Bioorganic & Medicinal Chemistry Letters 21 (2011) 5210–5213

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of rhodamine B–benzenesulfonamide conjugates and their inhibitory activity against human α- and bacterial/fungal β-carbonic anhydrases

Marouan Rami [a], Alessio Innocenti [b], Jean-Louis Montero [a], Andrea Scozzafava [b], Jean-Yves Winum [a,*], Claudiu T. Supuran [b,*]

[a] Institut des Biomolécules Max Mousseron (IBMM), UMR 5247 CNRS-UM1-UM2, Bâtiment de Recherche Max Mousseron, Ecole Nationale Supérieure de Chimie de Montpellier, 8 rue de l'Ecole Normale, 34296 Montpellier Cedex, France

[b] Università degli Studi di Firenze, Laboratorio di Chimica Bioinorganica, Rm. 188, Via della Lastruccia 3, I-50019 Sesto Fiorentino (Firenze), Italy

ARTICLE INFO

Article history:
Received 21 June 2011
Revised 11 July 2011
Accepted 12 July 2011
Available online 21 July 2011

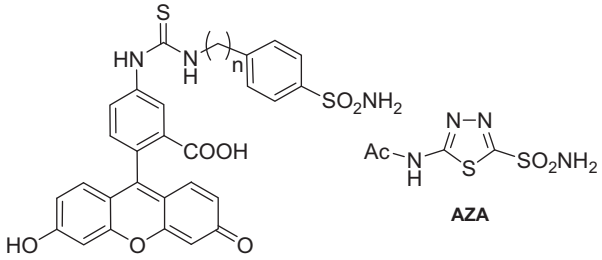
Keywords:
Carbonic anhydrase
Sulfonamide
Rhodamine B
Fluorescence
Tumor imaging
Bacterial/fungal carbonic anhydrase

ABSTRACT

A series of fluorescent sulfonamide carbonic anhydrase (CA, EC 4.2.1.1) inhibitors were obtained by attaching rhodamine B moieties to the scaffold of benzenesulfonamides. The new compounds have been investigated for the inhibition of 12 human α-CA isoforms (hCA I–hCA XIV), three bacterial and one fungal β-class enzymes from the pathogens *Mycobacterium tuberculosis* and *Candida albicans*. All types of inhibitory activities have been detected, with several compounds showing low nanomolar inhibition against the transmembrane isoforms hCA IX, XII (cancer-associated) and XIV. The β-CAs were inhibited in the micromolar range by these compounds which may have applications for the imaging of hypoxic tumors or bacteria due to their fluorescent moieties.

Rhodamine dyes are extensively used in biotechnological applications such as fluorescence microscopy, flow cytometry, fluorescence correlation spectroscopy,[1,2] as well as for staining microorganisms (such a *Mycobacterium tuberculosis*),[3] due to their high fluorescence quantum yields and rather simple chemical structure. Together with fluorescein,[4] these are the two dyes mostly used for attaching fluorescent tags to biomolecules, such as for example enzyme inhibitors, PET tracers, agents to visualize mitochondrial function, hypoxic tumors, etc.[5,6]

We have explored earlier[4] the use of fluorescein moieties for preparing inhibitors of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1).[7] Sulfonamides bearing such moieties, of types **A** and **B**, were showing enhanced affinity for the tumor-associated isoforms CA IX and XII compared to the cytosolic ones CA I and II, and were used in the proof-of-concept studies which demonstrated the involvement of CA IX in tumor acidification processes.[4b] Furthermore, in vivo, in animal models of hypoxic tumors, compounds **A** and **B** were observed to accumulate only in the tumor[6c,6d] making this type of derivatives (and the entire class of the CA IX-selective inhibitors) interesting imaging candidates of this type of tumors. More recently, it has been also shown that sulfonamide **B** inhibits in vivo the growth of primary tumors and metastases in a highly aggressive breast cancer cell line, in animals harboring such tumors.[8]



A: n = 1
B: n = 2

All these data clearly show the usefulness of fluorescently labeled CA inhibitors (CAIs) for both in vitro and in vivo studies with this class of pharmacological agents which have various applications as diuretics, antiglaucoma, antiobesity, antiepileptic and antitumor agents.[7,8] Furthermore, as CAs belonging to various classes (α-, β- and/or γ-CA family) are present in many pathogenic nematodes, bacteria and fungi,[7,9,10] fluorescently labeled CAIs may

* Corresponding authors. Tel.: +33 4 67 14 72 34; fax: +33 4 67 14 43 44 (J.Y.W.); tel.: +39 055 457 3005; fax: +39 055 457 3385 (C.T.S.).

