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N,*N*′-Dicyclohexylsulfamide and *N*,*N*′-diphenethylsulfamide are anticonvulsant sulfamides with affinity for the benzodiazepine binding site of the $GABA_A$ receptor and anxiolytic activity in mice

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ABSTRACT

A set of sulfamides designed, synthesized and evaluated against maximal electroshock seizure (MES) and pentilenetetrazol (PTZ) tests with promising results, were tested for their affinity for the benzodiazepine binding site of the $GABA_A$ receptor. The most active compounds, *N*,*N*′-dicyclohexylsulfamide (**7**) and *N*,*N*′-diphenethylsulfamide (**10**), competitively inhibited the binding of [^{3}H]-flunitrazepam to the benzodiazepine binding site with $K_i \pm$ SEM values of 27.7 ± 4.5 μM ($n = 3$) and 6.0 ± 1.2 μM ($n = 3$), respectively. The behavioral actions of these sulfamides, i.p. administered in mice, were examined in the plus-maze, hole-board and locomotor activity assays. Compound **7** exhibited anxiolytic-like effects in mice evidenced by a significant increase of the parameters measured in the hole-board test (at 1 and 3 mg/kg) and the plus-maze assay (at 1 and 3 mg/kg). Compound **10** evidenced anxiolytic activity in the plus-maze and the hole-board tests at 1 mg/kg. Locomotor activity of mice was not modified by compound **7** or **10** at the doses tested. Flumazenil, a non selective benzodiazepine binding site antagonist, was able to completely reverse the anxiolytic-like effects of these sulfamides, proving that the $GABA_A$ receptor is implicated in this action. Anxiety represents a major problem for people with epilepsy. The use of anxiolytic and anticonvulsant sulfamides would be beneficial to individuals who suffer from both disorders.

1. Introduction

Epilepsy is a common chronic neurological disorder of varied etiology characterized by recurrent unprovoked seizures that affects about 1% of the world population. It is the third most frequent neurological disorder, after cerebrovascular disease and dementia [1].

From the beginning of medical history, continuous efforts have been made to prevent and treat seizures, mainly focused on the development of new antiepileptic drugs. However, many patients do not achieve a complete response with the available therapy and they experience significant adverse effects, proving that there still is a clinical need for alternative drug treatments and novel compounds with higher potency, specificity and low toxicity.

It is now recognized that anxiety can have a profound influence on the quality of life of patients with epilepsy: up to 50 or 60% of the affected people with chronic epilepsy have various mood disorders including depression and anxiety. The relationship between anxiety disorders and epilepsy is complex [2] and there are no systematic studies for best treatment practice to reduce this psychiatric comorbidity in patients with epilepsy. Although several antiepileptic drugs have proven their value in the psychiatric treatment of mental disorders, many of these drugs have dose limiting side effects.

Marketed antiepileptic drugs predominantly target voltage-gated cation channels (the α subunits of voltage gated Na^+ channels and also T-type voltage-gated Ca^{2+} channels), attenuate excitatory neurotransmission (NMDA and kainate) or influence gamma-aminobutyric acid (GABA)-mediated inhibition.

Previous works from our laboratory described the synthesis of a series sulfamides, new bioisosteric compounds derived from valpromides, and their anticonvulsant action was confirmed through the evaluation against the maximal electroshock (MES) and the pentylenetetrazole (PTZ) tests, following the standard procedures of the Anticonvulsant Drug Development Program of the National Institute of Health [3,4].

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In this work the capacity of the synthetic sulfamides to bind to the benzodiazepine binding site of the $GABA_A$ receptor was evaluated considering that GABA is the most important inhibitory neurotransmitter and that it is implicated in both epilepsy and anxiety disorders. The most active compounds, *N*,*N*′-dicyclohexylsulfamide (**7**) and *N*,*N*′-diphenethylsulfamide (**10**), were studied for their anxiolytic effects in mice.

2. Materials and methods

2.1. Animals

Adult male Swiss mice weighing 25–30 g were used in the pharmacological assays and adult male rats (200–300 g) Wistar strain for biochemical studies, both were obtained from the Central Animal House of the School of Pharmacy and Biochemistry, University of Buenos Aires. For behavioral assays mice were housed in groups of five in a controlled environment (20–23 °C), with free access to food and water and maintained on a 12 h/12 h day/night cycle, light on at 06:00 AM. Housing, handling, and experimental procedures complied with the recommendations set forth by the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and the Institutional Committee for the Care and Use of Laboratory Animals, University of Buenos Aires, Argentina. All efforts were taken in order to minimize animal suffering. The number of animals used was the minimum number consistent with obtaining significant data. The animals were randomly assigned to any treatment groups and were used only once. The behavioral tests were evaluated by experimenters who were kept unaware of the treatment administered and were performed between 10:00 AM and 2:00 PM.

2.2. Chemistry

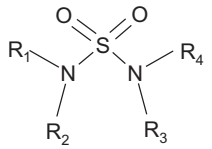
The synthesis of the sulfamide derivatives were performed as described previously [3,4] and the synthetic processes were selected according to the sulfamide substituents.

