5-(4-Chlorophenyl)-4-methyl-3-(1-(2-phenylethyl)piperidin-4-yl)isoxazole: A Potent, Selective Antagonist at Human Cloned Dopamine D4 Receptors

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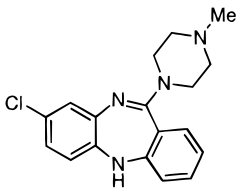
Schizophrenia is a mental illness for which there is still a great need for novel drug therapy. Classical neuroleptics, which are presumed to act by antagonism of dopamine D2 receptors,[1] are useful for the treatment of the positive symptoms, but suffer from various motor side effects[2] along with increases in serum prolactin levels.[3] The atypical neuroleptic clozapine[4] (**1**) can be used to treat both positive and negative symptoms of schizophrenia and does not induce extrapyramidal side effects. However, in 1−2% of patients the drug induces agranulocytosis, and its use is therefore limited and must be closely monitored.[5] In recent years the application of molecular biology techniques has seen the cloning of a number of different subtypes of dopamine receptors[6−10] which on the basis of their pharmacology can be divided into two classes, D1-like (D1, D5) and D2-like (D2, D3, D4). Clozapine, among its many actions, has higher affinity for the D4 subtype than for D2.[9] Recent reports[11] suggests that D4 receptor density is elevated in postmortem schizophrenic brain. For these reasons we decided to identify selective D4 receptor antagonists to investigate their potential as novel antipsychotics.



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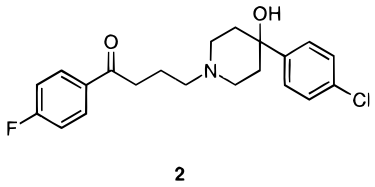
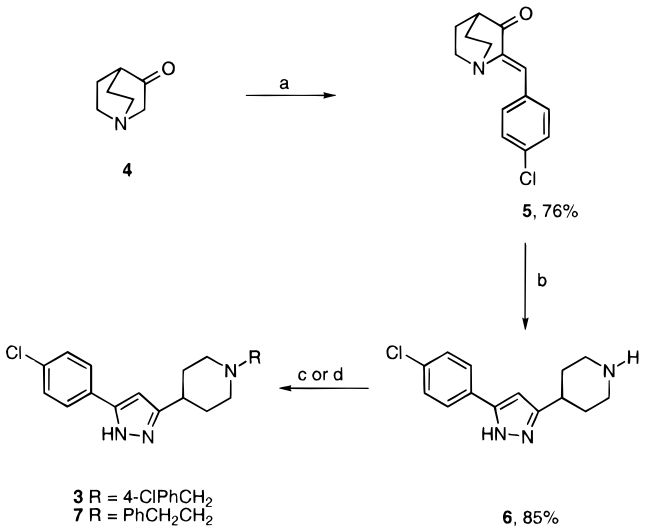


2

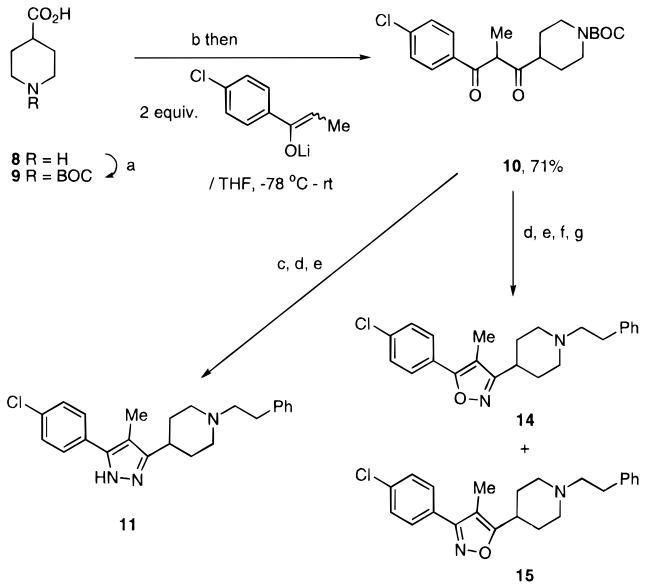
Our strategy began with the screening of our sample collection. Compounds were tested for their ability to displace [^{3}H]spiperone from human cloned receptors, D2 and D3 stably expressed in CHO cells[12] and D4 in HEK-293 cells.[13] A representative number of known dopamine antagonists were selected and, using the topological similarity probe method[14] as implemented in the in-house TOPOSIM[15] package, the collection was screened for chemicals that have affinity for dopamine receptors and may show selectivity for D4 over other subtypes. One of these runs, based on the structure of the classical neuroleptic haloperidol (**2**), gave rise to a number of dopamine subtype selective ligands, from which 5-(4-chlorophenyl)-3-(1-(4-chlorobenzyl)piperidin-4-yl)pyrazole (**3**) was chosen as a suitable lead. This compound has moderate affinity for cloned human dopamine D4 receptors (K_i 61 nM) and 4-fold selectivity over D2. Our initial approach to improve on the affinity and selectivity of this lead was to make variations to the substituent on the basic nitrogen, which can be done by alkylation of a common precursor and by the synthesis of alternative aromatic heterocycles to replace the pyrazole.

Scheme 1[a]



[a] Reagents: (a) 4-Chlorobenzaldehyde, NaOH, EtOH, reflux; (b) hydrazine hydrate, KOH, ethylene glycol, 130−200 °C; (c) 4-$ClPhCH_2Cl$, $EtPr_2N$, DMF, room temperature; (d) $PhCH_2CH_2Br$, Et^iPr_2N, DMF 60 °C.

