Solution-Phase Parallel Synthesis of Novel 1,2,3,4-Tetrahydroisoquinolin-1-ones as Anticonvulsant Agents

Rosaria Gitto,*[,a] Eleonora Francica,[a] Giovambattista De Sarro,[b] Francesca Scicchitano,[b] and Alba Chimirri[a]

[a] *Department of Medicinal Chemistry, Faculty of Pharmacy, Università di Messina; Viale Annunziata I-98168 Messina: and* [b]*, Department of Experimental and Clinical Medicine, Faculty of Medicine and Surgery, University of Catanzaro; Via T. Campanella, 115 I-88100 Catanzaro, Italy.*

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Following our previous studies in the field of anticonvulsant agents, we planned a one-pot solution-phase parallel synthesis (SPPS) of a small library of new 1,2,3,4-tetrahydroisoquinoline derivatives. The activity against audiogenic seizures in DBA/2 mice of the newly synthesized compounds has also been evaluated.

Key words solution-phase parallel synthesis; isoquinolinone; anticonvulsant agent

The 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)-propionate receptor (AMPAR) mediates fast glutamatergic synaptic transmission in the mammalian central nervous system. Overactivation of AMPAR plays a pivotal role in epileptogenesis and glutamate-induced neuronal death.[1,2] On this basis, particular attention has been devoted to selective AMPAR antagonists (*e.g.*, GYKI 52466, **1**, Fig. 1), as potential neuroprotective agents against neurological pathologies such as epilepsy, ischemia, Parkinson's disease, and multiple sclerosis.[1]

Our previous studies reported chemical and pharmacological data of some 2,3-benzodiazepine derivatives (*e.g.*, **2**, CFM-2, Fig. 1), that proved to be specific noncompetitive AMPAR antagonists and potent anticonvulsant agents.[3—5] Our challenge was also to decipher the main structural requirements for noncompetitive AMPAR antagonists and for this reason we developed a suitable 3D ligand-based pharmacophore model.[6] This study allowed the identification of the 2-acetyl-1-(4′-chlorophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**3**, Fig. 1) as a new very potent anticonvulsant agent acting as noncompetitive AMPAR antagonist.[7,8]

The model obtained was also used as a search query for virtual screening on three dimensional databases and some molecules were selected and tested. Among the discovered compounds, the *trans* isomer of 2-(4-chlorobenzyl)-3-(4-methoxyphenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (**4**, PD00735, Fig. 1) showed the most interesting anticonvulsant efficacy[6] encouraging us to explore some structural modifications of PD00735.[9]

Isoquinolinonic acids have previously been found to possess a vast range of pharmacological properties including psychotropic, antiallergic, antiinflammatory and estrogenic behaviour and for this reason the chemistry of isoquinolonic acids is extensively described. It is well known that the cycloaddition of homophthalic anhydride with aldimines provides a useful access to the preparation of this class of compounds. This reaction produces a mixture of *cis*- and *trans*-isomers and it has been observed that the reaction conditions (solvent polarity, temperature, *etc.*) and the nature of reagents employed influence their stereochemistry.[10—15]

We report here a simple methodology for the solution-phase parallel synthesis (SPPS) of PD00735 and its analogues with the aim to obtain a small library of isoquinolinonic acids for the evaluation in audiogenic seizure test and draw structure–activity relationship (SAR) considerations for this class of compounds.

Results and Discussion

Using PD00735 (**4**) as a scaffold we performed some modifications with the aim to study their influence on anticonvulsant activity. In particular, we inserted different substituents on the phenyl ring at C-3 to obtain SAR information. Furthermore, methyl ester and carboxamide derivatives of parent compound PD00735 (**4**) have been prepared to clarify the importance of carboxylic function on pharmacological properties.

A simple solution-phase parallel methodology was set up for the synthesis of 1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acids (Chart 1). The one-pot procedure was carried out stirring the amine and the aldehyde at room temperature to give the corresponding imine intermediate that in the same reactor was treated with homophthalic anhydride without the isolation procedure. This protocol was particularly appropriate to perform a solution phase parallel synthetic approach using a Buchi Syncore reactor. Starting from different amines **5**, aldehydes **6** and homophthalic anhydride, we ob-

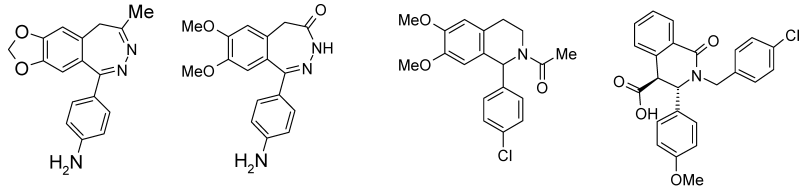
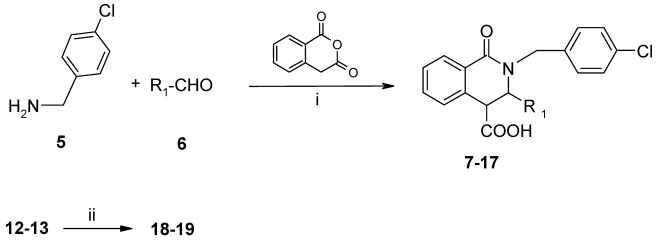


