Synthesis of (*R,S*)-*trans*-8-Hydroxy-2-[*N*-*n*-propyl-*N*-(3′-iodo-2′-propenyl)amino]tetralin (*trans*-8-OH-PIPAT): A New 5-HT_{1A} Receptor Ligand

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In order to develop tracers with higher specific activity to supplant the currently used [^{3}H]-8-OH-DPAT [8-hydroxy-2-(*N,N*-di-*n*-propylamino)tetralin] for *in vitro* and *in vivo* evaluation of 5-HT_{1A} receptors, a new radioiodinated ligand was prepared. (*R,S*)-*trans*-8-Hydroxy-2-[*N*-*n*-propyl-*N*-(3′-iodo-2′-propenyl)amino]tetralin (*trans*-8-OH-PIPAT), 8, was synthesized by a 10-step reaction. Binding studies with rat hippocampal membrane homogenates showed that 8 exhibited a K_i value of 0.92 nM against (*R,S*)-[^{3}H]-8-OH-DPAT. Radiolabeled [^{125}I]-8 was prepared from the corresponding tri-*n*-butyltin precursor via an oxidative iododestannylation reaction with sodium [^{125}I]iodide. Binding studies in the hippocampal homogenates revealed that [^{125}I]-8 bound to a single high-affinity site ($K_d = 0.38 \pm 0.03$ nM, $B_{max} = 310 \pm 20$ fmol/mg of protein). Competition binding experiments clearly indicated that the new ligand displayed the expected 5-HT_{1A} receptor binding profile. The rank order of potency was (*R,S*)-*trans*-8-OH-PIPAT > (*R,S*)-8-OH-DPAT > WB4101 > 5-HT > (*R,S*)-*trans*-7-OH-PIPAT > (*R,S*)-7-OH-DPAT > (*R,S*)-propranolol > spiperone >> ketanserin >> dopamine > atropine. This new ligand offers several unique advantages, including high specific activity, high binding affinity, and low nonspecific binding, all of which make it an excellent probe for the investigation and characterization of 5-HT_{1A} receptors.

The serotonin system is implicated in the modulation of various behaviors including anxiety and affective states.[1] One of the serotonin receptor subtypes, 5-HT_{1A}, plays an important function as the somatodendritic autoreceptor in the dorsal raphe nucleus and as a postsynaptic receptor for 5-HT in terminal field areas.[1,2] Azapirones, which are agonists and displayed high affinity to 5-HT_{1A} receptors, are being used as antianxiety agents.[3] Development of radioligands that are selective or relatively selective for specific 5HT_{1A} subtypes has facilitated the study and characterization of this binding site as a distinct entity. In the past few years, 8-hydroxy-2-(*N,N*-di-*n*-propylamino)tetralin (8-OH-DPAT) is the most potent 5-HT_{1A} agonist reported in the literature, and the tritium-labeled compound is the ligand of choice for binding studies of 5-HT_{1A} receptors (K_d = 0.65 nM, rat hippocampal membrane preparations).[4–10] Several recent reports have demonstrated that various analogs of 8-OH-DPAT displayed high agonist or antagonist activity, and they can be used as alternative tools to study 5-HT_{1A} receptors.[11–14]

Although [^{3}H]-8-OH-DPAT has been the most widely used radioligand to label selectively the 5-HT_{1A} binding site, other radioligands that have also been reported include: [^{3}H]WB4101,[15] [^{3}H]ipsapirone,[16] [^{3}H]PAPP,[17] and [^{3}H]spiroxatrine.[18] All of these radioligands exhibited high affinity for the 5-HT_{1A} binding sites and gave reasonable specific to nonspecific binding. However, a [^{125}I]-labeled radioligand with high specific activity (theoretically 2200 Ci/mmol, $T_{1/2}$ = 60 d, γ energy 35–60 keV) would offer advantages over tritiated radioligands with lower specific activity (60–80 Ci/mmol) for detecting low levels of binding sites, especially for quantitative autoradiographic studies. One other advantage of the iodinated ligand is the potential of labeling with I-123 ($T_{1/2}$ = 13 h, γ 1.59 keV) for *in vivo* single photon emission computed tomography (SPECT). In developing selective radioiodinated ligands for 5-HT_{1A} receptors, based on 8-OH-DPAT, the iodine atom was attached to an allyl group substituting for one of the *N*-propyl groups. This strategy stabilizes the iodine–carbon bond and preserves the selectivity and binding affinity to 5-HT_{1A} receptors. The synthesis, radiolabeling, and *in vitro* binding study of (*R,S*)-*trans*-8-hydroxy-2-[*N*-*n*-propyl-*N*-(3′-iodo-2′-propenyl)amino]tetralin, (*R,S*)-*trans*-8-OH-PIPAT, 8, are reported hereafter.

