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N-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butyl, Butenyl and Butynyl}arylcarboxamides as Novel Dopamine D_3 Receptor Antagonists

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Abstract—The dopamine D_3 receptor subtype has been targeted as a potential neurochemical modulator of the behavioral actions of psychomotor stimulants, such as cocaine. Previous synthetic studies provided structural requirements for high affinity binding to D_3 receptors which included a 2,3-dichloro-phenylpiperazine linked to an arylamido function via a butyl chain. To reduce lipophilicity of these agents and further investigate optimal conformation, a second series of 15 novel ligands was designed that included heteroaromatic substitution and unsaturated alkyl linkers. These compounds were synthesized and evaluated for binding at rat D_3 and D_2 receptors stably expressed in Sf9 cells. D_3 binding affinities ranged from $K_i = 0.6$–1080 nM, with a broad range of D_3/D_2 selectivities (2–97). The discovery of potent, selective and bioavailable D_3 receptor ligands will provide essential molecular probes to elucidate the role D_3 receptors play in the psychomotor stimulant and reinforcing effects of cocaine.

The dopamine D_3 receptor, a member of the D_2 receptor family, resides in brain regions associated with emotional and cognitive function, such as the nucleus accumbens (for review see refs 1 and 2). Efforts to further elucidate the function and potential therapeutic advantages of targeting D_3 receptors have resulted in compounds that may be useful in treating Parkinson's disease, schizophrenia and drug abuse.[2–4] The discovery of D_3 receptor selective antagonists and partial agonists has received particular attention for potential treatment of cocaine abuse since the compound BP 897 was first reported to block cue-controlled cocaine-seeking in rats.[5] Additional studies with BP 897[6] and the potent and D_3 selective antagonist SB-277011[7,8] further support the development of D_3 selective ligands as potential cocaine abuse medications.

As we have described[9] and others have more recently confirmed[10–12] optimal D_3 receptor binding affinities are obtained when a 2,3-dichlorophenylpiperazine is linked to an aryl amide via a butyl chain (Fig. 1). However, the 2- or 4-substituted fluorenyl analogues, which gave the highest D_3 binding affinities and selectivities rendered these molecules highly lipophilic and possibly limited their bioavailability.[9] In vivo investigation of these ligands will be required to elucidate mechanisms associated with reduction in cocaine-seeking behavior. Thus, improving physical properties of the molecules by reducing lipophilicity and further identifying structural modifications that would yield highly potent and selective D_3 ligands, was prioritized. In this pursuit, heteroaromatic replacement of the fluorenyl ring system and conformational optimization by adding unsaturation to the butyl linker was explored. The target molecules are shown in Figures 2 and 3.

Synthesis of novel amides **21**–**35** was achieved as depicted in Scheme 1. 2,3-Dichlorophenypiperazine was linked to the *N*-phthalimido-protected butyl (**11**), butenyl (*cis*; **16a** or *trans*; **16b**) or butynyl (**19**) amines using standard *N*-alkylation conditions followed by deprotection with hydrazine.[9] Amidation via the acid chloride using Schotten–Baumann conditions (Method A) or directly using CDI (Method B) gave the desired

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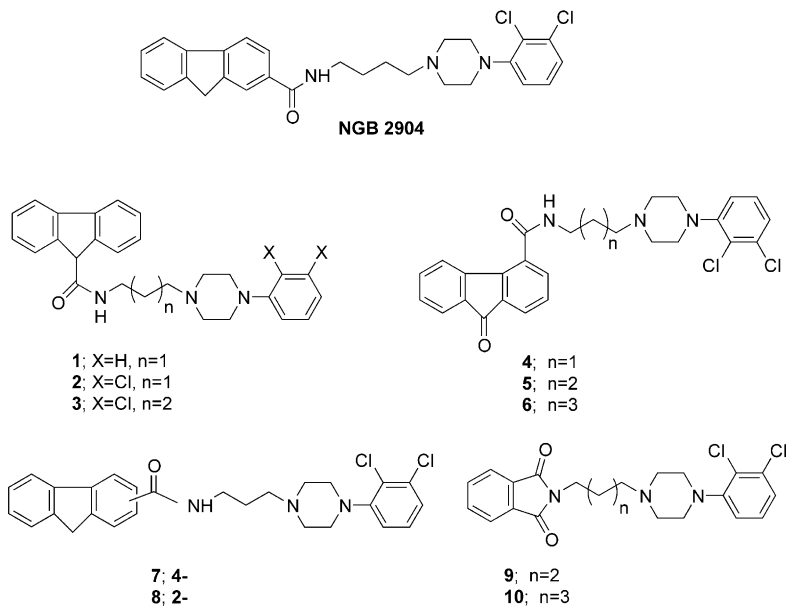


Figure 1. Previously synthesized D_3 ligands.