2.3. Biochemical assay ([^{3}H]-flunitrazepam binding assay)

A radioligand binding assay was used to evaluate the putative action of the compounds (**1**–**22**, Table 1) on the benzodiazepine binding site of the $GABA_A$ receptor complex. The binding of [^{3}H]-flunitrazepam (81.8 Ci/mmol; obtained from PerkinElmer Life and Analytical Sciences, Boston, MA, USA) to the benzodiazepine binding site was performed in washed crude synaptosomal membranes from rat cerebral cortex. Membranes were prepared according to [5]. Briefly, the brains were rapidly dissected out on ice and the different structures were homogenized in 10 volumes of 0.32 M sucrose and centrifuged at $900 \times g$ for 10 min. The resulting supernatant was centrifuged at $100{,}000 \times g$ for 30 min and the pellet washed twice in 25 mM Tris–HCl buffer pH 7.4 at $100{,}000 \times g$ for 30 min, and stored at −20 °C until used. Protein determination was carried out with Bradford's method [6].

The compounds were added to 0.2–0.3 mg membrane protein suspended in 1 ml of 25 mM Tris–HCl buffer in the presence of [^{3}H]-flunitrazepam 0.3 nM. In the screening assays each compound was tested at 300 μM in triplicate. In the competition assays, the incubations were done with 10–600 μM of compound **4**, 1–300 μM of compound **7** and 1–100 μM of compound **10**. Diazepam was used as positive control in concentrations between 1 and 100 nM. In saturation assays, increasing concentrations of [^{3}H]-flunitrazepam (0.2–11 nM) were incubated in the presence of vehicle, compound **7** 50 μM or compound **10** 10 μM. Non-specific

Table 1
Activities of the sulfamide derivatives on the benzodiazepine binding site, maximal electroshock seizure (MES) and pentilenetetrazol (PTZ) tests.



Compound	R_1	R_2	R_3	R_4	Binding inhibition[a]	MES[b]	PTZ[b]
1	nPropyl	H	nPropyl	H	–	2	1
2	nPropyl	nPropyl	nPropyl	nPropyl	+	3	3
3	nButyl	H	H	H	–	1	I
4	nButyl	H	nButyl	H	++	ED_{50} 61 mg/kg	I
5	nButyl	nButyl	nButyl	nButyl	–	3	1
6	Cyclopropyl	H	Cyclopropyl	H	–	3	3
7	Cyclohexyl	H	Cyclohexyl	H	++	ED_{50} 39 mg/kg	I
8	Cyclohexyl	H	H	H	–	1	ND
9	Benzyl	H	Benzyl	H	+	ED_{50} 66 mg/kg	I
10	Phenethyl	H	Phenethyl	H	+++	ED_{50} 80 mg/kg	I
11	Morpholin		Morpholin		–	I	
12	nButyl	H	*tert*-Butoxycarbonyl	H	–	1	3
13	nButyl	nButyl	*tert*-Butoxycarbonyl	H	–	1	3
14	nBenzyl	H	*tert*-Butoxycarbonyl	H	–	3	3
15	2-Propylpentyl	H	β-Alanine methyl ester	H	+	ED_{50} 23 mg/kg	1
16	2-Propylpentyl	H	Glycine methyl ester	H	–	ED_{50} 34 mg/kg	3
17	β-Alanine methyl ester	H	β-Alanine methyl ester	H	+	1	3
18	L-Alanine methyl ester	H	L-Alanine methyl ester	H	–	3	3
19	L-valine methyl ester	H	L-valine methyl ester	H	–	1	3
20	Glycine methyl ester	H	*tert*-Butoxycarbonyl	H	–	3	3
21	L-Alanine methyl ester	H	*tert*-Butoxycarbonyl	H	–	3	3
22	nCyclohexyl	H	*tert*-Butoxycarbonyl	H	+	ND	ND

ND, not determined; I, inactive up to 100 mg/kg.

[a] Capacity of the compounds, at 300 μM, to inhibit the binding of [^{3}H]-flunitrazepam to the benzodiazepine binding site of the $GABA_A$ receptor indicated as: inhibition > 80% (+++); inhibition; 40–80% (++); inhibition 20–40% (+); and inhibition < 20% (–).

[b] Values previously reported [3,4]: (1) anticonvulsant activity at 100 mg/kg or less; (2) anticonvulsant activity at doses higher than 100 mg/kg; (3) compound inactive at any doses up to 300 mg/kg.

binding was measured in the presence of flunitrazepam 10 μM and represented 5–15% of the total binding. The incubations were carried out at 4 °C for 1 h. After incubation, the assays were terminated by filtration under vacuum through Whatman GF/B glass-fiber filters followed by washing three times with 3 ml each of incubation medium. Individual filters were incubated overnight with scintillation cocktail (OptiPhase 'HiSafe' 3) before measuring radioactivity in a Wallac Rackbeta 1214 liquid scintillation counter.

2.4. Drugs and administration protocols

Synthetic starting material reagents and solvents were of analytical reagent grade and were purchased from Sigma–Aldrich and Fluka.

Diazepam (Roche Diagnostics, Argentina), flumazenil (Richet S.A., Argentina) and compounds **7** and **10** were dissolved by using the sequential addition of dimethylsulfoxide, a solution of 0.25% Tween 80 and saline; up to final concentrations of 5%, 20% and 75%, respectively. The mice were intraperitoneally (i.p.) injected 30 min before performing the pharmacological tests. In the blockade experiments flumazenil was i.p. injected 45 min prior to testing. The volume of i.p. injections was 0.15 ml/30 g of the body weight. In each session, a control group receiving only vehicle was tested in parallel. Vehicle control mice showed no significant differences in any of the tests assayed compared to mice treated with saline (data not shown).

