Analogues of the dopamine D2 receptor antagonist L741,626: Binding, function, and SAR

Peter Grundt,[a] Sarah Little Jane Husband,[a] Robert R. Luedtke,[b] Michelle Taylor[b] and Amy Hauck Newman[a],*

[a]*Medicinal Chemistry, National Institute on Drug Abuse—Intramural Research Program, National Institutes of Health, 5500 Nathan Shock Drive, Baltimore, MD 21224, USA*
[b]*Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107, USA*

Abstract—A series of analogues of the dopamine D2 receptor antagonist L741,626 were synthesized and evaluated for binding and function at D2 family receptor subtypes. Several analogues showed comparable binding profiles to the parent ligand, however, in general, chemical modification served to reduce D2 binding affinity and selectivity.
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The neurotransmitter dopamine has been associated with fine movement coordination, cognition, emotion, affect, memory, and the regulation of prolactin secretion by the pituitary reward system.[1] Alterations in dopaminergic function are not only involved in the pathogenesis of Parkinson's disease[2] and schizophrenia,[3] but also occur as a consequence of acute and chronic abuse of pyschostimulants.[4] Therefore, the D1-like (D1 and D5) and the D2-like (D2, D3, and D4) dopamine receptor families have been targets for the development of treatment medications for these disorders.[5–8]

The majority of antipsychotic medications are nonselective dopamine D2 receptor antagonists that frequently produce undesirable extrapyramidal side effects upon chronic exposure. As such, to date the discovery of highly selective dopamine D2 antagonists has been elusive in part because the therapeutic value of such agents has been perceived as minimal due to the association of this receptor exclusively with the unwanted side effects of nonselective D2 receptor antagonists. Furthermore, the high degree of amino acid homology within the binding sites of the D2-like receptors provides a formidable challenge to discovering highly selective and potent D2

(or D3) antagonists.[7,9] Nevertheless, the discovery of D2 receptor selective antagonists and partial agonists would provide important pharmacological tools to determine the role of the D2-like receptor subtypes in (1) the mechanism of action of antipsychotic agents, (2) the etiology of drug-induced extrapyramidal side effects, and (3) the contribution of the D2 and D3 receptor subtypes in the CNS reward system. In addition, such D2 receptor-selective compounds would also be a starting point for the development of radioligands.

A decade ago, a D2-selective antagonist, L741,626 (**1**) (Fig. 1) was reported amongst a series of D4-selective agents to bind with ~40-fold higher affinity to D2 over D3 receptors and is currently being used as a D2-selective antagonist in animal models of drug abuse.[10] During the course of our investigation, attempts to define the D2 receptor pharmacophore have led to the identification of D2-related SAR and several novel analogues
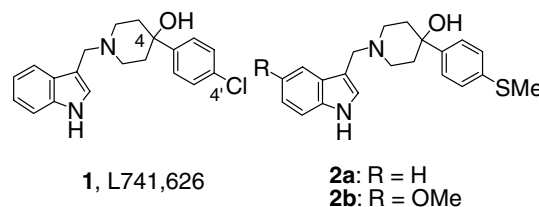
* Corresponding author. Tel.: +1 410 550 6568; fax: +1 410 550 6855; e-mail: anewman@intra.nida.nih.gov

Figure 1. Structure of L741,626 (**1**)[10] and compounds **2a**[11] and **2b**.[11]

