8,9-Dimethoxy-6-(4-bromophenyl)-11H-[1,2,4]triazolo[4,5-c][2,3]benzodiazepin-3(2H)-one (5b).
Mp >300°C, yield 60%. ¹H-NMR (CDCl₃): 3.70 (s, 3H, MeO-8), 3.84 (s, 2H, CH₂-11), 3.98 (s, 3H, MeO-9), 6.60 (s, 1H, H-7), 6.85 (s, 1H, H-10), 7.58 (d, 2H, J=8.4, H-3',5'), 7.75 (d, 2H, J=8.4, H-2',6'), 10.09 (bs, 1H, NH). Anal. (C₁₈H₁₂BrN₄O₃) C, H, N.

8,9-Dimethoxy-6-(4-chlorophenyl)-11H-[1,2,4]triazolo[4,5-c][2,3]benzodiazepin-3(2H)-one (5c).
Mp >300°C, yield 64%. ¹H-NMR (CDCl₃): 3.70 (s, 3H, MeO-8), 3.84 (s, 2H, CH₂-11), 3.97 (s, 3H, MeO-9), 6.60 (s, 1H, H-7), 6.86 (s, 1H, H-10), 7.42 (d, 2H, J=8.4, H-3',5'), 7.75 (d, 2H, J=8.4, H-2',6'), 10.28 (bs, 1H, NH). Anal. (C₁₈H₁₂ClN₄O₃) C, H, N.

2,12-Dihydro-9,10-dimethoxy-7-phenyl[1,2,4]triazino[4,3-c][2,3]benzodiazepine-3,4-dione (7a).
Mp >300°C, yield 52%. ¹H-NMR (CDCl₃): 3.75 (s, 3H, MeO-9), 3.68-3.82 (m, 2H, CH₂), 4.00 (s, 3H, MeO-10), 6.68 (s, 1H, H-8), 6.90 (s, 1H, H-11), 7.46-7.83 (m, 5H, Ar), 9.60 (bs, 1H, NH). Anal. (C₁₉H₁₆N₄O₄) C, H, N.

2,12-Dihydro-9,10-dimethoxy-7-(4-bromophenyl)[1,2,4]triazino[4,3-c][2,3]benzodiazepine-3,4-dione (7b).
Mp >300°C, yield 65%. ¹H-NMR: 3.76 (s, 3H, MeO-9), 3.65-3.82 (m, 2H, CH₂), 4.00 (s, 3H, MeO-10), 6.63 (s, 1H, H-8), 6.90 (s, 1H, H-11), 7.60-7.72 (m, 4H, Ar), 9.20 (bs, 1H, NH). Anal. (C₁₉H₁₆BrN₄O₄) C, H, N.

2,12-Dihydro-9,10-dimethoxy-7-(4-chlorophenyl)[1,2,4]triazino[4,3-c][2,3]benzodiazepine-3,4-dione (7c).
Mp >300°C, yield 65%. ¹H-NMR: 3.76 (s, 3H, MeO-9), 3.65-3.82 (m, 2H, CH₂), 4.00 (s, 3H, MeO-10), 6.63 (s, 1H, H-8), 6.90 (s, 1H, H-11), 7.60-7.72 (m, 4H, Ar), 9.20 (bs, 1H, NH). Anal. (C₁₉H₁₆ClN₄O₄) C, H, N.

2,12-Dihydro-9,10-dimethoxy-7-(4-fluorophenyl)[1,2,4]triazino[4,3-c][2,3]benzodiazepine-3,4-dione (7d).
Mp >300°C, yield 64%. ¹H-NMR (CDCl₃): 3.76 (s, 3H, MeO-9), 3.65-3.82 (m, 2H, CH₂), 4.00 (s, 3H, MeO-10), 6.64 (s, 1H, H-8), 6.90 (s, 1H, H-11), 7.17 (dd, J=8.8, J=8.5, H-3',5')-7.83 (dd, J=8.8, J=6.5, H-2',6'), 9.17 (bs, 1H, NH). Anal. (C₁₉H₁₅FN₄O₄) C, H, N.

