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Effects of Two *N*-arylpiperazinylmethylpyrazolo [1,5-*d*][1,2,4]triazine Derivatives in Pain and Antidepressant Tests in Mice

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Abstract

The antinociceptive and antidepressant effects of two pyrazolotriazine derivatives, 2-phenyl-3,3*a*-dihydro-4-oxo-5-(4-phenylpiperazin-1-yl)methyl-pyrazolo[1,5-*d*][1,2,4]-triazine (SM1) and 2-phenyl-3,3*a*-dihydro-4-oxo-5-[4-(4-fluorophenyl)piperazin-1-yl]methylpyrazolo[1,5-*d*][1,2,4] triazine (SM3) have been investigated in mice using classical pharmacological tests.

The intraperitoneal LD50 values of SM1 and SM3 were 253·4 and 218·8 mg kg^{-1} respectively. SM1 and SM3 showed analgesic properties in the phenylbenzoquinone-induced abdominal constriction test (ED50 ≈ 10–15 mg kg^{-1}, i.p.) and in the hot-plate test. The antinociceptive effects of the triazines were significantly reduced by administration of naloxone (1 and 3·2 mg kg^{-1}, s.c.) and yohimbine (1 mg kg^{-1}, p.o.). Acute intraperitoneal administration of both compounds (1 mg kg^{-1} SM1 or 1·5 mg kg^{-1} SM3) potentiated morphine (0·15 mg kg^{-1}, s.c.) analgesia in the phenylbenzoquinone test. Although this synergistic activity was not reversed by methysergide (0·5 mg kg^{-1}, i.p.), the analgesic activity of both compounds was enhanced by administration of 5-hydroxytryptophan (50 mg kg^{-1}, i.p.) in conjunction with carbidopa (25 mg kg^{-1}, i.p.). Furthermore, neither compound (at 100 mg kg^{-1}, i.p.) significantly reduced the duration of immobility of mice in the forced swimming test, and both (at 75 mg kg^{-1}, i.p.) were ineffective at enhancing the toxic effects of yohimbine (30 mg kg^{-1}, s.c.). Only SM3 (ED50 = 74·5 mg kg^{-1}, i.p.) significantly antagonized reserpine (2·5 mg kg^{-1}, i.p.)-induced ptosis.

Thus, the results suggest that SM1 and SM3 have antinociceptive properties related to co-involvement of opioidergic and α_2-adrenoceptor mechanisms without associated antidepressant properties.

We have previously reported the synthesis and preliminary pharmacological screening of a series of *N*-arylpiperazinylmethylpyrazolo[1,5-*d*][1,2,4]triazine derivatives designed as structural analogues of trazodone and nefazodone (Mavel et al 1993). It was shown that the pyrazolotriazine nucleus bearing an arylpiperazinyl group confers potent analgesic activity especially to compounds SM1 (2-phenyl-3,3*a*-dihydro-4-oxo-5(4-phenyl-piperazin-1-yl)methylpyrazolo[1,5-*d*][1,2,4] triazine) and SM3 (2-phenyl-3,3*a*-dihydro-4-oxo-5[4-(4-fluorophenyl) piperazin-1-yl] methylpyrazolo [1,5-*d*] [1,2,4]triazine) administered orally.

This work has been undertaken to determine the role of endorphin systems and monoamines (5-HT and adrenaline) in the antinociceptive effects of SM1 and SM3. Because of their chemical relationship with the atypical non-tricyclic antidepressants trazodone and nefazodone, we also investigated these compounds further for antidepressant activity. The structures of trazodone, nefazodone, SM1 and SM3 are given in Fig. 1.

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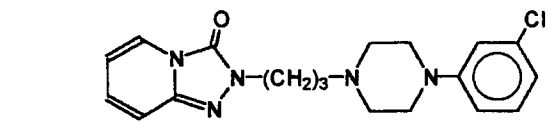
Materials and Methods

Animals and experimental procedure

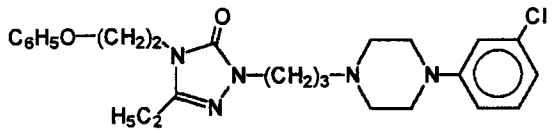
Swiss male mice, 18–22 g, purchased from Depre (Saint-Doulchard, France) were used in all experiments. Mice were kept in groups of ten in a temperature-controlled room with a 12-h light–dark cycle. Food and water were freely available until the time of the experiment. The allocation of animals to different groups was randomized and the experiments were performed under blind conditions. Test compounds were administered intraperitoneally in saline (0·9% NaCl).

