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Behavioural Profile of Two Potential Antidepressant Pyridazine Derivatives Including Arylpiperazinyl Moieties in Their Structure, in Mice

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Abstract

The potential antidepressant effects of two pyridazine derivatives, 5-benzyl 6-methyl 2-[4-(3-trifluoromethyl phenyl) piperazin-1-yl] methylpyridazin-3-one (PC4) and 5-benzyl 6-methyl 2-[4-(3-chlorophenyl) piperazin-1-yl] methylpyridazin-3-one (PC13), were evaluated using classical psychopharmacological tests in mice.

The intraperitoneal LD50 values of PC4 and PC13 were respectively 1125·8 and 429·6 mg kg^{-1}. Only at intraperitoneal doses of 100 mg kg^{-1} did PC4 or PC13 significantly decrease locomotor activity. Both compounds (5–20 mg kg^{-1}, i.p.) reduced the duration of immobility of mice in the forced swimming test, antagonized reserpine (2·5 mg kg^{-1}, i.p.)-induced ptosis, and potentiated reserpine (2·5 mg kg^{-1}, i.p.)-induced hypothermia. PC4 and PC13 (20 mg kg^{-1}, i.p.) partly reversed hypothermia induced by low dose apomorphine (5 mg kg^{-1}, s.c.) but were less effective for higher doses of apomorphine (16 mg kg^{-1}, s.c.). At 200 mg kg^{-1}, intraperitoneal PC13 enhanced the toxic effects of yohimbine (30 mg kg^{-1}, s.c.), while PC4 was inactive. Head twitches produced either by L-5-hydroxytryptophan (4 mg kg^{-1}, i.p.) in mice pretreated with pargyline (100 mg kg^{-1}, i.p.) or by 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (3 mg kg^{-1}, i.p.) were antagonized by both pyridazine derivatives (20 mg kg^{-1}, i.p.). PC4 and PC13 showed analgesic properties in the phenylbenzoquinone-induced abdominal constriction test ($5·0 < ED50 < 5·5$ mg kg^{-1}, i.p.) and in the hot-plate test (10 to 37% of analgesia at 10 mg kg^{-1}, i.p.). These antinociceptive effects were not significantly diminished by naloxone (1 mg kg^{-1}, i.p.). Furthermore, acute intraperitoneal administration of both compounds (20 mg kg^{-1} for PC4 and 5 mg kg^{-1} for PC13) potentiated morphine (7·5 mg kg^{-1}, s.c.) analgesia in the hot-plate test.

Thus, these results suggest that PC4 and PC13 possess potential antidepressant effects related to different aminergic mechanisms, especially at the 5-HT_2 receptor level.

Considerable interest has been paid to derivatives containing 1-arylpiperazinyl moieties, because of their effects on the central nervous system (Bosc et al 1992; Mokrosz et al 1992; Perregaard et al 1992; Sladowska 1993). 1-Arylpiperazine derivatives produce a variety of behavioural responses and pharmacological activities via a central 5-HT-ergic mechanism (Abou-Gharbia 1988; Simansky & Schechter 1988). Among these compounds, the two agents presently in clinical use, trazodone and buspirone, may form 1-arylpiperazines during biotransformation in-vivo (Diaz-Marot et al 1989; Caccia & Garattini 1990). These considerations prompted us to synthesize a series of new pyridazine derivatives including 1-arylpiperazinyl moieties in their structure (Rubat et al 1992). The first pharmacological results (Rubat et al 1992) revealed important analgesic properties for PC4 (5-benzyl 6-methyl 2-[4-(3-trifluoromethyl phenyl) piperazin-1-yl] methylpyridazin-3-one) and PC13 (5-benzyl 6-methyl 2-[4-(3-chlorophenyl) piperazin-1-yl] methylpyridazin-3-one) (see Fig. 1).

Taking into account the large number of antidepressant drugs which also have analgesic effects (Lee & Spencer 1977; Isenberg & Cicero 1984) and potentiate morphine analgesia (Malseed & Goldstein 1979), it seemed to be of interest to investigate these compounds further for antidepressant activity.

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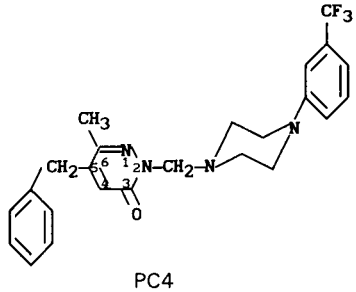
Materials and Methods

Animals and experimental procedure

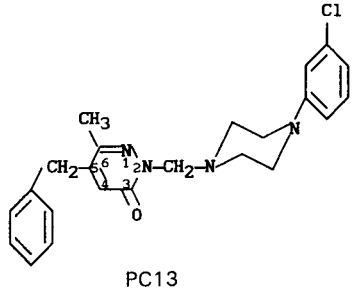
Male CD (Charles River) mice purchased from Charles River S.A. France (Cleon, France), 20–25 g, were used in the forced swimming test. Swiss male mice purchased from DEPRE (Saint-Doulchard, France), 18–22 g, were used in all other experiments. Groups of 10 mice were used. In the studies described below, test compounds were administered intraperitoneally in saline (0·9% NaCl).

