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Communications to the Editor

2-Phenyl-4(5)-[[4-(pyrimidin-2-yl)piperazin-1-yl]methyl]imidazole. A Highly Selective Antagonist at Cloned Human D_4 Receptors

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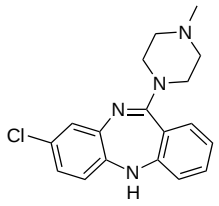
The neuroleptic agent clozapine (**1**) has been referred to as an atypical antipsychotic because it does not induce the extrapyramidal motor side effects characteristic of all other antischizophrenia medications.[1,2] The discovery that clozapine displays a higher affinity for dopamine D_4 receptors than D_2 receptors[3] coupled with the controversial[4] finding that D_4 receptor density is elevated in schizophrenic relative to nonschizophrenic postmortem brain tissue[5,6] has led to the postulate that dysfunction of the D_4 system is, at least in part, involved in the etiology of schizophrenia. A number of ligands selective for the D_4 receptor subtype have recently been reported.[7]

Previously we reported on a series of 2-phenyl-4-(aminomethyl)imidazoles (**2**) which displayed affinity at dopamine D_2 receptors.[8] Expansion of this work lead us to investigate modifications of the aminomethyl group within this series, eventually leading to the preparation of various aryl- and heteroarylpiperazine derivatives of general structure **3**. Receptor binding in cloned human receptors indicated such modification within this structural class produced a series of compounds with greatly improved D_4/D_2 binding ratios.

The synthetic methods used in the preparation of the compounds **3a**−**3g** are shown graphically in Scheme 1. The compounds (except **3h**) were prepared by the alkylation of the appropriate 1-aryl- or 1-heteroarylpiperazine (**4**) with 2-phenyl-4-(chloromethyl)imidazole (**5**) in the presence of triethylamine. Compound **3h** was prepared via a Mannich reaction between the commercially available reagents 2-phenyl-4-methylimidazole (**7**) and 1-(2-pyrimidinyl)piperazine (**6**). Methods for the preparation of **5** have been previously described.[8]



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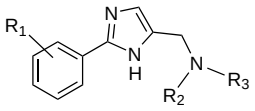

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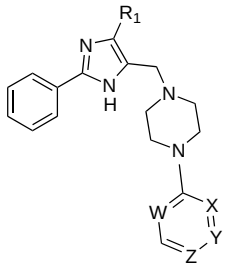

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The binding affinities of compounds **3a**−**h** for cloned human D_2, D_3, and D_4 receptors are shown in Table 1. Binding affinities (K_i) of **3a**−**h** at cloned D_1 and D_5 receptors were greater than 5000 nM.

The unsubstituted phenyl derivative **3a** displayed appreciable affinity at both D_2 and D_4 receptors and a D_2/D_4 ratio of approximately 50. Use of pyridyl, pyrazinyl, or pyrimidinyl heterocycles in place of phenyl resulted in attenuation of D_2 receptor binding in all cases except **3c**. Dopamine D_4 affinity was also affected by changes in the arylpiperazine portion of the molecule. Of the three possible pyridyl derivatives, only the 2-pyridyl (**3b**) retained significant affinity at the D_4 receptor. Among the diazaheterocycles, the 2-pyrazinyl (**3e**) and 4-pyrimidinyl (**3f**) compounds displayed diminished D_4 binding (similar to their direct monoaza analogs **3c** and **3d**), while the D_4 binding of the 2-pyrimidinyl compound **3g** was largely unaffected by the additional nitrogen. Its D_2/D_4 binding ratio of approximately 675 was the best among the compounds tested. Examination of the binding results indicates

