Novel Potent AMPA/Kainate Receptor Antagonists: Synthesis and Anticonvulsant Activity of a Series of 2-[(4-Alkylsemicarbazono)-(4-aminophenyl)methyl]-4,5-methylenedioxyphenylacetic Acid Alkyl Esters

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In this paper we describe the synthesis of a series of novel 2-[(4-alkylsemicarbazono)-(4-aminophenyl)-methyl]-4,5-methylenedioxyphenylacetic acid alkyl esters (**10**−**19**) carrying an alkylsemicarbazono moiety at a benzylic site. The influence of this group on the biological activity was evaluated by testing the corresponding derivatives **20**−**22** in which the 4-alkylsemicarbazono moiety was removed (compound **20**) or its alkylureido portion shifted at position 1 (compounds **21**−**22**). Furthermore, the involvement of the 4-aminobenzyl moiety in the anticonvulsant activity was evaluated by testing derivative **23**. The anticonvulsant activity of all compounds was assayed against audiogenic seizures induced in DBA/2 mice. Within this series of derivatives, 2-[(4-aminophenyl)-(4-methylsemicarbazono)-methyl]-4,5-methylenedioxyphenylacetic acid methyl ester (**10**) proved to be the most active compound. It displayed a potency 5-fold higher than that shown by 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (**1**, GYKI 52466), a well-known noncompetitive 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) receptor antagonist. Compound **10** was also effective in suppressing seizures induced in Swiss mice by maximal electroshock (MES) or pentylenetetrazole (PTZ). Furthermore, it antagonized in vivo seizures induced by icv administration of AMPA or kainate (KA). Using the patch-clamp technique in primary cultures of granule neurons we tested compounds **10** and **21** for their ability to modulate currents evoked by KA and 2-amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl)propionic acid (ATPA). These two derivatives reduced KA and ATPA currents to a larger extent than that shown by reference compound **1**. Compounds **10** and **21** were also able to reduce neuronal cell death induced by the application of KA (100 μM).

Introduction

Hyperactivity of ionotropic glutamate receptors, classified as *N*-methyl-D-aspartic acid (NMDA), 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA), and kainic acid (KA), is believed to be implicated in neuronal damage in humans suffering from acute degenerative disorders such as stroke and epilepsy, or chronic degenerative diseases including amyotrophic lateral sclerosis, Huntington's chorea, and Parkinson's and Alzheimer's diseases.[1] Consistent with this hypothesis, competitive and noncompetitive antagonists of these receptors are attractive therapeutic targets.[2] To date, interest in this area seems to be focused on antagonists acting selectively on AMPA and KA receptors due to their effectiveness in the treatment of epilepsy and cerebral ischaemia, and because, at variance with NMDA receptor antagonists, they appear to be relatively well tolerated and devoid of psychotomimetic effects.[3]

1-(4-Aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (**1**, GYKI 52466) (Chart 1), the prototype of noncompetitive AMPA receptor antagonists endowed with anticonvulsant and neuroprotective properties,[4] induced growing interest on 2,3-benzodiazepine derivatives. A significant improvement in the pharmacological profile of GYKI 52466 was obtained by appending an alkylcarbamoyl group at *N*-3 of the benzodiazepine ring.[5] As a matter of fact, the 3-*N*-methylcarbamoyl derivative **2** (GYKI 53655, LY 300168) (Chart 1) emerged as the most potent compound among this series of derivatives.[6]

As part of a program aimed at identifying potent and selective AMPA receptor antagonists, we[7] and other authors[8] previously reported investigations on a series of 1-aryl-3,5-dihydro-7,8-methylenedioxy-4*H*-2,3-benzodiazepin-4-ones where the iminohydrazone portion of **1** was replaced by the iminohydrazide moiety. In this context we noticed that derivative **3** (Chart 1) was roughly 2-fold more potent than **1** and was provided with a better protective index. Taking into account modifications previously made[5] on analogues of GYKI 52466, we prepared a number of 3-*N*-alkylcarbamoyl-derivatives (e.g., **4**−**7**) (Chart 1). The results indicated

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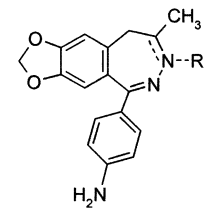
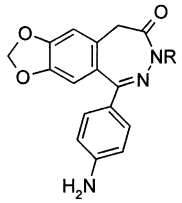
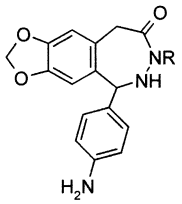
Chart 1



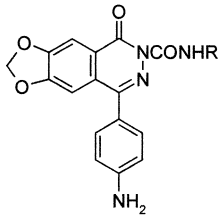
1, GYKI 52466 3,4-Δ
2, GYKI 53655 3,4-dihydro, R=CONHMe



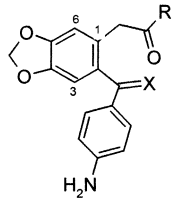
3, R=H
4, R=CONHMe
5, R=CONHEt
6, R=CONHPr
7, R=CONHBu



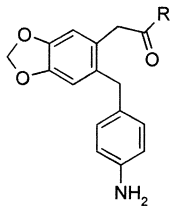
8, R=H, CONH-alkyl

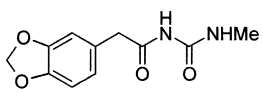


9, R=alkyl



	R	X
10	OMe	NNHCONHMe
11	OEt	NNHCONHMe
12	OPr	NNHCONHMe
13	OBu	NNHCONHMe
14	OMe	NNHCONHEt
15	OEt	NNHCONHEt
16	OPr	NNHCONHEt
17	OBu	NNHCONHEt
18	OMe	NNHCONHPr
19	OMe	NNHCONHBu
22	NHCONHMe	O





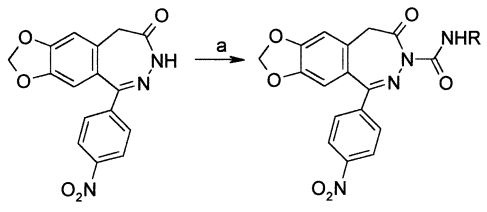
23

20, R=OMe
21, R=NHCONHMe

that the introduction of a methylcarbamoyl group at *N*-3, i.e., compound **4**, increased the anticonvulsant potency.[9] Worth noting, derivative **4** was also provided with a longer-lasting anticonvulsant activity.

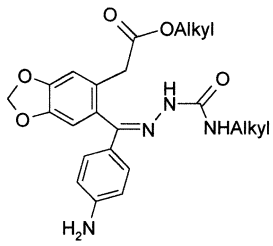
In our ongoing studies on the structure−activity relationships (SAR) of 2,3-benzodiazepine derivatives,[10] we have recently prepared[11] a number of 1,2,3,5-tetrahydro-2,3-benzodiazepine derivatives, i.e., **8**, as well as a series of novel phthalazin-1(2*H*)-ones, i.e., **9**, to check the influence of both the 1,2-azomethine moiety of the diazepine nucleus and the size of the heterocyclic ring on the anticonvulsant activity. The results showed that some derivatives, **8**, were provided with a noteworthy anticonvulsant activity which was longer-lasting when compared to that shown by the corresponding unsaturated analogues. On the other hand, the activity of the phthalazino derivatives **9** was strictly dependent upon the alkyl group of the carbamoyl moiety appended at *N*-2. The peak value was reached with the *n*-butyl group.

Scheme 1[a]

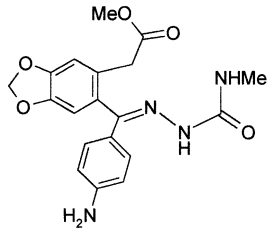


24

25, R=Me; **26**, R=Et
27, R=Pr; **28**, R=Bu



10-19



29

[a] Reagents: (a) CH_2Cl_2, Et_3N, RNCO, rt, 36 h; (b) anhydrous alcohols, $SnCl_2$, 80 °C, 2 h.

Encouraged by these results, we designed and biologically evaluated a series of alkyl esters of 4,5-methylendioxyphenylacetic acids **10**−**19** bearing a 4-alkylsemicarbazono moiety at position 2, that may be envisaged as "open models" of reference compounds **4**−**7** (Chart 1). The influence of this group on the biological activity was evaluated by testing the corresponding derivatives **20**−**22,** in which the 4-alkylsemicarbazono moiety was removed (compound **20**) or its alkylureido portion shifted at position 1 (compounds **21**−**22**). Furthermore, the involvement of the 4-aminobenzyl moiety in the anticonvulsant activity was evaluated by testing derivative **23**.

Chemistry

The synthesis of 2-[(4-alkylsemicarbazono)-(4-aminophenyl)-methyl]-4,5-methylenedioxyphenylacetic acid alkyl esters (**10**−**19**) was accomplished according to the reaction sequence reported in Scheme 1.

