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Original article

Butyrophenone analogues in the carbazole series as potential atypical antipsychotics: synthesis and determination of affinities at D_2, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors[#]

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Abstract – We describe practical and efficient routes for synthesis of 2-aminomethyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-ones using the Fischer indole synthesis or palladium-catalysed cyclization methodologies, as well as their affinities for D_2, 5-HT_{2A} and 5-HT_{2C} receptors, and their activity at the 5-HT_{2B} receptor. The most active compounds, **4b** (QF 2003B) and **4c** (QF 2004B), with a pK_i (5-HT_{2A}/D_2) ratio of 1.28 show a potential antipsychotic profile according to Meltzer's classification. © 2000 Éditions scientifiques et médicales Elsevier SAS

antipsychotics / serotonin 5-HT_2 receptors / dopamine D_2 receptors

1. Introduction

Schizophrenia is a complex psychological disorder of unclear aetiology which to some degree affects 0.5–1.5% of the world's population. It is widely accepted that the dopaminergic system plays a key role in schizophrenic illness. Affected individuals may exhibit a wide spectrum of behavioural and other symptoms, ranging from social withdrawal, catatonia and affective flattening of the personality ('negative' symptoms thought to be associated with dopaminergic hypoactivity in the prefrontal cortex) to hallucinations, paranoia and disorganized behaviour ('positive' symptoms thought to be associated with hyperactive dopaminergic transmission in the mesolimbic region of the brain) [1, 2].

The cell-borne receptors with which dopamine interacts are broadly classified as D_1-like or D_2-like. Only the D_2-like family, which includes receptors D_2, D_3 and D_4, appears to be involved in schizophrenia. The activity of classical antipsychotics such as haloperidol, and the intensity of their undesirable side effects (prolactin release and extrapyramidal symptoms (EPS)), are closely correlated with their ability to block dopamine receptor D_2. It has also been suggested that receptors D_3 [3] and D_4 may be involved in antipsychotic activity (the atypical antipsychotic clozapine has high affinity for D_4), but clinical assays have shown selective D_4 blockers (e.g. L-745,870 [4], CP-293,019 [5]) to be ineffective as antipsychotics [6].

Haloperidol (*figure 1*) and other classical butyrophenone-based antipsychotics, such as spiperone and fluanisone, not only have the side effects mentioned above, but are also ineffective against negative symptoms. Clozapine is the prototype of a new group of 'atypical' (non-classical) antipsychotics that cause no EPS and are effective against negative as well as positive symptoms. This superior activity profile of clozapine and other atypical antipsychotics, such as risperidone, olanzapine and quetiapine, may be due to their blocking not

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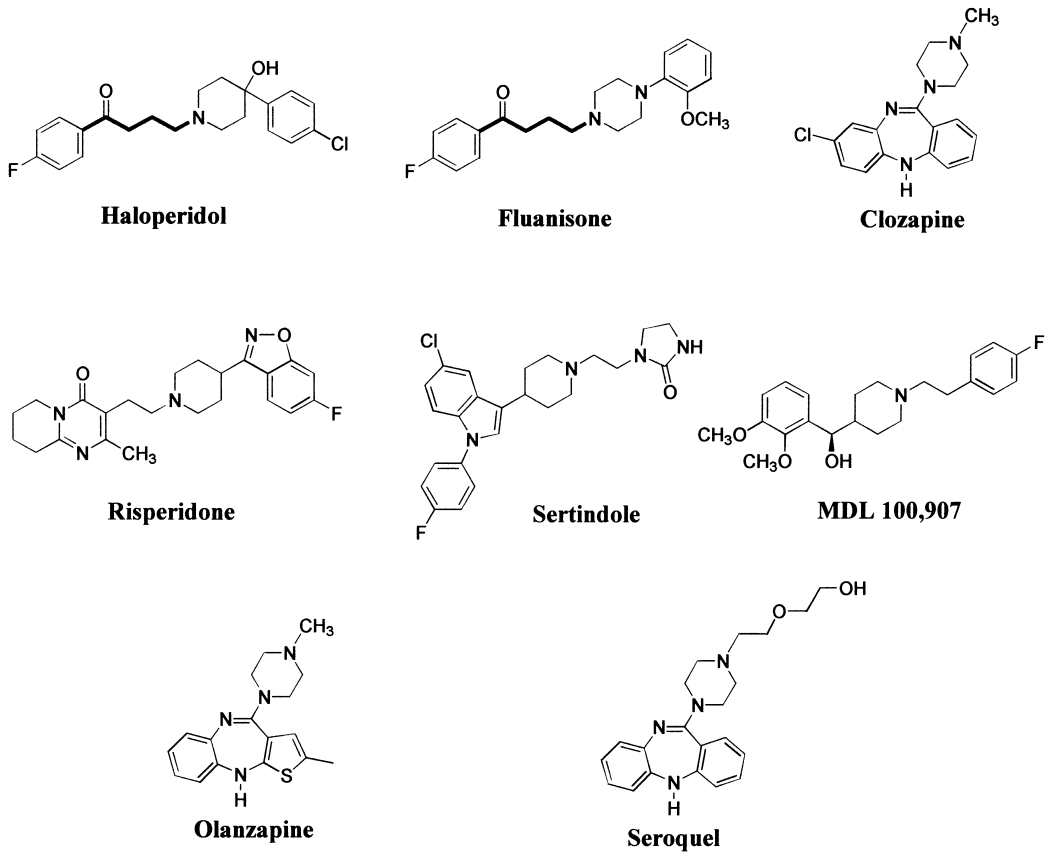


Figure 1. Structures of some typical and atypical antipsychotics.

only dopamine receptors but also serotonin receptors [7–11].

Atypical antipsychotics bind to 5-HT_2, 5-ht_6 and 5-HT_7 serotonin receptors [12]. In the case of the last two recombinant receptors, new supplementary studies are necessary in order to clarify their role as pharmacological receptors and their pathophysiological involvements [13, 14]. In any case, 5-HT_7 receptors are thought not to be involved in schizophrenia [15]. 5-HT_2 type receptors have been related with serotonin-mediated mechanisms not only in schizophrenia but also in anxiety, depression, anorexia nervosa, migrain, hypertension and other cardiovascular disorders [16].

Meltzer and coworkers [17, 18] have suggested that the ratio between the pK_i's of antipsychotic agents at 5-HT_{2A} and D_2 receptors reflect an atypical profile, this ratio appears to be > 1.12 for atypical antipsychotics and < 1.09 for classical antipsychotics.

