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Synthesis and activity of 2-[4-(4-[^{3}H]-2-cyanophenyl)piperazinyl]-*N*-(2,4,6-[^{3}H]$_3$-3-methylphenyl)acetamide: a selective dopamine D_4 receptor agonist and radioligand

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Abstract—The first selective dopamine D_4 agonist radioligand is described. The synthesis of these piperazine radioligands relied on the transformation of brominated precursors **4a** and **4b** with tritium gas in the presence of a sensitive cyano functional group. The specific activity of these two radioligands was measured and [^{3}H]**6b** found to be suitable for use in D_4 saturation and competition binding studies. The synthesis, biological, and radioactivity of this new agonist radioligand as well as preliminary SAR will be discussed.

Dopamine, the predominant catechol neurotransmitter in the brain, exerts its actions via two classes of G-protein coupled receptors: the G_s-coupled D_1-like family (D_1 and D_5) and the $G_{i/o}$-coupled D_2-like family (D_2, D_3, D_4).[1,2] These receptors have been characterized by cloned gene expression in stable cell lines, by immunohistochemistry and by use of D_4 selective ligands.[1–3] While the localization of the dopamine D_4 receptor in the cortex suggests an important function in psychiatric disorders like schizophrenia, this hypothesis has not been validated at the clinical level.[4,5] In addition, recent publications have demonstrated that selective D_4 agonists play a role in facilitating penile erection.[6,7] In view of these important pharmacological effects associated with this receptor, the availability of selective D_4 radioligands would be useful in understanding the localization of dopamine D_4 receptors in specific areas of the brain. These selective radioligands could be used not only to determine relevant affinity (competition binding assays) dependent on the functional profile of the targeted ligand but also to measure receptor abundance and localization in brain (saturation binding assays). Ultimately, the specific localization of D_4 receptors in brain could be determined either in vitro (autoradiography) or in vivo with positron emission tomography (PET) techniques using the proper radiolabels such as carbon (^{11}C) or fluorine (^{18}F).

Recently, published reports[8] have appeared utilizing both selective and nonselective antagonist radioligands in an effort to characterize the D_4 receptor. As a result of nonspecific ligand binding and low abundance of D_4 receptor in brain, characterization in vitro and in vivo has been difficult. Several groups report the need for higher affinity, higher specific activity, and more selective D_4 radioligands for these studies.[9–11]

In our previous efforts to identify new dopamine D_4 agonists,[12] we identified compound **5a** (Scheme 1) as a potent, selective[13] agonist ligand. Acetamide **5a** showed selectivity of >400-fold over D_{2L} and >700-fold overall other dopamine receptor subtypes. A general screen of

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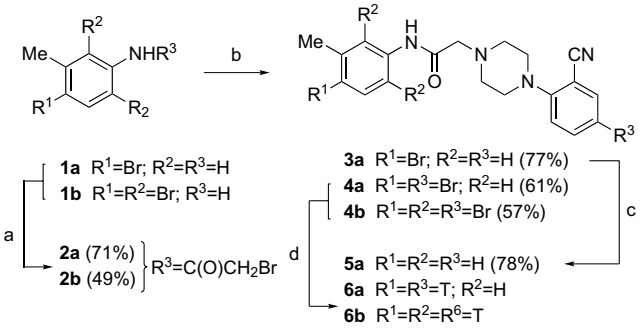
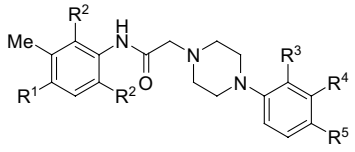


Scheme 1. Reagents and conditions: (a) **2a**: $ClC(O)CH_2Br$, 2 N NaOH, CH_2Cl_2, rt; **2b**: $ClC(O)CH_2Br$, PhMe, 100 °C; (b) piperazine, *i*-Pr_2EtN, PhMe, 60 °C; (c) 10% Pd/C, H_2 (50 psi), MeOH, rt, 1 h; (d) T_2, Pd/C, Et_3N, EtOAc, 2.5 h.

5a, in approximately 70 receptors,[14] showed little activity at other nondopaminergic receptors. There was significant activity at the 5-HT_{1A} receptor but even in that case, the dopamine D_4 selectivity was >350-fold. During the course of our studies, it was discovered that substitution on the aryl piperazine portion of the molecule had a significant effect on the functional activity. If functional groups such as the cyano group in **5a** were moved to the *para* position (e.g., R^5 in Table 1), a loss in agonist activity was observed. Additional characterization revealed that these *para*-substituted analogs displayed good binding affinities using [^{3}H]spiperone and were further shown to be potent antagonists. We were interested in developing an agonist radioligand in order to more accurately characterize competitive binding for agonists at the D_4 receptor. It was decided to incorporate tritium into our lead agonist in the acetamide series, **5a**. Tritium offers predictable biochemical activity, greater analog stability as well as rapid and facile synthesis. In addition, we would be able to easily modulate the required specific activity needed for binding studies by the substitution of multiple tritium atoms. This report describes the identification of the first selective dopamine D_4 agonist radioligand.

