Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 14 (2004) 3757–3762

Carbonic anhydrase inhibitors. Inhibition of cytosolic isozyme XIII with aromatic and heterocyclic sulfonamides: a novel target for the drug design

Jonna M. Lehtonen,[a] Seppo Parkkila,[a,b] Daniela Vullo,[c] Angela Casini,[c] Andrea Scozzafava[c] and Claudiu T. Supuran[c,*]

[a]*Institute of Medical Technology, University of Tampere, and Tampere University Hospital, Biokatu 6, 33520 Tampere, Finland*
[b]*Department of Clinical Chemistry, University of Oulu, Kajaanintie 50, 90220 Oulu, Finland*
[c]*Università degli Studi di Firenze, Laboratorio di Chimica Bioinorganica, Rm. 188, Via della Lastruccia 3, I-50019 Sesto Fiorentino (Firenze), Italy*

Received 19 February 2004; revised 26 April 2004; accepted 29 April 2004
Available online 4 June 2004

Abstract—The inhibition of the newly discovered cytosolic carbonic anhydrase isozyme XIII (CA XIII) has been investigated with a series of aromatic and heterocyclic sulfonamides, including some of the clinically used derivatives, such as acetazolamide, methazolamide, dichlorophenamide, dorzolamide, and valdecoxib. Inhibition data for the physiologically relevant isozymes I and II (cytosolic forms) and the tumor associated isozyme IX (transmembrane) were also provided for comparison. A very interesting and unusual inhibition profile against CA XIII with these sulfonamides has been observed. The clinically used compounds (except valdecoxib, which was a weak CA XIII inhibitor) potently inhibit CA XIII, with K_i's in the range of 17–23 nM, whereas sulfanilamide, halogenated sulfanilamides, homosulfanilamide, 4-aminoethylbenzenesulfonamide, and orthanilamide were slightly less effective, with K_i's in the range of 32–56 nM. Several low nanomolar (K_i's in the range of 1.3–2.4 nM) CA XIII inhibitors have also been detected, all of them belonging to the sulfanilyl–sulfonamide type of inhibitors, of which aminobenzolamide is the best known representative. Because CA XIII is an active isozyme predominantly expressed in salivary glands, kidney, brain, lung, gut, uterus, and testis, where it probably plays an important role in pH regulation, its inhibition by sulfonamides may lead to novel therapeutic applications for this class of pharmacological agents.

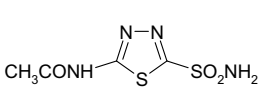
1. Introduction

The metallo-protein carbonic anhydrase (CA, EC 4.2.1.1) is one of the most widely spread biological catalysts all over the phylogenetic tree, with five distinct classes of such enzymes presently known:[1–5] the α-CAs (present in vertebrates, bacteria, algae, and cytoplasm of green plants), the β-CAs (predominantly in bacteria, algae, and chloroplasts of both mono- as well as dicotyledons), the γ-CAs (in Archaea and some bacteria), the δ-CAs, isolated in some marine diatoms[6,7] and the ε-CAs, recently found in cyanobacteria and some chemolithoautotrophic bacteria.[8] These enzymes catalyze the reversible hydration of carbon dioxide to bicarbonate, handling in this way one of the simplest and most important biomolecules (CO_2), generated in many physiological processes.[1–5] Many of the presently known CAs possess other catalytic activities (such as esterase, hydrolase, etc.) but their significance from the physiologic/pathologic point of view is unknown.[9,10] An increasing number of such enzymes are constantly being discovered both in higher vertebrates, humans included, as well as in many other organisms, such as archaea, bacteria, protozoa, plants, etc.[1–10] Modulating the activity of these enzymes via specific inhibitors or activators, as well as the study of their gene expression in different pathologies, allows for novel therapeutic approaches in many diseases.[9,10] Four sulfonamide CA inhibitors (CAIs), acetazolamide **AAZ**, methazolamide **MZA**, ethoxzolamide **EZA**, and dichlorophenamide **DCP**, have been used for more than 45 years as systemic CAIs, whereas two additional drugs dorzolamide **DZA** (clinically launched in 1995) and the structurally-related brinzolamide **BRZ** (used since 1999) are topically acting