Recent experimental and clinical studies appear to confirm the importance of 5-HT_{2A} for the activity profile of atypical antipsychotics [19–21]. Indeed, even the 5-HT_{2A}-selective serotonin antagonist MDL 100,907, which has no activity at dopamine receptors, has shown antipsychotic potential in experiments with neurochemical, electrophysiological and behavioural models [22]. Meltzer also reported [23] that affinities for 5-HT_{2C} receptors enhance the distinction between typical and atypical antipsychotic drugs. Clozapine and other atypical antipsychotics like olanzapine or seroquel have a higher affinity for 5-HT_{2C} receptors than for D_2 receptors [24]. Recently, Reavill et al. have suggested that 5-HT_{2C} receptor antagonism is likely to be the mechanism by which atypical antipsychotic drugs lack EPS [25]. In addition to that, clozapine exhibits high affinity at 5-HT_{2B} human receptors (K_i = 7.57 nM [26]); these receptors, with close operational and molecular similarity with 5-HT_{2C} receptors [27, 28], were found to have a low level of expression at the human and rat CNS [29–32]. However, the presence of these receptors in rat CNS and their implication in animal models of

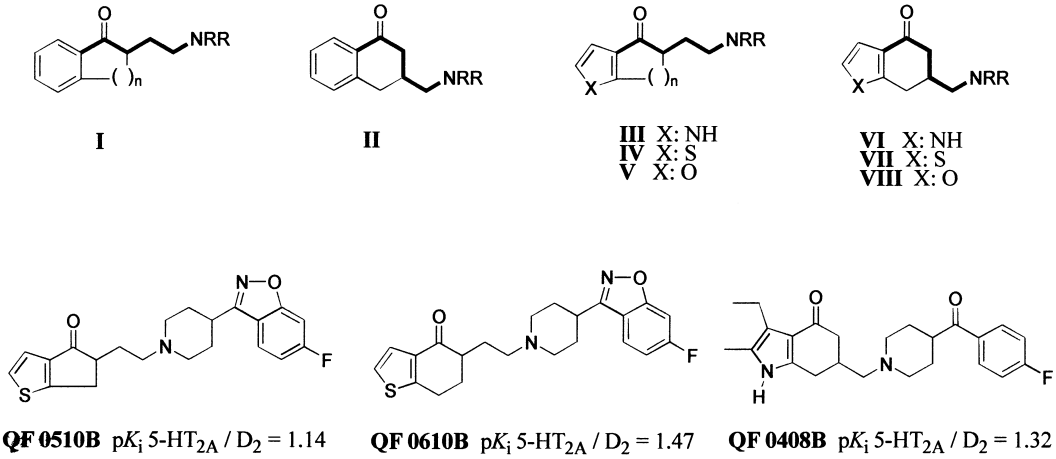


Figure 2. General structures of some butyrophenone analogues and pKi ratios (5-HT_{2A}/D_2) of some potential atypical antipsychotics in the heterocyclic series.

behaviour, such as has been shown by Duxon et al. [33], seems a controversial question.

The development of drugs such as risperidone, ocaperidone or sertindole which, like clozapine, block both 5-HT_{2A} and D_2, has been prompted in part by the finding that clozapine increases the risk of agranulocytosis [34]. However, since none of these drugs has proven to be as broadly effective as clozapine, the discovery of effective antipsychotics with no side effects is still a major research goal. Likewise, the discovery of new selective pharmacological tools acting on the different types of 5-HT_2 receptors is essential.

In previous papers [35, 36] we have reported the synthesis and antipsychotic activity of aminoalkylbenzocycloalkanones (**I** and **II**) which are conformationally restricted butyrophenone analogous of haloperidol, with the aminobutyl side chain incorporated in a semi-rigid structure (*figure 2*). Later, we prepared 5-aminoethyl and 6-aminomethyl-4,5,6,7-tetrahydroindole-4-ones (**III** and **VI**), 4,5,6,7-tetrahydrobenzo[b]thiophen-4-ones (**IV** and **VII**) and 4,5,6,7-tetrahydrobenzo[b]furan-4-ones (**V** and **VIII**) as conformationally constrained butyrophenone derivatives in the indole, benzothiophene and benzofuran series, respectively, as putative atypical antipsychotics [37–39]. Many of such heterocyclic restricted butyrophenones (e.g. QF 0408B [38], QF 0610B [39] and QF 0510B [39], *figure 2*) show a very favourable 5-HT_{2A}/D_2 balance and its cataleptogenic activity is low [40].

As a continuation, within a program of research of new CNS acting agents, we wish now to report practical and efficient synthetic strategies for preparing new 2-aminomethyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-ones (**4a–f**), cyclic butyrophenone derivatives in the carbazole series, as well as the results of studies of the affinities of the title compounds for D_2, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors. Some preliminary results from this work have been published in communication form [41]. Some of these compounds have two butyrophenone pharmacophores: the semirigid aminoalkyl indolone moiety and the 4-(*p*-fluorobenzoyl) or the 3-[4-(6-fluoro-1,2-benzisoxazole-3-yl)]piperidine fragments. The 4-(*p*-fluorobenzoyl)piperidine fragment may be considered as a butyrophenone pharmacophore constrained in a six membered ring; this fragment is also an important feature for 5-HT_{2A} binding [42, 43]. Moreover, the bioisosteric relationships between benzoyl and 1,2-benzisoxazol moieties are noteworthy [44, 45].

2. Chemistry

For the synthesis of the carbazolones **4a–c** (*figure 3*, method A) we started from the 1,4-dihydro-3,5-dimethoxybenzyl alcohol **1**, which was readily prepared from the cheap 3,5-dimethoxy- (or 3,4,5-trimethoxy-) benzoic acid in 85% yield as previously described [46, 47]. This alcohol has proven to be a versatile synthon in the preparation of condensed heterocyclic butyrophenone systems [38, 48]. Application of the Fischer indole methodology [49] with phenylhydrazine in 4% H_2SO_4 solution allowed the construction of the desired carbazole intermediate **2** (*figure 3*).

Reaction of the resulting 2-hydroxymethyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one **2** with *p*-toluenesulfonyl chloride in pyridine afforded the tosylate **3** as a white

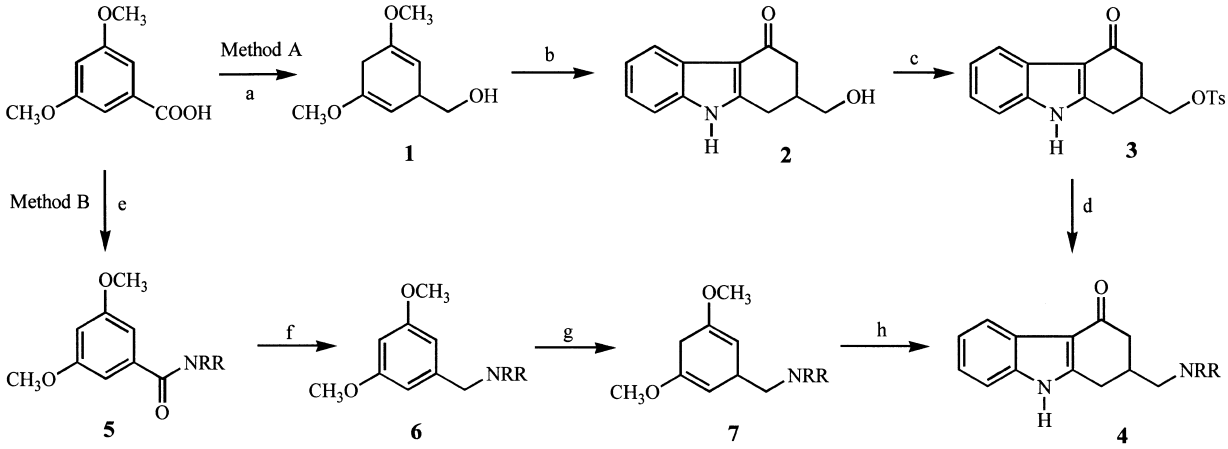
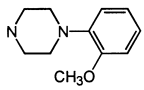
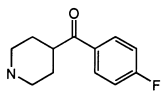
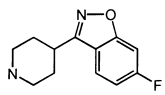


Figure 3. (a) see [46, 47]; (b) $PhNHNH_2$/4% H_2SO_4; (c) TsCl/Py; (d) HNRR/NMP; (e) HNRR/DCC/HOBt; (f) $LiAlH_4$/THF; (g) Li/NH_3; (h) $PhNR'NH_2$.

crystalline solid (70%). Nucleophilic displacement of the tosylate with amines (single or complex, heterocyclic amines such as piperazines or substituted piperidines, e.g. *p*-fluorobenzoylpiperidine, 4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidine [50]) in N-methyl-2-pyrrolidone (NMP) provided, after bulb to bulb distillation of NMP, the amines **4a–c** as white crystalline solids with yields ranging from 55–70% (*table I*).

