Bioorganic & Medicinal Chemistry 17 (2009) 3659–3664

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis and evaluation of pharmacological profile of 1-aryl-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamides

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ARTICLE INFO

Article history:
Received 27 January 2009
Revised 26 March 2009
Accepted 29 March 2009
Available online 7 April 2009

Keywords:
Isoquinolines
Anticonvulsants
DBA/2 mice
Carbonic anhydrase inhibitors

ABSTRACT

In previous studies we identified several 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline derivatives displaying potent anticonvulsant effects in different animal models of epilepsy. With the aim to deepen the structure–activity relationships (SAR) for this class of compounds and identify novel anticonvulsant agents we synthesized a series of 1-aryl-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamides. The new compounds incorporate the main features of the above-mentioned anticonvulsants and a sulfonamide function capable to inhibit the enzyme carbonic anhydrase (CA, EC 4.2.1.1), which represents an attractive target in epilepsy. Pharmacological effects were evaluated in vivo against audiogenic seizures in DBA/2 mice and in vitro against several CA isoforms. Some of the new molecules showed anticonvulsant properties better than topiramate, but weak inhibitory activity and low selectivity in enzymatic assay.

1. Introduction

Epilepsy affects ~1% of the population worldwide; even though most people become seizure-free with drug therapy there is a significant group of patients (30%) who are resistant to the currently available antiepileptic drugs (AEDs) and more effective therapies are needed. The efficacy of anticonvulsant drugs may be connected to different molecular targets to reduce the excitability of neurons involved in seizures on-set. For anticonvulsant activity several mechanisms can be considered and the different targets include not only voltage-gated ion channels (Na^+, Ca^{2+}, K^+, Cl^-) or ligand-gated ion channels (GABA, glutamate, glycine, etc.) but also neurotransmitter transporters (GAT1-4, EAAT1-5), and enzymes (GABA-transaminase, carbonic anhydrase, specific protein kinases).[1,2] The marketed anticonvulsants predominantly target voltage-gated cation channels, the improvement of GABA-mediated inhibition, and the blockade of excitatory glutamatergic neurotransmission.[3,4] Nevertheless, the newer agents currently in preclinical or clinical development are characterized by pioneering mechanisms of action.[5]

In previous papers, we planned the synthesis of N-substituted 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines and identified some potent anticonvulsant agents acting as noncompetitive antagonists by interaction with glutamate ionotropic AMPA receptor (AMPAR) complex.[6–12] In particular, the 2-acetyl-1-(4′-chlorophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**A**) and 1-(4′-bromophenyl)-6,7-dimethoxy-2-(piperidin-1-yl-acetyl)-3,4-dihydroisoquinoline (**B**), the most active compounds of the series (Fig. 1), showed stronger potency in in vivo and in vitro tests when compared with other known AMPAR antagonists.[8,11]

Starting from these promising results we synthesized a large series of analogues exploring the effect of the modifications both of the moiety at N-2 position and the substituent on the C-1 phenyl group, as well as on the benzene fused ring. Structure-activity relationship (SAR) studies highlighted that the substituent at the N-2

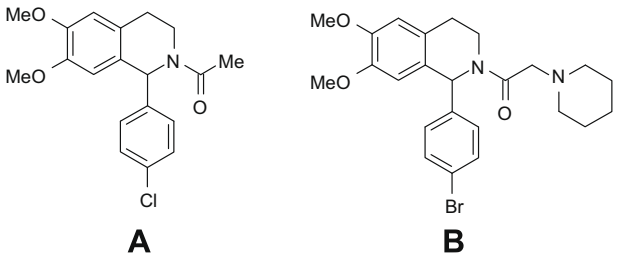

Figure 1. 1-Aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines as AMPAR antagonists.

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doi:10.1016/j.bmc.2009.03.066

position of tetrahydroisoquinoline system plays the principal role in the process of receptor recognition, as had been already suggested by our computational studies.[7,8]

On the basis of these findings to identify new anticonvulsant agents containing isoquinoline skeleton we herein report the synthesis of 1-aryl-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamides (**1a–j**, Fig. 2) in which we explored if the sulfonamide function could be a feature useful to improve the anticonvulsant efficacy.

We selected this moiety taking in account that sulfonamide or sulfamate functions are able to coordinate the metal ion of some metalloenzymes[13,14] such as carbonic anhydrase (CA) whose inhibition has been shown to play an important role in convulsion control due to CO_2 retention secondary to the inhibition of enzyme.[15] In fact the anticonvulsants zonisamide[16] and topiramate[17] (Fig. 3), currently in therapy, are CA inhibitors, although their mechanism of action cannot be completely attributed to this enzyme inhibition.

