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Synthesis and anticonvulsant evaluation of N-substituted isoquinoline AMPA receptor antagonists

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Abstract—In our previous studies a ligand-based approach led to the identification of noncompetitive AMPA receptor antagonists containing isoquinoline scaffold. In an attempt to perform a systematic SAR study, we synthesized new N-substituted-isoquinolines bearing the most salient features described by our 3D pharmacophore model. All compounds were screened against audiogenic seizures and some derivatives showed anticonvulsant properties. Compound **24**, the most active of the series, was also tested in vitro using the patch–clamp technique and proved to antagonize AMPA-mediated effects.

1. Introduction

Glutamate (Glu), the major stimulatory neurotransmitter in the central nervous system (CNS) of vertebrates, interacts with ionotropic (iGluRs) and metabotropic (mGluRs) receptors.[1] Within the large family of iGluRs, 2-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors (AMPARs) constitute a subfamily characterized by specific pharmacological and functional properties.[2,3] In particular, AMPARs play important roles in neurotransmission in the CNS and in synaptic plasticity that underlies learning processes and memory.[4] However, the AMPARs over-activation determines neuronal cell death related to various neurological diseases such as stroke, Huntington's chorea, epilepsy, etc. Therefore, AMPAR antagonists have been considered useful as therapeutic agents for these disorders.[5–7] Moreover, the unwanted side effects of competitive antagonists steered the search for new agents such as 2,3-benzodiazepine derivatives (e.g., GYKI 52466, **1**, and CFM-2, **2**) that noncompetitively inhibit AMPARs.[8–12]

In an attempt to describe the structural requirements for noncompetitive antagonism, we have previously developed a 3D pharmacophore model which led to the identification of a new class of AMPAR ligands containing the tetrahydroisoquinoline scaffold.[13] Then we synthesized a large series of tetrahydroisoquinolines and found that some derivatives, such as the 2-acetyl-1-(4′-chlorophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**3**, Fig. 1) and 1-(4′-bromophenyl)-6,7-dimethoxy-*N*-(piperidin-1-yl-acetyl)-1,2,3,4-tetrahydroisoquinoline (**4**, Fig. 1), demonstrated marked anticonvulsant effects in various seizure models and noncompetitive AMPAR antagonism.[14–19]

In our efforts to discover novel ligands with improved pharmacological profile and to gain more insights into the structure–activity relationships (SAR) for this class of compounds, we report the synthesis of new N-substituted-isoquinolines (Fig. 2) that maintain the main structural features suggested by our molecular modelling studies carried out by Catalyst software package and shown in Figure 3 for compound **4**.[19] In particular, we explored the effects of *N*-oxoacetamide chain containing variable sizes of alkyl or cycloalkyl amines as substituents in comparison with previously reported *N*-acetamide-isoquinolines (e.g., **4**). We evaluated their anticonvulsant properties against audiogenic seizures and studied noncompetitive AMPAR antagonist effects

Keywords: Isoquinolines; Microwave-assisted synthesis; Anticonvulsant agents; AMPA-antagonists; Molecular modelling.
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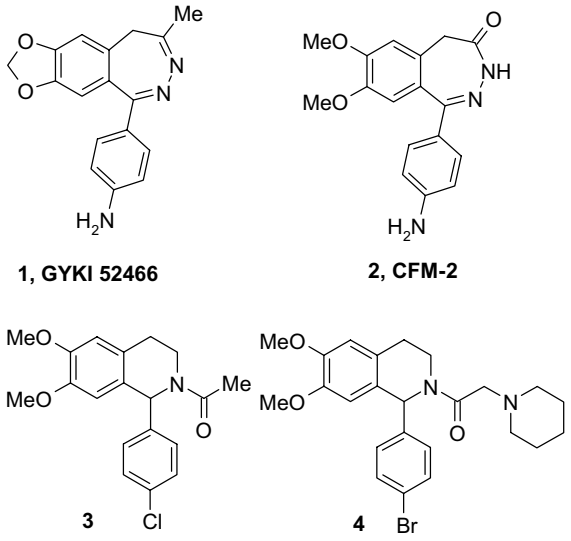


Figure 1. Noncompetitive AMPAR antagonists.

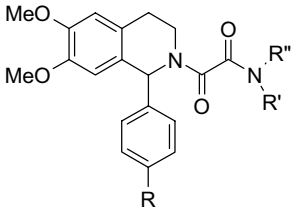


Figure 2. General structure of the designed compounds.