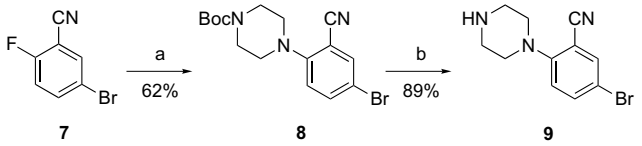
Our strategy was to incorporate the radioactive labels by dehalogenation of aryl bromides in the presence of tritium gas. Compound **5a** (Scheme 1) was chosen as the representative agonist for tritiation. A concern was the reductive conditions required in the presence of a sensitive cyano functional group. As a result, it was decided to attempt hydrogenation on a model system, bromide **3a**. If the reduction was successful, then the tritiation could be carried out on dibromide **4a** or perhaps tetrabromide **4b**. The synthesis of **3a**, **4a**, and **4b** is shown in Scheme 1. Commercially available anilines, **1a** and **1b**, were acylated with chloroacetyl bromide using either basic, biphasic conditions or refluxing toluene[15] to provide bromides **2a** and **2b** in moderate yields. Model compound **3a** was completed by reaction of **2a** with commercially available 1-(2-cyanophenyl)piperazine in

Table 1. Pharmacologic characterization of analogs **3**–**6a**,**b** and **10**–**17** in the human $D_{4.4}$ receptor



Compound	R^1	R^2	R^3	R^4	R^5	EC_{50}[a]	%E[b]	[^{3}H]**6b** K_i[c]	[^{3}H]spiperone K_i[d]
3a	Br	H	CN	H	H	96.0 ± 22.8	69	ND	ND
4a	Br	H	CN	H	Br	>10,000	—	14.0 ± 3.5	54.2 ± 5.9
4b	Br	Br	CN	H	Br	>10,000	—	113.5 ± 14.4	936 ± 118
5a	H	H	CN	H	H	7.5 ± 0.8	80	14.5 ± 2.1	2.7 ± 1.0
6a	T	H	CN	H	T	ND[e]	ND	ND	ND
6b	T	T	CN	H	T	ND	ND	4.0 ± 0.7[f]	ND
10	H	H	OMe	H	H	26.8 ± 6.7	60	1.8 ± 0.3	0.7 ± 0.1[g]
11	H	H	H	OMe	H	>10,000	—	39.1 ± 4.2	46 ± 2.2
12	H	H	H	H	OMe	>10,000	—	88.9 ± 9.1	101 ± 12
13	H	H	H	H	Ph	>10,000	—	>10,000	ND
14	H	H	H	H	Bn	>10,000	—	>10,000	ND
15	H	H	H	H	$CHPh_2$	>10,000	—	174.5 ± 37.8	ND
16	H	H	H	H	F	>10,000	—	90.7 ± 6.7	61.9 ± 1.6
17[h]	H	H	—	—	—	>10,000	—	>10,000	>10,000
Clozapine								30.4 ± 4.7	28.4 ± 0.4
Haloperidol						>10,000	—	1.9 ± 0.3	1.4 ± 0.04

[a] Mean values for agonists (EC_{50} in nM) calculated from at least three determinations ± standard error of the mean (SEM) in the calcium flux assay (agonist mode) using HEK-293 cells co-transfected with human $D_{4.4}$ receptor and $G\alpha_{qo5}$.
[b] Efficacy relative to 10 μM dopamine (100%).
[c] Mean values for binding affinity (K_i in nM) calculated from at least three determinations ± SEM versus [^{3}H]**6b**.
[d] Mean values for binding affinity (K_i in nM) calculated from at least three determinations ± SEM versus [^{3}H]spiperone.
[e] ND = not determined.
[f] K_d.
[g] In the human dopamine $D_{4.2}$ allele.
[h] 4-Pyridyl piperazine.



Scheme 2. Reagents and conditions: (a) Boc-piperazine, DMSO, Et_3N, 90 °C, 24 h; (b) concentrated HCl, rt, 15 min.

the presence of a tertiary amine base and toluene at elevated temperatures in 77% yield. In order to complete **4a** and **4b**, bromo piperazine **9** was required (Scheme 2). This compound was obtained from commercially available fluoride, **7**, by a two-step procedure. First, nucleophilic addition of *t*-butyl 1-piperazinecarboxylate in DMSO provided compound **8** in 62% yield. Deprotection of the piperazine nitrogen under acidic conditions gave the desired intermediate, **9**. Reaction of **9** with **2a** or **2b** at elevated temperatures in toluene in the presence of a tertiary amine base furnished acetamides **4a** and **4b** in 61% and 57% yield, respectively.