2.5. Pharmacological studies

2.5.1. Anticonvulsant activity

The anticonvulsant evaluation of the synthesized compounds was performed following the standard procedures proposed by The National Institute of Health anticonvulsant drug development program, via the anticonvulsant screening project (ASP) [7,8] and were previously reported [3,4] (Table 1). The initial evaluation (phase 1) includes the use of two convulsant tests: maximal electroshock seizure test (MES) and pentylenetetrazol test (PTZ), the most widely used [9]. The MES test is associated with the electrical induction of the seizure, whereas PTZ test involves a chemical induction to generate the convulsion. The compounds were administrated to animals (mice) intraperitoneally at three doses (30, 100, and 300 mg/kg), and all the assays were performed at 0.5 and 4 h.

Quantitative biological studies (phase II) were performed for the most promising compounds from phase I (in MES test). At this stage, the anticonvulsant activity was expressed as median effective dose, ED_{50}, which determines the drug concentration that is effective in the 50% of the tested animals. The evaluations were performed at the time of peak effect previously determined. The method of Litchfield and Wilcoxon was used to compute the ED_{50} values [10].

2.5.2. Hole-board assay

This assay was conducted in a walled black Plexiglass arena with a floor of 60 cm × 60 cm and 30 cm high walls, with four centered and equally spaced holes in the floor, 2 cm in diameter each. The holes housed an infrared emitting diode and an infrared detector oriented along a diameter and perfectly aligned. Each of these pairs forms a hole exploration sensor, as previously described [11]. The apparatus was illuminated by an indirect and dimly light of approximately 125 lx. Each animal was placed in the center of the hole-board and allowed to freely explore the apparatus for 5 min. The number of holes explored and the duration of the explorations were measured automatically and shown in real time to the observer. At the end of the experiment all the information was stored in a file for post-experiment study. The number of mouse "rearings" was detected visually and recorded by the observer, who was blinded to the drug treatments.

2.5.3. Elevated plus-maze test

The elevated plus-maze set-up consisted of a maze of two open arms, 25 cm × 5 cm, crossed by two closed arms of the same dimensions, with free access to all arms from the crossing point. The closed arms had walls 15 cm high all around. The maze was suspended 50 cm from the room floor. Mice were placed on the central part of the cross facing an open arm. The number of entries and time spent in open arms were counted during 5 min under red dim light. An arm entry was defined as all four paws having crossed the dividing line between an arm and the central area. The total exploratory activity (number of entries in both arms) was also determined [12]. The observer recording the plus-maze parameters was blinded to the drug treatments.

2.5.4. Locomotor activity test

Because all behaviors measured in the tests performed depend on locomotor activity that is probably the confounding issue; the spontaneous locomotor activity was recorded in separate experiments. The spontaneous locomotion activity was measured in a box made of Plexiglass, with a floor of 30 cm × 15 cm and 15 cm high walls as previously described [13]. The locomotor activity was expressed as total light beam counts per 5 min.

2.6. Statistical analyses

For the competition binding, data were analyzed by nonlinear regression of specific bound vs. radioligand concentration. Regressions for two (full model) and one (reduced model) binding site(s) were further compared by the extra sum-of-squares F test. According to the best fit model, the affinity constant (K_d) and number of binding sites (B_{max}) were estimated and compared (GraphPad Prism version 5.0® software). K_i values were calculated using the Cheng-Prusoff/Chou equation: $K_i = IC_{50}/[1 + (L/K_d)]$, where K_i refers to the inhibition constant of the unlabeled ligand, IC_{50} is the concentration of unlabeled ligand required to reach half-maximal binding, K_d refers to the equilibrium dissociation constant of the radioactive ligand and L refers to the concentration of radioactive ligand. For saturation binding, data were fitted by nonlinear regression using the equation $y = (B_{max})(x)/(K_d + x)$, where y is specifically bound [^{3}H]-ligand in dpm, B_{max} is maximal binding, and x is the concentration of [^{3}H]-ligand.

The effects of the compounds in mice were analyzed by one-way analysis of variance (ANOVA) and post hoc comparisons between treatments and vehicle were made using Dunnett multiple comparison test. The blockade experiments were analyzed by two-way ANOVA (pre-treatment vs. treatment) and post hoc comparison was made using Bonferroni post test. Significance levels were set at $P < 0.05$.

3. Results

3.1. Effects of the synthetic sulfamides on [^{3}H]-flunitrazepam binding

The capacity of the compounds, tested at 300 μM, to inhibit the binding of [^{3}H]-flunitrazepam to the benzodiazepine binding site of the $GABA_A$ receptor is shown in Table 1.