3,5-Dihydro-7,8-methylenedioxy-1-(4-nitrophenyl)-4*H*-2,3-benzodiazepin-4-one, **24**, used as the reagent, was prepared according to the procedure previously reported.[8] It was treated with an excess of the appropriate alkyl isocyanate in the presence of triethylamine to yield the corresponding 3-*N*-alkylcarbamoyl derivatives **25**−**28**. If the reduction of intermediates **25**−**28** was carried out under mild conditions, i.e., catalytic hydrogenation,

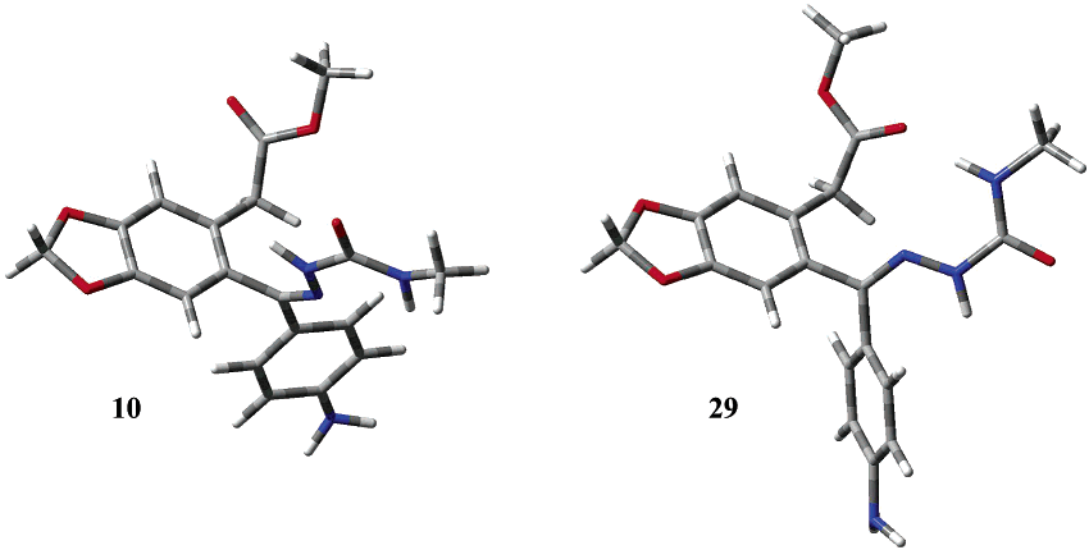


Figure 1. 3D Plot of a representative conformation of stereoisomers **10** and **29**.

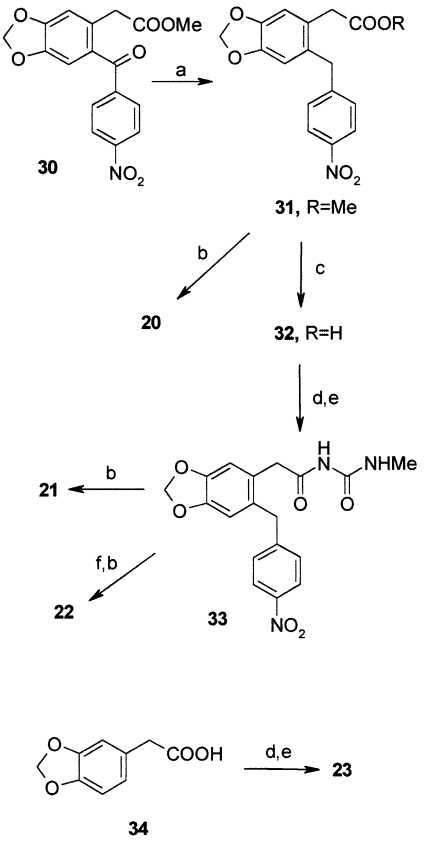
5% Pd/C-methanol at room temperature, the corresponding amino derivatives **4**−**7** were obtained in good yields and pure form.[9] Conversely, if the same reaction was conducted under harsh conditions, i.e., tin(II) chloride in various alcohols at reflux for 2 h, compounds **10**−**19** were obtained in reasonable yield. The expected reduction of the nitro group to the amino moiety was accompanied by the cleavage of the heterocyclic ring. Conceivably, the nucleophilic attack of the alcohol at C-4, which caused the cleavage of the C−N bond, was catalyzed by tin(II) chloride, the Lewis acid. As a matter of fact, heating at reflux of a methanol solution of both **4** and **25** did not cause the cleavage of the heterocyclic ring, but resulted in the removal of their side-chain.

The nucleophilic attack of the alcohol to the carbonyl moiety of bicyclic derivatives **25**−**28** yielded the corresponding monocyclic analogues **10**−**19** as single isomers. During the synthesis of (−)-3-acetyl-1-(4-aminophenyl)-3,4-dihydro-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (LY300164) some authors prepared intermediate hydrazones similar to the present ones and noticed that they were composed by an inseparable mixture of *E*/*Z* isomers.[12] A chloroform solution of **10** left aside for two weeks at room temperature gave a 70:30 mixture of **10** and **29**; the two isomers were easily separated by a silica gel column chromatography. HPLC−MS analysis of the mixture showed that **10** and **29** were isomers and they possessed the same fragmentation pattern (see Experimental Section). The stereochemistry around the C=N bond of semicarbazono derivatives **10**−**19** was investigated on the couple of stereoisomers, **10** and **29**, using a combination of modeling studies and ^{1}H NMR spectroscopy. The conformational preferences of both the compounds **10** and **29** were evaluated at the semiempirical AM1 level.[13] Both the compounds showed a large conformational freedom with some relevant differences. The rotation around the single bond connecting the semicarbazono moiety to C-2 appeared to be easier for the *E*-isomer (**29**) than for the corresponding *Z*-form (**10**). The energy barrier, corresponding to the planar arrangement of the groups, was about 6−9 kcal/mol for **29** and about 18−22 kcal/mol for **10**, making derivative **10** a chiral molecule in the NMR time scale. As a matter of fact, ^{1}H NMR spectra of derivatives **10** and **29,** recorded both in acetone-d_6 and DMSO-d_6, showed the splitting of some signals, thus confirming the above-reported observations. The two CH_2 groups, i.e., the one in the α position in respect to the ester function and the one of the dioxole ring, appeared as singlets in derivative **29** and as AB systems or multiplets in its *Z*-form (**10**). These data testify that the geminal hydrogens are diastereotopic in **10** because of the hindered rotation around the single bond connecting the semicarbazono moiety to C-2. The same does not hold true for derivative **29** because of the much easier rotation around the same single bond.

NOESY experiments, performed in DMSO-d_6, confirmed the proposed structural assignment. In fact, a strong cross-peak between N*H*-2 of the semicarbazono moiety and aromatic 2′ and 6′ protons was evidenced in derivative **29** and was not detected in its stereoisomer **10**. These experimental findings support the value of the distance between N*H*-2 and the nearest of the two 2′ and 6′ protons found in various conformations of **29** (2.4−2.6 Å); a value quite different from that observed in derivative **10** (4.4−4.8 Å). In Figure 1 is depicted a representative conformation of stereoisomers **10** and **29**. Conversely, the N*H*-2 proton gave a weak cross-peak with H-3 in derivative **10** but not in its isomer **29**; once again supporting the values of the corresponding distances (2.9−3.6 Å for **10** versus 4.5−4.7 Å for **29**) calculated for their most stable conformations.

Compound **20** was synthesized according to the reaction sequence reported in Scheme 2. The reduction of the keto group of **30**[8] to yield **31** was efficiently achieved with sodium cyanoborohydride in the presence of 3 equivalents of boron trifluoride diethyl etherate.[14] The nitro ester **31** was transformed into the corresponding amino derivative **20** under standard conditions. Ester **31** was also hydrolyzed to acid **32** and subsequently transformed into the corresponding acyl chloride with a standard methodology. Such an intermediate was not characterized but directly reacted with *N*-methylurea to afford compound **33** in reasonable yield. Reduction of the nitro group of **33** was accomplished with Raney−Ni/ammonium formate and gave final derivative 1-[2-

Scheme 2[a]



[a] Reagents: (a) $NaBH_3CN$, BF_3OEt_2, anhydrous THF, reflux, 16 h; (b) EtOH, Raney−Ni, ammonium formate; (c) H_2O/THF, HCl 6 N, reflux, 9 h; (d) $SOCl_2$; (e) methylurea, benzene, reflux, 6 h; (f) $KMnO_4$, acetone, rt, 3 h, Na_2SO_4.

(4-aminobenzyl)-4,5-methylenedioxyphenylacetyl]-3-methylurea (**21**) in good yield. 1-[2-(4-Aminobenzoyl)-4,5-methylenedioxyphenylacetyl]-3-methylurea (**22**) was obtained through an oxidation of intermediate **33** with potassium permanganate followed by a reduction of its nitro group carried out under standard conditions (Scheme 2). Finally, compound **23** was easily prepared by coupling 3,4-methylenedioxyphenylacetyl chloride with methylurea at reflux in benzene.

Results and Discussion

Novel 2-[(4-alkylsemicarbazono)-(4-aminophenyl)-methyl]-4,5-methylenedioxy-phenylacetic acid alkyl esters (**10**−**19**), 2-(4-aminophenyl)-4,5-methylenedioxyphenylacetic acid methyl ester (**20**), 1-[2-(4-aminobenzyl)-4,5-methylenedioxyphenylacetyl]-3-methylurea (**21**), 1-[2-(4-aminobenzoyl)-4,5-methylenedioxyphenylacetyl]-3-methylurea (**22**), and 1-[2-(3,4-methylenedioxyphenylacetyl)]-3-methylurea (**23**) were evaluated for their anticonvulsant properties in DBA/2 mice, a strain genetically susceptible to sound-induced seizures. This test has been considered an excellent animal model for generalized epilepsy and for screening of new anticonvulsant drugs.[15] Compounds **10**−**23** were administered intraperitoneally (ip) at doses spanning the range 3.3−200 μmol/kg, and their anticonvulsant properties, expressed as median effective dose (ED_{50}) (Table 1), were evaluated 30 min after injection.

The structure−activity relationships among this series of derivatives were examined in respect to the following structural modifications: (i) the size of the alkyl group appended at the semicarbazono moiety; (ii) the removal of the semicarbazono moiety or the shift of its alkylureido portion to position 1; (iii) the bulkiness of the ester alkyl group; and (iv) the presence of the 4-aminobenzyl group. The data will be discussed in comparison to those previously reported by us[9] for the corresponding bicyclic derivatives **3**−**7**, taken as models.

