Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 3302–3306
Bioorganic & Medicinal Chemistry Letters

Carbonic anhydrase inhibitors. Synthesis and inhibition of cytosolic/tumor-associated carbonic anhydrase isozymes I, II, and IX with boron-containing sulfonamides, sulfamides, and sulfamates: Toward agents for boron neutron capture therapy of hypoxic tumors

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

[a]*Università degli Studi di Firenze, Polo Scientifico, Laboratorio di Chimica Bioinorganica, Rm. 188, Via della Lastruccia 3, 50019 Sesto Fiorentino (Florence), Italy*

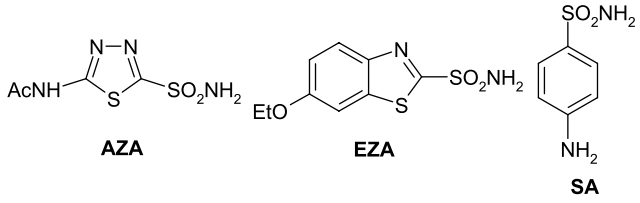
[b]*Université Montpellier II, Laboratoire de Chimie Biomoléculaire, FRE 5032, Ecole Nationale Supérieure de Chimie de Montpellier, 8 rue de l'Ecole Normale, 34296 Montpellier Cedex, France*

Received 7 March 2005; revised 31 March 2005; accepted 12 April 2005
Available online 23 May 2005

Abstract—A library of boron-containing carbonic anhydrase (CA, EC 4.2.1.1) inhibitors, including sulfonamides, sulfamides, and sulfamates is reported. The new compounds have been synthesized by derivatization reactions of 4-carboxy-/amino-/hydroxy-phenylboronic acid pinacol esters with amino/isothiocyanato-substituted aromatic/heteroaromatic sulfonamides or by sulfamoylation reactions with sulfamoyl chloride. The new derivatives have been assayed for the inhibition of three physiologically relevant CA isozymes, the cytosolic CA I and II, and the transmembrane, tumor-associated isozyme CA IX. Effective inhibitors were detected both among sulfonamides, sulfamates, and sulfamides. Against the human isozyme hCA I the new compounds showed inhibition constants in the range of 34–94 nM, against hCA II in the range of 3.1–48 nM, and against hCA IX in the range of 7.3–89 nM, respectively. As hypoxic tumors highly overexpress CA IX, the design of boron-containing inhibitors with high affinity for the tumor-associated CA isozymes may lead to important advances in boron neutron capture therapy (BNCT) applications targeting such tumors, which are non-responsive to both classical chemo- and radiotherapy.

1. Introduction

The carbonic anhydrases (CAs, EC 4.2.1.1) are widespread metalloenzymes, present in mammals in a multitude (at least 15) of isoforms, and catalyze the interconversion between carbon dioxide and bicarbonate at the physiological pH (a proton is also formed in this reaction).[1,2] Some of these isozymes are cytosolic (CA I, CA II, CA III, CA VII, and CA XIII), others are membrane-bound (CA IV, CA IX, CA XII, and CA XIV), CA VA, and CA VB are present in mitochondria throughout the body, whereas CA VI is secreted in the saliva and milk.[1–3] Three cytosolic acatalytic forms are also known (CARP VIII, CARP X, and CARP XI).[1] The catalytically active isoforms, which play important physiological and patho-physiological functions in all tissues/organs in which the three chemical species mentioned above are present (i.e., CO_2, bicarbonate, and



* Corresponding authors. Tel.: +33 4 67 14 72 34; fax: +33 4 67 14 43 44 (J.-Y.W.); tel.: +39 055 4573005; fax: +39 055 4573385 (C.T.S.); e-mail addresses: winumj@univ-montp2.fr; claudiu.supuran@unifi.it

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.04.058

H^+ ions), are also strongly inhibited by aromatic and heterocyclic sulfonamides, sulfamates, sulfamides, and some of their derivatives.[3–7] The catalytic and inhibition mechanisms of these enzymes are understood in great detail, and this was helpful for the design of potent inhibitors, some of which possess clinical applications for the treatment or prevention of a multitude of diseases.[1–3]

