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Pyridazine derivatives XII. Synthesis and antipsychotic-antidepressant activity of some butyrophenone derivatives of 6-phenylpyridazine

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Summary — We have synthesized several 3-amino-6-phenyl pyridazines in which the amino substituent is a linear butyrophenone moiety (compounds **8** and **10**), a cyclic butyrophenone moiety (compound **3**), or a phenylpiperazine fragment (compound **2**). Compound **8** potently inhibited [^{3}H]spiperone binding to striatal D_2 receptors and [^{3}H]SCH 23390 binding to striatal D_1 receptors (K_i in the nanomolar range but lower than that of haloperidol). Compounds **3**, **2** and **10** showed no affinity for dopamine (DA) receptors. Only 2 compounds (**3** and **8**) inhibited [^{3}H]ketanserin binding to cortical 5-HT_{2A} receptors; compound **8** strongly inhibited binding with a K_i similar to that of methysergide, while binding was only weakly inhibited by compound **3**. The DA and 5-HT_{2A} antagonist activity of compound **8** was evaluated *in vivo* and *in vitro*. The results in standard screening tests indicate that this compound possesses neuroleptic activity. However, in contrast to haloperidol, compound **8** did not modify DA and its metabolite levels in rat striatum, or induce catalepsy. It inhibited serotonin-induced contractions in endothelium-stripped aorta with a pA_2 of 8.26 and did not affect reserpine-induced palpebral ptosis, indicating that it does not have antidepressant activity; compound **10**, however, showed slight activity in this test.

pyridazine / butyrophenone / synthesis / antipsychotic activity / antidepressant activity

Introduction

The discovery in 1959 [1] that a series of butyrophenone derivatives had pronounced central nervous system (CNS) depressant activity has led to the preparation of a number of related compounds. Several of them, in particular haloperidol, have been found to possess useful neuroleptic activity in man [2]. Recently a large number of new tertiary and secondary amines derived from 4-aminobutyrophenones have been reported as D_2 antagonists and their CNS depressant activities evaluated [3]. More recently, D_2- and/or 5-HT_{2A}-receptor-blocking activity has been reported for certain structures, including cinuperone [4] and setoperone [5], in which the *p*-fluorobenzoylpiperidine fragment (which can be seen as a butyrophenone) is linked directly or indirectly to heterocycles (fig 1).

Wermuth *et al* [6] state that compounds with structures related to that of aminopyridazine may similarly exhibit a wide range of psychotropic effects and affect many neurotransmitter systems. Compounds of this type include useful therapeutic drugs, such as the commercially available minaprine, 3-[(β-morpholinoethyl)amino]-4-methyl-6-phenylpyridazine dihydrochloride (fig 1), which shows an atypical antidepressant profile but which, unlike classical tricyclic antidepressants, has no anticholinergic effects. In contrast, minaprine has cholinomimetic properties which could, at least in part, be mediated by its interaction with M_1 muscarinic receptors [7]. Biochemical and pharmacological studies *in vivo* have shown that minaprine enhances both serotonergic and dopaminergic transmission [6, 7], but does not affect noradrenergic transmission [8]. However, *in vitro* studies in rat frontal cortex with minaprine show that the drug

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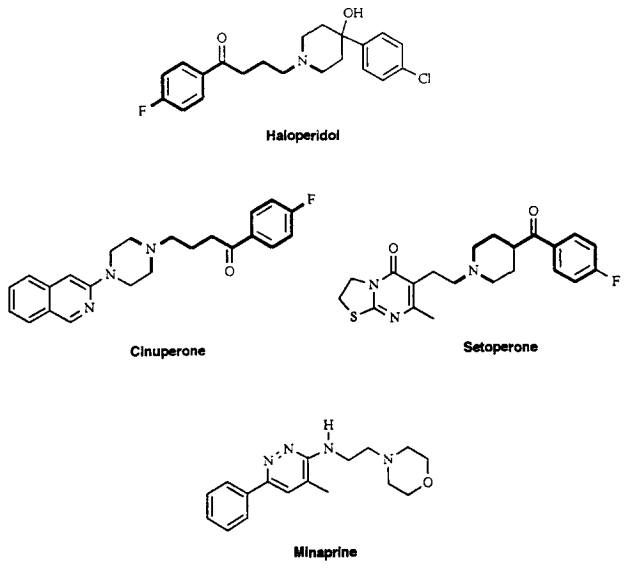


Fig 1.

reduces the accumulation of cAMP induced by noradrenaline, suggesting that minaprine (like other antidepressants) attenuates beta-adrenergic receptor function [9]. *In vitro*, minaprine does not affect the uptake, release or metabolism of either serotonin or dopamine (DA), and does not appear to interact with either serotonin or DA receptors [10]. However, as evidenced in some studies, minaprine inhibits the binding of [^{3}H]-ketanserin in rat hippocampus slices (K_i = 2.9 μM) [11] and in the rat striatum [12], and appears to interact with heterologous presynaptic 5-HT_{1B} receptors [13]. Thus, the mechanism, or mechanisms, through which minaprine exerts this dual serotoninomimetic and dopaminomimetic activity remain unclear.

Following our earlier reports on pyridazines [14–16] and butyrophenones [17, 18], we now describe the synthesis and potential antipsychotic-antidepressant activity of compounds **2**, **3**, **8** and **10**. These compounds bear a 6-phenylpyridazine fragment, which is also present in minaprine, and a linear flexible butyrophenone linked to the 6-phenylpyridazine by a piperazine bridge in compound **8**; a linear flexible butyrophenone linked directly to the 6-phenylpyridazine fragment in compound **10**; a *p*-fluorobenzoylpiperidine moiety in compound **3** [19]; or a phenylpiperazine fragment in compound **2**[1] (see scheme 1).

[1]The introduction of an arylpiperazine fragment by means of pharmacomodulation is well known (see reference [14] and references cited therein).

