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N-(SUBSTITUTED-PHENYL) PIPERAZINES: ANTAGONISTS WITH HIGH BINDING AND FUNCTIONAL SELECTIVITY FOR DOPAMINE D_4 RECEPTORS

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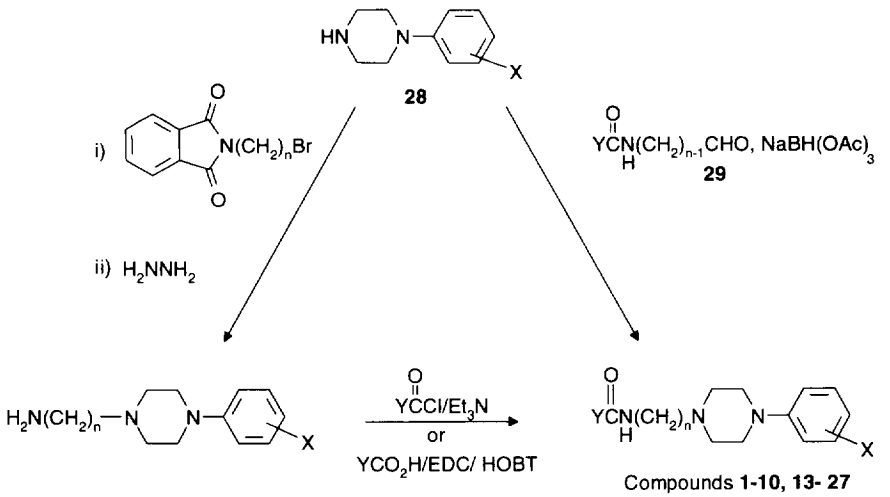
Abstract: A series of N-(substituted-phenyl) piperazine derivatives was prepared as selective antagonists of the dopamine D_4 receptor. Many analogues possessed a binding selectivity of over 100 fold for D_4 over D_2 receptors. In functional studies in the microphysiometer, compound **24** showed a selectivity over dopamine D_2 receptors of greater than 1000 fold. Copyright © 1996 Elsevier Science Ltd

Current drugs used for the treatment of schizophrenia demonstrate poor side-effect profiles causing, in particular, major movement disorders known as extrapyramidal side-effects (EPS).[1] A cornerstone of current neuroleptic therapy involves the use of agents that block (*via* D_2-like receptors) the up-regulation of the dopamine system that has been associated with schizophrenia.[2] In the past few years, advances in the molecular biology of dopamine receptors have shown that these D_2-like receptors may be divided into D_2, D_3 and D_4 subtypes.[3-5] The EPS caused by existing therapy are thought to be due to blockade of D_2 receptors in the striatum. Studies of mRNA distribution indicate that D_4 receptors are preferentially located in cortical and other regions of the brain associated with antipsychotic activity and have a low density in the striatum.[5,6] A selective D_4 antagonist thus has the potential to be an effective antipsychotic agent lacking the EPS of current therapy. Furthermore, it has been reported that D_4 receptor levels are elevated in schizophrenia,[7,8] although the evidence for this is currently under debate.[9] Moreover, it has been speculated that the modest D_4 selectivity[5] of the atypical antipsychotic agent clozapine may contribute to its higher efficacy compared to other neuroleptics and its lower propensity to cause EPS. There is therefore a need for a selective D_4 antagonist to evaluate the role of D_4 receptors in schizophrenia.[10] In this report, we describe the identification of a series of aryl piperazine derivatives with high selectivity for the D_4[11] over the D_2 receptor.

The 2-methoxyphenylpiperazine derivative **1** was identified as a non-selective high affinity lead (Table 1) following a programme of rapid parallel synthesis based on known dopaminergic structural motifs. An SAR

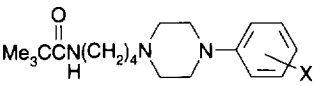
analysis around this structure was then carried out. Compounds were prepared by reaction of a range of piperazines **28** with either a haloalkyl phthalimide, followed by deprotection and acylation or by reductive amination of an amidoaldehyde **29** (Scheme 1).

Scheme 1



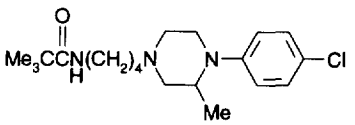
Variation of the aromatic substituent **X** in this structure proved highly worthwhile (Table 1).