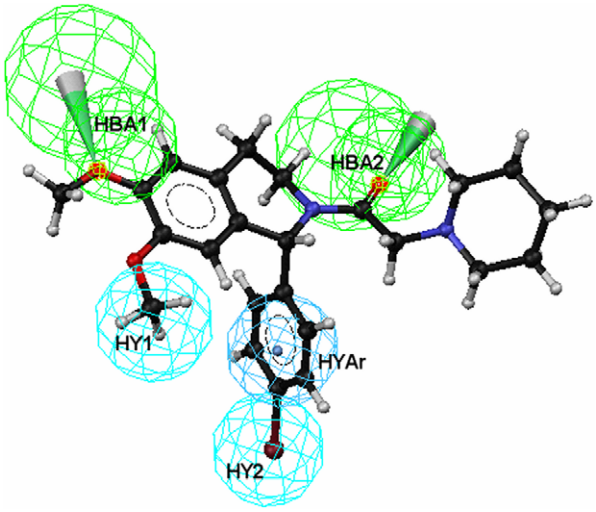
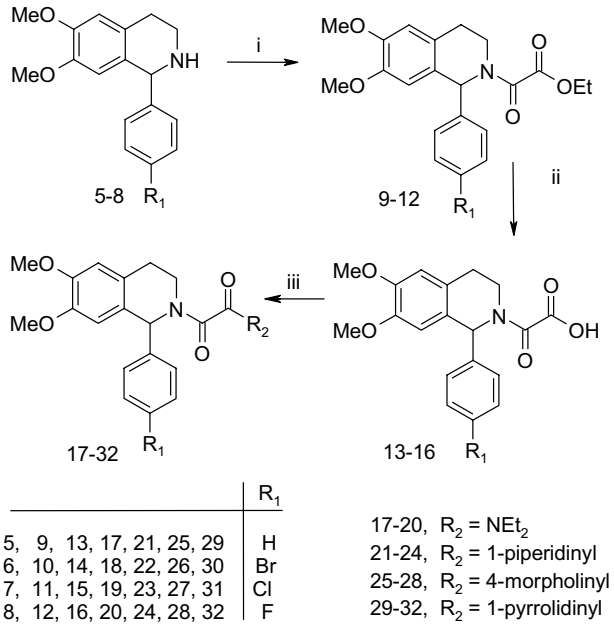


Figure 3. Best HypoGen pharmacophore hypothesis aligned to compound **4** (in the R configuration chosen by Catalyst). The pharmacophore features are colour coded as follows: green, hydrogen bond acceptors (HBA1 and HBA2); blue, hydrophobic aromatic region (HYAr); cyan, hydrophobic groups (HY1 and HY2).

through an electrophysiological study to establish the mechanism of action.

2. Results and discussion

A series of N-substituted-2-oxoacetamide-1,2,3,4-tetrahydroisoquinolines (**17**–**32**) were synthesized employing



Scheme 1. Reagents and conditions: (i) EtOCOCOCl, CH_2Cl_2, T.E.A, rt, 90 min or MW, 280 W, rt, 5 min; (ii) NaOH, EtOH/H_2O, rt, 2 h, then HCl 6 N 2 h; (iii) *N*,*N*-diethylamine or cycloalkylamines, HBTU, DMF, T.E.A, rt, 20 h or MW, 250 W, rt, 10 min.

a multistep approach starting from 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines (**5**–**8**) that were prepared[16] according to previously reported procedure. As drawn in Scheme 1 the reaction of ethyl oxalylchloride with 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines (**5**–**8**) gave the ethyl 2-oxoacetates **9**–**12**; these intermediates were successively converted into the corresponding 2-oxoacetic acid derivatives **13**–**16** which were coupled to appropriate amines using a standard amide coupling reaction to afford the corresponding 2-oxoacetamide derivatives **17**–**32**. We also carried out a high-speed microwave-assisted synthesis of intermediates **9**–**12** and desired compounds **17**–**32** thus optimizing the chemical yield as well as greatly reducing the reaction times more than 10-fold (see Scheme 1). The structures of the compounds obtained were supported by elemental analyses and spectroscopic measurements (^{1}H NMR).