Results

Chemistry

Compounds **2** and **3** were prepared by direct nucleophilic replacement of the chlorine atom of 3-chloro-6-phenylpyridazine [20] with the corresponding amines: *N*-phenylpiperazine for compound **2** and 4-*p*-fluorobenzoylpiperidine for compound **3**. Two alternative routes were considered for the preparation of **8**. In *Route A*, the reaction of 3-chloro-6-phenylpyridazine with *p*-fluoro-4-(*N*-piperazin-1-yl)butyrophenone **5** gave poor yields (about 30%), and the intermediates were difficult to manipulate. Higher yields were obtained in the reaction of 3-(*N*-piperazin-1-yl)-6-phenylpyridazine **6** with 4-chloro-1,1-ethylenedioxy-1-(4-fluorophenyl)butane in methyl isobutyl ketone and subsequent acidic hydrolysis of ethyleneketal (*Route B*). The latter procedure also allowed the intermediates to be more easily manipulated.

The best yields for compound **10** (65%) were obtained by refluxing 3-chloro-6-phenylpyridazine with 4-amino-1,1-ethylenedioxy-1-(4-fluorophenyl)butane **9** [21] after addition of potassium iodide, under strongly basic conditions and with prolonged heating.

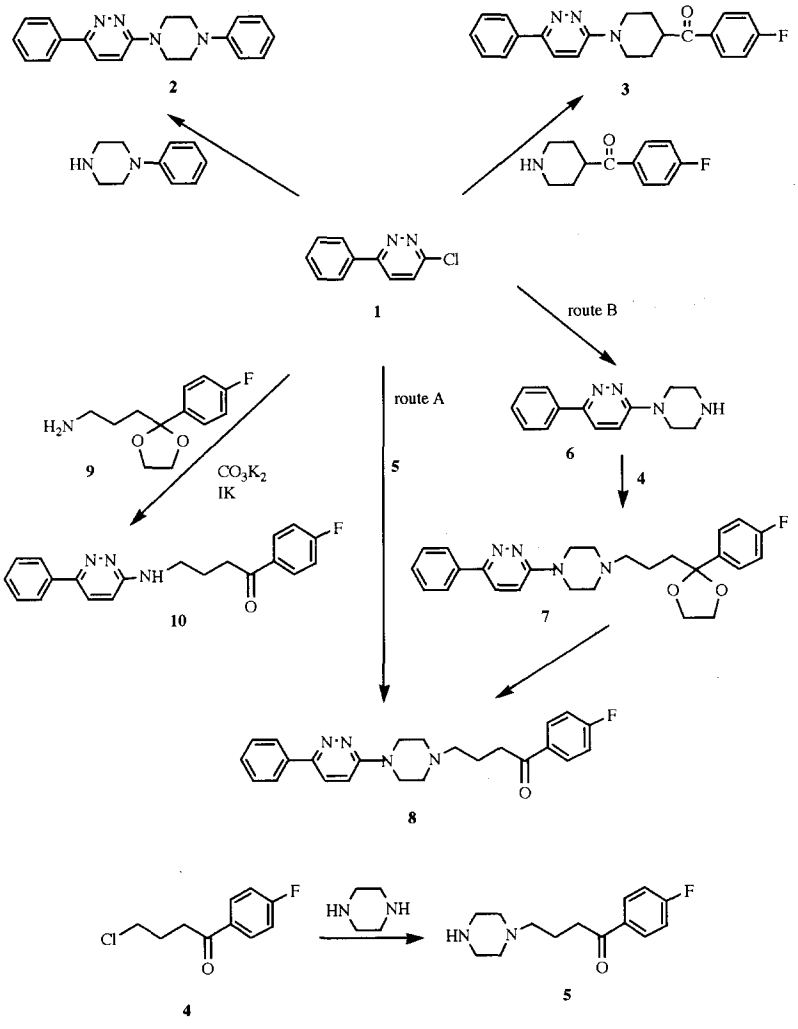
Pharmacology

In vitro *experiments*

Binding assays. Compound **8** inhibited [^{3}H]spiperone binding to D_2 receptors and [^{3}H]SCH 23390 binding to D_1 receptors in rat striatal tissue preparations. Affinity for D_2 and D_1 receptors was about 10 and 3 times lower than that of haloperidol. Interestingly, compounds **2**, **3** and **10** did not inhibit either [^{3}H]spiperone or [^{3}H]SCH 23390 binding to dopamine receptors in striatum in concentrations up to 10^{-5} M. Dilution in methanol precluded the use of higher concentrations.

Only 2 compounds (**3** and **8**) inhibited [^{3}H]ketanserin binding to 5-HT_{2A} receptors in the frontal cortex. The most active was compound **8**, which had a pK_i of 8.13 (similar to that of methysergide, pK_i = 8.84) and its affinity for these receptors was about 3 times higher than that of haloperidol (pK_i = 7.70). Compound **3** had a very low affinity for 5-HT_{2A} receptors, with a pK_i of 5.52 (table I).

Effects on DA and DA metabolite levels in rat striatum. Compound **8** (10 mg/kg) did not modify the levels of DA or its metabolites (DOPAC, 3-MT and HVA) in rat striatum 2 h after administration. Haloperidol (2 mg/kg), on the other hand, caused significant increases in the levels of both DOPAC and HVA (218% and 273%, respectively), although it had no significant effects on levels of DA or 3-MT (results not shown).



Scheme 1.

Effects on serotonin-induced contractions of rat-aorta rings. Compound **8** inhibited serotonin-induced contractions of endothelium-rubbed rat aorta with a pA_2 of 8.26 ± 0.74 (slope 0.61); this pA_2 value is slightly lower than that of ketanserin (8.87 ± 0.11, slope 0.82) (table I).

In vivo experiments

Locomotor activity. Compound **8** caused significant dose-dependent reductions in spontaneous motor activity of naive mice (91.5% at 10 mg/kg, 35.8% at 2 mg/kg), 60 min after administration, compared with the 94% reduction induced by haloperidol (2 mg/kg) (fig 2).

