Synthesis of (*R,S*)-2′-*trans*-7-Hydroxy-2-[*N*-*n*-propyl-*N*-(3′-iodo-2′-propenyl)-amino]tetralin (*trans*-7-OH-PIPAT): A New D3 Dopamine Receptor Ligand

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The central nervous system (CNS) dopaminergic system in mammalian brain is very important for normal brain function, and it is also the apparent action site for various neuroleptic drugs in the treatment of schizophrenia and other mental disorders.[1] Cloning of dopamine receptors has yielded at least six different subtypes: [1e-l] D1, $D2_L$, $D2_S$, D3, D4, and D5, a diversity far beyond the traditional classification of the two subtypes, D1 and D2, proposed by Kebabian in 1979.[1m] The D3 receptor differs from D1 and D2 receptors in several ways: amino acid sequence, pharmacological profiles (*in vivo* and *in vitro*), tissue distribution, and most likely, the receptor–effector coupling mechanism.[2]

Tritium-labeled quinpirole, a well-known agonist for the dopamine receptor, has been employed as a ligand for D2-like dopamine receptors in *in vitro* binding assay of rat brain.[3] Binding studies of this ligand using D3 dopamine receptors expressed in Chinese hamster ovary (CHO) cells suggested that quinpirole showed higher binding affinity for the D3 receptor than that for the D2 receptor, K_iD2/K_iD3 = 113.[1h] One of the iodinated benzamide derivatives, [^{125}I]NCQ298, (*S*)-(−)-3-iodo-*N*-[(1′-ethyl-2′-pyrrolidinyl)methyl]-2-hydroxy-5,6-dimethoxybenzamide, was initially reported as a selective D2 receptor ligand (K_d = 19 pM) in membrane homogenates of rat striatal tissues.[4] Subsequent binding studies, using this ligand with dopamine receptor subtypes expressed separately in CHO and *Spodoptera frugiperda* (Sf9) cells, gave similar K_d values for the D2 and D3 dopamine receptors, respectively.[5] However, both D2 and D3 dopamine receptors are present in striatum of rat brain. The lack of potent and selective ligands labeled with high specific acitivity hinders the progress of understanding the pharmacological function and regulation of the receptor subtypes in their native states. Recently, [^{3}H]-7-OH-DPAT (7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin) was identified as a selective ligand for the D3 receptor expressed in CHO cells, K_d = 0.67 nM.[6] Based on 7-OH-DPAT, we have designed an iodinated derivative, (*R,S*)-2′-*trans*-7-hydroxy-2-[*N*-*n*-propyl-*N*-(3′-iodo-2′-propenyl)amino]-tetralin (*trans*-7-OH-PIPAT, **5**) by placing the iodine atom on the *N*-propenyl side chain. This unique feature has led to a stable iodinated derivative with highly desirable properties: higher specific activity (theoretical specific activity for I-125 is 2200 Ci/mmol), higher selectivity, more potent binding affinity and lower nonspecific binding.

Scheme I

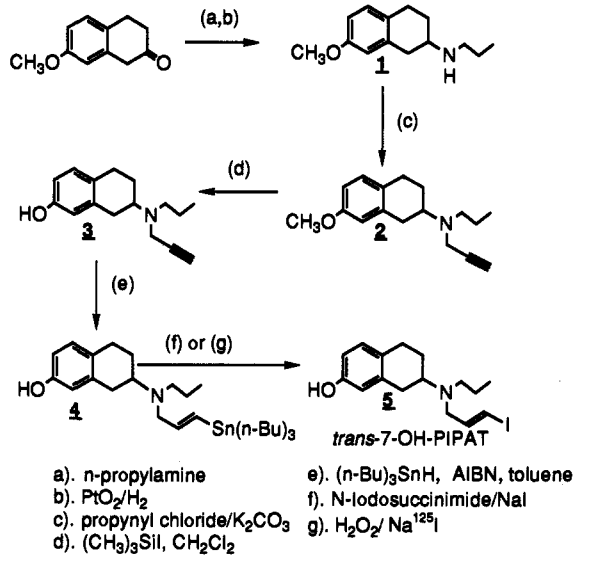

Table I. Inhibition Constants for the Binding of [^{125}I]NCQ298 toward D2 and D3 Dopamine Receptors Expressed in Sf9 Cells

	D2 K_i (nM ± SE)	D3 K_i (nM ± SE)	D2/D3 ratio
7-OH-DPAT	142 ± 14.2	2.90 ± 0.50	48.9
trans-7-OH-PIPAT, **5**	265 ± 36	1.85 ± 0.37	143

Synthesis of *trans*-7-OH-PIPAT (**5**) was achieved by a sequence of reactions listed in Scheme I. Amination of 7-methoxy-2-tetralone was carried out with *n*-propylamine followed by hydrogenation in the presence of PtO_2 under hydrogen (30 psi) to give **1** in good yield (61%).[7] Compound **1** was reacted with propynyl chloride; the reaction gave the desired compound **2** in excellent yield (87%). Deblocking of **2** with trimethylsilyl iodide gave **3** in 40% yield. When **3** was reacted with tributyltin hydride in the presence of 2,2′-azobis(2-methylpropionitrile) (AIBN) as the catalyst, the reaction gave *trans* isomer **4** as the main product in 20% overall yield (*cis*/*trans* ratio = 0.88). The corresponding 2′-*trans*-iodopropenyl derivative, **5**, was prepared by reacting the *trans*-tributyltin derivative with sodium iodide and *N*-iodosuccinimide. All compounds were characterized by FTIR, NMR, and high-resolution mass spectrometry. Radioiodination with I-125 (carrier-free, Na^{125}I) was successfully carried out starting with the corresponding tributyltin derivative, **4**, with hydrogen peroxide as the oxidant as reported before.[8] The desired product, [^{125}I]-*trans*-7-OH-PIPAT, was obtained after HPLC separation (radiochemical purity >98%, yield 85–90%; specific activity was assumed to be 2200 Ci/mmol).