Recently, the involvement of some CAs and their sulfonamide inhibitors in cancer has been investigated: many potent CA inhibitors (CAIs) derived from acetazolamide **AZA**, ethoxzolamide **EZA**, and sulfanilamide (**SA**) as lead molecules among others, were shown to inhibit the growth of several tumor cell lines in vitro and in vivo, constituting thus interesting candidates for developing novel antitumor therapies.[8–14] Indeed, Svastova et al.[15] showed that the acidic extracellular pH, which is a typical attribute of the tumor microenvironment, is generated by the activity of one of the tumor-associated CA isozymes, that is, CA IX, and that this acidification can be perturbed by deletion of the enzyme active site and inhibited by CA IX-selective inhibitors of the sulfonamide type (some thioureido- or pyridinium derivatives of **SA** among others have been employed in such experiments),[7,16] which bind only to hypoxic cells containing CA IX. The involvement of the other tumor-associated isozyme, that is, CA XII, in such processes has not been investigated in detail for the moment. Thus, it appears of critical importance to continue the development of CAIs targeting the tumor-associated CA isozymes CA IX and XII, eventually belonging to novel classes of compounds, less investigated up to now.[7,8] This may lead to novel possibilities for the management (imaging and treatment) of hypoxic tumors in which these enzymes are highly overexpressed.[17–19]

Boron neutron capture therapy (BNCT) is based on the preferential targeting of tumor cells with ^{10}B-containing compounds and subsequent activation with thermal neutrons to produce a highly localized radiation.[20,21] This therapy relies on a binary process in which the capture of a slow neutron by the ^{10}B nucleus leads to an energetic nuclear fission reaction, with the formation of one ^{7}Li nucleus and one $^{4}He^{2+}$ nucleus, accompanied by about 2.4 MeV of energy. The fleeting of these two nuclei travel a distance of only about the diameter of one cell, being deadly to any cell in which they have been produced.[22–24] Thus, provided that a boron-containing drug is being delivered preferentially to the tumor cells, there is a real possibility for the design of highly innovative techniques for the management of tumors. Hypoxic tumors highly overexpress CA IX[19,25] (and to some extent also CA XII),[14,15] isozymes showing high affinity for sulfonamide/sulfamate CA inhibitors.[1–8] As a consequence, the design of boron-containing CAIs with high affinity for the tumor-associated isozymes IX and XII, and eventually a lower affinity for other CA isozymes (such as the ubiquitous CA I and II) may lead to important advances in BNCT targeting hypoxic tumors, which are non-responsive to both classical chemo- and radiotherapy.[15,18]

2. Chemistry

Since it has previously been shown, mainly by this group that potent hCA I, II, and IX inhibitors can be designed possessing various zinc-binding groups, such as the sulfonamide, sulfamide, or sulfamate one,[1–4,6–8] we here report all these three types of derivatives incorporating boron-containing moieties, which, as stressed above, may be useful for BNCT applications of these compounds. The chemistry used for the design of these inhibitors is illustrated in Scheme 1.