Compound **8** and haloperidol caused a significant reduction in the hypermotility induced by amphetamine (5 mg/kg, ip) 60 min after administration in hyperactive mice. Percentage inhibition by compound **8** was 32.2, 52.8 and 70.5% at doses of 5, 10 and 20 mg/kg ip respectively (ED_{50} = 9.36 mg/kg), and percentage inhibition by haloperidol was 28, 33.5 and 72.6% at doses of 0.063, 0.094 and 0.125 mg/kg ip respectively (ED_{50} = 0.098 mg/kg) (fig 3).

Antagonism of apomorphine-induced stereotyped activity. Compound **8** antagonized stereotypies induced by apomorphine (a mixed D_1/D_2 agonist) during the period when apomorphine has most effect (20–30 min after administration), showing an ED_{50} value of 9.85 mg/kg ip. Compound **8** was not, however, as potent as haloperidol, which showed an ED_{50} value of 0.13 mg/kg ip (fig 4).

Catalepsy. The induction of catalepsy in mice by compound **8** was much less than that shown by halo-

Table I. pK_i values for inhibition of [^{3}H]ketanserin binding to rat frontal cortex membranes (5-HT_{2A}) and of [^{3}H]spiperone or [^{3}H]SCH 23390 binding to striatal membranes (D_2 or D_1). Results are means ± sem of 3–4 experiments.

Drug	*pK_i values*			*pK_i ratios*			*pA_2*
	D_1	*D_2*	*5-HT_{2A}*	*D_1/D_2*	*5-HT_{2A}/D_1*	*5-HT_{2A}/D_2*	*5-HT_{2A}*
Haloperidol	7.01	8.30	7.70	0.85	1.10	0.93	–
2	< 5	< 5	< 5	–	–	–	–
3	< 5	< 5	5.52	–	–	–	–
8	6.65	7.39	8.13	0.90	1.22	1.10	8.26
10	< 5	< 5	< 5	–	–	–	–
Ketanserin	–	–	–	–	–	–	8.87
Methysergide	–	–	8.84	–	–	–	–

pA_2 values were obtained against serotonin-induced contractions in rat-aorta rings; (–) not evaluated.

peridol. The ED_{50} value for compound **8**, 60 min after administration, was 110 mg/kg (confidence limits 56–215 mg/kg), and that of haloperidol 0.66 mg/kg (confidence limits 0.5–0.9 mg/kg) (fig 5).

Physostigmine (eserine)-induced mortality. Neither compound **8** (10 mg/kg) nor haloperidol (2 mg/kg) conferred any protection against the lethal effects of this cholinesterase inhibitor, compared with the full protection afforded by atropine (4 mg/kg) (data not shown).

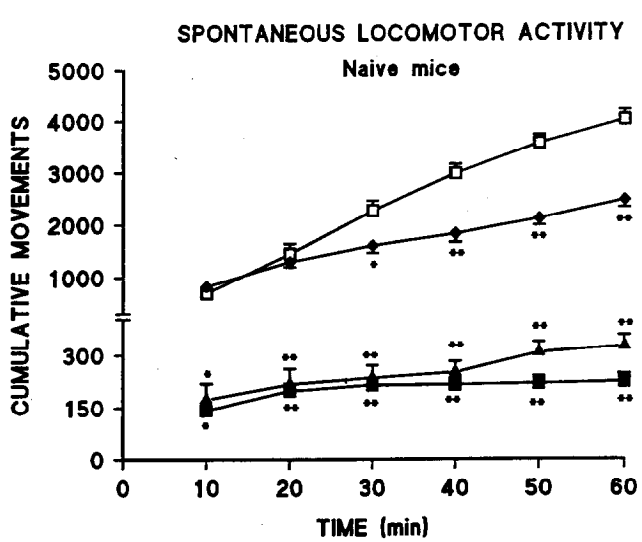


Fig 2. Spontaneous locomotor activity in mice treated with vehicle (□), haloperidol (■ 2 mg/kg) or compound **8** (◆ 2 mg/kg, ▲ 10 mg/kg). The data shown are means ± sem. Significant differences with respect to control indicated by * ($p < 0.05$) or ** ($p < 0.01$).

Antagonism of reserpine-induced ptosis. Of the new compounds only compound **10** (10 mg/kg) showed antidepressant activity, inhibiting ptosis by 30% (*cf* imipramine at 2.4 mg/kg inhibited ptosis by 55%, and minaprine dihydrochloride showed an ED_{50} value of 7.1 mg/kg (confidence limits 5.3–9.6 mg/kg).

Discussion

All the compounds synthesized in this study, except compound **2** bear either a flexible (compounds **8** and **10**) or rigid (compound **3**) butyrophenone fragment. Previous studies have shown that both rigid and flexible butyrophenone fragments display affinity for D_2 receptors [1, 2, 17, 19]. Of the compounds studied here, only compound **8** interacts with D_2 receptors (compounds having K_i values greater than 10 000 nM in the binding assays were considered to display no affinity for receptors).

The affinity for D_2 receptors for compound **8** and not for compounds **3** and **10** could be explained on the basis of the presence of a more basic aliphatic nitrogen atom in **8** than the aminopyridazinic nitrogen atom in **3** and **10** (all the reference compounds in figure 1 that are active as dopamine antagonists have an aliphatic nitrogen atom). The lack of binding by compound **10** may be attributed to the absence of a tertiary nitrogen, since binding to D_2 receptors generally seems to involve a basic tertiary amino group; only a few compounds with no tertiary nitrogen have been reported to possess antipsychotic activity [22].