Drugs

Apomorphine (Aguettant, Lyon), citalopram (Lundbeck, Copenhagen), clomipramine (Anafranil, Ciba-Geigy, Rueil-Malmaison), fluoxetine (Prozac, Lilly, St Cloud), haloperidol (Haldol, Janssen, Boulogn Billancourt), L-5-hydroxytryptophan (Sigma, Montlucon), imipramine (Tofranil, Ciba-Geigy), mianserin (Athymil, Organon, Saint-Denis), morphine hydrochloride (Coopération Pharmaceutique Française, Melun), naloxone (Narcan, Du Pont de Nemours, Paris), pargyline (Sigma), (±)-propranolol (Avlocardyl, ICI, Cergy) and trazodone (Pragmarel, UPSA, Rueil Malmaison) were dissolved in saline.



PC4



PC13

FIG. 1. PC4 and PC13 formulas.

1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (RBI) and yohimbine (Sigma) were dissolved in de-ionized water.

Phenylbenzoquinone (Eastman Kodak, Rochester, USA) was dissolved to give a 0·02% solution in 5% ethanol.

Reserpine (Sigma) was dissolved in glacial acetic acid (0·1 mL) and diluted to 100 mL in saline.

Acute toxicity

The compounds were administered at various doses (50–1600 mg kg^{-1}) intraperitoneally. The animals were kept under observation for eight days to detect any sign of toxicity.

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) (Boissier & Simon 1965).

Forced swimming test

Measurement of immobility in mice was carried out according to a modified 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; diam.: 10 cm) containing 8 cm water at 21–23°C.

Interaction with reserpine

Drugs were injected (i.p.) 1 h before reserpine (2·5 mg kg^{-1}, i.p.) administration. Palpebral ptosis (Gouret & Thomas 1973; Raynaud & Gouret 1973) was evaluated 2 h after administration. Rectal temperature (Raynaud & Gouret 1973) was measured with a thermistor probe (Ellab, DM 852, Carrieri) and recorded just before and 2 and 4 h after reserpine administration.

Interaction with apomorphine

Drugs were injected (i.p.) 30 min before apomorphine (5 and 16 mg kg^{-1}, s.c.) administration. Rectal temperature (Costentin et al 1975; Puech et al 1981) was measured with a thermistor probe (Ellab, DM 852, Carrieri) just before and every 15 min for 90 min after drug administration.

Yohimbine-induced toxicity

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

Inhibition or potentiation of head twitches induced by L-5-hydroxytryptophan (5-HTP)

Mice received the monoamine oxidase inhibitor pargyline at a dose of 100 mg kg^{-1}, i.p., 3 h before the test. Drugs were injected (i.p.) 30 min before administration of 5-HTP (4 mg kg^{-1}, i.p.). Immediately after 5-HTP administration, mice were observed for 1 min (Martin et al 1985, 1989); mice were observed for 1 min every 10 min during a 60-min period. Head movements (head twitches) were scored for each mouse as follows: 0 = absence of head twitches; 1 = sparse head twitches; 2 = weak head twitches; 3 = more or less intense head twitches; 4 = intense head twitches.

Inhibition or potentiation of head twitches induced by DOI

Drugs were injected (i.p.) 30 min before administration of DOI (3 mg kg^{-1}, i.p.). Head twitches were recorded between the 5th and the 15th minute after DOI administration and scored as described above (Doble et al 1992; Oka et al 1993).

Phenylbenzoquinone-induced abdominal constriction test

Phenylbenzoquinone solution, maintained at 37°C, was administered by intraperitoneal injection to mice, 30 min after intraperitoneal administration of drugs. The number of abdominal constrictions of each animal was counted between the 5th and the 15th minute after the 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. Two basal measurements of the nociceptive threshold were made before administration. Measurements were carried out 30 min later (Woolfe & McDonald 1944; O'Callaghan & Holtzman 1975). A standard 40-s cut-off time was used as a maximal effect.

For the potentiation of the morphine analgesia, morphine (7·5 mg kg^{-1}, s.c.) was injected at the same time as drugs, 30 min before the test (Fialip et al 1989).

Protocol used for the evaluation of the effect of naloxone on drug-induced analgesia was similar to that described above. Naloxone (1 mg kg^{-1}, i.p.) was injected at the same time as drugs (i.p.), 30 min before the test (Vaught et al 1990).

