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Design of 1-substituted 2-arylmethyl-4,5-methylenedioxybenzene derivatives as antiseizure agents

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Abstract—A series of 1-substituted 2-[(4-aryl)-methyl]-4,5-methylenedioxybenzene derivatives (**13**–**25**), structurally related to model compound **5** (2-[(4-aminophenyl)-(4-methylsemicarbazono)-methyl]-4,5-methylenedioxyphenylacetic acid methyl ester), were synthesized and tested as anticonvulsant agents in DBA/2 mice against sound-induced seizures. The new compounds possess anticonvulsant properties lower than those of prototype **5** but, in some instances, comparable to that of GYKI 52466, a well-known noncompetitive AMPA receptor antagonist.

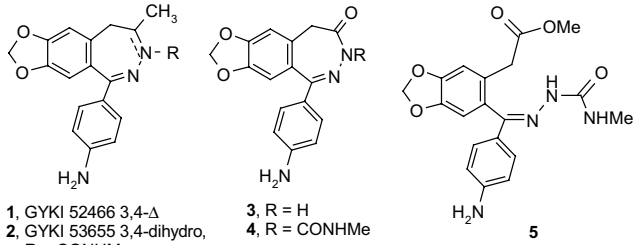
1. Introduction

Epilepsies are common neurological disorders, affecting approximately 2.5 million people solely in the United States. Epileptic seizures often cause transient impairment of consciousness, leaving the individual at risk of bodily harm. Therapy is symptomatic in that available drugs inhibit seizures, but they neither affect prophylaxis nor cure the cause. Compliance with medication is a major problem, due to the need of a long-term therapy, which is quite often associated with unwanted side effects.[1]

Drugs effective against the most common forms of epileptic seizures, appear to work by limiting the sustained repetitive firing of a neuron by one of the following mechanisms: (i) enhancement of GABA-mediated synaptic inhibition, (ii) stabilization of the inactivated state of voltage-operated Na^+ channels, (iii) inactivation of voltage-activated Ca^{2+} channels. Considerable interest has lately been focused on antagonists of the ionotropic glutamate receptors (NMDA, AMPA, and KA) having significant anticonvulsant activity thus providing the basis for an extensive research in this area. As a matter of fact, a number of 2,3-benzodiazepine derivatives, that is GYKI 52466 (**1**) and GYKI 53655 (**2**) (Fig. 1), acting as noncompetitive antagonists of the AMPA receptors,[2] displayed potent anticonvulsant properties[3,4] and behaved as neuroprotective agents in focal and global ischemia.[5]

As part of a program aimed at identifying potent and selective AMPA receptor antagonists, we have previously reported[6,7] an investigation on the anticonvulsant activity of a series of 1-aryl-3,5-dihydro-7,8-methylenedioxy-4*H*-2,3-benzodiazepin-4-ones, for example, **3**, and their 3-*N*-alkylcarbamoyl derivatives, for example, **4** (Fig. 1), which are structural analogues of **1** and **2**, respectively.

Compounds **3**–**4** proved to be roughly 2–3-fold more potent than GYKI 52466 and were provided with a



1, GYKI 52466 3,4-Δ
2, GYKI 53655 3,4-dihydro, R = CONHMe
3, R = H
4, R = CONHMe
5

Figure 1.

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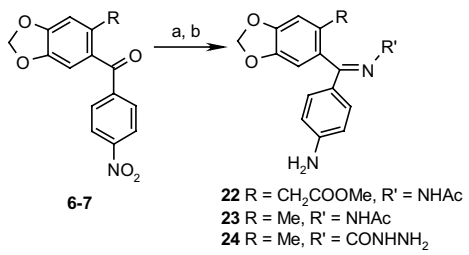
better protective index. Furthermore, analogously to **1** and **2**, they displayed anticonvulsant activity mainly by blocking the allosteric AMPA-receptor binding site.[8] In view of these results, we subsequently designed and tested[9] a series of alkyl esters of 4,5-methylenedioxyphenylacetic acid bearing an *N*-alkyl-semicarbazono moiety at position 2, which may be envisaged as 'open models' of reference compound **4**. Within this series of derivatives, 2-[(4-aminophenyl)-(4-methylsemicarbazono)-methyl]-4,5-methylenedioxyphenylacetic acid methyl ester **5** proved to be the most active compound. It displayed an anticonvulsant activity slightly higher than that of its parent **4** and 5-fold higher than that shown by reference compound **1**. It is worth pointing out that, despite a close structural similarity with **4**, derivative **5** possesses significant differences in the pharmacological profile. Compound **5** does not bind or only marginally interacts with either the GYKI 52466- or the AMPA-receptor binding sites. Furthermore, no interaction with either the glycine-binding site of the NMDA receptors or the inhibitory $GABA_A$-receptor complex was noticed.[8]