Among the compounds examined, derivatives **10**−**12**, **14**, **21**, and **29** possess an anticonvulsant activity equal to or higher than that displayed by **1**. In particular, compounds **10** and **21** are 5- and 4-fold more potent than **1** with ED_{50} values of 7.87 and 9.28 μmol/kg, respectively. It is worth noting that **10** carries the smallest alkyl group on both the semicarbazono and ester moieties. The same trend was previously noticed in the series of 3-*N*-alkylcarbamoyl-2,3-benzodiazepines where the methyl group gave the peak value (e.g., **4** vs **5**−**7**, Table 1). Compound **29,** the *E*-form of **10,** possesses a noteworthy activity even if slightly lower than that of **10**. In any case, we cannot exclude an in vivo interconversion among stereoisomers **10** and **29**. An increase in the bulkiness of the alkyl group at the ester side produces a decrease in activity, i.e., **10** > **11**−**13** and **14** > **15**−**17** (Table 1). The same trend holds true for the alkyl group appended at the semicarbazono moiety, i.e., **10** > **14, 18**−**19** (Table 1). These observations seem to indicate that lipophilicity plays a pivotal role in determining the anticonvulsant activity of compounds **10**−**19**. Usually, an increase of lipophilicity reduces the anticonvulsant activity, in analogy to the trend previously observed in the set of bicyclic derivatives, i.e., **4** > **5**−**7**. The sole exception is represented by compounds **21** and **22** where the higher lipophilicity of **21** (R_m −0.570 for **21** vs −0.689 for **22**) is reflected in a higher anticonvulsant activity (ED_{50} 9.28 for **21** vs 39.5 for **22**). The different hybridization of the carbon bearing the aryl group could modify the spatial arrangement of such a moiety. The lack of anticonvulsant activity of **23** could be attributed to the absence of the *p*-aminobenzyl group which seems to be an essential feature in this series of compounds since the most active noncompetitive AMPA antagonists, provided with the 2,3-benzodiazepine nucleus, bear the 4-aminophenyl moiety in position 1 of the bicyclic system.[10] A comparison of the anticonvulsant activity displayed by compounds **10**, **20**, and **21** indicates that the methylsemicarbazono moiety plays a role of utmost importance in determining the rank of activity. As a matter of fact, the shift of its methylureido portion from position 2 to position 1, i.e., on passing from **10** to **21**, marginally influences the activity, whereas its removal gives an inactive compound (**20**).

No adverse side effects, such as sedation or ataxia, were observed for novel compounds **10**−**23** and **29** at the doses used to test their anticonvulsant activity, in agreement with the results obtained with different series' of noncompetitive AMPA antagonists.[6,7,9] It is noteworthy that all compounds possess a protective index (PI) higher than that shown by **1** (Table 1).

Because of its potent anticonvulsant activity in the audiogenic seizure model, compound **10** was further investigated and the results are herewith compared with those of the related bicyclic derivatives **3**−**4** as well as model compound **1**.

Table 1. Anticonvulsant Activity of Compounds **1**, **3**−**7**, **10**−**23**, and **29** Against Audiogenic Seizures in DBA/2 Mice, TD_{50} Values on Locomotion Assessed by Rotarod Test, Relative Lipophilicity (R_m), and Calculated log *P*

compd	ED_{50}, μmol/kg[a] clonic phase	ED_{50}, μmol/kg[a] tonic phase	TD_{50}, μmol/kg,[a] locomotor deficit	PI,[b] TD_{50}/ED_{50}	R_m	log P[c]
1	35.8 (24.4−52.4)	25.3 (16.0−40.0)	76.1 (47.5−122)	2.1	−0.502	3.51
3	15.4 (10.1−23.5)	10.9 (4.60−24.6)	99.1 (72.4−135)	6.3	−0.634	2.58
4	12.4 (6.44−23.8)	8.70 (4.61−16.4)	48.6 (31.4−54.6)	3.9	−0.703	1.99
5	35.0 (18.5−66.3)	23.8 (13.4−42.1)	134 (70.7−255)	3.8	−0.620	2.41
6	69.4 (36.2−133)	49.2 (25.9−93.4)	182 (95.1−350)	2.6	−0.564	2.77
7	38.7 (21.2−70.8)	32.6 (18.2−58.4)	108 (82.2−143)	2.8	−0.428	3.34
10	7.87 (4.68−13.2)	4.62 (2.47−8.61)	28.3 (19.0−42.3)	3.6	−0.682	2.46
11	28.8 (16.9−49.1)	10.9 (5.76−20.8)	95.1 (65.6−137)	3.3	−0.452	2.89
12	18.0 (10.8−30.2)	8.93 (4.40−18.1)	68.5 (41.5−112)	3.8	−0.364	3.24
13	66.2 (43.1−101)	39.8 (28.8−55.1)	179 (125−256)	2.7	−0.244	3.81
14	30.3 (24.4−37.8)	32.8 (19.3−29.5)	113 (84.6−152)	3.7	−0.560	2.89
15	84.4 (64.3−110)	60.8 (44.9−82.2)	227 (174−229)	2.7	−0.490	3.31
16	55.5 (44.3−69.5)	40.3 (28.7−52.6)	187 (143−249)	3.4	−0.254	3.67
17	53.1 (39.1−72.0)	38.7 (28.5−52.6)	117 (93.9−145)	2.2	−0.135	4.24
18	57.1 (41.5−78.5)	42.8 (24.5−74.7)	177 (137−228)	3.1	−0.463	3.24
19	65.0 (54.7−77.2)	38.2 (23.8−61.4)	188 (164−216)	2.9	−0.225	3.81
20	54.3 (42.9−68.9)	47.7 (38.7−58.9)	169 (154−214)	3.1	−0.456	2.68
21	9.28 (4.82−17.8)	7.65 (3.79−46.3)	33.3 (18.8−19.0)	3.6	−0.570	2.23
22	39.5 (29.4−53.0)	28.9 (17.9−46.3)	134 (102−174)	3.4	−0.689	1.82
23	87.8 (63.4−121)	74.4 (41.9−132)	219 (159−303)	2.5	−0.916	1.09
29	10.4 (5.22−20.8)	9.51 (5.09−17.7)	38.6 (26.9−53.4)	3.7	−0.583	2.99

[a] All data were calculated according to the method of Litchfield and Wilcoxon.[29] 95% confidence limits are given in parentheses. At least 32 animals administered ip were used to calculate each ED_{50} and TD_{50} value. [b] PI, protective index, represents the ratio between TD_{50} and ED_{50} (from the clonic phase of the audiogenic seizures). [c] Calculated with the XLOGP 2.0 program.

Table 2. ED_{50} Values at Various Times Following Intraperitoneal Administration of Compounds **1**, **3**, **4**, and **10**

compd	ED_{50}, μmol/kg (±95% confidence limits),[a] clonic phase 15 min	30 min	45 min	60 min	90 min	120 min
1	10.8 (7.11−16.4)	35.8 (24.4−62.4)	37.3 (27.2−52−1)	39.5 (29.6−52.7)	> 50	> 50
3	7.55 (4.08−14.0)	15.4** (10.1−23.5)	18.7** (11.3−31.0)	21.3* (14.2−31.9)	> 50	> 50
4	14.3 (7.54−27.0)	12.4** (6.44−23.8)	12.1** (7.28−20.2)	13.0** (7.40−22.8)	19.4** (7.96−47.5)	40.8 (13.2−126)
10	24.1 (13.6−42.6)	7.87** (4.68−13.2)	7.75** (4.62−13.0)	10.2** (3.87−26.9)	25.5* (11.1−56.5)	36.8 (26.6−50.9)

[a] Significant differences among compounds **1**, **3**, **4**, and **10** were evaluated at the corresponding times and denoted as *$p < 0.05$ and **$p < 0.01$ using the method of Litchfield and Wilcoxon.[29]

Table 3. ED_{50} Values of **1**, **3**, **4**, and **10** Against MES- and PTZ-Induced Seizures in Swiss Mice, and Against AMPA- and KA-Induced Seizures in DBA/2 Mice

compd	ED_{50} μmol/kg[a] (± 95% confidence limits) MES tonus	PTZ clonus	AMPA[b] tonus	AMPA[b] clonus	KA[c]
1	35.7 (29.3−43.4)	68.3 (56.2−83.1)	57.5 (43.5−76.0)	40.5 (26.3−60.8)	27.8 (18.8−40.9)
3	32.1 (23.2−44.3)	71.8 (53.2−96.9)	37.6 (26.4−53.6)	27.0 (18.4−40.3)	19.6 (7.74−49.6)
4	18.6 (8.40−41.2)	16.3 (7.30−36.0)	18.6 (6.44−53.6)	10.3 (5.27−20.1)	8.61 (4.85−15.3)
10	15.7 (11.7−21.3)	14.7 (10.1−21.3)	13.9 (9.7−19.9)	8.9 (6.3−31.8)	16.6 (8.67−31.8)

[a] All data were calculated according to the method of Litchfield and Wilcoxon.[29] At least 32 animals were used to calculate each ED_{50} value. [b] AMPA was administered icv at the CD_{97} for either clonus (9.7 nmol) or forelimb tonic extension (11.7 nmol) 30 min after ip injection of tested compounds. [c] KA was administered sc at the CD_{97} (32 mg/kg) 15 min after ip injection of tested compounds.

The time-course of the anticonvulsant activity of derivative **10** is similar to that of 3-*N*-methylcarbamoyl derivative **4**, as they reach the maximum value of protection between 30 and 45 min with a return to control after 120 min from ip administration (Table 2). On the contrary, model compounds **1** and **3** displayed their maximum protection at 15 min from ip administration and returned to control at 90 min. These results suggest that the presence of the *N*-methylcarbamoyl group is responsible for the longer-lasting activity of **4** and **10** with respect to **3**.

As shown in Table 3, the tonic extension and the clonic phase of the seizures induced in Swiss mice by maximal electroshock (MES) and pentylenetetrazole (PTZ), respectively, were significantly reduced at 45 min after ip administration of compound **10**. Compound **10** is definitely more effective than GYKI 52466 and **3** in both tests and is equiactive with bicyclic derivative **4**.