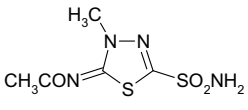
* Corresponding author. Tel.: +39-055-457-3005; fax: +39-055-457-3385; e-mail: claudiu.supuran@unifi.it

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.04.106

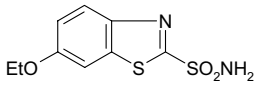
antiglaucoma agents.[1–5] Valdecoxib **1**, a COX-2 selective sulfonamide inhibitor, has also recently been shown to act as potent CAI, together with other clinically used such agents, for example, celecoxib.[11]



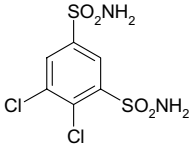
AAZ



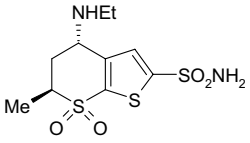
MZA



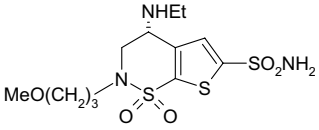
EZA



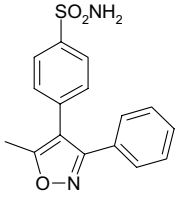
DCP



DZA



BRZ



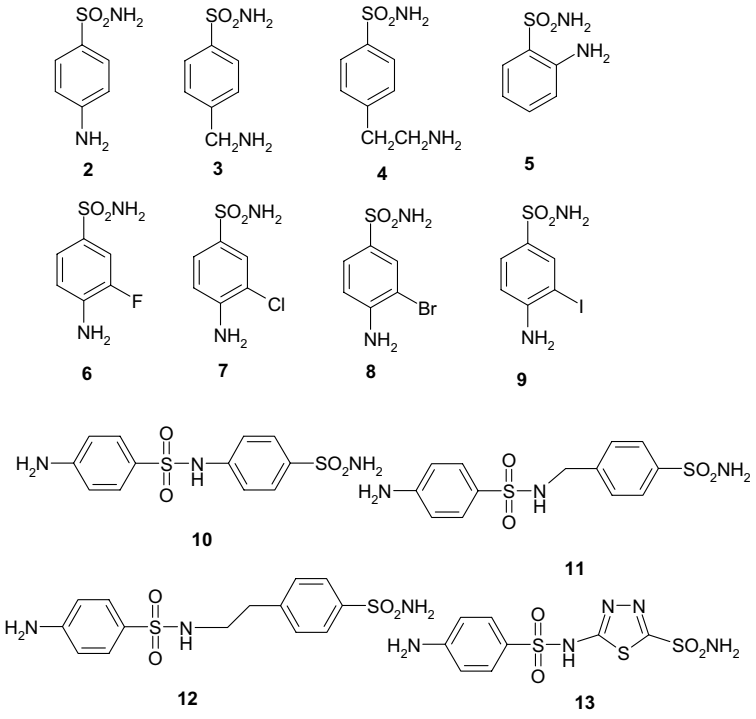
1: Valdecoxib

Isozyme CA XIII has only recently been purified and characterized in detail,[12] being demonstrated that this cytosolic isoform shows catalytic activity similar to that of the mitochondrial isozyme V and the cytosolic isozyme I, with k_{cat}/K_M of $4.3\times10^7\ M^{-1}\ s^{-1}$, and k_{cat} of $8.3\times10^4\ s^{-1}$, being also very susceptible to inhibition by acetazolamide and cyanate (an anionic inhibitor).[12] Immunohistochemical and PCR data indicated that the distribution of CA XIII is clearly unique when compared to the other cytosolic CA isozymes (mainly CA I and II).[12] The most distinct differences between CA XIII and II (the major cytosolic isoform)[1–5] were observed in the human testis and uterus, organs in which pH and ion balance has to be tightly regulated to ensure normal fertilization.[12] CA XIII was found to be expressed in all stages of developing human sperm cells. In contrast, CA II was confined to the mature sperm cells as shown earlier by some of us.[13] The bicarbonate present in the ejaculate has been proposed to maintain the sperm motility until the cells enter the lumen of the uterus through the cervical canal. In the female genital tract, the endometrial and oviductal epithelium produce an alkaline environment for maintaining the sperm motility. In the uterine endometrium and cervical glands, the presence of CA XIII could thus explain the early histochemical and biochemical results showing CA activity in the epithelial cells that was not due to CA I or II.[13] Thus, it has been hypothesized that CA XIII is a key factor contributing to the appropriate bicarbonate concentration in the cervical and endometrial mucus needed for normal fertilization processes.[12,13] Keeping such findings in mind it would be interesting to detect either CA XIII-specific inhibitors or CA XIII deficient animal models to determine the role of this novel enzyme in spermatogenesis and fertilization capacity. Based on the distribution of CA XIII, other major target tissues in physiological studies should include at least the brain, salivary glands, intestine, kidney, and lymphoid organs.