The anticonvulsant effects of (1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-oxoacetamides **17**–**32** containing different substituents both on *N*-2-oxoacetamide moiety and 1-aryl portion were evaluated against audiogenic seizures in DBA/2 mice. This is considered an excellent animal model for generalized epilepsy and for screening new anticonvulsant drugs.[20] The results of pharmacological screening were compared with those of other noncompetitive AMPA receptor antagonists such as GYKI 52466 (**1**) as well as isoquinoline derivative **4**. The data reported in Table 1 demonstrate that these compounds generally protect DBA/2 mice in audiogenic test after intraperitoneal (ip) administration at higher doses than those of reference compounds **4** and GYKI 52466 (**1**). Compound **24** was the most active derivative among this series of new N-substituted-iso-

Table 1. Anticonvulsant activity of compounds **1**, **4**, and **17**–**32** against audiogenic seizures in DBA/2 mice

	ED_{50}[a] (μmol/kg)		Log *P*[b]
	Clonus	Tonus	
1[c]	35.8 (24.4–52.4)	25.3 (16.0–40.0)	0.55
4[c]	12.7 (6.09–26.3)	8.17 (4.03–16.6)	4.93
17	65.1 (50.8–83.4)	53.3 (40.4–70.3)	2.54
18	>100	>100	3.31
19	75.9 (60.8–94.7)	67.7 (52.4–87.5)	3.13
20	>100	>100	2.59
21	98.1 (78.4–123)	55.3 (38.1–80.3)	2.25
22	60.5 (26.2–101)	41.6 (29.7–58.1)	3.02
23	88.4 (63.2–124)	65.3 (47.2–90.4)	2.84
24	44.6 (29.0–68.7)	38.7 (22.8–65.7)	2.30
25	>100	72.1 (55.9–93.0)	0.70
26	>100	>100	1.47
27	91.0 (68.4–121)	46.9 (33.3–66.2)	1.29
28	>100	90.2 (56.8–144)	0.75
29	>100	54.6 (41.5–71.9)	1.68
30	89.2 (64.1–124)	45.4 (29.7–69.4)	2.45
31	58.1 (37.3–90.5)	37.2 (24.0–57.7)	2.28
32	>100	73.0 (45.7–117)	1.73

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

[b] Log *P* data are predicted from a commercially available program (ACD/Lab).

[c] Ref. 19.

quinolines (**17**–**32**) in which the different anticonvulsant potencies were not apparently related to their relative lipophilicity (Log *P*, Table 1).[21] These results suggested that the nature of (cyclo)alkylamino substituents might influence the anticonvulsant effects and in particular the 1-piperidinyl moiety generally characterized the most active compounds (e.g., **24**). Significant activity was also shown by some *N*,*N*-diethylamine and 1-pyrrolidinyl derivatives, whereas the presence of the 4-morpholinyl moiety was detrimental for anticonvulsant efficacy. The trends were not clear when we considered the effects of the different substitution pattern of 4′-position of phenyl ring linked to the C-1 of isoquinoline skeleton.

In spite of the fact that these new derivatives fitted well the main structural features suggested by our 3D pharmacophore hypothesis (two hydrogen bond acceptors, hydrophobic aromatic region and hydrophobic groups shown in Fig. 3), their less activity with respect to the *N*-acetamide-isoquinolines (e.g., **4**) could be due to the presence of an additional carbonyl moiety which negatively influences their pharmacokinetic properties.

With the aim to define the mechanism of action of compound **24** electrophysiological study has also been performed.[22] The application of AMPA (5 μM, 5 s) induced in CA1 pyramidal neurons an inward current, the amplitude of which ranged between 30 ± 9 and 209 ± 13 pA in different neurons (mean ± SEM of 3–6 consecutive responses to AMPA). When compound **24** (50 μM, 5 min) was simultaneously applied through the bath perfusion system, AMPA-mediated current was reversibly reduced (Fig. 4A). The amplitude of AMPA-mediated current (at least 3 values from consecutive responses) was compared with amplitude values (at