Our hypothesis was that the introduction of the sulfonamide function into tetrahydroisoquinoline scaffold could determine a pharmacological additive effect. So the suitable synthetic approach has been set up and the new derivatives obtained **(1a–j)** compounds were evaluated as anticonvulsant agents against sound-induced seizures in DBA/2 mice. Moreover their inhibitory activity was assayed on several CA isoforms.

2. Results and discussion

2.1. Chemistry

As depicted in Scheme 1, the synthesis of 1-aryl-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamides (**1a–j**) was carried out through a multistep procedure with good yields. Following a previously reported method[11] and in Microwave Assisted Organic Synthesis (MAOS) conditions,[18] we prepared the 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines (**5a–h**). By reaction of the 6,7-dimethoxyphenethylamine (**2**) with suitable aromatic aldehydes **3a–h** we obtained the corresponding imine intermediates **4a–h**, which without purification were treated with trifluoroacetic acid (TFA) to provide the desired isoquinolines **5a–h**. Successively, the key intermediates **5a–h** reacted with a large excess of sulfamide leading to the 1-aryl-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamides (**1a–h**). In the first approach this step was performed using a conventional method,[19] then it was optimized by application of MAOS, allowing a significantly reaction time reduction and a considerable enhancement of the yields. Finally the aminophenyl derivatives **1i–j** were prepared by reduction of the corresponding nitrophenyl derivatives **1g–h**. The structures of all compounds obtained were supported by elemental analyses and spectroscopic measurements.

2.2. Anticonvulsant properties

The anticonvulsant effects of the designed 1-aryl-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamides (**1a–j**) were evaluated against audiogenic seizures in DBA/2 mice, considered an excellent animal model for generalized epilepsy and for the screening of new anticonvulsant drugs.[20] Table 1 reports the ED_{50} values measured after intraperitoneal (ip) administration of new synthesized compounds; these values were compared with those of parent unsubstituted 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines (**5a–j**) and topiramate as reference compound. The obtained results suggest that the 1-(4′-aminophenyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (**1j**) is 2.5-fold more potent than parent compound **5j**; in addition, the 1-(4′-chlorophenyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (**1c**) is able to protect in audiogenic seizure test at doses that are comparable to those of unsubstituted analogue **5c**. Furthermore, **1c** and **1j**, the most active compounds of the series, were more potent than topiramate. Moreover, compound **1a** displayed significant improvement of anticonvulsant activity in tonic phase of the test. Nevertheless, compounds **1c** and **1j** were less potent than our hits previously synthesized (i.e., compounds **A** and **B**).[8,11] The other sulfonamide derivatives **1a–b** and **1d–i** are characterized by poor anticonvulsant efficacy and lower activity than parent compounds **5a–b** and **5d–i**. These findings highlighted that the introduction of the sulfonamide moiety is not conducive to enhance the anticonvulsant effects except for compound **1j**. Furthermore, we explored the modifications on C-1 phenyl structural fragment. Pharmacological data showed that the best substitution pattern involves the presence of a chlorine atom or an amino group at 4′-position of phenyl group for compounds **1c** and **1j**, respectively. These results suggest that the anticonvulsant properties of this series of derivatives might be directly correlated neither to the electronic nor to lipophilic properties of the 4′-substituent on the phenyl ring. Interestingly, the 4-aminophenyl or 4-chlorophenyl substituents had been suggested, by our previous studies, as struc-

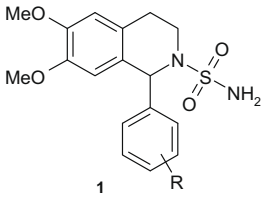


Figure 2. Designed 1-aryl-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamides (**1**).

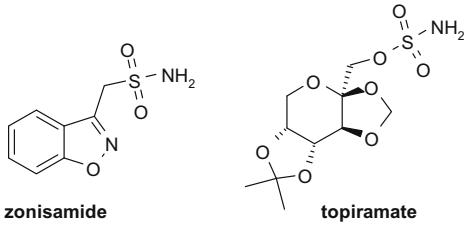


Figure 3. Chemical structure of CA inhibitors Zonisamide and Topiramate.

