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Bioorganic & Medicinal Chemistry Letters 14 (2004) 5781–5786
Bioorganic & Medicinal Chemistry Letters

Carbonic anhydrase inhibitors. Design of anticonvulsant sulfonamides incorporating indane moieties

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Received 16 June 2004; revised 3 September 2004; accepted 17 September 2004

Abstract—A series of aromatic sulfonamides incorporating indane moieties were prepared starting from commercially available 1- and 2-indanamine, and their activity as inhibitors of two carbonic anhydrase (CA, EC 4.2.1.1) isozymes, hCA I and II was studied. The new sulfonamides incorporating acetamido, 4-chloro-benzoyl, valproyl, tetra-, and pentafluorobenzoyl moieties acted as very potent inhibitors of the slow red blood cell isozyme hCA I (K_is in the range of 1.6–8.5 nM), which usually has a lower affinity for such inhibitors, as compared to isozyme II. Some derivatives also showed excellent hCA II inhibitory properties (K_is in the range of 2.3–12 nM), but the anticonvulsant activity of these sulfonamides was rather low as compared to that of other sulfonamide/sulfamate CA inhibitors, such as methazolamide. Furthermore, the 2-amino/acetamido-indane-5-sulfonic acids prepared during this work also showed interesting CA inhibitory properties, with inhibition constants in the range of 43–89 nM against the two isozymes, being among the most potent sulfonic acid CA inhibitors reported so far.

1. Introduction

Unsubstituted aromatic sulfonamides were known to inhibit the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1) since the beginning of research in this field.[1] Starting with the 1950s, potent CAIs belonging to the heterocyclic sulfonamide class have been developed that led to the benzothiadiazine and high-ceiling diuretics, as well as to the systemic antiglaucoma drugs acetazolamide **AAZ**, methazolamide **MZA**, ethoxzolamide **EZA**, and dichlorophenamide **DCP**.[2,3]

Discovering these drugs highly benefited the chemistry of sulfonamides, as thousands of derivatives belonging to the heterocyclic, aromatic, and bis-sulfonamide classes have been synthesized and investigated for their biological activity.[3] In the late 80s and early 90s, the topically effective antiglaucoma CA inhibitors (CAIs) have been discovered, with two such drugs—dorzolamide **DZA** and brinzolamide **BRZ**—now used clinically.[4,5] All these CAI drugs have been designed by the ring approach, that is, investigating a very large number of ring systems incorporating sulfamoyl moieties.[6] More recently, an alternative approach, the 'tail' one,[6] has been reported by our group for the design of antiglaucoma CAIs with topical activity, but this approach has then been extended for other applications of these pharmacological agents.[7–10] The tail approach consists of attaching tails, that is, moieties that will induce the desired physico-chemical properties to aromatic/heterocyclic sulfonamide scaffolds possessing derivatizable amino or hydroxy moieties. By using this approach, important progress has been achieved for evidencing inhibitors with higher affinity for a certain isozyme, although clear-cut isozyme-specific inhibitors are not available at this moment for the different 14 CA isozymes discovered so far in humans.[11] Inhibitors selective for the membrane associated (CA IV, IX, XII, and XIV) versus the cytosolic isozymes were recently described, belonging either to macromolecular compounds or to