Fig. 1. Noncompetitive AMPAR Antagonists

* To whom correspondence should be addressed. e-mail: rgitto@pharma.unime.it



Reagents and conditions: i) $(MeO)_3CH$, CH_2Cl_2, r.t., 24 h, 200 rpm; ii) H_2/Pd-C (5%) MeOH, r.t., 1h

	R_1		R_1
7[a]	C_6H_5	**14**[b]	4-MeC_6H_4
8[b]	3-FC_6H_4	**15**[b]	4-$CF_3C_6H_4$
9[b]	4-FC_6H_4	**16**[b]	3,4-$F_2C_6H_3$
10[b]	4-ClC_6H_4	**17**[b]	3,4-$(OMe)_2C_6H_3$
11[b]	4-BrC_6H_4	**18**[b]	3-$NH_2C_6H_4$
12[b]	3-$NO_2C_6H_4$	**19**[b]	4-$NH_2C_6H_4$
13[b]	4-$NO_2C_6H_4$		

[a] *cis/trans* diasteroisomer mixture, [b] *trans*-diasteroisomer.

Chart 1

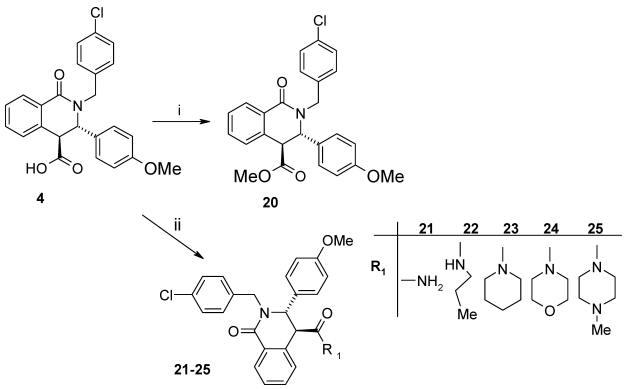


Chart 2

tained in good yields a large series of new 1,2,3,4-tetrahydroisoquinolinone derivatives **7**—**17** according to previous procedure reported for this class of compounds[16] with minor modifications.

The reaction between the benzylamine derivatives **5**, aldehydes **6** and homophthalic anhydride generally furnished the cycloadducts **7**—**17** as *trans*-disteroeisomers except for derivative **7** which gave mixture of *cis*/*trans*-diastereoisomers **7a**, **b** resolved by fractional crystallization from ethyl acetate.

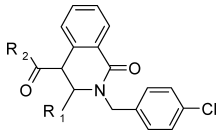
In previous papers the cycloaddition of homophthalic anhydrides with aldimine has been reported for the synthesis of tetrahydroisoquinolinonic acids by using different reaction conditions and catalysts in attempt to obtain an isomer selectivity, but a definitive definition of the factors that control the stereochemical outcomes of the reaction was not reached.

Aminophenyl analogues **18**, **19** were obtained by reduction of the corresponding nitro derivatives **12**, **13**.

By treatment of compound **4** with methyl iodide or suitable amines, methyl ester **20** and carboxamides **21**—**25** were obtained respectively (Chart 2) as *trans*-diastereoisomers. In particular, carboxamides **21**—**25** were easily prepared employing *S*-(1-oxido-2-pyridinyl)-1,1,3,3-tetramethylthiouronium tetrafluoroborate (TOTT) and *N*,*N*′-carbonyldiimidazole, as coupling agents, for amide **21** and *N*-substituted amides **22**—**25** respectively.

The configuration of the compounds synthesized was es-

Table 1. Anticonvulsant Activity of 1,2,3,4-Tetrahydroisoquinoline Derivatives against Audiogenic Seizures in DBA/2 Mice