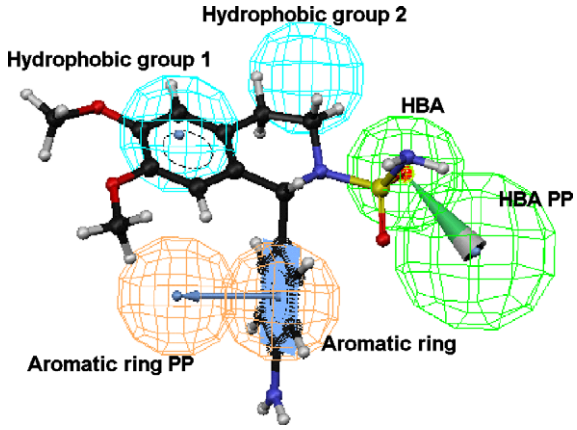
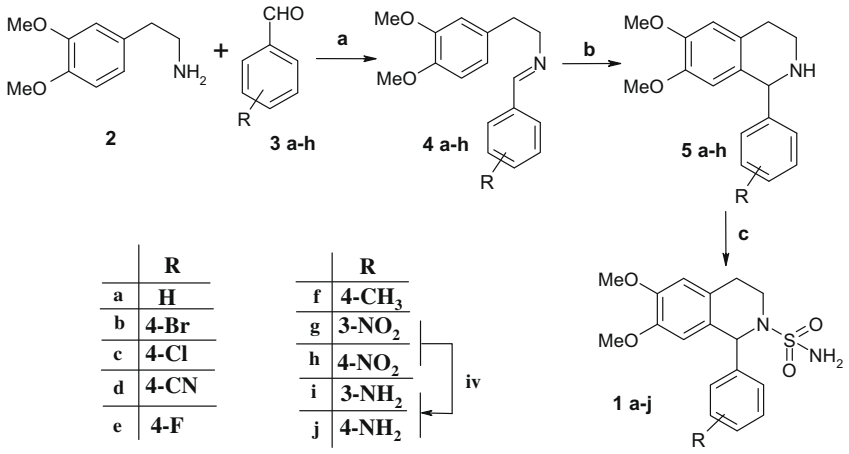
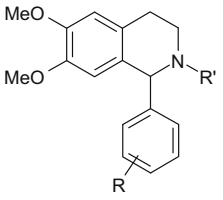


Figure 4. The highest-ranked four-point hypothesis for noncompetitive AMPAR antagonists derived using the Catalyst/HIPHOP program. Cyan: hydrophobic groups; green: hydrogen bond acceptor feature (HBA) with a vector in the direction of the putative hydrogen donor (HBA PP); orange: aromatic ring with proposed δ-stacking interaction shown by an arrow (aromatic ring PP). The alignment of **1j** on the pharmacophore is shown.



Scheme 1. Reagents and conditions: (a) MW: 5 min, 90 °C, 200 Psi, 150 W; (b) TFA MW: 5 min, 90 °C, 200 Psi, 150 W; (c) $CH_3CH(OCH_3)_2$, $NH_2SO_2NH_2$, Δ, 24–48 h or two steps in the same conditions: 20 min, 100 °C, 200 Psi, 150 W; (iv) Zn/HCl concd, Δ, 2 h.

Table 1
Anticonvulsant activity of 1-aryl-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamides (**1a–j**), 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines (**5a–j**) and reference compounds against audiogenic seizures in DBA/2 mice



