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Neuropharmacological study of hetero[2,1]benzothiazepine derivatives analogues of tianeptine

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Abstract

Neuropharmacological studies were conducted in mice with a number of hetero[2,1]benzothiazepine derivatives, analogues of tianeptine. Seven of the 12 compounds under study potentiated the actions of 5-hydroxytryptophan (5-HTP, 50 mg/kg i.p.) and/or antagonised the hypothermia induced by high doses of apomorphine. Moreover, some of them inhibited the head twitches induced by 5-HTP (250 mg/kg i.p.) and the stereotyped behaviour and/or climbing behaviour of low doses of apomorphine. These compounds also produced a slight inhibition of exploratory behaviour in the holeboard test. On the other hand, no significant muscle relaxant, anticonvulsant and anxiolytic activities were observed at any dose employed. Together, these data suggest that some of the compounds under study exert antidepressant and neuroleptic effects in mice with no muscle relaxant, anxiolytic and anticonvulsant activities.

Keywords: Heterobenzothiazepine derivatives; Antidepressant activity; Neuroleptic activity; Tianeptine analogues; Neuropharmacology

1. Introduction

Depressive disorders are among the most common diseases in humans, with ca. 10.3% of all adults afflicted during any 1 year [1,2]. Current therapies include the use of electroconvulsive (shock) therapy, psychiatric intervention, and antidepressant drugs such as tricyclic antidepressants, monoamine oxidase inhibitors, and serotonin-selective reuptake inhibitors. However, all of these drugs have been associated with particular adverse effects, which can range from being a mild nuisance or moderate discomfort to those causing intolerable distress and even life-threatening conditions [2,3].

In search of new potential antidepressant with fewer side effects than the classical tricyclic antidepressants, a number of new hetero[2,1]benzothiazepine derivatives were synthesised and pharmacologically studied [4]. These compounds belong to the series of the 10-(2-*N*,*N*-dialkylamino-ethoximino)-thieno[3,4-*c*][2,1]benzothiazepine 4,4-dioxides (compounds **1**–**4**), 10-(2-*N*,*N*-dialkylamino-ethoximino)-thieno[3,2-*c*][2,1]benzothiazepine 4,4-dioxides (compounds **5**–**8**), and 4-(2-*N*,*N*-dialkylamino-ethoximino)-2*H*-pyrazolo[3,4-*c*][2,1]benzothiazepine 10,10-dioxides (compounds **9**–**12**) (see Fig. 1). These ring systems have clear relationships of isosterism with the tricyclic structure of tianeptine, an antidepressant, which in contrast with tricyclic antidepressants or selective serotonin reuptake inhibitors, enhances presynaptic serotonin uptake [5,6]. In this previous report [4], it has been shown that some of these new compounds (specially compounds **5**, **6**, **8** and **9**) were orally effective in two animals models predictive of antidepressant activity like the antagonims of tetrabenazine-induced ptosis and motor depression and the forced swimming test, with slight sedative effects and no anticholinergic activity on mice. In general, these compounds were shown to have values of acute lethal toxicity (LD_{50}) superior to 300 mg/kg p.o., with the exception of compounds **5**, **6**, **7** and **11** which presented higher toxicities (LD_{50} of 300, 80, 146 and 200 mg/kg

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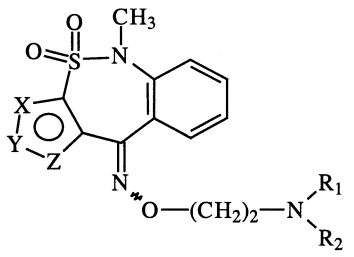

Compound (*Z* and *E* mixture)	X	Y	Z	R_1	R_2	Isomer	Ratio %
1	CH	S	CH	CH_3	CH_3	*Z*	49
						E	51
2	CH	S	CH	$(CH_2)_4$		*Z*	46
						E	54
3	CH	S	CH	$(CH_2)_5$		*Z*	46
						E	54
4	CH	S	CH	$(CH_2)_2$-O-$(CH_2)_2$		*Z*	47
						E	53
5	CH	CH	S	CH_3	CH_3	*Z*	77
						E	23
6	CH	CH	S	$(CH_2)_4$		*Z*	100
						E	0
7	CH	CH	S	$(CH_2)_5$		*Z*	75
						E	25
8	CH	CH	S	$(CH_2)_2$-O-$(CH_2)_2$		*Z*	81
						E	19
9	N	NCH_3	CH	CH_3	CH_3	*Z*	86
						E	14
10	N	NCH_3	CH	$(CH_2)_4$		*Z*	85
						E	15
11	N	NCH_3	CH	$(CH_2)_5$		*Z*	94
						E	6
12	N	NCH_3	CH	$(CH_2)_2$-O-$(CH_2)_2$		*Z*	94
						E	6

Fig. 1. Chemical structure of the hetero[2,1]benzothiazepine derivatives.

p.o., respectively) and were, therefore, assayed at a maximum of a quarter of this value.

