1-Aryl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones: Novel AMPA Receptor Antagonists

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Our previous publication (*Eur. J. Pharmacol.* **1995**, *294*, 411−422) reported preliminary chemical and biological studies of some 2,3-benzodiazepines, analogues of 1-(4-aminophenyl)-4-methyl-7,8-(methylenedioxy)-5*H*-2,3-benzodiazepine (**1**, GYKI 52466), which have been shown to possess significant anticonvulsant activity. This paper describes the synthesis of new 1-aryl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones and the evaluation of their anticonvulsant effects. The observed findings extend the structure−activity relationships previously suggested for this class of anticonvulsants. The seizures were evoked both by means of auditory stimulation in DBA/2 mice and by pentylenetetrazole or maximal electroshock in Swiss mice. 1-(4′-Aminophenyl)- (**38**) and 1-(3′-aminophenyl)-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one (**39**), the most active compounds of the series, proved to be more potent than **1** in all tests employed. In particular, the ED_{50} values against tonus evoked by auditory stimulation were 12.6 μmol/kg for derivative **38**, 18.3 μmol/kg for **39**, and 25.3 μmol/kg for **1**. Higher doses were necessary to block tonic extension induced both by maximal electroshock and by pentylenetetrazole. In addition these compounds exhibited anticonvulsant properties that were longer lasting than those of compound **1** and were less toxic. The novel 2,3-benzodiazepines were also investigated for a possible correlation between their anticonvulsant activities against convulsions induced by 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) and their affinities for benzodiazepine receptors (BZR). The 2,3-benzodiazepines did not affect the binding of [^{3}H]flumazenil to BZR, and conversely, their anticonvulsant effects were not reversed by flumazenil. On the other hand the 2,3-benzodiazepines antagonized seizures induced by AMPA and aniracetam in agreement with an involvement of the AMPA receptor. In addition, both the derivative **38** and the compound **1** markedly reduced the AMPA receptor-mediated membrane currents in guinea-pig olfactory cortical neurons *in vitro* in a noncompetitive manner. The derivatives **25** and **38**−**40** failed to displace specific ligands from *N*-methyl-D-aspartate (NMDA), AMPA/kainate, or metabotropic glutamate receptors.

Introduction

A large number of people worldwide suffer from epilepsy, and at least 25% of them have seizures that are resistant to available medical therapies.[2] Treatment of uncontrolled seizures therefore poses a continuing challenge in epilepsy management. Existing antiepileptic drugs and those in current development prevent seizures by a variety of mechanisms which include modulation of sodium channels or thalamic calcium currents, enhancement of GABA-mediated inhibition, or antagonist action at excitatory amino acid (EAA) receptors.

Much of the available evidences demonstrate that EAA receptor antagonists are potent anticonvulsant compounds. The more well-defined EAA receptors are the ionotropic channels activated by either *N*-methyl-D-aspartic acid (NMDA) or 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) and kainic acid (non-NMDA) and the metabotropic receptors activated by *trans*-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD).[3] Historically, NMDA receptor antagonists have received intense interest as therapeutic agents,[4] but the initial enthusiasm has been dampened by increasing awareness of their various toxicities.[5] Today, there is considerable interest in the development of potent and selective non-NMDA receptor antagonists because of their important role in the treatment of epilepsy as well as cerebral ischaemia and various forms of neurodegenerative diseases.[6,7] Studies utilizing potent AMPA/kainate receptor antagonists such as 6-nitro-7-sulfamoylbenzo[*f*]quinoxaline-2,3-dione (NBQX) have supplied experimental evidence that the design and the synthesis of AMPA receptor antagonists could help to elucidate the understanding of EAA receptor function in the pathogenesis and propagation of epileptic phenomena.

It was previously reported[8] that 1-(4-aminophenyl)-4-methyl-7,8-(methylenedioxy)-5*H*-2,3-benzodiazepine (**1**,

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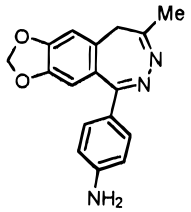
GYKI 52466) differs pharmacologically from conventional 1,4-benzodiazepines, such as diazepam, in that it lacks sedative−hypnotic activity and does not bind to benzodiazepine receptors (BZR). Compound **1** shows



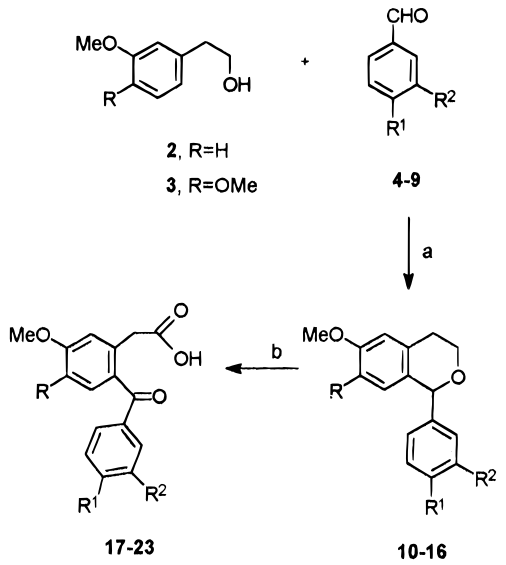
1 (GYKI 52466)

anticonvulsant and muscle-relaxant properties and proved to be a highly selective AMPA/kainate receptor antagonist; it does not affect NMDA, metabotropic glutamate, or $GABA_A$ receptor-mediated responses. With these considerations in mind and as a part of our continuing studies on potential anticonvulsant agents[9] (particularly benzodiazepine[10] and benzothiazepine[11] derivatives), we have recently reported[1] the synthesis and the anticonvulsant activity of some 2,3-benzodiazepine derivatives that are analogues of **1**. On the basis of the significant AMPA receptor antagonist activity of some of the compounds and in an attempt to increase their potency and selectivity and improve the definition of their structure−activity relationships (SAR), we now report the synthesis and anticonvulsant activity of several novel 1-aryl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-one derivatives. SAR of variation of N-3, C-8, and C-1 aromatic ring substitutions and the resulting modulation of anticonvulsant activity are described. Compounds were studied after intraperitoneal (ip) administration in DBA/2 mice, a strain genetically susceptible to sound-induced seizures, which has been considered an excellent animal model for generalized epilepsy and for screening new anticonvulsant drugs.[12] 2,3-Benzodiazepines were also examined for anticonvulsant activity both against pentylenetetrazole and maximal electroshock-induced seizures. In addition, we attempted to correlate the anticonvulsant efficacies of some of the 2,3-benzodiazepines with their respective affinities for the BZR or AMPA receptors. BZR affinities were assessed by the potencies of the 2,3-benzodiazepines to inhibit [^{3}H]flumazenil binding in a cortical membrane preparation, or by the ability of flumazenil, a "neutral" BZR antagonist, to reverse their anticonvulsant activity. Since **1** and related compounds are known not to bind competitively at the AMPA receptor,[13] the AMPA affinities of 2,3-benzodiazepines were assessed indirectly through their inhibitory action on AMPA-induced seizures. The abilities of aniracetam, a compound which potentiates the effects of AMPA,[14] to reverse the inhibition of AMPA-induced seizures by 2,3-benzodiazepines were also assessed. The results obtained were compared to the findings previously reported[1] for compounds **24**−**26** and **32**. Finally, we also conducted electrophysiological experiments on guinea pig cortical brain slice neurons to investigate the mode of inhibitory action of some 2,3-benzodiazepines at AMPA receptor sites.

Chemistry

1-Aryl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones **24**−**44** were obtained through the synthetic procedures

Scheme 1[a]

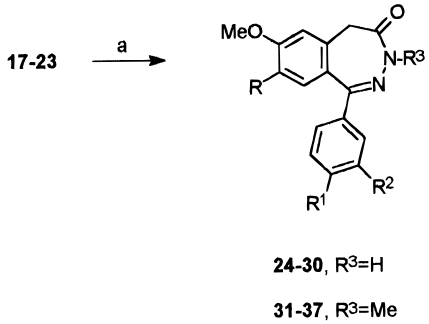


	R^1	R^2
4	H	H
5	Cl	H
6	Br	H
7	CN	H
8	NO_2	H
9	H	NO_2

	R	R^1	R^2
10,17	H	H	H
11,18	OMe	H	H
12,19	OMe	Cl	H
13,20	OMe	Br	H
14,21	OMe	CN	H
15,22	OMe	NO_2	H
16,23	OMe	H	NO_2

[a] Reagents: (a) HCl(g)-saturated dioxane, reflux, 1 h; (b) 35% H_2SO_4, CrO_3, acetone.