	R_1	R_2	ED_{50} μmol/kg[a]	
			Clonus	Tonus
4[b]	4-$OMeC_6H_4$	OH	19.7 (14.6—20.0)	16.3 (12.6—21.1)
7a	C_6H_5	OH	>100	21.8 (12.8—37.2)
7b	C_6H_5	OH	>100	>100
8	3-FC_6H_4	OH	76.2 (60.6—95.8)	31.7 (21.6—46.4)
9	4-FC_6H_4	OH	50.4 (28.2—90.3)	18.2 (11.9—27.8)
10	4-ClC_6H_4	OH	>100	>100
11	4-BrC_6H_4	OH	>100	>100
12	3-$NO_2C_6H_4$	OH	>100	59.9 (38.7—92.7)
13	4-$NO_2C_6H_4$	OH	>100	56.5 (38.0—84.0)
14	4-MeC_6H_4	OH	>100	25.0 (14.1—44.6)
15	4-$CF_3C_6H_4$	OH	43.3 (30.1—62.3)	15.0 (9.53—23.5)
16	3,4-$F_2C_6H_3$	OH	>100	48.1 (29.4—78.8)
17	3,4-$(OMe)_2C_6H_3$	OH	74.7 (60.4—92.4)	36.1 (24.5—53.4)
18	3-$NH_2C_6H_4$	OH	>100	>100
19	4-$NH_2C_6H_4$	OH	78.1 (55.1—110)	34.3 (21.4—54.8)
20	4-$OMeC_6H_4$	OCH_3	86.1 (67.3—110)	36.5 (24.5—54.2)
21	4-$OMeC_6H_4$	NH_2	>100	58.4 (39.6—86.2)
22	4-$OMeC_6H_4$	$NHnC_3H_7$	95.9 (69.3—132)	49.6 (32.8—75.0)
23	4-$OMeC_6H_4$	$N(C_2H_4)_2O$	>100	44.6 (32.4—61.5)
24	4-$OMeC_6H_4$	$N(C_2H_4)_2CH_2$	>100	56.2 (42.2—75.0)
25	4-$OMeC_6H_4$	$N(C_2H_4)_2NCH_3$	>100	51.6 (36.2—73.7)
1, GYKI 52466			35.8 (24.4—52.4)	25.3 (16.0—40.0)

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*) Ref. 9.

tablished on the basis of the value of the vicinal coupling constants between the H-3 and H-4 protons. In the *cis* configuration the $J_{3,4}$ values were of *ca.* 6 Hz, while in the *trans* isomers the H_3 and H_4 hydrogens appear as two singlets for compounds **7**—**22** and **24**, and *ca.* 3.5 Hz for carboxamides **23** and **25**.

In attempt to explore some structure–activity relationships (SAR) for this class of compounds, the anticonvulsant effects of new synthesized 1,2,3,4-tetrahydroisoquinolines were evaluated after intraperitoneal (i.p.) administration against audiogenic seizures in DBA/2 mice, which are considered an excellent animal model for generalized epilepsy and for screening new anticonvulsant drugs.[3]

In Table 1 we report the ED_{50} values of new synthesized compounds in comparison with the PD00735 (**4**). The results pointed out that the replacement of 4′-methoxy group with other substituents on the 4-aromatic frame of 1,2,3,4-tetrahydroisoquinoline scaffold was detrimental for the activity. Compounds **9** and **15** showed anticonvulsant effects comparable to those of GYKI 52466 (**1**), but their anticonvulsant activity was lower than that of parent compound **4**. When we evaluated the activity of *cis*/*trans* **7** diastereoisomers we confirmed that the *trans*-isomer **7a** was more potent than *cis*-isomer **7b** in line with our virtual screening that selected only PD00735 as lead compound and not its *cis*-isomer.[9]

As shown in Table 1 also the corresponding ester **20** and amides **21**—**25**, independently from the nature and bulk of the substituent, lost activity thus suggesting that the car-

boxylic moiety is essential for anticonvulsant efficacy of the parent compound **4**.

In conclusion, by employing solution-phase parallel synthesis we realized a small library of PD00735 (**4**) analogues as potential anticonvulsant agents. Even if the above reported structural modifications afforded derivatives less active than lead compound **4**, derivatives **9** and **15** showed anticonvulsant effects comparable to those of GYKI 52466 (**1**), the prototype of non-competitive AMPA-antagonists.

Experimental

Melting points were determined on a STUART SPM10 hot stage 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 F_{254} plates were used for analytical TLC. ^{1}H- and ^{13}C-NMR spectra were measured with a Varian Gemini 300 spectrometer; chemical shifts are expressed in δ (ppm) relative to tetramethyl saline (TMS) as internal standard and coupling constants (J) in Hz. All exchangeable protons were confirmed by addition of D_2O.

General One-Pot Procedure for the Synthesis of 1,2,3,4-Tetrahydroisoquinolinone-4-carboxylic Acid Derivatives (7—17) The mixtures of the suitable amine (6 mmol) and aldehyde (6 mmol) in 15 ml dichloromethane were prepared into each reaction vessel then shaked overnight at 200 rpm at room temperature in the presence of 1 ml trimethyl orthoformate (9.6 mmol). Then, the homophthalic anhydride (6 mmol) was added to each vessel and the reaction mixture was stirred at 200 rpm at room temperature for 16 h; the desired products were collected by filtration and crystallized from ethyl acetate to give **7**—**17**. The *cis*/*trans* mixture of **7a**, **b** was solved by fractional crystallization from ethyl acetate.