The results pointed out that sulfamides **2**, **4**, **7**, **9**, **10**, **15**, **17** and **22** exhibited low to high activity in the binding assay. The compounds with the highest activity in the binding assay; **4**, **7** and **10**; inhibited the binding of [^{3}H]-flunitrazepam with K_i values (mean ± S.E.M.) of 215 ± 6 μM (n = 3); 27.7 ± 4.5 μM (n = 3) and 6.0 ± 1.2 μM (n = 3), respectively (Fig. 1). Concentrations higher than

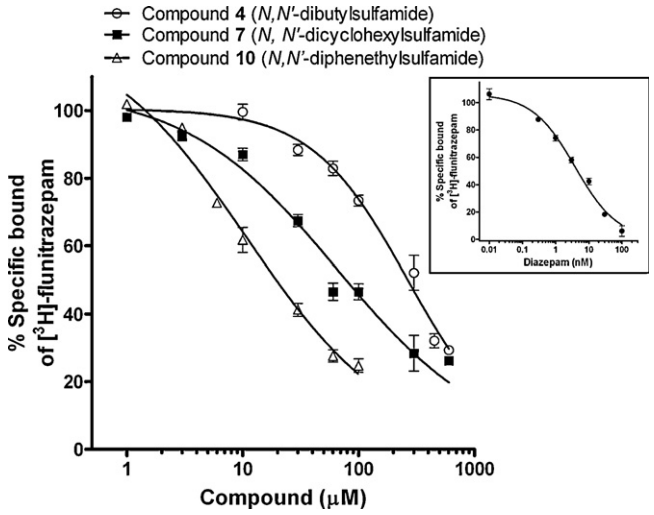


Fig. 1. Competition curves of compounds **4**, **7** and **10** for [^{3}H]-flunitrazepam binding to washed crude synaptosomal membranes of the rat cerebral cortex. Inset, competition curves of diazepam. Data shown represent the mean ± S.E.M. of three independent experiments performed in duplicate.

0.6 mM were not evaluated due to the limited solubility of the compounds. Diazepam, used as a positive control, gave a $K_i = 0.0070 \pm 0.0005$ μM ($n = 5$), as expected [13]. In all cases, data obtained were best fitted to one site binding hyperbola. Scatchard plot analysis of saturation curves (Fig. 2) showed that both most active compounds **7** (50 μM) and **10** (10 μM) competitively inhibited the binding of [^{3}H]-flunitrazepam with a significant decrease in [^{3}H]-flunitrazepam binding apparent affinity (K_d) [$F(2,14) = 18.57$, $P = 0.0001$] and no change in maximal binding (B_{max}) [$F(2,14) = 0.68$, $P = 0.5221$]. $K_d \pm$ S.E.M. and $B_{max} \pm$ S.E.M. values for vehicle and compound **7** were (1.38 ± 0.09) nM and (2.58 ± 0.06) pmol/mg protein, and (2.77 ± 0.25) nM and (2.58 ± 0.09) pmol/mg protein, respectively; while the values for compound **10** were (2.50 ± 0.32) nM and (2.46 ± 0.11) pmol/mg protein.

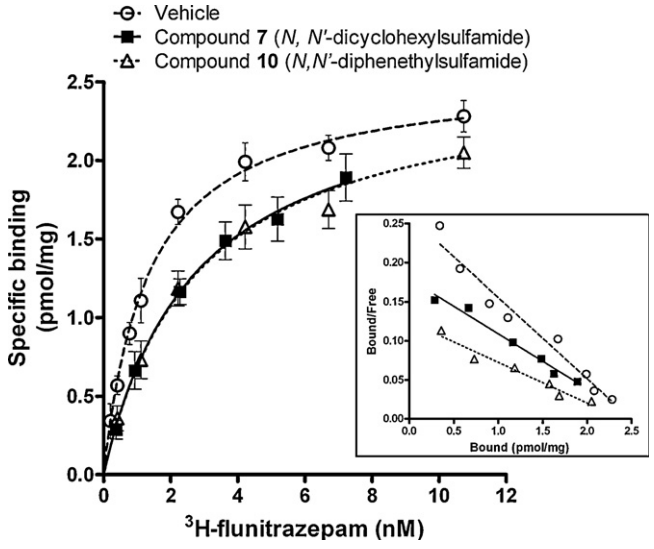


Fig. 2. Effect of compound **7** (50 μM) and **10** (10 μM) on [^{3}H]-flunitrazepam binding to washed crude synaptosomal membranes of the rat cerebral cortex. Saturation isotherms and Scatchard plot (inset) of [^{3}H]-flunitrazepam (0.2–11 nM) are shown. Data were fitted to one site binding hyperbolas and the apparent affinity constant (K_d) and the number of binding sites (B_{max}) were estimated. Data shown represent the mean ± S.E.M. of three independent experiments performed in duplicate. Inset, representative experiment done in duplicate and replicated three times.