The binding affinities of *trans*-7-OH-PIPAT (**5**) toward D2 and D3 receptors were compared with those of 7-OH-DPAT with the D2 and D3 dopamine receptors expressed separately in Sf9 cells.[5b] These tetralins were found to be selective with excellent affinity for the D3 receptor (Table I). The radioactive labeled compound, [^{125}I]-*trans*-7-OH-PIPAT, [^{125}I]-**5**, displayed saturable and high specific binding to the membranes. Nonspecific binding accounted for a small fraction of the total binding (approximately 10% at K_d). In addition, [^{125}I]-**5**, revealed one-site binding (Hill coefficient ≈1) with a K_d value of 0.13 nM (Figure 1). The B_{max} obtained for this iodinated ligand was 3–4 pmol/mg protein, comparable to that measured with [^{125}I]NCQ298 in the same system. In contrast to

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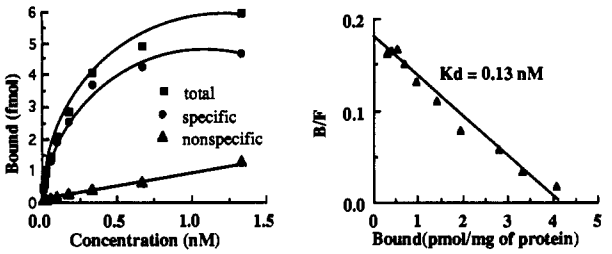

Figure 1. Saturation and Scatchard plots of [^{125}I]-*trans*-7-OH-PIPAT, **5**, in AcMNPVrD3-infected Sf9 cell membranes at 25 °C without NaCl.

Table II. Potencies of Various Compounds Competing with [^{125}I]-*trans*-7-OH-PIPAT, **5**, Binding to D3 Receptors Expressed in Sf9 Cells[a]

compound	receptor type	K_i (nM ± SE)	Hill co.
trans-7-OH-PIPAT, **5**	D3	0.99 ± 0.08	1.02
7-OH-DPAT	D3	1.81 ± 0.43	0.87
raclopride	D2/D3	9.25 ± 2.3	0.78
haloperidol	D2/D3	14.3 ± 3.1	0.68
(+)-butaclamol	D1/D2/D3	3.91 ± 0.86	0.76
dopamine	DA	49.3 ± 5.4	0.85
quinpirole	D3	5.65 ± 0.62	0.86
SCH23390	D1	768 ± 150	0.80
WB4101	α1	107 ± 13	0.96
8-OH-DPAT	5-HT$_{1A}$	248 ± 29	0.78
mianserin	5-HT$_2$/HT$_{1c}$	1524 ± 228	1.03
yohimbine	α2	2430 ± 146	0.97
5-HT	5-HT	2338 ± 420	0.90
(±)-propranolol	β	>5000	
naloxone	opiate	>20000	

[a] [^{125}I]-*trans*-7-OH-PIPAT [^{125}I]-**5**, (0.1–0.2 nM) was incubated at 37 °C with 120 mM NaCl in the presence of the indicated compounds at 9–11 concentrations, in membrane preparations of AcMNPVrD3-infected Sf9 cells. Values are from two to three independent determinations in duplicate.

[^{125}I]NCQ298, [^{125}I]-*trans*-7-OH-PIPAT showed no specific binding toward dopamine D2$_L$ and D2$_S$ receptors expressed in Sf9 cells (data not shown). Competition experiments performed with [^{125}I]-*trans*-7-OH-PIPAT in AcMNPVrD3 infected Sf9 cell membranes revealed that several known D2 and D3 ligands including 7-OH-DPAT, (+)-butaclamol, and haloperidol have high affinity for the D3 receptor. In contrast, the D1-selective antagonist, SCH23390, and ligands for other receptors such as WB4101, mianserin, yohimbine, and naloxone, displayed moderate to low affinity (Table II). The high affinity observed for the dopamine agonists, quinpirole (K_i = 5.6 nM) and dopamine (K_i = 49.3 nM), is consistent with a similar study using D3 receptors expressed in CHO cells,[6] suggesting the same binding characteristics for D3 receptors with these two ligands.

In conclusion, synthesis and initial binding characterization of the first novel iodinated D3 dopamine ligand, *trans*-7-OH-PIPAT, **5**, is reported. This tetralin derivative demonstrates unique high affinity and selectivity toward the D3 receptor. It is possible that the ligand may be used to study the D3 dopamine receptor in *in vivo* and *in vitro* systems. The information generated will be very important for understanding the pharmacology as well as the relevance of D3 dopamine receptors on the mechanism of action of neuroleptics for treatment and management of patients with mental illness.

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Supplementary Material Available: Experimental data and preparations for 1–5 (8 pages). Ordering information is given on any current masthead page.

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