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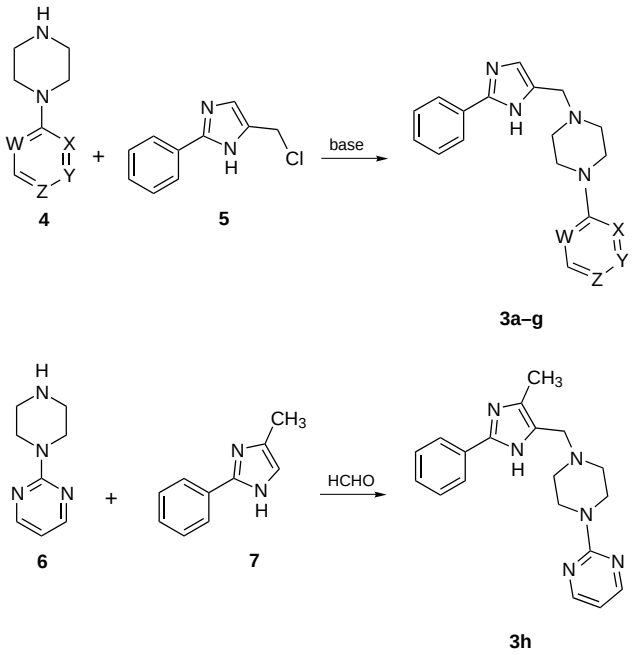
Scheme 1. Synthesis of 2-Phenyl-4-(1-[piperazin-1-yl])methylimidazoles.



that replacement of the phenyl group of **3a** with a 2-aza heterocycle such as 2-pyridyl or 2-pyrimidinyl is not only tolerated but leads to a significant improvement in the D_2/D_4 binding ratio. In contrast, replacement of the phenyl of **3a** by a 3- or 4-aza heterocycle reduces dopamine receptor binding. This is true even in those cases where a 2-aza moiety is also present in the same structure (i.e. **3e**, **3f**).

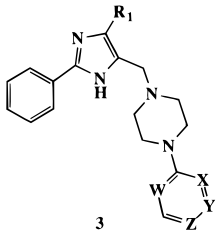
Compound **3h** was prepared as a conformationally restricted analog of **3g**. Molecular mechanics studies confirmed that the barriers to free rotation about the methylene group at the C-4 position on the imidazole of **3h** were higher than those of **3g**. The fact that good D_4 binding and selectivity were retained by **3h** suggests that the binding conformation of this series to D_4 receptors is as shown in structure **3** rather than the alternative extended conformation.

The favorable dopamine receptor binding results obtained for **3g** prompted a closer examination of the compound. Further receptor binding studies revealed no significant affinity (<1000 nM) for a wide variety of CNS and non-CNS receptor systems with the exception of 5-HT_{1a} (K_i = 181 nM).

In a series of biochemical experiments, the antagonistic properties of **3g** on the D_4 receptor were demonstrated. It has been shown that D_4 receptors are coupled to G-proteins.[9] The D_4 receptor is also negatively linked to adenylate cyclase such that agonists at D_4 receptors inhibit forskolin-stimulated cAMP production. Neither **3g** nor the dopamine agonist quinpirole had any effect on the basal (non forskolin stimulated) amount of cAMP formed in the D_4 receptor expression system. The addition of forskolin, which directly activates adenylate cyclase, caused a significant increase in cAMP levels. This increase was inhibited by the agonist quinpirole in a dose dependent fashion. Compound **3g** did not inhibit forskolin-stimulated cAMP production, but produced a 50% attenuation of the quinpirole-induced inhibition of cAMP production. Thus, **3g** exerts functional antagonism within the D_4 receptor system.

Behavioral testing for antipsychotic efficacy was then carried out on **3g**. At relatively low doses (0.1, 0.5 and1.0 mg/kg sc), **3g** significantly ($p < 0.05$) attenuated the disruptive effects of apomorphine (20 mg/kg sc) on prepulse inhibition. This effect is consistent with that of other antipsychotic drugs in this model.[10] In behavioral assays of extrapyramidal side effect (EPS) liability,[11,12] **3g** failed to block either apomorphine or amphetamine-induced stereotypy and did not induce catalepsy over a wide dose range (0.1−10.0 mg/kg sc). These tests suggest a low EPS liability for **3g** in humans. In behavioral assays of CNS depression and sedation,[13] **3g** displayed no consistent effects on spontaneous locomotor activity and did not affect the amplitude of the response in acoustic startle. All behavioral testing was carried out using male Sprague−Dawley rats.