Scheme 2[a]

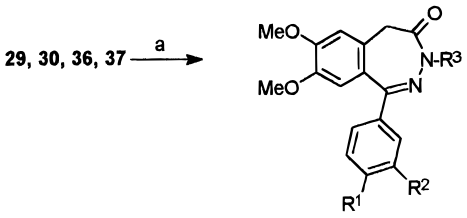


	R	R^1	R^2
24,31	H	H	H
25,32	OMe	H	H
26,33	OMe	Cl	H
27,34	OMe	Br	H
28,35	OMe	CN	H
29,36	OMe	NO_2	H
30,37	OMe	H	NO_2

[a] Reagents: (a) $NH_2NH_2 \cdot H_2O$ or $MeNHNH_2$, EtOH, reflux, 3−4 h.

outlined in Schemes 1−4. Derivatives **27**−**30** and **34**−**44** are new compounds, whereas the synthesis of **24**−**26** and **31**−**33** was previously reported by Gatta *et al.*[15] 3-Methoxy- (**2**) or 3,4-dimethoxyphenethyl alcohol (**3**) was condensed with aromatic aldehydes **4**−**9** to afford 1-arylisochromans **10**−**16**. Derivatives **10**−**16** were oxidized to 2-aroylphenylacetic acids **17**−**23** which, by treatment with hydrazine or monomethylhydrazine,

Scheme 3[a]

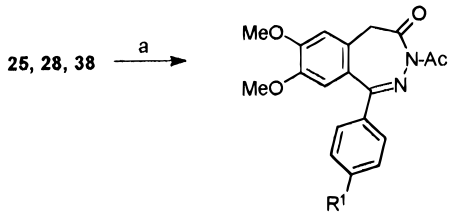


38-41

	R^1	R^2	R^3
38	NH_2	H	H
39	H	NH_2	H
40	NH_2	H	Me
41	H	NH_2	Me

[a] Reagents: (a) granulated tin, 37% HCl, reflux, 1 h.

Scheme 4[a]



42, R^1=H
43, R^1=CN
44, R^1=NAc_2

[a] Reagents: (a) Ac_2O, Et_3N, $CHCl_3$, room temperature, 1−2 h.

gave 2,3-benzodiazepin-4-ones **24**−**30** and 3-methyl-2,3-benzodiazepin-4-ones **31**−**37**, respectively. When an amino group is present on the phenyl ring at C-1 of the isochroman, the oxidation process fails to give the expected aroylphenylacetic acids. Thus, derivatives **38**−**41** were prepared by reduction with Sn/HCl of the corresponding nitro analogues **29**−**30** and **36**−**37**. Moreover, *N*-acetyl derivatives **42**−**44** were obtained from the analogous 2,3-benzodiazepin-4-ones **25**, **28**, and **38** by treatment with an excess of acetic anhydride. The structures of all the newly synthesized compounds were determined by analytical and spectral data (^{1}H NMR) and are reported in the Experimental Section.

Lipophilicity Measurements

The relative lipophilicity (R_m) (see the Experimental Section) of the 2,3-benzodiazepines studied are summarized in Table 1. As expected, the most lipophilic compounds were **33** and **34**, owing to the simulataneous presence of a methyl group and a halogen atom.

Biological Results

Anticonvulsant Activity against Audiogenic Seizures in DBA/2 Mice. The anticonvulsant properties of compounds **24**−**44** were evaluated 30 min after ip administration of several doses (dose range 3.3−200 μmol/kg) in DBA/2 mice. Table 1 reports the median effective dose (ED_{50}) values required to prevent clonic and tonic phases of sound-induced seizures. The rank order of anticonvulsant potency was as follows: **38** > **39** > **25** > **1** > **41** > **32** > **33** > **40** > **44** > **34** > **24** > **26** > **42** > **27**. The remaining compounds showed no activity. The wild running phase was significantly reduced after ip administration of **24**−**27**, **31**−**33**, **38**−**41**, and **44** at the highest doses tested.

Following ip administration of some active compounds such as **25**,[1] **32**,[1] **38**, **40**, and **44**, 33 μmol/kg, maximum protection was observed from 30 to 90 min for compound **38** and from 45 to 90 min for the others with subsequent return to control seizure response at 180 min for all tested derivatives, as shown in Figure 1 for **38**, **40**, and **44**. In contrast, **1**, 33 μmol/kg, displayed the maximum protection from 5 to 15 min followed by gradual return to control seizure response between 30 and 90 min (Figure 1).

Anticonvulsant Properties against Maximal Electroshock in Swiss Mice. As shown in Table 2, the

Table 1. ED_{50} Values (95% Confidence Limits) of **24**−**44** and **1** against the Clonic and Tonic Phases of the Audiogenic Seizures in DBA/2 Mice after 30 min Pretreatment[a] and Relative Lipophilicity (R_m) of the Same 2,3-Benzodiazepines

		ED_{50}, μmol/kg (±95% confidence limits)		ED_{50}, mg/kg (±95% confidence limits)		
compd	dose range (μmol/kg)	clonic phase	tonic phase	clonic phase	tonic phase	R_m
24[b]	33−200	75.5 (47.5−120)	64.8 (45.0−93.3)	20.0 (12.6−31.9)	17.2 (11.9−24.8)	−0.052
25[b]	10−100	33.9 (26.0−44.2)	31.8 (24.8−40.6)	10.0 (7.71−13.1)	9.41 (7.34−12.0)	−0.012
26[b]	33−200	102 (76.1−137)	75.3 (60.7−93.2)	33.7 (25.1−45.2)	24.8 (20.0−30.7)	0.017
27	10−133	110 (79.3−151)	82.5 (58.6−116)	41.2 (29.7−56.5)	30.7 (21.9−43.5)	0.052
28	10−120	>120	>120	>44	>44	−0.218
29	10−120	>120	>120	>44	>44	−0.096
30	10−120	>120	>120	>44	>44	−0.140
31	33−133	117 (93.5−146)	99.1 (82.2−119)	32.8 (26.2−40.9)	27.8 (23.0−33.3)	0.189
32[b]	10−100	37.8 (23.7−60.1)	26.7 (14.7−48.2)	11.7 (7.35−18.6)	8.28 (4.56−15.0)	0.000
33	33−66	41.2 (36.0−47.2)	38.6 (33.6−44.4)	14.2 (12.4−16.3)	13.3 (11.6−15.3)	0.250
34	10−100	63.0 (33−121)	38.0 (20.0−72.0)	24.5 (12.8−47.0)	14.8 (7.78−28.0)	0.288
35	10−120	>120	>120	>44	>44	−0.031
36	10−120	>120	>120	>44	>44	0.110
37	10−120	>120	>120	>44	>44	0.052
38	3.3−100	15.0 (9.01−24.0)	12.6 (8.01−19.0)	4.66 (2.80−7.46)	3.92 (2.49−5.91)	−0.602
39	10−66	19.3 (16.9−22.0)	18.3 (16.0−20.8)	6.00 (5.25−6.84)	5.69 (4.98−6.47)	−0.525
40	10−100	50.2 (34.6−73.0)	43.7 (31.3−61.0)	16.3 (11.2−23.7)	14.2 (10.2−19.8)	−0.374
41	10−66	36.8 (28.3−47.7)	30.6 (23.8−39.3)	11.9 (9.20−15.5)	9.94 (7.73−12.8)	−0.327
42	10−100	101 (52.0−194)	72.1 (47.6−109)	33.3 (17.6−65.6)	24.4 (16.1−36.9)	−0.052
43	10−120	>120	>120	>44	>44	−0.065
44	10−100	56.8 (39.3−82.1)	43.9 (31.2−61.9)	24.8 (17.2−35.8)	19.2 (13.6−27.0)	−0.673
1	3.3−66	35.8 (24.4−52.4)	25.3 (16−40.0)	10.5 (7.15−24.7)	7.41 (4.68−11.7)	−0.298

[a] All data were calculated according to the method of Litchfield and Wilcoxon.[41] [b] Reference 1.

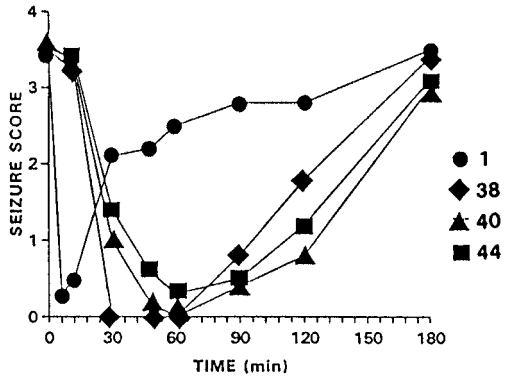


Figure 1. Anticonvulsant effects of **38**, **40**, **44**, and **1** (33 μmol/kg ip) against audiogenic seizures in DBA/2 mice. The ordinate shows seizure score; the abscissa shows the time after intraperiotoneal administration of drug in min. Ten animals were used for the determination of each point.

