Synthesis and Atypical Antipsychotic Profile of Some 2-(2-Piperidinoethyl)benzocycloalkanones as Analouges of Butyrophenone†

José A. Fontenla,* Javier Osuna,† Elizabeth Rosa,† Ma Elena Castro,† Tomás G-Ferreiro,† Isabel Loza-García,‡ José M. Calleja,* Ferrán Sanz,† Jesús Rodríguez,† Enrique Ravina,† Javier Fueyo,† Christian F-Masaguer,‡ Antonio Vidal,† and María L. de Ceballos†

Department of Pharmacology, Faculty of Pharmacy, and Department of Organic Chemistry, Laboratory of Pharmaceutical Chemistry, University of Santiago de Compostela, 15706 Santiago de Compostela, Spain, Department of Medical Informatics, Institut Municipal d'Investigació Mèdica (UAB), c/ Doctor Aiguader, 80, 08003 Barcelona, Spain, and Department of Neuropharmacology, Cajal Institute, CSIC, 28002 Madrid, Spain

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Four new 2-(2-piperidinoethyl)benzocycloalkanone derivatives, 20–23, were prepared and evaluated as potential antipsychotic agents in receptor binding assays for dopamine (DA) and 5-HT2A receptors and in functional and behavioral screens. Their affinities for D2 receptors (Kᵢ's in the nanomolar range: 46.7–70.7) and D1 receptors (Kᵢ's in the micromolar range: 1.09–2.81) were slightly lower than that showed by haloperidol (Kᵢ's in the nanomolar range: 5.01 and 97.72 for D2 and for D1 receptors, respectively). The ratio of pKᵢ's values D1/D2 showed that the new molecules are more D₂-selective than haloperidol. In contrast, in the [³H]-ketanserin binding assays the new compounds had greater affinity for 5-HT2A receptors (pKi's 7.89–8.60) than haloperidol (pKᵢ 7.70) and in functional studies, endothelium-stripped aorta rings, the pA4 values (6.75–8.12) were slightly lower than that of ketanserin (8.87) in suppressing serotonin-induced contractions. The pKi's for D2 binding (and to a lesser extent pKᵢ's for D1 binding) tend to be greater among typical (classical) than among atypical antipsychotics, while these two classes of antipsychotics exhibit no difference with regard to pKᵢ's for 5-HT2A receptors. The ratios of pKᵢ's for 5-HT2A/D2 receptors may be useful for rapid screening of new compounds, and its potential induction of extrapyramidal symptoms (ratio values >1.12 were predictive of an atypical antipsychotic profile). The new molecules had a ratio value in the range 1.08–1.20, while haloperidol showed a ratio of 0.93. In the behavioral screening tests, the new molecules showed antagonist activity of amphetamine-inducing hyperactivity and apomorphine-induced climbing (predictive tests for antipsychotic activity). In the catalepsy test (predictive test for induction of extrapyramidal symptoms), the values obtained were in accordance with an atypical antipsychotic drugs profile.

Introduction

Administration of neuroleptics such as chlorpromazine or haloperidol is still the only effective treatment of a variety of psychiatric disorders (schizophrenia, the manic phase of manic-depressive psychosis). However, most neuroleptics (also called "classical antipsychotics") have two major drawbacks. First, they can induce extrapyramidal symptoms (sometimes at the start of treatment or following months/years of therapy). Second, negative symptoms (such as blunted affectivity, emotional withdrawal, apathy, and motor retardation) respond poorly in most patients. Since the 1970s, atypical (nonclassical) antipsychotics which have no extrapyramidal side effects and are effective against negative symptoms have been available. The prototype of this group, clozapine, whose atypical activity has been attributed classically to its anticholinergic activity, was withdrawn because of its ability to induce agranulocytosis, but in view of the lack of alternatives has since been reintroduced into clinical practice, with the restriction that its use must be accompanied by rigorous blood monitoring.1–3