E-mail addresses: jean-yves.winum@univ-montp2.fr (J.-Y. Winum), claudiu.supuran@unifi.it (C.T. Supuran).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.07.045

have interesting applications as research tools for better understanding the role these enzymes play in the life cycle of these pathogens or for eventually developing alternative pharmacologic agents to the clinically used compounds which led in many cases to extensive drug resistance problems.[9,10]

As only fluorescein-tagged CAIs have been reported up until now,[4] in this Letter we report the synthesis and inhibition studies of CAIs of the sulfonamide type labeled with rhodamine B moieties.

For this scope, the carboxylic acid moiety of rhodamine B **1** has been transformed to the corresponding acyl chloride by reaction with phosphorus oxychloride, and then coupled with amino-benzenesulfonamide derivatives of type **2**, bearing a free amino, methylamino or ethylamino moiety. The sulfonamide–rhodamine B conjugates **3–6** obtained in this way (Scheme 1) were thoroughly characterized and their structures were confirmed.[11]

The new compounds reported here, of types **3–6** and acetazolamide **AZA** as standard drug have been investigated for the inhibition of 12 human α-CA isoforms (hCA I–hCA XIV),[7] three bacterial and one fungal β-class enzymes from the pathogens *Mycobacterium tuberculosis* (mtCA 1, 2 and 3) and *Candida albicans* (caNce 103).[9,10] Data of Table 1 show the following structure–activity relationship (SAR) for the inhibition of these enzymes with the investigated sulfonamides:

Table 1
Inhibition data of human α- and bacterial/fungal β-CAs with sulfonamides **3–6** and acetazolamide AAZ as standard, by a stopped-flow CO_2 hydrase assay.[12] hCA = human CA isoform, mtCA = *Mycobacterium tuberculosis* CA, caNce103 = *Candida albicans* enzyme

Isoform/inhibitor	K_I[a] (nM)				
	3	**4**	**5**	**6**	**AZA**
hCA I	512	311	714	692	250
hCA II	279	21.5	980	955	12
hCA III	32,000	34,500	21,300	18,900	20,000
hCA IV	7200	6740	7450	7500	74
hCA VA	413	347	1040	996	63
hCA VB	310	321	513	427	54
hCA VI	980	543	1035	814	11
hCA VII	60.2	12.1	34.9	91.7	2.5
hCA IX	18.5	24.0	10.2	19.4	25
hCA XII	9.1	10.5	8.6	25.3	5.7
hCA XIII	280	236	515	463	17
hCA XIV	7.7	8.4	4.9	14.6	41
mtCA 1	4410	4360	4480	3700	481
mtCA 2	414	443	411	409	9
mtCA 3	342	413	469	422	104
caNce103	4990	4095	5320	4730	132

[a] From three different determinations. Errors were in the range of ±5–10% of the reported value.

(i) The human isoforms hCA I, VA, VB, VI and XIII were inhibited by the new sulfonamides **3–6** moderately, with inhibition constants in the low micromolar–submicromolar range. Thus, for hCA I, the K_Is were in the range of 311–714 nM, for hCA VA in the range of 347–1040 nM; for hCA VB in the range of 310–513 nM; in the range of 543–1035 nM against hCA VI; and for hCA XIII in the range of 236–515 nM, respectively, (Table 1). It may be observed that most of the time the metanilamide derivative **4** was the most active in the series, followed by the sulfanilamide one **3**. The longer molecules incorporating aminomethyl and aminoethyl linkers (**5** and **6**) were generally the least active inhibitors in this small series of investigated compounds. Irrespective of the fact that some of these isoforms are cytosolic (hCA I and XIII), mitochondrial (hCA VA and VB) or secreted (hCA VI), their behavior against this class of inhibitors is rather similar.

