Bioorganic & Medicinal Chemistry Letters 21 (2011) 1334–1337

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

(*R*)-/(*S*)-10-Camphorsulfonyl-substituted aromatic/heterocyclic sulfonamides selectively inhibit mitochondrial over cytosolic carbonic anhydrases

Alfonso Maresca, Claudiu T. Supuran *

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

ARTICLE INFO

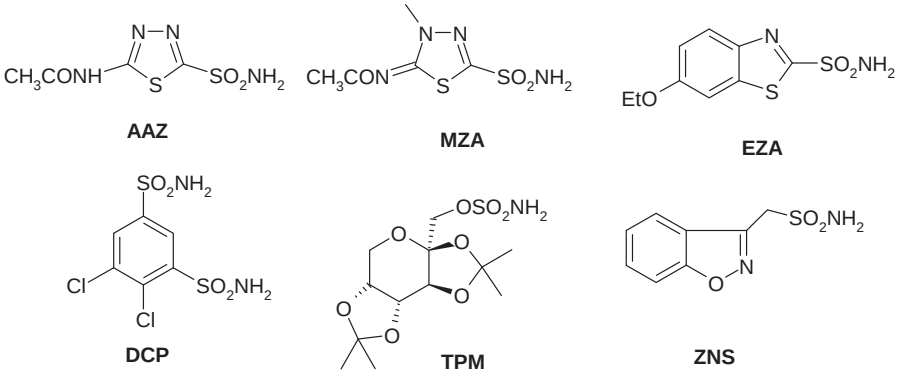
Article history:
Received 1 December 2010
Revised 11 January 2011
Accepted 13 January 2011
Available online 18 January 2011

Keywords:
Carbonic anhydrase
Mitochondrial isoforms
Sulfonamide
Camphorsulfonyl
Obesity
Isoform-selective enzyme inhibitor

ABSTRACT

A series of aromatic and heterocyclic sulfonamides incorporating *R*- and *S*-camphorsulfonyl moieties were synthesized and investigated for the inhibition of several mammalian isoforms of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1). The new sulfonamides selectively inhibited the mitochondrial isozymes hCA VA and VB (*h* = human isoform) over the cytosolic, off-target ones hCA I and II, with inhibition constants in the low nanomolar range. The chirality and position of the groups substituting the sulfonamide scaffold greatly influenced CA inhibitory properties. These compounds are excellent leads for designing isoform-selective enzyme inhibitors targeting mitochondrial CAs involved in lipogenesis and obesity.

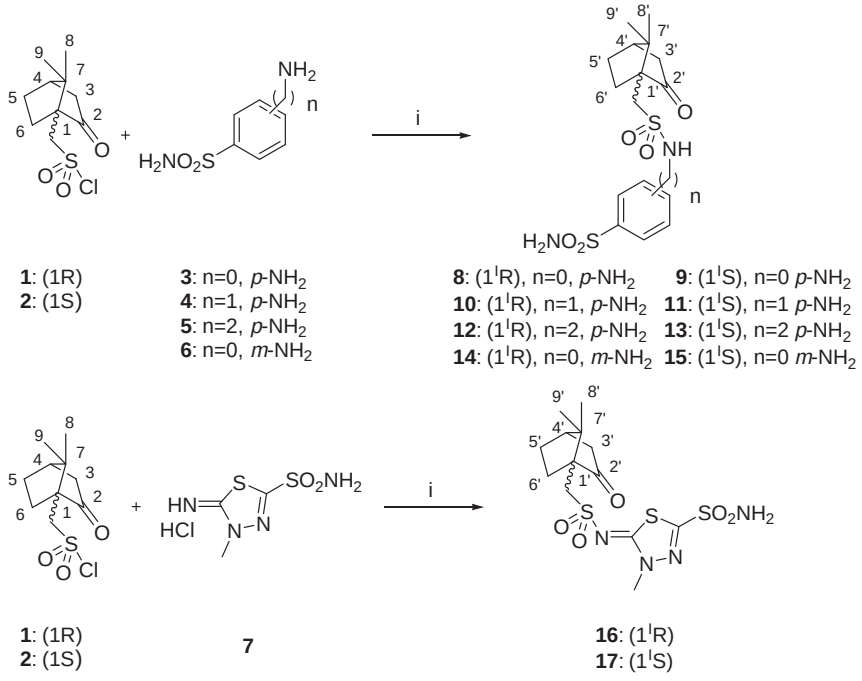
Carbonic anhydrase (CA, EC 4.2.1.1) inhibitors (CAIs) of the sulfonamide type, such as acetazolamide **AAZ**, methazolamide **MZA**, ethoxzolamide **EZA** or dichlorphenamide **DCP**, are clinically used for decades, for various classes of diuretics and systemically acting antiglaucoma agents.[1–4] In the last years novel applications emerged for this class of pharmacological agents, such as the topically acting antiglaucoma agents, anticonvulsants, antiobesity, antipain and antitumor drugs/diagnostic tools.[3–7] However critical barriers to the use of CAIs as therapeutic agents are related to the high number of isoforms in humans (i.e., 16 CAs, of which 13 have catalytic activity), their rather diffuse localization in many tissues/organs, and the lack of isozyme selectivity of the presently available inhibitors of the sulfonamide/sulfamate type.[3,8–10] Thus, there is a stringent need of CAIs with a more selective inhibition profile compared to the sulfonamides mentioned above (first generation CAIs) or their isosteres (such as sulfamates, of which topiramate **TPM** is a clinically used antiepileptic drug).[11]