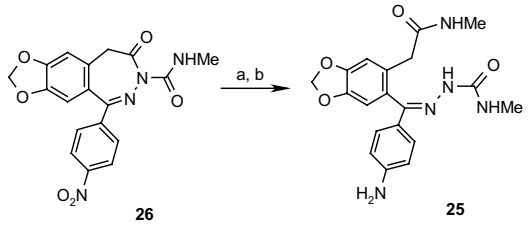
Since the semicarbazono moiety is an important structural feature of a number of anticonvulsant agents,[10] we designed the series of derivatives **13**–**25** as analogues of the lead compound **5**. The objective of this study was to investigate whether the ester group and the 4-aminophenyl moiety were involved in determining the anticonvulsant potency of the compounds. Therefore, the methyl ester functionality of **5** was converted into the bioisosteric amide, that is **25**, or removed, that is **21**. The importance of the 4-aminophenyl group was evaluated by replacing it with a 4-halophenyl moiety, that is **18**–**20**. Furthermore, in order to test the influence of the methylsemicarbazono group on the anticonvulsant activity, we converted such a moiety into its bioisosteric thio-analogue, that is **13**–**17**, inverted it, that is **24**, and transformed it into the acetylhydrazono moiety, that is **22**–**23**.

2. Chemistry

The synthesis of target compounds **13**–**25** is outlined in Schemes 1–3. Compounds **6**–**10**, used as starting material,



Scheme 2. (a) $AcNHNH_2$ or $NH_2NHCOOMe$, MeOH/HCl cat., reflux, 48 h; (b) Raney-Ni, ammonium formate, EtOH, reflux, 2 h.

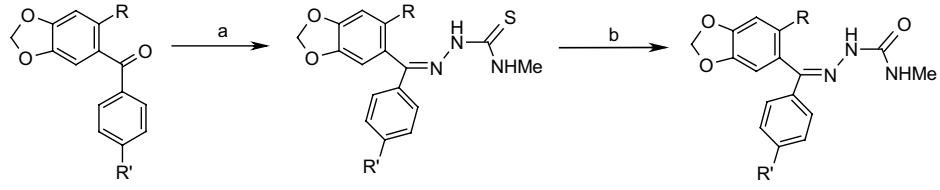


Scheme 3. (a) $MeNH_2$, THF, reflux, 1 h; (b) Raney-Ni, ammonium formate, EtOH, reflux, 2 h.

were prepared via a Friedel–Crafts acylation according to a previously reported methodology.[11] Derivatives **11**–**15** were prepared in good yields by reacting **6**–**10** with 4-methyl-3-thiosemicarbazide. The 4-nitrophenyl derivatives **11**–**12** were subsequently transformed into the 4-aminophenyl analogues **16**–**17** through a reduction process carried out with Raney-Ni/ammonium formate. On the other hand, thiosemicarbazono derivatives **13**–**16** were easily converted into the corresponding semicarbazono derivatives **18**–**21** by treatment with hydrogen peroxide and sodium methoxide (Scheme 1).

Compound **6** was also treated with acetylhydrazine or methyl hydrazinocarboxylate to give compounds **23** and **24**, respectively,whereas derivative **7** was reacted with acetylhydrazine to yield derivative **22** (Scheme 2).

Finally, with a procedure similar to that employed for compound **5**,[9] derivative **25** was prepared by reacting



	R	R'
6	Me	NO_2
7	CH_2COOMe	NO_2
8	CH_2COOMe	Br
9	CH_2COOMe	Cl
10	CH_2COOMe	F

	R	R'
11	Me	NO_2
12	CH_2COOMe	NO_2
13	CH_2COOMe	Br
14	CH_2COOMe	Cl
15	CH_2COOMe	F
16	Me	NH_2
17	CH_2COOMe	NH_2

	R	R'
18	CH_2COOMe	Br
19	CH_2COOMe	Cl
20	CH_2COOMe	F
21	Me	NH_2

Scheme 1. (a) $MeNHCSNHNH_2$, MeOH, HCl cat., reflux, 48 h; (b) H_2O_2, MeONa, MeOH, rt, 20′; (c) Raney-Ni, ammonium formate, EtOH, reflux, 2 h.

3,5-dihydro-3-*N*-methylcarbamoyl-7,8-methylenedioxy-1-(4-nitrophenyl)-4*H*-2,3-benzodiazepin-4-one (**26**) with methylamine and subsequently reducing its nitro group (Scheme 3).