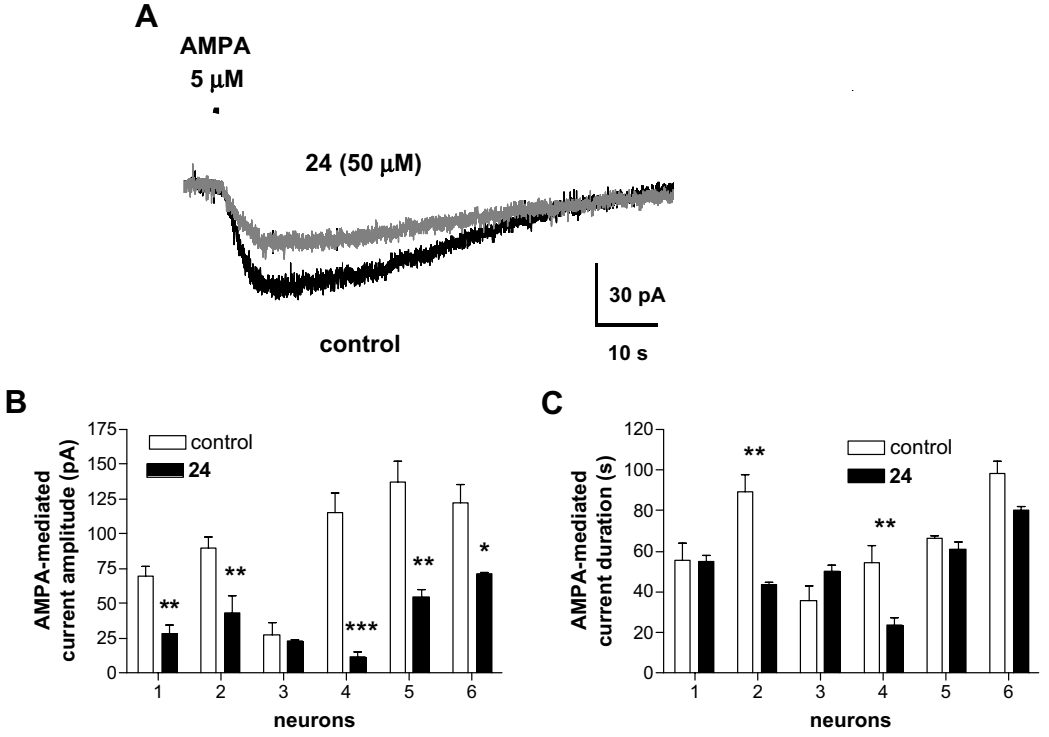


Figure 4. Effect of compound **24** on AMPA-mediated current. (A) Application of AMPA (5 μM, 2 s) induced in CA1 pyramidal neurons an inward current, the amplitude of which was reduced by application of the antagonist **24** (50 μM, 5 min). (B) The amount of reduction of AMPA-mediated current amplitude by **24** was statistically significant in 5 out of 6 neurons. (C) In 2 of the responsive neurons, AMPA-mediated current was also reduced in duration in the presence of compound **24** (***$P < 0.0001$; **$P < 0.001$; *$P < 0.01$; unpaired *t* test, two-tailed).

least 3) during antagonist application: the amount of decrease was statistically significant in 5 out of 6 neurons tested (Student's *t* test, Fig. 4B). In the presence of **24**, the amplitude of AMPA-mediated current was reduced on average by 60 ± 8% (mean ± SEM, $n = 5$, range 41–89, median 60.0); Wilcoxon matched pairs test, performed on data from all the tested neurons, confirmed a significant reduction of AMPA-mediated current. The duration of AMPA-mediated current was reduced during **24** application in 3 of the neurons studied (numbered as 2, 4 and 6 in Fig. 4C). In the remaining neurons, the duration of AMPA response was either not modified (neuron No. 1 and 5, Fig. 4C) or slightly increased (neuron No. 3, Fig. 4C). Recovery of AMPA-mediated current amplitude and duration was observed between 2 and 5 min after washout of **24**. In line with in vivo, study GYKI 52466 (**1**) demonstrated better effects than compound **24** in the same assay (see Supplementary material).

3. Conclusion

In conclusion, a series of new N-substituted isoquinoline **17**–**32** AMPA receptor antagonists was prepared and tested as anticonvulsant agents. In spite of maintenance of structural requirements for recognition of noncompetitive binding site of AMPAR, the results obtained suggest that the planned modification of the linkage between tetrahydroisoquinoline system and alkylamino moiety negatively affects the anticonvulsant efficacy. In fact only compound **24** proved to prevent audiogenic seizures in DBA/2 mice with a potency comparable to that of GYKI 52466 (**1**) and reduce AMPA-mediated current in electrophysiological assay.

