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Short communication

Synthesis and pharmacological evaluation of a new series of substituted benzoyl-γ-butyrolactone derivatives

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Summary — A series of substituted benzoyl-γ-butyrolactones (**1–3**) has been synthesized and tested for their ability to affect central dopaminergic and GABAergic function in comparison to γ-butyrolactone (GBL). Similarly to GBL, α-, β- and γ-substituted GBLs **1–3** with one or more chlorine on the phenyl ring were found to induce central depressant effects in rats, though at different degrees. However, the test compounds modified dopamine (DA) metabolism in rat striatum differently from GBL. In fact, whereas GBL increased both DA and dihydroxyphenylacetic acid (DOPAC) content, GBL derivatives **1–3** increased DA levels, but reduced the DOPAC concentration. Moreover, some of them, unlike GBL, effectively antagonized pentylenetetrazole (PTZ)-induced seizures in mice. In particular, α-3,5-dichlorobenzoyl-GBL (**1g**) was effective at a dose as low as 36 mg/kg in decreasing the number of animals having convulsions. However, *in vitro* addition and *in vivo* administration of the test compounds failed to modify [^{35}S]-*t*-butylbicyclophosphorothionate ([^{35}S]-TBPS) binding, which is a very sensitive tool for revealing changes in the GABAergic function.

γ-butyrolactone / benzoyl-γ-butyrolactone / dopamine / anticonvulsant effect

Introduction

γ-Butyrolactone (GBL) and its active metabolite γ-hydroxybutyrate (GHB) produce different behavioral and neurochemical effects in different animal species. Thus, GHB is known to induce spike and wave activity in the EEG and a profound central nervous system (CNS) depressant effect in both laboratory animals [1–3] and humans [4]. Anaesthetic doses of GHB produce changes in dopaminergic activity in the CNS, such as inhibition of dopaminergic neuronal activity [5, 6], increase in dopamine (DA) content [7] and synthesis rate [8] as well as inhibition of DA release [9]. Furthermore, while early studies reported a direct interaction of GHB with the 4-aminobutanoic acid ($GABA_A$) receptor function [10, 11], such an interaction was not confirmed by our recent study [12].

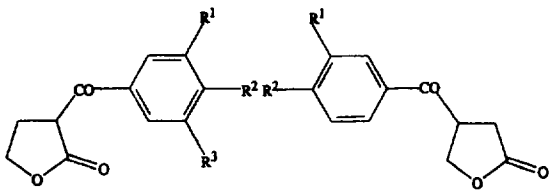
Alkyl-substituted γ-butyrolactones (GBLs) are known to have different neuropharmacological effects depending on the substitution pattern. In particular, β-alkyl GBLs have been shown to cause seizures in laboratory animals [13], while α-alkyl GBLs protect against pentylenetetrazole (PTZ)-induced seizures [14].

Although several aroyl-substituted-GBLs have been chemically described [15–17], to our knowledge no pharmacological studies have been performed on the aroyl-GBLs **1–3** represented in figure 1.

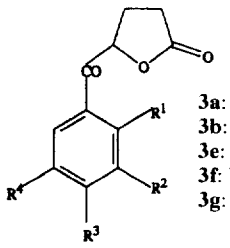
Preliminary experiments from our laboratories showed that some of them possessed sedative and hypnotic properties, which prompted us to further characterize their pharmacological profile. The present study describes the synthesis of a number of α-, β- and γ-aroyl-GBLs (**1–3**) and their pharmacological effects in comparison with GBL.