Biochemical evaluation of bromides **3a**, **4a**, and **4b** in the calcium flux assay duplicated (Table 1) the trend reported earlier.[12] Substitution in the *para* position of the benzonitrile ring caused a loss in D_4 agonist activity. Further evaluation of compounds **4a** and **4b** in a competition binding assay using [^{3}H]spiperone revealed that both analogs bound to the D_4 receptor with good affinity.

With the desired bromides in hand, hydrogenation studies of compound **3a**; aimed at nitrile stability and the potential of undesired side products; were initiated. Upon hydrogenation of **3a**, compound **5a** was isolated and found to match that of previously reported material.[12] We were confident that the tritiation of dibromide **4a** would proceed without disturbing the nitrile functionality or produce any undesired side products. Upon tritiation of **4a**, compound [^{3}H]**6a** was isolated and determined to have a specific activity of 23.7 Ci/mmol. This specific activity was inadequate for accurate cell tissue saturation and competition binding assays due to the low expression levels of the D_4 receptor in rat brain. Therefore, the tritiation of **4b** was carried out to provide tetra tritiated compound, [^{3}H]**6b**. This material had 88.1 Ci/mmol specific activity and performed well in a saturation binding assay using human D_4 receptor transfected HEK-293 cells (K_d = 4.0 nM). In addition, compound [^{3}H]**6b** showed equipotency for the three major human D_4 variants as well as rat D_4.[13]

Previous work by Van Vliet et al.[16] reported a dramatic difference in binding affinities with D_{2L} selective agonists using the antagonist radioligand, [^{3}H]spiperone and the D_{2L} agonist radioligand, **N-0437**. The conclusion was that because the D_{2L} receptor can exist in a high- and low-affinity binding state, an agonist radioligand more accurately measured relevant agonist binding affinities for the high-affinity receptor conformation. We speculated that the same observation might be made for the D_4 receptor. Competition binding studies were initiated with [^{3}H]**6b** on a number of analogs shown in Table 1. The *ortho* methoxy substituted agonist, **10**, showed good binding affinity (K_i = 1.8 nM) as did the regioisomers **11** and **12**. Previous work showed that **11** and **12** were antagonists.[12] Comparison of [^{3}H]**6b** and [^{3}H]spiperone with agonists **5a** and **10**, however, did not show significant differences in calculated K_i values for either D_4 agonist. As a result, we are currently expanding our investigation of this series as well as other structurally diverse D_4 agonists within our program in order to uncover potential differences in measured K_i values when using [^{3}H]**6b**.

Another aspect of our SAR study was to explore size limitations in the aryl piperazine portion of the D_4 receptor. We examined relatively encumbered ligands **13**, **14**, and **15** and less sterically hindered analogs **16** and **17**. The fluoro analog, **16**, showed moderate binding affinity for D_4 and was functionally active as an antagonist.[12] The *para*-substituted phenyl compound, **13**, showed poor binding affinity as did the benzyl analog, **14** thus suggesting a disfavored ligand–receptor interaction in this region of the molecule. The larger benzhydryl compound (**15**), however, displayed modest affinity (K_i = 174 nM) while no binding was observed for the much smaller pyridyl analog, **17**.

These preliminary results show that a careful examination of this region of the molecule is required to understand the critical factors associated with both functional activity and binding affinity. More subtle factors of ligand–receptor interaction (both steric and electronic), the possibility of different binding orientations within the same series of analogs and the possibility for high- and low-affinity binding conformations of each analog are clearly relevant.[17] As this acetamide series is expanded to address these questions, the use of both functional assays and competition binding studies using [^{3}H]**6b** will play a critical role. In addition to in vitro SAR development using [^{3}H]**6b**, efforts in our laboratories are currently underway to expand the scope of this radioligand in both brain tissue saturation binding studies and autoradiography to further characterize the localization and function of the D_4 receptor.[13]

In conclusion, we have synthesized and evaluated the first selective D_4 agonist radioligand. Brominated analogs **4a** and **4b** were successfully transformed into [^{3}H]**6a** and [^{3}H]**6b**. Subsequent biochemical evaluation of these radioligands showed [^{3}H]**6b** to possess sufficient selectivity and specific activity to be used in competition binding studies. Initial evaluation of acetamide D_4 agonists with both [^{3}H]**6b** and the [^{3}H]spiperone, did not show the same shift in binding affinities as reported for the D_{2L} receptor. Additional examples in the acetamide series are required to further evaluate use of [^{3}H]**6b** to determine agonist affinities for the putative high-affinity receptor conformations. Structure–activity relationships with [^{3}H]**6b** have shown that the aryl piperazine region of the D_4 receptor tolerates larger groups (like **15**) but the lack of binding affinity in **17** suggesting a subtle electronic element or larger changes in ligand binding orientation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.bmcl.2004.07.068.

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