Compound **8** inhibited [^{3}H]ketanserin binding to frontal cortex 5-HT_{2A} receptors and compound **3** slightly inhibited binding. Compounds **2** and **10** caused no inhibition. These differences can probably be attributed to the reasons outlined above for D_2 receptors, because the structural requirements for

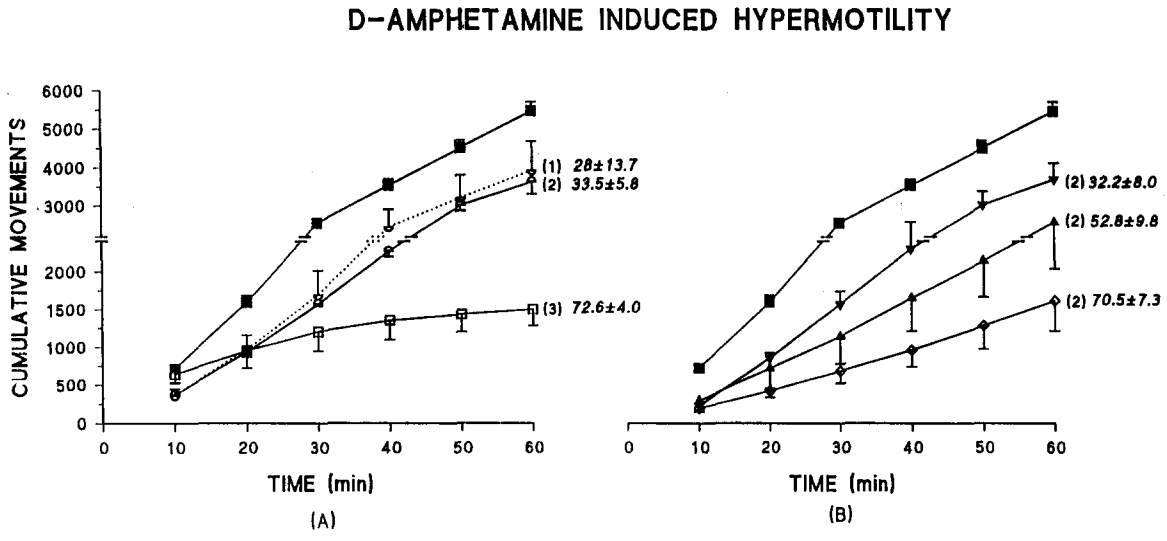


Fig 3. Antagonism of *d*-amphetamine-induced hypermotility in mice treated with: (A) vehicle (■), haloperidol (▫ 0.063, ▫ 0.094, □ 0.125 mg/kg) or (B) compound **8** (▼ 5, ▲ 10 or ◇ 20 mg/kg). The data shown are means ± sem. (1) Significant differences with respect to control ($p < 0.01$ at 10, 20, 30 min and $p < 0.05$ at 40, 50, 60 min after administration). (2) Significant differences with respect to control ($p < 0.01$) throughout the experiment. (3) No significant differences with respect to control 10 min after administration, significant differences with respect to control ($p < 0.05$ 20 min after administration and $p < 0.01$ at other times of measurement).

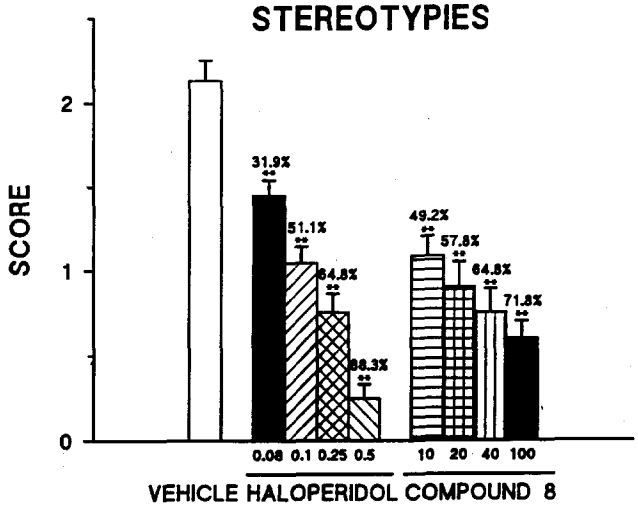


Fig 4. Stereotyped behaviour induced by administration of apomorphine (1 mg/kg, sc) 30 min after administration of vehicle, haloperidol (0.08, 0.1, 0.25 or 0.5 mg/kg) or compound **8** (10, 20, 40 or 100 mg/kg). Stereotyped behaviour was monitored 20 and 30 min after injection of apomorphine. The data shown are mean ± sem of averaged stereotypy scores obtained between 20–30 min. Significant differences with respect to control indicated by * ($p < 0.05$) or ** ($p < 0.01$). Numbers at the top of columns show the percentage reduction in stereotyped behaviour.

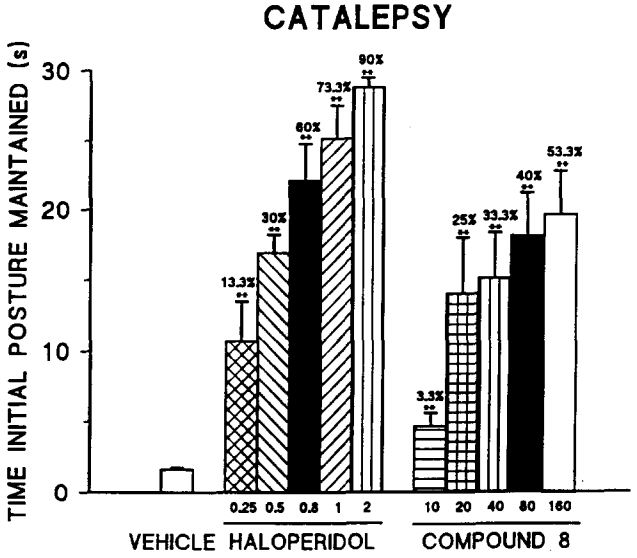


Fig 5. Time for which the initial posture was maintained in the catalepsy test (mean ± sem). Mice were treated with vehicle, haloperidol (0.25, 0.5, 0.8, 1 or 2 mg/kg) or compound **8** (10, 20, 40, 80 or 160 mg/kg). Times in excess of 30 s were recorded as 30 s. Significant differences with respect to control indicated by * ($p < 0.05$) or ** ($p < 0.01$). Numbers at the top of columns show the percentage of cataleptics.

binding to D_2 receptors and 5-HT_{2A} receptors are probably very similar, since there are many molecules with a butyrophenone pharmacophore which display affinity for both types of receptors.