Drugs

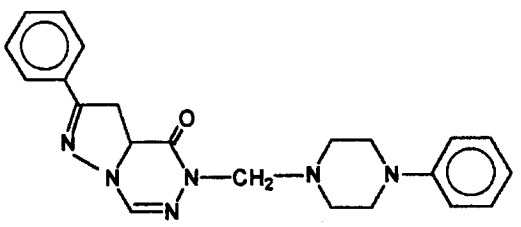
Carbidopa (ICN Biomedicals, Orsay, France), citalopram (Lundbeck, Copenhagen, Denmark), clonidine (Catapressan, Boehringer-Ingelheim, Paris, France), fluoxetine (Prozac, Lilly, St-Cloud, France), L-5-hydroxytryptophan (Sigma, Montluçon, France), methysergide (Desernil, Sandoz, Rueil-Malmaison, France), morphine hydrochloride (Cooperation Pharmaceutique Française, Melun, France), naloxone (Narcan, Du Pont de Nemours, Paris, France) and trazodone (Pragmarel, UPSA, Rueil-Malmaison, France) were dissolved in saline. Yohimbine (Sigma) was dissolved in de-ionized water. Phenylbenzoquinone (Eastman Kodak, Rochester, USA) was dis-



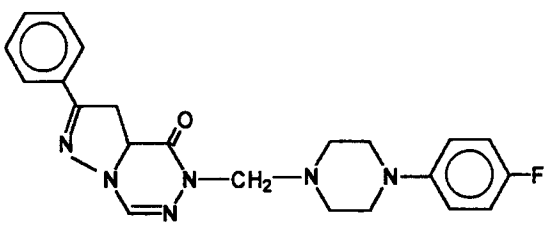
Trazodone



Nefazodone



SM1



SM3

FIG. 1. The structures of trazodone, nefazodone, SM1 and SM3.

solved in 5% ethanol to give a 0·02% solution. Reserpine (Sigma) was dissolved in glacial acetic acid (0·05 mL) and diluted to 50 mL with saline.

Acute toxicity studies

The compounds were administered at doses of 100, 200, 400, 600 and 800 mg kg^{-1}. Animals were observed continuously for 3 h for gross effects and then at 24-h intervals for up to 7 days. Behavioural, neurologic, autonomic and toxic effects were recorded.

Locomotor activity

The number of photocell beams crossed was recorded 30 min after drug administration (i.p.) in mice individually placed for 10 min in a photocell actimeter (Apelex).

Phenylbenzoquinone-induced abdominal constriction test

Phenylbenzoquinone solution, maintained at 37°C, was administered by intraperitoneal injection to mice 30 min after injection of the drugs. The number of abdominal constrictions of each animal was counted between 5 and 15 min after injection of the irritant (Siegmund et al 1957; Linée 1972).

Hot-plate test

Animals were placed on a copper plate (Apelex), maintained at a constant temperature of 56°C. The time necessary to induce the licking reflex of the forepaws was then recorded. Measurements were performed 30 min later. A cut-off withdrawal latency of 40 s was used to prevent tissue damage (Woolfe & McDonald 1944).

Potentiation of morphine analgesia

The protocol used was the same as in the phenylbenzoquinone test. Morphine (0·15 mg kg^{-1}, s.c.) was injected at the same time as the drugs, 30 min before the test (Fialip et al 1989).

Antagonism of drug antinociception by naloxone

The protocol used for the evaluation of the effect of naloxone on drug-induced analgesia was similar to that described in the phenylbenzoquinone test. Naloxone (1 or 3·2 mg kg^{-1}, s.c.) was injected 5 min before intraperitoneal administration of phenylbenzoquinone solution (Nevinson et al 1991).

Potentiation of drug antinociception by 5-hydroxytryptophan (5-HTP)

The protocol used was adapted from the technique of Von-voigtlander et al (1984). Experiments were performed in a manner similar to that used in the phenylbenzoquinone test. Carbidopa (25 mg kg^{-1}, i.p.) and, after 30 min, 5-HTP (50 mg kg^{-1}, i.p.) were administered then, after a further 15 min, the drugs. The analgesic test was performed 20 min later by administration of phenylbenzoquinone solution.