Encouraged by these results, the present investigation was carried out to further study the effects of these compounds on apomorphine, 5-hydroxytryptophan (5-HTP) and dexamphetamine induced symptoms, in order to understand the mechanism involved in the antidepressant activity. Additionally, we evaluated the potential anxiolytic and antipsychotic activities using 'plus-maze' and 'climbing' test, respectively. Moreover, their possible anticonvulsant and muscle relaxant activities as well as their effect on exploratory behaviour in mice were also investigated.

2. Experimental

2.1. Animals

Male and female albino Swiss mice (22–28 g) were purchased from Interfauna (Barcelona, Spain) and were housed in groups of ten per cage for a minimum of 3 days prior to behavioural studies with free access to standard laboratory food and tap water and maintained on a 12-h light:12-h dark cycle (light from 08:00 to 20:00 h). All animals were fasted overnight before dosing, tap water being available ad libitum. Each experimental dose group consisted of five randomly chosen animals

unless otherwise stated and the ambient temperature was 22 ± 1 °C. Procedures involving animals and their care were conducted in accordance with the European Union Regulations on use of animals for scientific purposes (CEE Council directives 86/609).

2.2. Drugs

The test compounds were obtained as mixtures of the *Z* and *E* isomers (which were not separated) and characterised as described in a previous paper [4]. All other drugs were obtained from standard commercial sources.

Compounds under study and reference drugs were dissolved in 0.9% saline and given orally 1 h before the experiments mentioned bellow in a dose volume of 0.5 ml/20 g body weight in mice. Control animals received, under the same conditions, 0.9% saline solution.

2.3. Tests for antidepressant activity

2.3.1. Effect on apomorphine (16 mg/kg)-induced hypothermia in mice

Apomorhine hydrochloride 16 mg/kg was injected subcutaneously 60 min after p.o. administration of the drugs to group of six male mice. Temperature was measured with a thermistor thermometer (Panlab 0331) before any treatment with drugs and 30 min after apomorphine administration. Male mice with a rectal temperature between 36 and 38 °C prior to experiment were used.

2.3.2. Effect on 5-hydroxytryptophan (5-HTP)-induced head twitches and syndrome in mice

Test drugs were administered p.o. 60 min before 50 mg/kg L-5-HTP (DE_{10}) or 250 mg/kg 5-HTP (DE_{90}) i.p. The female mice were then placed into glass bell jars and 14 min later the number of head twitches was counted in five 2 min intervals (between 14–16, 24–26, 34–36, 44–46 and 54–56 min). Mice were also observed at these intervals for the presence or absence of whole body tremor, forepaw treading, hind-lamb abduction and outstretched posture with the abdomen resting close to the cage floor. The total score for each mouse was calculated (maximal possible score = 25, including into the syndrome the presence or absence of head-twitches).

2.3.3. Effect on D-amphetamine-induced hypermotility in mice

Groups of six female mice received oral doses of test compounds or saline and 60 min later animals were administered 5 mg/kg D-amphetamine sulfate intraperitoneally. After 30 min motor activity was recorded for 15 min by means of a Digiscan Animal Activity Monitor (Omnitech Electronics, Inc.), which consists in several cages ($42 \times 42 \times 30$ cm) equipped with infrared light beams, and linked to a control unit and printer. The cages were divided into four departments ($10 \times 10 \times 30$ cm) and the animals were placed individually in opposite quadrants (two per cage). Three measures were considered: (1) horizontal activity (the total number of beam interruptions that occurred in the horizontal sensor during a given sample period); (2) total distance (the number indicates in centimetres the distance travelled by the animal in a given sample period; it is a more accurate indicator of ambulatory activity; (3) number of movements (this indicates the number of separate horizontal movements executed by the animal in a given sample period).

2.4. Neuroleptic activity: effect on apomorphine (3 mg/kg)-induced hypothermia, climbing and stereotyped behaviour in mice

Groups of six male mice were injected p.o. with the compounds to be tested for neuroleptic activity and 30 min later placed in individual wire mesh cages ($10 \times 10 \times 20$ cm high) for adaptation and exploration of the new environment. Apomorphine 3 mg/kg was injected subcutaneously 60 min after p.o. administration of the drugs and mice were rated for climbing and stereotyped behaviour 10, 20 and 30 min later. Stereotypy was scored (0–3) according to their intensity as follows: 0, absent of stereotypy or any abnormal movement; 1, discontinuous sniffing; 2, continuous sniffing; 3, continuous sniffing with biting, licking or gnawing. Climbing behaviour was rated according to the following scale: 0, four paws on the bottom of the cage (no climbing); 1, two paws on the wall; 2, four paws on the wall. Cumulative scores were obtained for the three readings (maximal possible score = 9 for the stereotyped behaviour and 6 for climbing behaviour). Temperature was measured with a thermistor thermometer (Panlab 0331) before any treatment with drugs and 30 min after apomorphine administration. Mice with a rectal temperature between 36 and 38 °C prior to experiment were used.