Table 2. ED_{50} Values (95% Confidence Limits) of Various 2,3-Benzodiazepines against the Maximal Electroshock and Pentyleneterazole-Induced Seizures in Swiss Mice after 45 min Pretreatment[a]

		ED_{50}, μmol/kg (±95% confidence limits)	
compd	dose range	maximal electroshock	pentylenetetrazole
24	33−200	73.4 (34.6−155)	98.2 (56.0−172)
25	10−100	35.8 (28.6−44.7)	68.2 (54.6−85.2)
26	33−200	115 (81.2−164)	145 (83.4−254)
27	10−150	123 (98.7−154)	>150
28	10−150	>150	>150
29	10−150	>150	>150
30	10−150	>150	>150
32	10−150	42.7 (26.5−68.8)	89.9 (51.5−157)
36	10−150	>150	>150
38	10−100	15.9 (7.3−33.5)	22.6 (11.7−43.8)
40	10−150	57.2 (41.5−78.8)	70.8 (44.7−112)
1	10−100	35.7 (29.3−43.4)	68.3 (56.2−83.1)

[a] All data were calculated according to the method of Litchfield and Wilcoxon.[41]

tonic extension of the seizures induced by maximal electroshock was significantly reduced 45 min after ip administration of **24**−**27**, **32**, **38**, **40**, and **1**, while compounds **28**−**30** and **36** at doses up to 150 μmol/kg ip had no anticonvulsant activity against maximal electroshock seizures.

Anticonvulsant Properties against Pentylenetetrazole-Induced Seizures in Swiss Mice. As shown in Table 2, the clonic phase of the seizures induced by pentylenetetrazole was significantly reduced 45 min after ip administration of **24**−**26**, **32**, **38**, **40**, and **1** at doses higher than those which protected against maximal electroshock. Compounds **27**−**30** and **36** at doses up to 150 μmol/kg ip had no anticonvulsant properties against seizures induced by pentylenetetrazole.

The most active compounds of the series were further evaluated, and the biological results are reported below.

Anticonvulsant Activity against AMPA-Induced Seizures in DBA/2 Mice. Intracerebroventricular (icv) administration of 10 μL of AMPA (1−10 nmol) induced generalized seizures in DBA/2 mice, with CD_{97} (the dose which induced convulsions in 97% of mice) values of 9.7 nmol for all-limb clonic seizures (latency 1.3 ± 0.3 min) and 11.7 nmol for forelimb tonic seizures (latency 1.9 ± 0.4 min), respectively. Table 3 reports the anticonvulsant ED_{50} values of a series of 2,3-benzodiazepines against AMPA-induced clonic (9.7 nmol AMPA icv) and tonic seizures (11.7 nmol AMPA icv). The clonic and tonic phases of the seizure induced by AMPA were significantly reduced 30 min after ip administration both of **25** and **32** as already reported[1] and of **38**−**40** and **1** as shown in Figure 2.

Table 3. ED_{50} Values (95% Confidence Limits) of Some 2,3-Benzodiazepines against the Clonic and Tonic Seizures Induced by icv Injection of AMPA in DBA/2 Mice[a]

		ED_{50}, μmol/kg (±95% confidence limits)	
compd	dose range	clonic phase	tonic phase
25[b]	10−200	66.0 (45.9−94.9)	42.6 (26.4−68.8)
32[b]	10−200	76.1 (47.5−122.1)	69.6 (44.1−110.1)
38	10−100	32.1 (23.2−44.3)	25.0 (16.5−30.0)
39	10−66	30.9 (23.9−39.9)	27.8 (21.5−35.9)
40	10−200	72.1 (47.5−109.4)	62.4 (44.7−87.3)
1	10−100	57.5 (43.5−76.0)	40.5 (26.3−60.8)

[a] AMPA was administered icv at the CD_{97} for either clonus (9.7 nmol) or forelimb tonic extension (11.7) 30 min after injection of 2,3-benzodiazepines. All data were calculated according to the method of Litchfield and Wilcoxon.[41] [b] Reference 1.

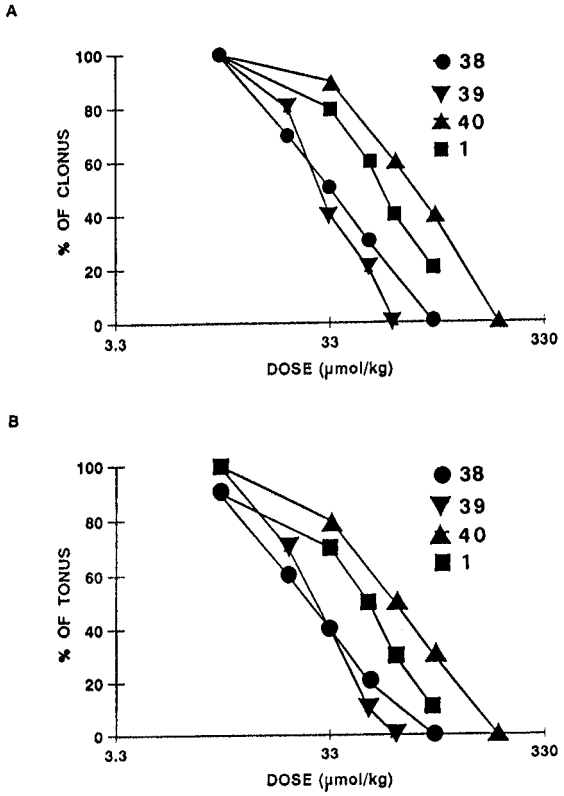


Figure 2. Anticonvulsant effects of **38**−**40** and **1** against seizures induced by AMPA in DBA/2 mice. The ordinate shows percent of response of clonic (A) or tonic (B) seizures; the abscissa shows the dose in μmol/kg ip. For the determination of each point, 10 animals were used.

The highest doses of 2,3-benzodiazepines studied induced ataxia, splayed hind limbs, and tremor in some animals during the period of maximal anticonvulsant activity.

Pretreatment with Aniracetam. The icv injection of aniracetam alone (12.5, 50, and 100 nmol), 60 min before testing, did not significantly modify the audiogenic seizure response in DBA/2 mice, but aniracetam (50 nmol icv), 60 min before testing, reversed the anticonvulsant effects of 2,3-benzodiazepines in DBA/2 mice. Aniracetam reduced the anticonvulsant efficacy of **25**, **32**, **38**, **40**, and **1** (Table 4), resulting in an increase of anticonvulsant ED_{50} values ranging from 2.6- to 7.3-

Table 4. ED_{50} Values (95% Confidence Limits) of Some 2,3-Benzodiazepines against the Clonic and Tonic Phases of the Audiogenic Seizures after Pretreatment with Aniracetam in DBA/2 Mice[a]

		ED_{50}, μmol/kg (±95% confidence limits)	
compd	dose range	clonic phase	tonic phase
25[b]	33−300	215 (142−324)*	157 (114−217)*
32[b]	33−300	259 (176−380)*	197 (137−281)*
38	10−200	65.4 (44.5−96.2)*	58.2 (43.4−77.9)*
40	10−200	141 (101−198)*	112 (83.8−150)*
1	10−200	134 (88.8−203)*	100 (63.4−158)*

[a] All data were calculated according to the method of Litchfield and Wilcoxon.[41] Significant differences between ED_{50} values of group treated with aniracetam + 2,3-benzodiazepine and group treated with 2,3-benzodiazepine alone (Table 1) are denoted *$P < 0.01$. [b] Reference 1.

Table 5. ED_{50} Values (95% Confidence Limits) of Some 2,3-Benzodiazepines against the Clonic and Tonic Phases of the Audiogenic Seizures after Concomitant Treatment with Flumazenil in DBA/2 Mice[a]

		ED_{50}, μmol/kg (±95% confidence limits)	
compd	flumazenil, μmol	clonic phase	tonic phase
25[b]	8.24	38.2 (28.5−51.2)	35.8 (26.2−49.0)
	24.72	50.9 (36.9−70.1)	41.3 (28.0−60.7)
38	8.24	14.1 (10.1−19.9)	12.0 (6.82−21.0)
	24.72	12.0 (6.82−21.0)	10.2 (7.61−13.7)
39	8.24	21.0 (17.2−25.8)	18.2 (8.90−48.2)
	24.72	29.2 (8.90−48.7)	17.5 (8.70−35.1)
40	8.24	51.2 (24.8−106)	38.5 (21.3−69.6)
	24.72	45.9 (22.5−93.7)	32.2 (17.7−58.8)
1	8.24	37.8 (23.7−60.1)	26.7 (14.7−48.2)
	24.72	39.5 (29.6−53.7)	29.7 (20.9−42.1)

[a] All data were calculated according to the method of Litchfield and Wilcoxon.[41] [b] Reference 1.

fold. The corresponding aniracetam-induced shifts to the right of the dose−response curves for protection by **38** and **40** against sound-induced clonic and tonic seizures are shown in Figure 3.

The highest doses of some 2,3-benzodiazepines studied induced ataxia, splayed hind limbs, and tremor in some animals during the period of maximal anticonvulsant activity.

Treatment with Flumazenil. As previously demonstrated,[16] flumazenil, administered ip at 8.24 or 24.7 μmol/kg, is not itself convulsant and does not significantly modify the phases of the audiogenic seizure response in DBA/2 mice, but antagonizes the anticonvulsant action of classical 1,4-benzodiazepines. In order to ascertain the possible involvement of benzodiazepine receptors in the antiseizure activity of 2,3-benzodiazepines, derivatives **25**, **38**−**40**, and **1** were administered concomitantly with flumazenil, but no significant modification of the antiseizure effects of these derivatives was observed. The ED_{50} values for the different phases of audiogenic seizures are reported in Table 5.