Seeman4–6 reported that antipsychotic activity is closely correlated with the ability to block D₂ dopamine receptors but not with D₁ receptor blockade, although the subsequent development of D₁-selective agonists and antagonists indicated that D₁ receptors may also be involved in the clinical response to antipsychotics.7–9 For 10 years or more, the two subtype classification could accommodate most of the activities attributed to the dopaminergic system. However, this has changed and the dopamine receptors have been reviewed recently.10,11 Molecular cloning techniques have permitted the identification of several dopamine receptors called D₁, D₂ (D₂L and D₂S), D₃, D₄, and D₁/D₁B, recently grouped together into two subfamilies: D₁-like (D₁A and D₁B) and D₂-like (D₂, D₃, and D₄) subfamilies. The D₁-like receptors exhibit very similar ligand binding properties: high affinity for benzazepines (SCH 23390, SKF 38393) and low affinity for butyrophenones (spiperone, haloperidol) and substituted benzamides (sulpiride). An interesting difference between the D₁-like receptors is that the D₃/D₁B receptor binds dopamine with a higher affinity than does the D₁A receptor.
All three D2-like receptors possess high affinity for butyrophenones such as spiperone and haloperidol and low affinity for benzazepines such as SKF 38393. The D4 receptor exhibits relatively high affinity for the atypical neuroleptic clozapine; this has led to speculation that the D4 receptor might be the relevant target of this atypical neuroleptic (for a review of DA receptors, see ref 12 and 13).

Clozapine blocks not only dopamine receptors but also 5-HT2A serotonin receptors, and recent findings suggest that its atypical activity may be due to this latter feature. This hypothesis is supported by clinical evidence that the 5-HT2A-blocking capacity of certain antipsychotics reduces extrapyramidal side effects and improves the outcome of negative symptoms. An example is setoperone, a drug with potent 5-HT2A-blocking and moderate D2-blocking activity which has proved effective in the treatment of negative symptoms in schizophrenics and also causes few extrapyramidal effects.

In previous papers we have reported the synthesis and antidopaminergic and antiserotonergic activities of 3-(aminomethyl)tetriones 1–3, which are conformationally restricted butyrophenone structures analogous to haloperidol. As a continuation of that work we have now prepared the 2-(2-aminoethyl)benzocycloalkanes 20–23, which also possess the essential requirements for interaction with DA and serotonin (5-HT) receptors. These compounds have two butyrophenone pharmacophores, the (aminomethyl)cycloalkane moiety and the 4-(p-fluorobenzoyl)piperidine fragment. This latter, which has been described as a neuroleptic pharmacophore of similar potency to the butyrophenone portion, is also an important feature for 5-HT2A binding.

On the other hand, it is known that ketanserin is the prototypic 5-HT2A receptor antagonist. In the ketanserin molecule is necessary to consider two structural aspects: (a) the quinazolinone moiety is not essential and may be replaced by a pyrimido pyrimidine (e.g., pirenperone 6) or a thienopyrimidine nucleus (e.g., setoperone 7) or abbreviated to a simpler structural aryl ketone or amide and (b) there is an aromatic binding site that accommodates the aryl portion of the heterocyclic ring. It is evident that the design of molecules under study satisfy these requirements because they have an aryl portion that binds the p-fluorobenzoyl fragment across an aminoethyl side chain partially incorporated in a conformationally restricted cycloalkanone structure.

Chemistry

Compounds 20–23 were synthesized via the sequence of reactions outlined in Scheme 1. Literature procedures for the preparation of tetralone- and indanone-acetic acids involve condensation with glyoxylic acid in alkaline medium and subsequent reduction with zinc and acetic acid. Other synthetic routes have been described for preparing these compounds. In this paper, indanone, 5-fluoroindanone, tetralone, and benzosuberone-2-acetic acids 12–15 were prepared by one of the following methods: (A) aldol condensation with glyoxylic acid in alkaline medium to give 1-oxo-2,3-dihydro-indanylidene acetic acid 8 and their 5-fluoro derivative 9, (1-oxo-1,2,3,4-tetrahydro-2-naphthylidene)-acetic acid 10, and (1-oxo-1,2,3,4-tetrahydro-5H-2-benzocyclohepteny1idene)acetic acid 11 in variable yields and (B) thermal condensation with glyoxylic acid at 160 °C in quantitative yields (Table 1). Subsequent reduction of the alkene acids with zinc and acetic acid gave the saturated acids 12–15 also in quantitative yields (Table 2). 1-Tetralone-2-acetic acid, 12, was also prepared with an overall yield of 75% by hydrolysis of the...
Scheme 1