Chemistry

The α-aroyl-GBLs **1** and β-aroyl-GBLs **2**, with the single exception of **2f**, have been previously described and were therefore prepared according to the reported methods [18–20]. The unknown derivates **3** were



1b: $R^1 = R^3 = H$; $R^2 = Cl$
1g: $R^1 = R^3 = Cl$; $R^2 = H$
2b: $R^2 = Cl$; $R^1 = H$
2c: $R^2 = NHCOCH_3$; $R^1 = H$
2d: $R^2 = OCH_3$; $R^1 = H$
2f: $R^2 = R^1 = Cl$



3a: $R^1 = R^3 = R^4 = H$; $R^2 = Cl$
3b: $R^1 = R^2 = R^4 = H$; $R^3 = Cl$
3e: $R^1 = R^3 = Cl$; $R^2 = R^4 = H$
3f: $R^1 = R^4 = H$; $R^2 = R^3 = Cl$
3g: $R^1 = R^3 = H$; $R^2 = R^4 = Cl$

Fig 1. Structures of compounds **1b,g**, **2b–d,e** and **3a,b,e–g**.

synthesized by adapting the method of Yamada *et al* [21], by base-catalyzed ring closure of the appropriate γ-bromo-γ-benzoylbutyric acids (see scheme 1). Accordingly, the acids **4a**, **b**, **e–g** were reacted with bromine in dioxane and directly cyclized in a one-pot reaction by adding 5% sodium hydrogen carbonate to the reaction mixture. The required **4** was then obtained by acylation of the appropriate substituted benzene (**5b**, **f**) with glutaric anhydride in the presence of aluminum chloride (to give **4b**, **f**) or from the corresponding aroylacetates **6a**, **e**, **g** which were alkylated with ethyl-3-bromopropionate followed by hydrolysis and decarboxylation (to give **4a**, **e**, **g**). The physical properties of compounds **2–4** and **6** are shown in table I.

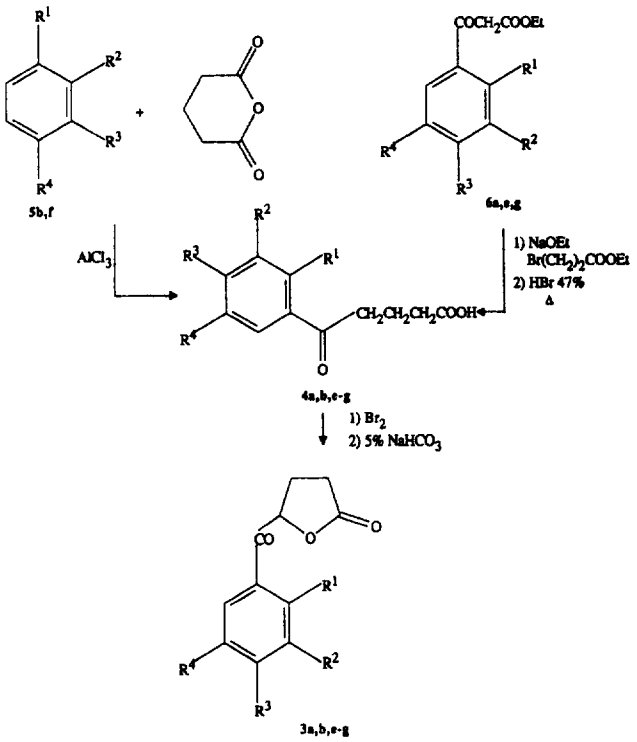
Pharmacology

The compounds were tested for their effects on DA and dihydroxyphenylacetic acid (DOPAC) concentration in rat striatum after systemic administration. The possible interaction of these compounds with the $GABA_A$ receptor complex in the mouse was also evaluated. For this purpose, compounds were tested for their ability to antagonize PTZ-induced convulsions (0.4 mM/kg) in mice. Moreover, to better clarify their molecular mechanisms of action the *in vitro* and *ex vivo* effects of the test compounds on [^{35}S]-*t*-butylbicyclophosphorothionate ([^{35}S]TBPS) binding were studied. This is a biochemical parameter often used to evaluate the function of GABAergic system. In addition, we evaluated the capability of these compounds to displace specific membranes binding sites for [^{3}H]-γ-hydroxybutyric acid ([^{3}H]-GHB) which are present in both human and rat brain [22, 23].