The structure and stereochemistry of new derivatives **11**–**25**, always obtained as as single isomer, rely on NMR data. The stereochemistry around the C=N bond of derivative **5** was previously investigated using a combination of modeling studies and ^{1}H NMR spectroscopy.[9] Molecular modeling studies showed that the rotation around the single bond connecting the semicarbazono moiety to C-2 is hindered, making derivative **5** a chiral molecule in the NMR time scale.

As a matter of fact, the geminal hydrogens of the two methylene groups of derivative **5**, that is the one in the α-position with respect to the ester function and the other of the dioxole ring, are diastereotopic and resonate as AB systems or multiplets. The stereochemistry around the C=N bond was assigned as *Z*. These spectroscopic features were always found in derivatives **11**–**25**. Therefore, by analogy with derivative **5**, we assigned the *Z* configuration to all new derivatives.

3. Results and discussion

The anticonvulsant activity of derivatives **13**–**25** against audiogenic seizures was evaluated 30 min after intraperitoneal administration to 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 new anticonvulsant drugs.[12] The results are compared with those previously reported for **5** and reference compound **1** (Table 1).[9]

As shown in Table 1, the new compounds possess anticonvulsant properties lower than those of prototype **5** but, in some instances, comparable to those of GYKI 52466.

In particular, with respect to compound **5**, the activity is significantly reduced by replacing the semicarbazono moiety with the corresponding thiosemicarbazono one (**17**) or the acetylhydrazono group (**22**). Noteworthy, at variance with derivative **5**, the anticonvulsant activity of compound **17** is enhanced if the audiogenic seizure test is carried out 15 min rather than 30 min after ip administration (see footnotes in Table 1), indicating that such a bioisosteric modification produces a short-time activity. A conceivable explanation could be that compound **17** is more easily diffused across the blood–brain barrier than its analogue **5**.

The replacement of the 4-aminophenyl group with a 4-halophenyl moiety (**18**–**20**) also negatively influences activity. However, contrarily to the trend observed in compound **17**, thioderivatives **13**–**15** are more active than their carbonyl analogues **18**–**20**.

Removal of the ester functionality (**21**) or its transformation into an amide (**25**) destroys or reduces the anticonvulsant activity (ED_{50} >100 μmol/kg for **21** and 31.8 μmol/kg for **25** vs 7.87 μmol/kg for **5**) except for acetylhydrazono derivative **23**, which is slightly more potent than its ester counterpart **22**. It is worth pointing out that the anticonvulsant activity of the compounds has been evaluated on a in vivo assay and, consequently, the observed variations in potency could simply be due to differences in the pharmacokinetic parameters, such as metabolism or diffusion across blood–brain barrier.

The anticonvulsant activity of the tested compounds was effective at doses, which did not cause sedation and

Table 1. Anticonvulsant activity of compounds **1**, **5**, and **13**–**25** against audiogenic seizures in DBA/2 mice and TD_{50} values on locomotion assessed by rotarod test[a]

Compds	ED_{50} (μmol/kg)		TD_{50} (μmol/kg) locomotor deficit	PI,[b] TD_{50}/ED_{50}
	Clonic phase	Tonic phase		
1	35.8 (24.4–52.4)	25.3 (16.0–40.0)	76.1 (47.5–122)	2.1
5[c]	7.87 (4.68–13.2)	4.62 (2.47-8.61)	28.3 (19.0–42.3)	3.6
13	37.8 (17.6–81.3)	26.2 (16.3–42.2)	121 (82.6–163)	3.2
14	24.7 (11.0–55.3)	19.6 (10.6–36.0)	69.2 (49.4–96.9)	2.8
15	36.5 (11.8–112)	20.5 (11.4–37.1)	113 (82–156)	3.1
16	42.0 (30.2–58.3)	29.1 (16.0–53.1)	110 (113–153)	2.8
17[d]	39.4 (26.5–58.8)	24.7 (14.1–43.3)	113 (82.5–156)	2.7
18	43.5 (22.1–85.5)	36.0 (19.9–64.9)	117 (85.6–151)	2.7
19	73.0 (28.6–186)	44.7 (21.2–94.5)	168 (135–208)	2.3
20	53.6 (24.2–117)	37.8 (17.6–81.3)	134 (110–163	2.5
21	>100	>100	197 (162–239)	ND
22	55.1 (21.9–138)	17.1 (7.02–42.4)	138 (111–171)	2.5
23	45.7 (18.9–110)	16.9 (6.94–41.5)	119 (84.6–167)	2.6
24	>100	>100	204 (164–254)	ND
25	31.8 (16.8–60.2)	27.2 (10.5–69.9)	57.3 (34.2–96.1)	1.8

[a] All compounds were given ip (at doses spanning the range 3.3–200 μmol/kg) 30 min before auditory stimulation. All data were calculated according to the method of Litchfield and Wilcoxon[19] 95% confidence limits are given in parentheses. At least 32 animals 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). ND, not detectable.