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 F_{254} plates were used for analytical TLC. ^{1}H NMR spectra were measured in $CDCl_3$ with a Varian Gemini 300 spectrometer; chemical shifts are expressed in δ (ppm) relative to TMS as internal standard and coupling constants (*J*) in Hz.

4.1.1. General procedure for the preparation of 2-(6,7-dimethoxy-1-aryl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-oxoacetamide derivatives 17–32. A mixture of suitable isoquinoline derivatives (**5**–**8**)[16] (1 mmol) and ethyloxalyl chloride (14 mmol) in CH_2Cl_2 (10 mL) was placed in a cylindrical quartz tube (∅ 2 cm), then stirred and irradiated in a microwave oven at 280 W for 5 min at 25 °C. After cooling the reaction was quenched by adding a saturated sodium bicarbonate solution, and the mixture extracted with EtOAc. The organic layer was dried over Na_2SO_4, and the solvent was removed until dryness under reduced pressure to give ethyl (1-aryl-6,7-dimethoxy-1,2,3,4-dihydroisoquinolin-2-yl)-oxoacetate intermediates (**9**–**12**). The crude product from the previous step was dissolved in 50% EtOH/water (5 mL) and stirred at 25 °C for 2 h. Then the mixture was made acidic by the addition of HCl (6.0 N); the resultant yellow solid was removed by filtration, washed with water to afford 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl-oxoacetic acids (**13**–**16**). To a solution in DMF (5 mL) of crude material obtained in previous step (1 mmol, **13**–**16**) and HBTU (1 mmol), the suitable alkyl- or cycloalkyl amine (1 mmol) was added; the reaction mixture was placed in a cylindrical quartz tube (∅ 2 cm), then stirred and irradiated in a microwave oven at 250 W for 10 min at 25 °C; the cooled organic layer was extracted with EtOAc, dried over Na_2SO_4 and the solvent was removed until dryness under reduced pressure; the resultant solid was crystallized from 50% diethyl ether/cyclohexane to give ethyl(1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-oxoacetamide derivatives **17**–**32**. ^{1}H NMR spectral data confirmed the structure of intermediates **9**–**16** (see Supplementary material).

4.1.2. 2-(6,7-Dimethoxy-1-phenyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-*N*,*N*-diethyl-2-oxoacetamide (17). Mp 136–138 °C. Yield 80%. ^{1}H NMR: δ 1.10 (t, 3H, $J = 7.14$, CH_3), 1.22 (t, 3H, $J = 7.14$, CH_3), 2.75–3.69 (m, 8H, CH_2), 3.80 (s, 3H, CH_3O-6), 3.94 (s, 3H, CH_3O-7), 6.54 (s, 1H, H-1), 6.70 (s, 1H, H-5), 6.82 (s, 1H, H-8), 7.31–7.34 (m, 5H, Ar). Anal. ($C_{23}H_{28}N_2O_4$) C, H, N.

4.1.3. 2-[1-(4-Bromophenyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]-*N*,*N*-diethyl-2-oxoacetamide (18). Mp 82–84 °C. Yield 83%. ^{1}H NMR: δ 1.09 (t, 3H, $J = 7.14$, CH_3), 1.18 (t, 3H, $J = 7.14$, CH_3), 2.70–3.61 (m, 8H, CH_2), 3.76 (s, 3H, CH_3O-6), 3.90 (s, 3H, CH_3O-7), 6.45 (s, 1H, H-1), 6.66 (s, 1H, H-5), 6.72 (s, 1H, H-8), 7.17 (d, 2H, $J = 7.7$, H2′–H6′), 7.43 (d, 2H, $J = 7.7$, H3′–H5′). Anal. ($C_{23}H_{27}BrN_2O_4$) C, H, N.

4.1.4. 2-[1-(4-Chlorophenyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]-*N*,*N*-diethyl-2-oxoacetamide (19). Mp 135–137 °C. Yield 82%. ^{1}H NMR: δ 1.13 (t, 3H, $J = 7.14$, CH_3), 1.23 (t, 3H, $J = 7.14$, CH_3), 2.74–3.66 (m, 8H, CH_2), 3.80 (s, 3H, CH_3O-6), 3.94 (s, 3H, CH_3O-7), 6.49 (s, 1H, H-1), 6.70 (s, 1H, H-5), 6.79 (s, 1H, H-8), 7.27 (d, 2H, $J = 8.2$, H2′–H6′), 7.32 (d, 2H, $J = 8.2$, H3′–H5′). Anal. ($C_{23}H_{27}ClN_2O_4$) C, H, N.