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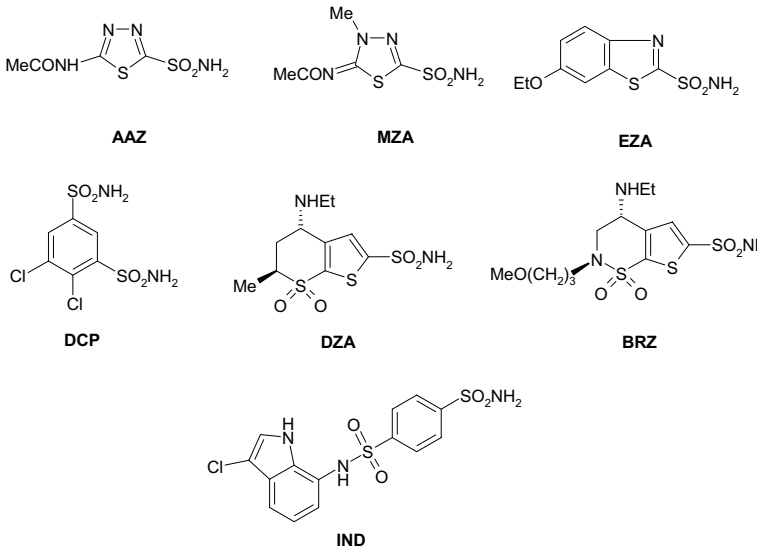
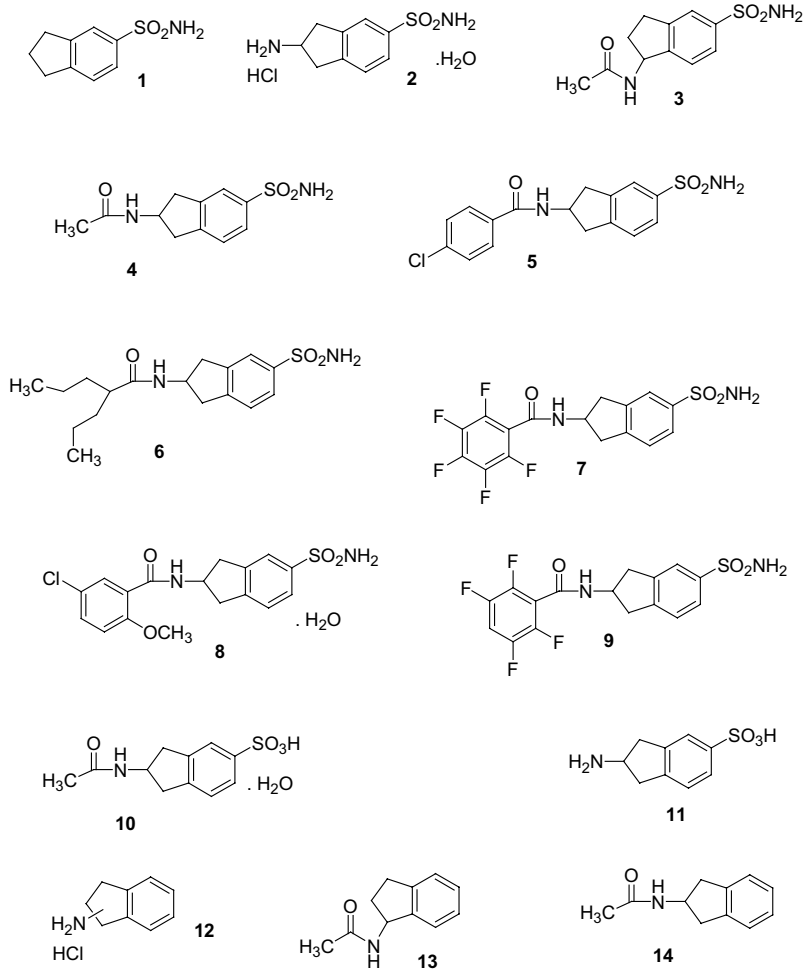
doi:10.1016/j.bmcl.2004.09.061



the positively charged derivatives (of low molecular weight).[3,12] CAIs possessing relevant antitumor properties, and thus potent inhibition of the tumor-associated isozyme CA IX were also discovered, with one such derivative—indisulam **IND**—in advanced clinical trials for the treatment of solid tumors.[13] CAIs with good anticonvulsant activity and several other biomedical applications have recently been reported by our groups.[14] This field is in constant progress and may lead to the discovery of highly interesting pharmacological agents. All the seven drugs in clinical use/trials belonging to the CAIs mentioned above, have been discovered



by the ring approach, whereas most of the work from our laboratory was dedicated to finding novel tails that will induce useful applications to the CAIs that we designed. Here we report sulfonamide derivatives belonging to a ring system that has never been investigated for its possible applications in the design of CAIs, that is, the indane ting. Sulfonamides **1**–**9** and sulfonic acids **10** and **11** were thus prepared and investigated for their CA inhibitory and anticonvulsant activity.