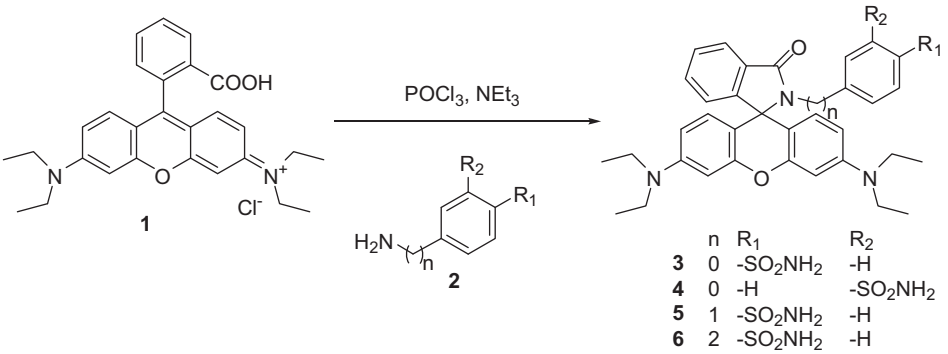
(ii) The physiologically dominant isoform hCA II was potently inhibited by the metanilamide rhodamide conjugate **4** (K_I of 21.5 nM) and weakly inhibited by the remaining compounds **3**, **5** and **6**, which showed K_Is in the range of 279–980 nM. The SAR is thus rather similar with what discussed above for the isoforms I, VA, VB and XIII.

(iii) The cytosolic low activity isoforms hCA III was poorly inhibited by all these sulfonamides (and **AZA**), with K_Is in the range of 18.9–34.5 μM. However, in this case, the least active compound was the metanilamide conjugate **4**, whereas the best inhibitor was the aminoethylbenzenesulfonamide derivative **6** (and acetazolamide **AZA**).

(iv) The membrane-associated hCA IV was also rather weakly inhibited by sulfonamides **3**–**6**, with K_Is in the range of 6.74–7.50 μM (Table 1). **AZA** on the other hand is a potent inhibitor of this isoform (K_I of 74 nM).

(v) The brain-associated, cytosolic isoform hCA VII was effectively inhibited by sulfonamides **3–6** incorporating rhodamine B moieties, with K_Is in the range of 12.1–91.7 nM. Again the best inhibitor was the metanilamide conjugate **4**, but followed by the aminomethyl derivative **5**, whereas the weakest one was the aminoethyl derivative **6**.

(vi) The most interesting inhibition profile with derivatives **3–6** has been observed against the transmembrane isoforms hCA IX, XII and XIV (two of them, hCA IX and XII are associated to tumors, whereas hCA XIV is not).[7] Indeed, against hCA IX these compounds showed K_Is in the range of 10.2–24.0 nM, with the metanilamide conjugate **4** being the least effective inhibitor and the aminomethyl one **5** the best. However, the SAR is rather flat as all these compounds are highly effective as CA IX inhibitors (similar to **AZA**). Against hCA XII the efficacy was again excellent, with K_Is in the range of 8.6–25.3 nM and a similar SAR. hCA XIV was inhibited with K_Is in the range of 4.9–14.6 nM, being the most sensitive isoform to this type of CA inhibitor.



Scheme 1. Preparation of rhodamine-substituted sulfonamides **3–6**.

(vii) The mycobacterial enzyme mtCA 1 was weakly inhibited by sulfonamides **3–6**, with K_Is in the range of 3.70–4.48 μM, whereas the remaining two β-CAs from this pathogen were at least one order of magnitude more sensitive to be inhibited by these compounds. Indeed, against mtCA 2 the inhibition constants were in the range of 409–443 nM, and against mtCA **3** in the range of 342–469 nM (Table 1). Thus, the new sulfonamides reported here are less effective than **AZA** as mtCA inhibitors.

(viii) The fungal enzyme from *C. albicans* caNce 103 was also weakly inhibited by the compounds investigated here, which showed K_Is in the range of 4.09–5.32 μM.

In conclusion, we report the synthesis of a series of fluorescent CA inhibitors, which were obtained by attaching rhodamine B moieties to the scaffold of benzenesulfonamides. The new compounds have been investigated for the inhibition of 12 human α-CA isoforms (hCA I–hCA XIV), three bacterial and one fungal β-class enzymes from the pathogens *M. tuberculosis* and *C. albicans*. All types of inhibitory activities have been detected, with several compounds showing low nanomolar inhibition against the transmembrane isoforms hCA IX, XII (cancer-associated) and XIV. The β-CAs were inhibited in the micromolar range by these compounds which may have applications for the imaging of hypoxic tumors or bacteria due to their fluorescent moieties.

Acknowledgment

This research was financed in part by a 7 FP EU project (Metoxia).