Table 1. Acute toxicity (LD50) and sedative activity of PC4, PC13, citalopram, fluoxetine and trazodone.

Compounds	LD50 (mg kg^{-1}, i.p.)	Decrease of motor activity (%)	
		50 mg kg^{-1} (i.p.)	100 mg kg^{-1} (i.p.)
PC4	1125·8(996·3–1272·1)	0 ± 5·9	16·2 ± 3·2*
PC13	429·6(361·0–511·2)	3·3 ± 7·7	22·9 ± 6·6*
Citalopram	178·9(171·0–187·1)	38·8 ± 5·9[a]	4·9 ± 1·3
Fluoxetine	100·0(91·7–109·1)	39·6(30·6–51·2)[b]	
Trazodone	223·4(215·0–232·1)	6·0(5·7–6·4)[b]	

LD50 values are expressed with their 95% confidence intervals. *$P < 0.05$. [a]This value indicates an increase of motor activity. [b] ED50 value with its 95% confidence interval.

Molecular modelling

The input geometry of PC13 was generated and minimized using the program Alchemy III (Tripos Associates, St Louis, MO, USA).

Data analysis

The LD50 and ED50 values, and their 95% confidence intervals were determined by the method of Litchfield & Wilcoxon (1949). In some experiments, doses of test drugs were compared with controls or other treatment groups using Student's *t*-test. $P < 0.05$ was accepted as the level 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).

The potentiation of morphine analgesia and antagonism of analgesia by naloxone were expressed as the time necessary to induce the licking reflex of the forepaws in mice.

Results

Acute toxicity

In preliminary experiments, intraperitoneal acute toxicity was investigated in mice (Table 1). Only at doses of 100 mg kg^{-1} did either test compound produce sedation in animals. PC13 induced tremors in mice after intraperitoneal administration of doses from 300 mg kg^{-1}. PC4 and PC13 did not induce significant changes (less than ± 0·5°C) in body temperature even at doses up to 75 mg kg^{-1} (i.p.). At a higher dose (200 mg kg^{-1}) a long-lasting (4 h) and significant decrease (–4°C) in body temperature appeared after PC13 administration. At this dose, PC4 also induced a significant but less important decrease (–1·5°C) in body temperature. Furthermore, it appeared that PC4 and PC13 were less toxic than reference drugs citalopram, fluoxetine and trazodone.

Locomotor activity

A significant decrease in locomotor activity was induced by PC4 and PC13 (Table 1).

Forced swimming test

Like imipramine, clomipramine, citalopram and fluoxetine, PC4 and PC13 reduced immobility of mice (Table 2). In the case of trazodone, the sedative effects observed from 5–10 mg kg^{-1}, intraperitoneally, appeared to mask the reduced immobility observed at 20 mg kg^{-1}, intraperitoneally.

Interaction with reserpine

Imipramine, clomipramine, PC4 and PC13 antagonized reserpine-induced palpebral ptosis (Table 2). Trazodone, citalopram and fluoxetine were less active.

At a dose of 20 mg kg^{-1}, PC4, PC13, trazodone, citalopram and fluoxetine potentiated reserpine-induced hypothermia (from 2·7 to 5·2°C) 4 h after reserpine administration (Fig. 2). In the same time, clomipramine (20 mg kg^{-1}) did not significantly potentiate reserpine-induced hypothermia; imipramine (20 mg kg^{-1}) reduced hypothermia from 1·6 to 2·6°C between the second and the fourth hour after reserpine injection.

Interaction with apomorphine

From the 30th minute following apomorphine (5 mg kg^{-1}, s.c.) injection, both PC4 and PC13 (20 mg kg^{-1}) produced a significant decrease (up to 5·7°C for PC4, 45 min after apomorphine injection) in apomorphine-induced hypothermia (Fig. 3). Similarly, haloperidol (0·5 mg kg^{-1}) reduced hypothermia induced by a low dose of apomorphine.

After injection of a higher dose of apomorphine (16 mg kg^{-1}, s.c.), PC4 (20 mg kg^{-1}) slightly reduced hypothermia but the increase in body temperature was only significant from the 60–90 min after drug administration (Fig. 4). With PC13, the antagonism of high dose apomorphine-induced hypothermia was significant from the 30th minute after intraperitoneal injection of the drug. Salbutamol (25 mg kg^{-1}) produced a strong decrease (from 2·0 to 4·5°C) in apomorphine-induced hypothermia.

Table 2. Forced swimming test and prevention of the reserpine-induced palpebral ptosis in mice.

