Synthesis and Anticonvulsant Activity of Novel and Potent 6,7-Methylenedioxyphthalazin-1(2*H*)-ones

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In this paper, we describe the synthesis of a series of novel substituted 4-aryl-6,7-methylenedioxyphthalazin-1(2*H*)-ones. The anticonvulsant activity of these compounds against audiogenic seizures was evaluated in DBA/2 mice after intraperitoneal (ip) injection. Most of these derivatives are more active than 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (**1**, GYKI 52466), a well-known noncompetitive AMPA receptor antagonist. As deduced by the rotarod test, all the compounds exhibit a toxicity lower than that of **1**. Within the series of derivatives submitted to investigation, 4-(4-aminophenyl)-2-butylcarbamoyl-6,7-methylenedioxyphthalazin-1(2*H*)-one (**21**) proved to be the most active compound and is 11-fold more potent than **1** (i.e., ED_{50} 3.25 μmol/kg for **21** versus ED_{50} 35.8 μmol/kg for **1**). When compared to **1,** compound **21** as well as its analogue 4-(4-aminophenyl)-6,7-methylenedioxyphthalazin-1(2*H*)-one (**16**) show a longer lasting anticonvulsant activity. Compound **21** also effectively suppresses seizures induced in Swiss mice by maximal electroshock (MES) and pentylenetetrazole (PTZ). Furthermore, it antagonizes in vivo seizures induced by 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA), 2-amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl)propionic acid (ATPA), and kainate (KA), and its anticonvulsant activity is reversed by pretreatment with aniracetam. Using the patch-clamp technique, the capability of derivatives **16** and **21** to antagonize KA-evoked currents in primary cultures of granule neurons was tested. They behaved as antagonists, but they proved to be less effective than **1** and 1-(4-aminophenyl)-3,4-dihydro-4-methyl-3-*N*-methylcarbamoyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (**2,** GYKI 53655) to reduce the KA-evoked currents.

Introduction

L-Glutamate is the major amino acid mediator of excitatory neurotransmission in the brain and spinal cord. Excessive stimulation of ionotropic glutamate receptors, *N*-methyl-D-aspartic acid (NMDA), 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA), and kainate (KA) receptors, could result in acute neurological disorders such as cerebral ischemia and epilepsy as well as chronic neurodegenerative pathologies such as amyotrophic lateral sclerosis, Huntington's chorea, and Alzheimer's disease.[1] Therefore glutamate receptor antagonists could have many potential therapeutic utilities. Since many known antagonists do not discriminate among the glutamate receptor subtypes, the development of more potent and selective ligands has become a crucial goal to assess the physiological and pathological roles played by the single receptor subtypes. On the other hand, a compound capable to interact simultaneously with different receptor subtypes could be important from a therapeutic point of view.[2]

1-(4-Aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (**1**, GYKI 52466) and its 3,4-dihydro-3-*N*-methylcarbamoyl derivative (**2,** GYKI 53655) (Chart 1), possess noteworthy anticonvulsant[3] and neuroprotective[4] properties, due to their AMPA antagonist activities. GYKI 52466 does not affect the NMDA receptors and marginally interacts with the kainate receptors.[5] It is now well established[6] that **1** is a noncompetitive AMPA receptor antagonist.

We[7,8] and other authors[9] have reported the AMPA antagonist activity of 2,3-benzodiazepin-4-one derivatives, e.g., **3**. The goal of the research in this field is aimed at finding new anticonvulsant compounds devoid of the side effects and a poor biodistribution profiles which are commonly associated with AMPA receptor antagonists and have hampered their clinical development.[10] We have recently shown[8] that the replacement of the azomethine moiety of **1** with a (thio)lactam functionality and the introduction of a methylcarbamoyl group at N-3 position of the heterocyclic nucleus resulted in an increase in potency. Furthermore, as deduced from the rotarod test, compounds **3** are all characterized by a toxicity lower than that of **1**.

Recently, a class of substituted 1,2-dihydrophthalazines, structurally related to **1** and **2**, was identified as anticonvulsant agents. Their biological activity is due to a selective and noncompetitive inhibition of the AMPA receptor complex.[11] In this context, 2-(*N*-but-

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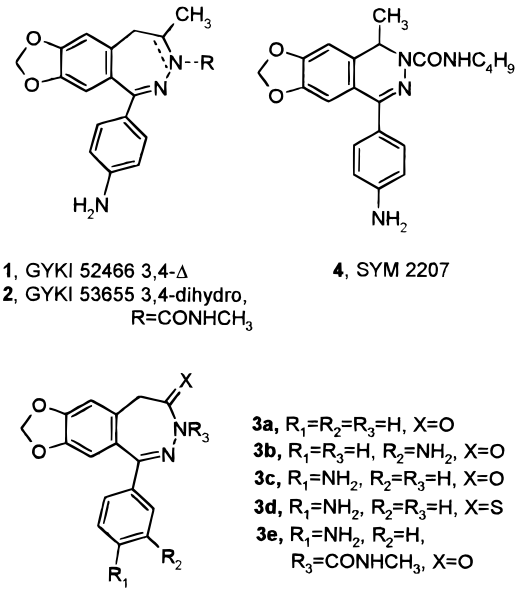
Chart 1

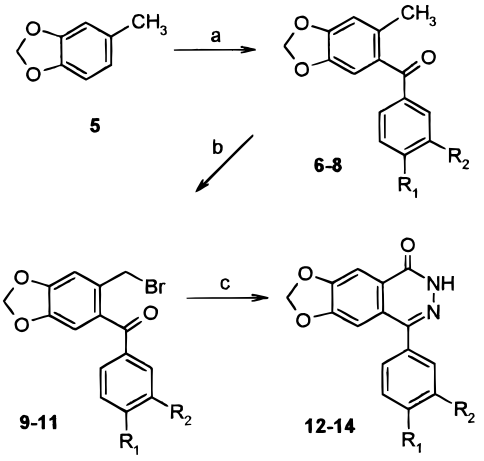


ylcarbamoyl)-1,2-dihydrophthalazine **4** (SYM 2207) emerged as the lead compound in this series of new anticonvulsant agents.

To evaluate the impact of ring size of 2,3-benzodiazepin-4-one derivatives on the anticonvulsant activity, we synthesized a series of novel phthalazin-1(2*H*)-ones. This report deals with the preparation of a number of 4-aryl-6,7-methylenedioxyphthalazin-1(2*H*)-ones (**12**−**16**) and their 2-*N*-alkylcarbamoyl derivatives (**17**−**23**). Furthermore, since the bioisosteric replacement of oxygen by sulfur in the carbonyl group in derivatives **3** gave a significant improvement in the pharmacological profile,[8c] we synthesized 4-aryl-6,7-methylenedioxyphthalazine-1(2*H*)-thiones (**24**, **25**). The new compounds (**12**, **14**−**25**) were evaluated for their anticonvulsant properties 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] We assessed the propensity of all compounds to induce neurological impairment by using the rotarod test. Compound **21**, the most active derivative in this series, and its parent compound **16** were also examined as antagonists against seizures induced in Swiss mice either by pentylenetetrazole (PTZ) or by maximal electroshock (MES) and the time course of anticonvulsant activity was also studied. The activity of derivatives **16** and **21** was also evaluated for their ability to antagonize seizures induced by the intracerebroventricular (icv) administration of AMPA and 2-amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl)propionic acid (ATPA) or subcutaneous (sc) administration of KA. The ability of aniracetam (Ro 13-5057), a potentiator of the AMPA receptors,[13] to reverse the anticonvulsant properties of these compounds was also studied.