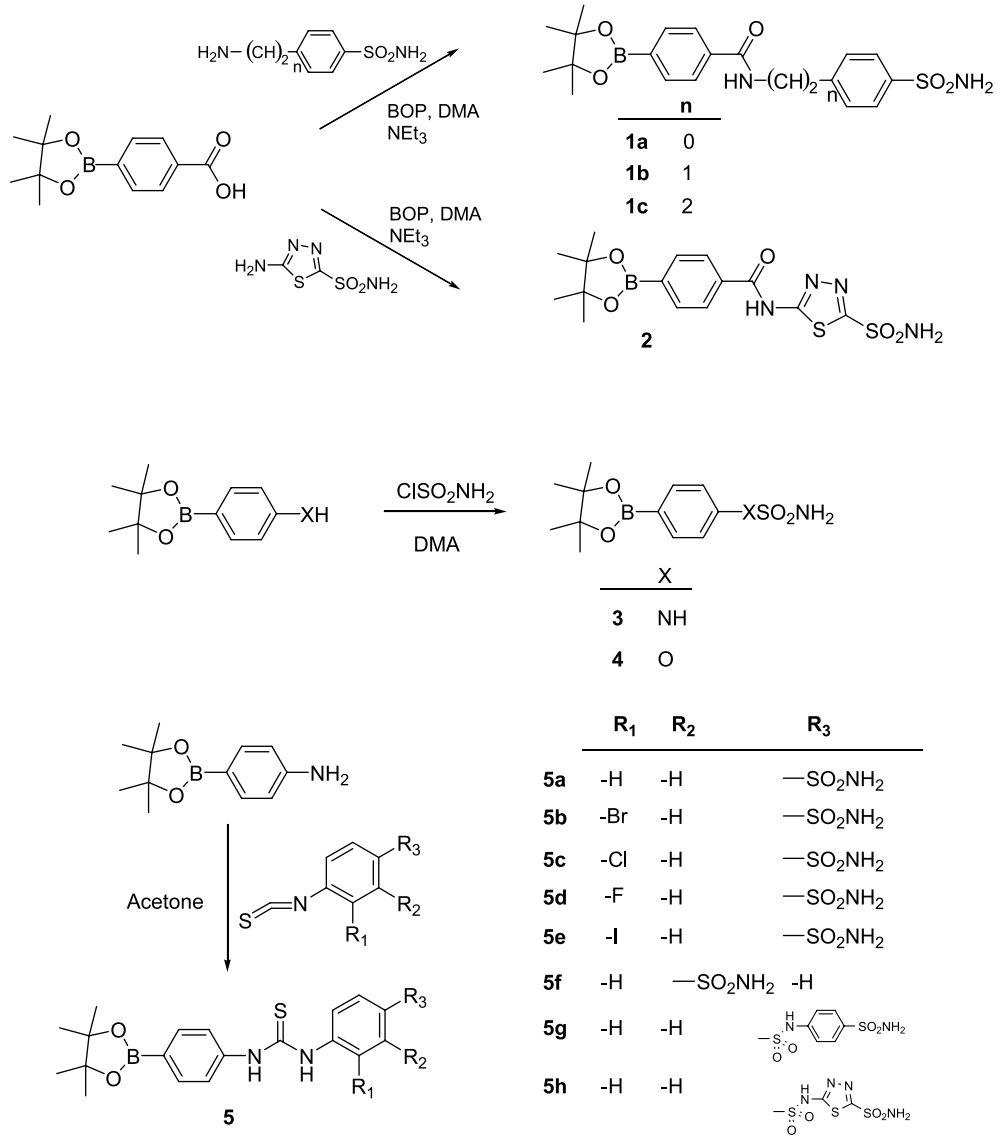
A first group of derivatives were obtained from the pinacol ester of 4-carboxy-phenylboronic acid, which were treated with aromatic or heteroaromatic amino-sulfonamides in the presence of BOP, leading to carboxamides of type **1a–c** and **2**. It has been previously shown that acylated such sulfanilamide, homosulfanilamide, 4-aminoethylbenzenesulfonamide, or 5-amino-1,3,4-thiadiazole-2-sulfonamide derivatives show enhanced hCA I, II, IV, and V inhibitory properties over the free amino sulfonamides from which they were obtained,[26,27] and this constitutes one of the rationales of performing this type of coupling reaction.

Sulfamoylation of the pinacol ester of 4-amino- or 4-hydroxy-phenylboronic acid with sulfamoyl chloride afforded the sulfamide derivative **3** and the corresponding sulfamate **4**, respectively, by the procedure already applied for the preparation of both aliphatic/aromatic sulfamide/sulfamate CAIs (Scheme 1).[5,12,13]

Finally, a third type of chemistry used for the preparation of some boron-containing CAIs, takes advantage of the coupling reactions between isothiocyanato-substituted sulfonamides and the pinacol ester of 4-amino-phenylboronic acid (Scheme 1). We have previously shown that this type of thioureido-containing sulfonamides show excellent CA I, II, IV, and IX inhibitory properties, both in the aromatic and heteroaromatic sulfonamides series.[16,28,29] In such work, we have investigated the reactions of isothiocyanato sulfonamides with diverse amino acids, some of their derivatives as well as simple amines, mainly in the search of water-soluble sulfonamides with applications as antiglaucoma drugs.[28,29] However, this type of chemistry is quite versatile and may be applied also for the design of inhibitors with applications in BNCT. Thus, reaction of the pinacol ester of 4-amino-phenylboronic acid with 4-isothiocyanato-sulfanilamide, its 3-halogenated derivatives, the isomeric metanilamide isothiocyanate as well as the sulfanilyl-sulfanilamide isothiocyanate or aminobenzolamide isothiocyanate, afforded the small library of thioureas **5a–h** (Scheme 1).[30]

3. CA inhibition data

Inhibition data against isozymes hCA I, II, and IX with compounds **1–5** as well as standard CA inhibitors are presented in Table 1.[31]



Scheme 1. Synthesis of the boron-substituted sulfonamides, sulfamides, and sulfamates **1**–**5**.