Results

Dopamine metabolism

The effects of GBL and aroyl-GBLs (**1–3**) on DA and DOPAC concentrations in the rat striatum are shown



5	R^1	R^2	R^3	R^4
b	H	H	Cl	H
f	H	Cl	Cl	H

3,4,6	R^1	R^2	R^3	R^4
a	H	Cl	H	H
b	H	H	Cl	H
e	Cl	H	Cl	H
f	H	Cl	Cl	H
g	H	Cl	H	Cl

Scheme 1.

Table I. Physical properties of compounds **2**, **3**, **4** and **6**.

Compound	*Yield (%)*	*Mp (°C); bp/mmHg (°C)*	*Molecular formula*	*^{1}H NMR (ppm)*
2f	92	89–91	$C_{11}H_8Cl_2O_3$	3.0 (t, 2H), 4.4–4.8 (m, 3H), 7.5–7.9 (m, 3H)
3a	64	97–98	$C_{11}H_9ClO_3$	2.2–2.7 (m, 4H), 5.6–5.9 (m, 1H), 7.3–8.1 (m, 4H)
3b	58	105–107	$C_{11}H_9ClO_3$	2.6 (d, 4H), 5.6–5.8 (m, 1H), 7.5 (d, 2H), 8.0 (d, 2H)
3e	41	76–77	$C_{11}H_8Cl_2O_3$	2.4–2.6 (m, 4H), 5.6–5.7 (m, 1H), 7.4 (dd, 1H), 7.5 (d, 1H), 7.6 (d, 1H)
3f	26	90–91	$C_{11}H_8Cl_2O_3$	2.5 (d, 4H), 5.5–5.8 (m, 1H), 7.5–8.1 (m, 3H)
3g	6	82–84	$C_{11}H_8Cl_2O_3$	2.6 (d, 4H), 5.6–5.8 (m, 1H), 7.6–7.7 (m, 1H), 7.9 (d, 2H)
4a	62	112–114	$C_{11}H_{11}ClO_3$	1.9–2.2 (m, 2H), 2.3–2.6 (m, 2H), 2.8–3.2 (m, 2H), 7.2–7.9 (m, 4H), 8.8 (br s, 1H, exchange with D_2O)
4b	22	118–120	Ref 31	
4e	83	86–87	$C_{11}H_{10}Cl_2O_3$	1.8–2.2 (m, 2H), 2.5 (t, 2H), 3.0 (t, 2H), 7.1–7.6 (m, 3H), 10.4 (br s, 1H, exchange with D_2O)
4f	10	128–130	$C_{11}H_{10}Cl_2O_3$	1.6–2.0 (m, 2H), 2.1–2.6 (m, 2H), 2.9–3.0 (m, 2H), 7.7–8.2 (m, 3H), 8.3 (br s, 1H, exchange with D_2O)
4g	70	120–122	$C_{11}H_{10}Cl_2O_3$	1.9–2.2 (m, 2H), 2.3–2.6 (m, 2H), 2.8–3.1 (m, 2H), 7.5–7.6 (m, 1H), 7.8 (d, 2H), 9.5 (br s, 1H, exchange with D_2O)
6a	30	130/0.5	$C_{11}H_{11}ClO_3$	0.9 (t, 3H), 3.9 (s, 2H), 4.2 (q, 2H), 7.3–7.9 (m, 4H)
6e	73	Oil	$C_{11}H_{10}Cl_2O_3$	0.8 (t, 3H), 3.5 (s, 2H), 3.9 (q, 2H), 7.2–7.5 (m, 3H)
6g	53	43–45	$C_{11}H_{10}Cl_2O_3$	1.2 (t, 3H), 3.9 (s, 2H), 4.2 (q, 2H), 7.3–7.6 (m, 2H), 7.7 (d, 1H)

in table II. GBL (4.5 mmol/kg) increased DA and DOPAC content by 54 and 63%, respectively. Anova revealed a significant drug effect on DA concentration ($F(6,28) = 12.45$, $P < 0.0001$) and DOPAC levels ($F(6,28) = 163.64$, $P < 0.0001$). Similarly to GBL, **1b**, **1g**, **3b** and **3g** significantly increased DA concentration (Newman–Keuls test), but contrary to GBL, they caused a dramatic decrease in DOPAC levels ($P <$ 0.001 with the Newman–Keuls test).