Table 1. Affinities of 2-Phenyl-4-(piperazin-1-ylmethyl)imidazoles at Cloned Human Dopamine Receptors



						K_i (nM)[a]		
compound	W	X	Y	Z	R_1	D_2	D_3	D_4
3a	C	C	C	C	H	254 ± 41	193 ± 31	5.2 ± 1.3
3b	C	N	C	C	H	2190 ± 190	1530 ± 680	8.5 ± 1.5
3c	C	C	N	C	H	296 ± 42	289 ± 31	73 ± 1
3d	C	C	C	N	H	>3500	>8000	>7300
3e	N	C	N	C	H	>4130	>6665	50 ± 9
3f	C	N	C	N	H	>4130	>8000	1400 ± 120
3g	N	N	C	C	H	2250 ± 440	>8000	3.8 ± 0.7
3h	N	N	C	C	Me	1440 ± 94	2980 ± 1390	6.0 ± 0.9
haloperidol						4.8 ± 1.2	nd	7.3 ± 1.3
clozapine						254 ± 19	454 ± 39	70 ± 11

[a] Binding data are the means of at least three independent experiments using standard displacement assays with [^{3}H]YM 09151 as the competitive ligand and human dopamine receptor subtypes expressed in CHO cells.

In conclusion, the relatively modest D_4 selectivity of 2-phenyl-4-[(4-phenylpiperazin-1-yl)methyl]imidazole (**3a**) could be greatly amplified by replacement of the phenylpiperazine with either 2-pyridylpiperazine (**3b**) or 2-pyrimidinylpiperazine (**3g**). The interesting binding and behavioral profile of **3g** has led to its selection as a clinical candidate for the treatment of schizophrenia.

Supporting Information Available: Synthetic procedures, melting points, and ^{1}H NMR data for all products plus a molecular modeling study (7 pages). Ordering information is given on any current masthead page.

References

(1) Cavallaro, R.; Smeraldi, E. Antipsychotic-Induced Tardive Dyskinesia. *CNS Drugs* **1995**, *4*, 278−293.

(2) Whitworth, A. B.; Fleishhacker, W. W. Adverse Effects of Antipsychotic Drugs. *Int. Clin. Psychopharm.* **1995**, 9, Suppl. 5, 21−27.

(3) Van Tol, H. H. M.; Bunzow, J. R.; Guan, H. C.; Sunahara, R. K.; Seeman, P.; Niznik, H. B.; Civelli, O. Cloning of the Gene for a Human Dopamine D_4 Receptor with High Affinity for the Antipsychotic Clozapine. *Nature* **1991**, *350*, 610−615.

(4) Reynolds, G. P.; Mason, S. L. Absence of Detectable Striatal Dopamine D4 Receptors in Drug-Treated Schizophrenia. *Eur. J. Pharmacol.* **1995**, *281*, R5-R6.

(5) Seeman, P.; Guan, H.-C.; Van Tol, H. H. M. Dopamine D_4 Receptors Elevated in Schizophrenia. *Nature* **1993**, *365*, 441−445.

(6) Murray, A. M.; Hyde, T. M.; Knable, M. B.; Herman, M. M.; Bigelow, L. B.; Carter, J. M.; Weinberger, D. R.; Kleinman, J. E. Distribution of Putative D_4 Dopamine Receptors in Postmortem Striatum from Patients with Schizophrenia. *J. Neurosci.* **1995**, *15*, 2186−2191.