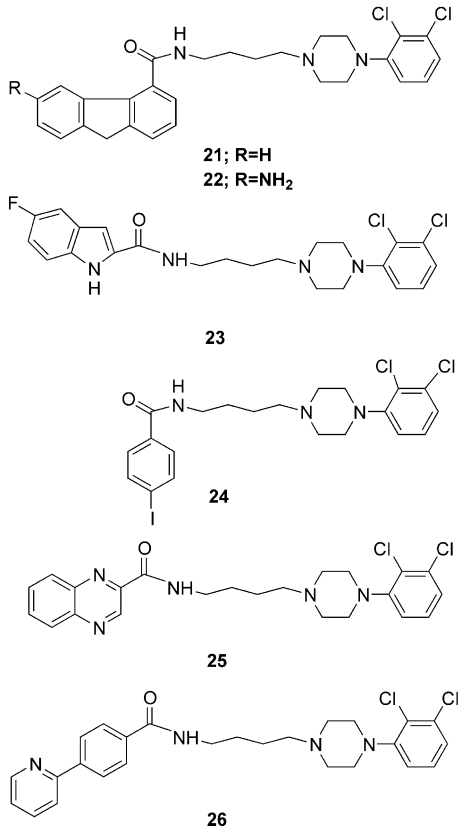


Figure 2. Novel D_3 ligands with saturated butyl chain link.

products (**21** and **23**–**35**). For compound **22**, the 5-nitrofluorenyl carboxylic acid was first reduced by catalytic hydrogenation (10% Pd/C) to the intermediate aniline that was then coupled to amine **13** using DCC and HOBt (Method C). All carboxylic acids were either commercially available or prepared by literature methods as indicated in Table 1 where physical properties of all final products are provided.

D_3 and D_2 receptor binding affinities for the 15 novel compounds were determined by competitive radioligand binding experiments using (a) the D_2-like receptor selective radioligand ^{125}I-IABN[13] and (b) either rat D_2 or D_3 receptors expressed in Sf9 cells using a recombinant baculovirus. The binding methods used are as previously reported[14] and briefly described in the Table 1 legend. Ten previously reported compounds **1**–**10** and NGB 2904, shown in Figure 1, were also evaluated for comparison purposes. cLogD values[15] are also shown in Tables 2 and 3.

The binding data in Table 2 revealed that when the aromatic ring system contains heteroatoms, high affinity binding at D_3 is retained, while lipophilicity is decreased, for example compounds **5** and **21** compared to **23**, **25**, and **26**. Compound **26** demonstrated the highest D_3 binding affinity ($K_i = 0.6$ nM) in this series and was 97-fold selective for D_3 receptors over D_2. Recently, similar findings have been reported with other heteroaromatic substitutions.[10,11] However, smaller aryl ring systems on the saturated butyl linker also demonstrate moderately high affinity binding at D_2 receptors, limiting their utility as selective D_3 compounds (e.g., **9** and **10** as compared to **5**). Substitution of a phenyl ring with the sterically bulky 4′-iodo atom in compound **24**, somewhat improved D_3-selectivity, nevertheless, this compound was still quite lipophilic (cLogD = 6.20). Alternatively, the investigation into structurally more rigid analogues obtained by unsaturation of the butyl linker led to a promising lead.

Whereas the alkyne linker (**33**–**35**) served to significantly reduce binding affinities at both D_3 and D_2, introduction of a *cis*, and particularly a *trans*, olefin resulted in retention of high affinity binding at D_3 receptors (Table 3). This was particularly true when the lipophilic fluorenyl ring system was replaced with a simple phenyl ring, where compound **31** showed high affinity ($K_i = 1.3$ nM), as well as 39-fold selectivity for D_3 over D_2 receptors.