4.1.5. *N*,*N*-Diethyl-2-[1-(4-fluorophenyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]-2-oxoacetamide (20). Mp 141–143 °C. Yield 81%. ^{1}H NMR: δ 1.08 (t, 3H, $J = 7.14$, CH_3), 1.18 (t, 3H, $J = 7.14$, CH_3), 2.70–3.59 (m, 8H, CH_2), 3.75 (s, 3H, CH_3O-6), 3.90 (s, 3H, CH_3O-7), 6.45 (s, 1H, H-1), 6.66 (s, 1H, H-5), 6.72 (s, 1H, H-8), 7.15–7.44 (m, 4H, Ar). Anal. ($C_{23}H_{27}FN_2O_4$) C, H, N.

4.1.6. 6,7-Dimethoxy-2-[oxo(piperidin-1-yl)acetyl]-1-phenyl-1,2,3,4-tetrahydroisoquinoline (21). Mp 115–117 °C. Yield 46%. ^{1}H NMR: δ 1.21–1.71 (m, 6H, CH_2), 2.72–3.64 (m, 8H, CH_2), 3.75 (s, 3H, CH_3O-6), 3.89 (s, 3H,

CH_3O-7), 6.49 (s, 1H, H-1), 6.66 (s, 1H, H-5), 6.76 (s, 1H, H-8), 7.28–7.30 (m, 5H, Ar). Anal. ($C_{24}H_{28}N_2O_4$) C, H, N.

4.1.7. 1-(4-Bromophenyl)-6,7-dimethoxy-2-[oxo(piperidin-1-yl)acetyl]-1,2,3,4-tetrahydroisoquinoline (22). Mp 94–96 °C. Yield 48%. ^{1}H NMR: δ 1.18–1.60 (m, 6H, CH_2), 2.69–3.65 (m, 8H, CH_2), 3.75 (s, 3H, CH_3O-6), 3.89 (s, 3H, CH_3O-7), 6.44 (s, 1H, H-1), 6.65 (s, 1H, H-5), 6.70 (s, 1H, H-8), 7.16 (d, 2H, J = 8.5, H2′–H6′), 7.43 (d, 2H, J = 8.5, H3′–H5′). Anal. ($C_{24}H_{27}BrN_2O_4$) C, H, N.

4.1.8. 1-(4-Chlorophenyl)-6,7-dimethoxy-2-[oxo(piperidin-1-yl)acetyl]-1,2,3,4-tetrahydroisoquinoline (23). Mp 88–90 °C. Yield 47%. ^{1}H NMR: δ 1.25–1.64 (m, 6H, CH_2), 2.70–3.65 (m, 8H, CH_2), 3.75 (s, 3H, CH_3O-6), 3.90 (s, 3H, CH_3O-7), 6.44 (s, 1H, H-1), 6.65 (s, 1H, H-5), 6.72 (s, 1H, H-8), 7.22 (d, 2H, J = 8.2, H2′–H6′), 7.29 (d, 2H, J = 8.2, H3′–H5′). Anal. ($C_{24}H_{27}ClN_2O_4$) C, H, N.

4.1.9. 1-(4-Fluorophenyl)-6,7-dimethoxy-2-[oxo(piperidin-1-yl)acetyl]-1,2,3,4-tetrahydroisoquinoline (24). Mp 140–142 °C. Yield 46%. ^{1}H NMR: δ 1.25–1.62 (m, 6H, CH_2), 2.70–3.60 (m, 8H, CH_2), 3.75 (s, 3H, CH_3O-6), 3.89 (s, 3H, CH_3O-7), 6.45 (s, 1H, H-1), 6.65 (s, 1H, H-5), 6.73 (s, 1H, H-8), 6.96–7.01 (m, 4H, Ar). Anal. ($C_{24}H_{27}FN_2O_4$) C, H, N.

4.1.10. 6,7-Dimethoxy-2-[morpholin-4-yl(oxo)acetyl]-1-phenyl-1,2,3,4-tetrahydroisoquinoline (25). Mp 153–155 °C. Yield 51%. ^{1}H NMR: δ 2.73–3.71 (m, 12H, CH_2), 3.75 (s, 3H, CH_3O-6), 3.90 (s, 3H, CH_3O-7), 6.48 (s, 1H, H-1), 6.67 (s, 1H, H-5), 6.76 (s, 1H, H-8), 7.25–7.31 (m, 5H, Ar). Anal. ($C_{23}H_{26}N_2O_5$) C, H, N.