Compound	ED50 (mg kg^{-1}, i.p.)	
	Forced swimming test	2 h after reserpine administration
PC4	10·8 (4·1–28·8)	7·0 (2·5–19·9)
PC13	18·3 (15·9–21·0)	11·5 (3·1–42·5)
Imipramine	14·2 (4·7–42·8)	4·8 (3·2–7·2)
Clomipramine	11·9 (8·1–17·5)	9·5 (3·5–25·6)
Citalopram	38·0 (21·3–67·9)	58·0 (52·0–64·8)
Fluoxetine	42·7 (36·9–49·4)	55·4 (34·3–89·4)
Trazodone	25·0 ± 17·9[a]	48·4 (46·4–50·5)

ED50 values are expressed with their 95% confidence intervals. [a] Tested at 20 mg kg^{-1} (i.p.).

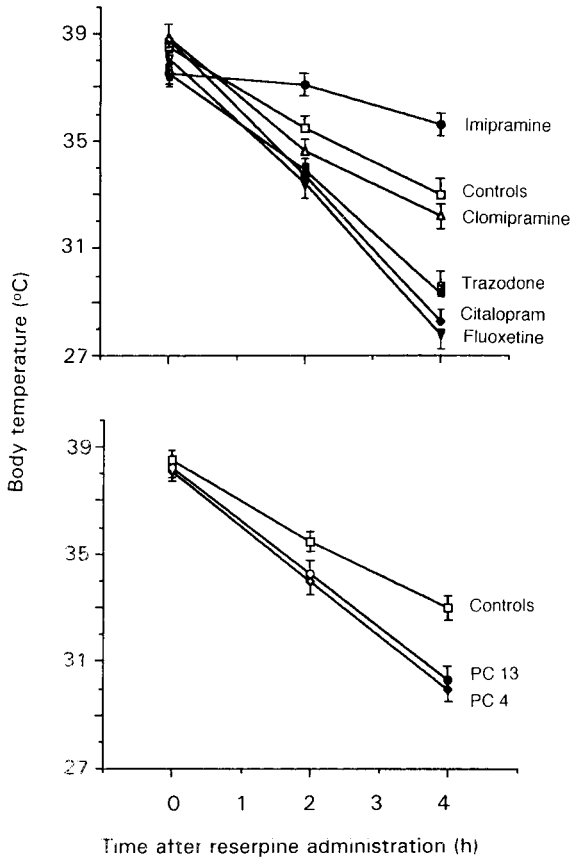


FIG. 2. Effects of PC4, PC13, imipramine, clomipramine, citalopram, fluoxetine and trazodone administered at the same dose of 20 mg kg^{-1} (i.p.) on reserpine-induced hypothermia in mice. Reserpine (2·5 mg kg^{-1}, i.p.) was administered 1 h after injection of drugs. Significant differences ($P < 0·05$) from respective control groups are represented by solid symbols.

Interaction with yohimbine

PC13 increased yohimbine-induced toxicity in mice in the same way as trazodone did (Table 3). On the other hand, even at a dose of 200 mg kg^{-1}, PC4 did not affect yohimbine-induced toxicity. Because of their acute toxicity in mice, citalopram and fluoxetine were not administered at doses up to 150 and 50 mg kg^{-1}, intraperitoneally, respectively. At these doses neither citalopram nor fluoxetine showed significant activity in this test. With regards to tricyclic antidepressants, imipramine and clomipramine, potentiation of this toxicity appeared at lower doses.

Inhibition or potentiation of 5-HTP- and DOI-induced head twitches

Head twitches elicited by 5-HTP were blocked by both PC4 and PC13 at the same dose of 20 mg kg^{-1} (Table 4). Mianserin was less potent with 41·7% of 5-HTP-induced head twitches inhibited in mice pretreated with pargyline. At the opposite extreme, trazodone potentiated 5-HTP-induced head twitches with 81·5% of activity at 20 mg kg^{-1}.

When head twitches were evoked by DOI, these were blocked by both PC4 and PC13.

Analgesic activity in the phenylbenzoquinone-induced abdominal constriction test and in the hot-plate test

In the phenylbenzoquinone-induced abdominal constriction test, PC4 and PC13 were several times more potent than noramidopyrine and equipotent to trazodone (Table 5).

In the hot-plate test, PC4 and PC13 were also active at 10–20 and 5 mg kg^{-1}, respectively, but they were less effective than morphine administered at a dose of 7·5 mg kg^{-1}, subcutaneously (Table 5). Trazodone did not present significant activity at 10 mg kg^{-1} in this test.

Potentiation of morphine analgesia

Administered simultaneously with morphine 30 min before testing, PC4 and PC13 induced a potent analgesia significantly greater than the sum of the individual effects induced by the drugs as illustrated in Fig. 5.