trans N-(4′-Chlorobenzyl)-1-oxo-3-phenyl-1,2,3,4-tetrahydroisoquinoline-4-carboxylic Acid (**7a**): 57% yield as white crystals, mp 260—262 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.88 and 5.22 (2H, 2d, J=14.83, CH_2), 4.10 (1H, s, H-3), 5.27 (1H, s, H-4), 7.03 (2H, m, ArH), 7.18—7.23 (8H, m, ArH), 7.39—7.43 (2H, m, ArH), 7.95—7.99 (1H, m, H-8), 11.50 (1H, bs, COO<u>H</u>). *Anal.* Calcd for $C_{23}H_{18}ClNO_3$ (391.86): C, 70.50; H, 4.63; N, 3.75. Found: C, 70.58; H, 4.64; N, 3.75.

cis N-(4′-Chlorobenzyl)-1-oxo-3-phenyl-1,2,3,4-tetrahydroisoquinoline-4-carboxylic Acid (**7b**): 38% yield as white crystals, mp 168—170 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.86 and 5.19 (2H, 2d, J=15.08, CH_2), 4.72 (1H, d, J=6.32, H-3,), 4.99 (1H, d, J=6.04, H-4), 6.95—7.55 (m, 12H, ArH), 8.07 (1H, d, J=7.69, H-8,), 12.00 (1H, bs, COO<u>H</u>). *Anal.* Calcd for $C_{23}H_{18}ClNO_3$ (391.85): C, 70.50; H, 4.63; N, 3.57. Found: C, 70.53; H, 4.64; N, 3.58

trans N-(4′-Chlorobenzyl)-3-(3″-fluorophenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic Acid (**8**): 51% yield as white crystals, mp 277—278 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.99 and 5.16 (2H, 2d, J=14.83, CH_2), 4.16 (1H, s, H-3), 5.34 (1H, s, H-4), 6.83—7.47 (m, 11H, ArH), 7.97 (s, 1H, H-8), 12.50 (1H, bs, COO<u>H</u>). *Anal.* Calcd for $C_{23}H_{17}ClFNO_3$ (409.09): C, 67.40; H, 4.18; N, 3.42. Found: C, 67.50; H, 4.19; N, 3.42.

trans N-(4′-Chlorobenzyl)-3-(4″-fluorophenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic Acid (**9**): 25% yield as white crystals, mp 280—282 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.93 and 5.15 (2H, 2d, J=15.11, CH_2), 4.08 (1H, s, H-3), 5.29 (1H, s, H-4), 7.04—7.07 (2H, m, ArH), 7.21—7.42 (9H, m, ArH), 7.97 (s, 1H, H-8), 12.00 (1H, bs, COO<u>H</u>). *Anal.* Calcd for $C_{23}H_{17}ClFNO_3$ (409.85): C, 67.40; H, 4.18; N, 3.42. Found: C, 67.45; H, 4.19; N, 3.41.

trans N-(4′-Chlorobenzyl)-3-(4″-chlorophenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic Acid (**10**): 47% yield as white crystals, mp 258—260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.94 and 5.16 (2H, 2d, J=15.11, CH_2), 4.10 (1H, s, H-3), 5.29 (1H, s, H-4), 7.03—7.05 (2H, m, ArH), 7.21—7.42 (9H, m, ArH), 7.97 (s, 1H, H-8), 12.00 (bs, 1H, COO<u>H</u>). *Anal.* Calcd for $C_{23}H_{17}Cl_2NO_3$ (426.30): C, 64.08; H, 4.02; N, 3.39. Found: C, 63.98; H, 4.00; N, 3.38.

trans 3-(4″-Bromophenyl)-*N*-(4′-chlorobenzyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic Acid (**11**): 56% yield as white crystals, mp 260—262 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.93 and 5.17 (2H, 2d, J=15.10, CH_2), 4.10 (1H, s, H-3), 5.27 (1H, s, H-4), 6.96—6.99 (2H, m, ArH), 7.19—7.21 (m, 1H, ArH), 7.30—7.31 (4H, m, ArH), 7.41—7.44 (4H, m, ArH), 7.95—7.97 (1H, m, H-8), 12.00 (1H, bs, COO<u>H</u>). *Anal.* Calcd for $C_{23}H_{17}BrClNO_3$ (470.75): C, 58.68; H, 3.64; N, 2.98. Found: C, 58.82; H, 3.65; N, 2.98.

trans N-(4′-Chlorobenzyl)-3-(3″-nitrophenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic Acid (**12**): 40% yield as yellow crystals, mp 254—256 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.19 and 5.04 (2H, 2d, J=15.11, CH_2), 4.23 (1H, s, H-3), 5.53 (1H, s, H-4), 7.19—7.48 (9H, m, ArH), 7.84 (1H, s, ArH, H-2″), 7.98—8.04 (2H, m, ArH), 12.50 (1H, bs, COO<u>H</u>). ^{13}C-NMR (DMSO-d_6) δ: 49.22 (CH_2), 50.38 and 60.88 (C-3 and C-4), 121.34, 122.64, 127.25, 128.14, 128.36, 128.75, 129.83, 130.27, 130.52, 131.96, 132.90, 133.31, 136.34, 141.80, 147.89, 163.00 (CO), 169.03 (<u>CO</u>OH). *Anal.* Calcd for $C_{23}H_{17}ClN_2O_5$ (436.86): C, 63.24; H, 3.92; N, 6.41. Found: C, 63.28; H, 3.92; N, 6.42.