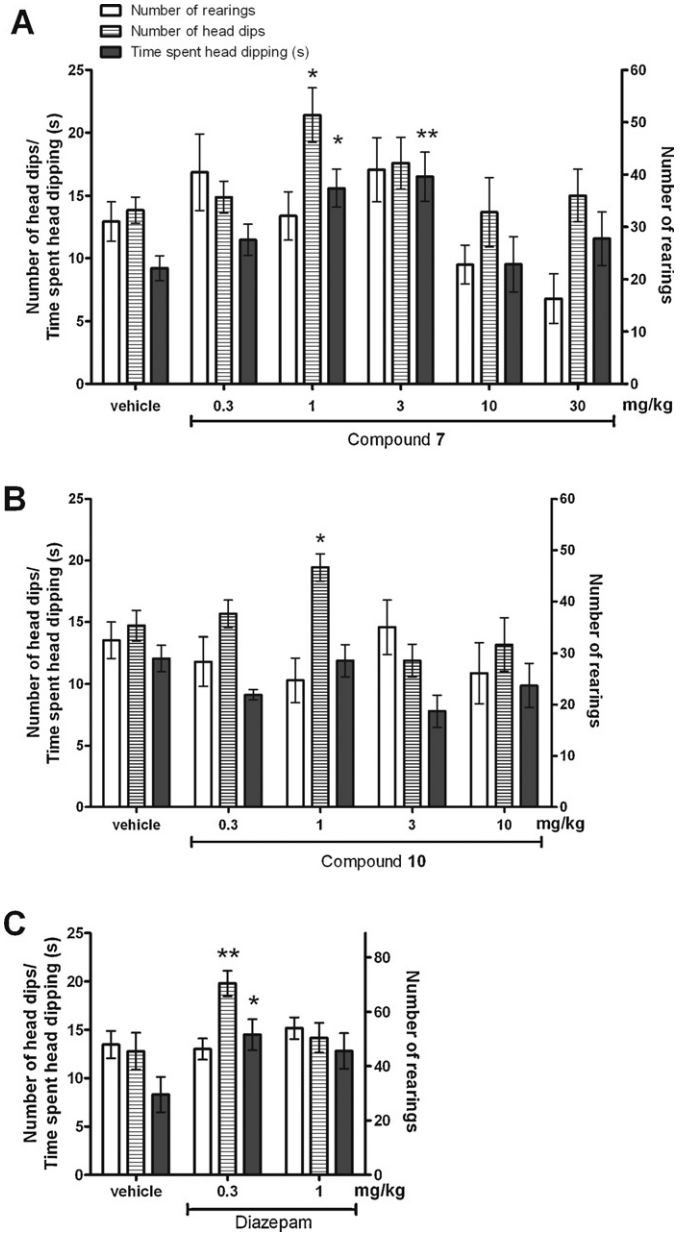


Fig. 3. Effect of the i.p. injection of compounds **7**, **10** and diazepam in the hole-board test in mice. Results are expressed as mean ± S.E.M. of the hole-board parameters registered in 5 min sessions for (A) compound **7**; (B) compound **10** and (C) diazepam. The symbols denote significance levels: $^*P < 0.05$, $^{**}P < 0.01$ significantly different from vehicle; Dunnett's multiple comparison test after one-way ANOVA. Number of animals per group ranged between 6 and 14, control animals were 18.

3.2. Behavioral effects of sulfamides 7 and 10 in the hole-board and the locomotor activity tests

The effects of compounds **7**, **10** and diazepam, used as a reference compound, in the hole-board and locomotor activity tests are shown in Figs. 3 and 4, respectively. For compound **7** ANOVA indicated a significant effect on the number of head dips [$F(5,60) = 2.438$, $P = 0.0445$], the time spent head dipping [$F(5,60) = 3.719$, $P = 0.0053$], the number of mouse rearings [$F(5,60) = 3.329$, $P = 0.0102$] (Fig. 3A) but not for the locomotor activity counts [$F(5,68) = 2.133$, $P = 0.0719$] (Fig. 4A). Comparisons between the vehicle control group and experimental groups (Dunnett's procedure) indicated that compound **7** increased the number of head dips at 1 mg/kg ($P < 0.05$) and the time spent head dipping at 1 mg/kg ($P < 0.05$) and 3 mg/kg ($P < 0.001$) (Fig. 3A).