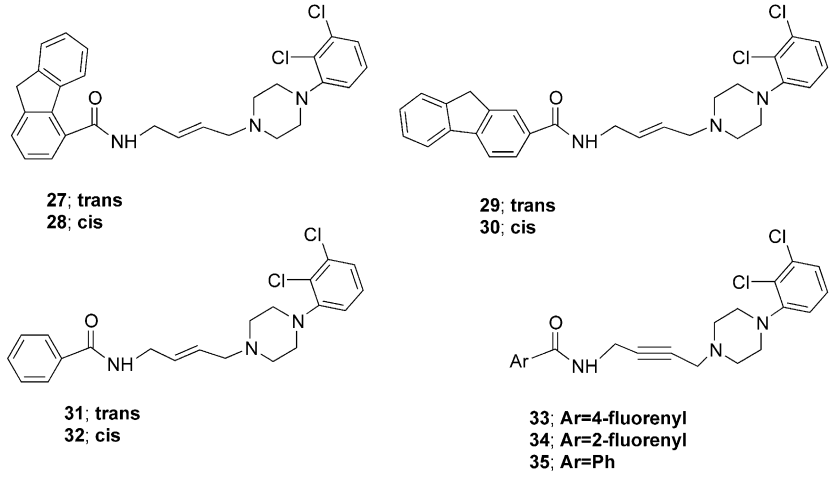
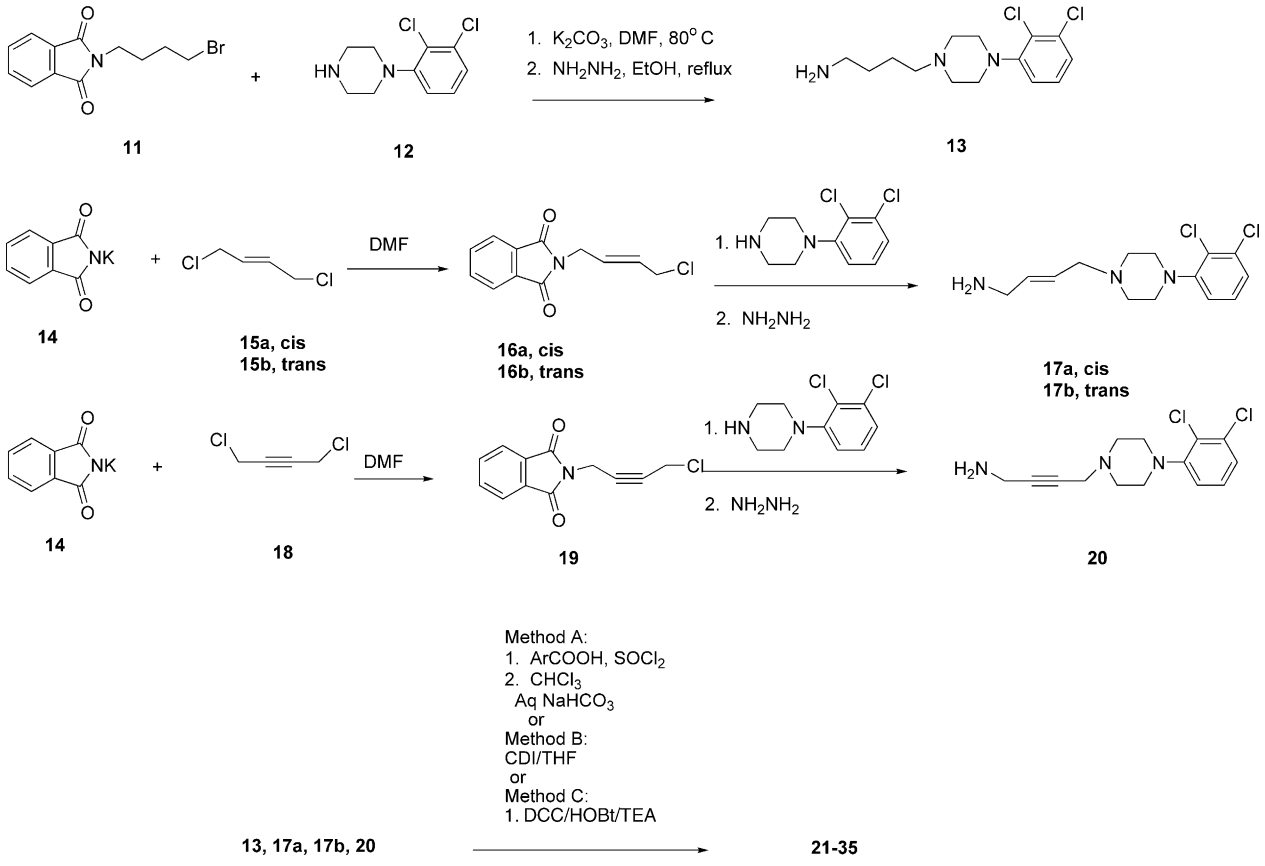


Figure 3. Structurally rigid D_3 receptor ligands with unsaturated chain link.