Inhibition of Binding, *in Vitro*. The potency of the 2,3-benzodiazepines as inhibitors of [^{3}H]flumazenil binding to membranes from cortex was evaluated, and no inhibition was observed (IC_{50} > 10000 nM).

By using crude cortical synaptic membranes prepared from the rat brain, the derivative **25** and **38**−**40** failed to displace [^{3}H]spiperone from dopamine and 5-hydroxytryptamine$_1$ receptors; [^{3}H]ketanserin from 5-hydroxytryptamine$_2$ receptors; [^{125}I]pindolol from β-adrenergic

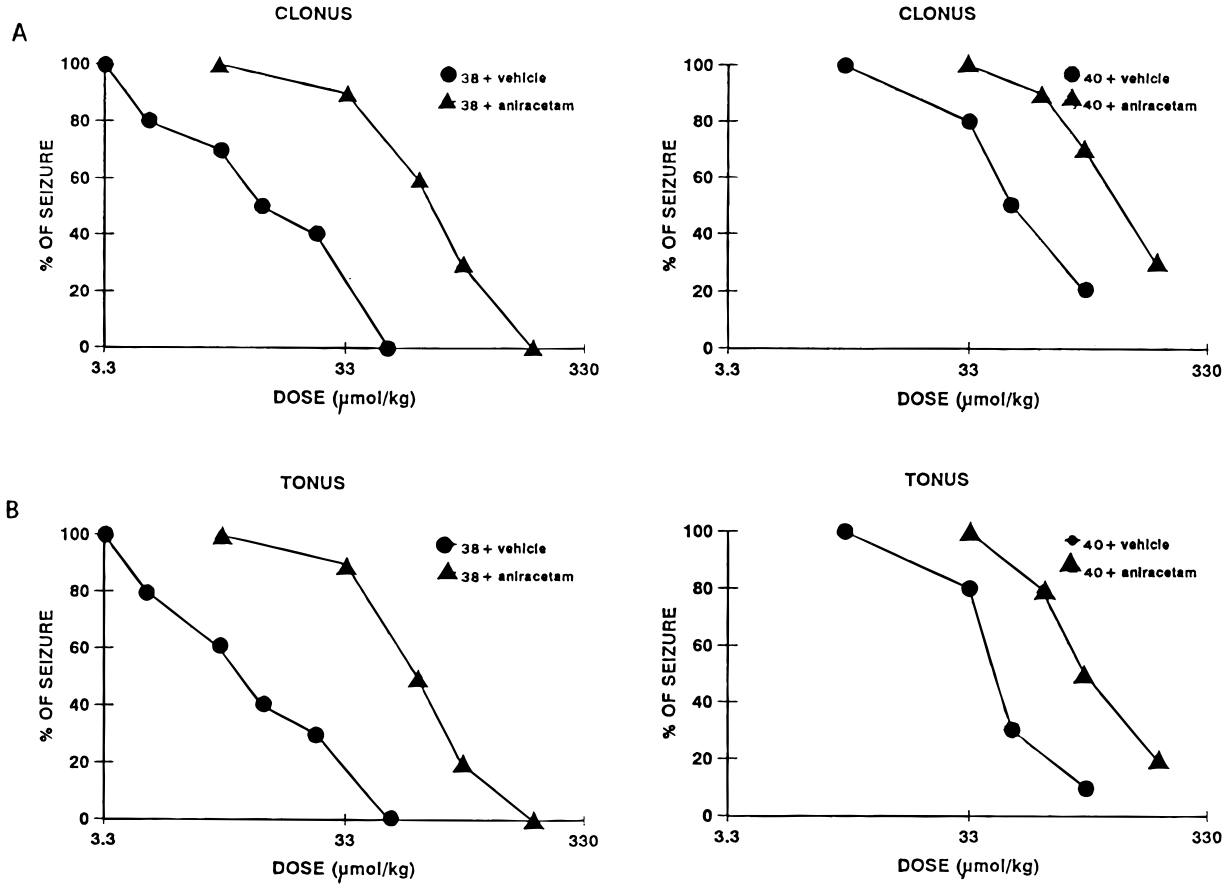


Figure 3. Antagonism by aniracetam (50 nmol icv) of the anticonvulsant effects of **38** and **40** against audiogenic seizures in DBA/2 mice. The ordinate shows percent of response of clonic (A) or tonic (B) seizures. The abscissa shows the dose in μmol/kg ip. For the determination of each point, 10 animals were used.

Table 6. ED_{50} and TD_{50} Values (95% Confidence Limits) of Various 2,3-Benzodiazepines on Locomotion Assessed by Rotarod Test following 30 min Pretreatment[a]

compd	ED_{50} clonic phase	TD_{50} locomotor deficit	TI[c] TD_{50}/ED_{50}
24[b]	75.5 (47.5−120)	197 (138−281)	2.6
25[b]	33.9 (26.0−44.2)	142 (87.3−231)	4.2
26[b]	102 (76.1−137)	240 (102−564)	2.3
27	110 (79.3−151)	226 (116−437)	2.0
28	>120	>150	ND
29	>120	>150	ND
30	>120	>150	ND
32[b]	37.8 (23.7−60.1)	154 (104−227)	4.1
35	>120	>150	ND
36	>120	>150	ND
38	15.0 (9.01−24.0)	56.8 (39.3−82.1)	3.8
39	19.3 (16.9−22.0)	51.5 (34.1−77.8)	2.7
40	50.2 (34.6−73.0)	159 (95−268)	3.2
42	101 (52.0−194)	181 (106−309)	1.8
43	>120	>150	ND
1	35.8 (24.4−52.4)	76.1 (47.5−122)	2.1

[a] All data are expressed as μmol/kg and were calculated according to the method of Litchfield and Wilcoxon.[41] [b] Reference 1. [c] TI = therapeutic index and represents the ratio between TD_{50} and ED_{50} (from the clonic phase of the audiogenic seizures); ND = not determined.

receptors; [^{3}H]-(*RS*)-3-(2-carboxypiperazin-4-yl)propane-1-phosphonic acid ([^{3}H]CPP) and [^{3}H]dizocilpine ([^{3}H]-MK-801) from NMDA receptors; [^{3}H]-5,7-dichlorokynurenic acid ([^{3}H]5,7-DCKA) from glycine site on NMDA receptors; [^{3}H]AMPA and [^{3}H]-6-cyano-7-nitroquinoxaline-2,3-dione ([^{3}H]CNQX) from AMPA/kainate receptors; mixture of [^{3}H](1S,3R)ACPD and [^{3}H](1R,-3S)ACPD from metabotropic glutamate receptors.

Effects on Motor Movements. Table 6 shows the doses which induced motor toxicity in 50% of mice (TD_{50} values) obtained 30 min following the ip administration of various 2,3-benzodiazepines. Compounds **24**, **26**−**27**, **39**, **42**, and **1** affected the rotarod test at doses approximately double those which showed anticonvulsant activity while derivatives **25**, **32**, **38**, and **40** had therapeutic index (TI) values in the range 3.2−4.2.

Effects of 2,3-Benzodiazepine Derivatives on AMPA Receptor-Mediated Membrane Currents, *in Vitro*. The effects of **38**, the most active compound of the series, and **1** were compared on AMPA receptor-mediated membrane currents measured under voltage clamp in guinea pig olfactory cortical neurons *in vitro*. Inward currents induced by bath application of AMPA were recorded in the presence of 1 μM tetrodotoxin (TTX) to prevent voltage-activated sodium currents and repetitive sodium spikes at the peak of AMPA responses. When applied to neurons voltage clamped at −80 mV holding potential, AMPA (0.25−5 μM; 4 min; $N = 7$ experiments) produced a slow dose-dependent inward current of large amplitude (mean amplitude at 1 μM; 0.73 ± 0.12 nA) that attained a plateau over 3−4 min of application and declined slowly to baseline current level over 5−10 min after drug washout (often with a slow outward "rebound" current phase, Figure 4). This response was reproducible, providing an adequate period (15−20 min) of intermediate washout was allowed. In the presence of a fixed concentration (50 μM) of **38** ($N = 4$ experiments; 15 min preincubation), the peak amplitude of AMPA-induced inward currents was markedly reduced at each agonist dose level (∼75−85% suppression) with a clear noncompetitive-type depression of the AMPA dose−response curve. A similar type and degree of depression was observed in the presence of 50 μM **1** (Figure 4). In each case, the depression of 1, 2, and 5 μM AMPA mean currents by the antagonists was significant relative to control ($P <$ 0.05, 0.01, and 0.001, respectively, by *t*-test). The blocking effects of **38** or **1** were fully reversible within 20−30 min of antagonist washout (data not shown). Neither agent tested alone showed any effect on steady holding current at −80 mV.

Discussion

The recent availability of centrally active antagonists of non-NMDA (AMPA/kainate) receptors has made it possible to evaluate the potential of such compounds as antiepileptic agents in animal seizure models. Several previous studies[4,6,17−19] have indicated that the systemic administration of AMPA receptor antagonists to experimental animals produces anticonvulsant activity; the present results would confirm these effects.

Several 2,3-benzodiazepine derivatives reported in the present study have marked anticonvulsant activity, in some cases higher than compound **1**, both in the audiogenic seizure test with DBA/2 mice (Table 1) and against the maximal electroshock- and pentylenetetrazole-induced seizures in Swiss mice (Table 2).

