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# Synthesis and biological profile of new 1,2,3,4-tetrahydroisoquinolines as selective carbonic anhydrase inhibitors

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ABSTRACT

In a previous paper we identified several 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-2-sulfonamides that displayed inhibitory effects toward selected carbonic anhydrase isozymes at micromolar concentration. In order to deepen the structure–activity relationships (SARs) and identify novel compounds with improved activity, we synthesized a series of monomethoxy analogues of the previously investigated dimethoxy derivatives. The evaluation of biological profile has been focused on in vitro effects against several CA isoforms. The new monomethoxy derivatives showed higher hCA inhibitory effects against several isoforms compared to the dimethoxy analogues. Particularly, some of these compounds (e.g., **1b** and **1h**) showed low nanomolar $K_I$ values and excellent selectivity for hCA IX and hCA XIV versus hCA I and II inhibition.



## 1. Introduction

The carbonic anhydrases (CAs, EC 4.2.1.1) are a family of zinc metalloenzymes that catalyze the reversible hydration of $CO_2$. Since this reaction regulates a broad range of physiological functions, the pharmacological modulation of CA activity could be useful for the treatment of several human diseases.[1] There are 16 human known CA (hCA) isoforms with different tissue distribution, expression levels, and subcellular locations; some of these isozymes (e.g., hCA II, IV, VA, VB, VII, IX, XII, XIII, and XIV) constitute valid targets for the development of anticancer,[2] antiglaucoma,[3] antiobesity,[4] or anticonvulsant drugs.[5] However, the CA diffuse localization in many tissues and organs limits their potential clinical applications. So the development of CA inhibitors (CAIs) possessing high potency and selectivity against specific isoforms represents an attractive strategy to obtain new active compounds thus avoiding side effects and improving therapeutic safety.[1,6] Most of known CAIs contain a specific moiety (sulfonamide, sulfamate or sulfamide) able to coordinate the zinc ion of catalytic binding site[7,8] (e.g., zonisamide, topiramate and its sulfamide analogue, Fig. 1) inhibiting in this way the enzymatic activity. These inhibitors also bear specific functional groups that interact with important amino acid residues thus driving the selectivity against the different isoforms as confirmed by X-ray crystallographic data of complexes between CAIs and isozymes available in the literature.[9–16]

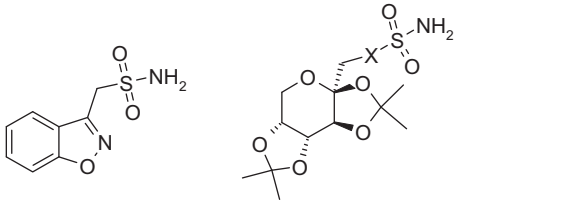


**Figure 1.** Carbonic anhydrase inhibitors.

There is a significant interest in the development of selective inhibitors targeting specific and druggable isoforms such as hCA IX and hCA XIV.[9,17] hCA IX is expressed in a limited number of normal tissues, whereas it is overexpressed in many solid tumors and considered involved in critical processes connected with cancer progression.[17] The expression of CA IX is strongly up-regulated by hypoxia via the hypoxia inducible factor-1 (HIF-1) transcription factor.[18,19] The overexpression of CA IX induces the pH imbalance of tumor issue contributing significantly to the extracellular acidification of solid tumor; thereby CA IX inhibitors could specifically bind hypoxic tumor cells expressing this isoform, consequently they have been proposed as antitumor agents.[20] CA XIV is a transmembrane

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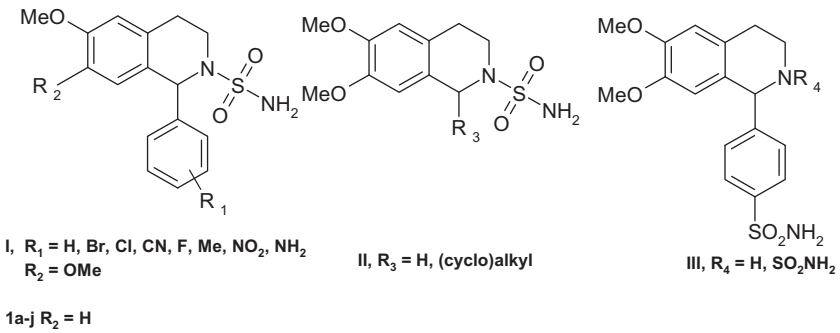