[12] Being such a widely expressed isozyme, CA XIII could compensate other CAs, and thus, needs to be considered when any CA-deficient animal model is tested in phenotypic analyses, or when inhibitors are clinically used in the treatment and prevention of diverse disorders.[1–5,12] It is also notable that positive signals for CA13 gene expression were detected in samples obtained from mouse embryos.[12] This finding suggests that CA XIII may play a role in embryogenesis, and perturbation of its function by genetic modification could potentially lead to developmental abnormalities.[12,13]

Here we report the first detailed CA XIII inhibition study, as in the previous work form our laboratories[12] only acetazolamide and cyanate have been used as inhibitors. The detection of potent inhibitors targeting this widely spread isozyme may lead to future applications of pharmacological agents belonging to the CAIs class.

2. Chemistry

Sulfonamides investigated for the inhibition of the new isozyme CA XIII, of types **1**–**13**, as well as the clinically used inhibitors **AZA**, **MZA**, **DCP**, and **DZA** are shown below. Compounds **1**–**5**, **AZA**, **MZA**, **DCP**, and **DZA** are commercially available, whereas **6**–**9**,[14] **10**–**12**,[15] and **13**[16] were prepared as reported earlier.



3. CA inhibition data

Inhibition data against four CA isozymes, that is, CA I, II, IX, and XIII,[17–19] with the above mentioned compounds **1**–**13** and four clinically used inhibitors, are shown in Table 1.

Table 1. CA I, II, IX, and XIII inhibition data with sulfonamides **1**–**13** and clinically used inhibitors, acetazolamide (**AAZ**), methazolamide (**MZA**), dichlorophenamide (**DCP**), and dorzolamide (**DZA**)

Inhibitor	K_I* (nM)			
	hAC I[a]	hCA II[a]	hCA IX[b]	mCA XIII[c]
AAZ	250	12	25	17
MZA	50	14	27	19
DCP	1200	38	50	23
DZA	50,000	9.0	52	18
1	54,000	43	27	425
2	28,000	300	294	32
3	25,000	170	103	41
4	21,000	160	33	43
5	45,400	295	33	43
6	8300	60	245	50
7	9800	110	264	56
8	6500	40	269	54
9	6000	70	285	50
10	164	46	34	2.4
11	109	33	31	1.9
12	95	30	24	1.3
13	6.0	2.0	38	2.0

* Errors in the range of 5–10% of the shown data, from three different assays. Data for hCA I, II, and IX are from Refs. 11,22.

[a] Human cloned isozymes, esterase assay method.[19]

[b] Catalytic domain of the human cloned isozyme, CO_2 hydrase assay method.[18]

[c] Murine cloned isozyme, CO_2 hydrase assay method.[18]