	R	R′	ED_{50} μmol/kg[a]	
			Clonic phase	Tonic phase
5a[b]	H	H	44.8 (23.9–84.0)	19.3 (9.05–41.3)
5b[b]	4-Br	H	30.6 (19.4–48.2)	12.5 (6.90–22.6)
5c[b]	4-Cl	H	20.1 (9.65–41.9)	19.3(11.8–31.5)
5d	4-CN	H	>100	48.6 (32.9–71.8)
5e[b]	4-F	H	57.8(25.0–133)	26.4(15.5–44.9)
5f	4-Me	H	48.6 (32.8–71.9)	36.5 (28.2–47.3)
5g[b]	3-NO_2	H	19.3 (6.10–61.2)	7.20 (2.45–21.2)
5i[b]	3-NH_2	H	40.4 (21.8–74.7)	18.2 (7.76–41.8)
5h[b]	4-NO_2	H	101 (52.0–194)	56.1 (26.9–117)
5j[b]	4-NH_2	H	46.6 (28.0–77.4)	28.6 (14.4–56.8)
1°	H	SO_2NH_2	>100	7.82 (6.66–9.19)
1b	4-Br	SO_2NH_2	>100	67.3 (44.6–102)
1c	4-Cl	SO_2NH_2	23.5 (14.4–38.5)	7.25 (4.54–11.6)
1d	4-CN	SO_2NH_2	>100	>100
1e	4-F	SO_2NH_2	>100	41.8 (19.7–88.9)
1f	4-Me	SO_2NH_2	97.1 (64.2–147)	51.7 (36.9–104)
1g	3-NO_2	SO_2NH_2	>100	>100
1h	4-NO_2	SO_2NH_2	>100	>100
1i	3-NH_2	SO_2NH_2	>100	65.8 (41.7–104)
1j	4-NH_2	SO_2NH_2	17.7 (9.80–30.1)	3.60 (1.45–8.97)
Compound **A**			4.18 (2.23–7.84)	2.39 (1.30–4.40)
Compound **B**			12.7 (6.09–26.3)	8.17 (4.03–16.6)
Topiramate			35.7 (20.4–62.3)	18.0 (10.2–31.6)

[a] All data were calculated according to the method of Litchfield and Wilcoxon. At least 32 animals were used to calculate each ED_{50}. ±95% confidence limits are given in parentheses.
[b] From Ref. 21.

tural requirements able to positively influence the anticonvulsant activity of AMPA receptor antagonists.[4] With the aim to corroborate this hypothesis we performed the overlapping of the most active derivative on our previously developed 3D-pharmacophore model. The fitting of the 1-(4′-aminophenyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (**1j**) demonstrated good alignment with all the 3D pharmacophoric features requested for AMPA receptor antagonism (Fig. 4). In particular the sulfonamide moiety generates the hydrogen bond acceptor (HBA) feature thus mimicking the carbonyl function of active compounds **A–B**.

2.3. Carbonic anhydrase inhibition

To determine the enzymatic inhibitory activity we assayed the new series of 1-aryl-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamides (**1a–j**) on four important carbonic anhydrase isoforms involved in several physiological and pathological process, hCA I, hCA II, hCA IX and hCA XIV (Table 2). This preliminary screening showed that all tested compounds have a moderate–weak inhibitory activity against these isozymes, with inhibition constants (K_I) in the range of 0.33–25.70 μM. The most interesting compound was 6,7-dimethoxy-1-(4′-nitrophenyl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (**1h**), which demonstrated K_I value lower than that of topiramate against hCA XIV; moreover, **1h** and topiramate inhibited hCA I with similar potency. Nevertheless, the inhibitory effects of **1h** were very similar against the different tested isozymes, exhibiting poor selectivity when compared with topiramate.

For the other tested compounds (**1a–g** and **1i–j**), the data reported in Table 2 show that the nature of 4′-substituent on the

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

	K_I (μM)[a]			
	hCA I	hCA II	hCA IX	hCA XIV
1a	8.98	15.70	8.44	3.86
1b	25.70	10.71	8.20	7.02
1c	9.44	5.30	7.54	8.98
1d	4.07	4.24	4.98	3.04
1e	4.43	14.98	5.59	6.93
1f	3.49	10.77	6.75	7.05
1g	7.69	3.82	6.45	5.35
1h	0.33	0.60	0.48	0.21
1i	3.83	9.31	8.13	6.67
1j	8.07	11.30	6.78	3.07
Topiramate	0.25	0.010	0.058	1.46

[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.[24–27]

phenyl ring does not significantly influence inhibitory effects against hCA I, hCA II, hCA IX and hCA XIV isoforms. Considering these results we could speculate that this molecular frame is not involved in the binding recognition process with the active site. Furthermore, with the aim to confirm that the introduction of sulfonamide moiety determines CA inhibitory effects, we also evaluated the activity of some selected free amines (e.g., **5c** and **5j**) and corresponding N-acetyl derivatives on hCA I, hCA II, hCA IX and hCA XIV isoforms. These compounds were not able to produce any inhibitory effects at higher tested doses (K_i > 200 μM).