Table 4. Percentage of Reduction of KA- and ATPA-evoked Currents Induced by **1**, **3**, **4**, **10**, and **21**[a]

	% reduction[b] (mean ± SE)	
compd	KA	ATPA
1	47 ± 4	45 ± 7
3	79 ± 4	94 ± 1
4	58 ± 4	50 ± 3
10	60 ± 1	75 ± 5
21	54 ± 7	32 ± 5

[a] KA, ATPA, compounds **1**, **3**, **4**, **10**, and **21** were tested at 100 μM. [b] Each value is the mean ± SE of at least 10 cells.

To investigate the relationship between the anticonvulsant activity of **10** and its activity in non-NMDA receptors, additional tests were performed (Table 3). Compound **10** produced a dose-dependent protection against seizures induced by the intracerebroventricular (icv) administration of AMPA and subcutaneous (sc) administration of KA. The ED_{50} values are higher than those needed to block audiogenic seizures (Table 1) and lower than or similar to those capable of protecting the animals against hind limb extension in the MES test (Table 3).

The activity of derivatives **10** and **21** on KA- and ATPA-evoked currents was assessed using the patch-clamp technique in cerebellar neurons grown in primary cultures. At variance of AMPA-response which is fast desensitizing,[16] KA elicits an inward nondesensitizing current that is mediated by the activation of both AMPA and KA receptors, whereas ATPA selectively activates GluR5, a subtype of the KA receptors. KA- and ATPA-evoked currents were reduced by the application of **10** and **21** (100 μM) to an extent that was comparable to that of **4** (100 μM) and lower than that of **3** (100 μM). The degree of block of the peak currents produced by **10**, expressed as the percent of reduction of the KA- or ATPA-evoked currents (100%), was higher than that elicited by **1** (Table 4) giving an explanation at receptor level of the higher capability of **10**, in comparison to **1**, to block seizures.

Because derivatives **10** and **21** reduced KA-evoked currents, their neuroprotective properties toward a KA-induced toxicity in primary cultures of cortical neurons were tested. The application of KA (100 μM) to eight DIV (days in vitro) neurons for 3 h induced a 70% cell death. The co-application of KA and derivative **10** or **21** (100 μM) reduced significantly neuronal cell death to 45% and 55%, respectively (Figure 2). Nevertheless, compounds **10** and **21** did not completely abolish neuronal cell death induced by KA.

In conclusion, novel 2-[(4-alkylsemicarbazono)-(4-aminophenyl)-methyl]-4,5-methylenedioxyphenylacetic acid alkyl esters reported in this study possess a remarkable anticonvulsant activity and exhibit lower toxicity than that shown by compound **1**. In particular, derivative **10** is 5-fold more potent than GYKI 52466 and is slightly more active than its structurally related bicyclic derivative **4**. As a consequence, the data reported in this paper indicate that the 2,3-diazepine nucleus is not an essential structural requirement for anticonvulsant activity and can be replaced by a side chain carrying a moiety capable to mimic the heterocyclic ring.

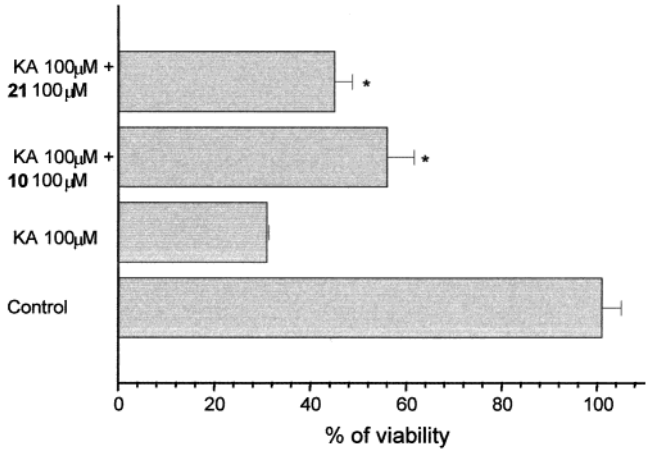


Figure 2. Protective effects of compounds **10** and **21** against neuronal cell death induced by 100 μM KA for 3 h. Toxicity experiment was performed on primary cultures of rat cortical neurons at 8 DIV. Bar plot showing the % of cell viability expressed in representative experiments. Bars represent mean values ± SE from 8 determinations. Asterisks mean significant differences (ANOVA $p < 0.01$) between KA alone and with **10** or **21**.

Monocyclic derivatives **10** and **21** will be taken by us as lead compounds for further investigations of new AMPA/KA antagonists which could find applications in the treatment of acute or chronic neurodegenerative diseases.

Experimental Section

Chemistry. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Elemental analyses were carried out on a Carlo Erba 1106 elemental analyzer for C, H, and N, and the results are within ±0.4% of the theoretical values. Merck silica gel 60 F_{254} plates were used for analytical TLC; column chromatography was performed on Merck silica gel 60 (70−230 mesh). ^{1}H NMR spectra were recorded in $CDCl_3$ by means of a Varian Gemini-300 spectrometer. ^{1}H NMR spectra of compounds **10** and **29** were also recorded in acetone-d_6 and DMSO-d_6. Chemical shifts are expressed in δ (ppm) relative to TMS as internal standard, and coupling constants (J) are in Hz. All exchangeable protons were confirmed by addition of D_2O. NOESY spectra were carried out by using the standard software package. LC−MS experiments were performed on a Finnigan MAT LCQ ion trap mass spectrometer equipped with an APCI interface (source current 5 mA; capillary temperature 150 °C; vaporizer temperature 600 °C) and connected to a Waters 616 chromatograph equipped with a Waters 996 photodiode array detector. The mass spectrometer operated in a positive-ion mode, with a scan range from $m:z$ 150 to 800. The HPLC separation was done by reverse-phase chromatography with a LC-18 column (250 × 4.6 mm, 5 μm): eluant 0.05% TFA in water/CH_3CN 68:32, flow rate 200 μL/min, λ 254 nm.

General Procedure for the Synthesis of 3-*N*-Alkylcarbamoyl-3,5-dihydro-7,8-methylenedioxy-1-(4-nitrophenyl)-4*H*-2,3-benzodiazepin-4-ones (25−28). To a solution of **24** (0.5 g, 1.54 mmol) in CH_2Cl_2 (60 mL) were added triethylamine (2 mL, 14.4 mmol) and the suitable isocyanate (7.7 mmol). The reaction mixture was stirred at room temperature for 36 h and then concentrated in vacuo. The resulting residue was purified by silica gel column chromatography with $CHCl_3$/EtOAc (70:30) as eluant and recrystallized from EtOAc to give **25**−**28**.

3,5-Dihydro-3-*N*-methylcarbamoyl-7,8-methylenedioxy-1-(4-nitrophenyl)-4*H*-2,3-benzodiazepin-4-one (25). Mp 194−196 °C (530 mg, 90%). ^{1}H NMR 2.94 (d, 3H, J = 4.7, CH_3), 3.54 (m, 2H, CH_2-5), 6.08 (m, 2H, OCH_2O), 6.59 (s, 1H, H-9), 6.91 (s, 1H, H-6), 7.95 (d, 2H, J = 8.9, H-2′,6′), 8.29 (d, 2H, J = 8.9, H-3′,5′), 8.63 (bs, 1H, NH). Anal. ($C_{17}H_{14}N_4O_6$) C, H, N.

3,5-Dihydro-3-*N*-ethylcarbamoyl-7,8-methylenedioxy-1-(4-nitrophenyl)-4*H*-2,3-benzodiazepin-4-one (26). Mp 176−178 °C (537 mg, 88%). ^{1}H NMR 1.22 (t, 3H, J = 7.2, CH_3), 3.39 (m, 2H, NHCH_2), 3.54 (m, 2H, CH_2-5), 6.08 (m, 2H, OCH_2O), 6.59 (s, 1H, H-9), 6.91 (s, 1H, H-6), 7.95 (d, 2H, J = 8.8, H-2′,6′), 8.28 (d, 2H, J = 8.8, H-3′,5′), 8.70 (bs, 1H, NH). Anal. ($C_{18}H_{16}N_4O_6$) C, H, N.

3,5-Dihydro-7,8-methylenedioxy-1-(4-nitrophenyl)-3-*N*-propylcarbamoyl-4*H*-2,3-benzodiazepin-4-one (27). Mp 155−157 °C (442 mg, 70%). ^{1}H NMR 0.92 (t, 3H, J = 7.2, CH_3), 1.58 (m, 2H, CH_2CH_3), 3.32 (m, 2H, NHCH_2), 3.54 (m, 2H, CH_2-5), 6.08 (m, 2H, OCH_2O), 6.59 (s, 1H, H-9), 6.91 (s, 1H, H-6), 7.95 (d, 2H, J = 8.8, H-2′,6′), 8.29 (d, 2H, J = 8.8, H-3′,5′), 8.75 (bs, 1H, NH). Anal. ($C_{19}H_{18}N_4O_6$) C, H, N.

3-*N*-Butylcarbamoyl-3,5-dihydro-7,8-methylenedioxy-1-(4-nitrophenyl)-4*H*-2,3-benzodiazepin-4-one (28). Mp 153−156 °C (438 mg, 67%). ^{1}H NMR 0.93 (t, 3H, J = 7.2, CH_3), 1.35 (m, 2H, CH_2CH_3), 1.58 (m, 2H, NHCH$_2CH_2$), 3.36 (m, 2H, NHCH_2), 3.54 (m, 2H, CH_2-5), 6.08 (m, 2H, OCH_2O), 6.59 (s, 1H, H-9), 6.91 (s, 1H, H-6), 7.95 (d, 2H, J = 9.0, H-2′,6′), 8.28 (d, 2H, J = 9.0, H-3′,5′), 8.73 (bs, 1H, NH). Anal. ($C_{20}H_{20}N_4O_6$) C, H, N.