To investigate the mechanism of action of these compounds, we used the patch-clamp technique in primary cultures of granule neurons. The capability of **16** and **21** to antagonize KA-evoked current was tested and compared with that of parent compounds **1** and **2**.

Scheme 1[a]



	R_1	R_2
6, 9, 12	H	H
7, 10, 13	H	NO_2
8, 11, 14	NO_2	H

[a] Reagents: (a) $C_6H_5COCl/SnCl_4/CH_2Cl_2$ or 3- or 4-$NO_2C_6H_4$-$COOH/P_2O_5/(CH_2)_2Cl_2$, rt, 12 h; (b) Br_2/250 W high pressure Hg lamp/CCl_4, reflux, 8 h, or NBS-AlBN/CCl_4, reflux, 2 h; (c) $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 16 h.

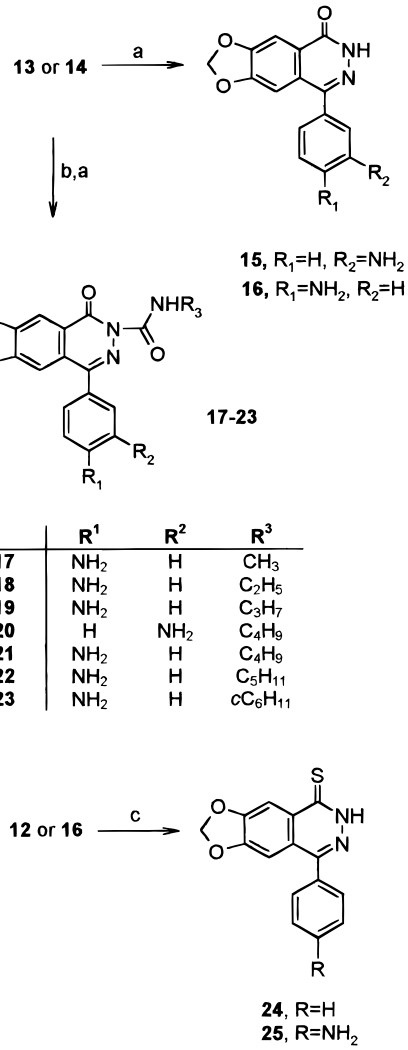
Chemistry

The synthesis of 4-aryl-6,7-methylenedioxyphthalazin-1(2*H*)-ones **12**−**14** was achieved as shown in Scheme 1. The Friedel−Crafts acylation[8a,b] of 3,4-methylenedioxytoluene **5** with benzoyl chloride was carried out in the presence of tin(IV) chloride to yield benzophenone derivative **6**. When 3- or 4-nitrobenzoic acid was used as the acylating reagent, phosphorus pentoxide was employed as the catalyst[14] to furnish compounds **7**−**8** in high yield. A solution of intermediates **6**−**8** in carbon tetrachloride was refluxed and irradiated with a 250 W high-pressure Hg lamp while a solution of bromine in the same solvent was added dropwise to yield unstable benzyl bromides **9**−**11**. Intermediates **9**−**11** were also prepared by reacting compounds **6**−**8** with *N*-bromosuccinimide (NBS) in the presence of trace amounts of α,α′-azoisobutyronitrile (AIBN). The structure of unstable benzyl bromides **9**−**11** was confirmed by ^{1}H NMR. The reaction of **9**−**11** with hydrazine produced directly derivatives **12**−**14** through a cyclocondensation followed by an oxidation process. Reduction of the nitro derivatives **13** and **14** by catalytic hydrogenation (5% Pd/C in methanol) gave amino derivatives **15** and **16** in excellent yield. Compounds **17**−**23** were obtained by reacting **13** and **14** with a series of isocyanates followed by a catalytic hydrogenation of the nitro group (Scheme 2). Compounds **12** and **16** were also converted into the corresponding thioderivatives **24** and **25** by treatment with Lawesson's reagent in refluxing toluene. The elemental analysis (C, H, N), ^{1}H NMR, ^{13}C NMR, and MS spectral data of all the synthesized compounds are in full agreement with the proposed structures and are reported in the Experimental Section.

Results and Discussion

Novel 4-aryl-6,7-methylenedioxyphthalazin-1(2*H*)-

Scheme 2[a]



	R^1	R^2	R^3
17	NH_2	H	CH_3
18	NH_2	H	C_2H_5
19	NH_2	H	C_3H_7
20	H	NH_2	C_4H_9
21	NH_2	H	C_4H_9
22	NH_2	H	C_5H_{11}
23	NH_2	H	cC_6H_{11}

[a] Reagents: (a) 5% Pd-C/H_2, MeOH, rt, 3 h; (b) $R^3NCO/Et_3N/CH_2Cl_2$, rt, 24 h; (c) Lawesson's reagent, toluene, reflux, 2 h.

ones (**12** and **14**−**16**), 2-(*N*-alkylcarbamoyl)-4-aryl-6,7-methylenedioxyphthalazin-1(2*H*)-ones (**17**−**23**), and 4-aryl-6,7-methylenedioxyphthalazine-1(2*H*)-thiones (**24**, **25**) were tested for anticonvulsant activity against audiogenic seizures in DBA/2 mice, and the results are compared with those previously reported[8c] for **1** and **3a**−**c**. The compounds 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. A dose-dependent and significant ($p < 0.01$ using Fischer's exact probability test) anticonvulsant activity was observed 30 min after ip administration of **12** (50, 66, and 100 μmol/kg), **15**−**18**, **23** (33, 50, and 66 μmol/kg), **19**, **20** (21, 33, and 50 μmol/kg), **21** (3.3, 6.6, 10, and 21 μmol/kg), and **22** (21 and 33 μmol/kg).

The structure−activity relationships in this series were examined by three types of structural changes: (i) insertion of an amino group in different positions of the phenyl ring at C-4; (ii) the introduction of a (cyclo)-alkylcarbamoyl group at *N*-2; (iii) the conversion of the lactam group into its bioisosteric thiolactam moiety.