(e.g., **2a** and **2b**) of L741,626 that show D2 selectivity over D3 and D4 receptors have been reported.[11,12] In one report, **2b** demonstrated a >100-fold D3/D2 selectivity that appeared to be related to the addition of a 5-OCH3 onto the indolyl moiety of **2a**.[11] This study and many others describing D3-selective ligands demonstrate the importance and potential differences in pendant aryl ring substitution that may provide separation between D2 and D3 receptor recognition. Thus, our approach to further define the D2 pharmacophore and design D2-selective and bioavailable ligands was to (1) examine the effects of various substituents on the pendant piperidinol-phenyl ring, (2) retain the sp³ piperidine C-4, as determined by others to retain D2 affinity but to investigate substitutions of the OH that are either isosteric or would be prone to elimination in vivo, (3) examine heteroatom replacements in and substitutions on the indole ring, and (4) combine some of these features for comparison with compounds recently reported.
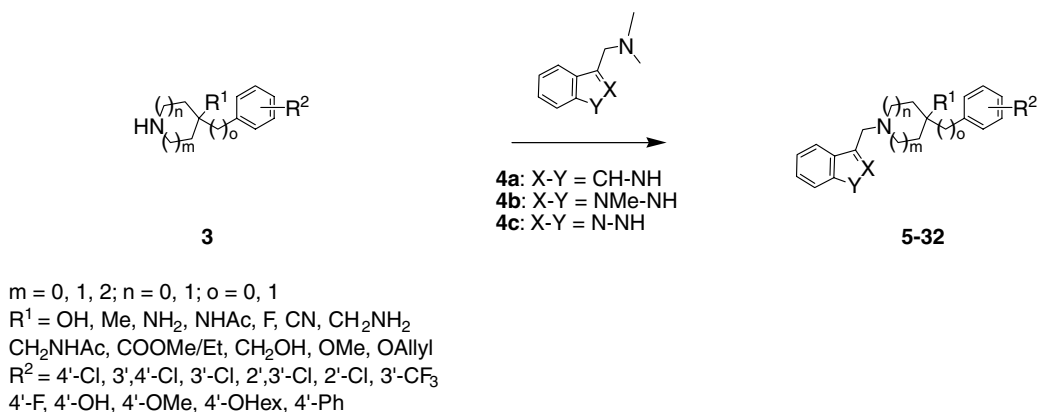
A general synthetic strategy for analogues **5–32** of L741,626 is depicted in Scheme 1. Phenyl-piperidines **3** were reacted with gramine (**4a**, X–Y = CH–NH), 2-methyl-gramine (**4b**, X–Y = CMe–NH), or (1*H*-indazol-3-ylmethyl)-dimethyl-amine (**4c**, X–Y = N–NH)[13] in refluxing pyridine overnight to give products **5–32**. 3-Chloromethyl-benzofuran[14] was converted to analogues **33–35** in the presence of sodium bicarbonate in refluxing acetonitrile. Regioselective alkylation of the indole nitrogen (**36** and **37**) was achieved by reaction of the dianion of L741,626 (generated by treatment with 2 equiv sodium hydride) with the methyl iodide or hexyl bromide in THF. The 2-indolyl analogue **38** of L741,626 was prepared from the corresponding amide of 2-indolecarboxylic acid by reduction with lithium aluminum hydride.

Thirty-four novel ligands were evaluated in competition binding assays in HEK 293 cells transfected with D2L, D3 or D4 human dopamine receptors[15] using [¹²⁵I]IABN[16] as the radiolabeled ligand. In addition, intrinsic activities for selected compounds were determined in a functional assay using stimulation (agonist) or inhibition of quinpirole stimulation (antagonist) of mitogenesis in human dopamine D2 or D3 receptors

transfected into Chinese hamster ovary (CHO) cells (Table 1). All of the compounds evaluated were antagonists in these functional tests. The parent molecule L741,626 was prepared by literature methods[10] (K_i (D2) = 11.2 nM) and displayed a D3/D2 and D4/D2 selectivity ratio of 15- and 136-fold, respectively. In the functional assay L741,626 was a potent antagonist (EC_{50} (D2) = 4.46 nM) with D2 selectivity (EC_{50} (D3) = 90.4 nM).