2,12-Dihydro-9,10-dimethoxy-7-(3-nitrophenyl)[1,2,4]triazino[4,3-c][2,3]benzodiazepine-3,4-dione (7e).
Mp >300°C, yield 60%. ¹H-NMR (CDCl₃): 3.75 (s, 3H, MeO-9), 3.72-3.81 (m, 2H, CH₂), 4.03 (s, 3H, MeO-10), 6.62 (s, 1H, H-8), 6.94 (s, 1H, H-11), 7.74-8.45 (m, 4H, Ar), 9.22 (bs, 1H, NH). Anal. (C₁₉H₁₅N₃O₆) C, H, N.

2,12-Dihydro-9,10-dimethoxy-7-(4-nitrophenyl)[1,2,4]triazino[4,3-c][2,3]benzodiazepine-3,4-dione (7f).
Mp >300°C, yield 57%. ¹H-NMR (CDCl₃): 3.75 (s, 3H, MeO-9), 3.69-3.84 (m, 2H, CH₂), 4.02 (s, 3H, MeO-10), 6.58 (s, 1H, H-8), 6.92 (s, 1H, H-11), 8.01-8.34 (m, 4H, Ar), 9.35 (bs, 1H, NH). Anal. (C₁₉H₁₅N₃O₆) C, H, N.

2,12-Dihydro-9,10-dimethoxy-7-(3-aminophenyl)[1,2,4]triazino[4,3-c][2,3]benzodiazepine-3,4-dione (7g).
Mp >300°C, yield 55%. ¹H-NMR (DMSO-d₆): 3.65 (s, 3H, MeO-9), 3.70 (s, 2H, CH₂), 3.85 (s, 3H, MeO-10), 5.96 (s, 2H, NH₂) 6.72 (s, 1H, H-8), 7.20 (s, 1H, H-11), 7.60-7.72 (m, 4H, Ar), 12.26 (s, 1H, NH). Anal. (C₁₉H₁₅N₃O₆) C, H, N.

2,12-Dihydro-9,10-dimethoxy-7-(4-aminophenyl)[1,2,4]triazino[4,3-c][2,3]benzodiazepine-3,4-dione (7h).
Mp >300°C, yield 50%. ¹H-NMR (DMSO-d₆): 3.62 (s, 3H, MeO-9), 3.74 (s, 2H, CH₂), 3.86 (s, 3H, MeO-10), 5.38 (s, 2H, NH₂), 6.68 (s, 1H, H-8), 7.23 (s, 1H, H-11), 6.72-7.18 (m, 4H, Ar), 10.57 (bs, 1H, NH). Anal. (C₁₉H₁₇N₅O₄) C, H, N.
Testing of Anticonvulsant Activity. 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-days-old) were purchased from Charles River (Calco, Como, 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 ip (0.1 mL/10 g of body weight of the mouse) as a freshly-prepared aqueous solution of 50% dimethyl sulfoxide (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 [1].

MES Test in Swiss Mice. Male Swiss mice (20-26 g, 42-48-days-old) were purchased from Charles River (Calco, Como, Italy). Electrical stimuli were applied via ear-clip electrodes to the mice (rectangular constant current impulses, amplitude 50 mA, width 20 ms, frequency 35 Hz, duration 400 ms) according to the method of Swinyard et al. [2] Abolition of tonic hindlimb extension after drug treatment was considered as the endpoint of protection. In general, the dose-response curves were estimated by testing four to five doses using eight to 10 mice for each dose.

PTZ-Induced Seizures in Swiss Mice. Male Swiss mice were pretreated with vehicle or drug 45 min before the subcutaneous (sc) administration of pentyleneetetrazole. For systemic injections, all tested compounds were given ip (0.1 mL/10 g of body weight of the mouse) as a freshly-prepared solution in 50% DMSO and 50% sterile saline (0.9% NaCl). The convulsive dose 97 (CD97) of PTZ (85 mg/kg) was applied and the animals observed for 30 min. A threshold convulsion was an episode of clonic spasms lasting for at least 5 s. The absence of this threshold convulsion over 30 min indicated that the tested substance had the ability to elevate PTZ seizure threshold [3].