trans N-(4′-Chlorobenzyl)-3-(4″-nitrophenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic Acid (**13**): 57% yield as yellow crystals, mp 234—236 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.06 and 5.15 (2H, 2d, J=15.11, CH_2), 4.20 (1H, s, H-3), 5.49 (1H, s, H-4), 7.18—7.34 (7H, m, ArH), 7.41—7.44 (m, 2H, ArH), 7.96—7.99 (1H, m, H-8), 8.08 (2H, d, J=8.51, ArH), 12.50 (1H, bs, COO<u>H</u>). *Anal.* Calcd for $C_{23}H_{17}ClN_2O_5$ (436.86): C, 63.24; H, 3.92; N, 6.41. Found: C, 63.45; H, 3.91; N, 6.42.

trans N-(4′-Chlorobenzyl)-3-(4″-methylphenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic Acid (**14**): 50% yield as white crystals, mp 253—255 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.79 (3H, s, CH_3), 4.12 and 5.54 (2H, 2d, J=15.11, CH_2), 4.36 (1H, s, H-3), 5.51 (1H, s, H-4), 7.22 (2H, d, J=8.24, ArH), 7.35 (2H, d, J=7.97, ArH), 7.47—7.50 (1H, m, ArH), 7.61—7.70 (4H, m, ArH), 7.71—7.73 (2H, m, ArH), 8.25—8.28 (1H, m, H-8), 12.00 (1H, bs, COO<u>H</u>). *Anal.* Calcd for $C_{24}H_{20}ClNO_3$ (405.88): C, 71.02; H, 4.97; N, 3.45. Found: C, 71.18; H, 4.98; N, 3.46.

trans N-(4′-Chlorobenzyl)-1-oxo-3-(4″-trifluoromethyphenyl)-1,2,3,4-tetrahydroisoquinoline-4-carboxylic Acid (**15**): 41% yield as white crystals, mp 240—242 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.01 and 5.16 (2H, 2d, J=14.72, CH_2), 4.17 (1H, s, H-3), 5.41 (1H, s, H-4), 7.21—7.30 (7H, m, ArH), 7.41—7.43 (2H, m, ArH), 7.60 (2H, d, J=8.24, ArH), 7.96—7.99 (1H, m, H-8), 13.00 (1H, bs, COO<u>H</u>). ^{13}C-NMR (DMSO-d_6) δ: 48.83 (CH_2), 50.32 and 60.88 (C-3 and C-4), 124.32, 125.36, 126.96, 127.06, 127.93, 128.04, 128.62, 129.60, 130.18, 131.75, 132.19, 133.28, 136.04, 136.21, 154.54, 162.98 (CO), 171.65 (<u>CO</u>OH). *Anal.* Calcd for $C_{24}H_{17}ClF_3NO_3$ (459.84): C, 62.69; H, 3.73; N, 3.05. Found C, 62.73; H, 3.73; N, 3.05.

trans N-(4′-Chlorobenzyl)-1-oxo-3-(3″,4″-difluorophenyl)-1,2,3,4-tetrahydroisoquinoline-4-carboxylic Acid (**16**): 39% yield as white crystals, mp 250—252 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.06 and 5.10 (2H, 2d, J=15.11, CH_2), 4.14 (1H, s, H-3), 5.33 (1H, s, H-4), 7.09 (1H, s, ArH), 7.13—7.47 (9H, m, ArH), 7.96 (1H, d, J=8.79, H-8,), 13.00 (1H, bs, COO<u>H</u>). *Anal.* Calcd for $C_{23}H_{16}ClF_2NO_3$ (427.08): C, 64.57; H, 3.77; N, 3.24. Found: C, 64.70; H, 3.78; N, 3.24.

trans N-(4′-Chlorobenzyl)-3-(3″,4″-dimethoxyphenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic Acid (**17**): 29% yield as white crystals, mp 252—253 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.61 (3H, s, OC<u>H</u>$_3$), 3.64 (3H, s, OC<u>H</u>$_3$), 3.89 and 5.21 (2H, 2d, J=15.11, CH_2), 4.11 (1H, s, H-3), 5.17 (1H, s, H-4), 6.41 (1H, d, J=8.24, ArH), 6.76 (1H, d, J=8.51, ArH), 6.69 (1H, s, ArH, H-5″), 7.19—7.22 (m, 1H), 7.32 (bs, 4H, ArH), 7.38—7.47 (m, 2H), 7.97 (1H, bs, H-8), 12.50 (bs, 1H, COO<u>H</u>). *Anal.* Calcd for $C_{25}H_{22}ClNO_5$ (451.12): C, 66.45; H, 4.91; N, 3.10. Found: C, 66.50; H, 4.92; N, 3.10.