4.1.11. 1-(4-Bromophenyl)-6,7-dimethoxy-2-[morpholin-4-yl(oxo)acetyl]-1,2,3,4-tetrahydroisoquinoline (26). Mp 161–163 °C. Yield 53%. ^{1}H NMR: δ 2.72–3.72 (m, 12H, CH_2), 3.75 (s, 3H, CH_3O-6), 3.90 (s, 3H, CH_3O-7), 6.43 (s, 1H, H-1), 6.66 (s, 1H, H-5), 6.69 (s, 1H, H-8), 7.16 (d, 2H, J = 8.2, H2′–H6′), 7.43 (d, 2H, J = 8.5, H3′–H5′). Anal. ($C_{23}H_{25}BrN_2O_5$) C, H, N.

4.1.12. 1-(4-Chlorophenyl)-6,7-dimethoxy-2-[morpholin-4-yl(oxo)acetyl]-1,2,3,4-tetrahydroisoquinoline (27). Mp 99–100 °C. Yield 52%. ^{1}H NMR: δ 2.76–3.72 (m, 12H, CH_2), 3.75 (s, 3H, CH_3O-6), 3.89 (s, 3H, CH_3O-7), 6.43 (s, 1H, H-1), 6.65 (s, 1H, H-5), 6.81 (s, 1H, H-8), 7.25 (d, 2H, J = 8.5, H2′–H6′), 7.29 (d, 2H, J = 8.5, H3′–H5′). Anal. ($C_{23}H_{25}ClN_2O_5$) C, H, N.

4.1.13. 1-(4-Fluorophenyl)-6,7-dimethoxy-2-[morpholin-4-yl(oxo)acetyl]-1,2,3,4-tetrahydroisoquinoline (28). Mp 191–193 °C. Yield 48%. ^{1}H NMR: δ 2.73–3.72 (m, 12H, CH_2), 3.75 (s, 3H, CH_3O-6), 3.90 (s, 3H, CH_3O-7), 6.45 (s, 1H, H-1), 6.66 (s, 1H, H-5), 6.69 (s, 1H, H-8), 6.73–7.27 (m, 4H, Ar). Anal. ($C_{23}H_{25}FN_2O_5$) C, H, N.

4.1.14. 6,7-Dimethoxy-2-[oxo(pyrrolidin-1-yl)acetyl]-1-phenyl-1,2,3,4-tetrahydroisoquinoline (29). Mp 114–116 °C. Yield 42%. ^{1}H NMR: δ 1.43–1.92 (m, 4H, CH_2), 2.72–3.64 (m, 8H, CH_2), 3.75 (s, 3H, CH_3O-6), 3.90 (s, 3H, CH_3O-7), 6.49 (s, 1H, H-1), 6.66 (s, 1H, H-5), 6.77 (s, 1H, H-8), 7.21–7.30 (m, 5H, Ar). Anal. ($C_{23}H_{26}N_2O_4$) C, H, N.

4.1.15. 1-(4-Bromophenyl)-6,7-dimethoxy-2-[oxo(pyrrolidin-1-yl)acetyl]-1,2,3,4-tetrahydroisoquinoline (30). Mp 97–99 °C. Yield 46%. ^{1}H NMR: δ 1.42–1.91 (m, 4H, CH_2), 2.69–3.66 (m, 8H, CH_2), 3.75 (s, 3H, CH_3O-6), 3.89 (s, 3H, CH_3O-7), 6.45 (s, 1H, H-1), 6.66 (s, 1H, H-5), 6.72 (s, 1H, H-8), 7.17 (d, 2H, J = 8.0, H2′–H6′), 7.43 (d, 2H, J = 8.0, H3′–H5′). Anal. ($C_{23}H_{25}BrN_2O_4$) C, H, N.