AMPA-induced seizures in DBA/2 mice. Seizures were also induced by icv injection of AMPA. The CD50 of AMPA for clonus was 1.76 (1.06-3.07) while that for tonic was 2.90 (1.83-4.58) nmol. For icv injection, mice were anesthetized with diethyl ether, and injections were made in the left or right lateral ventricle (coordinates 1 mm posterior and 1 mm lateral to the bregma; depth 2.4 mm) using a 10 μL Hamilton microsyringe (type 701N) fitted with a nylon cuff on the needle as previously described [4]. Injections of drugs by this procedure led to a uniform distribution throughout the ventricular system within 10 min. The animals were placed singly in a 30x30x30 cm box and the observation time was 30 min after the administration of AMPA. In particular, clonic seizures were induced by AMPA (9.7 nmol/mouse, icv.) and tonic seizures by AMPA (11.7 nmol/mouse, icv.).

Electrophysiology. Transverse slices of olfactory cortex (~450 μm thick) were obtained from 150-200g male Sprague-Dawley rats as previously described [5] and stored in oxygenated Krebs solution at 32 °C for at least 30 min before being transferred to an immersion chamber for recordings. The composition of the Krebs fluid was (mM) as follows: NaCl, 118; KCl, 3; CaCl2, 1.5; NaHCO3, 25; MgCl2·6H2O, 1; and D-glucose, 11 (bubbled with 95% O2:5% CO2, pH 7.4). Conventional intracellular recordings were obtained from the periamygdaloid area of the slices within the olfactory pyramidal cell layer II-III, using glass microelectrodes (tip resistances 40-60 MΩ) filled with 4M potassium acetate. Voltage clamp recordings were made at a holding membrane potential of -70 mV with the aid of an Axoclamp 2B sample-and-hold preamplifier (2-3 kHz switching frequency; 30% duty cycle). Sampled membrane currents (filtered at 30 Hz, low pass) and voltage were recorded on a Gould TA240 chart recorder. Data are presented as mean ± SEM, and statistical significance between data groups was assessed by unpaired t-test. In addition, slices were continuously superfused with 1 μM TTX, to block voltage-activated sodium currents and induced repetitive firing at the peak of AMPA responses. AMPA and TTX were freshly prepared in Krebs solution whereas tested compound was predissolved in dimethyl sulfoxide (DMSO) to give 1mM stock solutions, and subsequently diluted in Krebs solution (containing 0.1-
1% v/v DMSO), immediately prior to use. These concentrations of DMSO had no deleterious effects on neuronal membrane properties or AMPA-induced inward currents. All measurements were performed before, during, and after bath application of pharmacological agents so that each neuron served as its own control.

For compound 5b the following experimental procedure was carried out: short (1 min) bath-sup erfusions of AMPA (0.25-5 μM; N=8 experiments) to neurons voltage clamped at -70 mV holding potential induced slow, dose-dependent and reproducible membrane inward currents (mean response measured at peak amplitude for 1 μM AMPA = 1.44 ± 0.32 nA) that attained a plateau over 3-4 min application and declined slowly to baseline current level over 7-12 min after wash out, depending on current response magnitude. A slow outward “rebound” current phase (up to ~ 0.5 nA) was also usually seen during the agonist washout period, particularly following the larger AMPA responses, most likely due to activation of electrogenic Na⁺-K⁺ pump activity [6]. In initial trial experiments, application of 5b alone (50 or 10 μM; N=4) had no effect on steady holding current at -70 mV or input resistance; however, in the presence of this compound, 1 or 2 μM AMPA responses were consistently abolished. Compound 5b was thus more effective than the previously reported 1 or 3b as AMPA antagonist [7]. In the presence of a fixed concentration (1 μM) of 5b (N =7 experiments; 15 min preincubation), the mean peak amplitude of the AMPA-evoked inward currents were suppressed by ~7-57%, over the AMPA dose range of 0.25-5 μM, respectively (Fig. 1A). In each case, the depression of 1, 2, and 5 μM AMPA mean currents by the antagonist was significant relative to control (P< 0.05, 0.01, and 0.001, respectively, by t-test). An example of such an experiment carried out on a single neuron is shown in Fig. 1B. From the clear depression of the apparent maximum of the AMPA dose-response relation, it is likely that 5b was acting via a non-competitive-type blocking mechanism at the AMPA receptor/ion channel complex.

**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 ED₅₀ values of each phase of the audiogenic seizure were determined for each dose of compound administered, and dose-response curves were fitted using a computer program by Litchfield and Wilcoxon’s method [8]. The relative anticonvulsant activities were determined by comparison of respective ED₅₀ values. Statistical significance between control and test groups of data means was tested using a two-tailed Students t-test.

**References**