The following may be noted regarding mCA XIII inhibition with the sulfonamides investigated here (inhibition data against isozymes I, II, and IX have been published earlier, and will be not discussed in detail here): (i) all these sulfonamides, except the COX-2 selective inhibitor valdecoxib **1**, behave as potent CA XIII inhibitors, with inhibition constants in the range of 1.3–56 nM. Valdecoxib is a weak mCA XIII inhibitor, with an inhibition constant of 0.425 μM, whereas it behaves as a much more potent inhibitor of isozymes hCA IX and II. Valdecoxib on the other hand is a very weak hCA I inhibitor (K_I of 54 μM); (ii) a group of simple aromatic sulfonamides, of type **2**–**9**, including sulfanilamide and halogenated sulfanilamides, homosulfanilamide, 4-aminoethyl-benzenesulfonamide and orthanilamide, showed an appreciable inhibition of mCA XIII, with K_i's in the range of 32–56 nM, whereas generally, these compounds behaved as weaker inhibitors of the other investigated isozymes, I, II, and IX (the exceptions are constituted by compounds **4** and **5**, which act as potent hCA IX inhibitors, being more effective in inhibiting this tumor-associated isozyme as compared to the cytosolic isozyme XIII). For example sulfanilamide **2** was almost 10 times more potent CA XIII inhibitor as compared to its affinity for isozymes II and IX (inhibition constants around 300 nM), whereas against CA I this isozyme behaved as a really weak inhibitor (K_i of 28 μM). Thus, the next idea was to test sulfanilamide-based CAIs, and indeed, the most potent CA XIII inhibitors detected were derivatives of this lead molecule (see later in the text); (iii) the clinically used sulfonamides including acetazolamide, methazolamide, dichlorophenamide, and dorzolamide demonstrated potent CA XIII inhibition, with K_i's in the range of 17–23 nM. The affinity of mCA XIII for these inhibitors is rather similar to that of the major cytosolic isozyme, hCA II, whereas for the tumor-associated isozyme IX

these compounds acted as slightly less effective inhibitors. The most resistant isozyme to inhibition by these sulfonamides was hCA I (K_i's in the range of 50–50,000 nM); (iv) the sulfanilyl-derived compounds **10**–**13**, obtained by reaction of aminosulfonamides with 4-acetamido-benzenesulfonyl chloride (followed by deprotection of the amino group),[15,16] showed very potent CA XIII inhibitory properties, with K_i's in the range of 1.3–2.4 nM, whereas these compounds (except aminobenzolamide **13**) were generally much less effective as inhibitors of isozymes I, II, and IX. Indeed, for example the selectivity ratio (defined as the ratio of the inhibition constant against two different isozymes) of **12** (the best CA XIII inhibitor detected up to now) was of 23.0 against isozyme II, of 18.4 against isozyme IX, and of 87 against isozyme I. It may be thus assumed that this is a rather selective CA XIII inhibitor, considering the fact that it has 23 times higher affinity for this isozyme than for CA II, and 87 higher affinity for CA XIII than for CA I, and a 18 times higher affinity for isozyme XIII than for isozyme IX. It should also be noted that there are relatively small differences of activity of these four derivatives (**10**–**13**) as CA XIII inhibitors, proving that the scaffold of the sulfanilyl–sulfonamide type is a very effective one in generating potent and selective CA XIII inhibitors, although the affinity of these sulfonamides against other isozymes varied in a much wider range (Table 1).

In order to try to explain our data, one must consider the amino acid residues present in the CA XIII active site, as the X-ray crystal structure of this isozyme is not available yet (CA XIII has about 60% sequence identity with the other cytosolic isozymes, such as CA I, II, and III) (Fig. 1).[12] One of the most important characteristics of human and murine CA XIII is the presence of a valine residue in position 200, instead of the Thr one present in all other human isozymes except CA I, which has a His 200.[12] It is well-known that this residue is highly important for the binding of inhibitors and probably also for the binding of the substrates.[20,21] Considering the high difference of activity between hCA I (a low activity isozyme) and hCA II (a very high activity isozyme)[1–5,12] one should have expected that the Val 200 present in CA XIII should lead to a CA I—type of isozyme, with a low catalytic activity and a rather low affinity for sulfonamide inhibitors (but high affinity for anion inhibitors).[12] Data of Table 1 show that this guess is only partly true: indeed, CA XIII has a moderate catalytic activity, similar to that of the mitochondrial isozyme V and the cytosolic isozyme I, with k_{cat}/K_M of $4.3\times10^7\,M^{-1}\,s^{-1}$, and k_{cat} of $8.3\times10^4\,s^{-1}$ at 25 °C and pH 7.5,[12] but unexpectedly, the affinity for sulfonamide inhibitors is very high, with inhibition constants in the very low nanomolar range for many derivatives. Thus, among isozymes I, II, IX, and XIII included in our study, only hCA II has in some cases a higher affinity for some sulfonamide inhibitors (such as for example **AZA**, **MZA**, **DZA**, **1**, **13**) as compared to mCA XIII, whereas hCA I is much less sensitive to this class of inhibitors. From our data, it is clear that CA XIII is indeed a target for many sulfonamide inhibitors, both among the clinically used derivatives, as well as the compounds originally developed as antiglaucoma[1–5] or antitumor sulfonamides.[22–24]