Table 1. Physical Properties for Compounds 8–11

<table>
<thead>
<tr>
<th>compd</th>
<th>R</th>
<th>yield, %</th>
<th>method</th>
<th>mp, °C</th>
<th>recrystn</th>
<th>solv</th>
<th>formula</th>
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<tr>
<td>8</td>
<td>H</td>
<td>66</td>
<td>A</td>
<td>147–149</td>
<td>AcOEt</td>
<td>167-168</td>
<td>C_{12}H_{16}O_{3}</td>
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<tr>
<td>9</td>
<td>F</td>
<td>92</td>
<td>130–133</td>
<td>AcOEt</td>
<td>167-168</td>
<td>C_{13}H_{18}O_{3}</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>H</td>
<td>95</td>
<td></td>
<td>107–109</td>
<td>AcOEt</td>
<td>167-168</td>
<td>C_{14}H_{18}O_{3}</td>
</tr>
<tr>
<td>11</td>
<td>H</td>
<td>96</td>
<td></td>
<td>129–127</td>
<td>AcOEt</td>
<td>167-168</td>
<td>C_{15}H_{18}O_{3}</td>
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Table 2. Physical Properties for Compounds 12–15

<table>
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<th>yield, %</th>
<th>mp, °C</th>
<th>recrystn</th>
<th>solv</th>
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<tbody>
<tr>
<td>12</td>
<td>H</td>
<td>66</td>
<td>147–149</td>
<td>AcOEt</td>
<td>167-168</td>
<td>C_{12}H_{16}O_{3}</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>92</td>
<td>130–133</td>
<td>AcOEt</td>
<td>167-168</td>
<td>C_{13}H_{18}O_{3}</td>
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<tr>
<td>14</td>
<td>H</td>
<td>95</td>
<td>107–109</td>
<td>AcOEt</td>
<td>167-168</td>
<td>C_{14}H_{18}O_{3}</td>
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<tr>
<td>15</td>
<td>H</td>
<td>87</td>
<td>123–127</td>
<td>AcOEt</td>
<td>167-168</td>
<td>C_{15}H_{18}O_{3}</td>
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Table 3. Physical Properties for Compounds 16–19

<table>
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<td>H</td>
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<td>oil</td>
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<td></td>
<td></td>
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<tr>
<td>17</td>
<td>F</td>
<td>69</td>
<td>127–128</td>
<td>i-PrOH</td>
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<tr>
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<td>H</td>
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<td>oil</td>
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<tr>
<td>19</td>
<td>H</td>
<td>78</td>
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<td>C_{12}H_{20}O_{3}F</td>
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by reaction of the acid chlorides with 4-(p-fluorobenzoyl) piperidine (route A) or by direct acid amine coupling with carboxylate activation by dicyclohexylcarbodiimide in the presence of 1-hydroxybenzotriazole (HOBt) (route B). Ketalization of both the carbonyl groups with ethylene glycol and p-TsOH in toluene gave the bis-(ethylene ketalts) with variable yields. Later, lithium aluminum hydride reduction and subsequent deketalization then afforded to the amino ketones 20–23 in good to excellent yields (Table 4).
Table 4. Physical Properties for Compounds 20–23

<table>
<thead>
<tr>
<th>compd (code number)</th>
<th>n</th>
<th>R</th>
<th>yield, %</th>
<th>mp, °C</th>
<th>recrystn solv</th>
<th>formula</th>
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<tbody>
<tr>
<td>20 (QF 0307B)</td>
<td>1</td>
<td>H</td>
<td>91</td>
<td>264–266</td>
<td>MeOH</td>
<td>C20H24NO2F·HCl</td>
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<tr>
<td>21 (QF 0313B)</td>
<td>1</td>
<td>F</td>
<td>55</td>
<td>270–272</td>
<td>MeOH–Et2O</td>
<td>C20H24NO2F2·HCl</td>
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<tr>
<td>22 (QF 0303B)</td>
<td>2</td>
<td>H</td>
<td>85</td>
<td>176–179</td>
<td>MeOH</td>
<td>C20H24NO2F·HCl</td>
</tr>
<tr>
<td>23 (QF 0311B)</td>
<td>3</td>
<td>H</td>
<td>80</td>
<td>224.5–226</td>
<td>MeOH–Et2O</td>
<td>C20H24NO2F·HCl</td>
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Table 5a

<table>
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<th>drug</th>
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<th>pKi ratios</th>
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<tbody>
<tr>
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<td>D1</td>
<td>D2</td>
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<tr>
<td>haloperidol</td>
<td>7.01</td>
<td>8.30</td>
</tr>
<tr>
<td>20</td>
<td>5.55</td>
<td>7.15</td>
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<tr>
<td>21</td>
<td>5.95</td>
<td>7.32</td>
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<tr>
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</tr>
<tr>
<td>23</td>
<td>5.89</td>
<td>7.33</td>
</tr>
<tr>
<td>ketanserin</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>methysergide</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*pKi's were obtained from the inhibition of [³H]ketanserin binding to rat frontal cortex membranes (5-HT₂A) and [³H]spiperone or [³H]SCH 23390 binding to striatal membranes (D₂ or D₁) by new compounds. Results are means ± SEM of three or four separate experiments. D₁/D₂, pKi value of D₁ binding/D₂ binding; 5-HT₂A/D₁, pKi value of 5-HT₂A binding/D₁ binding; 5-HT₂A/D₂, pKi value of 5-HT₂A binding/D₂ binding. pKi values were obtained against serotonin-induced contractions in rat aorta rings. (–) not evaluated.

