3-[[4-(4-Chlorophenyl)piperazin-1-yl]-methyl]-1*H*-pyrrolo[2,3-*b*]pyridine: An Antagonist with High Affinity and Selectivity for the Human Dopamine D_4 Receptor

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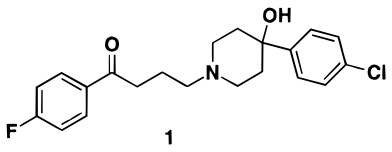
It is widely accepted that the dopaminergic system plays a key role in the manifestation of schizophrenic illness,[1] a belief supported by the observation that all clinically effective antipsychotic agents act as antagonists at the dopamine D_2 receptor[2] and that therapeutically relevant plasma concentrations of drug correlate closely to their affinity for this site.[3] Although classical neuroleptics such as haloperidol **1** constitute first-line antipsychotic therapy, their use is associated with severe, mechanism-related side effects including induction of acute extrapyramidal symptoms (EPS), tardive dyskinesia, and problems such as galactorrhea due to increased prolactin release.[4] In contrast, atypical antipsychotics such as clozapine **2** present a lower incidence of EPS, are effective in patients who are unresponsive to classical agents and may also offer advantages in treating the more resistant negative symptoms of schizophrenia.[5] The use of clozapine has, however,



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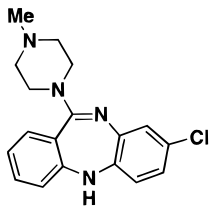

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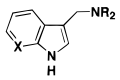
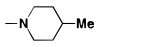
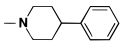
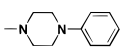
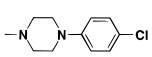
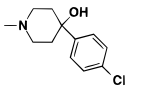
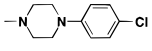
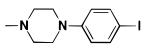
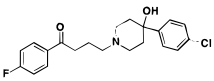
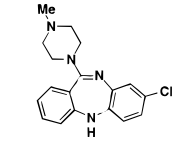
been compromised by a relatively high (up to 2%) incidence of the potentially fatal blood disorder agranulocytosis,[6] necessitating stringent monitoring of plasma levels. The high affinity of clozapine for a wide range of neurotransmitter receptors has frustrated attempts to rationalize the improved clinical properties of this drug.[7] It has been postulated that a combination of D_2 and 5-HT_2 antagonism is responsible for the beneficial properties of atypical neuroleptics,[8] and compounds with this profile, which will serve to support the hypothesis, are now emerging.[9] However, alternative approaches have also been proposed,[10] and the absence of a well-defined, unequivocal mechanism of action for clozapine has hitherto hampered the development of superior antipsychotic agents.

The discovery of two new dopamine receptor subtypes, designated D_3 and D_4, has elicited considerable interest due to their close homology to the D_2 receptor[11] and the disclosure of preferential binding of clozapine to the D_4 receptor.[12] Moreover, clinically relevant plasma levels of clozapine correlate more closely to its affinity for the D_4 rather than D_2 receptor, a property not shared by other antipsychotic agents.[13] Although controversial,[14] an association between the D_4 receptor and schizophrenia was recently suggested by Seeman,[15] who found a 6-fold increase in D_4 receptors in the caudate of schizophrenic patients relative to controls. These intriguing findings highlight the need for a selective ligand to aid elucidation of the pharmacological role played by the D_4 receptor. In particular, a D_4 receptor antagonist may have potential as an antipsychotic devoid of the undesirable side effects associated with agents in current use.

Table 1. Dopamine Receptor Subtype Affinity for Indoles and 7-Azaindoles



Compound[b]	X	NR_2	Ki(nM)[a] D_2	D_3	D_4
3	CH		5500	6700	570
4	CH		>1300	>3700	>2600
7	CH		110	95	25
8	CH		120	280	8.0
9	CH		71	150	1.6
10	CH		2.4	100	220
11	N		960	2300	0.43
12	N		>1700	>4500	0.51
1 Haloperidol			1.4	2.0	2.3
2 Clozapine			74	200	10

[a] Data are the mean of two to four independant determinations. [b] All new compounds were characterized by ^{1}H NMR and mass spectroscopy and gave satisfactory elemental analyses.