Alternatively (*figure 3*, method B), aminomethyl carbazolones with non-aromatic amine fragments have been prepared from the corresponding 1,4-dihydro-3,5-dimethoxybenzylamines **7**, obtained following our previously reported methodology [51] for the indole series. The direct conversion of these amines by the Fischer indole reaction into a new class of potential atypical antipsychotics, the 2-aminomethyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-ones **4d–f**, was achieved by reaction with phenylhydrazine or N-methyl-N-phenylhydrazine in 4% H_2SO_4 solution at reflux temperature, with yields from 60–70% (*table I*).

Table I. 2-Aminomethyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-ones **4a–f.**

Compound	NRR	R′	Method	M.p. (recr. solvent)	Yield (%)
4a		H	A	229–230 (AcOEt)	62
4b		H	A	217–219 (AcOET)	55
4c		H	A	222–223 (*iso*-PrOH)	70
4d	NEt_2	H	B	184–185 (CH_3CN)	66
4e		H	B	207–208 (AcOEt)	60
4f	NEt_2	CH_3	B	192–193 (acetone)	70

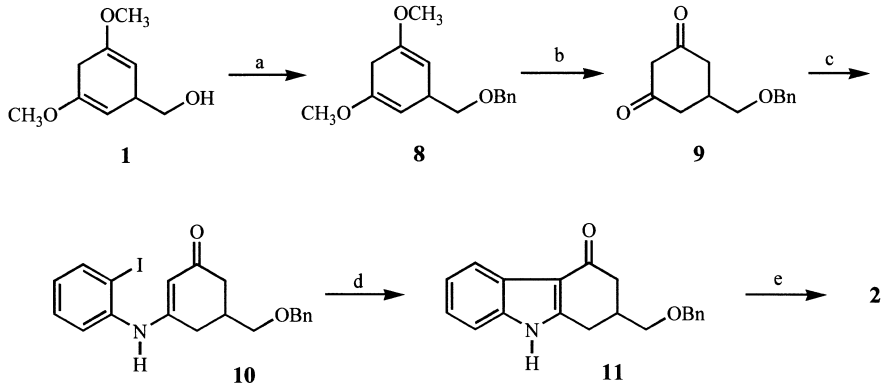


Figure 4. (a) BnBr/NaH/Bu_4NI; (b) 10% HCl; (c) 2-iodoaniline/pTsOH; (d) $Pd(AcO)_2/Et_3N$; (e) H_2/Pd.

Also, we have studied intramolecular palladium-catalysed cyclization of the N-(2-iodophenyl)enaminone of compound **1** to afford the corresponding carbazolone (*figure 4*). Iida et al. [52] reported the palladium-catalysed cyclization of the N-(2-bromophenyl)enaminone of dimedone to give the tetrahydrocarbazolone derivative, but in low yield. However, the cyclization was successfully achieved by Sakamoto et al. using the N-(2-iodophenyl)enaminone and $Pd(OAc)_2$ as catalyst [53]. The application of this methodology to the *O*-benzyl ether of alcohol **1** allows us to obtain the tetrahydrocarbazolone derivative **11** in good yield.

The 5-benzyloxymethyl-3-(2-iodophenyl)amino-2-cyclohexen-1-one was synthesized by dehydroxy-condensation of 2-iodoaniline with 5-benzyloxymethyl-1,3-cyclohexanedione **9**, obtained in turn by benzylation and subsequent acidic hydrolysis of enol-ether groups of alcohol **1** (*figure 4*). The palladium-catalysed carbazole-cyclization was carried out using $Pd(OAc)_2$ and triethylamine in DMF, with 87% yield. The benzyl-protecting group was quantitatively removed by hydrogenolysis with palladium on carbon in ethanol to give the desired intermediate **2**.

3. Results and discussion

The dopamine D_2 and serotonin 5-HT_{2A} and 5-HT_{2C} receptor affinities, and the 5-HT_{2A} and 5-HT_{2B} antagonistic affinities of compounds **4a–4f** are shown in *table II*. *Figure 5* shows the radioligand–competition binding curves recorded with compound **4b** and [^{3}H]ketanserine (5-HT_{2A} receptors, *figure 5A*) or [^{3}H]mesulergine (5-HT_{2C} receptors, *figure 5C*), and the dose–response curve for **4b** (5-HT_{2B} receptors, *figure 5B*).

Table II. Inhibition constants (pK_i) at D_2, 5-HT_{2A} and 5-HT_{2C} receptors, and antagonist activity (pA_2) at 5-HT_{2A} and 5-HT_{2C} receptors of compounds **4a–f**.

Compound	pK_i ± SEM[a]			pA_2 ± SEM[b]		pK$_i$ ratio ± SEM
	D_2	5-HT_{2A}	5-HT_{2C}	5-HT_{2A}	5-HT_{2B}	5-HT_{2A}/D_2
4a (QF 2006B)	5.81 ± 0.33	6.20 ± 0.61	5.92 ± 0.06	6.50 ± 0.20	6.80 ± 0.05	1.07 ± 0.05
4b (QF 2003B)	6.25 ± 0.24	8.04 ± 0.80	6.23 ± 0.09	9.20 ± 0.27	7.22 ± 0.23	1.29 ± 0.10
4c (QF 2004B)	6.85 ± 0.21	8.80 ± 0.88	7.24 ± 0.06	9.50 ± 0.32	6.35 ± 0.10	1.28 ± 0.10
4d (QF 2002B)	< 5	< 5	n.t.[c]	n.t.	n.t.	–
4e (QF 2014B)	< 5	< 5	n.t.	n.t.	n.t.	–
4f (QF 2001B)	< 5	< 5	n.t.	n.t.	n.t.	–
Haloperidol	8.48 ± 0.12	7.70 ± 0.22	5.60[d]	–	–	0.91
Clozapine	6.58 ± 0.05	8.12 ± 0.07	8.20[d]	8.62 ± 0.05	6.77 ± 0.20	1.23

[a] Binding affinity (striatal membranes: D_2 [^{3}H]spiperone; rat frontal cortex: 5-HT_{2A} [^{3}H]ketanserin; bovine choroid plexus: 5-HT_{2C} [^{3}H]mesulergine). [b] Antagonism of serotonin at 5-HT_{2A} (rat aorta) and 5-HT_{2B} (rat stomach fundus) receptors. [c] Not tested. [d]Data from reference [25].