In the aryl ring-substituted analogues (**5–14**) the chloro-substituent and its position on the phenyl-piperidinol moiety was pivotal for high affinity binding at the dopamine D2 receptor and for selectivities over the D3 and D4 receptors. Only the 3′,4′-dichloro-substituted derivative **8** demonstrated the same binding and selectivity profile as the parent compound L741,626, however in the functional assay **8** (EC_{50} (D2) = 15.3 nM) was 3-fold less potent at D2 than the parent molecule. Variation of the substituent of the phenyl-piperidinol moiety (**9–14**) resulted in decreased dopamine D2 receptor binding affinities. A 4′-OH group (**11**, K_i (D2) = 5360 nM) was particularly poorly tolerated, whereas the 4′-OCH3 analogue **12** (K_i (D2) = 177 nM) regained some affinity at the dopamine D2 receptor. Interestingly, the 4′-SCH3 derivative **2a** had a 7-fold higher affinity at the dopamine D2 receptor than the 4′-OCH3 analogue **12**, indicating that a more polarizable third row element may be required to achieve high dopamine D2 receptor binding affinity.

Retention of the C-4 piperidine sp³ carbon was previously reported to be preferred by the D2 receptor[12] and replacement of the 4-piperidinol function with other groups was explored (**15–29**). In general, none of these analogues showed higher affinity for D2 than the parent compound. Introduction of a methylene spacer between the C-4 and OH (**27**) or other functional groups (**25** and **26**) resulted in very low D2 affinities (<2 μM). Interestingly, the derivative **27** showed higher affinity for the dopamine D3 than D2 receptor, D2/D3 = 22 and D4/D3 = 18. Despite lower D2 affinities than L741,626, both the 4-carboxylic methyl ester (**23**) and its ethyl analogue (**24**) demonstrated similar D3/D2 selectivity. Isosteric replacement of the 4-OH group with a 4-methyl group (**18**), 4-amino group (**19**) or a 4-fluoro group

4a: X-Y = CH-NH
4b: X-Y = NMe-NH
4c: X-Y = N-NH

m = 0, 1, 2; n = 0, 1; o = 0, 1
R¹ = OH, Me, NH2, NHAc, F, CN, CH2NH2
CH2NHAc, COOMe/Et, CH2OH, OMe, OAllyl
R² = 4'-Cl, 3',4'-Cl, 3'-Cl, 2',3'-Cl, 2'-Cl, 3'-CF3
4'-F, 4'-OH, 4'-OMe, 4'-OHex, 4'-Ph

Scheme 1. General synthesis of the derivatives **5–32**.[18]
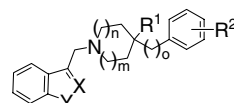
Table 1. Binding and functional data for D2 compounds