3. Conclusions

An innovative approach for the preparation of sulfonamide derivatives has been set up and used to obtain a new series of N-substituted 1-aryl-6,7-dimethoxy-3,4-dihydroisoquinolines (**1a–j**). Among the new synthesized compounds, derivatives **1c** and **1j** proved to be more potent than topiramate against audiogenic seizures in DBA/2 mice. The evaluation of their inhibitory effects on several CA isozymes demonstrated that anticonvulsant properties of **1c** and **1j** are not related to inhibition of carbonic anhydrase. On the contrary, compound **1h** provided good CA inhibitory activity, even if it lacked in vivo properties. Collecting the in vivo and in vitro results we can summarize that the introduction of sulfonamide function on isoquinoline skeleton provides potent anticonvulsant agents, but their activity does not appear correlated only to CA inhibition.

4. Experimental

4.1. Chemistry

Microwave-assisted reactions were carried out in a CEM focused Microwave Synthesis System. Melting points were determined on a Stuart SMP10 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. ^{1}H NMR and ^{13}C 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 hertz. All exchangeable protons were confirmed by addition of D_2O.

4.1.1. General procedure for the synthesis of 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines (5a–h)

A mixture of 2-(3′,4′-dimethoxyphenyl)ethylamine (**2**) (1.0 mmol, 181.24 mg) and suitable aldehyde derivative (1.2 mmol) **3a–h** was placed in a cylindrical quartz tube (Ø 2 cm), then stirred and irradiated in a microwave oven at 280 W for 5 min at 90 °C; after cooling to room temperature trifluoroacetic acid (2 mL) was added to crude intermediates **4a–h** obtained in the previous step and the mixture was irradiated at 280 W for 5 min at 90 °C. The reaction was quenched by adding water, and the mixture was basified (pH 8–9) with sodium hydroxide to give the isoquinoline derivatives as a solid. The crude product was collected by filtration and purified by crystallization with EtOH to afford compounds **5a–h**. Analytical data for compounds **5a–c**, **5e**, **5g–h** are in accordance with literature.[21]

4.1.1.1. 1-(4′-Cyanophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (5d). Yield 46%. Mp 217–219 °C. ^{1}H NMR ($CDCl_3$, δ): 2.85–3.18 (4H, m, CH_2CH_2), 3.63 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 5.42 (1H, s, CH), 6.07 (1H, s, ArH), 6.67 (1H, s, ArH), 7.44 (2H, d, J = 8.24, ArH), 7.68 (2H, d, J = 8.24, ArH). Anal. Calcd for $C_{18}H_{18}N_2O_2$: C, 73.45; H, 6.16; N, 9.52. Found: C, 73.68; H, 6.33; N, 9.24.

4.1.1.2. 6,7-Dimethoxy-1-(4′-methylphenyl)-1,2,3,4-tetrahydroisoquinoline (5f). Yield 72%. Mp 140–142 °C. ^{1}H NMR ($CDCl_3$, δ): 2.34 (3H, s, CH_3), 2.73–3.26 (4H, m, CH_2CH_2), 3.64 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 5.03 (1H, s, CH), 6.26 (1H, s, ArH), 6.62 (1H, s, ArH) 7.13 (4H, s, ArH). Anal. Calcd for $C_{18}H_{21}NO_2$: C, 76.30; H, 7.47; N, 4.94. Found: C, 76.52; H, 7.15; N, 5.13 .

4.1.2. General procedure for the synthesis of 1-aryl-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamides (1a–h)

A mixture of the appropriate 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**5a–h**) (1.0 mmol) and sulfamide (6 mmol, 576 mg) in dimethoxyethane (2 mL) was placed in a cylindrical quartz tube (Ø 2 cm), then stirred and irradiated in a microwave oven at 150 W for two steps of 20 min at 90 °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. The residue crystallized from diethyl ether to give the desired compounds **1a–h** as pure products.

4.1.2.1. 6,7-Dimethoxy-1-phenyl-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (1a). Yield 74%. Mp 186–188 °C. ^{1}H NMR ($CDCl_3$, δ): 2.69–2.77 (1H, m, CH_2CH_2), 3.15–3.29 (2H, m, CH_2CH_2), 3.73 (3H, s, CH_3O), 3.79–3.85 (1H, m, CH_2), 3.90 (3H, s, CH_3O), 4.06 (2H, br s, NH_2), 6.00 (1H, s, CH), 6.41 (1H, s, ArH), 6.68 (1H, s, ArH), 7.27–7.36 (5H, m, ArH). ^{13}C NMR ($CDCl_3$, δ): 26.4, 38.6, 55.9, 59.4, 111.8, 111.9, 125.8, 127.8, 128.9, 129.0, 140.2, 148.2, 148.3. Anal. Calcd for $C_{17}H_{20}N_2O_4S$: C, 58.60; H, 5.79; N, 8.04. Found: C, 58.86; H, 5.62; N, 8.21.