Binding studies

The effect of *in vitro* addition of the test compounds on [^{35}S]TBPS binding was measured in membranes from mouse cerebral cortex, in order to evaluate the possible interactions of these compounds with $GABA_A$ receptor complex. As expected [12], the *in vitro* addition of GBL did not change [^{35}S]TBPS binding at any concentration and similar results were obtained with the test compounds (see table III). In agreement with the *in vitro* data, *ip* administration of **1g** at a dose of 150 mg/kg failed to change [^{35}S]TBPS binding (measured 30 min after treatment in unwashed mouse cortical membranes) (see table IV). Similarly, neither GBL [12] nor the other test compounds could affect TBPS binding in the same test. Finally, the compounds failed to displace specific [^{3}H]GHB binding (data not shown).

Anticonvulsant activity in mice

As shown in table V, **1g** and **3g** were the most effective anticonvulsant agents, and could fully antagonize PTZ-induced convulsions in mice at a dose of 300 and 400 mg/kg, respectively. The other test compounds were less effective, whereas GBL was still completely inactive at a dose as high as 400 mg/kg. It should be noted that **1g** could significantly decrease the number

Table II. Effect of Cl-aroyl-GBLs on DA and DOPAC concentrations in the rat striatum.

Compound (mg/kg)	*DA (ng/mg)*	*%*	*DOPAC (ng/mg)*	*%*
Vehicle	13.44 ± 0.93	100	2.14 ± 0.14	100
GBL (400)	23.78 ± 2.09***	154	4.51 ± 0.07***	163
1b (1000)	18.05 ± 0.73**	134	0.77 ± 0.01***	34
1g (400)	16.09 ± 0.47	119	1.17 ± 0.07***	55
2b (1000)	14.38 ± 0.50	107	1.07 ± 0.10***	50
3b (1000)	17.74 ± 0.64*	132	0.92 ± 0.16**	43
3g (400)	16.53 ± 0.39*	123	1.52 ± 0.09**	71

Animals were treated 1 h before sacrifice. DA and DOPAC levels were determined in the striatum as reported in *Experimental protocols*. Values are the means ± SEM of 5 rats. Statistical evaluation was made by one-way Anova followed by Newman-Keuls test. ***$P < 0.0001$; **$P < 0.01$; *$P < 0.05$ with respect to vehicle-treated rats.

of animals having convulsion at a dose (36 mg/kg) markedly lower than **3g** (150 mg/kg) (see table VI).

Discussion

As reported in previous studies [1–8], high doses of GBL produced sedation and anaesthesia associated with increase in striatal DA and DOPAC levels in rats. The increase in DA content may result from the inhibition of the amine release as a consequence of the suppression of the firing of nigrostriatal DA neurons [8]. After an initial period of about 60 min, during which DA seems to be protected from metabolism, the accumulated DA is eventually metabolized by monoamine oxidase (MAO) to DOPAC, whose accumulation generally begins 60 min after GBL treatment [24].

GBL derivatives **1b**, **1g**, **3b** and **3g** were able to modify DA metabolism in the striatum of rat, but unlike GBL, the increase in DA concentration was associated with a marked decrease in DOPAC content. Whether this effect is due to inhibition of MAO is still unknown.