Compound	m	n	o	X–Y	R^1	R^2	K_i (nM) ± SEM			D3/D2	D4/D2	EC$_{50}$ (nM) ± SEM	
							D2[a]	D3[a]	D4[a]			D2 function[b]	D3 function[b]
5	1	1	0	CH–NH	OH	2′-Cl	382 ± 33	1860 ± 340	2820 ± 64	5	7	586 ± 210	ND
6	1	1	0	CH–NH	OH	2′,3′-Cl	26.1 ± 2.0	273 ± 42	1550 ± 190	10	59	34.3 ± 6.8	232 ± 7.7
7	1	1	0	CH–NH	OH	3′-Cl	129 ± 22	450 ± 80	3540 ± 660	3	27	83.4 ± 10	ND
8	1	1	0	CH–NH	OH	3′,4′-Cl	6.9 ± 1.0	111 ± 17	1100 ± 210	16	159	15.3 ± 0.8	41.5 ± 9.1
9	1	1	0	CH–NH	OH	3′-CF$_3$	106 ± 15	570 ± 44	3750 ± 140	5	35	69.1 ± 23	ND
10	1	1	0	CH–NH	OH	4′-F	127 ± 7.2	490 ± 74	6090 ± 580	4	48	ND	ND
11	1	1	0	CH–NH	OH	4′-OH	5360 ± 1100	16800 ± 6500	1360 ± 270	3	0.3	ND	ND
12	1	1	0	CH–NH	OH	4′-OMe	177 ± 61	2130 ± 300	679 ± 60	12	4	ND	ND
13	1	1	0	CH–NH	OH	4′-OHex	159 ± 60	1950 ± 350	1490 ± 150	12	9	216 ± 1.7	ND
14	1	1	0	CH–NH	OH	4′-Ph	98.4 ± 19	467 ± 38	129 ± 26	5	1	ND	ND
15	2	1	0	CH–NH	OH	4′-Cl	201 ± 21	2100 ± 490	3870 ± 770	10	19	260 ± 91	ND
16	1	1	1	CH–NH	OH	4′-Cl	2890 ± 560	5480 ± 2900	3930 ± 1200	2	1	ND	ND
17	2	0	0	CH–NH	OH	4′-Cl	4265 ± 2280	4270 ± 400	2440 ± 1400	1	1	ND	ND
18	1	1	0	CH–NH	Me	4′-Cl	992 ± 192	2100 ± 150	2750 ± 140	2	3	314 ± 4.2	347 ± 7.4
19	1	1	0	CH–NH	NH$_2$	4′-Cl	184 ± 40	382 ± 55	2293 ± 545	2	12	ND	ND
20	1	1	0	CH–NH	NHAc	4′-Cl	83.1 ± 47	193 ± 80	348 ± 39	2	4	24.8 ± 7.6	ND
21	1	1	0	CH–NH	F	4′-Cl	120 ± 34	211 ± 67	365 ± 63	2	3	ND	ND
22	1	1	0	CH–NH	CN	4′-Cl	199 ± 24	684 ± 180	2270 ± 350	3	11	225 ± 80	ND
23	1	1	0	CH–NH	COOMe	4′-Cl	60.0 ± 9.5	544 ± 128	3930 ± 2500	9	66	76.7 ± 21	ND
24	1	1	0	CH–NH	COOEt	4′-Cl	58.8 ± 20	724 ± 120	3520 ± 1320	12	60	ND	ND
25	1	1	0	CH–NH	CH$_2$NH$_2$	4′-Cl	>10000	>5000	>20000			ND	ND
26	1	1	0	CH–NH	CH$_2$NHAc	4′-Cl	2000 ± 640	12500 ± 1300	4450 ± 750	6	2	ND	ND
27	1	1	0	CH–NH	CH$_2$OH	4′-Cl	2370 ± 560	106 ± 19	1940 ± 250	0.04	1	ND	ND
28	1	1	0	CH–NH	OMe	4′-Cl	68.1 ± 22	262 ± 73	34.1 ± 6.0	4	1	34.7 ± 2.9	248 ± 84
29	1	1	0	CH–NH	OAllyl	4′-Cl	165 ± 91	718 ± 35	1190 ± 360	4	7	ND	ND
30	1	1	0	CMe–NH	OH	4′-Cl	15.4 ± 14	44.1 ± 11	2990 ± 180	3	194	2.13 ± 0.3	20.2 ± 4.2
31	1	1	0	N–NH	OH	3′,4′-Cl	438 ± 46	156 ± 22	677 ± 148	0.4	2	ND	ND
32	1	1	0	N–NH	OH	4′-Cl	548 ± 80	440 ± 88	2146 ± 616	1	4	ND	ND
33	1	1	0	CH–O	OH	3′,4′-Cl	21.0 ± 4	28.0 ± 6	167 ± 35	1	8	ND	ND
34	1	1	0	CH–O	OH	4′-Cl	21.0 ± 4	53.0 ± 12.5	404 ± 24	3	19	ND	ND
35	1	1	0	CH–O	OH	4′-SMe	54.0 ± 13	139 ± 7.9	153 ± 35	3	3	ND	ND
36	1	1	0	CH–NMe	OH	4′-Cl	29.6 ± 6.2	238 ± 100	5140 ± 3330	8	174	20.0 ± 4.5	98.6 ± 29
37	1	1	0	CH–NHex	OH	4′-Cl	188 ± 27	488 ± 280	8890 ± 5600	3	47	166.0 ± 35	661 ± 56
38	1	1	0	n/a[c]	OH	4′-Cl	>10000	ND	>20000			ND	ND
2a[11]	1	1	0	CH–NH	OH	4′-SMe	23.9 ± 5.5	638 ± 159	319 ± 58	27	13	ND	ND
2b[11]	1	1	0	CH–NH[d]	OH	4′-SMe	5.5 ± 0.1	580 ± 92	567 ± 140	105	103	ND	ND
1,L741,626[10]	1	1	0	CH–NH	OH	4′-Cl	11.2 ± 0.8	163 ± 32	1520 ± 280	15	136	4.46 ± 0.9	90.4 ± 15