Antagonism of drug + morphine antinociception by methysergide

The protocol used was adapted from the technique of Bhattacharya (1978). Methysergide (0·5 mg kg^{-1}) was injected intraperitoneally then, 30 min later, the drugs (i.p.) and morphine (0·15 mg kg^{-1}, s.c.) were administered simultaneously. The analgesic test was performed 30 min later, by administration of phenylbenzoquinone solution (i.p.) and the abdominal constriction response was observed as described above.

Antagonism of drug antinociception by yohimbine

The drugs (i.p.) and yohimbine (1 mg kg^{-1}, p.o.) were administered simultaneously and then, 30 min later, phenylbenzoquinone solution was given intraperitoneally. Beginning 5 min after injection of the irritant, mice were observed for abdominal constriction for a period of 10 min (Luttinger et al 1985).

Forced swimming test

Measurement of immobility in mice was performed by a modification of the method of Porsolt et al (1977). Acquired immobility of mice was evaluated 32–36 min after intraperitoneal drug or placebo treatment, by recording the time the animals remained immobile after being placed (at 30 min) in vertical glass cylinders (height, 25 cm; i.d. 10 cm) containing 8 cm water at 21–23°C.

Table 1. Acute toxicity, sedative activity and analgesic activity of SM1, SM3, citalopram, fluoxetine, trazodone and morphine.

Compound ($mg\,kg^{-1}$)	Acute toxicity†	Decrease of motor activity	Phenylbenzo-quinone test‡	Hot-plate test§
SM1	253·4 (199·5–321·8)	16·5 (11·6–23·4)	9·6 (5·9–15·6)	9·2 ± 0·9*,††
SM3	218·8 (116·4–411·3)	37·6 (23·6–59·8)	13·3 (5·6–31·7)	9·1 ± 0·8*,††
Citalopram	178·9 (171·0–187·1)	4·9 ± 1·3‡‡	16·5 (8·9–30·5)	Not tested
Fluoxetine	100·0 (91·7–109·1)	39·6 (30·6–51·2)	13·4 (8·8–20·5)	Not tested
Trazodone	223·4 (215·0–232·1)	6·0 (5·7–6·4)	9·0 (6·5–12·4)	8·5 ± 0·7*,§§
Morphine(s.c.)	Not tested	9·6 (8·0–11·5)	0·6 (0·3–1·1)	16·5 ± 1·5*,§§

†LD50. ‡ED50. LD50 and ED50 values are expressed with their 95% confidence intervals. §Reaction time (s), mean ± s.e.; the reaction time for saline was 5·9 ± 0·6 s. ††Administered at $20\,mg\,kg^{-1}$. ‡‡Percentage of activity at $100\,mg\,kg^{-1}$ (i.p.). §§Administered at $7·5\,mg\,kg^{-1}$. *$P < 0·05$, significant compared with controls.

Interaction with reserpine

Drugs were injected (i.p.) 1 h before administration of reserpine ($2·5\,mg\,kg^{-1}$, i.p.). Palpebral ptosis (Gouret & Thomas 1973; Raynaud & Gouret 1973) was evaluated 2 h after reserpine administration.

Yohimbine-induced toxicity

Drugs were injected (i.p) 30 min before administration of yohimbine ($30\,mg\,kg^{-1}$, s.c.) and mortality was assessed 24 h later (Quinton 1963).

Data analysis

The LD50 and ED50 values and their 95% confidence intervals were determined by the method of Litchfield & Wilcoxon (1949). The significance of pharmacological data expressed as means ± s.e. was analysed by use of Student's *t*-test. *P* values < 0·05 were accepted as indicative of statistical significance (Schwartz 1984).

In the hot-plate test, the results are expressed as the percentage of analgesia for the different groups (drugs + saline; saline + morphine).

Results and Discussion

In the acute toxicity studies no gross behavioural, neurologic or autonomic effects were observed in mice, except marked sedative action of intraperitoneal doses in excess of $100\,mg\,kg^{-1}$. The intraperitoneal LD50 values of SM1 and SM3 were 253·4 and $218·8\,mg\,kg^{-1}$, respectively, values comparable with that of trazodone (Table 1).

Furthermore, it appeared that both triazine derivatives were less toxic than the reference drugs citalopram and fluoxetine. A significant decrease in locomotor activity was induced by SM1 and SM3 (ED50 = 16·5 and $37·6\,mg\,kg^{-1}$, respectively) but it was less intense than that generated by citalopram or trazodone.