***(Z)*-2-[(4-Aminophenyl)-(4-methylsemicarbazono)-methyl]-4,5-methylenedioxyphenylacetic Acid Methyl Ester (10).** To a stirred solution of 3-*N*-methylcarbamoyl-3,5-dihydro-7,8-methylenedioxy-1-(4-nitrophenyl)-4*H*-2,3-benzodiazepin-4-one (**25**) (1.2 mmol) in anhydrous MeOH (50 mL), tin(II)-chloride (6 mmol) was added. The reaction mixture was heated at 80 °C for 2 h, and before cooling it was filtered over activated carbon. The solvent was removed under vacuum, and the resulting residue was dissolved in EtOAc and neutralized with 2 N NaOH (2 × 100 mL). The organic layer was dried (Na_2SO_4) and the solvent was removed at reduced pressure. The residue was purified by silica gel column chromatography using EtOAc/cyclohexane/*i*-PrOH (60:30:10) as eluant and recrystallized from EtOAc to give **10** (378 mg, 82%). R_f = 0.55; mp 209−212 °C; HPLC 5.03 min; MS 385[M + 1], 353, 328, 311, 296. ^{1}H NMR 2.94 (d, 3H, J = 4.9, NHCH_3), 3.30 (m, 2H, CH_2), 3.52 (s, 3H, OCH_3), 3.83 (bs, 2H, NH_2), 6.05 (m, 2H, OCH_2O), 6.41 (m, 1H, *NH*CH_3), 6.56 (s, 1H, H-3), 6.60 (d, 2H, J = 8.7, H-3′,5′), 6.90 (s, 1H, H-6), 7.29 (d, 2H, J = 8.7, H-2′,6′), 7.39 (bs, 1H, NH). Anal. ($C_{19}H_{20}N_4O_5$) C, H, N.

***(Z)*-2-[(4-Aminophenyl)-(4-methylsemicarbazono)-methyl]-4,5-methylenedioxy-phenylacetic Acid Ethyl Ester (11).** With a similar procedure, **11** was prepared from **25** and anhydrous EtOH. Mp 148−151 °C (382 mg, 80%). ^{1}H NMR 1.11 (t, 3H, J = 7.1, OCH_2CH_3), 2.93 (d, 3H, J = 4.9, NHCH_3), 3.28 (m, 2H, CH_2), 3.85 (bs, 2H, NH_2), 3.97 (m, 2H, OCH_2CH_3), 6.04 (m, 2H, OCH_2O), 6.23 (m, 1H, *NH*CH_3), 6.54 (s, 1H, H-3), 6.59 (d, 2H, J = 8.5, H-3′,5′), 6.91 (s, 1H, H-6), 7.28 (d, 2H, J = 8.5, H-2′,6′), 7.42 (bs, 1H, NH). Anal. ($C_{20}H_{22}N_4O_5$) C, H, N.

***(Z)*-2-[(4-Aminophenyl)−(4-methylsemicarbazono)-methyl]-4,5-methylenedioxyphenylacetic Acid Propyl Ester (12).** With a similar procedure, **12** was prepared from **25** and anhydrous PrOH. Mp 108−111 °C (381 mg, 77%). ^{1}H NMR 0.82 (t, 3H, J = 7.4, O(CH$_2$)$_2CH_3$), 1.51 (m, 2H, $OCH_2CH_2CH_3$), 2.93 (d, 3H, J = 4.9, NHCH_3), 3.29 (m, 2H, CH_2), 3.85 (bs, 2H, NH_2), 3.89 (m, 2H, O$CH_2CH_2CH_3$), 6.04 (m, 2H, OCH_2O), 6.21 (m, 1H, *NH*CH_3), 6.54 (s, 1H, H-3), 6.59 (d, 2H, J = 8.5, H-3′,5′), 6.92 (s, 1H, H-6), 7.29 (d, 2H, J = 8.5, H-2′,6′), 7.36 (bs, 1H, NH). Anal. ($C_{21}H_{24}N_4O_5$) C, H, N.

***(Z)*-2-[(4-Aminophenyl)−(4-methylsemicarbazono)-methyl]-4,5-methylenedioxyphenyl acetic Acid Butyl Ester (13).** With a similar procedure, **13** was prepared from **25** and anhydrous BuOH. Mp 77−80 °C (282 mg, 55%). ^{1}H NMR 0.87 (t, 3H, J = 7.1, O(CH$_2$)$_3CH_3$), 1.25 (m, 2H, $OCH_2CH_2CH_2CH_3$), 1.47 (m, 2H, $OCH_2CH_2CH_2CH_3$), 2.94 (d, 3H, J = 4.9, NHCH_3), 3.29 (m, 2H, CH_2), 3.84 (bs, 2H, NH_2), 3.92 (m, 2H, O$CH_2CH_2CH_2CH_3$), 6.05 (m, 2H, OCH_2O), 6.21 (m, 1H, *NH*CH_3), 6.55 (s, 1H, H-3), 6.59 (d, 2H, J = 8.5, H-3′,5′), 6.92 (s, 1H, H-6), 7.29 (d, 2H, J = 8.5, H-2′,6′), 7.35 (bs, 1H, NH). Anal. ($C_{22}H_{26}N_4O_5$) C, H, N.

***(Z)*-2-[(4-Aminophenyl)-(4-ethylsemicarbazono)-methyl]-4,5-methylenedioxyphenylacetic Acid Methyl Ester (14).** With a similar procedure, **14** was prepared from **26** and anhydrous MeOH. Mp 186−189 °C (382 mg, 80%). ^{1}H NMR 1.24 (t, 3H, J = 7.1, CH_3), 3.30 (m, 2H, CH_2), 3.39 (m, 2H, NHCH_2CH_3), 3.52 (s, 3H, OCH_3), 3.82 (bs, 2H, NH_2), 6.05 (m, 2H, OCH_2O), 6.23 (m, 1H, *NH*CH_2), 6.56 (s, 1H, H-3), 6.61 (d, 2H, J = 8.8, H-3′,5′), 6.90 (s, 1H, H-6), 7.29 (d, 2H, J = 8.8, H-2′,6′), 7.33 (bs, 1H, NH). Anal. ($C_{20}H_{22}N_4O_5$) C, H, N.

***(Z)*-2-[(4-Aminophenyl)-(4-ethylsemicarbazono)-methyl]-4,5-methylenedioxyphenylacetic Acid Ethyl Ester (15).** With a similar procedure, **15** was prepared from **26** and anhydrous EtOH. Mp 75−78 °C (386 mg, 78%). ^{1}H NMR 1.12 (t, 3H, J = 7.1, OCH_2CH_3), 1.24 (t, 3H, J = 7.2, NHCH$_2CH_3$), 3.28 (m, 2H, CH_2), 3.38 (m, 2H, NHCH_2CH_3), 3.85 (bs, 2H, NH_2), 3.98 (m, 2H, OCH_2CH_3), 6.05 (m, 2H, OCH_2O), 6.23 (m, 1H, *NH*CH_2), 6.55 (s, 1H, H-3), 6.60 (d, 2H, J = 8.8, H-3′,5′), 6.92 (s, 1H, H-6), 7.30 (d, 2H, J = 8.8, H-2′,6′), 7.32 (bs, 1H, NH). Anal. ($C_{21}H_{24}N_4O_5$) C, H, N.

***(Z)*-2-[(4-Aminophenyl)-(4-ethylsemicarbazono)-methyl]-4,5-methylenedioxyphenylacetic Acid Propyl Ester (16).** With a similar procedure, **16** was prepared from **26** and anhydrous PrOH. Mp 64−67 °C (297 mg, 58%). ^{1}H NMR 0.82 (t, 3H, J = 7.4, O(CH$_2$)$_2CH_3$), 1.24 (t, 3H, J = 7.1, NHCH$_2CH_3$), 1.51 (m, 2H, $OCH_2CH_2CH_3$), 3.29 (m, 2H, CH_2), 3.38 (m, 2H, NHCH_2CH_3), 3.86 (bs, 2H, NH_2), 3.87 (m, 2H, O$CH_2CH_2CH_3$), 6.04 (m, 2H, OCH_2O), 6.24 (m, 1H, *NH*CH_2), 6.55 (s, 1H, H-3), 6.60 (d, 2H, J = 8.8, H-3′,5′), 6.92 (s, 1H, H-6), 7.29 (d, 2H, J = 8.8, H-2′,6′), 7.32 (bs, 1H, NH). Anal. ($C_{22}H_{26}N_4O_5$) C, H, N.

***(Z)*-2-[(4-Aminophenyl)-(4-ethylsemicarbazono)-methyl]-4,5-methylenedioxyphenylacetic Acid Buthyl Ester (17).** With a similar procedure, **17** was prepared from **26** and anhydrous BuOH. Mp 62−65 °C (286 mg, 54%). ^{1}H NMR 0.87 (t, 3H, J = 7.4, O(CH$_2$)$_3CH_3$), 1.24 (t, 3H, J = 7.1, NHCH$_2CH_3$), 1.25 (m, 2H, $OCH_2CH_2CH_2CH_3$), 1.47 (m, 2H, $OCH_2CH_2CH_2CH_3$), 3.29 (m, 2H, CH_2), 3.39 (m, 2H, NHCH_2CH_3), 3.85 (bs, 2H, NH_2), 3.92 (m, 2H, O$CH_2CH_2CH_2CH_3$), 6.04 (m, 2H, OCH_2O), 6.23 (m, 1H, *NH*CH_2), 6.55 (s, 1H, H-3), 6.60 (d, 2H, J = 8.8, H-3′,5′), 6.92 (s, 1H, H-6), 7.30 (d, 2H, J = 8.8, H-2′,6′), 7.32 (bs, 1H, NH). Anal. ($C_{23}H_{28}N_4O_5$) C, H, N.