Results and Discussion

Pharmacology. Dopamine and 5-HT₂A antagonist activity of the compounds was evaluated by in vivo and in vitro experiments.

In Vitro Results. In [³H]spiperone binding assays, all the new compounds exhibited pKi values of 7.15–7.33, slightly lower than that of haloperidol, 8.3 (Table 5). In [³H]SCH 23390 binding assays, the new molecules again had very similar pKi values (5.55–5.96) which were again lower than that of haloperidol (7.01). These results indicate that the new compounds are D₂-selective rather than haloperidol.

In [³H]ketanserin binding experiments, the new molecules showed greater affinity for 5-HT₂A receptors (pKi 7.89–8.60) than haloperidol (pKi 7.70). The new compounds exhibited pKi values of 6.75–8.12,27 slightly lower than that of ketanserin (8.87) in suppressing serotonin-induced contractions in rat aorta rings stripped of endothelium (Table 5).

pKi's for D₂ binding (and to a lesser extent pKi's for D₁ binding) tend to be greater among typical (classical) than atypical antipsychotics, whereas these two classes of antipsychotic exhibit no difference as regards pKi's for 5-HT₂A binding.28,29

In Vivo Results. All the substances tested caused statistical significant reductions in spontaneous motor activity (SMA) at a dose of 2 mg/kg (Figure 3). Haloperidol and compounds 20, 23, and 21 were effective immediately after their administration, and 22, 40 min later.

Depending on the reductions of SMA obtained with 2 mg/kg doses, the substances were next assayed at doses 4 times greater or smaller. The percent reduction in SMA measured 60 min after administration of 8 mg/kg of compound 20 or 0.5 mg/kg of 23 or 21 was in all three cases very similar to that caused by 0.5 mg/kg of haloperidol, and 22 (8 mg/kg) was slightly less active (Figure 4).

The new compounds were also tested for activity against amphetamine-induced hyperactivity at doses which had caused at least 75% inhibition of SMA 60 min after their administration. Haloperidol (ED₅₀ = 0.098 mg/kg ip) or compound 21 at dosages of 0.5 mg/kg, 23 at 0.5 or 2 mg/kg, and 20 or 22 at 8 mg/kg all inhibited...
Differences in the affinity for either D1 or D2 receptors cannot account for the relative potencies of the compounds, since their Kᵰ's were almost identical. It appears that the existence of two complete butyrophenone groups, such as in 21, is necessary to show a similar profile to haloperidol in behavioral tests.

Certain experimental results suggest that D₂ selectivity may be a sign that a drug will induce catalepsy in experimental animals (and hence that it may cause extrapyramidal symptoms in human beings). Since our binding results showed that all the new substances are more D₂ selective than haloperidol, we hypothesized that they would be more cataleptogenic than haloperidol in vivo. Somewhat to our surprise, this was not so. Whereas haloperidol, as expected, was markedly cataleptogenic (ED₅₀ = 0.67 mg/kg ip, confidence limits 0.5–0.9) and induced an average increase in position retention time of 28.8 s (p < 0.01) when the larger dose was used, the new compounds, in general, exhibited a much lower propensity to produce catalepsy. Compounds 20 and 22 produced, at dosages of 2–8 mg/kg, a slight increase (no more than 5 s) in the time for which the imposed position was maintained and induced no catalepsy, while the molecules 21 and 23 (dosages of 0.5–8 mg/kg) increased the time in initial imposed posture (in the range 10–20 s) and catalepsy was induced in only 13.3–33.3% and 6.7–16.7% of the tested animals, respectively; however, these percentages are much lower than those obtained with haloperidol (Figure 7).

Furthermore, the effects of 21 and 23 in the catalepsy test differed qualitatively from those of haloperidol: whereas animals treated with the new molecules showed reduced muscle tone (appeared flaccid), the animals treated with haloperidol appeared rigid with high level of muscle tone.

