-(Dimethylamino)propyl]-3-ethyl carbodiimide hydrochloride (EDCI) was added portionwise (1 mmol) to a stirred and cooled (0 °C) solution of the carboxylic acid **16c**, **17b**,**c**, **18b** (1 mmol) and *O*-(*tert*-butyldimethylsilyl)hydroxylamine (1 mmol) in freshly distilled CH_2Cl_2 (18 mL). After stirring at rt for 20 h, the mixture was washed with water (20 mL) and the organic phase was dried and evaporated in vacuo. The crude residue was purified by flash chromatography on silica gel to yield pure **19c**, **20b**,**c**, **21b** as oils.

5.1.8.1. 2-(4-Chlorobenzyloxyamino)-*N*-(*tert*-butyldimethylsilyloxy)propanamide (19c). The title compound was prepared from **16c** following the general procedure. The crude product was purified by flash chromatography on silica gel (*n*-hexane/EtOAc 4:1) to give **19c** (55% yield) as a yellow oil; ^{1}H NMR ($CDCl_3$) δ: 0.16 (s, 6H), 0.95 (s, 9H), 1.20 (d, J = 7.0 Hz, 3H), 3.50 (m, 1H), 4.64 (s, 2H), 7.20–7.40 (m, 4H), 8.37 (br s, 1H).

5.1.8.2. 2-(Benzyloxyamino)-*N*-(*tert*-butyldimethylsilyloxy)hexanamide (20b). The title compound was prepared from **17b** following the general procedure. The crude product was purified by flash chromatography on silica gel (*n*-hexane/EtOAc 4:1) to give **20b** (63% yield) as oil; ^{1}H NMR ($CDCl_3$) δ: 0.16 (s, 3H), 0.17 (s, 3H), 0.78–0.92 (m, 3H), 0.95 (s, 9H), 1.20–1.38 (m, 4H), 1.40–1.52 (m, 2H), 3.35 (m, 1H), 4.65 (s, 2H), 7.30–7.42 (m, 5H), 8.25 (br s, 1H).

5.1.8.3. 2-(4-Chlorobenzyloxyamino)-*N*-(*tert*-butyldimethylsilyloxy)hexanamide (20c). The title compound was prepared from **17c** following the general procedure. The crude product was purified by flash chromatography on silica gel (*n*-hexane/EtOAc 4:1) to give **20c** (87% yield) as oil; ^{1}H NMR ($CDCl_3$) δ: 0.17 (s, 6H), 0.79–0.90 (m, 3H), 0.91 (s, 9H), 1.20–1.40 (m, 4H), 1.42–1.70 (m, 2H), 3.35 (m, 1H), 4.63 (s, 2H), 7.20–7.40 (m, 4H), 8.17 (br s, 1H).

5.1.8.4. 2-(Benzyloxyamino)-*N*-(*tert*-butyldimethylsilyloxy)-3-methylpentanamide (21b). The title compound was prepared from **18b** following the general procedure. The crude product was purified by flash chromatography on silica gel (*n*-hexane/EtOAc 4:1) to give **21b** (60% yield) as oil; ^{1}H NMR ($CDCl_3$) δ: 0.16 (s, 3H), 0.17 (s, 3H), 0.75–1.10 (m, 12H), 1.05–1.30 (m, 4H), 1.55–1.75 (m, 2H), 3.00–3.30 (m, 1H), 4.65 (s, 2H), 7.20–7.40 (m, 5H).

5.1.9. General procedure for the preparation of acid hydroxyamides (4c, 5b,c, 6b). TFA (4.4 mL, 57 mmol) was added dropwise to a stirred solution of **19c**, **20b**,**c**, **21b** (1 mmol) in freshly distilled CH_2Cl_2 (4.4 mL) cooled to 0 °C. The solution was stirred for 5 h at 0 °C and the solvent was removed in vacuo to give a solid. The crude products were recrystallized from Et_2O and *n*-hexane to yield **4c**, **5b**,**c**, **6b** as pure solids.