The structure−activity relationships in this series were examined by varying C-8, N-3, and C-1 aromatic ring substitutions in order to test the influence of physicochemical parameters on anticonvulsant activity. One or two methoxy groups were introduced in the 7- and 8-positions of the benzodiazepine moiety; H at N-3 was substituted with a methyl or an acetyl group, and in particular, a number of substitutions at the C-4′ position of the phenyl ring at C-1 were explored. Finally, since the 4′-aminophenyl derivative proved to be the most active compound of the series, the position of the NH_2 substituent was also varied.

The ability of the structural changes introduced into the 2,3-benzodiazepines to modify the anticonvulsant efficacies of the parent compounds is shown in Tables 1 and 2 and discussed below.

The presence of two methoxy groups on the benzodiazepine system increases anticonvulsant activity; in fact, the dimethoxy-substituted compound **25** is more active than the monomethoxy derivative **24** and shows an anticonvulsant activity comparable to that of **1**.

The introduction of a bromine or a chlorine atom and a cyano or a nitro group on the phenyl ring at C-1 negatively influences the activity, whereas the presence of an amino group, independently by its position, increases the anticonvulsant activity of the unsubstituted parent compound **25** (Tables 1 and 2), the most active compounds being the amino derivatives **38** and **39**, as with other amino derivatives.[20] It is interesting to note that **38** and **39** proved to be more potent than compound **1**.

The presence of a methyl and in particular an acetyl group at N-3 generally led to compounds with weaker anticonvulsant properties compared to the N-3 unsubstituted derivatives. The time course studied for derivatives **40** and **44** suggests (Figure 1) that a metabolic activation might take place *in vivo* and that the N-substituted derivatives undergo biotransformation in the parent compounds by loss of the N-3 substituent. We have chemical evidence from an HPLC study that

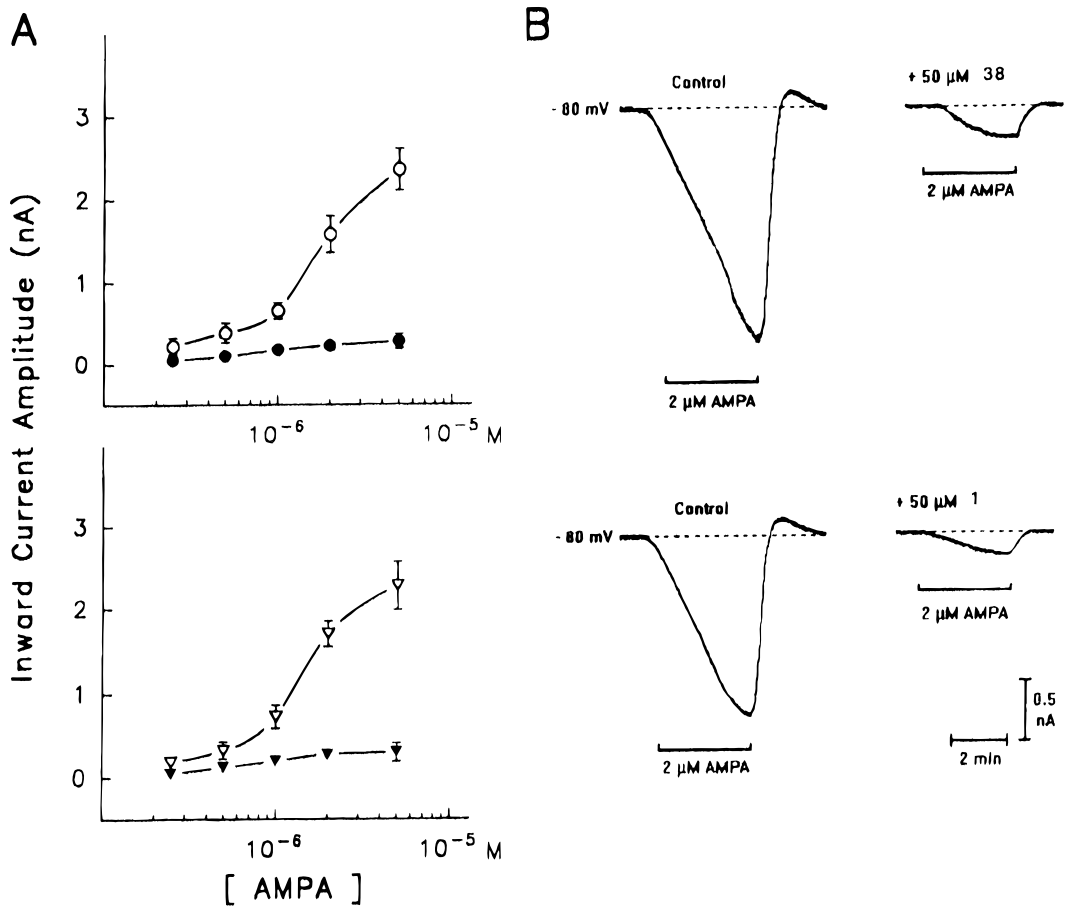


Figure 4. Noncompetitive type antagonism of AMPA-induced inward currents by 2,3-benzodiazepine derivatives, recorded in guinea pig olfactory cortical neurons voltage-clamped at −80 mV (in the presence of 1 μM TTX). (A) Pooled dose−response curves to AMPA measured in the absence (○; $N = 4$) and presence (●; $N = 4$) of 50 μM compound **38** (upper panel) or in absence (▽; $N = 3$) and presence (▼; $N = 3$) of 50 μM **1** (lower panel). Each point represents mean (±SEM) plotted against AMPA concentration (0.25−5 μM; log scale). Points without error bars had SEM less than the symbol size. (B) Chart records showing inward currents activated by a 4 min bath application of AMPA (2 μM) in control and after 15 min preincubation with 50 μM compound **38** (upper panel) or 50 μM **1** (lower panel); note the similar degree of response depression induced by both compounds. The depression of 1, 2, and 5 μM AMPA mean currents by either antagonist was significant relative to control ($P < 0.05$, 0.01, and 0.001, respectively; Students *t*-test). Scale bar applies to all traces. Drug washout periods were recorded at 0.4 × chart speed.

compounds **40** and **40** are converted to compound **38** in rats (unpublished data), and this could better explain the results of the behavioral time course effects. Unexpectedly, the presence of a methyl group at the N-3 position in compounds **33** and **34** enhances the activity with respect to the related unsubstituted **26** and **27** derivatives (Table 1). The reason for this different behavior with respect to all the other N-substituted derivatives is not presently understood. Further experiments on these compounds and their metabolites are in progress and will be detailed in future reports.

The different anticonvulsant potencies of the 2,3-benzodiazepines were only partially correlated to their relative lipophilicities (Table 1), indicating intrinsic differences in the anticonvulsant potencies of the compounds, rather than merely a different degree of penetration through the blood-brain barrier following systemic administration.

Figure 1 shows that when anticonvulsant activity was assessed 15 min after treatment with drug, compound **1** demonstrated an anticonvulsant potency higher than other 2,3-benzodiazepines, indicating faster blood−brain barrier transport, or higher affinities for the AMPA receptor,[6] whereas the 2,3-benzodiazepines tested, **38**, **40**, and **44**, become more potent 30 min after pretreatment and showed a more prolonged time course of action than **1**.

Data supporting the hypothesis that the 2,3-benzodiazepine derivatives tested interact with non-NMDA receptors are also reported. Specifically, the drugs were found to afford effective protection against seizures induced by AMPA, a chemical agent which stimulates the AMPA/kainate receptor complex (Table 3).

In the present study we also demonstrated that aniracetam, a potentiator of AMPA effect,[14] markedly antagonized the anticonvulsant effects of **25**, **32**, **38**, and **40** in DBA/2 mice (Table 4) with a pattern of activity similar to **1** and NBQX[7,18] and shifted to the right the dose−response curves for derivatives **38** and **40** (Figure 3). Since **1** and NBQX are considered as AMPA/kainate receptor antagonists,[6,17,18,21] we suggest that 2,3-benzodiazepines also act as antagonists at this receptor site. It has been shown that aniracetam is a positive allosteric modulator of AMPA/kainate-selective glutamate receptors by reducing the rate of rapid receptor autodensensitization[14] and that it reverses the anticonvulsant properties of some 2,3-benzodiazepines (Table 4), we therefore suggest that, by analogy to **1**,[20,22−23] the 2,3-benzodiazepines described here might antagonize

the AMPA/kainate receptor-mediated responses by an allosteric blocking mechanism. Indeed, the noncompetitive nature of the AMPA block exerted by the most potent of the 2,3-benzodiazepine derivative tested (**38**) was confirmed in electrophysiological experiments performed in olfactory cortical brain slice neurons. In these tests, both **38** and **1** consistently depressed the AMPA dose−response relation in a nonparallel manner, even at the highest agonist concentration used (5 μM). This noncompetitive mode of antagonism of AMPA responses by **1** detected here is in agreement with previous *in vitro* data obtained from cultured hippocampal neurones,[23,24] cortical wedges,[25] and hippocampal slices.[26] The similarity between **38** and **1** effects further implies that the 2,3-benzodiazepine analogues may all share a common noncompetitive mode of action at the AMPA receptor.