**Figure 2.** Target compounds.

isozyme with the active site oriented extracellularly; it is highly abundant in neurons and axons in the murine and human brain, where it seems to play an important role in modulating excitatory synaptic transmission.[21]

Our previous studies indicated that some sulfamide-based heterocyclic compounds such as 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline derivatives (**I**, Fig. 2) inhibited several CA isoforms at micromolar concentration but showing poor selectivity.[22] Two members of this series were also efficacious as anticonvulsants in audiogenic seizure test exhibiting more efficacy than topiramate. Successively we extended our study to other 1,2,3,4-tetrahydroisoquinoline-2-sulfonamides (**II** and **III**, Fig. 2)[23,24] containing some structural modifications in search of the enhancement both of activity and selectivity. The results of this study were the identification of potent and selective inhibitors towards hCA VII, hCA IX and hCA XIV isoforms, making them as ideal candidates for a potential treatment of several diseases in which these isozymes are involved. In addition, some molecules displayed low affinity for hCA II isoform that is considered responsible of unwanted side effects of CAIs currently in therapy. By means of docking experiments we suggested the main interactions that some selective 1,2,3,4-tetrahydroisoquinoline-2-sulfonamides can engage into catalytic pocket producing hCA IX and hCA XIV selectivity over hCA II.[23] Moreover, X-ray crystallographic studies of these inhibitors in complex with wide-expressed hCA II isoform displayed the main interactions between its catalytic pocket and some active 1,2,3,4-tetrahydroisoquinoline-2-sulfonamide derivatives.[23] From these crystallographic data, it seems that the inhibitor arrangement in catalytic pocket was also influenced by the methoxy-groups and suitable substituents at C-1 position on isoquinoline skeleton.[23,25]

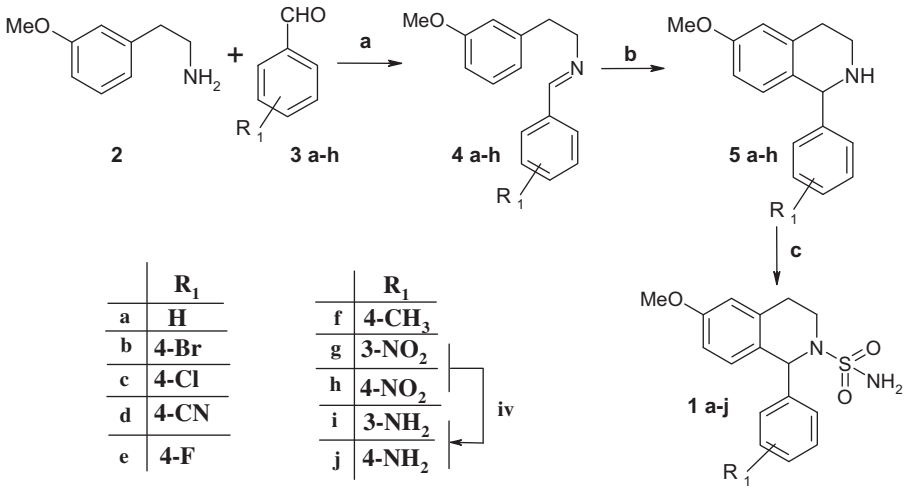
To obtain further SAR information about this class of compounds we herein report the synthesis of novel 1-aryl-6-methoxy-1,2,3,4-tetrahydroisoquinoline-2-sulfonamides ($R_2$ = H, see Fig. 2) in which we maintained the moiety able to coordinate the Zinc ion of metalloenzyme giving inhibitory effects,[8] and we explored the effects of 7-methoxysubstituent deletion as well as the role of the various substituents on arylgroup at C-1 position. The new synthesized compounds were evaluated as CAIs towards various isozymes (e.g., hCA I, hCA II, hCA IX, and hCA XIV).