The *ex vivo* binding of [^{35}S]TBPS to specific recognition sites coupled with the GABA-dependent chloride channel is a very sensitive tool for revealing changes in the function of the GABAergic synapses induced by *in vivo* administration of drugs which are known to alter the activity of central GABAergic transmission. Accordingly, we found that the *in vivo* administration of drugs which alter the cognitive, emotive and motor function, such as the benzodiazepine receptor inverse agonists, isoniazid and other inhibitors of the GABAergic transmission, enhanced in a dose-dependent manner the total number of [^{35}S]-TBPS binding sites in the rat cerebral cortex and in other brain areas, an effect opposite to that produced by benzodiazepines and GABA agonists [25–28]. The evidence that derivatives **1–3**, like GBL, could not strongly affect [^{35}S]TBPS binding suggests that these compounds do not interact with the $GABA_A$/benzodiazepine receptor complex in the rat cerebral cortex. However, contrary to GBL, some of the new derivatives (**1g**, **2b**, **3b** and **3g**) were effective in preventing PTZ-induced convulsions in mice. These results are in contradiction with those obtained by other investigations who showed that different α- and β-alkyl-substituted GBLs with anticonvulsant and convulsant activity, respectively, bind competitively to [^{35}S]TBPS binding sites [29]. Thus, the anticonvulsant activity of these compounds might be related to their interaction with other neurotransmitter systems. This conclusion may be supported by the evidence that they failed to interact *in vitro* with the specific binding sites for γ-

Table III. Effects of the substituted-benzoyl-γ-butyrolactones on [^{35}S]TBPS binding in the mouse cerebral cortex.

Compound	*[^{35}S]TBPS binding (% of control)*
Control	100 ± 1.0
GBL	98 ± 1.2
1g	97 ± 1.8
2b	105 ± 3.2
3b	100 ± 2.1
3e	87 ± 2.2
3f	90 ± 1.3
3g	90 ± 1.2

Drugs were tested at the concentration of 100 μM. Data are expressed as percentage of control and are the means ± SEM of three separate experiments performed in triplicate.

Table IV. [^{35}S]TBPS binding after administration of **1g**.

Experimental group	*[^{35}S]TBPS binding (fmol/mg protein)*
Vehicle	50 ± 1 (100%)
1g	51 ± 2 (102%)

Mice were killed 30 min after *ip* injection of 150 mg/kg **1g** or vehicle. [^{35}S]TBPS binding (2 nM) was measured as described in the *Experimental protocols*. Each value is the mean ± SEM of two separate experiments (eight animals for each group).

Table V. Effects of **1g**, **2b**, **3b** and **3g** on PTZ-induced seizures in mouse.

Experimental group (mg/kg)	*Convulsions*	
	Latency (min)	*No of animals presenting convulsions*
PTZ (55)	3.8 ± 0.8	18/20 (90%)
GBL (400)	3.6 ± 1	14/15 (93%)
1g (300)	–	0/10 (0%)*
2b (300)	40 ± 1	4/10 (40%)*
3b (300)	20 ± 0.5	3/15 (30%)**
3e (300)	4.1 ± 0.6	9/10 (90%)
3f (300)	3.9 ± 0.7	8/10 (80%)
3g (400)	–	0/10 (0%)*

Animals were injected (ip) with the drugs 30 min before PTZ (ip). *$P < 0.05$ *vs* PTZ-treated mice. **$P < 0.01$ *vs* PTZ-treated mice (Fisher's exact probability test).

hydroxybutyric acid. In conclusion, the present study shows that aroyl-GBLs (**1–3**) have pharmacological properties different from those of GBL itself, as indicated by their behavior on DA metabolism and their protecting ability against PTZ-induced seizures. In addition, biochemical results suggest that the anticonvulsant effects elicited by Cl-aroyl-GBLs are not mediated by the post-synaptic $GABA_A$ receptor complex.