2.5. Anxiolytic activity: plus-maze test

The plus-maze apparatus was made of plexiglas and consisted of two open arms (30×5 cm) and two enclosed ($30 \times 5 \times 15$ cm) arms connected by a central platform (5×5 cm). The open arms, the central platform and the floor of the closed arms were made of black plexiglas, and the sides of the closed arms were made of clear plexiglas. The apparatus was mounted on a base raising it 38.5 cm above the floor. The test consisted of injection p.o. mice with the drug or its vehicle 55 min before being tested individually in the holeboard for 5 min. Immediately after the holeboard test each animal was placed in the centre of the plus-

maze facing an open arm and allowed to freely explore for 5 min. The number of entries made on the open and closed arms and the time spent in each type of arm were recorded. Three measures were obtained from the test: the total number of arm entries; the percentage of arms entries made on the open arms; and the time spent on the open arms expressed as a percentage of the time spent on both the open and closed arms [7,8].

2.6. Exploratory activity: holeboard test

The holeboard apparatus consisted of a wooden board (40 × 40 cm) which had 16 equally spaced holes. The holeboard testing involved placing the mouse 55 min after p.o. injection with the control vehicle or test material in the centre of the floor and counting the number of head-dips during 5 min trials [9]. The holeboard test was made just before the plus-maze test in order to increase maze exploration.

2.7. Anticonvulsant activity: effect on chemically induced seizures in mice

Convulsions were induced by an i.p. injection of pentylenetetrazol (120 mg/kg) or strychine sulfate (2.5 mg/kg). After 60 min of the administration of the test drugs the animals were injected with the convulsants and immediately place in separate cages for observation. The times of onset of clonic and tonic seizures and death were noted.

2.8. Muscle relaxant activity

2.8.1. Traction test

Mice were forced to hang with their forelegs on a wire of 1 mm in diameter, which was stretched horizontally at a height of 35 cm. When they fell off the wire within 5 s or they failed to grasp the wire with their hind legs three times successively, muscle relaxation was judged to be positive. This test was conducted in groups of previously screened animals, 60 and 120 min after injection of control vehicle or test material.

2.8.2. Chimney test

In a pyrex-glass tube (30 cm long and 28 cm diameter) marked at 20 cm from the base, a mouse was introduced at the end of the tube near the mark. When the animal reached the other end of the tube, the tube was moved to the vertical position and immediately the mouse tried to climb the tube backwards. Only those mice who reached the mark within 30 s were selected for further testing. The operation was repeated 60 and 120 min p.o. administration of the test drug. When the mice did not reach the mark within 30 s, muscle relaxation was judged to be positive.

2.9. Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA) followed by Student's unpaired t-test [10]. A probability level of 0.05 or less was accepted as significant. The χ^2-test was used for the percentage of failures in the muscle relaxant activity and for the survival rate in the anticonvulsant activity. Stereotypy and climbing behaviour induced by apomorphine, and number of head twitches and syndrome induced by 5-HTP were analysed using the Mann–Whitney test for non-parametric data.

3. Results

3.1. Antidepressant activity

As shown in Table 1, only the compounds **4**, **5**, **7** and **12** at the dose of 25 mg/kg p.o. exhibited a significant antagonism of apomorphine (16 mg/kg s.c.)-induced

Table 1
Effect of the hetero[2,1]benzothiazepine derivatives on apomorphine (16 mg/kg s.c.) induced hypothermia in mice

Comp.	Dose (mg/kg p.o.)	Mean decrease in rectal temperature (°C)
Control		3.94 ± 0.17
1	25	4.23 ± 0.42
2	25	3.00 ± 0.57
3	25	3.48 ± 0.35
4	25	2.73 ± 0.67*
5	25	1.88 ± 0.53**
6	10	3.85 ± 0.27
7	25	2.40 ± 0.45**
8	25	3.85 ± 0.26
9	25	3.21 ± 0.44
10	25	4.16 ± 0.39
11	25	3.66 ± 0.35
12	25	2.53 ± 0.35**
Imipramine	25	2.55 ± 0.23**
Tianeptine	25	4.88 ± 0.29*
Control		2.76 ± 0.36
1	100	2.85 ± 0.53
2	66	1.91 ± 0.55
3	100	2.08 ± 0.22
4	100	1.86 ± 0.29
5	74	1.51 ± 0.25
6	20	2.35 ± 0.54
7	36	2.70 ± 0.56
8	100	3.53 ± 0.47
9	100	3.83 ± 0.38
10	100	3.30 ± 0.37
11	50	3.45 ± 0.43
12	100	4.33 ± 0.96
Imipramine	100	0.36 ± 0.29**
Tianeptine	100	2.33 ± 0.57

Each value represents the mean ± SEM of 12 animals. *$P < 0.05$; **$P < 0.01$ compared with control.

