Iodinated 2-Aminotetralins and 3-Amino-1-benzopyrans: Ligands for Dopamine D2 and D3 Receptors

Sumalee Chumpradit, Mei-Ping Kung, Janet Vessotskie,† Catherine Foulon, Mu Mu, and Hank F. Kung*,†

Departments of Radiology and Pharmacology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

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In developing selective ligands for dopamine D2 and D3 receptors, several iodinated 2-aminotetralins and 3-amino-1-benzopyrans, *trans*-7-hydroxy-2-[*N*-(3′-iodo-2′-propenyl)amino]tetralin (**1**), *trans*-monohydroxy-2-[*N*-propyl-*N*-(3′-iodo-2′-propenyl)amino]tetralin (7-, 5-, and 6-OH-PIPAT) (**2**, **3**, and **4**), and *trans*-monohydroxy-3,4-dihydro-3-[*N*-propyl-*N*-(3′-iodo-2′-propenyl)-amino]-2*H*-1-benzopyran (6- and 8-OH-benzopyrans) (**5** and **6**), were prepared. These compounds were evaluated for their binding profiles in several membrane preparations: *Spodoptera frugiperda* (Sf9) cells expressing dopamine D2 (non-GTP coupled, low-affinity states) and D3 receptors, HEK293 cells expressing dopamine D2 receptors in high-affinity states (D2H), rat hippocampal homogenates for 5-HT_{1A} receptors, and cerebellar homogenates for σ receptors. The mono-*N*-alkylated 2-aminotetralin, **1**, displayed high σ binding (K_i = 1.68 nM) with a moderate D3 binding (K_i = 30.2 nM). Derivatives with one *N*-propyl and one *N*-(3′-iodo-2′-propeny) group generally displayed high to moderate affinity to D3 receptors (K_i = 2.90, 1.85, 0.99, 2.20, 31.4, and 6.69 nM for 7-OH-DPAT [7-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin], **2**, **3**, **4**, **5**, and **6**, respectively). It is interesting to note that all of the active D3 ligands also displayed comparable binding to the high affinity states of D2 receptors in HEK293 cells (K_i = 6.6, 3.6, 9.7, and 10.8 nM for **2**, **3**, **4**, and **6**, respectively). Among all of the tetralin derivatives tested, 5-OH-PIPAT, **3**, showed the highest binding affinity to D3 receptors (K_i = 0.99 nM) and better selectivity (K_{iD2H}/K_{iD3}, K_{iD2}/K_{iD3}, $K_{i5\text{-}HT1A}/K_{iD3}$ and $K_{i\sigma}/K_{iD3}$ = 3.64, 327, 48.4, and 1250 nM, respectively), making it the best ligand for studying dopamine D2H and D3 receptors.

In recent years, application of molecular biology techniques to express receptors in cloned cells has significantly broadened the understanding of dopamine receptors in the central nervous system. At least five different dopamine receptor subtypes, D1, D2, D3, D4, and D5,[1-6] have been cloned. This diversity is far beyond the traditional classification of two subtypes, D1 and D2, proposed by Kebabian in 1979.[7]

It has been more than 18 years since the synthesis of 2-aminotetralins and their dopaminergic activity were reported.[8,9] A wealth of information on the structure–activity relationship of 2-aminotetralin derivatives has been illustrated.[10,11] Literature reports were mainly focused on the use of such agents as dopamine agonists and potential selective dopamine autoreceptor agents.[12-14] The compounds of the tetralin series which received the most attention are the *N*,*N*-di-*n*-propyl derivatives such as 5-, 6-, and 7-OH-DPAT (hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin). It is well-known that 5-, 6-, and 7-OH-DPAT and their related tetralin derivatives containing various substitutions have propensity for binding to dopamine receptors,[15-21] while the 8-OH derivative is selective for 5-HT_{1A} receptors.[22-24] Optically resolved *R*-(+)- and *S*-(−)-isomers of 5-, 6-, and 7-OH-DPAT derivatives displayed differential dopaminergic activity.[18,19,25-28] A large number of 2-aminotetralins, including fused ring systems, have also been reported in the literature. Based on the known dopaminergic activity, these tetralins may have high affinity to both D2 and D3 receptors. However, due to the fact that most of the compounds predate the discovery of various dopamine receptor subtypes, there have been no systematic efforts in preparing and screening for dopamine subtype selective compounds.