## 2. Results and discussion

Scheme 1 describes the synthetic pathway for obtaining the 1-aryl-6-methoxy-1,2,3,4-tetrahydroisoquinoline-2-sulfonamides **1a–j**. First, following a previously reported method in Microwave Assisted Organic Synthesis (MAOS) conditions,[22] we prepared the precursors **5a–h**. In particular, by reaction of the 2-(3′-methoxyphenyl)ethylamine (**2**) with suitable benzaldehydes **3a–h** and in solvent free condition, we obtained the corresponding imine intermediates **4a–h**, which were treated with trifluoroacetic acid (TFA) to provide the desired isoquinolines **5a–h**. Successively, these intermediates reacted with a large excess of sulfamide leading to the 1-aryl-6-methoxy-1,2,3,4-tetrahydroisoquinoline-2-sulfonamides **1a–h** as racemic mixture. Finally the aminophenyl derivatives **1i–j** were prepared by reduction of the corresponding nitrophenyl analogues **1g–h**. The structures of all obtained compounds were supported by elemental analyses and spectroscopic measurements.

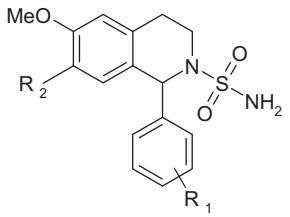
The new synthesized 1-aryl-6-methoxy-1,2,3,4-tetrahydroisoquinoline-2-sulfonamides (**1a–j**) were subjected to evaluation of their inhibition ability of the catalytic activity of hCA I, hCA II, hCA IX and hCA XIV isozymes (Table 1). The data obtained were compared with those of the previously reported dimethoxy analogues **Ia–j**;[22] topiramate and zonisamide were included as reference compounds in this study. The analysis of the screening results displayed in Table 1 highlighted that the new synthesized isoquinoline-*N*-sulfonamides **1a–j** were generally micromolar inhibitors ($K_I$ from 88 to 6003 nM values) of hCA I and hCA II isoforms, that are considered responsible of unwanted side-effects characterizing non-selective inhibitors currently in therapy. On the contrary, compounds **1a–j** were potent inhibitors of hCA IX and hCA XIV isoforms in the range of 5–78 nM and showed higher activity than dimethoxy analogues **Ia–j**. Given that the unique difference between these two classes of molecules is the presence/absence of 7-methoxy group we can speculate that this substituent is the main player influencing the interaction within catalytic site.

Collecting structure–activity relationship data we mainly found that the effect of the nature of $R_1$ substituent on the C-1 phenyl ring is rather variable. The unsubstituted derivative **1a** was a very potent hCA XIV inhibitor ($K_I$ value of 7.9 nM), whereas its inhibitory efficacy towards hCA IX was significant lower than other analogues bearing aryl substituted moiety. The presence of nitro group at 4′- or 3′-position of C-1 phenyl substituent optimizes the inhibitory effects of 1-aryl-6-methoxy-1,2,3,4-tetrahydroisoquinoline-2-sulfonamides (e.g., **1h** and **1g**) towards druggable hCA IX and hCA XIV isoforms. Particularly, the 4′-nitro derivative **1h** behaves as a very potent hCA IX inhibitor being about 15-fold more potent than unsubstituted compound **1a**. The 4′-fluorine derivative **1e** displayed a potency very similar to that of the highest active nitro-analogue **1g** as hCA XIV inhibitor (6.8 vs 6.3 nM), even if we observed that the introduction of halo-substituent generally was detrimental for the affinity towards all studied CA isoforms. The compounds incorporating hydrophilic $R_1$ substituents (e.g., amino or cyano groups) possessed a similar potency of analogs containing lipophilic ones (see Table 1). Overall, the majority of $K_I$ values was clustered within a restricted range thus suggesting a slight impact on SAR of the 1-aryl substituents; in fact sulfonamides **1g**, **1e**, **1i**, **1f**, **1a**, **1b**, **1j**, and **1h** were potent hCA XIV inhibitors showing $K_I$ values ranging from 6.3 to 9.5 nM. Notable, one of the most active compound **1g** displayed $K_I$ value of 6.3 nM against hCA XIV, that is about 1000-fold lower than corresponding $K_I$ value




**Scheme 1.** Reagents and conditions: (a) MW: 10 min, 90 °C, 150 W; (b) TFA MW: 10 min, 90 °C, 150 W; (c) $CH_3CH(OCH_3)_2$, $NH_2SO_2NH_2$, two steps 20 min, 100 °C, 150 W; iv) Zn/HCl conc., Δ, 2 h.