Table 2
Effect of the hetero[2,1]benzothiazepine derivatives on 5-HTP (50 and 250 mg/kg i.p.) behaviour in mice

Comp.	Dose (mg/kg p.o.)	5-HTP (50 mg/kg i.p.)		5-HTP (250 mg/kg i.p.)	
		Number of head twitches (mean ± SEM)	Syndrome score [a] (mean ± SEM)	Number of head twitches (mean ± SEM)	Syndrome score [a] (mean ± SEM)
Control		1.00 ± 0.39	0.90 ± 0.28	17.00 ± 3.16	11.60 ± 0.60
1	100	0.20 ± 0.20	0.40 ± 0.40	15.80 ± 6.98	7.80 ± 3.47
2	66	1.80 ± 1.31	1.00 ± 0.54	11.20 ± 5.26	6.80 ± 2.46
3	100	0.00 ± 0.00	4.00 ± 0.77*	25.20 ± 4.87	15.20 ± 1.96
4	100	1.80 ± 1.31	1.20 ± 0.73	20.80 ± 4.79	13.40 ± 2.96
5	74	0.00 ± 0.00	0.00 ± 0.00	11.60 ± 2.97	10.80 ± 1.62
6	20	2.20 ± 1.01	1.80 ± 0.80	13.00 ± 4.93	8.20 ± 1.93
7	36	1.00 ± 0.31	1.00 ± 0.31	6.40 ± 2.36*	6.40 ± 1.63**
8	100	0.80 ± 0.37	3.00 ± 0.45*	7.00 ± 4.29	5.40 ± 2.64
9	100	1.40 ± 0.51	1.20 ± 0.37	15.60 ± 6.18	10.40 ± 2.38
10	100	1.20 ± 0.97	0.80 ± 0.58	10.60 ± 5.39	6.00 ± 1.95**
11	50	0.60 ± 0.40	3.20 ± 0.20**	22.00 ± 6.25	12.40 ± 0.68
12	100	0.40 ± 0.40	2.80 ± 0.73**	9.00 ± 4.12	8.25 ± 3.42
Amitriptyline	50	NT	NT	6.60 ± 3.23*	20.80 ± 0.58**
Fluoxetine	100	5.60 ± 1.90*	18.70 ± 0.82**	NT	NT
Tianeptine	100	0.00 ± 0.00	0.00 ± 0.00	1.40 ± 0.98**	0.60 ± 0.50**

NT, not tested; *$P < 0.05$, **$P < 0.01$ compared with control. Each value represents the mean ± SEM of ten animals.

[a] The presence or absence of head-twitches, whole body tremor, forepaw treading, hind-lamb abduction and outstretched posture with the abdomen resting close to the cage floor was scored in five 2 min intervals 14 min after injection of 5-HTP. The total score for each mouse was calculated.

hypothermia with values ranging from 30 to 52%, being the compound **5**, in this regard, the most effective, with a percentage of antagonism (52.28%) superior to that found for imipramine at the same dose (35.28%). When they were tested at the highest dose, only compounds **4** and **5** antagonised the hypothermia by 32.61 and 45.29%, respectively, although without reaching a statistical significance as compared to control.

As regards the effects of these compounds on the head twitches and syndrome induced by 5-HTP (50 mg/kg i.p.), none of them significantly potentiated the head twitch responses induced by this serotonin precursor, but compounds **3** and, to a lesser extent, **8**, **11** and **12**, produced a significant potentiation of the syndrome induced by this drug when compared with control (Table 2), although they were less potent in this regard than fluoxetine (antidepressant of reference).

Concerning the effects of these compounds on the 5-HTP (250 mg/kg i.p.)-induced head twitches, only compound **7** significantly reduced the head twitches induced by this drug (62.35%), an effect also seen with amitriptyline and tianeptine (Table 2). Compounds **8** and **12** also antagonised this effect by a 58.82 and 47.06%, respectively, but not significantly. When the 5-HTP (250 mg/kg i.p.)-induced syndrome was considered, only compounds **7** and **10** produced a significant inhibition by more than 40%. Compounds **8** and, to a lesser extent, **1** and **2**, inhibited this syndrome with values ranging from 41 to 53%, but the results did not reach statistical significance.

On the other hand, the compounds under study did not alter significantly the hypermotility induced by D-amphetamine in mice (data not shown).