***(Z)*-2-[(4-Aminophenyl)-(4-propylsemicarbazono)-methyl]-4,5-methylenedioxyphenylacetic Acid Methyl Ester (18).** With a similar procedure, **18** was prepared from **27** and anhydrous MeOH. Mp 74−77 °C (257 mg, 52%). ^{1}H NMR 0.99 (t, 3H, J = 7.4, CH_3), 1.64 (m, 2H, NHCH$_2CH_2CH_3$), 3.30 (m, 4H, CH_2 and NHCH$_2CH_2CH_3$), 3.52 (s, 3H, OCH_3), 3.82 (bs, 2H, NH_2), 6.04 (m, 2H, OCH_2O), 6.26 (m, 1H, *NH*CH_2), 6.56 (s, 1H, H-3), 6.60 (d, 2H, J = 8.8, H-3′,5′), 6.90 (s, 1H, H-6), 7.28 (d, 2H, J = 8.8, H-2′,6′), 7.33 (bs, 1H, NH). Anal. ($C_{21}H_{24}N_4O_5$) C, H, N.

***(Z)*-2-[(4-Aminophenyl)-(4-butylsemicarbazono)-methyl]-4,5-methylenedioxyphenylacetic Acid Methyl Ester (19).** With a similar procedure, **19** was prepared from **28** and anhydrous MeOH. Mp 66−69 °C (256 mg, 50%). ^{1}H NMR 0.97 (t, 3H, J = 7.4, CH_3), 1.42 (m, 2H, NHCH$_2CH_2CH_2CH_3$), 1.59 (m, 2H, NHCH$_2CH_2CH_2CH_3$), 3.25−3.38 (m, 4H, CH_2 and NHCH_2), 3.52 (s, 3H, OCH_3), 3.82 (bs, 2H, NH_2), 6.05 (m, 2H, OCH_2O), 6.25 (m, 1H, *NH*CH_2), 6.56 (s, 1H, H-3), 6.61 (d, 2H, J = 8.5, H-3′,5′), 6.90 (s, 1H, H-6), 7.28 (d, 2H, J = 8.5, H-2′,6′), 7.32 (bs, 1H, NH). Anal. ($C_{22}H_{26}N_4O_5$) C, H, N.

***(E)*-2-[(4-Aminophenyl)−(4-methylsemicarbazono)-methyl]-4,5-methylenedioxyphenylacetic Acid Methyl Ester (29).** A $CHCl_3$ solution (25 mL) of compound **10** (100 mg) left aside for two week at room temperature yielded a 70:30 mixture of **10** and **29**. Compound **29** was obtained in a pure form by a silica gel column chromatography using EtOAc/cyclohexane/*i*-PrOH (60:30:10) as eluant and was then crystallized from EtOAc. R_f = 0.42; mp 207−210 °C; HPLC 7.25 min. MS 385[M + 1], 353, 328, 311, 296. ^{1}H NMR 2.90 (d, 3H, J = 4.7, NHCH_3), 3.68 (s, 3H, OCH_3), 3.77 (m, 2H, CH_2), 3.92 (bs, 2H, NH_2), 5.94 (m, 2H, OCH_2O), 6.53 (s, 1H, H-3), 6.54 (m, 1H, *NH*CH_3), 6.71 (d, 2H, J = 8.5, H-3′,5′), 6.79 (s, 1H, H-6), 7.02 (d, 2H, J = 8.5, H-2′,6′), 7.90 (bs, 1H, NH). Anal. ($C_{19}H_{20}N_4O_5$) C, H, N.

2-(4-Nitrobenzyl)-4,5-methylenedioxyphenylacetic Acid Methyl Ester (31). To a solution of 2-(4-nitrobenzoyl)-4,5-

methylenedioxyphenylacetic acid methyl ester (**30**) (1.0 g, 2.9 mmol) in anhydrous THF (40 mL), were added BF_3OEt_2 (1.1 mL, 8.7 mmol) and sodium cyanoborohydride (364 mg, 5.8 mmol). The reaction mixture was refluxed overnight, cooled at room temperature, and diluted with diethyl ether (40 mL). The organic solution was sequentially treated with a saturated $NaHCO_3$ solution and brine, and then dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by column chromatography using light petroleum/diethyl ether (70:30) as eluant to give 0.8 g (83%) of **31** as a white solid. Mp 119−122 °C. ^{1}H NMR 3.47 (s, 2H, CH_2COOCH_3), 3.60 (s, 3H, CH_3), 4.04 (s, 2H, PhCH_2), 5.96 (s, 2H, OCH_2O), 6.59 and 6.76 (2s, 2H, H-3 and H-6), 7.26 (d, 2H, J = 8.5, H-2′,6′), 8.13 (d, 2H, J = 8.5, H-3′,5′). Anal. ($C_{17}H_{15}NO_6$) C, H, N.

2-(4-Aminobenzyl)-4,5-methylenedioxyphenylacetic Acid Methyl Ester (20). A suspension of **31** (400 mg, 1.2 mmol) and Raney−Ni (60 mg) in EtOH (20 mL) was stirred with ammonium formate (120 mg) at room temperature. After completion of the reaction (monitored by TLC EtOAc/cyclohexane, 1:1), the mixture was filtered off. The organic layer was evaporated under reduced pressure, and the residue dissolved in $CHCl_3$ was washed with saturated NaCl to remove ammonium formate. The organic layer was evaporated under reduced pressure and the residue was purified by treatment with acetone to give 294 mg, 81% of **20**. Mp 150−153 °C. ^{1}H NMR 3.53 (s, 2H, CH_2CO), 3.64 (s, 3H, CH_3), 3.82 (s, 2H, PhCH_2), 3.86 (bs, 2H, NH_2), 5.92 (s, 2H, OCH_2O), 6.62 and 6.73 (2s, 2H, H-3 and H-6), 6.61 (d, 2H, J = 8.0, H-3′,5′), 6.89 (d, 2H, J = 8.0, H-2′,6′). Anal. ($C_{17}H_{17}NO_4$) C, H, N.

2-(4-Nitrobenzyl)-4,5-methylenedioxyphenylacetic Acid (32). To a suspension of **31 (**800 mg, 2.4 mmol) in H_2O (60 mL) were added THF (15 mL) and HCl 6 N (4 mL). The mixture was refluxed for 9 h, cooled at room temperature, and then diluted with H_2O (60 mL) and extracted with EtOAc (2 × 60 mL). The combined extracts were evaporated under reduced pressure, and the residue was purified by treatment with diethyl ether to give 500 mg, 65% of **32**. Mp 222−224 °C. ^{1}H NMR 3.51 (s, 2H, CH_2COOH), 4.04 (s, 2H, PhCH_2), 5.97 (s, 2H, OCH_2O), 6.60 and 6.77 (2s, 2H, H-3 and H-6), 7.26 (d, 2H, J = 8.5, H-2′,6′), 8.13 (d, 2H, J = 8.5, H-3′,5′). Anal. ($C_{16}H_{13}NO_6$) C, H, N.

1-[2-(4-Nitrobenzyl)-4,5-methylenedioxyphenylacetyl]-3-methylurea (33). A mixture of compound **32** (500 mg, 1.6 mmol) and excess of thionyl chloride (8 mL) was heated at reflux for 6 h. Excess of thionyl chloride was removed under reduced pressure, and the residue was dissolved in benzene (50 mL) and treated with methylurea (176 mg, 2.4 mmol) at reflux (6 h). The resulting mixture was poured into saturated aqueous $NaHCO_3$ and extracted with EtOAc (2 × 50 mL). The combined extracts were dried over Na_2SO_4 and purified by column chromatography with CCl_4/EtOAc/*i*-PrOH (70:20:10) as eluant to give 354 mg (60%) of **33**. Mp 207−210 °C. ^{1}H NMR 2.79 (d, 3H, J = 4.7, CH_3), 3.50 (s, 2H, CH_2CO), 4.02 (s, 2H, PhCH_2), 6.00 (s, 2H, OCH_2O), 6.67 (s, 1H, H-3), 6.76 (s, 1H, H-6), 7.24 (d, 2H, J = 8.5, H-2′,6′), 8.12 (d, 2H, J = 8.5, H-3′,5′), 8.14 (bs, 1H, $NHCH_3$), 8.26 (bs, 1H, NH). Anal. ($C_{18}H_{17}N_3O_6$) C, H, N.

1-[2-(4-Aminobenzyl)-4,5-methylenedioxyphenylacetyl]-3-methylurea (21). To a solution of **33** (354 mg, 0.95 mmol) in EtOH (30 mL) was added ammonium formate (96 mg, 1.5 mmol) and Raney−Ni (50 mg), and the mixture was heated at reflux for 2 h. The mixture was filtered off on a Celite pad and the solvent was removed under reduced pressure. The residue, dissolved in $CHCl_3$, was washed with saturated NaCl to remove ammonium formate. The organic layer, after evaporation of the solvent, gave the desired amino derivative **21** which was purified by treatment with acetone (273 mg, 84%). Mp 199−202 °C. ^{1}H NMR 2.82 (d, 3H, J = 4.7, CH_3), 3.51 (s, 2H, CH_2CO), 3.59 (bs, 2H, NH_2), 3.76 (s, 2H, PhCH_2), 5.87 (s, 2H, OCH_2O), 6.61 (d, 2H, J = 8.2, H-3′,5′), 6.67 (s, 1H, H-3), 6.71 (s, 1H, H-6), 6.86 (d, 2H, J = 8.2, H-2′,6′), 7.16 (bs, 1H, NH), 8.12 (bs, 1H, $NHCH_3$). Anal. ($C_{18}H_{19}N_3O_4$) C, H, N.