In order to investigate the pharmacology directly associated with various subtypes of dopamine receptors, there is an urgent need for subtype selective ligands. Recently, [^{3}H]-7-OH-DPAT was identified as a selective ligand for D3 receptors expressed in CHO cells, with a K_d = 0.67 nM.[29,30] Based on 7-OH-DPAT, we have reported the synthesis and initial evaluation of an iodinated derivative of 7-OH-DPAT, namely *trans*-7-OH-PIPAT, by placing the iodine atom on the *N*-propenyl side chain.[31] This unique feature has led to a stable iodinated derivative with highly desirable properties: high specific activity and high binding affinity for dopamine D3 receptors. Resolution of (*R*,*S*)-*trans*-7-OH-PIPAT into individual *R*-(+)- and *S*-(−)-isomers has led to the identification of the *R*-(+)-*trans*-7-OH-PIPAT as the active isomer for binding to dopamine D3 receptors.[32,33] Binding of [^{125}I]-*R*-(+)-*trans*-7-OH-PIPAT to high-affinity states of dopamine D2 receptors has been demonstrated in transfected HEK293 cells expressing guanine nucleotide sensitive dopamine D2 receptors (K_d = 0.1 nM).[34] In addition, this iodinated ligand binds with high affinity to 5-HT_{1A} receptors (K_d = 1.4 nM, rat hippocampus) and to σ sites[35] (K_d = 10.8 nM, rat cerebellum). Under suitable assay conditions, [^{125}I]-*R*-(+)-*trans*-7-OH-PIPAT displays D3 receptor binding in its native state.[34,35] In order to further extend our search for more selective ligands, a series of aminotetralin derivatives, mono-*N*-alkylated derivative, 7-OH-IPAT, (*R*,*S*)-*trans*-7-hydroxy-2-[*N*-(3′-iodo-2-propenyl)-amino]tetralin (1), 5-OH- and 6-OH-PIPAT, (*R*,*S*)-*trans*-5-hydroxy-2-[*N*-propyl-*N*-(3′-iodo-2′-propenyl)amino]-

* Address for correspondence: Hank F. Kung, Ph.D., Departments of Radiology and Pharmacology, University of Pennsylvania, 3700 Market Street, Room 305, Philadelphia, PA 19104. Tel.: (215) 662-3096. Fax: (215) 349-5035. e-mail: kunghf@sunmac.spect.upenn.edu.
† Department of Pharmacology.
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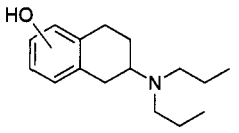
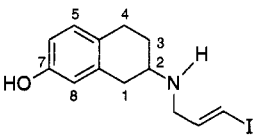
Table 1. Comparison of Inhibition Constants K_i (nM) of (*R,S*)-7-OH-DPAT and (*R,S*)-**1**–**6** with Dopamine D2H, D2, D3, $5HT_{1A}$, and σ Receptors

compd	D2H HEK293	D2 (Sf9)	D3 (Sf9)	5-HT_{1A} (rat hipp)	σ (rat cereb)
7-OH-DPAT		142 ± 14.2	2.90 ± 0.5	72.7 ± 8.0	23.2 ± 3.2
1			30.2 ± 2.1		1.68 ± 0.18
2	6.6 ± 5.9	265 ± 36	1.85 ± 0.37	5.1 ± 1.4	3.2 ± 0.2
3	3.6 ± 1.3	324 ± 23.6	0.99 ± 0.16	47.9 ± 11	1238 ± 334
4	9.7 ± 3.0	547 ± 40	2.20 ± 0.45	297 ± 21.9	28.4 ± 8
5		>5000	31.4 ± 6.2	1273 ± 156	492 ± 96
6	10.8 ± 3.8	784 ± 78	6.69 ± 1.87	619 ± 172	>10,000

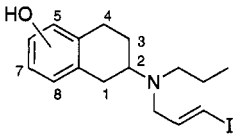
tetralin ((*R,S*)-*trans*-5-OH-PIPAT) (**3**), (*R,S*)-*trans*-6-hydroxy-2-[*N*-propyl-*N*-(3′-iodo-2′-propenyl)amino]tetralin ((*R,S*)-*trans*-6-OH-PIPAT) (**4**), and 6-OH- and 8-OH-benzopyran derivatives (*R,S*)-*trans*-8-hydroxy-3,4-dihydro-3-[*N*-propyl-*N*-(3′-iodo-2′-propenyl)amino]-2*H*-1-benzopyran (**5**), and (*R,S*)-*trans*-6-hydroxy-3,4-dihydro-3-[*N*-propyl-*N*-(3′-iodo-2′-propenyl)amino]-2*H*-1-benzopyran (**6**) were synthesized. This paper will specifically address the synthesis and *in vitro* binding evaluation of compounds with hydroxy-2-aminotetralin and 3-hydroxy-1-benzopyran backbone structures.