The spontaneous motor activity test is useful for classifying molecules with suspected CNS activity as stimulants or depressants [23]. In this test, we found compound **8** to be about 5 times less potent than haloperidol.

The induction of hypermotility by amphetamine at low doses (5 mg/kg) is largely due to the stimulation of dopaminergic neurotransmission in the nucleus accumbens [24, 25]. Haloperidol strongly inhibited both spontaneous motor activity and amphetamine-induced hypermotility, whereas compound **8** inhibited amphetamine-induced hypermotility much less strongly than spontaneous motor activity. Since the hypermotility test specifically evaluates blockade of postsynaptic DA receptors in the nucleus accumbens, this suggests that the reduction of spontaneous motor activity by compound **8** involves other effects (for example, sedation due to blockade of α_1-adrenergic, H_1 or other central receptors). There was some inhibition of amphetamine-induced hypermotility by compound **8**, which indicates that it can be classified as a neuroleptic. Compound **8** also reduced apomorphine-induced stereotypes.

In the catalepsy test, compound **8** caused a slight increase in the time for which the initial posture was maintained and the percentage of animals showing catalepsy was much lower than that exhibited by haloperidol. The 'atypical' neuroleptic profile is characterized by a large margin between the doses inhibiting amphetamine or apomorphine-induced behavioural effects (index for antipsychotic activity) and the doses inducing catalepsy (index for acute extrapyramidal side effects). The ED_{50} ratios for the new compound were about twice as high as that of haloperidol (table II).

Given that the induction of catalepsy in mice is strongly correlated with the appearance of extrapyramidal symptoms (EPS) in humans [26], the results of the catalepsy test suggest that compound **8** may have a weak propensity to induce EPS in man.

The catalepsy test results at the dose of 10 mg/kg are also in agreement with the determinations of endogenous DA and its metabolites in rat striatum, which suggests that compound **8** has no effect on striatal dopaminergic neurons (or that its effects are masked).

It is well known that administration of an anticholinergic at the same time as a neuroleptic may attenuate or abolish the EPS caused by the latter. It has been suggested that neuroleptics may provoke EPS by causing an imbalance between the dopaminergic and cholinergic systems [27]. In accordance with this, neuroleptics, such as clozapine (which possesses both antidopaminergic and anticholinergic activity), do not cause EPS. However, compound **8** did not protect against physostigmine (eserine)-induced mortality, suggesting that it does not have anticholinergic activity.

Recent studies [28–31] have indicated that 5-HT_{2A} blockers not only reduce the capacity of neuroleptics to induce catalepsy but also contribute to their antipsychotic activity. Setoperone and ritanserin, for example, are both more selective for 5-HT_{2A} receptors than for D_2 receptors, and have been reported to have few EPS and to be more effective than 'typical' neuroleptics at reducing the negative symptoms of schizophrenia [32, 33]. These findings have led to the development of a number of compounds with combined 5-HT_{2A}- and D_2-blocking activity. Compound **8** inhibited serotonin-induced contractions of endothelium-rubbed rat-aorta rings (see table I). Furthermore, the determination of pK_i values for 5-HT_{2A} and D_2 binding sites also provides a basis for classifying a compound as a 'typical' or 'atypical' antipsychotic: the ratio of pK_i 5-HT_{2A} to pK_i D_2 is characteristically in the range 0.79–1.09 for 'typical' antipsychotics and 1.12–1.43 for 'atypical' antipsychotics [34]. The ratio for compound **8** was 1.10.

Neuroleptics bind to D_2 receptors or to both D_2 and D_1 receptors, and it has been suggested that drugs that have affinity for both receptor types cause fewer EPS than drugs that are more selective for D_2 receptors [35–38]. The difference between pK_i D_2 and pK_i D_1 for compound **8** (0.74) is much lower than that for haloperidol (1.29), indicating that compound **8** is less D_2-selective than haloperidol. The weak induction of catalepsy by compound **8** may be a result of a relatively high pK_i 5-HT_{2A}/pK_i D_2 ratio and/or a relatively

Table II. ED_{50} values and ratios.

Compound	*ED_{50} (mg/kg, ip)*				
	Inhibition hyperactivity[a]	*Inhibition stereotypies*[b]	*Induction catalepsy*[c]	*ED_{50}^c/ED_{50}^a*	*ED_{50}^c/ED_{50}^b*
Haloperidol	0.098	0.13	0.67 (0.5–0.9)	6.8	5.2
8	9.36	9.85	110 (56–215)	11.8	11.2

low selectivity for D_2 receptors compared to D_1 receptors.

Finally, compounds **8**, **3** and **2** did not inhibit palpebral ptosis induced by reserpine. Compound **10**, which bears a secondary nitrogen atom, slightly inhibited reserpine-induced ptosis.

In conclusion, all the new compounds (except compound **2**) possess a flexible or rigid butyrophenone fragment, linked to the 6-phenylpyridazine moiety. Of the 3 compounds that carry the butyrophenone fragment only compound **8** possessed affinity for DA receptors which can probably be explained by the basic character of the piperazine bridge.

Experimental protocols

Chemistry

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer 1600 FTIR spectrophotometer (KBr discs and NaCl film). ^{1}H-NMR spectra were recorded in $CDCl_3$ with a Bruker WM-250 (250 MHz) spectrometer; chemical shifts are given in ppm with respect to TMS as an internal standard. Elemental analyses for C, H, N were performed by the Microanalysis Service of the University of Santiago de Compostela using a Perkin-Elmer 240 apparatus, and were within ± 0.4% of the theoretical values. The compound *p*-fluoro-4-(*N*-piperazin-1-yl)butyrophenone **5** was prepared as previously described [17].