Chemistry

Synthesis of the desired *trans*-8-OH-PIPAT (8) was achieved by reactions described in Scheme I. The starting material, 8-methoxy-2-tetralone (2)[19] was condensed with 2-propynylamine followed by reduction with sodium cyanoborohydride to give 8-methoxy-2-(*N*-propynylamino)tetralin (3) in 64% yield. The acetylene derivative, 3, was converted to a mixture if cis and trans isomers, 4 (85% yield), with tri-*n*-butyltin hydride in the presence of AIBN [2,2′-azobis(2-methylpropionitrile)] as the catalyst. The corresponding iodo derivative, 5, also a cis/trans mixture, was obtained in 82% yield by reacting 4 with iodine in chloroform. Compound 5 was successfully demethylated by BBr_3 to give a mono-N-alkylated compound 6 in 40% yield. After separating the trans isomer of 5, subsequent reactions were carried out with the trans isomer only. For N,N-dialkylated derivatives, *trans*-5 was N-propylated first with 1-iodopropane to give 7 (80% yield), followed by O-demethylation of 7 using BBr_3 to give the desired product 8 (70% yield). Using this method, we have made all four unlabeled compounds 5–8. The K_i values showed that 8 was the most potent compound. However, the reaction scheme described above is not suitable for

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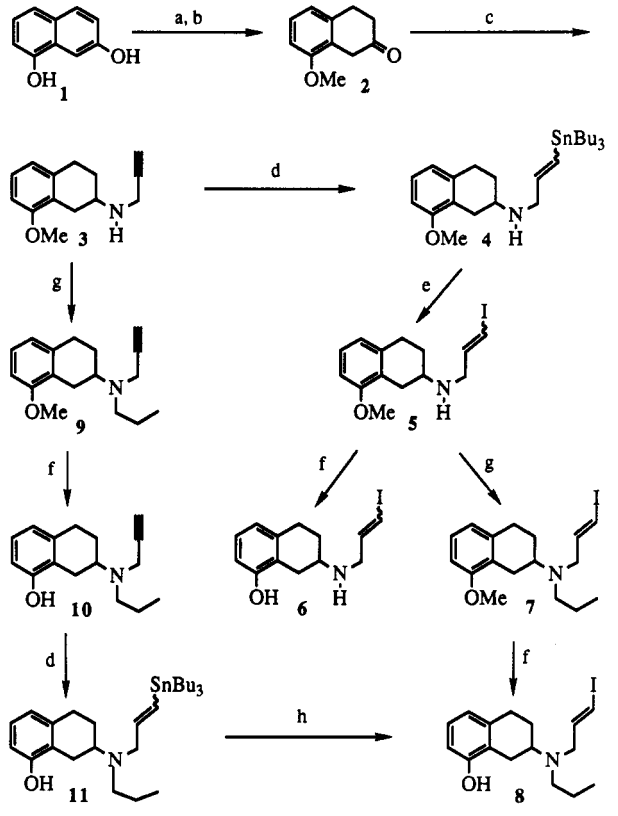
Scheme I[a]



[a] (a) Me_2SO_4, NaOH; (b) Na, EtOH; HCl; (c) propynylamine, *p*-TsOH, benzene; $NaCNBH_3$, MeOH; (d) $HSNBu_3$, AIBN, toluene; (e) I_2, $CHCl_3$; (f) BBr_3, CH_2CL_2; (g) 1-iodopropane, K_2CO_3, EtOH; (h) Na^*I/H_2O_2.

preparation of radioactive iodinated compound 8. Therefore, a new scheme was designed and carried out.