Thus, this isozyme shows a catalytic activity similar to that of CA V and CA I, and high affinities for both sulfonamide inhibitors (similar to that of CA II and CA IX) and anion inhibitors (similar to that of CA I).[12] We presume that the main feature responsible for this particular behavior is the presence of Val 200, unique to this isozyme, while all other isozymes known to date possess Thr 200 (CA I is an exception, possessing His 200). It should be mentioned that Val is more bulky than Thr, but not as bulky as His, and this may constitute part of the explanation regarding this unexpected behavior of CA XIII toward the sulfonamide inhibitors investigated here.

4. Conclusion

We report here the first detailed inhibition study of the newly isolated cytosolic isozyme CA XIII, predominantly expressed in salivary glands, kidney, brain, lung, gut, uterus, and testis, where it probably plays an important role in pH regulation. This enzyme has an unexpectedly high affinity for most sulfonamide inhibitors of the aromatic or heterocyclic type, whereas only the COX-2 selective inhibitor valdecoxib behaved as a weak CA XIII inhibitor. The clinically used compounds acetazolamide, methazolamide, dichlorophenamide and dorzolamide potently inhibited CA XIII, with K_i's in the range of 17–23 nM, whereas sulfanilamide, halogenated sulfanilamides, homosulfanilamide, 4-aminoethyl-benzenesulfonamide, and orthanilamide were slightly less effective, with K_i's in the range of 32–56 nM. Several low nanomolar (K_i's in the range of 1.3–2.4 nM) CA XIII

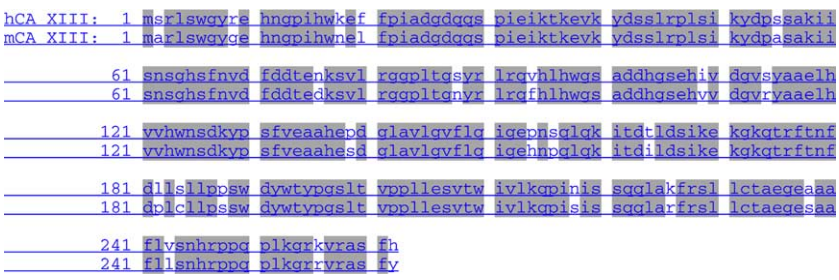


Figure 1. Amino acid sequences of human and murine CA XIII enzymes obtained from databases (hCA XIII; accession number NP_940986 and mCA XIII; accession number AAK16672). The conserved residues are shaded.

inhibitors have also been detected, all of them belonging to the sulfanilyl–sulfonamide type of inhibitors, of which aminobenzolamide is the best known representative. Targeting of CA XIII by selective inhibitors may lead to novel therapeutic applications for this class of pharmacological agents.

Acknowledgements

This research was financed in part by a grant from the Italian CNR—target project Biotechnology (to A.S. and C.T.S.), and by grants from Sigrid Juselius Foundation and Academy of Finland (to S.P.).