All new compounds possess remarkable anticonvulsant activity which, with the exception of derivatives **12**, **14**, and **18**, is higher than that displayed by **1**. The unsubstituted 4-phenyl derivative **12**, similarly to **3a**, is less active than **1**. The introduction of a *p*-aminophenyl group at C-4 produced an enhancement in the anticonvulsant activity in analogy to the trend observed in the benzodiazepine series. For example, compound **16** is more active than **12** (ED_{50} 21.2 μmol/kg for **16** versus ED_{50} 36.7 μmol/kg for **12**). The introduction of a nitro group on the aromatic group (**14**) is detrimental to the activity (ED_{50} >100 μmol/kg). When an alkylcarbamoyl group was appended at N-2 of **16**, the anticonvulsant activity increased and reached its maximum value with a *n*-butyl side chain (**21**). Compound **21** was 11-fold more potent than **1** with an ED_{50} value of 3.25 μmol/kg. It is particularly interesting to note that the *n*-butylcarbamoyl group afforded the most potent derivative both in this series and in the corresponding 1,2-dihydrophthalazines (i.e., **4**)[11] whereas a different trend was observed in the series of 3-(*N*-alkylcarbamoyl)-2,3-benzodiazepines.[8c,15] The same modification at *N*-2, carried out on the corresponding 3-aminophenyl derivative **15**, did not produce any increase in activity (compound **20**, Table 1). Replacement of the carbonyl group with a thiocarbonyl moiety once again yielded an increase in potency (e.g., ED_{50} 15.2 μmol/kg for **25** versus ED_{50} 21.2 μmol/kg for **16**). This result matches that previously reported for related compounds.[8c] A possible explanation could be that compound **25** has a better ip absorption and a more favorable diffusion across the blood-brain barrier (BBB). In fact, compounds **24** and **25** were found to have a higher lipophilicity (expressed as R_m value) than parent derivatives **12** and **16** (e.g., $R_m = -0.205$ for **25** versus $R_m = -0.454$ for **16**; Table 1). Similarly, the higher anticonvulsant activity of **21**, when compared to **16**, can be explained by an increased BBB penetration of **21** due to its higher lipophilicity (Table 1). It is worth pointing out that R_m values, determined experimentally by reversed-phase high-performance thin-layer chromatography (RP-HPTLC), correlate linearly (r^2 of 0.86 at 95% of confidence limits) with log *P* values of compounds **3a**−**c**, **12**, **15**−**25**, calculated with the Pallas program (Table 1). The sole exception is represented by GYKI 52466 (**1**) where the calculated log *P* is significantly higher than that predicted by the correlation. However, lipophilicity is not the only parameter that affects the potency of these compounds. For example, compound **21** is much more active than **20** despite their similar values of R_m and log *P*.

The anticonvulsant activity of compounds **12** and **15**−**25** was effective at doses which did not cause sedation and ataxia, in agreement with other series of noncompetitive AMPA antagonists.[3b,c,7,8] It is noteworthy that compound **21** possesses protective index (PI) 3-fold higher than that of **1** (Table 1).

Due to their potent anticonvulsant activity in the audiogenic seizure model, derivative **21** and its N-2 unsubstituted analogue **16** were further investigated, and the results were compared to compound **1**.

The time course of the anticonvulsant activity of compounds **16** and **21** showed the peak effect at 30 min and a return to the control seizure response at 120 min from administration whereas compound **1** displayed its

Table 1. Anticonvulsant Activity of Compounds **1**, **3a**−**c**, **12,** and **14**−**25** 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	−0.304	3.150
3a	43.3 (34.4−54.6)	40.6 (30.1−54.9)	159 (82.6−306)	3.7	−0.142	2.600
3b	18.0 (10.0−32.5)	12.7 (6.13−26.2)	101 (52.0−194)	5.6	−0.372	1.700
3c	15.4 (10.1−23.5)	10.9 (4.60−24.6)	99.1 (72.4−135)	4.5	−0.430	1.700
12	36.7 (24.4−55.1)	31.3 (19.4−50.6)	112 (74.8−169)	3.1	−0.158	2.860
14	>100	>100				
15	23.3 (15.4−35.3)	15.7 (8.98−27.3)	72.4 (59.5−88.2)	3.1	−0.399	1.960
16	21.2 (9.04−49.8)	7.56 (2.47−23.1)	60.1 (44.3−81.6)	2.8	−0.454	1.960
17	23.4 (15.5−35.4)	16.8 (12.0−23.6)	59.4 (45.2−78.2)	2.5	−0.364	1.560
18	42.6 (25.6−70.9)	35.1 (20.2−60.9)	91.1 (72.8−114)	2.1	−0.235	1.970
19	15.8 (8.53−29.3)	6.82 (4.02−11.6)	52.8 (31.6−88.2)	3.3	−0.126	2.480
20	31.6 (18.1−55.3)	22.4 (11.5−43.6)	101 (76.1−134)	3.2	0.010	2.990
21	3.25 (1.61−6.56)	2.23 (1.32−3.79)	20.4 (13.3−31.0)	6.3	0.002	2.990
22	13.2 (6.42−27.3)	5.34 (2.43−11.7)	56.6 (39.3−81.6)	4.3	0.101	3.500
23	28.4 (18.0−45.0)	26.2 (17.8−38.6)	63.8 (45.4−89.7)	2.2	0.108	4.000
24	27.8 (22.0−35.1)	22.5 (16.1−31.5)	89.0 (59.6−132)	3.2	0.050	3.030
25	15.2 (6.52−35.4)	8.94 (4.02−19.9)	54.7 (36.1−82.9)	3.6	−0.205	2.130

[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 PALLAS 2.0 program.

Table 2. ED_{50} Values at Various Times Following ip Administration of Compounds **1**, **16**, and **21**

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
16	32.7 (25.1−42.6)	21.2* (9.04−49.8)	23.8* (13.8−41.2)	29.7 (18.8−47.0)	43.6 (30.5−62.3)	>50
21	13.9 (7.93−24.5)	3.25** (1.61−6.56)	8.63** (4.14−18.0)	11.2** (6.27−20.1)	26.6** (15.6−45.2)	44.4 (25.5−77.3)

[a] Significant differences among compounds **1** and **16** or **21** 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**, **16**, and **21** against MES- and PTZ-Induced Seizures in Swiss Mice

compd	ED_{50}, μmol/kg,[a] (±95% confidence limits) MES tonus	PTZ clonus
1	35.7 (29.3−43.4)	68.3 (56.2−83.1)
16	41.4 (29.9−57.3)	49.4 (30.9−78.8)
21	33.1 (25.9−42.3)	41.9 (31.3−55.9)

[a] All data were calculated according to the method of Litchfield and Wilcoxon.[29] At least 32 animals administered ip were used to calculate each ED_{50} value.

maximum protection at 15 min from ip administration and its effect disappeared at 90 min (Table 2).

As shown in Table 3, the tonic extension and the clonic phase of the seizures induced by MES and PTZ, respectively, were significantly reduced at 45 min after ip administration of compounds **16** and **21**. These two compounds are nearly as potent as GYKI 52466 in both tests.

To investigate the relationship between the anticonvulsant activity of **16** and **21** and their activity in non-NMDA receptors, additional tests were performed (Table 4). Compounds **16** and **21** produced a dose-dependent protection against AMPA-, ATPA-, and KA-induced seizures. 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 influence of aniracetam, a potentiator of the AMPA receptors,[13] on the anticonvulsant activity of derivatives **16** and **21** in DBA/2 mice was also tested. An icv injection of aniracetam (50 nmol/mouse) on its own had no convulsant activity; nevertheless, the administration of aniracetam 60 min before the injection of the tested compounds reversed their anticonvulsant effects and shifted the dose−response curves to the right with a pattern of activity similar to that of **1**.