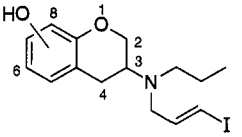
OH-DPAT



1: 7-OH-IPAT



2: 7-OH
3: 5-OH
4: 6-OH

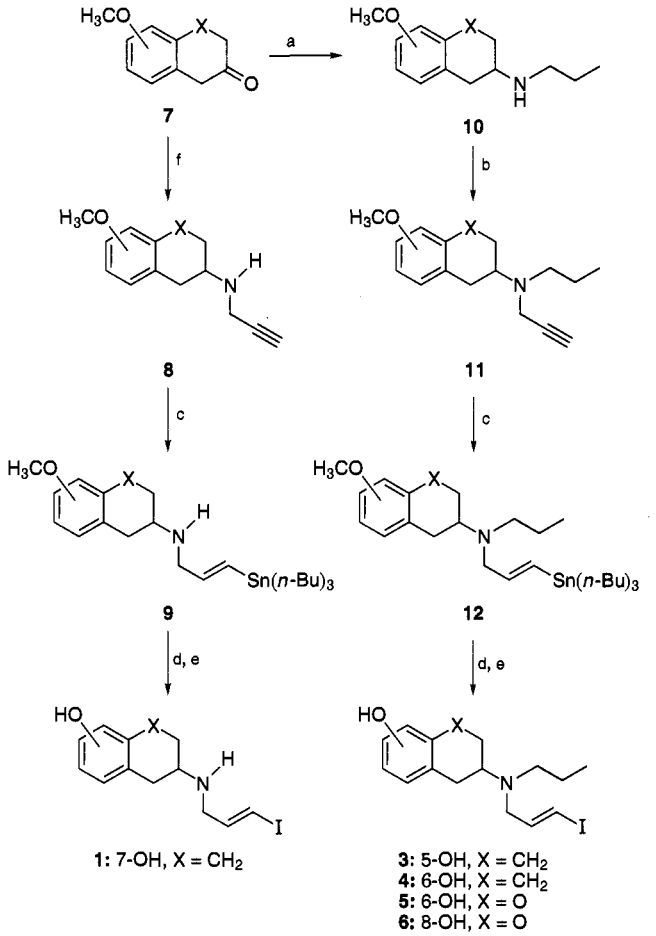


5: 6-OH
6: 8-OH

Chemistry

Syntheses of compounds 1 and **3-6** were achieved by reactions described in Scheme 1. Compound **2**, (*R,S*)-7-OH-PIPAT, was reported previously, and the optical resolution of 1 was also reported recently.[33] Using similar reaction schemes previously reported, appropriately substituted tetralones were the key starting material. The methoxy tetralones, **7**, were either commercially available or synthesized according to the literature.[36] Reductive amination of tetralones, **7**, with *n*-propynylamine gave 7-methoxy-2-(*N*-propynylamino)-tetralones, **8**. Reduction of the triple bond with tri-*n*-butyltin hydride produced the desired tri-*n*-butyl derivatives, **9**. After demethylation and replacement of the tri-*n*-butyltin group with iodine, compound **1** was produced in good yield (70%). For other *N,N*-dialkylated 2-aminotetralins or 3-hydroxy-1-benzopyrans, the reaction sequence started with a reductive amination of **7** with *n*-propylamine and sodium cyanoborohydride. The resulting *N*-*n*-propyl substituted compound, **10**, was alkylated with *n*-propynyl chloride to give **11**, which was reduced with tri-*n*-butyltin hydride to give the desired tri-n-butyl derivative, **12**. Iododemetalation of the tin derivative **12** with iodine in chloroform, followed by demethylation with boron tribromide, gave the desired products, **3**–**6**. All of the new 2-aminotetralins or 3-hydroxy-1-benzopyrans reported herein are in their racemic forms.

Scheme 1[a]



[a] (a) *n*-Propylamine/$NaBH_3CN$: (b) 2-propynyl chloride, K_2CO_3; (c) $(n\text{-Bu})_3SnH$, AIBN, toluene; (d) I_2, $CHCl_3$; (e) BBr_3, CH_2Cl_2; (f) propynyl amine, $NaBH_3CN$.