References and notes

1. Supuran, C. T.; Vullo, D.; Manole, G.; Casini, A.; Scozzafava, A. *Curr. Med. Chem.—Cardiovasc. Hematol. Agents* **2004**, *2*, 49–68.
2. Supuran, C. T.; Scozzafava, A.; Casini, A. *Med. Res. Rev.* **2003**, *23*, 146–189.
3. Supuran, C. T.; Scozzafava, A. *Expert Opin. Ther. Patents* **2002**, *12*, 217–242.
4. Supuran, C. T.; Scozzafava, A. *Curr. Med. Chem.-Imm.; Endoc. Metab. Agents* **2001**, *1*, 61–97.
5. Supuran, C. T.; Scozzafava, A. *Expert Opin. Ther. Pat.* **2000**, *10*, 575–600.
6. Cox, E. H.; McLendon, G. L.; Morel, F. M.; Lane, T. W.; Prince, R. C.; Pickering, I. J.; Gorge, G. N. *Biochemistry* **2000**, *39*, 12128–12130.
7. Lane, T. W.; Morel, F. M. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 4627–4631.
8. So, A. K.; Espie, G. S.; Williams, E. B.; Shively, J. M.; Heinhorst, S.; Cannon, G. C. *J. Bacteriol.* **2004**, *186*, 623–630.
9. *Carbonic anhydrase—Its inhibitors and activators*; Supuran, C. T., Scozzafava, A., Conway, J., Eds.; CRC: Boca Raton (FL), USA, 2004; pp 1–376, and references cited therein.
10. Pastorekova, S.; Parkkila, S.; Pastorek, J.; Supuran, C. T. *J. Enzym. Inhib. Med. Chem.*, **2004**, *19*, in press.
11. Weber, A.; Casini, A.; Heine, A.; Kuhn, D.; Supuran, C. T.; Scozzafava, A.; Klebe, G. *J. Med. Chem.* **2004**, *47*, 550–557.
12. Lehtonen, J.; Shen, B.; Vihinen, M.; Casini, A.; Scozzafava, A.; Supuran, C. T.; Parkkila, A. K.; Saarnio, J.; Kivela, A. J.; Waheed, A.; Sly, W. S.; Parkkila, S. *J. Biol. Chem.* **2004**, *279*, 2719–2727.
13. Parkkila, S.; Kaunisto, K.; Kellokumpu, S.; Rajaniemi, H. *Histochemistry* **1991**, *95*, 477–482.
14. Ilies, M. A.; Vullo, D.; Pastorek, J.; Scozzafava, A.; Ilies, M.; Caproiu, M. T.; Pastorekova, S.; Supuran, C. T. *J. Med. Chem.* **2003**, *46*, 2187–2196.
15. Clare, B. W.; Supuran, C. T. *Eur. J. Med. Chem.* **1999**, *34*, 463–474.
16. (a) Supuran, C. T.; Ilies, M. A.; Scozzafava, A. *Eur. J. Med. Chem.* **1998**, *33*, 739–752; (b) Supuran, C. T.; Clare, B. W. *Eur. J. Med. Chem.* **1999**, *34*, 41–50.
17. The expression of human and mouse CA XIII mRNAs were examined using cDNA kits purchased from BD Biosciences (Palo Alto, CA). The cDNAs included in MTC™ panels were used as templates for polymerase chain reaction (PCR) using CA XIII gene-specific primers. The human MTC™ panel II and mouse MTC™ panel I (BD Biosciences) contained first strand cDNA preparations produced from total poly(A) RNAs isolated from a number of different tissues. The primers were produced by Sigma Genosys. To amplify mouse CA13 cDNA, three primers (Sigma Genosys) were chosen based on the mouse CA13 sequence published in GenBank™ (AF231123); forward (F1) 5′-GTCCCTGCCACAGGCTCT-3′ (nucleotides 3–20) and reverse (R1) 5′-TGCACAA-GAGGCTTCGGA-3′ (nucleotides 712–729), which generated a 730-bp product. Forward primer F1 together with reverse primer (R2) 5′-ATACATTGGGG-CAAATCT-3′ (nucleotides 993–1010) generated a full-length 1008-bp amplification product. The PCR amplification for mouse CA13 with primer F1 and R1 was performed using a two-step method recommended by the manufacturer. The PCR cycling parameters consisted of denaturation at 94 °C for 2 min, followed by 33 cycles of denaturation at 94 °C for 30 s and extension and annealing at 68 °C for 2 min, followed by final extension at 68 °C for 3 min. The PCR amplifications for mouse spleen and 17-days-old embryo with primers F1 and R2 were also performed using a three-step method consisting cycling parameters of denaturation at 94 °C for 2 min, followed by 33 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min 30 s, followed by final extension at 72 °C for 10 min. The PCR products were analyzed by electrophoresis on 1,2% agarose gel containing 0.1 μg/mL ethidium bromide with DNA standard (100 bp DNA Ladder, New England Biolabs, Beverly, MA). *E. coli* TOP 10 strain transformed with pTrcHis plasmid containing mCA XIII cDNA was grown at 37 °C in 50 mL LB medium containing 50 μg/mL ampicillin and 0,5% glucose to an O.D of 0.6 at 600 nM. The inducer, IPTG, was then added to a final concentration of 1 mM and then the culture was incubated at 37 °C. The cells were harvested after 5 h by centrifugation and the pellet was frozen at −20 °C for later use. For large scale expression the bacteria were grown in 1 L of LB medium. The bacterial cell pellet was resuspended in 160 mL of lysing buffer (50 mM Na_2HPO_4, 0,5 M NaCl, pH 8.0) and 160 mg lysozyme (Sigma) was added. The resuspended cells were incubated on ice 30 min and lysed by pipetting. Bacterial cell components were removed by centrifugation (13,000 rpm, 15 min, +4 °C). The supernatant was directly applied onto either Invitrogen's ProBond™*sans*-purification system or conventional CA-inhibitor affinity chromatography purification. The ProBond™*sans*-purification was performed under native conditions according to Invitrogen's protocol using the ProBond™*sans*-resin. The inhibitor affinity chromatography was performed using the carboxymethyl (CM) Bio-Gel A (Bio-Rad Laboratories, Richmond, CA) coupled to *p*-aminomethyl-benzenesulfonamide.[18] The gel was washed using 10 mM HEPES buffer (pH 7.5) containing 150 mM NaCl and the bound enzyme was eluted using 0.1 M sodium acetate, pH 5.6, containing 0.5 M sodium perchlorate. The purified His-tagged CA XIII was analyzed by two methods: colloidal Coomassie blue protein stain (Invitrogen) and western blotting. The His-tagged and untagged mCA XIII showed practically identical catalytic activity and affinity for sulfonamide inhibitors (data not shown).
18. Khalifah, R. G. *J. Biol. Chem.* **1971**, *246*, 2561–2573. An SX.18MV-R Applied Photophysics stopped-flow instrument has been used. 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. Saturated CO_2 solutions in water at 20 °C were used as substrate. Stock solutions of inhibitors were prepared at a concentration of 1–3 mM (in DMSO–water 1:1, v/v) and dilutions up to 0.1 nM done with the assay buffer mentioned above. Inhibitor and enzyme solutions were preincubated together for 10 min at room temperature prior to assay, in order to allow for the formation of the E–I complex. Triplicate experiments were done for each inhibitor concentration, and the values reported throughout the paper are the mean of such results.