[a] Inhibition of binding assay in HEK 293 cells transfected with either hD2$_L$, hD3, or hD4 dopamine receptors, radioligand ^{125}I-IABN.[15,16]

[b] Functional assays using inhibition of quinpirole stimulation in hD2 and hD3 receptors transfected into CHO cells. These data were obtained through the service of CTDP, Division of Treatment Research and Development, NIDA, using a Contract (N01DA-1-8816) protocol. ND, not determined.

[c] 1-((1H-indol-2-yl)methyl)-4-(4′-chlorophenyl)piperidin-4-ol.

[d] 1-((5-Methoxy-1H-indol-3-yl)methyl)-4-(4′-(methylthio)phenyl)piperidin-4-ol.

(**21**) resulted in a loss of affinity at the dopamine D2 receptor up to 89-fold. All of these derivatives were non-selective over the dopamine D3 receptor, only the 4-amino derivative **19** was to some extent selective over D4 (D4/D2 = 12). The 4-amino acetyl analogue **20** (K_i (D2) = 83.1 nM) displayed a slightly higher binding affinity at the dopamine D2 receptor than the 4-amino derivative **19** (K_i (D2) = 184 nM) but the D4/D2 selectivity was 3-fold lower.

Haloperidol and its homopiperidine analogue have been reported to be equipotent at the dopamine D2 receptor,[17] however the corresponding azepine analogue of L741,626, **15**, showed an 18-fold lower binding affinity at the dopamine D2 receptor and reduced D4/D2 selectivity (7-fold). In addition, the replacement of the 4′-chloro-phenyl ring of L741,626 by a 4′-chloro-benzyl group (**16**) was not tolerated (K_i (D2) = 2890 nM). The 3-phenyl-piperidol analogue **17** was inactive at dopamine D2-like receptor subtypes.

Substitution or heteroatom replacement in the indole moiety was also explored (**30–38**). A methyl group in the 2-position of the indole moiety (**30**) was well-tolerated at the dopamine D2 receptor (K_i (D2) = 15.4 nM, EC_{50} (D2) = 2.13 nM), however this modification resulted in a 5-fold reduction of D3/D2 selectivity, while D4/D2 selectivity was unaffected. In contrast, moving the piperidinol moiety to the 2-position of the indole was not tolerated. Binding affinities of >10,000 nM were determined at both D2 and D4 dopamine receptor subtypes for the 1*H*-indol-2-yl analogue **38**, and no further testing was performed. Both the indazole derivatives **31** and **32** had low-binding affinities at D2 receptors (K_i (D2) = 438 and 548 nM, respectively). Notably, the 3′,4′-dichloro analogue **31** was nonselective over the dopamine D2-like subtypes. The benzofurans **33–35** showed moderate binding affinity at the dopamine D2 receptor but the D3/D2 and D4/D2 selectivity was low (< 20-fold) even when substitution on the piperidinol phenyl ring was made optimal for D2 (**33** and **35** compared to **8** and **2a,b**, respectively).