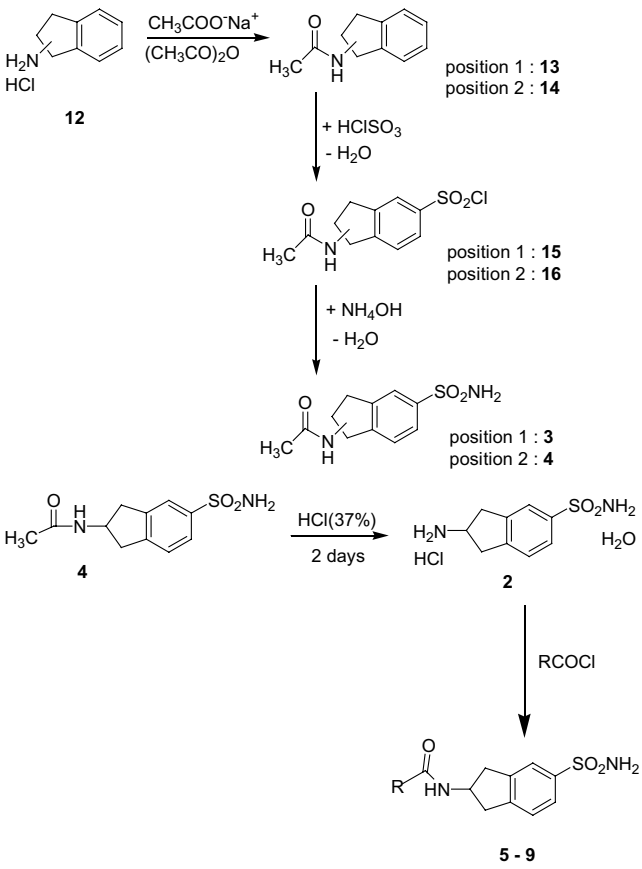
2. Chemistry

In a preliminary experiment (data not published) we observed that the indanesulfonamide derivative **1** showed good CA I and II inhibitory properties (see later in the text). Thus, we decided to investigate some derivatives incorporating this ring system. For this reason, we needed to prepare the amino-indanesulfonamide derivative(s) of **1**, which were not described in the literature as CAIs. Commercially available 1- and 2-aminoindanes **12** were used for this purpose: acetylation of **12** with acetic anhydride/sodium acetate, led to the acetamido derivatives **13** and **14** (Scheme 1).[15] Both of them were then reacted with chlorosulfonic acid, leading to the sulfonyl chlorides **15** and **16**, which were converted to the two isomeric sulfonamides **3** and **4** (the main product of this reaction is the 5-sulfonyl chloride of type **15** and **16**, respectively).[15] The acetamido intermediate **3** was obtained pure after several recrystallizations from acetonitrile/water (1/1). In the case of the acetamido intermediate **4**, only one compound is formed by treatment of **14** with chlorosulfonic acid, and subsequent amidation, due to the symmetric nature of the compound. This procedure also allowed us to isolate and characterize the hydrolysis product of the sulfonylchloride intermediate **16**, the sulfonic acid **10**. Its amino function was then deprotected, leading to the aminosulfonic acid **11**. Deprotection of the amino moiety of the key intermediate **4**, with concentrated hydrochloric acid, afforded the aminosulfonamide **2** (as hydrochloride) which was thereafter used for the preparation of the derivatives **5**–**9**, by reaction with acyl halides, as previously reported.[7–10,16]

The nature of the R moieties in derivatives **5**–**9** was chosen considering the good activity of compounds incorporating chloro-benzoyl, valproyl, tetra-, and pentafluorobenzoyl moieties and other amino-sulfonamide ring systems, such as the sulfanilamide, homosulfanilamide, or 5-amino-1,3,4-thiadiazole-2-sulfonamide derivatives, previously reported by this group (Scheme 1).[9,14]