(7) Kulagowski, J. J.; Broughton, H. B.; Curtis, N. R.; Mawer, I. M.; Ridgill, M. P.; Baker, R.; Emms, F.; Freedman, S. B.; Marwood, R.; Patel, S.; Patel, S.; Ragan, C. I.; Leeson, P. D. 3-[[4-(4-Chlorophenyl)piperazin-1-yl]methyl]-1H-pyrrolo[2,3-b]pyridine: An Antagonist with High Affinity and Selectivity for the Human Dopamine D_4 Receptor. *J. Med. Chem.* **1996**, *39*, 1941−1942. Rowley, M.; Broughton, H. B.; Collins, I.; Baker, R.; Emms, F.; Marwood, R.; Patel, S.; Patel, S.; Ragan, C. I.; Freedman, S. B.; Leeson, P. D. 5-(4-Chlorophenyl)-4-methyl-3-(1-(2-phenylethyl)piperidin-4-yl)isoxazole: A Potent, Selective Antagonist at Human Cloned Dopamine D_4 Receptors. *J. Med. Chem.* **1996**, *39*, 1943−1945. Boyfield, I.; Brown, T. H.; Coldwell, M. C.; Cooper, D. G.; Hadley, M. S.; Hagan, J. J.; Healy, M. A.; Johns, A.; King, R. J.; Middlemiss, D. N.; Nash, D. J.; Riley, G. J.; Scott, E. E.; Smith, S. A.; Stemp, G. Design and Synthesis of 2-Naphoate Esters as Selective Dopamine D_4 Antagonists. *J. Med. Chem.* **1996**, *39*, 1946−1948. TenBrink, R. E. Bergh, C. L.; Duncan, J. N.; Harris, D. W.; Huff, R. M.; Lahti, R. A.; Lawson, C. F.; Lutzke, B. S.; Martin, I. J.; Rees, S. A.; Schlachter, S. K.; Sih, J. C.; Smith, M. S. (S)-(-)-4-[4-[2-(Isochroman-1-yl)ethyl]-piperazin-1-yl]benzenesulfonamide, a Selective Dopamine D_4 Antagonist. *J. Med. Chem.* **1996**, *39*, 2435−2437.

(8) Thurkauf, A.; Hutchison, A.; Peterson, J.; Cornfield, L.; Meade, R.; Huston, K.; Harris, K.; Ross, P. C.; Gerber, K.; Ramabhadran, T. V. 2-Phenyl-4-(aminomethyl)imidazoles as Potential Antipsychotic Agents. Synthesis and Dopamine D_2 Receptor Binding. *J. Med. Chem.* 1**995**, *38*, 2251−2255.

(9) McAllister, G.; Knowles, M. R.; Ward-Booth, S. M.; Sinclair, H. A.; Patel, S.; Marwood, R.; Emms, F.; Patel, S.; Smith, G. R.; Seabrook, G. R.; Freedman, S. B. Functional Coupling of Human D_2, D_3 and D_4 Dopamine Receptors in HEK293 Cells. *J. Recep. Sig. Trans. Res.* **1995**, *15*, 267−281.

(10) Rigdon, G. C.; Viik, K. Prepulse inhibition as a Screening Test for Potential Antipsychotics. *Drug Develop. Res.* **1991**, *23*, 91−99.

(11) Ljongberg, T.; Ungerstedt, U. Classification of Neuroleptic Drugs According to their Ability to Inhibit Apomorphine-induced locomotion and gnawing: Evidence for Two Different Modes of Action. *Psychopharmacology* **1978**, *56*, 239−247.

(12) Ellenbrock, B. A. Treatment of Schizophrenia: A Clinical and Preclinical Evaluation of Neuroleptic Drugs. *Pharmacol. Ther.* **1993**, *52*, 1−78.

(13) Davis, M. Neurochemical Modulation of Sensory-motor Reactivity: Acoustic and Tactile Startle Reflexes. *Neuroscience Biobehav. Rev.* **1980**, *4*, 241−263.

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