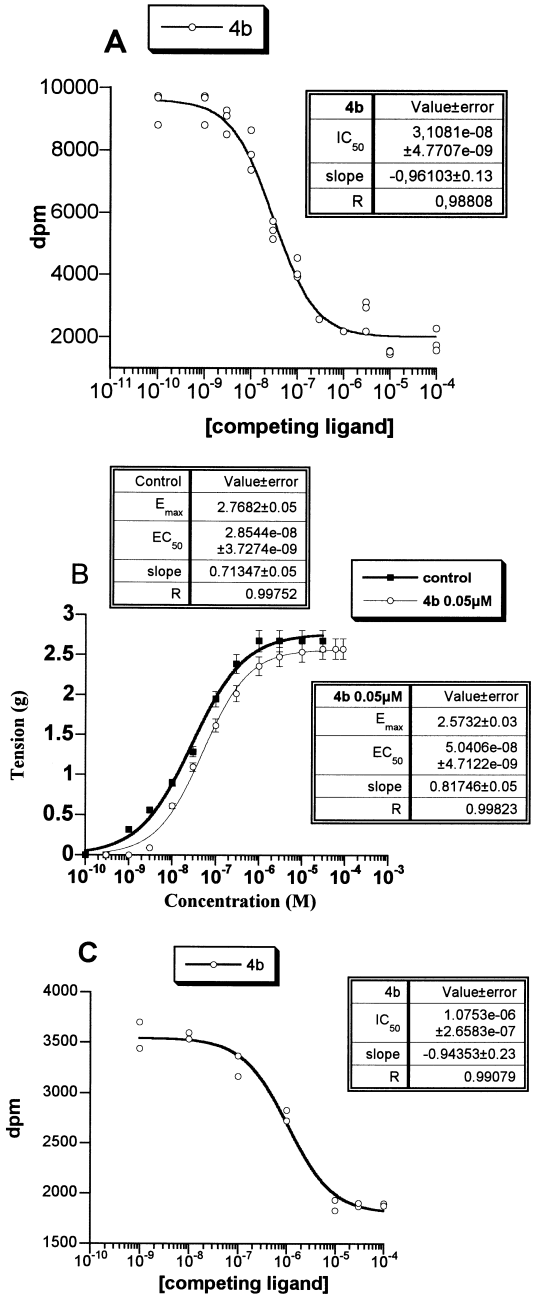


Figure 5. A) Inhibition by **4b** of [^{3}H]ketanserin-binding by 5-HT$_{2A}$ receptors in rat frontal cortex membrane preparations. **B)** Inhibition by **4b** of serotonin-induced contractions of rat stomach fundus (points show the average of 2–3 replicate experiments; vertical bars indicate SEM). **C)** Inhibition by **4b** of [^{3}H]mesulergine-binding by 5-HT$_{2C}$ receptors in bovine choroid plexus membrane preparations. Graphs **A** and **C** show duplicate and triplicate, respectively, points from a single experiment; a total of two replicate experiments were performed in each case.

Compounds **4a–c** inhibited the binding of [^{3}H]-spiperone to D_2 receptors with pK_i values ranging from 5.81–6.85, and the binding of [^{3}H]-ketanserine to 5-HT$_{2A}$ receptors with pK_i values from 6.20–8.80. Compounds **4d–f**, bearing simple amines, did not show affinity for either D_2 or 5-HT$_{2A}$ receptors. The most active compounds, **4b** (QF 2003B) and **4c** (QF 2004B), exhibited a high affinity for 5-HT$_{2A}$ receptors, while the pK_i value for the D_2 receptor was lower. Both of them show a pK_i (5-HT$_{2A}$/D_2) ratio of 1.28. In keeping with the hypotheses suggested by Meltzer et al. [17, 18] regarding the combination of 5-HT$_{2A}$-blocking and D_2-blocking activities, the compounds **4b** and **c** are thought to have an atypical antipsychotic profile from Meltzer's, pK_i ratio > 1.12.

The *p*-fluorobenzoyl derivative **4b** has a pK_i (5-HT$_{2A}$/D_2) ratio for compound **4b** (1.28), higher than that for haloperidol (0.91), risperidone (1.20) or clozapine (1.23). This compound also exhibited a pA_2 value of 9.2 in suppressing serotonin-induced contractions in rat aorta ring stripped of endothelium [54]. According to Meltzer's classification **4b** shows a profile of an atypical antipsychotic.

The replacement of the benzoylpiperidine fragment of **4b** by a 1,2-benzisoxazolyl-piperidine moiety (compound **4c**) increased the affinity for both D_2 and 5-HT$_{2A}$ receptors, with pK_i values of 6.85 and 8.80, respectively. The affinity for 5-HT$_{2A}$ receptors (pK_i and pA_2) is higher than that exhibited by clozapine.

The introduction of *o*-methoxyphenylpiperazine or amines with non-aromatic fragments does not improve its affinity for 5-HT$_{2A}$ and D_2 receptors.

Compounds **4a–c** did not show a significant difference of affinity between 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors. The compounds **4b** and **4c** showed higher affinity at 5-HT$_{2A}$ receptors than at 5-HT$_{2B}$ receptors: the *p*-fluorobenzoylpiperidine derivative **4b** exhibits a 100-fold higher antagonist activity (K_i vs. K_b) at 5-HT$_{2A}$ receptors than at 5-HT$_{2B}$ receptors. This selectivity increases up to 1 000-fold in the benzisoxazolylpiperidine derivative **4c**. Both **4b** and **4c** display higher affinity (K_i) at 5-HT$_{2A}$ than at 5-HT$_{2C}$ receptors, about 60-fold for **4b** and 35-fold for **4c**.

The presence of an *o*-methoxyphenylpiperazine strongly modifies this selectivity, conserving 5-HT$_{2B}$ activity, slightly decreasing 5-HT$_{2C}$ affinity and dramatically decreasing 5-HT$_{2A}$ affinity and activity.

4. Conclusions

We have developed a practical and efficient 4 (for amines with non-aromatic fragments) or 5 (for amines

with aromatic cycles) step synthesis for new derivatives in the carbazole series as atypical antipsychotics from cheap and readily available starting materials using Fischer indole methodology. Also, we have studied and successfully applied the palladium-catalysed cyclization as an alternative route for the preparation of carbazole intermediates. The promising affinity for both D_2 and 5-HT_{2A} receptors shown by compound **4c** (QF 2004B), its high Meltzer ratio (1.28) and its good 5-HT_2 selectivity has prompted us to choose this compound for further development.

5. Experimental protocols

5.1. Chemistry

Melting points were determined with a Kofler hot stage instrument or a Gallenkamp capillary melting point apparatus and are uncorrected. Infrared spectra were recorded with a Perkin Elmer 1600 FTIR spectrophotometer; the main bands are given in cm^{-1}. Proton NMR spectra (^{1}H-NMR) were recorded with a Brucker WM AMX (300 MHz); chemical shifts are recorded in parts per million (δ) downfield from tetramethylsilane (TMS). Mass spectra were performed on Kratos MS-50 or Varian Mat-711 mass spectrometers in fast atom bombardment (FAB) mode (with 2-hydroxyethyl disulphide as matrix) or by electron impact (EI). Flash column chromatography was performed using Kieselgel 60 (60–200 mesh, E. Merck AG, Darmstadt, Germany). Reactions were monitored by thin layer chromatography (TLC) on Merck 60 GF_{254} chromatogram sheets using iodine vapour and/or UV light for detection; unless otherwise stated the purified compounds each showed a single spot. Elemental analyses were performed on a Perkin Elmer 240B apparatus at the Microanalyses Service of the University of Santiago; unless otherwise stated all reported values are within ± 0.4% of the theoretical compositions.