Table 1[12]



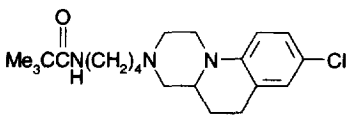
Compound	X	D_4 (pKi)	D_2 (pKi)	Selectivity
1	2-OMe	7.9	7.7	2
2	2-Cl	7.1	7.2	1
3	3-Cl	7.0	6.6	3
4	4-Cl	7.9	5.6	200
5	4-OMe	6.5	<5.0	>30
6	H	6.5	6.1	3
7	4-F	6.9	5.7	15
8	4-Br	7.5	5.8	50
9	4-CF_3	7.3	5.4	80
10	2-OMe, 4-Cl	7.8	6.8	10

Whilst replacement of the 2-methoxy substituent with 2- or 3-chloro did not prove beneficial (cf **1** with **2,3**), replacement by a 4-chloro group gave compound **4**, which showed a greater than 100 fold increase in D_4 selectivity due to a marked reduction in affinity for the D_2 receptor compared to **1**. The related 4-methoxy derivative **5**, although selective, was considerably less potent at D_4 receptors. Indeed, 4-chloro proved the optimum substituent at this position (see compounds **6** - **9**). The 20-fold lower selectivity of the 2-methoxy, 4-chloro derivative **10** compared to the 4-chloro derivative **4** indicates an important role for the 2-methoxy group in increasing affinity for the D_2 receptor. This role is more likely to be electronic rather than steric as compound **11** in which the aromatic ring of **4** is held out of the plane of the piperazine ring, showed low D_2 affinity whilst compound **12**, in which the aromatic ring of **4** is held in the plane of the piperazine ring, showed higher D_2 affinity.



11

pKi D_4 = 7.2, pKi D_2 = 5.8

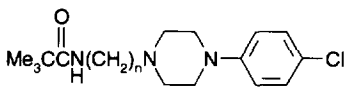


12

pKi D_4 = 7.2, pKi D_2 = 6.4

We next turned our attention to the length of the carbon chain linking the piperazine and amide moieties (Table 2). A two or four methylene linker (**13** and **4**) proved to be considerably better than either three or five (**14** and **15**).

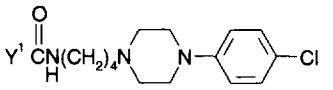
Table 2



Compound	n	D_4 (pKi)	D_2 (pKi)	Selectivity
13	2	7.6	5.6	100
14	3	6.7	<5.0	>50
4	4	7.9	5.6	200
15	5	6.9	6.4	3

As a result of the similar high binding affinities and selectivities of compounds **13** and **4** (perhaps due to folding of the linking chain), optimisation of the amide moiety was undertaken in both the two and four methylene series. Within the latter series, it quickly became apparent that a hindered tertiary aliphatic centre was a strong requirement for high D_4 receptor binding affinity (cf **4** with **16-18**, Table 3). Increases to the size of the pivaloyl group in **4** showed no further advantage to binding affinity (compounds **19-22**).

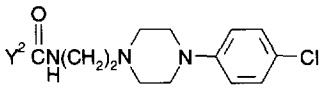
Table 3



Compound	Y^1	D_4 (pKi)	D_2 (pKi)	Selectivity
16	Me	5.9	5.8	1
17	Me_2CH	6.9	5.7	16
4	Me_3C	7.9	5.6	200
18	Phenyl	6.8	6.4	3
19	$EtMe_2C$	7.8	6.0	63
20	$nPrMe_2C$	7.9	5.8	125
21	1-(1-Me,cC_6H_{11})	8.0	6.0	100
22	1-Adamantyl	7.7	6.2	30

Broadly similar results were obtained in the two methylene chain series. However, perhaps as a result of lowered steric constraint, extending the pivaloyl group proved more advantageous (Table 4).

Table 4



Compound	Y^2	D_4 (pKi)	D_2 (pKi)	Selectivity
13	Me_3C	7.6	5.6	100
23	$EtMe_2C$	8.1	5.6	320
24	$nPrMe_2C$	8.1	5.6	320
25	$nBuMe_2C$	7.5	5.8	50
26	1-(1-Me,cC_6H_{11})	8.1	6.1	100
27	1-Adamantyl	8.3	6.4	80

Indeed, both the ethyl dimethyl and propyl dimethyl derivatives (compounds **23** and **24** respectively) not only showed high D_4 receptor binding affinities but also very high selectivities over D_2 receptors.[13] Further extension to the propyl group of **24** unfortunately gave a compound **25**, of reduced D_4 affinity and selectivity. Cyclisation of this propyl moiety on the other hand, gave compounds (**26** and **27**) which displayed higher D_2 affinity and thereby reduced selectivity. These results emphasise that subtle differences in shape in this part of the structure can modify binding to D_2 and D_4 receptors.