3. Carbonic anhydrase inhibition and anticonvulsant activity

All the compounds reported here were assayed for inhibition of two CA isozymes involved in important physiological processes, hCA I and hCA II.[17] It is well known that these isozymes play a crucial role in the transport of CO_2 in a large variety of tissues as well as for the pH regulation in many organs.[2–6] Furthermore,



Scheme 1.

they seem to be involved in the anticonvulsant activity shown by many sulfonamide drugs with CA inhibitory properties.[14] The inhibitory activity was evaluated measuring the CO_2 hydrase activity of these two isozymes with the physiological substrate CO_2.[18,19] The inhibition data are shown in Table 1.

The following should be noted regarding data of Table 1: (i) compounds **1**–**11** investigated here act as quite potent hCA I inhibitors, a feature which is noteworthy, since this is an isozyme with a rather reduced affinity for sulfonamide inhibitors, as compared to sulfonamide-avid isozymes such as CA II, IV, VII, or IX among others.[3–5] Thus, compounds **1**–**11** show inhibition constants in the range of 1.6–215 nM against this isozyme, being much more effective than the clinically used derivatives acetazolamide and methazolamide, which show inhibition constants in the range of 780–900 nM. Among derivatives **1**–**11**, the sulfonamide **1** and the two sulfonic acids **10** and **11** are moderate hCA I inhibitors (K_is of 43–215 nM), whereas the sulfonamides **2** and **5** already show an increased affinity (K_is of 33–36 nM). But the other sulfonamides, that is, **3**, **4**, **6**–**9** are indeed very potent hCA I inhibitors, with K_is in the range of 1.6–8.5 nM, being among the most potent hCA I inhibitors ever reported. Thus, this ring system incorporating either an 1-acetamido- or 2-alkyl/arylcarboxamido tails, induces very potent hCA I inhibitory properties to the corresponding sulfonamides, whereas the simple unsubstituted sulfonamide **1** and the sulfonic acids are weaker CA I inhibitors.

Table 1. CA inhibition and MES data for compounds **1**–**11** reported in the paper, and standard sulfonamide CA inhibitors, acetazolamide (**AAZ**), and methazolamide (**MZA**)

Compound	K_i (nM)		MES-test[b] % of protected mice
	hCA I[a]	hCA II[a]	
AAZ	900	12	NT[d]
MZA	780	14	100 (8/8)[c]
1	215	52	NT[d]
2	33	28	0 (0/8)
3	8.5	19	NT[d]
4	7.1	2.3	38 (3/8)
5	36	7.5	31 (2.5/8)
6	1.6	3.4	13 (1/8)
7	4.1	6.9	38 (3/8)
8	3.5	17	38 (3/8)
9	7.2	12	13 (1/8)
10	89	65	0 (0/8)
11	43	84	0 (0/8)

[a] Human, cloned isozyme.
[b] Determined as described in Ref. 14.
[c] % of mice protected ($n = 8$) against seizures induced by a maximal electroshock (50 mA, 0.2 s). Drug (30 mg/kg) was injected ip 3 h prior to electroshock.
[d] NT = not tested.

These sulfonic acids are also among the most potent such nonsulfonamide inhibitors detected so far;[2–5] (ii) Against the major red cell isozyme hCA II, again the lead molecule **1** as well as the sulfonic acids **10** and **11** show weak inhibitory activity, with K_is in the range of 52–84 nM. The sulfonamides **2**, **3**, and **8** on the other hand are medium-potency–strong inhibitors, with K_is in the range of 17–28 nM, comparable to that of the clinically used sulfonamides acetazolamide and methazolamide. Excellent CA II inhibitory activity was shown by sulfonamides **4**–**7** and **9**, incorporating aliphatic (acetamido and valproamido) and aromatic (4-chlorobenzamido-, pentafluorobenzoylamido, and tetrafluorobenzoylamido) moieties, which showed inhibition constants in the range of 2.3–12 nM. Thus, clearly the indanesulfonamide derivatives with good affinity for hCA II can be designed rather easy; (iii) several of the new derivatives, more precisely **4**, **5**, **7**, and **8**, showed a moderate anticonvulsant activity (% of protected mice in the MES test of 31–38%), which was much inferior to that of metahzolamide, which led to a 100% protection in the same animal model. Compounds **6** and **9** were on the other hand less effective anticonvulsants in the same model, whereas the other investigated derivatives were devoid of this property. Thus, indanesulfonamide derivatives are not good lead molecules for the design of anticonvulsants belonging to the class of the CA inhibitors.