Binding Studies

Similar to that reported previously for 7-OH-PIPAT,[31,33] two regioisomers, 5-OH and 6-OH-PIPAT, also displayed high binding affinity to D3 receptors expressed in Sf9 cells (K_i = 0.99 and 2.20 nM, respectively) (Table 1). Initial evaluation of these two compounds for the D2 receptor binding in Sf9 cells revealed low affinity, with K_i values of 324 and 547 nM for 5-OH- and 6-OH-PIPAT, respectively. Thus, comparable selectivities for D2 *vs* D3 receptor binding were obtained for 5-OH-, 6-OH-, and 7-OH-PIPAT; however, due to the limitation of using Sf9 cells (Baculovirus expression system) for screening of dopamine D2 subtype (only at low affinity states),[35] binding affinity was further evaluated with HEK293-D2 receptors which contain G-protein coupled states of D2 receptors (D2 high affinity states).[34]

Using [^{125}I]-*R*-(+)-*trans*-7-OH-PIPAT as the radioligand for binding to the high-affinity states of dopamine

Table 2. Comparison of Binding of Dopamine D3 *vs* Other Receptor (values of K_{ix}/K_{iD3})

compd	D2H/D3	D2/D3	5-HT_{1A}/D3	σ/D3
7-OH-DPAT		49.0	25.1	8.0
1				0.056
2	3.57	143	2.76	1.73
3	3.64	327	48.4	1250
4	4.40	249	135	12.9
5		>159	40.5	15.7
6	1.61	117	92.5	1495

D2 receptors (D2H) expressed in HEK293 cells, 5-OH-, 6-OH-, and 7-OH-PIPAT competed with the radioligand binding with similar potency (K_i = 3.6, 9.7, and 6.6 nM, respectively). The difference between the binding selectivity of high-affinity states of D2 receptors (in HEK293 cells) and D3 receptors is significantly diminished (less than 5-fold) for all three compounds. Two 3-hydroxy-1-benzopyran derivatives, **5** and **6**, displayed slightly lower binding affinity for D3 receptors (K_i = 31.4 and 6.69 nM, respectively) but retained similar selectivity between D2 and D3 receptors expressed in Sf9 cells. Similarly, the K_i value (10.8 nM) of **6** obtained with HEK293-D2H receptors was close to that observed for D3 receptors.

Comparing the binding affinity of these new aminotetralin derivatives to 5-HT_{1A} receptors, 7-OH-PIPAT appeared to be the most potent, with a K_i value of 5.1 nM, while 5-OH- and 6-OH-PIPAT displayed significantly less binding to 5-HT_{1A} receptors (K_i = 47.9 and 297 nM, respectively). Neither 6-OH nor 8-OH benzopyran derivatives revealed significant 5-HT_{1A} receptor binding (Table 1); K_i values were high. The results of σ binding for these derivatives displayed varying degrees of potency: 7-OH-PIPAT > 6-OH-PIPAT > 6-OH-benzopyran > 8-OH-benzopyran > 5-OH-PIPAT. The mono-*N*-alkylated derivative, 1, differed from other derivatives, displaying very high binding to σ sites and only a moderate affinity toward dopamine D3 receptors.

Table 2 summarizes the relative binding affinity of compounds 7-OH-DPAT and 1–**7** to various receptors. The binding affinity ratios for D2H/D3 are generally low (<5), suggesting that none of the compounds displayed selectivity. D2/D3 ratios are higher for compounds **2**, **3**, **4**, and **5**. Since the D2 receptors expressed in Sf9 cells have not been shown to be coupled to G-proteins (low-affinity states), these values may not be as important as those of D2H/D3. The ratios of 5-HT_{1A}/D3 showed distinctive divergence, with compounds **3**, **4**, **5**, and **6** displaying higher values (48.4, 135, 40.5, and 92.5, respectively), and compound **2**, 7-OH-PIPAT, showing the least selectivity. The σ/D3 ratio (1250) of 5-OH-PIPAT, **3**, suggested that this compound is highly exceptional in its selectivity.

Discussion

The *in vitro* binding data reported for 7-OH-PIPAT, **2**, demonstrated that its binding profile is complex, displaying high affinity to dopamine D2 and D3 receptor subtypes, as well as other receptors, such as 5-HT_{1A}[34,35] and σ sites.[37] The different binding profiles associated with each enantiomer were particularly distinctive and unexpected.[33] [^{125}I]-*R*-(+)-*trans*-7-OH-PIPAT, **2**, displayed equally high affinity toward dopamine D3 receptors as toward the high-affinity states of dopamine D2 receptors.[34] The unexpected binding affinity of [^{125}I]-*R*-(+)-*trans*-7-OH-PIPAT, **2**, toward 5-HT_{1A} receptors further demonstrated the nonselective nature of this iodinated ligand for dopamine receptor binding.[35] Although high σ site binding (K_d = 1.5 nM) was found to be associated with [^{125}I]-*S*-(−)-*trans*-7-OH-PIPAT,[37] significant σ site binding was also observed with the other optical isomer, [^{125}I]-*R*-(+)-*trans*-7-OH-PIPAT (K_i = 10.8 nM), **2**.[35]