Two of the 16 mammalian CA isoforms are mitochondrial ones, CA VA and VB.[12] They were shown to be involved among others in several biosynthetic processes, such as ureagenesis,[12a]

* Corresponding author. Tel.: +39 055 457 3005; fax: +39 055 4573385.
E-mail address: claudiu.supuran@unifi.it (C.T. Supuran).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.01.050



Scheme 1. Synthesis of sulfonamide derivatives **8**–**17**. Reagents and conditions: (i) dry DMF, dry TEA, N_2, 0° to rt.

gluconeogenesis,[13] and lipogenesis, both in vertebrates (rodents) as well as invertebrates (locust).[14,15] Indeed, in several important biosynthetic processes involving pyruvate carboxylase, acetyl CoA carboxylase, and carbamoyl phosphate synthetases I and II, bicarbonate, it is not carbon dioxide the real substrate for these carboxylating enzymes, but its hydration product, bicarbonate, and the provision of enough bicarbonate is assured by the catalysis involving the mitochondrial isozymes CA VA and VB.[3,13–15]

As obesity represents a serious medical problem,[7] mitochondrial CA inhibition has been proposed as an anti-obesity approach for designing novel pharmacological agents.[3,7] Indeed, topiramate **TPM** and zonisamide **ZNS** (another clinically used antiepileptic drug),[3] showed a steadfast and important weight loss as 'side-effects' in obese epileptic patients.[16] It is actually considered that this effect is due to the inhibition of lipogenesis mediated by these agents, as a consequence of mitochondrial CA inhibition.[3,7] However, both **TPM** and **ZNS**, but also sulfonamides **AAZ–DCP** mentioned above, do not show any selectivity for inhibiting the mitochondrial over the ubiquitous cytosolic CA isoforms. In this Letter, we report a novel class of lipophilic CAIs incorporating *R*- and *S*-10-camphorsulfonyl moieties, which show high selectivity and excellent potency for the inhibition of the mitochondrial isoforms CA VA and CA VB.

Sulfonamides[1–3] and sulfamates[3,11] are among the most potent CAIs reported up to now against all the physiologically relevant CA isozymes, including the mitochondrial ones CA VA and VB.[3,12] Thus, we decided to explore new series of aromatic as well as heterocyclic sulfonamides which should also incorporate lipophilic moieties in their molecule, in order to endow them with good membrane permeability and thus access to mitochondria, where these isoforms are located. We have considered the reactions of (1*R*)-(−)-10-camphorsulfonyl chloride **1** and (1*S*)-(+)-10-camphorsulfonyl chloride **2** with aromatic/heterocyclic sulfonamides incorporating amino or imino moieties, as a facile way to generate such new compounds (Scheme 1).