**Table 1**
Inhibition of hCA I, hCA II, hCA IX and hCA XIV isoforms by 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines-2-sulfonamides (**Ia–j**), 1-aryl-6-methoxy-1,2,3,4-tetrahydroisoquinoline-2-sulfonamides (**1a–j**), topiramate and zonisamide




| | $R_1$ | $R_2$ | $K_I$[a] (nM) | | | |
|---|---|---|---|---|---|---|
| | | | hCA I | hCA II | hCA IX | hCA XIV |
| **Ia**[b] | H | MeO | 8980 | 15700 | 8440 | 3860 |
| **Ib**[b] | 4′-Br | MeO | 25700 | 10710 | 8200 | 7020 |
| **Ic**[b] | 4′-Cl | MeO | 9440 | 5300 | 7540 | 8980 |
| **Id**[b] | 4′-CN | MeO | 4070 | 4240 | 4980 | 3040 |
| **Ie**[b] | 4′-F | MeO | 4430 | 14980 | 5590 | 6930 |
| **If**[b] | 4′-Me | MeO | 3490 | 10770 | 6750 | 7050 |
| **Ig**[b] | 3′-$NO_2$ | MeO | 7690 | 3820 | 6450 | 5350 |
| **Ih**[b] | 4′-$NO_2$ | MeO | 330 | 600 | 480 | 210 |
| **Ii**[b] | 3′-$NH_2$ | MeO | 3830 | 9310 | 8130 | 6670 |
| **Ij**[b] | 4′-$NH_2$ | MeO | 8070 | 11300 | 6780 | 3070 |
| **1a** | H | H | 6003 | 422 | 78 | 7.9 |
| **1b** | 4′-Br | H | 4790 | 4184 | 60.7 | 8.3 |
| **1c** | 4′-Cl | H | 101 | 104 | 21.8 | 53.9 |
| **1d** | 4′-CN | H | 995 | 91 | 7.0 | 19.8 |
| **1e** | 4′-F | H | 5134 | 476 | 31 | 6.8 |
| **1f** | 4′-Me | H | 957 | 88 | 7.5 | 7.7 |
| **1g** | 3′-$NO_2$ | H | 3514 | 293 | 16 | 6.3 |
| **1h** | 4′-$NO_2$ | H | 5213 | 320 | 5.0 | 9.5 |
| **1i** | 3′-$NH_2$ | H | 4815 | 99.7 | 18 | 7.1 |
| **1j** | 4′-$NH_2$ | H | 4793 | 222 | 12 | 8.6 |
| Topiramate[b] | | | 250 | 10 | 58 | 1460 |
| Zonisamide[b] | | | 56 | 35 | 5.1 | 5250 |

[a] Errors in the range of ±10% of the reported value, from three different assays. Recombinant full length hCA I, II and XIV and catalytic domain of hCA IX were used.
[b] Ref. 22

of 5350 nM measured for analogue **Ig**. Furthermore, compounds **1a–j** were also more selective against hCA IX and hCA XIV over the off-target hCA II isoform. The most active hCA IX inhibitor 4′-nitro derivative **1h** ($K_I$ value of 5.0 nM) had >1000-fold selectivity over hCA I as well as >60-fold selectivity over hCA II. Compound **1b** ($K_I$ value of 8.3 nM) showed excellent hCA XIV selectivity displaying $K_I$ values more than 4000 against hCA I and hCA II.