Based on the findings obtained with [^{125}I]-*R*-(+)-*trans*-7-OH-PIPAT and its *S*-isomer, we continued to search for other iodinated tetralin derivatives in order to identify compounds with a better binding profile (higher affinity and greater selectivity). All of the tetralin derivatives prepared for testing are in their racemic forms. As shown in Table 1, 5-OH-PIPAT, **3**, displayed the highest binding affinity (0.99 nM) toward dopamine D3 receptors in this series, followed by 7-OH-PIPAT (1.85 nM), **2**. Moving the hydroxyl group from the 7-position to the 5-position significantly decreased the binding affinity for other receptors, such as 5-HT_{1A} receptors (K_i = 5.1 nM *vs* 47.9 nM for 7-OH-PIPAT, **2**, and 5-OH-PIPAT, respectively). σ site binding affinity for 5-OH-PIPAT, **3**, displayed the same trend of reduced affinity (400-fold lower) compared to that of 7-OH-PIPAT.

In this series of tetralin derivatives, 5-OH-PIPAT, **3**, appears to be the best candidate as a dopamine D2H/D3 receptor ligand. Since divergent binding profiles were observed for different enantiomers of 7-OH-PIPAT, the differences in the binding of enatiomers (*R*- or *S*-isomer) of 5-OH-PIPAT, especially for dopamine D3 receptors, were expected. Initial studies suggested that [^{125}I]-*S*-(−)-*trans*-5-OH-PIPAT is at least 10 times more active for binding to dopamine D3 receptors than [^{125}I]-*R*-(+)-*trans*-5-OH-PIPAT (data not shown). Detailed studies of separation, radiolabeling, and characterization of each enantiomer of 5-OH-PIPAT will be published elsewhere.

As shown in this study, all tetralin derivatives exhibited similar binding affinity toward dopamine D3 receptors and high-affinity states of dopamine D2 receptors, with no selectivity for these two subtypes. It will be interesting to fine tune the structures to identify the binding requirements for D3 receptors only, without concomitant binding to D2 receptors. The selective D2 and D3 ligands may provide powerful tools to delineate the distinct pharmacology associated with these dopamine receptor subtypes.

In conclusion, iodine-substituted derivatives of hydroxy-2-aminotetralin and 9-hydroxy-1-benzopyran were synthesized. The binding profiles to dopamine D2, D2H, D3, and 5-HT_{1A} receptors as well as to σ sites were evaluated. Among these compounds, 5-OH-PIPAT, **3**, displayed the most desirable binding affinity and pharmacological profile. It bound with equally high-affinity to dopamine D3 receptors and the high affinity states of dopamine D2 receptors. The binding profile of 5-OH-PIPAT (less 5-HT_{1A} receptor and σ site binding) makes it an attractive candidate for use as a tool for the evaluation of dopamine receptor function.

Experimental Section

NMR were recorded on a Varian EM 360A, a Bruker WM-250 (250 MHz), or a Bruker AM 500 (500 MHz) spectrometer. The chemical shifts were reported in ppm downfield from an internal tetramethylsilane standard. Infrared spectra were

obtained with a Mattson Polaris FT-IR spectrophotometer. Melting points were determined on a Meltemp apparatus and are reported uncorrected. HPLC was performed on a Model Rabbit HP with dual pumps (Rainin Instrument Co., Emeryville, CA) using a chiral column (chiracel-OD, 4.1 × 250 mm; Diacel Inc., Los Angeles, CA) or a silica column (Hamilton Co., Reno, Nevada). The HPLC system was equipped with a UV and a γ detector. The data were collected and analyzed with Dynamax software on Macintosh computers. Mass spectra were performed on a masspectrometer VG 70-70 HS with chemical ionization (CI), using methane or ammonia gas. Elemental analyses were performed by Atlantic Microlabs, Inc., Norcross, GA, and values were within 0.4% of the theoretical values. All chemicals were obtained from commercial sources. The starting material 7-methoxy-2-tetralone was purchased from Aldrich Chemical, 6-methoxy-2-tetralone was purchased from Narchem Corp., and 5-methoxy-2 tetralone,[36] 6-methoxy-3,4-dihydro-2*H*-1-benzopyran-3-one, and 8-methoxy-3,4-dihydro-2*H*-1-benzopyran-3-one[38] were synthesized according to reported procedures.