[c] ED_{50} for clonus 24.1 (13.6–42.6) μmol/kg at 15 min after ip administration.[9]

[d] ED_{50} for clonus 24.7 (15.3–48.2) μmol/kg at 15 min after ip administration.

Table 2. Reduction of KA-evoked currents induced by **1**, **5**, **16**–**17**, and **21**–**24**[a]

Compds	% Reduction[b]	Compds	% Reduction[b]
1	47 ± 4 ($n = 5$)	**21**	25 ± 3 ($n = 7$)
5	60 ± 1 ($n = 5$)	**22**	55 ± 4 ($n = 16$)
16	47 ± 5 ($n = 5$)	**23**	28 ± 4 ($n = 3$)
17	30 ± 5 ($n = 44$)	**24**	32 ± 5 ($n = 8$)

[a] All drugs were tested at 100 μM.
[b] Each value is the mean ± SE. The number of cells is given in parentheses.

ataxia. It is noteworthy that several derivatives possess a protective index (PI) higher than that of **1** (Table 1).

Recent binding studies[8] indicate that the lead compound **5** does not interact with neither the GYKI 52466- nor the AMPA-receptor binding site whereas electrophysiological experiments demonstrated that compound **5** was able to reduce KA-evoked currents.[9] Consequently, the ability of derivatives **16**, **17**, and **21**–**24** to affect KA-evoked currents was assessed in cerebellar granule neurons grown in primary cultures by using the patch clamp technique. As shown in Table 2, KA-evoked currents are marginally affected by the application of compounds **17**, **21**, **23**, and **24** (100 μM) and reduced by the application of compounds **16** and**22**. However, the high concentration (100 μM) needed to detect, for compounds **16** and **22**, a sizeable reduction in the KA-evoked currents does not allow to relate their anticonvulsant activity to the involvement of KA receptors.

Further investigations on the mechanism of action of these compounds have been undertaken and the results will be presented in due course.

To sum up, the results of the present investigation reveal that the methyl ester at position 1 as well as the semicarbazono moiety and the 4-aminophenyl group at position 2 seem to play a crucial role in determining the anticonvulsant activity of the lead compound **5**.

4. Experimental

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. 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. Mass spectra of the compounds were recorded under positive electrospray ionization (ESI+) with a ThermoQuest LCQ mass spectrometer. The synthesis, physical, and analytical properties of compound **22** have been previously described.[13]

4.1. (*Z*)-2-[(4-Methylthiosemicarbazono)-(4-nitrophenyl)-methyl]-4,5-methylenedioxytoluene (11)

4-Methyl-3-thiosemicarbazide (1.4 mmol) and HCl 10 N (0.2 mL) were added to a stirred solution of **6** (0.7 mmol) in MeOH (40 mL). The reaction mixture was refluxed for 48 h, cooled to room temperature to afford a yellow solid that was recrystallized from methanol. Mp 111–114 °C; $R_f = 0.36$ (diethyl ether/petroleum ether, 6:4); yield 75%. ^{1}H NMR 1.99 (s, 3H, CH_3-1), 3.31 (d, 3H, $J = 4.9$ Hz NHCH_3), 6.07 (m, 2H, OCH_2O), 6.52 (s, 1H, H-3), 6.86 (s, 1H, H-6), 7.66 (m, 1H, *NH*CH_3), 7.68 (d, 2H, $J = 8.8$ Hz, H-2′,6′), 8.20 (d, 2H, $J = 4.9$ Hz, H-3′,5′), 8.59 (bs, 1H, NH). MS (ESI+) 373 (M^++1). Anal. Calcd for $C_{17}H_{16}N_4O_4$ S: C, 54.83; H, 4.33; N, 15.04. Found C, 55.06; H, 4.28; N, 14.88.