In order to produce a tri-*n*-butyltin derivative, 11, for radioiodination, a separate reaction sequence was used. Compound 3 was N-alkylated with 1-iodopropane to give the N,N-dialkylated derivative, 9 (87% yield). Demethylation with BBr_3 (89% yield) and radical reaction with tri-*n*-butyltin hydride, in the presence of AIBN as the catalyst, gave the desired tri-*n*-butyltin precursor 11 (48% yield) for radioiodination. Radioiodination with I-125 (no carrier added, $Na^{125}I$) was successfully carried out starting with the corresponding tri-*n*-butyltin derivative, 11, with hydrogen peroxide as the oxidant as reported before.[20-22] The desired product, racemic [^{125}I]-*trans*-8-OH-PIPAT, 8, was obtained after HPLC purification (radiochemical purity >98%, yield 70–80%). Co-injection with "cold" *trans*-8-OH-PIPAT showed that UV and γ profiles displayed an identical retention time, suggesting that the radiolabeled material is the same as that of the "cold" authentic sample.

Binding Studies

Initial binding characterization was carried out in membrane homogenates of hippocampal tissue of rat brain. Since the 5-HT_{1A} binding is strongly influenced by the presence of endogenous serotonin, it is necessary to preincubate the membrane preparation to remove the endogenous serotonin.[10] Potencies of K_i values of various tetralin derivatives to inhibit [^{3}H]-8-OH-DPAT binding to 5-HT_{1A} receptors in the homogenates of rat hippocampal membranes are presented in Table I. The inhibition

Table I. Potencies of Compounds To Inhibit [^{3}H]-8-OH-DPAT Binding to 5-HT_{1A} Receptors in the Homogenates of Rat Hippocampal Membranes[a]

compound	K_i (nM ± SEM)
(*R,S*)-OH-DPAT	0.63 ± 0.12
(*R,S*)-*trans*-8-OH-PIPAT, 8	0.92 ± 0.13
(*R,S*)-*trans*-8-OMe-PIPAT, 7	12.6 ± 1.50
(*R,S*)-*trans*-8-OH-IPAT, 6	10.0 ± 2.0
(*R,S*)-*trans*-8-OMe-IPAT, 5	17.0 ± 2.2

[a] [^{3}H]-8-OH-DPAT (0.3–0.5 nM) was incubated with the membrane preparations of rat hippocampus at 37 °C for 15 min in the presence of the indicated compounds. Values are from two to three independent determinations in duplicate.

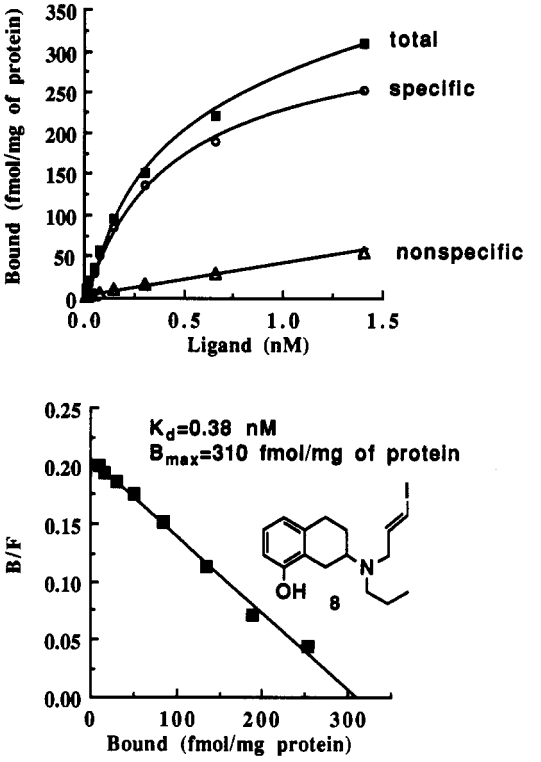


Figure 1. Saturation and Scatchard plots of [^{125}I]-*trans*-8-OH-PIPAT, [^{125}I]-8, in rat hippocampal membranes. The plots were constructed from values obtained by a nonlinear least squares analysis with the program LIGAND (K_d = 0.38 ± 0.03 nM; B_{max} = 310 fmol/mg of protein).