Synthesis of *trans*-3-Aminophenyl- and 4-Aminophenyl-*N*-(4′-chlorobenzyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic Acids (18, 19) The solution of suitable *trans* 1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (**12** or **13**) (0.5 mmol) in MeOH (30 ml) was stirred in a flask under a light stream of H_2 in presence of Pd/C 5% as catalyst for 1 h; then the mixture was filtered off and the filtrate evaporated *in vacuo* to give an oil residue that was treated with AcOEt to afford desired products **18** or **19**.

trans 3-(3″-Aminophenyl)-*N*-(4′-chlorobenzyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic Acid (**18**): 20% yield as pale crystals, mp 191—193 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.50 (2H, bs, NH_2), 3.83 and 5.27 (2H, 2d, J=14.68, CH_2), 4.08 (1H, s, H-3), 5.21 (1H, s, H-4), 6.73—6.87 (3H, m, ArH), 7.15—7.43 (8H, m, ArH), 7.98—7.99 (1H, m, H-8), 12.30 (1H, bs, COO<u>H</u>). *Anal.* Calcd for $C_{23}H_{19}ClN_2O_3$ (406.86): C, 67.90; H, 4.71; N, 6.89. Found: C, 67.85; H, 4.71; N, 6.90.

trans 3-(4″-Aminophenyl)-*N*-(4′-chlorobenzyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic Acid (**19**): 69% yield as pale crystals, mp 224—226 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.00 (2H, bs, NH_2), 4.36 and 5.73 (2H, 2d, J=14.83, CH_2), 4.55 (1H, s, H-3), 5.68 (s, 1H, H-4), 7.30—7.43 (4H, m, ArH), 7.69—7.96 (8H, m, ArH), 12.32 (1H, bs, COO<u>H</u>). *Anal.* Calcd for $C_{23}H_{19}ClN_2O_3$ (406.86): C, 67.90; H, 4.71; N, 6.89. Found: C, 67.81; H, 4.71; N, 6.88.

Synthesis of *trans* Methyl *N*-(4′-Chlorobenzyl)-3-(4″-methoxyphenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylate (20) A mixture of the appropriate 1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (1.6 mmol), methyl iodide (3.2 mmol, 0.2 ml), K_2CO_3 (0.22 g) and dry acetone (20 ml) was refluxed for 4 h and filtered. The filtrate was cooled to room temperature and evaporated under reduced pressure; the residual oil was

crystallized by treatment with Et_2O to afford methyl carboxylate derivative. 58% yield as white crystals; mp 168—169 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.43 (3H, s, OC$\underline{H_3}$), 3.64 and 5.59 (2H, 2d, J=14.83, CH_2), 3.74 (3H, s, OC$\underline{H_3}$), 3.85 (1H, s, H-3), 5.04 (1H, s, H-4), 6.75 (2H, d, J=8.8, ArH), 6.95 (2H, d, J=8.8, ArH), 7.00—7.06 (1H, m, ArH), 7.21—7.30 (4H, m, ArH), 7.42—7.46 (2H, m, ArH), 8.23 (1H, d, J=6.87, H-8). ^{13}C-NMR ($CDCl_3$): δ 48.26 (CH_2), 52.40 (COO$\underline{CH_3}$), 51.80 and 60.16 (C-3 and C-4), 55.00 (CH_3O), 114.21, 124.00, 127.44, 128.16, 128.49, 129.00, 130.18, 130.45, 130.20, 132.10, 132.21, 134.40, 135.91, 159.00, 164.10 (CO), 170.10 (CO$\underline{O}$H). *Anal.* Calcd for $C_{25}H_{22}ClNO_4$ (435.91): C, 68.89; H, 5.09; N, 3.21. Found: C, 69.00; H, 5.10; N, 3.21.