In conclusion, this study allowed us to identify new CAIs containing the isoquinoline scaffold, several of which proved to be very efficient ($K_I$ <10 nM) inhibitors exhibiting good selectivity towards the established antitumor target hCA IX and the potentially druggable one hCA XIV. Considering the structure–property relationships we found that (a) the introduction of an additional 7-methoxy group dramatically decreases the inhibitory efficacy; (b) the nature of the substituent on 1-aryl moiety plays a key role in selectivity over off-target hCA I and hCA II isozymes; (c) whereas this substituent seems to be not extremely relevant to control the potency of inhibitory effects against hCA IX and hCA XIV. Finally, the most active compounds were found to be more potent and selective inhibitors than well known CAIs such as topiramate and zonisamide. Overall, these findings contribute to improve our knowledge on SAR of the CAIs containing this sulfamide-based heterocyclic scaffold which may allow interesting future developments for obtaining isoform-selective inhibitors of these enzymes.

## 3. Experimental section

### 3.1. Chemistry

All starting materials and reagents commercially available (Sigma–Aldrich Milan, Italy) were used without further purification. Microwave-assisted reactions were carried out in a CEM focused Microwave Synthesis System. Melting points were determined on a Buchi B-545 apparatus and are uncorrected. Elemental analyses (C, H, N) were carried out on a Carlo Erba Model 1106 Elemental Analyzer and the results are within ±0.4% of the theoretical values. Merck silica gel 60 F254 plates were used for analytical TLC. $R_f$ values were determined on TLC plates using a mixture of $CH_2Cl_2$/$CH_3OH$ (96/4) as eluent. Flash Chromatography (FC) was carried out on a Biotage $SP_1$ EXP. $^1H$ NMR spectra were measured in $CDCl_3$ (TMS as internal standard) or DMSO-$d_6$ with a Varian Gemini 300 spectrometer; chemical shifts are expressed in δ (ppm) and coupling constants (*J*) in Hz. All exchangeable protons were confirmed by addition of $D_2O$.

#### 3.1.1. General procedure for the synthesis of 1-aryl-6-methoxy-1,2,3,4-tetrahydroisoquinolines (5a–h)

A mixture of 2-(3′-methoxyphenyl)ethylamine (**2**) (2.0 mmol) and suitable aldehyde **3a–h** (2.4 mmol), was placed in a cylindrical quartz tube (ø2 cm), in solvent free condition, then stirred and

irradiated in a microwave oven at 150 W for 10 min at 90 °C; after cooling to room temperature trifluoroacetic acid (2.5 mL) was added to crude intermediates **4a–h** obtained in the previous step and the mixture was irradiated at 150 W for 10 min at 90 °C. The reaction was quenched by adding water and the mixture was basified (pH 8–9) with sodium hydroxide 2 N (10 mL). The crude product was purified by crystallization with EtOH to afford compounds **5a–h** as white powder. Compounds **5a–c**, **5e**, **5g–h** were already synthesized; by means of the application of microwave irradiation as well as the employment of a different synthetic pathway these compounds were re-synthesized thus reducing reaction times and improving in same cases the yields; their analytical data are in accordance with literature.[26]

**3.1.1.1. (R,S)-1-(4′-Cyanophenyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline (5d).** Yield 40%. Mp 211–215 °C. $R_f$ = 0.30. $^1$H NMR (DMSO-$d_6$) $\delta$: 2.66–3.06 (4H, m, $CH_2CH_2$), 3.63 (3H, s, $OCH_3$), 4.96 (1H, s, CH), 6.50 (1H, d, $J$ = 8.5, ArH), 6.56–6.60 (1H, dd, $J$ = 3.2, $J$ = 7.5, ArH), 6.68 (1H, d, $J$ = 3.2, ArH), 7.29 (2H, d, $J$ = 7.5, ArH), 7.78 (2H, d, $J$ = 7.5, ArH), 7.91 (br s, 1H, NH). Anal. Calcd for $C_{17}H_{16}N_2O$: C, 77.25; H, 6.10; N, 10.60. Found: C, 76,85; H, 6.50; N, 10.26.

