Substituted [(4-Phenylpiperazinyl)-methyl]benzamides: Selective Dopamine D_4 Agonists

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The drugs currently available for treating schizophrenia are limited by disabling side effects such as extrapyramidal syndrome (EPS) and tardive dyskinesia. The blockade of dopamine (DA) D_2-like receptors, in limbic as well as striatal regions of the brain, is thought to be responsible for both their efficacy and neurological side effects.[1] Recent advances in molecular biology have shown that the DA D_2 family of receptors is comprised of D_2, D_3, and D_4 subtypes.[2] The D_3 and D_4 receptors are much less abundant in the brain than are D_2 receptors and are much more localized in the limbic regions that are thought to be responsible for cognitive and emotional functions.[3] The clinical potency of most antipsychotic drugs shows a high correlation with the DA D_2 receptor affinity.[4] An exception is clozapine, which has an almost 10-fold higher clinical potency than would be expected from its DA D_2 receptor affinity. This, and its low propensity to induce EPS, may be explained by clozapine's reported 2−10-fold higher affinity for the DA D_4 receptor.[5] However, clozapine's ability to bind with high affinity to several additional nondopaminergic receptors makes it difficult to identify the contribution of D_4 receptor function to clinical efficacy. Therefore, identification of a D_4 receptor subtype selective agonist or antagonist is necessary to determine the biological significance of this receptor.

In our search for a DA D_4 selective antagonist, we discovered a series of *N*-methylpiperazinyl benzamides (Figure 1) that consistently showed partial agonist activity. Optimization of the structure−activity relationship (SAR) for D_4 receptor affinity, selectivity, and agonist activity resulted in the identification of several compounds that may be useful tools for pharmacological testing.

Several analogs with various substituents on the phenyl rings of the arylpiperazine and benzamide were synthesized as shown in Scheme 1. The synthesis of many of these analogs started with oxidative decarboxylation of commercially available hippuric acids. The hippuric acids that were not commercially available were readily prepared from substituted acid chlorides and glycine. The acetates, resulting from oxidative decarboxylation,[6] were displaced with arylpiperazines[7] giving *N*-methylpiperazinyl benzamides in good overall yield.

The binding affinities for the DA D_4, D_3, and D_2 receptors[8] and the agonist activity[9] at the D_4 receptor are shown in Table 1. Agonist activation of dopamine receptors stimulates mitogenesis in D_4-transfected CHO pro-5 cells. This response was estimated by measuring the cellular uptake of [^{3}H]thymidine and comparing the response to the full agonist quinpirole (defined as 100%).

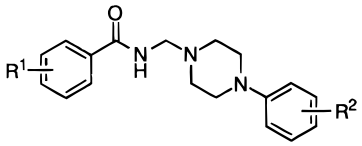
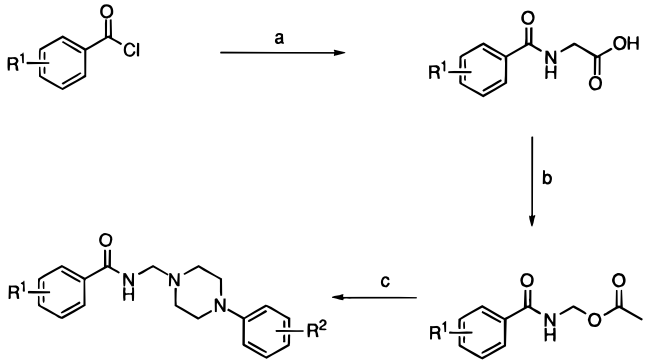


Figure 1.

Scheme 1[a]



[a] (a) Glycine, 2 N NaOH, CH_2Cl_2, 25 °C, 4 h, 60−90%; (b) $Pb(OAc)_4$, $Cu(OAc)_2$, C_6H_6, reflux, 4 h, 50−90%; (c) arylpiperazine, Et_3N, CH_3CN, 25 °C, 4 h, 75−98%.