4. Conclusion

We report a small library of indane-5-sulfonamide derivatives possessing either 1-acetamido- or 2-alkyl/arylcarboxamido moieties in their molecules, that were chosen in such a way as to incorporate tails leading to potent CA inhibitory properties. The new sulfonamides acted as very potent inhibitors of the slow red blood cell isozyme hCA I, which usually has a lower affinity for sulfonamide inhibitors, as compared to isozyme II. Some derivatives also showed excellent hCA II inhibitory properties, but the anticonvulsant activity of these derivatives was rather low as compared to that of other sulfonamide/sulfamate CA inhibitors.

References and notes

1. Mann, T.; Keilin, D. *Nature* **1940**, *146*, 164–165.
2. Maren, T. H. *Physiol. Rev.* **1967**, *47*, 595–781.
3. Supuran, C. T.; Casini, A.; Scozzafava, 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), USA, 2004; pp 67–148.
4. Supuran, C. T.; Scozzafava, A. *Expert Opin. Ther. Pat.* **2000**, *10*, 575–600.
5. Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. *Expert Opin. Ther. Pat.* **2004**, *14*, 667–702.
6. Supuran, C. T.; Scozzafava, A. *Curr. Med. Chem.—Imm., Endoc. Metab. Agents* **2001**, *1*, 61–97.
7. 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.
8. Winum, J.-Y.; Vullo, D.; Casini, A.; Montero, J.-L.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2003**, *46*, 5471–5477.
9. de Leval, X.; Ilies, M.; Casini, A.; Dogné, J.-M.; Scozzafava, A.; Masini, E.; Mincione, F.; Starnotti, M.; Supuran, C. T. *J. Med. Chem.* **2004**, *47*, 2796–2804.
10. Vullo, D.; Franchi, M.; Gallori, E.; Antel, J.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2004**, *47*, 1272–1279.
11. 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.
12. Casey, J. R.; Morgan, P. E.; Vullo, D.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. *J. Med. Chem.* **2004**, *47*, 2337–2347.
13. Abbate, F.; Casini, A.; Owa, T.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 217–223.
14. (a) Masereel, B.; Rolin, S.; Abbate, F.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2002**, *45*, 312–320; (b) Ilies, M. A.; Masereel, B.; Rolin, S.; Scozzafava, A.; Câmpeanu, G.; Cîmpeanu, V.; Supuran, C. T. *Bioorg. Med. Chem.* **2004**, *12*, 2717–2726.
15. Bondinell, W. E.; Pendleton, R. G. U.S. Patent 4,192,888, 1980.
16. 2-Acetamido-5-sulfonamidoindane **4**: 2-aminoindane hydrochloride (25 g), sodium acetate (12 g), and acetic anhydride (200 mL) were stirred one night at room temperature. The evaporation of the mixture under reduce pressure led to a white powder, which was extracted from the aqueous phase using chloroform. The chloroform was washed with HCl 10% and $NaHCO_3$ 5%, and dried upon $MgSO_4$. The evaporation of the solvent yields a white powder of **14** whose NMR characteristics are given: ^1H NMR (DMSO) δ ppm: 1.79 (s, 3H, CH_3); 2.71–3.17 (m, 4H, CH_2(1) and (3)); 4.42 (m, 1H, CH(2)); 7.13–7.22 (m, 4H, CH(4), (5), (6), and (7)); 8.14 (d, 1H, NH). The powder of **14** was added to chlorosulfonic acid (18 equiv) at −80 °C. The mixture remained under stirring for 1 h at room temperature. The excess of acid was then hydrolyzed with ice water. The product was quickly extracted with ethyl acetate, yielding the chlorosulfonyl compound after evaporation. This was quickly dissolved in a minimum