Derivatives SM1 and SM3 showed analgesic properties in the 'abdominal constriction' test in mice, SM3 was active at a dose of one sixteenth the LD50 whereas SM1 was effective at doses up to one twenty-sixth the LD50. They have the same order of activity as antidepressant drugs ($9·0 \leq ED50 \leq 16·5\,mg\,kg^{-1}$, i.p.). In the hot plate test, reaction time was significantly increased 30 min after intraperitoneal administration of $20\,mg\,kg^{-1}$ SM1 and SM3 (Table 1). However, the magnitude of the effect was less than that of morphine.

When administered together with $0·15\,mg\,kg^{-1}$ morphine (s.c.), a borderline dose for antinociceptive effect in the phenylbenzoquinone test, SM1 ($1\,mg\,kg^{-1}$, i.p.) potentiated the analgesia induced by the opioid agonist (Table 2).

Under the same conditions, neither SM3 nor fluoxetine showed significant activity. Reduced responsiveness to noxious stimuli was observed with weak doses of citalopram and trazodone when the drugs were administered in combination with a subanalgesic dose of morphine. This result confirms the enhancement, demonstrated by several authors (Malseed & Goldstein 1979; Botney & Fields 1983; Taiwo et al 1985; Fialip et al 1989; Valeri et al 1991), of morphine analgesia in animals by antidepressant drugs.

The interaction between SM1 or SM3 and morphine might be explained by interference with opiate receptors (Biegon & Samuel 1980; Somoza et al 1981; Isenberg & Cicero 1984) or possibly by modulation of central serotonergic or noradrenergic pathways, or both (Lidbrink et al 1971; Deakin & Dostrovsky 1978; Yaksch 1979; Kuraishi et al 1983).

Table 2. Analgesic effect of morphine ($0·15\,mg\,kg^{-1}$, s.c.) in the phenylbenzoquinone test after acute intraperitoneal administration of SM1, SM3, citalopram, fluoxetine and trazodone.

		Analgesia (%)		
	Dose	Morphine + saline	Drug + saline	Drug + morphine
SM1	$1\,mg\,kg^{-1}$	14·2 ± 9·2	–	–
SM3	$1·5\,mg\,kg^{-1}$	–	10·8 ± 8·1	84·7 ± 8·2*
Fluoxetine	$3\,mg\,kg^{-1}$	–	19·8 ± 8·2	35·1 ± 8·7
Citalopram	$10\,mg\,kg^{-1}$	–	20·2 ± 3·5	30·5 ± 4·2
Trazodone	$2\,mg\,kg^{-1}$	–	32·4 ± 6·1	72·9 ± 6·2*
		–	10·1 ± 6·0	43·3 ± 12·0*

Results are means ± s.e.m. *$P < 0·05$, significant compared with saline + morphine.

Table 3. Effect of naloxone (1 mg kg^{-1}, s.c.) on SM1-, SM3-, morphine-, fluoxetine-, citalopram- and trazodone-induced analgesia in the phenylbenzoquinone test.

	Dose	Route	Analgesia (%) Drug + saline	Drug + naloxone
SM1	20 mg kg^{-1}	i.p	61·7 ± 6·8	35·9 ± 5·9*
SM3	30 mg kg^{-1}	i.p	66·3 ± 7·5	35·1 ± 6·1
Morphine	1·5 mg kg^{-1}	s.c	99·2 ± 0·5	5·4 ± 4·6*
Fluoxetine	30 mg kg^{-1}	i.p	68·1 ± 7·4	67·5 ± 6·5
Citalopram	25 mg kg^{-1}	i.p	75·1 ± 6·9	66·3 ± 7·6
Trazodone	5 mg kg^{-1}	i.p	41·2 ± 6·5	58·6 ± 5·1

Results are means ± s.e.m. *$P < 0.05$, significant compared with drug + saline.

Table 4. Effect of naloxone (3·2 mg kg^{-1}, s.c.) on SM1-, SM3-, morphine-, fluoxetine-, citalopram- and trazodone-induced analgesia in the phenylbenzoquinone test.

	Dose	Route	Analgesia (%) Drug + saline	Drug + naloxone
SM1	20 mg kg^{-1}	i.p	85·4 ± 2·7	42·7 ± 7·5*
SM3	30 mg kg^{-1}	i.p	59·3 ± 12·4	36·8 ± 10·4
Morphine	0·75 mg kg^{-1}	s.c	73·6 ± 10·5	5·8 ± 5·4*
Fluoxetine	30 mg kg^{-1}	i.p	84·6 ± 3·7	57·4 ± 6·0*
Citalopram	25 mg kg^{-1}	i.p	73·8 ± 7·7	41·3 ± 7·0*
Trazodone	10 mg kg^{-1}	i.p	59·4 ± 7·0	76·5 ± 6·7

Results are means ± s.e.m. *$P < 0.05$, significant compared with drug + saline.