Scheme 1. Synthesis of novel D_3 ligands.

This modification reduced lipophilicity in this series of analogues by 2 orders of magnitude (e.g., compounds **27**–**30** compared to **31** and **32**). In fact, the smaller ring system improved binding affinity at D_3 significantly. Furthermore, for the phenyl-ring substituted compounds, **31** compared to **32**, the *trans* olefin is the preferred conformation for optimal D_3 binding affinity and selectivity over D_2. This conformational preference is in accord with *trans*-cyclohexamides previously reported as high affinity D_3 ligands.[16,17] Investigation into optimal substitution of the phenyl ring as well as heteroaryl substitutions in the *trans* butenyl series, is currently underway.

Evaluation of compounds **23**–**26**, **29**, **31**, and **32** in a functional assay using stimulation (agonist) or inhibition of quinpirole stimulation (antagonist) of mitogenesis in human D_3 transfected CHO cells is shown in Table 4. Data to support an antagonist profile for compounds **23**, **25**, **31** and **32**, which was predicted based on NGB 2904[18] and other more recently reported D_3 antagonists having the 2,3-dichlorophenyl moiety[10] is

Table 1. Synthetic methods and physical properties of novel D_3 ligands

Compd	Method[e]	Yield	Salt/rec solv.	Mp °C	Formula[c]
21	B	10[b]	Fumarate/MeOH	187∼190	$C_{28}H_{29}N_3OCl_2{\cdot}C_4H_4O_4{\cdot}0.5H_2O$
22	C	81	Oxalate/2-PrOH	140∼142	$C_{28}H_{28}Cl_2N_4O{\cdot}2C_2H_2O_4{\cdot}2H_2O$
23	B	46[b]	HCl/MeOH	d 271∼274	$C_{23}H_{25}N_4OFCl_2{\cdot}HCl$
24	B	25[b]	HCl/2-PrOH	227∼229	$C_{21}H_{24}N_3OCl_2I{\cdot}HCl$
25	B	20[b]	HCl/MeOH/ether	209∼211	$C_{23}H_{25}N_5OCl_2{\cdot}HCl$
26	A[a]	40[b]	diHCl/2-PrOH	234∼237	$C_{26}H_{28}N_4OCl_2{\cdot}2HCl$
27	B	87	Oxalate/MeOH/ether	187∼190	$C_{28}H_{27}Cl_2N_3O{\cdot}C_2H_2O_4{\cdot}1.5H_2O$
28	B	89	Oxalate/MeOH	170∼172	$C_{28}H_{27}Cl_2N_3O{\cdot}C_2H_2O_4{\cdot}0.5H_2O$
29	B[d]	99	Oxalate/MeOH/ether	174∼176	$C_{28}H_{27}Cl_2N_3O{\cdot}C_2H_2O_4{\cdot}0.25H_2O$
30	B[d]	94	Oxalate/MeOH	176∼178	$C_{28}H_{27}Cl_2N_3O{\cdot}C_2H_2O_4{\cdot}0.5H_2O$
31	A	99	Oxalate/Acetone	118∼122	$C_{21}H_{23}Cl_2N_3O{\cdot}C_2H_2O_4{\cdot}0.5H_2O$
32	A	99	HCl/2-PrOH	100∼102	$C_{21}H_{23}Cl_2N_3O{\cdot}HCl$
33	B	69	Oxalate/2-PrOH	180∼181	$C_{28}H_{25}Cl_2N_3O{\cdot}C_2H_2O_4{\cdot}0.25H_2O$
34	B[d]	96	Oxalate/MeOH	194∼195	$C_{28}H_{25}Cl_2N_3O{\cdot}C_2H_2O_4$
35	A	98	Oxalate/2-PrOH	126∼130	$C_{21}H_{21}Cl_2N_3O{\cdot}C_2H_2O_4$

[a]4-Pyridin-2-yl benzoic acid.[21]
[b]%Yield is based on the purified salt, all others based on crude free base.
[c]All compounds were purified through their respective salts and the free bases were characterized using ^1H and ^{13}C NMR, FT-IR and GC–MS. Combustion analysis results agreed to ±0.4% of C, H and N with theoretical values.
[d]2-Fluorenylcarboxylic acid.[9]
[e]Method A[9]; B[18]; C.[22]