amount of THF and poured in an excess of an aqueous ammonia solution. After one night stirring at room temperature, the white precipitate of **4** obtained was filtered and recrystallized from CH_3CN/H_2O 1/1. Yield: 45%. Mp: 218–220 °C. ^1H NMR (DMSO) δ ppm: 1.81 (s, 3H, CH_3); 2.80–3.26 (m, 4H, CH_2(1) and (3)); 4.49 (m, 1H, CH(2)); 7.23 (s, 2H, SO_2NH_2); 7.41 (d, 1H, CH(7)); 7.65 (d, 1H, CH(6)); 7.70 (s, 1H, CH(4)); 8.21 (d, 1H, NH). MS (electrospray): $MH^+ = 255$; $MMH^+ = 509$; $MNa^+ = 277$; $MMNa^+ = 531$. Analysis: calcd: C 51.95, H 5.55, N 11.02, S 12.61; found: C 51.85, H 5.68, N 11.06, S 11.59.

Further recrystallization also led to the hydrolysis product of the chlorosulfonyl intermediate, compound **10**. Mp: 159–161 °C. ^1H NMR (DMSO) δ ppm: 1.84 (s, 3H, CH_3); 2.72–2.78 (m, 2H, CH_2(1) or (3)); 3.11–3.18 (m, 2H, CH_2(1) or (3)); 4.45 (m, 1H, CH(2)); 7.19 (d, 1H, CH(7)); 7.45 (d, 1H, CH(6)); 7.48 (s, 1H, CH(4)); 7.62 (s, 1H, OH); 8.42 (d, 1H, NH). MS (electrospray): $MH^+ = 254$. Analysis: calcd: C 48.34, H 5.53, N 5.12, S 11.73; found: C 48.39, H 6.12, N 5.10, S 11.75.

Compound **10** was stirred under reflux in an excess of HCl 37%. The evaporation of water led to a white powder of **11**, which was recrystallized from a mixture $EtOH/H_2O$ 1/1. Yield: 85%. Mp: 187–188 °C. ^1H NMR (DMSO) δ ppm: 2.87–2.93 (m, 2H, CH_2(1) or (3)); 3.23–3.27 (m, 2H, CH_2(1) or (3)); 4.01 (m, 1H, CH(2)); 7.21 (d, 1H, CH(7)); 7.46 (d, 1H, CH(6)); 7.50 (s, 1H, CH(4)); 7.99 (s, 3H, NH_2 and OH). MS (electrospray): $MH^+ = 214$. Analysis: calcd: C 50.69, H 5.20, N 6.57, S 15.04; found: C 51.09, H 5.33, N 6.66, S 15.54.

2-Amino-5-sulfonamido-indane hydrochloride **2**: compound **4** was stirred under reflux in an excess of HCl 37%. The evaporation of water led to a white powder of **2**, which was recrystallized from a mixture $EtOH/H_2O$ 1/1. Yield: 79%. Mp: decomposition around 350 °C. ^1H NMR (DMSO) δ ppm: 3.12 (m, 2H, CH_2(1) or (3)); 3.36 (m, 2H, CH_2(1) or (3)); 4.04 (m, 1H, CH(2)); 7.37 (s, 2H, SO_2NH_2); 7.46 (d, 1H, CH(7)); 7.68 (d, 1H, CH(6)); 7.74 (s, 1H, CH(4)); 8.63 (s, 3H, NH_3^+). MS (electrospray): $MH^+ = 213$. Analysis: calcd: C 40.52, H 5.67, N 10.50, S 12.02; found: C 40.89, H 5.64, N 10.47, S 12.14.