4.1.2.2. 1-(4′-Bromophenyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (1b). Yield 86%. Mp 158–160 °C. ^{1}H NMR ($CDCl_3$, δ): 2.69–2.74 (1H, m, CH_2CH_2), 3.13–3.21 (2H, m, CH_2CH_2), 3.75 (3H, s, CH_3O), 3.80–3.86 (1H, m, CH_2CH_2), 3.89 (3H, s, CH_3O), 4.16 (2H, br s, NH_2), 5.96 (1H, s, CH), 6.39 (1H, s, ArH), 6.68 (1H, s, ArH), 7.15 (2H, d, J = 8.5, ArH), 7.45 (2H, d, J = 8.5, ArH). ^{13}C NMR ($CDCl_3$, δ): 26.8, 39.1, 55.9, 58.7, 110.6, 111.3, 122.2, 125.0, 125.8, 130.7, 131.7, 140.1, 147.8, 148.5. Anal. (C, H, N). Anal. Calcd for $C_{17}H_{19}BrN_2O_4S$: C, 47.78; H, 4.48; N, 6.56. Found: C, 47.56; H, 4.78; N, 6.86.

4.1.2.3. 1-(4′-Chlorophenyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (1c). Yield 51%. Mp 178–180 °C. ^{1}H NMR ($CDCl_3$, δ): 2.69–2.74 (1H, m, CH_2CH_2), 3.13–3.21 (2H, m, CH_2CH_2), 3.75 (3H, s, CH_3O), 3.79–3.85 (1H, m, CH_2CH_2), 3.90 (3H, s, CH_3O), 4.16 (2H, br s, NH_2), 5.97 (1H, s, CH), 6.39 (1H, s, ArH), 6.68 (1H, s, ArH), 7.21 (2H, d, J = 8.5, ArH), 7.30 (2H, d, J = 8.5, ArH). ^{13}C NMR ($CDCl_3$, δ): 27.1, 40.0, 56.1, 58.7, 110.8, 111.5, 125.0, 125.8, 129.3, 129.8, 131.7, 140.8, 147.7, 148.6. Anal. Calcd for $C_{17}H_{19}ClN_2O_4S$: C, 53.33; H, 5.00; N, 7.32. Found: C, 53.68; H, 5.23; N, 7.43.

4.1.2.4. 1-(4′-Cyanophenyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (1d). Yield 98%. Mp 195–197 °C. ^{1}H NMR ($CDCl_3$, δ): 2.68–2.78 (1H, m, CH_2CH_2), 3.14–3.17 (2H, m, CH_2CH_2), 3.76 (3H, s, CH_3O), 3.83–3.88 (1H, m, CH_2CH_2), 3.90 (3H, s, CH_3O), 4.30 (2H, br s, NH_2), 6.02 (1H, s, CH), 6.41 (1H, s, ArH), 6.70 (1H, s, ArH), 7.39 (2H, d, J = 8.1, ArH), 7.62 (2H, d, J = 8.1, ArH). ^{13}C NMR ($CDCl_3$, δ): 26.8, 40.0, 56.4, 59.2, 110.9, 111.9, 112.3, 118.9, 124.5, 126.4, 129.9, 132.7, 146.9, 148.3, 149.2. Anal. Calcd for $C_{18}H_{19}N_3O_4S$: C, 57.90; H, 5.13; N, 11.25. Found: C, 57.75; H, 5.33; N, 11.43.

4.1.2.5. 6,7-Dimethoxy-1-(4′-fluorophenyl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (1e). Yield 70%. Mp 116–118 °C. ^{1}H NMR ($CDCl_3$, δ): 2.73–2.78 (1H, m, CH_2CH_2), 3.18–3.30 (2H, m, CH_2CH_2), 3.79 (3H, s, CH_3O), 3.84–3.90 (1H, m, CH_2CH_2), 3.94 (3H, s, CH_3O), 4.21 (2H, br s, NH_2), 6.03 (1H, s, CH), 6.44 (1H, s, ArH), 6.72 (1H, s, ArH), 7.02–7.08 (2H, m, ArH), 7.27–7.31 (2H, m, ArH). ^{13}C NMR ($CDCl_3$, δ): 27.3, 39.0, 56.2, 57.8, 110.7, 111.2, 116.0, 125.7, 129.0, 129.8, 140.0, 147.7, 148.6, 158.0. Anal. Calcd for $C_{17}H_{19}FN_2O_4S$: C, 55.73; H, 5.23; N, 7.65. Found: C, 55.64; H, 5.48; N, 7.56.