Experimental protocols

Chemistry

Melting points were determined on a Büchi 510 capillary melting point apparatus and are uncorrected. Analyses indicated by the symbols were within ±0.4% of theoretical values. ^1H NMR spectra were recorded on a Hitachi–Perkin Elmer R 600 FR spectrometer; $CDCl_3$ was used as solvent (unless otherwise noted); chemical shifts are reported as δ (ppm) relative to tetramethylsilane as internal standard. TLC was performed on silica gel plates. Silica gel 60 (Merck; 70–230 mesh) was used for column chromatography. Lactones **1b**, **g** and **2b–d** are known compounds [18–20]. Compound **2f** was prepared according to the same procedure employed for **2b–d**.

*γ-Substituted aroylbutyric acids **4b, f**. General method*

To a solution of glutaric anhydride (50 g; 0.44 mol) in the appropriate substituted benzene (300 ml) cooled at 0°C, was added aluminum chloride (134 g; 1 mol) portionwise under vigorous stirring and the mixture refluxed for 1 h. After cooling to 0°C, water (200 ml) and then hydrochloric acid (65 ml) were added. The excess of the solvent was steam-distilled and the precipitate which appeared after cooling was collected by filtration and treated with 2 N sodium hydroxide. The insoluble part was filtered off and the solution was acidified with 6 N hydrochloric acid. The resulting precipitate was filtered and triturated with isopropyl ether (see table I for data).

*γ-Substituted aroylbutyric acids **4a, e, g**. General method*

To a solution of sodium ethoxide (18.7 g; 0.275 mol) in ethanol (118 ml) the appropriate ester **6** (0.118 mol) was added and the mixture was refluxed for 30 min. The temperature was then adjusted to 40°C, ethyl 3-bromopropionate (5 ml; 0.275 mol) was added dropwise and the mixture was refluxed for 7 h. After cooling, the inorganic salts were filtered off, the solvent evaporated, the residue taken up in chloroform (150 ml) and the organic layer was washed with water. After evaporation of the solvent, the residue was purified by silica gel chromatography, eluting with cyclohexane/ethyl acetate 9:1, to give the intermediate diesters.

A solution of the required diester (0.092 mol) in 47% HBr (196 ml) was refluxed over night. After cooling, the mixture was extracted with dichloromethane (3 × 50 ml) and the organic layer reextracted with 10% aqueous sodium carbonate. Acidification with 6 N hydrochloric acid gave the desired **4** (see table I for data).

*Ethyl (substituted aroyl)acetates **6a, e, g**. General method*

To a suspension of Mg turnings (6.3 g; 0.26 mol) in ethanol (27 ml) and carbon tetrachloride (3 ml), a solution of ethyl acetoacetate (33 ml; 0.26 mol) in ethanol (40 ml) and diethyl ether (30 ml) was added dropwise. After the reaction subsided, the mixture was cooled to 0°C and the required benzoyl chloride (0.26 mol) was added. The mixture was then stirred for 2 h at 60°C and after cooling, poured into ice (150 ml) and acidified to pH 4 with acetic acid. The organic layer was separated, thoroughly washed in succession with 5% $NaHCO_3$ and water and dried over sodium sulfate. After evaporation of the solvent the residue was distilled under vacuum or purified by silica gel chromatography to give ethyl-α-aroylacetoacetates. An ethanolic solution of the latter was then added dropwise to a solution of potassium hydroxide (26.9 g; 0.48 mol) in ethanol (150 ml)

Table VI. Effects of **1g** and **3g** on PTZ-induced seizures in mouse.

Experimental group (mg/kg)	*Convulsions*	
	Latency (min)	*No of animals presenting convulsions*
PTZ (55)	2.6 ± 0.8	19/20 (95%)
1g (36)	5.1 ± 0.3	5/10* (50%)
1g (75)	4.1 ± 0.2	2/10* (20%)
1g (150)	5	1/10* (10%)
1g (300)	–	0/10* (0%)
3g (150)	6.0 ± 1	6/10* (20%)
3g (300)	4.0 ± 0.6	2/10* (20%)
3g (400)	–	0/10* (0%)

Mice were injected with **1g** (ip) 30 min before PTZ (ip). *$P < 0.025$ *vs* PTZ-treated mice (Fisher's exact probability test).