Effect of naloxone on drug-induced analgesia

At an intraperitoneal dose of 1 mg kg^{-1}, naloxone caused a 50% reduction in analgesic effects of morphine (7·5 mg kg^{-1}, s.c.) (Fig. 6). On the other hand, naloxone failed to suppress analgesic activity of either PC4 or PC13 (20 mg kg^{-1}).

Molecular modelling

To corroborate experimental evidence on the 5-HT_2 antagonist effect of the most active derivative PC13, we decided to explore its possible interactions with the 5-HT_2 receptor

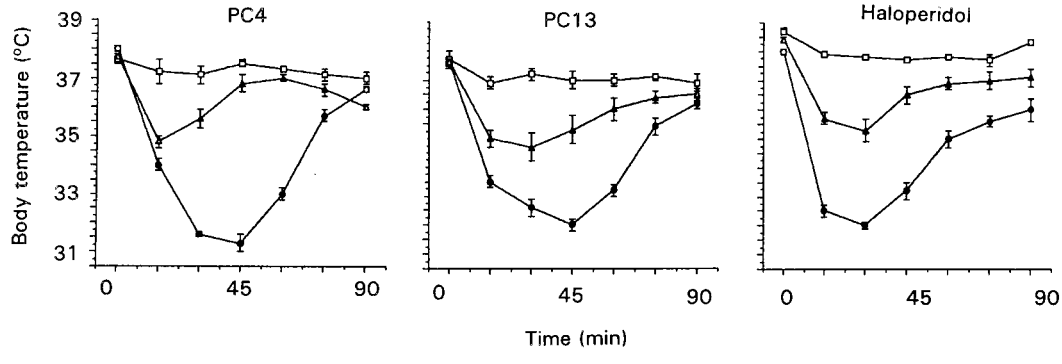


FIG. 3. Effects of PC4, PC13 (20 mg kg^{-1}, i.p.) and haloperidol (0·5 mg kg^{-1}, i.p.) on apomorphine-induced hypothermia in mice. Apomorphine (5 mg kg^{-1}, s.c.) was administered 30 min after injection of drugs. Significant differences ($P < 0·05$) from respective control groups are represented by solid symbols. □ Controls, ○ apomorphine, △ drug + apomorphine.

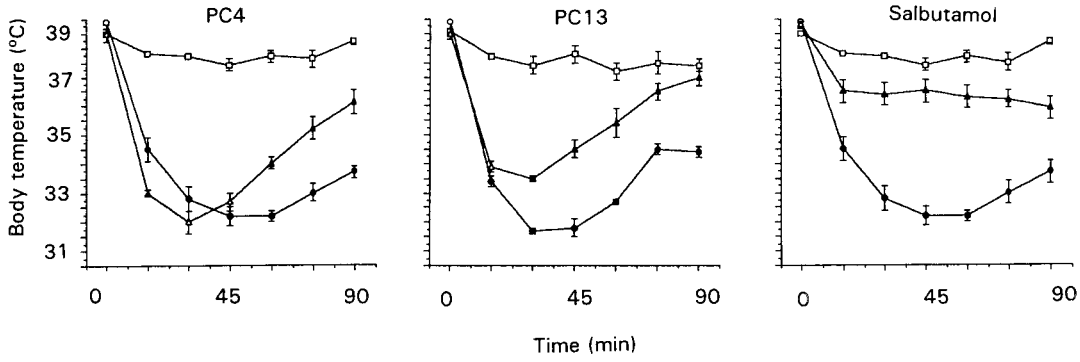


FIG. 4. Effects of PC4, PC13 (20 mg kg^{-1}, i.p.) and salbutamol (25 mg kg^{-1}, i.p.) on apomorphine-induced hypothermia in mice. Apomorphine (16 mg kg^{-1}, s.c.) was administered 30 min before injection of drugs. Significant differences ($P < 0.05$) from respective control groups are represented by solid symbols. □ Controls, ○ apomorphine, △ drug + apomorphine.

by molecular modelling analysis. The low-energy conformation of PC13 was compared with the essential structural features of the pharmacophoric model of Hibert (1988) (Fig. 7), i.e. a nitrogen atom placed at about 51 nm from the centroid B of an aromatic nucleus and located at about 3 nm over the plane defined by this nucleus, with this nitrogen atom placed at about 63 nm from a second aromatic nucleus A and located at about 33 nm over the plane defined by this last nucleus.

To interact with the 5-HT$_2$ site, a compound must possess at least two of the elements described above (nitrogen atom and aromatic ring) in the positions previously defined (Hibert 1988). In the case of PC13, the *m*-chlorophenylpiperazinyl moiety could impinge on the receptor as illustrated in Fig. 8 ($N_1 \leftrightarrow B = 56$ nm). Although the phenyl ring of the benzyl group was 78 nm distant from the nitrogen atom N_1, it was placed in an orthogonal plane with regard to the phenyl group attached to the piperazinyl group and so might also interact with the 5-HT$_2$ receptor.