Indeed, reaction of sulfonyl chlorides **1** and **2** with amino/imino-sulfonamides **3–7** afforded a series of chiral new sulfonamides of type **8**–**17**, which incorporate the highly lipophilic camphorsulfonyl moieties. Such derivatizations (the 'tail approach') have been investigated earlier extensively by this group,[17] being shown that they may lead to effective CAIs. All new compounds **8–17** have been thoroughly characterized by physico-chemical methods which confirmed their structure.[18]

The new sulfonamides reported here, of types **8**–**17**, as well as the classical CAIs in clinical use **AAZ–ZNS**, have been assayed[19] for the inhibition of four physiologically relevant CA isoforms, the cytosolic, off-target hCA I and II, as well as the mitochondrial hCA VA and hCA VB (Table 1). The following should be observed regarding CA inhibition data with these compounds:

(i) Sulfonamides **8–17** incorporating the lipophilic moieties of the *R*- and *S*-10-camphorsulfonyl type, behaved as weak inhibitors

Table 1
hCA I, II, VA and VB inhibition data with sulfonamides **8**–**17** and the standard inhibitors **AAZ**–**ZNS**, by a stopped-flow, CO_2 hydration assay method[19]

Compound	K_I (nM)[c]			
	hCA I[a]	hCA II[a]	hCA VA[b]	hCA VB[b]
8	1241	1207	17.1	26.4
9	3130	1165	68.6	30.0
10	920	1563	16.6	40.3
11	1042	2459	27.5	67.3
12	4964	963	64.5	36.2
13	5513	2254	33.2	54.1
14	9711	78.9	35.3	38.4
15	81.5	2304	48.7	44.3
16	5246	1773	5.9	7.8
17	4382	398	21.0	7.3
AAZ	250	12	63	54
MZA	50	14	65	62
EZA	25	8	25	19
DCP	1200	38	630	21
TPM	250	10	63	30
ZNS	56	35	20	6033

Data for the standard inhibitors are from Ref. 3a.
[a] Full length, cytosolic recombinant isoform.
[b] Full length, mitochondrial recombinant enzyme.
[c] Errors in the range of ±5–10% of the reported value, from three different assays.

of the cytosolic, slow isoform hCA I, with inhibition constants in the micromolar range (K_Is of 0.92–9.71 μM). Only one of these derivatives, **15**, showed more efficient inhibition of this isoform, with K_I of 81.5 nM (Table 1). It is interesting to note the huge difference in inhibitory power between the two enantiomers **14** and **15**, with the last one being approximately 120 times a better hCA I inhibitor compared to **14**. Thus, the nature of the moiety on which the sulfonamide group is grafted (aromatic or heterocyclic) as well as the spacer between this moiety and the camphorsulfonyl group, do not influence markedly the activity of these compounds. It is however difficult to explain the rather good inhibitory activity of **15**, but recent work showed a very variable binding pattern within the enzyme active site even for structurally very similar congeners belonging to the sulfonamides.[20] It may be observed on the other hand that the clinically used derivatives **AAZ–ZNS** showed more potent inhibitory activities against these isoforms, with inhibition constants in the range of 25 nM–1.2 μM (Table 1).

(ii) A rather similar inhibition pattern with compounds **8–17** (as for hCA I) has been also observed against the ubiquitous, physiologically dominant[3] isoform hCA II (Table 1). Thus, weak inhibition, in the micromolar range (K_Is of 0.96–2.45 μM) was observed for all these compounds except **14** and **17**, which were more effective hCA II inhibitors (K_Is of 78.9–398 nM). Again important differences of activity have been observed for diverse enantiomers of the same sulfonamide (e.g., **14** vs **15**, or **16** vs **17**), and this has been documented by crystallographic work on different classes of chiral sulfonamides.[21] The clinically used derivatives were on the other hand low nanomolar inhibitors of this isoform, with K_Is in the range of 8–38 nM (Table 1).