1-[2-(4-Aminobenzoyl)-4,5-methylenedioxyphenylacetyl]-3-methylurea (22). To a solution of **33** (354 mg, 0.95 mmol) in acetone (100 mL), potassium permanganate (1.42 mg) and anhydrous sodium sulfate (708 mg) were added, and the mixture was stirred at room temperature until the disappearance of the starting material (TLC monitoring). Excess of potassium permanganate was decomposed by adding EtOH. The reaction mixture was filtered off on a Celite pad and the filtrate was evaporated. The crude product was purified by column chromatography with CCl_4/EtOAc/*i*-PrOH as eluant to give 220 mg (60%). The subsequent treatment with ammonium formate and Raney−Ni as reported for compound **21**, gave a crude product that was purified by column chromatography using EtOAc/cyclohexane/*i*-PrOH, 60:30:10 as eluant to give 162 mg (80%) of **22**. Mp 109−112 °C. ^{1}H NMR 2.85 (d, 3H, J = 4.7, CH_3), 3.55 (s, 2H, CH_2CO), 4.38 (bs, 2H, NH_2), 6.04 (s, 2H, OCH_2O), 6.65 (d, 2H, J = 8.5, H-3′,5′), 6.89 (s, 1H, H-3), 6.92 (s, 1H, H-6), 7.70 (d, 2H, J = 8.5, H-2′,6′), 8.15 (bs, 1H, $NHCH_3$), 10.1 (bs, 1H, NH). Anal. ($C_{18}H_{17}N_3O_5$) C, H, N.

1-(3,4-Methylenedioxyphenylacetyl)-3-methylurea (23). 3,4-Methylenedioxyphenylacetic acid (**34**) (500 mg, 2.78 mmol) was refluxed with excess of thionyl chloride (10 mL) for 6 h. Excess of $SOCl_2$ was evaporated under reduced pressure, and the residue was dissolved in dry benzene and treated with methylurea (309 mg, 4.17 mmol). The reaction mixture was refluxed for 6−7 h, then washed with 2% $NaHCO_3$ and extracted with EtOAc (2 × 100 mL). The combined extracts were dried over Na_2SO_4, and the solvent was removed under pressure to yield a crude product which, by treatment with acetone, afforded 492 mg (75%) of **23**. Mp 162−165 °C. ^{1}H NMR 2.87 (d, 3H, J = 4.7, CH_3), 3.56 (s, 2H, CH_2), 5.96 (s, 2H, OCH_2O), 6.75−6.80 (m, 3H, Ar), 8.33 (bs, 1H, $NHCH_3$), 9.30 (bs, 1H, NH). Anal. ($C_{11}H_{12}N_2O_4$) 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.[17] Briefly, Whatman KC 18F 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 3:1 (v/v) mixture of acetone and water. Each compound was dissolved in $CHCl_3$ (2 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 are expressed as the mean values of five determinations. The R_m values were calculated from the experimental R_f values according to the equation $R_m = \log[(1/R_f) - 1]$. Higher R_m values indicate higher lipophilicity. The log P values reported in Table 1 were calculated by using the XLOGP v 2.0 program.[18] Calculated log P and experimental R_m values are linearly correlated with an r^2 value of 0.86 at 95% confidence limits.

Modeling Studies. Calculations on compounds **10** and **29** were performed with the PC Spartan Pro molecular modeling program (Wavefunction, Inc., Irvine, CA) using the semiempirical AM1 method[13] and were carried out at the RHF level. The molecules were built from the model kit containing the atomic fragments and were subjected to conformational search through the conformational distribution option. Both compounds showed several minimum energy conformations of comparable energy. The energy barriers for rotation around the single bond connecting the semicarbazono moiety to C-2 were determined through location of the transition states corresponding to the maxima of the energy profiles.

Testing of Anticonvulsant Activity Against Audiogenic Seizures in DBA/2 Mice. All experiments were performed with DBA/2 mice which are genetically susceptible to sound-induced seizures.[19] DBA/2 mice (8−12 g, 22−25 days old) were purchased from Charles River (Calco, Como, Italy). Groups of 10 mice 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 solution in 50% DMSO and 50% sterile saline (0.9% NaCl). Individual mice were placed under a hemispheric Perspex dome (diameter 58

cm) and were allowed 60 s for habituation. Assessment of locomotor activity was also made during this time interval. 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 audiogenic seizures.[20] The time course of the anticonvulsant action of **10** was determined following the administration of 33 μmol/kg of compound to groups of 10 mice for each time. The animals were tested for sound-induced seizure responses from 15 to 120 min after drug administration.

MES 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.[21] Abolition of tonic hindlimb extension after drug treatment was considered as the endpoint of protection. The dose−response curves were estimated by testing four to five doses using 8−10 mice for each dose.

PTZ-Induced Seizures in Swiss Mice. Male Swiss mice (20−26 g, 42−48 days old) were purchased from Charles River (Calco, Como, Italy) and pretreated with vehicle or drug 45 min before the sc administration of pentylenetetrazole (PTZ). 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 (CD_{97}) of PTZ (85 mg/kg) was applied, and the animals were 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.[22]

AMPA-Induced Seizures in DBA/2 Mice. The CD_{50} (± 95% confidence limits) of icv microinjected AMPA was 1.76 (1.06−3.07) nmol for clonus and 2.90 (1.83−4.58) nmol for tonus. For icv injection, the 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;[23] 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 30 × 30 × 30 cm box, and the observation time was 30 min after the administration of AMPA.

KA-Induced Seizures in Swiss Mice. KA was administered sc at a dose of 32 mg/kg (previously determined CD_{97} value) 15 min after ip administration of compound **10**. Animals showing 5 s or more of clonic activity were scored as not protected according to Donevan et al.[6] The period of observation was 60 min.

Electrophysiology. Primary cultures of cerebellar granule neurons were prepared from 7 to 8 days old Sprague−Dawley rats as previously described.[24] Cells from cerebella were dispersed with trypsin (0.24 mg/mL) and plated at a density of 10^6 cells/mL on 35-mm Falcon dishes coated with poly-L-lysine (10 μg/mL). The cells were grown in basal Eagle's medium, supplemented with 10% fetal bovine serum, 2 mM glutamine, and 100 μg/mL gentamycin, and maintained at 37 °C in 5% CO_2. Cytosine arabinofuranoside (10 μM) was added to the cultures 24 h after plating to prevent astroglia proliferation.

Electrophysiological Recordings. Recordings were performed on single cerebellar granule neurons after 7 days in culture[24] using the voltage-clamp technique in the whole-cell configuration.[25] Electrodes were pulled from borosilicate glass on a vertical puller and had a resistance of 5−7 MΩ when filled with KCl internal solution. Currents were amplified with an Axopatch 1D amplifier, filtered at 5 kHz, and digitized at 10 kHz by using pClamp software. Intracellular solution contained (mM): KCl 140, $MgCl_2$ 3, ethylene glycol-bis-(β-aminoethyl ether)*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) 5, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES) 5, ATP-Na 2, pH 7.3 with KOH. The cells were continuously perfused with the external solution (mM): NaCl 145, KCl 5, $CaCl_2$ 1, HEPES 5, glucose 5, sucrose 20, pH 7.4 with NaOH. All drugs were dissolved in DMSO and diluted at the final concentration (<1%) in extracellular solution. KA was also dissolved in the extracellular solution. All drugs were applied directly by gravity through a Y-tube perfusion system[26] at same concentration (100 μM). After a wash-out of compounds **10** and **21** from the cell culture, the responses to KA returned to the control level.

Effects on Motor Movements. Male Swiss mice (20−26 g, 48−54 days old) were purchased from Charles River. Groups of 10 mice were trained to do coordinated motor movements continuously for 2 min on a rotarod, 3 cm in diameter, at 8 rpm (U. Basile, Comerio, Varese, Italy). Impairment of coordinated motor movements was defined as the inability of the mice to remain on the rotarod for a 2-min test period.[27] The ability of the mice to remain on the rotarod was tested 30 min after administration of various compounds.

Excitotoxic Experiments.[28] Drugs were dissolved in Mg-free Loke's buffer (NaCl 154 mM, KCl 5.6 mM, $CaCl_2$ 2.3 m, $NaHCO_3$ 3.6 mM, D-glucose 5.5 mM, Hepes 5 mM, pH 7.4). The medium was removed from the cell culture and a solution of the test drug (KA 100 μM without or with 100 μM **10** or **21**) was added. The cultures were incubated for 3 h at 37 °C. The experiment was stopped by washing the cultures with the Loke's Buffer later replaced with the culture medium. Neuronal cell death was assessed 24 h later and was estimated by the MTT tetrazolium salt assay.[28] The adsorbance were recorded at 570 nm and 630 nm.

Statistical Analysis. The ED_{50} values of each phase of the audiogenic seizure or seizures induced by MES, PTZ, AMPA, or KA were determined for each compound administered, and dose−response curves were fitted using a computer program by the method of Litchfield and Wilcoxon.[29] The relative anticonvulsant activities were determined by comparison of respective ED_{50} values. The median toxic dose (TD_{50}) values were estimated using the method of Litchfield and Wilcoxon.[29] The relative activities were determined by comparison of respective TD_{50} values. Statistical significance between control and test groups of data means was tested using a two-tailed Student's *t* test. Electrophysiological data were analyzed using the software Clampex (Axon Instrument). Results are expressed as mean ± SE. Origin (Microcal Software, Northampton, MA) was used for figure preparation and statistical analysis.

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References

(1) (a) Ikonomidou, C.; Turski, L. Glutamate in Neurodegenerative Disorders. In *CNS Neurotransmitters and Neuromodulators: Glutamate;* Stone, T. W., Ed.; CRC Press: New York, 1995; pp 253−266. (b) Danysz, W.; Parsons, C. G.; Bresink, I.; Quack, G. Glutamate in CNS Disorders. *Drug News Perspect.* **1995**, *8*, 261−277.

(2) (a) Gill, R.; Lodge, D. Pharmacology of AMPA Antagonists and Their Role in Neuroprotection. *Int. Rev. Neurobiol.* **1997**, *40*, 197−232. (b) Bräuner-Osborne, H.; Egebjerg, J.; Nielsen, E.; Krogsgaard-Larsen, P. Ligands for Glutamate Receptors: Design and Therapeutic Prospects. *J. Med. Chem.* **2000**, *43*, 2609−2645. (c) Lees, G. J. Pharmacology of AMPA/Kainate Receptor Ligands and Their Therapeutic Potential in Neurological and Psychiatric Disorders. *Drugs* **2000**, *59*, 33−78.