6-Phenyl-3-(4-phenylpiperazin-1-yl)pyridazine ***2***
A mixture of 3-chloro-6-phenylpyridazine [39–40] (0.5 g, 2.6 mmol) and 1-phenylpiperazine (0.85 g, 5.2 mmol) in 15 ml toluene was refluxed for 24 h. The precipitate was filtered off, dissolved in water and extracted with CH_2Cl_2. The organic fractions were dried (Na_2SO_4) and evaporated under reduced pressure to yield 0.35 g (42%) of compound **2**; mp 226–228°C (EtOH); NMR: δ 3.36 (t, J = 5.1 Hz, 4H, $(CH_2)_2$-N-Ph), 3.89 (t, J = 5.1 Hz, 4H, $(CH_2)_2$-*N*-pyridazine), 6.92 (t, J = 7.3 Hz, *p*-Ph-piperazine), 6.98–7.02 (m, 2H, *o*-Ph-piperazine), 7.05 (d, J = 9.6 Hz, 1H, pyridazine), 7.28–7.32 (m, 2H, *m*-Ph-piperizine), 7.41–7.52 (m, 3H, *m*- and *p*-Ph-pyridazine), 7.69 (d, J = 9.6 Hz, 1H, pyridazine), 7.99–8.03 (m, 2H, *o*-Ph-pyridazine).

6-Phenyl-3-[4-(p-fluorobenzoyl)piperidin-1-yl]pyridazine ***3***
A mixture of 3-chloro-6-phenylpyridazine (0.62 g, 3.25 mmol) and 4-*p*-fluorobenzoylpiperidine (1.36 g, 6.5 mmol) in 20 ml xylene was refluxed for 48 h. The precipitate was filtered off, dissolved in water and extracted with CH_2Cl_2. The organic fractions were dried (Na_2SO_4) and evaporated under reduced pressure to yield 0.7 g (65%) of compound **3**; mp 173–174°C (*i*-Pro); NMR: δ 1.84–2.05 (m, 4H $(CH_2\text{-}CH_2)_2$N-), 3.22 (dt, J = 3.3, 12.3 Hz, 2H, $(HCH)_2$-N ax), 3.47–3.60 (m, 1H, >C*H*-), 4.49–4.54 (m, 2H, $(HCH)_2$N eq), 7.03 (d, J = 9.5 Hz, 1H, pyridazine), 7.17 (t, J = 8.5 Hz, 2H, *o*-F), 7.39–7.50 (m, 3H, *m*-Ph and *p*-Ph), 7.66 (d, J = 9.5 Hz, 1H, pyridazine), 7.98–8.04 (m, 4H, 2 *o*-Ph-CO and 2 *o*-Ph-pyridazine).

3-(N-Piperazin-1-yl)-6-phenylpyridazine ***6***
A solution of 3-chloro-6-phenylpyridazine (0.96 g, 5 mmol) and anhydrous piperazine (2.58 g, 30 mmol) in 25 ml methyl isobutyl ketone was refluxed with stirring for 12 h. The solvent was removed under reduced pressure and the residue dissolved in CH_2Cl_2. The organic phase was washed with water, dried (Na_2SO_4) and concentrated to yield 1.10 g (91%) of compound **6**; mp 155–156°C (MeOH); NMR: δ 1.69 (s, 1H, NH), 3.03 (t, J = 5.1 Hz, 4H, $(CH_2)_2$-NH), 3.69 (t, J = 5.1 Hz, 4H, $(CH_2)_2$-N-pyridazine), 6.98 (d, J = 9.5 Hz, 1H, pyridazine), 7.40–7.51 (m, 3H, *m*-Ph, *p*-Ph), 7.66 (d, J = 9.5 Hz, 1H, pyridazine), 7.98–8.02 (m, 2H, *o*-Ph-pyridazine).

4-[(4-Phenylpyridazinyl)piperazin-1-yl]-1,1-ethylenedioxy-1-(4-fluorophenyl)butane ***7***
A mixture of compound **6** (1 g, 4.2 mmol), 4-chloro-1,1-ethylenedioxy-1-(4-fluorophenyl)butane (1.02 g, 4.2 mmol), Na_2CO_3 (2.04 g) and KI (50 mg) in 70 ml methyl isobutyl ketone was refluxed with stirring for 8 h. The precipitate was filtered off and the solvent removed *in vacuo*. The crude residue was dissolved in water and extracted with CH_2Cl_2, dried (Na_2SO_4) and concentrated to yield 1.8 g of **7** (96%).

3-[4-[3-(p-Fluorobenzoyl)-1-propylpiperazin-1-yl]-6-phenylpyridazine ***8***
Route A. To a solution of compound **5** (1 g, 4 mmol) in 50 ml methyl isobutyl ketone, Na_2CO_3 (1.41 g), 3-chloro-6-phenylpyridazine (0.76 g, 4 mmol) and catalytic amounts of KI were added. The mixture was refluxed for 48 h. The precipitate was filtered off and the solvent removed *in vacuo*. Compound **8** was difficult to isolate and purify from the filtrate, and was obtained in low yield; this prompted us to prepare **8** by the following procedure.

Route B. A solution of compound **7** (1.8 g, 4.24 mmol) and concentrated HCl (2 ml) in 25 ml methanol was refluxed for 2 h. The reaction mixture was diluted with ethyl acetate and successively washed with 10% aqueous $NaHCO_3$ and water. The solution was dried (Na_2SO_4) and the solvents were removed by evaporation under reduced pressure. The resulting solid was recrystallized from isopropanol to yield 1.1 g (68%) of solid product **8**; mp 164–166°C (*i*-Pro); IR (KBr): ν 1680 (C=O), 1600 (Ph); NMR: δ 2.00 (q, J = 7.0 Hz, 2H, -C-CH_2-C-), 2.49 (t, J = 7.0 Hz, 2H, N-CH_2), 2.59 (t, J = 5.0 Hz, 4H, $(CH_2)_2$-N-butyrophenone), 3.03 (t, J = 7.0 Hz, 2H, CH_2-CO), 3.66 (t, J = 5.0, 4H, $(CH_2)_2$-N-pyridazine), 6.97 (d, J = 9.6 Hz, 1H, pyridazine), 7.14 (t, J = 8.5 Hz, 2H, *m*-Ph-CO), 7.39–7.49 (m, 3H, *m*- and *p*-Ph-pyridazine), 7.65 (d, J = 9.5 Hz, 1H, pyridazine), 8.01 (m, 4H, *o*-Ph-pyridazine).