1-Acetamido-5-sulfonamidoindane **3**: according to method described for **4**: 1-aminoindane (2.03 g), sodium acetate (1.25 g), and acetic anhydride (5 mL) led to a white powder of **13** (yield: 85%) whose NMR characteristics are given: ^1H NMR (DMSO) δ ppm: 1.71–1.77 (m, 1H, CH(2) or (3)); 1.87 (s, 3H, CH_3); 2.34–2.38 (m, 1H, CH(2) or (3)); 2.78–2.91 (m, 2H, CH(2) or (3)); 5.25 (m, 1H, CH(1)); 7.17–7.24 (m, 4H, CH(4), (5), (6), and (7)); 8.21 (d, 1H, NH). Compound **13** was treated with an excess of chlorosulfonic acid and the intermediate sulfonyl chloride was then treated with an aqueous solution of ammonia yielding **3**. Mp: 199–201 °C. ^1H NMR (DMSO) δ ppm: 1.81–1.89 (m, 1H, CH(2) or (3)); 2.41–2.44 (m, 1H, CH(2) or (3)); 2.80–3.10 (m, 2H, CH(2) or (3)); 5.34 (m, 1H, CH(1)); 7.33 (s, 2H, SO_2NH_2); 7.41 (d, 1H, CH(7)); 7.66 (s, 1H, CH(6)); 7.68 (s, 1H, CH(4)); 8.37 (d, 1H, NH). MS (electrospray): $MH^+ = 255$; $MMH^+ = 509$; $MNa^+ = 277$; $MMNa^+ = 531$; $MNH_4^+ = 272$.

2-(4′-Chlorobenzenecarbonyl)amino-5-sulfonamidoindane **5**: To 4′-chlorobenzoic acid (0.63 g) was added $SOCl_2$ (10 mL). The mixture was stirred under reflux for 2 h. The brown oil obtained after evaporation of the excess of $SOCl_2$ was poured into 2 mL of anhydrous THF and added to **2** in 2 mL anhydrous pyridine at 0 °C. The mixture was stirred for one night at room temperature. After evaporation of the solvents, an acid solution (3 mL HCl 37% in 50 mL H_2O) was added and the formed precipitate was filtered. The solid obtained was washed with pentane and recrystallized in CH_3CN/H_2O 1/1 affording **5** as a white powder. Yield: 33%. Mp: 252 °C. ^1H NMR (DMSO) δ ppm: 2.99–3.05 (m, 2H, CH_2(1) or (3)); 3.29–3.34 (m, 2H, CH_2(1) or (3)); 4.74 (m, 1H, CH(2)); 7.27 (s, 2H, SO_2NH_2); 7.41 (d, 1H, CH(7)); 7.54 (d, 2H, CH(2′)); 7.65 (d, 1H, CH(6)); 7.70 (s, 1H, CH(4)); 7.89 (d, 2H, CH(3′)); 8.79 (d, 1H, NH). MS (electrospray): $MH^+ = 251$; $MNa^+ = 373$. Analysis: calcd: C 54.78, H 4.31, N 7.99, S 9.14; found: C 54.93, H 4.43, N 7.83, S 9.00.

2-(Valproylcarboxamido-5-sulfonamidoindane **6**: According to the procedure used for **5**: valproic acid (0.58 g) and **2** (1 g) afforded **6** as a lightly brown powder. Yield: 45%. Mp: 116 °C. ^1H NMR (DMSO) δ ppm: 1.18–1.47 (m, 14H, $CH_3(CH_2)_2$); 2.12 (m, 1H, CH); 2.81 (m, 2H, CH_2(1) or (3)); 3.22 (m, 2H, CH_2(1) or (3)); 4.52 (m, 1H, CH(2)); 7.27–7.67 (m, 5H, CH(4), CH(6), CH(7), and SO_2NH_2); 8.18 (d, 1H, NH). MS (electrospray): $MH^+ = 339$; $MMH^+ = 677$. Analysis: calcd: C 58.76, H 7.83, N 8.06, S 9.23; found: C 58.71, H 7.78, N 8.13, S 9.66.