5.1.9.1. 2-(4-Chlorobenzyloxyamino)-*N*-hydroxypropanamide (4c). The title compound was prepared from **19c** following the general procedure. Recrystallization from Et_2O/*n*-hexane gave **4c** as white solid (53% yield); mp 137–138 °C; ^{1}H NMR (DMSO-d_6) δ: 1.03 (d, J = 6.8 Hz, 3H), 3.40–3.60 (m, 1H), 4.64 (s, 2H), 7.30–7.45 (m, 4H). Anal. Calcd for $C_{10}H_{13}ClN_2O_3$: C, 49.09; H, 5.36; N, 11.45. Found: C, 50.02; H, 5.39; N, 11.40.

5.1.9.2. 2-(Benzyloxyamino)-*N*-hydroxyhexanamide (5b). The title compound was prepared from **20b** following the general procedure. Recrystallization from Et_2O/*n*-hexane gave **5b** as white solid (60% yield); mp 101–103°C; ^{1}H NMR (DMSO-d_6) δ: 0.85–0.95 (m, 3H), 1.00–1.22 (m, 6H), 3.20–3.40 (m, 1H), 4.59 (s, 2H), 7.31 (s, 5H), 10.58 (br s, 1H). MS, *m*/*z*: 44 (5.71), 60 (3.64), 65 (10.6), 77 (14.91), 89 (2.61), 91 (100). Anal. Calcd for $C_{13}H_{20}N_2O_3$: C, 61.88; H, 7.99; N, 11.10. Found: C, 61.92; H, 8.06; N, 11.01.

5.1.9.3. 2-(4-Chlorobenzyloxyamino)-*N*-hydroxyhexanamide (5c). The title compound was prepared from **20c** following the general procedure. Recrystallization from Et_2O/*n*-hexane gave **5c** as white solid (60% yield); mp 125–126°C; ^{1}H NMR (DMSO-d_6) δ: 0.80–1.40 (m, 9H), 3.29 (m, 1H), 4.61 (s, 2H), 6.62 (br s, 2H), 7.15–7.60 (m, 4H), 10.62 (br s, 1H). MS, *m*/*z*: 44 (11.54), 58 (37.43), 77 (9.82), 89 (11.39), 125 (100), 127 (33.11). Anal. Calcd for $C_{13}H_{19}ClN_2O_3$: C, 54.45; H, 6.68; N, 9.77. Found: C, 54.50; H, 6.71; N, 9.70.

5.1.9.4. 2-(Benzyloxyamino)-*N*-hydroxy-3-methylpentanamide (6b). The title compound was prepared from **21b** following the general procedure. Recrystallization from Et_2O/*n*-hexane gave **6b** as white solid (47% yield); mp 145–146 °C; ^{1}H NMR (DMSO-d_6) δ: 0.60–0.85 (m, 6H), 0.85–1.55 (m, 5H), 3.05–3.20 (m, 1H), 4.56 (s, 2H), 7.31 (s, 5H), 10.53 (br s, 1H). MS, *m*/*z*: 44 (2.35), 65 (5.46), 77 (9.41), 91 (100). Anal. Calcd for $C_{13}H_{20}N_2O_3$: C, 61.88; H, 7.99; N, 11.10. Found: C, 61.90; H, 8.02; N, 11.06.

5.1.10. General procedure for the preparation of *N*-alkyloxyaminoacetic acids (22b,c). A cooled (0 °C) solution of the appropriate *N*-(alkyloxy)iminoacetic acids **15b**,**c** (11.17 mmol) and borane–triethylamine complex (7.78 mL, 52.65 mmol) in EtOH (21 mL) was treated dropwise under stirring with HCl 10% (37.5 mL).

After stirring for 18 h at room temperature, the resulting mixture was washed with $CHCl_3$ and then treated at 0 °C with NaOH 1 N until a white precipitate was formed (pH ~4). The aqueous phase was extracted with CH_2Cl_2 and the organic extracts, dried and evaporated, provided **22b**, **c** as solids.