3-[3-(p-Fluorobenzoyl)-1-propylamino]-6-phenylpyridazine ***10***
A mixture of the chloropyridazine **1** (0.42 g, 2.2 mmol), 4-amino-1,1-ethylenedioxy-1-(4-fluorophenyl)butane **9** (0.5 g, 2.2 mmol), K_2CO_3 (0.84 g, 6.1 mmol) and KI (18 mg) in 3.5 ml DMF was heated at reflux for 24 h and concentrated *in vacuo*. The mixture was partitioned between Et_2O and water. The organic phase was washed with water, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was dissolved in MeOH and treated with HCl/MeOH to give 0.55 g (65%) of **10** as hydrochloride; mp 207–208°C; IR (KBr): ν 3215 (N-H), 1680 (C=O), 1600 (C=C arom). NMR: δ 2.17 (q, J = 6.8 Hz, 2H, -CH_2-CH_2-CH_2-), 3.13 (t, J = 6.8 Hz, 2H, -CH_2-CO), 3.62 (c, J = 6.6 Hz, 2H, -CH_2-NH-), 4.95 (s, 1H, NH), 6.74 (d, J = 9.14 Hz, 1H, pyridazine), 7.13 (t, J = 8.6 Hz, 2H, *m*-Ph-CO), 7.40–7.50 (m, 3H, *m*- and *p*-Ph-pyridazine), 7.60 (d, J = 9.3 Hz, 1H, pyridazine), 7.95–8.02 (m, 4H, *o*-Ph-pyridazine and *o*-Ph-CO).

Pharmacology

Experimental animals and conditions
Adult male Charles River CD1 albino mice weighing 25 ± 2 g, adult male Wistar rats (275 ± 25 g) and adult male Sprague–

Dawley rats (300 ± 50 g) were brought to the laboratory at least one week before use and were housed under a 12:12 h light/dark cycle (08.00–20.00 h light) at 20–21°C. Food and tap water were freely available in the home cage. *In vivo* experiments were performed between 09.00 and 14.00 h.

Drugs and chemicals

All compounds were administered in 0.01 ml/g injections (apomorphine subcutaneously and all others intraperitoneally). All test compounds were administered as suspensions in 0.5% (W/V) sodium carboxymethylcellulose (Merck), except compound **10** which was dissolved in 1% lactic acid in water. Haloperidol and reserpine (Sigma) were dissolved in 1% lactic acid in water and 1% acetic glacial acid in water, respectively. Apomorphine hydrochloride, dextroamphetamine sulphate, physostigmine (eserine) hemisulphate, imipramine hydrochloride, minaprine dihydrochloride and atropine sulphate (Sigma) were prepared in saline. Methanol, citric acid monohydrate, perchloric acid, sodium bisulphite and sodium chloride were all of reagent grade. Dopamine HCl, 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine HCl (3-MT), homovanillic acid (HVA) and 1-hexanesulphonic acid (sodium salt) were purchased from Sigma. The water was deionized and then double quartz-distilled. All other reagents were of analytical grade.

In vitro experiments

Binding assays. Wistar rats were killed by cervical dislocation and decapitation. Both striata were quickly dissected out on a cold plate, weighed and stored at –20°C until assay.

For [^{3}H]spiperone binding assays, paired striata were homogenized in 50 vol of ice-cold 50 mM Tris HCl with a Polytron (setting 6 for 5 s), and centrifuged at 40 000 *g* for 10 min in a Sorvall centrifuge at 4°C. The pellet was resuspended and the process repeated. The final pellet was resuspended in 200 vol of 50 mM Tris HCl buffer containing 120 mM NaCl. Samples (200 µl) of the final suspension were incubated for 10 min at 37°C with 25 µl of displacing agent or vehicle (10% methanol) and 25 µl of a solution of [^{3}H]spiperone; the reaction was terminated by rapid vacuum filtration through Whatman GF/C filters, which were washed with 3 x 5 ml cold buffer. For equilibrium saturation analysis, 6 ligand concentrations from 0.05 to 1 nM were used. Non-specific binding was determined by addition of 10^{-5} M of (+)-sulpiride. For determination of the IC_{50} values of drugs inhibiting [^{3}H]spiperone (0.25 nM) binding, at least 6 ascending concentrations of each drug were used (10^{-9}–10^{-4} M). Assays were carried out in triplicate at each ligand or displacing drug concentration.

For [^{3}H]SCH-23390 binding assays paired striata were homogenized in 200 vol of 50 mM Tris HCl buffer and centrifuged at 1000 *g* for 10 min, and the supernatant was centrifuged at 20 000 *g* for 10 min. The pellet was resuspended and the process repeated. The final pellet was resuspended in 50 mM Tris HCl containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$. Saturation curves were constructed with 6 ligand concentrations from 0.15–2.5 nM. Non-specific binding was determined by addition of unlabelled SCH-23390 (10^{-6} M). Samples were incubated at 25°C for 30 min.

For [^{3}H]ketanserin binding, the frontal cortex tissue was dissected on a cold plate, weighed and homogenized (Ultraturrax, 5 s at 20 000 rpm) in 50 vol of Tris HCl 50 mM, pH 7.4, and centrifuged at 30 000 *g* for 10 min at 4°C. The pellet was rehomogenized and centrifuged again. The final pellet was reconstituted in 200 vol of buffer. Aliquots of membrane preparations (200 µl) were incubated with 25 µl of 1 nM [^{3}H]ketanserin (NEN 60 Ci/mmol), specific binding was defined by the incorporation of 25 µl methysergide (1 µM final concentration). Samples were incubated for 15 min at 37°C, and incubation was terminated by vacuum filtration.