Table 2. D_3 and D_2 receptor binding data and clog D values for non-rigid molecules

Compd	D_3 (nM)±SEM[a]	D_2 (nM)±SEM[a]	D_2/D_3	clog D[b]
1	84.5±19	1020±110	12	4.16
2	34.0±11	250±82	7	6.08
3	11.1±3.8	55.2±22	5	6.36
4	43.0±23	153±57	4	6.36
5	1.6±0.9	150±20	94	6.64
6	2.0±1.1	35.3±9.6	18	6.85
7	139±5.0	376±170	3	6.66
8	199±52	396±170	2	6.66
9	4.8±2.2	102±33	21	5.70
10	5.6±3.9	86.4±20	15	5.91
21	4.5±2.2	134±45	30	6.95
22	3.5±2.7	79.3±18	23	5.66
23	2.6±1.4	83.2±20	32	5.45
24	1.4±0.5	87.5 ±33	63	6.20
25	1.9 ±0.7	109.4±5.3	58	4.92
26	0.6±0.2	57.9 ±5.1	97	5.30
NGB 2904	1.1±0.2	911±190	830	6.94

[a]The methods for the binding assays have been previously described.[14] K_i values are the mean of at least three independent determinations. The IC_{50} values obtained from competition experiments were converted to K_i values using the Cheng and Prusoff correction.[23] The radioligand used for the competitive radioligand binding studies was ^{125}I-IABN. A recombinant baculovirus (Bv) expression system was used to express either rat D_2 (BvD_2) or rat D_3 (BvD_3) receptors in Sf9 cells. Competition curves were modeled as a one site fit using the TABLECURVE program. Human D_3 and D_2 receptor binding in CHO cells has previously been reported for compounds **1**–**10** and NGB 2904.[9]
[b]See ref 15.

Table 3. D_3 and D_2 receptor binding data and ClogD values for rigid analogues

Compd	D_3 (nM)±SEM[a]	D_2 (nM)±SEM[a]	D_2/D_3	clog D
27	19±7.8	106±22	6	7.07
28	6.5±1.8	10.7±5	1.6	7.07
29	5.9±1.8	198±39	34	7.07
30	6.0±1.6	87.3±19	15	7.07
31	1.3±0.4	50.1 ±6	39	5.13
32	4.9±1.9	20.0±1.8	4	5.13
33	1080±380	891±250	1	7.04
34	214±63	1000±180	5	7.04
35	394±140	1700±450	4	5.10

[a]K_i values were obtained as described in Table 1.

Table 4. D_3 functional assay using stimulation or inhibition of quinpirole stimulation of mitogenesis in CHO cells (hD_3)

Compd	Agonist EC_{50} (nM)±SEM[a]	%Max Stim.±SEM[a]	Antagonist IC_{50} (nM)±SEM[a]
23	>10,000	—	52.4±1.2
24	31.7±10	30.0±0.1	—
25	>10,000	—	26.9±7.7
26	6.31±1.7	29.7±0.2	—
29	173±9.6	44.1±0.9	—
31	>10,000	—	7.72±1.6
32	>10,000	—	6.00±0.59

[a]These data were obtained through the service of CTDP, Division of Treatment Research and Development, NIDA, using a contract (N01DA-1-8816) protocol.

shown. The *trans* olefins are particularly potent antagonists in this assay with IC_{50} values in the low nanomolar range. Nevertheless, compounds **24**, **26**, and **29** show a partial agonist profile, which is consistent with the 2-methoxy phenylpiperazine compound BP 897 that was initially tested in a similar in vitro model of D_3 receptor function.[5] However, subsequently BP 897 was shown to have an antagonist profile in other in vitro models of D_3 function.[19,20] Although the initial report on BP 897 suggested that D_3 partial agonists would be predicted to block cocaine-seeking, recent reports have shown that the potent and selective D_3 antagonist SB-277011 showed remarkable inhibition of cocaine seeking and cocaine-enhanced brain reward in rat.[8] These studies point to potential inconsistencies between in vitro and in vivo models of D_3 receptor function and support further investigation of novel D_3 compounds in vivo. Hence, the development of potent and selective D_3 receptor ligands, such as compounds **26** and **31**, will provide the pharmacological tools to clarify these

mechanistic questions and relate chemical structure, D_3 receptor function and behavior in models of cocaine abuse.

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