4.1.2.6. 6,7-Dimethoxy1-(4′-methylphenyl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (1f). Yield 56%. Mp 162–164 °C. ^{1}H NMR ($CDCl_3$, δ): 2.33 (3H, s, CH_3), 2.71–2.77 (1H, m, CH_2CH_2), 3.14–3.25 (2H, m, CH_2CH_2), 3.72 (3H, s, CH_3O), 3.76–3.82 (1H, m, CH_2CH_2), 3.89 (3H, s, CH_3O), 4.00 (2H, br s, NH_2), 5.97 (1H, s, CH), 6.39 (1H, s, ArH), 6.67 (1H, s, ArH), 7.12–7.19 (4H, m, ArH). ^{13}C NMR ($CDCl_3$, δ): 21.1, 27.2, 38.8, 55.9, 59.2, 110.7, 111.1, 125.7, 126.0, 128.9, 129.2, 137.8, 138.0, 147.9, 148.2. Anal. Calcd for $C_{18}H_{22}N_2O_4S$: C, 59.65; H, 6.12; N, 7.73. Found: C, 59.34; H, 6.38; N, 7.45.

4.1.2.7. 6,7-Dimethoxy-1-(3′-nitrophenyl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (1g). Yield 90%. Mp 173–175 °C. ^{1}H NMR ($CDCl_3$, δ): 2.68–2.76 (1H, m, CH_2CH_2), 3.14–3.25 (2H, m, CH_2CH_2), 3.76 (3H, s, CH_3O), 3.81–3.88 (1H, m, CH_2CH_2), 3.91 (3H, s, CH_3O), 4.38 (2H, bs, NH_2), 6.08 (1H, s, CH), 6.44 (1H, s, ArH), 6.72 (1H, s, ArH), 7.52 (1H, t, J = 8.1, H-5′), 7.71 (1H, d, J = 8.1, H-6′), 8.03 (1H, s, H-2′), 8.16 (1H, d, J = 8.1, H-4′). ^{13}C NMR (DMSO-d_6, δ): 25.9, 38.7, 55.4, 55.6, 57.4, 111.5, 111.8, 122.3, 122.7, 125.1, 126.8 129.7, 134.8, 145.4, 147.1, 147.6, 148.0. Anal. Calcd for $C_{17}H_{19}N_3O_6S$: C, 51.90; H, 4.87; N, 10.68. Found: C, 51.72; H, 4.62; N, 10.85.

4.1.2.8. 6,7-Dimethoxy-1-(4′-nitrophenyl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (1h). Yield 97%. Mp 194–196 °C. ^{1}H NMR ($CDCl_3$, δ): 2.68–2.78 (1H, m, CH_2CH_2), 3.10–3.25 (2H, m, CH_2CH_2), 3.77 (3H, s, CH_3O), 3.84–3.87 (1H, m, CH_2CH_2), 3.91 (3H, s, CH_3O), 4.33 (2H, br s, NH_2), 6.06 (1H, s, CH), 6.42 (1H, s, ArH), 6.71 (1H, s, ArH), 7.45 (2H, d, J = 8.5, ArH), 8.17 (2H, d, J = 8.5, ArH). ^{13}C NMR ($CDCl_3$, δ): 29.1, 39.8, 55.83, 59.8, 110.5, 111.6, 123.6, 127.7, 128.2, 129.8, 145.4, 146.3, 147.9, 148.6. Anal. Calcd for $C_{17}H_{19}N_3O_6S$: C, 51.90; H, 4.87; N, 10.68. Found: C, 52.04; H, 4.71; N, 10.55.

4.1.3. General procedure for the synthesis of 6,7-dimethoxy-1-(3′-aminophenyl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (1i) and 6,7-dimethoxy-1-(4′-aminophenyl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (1j)

A solution of appropriate 6,7-dimethoxy-1-(4′-aminophenyl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (0.6 mmol) in 3 mL of HCl and 4 mL of EtOH was stirred vigorously 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, and then extracted with ethyl acetate. 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–1j**).