The anticonvulsant activity of these compounds was evident at doses which generally did not cause sedation and ataxia. It has long been known that antagonists of the EAA receptors, especially those which block ion channels [e.g. dizocilpine (MK-801)], can induce cognitive deficits and a variety of other neurological and behavioral side effects.[22,27] There are, however, studies showing that potent antagonists at the AMPA/kainate receptor have anticonvulsant effects at doses below those impairing behavior.[28] The anticonvulsant profiles and improved therapeutic indices of some of the new 2,3-benzodiazepines provide an interesting illustration of the research effort which is being made to discover new drugs interacting with EAA receptors and possessing therapeutic potential with lower side effects.[4,22,29] The therapeutic indices (TI) of the present compounds were similar to that of **1** with the exception of **25**, **32**, **38**, and **40**, which caused significantly less motor impairment in the rotarod test and had TI values approximately twice that of **1** (Table 6). Since the anticonvulsant effects of **25**, **38**−**40**, and **1** were not reversed by flumazenil under an identical dose regimen (Table 5), it seems likely that the actions of these derivatives were not mediated by benzodiazepine receptor sites. The latter consideration derives also from the following observations: (a) the amounts of 2,3-benzodiazepines used in this study had not significant effects on the GABA/benzodiazepine receptor complex; (b) the 2,3-benzodiazepines showed significant anticonvulsant activity against AMPA; (c) the anticonvulsant effects were reversed by aniracetam but not by flumazenil. Since AMPA/kainate receptor antagonists have been claimed to be more effective than NMDA receptor antagonists in reducing damage in models of global ischaemia,[30] the present data would support the suggestion that the neuroprotective effects of 2,3-benzodiazepines are mediated through the AMPA/kainate receptor complex.

Despite the lack of activity of the 2,3-benzodiazepines at dopamine, serotonin, noradrenaline, NMDA, glycine site on NMDA and metabotropic glutamate binding sites, an interaction at other sites involved in the generation or expression of seizures cannot presently be ruled out. Since no reliable ligand-binding assay has been reported for noncompetitive agonists and antagonists affecting the AMPA receptor,[13] our binding data do not exclude that these new series of 2,3-benzodiazepines may act as noncompetitive AMPA antagonists. Electrophysiological experiments with the derivative **38** confirm this hypothesis.

In conclusion, the novel 2,3-benzodiazepine derivatives reported in this study have marked anticonvulsant activity both in the audiogenic seizures test with DBA/2 mice and against the maximal electroshock- and pentylenetetrazole-induced seizures in Swiss mice. In particular, derivatives **25** and **32** showed activities comparable to that of **1**, and compounds **38** and **39** were more potent than **1** *in vivo*. They also showed longer lasting anticonvulsant action and less toxicity than this compound. Moreover, they showed significant anticonvulsant activity against seizures induced by AMPA, thus suggesting an involvement of AMPA receptors, whereas they had no significant effects on BZRs. Electrophysiological data suggest a noncompetitive blocking mechanism of the 2,3-benzodiazepines at the AMPA receptor site at the doses studied. The structural features which determine the best biological profile in this class of compounds seem to be as follows: two methoxy groups at C-7 and C-8, an amino group on the phenyl ring at C-1, and a hydrogen atom at N-3 position. Further studies aimed at better elucidating the distribution, metabolism, and mechanism of action of 2,3-benzodiazepines are currently in progress.

Experimental Section

Chemistry. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Elemental analyses were made on a Carlo Erba 1106 elemental analyzer for C, H, and N, and the results are within ±0.4% of the theoretical values. ^{1}H NMR spectra were recorded in $CDCl_3$ on a Varian Gemini-300 spectrometer. Chemical shifts were expressed in δ (ppm) relative to TMS as internal standard. All exchangeable protons were confirmed by addition of D_2O.

Compounds **10**−**12**, **17**−**19**, **24**−**26**, and **31**−**33** were prepared according to a procedure previously described.[15]

General Procedure for the Synthesis of 2-Arylisochromans 13−16. A solution of 3,4-dimethoxyphenethyl alcohol **3** (1.82 g 10 mmol) and the suitable aldehyde **6**−**9** (12 mmol) in anhydrous dioxane (30 mL) was first saturated with gaseous HCl and then refluxed for 1 h. After cooling, the reaction mixture was poured into water (100 mL), made alkaline with 2 N NaOH (20 mL), and extracted with EtOAc (3 × 60 mL). The organic phase was dried over $MgSO_4$, the solvent was removed under reduced pressure, and the resulting residue was crystallized from EtOH to provide compounds **13**−**16**.

1-(4′-Bromophenyl)-6,7-dimethoxyisochroman (13). Mp: 109−111 °C. Yield: 44%. ^{1}H NMR: 2.73 and 3.00 (2m, 2H, CH_2-4), 3.67 and 3.88 (2s, 6H, OCH_3-7 and OCH_3-8), 3.89 and 4.10 (2m, 2H, CH_2-3), 5.64 (s, 1H, H-1), 6.18 (s, 1H, H-6), 6.65 (s, 1H, H-9), 7.17−7.50 (m, 4H, Ar). Anal. ($C_{17}H_{17}BrO_3$) C, H.

1-(4′-Cyanophenyl)-6,7-dimethoxyisochroman (14). Mp: 116−118 °C. Yield: 92%. ^{1}H NMR: 2.73 and 3.04 (2m, 2H, CH_2-4), 3.66 and 3.88 (2s, 6H, OCH_3-7 and OCH_3-8), 3.89 and 4.09 (2m, 2H, CH_2-3), 5.71 (s, 1H, H-1), 6.14 (s, 1H, H-6), 6.67 (s, 1H, H-9), 7.42−7.67 (m, 4H, Ar). Anal. ($C_{18}H_{17}NO_3$) C, H, N.

1-(4′-Nitrophenyl)-6,7-dimethoxyisochroman (15). Mp: 138−140 °C. Yield: 77%. ^{1}H NMR: 2.76 and 3.07 (2m, 2H, CH_2-4), 3.66 and 3.88 (2s, 6H, OCH_3-7 and OCH_3-8), 3.89 and 4.12 (2m, 2H, CH_2-3), 5.76 (s, 1H, H-1), 6.14 (s, 1H, H-6), 6.68 (s, 1H, H-9), 7.48−8.23 (m, 4H, Ar). Anal. ($C_{17}H_{17}NO_5$) C, H, N.

1-(3′-Nitrophenyl)-6,7-dimethoxyisochroman (16). Mp: 110−111 °C. Yield: 85%. ^{1}H NMR: 2.76 and 3.07 (2m, 2H, CH_2-4), 3.66 and 3.89 (2s, 6H, OCH_3-7 and OCH_3-8), 3.67 and 4.13 (2m, 2H, CH_2-3), 5.78 (s, 1H, H-1), 6.16 (s, 1H, H-6), 6.68 (s, 1H, H-9), 7.50−8.20 (m, 4H, Ar). Anal. ($C_{17}H_{17}NO_5$) C, H, N.

General Procedure for the Synthesis of 2-Aroylphenylacetic Acids 20−23. To a cooled stirred solution of **13**−**16** (0.002 mol) in acetone was added dropwise under 5 °C a solution of CrO_3 in 35% H_2SO_4. After the addition, the mixture was stirred for 2 h. The reaction mixture was poured into ice water. The resulting precipitate was filtered, washed with water, and crystallized from benzene to give **20**−**23**.

2-(4′-Bromophenyl)-4,5-dimethoxyphenylacetic Acid (20). Mp: 228−230 °C. Yield: 72%. ^{1}H NMR: 3.76 (s, 2H, CH_2), 3.78 and 3.97 (2s, 6H, OCH_3-7 and OCH_3-8), 6.89 (s, 1H, H-6), 6.96 (s, 1H, H-9), 7.63−7.71 (m, 4H, Ar). Anal. ($C_{17}H_{15}BrO_5$) C, H, N.

2-(4′-Cyanophenyl)-4,5-dimethoxyphenylacetic acid (21). Mp: 153−155 °C. Yield: 24%. ^{1}H NMR: 3.78 and 3.99 (2s, 6H, OCH_3-7 and OCH_3-8), 3.81 (s, 2H, CH_2), 6.86 (s, 1H, H-6), 6.99 (s, 1H, H-9), 7.80−7.94 (m, 4H, Ar). Anal. ($C_{18}H_{15}NO_5$) C, H, N.

2-(4′-Nitrophenyl)-4,5-dimethoxyphenylacetic acid (22). Mp: 170−172 °C. Yield: 25%. ^{1}H NMR: 3.77 and 3.99 (2s, 6H, OCH_3-7 and OCH_3-8), 3.86 (s, 2H, CH_2), 6.86 (s, 1H, H-6), 6.95 (s, 1H, H-9), 7.96−8.36 (m, 4H, Ar). Anal. ($C_{17}H_{15}NO_7$) C, H, N.