The following should be noted regarding the CA inhibitory properties of the boron-containing inhibitors **1**–**5**: (i) against the slow red blood cells isozyme hCA I, the new compounds **1**–**5** showed inhibition constants in the range of 34–94 nM, being slightly less effective than ethoxzolamide (K_I of 25 nM) but much more effective than acetazolamide (K_I of 250 nM). Sulfanilamide is on the other hand, a rather weak hCA I inhibitor (K_I of 28 μM). Except for the two 1,3,4-thiadizole-2-sulfonamide derivatives **2** and **5h**, which were the most efficient hCA I inhibitors (K_I-s in the range of 34–36 nM), all the other compounds, irrespective of whether they contained sulfonamide, sulfamide, or sulfamate zinc-binding groups showed a rather similar inhibitory efficacy against this isozyme, with inhibition constants in the range of 69–94 nM. Thus, all the substitution patterns/zinc-binding groups explored here for the boron-containing derivatives **1**–**5** lead to efficient inhibitors of hCA I, and the recent claim of Maryanoff et al.[32] that sulfamides are ineffective as CA inhibitors is refuted once again; (ii) against the physiologically relevant high activity cytosolic isozyme hCA II, the new boron-containing CAIs showed very good activity, with inhibition constants in the range of 3.1–48 nM. The most ineffective inhibitors were the sulfamide **3** and the corresponding sulfamate **4**, which are anyhow one order of magnitude better hCA II inhibitors than sulfanilamide (K_I of 300 nM). All the other CAIs, of the sulfonamide type, showed an inhibition power in the same range as that of the clinically used derivatives acetazolamide and ethoxzolamide (K_I-s of 8–12 nM). Thus, SAR is rather simple for this series of compounds since the phenylboronic acid pinacol ester tail induces excellent hCA II inhibitory properties to both aromatic as well as heteroaromatic sulfonamides incorporating them. Indeed, both the 1,3,4-thiadiazole-2-sulfonamide, sulfanilamide, homosulfanilamide, or halogenated sulfanilamide derivatives investigated here, of types **1**, **2**, and **5**, showed a compact behavior as hCA II inhibitors, with inhibition constants varying slightly, in the range of

Table 1. Inhibition of isozymes hCA I, II, and IX the boron-containing inhibitors **1**–**5** and standard sulfonamides

Inhibitor	K_I[a] (nM)		
	hCA I[b]	hCA II[b]	hCA IX[c]
AZA	250	12	25
EZA	25	8	50
SA	28,000	300	294
1a	70	5.2	53
1b	69	5.4	84
1c	70	5.1	42
2	36	3.1	7.6
3	92	48	81
4	94	32	80
5a	69	5.1	80
5b	72	5.1	89
5c	70.5	5.0	81
5d	77	5.1	10.1
5e	91	9.8	9.3
5f	70	5.5	81
5g	49	6.3	84
5h	34	5.1	7.3

[a] Errors in the range of 5–10% of the shown data from three different assays.
[b] Human recombinant isozymes.
[c] Catalytic domain of the human recombinant isozyme, CO_2 hydrase assay method.[30]

3.1–9.8 nM; (iii) the tumor-associated isozyme hCA IX was also inhibited by the boron-containing compounds **1–5**, with K_I-s in the range of 7.3–89 nM (Table 1). Several of the new compounds including **2**, **5d**, **5e**, and **5h** showed excellent hCA IX inhibitory efficacy, with inhibition constants of 7.3–10.1 nM, being much more effective as compared to the clinically used compounds acetazolamide or ethoxzolamide (K_I-s of 25–50 nM). It may be observed that these compounds incorporate (in addition to the phenylboronic acid pinacol ester group) the 1,3,4-thiadiazole-2-sulfonamide- or bromo/iodo-sulfanilamide moieties. The other derivatives investigated here were less effective as hCA IX inhibitors (K_I-s in the range of 42–89 nM) but much more effective as compared to sulfanilamide, which is a moderately weak inhibitor (K_I of around 300 nM); (iv) the three isozymes showed different affinities for this class of CAIs. Generally, hCA II showed the highest affinity, followed by hCA IX, whereas hCA I was less inhibited. However, no specificity of these compounds for a certain isozyme could be observed.