4.2. (*Z*)-2-[(4-Methylthiosemicarbazono)-(4-nitrophenyl)-methyl]-4,5-methylenedioxyphenylacetic acid methyl ester (12)

Compound **12** was prepared from **7** with a similar procedure. Mp 126–129 °C; $R_f = 0.58$ (cyclohexane/EtOAc, 5:5); yield 85%. ^{1}H NMR 3.26 (m, 2H, CH_2), 3.31 (d, 3H, $J = 4.7$ Hz NHCH_3), 3.55 (s, 3H, OCH_3), 6.12 (m, 2H, OCH_2O), 6.57 (s, 1H, H-3), 6.96 (s, 1H, H-6), 7.68 (d, 2H, $J = 8.5$ Hz, H-2′,6′), 7.69 (m, 1H, *NH*CH_3) 8.20 (d, 2H, $J = 8.5$ Hz, H-3′,5′), 8.63 (bs, 1H, NH). MS (ESI+) 431 (M^+ +1). Anal. Calcd for $C_{19}H_{18}N_4O_6S$: C, 53.02; H, 4.22; N, 13.02. Found: C, 52.87; H, 4.31; N, 13.11.

4.3. (*Z*)-2-[(4-Bromophenyl)-(4-methylthiosemicarbazono)-methyl]-4,5-methylenedioxyphenylacetic acid methyl ester (13)

Derivative **13** was prepared from **8** with a similar procedure. Mp 99–102 °C; $R_f = 0.39$ (cyclohexane/EtOAc, 7:3); yield 78%. ^{1}H NMR 3.27 (m, 2H, CH_2), 3.28 (d, 3H, $J = 4.9$ Hz, NHCH_3), 3.53 (s, 3H, OCH_3), 6.09 (m, 2H, OCH_2O), 6.56 (s, 1H, H-3), 6.93 (s, 1H, H-6), 7.37 (d, 2H, $J = 8.5$ Hz, H-3′,5′), 7.47 (d, 2H, $J = 8.5$ Hz, H-2′,6′), 7.52 (m, 1H, *NH*CH_3), 8.51 (bs, 1H, NH). MS (ESI+) 464 and 466 (M^++1). Anal. Calcd for $C_{19}H_{18}BrN_3O_4S$: C, 49.15; H, 3.91; N, 9.05. Found: C, 48.93; H, 3.96; N, 9.19.

4.4. (*Z*)-2-[(4-Chlorophenyl)-(4-methylthiosemicarbazono)-methyl]-4,5-methylenedioxyphenylacetic acid methyl ester (14)

Derivative **14** was prepared from **9** with a similar procedure. Mp 93–96 °C; $R_f = 0.42$ (cyclohexane/EtOAc, 7:3); yield 80%. ^{1}H NMR 3.26 (m, 2H, CH_2 and d, 3H, $J = 4.7$ Hz, NHCH_3), 3.54 (s, 3H, OCH_3), 6.08 (m, 2H, OCH_2O), 6.55 (s, 1H, H-3), 6.93 (s, 1H, H-6), 7.30 (d, 2H, $J = 8.5$ Hz, H-3′,5′), 7.45 (d, 2H, $J = 8.5$ Hz, H-2′,6′), 7.69 (m, 1H, *NH*CH_3), 8.51 (bs, 1H, NH). MS (ESI+) 420 (M^++1). Anal. Calcd for $C_{19}H_{18}ClN_3O_4$ S: C, 54.35; H, 4.32; N, 10.01. Found: C, 54.51; H, 4.21; N, 10.09.

4.5. (*Z*)-2-[(4-Fluorophenyl)-(4-methylthiosemicarbazono)-methyl]- 4,5-methylenedioxyphenylacetic acid methyl ester (15)

Compound **15** was prepared from **10** with a similar procedure. Mp 79–82 °C; $R_f = 0.41$ (cyclohexane/EtOAc, 7:3); yield 80%. ^{1}H NMR 3.27 (m, 2H, CH_2 and d, 3H, $J = 4.4$ Hz, NHCH_3), 3.54 (s, 3H, OCH_3), 6.08 (m, 2H, OCH_2O), 6.56 (s, 1H, H-3), 6.93 (s, 1H, H-6), 7.03 (dd, 2H, J_{H-H} and $J_{H-F} = 8.5$ Hz, H-3′,5′), 7.51 (dd, 2H, $J_{H-H} = 8.5$ Hz and $J_{H-F} = 5.2$ Hz, H-2′,6′), 7.65 (m, 1H, *NH*CH_3), 8.49 (bs, 1H, NH). MS (ESI+) 404 (M^++1). Anal. Calcd for $C_{19}H_{18}FN_3O_4S$: C, 56.57; H, 4.50; N, 10.42. Found: C, 56.66; H, 4.58; N, 10.25.