and the mixture stirred at room temperature for 15 h. The desired potassium salts separated after concentration of the solvent and were filtered, triturated with isopropyl ether and then dissolved in water (390 ml; 0.28 mol). To the hot mixture, ammonium chloride (29.7 g; 0.556 mol) and 30% ammonia were added and the mixture stirred for 2.5 h. After cooling, the solution was extracted with dichloromethane (3 × 100 ml), the solvent dried over sodium sulfate and evaporated under vacuum. The residue was then purified by silica gel chromatography eluting with cyclohexane/ethyl acetate 80:20 to give **6a**, **e**, **g** (see table I for data),

γ-(Substituted-benzoyl)-γ-butyrolactones ***3a, b, e–g****. General method*

To a solution of the appropriate acid **4** (0.063 mol) in dioxane (110 ml) and diethyl ether (35 ml), was added bromine (3.85 ml; 0.075 mol) dropwise. The mixture stirred for 3 h and then poured into 5% aqueous $NaHCO_3$ (90 ml) and stirred for 1 h. The mixture was then extracted with dichloromethane, the solvent evaporated under vacuum and the residue purified by silica gel chromatography, eluting with dichloromethane (see table I for data).

Pharmacology

Dopamine metabolism

In a separate experiment, the effect of acute injections of GBL and GBL analogues on DA and DOPAC was studied in Sprague–Dawley male rats (Morini, San Polo d'Enza, Italy) weighing 150–200 g. Animals were housed four per cage at 22–24°C, humidity 50–60%, with a 12 h light–dark cycle (light on at 8 am). Standard laboratory chow and water were always available. One hour after treatment the animals were killed by decapitation and their brains rapidly removed. Striata were dissected on ice, stored at 30°C until analyzed, and later homogenized in perchloric acid 0.1 N containing 3,4-dihydroxybenzylamine as an internal standard. Following centrifugation at 10 000 *g* for 20 min, the supernatants were filtered in a Millipore apparatus. DA and DOPAC were assayed using high pressure liquid chromatography coupled to electrochemical detection (HPLC-EC) [30]. Statistical analyses were performed using one-way Anova followed by Newman–Keuls test.

[35S]TBPS binding

Male CD-1 mice (Charles River, Como, Italy) with body masses of 20–30 g were maintained at 22–24°C, 50–60% humidity, with a 12 h light–dark cycle (light on at 8 am). Standard laboratory chow and water were always available. Mice were killed in the middle of the light phase, their brains rapidly removed and the cerebral cortex separated and used for the measurement of [35S]TBPS binding.

Cerebral cortices were homogenized in 50 volumes of ice-cold 50 mM Tris-citrate buffer (pH 7.4 at 24°C) containing 100 mM NaCl. The homogenate was centrifuged at 20 000 *g* for 20 min and the pellet was reconstituted in 50 volumes of 50 mM Tris-citrate buffer without salt for the binding assay. [35S]TBPS binding was determined in a final volume of 500 µl of [35S]TBPS (final concentration, 2 nM), 50 µl of 2 M NaCl, 50 µl of drugs or solvent and buffer to volume. Incubation (25°C) was initiated by the addition of membranes and was terminated after 90 min by rapid filtration through glass fiber filter strips (Whatman GFIB), which were then rinsed twice with 4 ml of ice-cold Tris-citrate buffer as described above. Filter-bound radioactivity was assayed by liquid scintillation spectroscopy. Non-specific binding was defined as binding in the presence of 100 µM picrotoxin.

In vivo studies. Drugs were suspended in distilled water with one drop of Tween 80 per 5 ml of water, sonicated for 30 min and injected ip (10 ml/kg of body mass). PTZ was injected ip 30 min after the drugs. Animals were observed for 1 h after PTZ injection to determine the incidence of convulsions.