5.1.1. 2-Hydroxymethyl-1,2,3,9-tetrahydro-4H-carbazol-4-one **2**

A solution of phenylhydrazine (5 mL, 3.52 mmol) in 4% H_2SO_4 (5 mL) was added dropwise to a solution of 1,4-dihydro-3,5-dimethoxybenzyl alcohol **1** [46, 47] (0.6 g, 3.5 mmol) in 4% H_2SO_4 (5 mL) at 40 °C. The resulting solution was refluxed under Argon for 4 h. After cooling, the solution was basified with an aqueous 25% NH_3 solution and extracted with AcOEt (3 × 15 mL). The organic layer was dried, filtered and concentrated to give a brown solid which was purified by column chromatography (silica gel, eluent: AcOEt): 0.34 g (45% yield) of a white solid, m.p. 210–211 °C (EtOAc). IR (KBr): ν_{max} = 3 151, 2 932, 1 629, 1 611 cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 2.26–2.43 (m, 3H), 2.77 (dd, 1H, J_1 = 8.9 Hz, J_2 = 16.5 Hz), 2.99–3.05 (m, 1H), 3.40–3.51 (m, 2H), 4.70 (br. s, 1H), 7.09–7.17 (m, 2H), 7.34–7.40 (m, 1H), 7.91–7.94 (m, 1H), 11.82 (s, 1H). MS (EI, *m/z*): 215 (M^+, 67%). Anal. $C_{13}H_{13}NO_2$ (C, H, N).

5.1.2. 2-(p-Toluenesulfonyl)oxymethyl-1,2,3,9-tetrahydro- 4H-carbazol-4-one **3**

To a solution of carbazolone **2** (0.35 g, 1.63 mmol) and a catalytic amount of dimethylaminopyridine in dry pyridine (7 mL) at 0 °C, *p*-toluenesulfonyl chloride (0.56 g, 2.93 mmol) was added. The mixture was stirred at 0 °C for 24 h and water (20 mL) was added. The precipitate was collected by filtration, washed with water and dried to give 0.54 g (89% yield) of a white solid, m.p. 215–216 °C (CH_3CN). IR (KBr) ν_{max}: 3 228, 1 625, 1 610 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.25–2.38 (m, 2H), 2.41 (s, 3H), 2.59–2.68 (m, 1H), 2.78 (d, 1H, J_1 = 10.2 Hz, J_2 = 16.4 Hz), 3.02 (dd, 1H, J_1 = 4.3 Hz, J_2 = 16.4 Hz), 4.07–4.16 (m, 2H), 7.08–7.18 (m, 2H), 7.37–7.40 (m, 1H), 7.48 (d, 2H, J = 8.2 Hz), 7.81 (d, 2H, J = 8.3 Hz), 7.88–7.91 (m, 1H), 11.87 (s, 1H). MS (EI, *m/z*): 369 (M^+, 11%). Anal. $C_{20}H_{19}NO_4S$ (C, H, N).

5.1.3. 1-Benzyloxymethyl-3,5-dimethoxy-1,4-dihydrobenzene **8**

To a 60% suspension of NaH in mineral oil (0.17 g, 7.1 mmol) in dry THF (10 mL), a solution of 1,4-dihydro-3,5-dimethoxybenzyl alcohol **1** (1.0 g, 5.9 mmol) in dry THF (10 mL) was added dropwise. Then a catalytic amount of tetrabutylammonium iodide and benzyl bromide (0.83 mL, 7.1 mmol) were added. The mixture was stirred at room temperature under argon for 36 h and methanol (4 mL) was added. The mixture was stirred for 30 min and then partitioned between AcOEt (40 mL) and water (40 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo to give a yellow oil which was purified by column chromatography (silica gel, eluent: AcOEt/hexane 1:15) to afford 1.15 g (75% yield) of a colourless oil. IR (KBr) ν_{max}: 3 029, 2 846, 1 694, 1 661, 1 599 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 2.80–2.83 (m, 2H), 3.29–3.31 (m, 1H), 3.35–3.37 (m, 2H), 3.59 (s, 6H), 4.58 (s, 2H), 4.71–4.72 (m, 2H), 7.29–7.39 (m, 5H). Anal. $C_{16}H_{20}O_3$ (C, H).

5.1.4. 5-Benzyloxymethyl-1,3-cyclohexanedione **9**

A solution of **8** (1 g, 3.8 mmol) and 10% HCl (3.5 mL) in THF (35 mL) was stirred at room temperature for 12 h. Then the solvent was removed in vacuo and the residue was dissolved in CH_2Cl_2, dried (Na_2SO_4), filtered and concentrated under reduced pressure to give 0.71 g (80% yield) of a white solid, m.p. 96–98 °C (AcOEt/hexane).

IR (KBr) ν_{max} = 2 856, 1 592, 1 532 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 2.40–2.46 (m, 1H), 2.61 (dd, 2H, J_1 = 6.1 Hz, J_2 = 15.7 Hz), 2.70 (dd, 2H, J_1 = 5.6 Hz, J_2 = 15.7 Hz), 3.25 (d, 1H, J = 18.0 Hz), 3.34 (d, 1H, J = 18.0 Hz), 3.49 (d, 2H, J = 3.6 Hz), 4.44 (s, 2H), 7.22–7.37 (m, 5H). Anal. $C_{14}H_{16}O_3$ (C, H).

5.1.5. 5-Benzyloxymethyl-3-(2-iodophenyl)amino-2-cyclohexen-1-one **10**

A mixture of 2-iodoaniline (0.48 g, 2.2 mmol), **9** (0.5 g, 2.1 mmol) and a catalytic amount of *p*-toluenesufonic acid in benzene (25 mL) was refluxed for 24 h using a Dean-Stark apparatus for azeotropic removal of water. The solution was washed with water (25 mL), dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography (eluent: AcOEt/hexane 2:1) to give 0.74 g (79% yield) of a slighty yellow oil. IR (film) ν_{max} = 3 285, 2 970, 1 603 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 2.18–2.28 (m, 1H), 2.44 (d, 1H, J = 16.2 Hz), 2.58 (br. s, 3H), 3.42–3.56 (m, 2H), 4.55 (s, 2H), 5.38 (s, 1H), 5.96 (br. s, 1H), 6.89–6.96 (m, 1H), 7.32–7.35 (m, 7H), 7.85 (d, 1H, J = 7.9 Hz).

5.1.6. 2-Benzyloxymethyl-1,2,3,9-tetrahydro-4H-carbazol- 4-one **11**

A solution of **10** (440 mg, 1 mmol), $Pd(OAc)_2$ (12 mg, 0.05 mmol), Et_3N (170 μL, 1.2 mmol) in DMF (1.5 mL) was heated in a sealed tube at 120 °C for 2 h. After removing the solvent in vacuo, the residue was dissolved in CH_2Cl_2 (30 mL) and washed with water (30 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated to give a brown solid which was chromatographed on a silica gel column (eluent: AcOEt/hexane 1:2) to give 270 mg (87% yield) of a white solid, m.p. 186–188 °C (AcOEt). IR (KBr) ν_{max} = 3 179, 2 930, 1 629, 1 584 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 2.48 (dd, 1H, J_1 = 11.1 Hz, J_2 = 16.4 Hz), 2.61–2.77 (m, 2H), 2.90 (dd, 1H, J_1 = 9.6 Hz, J_2 = 16.5 Hz), 3.12 (dd, 1H, J_1 = 4.8 Hz, J_2 = 16.5 Hz), 3.47–3.60 (m, 2H), 4.53 (dd, 2H, J_1 = 2.1 Hz, J_2 = 13.7 Hz), 7.20–7.36 (m, 8H), 8.19–8.22 (m, 1H), 8.58 (s, 1H). Anal. $C_{20}H_{19}NO_2$ (C, H, N).