4. Conclusions

A library of boron-containing CA inhibitors, including sulfonamides, sulfamides, and sulfamates is reported in this paper. The new compounds have been synthesized by derivatization reactions of 4-carboxy-/amino-/hydroxy-phenylboronic acid pinacol esters with amino/isothiocyanato-substituted aromatic/heteroaromatic sulfonamides or by sulfamoylation reactions with sulfamoyl chloride. The new derivatives have been assayed for the inhibition of three physiologically relevant CA isozymes, the cytosolic CA I and II, and the transmembrane, tumor-associated isozyme CA IX. Effective inhibitors were detected both among sulfonamides, sulfamates, and sulfamides. Against the human isozyme hCA I the new compounds showed inhibition constants in the range of 34–94 nM, against hCA II in the range of 3.1–48 nM, and against hCA IX in the range of 7.3–89 nM, respectively. As hypoxic tumors highly overexpress CA IX, the design of boron-containing inhibitors with high affinity for the tumor-associated CA isozymes may lead to important advances in boron neutron capture therapy applications targeting hypoxic tumors, which are non-responsive to both classical chemo- and radiotherapy

Acknowledgments

This research was financed in part by a grant of the 6th Framework Programme of the European Union (EUROXY project).

References and notes

1. *Carbonic Anhydrase—Its Inhibitors and Activators*; Supuran, C. T., Scozzafava, A., Conway, J., Eds.; CRC: Boca Raton, London, New York, 2004.
2. Pastorekova, S.; Parkkila, S.; Pastorek, J.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2004**, *19*, 199–229.
3. Supuran, C. T.; Scozzafava, A.; Casini, A. *Med. Res. Rev.* **2003**, *23*, 146–189.
4. Scozzafava, A.; Owa, T.; Mastrolorenzo, A.; Supuran, C. T. *Curr. Med. Chem.* **2003**, *10*, 925–953.
5. Casini, A.; Winum, J. Y.; Montero, J. L.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 837–840.
6. Winum, J. Y.; Pastorekova, S.; Jakubickova, L.; Montero, J. L.; Scozzafava, A.; Pastorek, J.; Vullo, D.; Innocenti, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 579–584.
7. Winum, J. Y.; Scozzafava, A.; Montero, J. L.; Supuran, C. T. *Med. Res. Rev.* **2005**, *25*, 186–228.
8. Casini, A.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, L. T. *Curr. Cancer Drug Targets* **2002**, *2*, 55–75.
9. Abbate, F.; Casini, A.; Owa, T.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 217–223.
10. Pastorekova, S.; Casini, A.; Scozzafava, A.; Vullo, D.; Pastorek, J.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 869–873.
11. Supuran, C. T.; Briganti, F.; Tilli, S.; Chegwidden, W. R.; Scozzafava, A. *Bioorg. Med. Chem.* **2001**, *9*, 703–714.
12. Winum, J. Y.; Vullo, D.; Casini, A.; Montero, J. L.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2003**, *46*, 5471–5477.
13. Winum, J. Y.; Vullo, D.; Casini, A.; Montero, J. L.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2003**, *46*, 2197–2204.
14. Vullo, D.; Innocenti, A.; Nishimori, I.; Pastorek, J.; Scozzafava, A.; Pastorekova, S.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 963–969.
15. Svastova, E.; Hulikova, A.; Rafajova, M.; Zat'ovicova, M.; Gibadulinova, A.; Casini, A.; Cecchi, A.; Scozzafava, A.; Supuran, C. T.; Pastorek, J.; Pastorekova, S. *FEBS Lett.* **2004**, *577*, 439–445.
16. Cecchi, A.; Winum, J. Y.; Innocenti, A.; Vullo, D.; Montero, J. L.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5775–5780.