Discussion

In behavioural mouse tests generally used in experimental psychopharmacology for antidepressants (Bourin 1990), PC4 and PC13 displayed similar effects which are shared by many antidepressant drugs (reduction of immobility duration in the forced swimming test, prevention of reserpine-induced ptosis, potentiation of both yohimbine-induced toxicity and reserpine-induced hypothermia, and inhibition of 5-HTP head twitches.

Contrary to imipramine, desipramine and mianserin however (Bourin 1990), PC4 and PC13 decreased locomotor activity in mice only at high doses).

Although it was less important than that of imipramine or clomipramine, activity of PC13 in the yohimbine test confirmed its potential antidepressant properties. If neuroleptics and anxiolytics were inactive in this test, tricyclic antidepressants as well as atypical antidepressants such as mianserin, viloxazine or nomifensine potentiated yohimbine toxicity (Bourin 1990). As PC13 potentiated the lethal effect of yohimbine in a similar manner to trazodone, a central origin ought to be attributable to this effect as suggested by the data of Lapin (1980).

The hypothermic action of low doses of apomorphine (< 8 mg kg^{-1}, i.p.) is usually considered to be mediated through the stimulation of dopaminergic receptors (Kulkarni 1980). Therefore, the fact that PC4 and PC13 were able to reduce low-dose apomorphine hypothermia suggested involvement of D_1/D_2 receptors in their effects. On the other hand, apomorphine hypothermia induced by higher doses (> 8 mg kg^{-1}, i.p.) involved the interaction of more than one type of receptor (Kulkarni 1980; Bourin 1990). This latter action was not specifically dopaminergic but was also due to interactions with the β-adrenergic system (Bourin 1990). Thus, salbutamol causes a strong decrease

Table 3. Effects of PC4, PC13, imipramine, clomipramine, citalopram, fluoxetine and trazodone on yohimbine-induced toxicity.

Compound	ED50 (mg kg^{-1}, i.p.)
PC4	Inactive at 200 mg kg^{-1}, (i.p.)
PC13	190·0 (170·4–211·9)
Imipramine	77·6 (60·4–99·7)
Clomipramine	112·5 (91·9–137·8)
Citalopram	30·0 ± 15·0[a]
Fluoxetine	20·0 ± 13·0[b]
Trazodone	183·1 (172·8–194·0)

Mice were injected with drugs 30 min before yohimbine (30 mg kg^{-1}, s.c.). The number of dead mice were recorded for 24 h. [a] Percentage of dead mice at 150 mg kg^{-1} (i.p.). [b] Percentage of dead mice at 50 mg kg^{-1} (i.p.).

Table 4. Inhibition (–) or potentiation (+) of 5-HTP- and DOI-induced head twitches by PC4, PC13, mianserin and trazodone in mice.

Compounds	5-HTP (% of activity at 20 mg kg^{-1}, i.p.)	DOI ED50 (mg kg^{-1}, i.p.)
PC4	–91·1 ± 8·9*	–14·0 (5·0–39·1)
PC13	–73·5 ± 20·6*	–12·5 (10·3–15·1)
Mianserin	–41·7 ± 8·0*	–9·2 (5·9–14·4)
Trazodone	+81·5 ± 5·7*	–13·5 ± 5·4[b]

*$P < 0.05$. ED50 values are expressed with their 95% confidence intervals. [b] Percentage of inhibition at 20 mg kg^{-1} (i.p.).

Table 5. Analgesic activity of PC4, PC13, trazodone, noramidopyrine and morphine in the phenylbenzoquinone-induced abdominal constriction test and in the hot-plate test.

Compound	Phenylbenzoquinone ED50 (mg kg^{-1}, i.p.)	Hot-plate test (% of analgesia at 10 mg kg^{-1}, i.p.)
PC4	5·0 (3·0–8·4)	10·6 ± 2.1*
PC13	5·5 (3·1–9·6)	36·7 ± 2·9*
Trazodone	9·0 (6·5–12·4)	7·4 ± 4·9
Noramidopyrine	60·0 (25·1–143·6)	Not tested
Morphine[a]	Not tested	27·5 ± 2·9*

ED50 values are expressed with their 95% confidence intervals. [a] Administered at 7·5 mg kg^{-1}(s.c.). *$P < 0.05$.

in high dose apomorphine-induced hypothermia. Moreover, thermal effects of apomorphine also appear to be mediated through a 5-HT-ergic mechanism (Grabowska et al 1973; Yamawaki et al 1983). Thus, the weak antagonism of high dose apomorphine-induced hypothermia observed after administration of PC4 or PC13 probably did not involve a β-adrenergic mechanism but a dopaminergic one, possibly associated with a 5-HT-ergic mechanism.