4.1.16. 1-(4-Chlorophenyl)-6,7-dimethoxy-2-[oxo(pyrrolidin-1-yl)acetyl]-1,2,3,4-tetrahydroisoquinoline (31). Mp 91–93 °C. Yield 45%. ^{1}H NMR: δ 1.43–1.93 (m, 4H, CH_2), 2.70–3.65 (m, 8H, CH_2), 3.75 (s, 3H, CH_3O-6), 3.89 (s, 3H, CH_3O-7), 6.45 (s, 1H, H-1), 6.67 (s, 1H, H-5), 6.73 (s, 1H, H-8), 7.21 (d, 2H, J = 8.2, H2′–H6′), 7.30 (d, 2H, J = 8.2, H3′–H5′). Anal. ($C_{23}H_{25}ClN_2O_4$) C, H, N.

4.1.17. 1-(4-Fluorophenyl)-6,7-dimethoxy-2-[oxo(pyrrolidin-1-yl)acetyl]-1,2,3,4-tetrahydroisoquinoline (32). Mp 76–78 °C. Yield 43%. ^{1}H NMR: δ 1.42–1.91 (m, 4H, CH_2), 2.76–3.65 (m, 8H, CH_2), 3.75 (s, 3H, CH_3O-6), 3.89 (s, 3H, CH_3O-7), 6.46 (s, 1H, H-1), 6.66 (s, 1H, H-5), 6.74 (s, 1H, H-8), 6.99–7.28 (m, 4H, Ar). Anal. ($C_{23}H_{25}FN_2O_4$) C, H, N.

4.2. Pharmacology

4.2.1. Testing of anticonvulsant activity. All experiments were performed with DBA/2 mice which are genetically susceptible to sound-induced seizures.[20] DBA/2 mice (8–12 g; 22 to 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 (ip) (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 was 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 were determined for each dose of compound administered, and dose–response curves were fitted using a computer program by Litchfield and Wilcoxon's method.[23]

4.2.3. Electrophysiology. Young Wistar rats (age 9–17 days) were decapitated under ether anaesthesia; brains were rapidly removed, placed in oxygenated ice-cold artificial cerebrospinal fluid (ACSF; composition in mM: NaCl 124; KCl 3.0; NaH_2PO_4 1.2; $MgSO_4$ 1.2; $CaCl_2$ 2.0; $NaHCO_3$ 26; D-glucose 10, pH 7.3) and cut into 300 μm slices with a vibratome. One slice containing the hippocampus was then transferred to the recording chamber of a patch–clamp set-up, continually perfused with oxygenated ACSF and viewed under an infrared differential interference contrast microscope (Leica DMLFS). CA1 pyramidal neurons were visually identified by their location and by their typical shape and dimension. Membrane current was recorded from single CA1 pyramidal neurons with the patch–clamp technique in the whole-cell configuration, using an L/M-EPC7 amplifier (List Electronic, Germany). The recording electrode was a glass micropipette having a final tip resistance between 1.5 and 3 MΩ, filled with intracellular solution (composition in mM: K-gluconate 170; HEPES 10; NaCl 10; $MgCl_2$ 2; EGTA 0.2; Mg-ATP 3.5; Na-GTP 1; pH 7.3).

Capacitive currents were electronically cancelled and series resistance was compensated by 60–80%. Data were acquired and analysed using EPC and Signal softwares (Cambridge Electronic Design, England). Statistical analysis was performed with Graph Pad software. (−)-Bicuculline methiodide (5 μm, Tocris) was routinely added to extracellular ACSF solution in order to prevent $GABA_A$-mediated inhibition by local interneurons.

The AMPA receptor antagonist **24** was dissolved in ACSF and applied by bath perfusion at a flow rate of 1 mL/min. The holding potential of CA1 pyramidal neurons was set at −70 mV and membrane current was continually recorded at an acquisition rate of 4 kHz. (*S*)-AMPA (5 μm, Tocris) was applied during 5 s using a computer-controlled electrovalve solution changer (MSC-200, Bio-Logic). Several pulses of AMPA were applied for each neuron, respectively, in control conditions and during application of **24** (50 μM, 5 min) through the bath perfusion system. AMPA-induced current amplitude was measured as the difference between peak current amplitude and baseline; duration of AMPA-induced current was measured as the time interval between the current onset (variation from baseline at least three times larger than background noise) and complete return to baseline. For each neuron studied, the amplitude and duration of at least three consecutive AMPA-induced currents were measured, respectively, in control conditions and in the presence of an antagonist and the two sets of raw values were compared using Student's unpaired t test. In addition, mean current amplitude and duration values in control conditions were confronted with mean amplitude and duration values in the presence of an antagonist using Wilcoxon matched pairs test

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.11.071.

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