The activity of **16**, **20**, and **21** on KA-evoked currents was assessed using the patch-clamp technique in cerebellar granule neurons grown in primary cultures. At variance of AMPA-response which is fast desensitizing,[6a,16] KA elicits an inward nondesensitizing current that is mediated by the activation of both AMPA and KA receptors. KA-evoked current is marginally affected by compound **20** (100 μM) and is significantly reduced by the application of **16** or **21** (100 μM) and to a bigger extent by **1** and **2** (100 μM) (Figure 1). The degree of block of the peak currents produced by **16** and **21**, expressed as the percent of reduction of the KA currents (100%), is dose-related and is compared to that elicited by 100 μM of **1** (−47 ± 4%, $n = 6$) and **2** (−80 ± 5%, $n = 11$) (Figure 2); the extent of the current reduction at 100 μM was higher for **16** (−32 ± 4%, $n = 19$) than for **21** (−24 ± 3%, $n = 19$). On the basis of the structural similarity of **16** and **21** with both 2,3-benzodiazepin-4-ones **3c** and **3e**[8c] and 1,2-dihydrophthalazine **4**,[11] it is reasonable to assess that the reduction of the current is due to the block of the AMPA-mediated component.

We also tested the modulation induced by **16**, **21**, and **1** on the current produced by the application of ATPA (100 μM), a selective agonist of GluR5, a subtype of KA

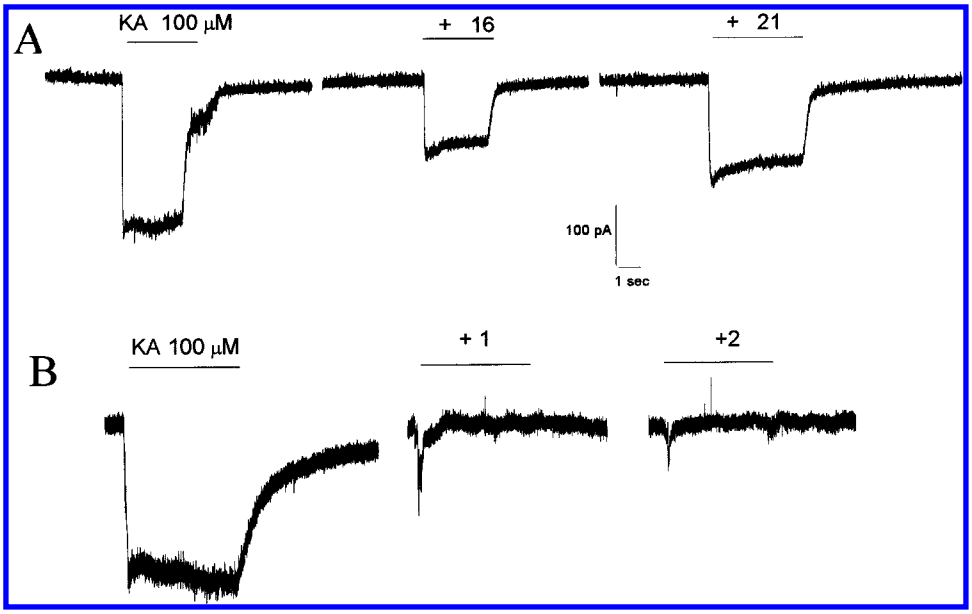


Figure 1. Representative traces of a patch-clamp experiment showing the inward current evoked by the application of 100 μM Ka and its reduction in the presence of compounds **16** or **21** (A**)**, and **1** or **2** (B). Horizontal bars show the duration of drug application. The middle and right traces refer to the coapplication of KA and **16** or **21** (A**)**, and **1** or **2** (B). All tested compounds were applied at 100 μM. After washout of compounds **1**, **2**, **16**, and **21**, the KA responses came back to the control level. The cell was voltage-clamped at −60 mV.

Table 4. ED_{50} Values of **1**, **16**, and **21** against AMPA-, ATPA-, and KA-Induced Seizures and against Audiogenic Seizures after Pretreatment with Aniracetam in DBA/2 Mice

	ED_{50}, μmol/kg,[a] (±95% confidence limits)						
	AMPA[b]		ATPA[c]			pretreatment with aniracetam[e]	
compd	tonus	clonus	clonus	tonus	KA[d]	clonus	tonus
1	57.5 (43.5−76.0)	40.5 (26.3−60.8)	63.8 (45.4−89.7)	43.1 (32.4−57.3)	27.8 (18.8−40.9)	134* (88.8−203)	100* (63.4−158)
16	45.2 (32.6−62.7)	35.8 (27.1−47.3)	38.6 (25.8−57.7)	31.9 (22.7−44.8)	38.9 (30.3−50.1)	56.9* (40.9−79.2)	32.0* (21.9−46.8)
21	47.4 (35.1−64.2)	37.0 (35.1−64.2)	36.2 (29.4−44.6)	30.1 (22.1−41.0)	36.2 (26.3−50.0)	17.6** (12.1−25.6)	12.3** (8.11−18.5)

[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] ATPA was administered icv at the CD_{97} for either clonus (1 nmol) or forelimb tonic extension (7 nmol) 30 min after ip injection of tested compounds. [d] KA was administered sc at the CD_{97} (32 mg/kg) 15 min after ip injection of tested compounds. [e] Significant differences between ED_{50} values of the group treated with aniracetam + phthalazine and the group treated with phthalazine alone (Table 1) are denoted: $*p < 0.05$ and $**p < 0.01$.

receptors.[17] The ATPA-evoked current was reduced by 100 μM of **16** (−45 ± 3%, $n = 4$), **21** (−30 ± 3%, $n = 4$) and **1** (−45 ± 7%, $n = 5$). It is noteworthy that, conversely to our expectations, compound **1** significantly reduced ATPA responses in a manner similar to derivatives **16** and **21**. This result could be interpreted either as ATPA at 100 μM activates both AMPA and kainate receptors or compound **1** inhibits both AMPA-preferring and kainate receptors.[16b]

To rule out the possibility that the anticonvulsant effect of **16** and **21** could be mediated through $GABA_A$-receptor activation, the two compounds were also tested on GABA-evoked currents. Derivatives **16** and **21** not only were devoid of any positive modulatory activity on GABA-currents but produced a marginal decrease of the current (**16**: −13 ± 7%, $n = 8$, **21**: −8 ± 4, $n = 9$) which could be ascribed to the solvent. When administered by itself, DMSO produced a similar effect (−8 ± 5%, $n = 3$).

Thus the in vitro data suggest that, despite a lower ability of **16** and **21** to reduce the KA-evoked currents with respect to **1** and **2**, the remarkable anticonvulsant activity of **16** and **21** can be accounted for by a blockade of both AMPA and kainate receptors. The higher than expected potency of **21** in vivo may reflect its higher bioavailability.