(iii) The mitochondrial isoform hCA VA was much more susceptible to inhibition by compounds **8–17** compared to the cytosolic ones discussed above. Indeed, K_Is in the range of 5.9–68.6 nM have been measured for these derivatives (Table 1). SAR is here more intriguing compared to the inhibition of the cytosolic isoforms. Thus, generally the *R*-enantiomer was more effective as a hCA VA inhibitor compared to the corresponding *S*-enantiomer (except for the pair **12–13** where the reverse was true). The most effective inhibitor was thus the heterocyclic derivative **16** (K_I of 5.9 nM), which as far as we know, is the most effective hCA VA inhibitor ever reported. Its enantiomer, **17**, was 3.5 times less effective an inhibitor, being anyhow equipotent to **ZNS**, one of the best hCA VA inhibitors among the clinically used drugs (Table 1 and Ref. 3). The benzenesulfonamide derivatives **8–15** showed rather comparable activity irrespective whether the two substituents on the ring were in *meta* (**14** and **15**) or *para* (**8–13**) positions, and of the length of the spacer separating the benzenesulfonamide and the camphorsulfonamide moieties.

(iv) The second mitochondrial isoform, hCA VB was also significantly inhibited by sulfonamides **8–17**, with inhibition constants in the range of 7.3–67.3 nM, orders of magnitude better than the inhibition of the cytosolic isoforms hCA I and II discussed above. Again the best inhibitors were the heterocyclic sulfonamides **16** and **17**, and generally the *R*-enantiomer was more active compared to the corresponding *S* one, as for the inhibition of hCA VA discussed above (the only exception is the pair **16** and **17**).

(v) The selectivity ratios for the inhibition of the mitochondrial over the cytosolic isoforms, for the new compounds reported here, are very good. For example, **16**, a low nanomolar hCA VA and VB inhibitor, had a selectivity ratio of 889 for the inhibition of hCA VA over hCA I, and of 300.5 for the inhibition of hCA VA over hCA II. The selectivity ratios for the inhibition of hCA VB over hCA I and of hCA VB over hCA II are of 672 and 227, respectively. For ethoxzolamide **EZA**, one of the best classical mitochondrial CA inhibitors, these parameters are of 1 (hCA VA over hCA I), 0.32 (hCA VA over hCA II), 1.31 (hCA VB over hCA I) and 0.42 (hCA VB over hCA II), respectively. Thus, most of the classical sulfonamides/sulfamates are much better hCA I/II inhibitors than hCA VA/VB inhibitors, whereas exactly the reverse is true for the new sulfonamides **8–17** reported here.

Work is in progress to evaluate pharmacologically these compounds as lipogenesis inhibitors. Their lipophilic character makes them interesting candidates for such an action, and it should be mentioned that their inhibition of other CA isoforms (e.g., CA IV, VI, IX and XII, data not shown) with important physiological roles is not in the low nanomolar range, being thus more similar to that of CA II (described here). Thus, there would be of little concern their activity against these off-target CAs.

In conclusion, we report in the present paper a series of aromatic and heterocyclic sulfonamides incorporating *R*- and *S*-10-camphorsulfonyl moieties, which were synthesized by reaction of the sulfonyl chlorides with amino/imino-containing sulfonamides. The new derivatives were investigated for the inhibition of several mammalian isoforms the zinc enzyme carbonic anhydrase, such as CA I, II, VA and VB. The new sulfonamides selectively inhibited the mitochondrial isozymes hCA VA and VB over the cytosolic, off-target ones hCA I and II, with inhibition constants in the low nanomolar range (5.9–68.6 nM). The nature, chirality and position of the groups substituting the sulfonamide greatly influenced the CA inhibitory properties. These compounds are excellent leads for designing isoform-selective enzyme inhibitors targeting mitochondrial CAs involved in lipogenesis and obesity.

Acknowledgment

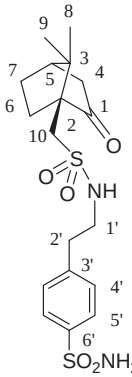
This research was financed in part by a grant of the 7th FP of EU (Metoxia project).