References and notes

1. (a) Karstens, T.; Kobs, K. *J. Phys. Chem.* **1980**, *84*, 1871; (b) Setiawan, D.; Kazaryan, A.; Martoprawiro, M. A.; Filatov, M. *Phys. Chem. Chem. Phys.* **2010**, *12*, 11238; (c) Kubin, R. *J. Luminesc.* **1983**, *27*, 455.
2. (a) Würth, C.; Grabolle, M.; Pauli, J.; Spieles, M.; Resch-Genger, U. *Anal. Chem.* **2011**, *83*, 3431; (b) Hossen, M. N.; Kajimoto, K.; Akita, H.; Hyodo, M.; Ishitsuka, T.; Harashima, H. *J. Controlled Release* **2010**, *147*, 261.
3. (a) Stancu, M. M.; Grifoll, M. *J. Gen. Appl. Microbiol.* **2011**, *57*, 1; (b) Hoff, D. R.; Ryan, G. J.; Driver, E. R.; Ssemakulu, C. C.; De Groote, M. A.; Basaraba, R. J.; Lenaerts, A. J. *PLoS One* **2011**, *6*, e17550.
4. (a) Cecchi, A.; Supuran, C. T. *Curr. Pharm. Des.* **2008**, *14*, 699; (b) Švastová, E.; Hulíková, A.; Rafajová, M.; Zatovičová, M.; Gibadulinová, A.; Casini, A.; Cecchi, A.; Scozzafava, A.; Supuran, C.; Pastorek, J. *FEBS Lett.* **2004**, *577*, 439; (c) Cecchi, A.; Hulikova, A.; Pastorek, J.; Pastoreková, S.; Scozzafava, A.; Winum, J.-Y.; Montero, J.-L.; Supuran, C. T. *J. Med. Chem.* **2005**, *48*, 4834; (d) Alterio, V.; Vitale, R. M.; Monti, S. M.; Pedone, C.; Scozzafava, A.; Cecchi, A.; De Simone, G.; Supuran, C. T. *J. Am. Chem. Soc.* **2006**, *128*, 8329.
5. a Zhou, Y.; Kim, Y. S.; Yan, X.; Jacobson, O.; Chen, X.; Liu, S. *Mol. Pharm.* in press.; (b) Koide, Y.; Urano, Y.; Hanaoka, K.; Terai, T.; Nagano, T. *ACS Chem. Biol.* **2011**, *6*, 600.
6. (a) Best, Q. A.; Xu, R.; McCarroll, M. E.; Wang, L.; Dyer, D. J. *Org. Lett.* **2010**, *12*, 3219; (b) Gottumukkala, V.; Heinrich, T. K.; Baker, A.; Dunning, P.; Fahey, F. H.; Treves, S. T.; Packard, A. B. *Nucl. Med. Biol.* **2010**, *37*, 365; (c) Dubois, L.; Douma, K.; Supuran, C. T.; Chiu, R. K.; van Zandvoort, M. A. M. J.; Pastoreková, S.; Scozzafava, A.; Wouters, B. G.; Lambin, P. *Radiother. Oncol.* **2007**, *83*, 367; (d) Dubois, L.; Lieuwes, N. G.; Maresca, A.; Thiry, A.; Supuran, C. T.; Scozzafava, A.; Wouters, B. G.; Lambin, P. *Radiother. Oncol.* **2009**, *92*, 423.
7. (a) Supuran, C. T. *Nat. Rev. Drug Disc.* **2008**, *7*, 168; (b) Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3467.
8. (a) Lou, Y.; McDonald, P. C.; Oloumi, A.; Chia, S. K.; Ostlund, C.; Ahmadi, A.; Kyle, A.; Auf dem Keller, U.; Leung, S.; Huntsman, D. G.; Clarke, B.; Sutherland, B. W.; Waterhouse, D.; Bally, M. B.; Roskelley, C. D.; Overall, C. M.; Minchinton, A.; Pacchiano, F.; Carta, F.; Scozzafava, A.; Touisni, N.; Winum, J. Y.; Supuran, C. T.; Dedhar, S. *Cancer Res.* **2011**, *71*, 3364; (b) Pacchiano, F.; Carta, F.; McDonald, P. C.; Lou, Y.; Vullo, D.; Scozzafava, A.; Dedhar, S.; Supuran, C. T. *J. Med. Chem.* **2011**, *54*, 1896.
9. (a) Hall, R. A.; Mühlschlegel, F. A. Fungal and Nematode Carbonic Anhydrases: Their Inhibition in Drug Design. In *Drug Design of Zinc-Enzyme Inhibitors: Functional, Structural, and Disease Applications*; Supuran, C. T., Winum, J. Y., Eds.; John Wiley & Sons: Hoboken, 2009; pp 301–322; (b) Ohndorf, U. M.; Schlicker, C.; Steegborn, C. Crystallographic Studies on Carbonic Anhydrases from Fungal Pathogens for Structure-Assisted Drug Development. In *Drug Design of Zinc-Enzyme Inhibitors: Functional, Structural, and Disease Applications*; Supuran, C. T., Winum, J. Y., Eds.; John Wiley & Sons: Hoboken, 2009; pp 323–334; (c) Schlicker, C.; Hall, R. A.; Vullo, D.; Middelhaufe, S.; Gertz, M.; Supuran, C. T.; Muhlschlegel, F. A.; Steegborn, C. *J. Mol. Biol.* **2009**, *385*, 1207.