Synthesis of *trans* *N*-(4′-Chlorobenzyl)-3-(4″-methoxyphenyl)-1-oxo-1,2,3,4-tetrahydro-isoquinoline-4-carboxylamide (21) To a solution of *trans* *N*-(4′-chlorobenzyl)-3-(4″-methoxyphenyl)-1-oxo-1,2,3,4-tetrahydro-isoquinoline-4-carboxylic acid (**4**, 422 mg, 1 mmol) in *N*,*N*-dimethylformamide (DMF) (3 ml), diisopropylethylamine (DIEA) (0.17 ml), NH_4Cl (1 mmol) and TOTT (235 mg, 0.75 mmol) were added. The reaction mixture was stirred for 30 min at room temperature; then brine was added and the mixture was extracted with EtOAc. The organic layer was washed with HCl (2 N), water, saturated aqueous $NaHCO_3$ and water again; then dried, filtered off and evaporated under reduced pressure to afford pure amide derivative **21** 20% yield as as white crystals; mp 240—242 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.73 (3H, s, OC$\underline{H_3}$), 3.83 and 5.43 (2H, 2d, J=14.56, CH_2), 5.41 (1H, s, H-4), 5.57 (1H, s, H-3), 5.46 (2H, bs, NH_2), 6.75 (2H, d, J=8.52, ArH, H-3″, H-5″), 6.97 (2H, d, J=8.52, ArH, H-2″, H-6″), 7.13—7.19 (1H, m, H-5), 7.24—7.29 (4H, m, ArH), 7.51—7.54 (2H, m, H-6, H-7), 8.30—8.33 (1H, m, H-8). *Anal.* Calcd for $C_{24}H_{21}ClN_2O_3$ (420.89): C, 68.49; H, 5.03; N, 6.66. Found: C, 68.54; H, 5.05; N, 6.68.

General Procedure for the Synthesis of 1,2,3,4-Tetrahydro-1-oxoisoquinoline-4-carboxamides (22—25) To a solution of compound **4** (1.25 mmol) in anhydrous DMF (10 ml) *N*,*N*′-carbonyldiimidazole (CDI, 1.25 mmol) was added and under magnetic stirring in nitrogen atmosphere; then a solution of the appropriate amine (1.25 mmol) in anhydrous DMF (5 ml) was added dropwise and the reaction mixture was stirred at room temperature for 3 h. The resulting mixture was evaporated to dryness leaving oily residue; the residue was treated with saturated sodium hydrogen carbonate aqueous solution and then with water. The organic layer was concentrated to dryness and the crude product was purified by column-chromatography ($CHCl_3$/MeOH 98 : 2 as eluant) to yield amides **22**—**25**.

trans *N*-(4′-Chlorobenzyl)-3-(4″-methoxyphenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-propyl-carboxamide (**22**): 25% yield as white crystals; mp 173—174 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.70 (3H, J=7.40, t, CH_3), 1.19—1.26 (2H, m, CH_2), 2.93—3.02 (2H, m, CH_2), 3.73 (3H, s, OC$\underline{H_3}$), 3.77 and 5.37 (2H, 2d, J=14.56, CH_2), 3.75 (1H, s, H-3) , 5.35 (1H, s, H-4), 5.02 (1H, bs, NH), 6.73 (2H, d, J=8.78, ArH), 6.97 (2H, d, J=8.51, ArH), 7.07—7.09 (1H, m, ArH), 7.24—7.26 (4H, m, ArH), 7.49—7.51 (2H, m, ArH), 8.27—8.29 (1H, m, H-8). ^{13}C-NMR ($CDCl_3$) δ: 17.2 (NHCH$_2$CH$_2$C$\underline{H_3}$), 22.35 (NHCH$_2$C$\underline{H_2}$CH$_3$), 41.62 (NH$\underline{CH_2}$CH$_2$CH$_3$), 47.50 (CH_2), 49.38 and 61.78 (C-3 and C-4), 53.87 (OCH_3), 114.14, 124.10, 127.35, 127.91, 128.12, 128.54, 129.11, 129.92, 132.00, 132.06, 132.10, 132.21, 132.83, 135.90, 159.00, 164.10 (CO), 168.41 (CO$\underline{O}$H). *Anal.* Calcd for $C_{27}H_{27}ClN_2O_3$ (462.97): C, 70.05; H, 5.88; N, 7.66. Found: C, 70.20; H, 5.90; N, 7.68.

trans *N*-(4′-Chlorobenzyl)-3-(4″-methoxyphenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-(4-piperidin-1-yl)carboxamide (**23**): 27% yield as white crystals; mp 178—179 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.18—1.21 (6H, m, CH_2), 3.12—3.22 (4H, m, CH_2), 3.57 and 5.63 (2H, 2d, J=15.10, CH_2), 3.77 (3H, s, OC$\underline{H_3}$), 4.24 (1H, d, J=3.57, H-3), 4.61 (1H, d, J=3.57, H-4), 6.77 (2H, d, J=8.79, ArH), 6.90—6.97 (3H, m, ArH), 7.13—7.45 (6H, m, ArH), 8.26—8.29 (1H, m, H-8). *Anal.* Calcd for $C_{29}H_{29}ClN_2O_3$ (489.01): C, 71.23; H, 5.98; N, 5.73. Found: C, 71.28; H, 6.00; N, 5.75.