Table 1. Effect of Substitution on Binding Affinity and hD_4 Receptor Mitogenesis

			binding affinity[a] (K_i, nM)[b]			[^{3}H]thymidine uptake[c]	
compd	R^1	R^2	hD_4	hD_3	hD_2	% max effect[d]	EC_{50}, nM[e]
1	H	2-OMe	22	2470	1880	60	31
2	H	2-Cl	32	177	1500	100	106
3	H	2-CN	27	>3000	4180	92	24
4	3-Me	2-OMe	6.7	2480	835	50	13
5	3-Me	2-Cl	27	464	1330	50	16
6	3-Me	2-CN	8.7	2810	3740	80	17
7	4-Me	2-OMe	7.7	174	291	49	3.4
8	4-Me	2-Cl	12	49	466	45	218
9	4-Me	2-CN	6.5	377	1150	54	1.2
10	3-Cl	2-OMe	15	1450	451	100	15
11	3-Cl	2-Cl	41	364	2420		
12	3-Cl	2-CN	34	2270	897	98	5.3
13	4-Cl	2-OMe	14	268	349	41	3.5
14	4-Cl	2-Cl	5.3	66	495	47	8.4
15	4-Cl	2-CN	5.5	600	929	≥70	≥3.9

[a] [^{3}H]Spiperone as ligand; human D_4, D_3, and D_2 receptors expressed in CHO K1 transfected cells. [b] K_i values were obtained from four to six concentrations, run in triplicate, by a nonlinear regression analysis. [c] Stimulation of [^{3}H]thymidine uptake on CHO pro-5 cells expressing human D_4 receptors. [d] Percent maximal effect compared to that of the full DA agonist quinpirole (100%). [e] EC_{50} values (95% confidence intervals) were generated from 10 concentrations, $n = 4$.

When the benzamide phenyl was unsubstituted (R^1 = H) it was found that only ortho substitution on the phenylpiperazine (R^2) gave compounds with at least moderate D_4 affinity. Of the ortho substituents investigated methoxy (**1**), chloro (**2**), and cyano (**3**) resulted in compounds with the best D_4 receptor affinity, selectivity, and agonist activity. To further optimize these properties various substituents were introduced on the benzamide phenyl (R^1). Of the substituents examined at R^1, *m*- and *p*-chloro and *m*- and *p*-methyl afforded the most significant improvement.

These functional group manipulations resulted in compound **6** (R^1 = 3-Me, R^2 = 2-CN), an agonist with

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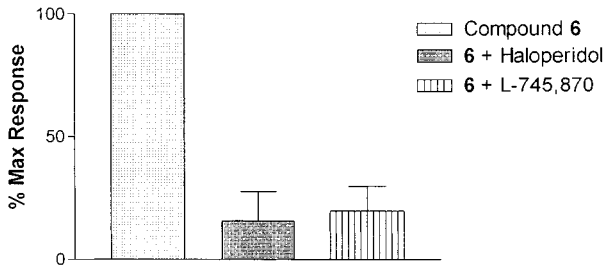

Figure 2. Compound **6** (100 nM) increases [^{3}H]thymidine uptake in CHO pro-5 cells expressing the human dopamine D_4 receptor. This effect is antagonized by 1000 nM haloperidol and L-745,870.

8.7 nM affinity for the DA D_4 receptor with >300-fold selectivity over D_3 and >400-fold selectivity over D_2. The binding affinities (K_i) for compound **6** for the α-1 adrenergic, α-2 adrenergic, 5-HT_{1A}, and 5-HT_{2A} receptors are 168, 177, 385, and 4010 nM, respectively.[10] We would therefore conclude that the activity of compound **6** would not be exerted through these receptors. Figure 2 shows that the agonist effect was blocked by the nonselective DA antagonist haloperidol (16 ± 12%) and the DA D_4 selective antagonist L-745,870[11] (20 ± 10%).

It has been demonstrated that N-Mannich bases of amides and other compounds containing acidic NH groups may be useful prodrugs. These N-Mannich bases were shown to decompose very rapidly in aqueous solution with formation of formaldehyde, amine, and parent compound (amide).[12] Since compounds **1**−**15** closely resemble these prodrugs, several were examined for their aqueous stability and were found to be stable in water for up to 24 h. They were also found to be stable in dilute acid (0.1 N HCl) for several hours but began to degrade in 3 N HCl within 10 min. This acid lability may exclude their use for orally administered *in vivo* experiments.

In conclusion, optimization of R^1 and R^2 functional groups resulted in **6**, a D_4 agonist with >400-fold selectivity over the D_2 receptor and >300-fold selectivity versus D_3. To our knowledge compound **6** is the first D_4 selective agonist reported in the literature. This compound may provide a useful tool in determining the contribution of D_4 receptors to schizophrenia.

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Supporting Information Available: Experimental data for compounds in this paper (4 pages). Ordering information is given on any current masthead page.

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(10) The reference agents for the α-1 adrenergic, α-2 adrenergic, 5-HT_{1A}, and 5-HT_{2A} assays had K_i values of 0.18 nm (prazosin), 1 nM (idazoxan), 0.65 nM (8-OH-DPAT), and 3.39 nM (ketanserine), respectively.

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