**3.1.1.2. (R,S)-6-Methoxy-1-(4′-methylphenyl)-1,2,3,4-tetrahydroisoquinoline (5f).** Yield 45%. Mp 105–108 °C. $R_f$ = 0.06. $^1$H NMR ($CDCl_3$) $\delta$: 2.45 (3H, s, $Ch_3$), 2.95–3.02 (1H, m, $CH_2CH_2$), 3.19–322 (2H, m, $CH_2CH_2$), 3.30–3.38 (1H, m, $CH_2CH_2$), 3.90 (3H, s, $OCH_3$), 5.47 (1H, s, CH), 6.73–6.82 (3H, m, ArH), 7.25–7.38 (4H, m, ArH). Anal. Calcd for $C_{17}H_{19}NO$: C, 80.60; H, 7.56; N, 5.53. Found: C, 81.02; H, 7.96; N, 5.13.

### 3.1.2. General procedure for the synthesis of 1-aryl-6-methoxy-1,2,3,4-tetrahydroisoquinoline-2-sulfonamides (1a–h)

A solution of the appropriate 1-aryl-6-methoxy-1,2,3,4-tetrahydroisoquinoline **5a–h** (1.0 mmol) and sulfamide (6 mmol) in dimethoxyethane (2 mL) was placed in a cylindrical quartz tube (ø2 cm), then stirred and irradiated in a microwave oven at 150 W for two steps of 20 min at 100 °C. The reaction was quenched by addition of water (5 mL) and extracted with ethyl acetate (3 × 5 mL). The organic layer was washed with an aqueous saturated solution of $NaHCO_3$ (2 × 5 mL), dried over $Na_2SO_4$ and concentrated until dryness under reduced pressure. From the obtained residue compound **1d** and **1g** were crystallized using diethyl ether to give white powders. The other compounds were purified by flash chromatography using a mixture of $CH_2Cl_2/CH_3OH$ (96/4) as eluent.

**3.1.2.1. (R,S)-6-Methoxy-1-phenyl-1,2,3,4-tetrahydroisoquinoline-2-sulfonamide (1a).** Yield 55%. Mp 124–128 °C. $\boldsymbol{R_f}$ = 0.80. $^1$H NMR ($CDCl_3$) $\boldsymbol{\delta}$ :2.75–3.78 (4H, m, $CH_2CH_2$), 3.81 (3H, s, $OCH_3$), 4.11 (2H, br s, $NH_2$), 6.02 (1H, s, CH), 6.73 (2H, m, ArH), 6.88 (1H, m, ArH), 7.27–7.35 (5H, m, ArH). Anal. Calcd for $C_{16}H_{18}N_2O_3S$: C, 60.36; H, 5.70; N, 8.80. Found: C, 59.96; H, 6.12; N, 9.21.

**3.1.2.2. (R,S)-1-(4′-Bromophenyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-2-sulfonamide (1b).** Yield 40%. Mp 110–114 °C. $R_f$ = 0.81. $^1$H NMR (DMSO-$d_6$) $\delta$: 2.64–2.69 (1H, m, $CH_2CH_2$), 3.02–3.17 (2H, m, $CH_2CH_2$), 3.52–3.57 (1H, m, $CH_2CH_2$), 3.75 (3H, s, $OCH_3$), 5.83 (1H, s, CH), 6.75–6.79 (2H, m, ArH), 6.92 (2H, br s, $NH_2$), 6.96–6.99 (1H, d, ArH), 7.13 (2H, d, $J$ = 8.2, ArH), 7.48 (2H, d, $J$ = 8.2, ArH). Anal. (C, H, N). Anal. Calcd for $C_{16}H_{17}BrN_2O_3S$: C, 48.37; H, 4.31; N, 7.05. Found: C, 47.97; H, 3.91; N, 7.45.

**3.1.2.3. (R,S)-1-(4′-Chlorophenyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-2-sulfonamide (1c).** Yield 60%. Mp 100–104 °C. $R_f$ = 0.82. $^1$H NMR ($CDCl_3$) $\delta$: 2.64–2.69 (1H, m, $CH_2CH_2$), 3.23–3.80 (3H, m, $CH_2CH_2$), 3.82 (3H, s, $OCH_3$), 4.12 (2H, br s, $NH_2$), 6.01 (1H, s, CH), 6.75–6.79 (2H, m, ArH), 6.88 (1H, d, ArH), 7.20 (2H, d, $J$ = 8.5, ArH), 7.29 (2H, d, $J$ = 8.5, ArH). Anal. (C, H, N). Anal. Calcd for $C_{16}H_{17}ClN_2O_3S$: C, 54.47; H, 4.86; N, 7.94. Found: C, 54.10; H, 4.46; N, 7.54.