4.6. (*Z*)-2-[(4-Aminophenyl)-(4-methylthiosemicarbazono)-methyl]-4,5-methylenedioxytoluene (16)

Raney-Ni (40 mg) and an excess of ammonium formate (1.00 mmol) were added to a slurry of **11** (0.50 mmol) in ethanol (40 mL). The reaction mixture was stirred under reflux for 2 h, then 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 was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using the same eluent employed for TLC. Mp 117–120 °C; $R_f = 0.44$ (cyclohexane/EtOAc, 5:5); yield 80%. ^{1}H NMR 2.00 (s, 3H, CH_3-1), 3.27 (d, 3H, $J = 4.7$ Hz NHCH_3), 3.90 (bs, 2H, NH_2) 6.02 (m, 2H, OCH_2O), 6.52 (s, 1H, H-3), 6.61 (d, 2H, $J = 8.8$ Hz, H-3′,5′), 6.81 (s, 1H, H-6), 7.32 (d, 2H, $J = 8.8$ Hz, H-2′,6′), 7.63 (m, 1H, *NH*CH_3) 8.37 (bs, 1H, NH). MS (ESI+) 343 (M^++1). Anal. Calcd for $C_{17}H_{18}N_4O_2S$: C, 59.63; H, 5.30; N, 16.36. Found: C, 59.49; H, 5.38; N, 16.49.

4.7. (*Z*)-2-[(4-Aminophenyl)-(4-methylthiosemicarbazono)-methyl]-4,5-methylenedioxyphenylacetic acid methyl ester (17)

Compound **17** was prepared from **12** with a similar procedure. Mp 110–112 °C; $R_f = 0.65$ (EtOAc/cyclohexane/*i*-PrOH, 6:3:1); yield 74%. ^{1}H NMR 3.26 (d, 3H, $J = 4.9$ Hz, NHCH_3), 3.29 (m, 2H, CH_2), 3.56 (s, 3H, OCH_3), 3.98 (bs, 2H, NH_2), 6.07 (m, 2H, OCH_2O), 6.56 (s, 1H, H-3), 6.59 (d, 2H, $J = 8.5$ Hz, H-3′,5′), 6.90 (s, 1H, H-6), 7.30 (d, 2H, $J = 8.5$ Hz, H-2′,6′), 7.63 (m, 1H, *NH*CH_3) 8.40 (bs, 1H, NH). MS (ESI+) 401 (M^+ +1). Anal. Calcd for $C_{19}H_{20}N_4O_4S$: C, 56.99; H, 5.03; N, 13.99. Found: C, 57.10; H, 4.84; N, 13.82.

4.8. (*Z*)-2-[(4-Bromophenyl)-(4-methylsemicarbazono)-methyl]-4,5-methylenedioxyphenylacetic acid methyl ester (18)

An excess of 30% H_2O_2 (10 mL) and MeONa (100 mg) were added to a solution of **13** (0.4 mmol) in MeOH (30 mL); the mixture was stirred at room temperature until disappearance of the starting material (TLC monitoring). The solvent was evaporated in vacuo and the residue, dissolved in EtOAc, was washed with H_2O to remove MeONa. The organic layer was dried (Na_2SO_4) and purified by silica gel column chromatography using EtOAc as eluent. Mp 113–116 °C; $R_f = 0.54$ (EtOAc); yield 83%. ^{1}H NMR 2.96 (d, 3H, $J = 4.9$ Hz, NHCH_3), 3.27 (s, 2H, CH_2), 3.51 (s, 3H, OCH_3), 6.07 (m, 2H, OCH_2O), 6.19 (m, 1H, *NH*CH_3), 6.54 (s, 1H, H-3), 6.92 (s, 1H, H-6), 7.36 (d, 2H, $J = 8.8$ Hz, H-3′,5′), 7.46 (d, 2H, $J = 8.8$ Hz, H-2′,6′), 7.52 (bs, 1H, NH). MS (ESI+) 448 and 450 (M^++1). Anal. Calcd for $C_{19}H_{18}BrN_3O_5$: C, 50.91; H, 4.05; N, 9.37. Found: C, 50.78; H, 4.11; N, 9.21.

4.9. (*Z*)-2-[(4-Chlorophenyl)-(4-methylsemicarbazono)-methyl]-4,5-methylenedioxyphenylacetic acid methyl ester (19)

Compound **19** was prepared from **14** with a similar procedure. Mp 109–111 °C; $R_f = 0.59$ (EtOAc); yield 82%. ^{1}H NMR 2.95 (d, 3H, $J = 4.9$ Hz, NHCH_3), 3.27 (m, 2H, CH_2), 3.51 (s, 3H, OCH_3), 6.07 (m, 2H, OCH_2O), 6.21 (m, 1H, *NH*CH_3), 6.55 (s, 1H, H-3), 6.92 (s, 1H, H-6), 7.29 (d, 2H, $J = 8.8$ Hz, H-3′,5′), 7.43 (d, 2H, $J = 8.8$ Hz, H-2′,6′), 7.54 (bs, 1H, NH). MS (ESI+) 404 (M^++1). Anal. Calcd for $C_{19}H_{18}ClN_3O_5$: C, 56.51; H, 4.49; N, 10.41. Found: C, 56.34; H, 4.59; N, 10.55.