2-(Pentafluorobenzoyl)amino-5-sulfonamidoindane **7**: according to the procedure used for **5**: pentafluorobenzoic acid (0.58 g) and **2** (1 g) afforded **7** as a white powder. Yield: 49%. Mp: decomposition around 350 °C. ^1H NMR (DMSO) δ ppm: 2.89–2.95 (m, 2H, CH_2(1) or (3)); 3.33–3.42 (m, 2H, CH_2(1) or (3)); 4.72 (m, 1H, CH(2)); 7.29 (s, 2H, SO_2NH_2); 7.45 (d, 1H, CH(7)); 7.65 (d, 1H, CH(6)); 7.72 (s, 1H, CH(4)); 9.33 (d, 1H, NH). MS (electrospray): $MH^+ = 407$; $MNH4^+ = 424$.

2-(2′-Methoxy-5′-chlorobenzenecarbonyl)amino-5-sulfonamidoindane **8**: according to the procedure used for **5**: 2-methoxy-5-chlorobenzoic acid (0.75 g) and **2** (1 g) afforded **8** as a white powder. Yield: 39%. Mp: 231 °C. ^1H NMR (DMSO) δ ppm: 2.90–3.02 (m, 2H, CH_2(1) or (3)); 3.28–3.32 (m, 2H, CH_2(1) or (3)); 3.83 (s, 3H, CH_3); 4.73 (m, 1H, CH(2)); 7.15 (d, 1H, CH(3′)); 7.28 (s, 2H, SO_2NH_2); 7.41 (d, 1H, CH(7)); 7.50 (dd, 1H, CH(4′)); 7.58 (d, 1H, CH(6′)); 7.64 (d, 1H, CH(6)); 7.69 (s, 1H, CH(4)); 8.50 (d, 1H, NH). MS (electrospray): $MH^+ = 381$. Analysis: calcd: C 53.61, H 4.50, N 7.36, S 8.42; found: C 53.49, H 4.46, N 7.12, S 7.31.

2-(2′,3′,5′,6′-Fluorobenzenecarbonyl)amino-5-sulfonamidoindane **9**: according to the procedure used for **5**: 2,3,5,6-fluorobenzoic acid (0.78 g) and **2** (1 g) afforded **9** as a white powder. Yield: 46%. Mp: 245 °C. ^1H NMR (DMSO) δ ppm: 2.89–2.95 (m, 2H, CH_2(1) or (3)); 3.34–3.42 (m, 2H, CH_2(1) or (3)); 4.73 (m, 1H, CH(2)); 7.30 (s, 2H, SO_2NH_2); 7.44 (d, 1H, CH(7)); 7.66 (d, 1H, CH(6)); 7.72 (s, 1H, CH(4)); 7.80 (m, 1H, CH(ar)); 9.33 (d, 1H, NH). MS (electrospray): $MH^+ = 389$; $MNH4^+ = 406$.

17. Human CA I and CA II cDNAs were expressed in *Escherichia coli* strain BL21 (DE3) from the plasmids pACA/pCA I and pACA/hCA II as previously described.[14] Cell growth conditions were those described by this group[14] and enzymes were purified by affinity chromatography according to the method of Khalifah et al.[18] Enzyme concentrations were determined spectrophotometrically at 280 nm, using a molar absorptivity of $49\,mM^{-1}cm^{-1}$ for CA I and $54\,mM^{-1}cm^{-1}$ for CA II, respectively, based on $Mr = 28.85$ kDa for CA I and $Mr = 29.30$ kDa for CA II.
18. Khalifah, R. G.; Strader, D. J.; Bryant, S. H.; Gibson, S. M. *Biochemistry* **1977**, *16*, 2241–2247.
19. 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 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 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. Triplicate experiments were done for each inhibitor concentration, and the values reported throughout the paper are the mean of such results.