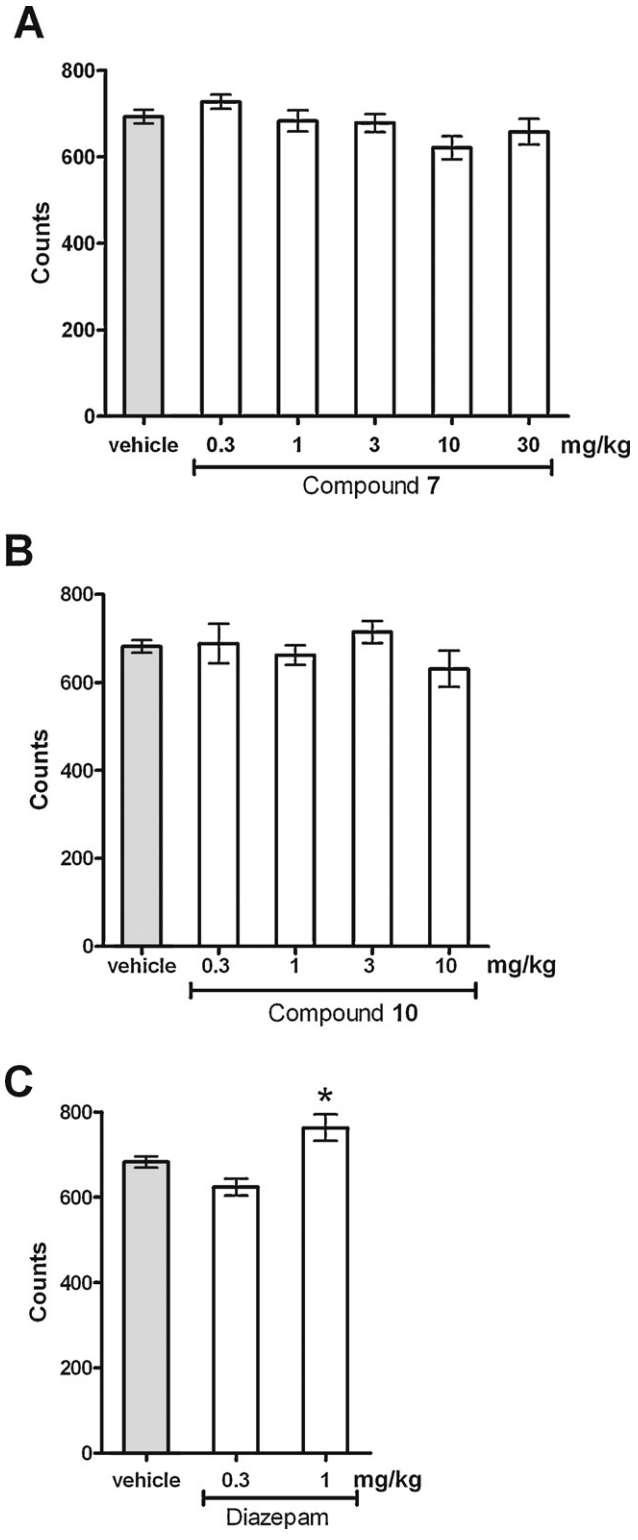


Fig. 4. Effect of the i.p. injection of compounds **7**, **10** and diazepam in the locomotor activity test in mice. Results are expressed as mean ± S.E.M. of the spontaneous locomotor activity counts; registered in 5 min sessions for (A) compound **7**; (B) compound **10** and (C) diazepam. The symbols denote significance levels: $^{*}P < 0.05$ significantly different from vehicle; Dunnett's multiple comparison test after one-way ANOVA. Number of animals per group ranged between 6 and 17, control animals were 21.

In the case of compound **10** ANOVA indicated a significant effect only in the number of head dips [$F(4,58) = 4.134$, $P = 0.0052$]. Control and experimental groups compared with Dunnett's procedure revealed that sulfamide **10** significantly increased the

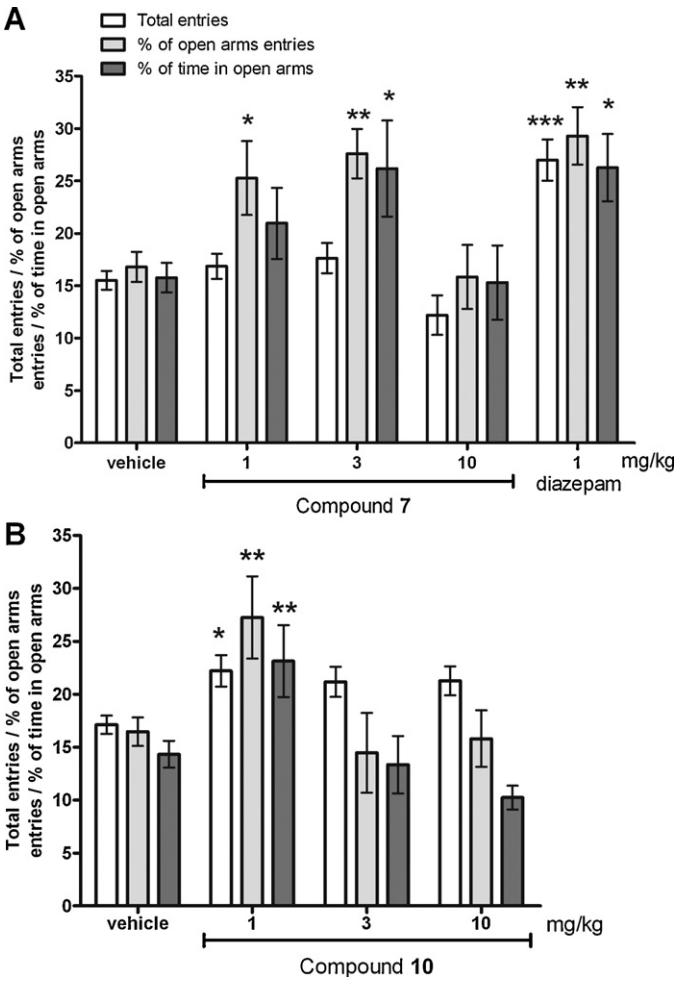


Fig. 5. Effect of the i.p. injection of compound **7**, **10** and diazepam in the plus-maze test in mice. Results are expressed as mean ± S.E.M. of total arm entries, percentage of open arm entries and percentage of time spent in open arms; registered in 5 min sessions. The symbols denote significance levels: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ significantly different from vehicle; Dunnett's multiple comparison test after one-way ANOVA. Number of animals per group ranged between 10 and 14, control animals were 33.

number of head dips at 1 mg/kg ($P < 0.05$, Fig. 3B). Locomotor activity of mice was not modified by compound **10** at the doses tested (Fig. 4B).

An anxiolytic-like behavior could be observed with mice treated with diazepam at 0.3 mg/kg (number of head dips, $P < 0.001$, and time spent head dipping, $P < 0.05$) (Fig. 3C). Diazepam, instead, increased locomotor activity at 1 mg/kg ($P < 0.05$) (Fig. 4C).

3.3. Effects of sulfamides 7 and 10 in the plus-maze test

The effect of compound **7** in the plus-maze test is shown in Fig. 5A. ANOVA of the results obtained yielded statistically significant differences in the percentage of open arms entries [$F(4,69) = 6.197$, $P = 0.0003$], percentage of time spent in open arms [$F(4,69) = 3.336$, $P = 0.0148$], total entries [$F(4,69) = 12.46$, $P < 0.0001$]. Compound **7**, at 1 and 3 mg/kg, significantly increased the percentage of open arm entries (Dunnett's procedure; $P < 0.05$, $P < 0.01$, respectively) and at 3 mg/kg the time spent in open arms was also augmented compared to control mice (Dunnett's procedure; $P < 0.05$). Diazepam (1 mg/kg) increased the percentage of open arm entries ($P < 0.001$), the percentage of time spent in open arms ($P < 0.05$) and the total arm entries ($P < 0.001$).