4.10. (*Z*)-2-[(4-Fluorophenyl)-(4-methylsemicarbazono)-methyl]-4,5-methylenedioxyphenylacetic acid methyl ester (20)

Derivative **20** was prepared from **15** with a similar procedure. Mp 190–193 °C; $R_f = 0.59$ (EtOAc); yield 85%. ^{1}H NMR 2.95 (d, 3H, $J = 4.9$ Hz, NHCH_3), 3.28 (m, 2H, CH_2), 3.51 (s, 3H, OCH_3), 6.06 (m, 2H, OCH_2O), 6.19 (m, 1H, *NH*CH_3), 6.55 (s, 1H, H-3), 6.92 (s, 1H, H-6), 7.02 (dd, 2H, $J_{H-H} = 8.8$ Hz and $J_{H-F} = 8.5$ Hz, H-3′,5′), 7.48 (dd, 2H, $J_{H-H} = 8.8$ Hz and $J_{H-F} = 5.5$ Hz, H-2′,6′ and bs, 1H, NH). MS (ESI+) 388 (M^++1). Anal. Calcd for $C_{19}H_{18}FN_3O_5$: C, 58.91; H, 4.68; N, 10.85. Found: C, 58.77; H, 4.75; N, 10.73.

4.11. (*Z*)-2-[(4-Aminophenyl)-(4-methylsemicarbazono)-methyl]-4,5-methylenedioxytoluene (21)

Compound **21** was prepared from **16** with a similar procedure. Mp 112–115 °C; $R_f = 0.51$ (EtOAc); yield 83%. ^{1}H NMR 1.99 (s, 3H, CH_3-1), 2.94 (d, 3H, $J = 4.9$ Hz, NHCH_3), 4.00 (bs, 2H, NH_2) 6.00 (m, 2H, OCH_2O), 6.27 (m, 1H, *NH*CH_3), 6.50 (s, 1H, H-3), 6.61 (d, 2H, $J = 8.2$ Hz, H-3′,5′), 6.78 (s, 1H, H-6), 7.31 (d, 2H, $J = 8.2$ Hz, H-2′,6′), 7.47 (bs, 1H, NH). MS (ESI+) 327 (M^+ +1). Anal. Calcd for $C_{17}H_{18}N_4O_3$:C, 62.57; H, 5.56; N, 17.17. Found: C, 62.81; H, 5.46; N, 17.21.

4.12. (*Z*)-2-[(4-Acetylhydrazono)-(4-aminophenyl)-methyl]-4,5-methylenedioxytoluene (23)

Acetylhydrazine (104 mg, 1.4 mmol) and 10 N HCl (0.2 mL) were added to a solution of **6** (200 mg,

0.7 mmol) in MeOH (25 mL). The reaction mixture was refluxed for 48 h. After cooling, the solvent was removed under reduced pressure and the residue was subjected to chromatography eluting with EtOAc/cyclohexane, 1:1 to afford the acetylhydrazono derivative, which was successively reduced, with the procedure reported for compound **16**, to give compound **23**. Mp 103–106 °C; $R_f = 0.67$ (EtOAc); yield 46%. ^{1}H NMR 2.00 (s, 3H, CH_3-1), 2.41(s, 3H, $COCH_3$), 3.87 (bs, 2H, NH_2), 6.01 (m, 2H, OCH_2O), 6.51 (s, 1H, H-3), 6.62 (d, 2H, $J = 8.8$ Hz, H-3′,5′), 6.80 (s, 1H, H-6), 7.36 (d, 2H, $J = 8.8$ Hz, H-2′,6′), 8.17 (bs, 1H, NH). MS (ESI+) 312 (M^+ +1). Anal. Calcd for $C_{17}H_{17}N_3O_3$: C, 65.58; H, 5.50; N, 13.50. Found: C, 65.36; H, 5.38; N, 13.74.