DA, DOPAC, 3-MT and HVA measurements in rat striatum. Sprague–Dawley rats were killed by decapitation 120 min after injection of vehicle, haloperidol (2 mg/kg) or compound **8** (10 mg/kg). The brain was removed within 30 s of decapitation and dissected on ice; the striatum was removed as described by Glowinski and Iversen [41] and immediately submitted to analysis. Samples of striatal tissue were weighed in 1.5 ml conical test-tubes, and 1000 µl of PCA solution (0.1 M perchloric acid, 4×10^{-5} M sodium bisulphite) was then added. The mixture was sonicated on ice (200 W for about 5 s), and the homogenate was centrifuged twice for 5 min at 10 500 rpm. A 20-µl aliquot of the supernatant was injected onto the chromatographic system (Kontron HPLC system 600 and Coulochem model 5100A). The mobile phase (citrate buffer pH 4.25, ionic strength 0.1, mixed 92:8 v/v with methanol, and containing 1-hexanesulphonic acid, sodium salt, at 1.7×10^{-3}, M was filtered (0.22 µm pore size) and then degassed with ultrasound before use. The flow rate was 1 ml/min and ambient temperature was maintained [42].

Aorta-ring experiments. Endothelium-stripped aorta rings from Sprague–Dawley rats were mounted under a resting tension of 1.5 g in a 20 ml organ bath containing Krebs solution (composition (mM): NaCl, 118.07; KCl, 4; $CaCl_2 \cdot H_2O$, 2.5; $MgSO_4 \cdot 7H_2O$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 1 l) at 37°C bubbled with carbogen (95% O_2, 5% CO_2). Isometric contraction forces were measured with a CPUL 0–25 g transducer connected to a Celaster IOS-1 apparatus. After stabilization for 60 min, cumulative concentration–response curves were constructed as described by Van Rossum [43], increasing serotonin concentration from 30 nM to 10 mM in the absence or the presence of increasing concentrations of ketanserin or compound **8**.

In vivo *experiments*

Locomotor activity. Locomotor activity during the hour following drug administration was measured in an activity cage (Panlab Actisystem DAS 16 V.1), which contained an electromagnetic field sensitive to any motion within it. Groups of 3 mice were placed in the cage immediately after administration of the control or test compounds.

The animals received either: a) vehicle, haloperidol (2 mg/kg) or compound **8** (2 or 10 mg/kg) (naive mice); or b) vehicle, haloperidol (0.125, 0.094 or 0.063 mg/kg) or compound **8** (5, 10 or 20 mg/kg) followed 30 min later by amphetamine (5 mg/kg) (hyperactive mice).

Antagonism of apomorphine-induced stereotyped behaviour. Mice (n = 10–34) received vehicle, haloperidol (0.08, 0.1, 0.25 or 0.5 mg/kg) or compound **8** (10, 20, 40 or 100 mg/kg), and then 30 min later apomorphine (1 mg/kg). Stereotyped behaviour was monitored 20 and 30 min after administration of apomorphine, and recorded on the following scale: **0** absence of stereotypes; **1** some stereotypes; **2** frequent sniffing with some nibbling and licking of paws; **3** near-continuous nibbling and licking, with mice usually climbing up the wall of the cage [44].

Catalepsy. Signs of catalepsy in mice (n = 15–30) were evaluated 60 min after administration of vehicle, haloperidol (0.25, 0.5, 0.8, 1 or 2 mg/kg) or compound **8** (10, 20, 40, 80 or 160 mg/kg). The mice were placed with their forepaws on one

horizontal wire and their hindpaws on another 6 cm away and 2 cm lower. The time during which the mouse maintained this position was recorded; more than 30 s was considered to indicate catalepsy [45].

Physostigmine (eserine)-induced mortality. Thirty minutes before injection of eserine (2 mg/kg), groups of 10 mice (2 groups for each substance) received vehicle, atropine (4 mg/kg), haloperidol (2 mg/kg) or compound **8** (10 mg/kg). Mice were then placed in cages and deaths were recorded 60 min after injection of eserine [46].

Antagonism of reserpine-incluced ptosis. Vehicle, imipramine (2.4 mg/kg), minaprine dihydrochloride (5, 7 or 9 mg/kg) or test compound (10 mg/kg) was administered 30 min before reserpine administration (2 mg/kg). One hour later, mice were individually monitored for palpebral ptosis on an all-or-none basis (ptosis being defined as closing of half or more of the palpebra) [47]. All control animals exhibited ptosis.

Expression of results and statistical analysis. Calculations were carried out with the PCS program (pharmacological calculation system) [48]. The statistical significance of differences between means was determined by Student's test for unpaired data; differences with $p < 0.05$ were considered statistically significant. The percentage of animals showing catalepsy (%C) was calculated as: %C = (number of animals showing catalepsy/total number of animals) x 100. Percentage change in locomotor activity (%LC) was calculated as: %LC = [(mean number of movements by control animals – mean number of movements by treated animals) / mean number of movements by control animals] x 100. ED_{50} values for the catalepsy and reserpine-induced ptosis tests were calculated by Litchfield and Wilcoxon's method.

Inhibition constant (K_i) values were calculated from the Cheng–Prussof equation: $K_i = IC_{50}/[1 + (F/K_d)]$, where F is the total concentration of [^{3}H]ligand used, K_d is the equilibrium dissociation constant and IC_{50} is the drug concentration required to inhibit 50% of specific binding [49]. Percentage specific binding was calculated as: [(dpm sample – dpm non-specific binding) / (dpm total binding – dpm non-specific binding)] x 100 (dpm = disintegrations per minute). Competitive antagonism was quantified as pA_2, which was calculated from a Schild plot of log (dose ratio – 1) for 3 antagonist concentrations; 6 replicate experiments were performed.

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