10. (a) Syrjänen, L.; Tolvanen, M.; Hilvo, M.; Olatubosun, A.; Innocenti, A.; Scozzafava, A.; Leppiniemi, J.; Niederhauser, B.; Hytönen, V. P.; Gorr, T. A.; Parkkila, S.; Supuran, C. T. *BMC Biochem.* **2010**, *11*, 28; (b) Minakuchi, T.; Nishimori, I.; Vullo, D.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2009**, *52*, 2226; (c) Nishimori, I.; Minakuchi, T.; Vullo, D.; Scozzafava, A.; Innocenti, A.; Supuran, C. T. *J. Med. Chem.* **2009**, *52*, 3116; (d) Burghout, P.; Vullo, D.; Scozzafava, A.; Hermans, P. W. M.; Supuran, C. T. *Bioorg. Med. Chem.* **2011**, *19*, 243.
11. The rhodamine B–benzenesulfonamides conjugates **3–6** were synthesized as follows: Rhodamine B (1 mmol) **1** was dissolved in phosphorus oxychloride $POCl_3$ (27 mmol). The mixture was refluxed overnight. After cooling at room temperature, the solvent was evaporated under reduced pressure. The obtained rhodamine B acid chloride was dissolved in acetonitrile. (10 mL). Benzenesulfonamide (2.5 mmol) derivatives **2** and triethylamine (3.6 mmol) were added to the solution and stirred at room temperature overnight under N_2. The mixture was then concentrated under reduced pressure, water was added to the residue, and the aqueous phase was extracted with methylene chloride (2 × 40 mL). The organic layer was washed twice with water, dried over anhydrous Na_2SO_4, and concentrated under vacuum. The residue was subjected to column chromatography (silica, CH_2Cl_2/MeOH, 99:1).
Rhodamine B-sulfanilamide conjugate **3**: Yield 29%, mp: 135–137 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.05 (t, 12H, J = 6.8 Hz), 3.29 (m, 8H), 6.35–6.59 (m, 6H), 7.06–7.07 (m, 1H), 7.13 (d, 2H, J = 8.2 Hz) 7.26 (s, 2H, SO_2NH_2), 7.55–7.59 (m, 2H), 7.6 (d, 2H, J = 8.2 Hz), 7.9 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 12.23, 43.59, 66.40, 123.0, 123.64, 125.43, 124.87, 125.96, 126.08, 128.14, 128.55, 128.67, 133.77, 139.96, 140.89, 141.41, 151.42, 151.95, 167.05, MS (ESI$^+$/ESI$^-$) m/z: 597.44 [M+H]$^+$, 619.21 [M+Na]$^+$, 595.37 [M−H]$^-$.
Rhodamine B-3-aminobenzenesulfonamide conjugate **4**: Yield 32%, mp: 137–139 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.05 (t, 12H, J = 6.8 Hz), 3.31 (m, 8H), 6.36–6.38 (m, 2H), 6.57 (s, 2H), 6.87–6.89 (m, 2H), 7.07 (d, 1H, J = 6.7 Hz), 7.38 (s, 2H), 7.36 (m, 2H), 7.55–7.65 (m, 4H), 7.91 (d, 1H, J = 6.7 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 12.06, 45.23, 66.40, 115.81, 122.96, 123.19, 123.49, 128.83, 129.07, 129.23, 133.72, 133.92, 136.67, 137.17, 144.48, 151.95, 166.94, MS (ESI$^+$/ESI$^-$) m/z: 597.44 [M+H]$^+$, 595.44 [M−H]$^-$.
Rhodamine B-4-aminomethylbenzenesulfonamide conjugate **5**: Yield 16%, mp >205 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.06 (t, 12H, J = 6.8 Hz), 3.29 (q, 8H, J = 6.9 Hz), 4.15 (s, 2H), 6.18 (m, 4H), 6.23 (s, 2H), 7.0 (d, 2H, J = 8.2 Hz), 7.09 (s, 1H), 7.15 (s, 2H), 7.44 (d, 2H, J = 8.2 Hz), 7.53–7.54 (m, 2H) 7.81–7.84 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 12.27, 42.85, 43.62, 64.37, 104.49, 96.92,108. 122.34, 123.73, 124.90, 128.06, 128.37, 128.52, 130.55, 132.81, 141.42, 142.11, 148.29, 152.72, 166.76, MS (ESI$^+$/ESI$^-$) m/z: 611.44 [M+H]$^+$, 633.46 [M+Na]$^+$, 609.39 [M−H]$^-$, 645.39 [M+Cl]$^-$.