4.13. (*Z*)-2-[(4-Aminophenyl)-(4-semicarbazono)-methyl]-4,5-methylenedioxytoluene (24)

Methylhydrazino-carboxylate (1.4 mmol) and 10 N HCl (0.2 mL) were added to a stirred solution of **6** (0.7 mmol) in MeOH (40 mL) The reaction mixture was refluxed for 48 h. After removal of the solvent under reduced pressure, the oily residue was treated with diethyl ether to afford a solid, which by successive reduction with the procedure reported for **16**, gave compound **24**. Mp 111–114 °C; $R_f = 0.80$; yield 68% (EtOAc); ^{1}H NMR 2.01 (s, 3H, CH_3), 3.83 (m, 4H, NH_2-4′ and $NHNH_2$), 6.02 (m, 2H, OCH_2O), 6.53 (s, 1H, H-3), 6.60 (d, 2H, $J = 8.2$ Hz, H-3′,5′), 6.81 (s, 1H, H-6), 7.38 (d, 2H, $J = 8.2$ Hz, H-2′,6′), 7.58 (bs, 1H, NH). MS (ESI+) 313 (M^+ +1). Anal. Calcd for $C_{16}H_{16}N_4O_3$: C, 61.53; H, 5.16; N, 17.94. Found: C, 61.69; H, 5.01; N, 17.81.

4.14. (*Z*)-2-{2-[(4-Aminophenyl)-(4-methylsemicarbazono)-methyl]-4,5-methylenedioxyphenyl}-*N*-methylacetamide (25)

An excess methylamine (1.5 mmol) was added to a stirred solution of **26**[9] (0.5 mmol) in dry THF (30 mL); the reaction mixture was refluxed for 1 h. After removal of the solvent under reduced pressure, the crude was purified by silica gel column chromatography using EtOAc/MeOH 90:10 as eluent to give the intermediate nitro derivative. The successive reduction was accomplished with the procedure reported for **16**. The oily residue, dissolved in 2 mL of acetone, by treatment with light petroleum gave **25** as pale yellow solid. Mp 166–168 °C; $R_f = 0.28$ (EtOAc/MeOH); yield 75%. ^{1}H NMR 2.57 (d, 3H, $J = 4.9$ Hz, NH*C*H_3-1), 2.92 (d, 3H, $J = 4.9$ Hz, NH *C*H_3-2), 3.13, and 3.20 (dd, 2H, $J = 15.4$ Hz, CH_2CO), 3.88 (bs, 2H, NH_2), 5.37 (m, 1H, *NH*CH_3-1), 6.04 (m, 2H, OCH_2O), 6.22 (m, 1H, *NH*CH_3-2), 6.57 (s, 1H, H-3), 6.61 (d, 2H, $J = 8.5$ Hz, H-3′,5′), 7.00 (s, 1H, H-6), 7.28 (d, 2H, $J = 8.5$ Hz, H-2′,6′), 7.63 (bs, 1H, NH). MS (ESI+) 384 (M^+ +1). Anal. Calcd for $C_{19}H_{21}N_5O_4$: C, 59.52; H, 5.52; N, 18.27. Found: C, 59.32; H, 5.68; N, 18.42.

4.15. Audiogenic seizures test in DBA/2 mice[14]

DBA/2 mice (8–12 g, 22–25 days old) were purchased from Charles River (Calco, Como, Italy). Groups of 10 mice of either sex were exposed to auditory stimulation 30 min following administration of vehicle or each dose of drugs studied. The compounds were given ip (0.1 mL/10 g of body weight of the mouse) as a freshly prepared solution in 50% DMSO and 50% sterile saline (0.9% NaCl). Individual mice were placed under a hemispheric perspex dome (diameter 58 cm) and 60 s were allowed for habituation and assessment of locomotor activity. Auditory stimulation (12–16 kHz, 109 dB) was applied for 60 s or until tonic extension occurred and induced a sequential seizure response in control DBA/2 mice, consisting of an early wild running phase, followed by generalized myoclonus and tonic flexion and extension, sometimes followed by respiratory arrest. The control and drug-treated mice were scored for latency to and incidence of the different phases of the seizures.[15]

4.16. Electrophysiological recordings

Recordings were performed on single cerebellar granule neurons after 7 days in culture[16] using the voltage-clamp technique in the whole-cell configuration.[17] 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-aminoethylether)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[18] at the same concentration (100 μM). After a wash-out of test compounds from the cell culture, the responses to KA returned to the control level.

4.17. Statistical analysis

The ED_{50} values of each phase of the audiogenic seizure were determined for each dose of compound administered, and dose–response curves were fitted using a computer program by the method of Litchfield and Wilcoxon.[19] The median toxic dose (TD_{50}) values were estimated using the method of Litchfield and Wilcoxon.[19] Electrophysiological data were analyzed using the software Clampex (Axon Instrument). Results are expressed as mean ± SE.

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