Ex vivo studies. Compound **1g** was suspended in distilled water with one drop of Tween 80 per 5 ml water, sonicated for 30 min and injected ip (10 ml/kg of body mass). The animals were observed until time of killing (30 min). Brains were removed rapidly, and the cerebral cortex was separated and used for the measurement of [35S]TBPS binding which was performed as described above. Data were analyzed by Fisher's exact probability test and Student's *t* test.

References

1 Godschalk M, Dzolzic MR, Bonta II (1977) *Eur J Pharmacol* 44, 105–111
2 Snead OC (1988) *Epilepsia* 29, 361–368
3 Snead OC, Yu RK, Huttelocker PR (1976) *Neurology* 26, 51–56
4 Laborit H, Jonany JM, Gerald J, Fabian F (1960) *Press Med* 68, 1867–1869
5 Walters JR, Roth RH, Aghajanian GK (1973) *J Pharmacol Exp Ther* 186, 630–639
6 Roth RM, Doherty JD, Walters JR (1980) *Brain Res* 556–560
7 Gessa GL, Vargiu L, Crabai F, Boero GC, Caboni F, Camba R (1966) *Life Sci* 5, 1921–1930
8 Walters JR, Roth RH (1974) *J Pharmacol Exp Ther* 19, 82–91
9 Echler V, Gobaille S, Bourguignon JJ, Maitre M (1991) *Neurochemistry* 56, 938–944
10 Roth RH, Nowycky MC (1977) *Biochem Pharmacol* 26, 2079–2082
11 Hösli L, Hösli E, Lehmann R, Schneider J, Borner M (1983) *Neurosci Lett* 37, 257–260
12 Serra M, Sanna E, Foddi C, Concas A, Biggio G (1991) *Psychopharmacology* 104, 351–355
13 Klunk WE, Covey DF, Ferrendelly JA (1982) *Mol Farm* 22, 431–437
14 Klunk WE, Covey DF, Ferrendelly JA (1982) *Mol Farm* 22, 438–443
15 Heymann H, Fieser LF (1951) *J Am Chem Soc* 73, 5252–5265
16 Wyss H, Vogeli U, Sheffold R (1981) *Helv Chim Acta* 64, 775–786
17 Cooper RD, Jigajinni VB, Wightman RH (1984) *Tetrahedron Lett* 25, 5215–5218
18 Barlocco D, Martini A, Pinna GA, Curzu MM, Sala F, Germini M (1987) *Il Farmaco Ed Sci* 42, 585–594
19 Curzu MM, Pinna GA, Barlocco D, Cignarella G (1990) *J Heterocycl Chem* 27, 205–208
20 Cignarella G, Grella G, Curzu MM (1980) *Synthesis* 825–827
21 Yamada U, Togawa Y, Kato T, Hirata Y (1971) *Tetrahedron* 27, 5445–5451
22 Benavides J, Rumigny JF, Bourguignon JJ *et al* (1982) *Life Sci* 30, 953–961
23 Snead OC, Liu CC (1984) *Biochem Pharmacol* 33, 2587–2590
24 Walter JR, Roth RH (1972) *Biochem Pharmacol* 21, 2111–2121
25 Sanna E, Concas A, Serra M, Biggio G (1989) *Eur J Neurosci* 2 (Suppl) 287
26 Sanna E, Serra M, Pepitoni S, Biggio G (1989) *Brain Res* 501, 144–149
27 Serra M, Sanna E, Biggio G (1989) *Eur J Pharmacol* 164, 385–388
28 Biggio G, Concas A, Corda MG, Giorgi O, Sanna E, Serra M (1990) *Pharmacol Ther* 48, 121–142
29 Levine JA, Ferrendelli JA, Covey DF (1985) *Biochem Pharmacol* 34, 4187–4190
30 Rossetti ZL, Mercuro G, Rivano AC (1983) *Life Sci* 33, 2387–2395
31 Randad RS, Kulkarni GH (1984) *Indian J Chem* 23B, 287–288