**3.1.2.4. (R,S)-1-(4′-Cyanophenyl)-6-methoxy–1,2,3,4-tetrahydroisoquinoline-2-sulfonamide (1d).** Yield 60%. Mp 158–162 °C. $R_f$ = 0.35. $^1$H NMR (DMSO-$d_6$) $\delta$: 2.66–2.71 (1H, m, $CH_2CH_2$), 3.14–3.31 (2H, m, $CH_2CH_2$), 3.52–3.58 (1H, m, $CH_2CH_2$), 3.82 (3H, s, $OCH_3$), 5.88 (1H, s, CH), 6.75–6.80 (2H, m, ArH), 6.94 (2H, br s, $NH_2$), 6.97–7.00 (1H, m, ArH), 7.25 (2H, d, $J$ = 7.4, ArH), 7.78 (2H, d, $J$ = 7.4, ArH). Anal. Calcd for $C_{17}H_{17}N_3O_3S$: C, 59.46; H, 4.99; N, 12.24. Found: C, 59.16; H, 5.39; N, 12.64.

**3.1.2.5. (R,S)-1-(4′-Fluorophenyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-2-sulfonamide (1e).** Yield 30%. Mp 46–50 °C. $R_f$ = 0.81. $^1$H NMR ($CDCl_3$) $\delta$: 2.74–2.80 (1H, m, $CH_2CH_2$), 3.12–3.29 (2H, m, $CH_2CH_2$), 3.81–3.83 (3H, m, $OCH_3$ and 1H, $CH_2CH_2$), 4.12 (2H, br s, $NH_2$), 6.01 (1H, s, CH), 6.73–6.77 (2H, m, ArH), 6.87–6.89 (1H, m, ArH), 6.97–7.23 (4H, m, ArH). Anal. (C, H, N). Anal. Calcd for $C_{16}H_{17}FN_2O_3S$: C, 57.13; H, 5.09; N, 8.33. Found: C, 57.53; H, 5.49; N, 8.73.

**3.1.2.6. (R,S)-6-Methoxy-1-(4′-methylphenyl)-1,2,3,4-tetrahydroisoquinoline-2-sulfonamide (1f).** Yield 45%. Mp 111–115 °C. $\boldsymbol{R_f}$ = 0.83. $^1$H NMR (DMSO-$\boldsymbol{d_6}$) $\boldsymbol{\delta}$: 2.25 (3H, s, $CH_3$), 2.62–2.67 (1H, m, $CH_2CH_2$), 3.03–3.14 (2H, m, $CH_2CH_2$), 3.54–3.58 (1H, m, $CH_2CH_2$), 3.74 (3H, s, $OCH_3$), 5.81 (1H, s, CH), 6.73–6.78 (2H, m, ArH), 6.86 (2H, br s, $NH_2$), 6.91–6.94 (1H, m, ArH), 7.03 (2H, d, $\boldsymbol{J}$ = 7.9, ArH), 7.09 (2H, d, $\boldsymbol{J}$ = 7.9, ArH). Anal. (C, H, N). Anal. Calcd for $C_{17}H_{20}N_2O_3S$: C, 61.42; H, 6.06; N, 8.43. Found: C, 61.03; H, 5.66; N, 8.03.

**3.1.2.7. (R,S)-6-Methoxy-1-(3′-nitrophenyl)-1,2,3,4-tetrahydroisoquinoline-2-sulfonamide (1g).** Yield 60%. Mp 140–144 °C. $\boldsymbol{R_f}$ = 0.71. $^1$H NMR (DMSO-$\boldsymbol{d_6}$) $\boldsymbol{\delta}$: 2.70–2.77 (1H, m, $CH_2CH_2$), 3.06–3.27 (2H, m, $CH_2CH_2$), 3.51–3.57 (1H, m, $CH_2CH_2$), 3.76 (3H, s, $OCH_3$), 5.99 (1H, s, CH), 6.78–6.84 (2H, m, ArH), 7.01 (2H, br s, $NH_2$), 7.05–7.08 (1H, m, ArH), 7.59–7.68 (2H, m, ArH), 8.01–8.14 (2H, m, ArH). Anal. (C, H, N). Anal. Calcd for $C_{16}H_{17}N_3O_5S$: C, 52.88; H, 4.72; N, 11.56. Found: C, 52.55; H, 4.38; N, 11.16.