5.1.7. Removal of the benzyl protecting group: 2-hydroxymethyl-1,2,3,9-tetrahydro-4H-carbazol-4-one **2**

A mixture of carbazolone **11** (220 mg, 0.72 mmol) and 10% Pd on charcoal (25 mg) in ethanol (20 mL) was stirred under H_2 at 45 °C for 48 h. After cooling, the mixture was filtered through Celite and the solvent was evaporated under reduced pressure to give 150 mg (97%) of carbazolone **2** as a white solid.

5.1.8. General procedure for the preparation of the 2-aminomethyl-1,2,3,9-tetrahydro-4H-carbazol-4-ones **4a–c**

A solution of the tosylate **3** (130 mg, 0.35 mmol) and the amine (0.70 mmol) in (1-methyl)-2-pyrrolidinone (3mL) was stirred at 85 °C for 48 h. The solvent was evaporated in vacuo, and the residue was dissolved in AcOEt and washed with water. The organic phase was dried, filtered and concentrated at reduced pressure to afford a solid which was purified by column chromatography (silica gel, eluent: AcOEt) to give the desired amine.

5.1.8.1. 2-[4-(o-Methoxyphenyl)piperazinyl]methyl-1,2,3,4-tetrahydrocarbazol-4-one **4a**

62% yield, m.p. 228–230 °C (AcOEt). IR (KBr) ν_{max} = 3 218, 2 811, 1 625, 1 610 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 2.38 (dd, 1H, J_1 = 9.7 Hz, J_2 = 15.8 Hz), 2.46–2.51 (m, 2H), 2.55–2.71 (m, 6H), 2.73–2.82 (m, 1H), 3.09 (br. s, 4H), 3.17 (dd, 1H, J_1 = 3.6 Hz, J_2 = 16.3 Hz), 3.86 (s, 3H), 6.85–7.04 (m, 4H), 7.21–7.28 (m, 2H), 7.33–7.36 (m, 1H), 8.21–8.24 (m, 1H), 8.71 (s, 1H). MS (EI, *m/z*): 389 (M^+, 35%). Anal. $C_{24}H_{27}N_3O_2$ (C, H, N).

5.1.8.2. 2-[4-(p-Fluorobenzoyl)piperidinyl]methyl-1,2,3,4-tetrahydrocarbazol-4-one **4b**

55% yield, m.p. 217–219 °C. IR (KBr): ν_{max} = 3 220, 2 930, 1 677, 1 627, 1 610 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.75–1.86 (m, 4H), 1.96–2.17 (m, 2H), 2.24–2.43 (m, 3H), 2.50–2.57 (m, 1H), 2.60–2.82 (m, 2H), 2.89–3.04 (m, 2H), 3.15–3.28 (m, 2H), 7.14 (t, 2H, J = 8.6 Hz), 7.20–7.27 (m, 2H), 7.31–7.37 (m, 1H), 7.97 (dd, 2H, J_1 = 5.4 Hz, J_2 = 8.8 Hz), 8.18–8.21 (m, 1H), 8.87 (s, 1H). MS (EI, *m/z*): 404 (M^+, 1%). Anal. $C_{25}H_{25}FN_2O_2$ (C, H, N).

5.1.8.3. 2-[4-(6-Fluorobenzisoxazol-3-yl)piperidinyl]-methyl-1,2,3,4-tetrahydrocarbazol-4-one **4c**

70% yield, m.p. 222–223 °C (iso-PrOH). IR (KBr) ν_{max} = 3 159, 2 946, 1 627, 1 608 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 1.78–2.05 (m, 4H), 2.09–2.20 (m, 2H), 2.26 (dd, 1H, J_1 = 10.5 Hz, J_2 = 16.3 Hz), 2.34–2.53 (m, 4H), 2.72 (dd, 1H, J_1 = 9.5 Hz, J_2 = 16.6 Hz), 2.90–3.00 (m, 2H), 3.07–3.16 (m, 2H), 7.09–7.17 (m, 2H), 7.25 (dt, J_1 = 2.1 Hz, J_2 = 9.1 Hz), 2.34–2.41 (m, 1H), 7.65 (dd, 1H, J_1 = 2.1 Hz, J_2 = 9.1 Hz), 7.91–8.00 (m, 2H), 11.81 (s, 1H). Anal. $C_{25}H_{24}FN_3O_2$ (C, H, N).

5.1.9. General procedure for the synthesis of the 3,5-dimethoxybenzamides **5a** *and* **5b**

A solution of 3,5-dimethoxybenzoic acid (1.00 g, 5 mmol), the amine (5 mmol) and 1-hydroxybenzotriazole (0.74 g, 5 mmol) in DMF (10 mL) was allowed to stir at 0 °C under argon atmosphere. N,

N´-Dicyclohexylcarbodiimide (1.13 g, 5 mmol) was added and the mixture stirred for 30 min at 0 °C and 20 h at room temperature. The precipitate was filtered off and the solution was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and washed with 5% HCl and 5% $NaHCO_3$. The organic layer was dried, filtered and concentrated at reduced pressure. The crude compound was purified by column chromatography (silica gel, eluent: AcOEt) to afford the pure amide.

5.1.9.1. N,N-Diethyl-3,5-dimethoxybenzamide **5a**

95% yield. IR (film): ν_{max} = 2 936, 1 676 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.09 (s, 3H); 1.20 (s, 3H); 3.23 (5.a., 2H); 3.48 (5.a., 2H); 6.43 (1H, t, *J* = 2.2 Hz); 6.45 (2H, d, *J* = 2.2 Hz).

5.1.9.2. N-(3,5-Dimethoxybenzoyl)morpholine **5b**

98% yield, m.p. 67–69 °C. IR (KBr) ν_{max} = 2 971, 1 633 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 3.46–3.56 (2× s. a., 8H); 3.70 (s, 6H); 6.49 (d, 1H, *J* = 2.2 Hz); 6.51 (d, 2H, *J* = 2.2 Hz).

5.1.10. General procedure for the synthesis of the 3,5-dimethoxybenzylamines **6a** *and* **6b**

To a suspension of $LiAlH_4$ (0.93 g, 25 mmol) in THF (50 mL) at 0 °C, was added dropwise a solution of the amide **5a** or **5b** (5 mmol) in THF (50 mL) under argon. The resulting mixture was stirred at room temperature for 24 h. Excess of $LiAlH_4$ was decomposed at 0 °C by the successive dropwise addition of water (1.5 mL), 10% NaOH (1.5 mL) and water (6 mL). The insoluble salts were removed by filtration and washed with AcOEt. The organic portions were combined, and the solvent was removed under reduced pressure to afford the free base.

5.1.10.1. N,N-Diethyl-3,5-dimethoxybenzylamine **6a**

84% yield, b.p. 95–100 °C/0.1 mm Hg. IR (film): ν_{max} = 2 968, 1 596 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.04 (t, 6H, *J* = 7.1 Hz); 2.52 (q, 4H, *J* = 7.1); 3.51 (s, 2H); 3.78 (s, 6H); 6.34 (t, 1H, *J* = 2.3 Hz); 6.53 (d, 2H, *J* = 2.3 Hz). Hydrochloride salt: white needles, m.p. 169–170 °C (iso-PrOH). Anal. $C_{13}H_{21}NO_2$·HCl (C, H, N).