2-(3′-Nitrophenyl)-4,5-dimethoxyphenylacetic Acid (23). Mp: 203−205 °C. Yield: 54%. ^{1}H NMR: 3.78 and 3.99 (2s, 6H, OCH_3-7 and OCH_3-8), 3.84 (s, 2H, CH_2), 6.89 (s, 1H, H-6), 6.97 (s, 1H, H-9), 7.70−8.64 (m, 4H, Ar). Anal. ($C_{17}H_{15}NO_7$) C, H, N.

General Procedure for the Synthesis of 2,3-Benzodiazepin-4-ones 27−30 and 34−37. The appropriate 2-aroylphenylacetic acid (0.003 mol) was refluxed with hydrazine hydrate or monomethylhydrazine (0.008 mol) for 2−3 h in EtOH (50 mL). After cooling, the solvent was removed under reduced pressure and the residue crystallized from MeOH.

1-(4′-Bromophenyl)-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one (27). Mp: 221−223 °C. Yield: 75%. ^{1}H NMR: 3.50 (s, 2H, CH_2), 3.73 and 3.96 (2s, 6H, OCH_3-7 and OCH_3-8), 6.62 (s, 1H, H-6), 6.84 (s, 1H, H-9), 7.50−7.58 (m, 4H, Ar), 8.43 (br s, 1H, NH). Anal. ($C_{17}H_{15}BrN_2O_3$) C, H, N.

1-(4′-Cyanophenyl)-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one (28). Mp: 245−247 °C. Yield: 38%. ^{1}H NMR: 3.51 (s, 2H, CH_2), 3.73 and 3.97 (2s, 6H, OCH_3-7 and OCH_3-8), 6.56 (s, 1H, H-6), 6.85 (s, 1H, H-9), 7.71−7.79 (m, 4H, Ar), 8.63 (br s, 1H, NH). Anal. ($C_{18}H_{15}N_3O_3$) C, H, N.

3,5-Dihydro-7,8-dimethoxy-1-(4′-nitrophenyl)-4*H*-2,3-benzodiazepin-4-one (29). Mp: 250−252 °C. Yield: 77%. ^{1}H NMR: 3.53 (s, 2H, CH_2), 3.72 and 3.98 (2s, 6H, OCH_3-7 and OCH_3-8), 6.56 (s, 1H, H-6), 6.86 (s, 1H, H-9), 7.82−8.30 (m, 4H, Ar), 8.60 (br s, 1H, NH). Anal. ($C_{17}H_{15}N_3O_5$) C, H, N.

3,5-Dihydro-7,8-dimethoxy-1-(3′-nitrophenyl)-4*H*-2,3-benzodiazepin-4-one (30). Mp: 263−265 °C. Yield: 88%. ^{1}H NMR: 3.53 (s, 2H, CH_2), 3.72 and 3.98 (2s, 6H, OCH_3-7 and OCH_3-8), 6.59 (s, 1H, H-6), 6.88 (s, 1H, H-9), 7.60−8.53 (m, 4H, Ar), 8.67 (br s, 1H, NH). Anal. ($C_{17}H_{15}N_3O_5$) C, H, N.

1-(4′-Bromophenyl)-3,5-dihydro-7,8-dimethoxy-3-methyl-4*H*-2,3-benzodiazepin-4-one (34). Mp: 154−156 °C. Yield: 79%. ^{1}H NMR: 3.43 (s, 5H, N-CH_3 and CH_2), 3.74 and 3.96 (2s, 6H, OCH_3-7 and OCH_3-8), 6.62 (s, 1H, H-6), 6.86 (s, 1H, H-9), 7.51−7.59 (m, 4H, Ar). Anal. ($C_{18}H_{17}BrN_2O_3$) C, H, N.

1-(4′-Cyanophenyl)-3,5-dihydro-7,8-dimethoxy-3-methyl-4*H*-2,3-benzodiazepin-4-one (35). Mp: 206−208 °C. Yield: 45%. ^{1}H NMR: 3.46 (s, 5H, NCH_3 and CH_2), 3.73 and 3.96 (2s, 6H, OCH_3-7 and OCH_3-8), 6.57 (s, 1H, H-6), 6.87 (s, 1H, H-9), 7.71−7.80 (m, 4H, Ar). Anal. ($C_{19}H_{17}N_3O_3$) C, H, N.

3,5-Dihydro-7,8-dimethoxy-3-methyl-1-(4′-nitrophenyl)-4*H*-2,3-benzodiazepin-4-one (36). Mp: 230−232 °C. Yield: 69%. ^{1}H NMR: 3.48 (s, 5H, N-CH_3 and CH_2), 3.73 and 3.97 (2s, 6H, OCH_3-7 and OCH_3-8), 6.57 (s, 1H, H-6), 6.88 (s, 1H, H-9), 7.84−8.30 (m, 4H, Ar). Anal. ($C_{18}H_{17}N_3O_5$) C, H, N.

3,5-Dihydro-7,8-dimethoxy-3-methyl-1-(3′-nitrophenyl)-4*H*-2,3-benzodiazepin-4-one (37). Mp: 263−265 °C. Yield: 65%. ^{1}H NMR: 3.48 (s, 5H, N-CH_3 and CH_2), 3.72 and 3.97 (2s, 6H, OCH_3-7 and OCH_3-8), 6.59 (s, 1H, H-6), 6.89 (s, 1H, H-9), 7.60−8.54 (m, 4H, Ar). Anal. ($C_{18}H_{17}N_3O_5$) C, H, N.

General Procedure for the Synthesis of Compounds 38−41. To a mixture of the appropriate nitro derivative **29**, **30**, **36**, or **37** (0.002 mol) and granulated tin (0.004 mol) was dropwise added 37% HCl (3 mL). The reaction mixture was heated on a boiling water bath for 1 h. The reaction mixture was cooled, treated with NaOH, and extracted with $CHCl_3$. The organic phase was dried over Na_2SO_4, the solvent was evaporated, and the crude residue was purified by column chromatography (EtOAc/CCl_4, 70:30, as eluent).

1-(4′-Aminophenyl)-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one (38). Mp: 178−180 °C. Yield: 59%. ^{1}H NMR: 3.47 (s, 2H, CH_2), 3.74 and 3.96 (2s, 6H, OCH_3-7 and OCH_3-8), 3.97 (br s, 2H, NH_2), 6.69−7.46 (m, 6H, Ar), 8.32 (br s, 1H, NH). Anal. ($C_{17}H_{17}N_3O_3$) C, H, N.

1-(3′-Aminophenyl)-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one (39). Mp: 253−255 °C. Yield: 56%. ^{1}H NMR: 3.49 (s, 2H, CH_2), 3.74 and 3.96 (2s, 6H, OCH_3-7 and OCH_3-8), 3.82 (br s, 2H, NH_2), 6.71−7.22 (m, 6H, Ar), 8.58 (br s, 1H, NH). Anal. ($C_{17}H_{17}N_3O_3$) C, H, N.

1-(4′-Aminophenyl)-3,5-dihydro-7,8-dimethoxy-3-methyl-4*H*-2,3-benzodiazepin-4-one (40). Mp: 223−225 °C. Yield: 78%. ^{1}H NMR: 3.39 (s, 3H, NCH_3) 3.47 (br s, 2H, CH_2), 3.75 and 3.95 (2s, 6H, OCH_3-7 and OCH_3-8), 3.93 (br s, 2H, NH_2), 6.68−7.49 (m, 6H, Ar). Anal. ($C_{18}H_{19}N_3O_3$) C, H, N.

1-(3′-Aminophenyl)-3,5-dihydro-7,8-dimethoxy-3-methyl-4*H*-2,3-benzodiazepin-4-one (41). Mp: 143−145 °C. Yield: 80%. ^{1}H NMR: 3.43 (s, 3H, NCH_3) 3.52 (br s, 2H, CH_2), 3.75 and 3.96 (2s, 6H, OCH_3-7 and OCH_3-8), 6.71−7.23 (m, 6H, Ar). Anal. ($C_{18}H_{19}N_3O_3$) C, H, N.

General Procedure for the Synthesis of 3-Acetyl Derivatives 42−44. To a cooled stirred solution of compound **25**, **28**, or **38** (0.001 mol) in $CHCl_3$ (30 mL) and Et_3N (5 mL) was dropwise added Ac_2O (5 mL). The mixture reaction was stirred at room temperature for 1−2 h, poured into water, extracted with $CHCl_3$, and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure afforded a crude product, which was purified by treatment with Et_2O.

3-Acetyl-3,5-dihydro-7,8-dimethoxy-1-phenyl-4*H*-2,3-benzodiazepin-4-one (42). Mp: 154−156 °C. Yield: 65%. ^{1}H NMR: 2.58 (s, 3H, $COCH_3$), 3.59 (s, 2H, CH_2), 3.75 and 3.98 (2s, 6H, OCH_3-7 and O CH_3-8), 6.69 (s, 1H, H-6), 6.90 (s, 1H, H-6), 7.42−7.73 (m, 4H, Ar). Anal. ($C_{19}H_{18}N_2O_4$) C, H, N.