Rhodamine B-4-aminoethylbenzenesulfonamide conjugate ***6:*** Yield 61%, mp: 117–119 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.08 (t, 12H, J = 6.8 Hz), 2.48 (t, 2H, J = 8.2 Hz), 3.33 (m, 10H), 6.27–6.42 (m, 6H), 7.03 (d, 2H, J = 8.2 Hz) 7.07–7.09 (m, 1H), 7.26 (s, 2H), 7.52–7.55 (m, 2H), 7.62 (d, 2H, J = 8.2 Hz), 7.78–7.81 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 12.27, 33.69, 43.61, 45.49, 64.10, 97.10, 108.16,122.22, 104.94, 123.63, 125.64, 128.34, 128.57, 130.84, 132.60, 132.96, 142.01, 142.93, 148.34, 152.81, 166.33, MS (ESI$^+$/ESI$^-$) m/z: 625.44 [M+H]$^+$, 647.48 [M+Na]$^+$, 623.39 [M−H]$^-$.
12. Khalifah, R. G. *J. Biol. Chem.* **1971**, *246*, 2561. An Applied Photophysics 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 10–20 mM Hepes (pH 7.5, for the α-CAs) or TRIS (pH 8.3, for the β-CAs) as buffers, and 20 mM Na_2SO_4 or 20 mM $NaClO_4$ (for maintaining constant the ionic strength), following the initial rates of 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 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, and the Cheng-Prosogg equation, as reported earlier,[8,10] and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in house as reported earlier.[13–16]
13. (a) Lehtonen, J. M.; Shen, B.; Vihinen, M.; Casini, A.; Scozzafava, A.; Supuran, C. T.; Parkkila, A.-K.; Saarnio, J.; Kivelä, A. J.; Waheed, A.; Sly, W. S.; Parkkila, S. *J. Biol. Chem.* **2004**, *279*, 2719; (b) Pastorekova, S.; Parkkila, S.; Pastorek, J.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2004**, *19*, 199; (c) Abbate, F.; Winum, J. Y.; Potter, B. V. L.; Casini, A.; Montero, J. L.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 231; (d) Clare, B. W.; Supuran, C. T. *J. Pharm. Sci.* **1994**, *83*, 768.
14. (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) Casini, A.; Scozzafava, A.; Mincione, F.; Menabuoni, L.; Ilies, M. A.; Supuran, C. T. *J. Med. Chem.* **2000**, *43*, 4884; (c) Casey, J. R.; Morgan, P. E.; Vullo, D.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. *J. Med. Chem.* **2004**, *47*, 2337.

15. (a) Nishimori, I.; Vullo, D.; Innocenti, A.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. *J. Med. Chem.* **2005**, *48*, 7860; (b) Nishimori, I.; Minakuchi, T.; Onishi, S.; Vullo, D.; Cecchi, A.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem.* **2007**, *15*, 7229; (c) Supuran, C. T. *Curr. Pharm. Des.* **2008**, *14*, 641; (d) Supuran, C. T.; Vullo, D.; Manole, G.; Casini, A.; Scozzafava, A. *Curr. Med. Chem.—Cardiovasc. Hematol. Agents* **2004**, *2*, 49.

16. (a) Nishimori, I.; Vullo, D.; Innocenti, A.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3828; (b) Vullo, D.; Voipio, J.; Innocenti, A.; Rivera, C.; Ranki, H.; Scozzafava, A.; Kaila, K.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 971; (c) Clare, B. W.; Supuran, C. T. *Eur. J. Med. Chem.* **1999**, *34*, 463; (d) Supuran, C. T.; Popescu, A.; Ilisiu, M.; Costandache, A.; Banciu, M. D. *Eur. J. Med. Chem.* **1996**, *31*, 439.