17. Watson, P. H.; Chia, S. K.; Wykoff, C. C.; Han, C.; Leek, R. D.; Sly, W. S.; Gatter, K. C.; Ratcliffe, P.; Harris, A. L. *Br. J. Cancer* **2003**, *88*, 1065–1070.
18. Potter, C.; Harris, A. L. *Cell Cycle* **2004**, *3*, 164–167.
19. Robertson, N.; Potter, C.; Harris, A. L. *Cancer Res.* **2004**, *64*, 6160–6165.
20. Coderre, J. A.; Turcotte, J. C.; Riley, K. J.; Binns, P. J.; Harling, O. K.; Kiger, W. S., 3rd *Technol. Cancer Res. Treat.* **2003**, *2*, 355–375.
21. Yang, W.; Gao, X.; Wang, B. *Med. Res. Rev.* **2003**, *23*, 346–368.
22. Hawthorne, M. F. *Mol. Med. Today* **1998**, *4*, 174–181.
23. Hawthorne, M. F.; Lee, M. W. *J. Neurooncol.* **2003**, *62*, 33–45.
24. Lu, D. R.; Mehta, S. C.; Chen, W. *Adv. Drug Delivery Rev.* **1997**, *26*, 231–247.
25. Kivela, A. J.; Parkkila, S.; Saarnio, J.; Karttunen, T. J.; Kivela, J.; Parkkila, A. K.; Pastorekova, S.; Pastorek, J.; Waheed, A.; Sly, W. S.; Rajaniemi, H. *Histochem. Cell. Biol.* **2000**, *114*, 197–204.
26. Vullo, D.; Franchi, M.; Gallori, E.; Antel, J.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2004**, *47*, 1272–1279.
27. Vullo, D.; Scozzafava, A.; Pastorekova, S.; Pastorek, J.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2351–2356.
28. Scozzafava, A.; Supuran, C. T. *J. Enzyme Inhib.* **1998**, *13*, 103–123.
29. Casini, A.; Scozzafava, A.; Mincione, F.; Menabuoni, L.; Ilies, M. A.; Supuran, C. T. *J. Med. Chem.* **2000**, *43*, 4884–4892.
30. *Synthesis of compounds* **1** *and* **2**: General procedure: 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid (1 equiv) was dissolved in dimethylacetamide (DMA) in the presence of the amino-aryl sulfonamide (1 equiv), BOP (1 equiv) and triethylamine (3 equiv). The mixture was stirred at room temperature for one night, then dissolved in ethyl acetate. The organic phase was washed several times with water, dried over anhydrous sodium sulfate, and then concentrated under vacuum. The residue was triturated with diethyl ether, then filtrated to give the expected compound as white powder.
Compound **1a**: ^{1}H NMR (DMSO d^6, 250 MHz) δ: 10.65 (s, 1H), 8 (m, 4H), 7.85 (m, 4H), 7.3 (s, 2H), 1.35 (s, 12H). MS ESI$^+$ *m/z*: 403 $[M+H]^+$, 425 $[M+Na]^+$. MS ESI$^-$ *m/z*: 401 $[M-H]^-$.
Synthesis of compounds **3** *and* **4**: these compounds were prepared by direct sulfamoylation of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol, respectively, as described previously.[12,13]
Compound **3**: ^{1}H NMR (DMSO d^6, 250 MHz) δ: 9.8 (s, 1H), 7.6 (d, 2H, J = 7.7 Hz), 7.2 (s, 2H), 7.15 (d, 2H, J = 7.7 Hz), 1.3 (s, 12H). MS ESI$^+$ *m/z*: 321 $[M+Na]^+$, 619 $[2M+Na]^+$. MS ESI$^-$ *m/z*: 297 $[M-H]^-$.
Compound **4**: ^{1}H NMR (DMSO d^6, 250 MHz) δ: 8.1 (s, 2H), 7.7 (d, 2H, J = 8.2 Hz), 7.3 (d, 2H, J = 8.2 Hz), 1.3 (s, 12H). MS ESI$^+$ *m/z*: 322 $[M+Na]^+$. MS ESI$^-$ *m/z*: 298 $[M-H]^-$.
Synthesis of compounds **5**: General procedure: 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (1 equiv) and the isothiocyanate derivative of the sulfonamide (1 equiv) were dissolved in dry acetone and stirred for one night at room temperature. The mixture was then concentrated, and the residue triturated in diethyl ether, then filtrated to give the expected compound as white powder.
Compound ***5a***: ^{1}H NMR (DMSO d^6, 250 MHz) δ: 10.2 (s, 2H), 7.7 (m, 8H), 7.3 (s, 2H), 1.35 (s, 12H). MS ESI$^+$ *m/z*: 456 $[M+Na]^+$, 889 $[2M+Na]^+$. MS ESI$^-$ *m/z*: 432 $[M-H]^-$, 865 $[2M-H]^-$.
31. Khalifah, R. G. *J. Biol. Chem.* **1971**, *246*, 2561–2573, An SX.18MV-R Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed 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 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 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 0.01 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,[16,26] and represent the mean from at least three different determinations. The cloned enzymes were obtained as reported earlier by this group.[12,13]
32. Maryanoff, B. E.; McComsey, D. F.; Costanzo, M. J.; Hochman, C.; Smith-Swintowsky, V.; Shank, R. P. *J. Med. Chem.* **2005**, *48*, 1941–1947.