3.2. Neuroleptic activity

The majority of the tested compounds significantly antagonised the stereotyped behaviour induced by low doses (3 mg/kg s.c.) of the dopamine agonist apomorphine, with values equal to or above 20% (Table 3). The compounds with the most pronounced effects were **7** at the dose of 36 mg/kg p.o. (44.13% of antagonism) and **12** at 100 mg/kg p.o. (41.89%), although they were less potent than haloperidol (100%). Concerning the effects of the compounds under study on the apomorphine (3 mg/kg s.c.)-induced climbing behaviour, it was found that only compound **7** at the highest dose assayed produced a significant inhibition of this effect (25.73%), but without reaching the values elicited by haloperidol (100%). Compound **12** also produced a 25.73% of antagonism, but it was not statistically significant. On the other hand, even at the highest dose of the compound tested, none of them antagonised significantly the hypothermia induced by a low dose of apomorphine.

3.3. Anxiolytic activity

The results obtained (Table 4) indicated that none of the compounds under study at the doses assayed significantly increased both the percentage of entries

Table 3
Effect of the hetero[2,1]benzothiazepine derivatives on apomorphine (3 mg/kg s.c.) induced stereotypy, climbing and hypothermia in mice

Comp.	Dose (mg/kg p.o.)	Stereotypy mean score	Climbing mean score	Mean decrease in rectal temperature (°C)
Control		7.70±0.28	5.85±0.08	1.83±0.17
1	25	8.33±0.42	6.00±0.00	3.33±0.42**
2	25	6.66±0.49	5.83±0.16	2.78±0.46*
3	25	6.83±0.54	5.66±0.21	2.36±0.31
4	25	7.00±0.51	5.66±0.33	2.91±0.42**
5	25	8.00±0.63	6.00±0.00	1.65±0.34
6	10	6.33±0.56*	5.50±0.34	2.48±0.37
7	25	6.33±0.21*	6.00±0.00	1.15±0.11
8	25	6.16±0.41**	6.00±0.00	2.03±0.57
9	25	6.16±0.41**	6.00±0.00	2.41±0.34
10	25	6.16±0.41**	5.50±0.34	2.90±0.52*
11	25	6.50±0.22	5.66±0.21	2.00±0.82
12	25	5.33±0.33**	5.16±0.54	2.83±0.48*
Haloperidol	1	0.00±0.00**	0.00±0.00**	0.57±0.37**
Control		7.16±0.38	5.83±0.16	2.85±0.35
1	100	7.00±0.63	5.50±0.34	3.61±0.45
2	66	5.16±0.54**	5.66±0.21	4.23±0.41*
3	100	5.83±0.70	5.83±0.16	3.58±0.60
4	100	4.50±0.56**	5.00±0.51	4.30±0.79
5	74	4.50±0.72**	5.33±0.49	2.75±0.42
6	20	5.16±0.75	5.16±0.54	2.13±0.62
7	36	4.00±0.51**	4.33±0.61*	3.11±0.53
8	100	5.50±0.56	5.16±0.40	3.46±0.19
9	100	5.16±0.60*	4.83±0.54	2.58±0.53
10	100	4.66±0.61**	5.50±0.34	2.43±0.42
11	50	5.16±0.48**	5.50±0.05	2.43±0.48
12	100	4.16±0.60**	4.33±0.99	4.01±0.17*
Haloperidol	1	0.00±0.00**	0.00±0.00**	0.35±0.20**

Results are expressed as mean±SEM of 12 mice. *$P < 0.05$; **$P < 0.01$ compared with control.

and percentage of time on the open arms of the plus-maze compared with the control and, therefore, they can not be considered as anxiolytic compounds. On the contrary, compounds **1** and **2** at both doses assayed, together with **3** at 100 mg/kg p.o. seem to have an anxiogenic profile since they reduced both measures (the two indices of anxiety used in this experiment).

3.4. Exploratory activity

It can be seen in Table 4 that there was a significant decrease in the number of head dips in mice treated with the compound **3** at both doses assayed, as well as the compounds **2** (66 mg/kg p.o.), **6** (10 mg/kg p.o.), **11** (25 mg/kg p.o.) and **12** (100 mg/kg p.o.) as compared with controls, with values ranging from 19 to 39%. Nevertheless, these values were not superior in any case to that shown by diazepam (60%).

3.5. Anticonvulsant activity

As shown in Table 5, in contrast to the benzodiazepine used as reference drug (diazepam), the tested compounds did not offer any protection either against seizures and mortality induced by pentylenetetrazol and strychnine. Exception to this rule was compound **11** at a dose of 50 mg/kg p.o., which significantly prolonged the onset of the tonic convulsions and death induced by strychnine, but it did not significantly reduced mortality. On the other hand, an increase of the latency of the clonic and tonic convulsions and death induced by pentylenetrazol as well as a 40% of protection of the animals from the lethal effects of this convulsant were observed with the compound **9** at 100 mg/kg p.o., although the results did not reach in any case statistical significance when compared with the control.

On the contrary, compound **3** (100 mg/kg p.o.) significantly shortened the time of onset of the tonic seizures and death induced by strychnine, which may suggest a proconvulsant action. Additional studies with a subconvulsant dose of strychnine sulfate will be required to confirm this suggestion.