In conclusion, the novel 6,7-methylenedioxyphthalazin-1(2*H*)-ones reported in this study, analogously to the corresponding 1,2-dihydrophthalazine **4**, possess a marked anticonvulsant activity and exhibit lower toxicity than compound **1**. In particular, derivative **21** shows an in vivo potency 11-fold higher than that of **1**, the reference compound. The marked anticonvulsant effects, observed after a systemic administration of derivative **21** in DBA/2 mice, can be related to its good bioavailability. It is well known that seizures induced by audiogenic stimulations are particularly sensitive to compounds acting as antagonists of glutamate receptors.[3a,b,8c,9,18] In electrophysiological experiments, compounds **16** and **21** were less effective than GYKI 52466 and GYKI 53655 to reduce currents mediated by the activation of AMPA and kainate receptors.

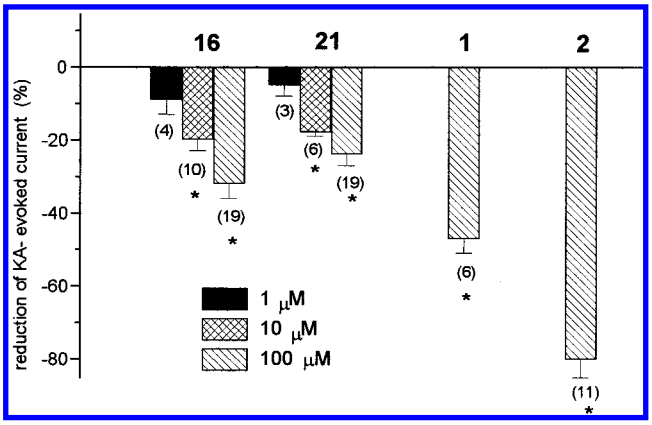


Figure 2. Histogram showing the percent reduction of KA (100 μM)-evoked current after the application of increasing concentrations of **16** and **21** (1−10−100 μM) or of **1** (100 μM) and **2** (100 μM). The numbers in parentheses indicate the number of cells tested. The percent reduction was calculated compared to the control (KA alone). Asterisks indicate significative differences (Student's *t*-test $p < 0.01$) compared to the control.

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 and ^{13}C NMR spectra were recorded in $CDCl_3$ by means of a Varian Gemini-300 spectrometer. Complete ^{1}H and ^{13}C assignments were made by using direct and long-range heteronuclear chemical shift correlation experiments (HETCOR and LR-HETCOR) carried out by using the standard software package. Chemical shifts were 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. Mass spectra of the compounds were recorded under positive electrospray ionization (ESI+) with a ThermoQuest LCQ mass spectrometer. The relative abundance of the ions are reported in brackets.

Synthesis of 2-Benzoyl-4,5-methylenedioxytoluene 6. To a cooled (0−5 °C) and stirred solution of 3,4-methylenedioxytoluene (**5**) (1 mL, 1.13 g, 8.33 mmol) in CH_2Cl_2 (40 mL) was added benzoyl chloride (1.26 mL, 1.52 g, 10.8 mmol) and 16.5 mL of 0.1 M tin(IV) chloride (16.5 mmol) in the same solvent. The ice-bath was removed, and the mixture was stirred at room temperature for 12 h. The resulting mixture was poured into water and extracted with diethyl ether (2 × 100 mL). The combined extracts were washed with water and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the resulting residue was purified by column chromatography using diethyl ether/light petroleum (50/50) as eluant to give red oil (1.92 g, 96%): ^{1}H NMR 2.30 (s, 3H, CH_3), 6.01 (s, 2H, OCH_2O), 6.77 and 6.83 (2s, 2H, H-3 and H-6), 7.44−7.80 (m, 5H, Ar). Anal. ($C_{15}H_{12}O_3$) C, H.

General Procedure for the Synthesis of 4,5-Methylenedioxy-2-(3- or 4-nitrobenzoyl)toluene 7−8. To a stirred solution of 3,4-methylenedioxytoluene (**5**) (1 mL, 1.13 g, 8.33 mmol) in 1,2-dichloroethane (100 mL) were added 3- or 4-nitrobenzoic acid (1.81 g, 10.83 mmol) and phosphorus pentoxide (5.7 g, 40 mmol), and the mixture was stirred for 16 h. The resulting mixture was neutralized with 10% NaOH and extracted with chloroform (2 × 100 mL). The combined extracts were washed with water and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the resulting residue was purified by column chromatography using diethyl ether/light petroleum (60:40) as eluant.

4,5-Methylenedioxy-2-(3-nitrobenzoyl)toluene (7): mp 98−100 °C (2.02 g, 85%); ^{1}H NMR 2.35 (s, 3H, CH_3), 6.04 (s, 2H, OCH_2O), 6.80 and 6.81 (2s, 2H, H-3 and H-6), 7.68 (t, 1H, J = 8.1, H-5′), 8.13 (dd, 1H, J = 1.9 and 8.1, H-6′), 8.42 (dd, 1H, J = 1.9 and 8.1, H-4′), 8.57 (t, 1H, J = 1.9, H-2′). Anal. ($C_{15}H_{11}NO_5$) C, H, N.

4,5-Methylenedioxy-2-(4-nitrobenzoyl)toluene (8): mp 121−124 °C (2.14 g, 90%); ^{1}H NMR 2.36 (s, 3H, CH_3), 6.04 (s, 2H, OCH_2O), 6.80 and 6.81 (2s, 2H, H-3 and H-6), 7.92 (d, 2H, J = 8.9, H-2′,6′), 8.31 (d, 2H, J = 8.9, H-3′,5′). Anal. ($C_{15}H_{11}NO_5$) C, H, N.

General Procedure for the Synthesis of 4-Aryl-6,7-methylenedioxyphthalazin-1(2*H*)-one 12−14. A solution of **6**−**8** (7.0 mmol) in CCl_4 (80 mL) was refluxed for 8 h and irradiated with a 250 W high-pressure Hg lamp while a solution of bromine (0.67 g, 8.4 mmol) in CCl_4 (8 mL) was added. Alternatively, a solution of **6**−**8** was heated under reflux for 2 h with NBS (7.0 mmol) and trace amounts of AIBN. Evaporation of the solvent gave a red oil whose ^{1}H NMR spectrum (CCl_4) showed signals at 4.61−4.70 ppm attributable to the methylene protons of the benzyl bromide, in addition to the methyl singlet of the starting compounds appearing at 2.30−2.36 ppm. The reaction mixture, whose ratio of the benzyl bromide and the starting material determined by ^{1}H NMR spectra ranged from 7.3 to 8.2, was used in the next step without any further purification, since all attempts to isolate the benzyl bromide afforded unidentified degradation products. Thus, a solution of the brominated mixture in ethanol (100 mL) was treated with hydrazine hydrate (1.09 mL, 22.4 mmol) and refluxed for 16 h. The solvent was removed under reduced pressure, and the residue was purified by treatment with light petroleum and recrystallized from acetone.