**3.1.2.8. (R,S)-6-Methoxy-1-(4′-nitrophenyl)-1,2,3,4-tetrahydroisoquinoline-2-sulfonamide (1h).** Yield 40%. Mp 55–59 °C. $R_f$ = 0.77. $^1$H NMR ($CDCl_3$) $\delta$: 2.73–3.79 (4H, m, $CH_2CH_2$), 3.81 (3H, s, $OCH_3$), 4.42 (2H, br s, $NH_2$), 6.06 (1H, s, CH), 6.76–6.93 (3H, m, ArH), 7.43 (2H, d, $J$ = 8.8, ArH), 8.13 (2H, d, $J$ = 8.8, ArH). Anal. (C, H, N). Anal. Calcd for $C_{16}H_{17}N_3O_5S$: C, 52.88; H, 4.72; N, 11.56. Found: C, 52.48; H, 4.30; N, 11.18.

### 3.1.3. General procedure for the synthesis of 1-(3′-aminophenyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-2-sulfonamide (1i) and 1-(4′-aminophenyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-2-sulfonamide (1j)

A solution of appropriate nitro-derivatives (**1g** or **1h**, 0.6 mmol) in 3 mL of HCl and 4 mL of EtOH was vigorously stirred and zinc dust (20 mmol) was added in several portions at room temperature. The reaction mixture was heated in a water bath for 1 h, cooled, made alkaline with a solution of NaOH 2 N (10 mL), and then extracted with ethyl acetate (3 × 5 mL). The organic phase washed with water, dried over $Na_2SO_4$ and evaporated. The residue was crystallized from ethanol to give the desired products **1i** and **1j**.

**3.1.3.1. (R,S)-1-(3′-Aminophenyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-2-sulfonamide (1i).** Yield 42%. Mp 116–120 °C. $R_f$ = 0.49. $^1$H NMR (DMSO-$d_6$) $\delta$: 2.62–2.87 (1H, m, $CH_2CH_2$),

3.12–3.29 (2H, m, $CH_2CH_2$), 3.54–3.68 (1H, m, $CH_2CH_2$), 3.73 (3H, s, $OCH_3$), 5.84 (1H, s, CH), 6.73–6.76 (2H, m, ArH), 6.77 (2H, br s, $NH_2$), 6.90–6.96 (1H, m, ArH), 7.07–7.20 (6H, m, ArH). Anal. Calcd for $C_{16}H_{19}N_3O_3S$: C, 57.64; H, 5.74; N, 12.60. Found: C, 58.04; H, 6.14; N, 12.26.

**3.1.3.2. (R,S)-1-(4′-Aminophenyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-2-sulfonamide (1j).** Yield 70%. Mp 175–179 °C. $R_f$ = 0.50. $^1$H NMR (DMSO-$d_6$) $\delta$: 2.59–2.63 (1H, m, $CH_2CH_2$), 3.02–3.08 (2H, m, $CH_2CH_2$), 3.54 (1H, m, $CH_2CH_2$), 3.72 (3H, s, $OCH_3$), 4.98 (2H, br s, $NH_2$), 5.69 (1H, s, CH), 6.42–6.87 (9H, m, ArH). Anal. Calcd for $C_{16}H_{19}N_3O_3S$: C, 57.64; H, 5.74; N, 12.60. Found: C, 58.04; H, 6.14; N, 12.36.

### 3.2. CA inhibition assay

An 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 10–20 mM Hepes (pH 7.5) or Tris (pH 8.3) as buffers, and 20 mM $Na_2SO_4$ or 20 mM $NaClO_4$ (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed $CO_2$ hydration reaction for a period of 10–100 s. The $CO_2$ concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (10 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, and represent the mean from at least three different determinations. CA isoforms were recombinant ones obtained as reported earlier by this group.[27–30]

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