3.6. Muscle relaxant activity

In general, no muscle relaxant activity could be observed with the different compounds assayed when tested with the traction and chimney tests (data not shown). Only compounds **5** (74 mg/kg p.o.), **10** (100 mg/kg p.o.) and **11** (25 and 50 mg/kg p.o.) showed some degree of myorelaxant activity in the traction test with percentage of failures ranging from 30 to 50%, although

Table 4
Effect of the hetero[2,1]benzothiazepine derivatives on holeboard and elevated plus-maze test in mice

Comp. [a]	Holeboard [b]		Plus-maze	
	Number of head-dips	Entries into open arms (%)	Time on open arms (%)	Total entries
Control	19.86±1.43	24.64±3.12	13.92±1.94	9.19±0.76
1 (25)	15.50±2.44	22.44±7.29	5.44±1.83**	6.00±0.95
2 (25)	14.75±3.64	10.55±4.09*	1.84±0.62**	7.00±1.15
3 (25)	14.12±1.64*	30.78±5.18	16.15±4.12	8.75±1.01
4 (25)	16.12±1.84	22.27±4.42	8.25±2.29	8.50±1.31
5 (25)	18.71±2.75	17.47±5.10	6.78±2.55	9.71±1.21
6 (10)	15.12±1.29*	27.71±4.93	18.36±4.47	10.75±1.41
7 (25)	20.12±2.31	24.68±5.60	15.53±6.70	9.75±0.45
8 (25)	20.75±1.99	35.08±4.12	19.53±3.67	12.50±0.75*
9 (25)	23.33±3.19	22.52±5.05	8.17±3.01	9.40±0.81
10 (25)	14.50±2.84	17.14±7.85	7.06±3.50	7.75±2.28
11 (25)	12.50±1.66*	15.00±6.45	4.96±2.31*	7.25±1.60
12 (25)	17.00±1.56	26.01±6.05	10.87±3.86	10.25±1.41
Diazepam (5)	7.90±0.76**	64.71±4.02**	73.49±4.35**	9.75±0.70
Control	22.65±1.32	21.63±1.92	11.70±1.86	9.22±0.35
1 (100)	20.75±2.12	13.62±1.87**	8.33±2.57	10.37±1.07
2 (66)	14.75±2.13**	13.69±4.05	5.81±1.73*	9.37±1.52
3 (100)	18.25±1.22*	11.73±3.64*	4.32±1.56**	7.37±1.36
4 (100)	18.50±2.11	25.70±9.15	18.66±7.78	9.12±1.85
5 (74)	19.33±2.17	17.71±3.72	7.08±2.03	10.00±0.86
6 (20)	17.00±2.07	19.71±7.71	9.02±4.20	9.80±1.77
7 (36)	19.00±3.03	22.02±1.23	9.72±1.94	11.50±1.55
8 (100)	18.87±1.69	21.47±9.77	13.82±6.91	9.75±0.88
9 (100)	19.37±1.95	21.08±4.10	9.42±3.35	9.75±0.77
10 (100)	17.40±3.11	15.58±6.91	11.63±5.58	8.60±1.50
11 (50)	19.12±2.31	13.22±4.30	5.31±2.35	8.50±1.57
12 (100)	13.75±0.48**	14.57±7.98	7.57±5.64	9.80±1.46
Diazepam (5)	6.64±1.03**	64.46±3.58**	64.64±4.29**	8.10±1.08

Results are expressed as mean±SEM of six mice. *$P < 0.05$, **$P < 0.01$ compared with control.
[a] The values in parentheses represent the dose in mg/kg p.o.
[b] The holeboard test was made just before the plus-maze test.

only the value of 50% of failures (120 min after administration of product **11**) reached statistical significance ($P < 0.05$). These percentage of failures, nevertheless, were far from that found for the standard diazepam (100%, $P < 0.05$) at the dose of 20 mg/kg p.o.

4. Discussion

In a previous paper [4], we have found that a series of hetero[2,1]benzothiazepine derivatives showed significant antidepressant activity in the antagonism of tetrabenazine-induced ptosis and motor depression as well as in the forced swimming test in mice. In the present study we wanted to further examine the effects of these benzothiazepine derivatives on other pharmacological assays frequently used for the screening of potential antidepressants such as the potentiation of amphetamine and serotonin actions or the inhibition of the apomorphine-induced hypothermia. Although the specificity and sensitivity of these pharmacological screening models remain doubtful, they continue to be part of numerous drug discovery projects for antidepressants and often provide information on the possible mechanism of action of the tested agents [11,12].