6,7-Methylenedioxy-4-phenylphthalazin-1(2*H*)-one (12): mp >300 °C (1.09 g, 59%); ^{1}H NMR 6.16 (s, 2H, OCH_2O), 7.06 (s, 1H, H-5), 7.85 (s, 1H, H-8), 7.53 (m, 5H, Ar), 9.98 (bs, 1H, NH); ^{13}C NMR 103.93 (OCH_2O), 104.67 (C-5), 105.03 (C-8), 125.62 (C-8a), 126.98 (C-4a), 129.57 (C-3′,5′), 129.89 (C-4′), 130.16 (C-2′,6′), 136.28 (C-1′), 146.74 (C-4), 151.77 (C-7), 153.07 (C-6), 159.76 (C-1); MS (ESI+) 267 (M^++1, 36), 251 (100). Anal. ($C_{15}H_{10}N_2O_3$) C, H, N.

6,7-Methylenedioxy-4-(3-nitrophenyl)-phthalazin-1(2*H*)-one (13): mp >300 °C (1.14 g, 52%); ^{1}H NMR ($CDCl_3$) 6.2 (s, 2H, OCH_2O), 6.96 (s, 1H, H-5), 7.73 (t, 1H, J = 8.0, H-5′), 7.85 (s, 1H, H-8), 7.92 (dd, 1H, J = 1.9 and 8.0, H-6′), 8.12 (dd, 1H, J = 1.9 and 8.0, H-4′), 8.45 (t, 1H, J = 1.9, H-2′), 10.3 (bs, 1H, NH); ^{13}C NMR 102.57 (OCH_2O), 104.18 (C-5), 104.92 (C-8), 125.38 (C-2′), 126.40 (C-4′), 129.56 (C-5′), 135.86 (C-6′), 124.34 (C-8a), 126.11 (C-4a), 138.42 (C-1′), 146.01 (C-4), 148.20 (C-3′), 151.02 (C-7), 152.65 (C-6), 158.76 (C-1). Anal. ($C_{15}H_9N_3O_5$) C, H, N.

6,7-Methylenedioxy-4-(4-nitrophenyl)-phthalazin-1(2*H*)-one (14): mp >300 °C (1.21 g, 55%); ^{1}H NMR 6.2 (s, 2H, OCH_2O), 6.96 (s, 1H, H-5), 7.76 (d, 2H, J = 8.7, H-2′,6′), 7.87 (s, 1H, H-8), 8.40 (d, 2H, J = 8.7, H-3′,5′), 9.94 (bs, 1H, NH); ^{13}C NMR 103.11 (OCH_2O), 104.26 (C-5), 106.23 (C-8), 123.93 (C-3′,5′), 125.21 (C-8a), 126.67 (C-4a), 130.67 (C-2′,6′), 142.39 (C-1′), 146.52 (C-4), 149.89 (C-4′), 151.21 (C-7), 152.64 (C-6), 158.84 (C-1). Anal. ($C_{15}H_9N_3O_5$) C, H, N.

General Procedure for the Synthesis of 4-(3- or 4-Aminophenyl)-6,7-methylenedioxyphthalazin-1(2*H*)-one 15, 16. To a solution of **13** or **14** (300 mg, 0.96 mmol) in methanol (25 mL) was added 5% Pd/C (30 mg). The mixture was shaken under hydrogen at atmospheric pressure for 3 h, and the Pd/C was filtered out. The solvent was removed in vacuo, and the resulting residue was purified by column chromatography with EtOAc/MeOH (98:2) as eluant and recrystallized from EtOAc to give **15** or **16**.

4-(3-Aminophenyl)-6,7-methylenedioxyphthalazin-1(2*H*)-one (15): mp >300 °C (236 mg, 87%); ^{1}H NMR 3.88 (bs, 2H, NH_2) 6.17 (s, 2H, OCH_2O), 6.82 (dd, 1H, J = 1.6 and 7.9, H-6′), 6.84 (t, 1H, J = 1.6, H-2′), 6.89 (dd, 1H, J = 1.6 and 7.9, H-4′), 7.11 (s, 1H, H-5), 7.30 (t, 1H, J = 7.9, H-5′), 7.81 (s, 1H, H-8), 10.2 (bs, 1H, NH); ^{13}C NMR 100.27 (OCH_2O), 104.33 (C-5), 104.94 (C-8), 114.98 (C-2′), 115.71 (C-4′), 117.20 (C-6′), 129.75 (C-5′), 124.71 (C-8a), 126.82 (C-4a), 136.56 (C-1′), 147.08

(C-4), 149.59 (C-3′), 151.44 (C-7), 151.53 (C-6), 157.96 (C-1); MS (ESI+) 282 (M^++1, 100), 240 (92). Anal. ($C_{15}H_{11}N_3O_3$) C, H, N.

4-(4-Aminophenyl)-6,7-methylenedioxyphthalazin-1(2*H*)-one (16): mp >300 °C (241 mg, 89%); ^{1}H NMR ($CDCl_3$): 3.88 (bs, 2H, NH_2) 6.16 (s, 2H, OCH_2O), 6.81 (d, 2H, J = 8.2, H-3′,5′), 7.15 (s, 1H, H-5), 7.35 (d, 2H, J = 8.2, H-2′,6′), 7.83 (s, 1H, H-8), 9.82 (bs, 1H, NH); ^{13}C NMR 102.82 (OCH_2O), 103.59 (C-5), 104.38 (C-8), 113.42 (C-3′,5′), 122.31 (C-1′), 124.61 (C-8a), 126.40 (C-4a), 130.00 (C-2′,6′), 146.31 (C-4), 149.39 (C-4′), 150.51 (C-7), 151.88 (C-6), 158.69 (C-1); MS (ESI+) 282 (M^++1, 100). Anal. ($C_{15}H_{11}N_3O_3$) C, H, N.

General Procedure for the Synthesis of 2-(Cyclo)-alkylcarbamoyl-4-(3- or 4-aminophenyl)-6,7-methylenedioxyphthalazin-1(2*H*)-ones 17−23. To a solution of **13** or **14** (0.3 g, 0.96 mmol) in CH_2Cl_2 (60 mL) were added triethylamine (1.2 mL, 8.6 mmol) and the suitable isocyanate (4.8 mmol). The reaction mixture was stirred at room temperature for 36 h, then was neutralized with 0.1 N HCl and washed with water. The organic phase was concentrated in vacuo, and the resulting residue was purified by column chromatography with $CHCl_3$/EtOAc (70:30) as eluant. The subsequent hydrogenation was carried out according to the procedure reported for compounds **15**−**16**. All compounds were recrystallized from EtOAc.