5.1.10.1. 2-(Benzyloxyamino)acetic acid (22b). The title compound was prepared from **15b** following the general procedure. Compound **22b** was used without further purification: white solid (42% yield); mp 114–116°C; ^{1}H NMR ($CDCl_3$) δ: 3.57 (s, 2H), 4.66 (s, 2H), 4.89 (br s, 2H), 7.28 (s, 5H). MS, *m*/*z*: 45 (9.46), 60 (1.05), 89 (1.26), 91 (100), 105 (0.78), 108 (0.83). Anal. Calcd for $C_9H_{11}NO_3$: C, 59.66; H, 6.12; N, 7.73. Found: C, 59.69; H, 6.19; N, 7.70.

5.1.10.2. 2-(4-Chlorobenzyloxyamino)acetic acid (22c). The title compound was prepared from **15c** following the general procedure. Compound **22c** was used without further purification: white solid (27% yield); mp 107–109°C; ^{1}H NMR ($CDCl_3$) δ: 2.48 (br s, 2H), 3.64 (s, 2H), 4.69 (s, 2H), 7.26–7.36 (m, 4H). MS, *m*/*z*: 45 (22.20), 60 (4.65), 89 (10.22), 125 (100), 126 (7.63), 127 (31.13), 142 (6.77). Anal. Calcd for $C_9H_{10}ClNO_3$: C, 50.13; H, 4.67; N, 6.50. Found: C, 50.25; H, 4.77; N, 6.48.

5.1.11. General procedure for the preparation of *O*-TBDMS acid hydroxyamides (23b,c). 1-[3-(Dimethylamino)propyl]-3-ethyl carbodiimide hydrochloride (EDCI) was added portionwise (1 mmol) to a stirred and cooled (0 °C) solution of the carboxylic acid **22b**,**c** (1 mmol) and *O*-(*tert*-butyldimethylsilyl)hydroxylamine (1 mmol) in freshly distilled CH_2Cl_2 (18 mL). After stirring at rt for 20 h, the mixture was washed with water (20 mL) and the organic phase was dried and evaporated in vacuo to provide **23b**,**c** as oils.

5.1.11.1. 2-(Benzyloxyamino)-*N*-(*tert*-butyldimethylsilyloxy)acetamide (23b). The title compound was prepared from **22b** following the general procedure. Compound **23b** was used without further purification: oil (73% yield); ^{1}H NMR ($CDCl_3$) δ: 0.12 (s, 3H), 0.17 (s, 3H),0.95 (s, 9H), 3.53 (br s, 2H), 4.71 (s, 2H), 7.25–7.45 (m, 5H), 8.42 (br s, 1H).

5.1.11.2. 2-(4-Chlorobenzyloxyamino)-*N*-(*tert*-butyldimethylsilyloxy)acetamide (23c). The title compound was prepared from **22c** following the general procedure. Compound **23c** was used without further purification: oil (80% yield); ^{1}H NMR ($CDCl_3$) δ: 0.12 (s, 3H), 0.17 (s, 3H), 0.95 (s, 9H), 3.52 (br s, 2H), 4.66 (s, 2H), 7.42–7.80 (m, 4H), 8.33 (br s, 1H).

5.1.12. General procedure for the preparation of acid hydroxyamides (3b, c). TFA (4.4 mL, 57 mmol) was added dropwise to a stirred solution of **23b**,**c** (1 mmol) in freshly distilled CH_2Cl_2 (4.4 mL) cooled to 0 °C. The solution was stirred for 5 h at 0 °C and the solvent was removed in vacuo to give a solid. The crude products were recrystallized from Et_2O and *n*-hexane to yield **3b**,**c** as pure solids.