trans *N*-(4′-Chlorobenzyl)-3-(4″-methoxyphenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-(morpholin-4-yl)-carboxamide (**24**): 42% yield as white crystals; mp 189—190 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.42—3.62 (8H, m, $(CH_2)_2$), 3.67 and 5.69 (2H, 2d, J=15.11, CH_2), 4.27 (1H, s, H-3), 4.65 (1H, s, H-4), 6.83—6.86 (2H, m, ArH), 6.97—6.99 (3H, m, ArH), 7.01—7.02 (2H, m, ArH), 7.17—7.20 (2H, m, ArH), 7.48—7.52 (2H, m, ArH), 8.33—8.55 (1H, m, H-8). *Anal.* Calcd for $C_{28}H_{27}ClN_2O_4$ (490.98): C, 68.50; H, 5.54; N, 5.71. Found: C, 68.57; H, 5.56; N, 5.72.

trans *N*-(4′-Chlorobenzyl)-3-(4″-methoxyphenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-(4-methylpiperazin-1-yl)carboxamide (**25**): 30% yield as white crystals; mp 173—174 °C. ^{1}H-NMR ($CDCl_3$) δ: 10% yield as white crystals; mp 145—146 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.58—1.76 and 1.80—1.96 (4H, 2m, CH_2), 2.32—2.50 (2H, m, CH_2), 2.24 (3H, s, CH_3), 3.22—3.38 (2H, m, CH_2), 3.55 and 5.66 (2H, 2d, J=15.10, CH_2), 3.77 (3H, s, OC$\underline{H_3}$), 4.21 (1H, d, J=3.57, H-3), 4.57 (1H, d, J=3.57, H-4), 6.71—6.96 (4H, m, ArH), 7.12—7.32 (5H, m, ArH), 7.40—7.48 (2H, m, ArH), 8.27—8.30 (1H, m, H-8). *Anal.* Calcd for $C_{29}H_{30}ClN_3O_3$ (504.02): C, 69.11; H, 5.03; N, 8.34. Found: C, 69.17; H, 5.04; N, 8.36.

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. DBA/2 mice (8—12 g; 22—25-d-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.[17] 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 erspex 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.

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.[18]

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References

1) De Sarro G., Gitto R., Russo E., Ibbadu G. F., Barreca M. L., De Luca L., Chimirri A., *Curr. Top. Med. Chem.*, **5**, 31—42 (2005).
2) Brauner-Osborne H., Egebjerg J., Nielsen E. O., Madsen U., Krogsgaard-Larsen P., *J. Med. Chem.*, **43**, 2609—2645 (2000).
3) Chimirri A., De Sarro G., De Sarro A., Gitto R., Grasso S., Quartarone S., Zappala M., Giusti P., Libri V., Constanti A., Chapman A. G., *J. Med. Chem.*, **40**, 1258—1269 (1997).
4) Chimirri A., De Sarro G., De Sarro A., Gitto R., Quartarone S., Zappala M., Constanti A., Libri V., *J. Med. Chem.*, **41**, 3409—3416 (1998).
5) Gitto R., Orlando V., Quartarone S., De Sarro G., De Sarro A., Russo E., Ferreri G., Chimirri A., *J. Med. Chem.*, **46**, 3758—3761 (2003).
6) Barreca M. L., Gitto R., Quartarone S., De Luca L., De Sarro G., Chimirri A., *J. Chem. Inf. Comput. Sci.*, **43**, 651—655 (2003).
7) Gitto R., Barreca M. L., De Luca L., De Sarro G., Ferreri G., Quartarone S., Russo E., Constanti A., Chimirri A., *J. Med. Chem.*, **46**, 197—200 (2003).
8) Gitto R., Caruso R., Orlando V., Quartarone S., Barreca M. L., Ferreri G., Russo E., De Sarro G., Chimirri A., *Farmaco*, **59**, 7—12 (2004).
9) Gitto R., Barreca M. L., Francica E., Caruso R., De Luca L., Russo E., De Sarro G., Chimirri A., *ARKIVOC* (Gainesville), **2004**, 170—180 (2004).
10) Cushman M., Madaj E. J., *J. Org. Chem.*, **52**, 907—915 (1987).
11) Azizian J., Mohammadi A. A., Karimi A. R., Mohammadizadeh M. R., *J. Org. Chem.*, **70**, 350—352 (2005).
12) Yadav J. S., Reddy B. V. S., Saritha Raj K., Prasad A. R., *Tetrahedron*, **59**, 1805—1809 (2003).
13) Stoyanova M. P., Kozekov I. D., Palamareva M. D., *J. Heterocyclic Chem.*, **40**, 795—803 (2003).
14) Kozekov I. D., Koleva R. I., Palamareva M. D., *J. Heterocyclic Chem.*, **39**, 229—236 (2002).
15) Bonnaud B., Carlessi A., Bigg D. C. H., *J. Heterocyclic Chem.*, **30**, 257—256 (1993).
16) Yu N., Poulain R., Gesquiere J. C., *Synlett*, **2000**, 355—356 (2000).
17) Chapman A. G., Croucher M. J., Meldrum B. S., *Arzneim.-Forsch.*, **34**, 1261—1264 (1984).
18) Litchfield J. T. J., *J. Pharmacol. Exp. Ther.*, **97**, 399—408 (1949).