19. A stopped flow variant of the Pocker and Stone spectrophotometric method, Pocker, Y.; Stone, J. T. *Biochemistry* **1967**, *6*, 668–678, has been employed, using an SX.18MV-R Applied Photophysics stopped flow instrument, as described previously.[14]
20. (a) Ferraroni, M.; Briganti, F.; Chegwidden, W. R.; Supuran, C. T.; Wiebauer, K. E.; Tashian, R. E.; Scozzafava, A. *Biochemistry* **2002**, *41*, 6237–6244; (b) Ferraroni, M.; Briganti, F.; Chegwidden, W. R.; Supuran, C. T.; Scozzafava, A. *Inorg. Chim. Acta* **2002**, *339*, 135–144.
21. Behravan, G.; Jonsson, B. H.; Lindskog, S. *Eur. J. Biochem.* **1991**, *195*, 393–396.
22. Vullo, D.; Franchi, M.; Gallori, E.; Pastorek, J.; Scozzafava, A.; Pastorekova, S.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1005–1009.
23. Franchi, M.; Vullo, D.; Gallori, E.; Pastorek, J.; Russo, A.; Scozzafava, A.; Pastorekova, S.; Supuran, C. T. *J. Enzym. Inhib. Med. Chem.* **2003**, *18*, 333–338.
24. Pastorekova, S.; Casini, A.; Scozzafava, A.; Vullo, D.; Pastorek, J.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 869–873.