The antagonistic effects of PC4 and PC13 on reserpine-induced ptosis was markedly weaker than that of imipramine, but comparable with that of clomipramine and much more important than that of trazodone. Likewise, the two pyridazine derivatives potentiated hypothermia induced by reserpine as did the 5-HT-reuptake inhibitors, trazodone and clomipramine. These results suggested that the activities of PC4 and PC13 in the reserpine test might be related to an α-adrenergic action or a 5-HT-ergic action (Bourin et al 1983). Taking into account the similar behavioural profile of pyridazine derivatives, trazodone, citalopram and fluoxetine, a 5-HT-ergic mechanism appeared evident, more especially as PC4 and PC13 included in their structure the 5-HT-ergic arylpiperazinyl moieties (Fuller et al 1978; Kahn & Wetzler 1991). In addition, administration of *m*-trifluoromethylpiperazine and *m*-chlorophenylpiperazine in mice produced dose-proportionate body temperature reductions (Maj et al 1988; Murphy et al 1991), as was also found after high intraperitoneal doses of PC4 and PC13.

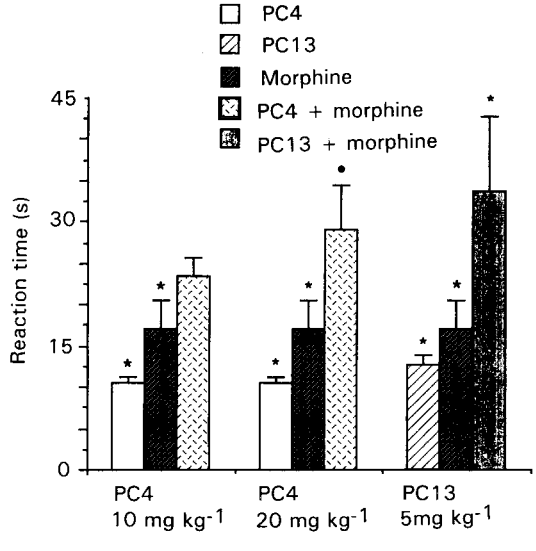


FIG. 5. Analgesic effect of morphine after acute intraperitoneal administration of PC4 and PC13. The antinociceptive effects of different groups were expressed as the time necessary to induce the licking reflex of the forepaws in mice. The baseline hot-plate latencies (saline, saline + saline) were 9·5 ± 1·4 and 9·8 ± 0·9 s, respectively. The bars represent s.e.m. *$P < 0.05$ compared with saline + morphine.

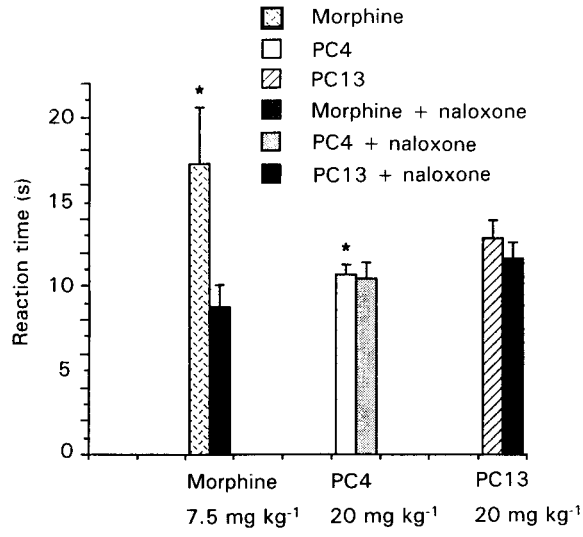


FIG. 6. Effect of naloxone on PC4-, PC13- and morphine-induced analgesia. The antinociceptive effects of different groups were expressed as the time necessary to induce the licking reflex of the forepaws in mice. The baseline hot-plate latencies (saline, saline + saline) were 8·4 ± 0·7 and 8·7 ± 0·7 s, respectively. The bars represent s.e.m. *$P < 0.05$ compared with saline + morphine, PC4 + saline and PC13 + saline.