The results obtained with these investigations demonstrated that the compounds **4**, **5**, **7** and **12** proved to be effective in antagonising the apomorphine (16 mg/kg s.c.)-induced hypothermia. It is known that this pharmacological screening model would seem to be specific for antidepressants such as imipramine and viloxazine, which inhibit norepinephrine reuptake, as well as for substances such as salbutamol which have direct or indirect beta-adrenergic activity [13,14]. It has been postulated that hypothermia induced by high dose of apomorphine seems to involve not only the dopaminergic system but also beta-adrenergic receptors. Apomorphine, by an action at presynaptic D_2 receptors situated on noradrenergic nerve terminals, would, therefore, prevent the release of noradrenaline. These receptors are only sensitive to high doses of apomorphine, since they are either non-functional or limited in number. It is also possible that high doses of apomorphine may stimulate presynaptic noradrenergic receptor as well as

Table 5
Effect of the hetero[2,1]benzothiazepine derivatives on convulsions and deaths induced by pentylenetetrazol (120 mg/kg i.p.) and strychnine (2.5 mg/kg i.p.) in mice

Comp.	Dose (mg/kg p.o.)	t_1 (min) (mean ± SEM)	t_2 (min) (mean ± SEM)	t_3(min) (mean ± SEM)	Survival
Pentylenetetrazol					
Control		1.13 ± 0.08	5.49 ± 0.76	5.84 ± 0.75	0
1	100	1.35 ± 0.20	4.90 ± 0.80	5.14 ± 0.81	0
2	66	1.32 ± 0.15	4.52 ± 1.30	4.73 ± 1.27	0
3	100	1.35 ± 0.16	5.10 ± 0.72	5.33 ± 0.24	0
4	100	1.06 ± 0.07	4.95 ± 1.83	5.44 ± 1.77	0
5	74	1.29 ± 0.17	3.46 ± 0.77	4.17 ± 0.70	0
6	20	1.24 ± 0.07	5.71 ± 1.69	6.08 ± 1.70	0
7	36	1.16 ± 0.07	6.96 ± 1.04	7.20 ± 1.00	0
8	100	1.59 ± 0.22	6.57 ± 2.43	8.44 ± 2.83	0
9	100	4.48 ± 1.50	11.72 ± 3.44	11.92 ± 3.38	40
10	100	1.29 ± 0.07	5.60 ± 1.00	5.77 ± 1.29	20
11	50	1.50 ± 0.18	6.78 ± 1.36	7.17 ± 1.39	0
12	100	1.28 ± 0.18	3.12 ± 0.65*	7.29 ± 2.53	0
Diazepam	20				100*
Strychnine					
Control		3.66 ± 0.54	4.05 ± 0.27	4.34 ± 0.28	0
1	100	4.92 ± 1.30	7.41 ± 2.28	7.95 ± 2.27	0
2	66	3.15 ± 0.19	3.56 ± 0.29	4.15 ± 0.30	0
3	100	2.58 ± 0.14	2.99 ± 0.12**	3.39 ± 0.12*	0
4	100	3.31 ± 0.18	3.59 ± 0.24	4.10 ± 0.16	0
5	74	3.32 ± 0.80	3.84 ± 0.71	4.53 ± 0.66	0
6	20	3.86 ± 0.32	4.31 ± 0.33	5.56 ± 0.85	0
7	36	4.32 ± 0.52	4.97 ± 0.63	6.28 ± 0.82	20
8	100	3.65 ± 0.64	4.51 ± 1.06	5.37 ± 1.33	0
9	100	3.19 ± 0.20	3.97 ± 0.38	5.36 ± 0.53	0
10	100	3.80 ± 0.67	4.75 ± 0.93	6.21 ± 1.45	0
11	50	4.29 ± 0.47	6.21 ± 0.80*	8.49 ± 1.40*	20
12	100	3.69 ± 0.37	4.75 ± 0.58	5.67 ± 1.01	0
Diazepam	20	5.17 ± 0.68	5.94 ± 0.74*		100*

Values are means of five mice. t_1 (clonic convulsion onset); t_2 (tonic convulsion onset), t_3 (time of death). *$P < 0.05$, **$P < 0.01$ compared with control.

D_2 receptors [12]. Thus, antagonism of the hypothermic effect of apomorphine (16 mg/kg) by some of the compounds assayed suggest that they may be acting at noradrenergic receptors.

In order to investigate their performance in the serotonin system, the potentiation of 5-HTP-induced head twitches and syndrome in mice was also examined. It is generally accepted that the ability of a compound to induce head twitches and syndrome in mice following a threshold dose of 5-HTP (50 mg/kg) indicates the compound is increasing the amount of serotonin available at brain synpases [15,16]. In these experiments it was observed that only compound **3**, **8**, **11** and **12** significantly potentiated the syndrome induced by 5-HTP, indicating either their possible serotonin reuptake or monoamine oxidase inhibition [15,16].

On the other hand, the compounds seem to be devoid of stimulating activity upon the dopaminergic receptor as it does not increase the hypermotility induced by D-amphetamine, a different profile from that seen with antidepressants such as the dopamine reuptake inhibitor nominfesine or the MAO inhibitor tranylcypromine, which cause marked increases in dopaminergic function [11].