Intermediates **10**, **11**, and **12** and final products **3**, **4**, **5**, and **6** from Scheme 1 were synthesized in the same manner as previously reported[33] using the appropriate tetralone or benzopyranone as starting material. Reported herein will be the physical data of the final products. Only the procedures different from those in the previous paper[33] will be described.

***(R,S)-trans*-7-Hydroxy-2-[*N*-(3′-iodo-2-propenyl)amino]-tetralin (1).** Reductive amination of 7-methoxy-2-tetralone with propargylamine[33] gave **8** (73.9%): FT-IR (neat) ν 3300 (strong, NH), 3000–2800 (strong, nonaromatic CH), 1620, 1500, 1280; ^{1}H NMR ($CDCl_3$) δ 6.92 (d, J = 8.3 Hz, 1H, ArH), 6.62 (dd, J = 8.3 Hz, J = 2.7 Hz, 1H, ArH), 6.54 (d, J = 2.7 Hz, 1H, ArH), 3.70 (s, 3H, OCH_3), 3.47 (d, J = 2.3 Hz, 2H, NCH_2), 3.17–3.08 (m, 1H, CHN), 2.90 (dd, J = 15.9 Hz, J = 4.6 Hz, 1H, of $ArCH_2$) 2.81–2.63 (m, 2H, CH_2Ar), 2.51 (dd, J = 15.9 Hz, J = 9.1 Hz, 1H, of $ArCH_2$), 2.14 (t, 1H, C≡CH), 1.99–1.89 and 1.66–1.47 (m, 2H, CH_2), 1.45 (br, 1H, NH). Anal. Calcd for $C_{14}H_{17}NO$: C, H, N.

A mixture of **8** (0.829 g, 3.08 mmol) in dry toluene (65 mL), tributyltin hydride (3.30 mL, 12.2 mmol), and AIBN (133 mg, 0.91 mmol) was refluxed for 4 h under a nitrogen atmosphere. The solvent was evaporated, and the residue was purified by column chromatography (silica gel, CH_2Cl_2, MeOH, NH_4OH = 95/5/1) to give 0.892 g (77%) of *cis* and *trans* isomers. From 0.892 g of product, only 0.613 g of pure trans **9** was isolated; 0.279 g was a mixture of *cis* and *trans* isomers. FT-IR (neat) ν 3400 (w, NH), 3000–2800 (strong, nonaromatic CH), 1620, 1500, 1450, 1250. ^{1}H NMR ($CDCl_3$) δ 6.94 (d, J = 8.4 Hz, 1H, ArH), 6.64 (dd, J = 8.4 Hz, J = 2.7 Hz, 1H, ArH), 6.56 (d, J = 2.4 Hz, 1H, ArH), 6.09 (d, J =18.9 Hz, 1H, C=CHSn), 5.94 (dt, J = 18.9 Hz, 1H, HC=CSn), 3.72 (s, 3H, OCH_3), 3.37 (d, J = 3.6 Hz, 2H, NCH_2), 2.98–2.87 (m, 2H, CHN and 1H of $ArCH_2$), 2.80–2.61 (m, 2H, CH_2Ar), 2.60–2.51 (m, 1H, of $ArCH_2$), 2.03–1.98 and 1.58–1.49 (m, 2H, CH_2), 1.47–1.39 (m, 6H, $(CH_2)_3$) 1.31–1.19 (hex, 6H, $(CH_2)_3$) 0.83 (t, 16H, $Sn(CH_2)_3$, $(CH_3)_3$ and NH). Anal. Calcd for $C_{26}H_{45}NOSn$: C, H, N.

Iododemetalation of **9** (75%), followed by demethylation with BBr_3, gave **1** (80%): MS *m/z* 330 (M + H); FT-IR (KBr) ν 3290 and 3100 (w, OH, NH), 3000–2800 (strong, nonaromatic CH), 1600, 1500, 1250; ^{1}H NMR ($CDCl_3$) δ 6.79 (d, J = 8.2 Hz, 1H, ArH), 6.50 (dd, J = 8.2 Hz, J = 2.8 Hz, 1H, ArH), 6.49 (dt, J = 14.2 Hz, 1H, CH_2CH=C), 6.41 (d, J = 2.4 Hz, 1H, ArH), 6.20 (d, J = 14.4 Hz, 1H, C=CHI), 3.68 (s, br, 3H, OH), 3.19 (dd, 2H, NCH_2), 2.87–2.77 (m, 2H, CHN and 1H of $ArCH_2$), 2.72–2.54 (m, 2H, CH_2Ar), 2.41 (dd, J = 17.2 Hz, 1H, of $ArCH_2$), 1.93–1.86 and 1.51–1.38 (m, 2H, CH_2). Anal. Calcd for $C_{16}H_{17}NOICl$: C, H, N.