3-Acetyl-1-(4′-cyanophenyl)-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one (43). Mp: 98−100 °C. Yield: 72%. ^{1}H NMR: 2.59 (s, 3H, $COCH_3$), 3.59 (s, 2H, CH_2), 3.75 and 3.99 (2s, 6H, OCH_3-7 and OCH_3-8), 6.60 (s, 1H, H-6), 6.92 (s, 1H, H-9), 7.73−7.87 (m, 4H, Ar). Anal. ($C_{20}H_{17}N_3O_4$) C, H, N.

3-Acetyl-1-(4′-(diacetylamino)phenyl)-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one (44). Mp: 148−150 °C. Yield: 68%. ^{1}H NMR: 2.22 (2s, 6H, $NCOCH_3$) 2.57 (s, 3H, $COCH_3$), 3.58 (s, 2H, CH_2), 3.76 and 3.98 (2s, 6H, OCH_3-7 and OCH_3-8), 6.70 (s, 1H, H-6), 6.89 (s, 1H, H-9), 7.63−7.71 (m, 4H, Ar). Anal. ($C_{23}H_{23}N_3O_6$) C, H, N.

Lipophilicity Measurements. The relative lipophilicity (R_m) of the compounds was measured by reversed-phase high-performance thin-layer chromatography (RP-HPTLC) according to the method previously described.[31] Briefly, Whatman KC18F plates were used as the nonpolar stationary phase. The plates were dried at 105 °C for 1 h before use. The polar mobile phase was a 2:1 (v/v) mixture of acetone and water. Each compound was dissolved in $CHCl_3$ (3 mg/mL), and 1 μL of solution was applied onto the plate. The experiments were repeated five times with different disposition of the compounds on the plate. The R_f values were expressed as the mean values of the five determinations. The R_m values were calculated from the experimental R_f values according to the formula $R_m = \log[(1/R_f) - 1]$. Higher R_m values indicate higher lipophilicity.

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.[32] DBA/2 mice (8−12 g; 22−25-days-old) were pur-

chased 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 drugs. The compounds were given ip (0.1 mL/10 g of body weight of the mouse) as a freshly prepared solution in 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 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.[33] The time course of the anticonvulsant action of **38**, **40**, **44**, and **1** was determined following the administration of 33 μmol/kg of 2,3-benzodiazepines to groups of 10 mice that were tested for sound-induced seizure responses at 5−180 min after drug administration.

Maximal Electroshock Seizure Test in Swiss Mice. Electrical stimuli were applied *via* ear-clip electrodes to Swiss 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.[34] 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.

Pentylenetetrazole-Induced Seizures in Swiss Mice. Male Swiss mice (20−26 g, 42−48-days-old) were purchased from Charles River (Calco, Como, Italy) and were pretreated with vehicle or drug 45 min before the subcutaneous (sc) administration of pentylenetetrazole. For systemic injections, all 2,3-benzodiazepines 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 (CD_{97}) of pentylenetetrazole (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 pentylenetetrazole seizure threshold.[35]

AMPA-Induced Seizures in DBA/2 Mice. Seizures were also induced by icv injection of AMPA. The CD_{50} of AMPA for clonus was 1.76 (1.06−3.07) while that for tonus 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:[36] injections of drugs by this procedure led to an uniform distribution throughout the ventricular system within 10 min. The animals were placed singly in a 30 × 30 × 30 cm box, and the observation time was 30 min after the administration of AMPA.

Membrane Preparation and [^{3}H]Flumazenil and Other Binding Studies. Male SD/Rij rats (FRAR, S.Pietro al Natisone, UD, Italy) weighing 200−250 g were decapitated, and different brain areas were rapidly dissected on ice. Brain regions were homogenized in 20 mL of ice-cold 0.32 M sucrose pH 7.4 by using a glass homogenizer with a Teflon pestle (10 up and down strokes). The homogenate was centrifuged at 1000*g* at 4 °C for 10 min, the P_1 pellet was discarded, and the supernatant was collected and recentrifuged at 20000*g* at 4 °C for 20 min. The resulting crude mitochondrial pellet (P_2) was resuspended in 20 mL of ice-cold distilled water and homogenized. The homogenate was centrifuged at 8000*g* at 4 °C for 20 min, the supernatant was collected and recentrifuged at 48000*g* at 4 °C for 20 min, and the final crude microsomal pellet (P_3) was frozen for at least 24 h. The pellet was resuspended in 10 mL of 50 mM Tris-HCl pH 7.4, centrifuged at 48000*g* at 4 °C for 20 min, and then resuspended in 10 vol of the same buffer for the standard binding assay. Aliquots of membrane suspensions (100 μL, or 0.15 mg of protein) were added to incubation medium containing 1 nmol of [^{3}H]flumazenil (specific activity 72.4 Ci/mmol) in a final volume of 1 mL of 50 mM Tris-HCl, 120 mM NaCl, and 5 mM KCl, pH 7.4. All 2,3-benzodiazepines were dissolved in DMSO at a final concentration of 1%. Incubations were carried out for 60 min at 4 °C in triplicate, and nonspecific binding was measured in the presence of 10 μM diazepam. Reactions were stopped by the addition of 5 mL of ice-cold Tris-HCl followed by rapid filtration through Whatman GF/C glass fiber filters (Whatman Inc. Clifton, NJ) and two additional washes. The radioactivity trapped on the filters was counted after the addition of 8 mL of Filter Count (Packard), by liquid scintillation spectrometry. The experiments were run in triplicate with eight different concentrations of competing ligand. The possibility that 2,3-benzodiazepines **25** and **38**−**40** bind to receptors other than BZR was studied in crude rate brain synaptic membranes according to established protocols[37] by using [^{3}H]spiperone, [^{3}H]ketanserin, [^{125}I]pindolol, [^{3}H]CPP, [^{3}H]MK-801, and [^{3}H]AMPA. In other binding studies aliquots of membrane suspension were incubated at the appropriate temperature for 60 min with 50 nM [^{3}H]CNQX (specific activity, 18.3 Ci/mmol), 10 nM [^{3}H]-5,7-DCKA (specific activity, 18.3 Ci/mmol), or [^{3}H]ACPD (mixture of [^{3}H](1*S*,3*R*)ACPD and [^{3}H](1*R*,3*S*)ACPD, specific activity 30−50 Ci/mmol). Nonspecific binding was defined as the binding measured in the presence of 0.2 mM quisqualate, 0.1 mM D-serine, or 0.2 mM (1*S*,3*R*)ACPD, respectively.

Effects on Motor Movements. Male Swiss mice (20−26 g, 48−54-days-old) were purchased from Charles River (Calco, Como, Italy). Groups of 10 mice were trained to do coordinated motor movements continuously for 2 min on a rotarod, 3 cm diameter, at 8 rpm (U. Basile, Comerio, Varese, Italy). Impairment of coordinated motor movements was defined as inability of the mice to remain on the rotarod for a 2 min test period.[38] The ability of the mice to remain on the rotarod was tested 30 min after administration of various 2,3-benzodiazepines.

Electrophysiology. Transverse slices of olfactory cortex (~450 μm thick) were obtained from adult guinea pigs (250−400 g; either sex) as previously described[39] 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; $CaCl_2$, 1.5; $NaHCO_3$, 25; $MgCl_2 \cdot 6H_2O$, 1; and D-glucose, 11 (bubbled with 95% O_2:5% CO_2, pH 7.4). Conventional intracellular recordings were obtained from the periamygdaloid area of the slices within the olfactory pyramidal cell layer II−III,[40] using glass microelectrodes (tip resistances 40−60 MΩ) filled with 4 M potassium acetate. Voltage clamp recordings were made at a holding membrane potential of −80 mV with the aid of a DAGAN 8100 sample-and-hold voltage clamp preamplifier (2−3 kHz switching frequency; 25% duty cycle). Sampled membrane currents (filtered at 30 Hz, low pass) and voltage were recorded on a Gould 2400 ink-jet chart recorder. The following compounds were tested: AMPA, **38**, and **1**. In addition, slices were continuously superfused with 1 μM TTX, to block voltage-activated sodium currents and underlying repetitive firing at the peak of AMPA responses. AMPA and TTX were freshly prepared in Krebs solution whereas compounds **38** and **1** were predissolved in dimethyl sulfoxide (DMSO) to give 10 mM 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.

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) or ANOVA followed by post-hoc Dunnett's *t*-test (rectal temperatures). The ED_{50} values of each phase of the audiogenic seizure or seizures induced by electroshock or pentylenetetrazole was determined for each dose of compound administered, and dose−response curves were fitted using a computer program by the method of Litchfield and Wilcoxon.[41] The

relative anticonvulsant activities were determined by comparison of respective ED_{50} values. The dose which induced 50% of mice to fall from the rotarod (TD_{50} values) was estimated using the method of Litchfield and Wilcoxon.[41] The relative activities were determined by comparison of respective TD_{50} values. For the binding experiments IC_{50} values for the [^{3}H]flumazenil or other ligands displacement were determined by the nonlinear curve-fitting program based on Ligand.[42] All data of electrophysiology are expressed as mean ± standard error of the mean (SEM). Statistical significance between control and test groups of data means was tested using a two-tailed Students *t*-test.

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