5.1.12.1. 2-(Benzyloxyamino)-*N*-hydroxyacetamide (3b). The title compound was prepared from **23b** following the general procedure. Recrystallization from Et_2O gave **3b** as white solid (45% yield); mp 117–119°C; ^{1}H NMR (DMSO-d_6) δ: 3.28 (s, 2H), 4.60 (s, 2H), 7.32 (m, 5H), 10.51 (s, 1H). MS, *m*/*z*: 44 (10.38), 65 (7.83), 77 (16.21), 91 (100). Anal. Calcd for $C_9H_{12}N_2O_3$: C, 55.09; H, 6.16; N, 14.28. Found: C, 55.19; H, 6.18; N, 14.19.

5.1.12.2. 2-(4-Chlorobenzyloxyamino)-*N*-hydroxyacetamide (3c). The title compound was prepared from **23c** following the general procedure. Recrystallization from $CHCl_3$/*n*-hexane gave **3c** as white solid (95% yield); mp 67–69°C; ^{1}H NMR (DMSO-d_6) δ: 3.37 (s, 2H), 4.66 (s, 2H), 6.30 (br s, 2H), 7.25–7.50 (m, 4H), 10.13 (s, 1H). MS, *m*/*z*: 45 (100), 51 (78.5), 69 (53.7), 77 (85.13), 125 (56.38), 155 (2.65). Anal. Calcd for $C_9H_{11}ClN_2O_3$: C, 46.87; H, 4.81; N, 12.15. Found: C, 46.95; H, 4.89; N, 12.05.

5.2. CA inhibition assay

Recombinant human CA isoforms I, II, and IX have been prepared as reported earlier by our group,[23,26] and their activity assayed by a stopped flow CO_2 hydration assay.[24] An Applied Photophysics (Oxford, UK) stopped-flow instrument has been used for assaying the CA-catalysed CO_2 hydration activity. 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 7.5) as buffer, 0.1 M Na_2SO_4 (for maintaining constant the ionic strength), following the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (1 mM) were prepared in distilled-deionized water with 10–20% (v/v) DMSO (which is not inhibitory at these concentrations) and dilutions up to 0.1 nM were done thereafter with distilled-deionized water. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the *E*–*I* complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3, from Lineweaver–Burk plots, as reported earlier, and represent the mean from at least three different determinations.[23,26]

5.3. MMP inhibition assays[27]

Human recombinant progelatinase A (pro-MMP-2) and B (pro-MMP-9) from transfected mouse myeloma cells were supplied by Prof. Gillian Murphy (Department of Oncology, University of Cambridge, UK). Proenzymes were activated immediately prior to use with *p*-aminophenylmercuric acetate (APMA 2 mM for 1 h at 37 °C for MMP-2 and 1 mM for 1 h at 37 °C for MMP-9). For assay measurements, the inhibitor stock solutions (DMSO, 100 mM) were further diluted, at sev-

en different concentrations (0.01 nM–300 μM) for each MMP in the fluorimetric assay buffer (FAB:Tris 50 mM, pH 7.5, NaCl 150 mM, $CaCl_2$ 10 mM, Brij 35 0.05%, and DMSO 1%). Activated enzyme (final concentration 2.7 nM for MMP-9 and 2.9 nM for MMP-2) and inhibitor solutions were incubated in the assay buffer for 4 h at 25 °C. After the addition of 200 μM solution of the fluorogenic substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 (Sigma) in DMSO (final concentration 2 μM), the hydrolysis was monitored every 15 s. for 20 min. recording the increase in fluorescence (λ_{ex} = 328 nm, λ_{em} = 393 nm) using a Molecular Device SpectraMax Gemini XS plate reader. The assays were performed in triplicate in a total volume of 200 μl per well in 96-well microtiter plates (Corning, black, NBS). Control wells lack inhibitor. The MMP inhibition activity was expressed in relative fluorescent units (RFU). Percent of inhibition was calculated from control reactions without the inhibitor. IC_{50} was determined using the formula: $V_i/V_o = 1/(1 + [I]/IC_{50})$, where V_i is the initial velocity of substrate cleavage in the presence of the inhibitor at concentration $[I]$ and V_o is the initial velocity in the absence of the inhibitor. Results were analyzed using SoftMax Pro software and GraFit software.[28]

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