constant of 8-OH-PIPAT, 8 (K_i = 0.92 nM), suggests that the ligand is an excellent analog of 8-OH-DPAT for binding 5-HT_{1A} receptors. In order to evaluate the structure–activity relationship of related DPAT derivatives on 5-HT_{1A} binding, an additional three compounds, 5–7, were tested (Table I). The binding data reflect the requirement of at least one *N*-*n*-propyl group, and the affinity is higher for compounds with di-N,N-substitution and a free 8-OH group. Furthermore, the specific binding of the radioiodinated ligand [^{125}I]-8 to rat hippocampal membranes was saturable and had high affinity (K_d = 0.38 ± 0.03 nM). The density of binding sites (B_{max} = 310 fmol/mg of protein) labeled by [^{125}I]-*trans*-8-OH-PIPAT, 8, is comparable to that obtained with [^{3}H]-8-OH-DPAT. This result suggested that the iodinated ligand labeled the same binding sites as those of 8-OH-DPAT, a well-known 5-HT_{1A} ligand. Competitive studies with different compounds also indicated that the specific binding of [^{125}I]-*trans*-8-OH-PIPAT, 8, in hippocampal homogenates is consistent with the profile of 5-HT_{1A} receptors. Previously known high-affinity ligands for 5-HT_{1A} receptors, such as 8-OH-DPAT, 5-HT, and WB4101, displayed high potency for

Table II. Inhibition Constants of Compounds on the Binding of [^{125}I]-(*R,S*)-*trans*-8-OH-PIPAT, 8, to the Rat Hippocampal Homogenates[a]

compound	K_i (nM, mean ± SE)	Hill coefficient
(*R,S*)-8-OH-DPAT	0.91 ± 0.12	0.83
(*R,S*)-*trans*-8-OH-PIPAT, 8	0.30 ± 0.08	0.85
(*R,S*)-7-OH-DPAT	72.7 ± 8.0	0.82
(*R,S*)-*trans*-7-OH-PIPAT	5.10 ± 1.4	0.90
5-HT	4.31 ● 0.52	1.04
WB4101	2.68 ● 0.27	0.96
(*R,S*)-propranolol	113 ± 16	0.99
spiperone	602 ± 90	0.86
ketanserin	1817 ± 178	1.04
dopamine	>10000	
atropine	>20000	

[a] [^{125}I]-*trans*-8-OH-PIPAT (0.2–0.3 nM) was incubated with the membrane preparations of rat hippocampus at 37 °C in the presence of indicated compounds. Values are from two to three independent determinations in duplicate.

the binding of [^{125}I]-*trans*-8-OH-PIPAT, 8, while ligands selective for other receptors, such as spiperone (D2), ketanserin (5-HT$_2$), dopamine, and atropine, all showed very low binding affinity (Table II).

Since the new 5-HT$_{1A}$ ligand, [^{125}I]-*trans*-8-OH-PIPAT, 8, is a close structural analog of [^{125}I]-*trans*-7-OH-PIPAT, a new dopamine D$_3$ receptor ligand,[20,23] the affinity of 8 to dopamine D$_2$ and D$_3$ receptors expressed in *Spodoptera frugiperda* (Sf9) cells was evaluated. Table III shows the comparison of *trans* 8-OH-PIPAT and the corresponding 7-OH derivative using the same membrane preparations from the cloned dopamine D$_2$ and D$_3$ receptors with [^{125}I]-NCQ298 as the ligand. As expected, 7-OH-DPAT and 7-OH-PIPAT exhibit higher dopamine D$_3$ receptor binding affinity (2.90 and 1.85 nM, respectively) than dopamine D$_2$ receptor binding (142 and 265 nM, respectively),[20] whereas the corresponding 8-OH derivatives, 8-OH-DPAT and 8-OH-PIPAT, displayed much lower affinities toward dopamine D$_3$ receptors (251 and 125 nM, respectively) and dopamine D$_2$ receptors (3243 and 1072 nM, respectively). The higher binding affinity of 8 to 5-HT$_{1A}$ (K_i = 0.92 nM against [^{3}H]-8-OH-DPAT) is consistent with the fact that the binding of this ligand is more selective to 5-HT$_{1A}$ than that to the dopamine D$_2$ and D$_3$ receptors. The data strongly suggest that the 7-OH derivatives preferentially bind to dopamine receptors, while the 8-OH tetralin compounds preferentially bind to 5-HT$_{1A}$ receptors.