In summary, we have used the D2-selective antagonist L741,626 as a lead compound to further elucidate D2 receptor SAR to identify novel D2-selective agents that can be used as research tools to more precisely define the physiological role of the dopamine D2 receptor in psychiatric and neurodegenerative disorders. However, the high degree of amino acid homology between the D2 and D3 dopamine receptor subtypes has thus far precluded our discovery of compounds that bind with a high degree of selectivity at the D2 receptor. Nevertheless, clues to D2/D3 separability continue to be revealed and some of these compounds may still prove useful for in vivo investigation as they have comparable profiles to L741,626.

Acknowledgments

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18. *Note*: The 4-substituted piperidines required for compounds **18–29** were synthesized in adaptation of literature methods: (a) Harriman, G. C. B.; Shao, J. X.; Luly, J. R. *Tetrahedron Lett.* **2000**, *41*, 8853; (b) Bernstein, P.; Cacciola, J.; Dedinas, R.; Shen, L.; Warwick, P. WO 2004022539, *Chem. Abstr.* **2004**, *140*, 270744; (c) Ghosh, S.; Patane, M. A.; Carson, K. G.; Chi, I.-C. S.; Ye, Q.; Elder, A. M.; Jenkins, T. J. WO 2003037271. *Chem. Abstr.* **2003**, *138*, 368921; (d) Luly, J. R.; Nakasato, Y.; Ohshima, E.; Harriman, G. C. B.; Carson, K. G.; Ghosh, S.; Elder, A. M.; Mattia, K. M. US 2005070549. *Chem. Abstr.* **2005**, *142*, 355256. All final compounds were characterized by NMR. Microanalysis data were determined to ± 0.4 % of the calculated values. Representative spectral data **30**: ¹H NMR (400 MHz, CDCl₃): δ 1.67 (d, *J* 12.1, 2H), 2.06 (td, *J* 12.9, 4.1, 2H), 2.35 (s, 1H), 2.39 (s, 3H), 2.45 (td, *J* 10.6, 1.8, 2H), 2.85 (d, *J* 11.3, 2H), 3.71 (s, 2H), 7.07–7.19 (m, 2H), 7.24–7.30 (m, 3H), 7.40 (d, *J* 8.6, 2H), 7.67 (d, *J* 6.6, 1H), 7.90 (s, 1H). Compound **32**: ¹H NMR (400 MHz, CD₃OD): δ 1.66 (d, *J* 12.1, 2H), 2.05 (m, 2H), 2.66 (m,

2H), 2.85 (d, *J* 11.3, 2H), 3.97 (s, 2H), 7.12 (m, 1H), 7.24 (d, *J* 8.6, 2H), 7.34 (m, 1H), 7.41 (d, *J* 8.6, 2H), 7.47 (d, *J* 8.2, 1H), 7.86 (d, *J* 7.6, 1H). Compound **34**: ^{1}H NMR (400 MHz, CDCl$_3$): δ 1.68 (d, *J* 14.1, 2H), 1.82 (s br, 1H), 2.07 (td, *J* 12.9, 4.5, 2H), 2.48 (td, *J* 12.1, 2H), 2.83 (d, 11.0, 2H), 3.69 (d, 0.8, 2H), 7.22–7.31 (m, 4H), 7.41 (d, *J*

9.0, 2H), 7.55 (s, 1H). Compound **36**: ^{1}H NMR (400 MHz, CDCl$_3$): δ 1.71 (d, *J* 12.8, 2H), 2.18 (t, *J* 11.4, 2H), 2.56 (t, *J* 11.8, 2H), 2.93 (d, *J* 10.8, 2H), 3.78 (s, 3H), 3.84 (s, 3H), 7.10 (s, 1H), 7.14 (m, 1H), 7.25 (m, 1H), 7.29 (d, *J* 8.8, 2H), 7.33 (m, 1H), 7.43 (d, *J* 8.4, 2H), 7.72 (d, *J* 8.0, 1H).