The possibility that activation of opioid receptors is involved in the analgesic activity of triazine derivatives was first explored. Naloxone is a non-specific opioid antagonist with a higher affinity for μ-receptors. It was administered at a low dose (1 mg kg^{-1}, s.c.) to antagonize these receptors and at a higher dose (3·2 mg kg^{-1}, s.c.) capable of blocking κ-receptors (Granados-Soto et al 1995). SM1 and SM3 analgesia to noxious stimuli was naloxone-sensitive (Tables 3 and 4) but statistical analysis failed to show any significant difference between the effects of 1 and 3·2 mg kg^{-1} naloxone. Accordingly, these data suggest involvement of an μ-opioidergic mechanism in the antinociceptive action of triazines. When animals were given an injection of morphine + naloxone, the effect of the morphine was practically abolished whatever the dose of naloxone used. As was previously described by Messing et al (1975) and Eschalier et al (1981), the antinociceptive activity of fluoxetine in the phenylbenzoquinone test was not affected by injection of naloxone (1 mg kg^{-1}). However, at 3·2 mg kg^{-1} significant blockade of its analgesic effect was observed. Similar results were obtained with citalopram, a specific inhibitor of 5-HT uptake, in contrast with trazodone, the analgesic properties of which were enhanced by naloxone (Tables 3 and 4).

Co-administration of subanalgesic dose of SM1 or SM3 with 5-HTP in combination with carbidopa resulted in potentiation of the analgesic effects of the triazines (Table 5). A similar observation was also made with the 5-HT-uptake inhibitors, fluoxetine, citalopram and trazodone, suggesting involvement of a 5-HTergic pathway in SM1 and SM3 analgesia. But, in contrast to citalopram, administration of methysergide, a 5-HT receptor inhibitor, did not modify the antinociceptive activity of triazines associated with morphine in the phenylbenzoquinone test (Table 6). Thus it seems that SM1 and SM3 did not interfere directly with 5-HTergic receptors but by an increase

Table 5. Potentiation of SM1-, SM3-, fluoxetine-, citalopram- and trazodone-induced analgesia by 5-hydroxytryptophan (50 mg kg^{-1}, i.p.) + carbidopa (25 mg kg^{-1}, i.p.) in the phenylbenzoquinone test.

	Dose	Route	Analgesia (%) Drug + saline	5-Hydroxytryptophan + carbidopa	Drug + 5-Hydroxytryptophan + carbidopa
None	–		–	10·4 ± 4·1	–
SM1	1 mg kg^{-1}	i.p	7·0 ± 3·1	–	67·5 ± 8·2
SM3	1·5 mg kg^{-1}	i.p	19·8 ± 8·2	–	49·9 ± 4·9*
Fluoxetine	3 mg kg^{-1}	i.p	20·0 ± 3·1	–	51·4 ± 5·6*
Citalopram	10 mg kg^{-1}	i.p	32·4 ± 6·1	–	73·1 ± 9·6*
Trazodone	2 mg kg^{-1}	i.p	10·1 ± 6·1	–	47·1 ± 9·5*

Results are means ± s.e.m. *$P < 0.05$, significant compared with drug + saline.

Table 6. Effect of methysergide (0·5 mg kg^{-1}, i.p.) on analgesia induced by SM1-, SM3- and citalopram in combination with morphine (0·15 mg kg^{-1}, s.c.) in the phenylbenzoquinone test.

	Dose	Route	Analgesia (%) Drug + saline	Morphine + saline	Drug + morphine	Drug + morphine + methysergide
None	–	–	–	4·4 ± 3·9		
SM1	1 mg kg^{-1}	i.p		16·3 ± 4·8	–	63·3 ± 9·5
SM3	1·5 mg kg^{-1}	i.p		20·4 ± 6·9	–	34·7 ± 6·9
Citalopram	10 mg kg^{-1}	i.p		32·4 ± 6·1	–	53·1 ± 5·0*

Results are means ± s.e.m. *$P < 0.05$, significant compared with drug + saline.

Table 7. Effect of yohimbine (1 mg kg^{-1}, p.o.) on SM1-, SM3- and clonidine-induced analgesia in the phenylbenzoquinone test.