Bruhwylere et al. suggested that the induction of extrapyramidal symptoms by neuroleptics may be due to an imbalance between their effects on dopaminergic and cholinergic systems, and that atypical antipsychotics, such as clozapine, may lack extrapyramidal effects because they have both antidopaminergic and anticholinergic activity. To determine whether the weak cataleptogenic activity exhibited by our new compounds might be due to anticholinergic activity, we evaluated the protection they afforded against eserine-induced lethality. Since none of the new compounds afforded such protection, even at the highest dose used (results not shown), their weak cataleptogenic activity cannot, in fact, be attributed to concomitant anticholinergic activity.

Recent studies have suggested that 5-HT₂A-blocking activity can reduce the cataleptogenic activity of neuroleptics while enhancing their antipsychotic activity. Setoperone and ritanserin, for example, both of which are more active as 5-HT₂A blockers than as D₂ blockers, have been reported to be more effective than selective DA receptors blockers in reducing the negative symptoms of schizophrenia and induce few extrapyramidal side effects. In view of such reports, other compounds with both 5-HT₂A-blocking and D₂-blocking activity have also been developed.

According to the binding studies the new compounds interacted with both D₁ and D₂ receptors like haloperidol, although they had less affinity. However, they were slightly more D₂ selective compared to haloperidol, as
Figure 6. Climbing behavior induced by administration of apomorphine (2 mg/kg sc) 30 min after administration of vehicle, haloperidol, 20, 21, 22, or 23. All values show means ± SEM of the scores obtained between 20–30 min postapomorphine administration. Significant differences with respect to control indicated by *p < 0.05 or **p < 0.01.

Figure 7. Results of catalepsy test, showing the time for which initial posture was maintained (mean ± SEM) by mice treated with vehicle, haloperidol, 20, 21, 22, or 23. 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. Numerical values at column heads represent the percentage of cataleptics.

Figure 8. Molecular fragments considered in the molecular modeling analysis. (QF 0303B) may be considered as a borderline case (Table 5). Our results with QF 0303B, QF 0307B, and QF 0311B are in accordance with Meltzer et al. In addition, QF 0313B having an appropriate pKᵢ 5-HT₂A/D₂ ratio induced weak catalepsy in a small percentage of animals.

In summary, these new synthetic molecules had pharmacological properties compatible with a profile of atypical antipsychotics.

Molecular Modeling. Structures of the compounds (20, 22, 23) were represented as X-Y (Figure 8) where Xs are the fused cyclic moieties (indanone, tetralone, and benzosuberone; X₁, X₂, and X₃ in Figure 8) and Y is the 4-(p-fluorobenzoyl)piperidine fragment common to all the compounds.

Structural fittings of X and Y fragments and the butyrophenone pharmacophore of haloperidol were performed by starting from their most stable conformations, superimposing the carbonyl groups, effecting torsions bringing the terminal nitrogen as close as possible to this of the template molecule, and then optimizing with these contraints. All the torsions affected only linear aliphatic chains and involved very little change in energy.

Fitting the butyrophenone pharmacophore of haloperidol to Y as above resulted in almost perfect overlap, with N atoms at only 0.09 Å (Figure 9). Since both structures block dopamine receptors (see Pharmacology section), this suggests that the active conformation of haloperidol fragment may not be its minimum-energy conformation (Figure 9a) but a more twisted for closer to Y (Figure 9b). In this specific case, the energetic cost to acquire this final conformation is only 0.85 kcal/mol; that it to say, nearly a free rotation.
Following the same previously described procedure, the rigid 4-(p-fluorobenzoyl)piperidine fragment (Y) was used as a template to which the three X fragments were fitted (Figure 10). Since the goodness of the fit was unaffected by the size of the rings, and since all the X fragments appear to be pharmacologically effective, planarity of the aryl systems related to their size seems not to influence the dopamine-blocking activity of these structures. This finding contrasts with those of a parallel study of their 5-HT2~a-blocking activity.27

Experimental Section

Chemistry. Melting points were determined with a Kofler hot-stage instrument or a Gallenkamp capillary melting points and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 1600 FTIR spectrophotometer; main bands are given in cm⁻¹. Proton magnetic resonance spectra were obtained with a Brucker WM-250 (250 MHz) with tetramethylsilane as an internal standard. Mass spectra (FAB) were performed with a Kratos MS-50 mass spectrometer using 2-hydroxyethyl disulfide as a matrix. Elemental analyses were performed in a Perkin-Elmer 240B apparatus at the Microanalyses Service of the University of Santiago de Compostela (Spain); all reported values are within ±0.4% of the theoretical compositions.