Taking together, our results in the antidepressant assays indicate that some of the compounds tested (**4**, **5** and **7**) may exert their antidepressant effects by enhancing directly or indirectly the quantity of norepinephrine in acute administration and others (**3**, **8** and **11**) by increasing the quantity of serotonin in acute administration. Compound **12** seems to affect both neurotransmitter, since it was found to be active in preventing apomorphine-induced hypothermia as well as potentiating the 5-HTP-induced syndrome in mice.

To complete its pharmacological profile, the potential antipsychotic action of these compounds was examined by using the antagonism of apomorphine (3 mg/kg)-induced hypothermia and other behavioural effects in mice. It is well known that low doses of apomorphine (3 mg/kg) produce hypothermia and other effects in mice by stimulation of central dopamine receptors [13,17]. Consequently these effects are prevented by dopamine receptor blockers such as the neuroleptics. In our study some of the tested products antagonised the stereotyped

and/or climbing behaviour without affecting hypothermia induced by 3 mg/kg apomorphine, in contrast to the classical neuroleptic haloperidol, but in line with other neuroleptics, such as chlorpromazine, clozapine and thioridazine [13]. Antagonism of apomorphine-induced stereotypy is generally considered to reflect postsynaptic dopamine receptor antagonist properties preferentially in the nigrostriatal areas, and, therefore, the majority of the compounds studied may be expected to produce extrapyramidal effects in humans [17,18]. On the other hand, compound **7** at the highest dose assayed also blocked climbing behaviour, suggesting that this compound may also interact with the dopamine receptor in the mesolimbic areas and it may possess antipsychotic activity in man [19,20].

It must be pointed out that four of the compounds studied (**4**, **5**, **7** and **12**) antagonised both hypothermia produced by 16 mg/kg apomorphine and stereotyped movements induced by 3 mg/kg, a profile also seen with the antidepressant drug amoxapine [13]. These data would suggest that they seem able both to block the dopaminergic system and activate the noradrenergic system.

Furthermore, our data show that compounds **7**, **8** and **12** may possess 5-HT_2 antagonist properties since they inhibited the head twitches induced by high doses of 5-HTP [21,22]. It is interesting to note that compounds **8** and **12** not only block the appearance of head twitches induced by an ED_{90} dose of L-5-HTP, supporting the concept that they are 5-HT_2 receptor antagonist, but also potentiate an ED_{10} dose of 5-HTP, suggesting that they may have 5-HT uptake or monoamine oxidase inhibition properties in vivo. The consequences of the fact that these compounds have two apparently antagonistic properties in terms of its potential antidepressant efficacy are unknown. However, the efficacy of clomipramine as antidepressants suggests that both properties may contribute to the effectiveness as an antidepressant [21]. In fact, there is an increasing body of evidence that suggests that the additional antiserotonergic activities, which possess some new antidepressant or antipsychotic drugs may contribute to the better efficacy and lower incidence of side effects observed with them [23–26].

On the other hand, the potential anxiolytic action of the products was assessed in the elevated plus-maze test, which is based on a conflict situation between the exploratory drive of rodents and their aversion from high and open spaces. Anxiolytic compounds selectively elevate the percentage of open arms entries into, and the time spent on, the open arms of the maze. In contrast, anxiogenic compounds diminish these two measures [7,8]. Unfortunately, we did not find anxiolytic activity for these compounds, on the contrary three of them (**1**, **2** and **3**) exerted an anxiogenic profile. It must be pointed out that this anxiogenic profile detected in the plus-maze test is not due to sedative properties, because there was no significant reduction of the number of arm entries of mice in the dose range within which compounds were found to be anxiogenic.

Finally, we investigated the effects of these compounds on other common psychopharmacological tests such as the exploratory behaviour, anticonvulsant and muscle relaxant activities. In general, some of these compounds produced a slight decrease in exploratory behaviour pattern as evident from the results of head-dip test, and exert practically no myorelaxant and anticonvulsant effects in mice.

In conclusion, the behavioural data obtained in the present and previous studies [4] demonstrate that these compounds appear to have an antidepressant-like effect in mice with little or no propensity of causing sedative, anticholinergic and muscle relaxant effects. Although the precise mechanism involved in the observable antidepressant activity is not clear, the experimental observations suggest a possible direct or indirect facilitation of the central serotonergic and/or noradrenergic transmission for seven of the 12 compounds studied. Additionally, some of them (specially compound **7**) possess antidopaminergic activity, suggesting their potential use as antipsychotic agents. From the point of view of the structure–activity relationships, no clear-cut correlation between the heterocyclic rings or the substituents of the aminoethoximino side-chains used in the three series and their respective pharmacological activities could be established. In view of the results obtained in these assays, further pharmacological studies are needed with the most interesting compounds in order to investigate the exact mechanisms involved in the effects observed.

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