	Dose	Route	Analgesia (%) Drug + saline	Drug + yohimbine
SM1	20 mg kg^{-1}	i.p	76·6 ± 3·3	59·0 ± 3·9*
SM3	30 mg kg^{-1}	i.p	80·4 ± 5·7	54·9 ± 1·9*
Clonidine	0·05 mg kg^{-1}	i.p	96·3 ± 7·1	37·8 ± 5·6*

Results are means ± s.e.m. *$P < 0.05$, significant compared with drug + saline.

in 5-HTergic activity with activation of one or several endorphin systems. Neuronal connections of 5-HTergic and endorphin-releasing fibres are known to exist (Yaksch & Elde 1980; Yaksch & Tyce 1980). Inhibition of the antinociceptive effects of both SM1 and SM3 by naloxone and not by methysergide favours such an interpretation.

With regard to the involvement of monoamines in pain regulation, analgesia was facilitated by noradrenaline in the spinal cord (Kuraishi et al 1983; Sawynok et al 1995).

Activation of α_2-adrenoceptors in the central nervous system produces analgesia or antinociception or both in a number of animal models (Pertovaara 1993). Thus clonidine, an α-adrenergic agonist induces a wide variety of effects including antihypertensive activity but also potent antinociceptive action (Schmitt et al 1974; Luttinger et al 1985; Laitin & Wallace 1996). These data prompted us to investigate triazine derivatives in this area and so to study effect of yohimbine, an α_2-adrenoceptor antagonist, on SM1- and SM3-induced antinociception in the phenylbenzoquinone test. Oral administration of yohimbine significantly attenuated the analgesic response produced by injection of SM1 or SM3 (Table 7). Although this last effect was less intense than that demonstrated with clonidine, it supported partial involvement of α_2-adrenoceptor mechanism in triazine antinociceptive action.

Considering that previously described pyridazinones with arylpiperazinyl groups had concomitant analgesic and antidepressant properties (Rubat et al 1995), it seemed of interest to determine whether the same was true for SM1 and SM3. In this eventuality, triazines might be active not only in the treatment of pain but also on its psychological component. Moreover, antidepressants which inhibit 5-HT or noradrenaline re-uptake or enhance monoaminergic neurotransmission induce an analgesic effect (Ardid et al 1992). On the basis of these considerations, we chose to use SM1 and SM3 in traditional tests effective at predicting the activity of a wide variety of antidepressants—the Porsolt forced-swimming induced behavioural despair model, the antagonism of reserpine-induced effects and the potentiation of yohimbine toxicity. In the three tests used, triazines were not efficient or barely active, except that SM3 antagonized reserpine-induced palpebral ptosis (ED50 = 74·5 mg kg^{-1}, i.p.; Table 8). Both triazines were unable to potentiate yohimbine lethality at 75 mg kg^{-1}. Higher doses were not administered because of the acute toxicity of the two molecules (LD0 ≈ 75 mg kg^{-1}, i.p.).

In conclusion, SM1 and SM3 had potent antinociceptive properties which seemed to be related to co-involvement of opioidergic and α_2-adrenoceptor mechanisms without associated antidepressant activity. The high LD50 values for SM1 and SM3 resulted in good therapeutic indices (LD50/ED50 in the phenylbenzoquinone test) of 26·4 and 16·5, respectively. However due to its better index, SM1 seems to offer greater therapeutic potential for the treatment of pain.

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Table 8. Forced swimming test, prevention of the reserpine-induced palpebral ptosis in mice and effects of SM1, SM3, fluoxetine, citalopram and trazodone on yohimbine-induced toxicity.

Compound	Forced swimming test*	2 h after reserpine administration*	Effect on yohimbine-induced toxicity*
SM1	> 100	> 100	Inactive at 75 mg kg^{-1} (i.p.)
SM3	> 100	74·5 (41·6–133·4)	Inactive at 75 mg kg^{-1} (i.p.)
Fluoxetine	42·7 (36·9–49·4)	55·4 (34·3–89·4)	20·0 ± 13·0†
Citalopram	38·0 (21·3–67·9)	58·0 (52·0–64·8)	30·0 ± 15·0‡
Trazodone	25·0 ± 17·9§	48·4 (46·4–50·5)	183·1 (172·8–194·0)

*ED50 values (mg kg^{-1}, i.p.) with their 95% confidence intervals. †Percentage of dead mice at 50 mg kg^{-1} (i.p.). ‡Percentage of dead mice at 150 mg kg^{-1} (i.p.). §Percentage of activity at 20 mg kg^{-1} (i.p.).

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