The general method of synthesis described are illustrative of these analogous compounds.

1-Oxo-1,2,3,4-tetrahydro-2-naphthaleneacetic Acid (10) (Table 1). Method B. A stirred mixture of 2.2 g (15 mmol) of α-tetralone and 1.48 g (15 mmol) of glyoxylic acid monohydrate was heated in a Dean–Stark apparatus at 160 °C for 40–45 min. After cooling, the solid which formed was thoroughly triturated with ethyl acetate (2 × 50 mL). After filtration to separate undissolved materials, the liquid filtrate was washed several times with water and dried (Na₂SO₄), and the solvent was evaporated to give 2.90 g (95%) of 10. A sample for analysis was obtained by recrystallization from EtOH. Mp: 188–190 °C. IR (KBr): 3400–2500 (OH), 1700 (COOH), 1670 (CO), 1640–1600 (C=C). ¹H NMR (CDCl₃): δ 8.11–8.08 (d, 1H, H-8), 7.63–7.57 (dt, 1H, H-6), 7.44–7.38 (t, 1H, H-7), 7.35–7.31 (d, 1H, H-5) 6.92 (s, 1H, =CHCOO), 3.48–3.42 (m, 2H, PhCH₂), 3.17–3.00 (m, 2H, PhCH₂CH₂).

1-Oxo-1,2,3,4-tetrahydro-2-naphthaleneacetic Acid (14) (Table 2). 14 was prepared by reduction of 10 with zinc dust and acetic acid as previously described.38 Mp: 107–109 °C. The acetic acid 14 was also prepared by hydrolysis of the corresponding ethyl ester as indicated below.

A solution of 1.06 mL (7.5 mmol) of disopropylamine in 40 mL of dried THF was cooled to −20 °C under N₂ with stirring. n-Butyllithium (3.06 mL of a 2.5 M solution in n-C₆H₁₄; 7.5 mmol) was added, and stirring was continued for 0.5 h between −20 and −10 °C. The solution was then cooled to −70 °C and stirred 1 h more. A solution of 1.10 g (7.5 mmol) of α-tetralone in 8 mL of THF was added dropwise over 5–10 min. After 15 min at −70 °C the cooling bath was removed and the reaction mixture was allowed to warm to room temperature under N₂ over 18 h. The THF was evaporated off, and the residue was dissolved in AcOEt/H₂O. The solution was washed with 5% NaHCO₃ and 5% HCl and dried (Na₂SO₄), and the AcOEt was evaporated off to give 1.71 g of crude product, which was purified by chromatography on silica gel with CH₂Cl₂ as eluent,
yielding 1.34 g (77%) of the desired 1-o xo-1,2,3,4-tetrahydro-2-naphthaleneacetic acid, ethyl ester. Mp: 47–49 °C. IR (KBr): 1730 (COO), 1682 (CO), 1600 (C=C). 1H-NMR (CDCl3): δ 8.04–7.93 (m, 2H, H-5, H-7), 7.50–7.44 (dt, 1H, H-6), 7.33–7.23 (m, 2H, H-5, H-7), 4.23–4.12 (dd, 1H, H-5, H-7, =CH), 2.30–1.88 (m, 2H, HCH=CH~), 1.31–1.22 (dt, 3H, CH3).

Acid hydrolysis of this ethyl ester with concentrated HCl gave 14 in quantitative yield. Mp: 107–109 °C (EtOH). IR (KBr): 3100–2500 (OH), 1710 (COO), 1650 (CO) (see Table 2).

1-Oxo-1,2,3,4-tetrahydro-2-naphthaleneacetic Acid Chloride. A solution of 76.2 g (0.6 mol) of oxaly chloride in 40 mL of anhydrous toluene was added dropwise to a stirred solution of 12.02 g (0.06 mmol) of 13 in 260 mL of anhydrous toluene under an atmosphere. The resulting reaction mixture was allowed to stir overnight at room temperature. The solvent was removed under reduced pressure, and the orange solid residue was used in the next step without further purification. IR: strong band at 1749 cm⁻¹ (O=C=O).

Quanti tative yield.