4-(4-Aminophenyl)-2-methylcarbamoyl-6,7-methylenedioxyphthalazin-1(2*H*)-one (17): mp >300 °C (239 mg, 87%); ^{1}H NMR 3.06 (d, 3H, J = 4.8, CH_3), 3.96 (bs, 2H, NH_2) 6.18 (s, 2H, OCH_2O), 6.78 (d, 2H, J = 8.5, H-3′,5′), 7.15 (s, 1H, H-5), 7.40 (d, 2H, J = 8.5, H-2′,6′), 7.85 (s, 1H, H-8), 9.52 (bs, 1H, NH); ^{13}C NMR 26.76 (CH_3), 102.93 (OCH_2O), 104.32 (C-5), 104.93 (C-8), 114.24 (C-3′,5′), 122.57 (C-1′), 125.12 (C-8a), 126.88 (C-4a), 131.85 (C-2′,6′), 147.15 (C-4), 149.35 (C-4′), 151.15 (C-7), 152.35 (C-6), 158.12 (C-1) 163.45 (C=O); MS (ESI+) 339 (M^++1, 68), 282 (100). Anal. ($C_{17}H_{14}N_4O_4$) C, H, N.

4-(4-Aminophenyl)-2-ethylcarbamoyl-6,7-methylenedioxyphthalazin-1(2*H*)-one (18): mp >300 °C (272 mg, 80%); ^{1}H NMR 1.30 (t, 3H, J = 7.2, CH_3), 3.52 (m, 2H, CH_2), 3.90 (bs, 2H, NH_2) 6.18 (s, 2H, OCH_2O), 6.78 (d, 2H, J = 8.3, H-3′,5′), 7.15 (s, 1H, H-5), 7.41 (d, 2H, J = 8.3, H-2′,6′), 7.85 (s, 1H, H-8), 9.60 (bs, 1H, NH); ^{13}C NMR 14.34 (CH_3), 35.37 (CH_2), 103.25 (OCH_2O), 104.29 (C-5), 104.76 (C-8), 113.37 (C-3′,5′), 121.36 (C-1′), 124.56 (C-8a), 126.10 (C-4a), 130.19 (C-2′,6′), 146.52 (C-4), 149.86 (C-4′), 151.13 (C-7), 152.57 (C-6), 157.24 (C-1), 165.50 (C=O); MS (ESI+) 353 (M^++1, 100), 282 (84). Anal. ($C_{18}H_{16}N_4O_4$) C, H, N.

4-(4-Aminophenyl)-6,7-methylenedioxy-2-propylcarbamoylphthalazin-1(2*H*)-one (19): mp >300 °C (268 mg, 76%); ^{1}H NMR 1.02 (t, 3H, J = 7.4, CH_3), 1.70 (m, 2H, CH_2), 3.46 (m, 2H, CH_2), 3.91 (bs, 2H, NH_2) 6.18 (s, 2H, OCH_2O), 6.78 (d, 2H, J = 8.4, H-3′,5′), 7.15 (s, 1H, H-5), 7.41 (d, 2H, J = 8.4, H-2′,6′), 7.85 (s, 1H, H-8), 9.62 (bs, 1H, NH); ^{13}C NMR 11.46 (CH_3), 22.55, 42.40 (CH_2), 102.78 (OCH_2O), 104.18 (C-5), 105.30 (C-8), 118.31 (C-3′,5′), 124.23 (C-1′), 124.37 (C-8a), 126.05 (C-4a), 129.88 (C-2′,6′), 146.43 (C-4), 149.81 (C-4′), 151.25 (C-7), 152.34 (C-6), 158.17 (C-1) 166.12 (C=O); MS (ESI+) 367 (M^++1, 100), 282 (73). Anal. ($C_{19}H_{18}N_4O_4$) C, H, N.

4-(3-Aminophenyl)-2-butylcarbamoyl-6,7-methylenedioxyphthalazin-1(2*H*)-one (20): mp 138−141 °C (250 mg, 68%); ^{1}H NMR 0.97 (t, 3H, J = 7.3, CH_3), 1.46 (m, 2H, CH_2), 1.67 (m, 2H, CH_2), 3.49 (m, 2H, CH_2), 3.88 (bs, 2H, NH_2) 6.18 (s, 2H, OCH_2O), 6.80−7.00 (m, 4H, ArH), 7.13 (s, 1H, H-5), 7.84 (s, 1H, H-8), 9.65 (bs, 1H, NH); ^{13}C NMR 13.76 (CH_3), 20.12, 31.36, 40.98 (CH_2), 102.82 (OCH_2O), 104.92 (C-5), 105.69 (C-8), 115.98 (C-2′), 116.06 (C-4′), 119.61 (C-6′), 129.31 (C-5′), 124.57 (C-8a), 126.50 (C-4a), 135.85 (C-1′), 146.70 (C-4), 149.22 (C-3′), 151.42 (C-7), 151.97 (C-6), 158.26 (C-1), 165.33 (C=O); MS (ESI+) 381 (100), 282 (84). Anal. ($C_{20}H_{20}N_4O_4$) C, H, N.

4-(4-Aminophenyl)-2-butylcarbamoyl-6,7-methylenedioxyphthalazin-1(2*H*)-one (21): mp >300 °C (261 mg, 71%); ^{1}H NMR 0.97 (t, 3H, J = 7.3, CH_3), 1.46 (m, 2H, CH_2), 1.67 (m, 2H, CH_2), 3.49 (m, 2H, CH_2), 3.88 (bs, 2H, NH_2) 6.18 (s, 2H, OCH_2O), 6.78 (d, 2H, J = 7.5, H-3′,5′), 7.16 (s, 1H, H-5), 7.42 (d, 2H, J = 7.5, H-2′,6′), 7.85 (s, 1H, H-8), 9.62 (bs, 1H, NH); ^{13}C NMR 13.77 (CH_3), 20.26, 31.85, 41.21 (CH_2), 103.39 (OCH_2O), 103.64 (C-5), 104.20 (C-8), 117.23 (C-3′,5′), 123.82 (C-1′), 124.63 (C-8a), 126.20 (C-4a), 129.64 (C-2′,6′), 146.35 (C-4), 148.81 (C-4′), 151.36 (C-7), 152.04 (C-6), 158.88 (C-1) 166.33 (C=O); MS (ESI+) 381 (M^++1, 93), 282 (100). Anal. ($C_{20}H_{20}N_4O_4$) C, H, N.

4-(4-Aminophenyl)-6,7-methylenedioxy-2-pentylcarbamoylphthalazin-1(2*H*)-one (22): mp 137−139 °C (255 mg, 67%); ^{1}H NMR 0.93 (t, 3H, J = 7.0, CH_3), 1.31−1.43 (m, 4H, CH_2CH_2), 1.67 (m, 2H, CH_2), 3.48 (m, 2H, CH_2), 3.90 (bs, 2H, NH_2), 6.18 (s, 2H, OCH_2O), 6.78 (d, 2H, J = 8.5, H-3′,5′), 7.16 (s, 1H, H-5), 7.41 (d, 2H, J = 8.5, H-2′,6′), 7.85 (s, 1H, H-8), 9.63 (bs, 1H, NH); ^{13}C NMR 13.97 (CH_3), 22.34, 29.00, 29.08, 41.07 (CH_2), 102.75 (OCH_2O), 105.46 (C-5), 105.75 (C-8), 114.73 (C-3′,5′), 121.47 (C-1′), 124.66 (C-8a), 126.76 (C-4a), 130.72 (C-2′,6′), 147.71 (C-4′), 148.09 (C-4), 151.26 (C-7), 152.51 (C-6), 157.09 (C-1), 161.26 (C=O); MS (ESI+) 395 (M^++1, 100), 282 (48). Anal. ($C_{21}H_{22}N_4O_4$) C, H, N.