The anxiolytic-like effect of compound **10** is shown in Fig. 5B. ANOVA evidenced statistically significant differences in the total arm entries [$F(3,61) = 3.597$, $P = 0.0184$], the percentage of open arms entries [$F(3,61) = 4.055$, $P = 0.0108$] and the percentage of time spent in open arms [$F(3,61) = 5.275$, $P = 0.0027$]. At the dose of 1 mg/kg there was a significant increase in the percentage of open arm entries ($P < 0.01$), the percentage of time spent in open arms ($P < 0.01$) and the total arm entries ($P < 0.05$).

3.4. Effects of the administration of flumazenil on the anxiolytic activity of sulfamides 7 and 10

Flumazenil (5 mg/kg, i.p.), a specific antagonist for the benzodiazepine binding site of the $GABA_A$ receptor, did not evoked any response by itself in the hole-board, locomotor activity and plus-maze tests. Several doses of this antagonist were tested alone to find the maximal dose devoid of intrinsic action to be used in the blockade experiments.

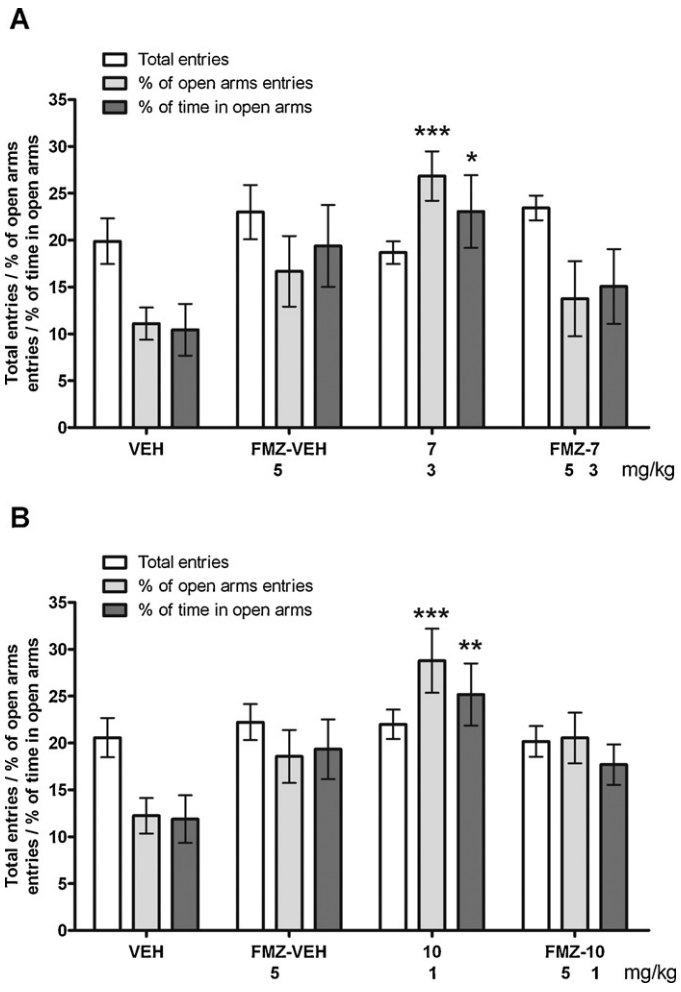


Fig. 6. Effect of flumazenil on the anxiolytic activity of sulfamides **7** and **10**. Mice were pretreated with vehicle (VEH) or flumazenil (FMZ) 15 min before the i.p. injection of compound **7** or compound **10**. Bars indicate mean ± S.E.M of: (A) the total arm entries, the percentages of open arm entries and time spent in open arms evaluated in the plus-maze assay for compound **7** [$F(1,29) = 0.1778$, $P = 0.6764$; $F(1,29) = 8.966$, $P = 0.0056$; $F(1,29) = 4.394$, $P = 0.0449$ two-way ANOVA, respectively]. $^{***}P < 0.001$, $^{*}P < 0.05$, significantly different from VEH/VEH (Bonferroni post hoc test); (B) the total arm entries, the percentages of open arm entries and time spent in open arms evaluated in the plus-maze assay for compound **10** [$F(1,38) = 0.9432$, $P = 0.3376$; $F(1,38) = 6.226$, $P = 0.0170$; $F(1,38) = 6.832$, $P = 0.0128$ two-way ANOVA, respectively]. $^{***}P < 0.001$, $^{**}P < 0.01$, significantly different from VEH/VEH (Bonferroni post hoc test).

As shown in Fig. 6A, the anxiolytic-like effect of compound **7** (3 mg/kg), evidenced in the plus maze test, was totally abolished by the pre-treatment of mice with flumazenil. Similarly, the administration of this specific antagonist could completely reverse the percentage of open arm entries and the percentage of time in open arms elicited by compound **10** (1 mg/kg) in the elevated plus-maze assay, as depicted in Fig. 6B.

4. Discussion

Anxiety disorders represent a frequent and clinically important comorbid disorder in patients with epilepsy. In this report we described the anxiety-like activity of two synthetic sulfamides with proved anticonvulsant effects on the MES test.