Scheme 2[a]



[a] Reagents: (a) BOC_2O, CH_2Cl_2, room temperature; (b) carbonyldiimidazole, THF, room temperature; (c) hydrazine hydrate, EtOH, room temperature; (d) CF_3CO_2H; (e) $PhCH_2CH_2Br$, Et^iPr_2N, DMF, 60 °C; (f) $H_2NOH{\cdot}HCl$, Et_3N, MeOH; (g) CH_3SO_2Cl, Et_3N, CH_2Cl_2.

The pyrazole (**3**) was synthesized (Scheme 1) starting from 3-quinuclidinone (**4**). Condensation with 4-chlorobenzaldehyde gave the unsaturated ketone **5**, which on treatment with hydrazine and base[16] ring opened to give pyrazole **6**. Alkylation with 4-chlorobenzyl chloride gave **3**. The *N*-phenylethyl analogue **7** was made by using 2-phenethyl bromide as the alkylating reagent. A more general route (Scheme 2) allowing variations to the pyrazole portion of the molecule started with

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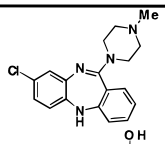
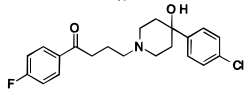
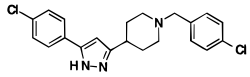
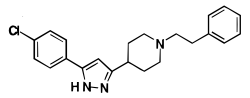
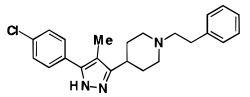
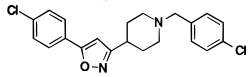
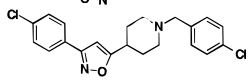
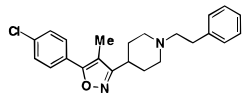
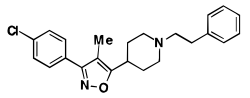
Table 1. Affinities for Cloned Human Dopamine Receptors

Number[a]	Structure	K_i (nM) [b] hD2	hD3	hD4
1		74	200	10
2		1.4	2.0	2.3
3		240	820	61
7		520	>4000	5.5
11		290	1200	1.2
12		160	430	44
13		130	81	3.6
14		>1700	770	3.5
15		>1700	480	2.5

[a] All compounds were characterized by proton NMR and mass spectra and gave satisfactory elemental analyses. [b] Binding data are the means of two to four independent determinations.

isonipecotic acid (**8**). N-Protection with di-*tert*-butyl dicarbonate gave acid **9**, which was activated with carbonyldiimidazole and treated with 2 equiv of the enolate derived from 4-chloropropiophenone to give the diketone **10**. Condensation with hydrazine converted the diketone to the pyrazole, and then deprotection of the nitrogen with trifluoroacetic acid, followed by alkylation with 2-phenethyl bromide, gave **11**. Alternatively, the nitrogen of **10** was deprotected and alkylated, followed by formation of a mixture of regioisomeric isoxazoles by treatment with hydroxylamine and dehydration of the intermediate hydroxyisoxazoline with methanesulfonyl chloride. The isomeric isoxazoles (**14**, L-741,742, and **15**) were formed in similar proportions, depending on precise reaction conditions, and separated chromatographically, and their regiochemistry was proved by X-ray analysis. Isoxazoles **12** and **13** were made in an analogous fashion using 4-chloroacetophenone and alkylating the piperidine nitrogen with 4-chlorobenzyl bromide.

Extension of the alkyl chain in the substituent on the basic nitrogen from benzyl to phenethyl (**7**, Table 1) gave a 10-fold improvement in affinity for the D4 receptor, along with a small reduction in binding to D2, leading overall to a compound with 100-fold selectivity. Introduction of a methyl group to the 4-position of the pyrazole **11** gave another increase in D4 affinity, possibly due to an alteration in the preferred conformation around the phenyl−heterocycle bond. On alteration of the pyrazole heterocycle to isoxazole, two regioisomers are possible. When this change is made on its own to the lead structure, two compounds with markedly different biological activities were obtained. One of the isomers, **12**, was very similar in its dopamine receptor binding profile to the lead structure, whereas the other, **13**, showed much higher affinity for D4 receptors than did **3**. The reasons for this are not clear, although it is interesting to note that the solid state conformations, as determined by X-ray crystallography, show that the isoxazole ring is turned by approximately 180° in one isomer relative to the other. The differences in D4 affinity may therefore be due again to differences in the preferred conformation around the bond between the two aromatic rings. In the last two compounds in Table 1, the changes made to the lead structure above were all incorporated. The two isoxazoles (**14** and **15**) with methyl groups in the 4-position and phenethyl groups attached to the piperidine nitrogen both show low nanomolar affinity for human dopamine D4 receptors and have greater than micromolar affinity for D2, leading to selectivities of over 500-fold. There is a small difference in their binding to D3, with the former compound, **14**, having >200-fold selectivity for D4 over D3, as well as its high selectivity over D2. In D4 HEK cells, compound **14** (1 μM) alone had no effect, but antagonized the dopamine (1 μM)-mediated inhibition of forskolin (10 μM)-induced elevation of cAMP levels.[13] Thus **14** is an antagonist at the D4 receptor.

With these tools in hand it is now possible to move forward to explore the relevance and importance of the D4 subtype of dopamine receptor, particularly in the possible treatment of schizophrenia. Full structure−activity relationships and experimental detail on this series of compounds will be published later.

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Supporting Information Available: Analytical data for compounds (3 pages). Ordering information is given on any current masthead page.

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