References and notes

1. Young, R. W.; Wood, K. H.; Eichler, J. A.; Vaughan, J. R., Jr.; Anderson, G. W. *J. Am. Chem. Soc.* **1956**, *78*, 4649.
2. (a) Svastova, E.; Hulikova, A.; Rafajova, M.; Zatovicova, M.; Gibadulinova, A.; Casini, A.; Cecchi, A.; Scozzafava, A.; Supuran, C. T.; Pastorek, J.; Pastorekova, S. *FEBS Lett.* **2004**, *577*, 439; (b) Cecchi, A.; Hulikova, A.; Pastorek, J.; Pastorekova, S.; Scozzafava, A.; Winum, J. Y.; Montero, J. L.; Supuran, C. T. *J. Med. Chem.* **2005**, *48*, 4834; (c) Dubois, L.; Lieuwes, N. G.; Maresca, A.; Thiry, A.; Supuran, C. T.; Scozzafava, A.; Wouters, B. G.; Lambin, P. *Radiother. Oncol.* **2009**, *92*, 423; (d) Hilvo, M.; Baranauskiene, L.; Salzano, A. M.; Scaloni, A.; Matulis, D.; Innocenti, A.; Scozzafava, A.; Monti, S. M.; Di Fiore, A.; De Simone, G.; Lindfors, M.; Janis, J.; Valjakka, J.; Pastorekova, S.; Pastorek, J.; Kulomaa, M. S.; Nordlund, H. R.; Supuran, C. T.; Parkkila, S. *J. Biol. Chem.* **2008**, *283*, 27799.
3. (a) Supuran, C. T. *Nat. Rev. Drug Disc.* **2008**, *7*, 168; (b) Supuran, C. T.; Scozzafava, A.; Casini, A. Development of Sulfonamide Carbonic Anhydrase Inhibitors (CAIs). In *Carbonic Anhydrase—Its Inhibitors and Activators*; Supuran, C. T., Scozzafava, A., Conway, J., Eds.; CRC Press: Boca Raton, FL, 2004; pp 67–147.
4. (a) Supuran, C. T. Carbonic Anhydrases as Drug Targets—General Presentation. In *Drug Design of Zinc-Enzyme Inhibitors: Functional, Structural, and Disease Applications*; Supuran, C. T., Winum, J. Y., Eds.; Wiley: Hoboken, NJ, 2009; pp 15–38; (b) Winum, J. Y.; Rami, M.; Scozzafava, A.; Montero, J. L.; Supuran, C. *Med. Res. Rev.* **2008**, *28*, 445; (c) Supuran, C. T.; Scozzafava, A.; Casini, A. *Med. Res. Rev.* **2003**, *23*, 146.
5. Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C. T.; De Simone, G. X-ray Crystallography of CA Inhibitors and Its Importance in Drug Design In *Drug Design of Zinc-Enzyme Inhibitors: Functional, Structural, and Disease Applications*; Supuran, C. T., Winum, J. Y., Eds.; Wiley: Hoboken, 2009; pp 73–138.
6. (a) Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3467; (b) Supuran, C. T. *Curr. Pharm. Des.* **2010**, *16*, 3233.
7. (a) Supuran, C. T. *Curr. Pharm. Des.* **2008**, *14*, 641; (b) Supuran, C. T.; Di Fiore, A.; De Simone, G. *Expert Opin. Emerg. Drugs* **2008**, *13*, 383; (c) De Simone, G.; Di Fiore, A.; Supuran, C. T. *Curr. Pharm. Des.* **2008**, *14*, 655; (d) Mincione, F.; Scozzafava, A.; Supuran, C. T. Antiglaucoma Carbonic Anhydrase Inhibitors as Ophthalomologic Drugs. In *Drug Design of Zinc-Enzyme Inhibitors: Functional, Structural, and Disease Applications*; Supuran, C. T., Winum, J. Y., Eds.; Wiley: Hoboken, NJ, 2009; pp 139–154; (e) Krungkrai, J.; Supuran, C. T. *Curr. Pharm. Des.* **2008**, *14*, 631; (f) Borras, J.; Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. *Bioorg. Med. Chem.* **1999**, *7*, 2397.
8. (a) Thiry, A.; Dogné, J. M.; Masereel, B.; Supuran, C. T. *Trends Pharmacol. Sci.* **2006**, *27*, 566; (b) Alterio, V.; Hilvo, M.; Di Fiore, A.; Supuran, C. T.; Pan, P.; Parkkila, S.; Scaloni, A.; Pastorek, J.; Pastorekova, S.; Pedone, C.; Scozzafava, A.; Monti, S. M.; De Simone, G. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 16233.