N-[(1-Oxo-1,2,3,4-tetrahydro-2-naphthyl)acetyl]-(4-p-fluorobenzoyl)piperidine (18). Route A. A solution of 1.91 g (22 mmol) of (p-fluorobenzoyl)piperidine in 30 mL of anhydrous toluene was slowly added to a stirred solution of 1.97 g (8.99 mmol) of acid chloride in 130 mL of anhydrous toluene under argon atmosphere. The resulting mixture was allowed to stir for 1 h and then heated under reflux for 24 h. After cooling, the solvent was removed under reduced pressure to give a brown oil which was dissolved in dichloromethane, the resulting solution was washed with 10% Na2CO3 and H2O and dried (Na2SO4), and the solvent was removed in vacuo. The resulting orange oil was purified by flash chromatography (silica gel, AcOEthexane, 1:1) to give 1.1 g (95%) of a yellow oil that does not crystallize on standing. Attempted Kugelrohr distillation caused decomposition. IR (film): 1715 (CO cycloalkanone), 1678 (CO benzoyl). 1H-NMR (CDCl3): δ 8.04–7.93 (m, 2H, H-5, H-7), 7.49–7.42 (dt, 1H, H-6), 7.33–7.37 (m, 2H, H-5, H-7), 7.16–7.09 (dd, 2H, H-ChF), 3.06–2.98 (m, 4H, CH2CH2N), 2.62–1.62 (complex multiplet). Anal. (C21H18N6O4FCl) C, H, N.

Pharmacology. Drugs and Chemicals. All compounds were administered in 0.01 mL/g injections in amphetamine subcutaneously and all others intraperitoneally. Compound 22 was administered as a suspension in 0.5% (w/v) sodium carboxymethyl cellulose (Merck); haloperidol (Sigma) and compounds 21 and 23 were dissolved in 1% lactic acid in water; dextroamphetamine sulfate (Sigma), atropine hydrochloride (Sigma), eserine (physostigmine) hemisul phate (Sigma), and compound 23 were prepared in saline; and apomorphine hydrochloride (Sigma) was dissolved in 0.9% saline solution with 1% ascorbic acid (w/v) to prevent oxidation. Chemicals used for physiological solutions were of analytical grade.

Experimental Animals and Conditions. Male Charles River CD1 albino mice weighing 25 ± 2 g, male Wistar rats (275 ± 25 g), and male Sprague–Dawley rats (275 ± 25 g) were used. "In vivo" assays were carried out in a quiet room thermostated at 22 ± 1 °C with a 12-h light/dark cycle (08:00–20:00) and always at the same time of day (so as to avoid variation due to circadian rhythms). Food and tap water were free available in the home cage.

"In Vivo" Experiments. Locomotor Activity. Spontaneous locomotor activity was monitored in six groups of five mice during 1 h following administration of haloperidol, 21, or 23 at doses of 0.5 or 2 mg/kg or 20 or 0.25 mg/kg vehicle alone. In experiments to determine amphetamine-induced hyperactivity, 5 mg/kg of dextroamphetamine sulfate was administered to 4–10 groups of three mice 30 min after administration of vehicle, haloperidol (0.063, 0.094, 0.125, or 0.5 mg/kg), compound 23 at doses of 0.5 or 2 mg/kg, 21 at a dose of 0.5 mg/kg, or 20 or 22 at a dose of 8 mg/kg. Both spontaneous and amphetamine-induced motor activity were recorded during the following 2 h in an activity cage (Panlab Actisystem D.A.S. 16.V1), which contains an electromagnetic field that is sensitive to any motion within it.

Catalepsy. This was tested in mice (n = 15–30) 60 min after administration of vehicle, haloperidol (0.25, 0.5, 0.8, 1, or 2 mg/kg), compounds 21 or 23 (0.5, 2, or 8 mg/kg), or 20 or 22 (2 or 8 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, and more than 30 s was considered to indicate catalepsy.

Eserine (Physostigmine)-Induced Mortality. Groups of 10 mice (two groups per compound and dosage level) were treated, 30 min before eserine injection (2 mg/kg), with vehicle, atropine (4 mg/kg), haloperidol, compounds 21 or 23 (2 mg/kg), or 20 or 22 (8 mg/kg). The mice were placed in cages and deaths were counted 60 min after injection of eserine.

Apomorphine-Induced Climbing. The method of Prota et al. was used with modifications. Long behavior of mice was observed in individual cylindrical stainless-steel wire cages (diameter, 12 cm; height, 14 cm). Mice (six per compound and dosage, except for the control group, for which 12 animals were used) were treated with vehicle, haloperidol (0.125–0.25 mg/kg), or compounds 20, 21, or 23 (0.5, 2, or 8 mg/kg) or compound 22 (2, 6, or 16 mg/kg), and 30 min later with 2 mg/kg of apomorphine (subcutaneously). During the next 30 min, climbing activity was recorded every 10 min using the following scale: 0, four paws were on the floor; 1, one or two paws were against the grid wall; and 2,
the mouse clung to the grid wall with three or four paws. The scores recorded 20 and 30 min postapomorphine were added, and the mean of this sum was calculated for each group.