4.1.3.1. 1-(3′-Aminophenyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (1i). Yield 35%. Mp 166–168 °C. ^{1}H NMR ($CDCl_3$, δ): 2.72–2.77 (1H, m, CH_2CH_2), 3.13–3.34 (2H, m, CH_2CH_2), 3.69–3.84 (4H, m, CH_3O and CH_2CH_2), 3.89 (3H, s, CH_3O), 4.03 (2H, br s, NH_2), 5.89 (1H, s, CH), 6.42–7.13 (8H, m, ArH). ^{13}C NMR ($CDCl_3$, δ): 29.3, 39.5, 55.8, 55.9, 57.4, 111.0, 111.3, 114.2, 115.5, 119.3, 127.5, 129.2, 129.8, 146.0, 146.5, 147.6, 148.0. Anal. Calcd for $C_{17}H_{21}N_3O_4S$: C, 56.18; H, 5.82; N, 11.56. Found: C, 56.32; H, 5.71; N, 11.75.

4.1.3.2. 1-(4′-Aminophenyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (1j). Yield 77%. Mp 197–199 °C. ^{1}H NMR ($CDCl_3$, δ): 2.72–2.77 (1H, m, CH_2CH_2), 3.09–3.30 (2H, m, CH_2CH_2), 3.68–3.81 (4H, m, CH_3O and CH_2CH_2), 3.88 (3H, s, CH_3O), 3.96 (2H, br s, NH_2), 5.90 (1H, s, CH), 6.38 (1H, s, ArH), 7.62 (2H, d, J = 8.5, ArH), 6.65 (1H, s, ArH), 7.07 (2H, d, J = 8.5, ArH). ^{13}C NMR ($CDCl_3$, δ): 27.6, 39.8, 56.2, 57.4, 110.7, 111.2, 116.8, 125.7, 127.3, 129.8, 132.7, 145.9, 147.3, 148.6. Anal. Calcd for $C_{17}H_{21}N_3O_4S$: C, 56.18; H, 5.82; N, 11.56. Found: C, 56.31; H, 5.56; N, 11.72.

4.2. Pharmacology

4.2.1. Testing of anticonvulsant activity against audiogenic seizures in DBA/2 mice

All experiments were performed with DBA/2 mice which are genetically susceptible to sound-induced seizures.[20] DBA/2 mice (8–12 g; 22–25-days-old) were purchased from Harlan Italy (Corezzano, Italy). Groups of 10 mice of either sex were exposed to auditory stimulation 30 min following administration of vehicle or each dose of drugs studied. The compounds were given intraperitoneally (i.p.) (0.1 mL/10 g of body weight of the mouse) as a freshly-prepared solution in 50% dimethylsulfoxide (DMSO) and 50% sterile saline (0.9% NaCl). Individual mice were placed under a hemispheric perspex dome (diameter 58 cm), and 60 s were allowed for habituation and assessment of locomotor activity. Auditory stimulation (12–16 kHz, 109 dB) was applied for 60 s or until tonic extension occurred, and induced a sequential seizure response in control DBA/2 mice, consisting of an early wild running phase, followed by generalized myoclonus and tonic flexion and extension sometimes followed by respiratory arrest. The control and drug-treated mice were scored for latency to and incidence of the different phases of the seizures. The experimental protocol and all the procedures involving animals and their care were conducted in conformity with the institutional guidelines and the European Council Directive of laws and policies.

4.2.2. Statistical analysis

Statistical comparisons between groups of control and drug-treated animals were made using Fisher's exact probability test (incidence of the seizure phases). The ED_{50} values of each phase of audiogenic seizures was determined for each dose of compound administered, and dose–response curves were fitted using a computer program by Litchfield and Wilcoxon's method.[22]

4.3. CA inhibition assay

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalysed CO_2 hydration activity.[23] 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 to-

gether 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.[24–27]

4.4. Computational studies

The structure of compound **1j** was generated using 2D/3D editor sketcher in CATALYST 4.10.[28] software package and minimized to their closest local energy minimum using a molecular mechanics approach. Regarding the chirality of the asymmetric carbon, as no experimental data on the biologically relevant conformations of this molecule is available, it was arbitrarily decided to assign 'undefined' chirality.

To build conformational models of up to 250 conformers for each molecule, the 'best conformer generation' option and a 10 kcal/mol energy cutoff were chosen.

Acknowledgments

Financial support for this research by Fondo di Ateneo per la Ricerca (PRA 2005—Università di Messina) and MiUR (FIRB_RBNE03YA3L_007) is gratefully acknowledged. The work was also partially supported by grants from the EU projects DeZnIT and Metoxia (to C.T.S.).

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