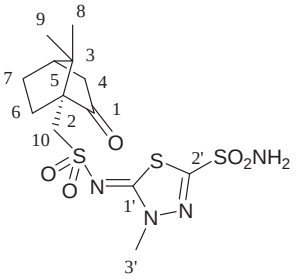
9. (a) Supuran, C. T.; Scozzafava, A. *Bioorg. Med. Chem.* **2007**, *15*, 4336; (b) Temperini, C.; Cecchi, A.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2567; (c) Supuran, C. T. *Curr. Pharm. Des.* **2008**, *14*, 603; (d) Temperini, C.; Cecchi, A.; Scozzafava, A.; Supuran, C. T. *Org. Biomol. Chem.* **2008**, *6*, 2499; (e) Temperini, C.; Cecchi, A.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2009**, *52*, 322; (f) Temperini, C.; Cecchi, A.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem.* **2009**, *17*, 1214.
10. (a) Ebbesen, P.; Pettersen, E. O.; Gorr, T. A.; Jobst, G.; Williams, K.; Kienninger, J.; Wenger, R. H.; Pastorekova, S.; Dubois, L.; Lambin, P.; Wouters, B. G.; Supuran, C. T.; Poellinger, L.; Ratcliffe, P.; Kanopka, A.; Görlach, A.; Gasmann, M.; Harris, A. L.; Maxwell, P.; Scozzafava, A. *J. Enzyme Inhib. Med. Chem.* **2009**, *24*, 1; (b) Ahlskog, J. K. J.; Dumelin, C. E.; Trüssel, S.; Marlind, J.; Neri, D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4851.
11. (a) Casini, A.; Antel, J.; Abbate, F.; Scozzafava, A.; David, S.; Waldeck, H.; Schäfer, S.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 841; (b) Vitale, R. M.; Pedone, C.; Amodeo, P.; Antel, J.; Wurl, M.; Scozzafava, A.; Supuran, C. T.; De Simone, G. *Bioorg. Med. Chem.* **2007**, *15*, 4152; (c) Alterio, V.; Monti, S. M.; Truppo, E.; Pedone, C.; Supuran, C. T.; De Simone, G. *Org. Biomol. Chem.* **2010**, *8*, 3528.
12. (a) Dodgson, S. J. *J. Appl. Physiol.* **1987**, *63*, 2134; (b) Nishimori, I.; Vullo, D.; Innocenti, A.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. *J. Med. Chem.* **2005**, *48*, 7860.
13. Dodgson, S. J.; Cherian, K. *Am. J. Physiol.* **1989**, *257*, E791.
14. (a) Spencer, I. M.; Hargreaves, I.; Chegwidden, W. R. *Biochem. Soc. Trans.* **1988**, *16*, 973; (b) Chegwidden, W. R.; Spencer, I. M. *Comp. Biochem. Physiol.* **1996**, *115B*, 247; (c) Chegwidden, W. R.; Dodgson, S. J.; Spencer, I. M. The Roles of Carbonic Anhydrase in Metabolism, Cell Growth and Cancer in Animals. In *The Carbonic Anhydrases—New Horizons*; Chegwidden, W. R., Edwards, Y., Carter, N., Eds.; Birkhäuser: Basel, 2000; pp 343–363.
15. (a) Lynch, C. J.; Fox, H.; Hazen, S. A.; Stanley, B. A.; Dodgson, S. J.; Lanoue, K. F. *Biochem. J.* **1995**, *310*, 197; (b) Hazen, S. A.; Waheed, A.; Sly, W. S.; Lanoue, K. F.; Lynch, C. J. *FASEB J.* **1996**, *10*, 481.
16. (a) Picard, F.; Deshaies, Y.; Lalonde, J.; Samson, P.; Richard, D. *Obes. Res.* **2000**, *8*, 656; (b) Supuran, C. T. *Exp. Opin. Ther. Patents* **2003**, *13*, 1545; (c) Kim, C. S. *J. Fam. Pract.* **2003**, *52*, 600; (d) Gadde, K. M.; Franciscy, D. M.; Wagner, H. R., 2nd; Krishnan, K. R. *JAMA* **2003**, *289*, 1820.
17. (a) Supuran, C. T.; Ilies, M. A.; Scozzafava, A. *Eur. J. Med. Chem.* **1998**, *33*, 739; (b) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. *J. Med. Chem.* **2000**, *43*, 4542; (c) Ilies, M.; Supuran, C. T.; Scozzafava, A.; Casini, A.; Mincione, F.; Menabuoni, L.; Caproiu, M. T.; Maganu, M.; Banciu, M. D. *Bioorg. Med. Chem.* **2000**, *8*, 2145.
18. Sulfonamides **3**–**6** and sulfonyl chlorides **1**, **2** used for the synthesis were commercially available from Sigma–Aldrich (Milan, Italy), and were used without further purification. The synthesis of compound **7** was carried out as reported in literature.[1] All CA isozymes were recombinant ones produced and purified in our laboratory as described earlier.[2,3] Reactions of (1*R*)-(−)-10-camphorsulfonyl chloride **1** and (1*S*)-(+)-10-camphorsulfonyl chloride **2** (0.3 g, 1.0 equiv) with amino derivatives **3**–**7** (1.0 equiv) were carried out at 0° to room temperature in Schotten–Baumann conditions under nitrogen atmosphere, in the presence of a stoichiometric amount of dropwised dry TEA (1.0 equiv for **3**, **5**, **6** and 2.0 equiv for **4**, **7**), in dry DMF as solvent (2 ml). When complete (monitoring by TLC), reactions were quenched with crushed ice, extracted with ethyl acetate (20 ml), washed with 1 N HCl (2 × 10 ml) and brine (2 × 10 ml). The collected organic phase was dried on anhydrous Na_2SO_4, filtered and evaporated under vacuum. The crude was purified by silica gel column chromatography eluting with *n*-hexane/ethyl acetate or DCM/methanol to afford compounds **8**–**17** as white solids in medium yields. Examples of several compounds prepared in this way are provided below:

4-(2-(((1S)-7,7-Dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl)methylsulfonamido)ethyl)benzene sulfonamide (**13**)



Compound **13** was synthesised by reacting (1*S*)-(+)-10-camphorsulfonyl chloride **2** (1.19 mmol, 300 mg) with 4-(2-Aminoethyl)benzenesulfonamide **5** (1.19 mmol, 238.3 mg) following the general procedure mentioned above. The crude was purified by silica gel column chromatography eluting with 2.5% MeOH in DCM to afford compounds **13** as white solid in 70% yield. Mp 140–142 °C, $[\alpha]_D^{20}$ +11.1, silica gel TLC R_f 0.27 (MeOH/DCM 2.5%), ν_{max} (KBr) cm^{-1}, 3250 (N–H), 2954 (C–H), 1741 (C=O), 1599 (aromatic), 1328 (SO_2–NH), δ_H (400 MHz, DMSO-d_6) 0.83 (3H, s, 9-H_3), 1.03 (3H, s, 8-H_3), 1.39–1.44 (1H, m, 6-H), 1.49–1.56 (1H, m, 7-H), 1.92–1.93 (1H, m, 4-H), 1.94–2.01 (1H, m, 6-H), 2.06–2.08 (1H, m, 4-H), 2.31–2.38 (1H, m, 7-H), 2.32–2.39 (1H, m, 5-H), 2.86–2.92 (3H, m, 2′-H_2, 10-H), 3.26–3.32 (3H, m, 1′-H_2, 10-H), 7.29 (2H, s, SO_2NH_2, exchange with D_2O), 7.46–7.50 (2H, m, 2 × 4′-H), 7.78–7.80 (2H, m, 2 × 5′-H), δ_C (100 MHz, DMSO-d_6) 215.6 (C-1), 144.2 (ipso), 143.1 (ipso), 130.3 (C-4′), 126.8 (C-5′), 58.7 (C-2), 48.5 (C-10), 48.4 (C-3), 44.5 (C-1′), 42.9 (C-4), 42.8 (C-5), 36.5 (C-2′), 27.2 (C-6), 25.3 (C-7), 20.3 (C-8), 20.3 (C-9), *m*/*z* (ESI-) 413.1 ($[M-H]^-$ 100%), 827.3 ($[2M-H]^-$ 18%), *m*/*z* (ESI+) 415.2 ($[M+H]^+$ 100%), 829.3 ($[2M+H]^+$ 50%).