Pretreatment of mice with the monoamine oxidase inhibitor pargyline induced head twitches in a dose-dependent manner, whereas administration of 5-HTP alone produces weak and less intense head twitches (Martin et al 1985, 1989; Nabeshima et al 1992). Use of high doses of 5-HTP generates tremors which seem to mask the head twitches (Martin et al 1985). Our observation that both PC4 and PC13 blocked the appearance of head twitches induced by 5-HTP in mice pretreated with pargyline suggested that these two compounds might possess antagonist activity at 5-HT$_2$ receptors. It is known that 5-HT$_2$ receptor binding sites mediate several 5-HT-dependent responses such as 5-HTP-induced head twitches in-vivo (Colpaert & Janssen 1983; Green et al 1983; Heal et al 1985; Kitatani et al 1993). The anti-DOI (5-HT$_2$ agonist) effects of PC4 and PC13 and molecular modelling confirmed their 5-HT$_2$ antagonistic

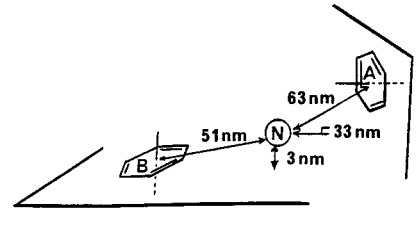


FIG. 7. Pharmacophoric model of the 5-HT$_2$ receptor of Hibert (1988).

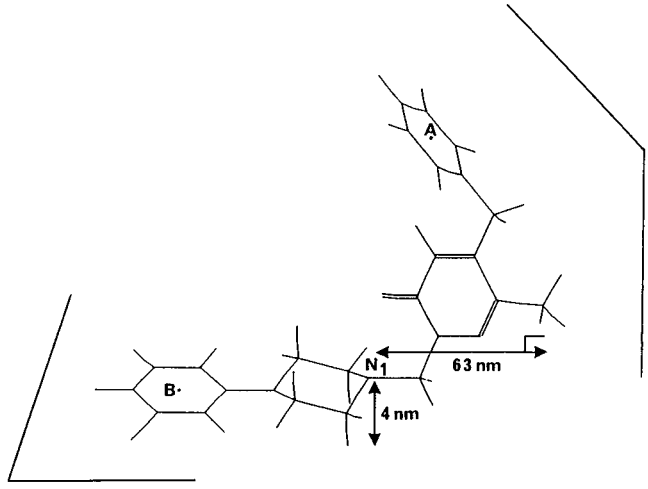


FIG. 8. Perspective view of PC13. $N_1 \leftrightarrow B = 56$ nm, $N_1 \leftrightarrow A = 78$ nm.

effect. Antagonism of DOI-induced head twitches by the two compounds was obtained in a similar dose-range potency as found for trazodone, whereas mianserin was slightly more active in this test.

Like many antidepressant drugs (citalopram, clomipramine, amitriptyline, maprotiline (Ardid et al 1992) and trazodone), PC4 and PC13 showed potent antinociceptive effect in the phenylbenzoquinone-induced abdominal constriction test. Analgesic activity was also found with PC4 and PC13 in the hot-plate test. In this last test, analgesia produced by our derivatives was equipotent to that described by Ardid et al (1992), with 5-HT-reuptake inhibitors tested at the same dose of 20 mg kg^{-1} intraperitoneally, whereas noradrenaline-reuptake inhibitors (desipramine and maprotiline) were less active. These results suggested that some properties of both PC4 and PC13 involve inhibition of 5-HT reuptake. In consequence, even if the 5-HT_2 antagonistic property of pyridazine derivatives was more dominant in-vivo than their possible 5-HT-uptake inhibitory property, the result of such effects might contribute to their effectiveness as antidepressant drugs. It is interesting to point out that two such apparently antagonistic properties have been previously reported for McN-5707 (Shank et al 1987) and clomipramine (Pinder et al 1980).

The interaction between PC4 or PC13 and morphine corresponded to a potentiation rather than a simple addition of the effects of the individual drugs. This potentiation of morphine analgesia might be explained by interference between both pyridazine derivatives and opiate receptors (Biegon & Samuel 1980; Somoza et al 1981; Isenberg & Cicero 1984), or by a possible inhibiting action of the new derivatives on the reuptake of 5-HT (Carlsson 1970; Lidbrink et al 1971). However, analgesic activity of PC4 and PC13 was not reversed by the opioid antagonist naloxone suggesting a lack of opioid involvement (Vaught et al 1990), and therefore a possible central 5-HT-ergic pathway as it was observed in narcotic antinociception (Deakin & Dostrovsky 1978; Yaksh 1979).

In conclusion, PC4 and particularly PC13 appeared to fit the biological activity profile for antidepressant efficacy in view of their effectiveness in the majority of mouse tests used generally for antidepressant trials. These two derivatives showed potential antidepressant properties at doses which were not sedative. This potential antidepressant activity seemed to be greatly mediated by a 5-HT-ergic mechanism and probably related to 5-HT_2 blockade. In the clinic, the use of 5-HT_2-receptor antagonists such as ritanserin has already been demonstrated (Idzikowsky et al 1986; Paiva et al 1988) and proposed in the treatment of anxiety and depression. PC4 and PC13 exhibited potent antinociceptive properties which appeared not to be mediated through an opioid mechanism but might be related to a 5-HT-ergic involvement.

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