5.1.10.2. N-(3,5-Dimethoxybenzyl)morpholine **6b**

88% yield. IR (film): ν_{max} = 2 955, 2 805, 1 597, 1 460 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 2.47 (t, 3H, *J* = 4.6 Hz); 3.46 (s, 2H); 3.71–3.74 (m, 4H); 3.79 (s, 6H); 6.36 (t, 1H, *J* = 2.3 Hz); 6.51 (d, 2H, *J* = 2.3 Hz). Hydrochloride salt: m.p. 188–189 °C (iso-PrOH). Anal. $C_{13}H_{19}NO_3$·HCl (C, H, N).

5.1.11. General procedure for the synthesis of the 3,5-dimethoxy-1,4-dihydrobenzylamines **7a** *and* **7b**

A solution of the amine **6a** or **6b** (6.45 mmol) in a mixture of *tert*-butanol (45 mL) and anhydrous ether (45 mL) was added dropwise to liquid NH_3 (30 mL) to –35 °C. When the addition was complete, Li (1.00 g, 150 mmol) was added portionwise, the mixture was allowed to warm to room temperature and was stirred for 4 h at that temperature. The reaction mixture was quenched by adding of NH_4Cl (6.10 g) and the excess of NH_3 allowed to evaporate by stirring at room temperature. The resulting white solid was dissolved in water (40 mL), the alcoholic phase was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were concentrated under reduced pressure to give the desired amine.

5.1.11.1. N,N-Diethyl-3,5-dimethoxy-1,4-dihydrobenzylamine **7a**

99% yield. IR (film): ν_{max} = 2 926, 1 694, 1 663 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.02 (t, 6H, *J* = 7.1 Hz); 2.29 (d, 2H, *J* = 7.1 Hz); 2.54 (q, 4H, *J* = 7.1 Hz); 2.77 (dd, 2H, J_1 = 1.0 Hz, J_2 = 6.8 Hz); 3.01–3.08 (m, 1H); 3.56 (s, 6H); 4.71–4.72 (m, 2H).

5.1.11.2. N,N-(3,5-Dimethoxy-1,4-dihydrobenzyl)morpholine **7b**

95%, yield, m.p. 158–160 °C. IR (KBr) ν_{max} = 2 939, 2 802, 1 693, 1 660 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 2.25 (d, 2H, *J* = 7.3 Hz); 2.46 (t, 4H, *J* = 4.6 Hz); 2.78 (dd, 2H, J_1 = 0.9 Hz, J_2 = 6.4 Hz); 3.11–3.18 (m, 1H); 3.57 (s, 6H); 3.72 (t, 4H, *J* = 4.6 Hz); 4.69 (dd, 2H, J_1 = 1.4 Hz, J_2 = 2.0 Hz).

5.1.12. General procedure for the preparation of the 2-aminomethyl-1,2,3,9-tetrahydro-4H-carbazol-4-ones **4d–f**

A solution of phenylhydrazine hydrochloride (0.39 g, 1.6 mmol) and the amine **7a** or **7b** (1.8 mmol) in 4% H_2SO_4 (12 mL) was heated at reflux temperature for 3 h. The resulting solution was basified with 25% NH_3 and extracted with AcOEt (3 × 15 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated to give a brown solid which was purified by column chromatography (silica gel, eluent: CH_2Cl_2/MeOH, 9:1) to give the carbazolone as a white solid.

5.1.12.1. 2-Diethylaminomethyl-1,2,3,4-tetrahydrocarbazol-4-one **4d**

66% yield, m.p. 184–185 °C (CH_3CN). IR (KBr): ν_{max} = 3 182, 2 966, 1 628, 1 617 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 0.92 (t, 6H, *J* = 7.1 Hz), 2.18–2.27 (m, 1H), 2.33–2.53 (m, 8H), 2.69 (dd, 1H, J_1 = 7.9 Hz, J_2 = 16.8 Hz),

3.04–3.10 (m, 1H), 7.08–7.17 (m, 2H), 7.35–7.39 (m, 1H), 7.91–7.94 (m, 1H), 11.79 (s, 1H). Anal. $C_{17}H_{22}N_2O$ (C, H, N).

5.1.12.2. 2-Morpholinylmethyl-1,2,3,4-tetrahydrocarbazol-4-one **4e**

60% yield, m.p. 207–208 °C (AcOEt). IR (KBr) ν_{max} = 3 177, 2 956, 1 618 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 2.30–2.50 (m, 7H), 2.55–2.65 (m, 1H), 2.67–2.79 (m, 2H), 3.14 (dd, 1H, J_1 = 4.4 Hz, J_2 = 16.3 Hz), 3.70 (t, 4H, J_1 = 4.7 Hz), 7.19–7.25 (m, 2H), 7.31–7.36 (m, 1H), 8.19–8.22 (m, 1H), 8.90 (s, 1H). MS (EI, *m/z*): 284 (M^+, 1%). Anal. $C_{17}H_{20}N_2O_2$.

5.1.12.3. 2-Diethylaminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazol-4-one **4f**

This compound was prepared following the same procedure but using N-methyl-N-phenylhydrazine as starting material; yield 70%; m.p. 192–193 °C (acetone). IR (KBr): ν_{max} = 2 967, 2 792, 1 636 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.01 (t, 6H, *J* = 7.1 Hz), 2.31 (dd, 1H, J_1 = 9.5 Hz, J_2 = 16.0 Hz), 2.41–2.69 (m, 9H), 3.15 (dd, 1H, J_1 = 3.1 Hz, J_2 = 16.0 Hz), 3.72 (s, 3H), 7.24–7.33 (m, 3H), 8.23–8.26 (m, 1H). MS (FAB, *m/z*): 285 (M^+ + 1, 100%). Anal. $C_{18}H_{24}N_2O$ (C, H, N).

5.2. Pharmacology

5.2.1. D_2 receptor binding assays

Male Sprague-Dawley rats were killed by decapitation and their brains were rapidly removed and dissected on an ice-cold plate. Striatal membrane preparations were obtained by homogenization (Polytron homogenizer, setting 6.10 s) in 50 mM Tris-HCl (pH 7.7 at 25 °C; about 100 μL per mg of tissue) containing 5 mM EDTA; the homogenates were centrifuged (49 000 *g* for 15 min at 4 °C, Sorvall RC-26 plus), resuspended in 50 mM Tris-HCl buffer (pH 7.4 at 25 °C) and centrifuged again (same conditions), and the final pellets were stored at –80 °C pending use. Just before binding assays, the pellets were resuspended (1.25 mg original wet weight per 750 μL for D_2 assays, 1.00 mg per 750 μL for D_1) in 50 mM Tris-HCl buffer (pH 7.4 at 25 °C) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$. For D_2 binding assays, 750 μL aliquots of striatal membrane preparation were added to ice-cold tubes containing (a) 100 μL of [^{3}H]spiperone, (b) 50 μL of ketanserin (final concentration 50 nM) to block 5-HT_{2A} receptors, and either (c) 100 μL of buffer (for total binding assay) or (d) 100 μL of sulpiride (final concentration 10 μM) to allow quantification of nonspecific binding by [^{3}H]spiperone, or (e) 100 μL of the compounds to be tested. The final assay volume was thus 1 mL in all cases. All assays were performed in duplicate. Incubations (15 min at 37 °C) were stopped by rapid vacuum filtration through GF-52 glass fibre filters (Schleicher and Schuell) in a Brandel M-30 cell harvester. The filters were rinsed three times with 3 mL of ice-cold 50 mM Tris-HCl buffer (pH 7.4) and radioactivity was determined by liquid scintillation counting in a Beckman LS-6000LL apparatus (counting efficiency approximately 50%). Competition analyses were carried out with the aid of the Prism program (GraphPad); K_i values were calculated from the Cheng-Prusoff equation [55]: $K_i = IC_{50}/(1 + D/K_d)$, where D is the concentration and K_d the apparent dissociation constant of the ligand.