(Z)-5-(((1R)-7,7-Dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl)methylsulfonylimino)-4-methyl-4,5-dihydro-1,3,4-thiadiazole-2-sulfonamide (**16**)



Compound **16** was synthesised by reacting (1*R*)-(−)-10-camphorsulfonyl chloride **1** (0.08 mmol, 200 mg) with derivative **7**[1] (0.08 mmol, 185 mg) following the general procedure mentioned above. The crude was purified by silica gel column chromatography eluting with 3% MeOH in DCM to afford compounds **16** as white solid in 46% yield. Mp 248–250 °C, $[\alpha]_D^{20}$ −47.5, silica gel TLC R_f 0.27 (MeOH/DCM 3%), ν_{max} (KBr) cm^{-1}, 3265 (N–H), 2962 (C–H), 1736 (C=O), 1654 (C=N), 1372 (SO_2–NH), δ_H (400 MHz, DMSO-d_6) 0.82 (3H, s, 9-H_3), 1.06 (3H, s, 8-H_3), 1.42–1.47 (1H, m, 6-H), 1.59–1.66 (1H, m, 7-H), 1.94–1.98 (1H, m, 4-H), 1.94–2.02 (1H, m, 6-H), 2.07–2.10 (1H, m, 4-H), 2.32–2.44 (1H, m, 7-H), 2.33–2.43 (1H, m, 5-H), 3.14–3.17 (1H, d, *J* 14.8, 10-H), 3.43–3.47 (1H, d, *J* 14.8, 10-H), 3.74 (1H, s, 3′-H), 8.60 (2H, s, SO_2NH_2, exchange with D_2O), δ_C (100 MHz, DMSO-d_6) 215.4 (C-1), 165.7 (C-2′), 157.0 (C-1′), 58.5 (C-2), 50.5 (C-10), 48.7 (C-3), 42.9 (C-4), 42.8 (C-5), 38.6 (C-3′), 27.2 (C-6), 25.2 (C-7), 20.1 (C-8), 20.1 (C-9), *m*/*z* (ESI-) 407.0 ($[M-H]^-$ 100%), 815.0 ($[2M-H]^-$ 8%), *m*/*z* (ESI+) 409.1 ($[M+H]^+$ 100%), 817.2 ($[2M+H]^+$ 5%).

19. 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.[18] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na_2SO_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, in triplicate measurements. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water 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, as reported earlier, [11,17] and represent the mean from at least three different determinations. CA isoforms were recombinant ones obtained in house as reported earlier.[11,17]
20. Pacchiano, F.; Aggarwal, M.; Avvaru, B. S.; Robbins, A. H.; Scozzafava, A.; McKenna, R.; Supuran, C. T. *Chem. Commun.* **2010**, *46*, 8371.
21. (a) D'Ambrosio, K.; Masereel, B.; Thiry, A.; Scozzafava, A.; Supuran, C. T.; De Simone, G. *Chem. Med. Chem.* **2008**, *3*, 473; (b) Güzel, Ö.; Temperini, C.; Innocenti, A.; Scozzafava, A.; Salman, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 152.