4-(4′-Aminophenyl)-2-cyclohexylcarbamoyl-6,7-methylenedioxyphthalazin-1(2*H*)-one (23): mp 141−144 °C (231 mg, 59%); ^{1}H NMR 0.87−1.44 (m, 10H, CH_2), 3.65 (m, 1H, CH), 3.91 (bs, 2H, NH_2), 6.18 (s, 2H, OCH_2O), 6.78 (d, 2H, J = 7.9, H-3′,5′), 7.15 (s, 1H, H-5), 7.41 (d, 2H, J = 7.9, H-2′,6′), 7.85 (s, 1H, H-8), 9.67 (bs, 1H, NH); ^{13}C NMR 24.90, 25.57, 33.90 (CH_2-cyclohexyl), 49.17 (CH-cyclohexyl), 102.18 (OCH_2O), 104.88 (C-5), 105.77 (C-8), 114.38 (C-3′,5′), 122.10 (C-1′), 124.36 (C-8a), 126.18 (C-4a), 130.42 (C-2′,6′), 147.32 (C-4′), 148.31 (C-4), 151.15 (C-7), 152.67 (C-6), 157.34 (C-1), 161.30 (C=O). Anal. ($C_{22}H_{22}N_4O_4$) C, H, N.

General Procedure for the Synthesis of 4-Aryl-6,7-methylenedioxyphthalazine-1(2*H*)-thiones 24, 25. A solution of **12** or **16** (1 mmol) and Lawesson's reagent (0.22 g, 0.55 mmol) in dry toluene (50 mL) was heated to reflux for 2 h. The solution was then cooled at room temperature and filtered. The toluene was removed in vacuo and the residue was crystallized from EtOH.

6,7-Methylenedioxy-1-phenylphthalazine-1(2*H*)-thione (24): mp >300 °C (270 mg, 85%); ^{1}H NMR 6.20 (s, 2H, OCH_2O), 7.10 (s, 1H, H-5), 7.55 (m, 5H, ArH), 8.40 (s, 1H, H-8), 11.56 (bs, 1H, NH); ^{13}C NMR 102.68 (OCH_2O), 104.13 (C-5), 104.63 (C-8), 124.88 (C-8a), 126.37 (C-4a), 128.83 (C-3′,5′), 129.24 (C-2′,6′), 129.71 (C-4′), 134.33 (C-1′), 146.46 (C-4), 151.65 (C-7), 152.02 (C-6), 197.28 (C-1); MS (ESI+) 283 (M^++1, 0), 251 (100). Anal. ($C_{15}H_{10}N_2O_2S$) C, H, N.

4-(4-Aminophenyl)-6,7-methylenedioxyphthalazine-1(2*H*)-thione (25): mp >300 °C (260 mg, 82%); ^{1}H NMR 3.88 (bs, 2H, NH_2), 6.18 (s, 2H, OCH_2O), 6.81 (d, 2H, J = 8.2, H-3′,5′), 7.14 (s, 1H, H-5), 7.35 (d, 2H, J = 8.2, H-2′,6′), 8.40 (s, 1H, H-8), 11.56 (bs, 1H, NH); ^{13}C NMR 101.57 (OCH_2O), 103.05 (C-5), 104.08 (C-8), 113.12 (C-3′,5′), 122.58 (C-1′), 124.31 (C-8a), 126.12 (C-4a), 129.84 (C-2′,6′), 146.12 (C-4), 148.16 (C-4′), 150.92 (C-7), 152.15 (C-6), 199.13 (C-1); MS (ESI+) 298 (M^++1, 0), 266 (100). Anal. ($C_{15}H_{11}N_3O_2S$) 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.[19] 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 2:1 (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 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. The log P values reported in Table 1 were calculated by using the PALLAS 2.0 program (Compudrug Chemistry Ltd.). Calculated log P and experimental R_m values are linearly correlated with an r^2 value of 0.86 at 95% confidence limits.

Testing of Anticonvulsant Activity against Audio-

genic Seizures in DBA/2 Mice. All experiments were performed with DBA/2 mice which are genetically susceptible to sound-induced seizures.[20] 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% dimethyl sulfoxide (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 period. 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.[21] The time course of the anticonvulsant action of **16** and **21** was determined following the administration of 33 μmol/kg of each phthalazine derivative to groups of 10 mice for each time. The animals were tested for sound-induced seizure responses at 5−120 min after drug administration.

Maximal Electroshock 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.[22] 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 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 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.[23]

AMPA-Induced Seizures in DBA/2 Mice. The CD_{50} (±95% confidence limits) of AMPA icv microinjected was 1.76 (1.06−3.07) nmol for clonus and 2.90 (1.83−4.58) nmol for tonus. 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;[24] 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^3 box, and the observation time was 30 min after the administration of AMPA.

ATPA-Induced Seizures in DBA/2 Mice. The CD_{97} of ATPA for clonus was 1.00 nmol, while that for tonus was 7.00 nmol. The icv microinjection of ATPA was performed according with experimental procedures previously described for AMPA microinjection.[3c] The observation time was 30 min after the administration of ATPA.

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 phthalazine derivative. Animals showing 5 s or more of clonic activity were scored as not protected according to Donevan et al.[3c] The period of observation was 60 min.

Pretreatment with Aniracetam. The icv microinjection of aniracetam was performed according with experimental procedures previously described for AMPA microinjection.[3c] The dose of aniracetam (50 nmol icv) was administered 60 min before auditory stimulation or 30 min before each compound in DBA/2 mice.

Electrophysiology. Primary cultures of cerebellar granule neurons were prepared from 7 to 8 days old Sprague−Dawley rats as previously described.[25] Briefly, 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). 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[25] using the voltage-clamp technique in the whole-cell configuration.[26] 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. 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. KA was purchased from Sigma, GYKI 52466 (**1**) was from RBI, and GYKI 53655 (**2**) was a gift from Lilly Research Lab. Compounds **1**, **2**, **16**, and **21** were dissolved in DMSO and diluted at the final concentration (<1%) in extracellular solution. KA was also dissolved in the extracellular solution. All tested compounds were applied at 100 μM. After a washout of compounds **1**, **2**, **16**, and **21** the responses to KA returned to the control level. All drugs were applied directly by gravity through a Y-tube perfusion system.[27]

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 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.[28] The ability of the mice to remain on the rotarod was tested 30 min after administration of various compounds.

Statistical Analysis. Statistical comparisons between groups of control and drug-treated animals were made using Fisher's exact probability test (incidence of the seizure phases). The ED_{50} values of each phase of the audiogenic seizure or seizures induced by MES, PTZ, AMPA, ATPA, or KA were determined for each dose of 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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