(3) Umemura, K.; Kondo, K., Ikeda, Y.; Teraya, Y.; Yoshida, H.; Homma, M.; Uematsu, T.; Nakashima, M. Pharmacokinetic and Safety of the Novel Amino-3-hydroxy-5-methylisoxazole-4-propionate Receptor Antagonist YM90K in Healthy Men. *J. Clin. Pharmacol.* **1997**, *37*, 719−728.

(4) (a) Chapman, A. G.; Smith, S. E.; Meldrum, B. S. The Anticonvulsant Effect of the Non-NMDA Antagonists, NBQX and GYKI 52466, in Mice. *Epilepsy Res.* **1991**, *9*, 92−96. (b) Smith, S. E.; Meldrum, B. S. Cerebroprotective Effect of a Non-*N*-Methyl-D-Aspartate Antagonist, GYKI 52466, After Focal Ischemia in the Rat. *Stroke* **1992**, *23*, 861−864.

(5) Tarnawa, I.; Berzsenyi, P.; Andrasi, F.; Botka, P.; Hamori, T.; Ling, I.; Körösi, J. Structure−Activity Relationships of 2,3-Benzodiazepine Compounds with Glutamate Antagonistic Action. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 99−104.

(6) Donevan, S. D.; Yamaguchi, S. I.; Rogawski, M. A. Non-*N*-methyl-D-aspartate Receptor Antagonism by 3-N-Substituted 2,3-Benzodiazepines: Relationship to Anticonvulsant Activity. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 25−29.

(7) (a) De Sarro, A.; De Sarro, G.; Gitto, R.; Grasso, S.; Micale, N.; Quartarone, S.; Zappalà M. 7,8-Methylenedioxy-4*H*-2,3-benzodiazepin-4-ones as Novel AMPA Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 971−976. (b) De Sarro, A.; De Sarro, G.; Gitto, R.; Grasso, S.; Micale, N.; Zappalà M. Synthesis and Anticonvulsant Activity of New 2,3-Benzodiazepines as AMPA Receptor Antagonists. *Farmaco* **1999**, *54*, 179−189.

(8) Wang, Y.; Konkoy, C. S.; Ilvy, V. I.; Vanover, K. E.; Carter, R.; Weber, E.; Keana, J. F. W.; Woodward, R. M.; Cai, S. X. Synthesis of 7,8-(Methylenedioxy)-1-phenyl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones as Novel and Potent Noncompetitive AMPA Receptor Antagonists. *J. Med. Chem.* **1998**, *41*, 2621−2625.

(9) Grasso, S.; De Sarro, G.; De Sarro, A.; Micale, N.; Zappalà, M.; Puja, G.; Baraldi, M.; De Micheli, C. Synthesis and Anticonvulsant Activity of Novel and Potent 2,3-Benzodiazepine Noncompetitive AMPA/Kainate Receptor Antagonists. *J. Med. Chem.* **1999**, *42*, 4414−4421.

(10) Zappalà, M.; Grasso, S.; Micale, N.; Polimeni, S.; De Micheli, C. Synthesis and Structure−Activity Relationships of 2,3-Benzodiazepines as AMPA Receptor Antagonists. *Mini Rev. Med. Chem.* **2001**, *1*, 243−253.

(11) (a) Grasso, S.; De Sarro, G.; De Sarro, A.; Micale, N.; Zappalà, M.; Puja, G. Baraldi, M.; De Micheli, C. Synthesis and Anticonvulsant Activity of Novel and Potent 6,7-Methylenedioxyphthalazin-1(2*H*)-ones. *J. Med. Chem.* **2000**, *43*, 2851−2860. (b) Grasso, S.; De Sarro, G.; De Sarro, A.; Micale, N.; Zappalà, M.; Puja, G.; Baraldi, M.; De Micheli, C. Synthesis and Anticonvulsant Activity of Novel and Potent 1-Aryl-7,8-methylenedioxy-1,2,3,5-tetrahydro-4*H*-2,3-benzodiazepin-4-ones. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 463−466.

(12) Anderson, B. A.; Hansen, M. M.; Harkness, A. R.; Henry, C. L.; Vicenzi, J. T.; Zmijewski, M. J. Application of a Practical Biocatalytic Reduction to an Enantioselective Synthesis of the 5*H*-2,3-Benzodiazepine LY300164. *J. Am. Chem. Soc.* **1995**, *117*, 12358−12359.

(13) Dewar, M. J. S.; Zoerbish, E. G.; Healy, E. F.; Stewart, J. J. P. Development and Use of Quantum Mechanical Molecular Models. 76. AM1: A New General Purpose Quantum Mechanical Molecular Model. *J. Am. Chem. Soc.* **1985**, *107*, 3902−3909.

(14) Srikrishna, A.; Sattigeri, J. A.; Viswajanani, R., Yelamaggad, C. V. A Simple and Convenient One Step Method for the Reductive Deoxygenation of Aryl Ketones to Hydrocarbons. *Synlett* **1995**, 93−94.

(15) (a) Chapman, A. G.; Croucher, M. J.; Meldrum, B. S. Evaluation of Anticonvulsant Drugs in DBA/2 Mice with Sound-Induced Seizures. *Arzneim. Forsch.* **1984**, *34*, 1261−1264. (b) Engstrom, F. L.; Woodbury, D. M. Seizure Susceptibility in DBA/2 and C57 Mice: the Effects of Various Convulsants. *Epilepsia* **1988**, *29*, 389−395.

(16) (a) Donevan, S. D.; Rogawski, M. A. GYKI 52466, a 2,3-Benzodiazepine, is a Highly Selective, Noncompetitive Antagonist of AMPA/Kainate Receptor Responses. *Neuron* **1993**, *10*, 51−59. (b) Patneau, D. K.; Mayer, M. L. Kinetic Analysis of Interactions between Kainate and AMPA: Evidence for Activation of a Single Receptor in Mouse Hippocampal Neurons. *Neuron* **1991**, *6*, 785−798. (c) Wilding, T. J.; Huettner, J. E. Differential Antagonism of α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid-preferring and Kainate-preferring Receptors by 2,3-Benzodiazepines. *Mol. Pharmacol.* **1995**, *47*, 582−587.

(17) (a) Boyce, C. B. C.; Milborrow, B. V. A Simple Assessment of Partition Data for Correlating Structure and Biological Activity Using Thin-Layer Chromatography. *Nature* **1965**, *208*, 537−539. (b) Pietrogrande, M. C.; Borea, P. A.; Biagi, G. L. Lipophilicity Measurements of Benzodiazepine-Receptor Ligands by Reversed-Phase Liquid Chromatography. Comparison between High-Performance Liquid and Thin-Layer Chromatography. *J. Chromatogr.* **1988**, *447*, 404−409.

(18) Wang, R.; Gao, Y., Lai, L. Calculating Partition Coefficient by Atom-additive Method. *Perspect. Drug Discovery Des.* **2000**, *19*, 47−66.

(19) Collins, R. L. Audiogenic Seizures. In *Experimental Models of Epilepsy;* Purpura, P., Penry, J. K., Tower, D., Woodbury, D. M., Walter, R., Eds.; Raven Press: New York, 1972; pp 347−372.

(20) De Sarro, G. B.; Croucher, M. J.; Meldrum, B. S. Anticonvulsant Actions of DS 103-282: Pharmacological Studies in Rodents and the Baboon *Papio papio*. *Neuropharmacology* **1984**, *23*, 526−530.

(21) Swinyard, E. A.; Brown, W. C.; Goodman, L. S. Comparative Assays of Antiepileptic in Mice and Rats. *J. Pharmacol. Exp. Ther.* **1952**, *106*, 319−330.

(22) Swinyard, E. A.; Woodhead, J. M. Experimental Detection, Quantification and Evaluation of Anticonvulsants. In *Epileptic Drugs;* Woodbury, D. M., Penry, J. K., Pippenger, C. E., Eds.; Raven Press: New York, 1982; pp 111−126.

(23) De Sarro, G. B.; Ongini, E.; Bertorelli, R.; Aguglia, U.; De Sarro, A. Excitatory Amino Acid Neurotransmission through both NMDA and Non-NMDA Receptors is Involved in the Anticonvulsant Activity of Felbamate in DBA/2 mice. *Eur. J. Pharmacol.* **1994**, *262*, 11−19.

(24) Gallo, V.; Ciotti, M. T.; Coletti, A.; Aloisi, F.; Levi, G. Selective Release of Glutamate from Cerebellar Granule Cells Differentiating in Culture. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 7919−7923.

(25) Hamil, O. P.; Marty, A.; Neher, E.; Sakmann, B.; Sigworth, F. J. Improved Patch Clamp Techniques for High-Resolution Current Recording from Cells and Free-membrane Patches. *Pflugers Arch.* **1981**, *414*, 85−100.

(26) Murase, K.; Ryu, P. D.; Randic, M. Excitatory and Inhibiting Amino Acids and Peptide-induced Responses in Acutely Isolated Rat Spinal Horn Neurons. *Neurosci. Lett.* **1989**, *103*, 56−63.

(27) Dunham, N. W.; Miya, T. S. A Note on a Simple Apparatus for Detecting Neurological Deficit in Rats and Mice. *J. Am. Pharm. Assoc.* **1957**, *46*, 208−209.

(28) Ankarcrona, M.; Dypbukt, J. M.; Bonfoco, E.; Zhivotovsky, B.; Orrenius, S.; Lipton, S. A.; Nicotera, P. Glutamate-induced Neuronal Death: a Succession of Necrosis or Apoptosis Depending on Mitocondrial Function. *Neuron* **1995**, *15*, 961−973.

(29) Litchfield, J.; Wilcoxon, F. A Simplified Method of Evaluating Dose−Effect Experiments. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99−113.

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