***(R,S)-trans*-5-Hydroxy-2-[*N*-propyl-*N*-(3′-iodo-2′-propenyl)amino]tetralin [(*R,S*)-3]:** ^{1}H NMR ($CDCl_3$) δ 6.99 (t, J = 7.7 Hz, 1H, ArH), 6.68 (d, J = 7.6 Hz, 1H, ArH), 6.59 (dt, J = 14.3 Hz, 1H, C=CH), 6.58, (d, J = 8.1 Hz, 1H, ArH), 6.20 (dt, J = 14.3 Hz, 1H, C=CHI), 3.19 (d, J = 6.0 Hz, 2H, CH_2C=C), 2.98–2.57 (m, 5H, CH_2ArCH_2 and CHN), 2.49 (t, J = 7.5 Hz, 2H, NCH_2), 2.10–2.02 and 1.65–1.53 (m, 2H, CH_2), 1.51–1.39 (hex, 2H, CH_2), 0.88 (t, 3H, CH_3). Anal. Calcd for $C_{16}H_{23}NOICl$: C, H, N.

***(R,S)-trans*-6-Hydroxy-2-[*N*-propyl-*N*-(3′-iodo-2′-propenyl)amino]tetralin [(*R,S*)-4]:** ^{1}H NMR ($CDCl_3$) δ 6.93 (d, J = 8.1 Hz, 1H, ArH), 6.60 (dd J = 9.0 Hz, 1H, ArH), 6.59 (dt, J = 14.2 Hz, 1H, C=CH), 6.54 (d, J = 2.6 Hz, 1H, ArH), 6.23 (dt, J = 14.3 Hz, 1H, C=CHI), 3.19 (d, J = 6.3 Hz, 2H, CH_2C=C), 3.07–2.60 (m, 5H, CH_2ArCH_2 and CHN), 2.49 (t, J = 5.9 Hz, 2H, NCH_2), 2.01–1.95 and 1.65–1.53 (m, 2H, CH_2), 1.52–1.42 (hex, 2H, CH_2), 0.88 (t, 3H, CH_3). Anal. Calcd for $C_{16}H_2NOI^2/_3HCl$: C, H, N.

***(R,S)-trans*-6-Hydroxy-3,4-dihydro-3-[*N*-propyl-*N*-(3′-iodo-2′-propenyl)amino]-2*H*-1-benzopyran [(*R,S*)-5]:** ^{1}H NMR ($CDCl_3$) δ 6.66 (d, J = 8.5 Hz, 1H, ArH), 6.57 (dd J = 8.5 Hz, 1H, ArH), 6.56 (dt, J = 14.4 Hz, 1H, C=CH), 6.54 (d, J = 2.7 Hz, 1H, ArH), 6.24 (dt, J = 14.4 Hz, 1H, C=CHI), 4.23–4.18 (m, 1H of CH_2O), 3.77 (t, J = 10.3 Hz, 1H of CH_2O), 3.20 (d, J = 6.3 Hz, 2H, CH_2C=C), 3.17–3.11 (m, 1H, CHN), 2.79 (d, J = 8.2 Hz, 2H, $ArCH_2$), 2.51 (t, J = 7.4 Hz, 2H, NCH_2) 1.50–1.38 (hex, 2H, CH_2), 0.87 (t, 3H, CH_3). Anal. Calcd for $C_{15}H_{21}NO_2ICl$: C, H, N.

***(R,S)-trans*-8-Hydroxy-3,4-dihydro-3-[*N*-propyl-*N*-(3′-iodo-2′-propenyl)amino]-2*H*-1-benzopyran [(*R,S*)-6]:** ^{1}H NMR ($CDCl_3$) δ 6.77–6.75 and 6.62–6.59 (m, 3H, ArH), 6.57 (dt, J = 15.0 Hz, 1H, C=CH), 6.25 (dt, J = 14.0 Hz, 1H, C=CHI), 4.36–4.29 (m, 1H of CH_2O), 3.88 (t, J = 10.0 Hz, 1H of CH_2O), 3.26–3.16 (m, 3H, CH_2C=C and CHN), 2.83 (t, J = 5.8 Hz, 2H, $ArCH_2$), 2.53 (t, J = 7.0 Hz, 2H, NCH_2), 1.51–1.35 (hex, 2H, CH_2), 0.88 (t, 3H, CH_3); high-resolution mass spectroscopy (M − CI) 373.0539, observed 373.0539 (error < 1ppm).