Compound **24** not only showed a very exciting binding profile, but proved in functional studies[14] to be a very potent antagonist of D_4 receptors (pKb D_4= 9.2 ± 0.2, n = 4) with a *1250 fold selectivity* over D_2 receptors (pKb D_2= 6.1 ± 0.4, n = 3).

In conclusion, structural modification of the non selective 2-methoxyphenyl piperazine ligand **1**, has given a number of highly potent D_4 ligands with greater than 100 fold selectivity over D_2 receptors. Compounds **23** and **24** are more D_4/D_2 selective than any structures currently described in the literature.[10] Furthermore, compound **24** has been shown to be a highly potent functional antagonist of the D_4 receptor and as such may prove a useful tool in the evaluation of the role of D_4 receptors in schizophrenia.

Acknowledgements: We thank the members of the Analytical Sciences department for spectral and analytical data.

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10. The only compound currently reported to show high D_4 selectivity is NGD 94-1, but no structure has been revealed - Tallman, J.F. American College of Neuropsychopharmacology 33rd Annual Meeting, San Juan, Puerto Rico, December 12-16, 1994.
11. Compounds were screened on cloned human D_2 (long) and $D_{4.4}$ receptors using ^{125}I-iodosulpiride and ^{3}H-nemonapride, respectively, as the radioligands and results are reported as pKi values. Cloned human dopamine $D_{4.4}$ receptors were expressed in HEK 293 cells; see McHale, M.; Coldwell, M.C.; Herrity, N.; Boyfield, I.; Winn, F.M.; Ball, S.; Cook, T.; Robinson, J.H.; Gloger, I.S. *FEBS Letters*, 1994, **345**, 147-150. Cloned human dopamine D_2 (long) receptors expressed in CHO cells were obtained from the Garvan Institute (Melbourne). The quoted figures are the means of at least two determinations at each receptor.
12. All compounds of Tables 1 - 4 gave satisfactory spectroscopic /analytical data. See also reference 15.
13. High selectivities for D_4 over D_3 receptors were also observed. For example compound **23** (pKi D_3 = 5.8) showed a 200 fold selectivity for D_4 over D_3 receptors. Similarly compound **24** (pKi D_3 = 5.6) showed a 320 fold selectivity for D_4 over D_3 receptors.
14. Functional studies using cloned human D_2 (long) and $D_{4.4}$ receptors were carried out *in vitro* using a Cytosensor Microphysiometer (Molecular Devices). Cells were seeded into 12mm Transwell inserts at 300000 cells/cup in culture medium containing foetal calf serum (FCS). The cells were incubated for 6h at 37 ^{o}C in 5% CO_2, before changing to medium without FCS. After a further 16-18h, cups were loaded into the sensor chambers of the microphysiometer and the chambers perfused with running medium (bicarbonate-free Dulbecco's modified Eagles medium containing 2 mM glutamine and 44 mM NaCl). For agonist experiments, cells were exposed to increasing concentrations of agonist at half hour intervals. For antagonist experiments, cells were exposed five times (at half hour intervals) to a single concentration of quinpirole (30 nM) before addition of the first antagonist concentration. After a 30 min interval, cells were again stimulated with quinpirole (in the continued presence of the antagonist), before the second (higher) antagonist concentration was applied. In all, responses in the presence of five increasing concentrations of antagonist were determined. Peak acidification rate to each agonist concentration was determined and concentration-response curves fitted using RoboFit (Tilford N.S.; Bowen, W.P.; Baxter, G.S. RoboFit: A Versatile Macro-Driven Template for Curve Fitting, Analysis and Presentation in Microsoft Excel. *Br. J. Pharmacol.* 1995, **115**, 160P).
15. Data for compound **24** (.2HCl) NMR (d_6-DMSO) (400MHz) δ 0.84 (3H, t, J=7Hz), 1.08 (6H, s), 1.08 - 1.20 (2H, m), 1.41 - 1.45 (2H, m), 3.10 - 3.28 (6H, m), 3.50 - 3.59 (4H, m), 3.80 (2H, m), 7.02 (2H, d, J = 9Hz), 7.28 (3H, overlapping br s and d, J=9Hz), 7.95 (1H, m), 11.48 (1H, br. s). Mass spectrum (m/z) $[M+H]^+$ = 352 (100%). Analysis: Found: C, 53.45; H, 7.2; N, 9.8%. $C_{19}H_{30}ClN_3O$. 2HCl requires C, 53.7; H, 7.6; N, 9.9%. Melting Point: 162-164°C.

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