Our search for a selective D_4 ligand commenced with a topological similarity (TOPOSIM) search[16] of the Merck sample collection using a number of known dopamine agonists and antagonists as probe structures. Receptor binding was determined by displacement of [^{3}H]spiperone from cloned human receptors, D_2 and D_3 being stably expressed in CHO cells[17] and D_4 in HEK293 cells.[18] This strategy led to the identification of the indole **3** which exhibited modest selectivity for the D_4 receptor (Table 1). In contrast, the unsubstituted piperidine **4**, also uncovered in the search, had no significant affinity for the D_4 receptor. This key finding led us to examine a number of piperidines and related cyclic amines substituted with lipophilic groups at the 4-position, while retaining the 3-indolylmethyl moiety on nitrogen. The compounds were readily prepared in 45−73% yield by displacement of gramine **5** or 7-azagramine **6**[19] with the appropriate commercially available amine in toluene or xylene at reflux (Scheme 1).

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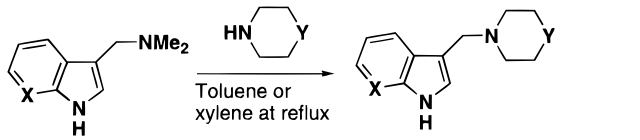
Scheme 1



5; X = CH
6; X = N

7; X = CH, Y = CHPh
8; X = CH, Y = NPh
9; X = CH, Y = N(4-Cl)Ph
10; X = CH, Y = COH(4-Cl)Ph
11; X = N, Y = N(4-Cl)Ph
12; X = N, Y = N(4-I)Ph

Replacement of the 4-methyl group of **3** with phenyl resulted in **7**, having substantially higher affinity for all three receptors, although selectivity for the D_4 receptor was reduced. Selectivity was restored with the corresponding piperazine **8**, which exhibited a 3-fold improvement in affinity for the D_4 receptor over **7**, with a corresponding reduction in D_3 receptor binding. Substitution of the phenyl ring of **8** was also found to confer higher selectivity, with location of the substituent at the 4-position being optimum, as demonstrated by the chloro derivative **9**. Although haloperidol itself displays no subtype selectivity in binding to dopamine receptors, replacement of the fluorobutyrophenone moiety with indolylmethyl gave **10** (L-741,626), which, remarkably, exhibited a complete reversal of the previously observed selectivity, now having *100-fold selectivity for the D_2 receptor*.

In addition to optimization of the cyclic amine side chain, changes were also made to the indole nucleus. The crucial modification in this area was the introduction of a further heteroatom by replacement of the indole of **9** with 7-azaindole (1*H*-pyrrolo[2,3-*b*]pyridine). This change resulted in a dramatic improvement in selectivity, with **11** (L-745,870) having K_i of 0.43 and 960 nM for D_4 and D_2 receptors, respectively, with considerably weaker (K_i 2300 nM) D_3 receptor affinity. Since most antipsychotic agents also possess high affinity for 5-HT_2 receptors, **11** was evaluated against this receptor and found to be >1000-fold selective. Moreover, no appreciable binding (K_i >10 000 nM) was observed to human cloned D_1 and D_5 receptors. Furthermore, **11** attenuated the dopamine mediated inhibition of forskolin-elevated cAMP in functionally-coupled HEK cells expressing the D_4 receptor,[18] while alone having no effect, thereby confirming it to be a functional antagonist. The potential for obtaining a D_4 selective radioligand from this series was demonstrated by preparation of the 4-iodo analogue **12**, which similarly bound with high affinity and selectivity to the D_4 receptor and was also shown to exhibit D_4 antagonist activity in the adenylate cyclase assay.

In conclusion, the modestly selective indole **3**, identified by directed screening of the Merck sample library, has been optimized to piperazinylazaindole **11**, resulting in a 1000-fold improvement in affinity for the D_4 receptor and providing an antagonist with 2200- and >5000-fold binding selectivity relative to D_2 and D_3 receptors, respectively. During the course of these studies, the D_2 selective ligand **10** and the iodo analogue **12**, a potential radioligand precursor, were also identified. The unique binding profile of **11** renders it an ideal ligand with which to begin to unravel the biological significance of the dopamine D_4 receptor.

Supporting Information Available: ^{1}H NMR and mass spectral data for compounds **3**, **4**, and **7**−**12** (3 pages). Ordering information is given on any current masthead page.

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