5.2.2. 5-HT_{2A} receptor binding assays

Male Sprague-Dawley rats (200–250 g) were asphyxiated with CO_2 and decapitated. The frontal cortex, containing 5-HT_{2A} receptors [56, 57], was dissected free on ice, frozen on dry ice and stored at –70 °C until use (generally less than 1 week later). All membrane preparation procedures were carried out at 4 °C. The tissue was thawed on ice and homogenized with 10 volumes of 0.32 M sucrose in a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). The homogenate was centrifuged twice at 4 °C (900 *g* for 10 min followed by 40 000 *g* for 30 min). The supernatant was discarded and the pellet resuspended in Tris-HCl buffer (pH 8.07) in a Teflon/glass homogenizer (10 strokes by hand). The homogenate was incubated at 37 °C for 15 min to remove endogenous 5-HT and centrifuged for 30 min at 40 000 *g*. The final pellet was resuspended in Tris-HCl buffer of pH 8.07 containing 4 mM $CaCl_2$ and 0.1% ascorbic acid. Competition at [^{3}H]ketanserin-binding sites was assayed in triplicate in assay mixtures consisting of 750 μl of membrane homogenate, 50 μL of [^{3}H]ketanserin, 50 μL of either buffer or the compound under test, 50 μL of masking ligand solution (1 μM methysergide) as required, and buffer to a final volume of 1 mL. Mixtures were incubated for 30 min at 37 °C. The assay was terminated by rapid filtration through Whatman GF/C filter strips (pre-soaked in 3% polyethylenylimine) in a Brandel cell harvester (Gaithersburg, MD) followed by washing with ice-cold Tris-HCl buffer (pH 6.6) to remove unbound radioligand. The radioactivity retained on filters was determined by liquid scintillation counting in a beta counter (Beckman, LS-1800).

The non-linear curve-fitting program Kaleidagraph (Synergy Software, Reading, PA) was used to fit the equation $E = E_{max} - [E_{max} - E_{min}/(1 + (IC_{50}/C)^n)]$, where E_{max} and E_{min} are dpm at the beginning and the end of the competition experiment, respectively, IC_{50} is the drug concentration required to inhibit binding by 50%, C is the

concentration of the inhibitor and n is the slope of the decay. Non-specific binding was determined independently in the presence of unlabelled methysergide. IC_{50} and pK_i values were calculated as for D_2 receptors.

5.2.3. 5-HT$_{2C}$ receptor binding assays

Bovine choroid plexus containing 5-HT_{2C} receptors [58] was treated as described in the previous assay. A suspension of the resulting pellet in the same buffer was stored on ice while not being manipulated. Competition at [^{3}H]mesulergine-binding sites was determined by a protocol analogous to that described above for the 5-HT_{2A} binding assay, using a final [^{3}H]mesulergine concentration of 2 nM and 1 mM mianserine as a 5-HT_{2A} receptor masking ligand. The mixtures were incubated for 1 h at room temperature. Membranes were harvested on Whatman GF/B filter paper. Non-specific binding was determined in the presence of unlabelled mianserine. IC_{50} and pK_i values were calculated as for D_2 and 5-HT_{2A} receptors.

5.2.4. Antagonism of serotonin at 5-HT$_{2A}$ receptors from rat aorta

Antagonism of serotonin at 5-HT_{2A} receptors [13, 14, 56] was assayed using thoracic aorta from male Sprague-Dawley rats (250–350 g) killed by cervical dislocation. The descending aorta was removed, cleaned, stripped of endothelium and cut into rings 4 mm in length [59] that were then mounted under a tension of 2 g in a CELASTER-IOS 1 computerized organ bath containing 20 mL of Krebs solution of the following composition (mM): NaCl, 119; KCl, 4.7; $MgSO_4 \cdot 7H_2O$, 1.2; $CaCl_2 \cdot 2H_2O$, 1.5; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11. Clorpheniramine (1 μM) was added to block uptake of serotonin [60, 61]. The bath solution was maintained at 37 °C and aerated with oxygen and carbon dioxide, (95% O_2, 5% CO_2). Isometric contraction force was monitored via a CPOL 0–25 g transducer. Following equilibration for 60 min under a load of 2 g, the rings were sensitized by addition of 10 μM 5-HT for 8 min. Equilibration periods (60 min) [62] were then alternated with the construction of cumulative 5-HT concentration–effect curves (from 30 nM to 100 μM). Two control runs giving identical curves were followed by test runs with ketanserin or the new compounds which were added to the bath solution 20 min before the end of the preceding equilibration period. Antagonist potency was measured, following Arunlaksana and Schild [63], in terms of pA_2 (–log concentration of antagonist required to maintain a constant response when agonist concentration is doubled).

5.2.5. Antagonism of serotonin at 5-HT$_{2B}$ receptors from rat stomach fundus

Male Sprague-Dawley rats (250–300 g) were killed by cervical dislocation. The stomach was dissected free from the abdomen and immersed in modified Krebs solution of the following composition (mM): NaCl, 118; KCl, 4.7; $MgSO_4 \cdot 7H_2O$, 1.2; $CaCl_2 \cdot 2H_2O$, 2.5; KH_2PO_4, 1.18; $NaHCO_3$, 25; glucose, 11. Strips of stomach fundus were prepared by Vane's method [64] and mounted in organ baths containing 10 mL of the same Krebs solution as above, maintained at 37 °C with aeration using oxygen, carbon dioxide (95% O_2, 5%CO_2). Before addition of drugs, the tissue strips were equilibrated for 1 h under a 1 g load. Isometric contractions were recorded during cumulative addition of serotonin using a Grass transducer FTO3C and a Grass polygraph 7D.

Concentration–response curves for serotonin were constructed as per Van Rossum. [65]. In the initial control runs, stable contractions were achieved over the concentration range of 0.01 nM–10 μM. Following the initial control run, each tissue strip was run alternately with and without antagonist. Between runs, the tissues were washed and allowed to rest for 60 min. Antagonist potency was measured according to Mackay [66] in terms of pA_2 (–log concentration of antagonist required to maintain a constant response when the agonist concentration is doubled).

5-Hydroxytryptamine·HCl was supplied by Sigma, and mianserine and methysergide by R.B.I. Aqueous solutions of all drugs as their hydrochlorides were prepared daily using distilled water. All drug concentrations mentioned above are final molar concentrations in the tissue bath. [^{3}H]ketanserin (60.08 Ci/mmol) and [^{3}H]mesulergine (76 Ci/mmol) were obtained from DuPont NEN (Boston, MA) and Amersham (UK), respectively. All other drugs and chemicals were reagent grade products from Sigma (St Louis, MO).

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