The main inhibitory neurotransmitter system in the brain, the GABA system, is the target for many clinically used drugs to treat anxiety and epilepsy disorders. This receptor possesses remarkable structural diversity. It comprises 5 subunits with the usual stoichiometry of the receptor: 2α, 2β and 1γ or δ subunits, but multiple subtypes of subunits exist, creating a vast pool of possible receptor isoforms. These different subunit combinations result in receptors with strikingly different pharmacological and biophysical properties resulting in distinctive functional effects on excitability of central nervous system circuits [14].

The sedation, anterograde amnesia, muscle relaxant effects and in part the anticonvulsant action are thought to be mediated by α1 subunit containing receptors [15–17], anxiolytic effect by α2- or α3-containing receptors [18–21], whereas α5-containing receptors may play an important role in learning and memory [22].

Most of the procedures used to assess anxiety in rodents are particularly adapted to detect anxiolytic activities. In general, animals are exposed to anxiogenic conditions, i.e., either a novel environment (as the elevated plus-maze test, social interaction test, open field test, hole-board test) or a conflict situation (as the Vogel punishment drinking test) [23]. The elevated plus-maze stands as one of the most popular in vivo animal tests currently in use to screen anxioselective effects of drugs. It is claimed to be an ethologically valid animal model of anxiety because it uses natural stimuli that can induce anxiety in human. Then fear to novel, elevated open arms may be similar to agoraphobia, vertigo and xenophobia [24]. This assay provides an excellent tool for detecting benzodiazepine/GABA and glutamate-related compounds, but inconsistent results have been reported for those drugs which modulate the serotoninergic or some of the neuropeptide systems [25]. The hole-board test offers a simple method for measuring the response of an animal to an unfamiliar environment and it has been used to assess emotionality, anxiety and/or responses to stress in animals. In this test several behaviors can be readily observed and quantified, which makes possible a comprehensive description of the animals' behavior [23]. Here, we have also employed a supplementary test, the locomotor activity assay.

Compounds **4**, **7** and **10** displayed low to medium affinity for the benzodiazepine site of the $GABA_A$ receptor, with **7** and **10** being the most active sulfamides. In the saturation experiments, the ability of these compounds to modify the K_d without any significant change in the B_{max} suggests that these compounds interact competitively at only one recognition site, probably the benzodiazepine binding site.

Compound **7** showed an anxiolytic-like action at 1 mg/kg and 3 mg/kg and compound **10** at 1 mg/kg, doses closely related to those elicited by diazepam, although they exhibited a moderate affinity for the benzodiazepine binding site (in the μM range), in contrast to diazepam (nM). It could be hypothesize that the pharmacological properties of these sulfamides in vivo might be influenced by an active biotransformation product with greater affinity or different subtype selectivity for the $GABA_A$-receptor.

Additionally, these compounds produced no disturbances in the locomotor activity of mice up to 30 mg/kg and 10 mg/kg, respectively, evidencing a lack of sedative effect. In a previous work, these compounds showed, also, no neurotoxicity assessed by the RotoRod test, at doses up to 100 mg/kg [3].

It is notable that the compounds tested showed a loss of anxiolytic effect at higher doses. Anxiolytic drugs usually display hormetic-like biphasic dose responses, independent of the test and animal model employed [26].

Flumazenil, a non selective benzodiazepine binding site antagonist was able to completely reverse the anxiolytic-like effects of these sulfamides, proving that the $GABA_A$ receptor is implicated in this action.

In an attempt to further study the structural features that could explain the binding characteristics of the active compounds, some preliminary interpretation of the results is suggested. The most active structures (**4**, **7** and **10**, Table 1) are *N*,*N*′-disubstituted derivatives, with at least four carbon atoms attached to the sulfamide function. In these compounds, the *N* atoms can act as hydrogen bond donors and they are placed near the *O* atoms of sulfamide function that can be hydrogen bond acceptors. The low activity of tri-substituted and tetrasubstituted sulfamides suggests that there are hydrogen bonding interactions between the NH group of the sulfamide function and the benzodiazepine binding site. Additionally, the lack of activity of aminoacid-derived sulfamides and monosubstituted compounds proposes the necessity of two hydrocarbon chains long enough to fulfill the lipophilic pockets in the active site. All these common characteristics are in agreement with the general pharmacophoric pattern proposed by some of us in previous investigations [27].

Compounds **4**, **7** and **10**, also, displayed an anticonvulsant profile that most closely resembles that of phenytoin: they are effective against tonic extension seizures induced by MES and ineffective against clonic seizures induced by PTZ [3]. Moreover, these sulfamides displayed an anxiolytic like activity at doses lower than those that exhibited protective effects in the MES test.

The results obtained suggest that sulfamides **7** and **10** possess a broader mechanistic profile than would be expected from the initial studies. One possibility is that the anxiolytic like action may be mediated by $GABA_A$ receptors, whereas the origin of their anticonvulsant effects could be associated with a blockade of voltage gated sodium channels. Antiepileptic drugs like phenytoin, carbamazepine and lamotrigine inhibit the voltage-dependant sodium channels, they decrease the cellular excitability by reducing the penetration of sodium into neurons [28].

The combined anticonvulsant and anxiolytic like profiles of the tested sulfamides may represent a valuable tool for the treatment of both disorders. Further studies might be necessary to determine if these compounds could exhibit enhanced pharmacokinetic or pharmacodynamic properties with advantages as pharmacological therapeutic agents for the treatment of various neuropsychiatric disorders.

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