Dopamine D2 or D3 Receptor Binding Assay. The membrane homogenates of AcMNPVrD3- or AcMNPVrD2-infected Sf9 cells were prepared as described.[35,39] Briefly, the harvested cells were centrifuged, resuspended, and homogenized in buffer containing 50 mM Tris, pH 7.4, 150 mM NaCl, 10 mM EDTA, and a mixture of protease inhibitors (1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL soybean trypsin inhibitor). The final pellets were resuspended in the same buffer and kept at −20 °C for radioligand binding assays. Binding assays were performed in glass tubes (12 × 75 mm) in a final volume of 0.2 mL. The buffer used for the dilution contained 50 mM Tris, pH 7.4, 120 mM NaCl, the protease inhibitors mixture, and BSA (1 mg/mL). Each drug, at concentrations up to 10^{-5} M, was examined for its ability to displace [^{125}I]-NCQ298 [(*S*)-(−)-3-iodo-*N*-[(1′-ethyl-2′-pyrrolidinyl)methyl]-2-hydroxy-5,6-dimethoxybenzamide] binding at a ligand concentration of 0.1 nM. After incubation for 30 min at 37 °C, the bound ligand was separated from the free ligand by filtration through glass fiber filters (No. 25, Schleicher & Schuell, Keene, NH) soaked with 1% polyethylenimine. The filters were then washed twice with 4 mL of ice-cold buffer (containing 50 mM Tris-HCl, pH 7.4), and the radioactivity on the filters was counted in a γ counter (Packard 5000) with 70% efficiency. The nonspecific binding was defined with either 10 μM (+)-butaclamol or 1 μM 7-OH-DPAT. Binding assays in HEK293 cells were carried out as described.[34] [^{125}I]-*R*-(+)-*trans*-7-OH-PIPAT was used as the radiolabeled ligand for the competition experiments, and the buffer used for the assay included 50 mM Tris-HCl, pH 7.4 and 2 mM $MgCl_2$ to warrant the binding of [^{125}I]-*R*-(+)-*trans*-7-OH-PIPAT to the high-affinity states of D2 receptors. The data was analyzed using the iterative nonlinear least-square curve-fitting program LIGAND.[40]

5-HT$_{1A}$ Binding Assay. The measurements of 5-HT$_{1A}$ binding sites with [^{125}I]-*trans*-8-OH-PIPAT were carried out as described previously.[24] The hippocampal homogenates were prepared in 100 volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.4) and centrifuged at 20000*g* for 20 min. The resulting pellets were resuspended in ice-cold water to lyse vesicles, subsequently preincubated at 37 °C, and re-centrifuged to remove the endogenous serotonin. The final pellets were resuspended in the Tris buffer containing 2 mM $MgCl_2$. The binding assays were carried out in a total volume of 0.25 mL containing 100 μL of tissue preparations (20–30 μg of protein), appropriate amounts of labeled ligand (0.2–0.5 nM), and different concentrations of inhibitors. The tubes were incubated at 37 °C for 15 min and then terminated by vacuum filtration through glass fiber filters (No. 25, Schleicher &

Schuell, Keene, NH) presoaked with 1% polyethylenimine. The filters were then washed three times with 3 mL of ice-cold buffer, and the radioactivity on the filters was counted in a γ counter (Packard 5000). Nonspecific binding was defined with 10 μM 5-HT. Competition experiments were analyzed using the iterative nonlinear least-square curve-fitting program LIGAND.[40]

σ Binding Assays. Binding assays were carried out by incubating 65–75 μg of cerebellar homogenates with 0.3–0.5 nM of [^{125}I]-*S*-(−)-*trans*-7-OH-PIPAT,[37] 15 nM spiperone, and various concentrations of inhibitors in a total volume of 0.2 mL. Nonspecific binding was determined in the presence of 10 μM haloperidol. Incubation was carried out for 20 min at 25 °C, and the samples were rapidly filtered with a cell Harvester (M-24RT) under vacuum through glass fiber filters (No. 25, Schleicher & Schuell, Keene, NH) presoaked in 1% polyethylenimine, and the filter papers were washed with 3 × 5 mL of ice-cold 50 mM Tris-HCl (pH 7.4). Radioactivity bound to the filter paper was counted in a γ counter (Packard 5000) at an efficiency of 70%. The data was analyzed using the iterative nonlinear least-squares curve-fitting program LIGAND.[40]

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