In vitro experiments. Male Wistar rats were killed by cervical dislocation and decapitation. Both striata and the frontal cortex were quickly dissected out on a cold plate, weighed, and stored at -20 °C until assay.

D. Binding. For [3H]spiperone binding assays, paired striata were homogenized in 50 volumes of ice-cold 50 mM Tris-HCl with a Polytron (setting 6 for 5 s) and then centrifuged at 4000g 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 volumes of 50 mM Tris-HCl 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 its vehicle (10% methanol) and 25 μL of a solution of [3H]spiperone; the reaction was terminated by rapid vacuum filtration through Watman GF/C filters, which were washed with 3 × 5 mL of cold buffer. For equilibrium saturation analysis, six ligand concentrations from 0.05–1 nM were used. Nonspecific binding was determined by addition of 10−5 M (+)-sulpiride. For determination of the IC50 values of drugs displacing [3H]spiperone (0.25 nM) binding, at least six 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.

E. [3H]DA Binding. Frontal cortex tissue was homogenized (UltraTurrax 5 s at 20 000 rpm) in 50 volumes of 50 mM Tris-HCl buffer and centrifuged at 1000g for 10 min, and the supernatant was centrifuged at 20000g 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 CaCl2, and 1 mM MgCl2. Saturation curves were constructed with six ligand concentrations from 0.15 to 2.5 nM. Nonspecific binding was determined by addition of unlabeled SCH 23390 (10−5 M). Samples were incubated at 25 °C for 30 min.

5-HT2A Binding. Frontal cortex tissue was homogenized (Ultraturrax 5 s at 20 000 rpm) in 50 volumes of 50 mM Tris-HCl pH 7.4, and centrifuged at 3000g for 10 min at 4 °C. The pellet was rehomogenized and centrifuged again. The final pellet was reconstituted in 200 volumes of buffer. Aliquots of membrane preparations (200 μL) were incubated with 25 μL of 1 nM [3H]ketanserin (NEN 60 Ci/mmole). Specific binding was defined by incorporation of 25 μL of methysergide (final concentration 1 μM). Samples were incubated for 15 min at 37 °C, and incubation was terminated by vacuum filtration.

In Vitro. Experiments. Male Wistar rats were killed by cervical dislocation and decapitation. Both striata and the frontal cortex were quickly dissected out on a cold plate, weighed, and stored at -20 °C until assay.

D. Binding. For [3H]spiperone binding assays, paired striata were homogenized in 50 volumes of ice-cold 50 mM Tris-HCl with a Polytron (setting 6 for 5 s) and then centrifuged at 4000g 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 volumes of 50 mM Tris-HCl 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 its vehicle (10% methanol) and 25 μL of a solution of [3H]spiperone; the reaction was terminated by rapid vacuum filtration through Watman GF/C filters, which were washed with 3 × 5 mL of cold buffer. For equilibrium saturation analysis, six ligand concentrations from 0.05–1 nM were used. Nonspecific binding was determined by addition of 10−5 M (+)-sulpiride. For determination of the IC50 values of drugs displacing [3H]spiperone (0.25 nM) binding, at least six 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.

E. [3H]DA Binding. Frontal cortex tissue was homogenized (UltraTurrax 5 s at 20 000 rpm) in 50 volumes of 50 mM Tris-HCl and centrifuged at 1000g for 10 min, and the supernatant was centrifuged at 20000g 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 CaCl2, and 1 mM MgCl2. Saturation curves were constructed with six ligand concentrations from 0.15 to 2.5 nM. Nonspecific binding was determined by addition of unlabeled SCH 23390 (10−5 M). Samples were incubated at 25 °C for 30 min.

5-HT2A Binding. Frontal cortex tissue was homogenized (UltraTurrax 5 s at 20 000 rpm) in 50 volumes of 50 mM Tris-HCl and centrifuged at 1000g for 10 min, and the supernatant was centrifuged at 20000g 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 CaCl2, and 1 mM MgCl2. Saturation curves were constructed with six ligand concentrations from 0.15 to 2.5 nM. Nonspecific binding was determined by addition of unlabeled SCH 23390 (10−5 M). Samples were incubated at 25 °C for 30 min.

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