In conclusion, synthesis, radiolabeling, and *in vitro* binding study of [^{125}I]-*trans*-8-OH-PIPAT, 8, as a potent and selective ligand for 5-HT$_{1A}$ receptors are reported. The new ligand offers several unique advantages, including high specific activity (2200 Ci/mmol), high binding affinity (K_d = 0.38 nM), and low nonspecific binding, all of which make it an excellent tool for investigation and characterization of 5-HT$_{1A}$ receptors.

Experimental Section

Proton NMR spectra were obtained with Bruker AmX300 or 500 spectrometers. The chemical shifts were reported in ppm downfield from the tetramethylsilane standard. Infrared spectra were recorded on the Mattson Polaris FT-IR spectrometer. Low-resolution mass spectra were carried out on the VG mass spectrometer (Model ZAB-E). Elemental analyses were performed by Atlantic Microlabs, Inc., Atlanta, GA, and the deviation was within 0.3% of the theoretical values. No-carrier-added sodium [^{125}I]iodide (in 0.1 N NaOH solution; specific activity 2200 Ci/mmol) was purchased from Du Pont, NEN Research Products (Boston, MA). Haloperidol was a gift from Jassen Pharmaceutica (Beerse, Belgium); dopamine, WB4101, 5-HT, and (*R,S*)-8-OH-DPAT were obtained from Research Biochemicals Inc. (Natick, MA); (*R,S*)-propranol was obtained from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals used were of reagent grade.

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

compound	K_i (nM ± SEM) D2	K_i (nM ± SEM) D3
(*R,S*)-7-OH-DPAT	142 ± 14	2.90 ● 0.5
(*R,S*)-*trans*-7-OH-PIPAT	265 ± 48	1.85 ± 0.4
(*R,S*)-8-OH-DPAT	3243 ± 1259	251 ± 40
(*R,S*)-*trans*-8-OH-PIPAT, 8	1072 ● 87	125 ± 8.7

8-Methoxy-2-(*N*-2′-propynylamino)tetralin (3). A mixture of 8-methoxy-2-tetralone (**2**, 2.5 g, 14.2 mmol), prepared from 1,7-dihydroxynaphthalene in two steps,[19] 2-propynylamine (3.9 g, 4.9 mL, 5 equiv), and *p*-TsOH (150 mg, 0.8 mmol) in benzene (30 mL) was refluxed with a Dean–Stark trap for 2 h. The solvent was evaporated, and the residue was dissolved in MeOH (30 mL), to which NaCNBH$_3$ (540 mg, 8.7 mmol) was added in solid form. The solution was stirred at room temperature for 30 min and acidified with HCl (10%) to pH = 1. The mixture was extracted with ether, and the aqueous phase was treated carefully with solvent NaHCO$_3$ to pH ≥ 8. The resulting mixture was extracted with CH$_2$Cl$_2$. The combined organic layers were dried, evaporated to give a dark oil which was purified by flash chromotography (silica gel; eluent: CH$_2$Cl$_2$–MeOH, 96:4) to give the desired product (1.96 g) in 64% yield. IR (film, ν_{max}): 3300, 3000, 2900, 2200, 1620, 1600, 1250 cm^{-1}. ^{1}H NMR (CDCl$_3$, δ ppm): 1.57, 1.61 (1H, dt, J = 10.3, 6.2 Hz, NHCHCH_aH$_b$CH$_2$), 1.71 (1H, br, NH), 2.01 (1H, m, NHCHCH$_a$H$_b$CH$_2$), 2.21 (1H, t, J = 2.3 Hz, propynyl), 2.33 (1H, dd, J = 16.6, 9.1 Hz, ArCH$_a$H$_b$CHN), 2.84 (2H, m, ArCH$_2$CH$_2$CHN), 3.05 (1H, dd, J = 16.6, 4.8 Hz, ArCH$_a$H$_b$CHN), 3.18 (1H, m, NCH), 3.58 (2H, d, J = 2.4 Hz, NHCH$_2$), 3.80 (3H, s, OCH$_3$), 6.66 (1H, d, J = 8.1 Hz, Ar-H), 6.72 (1H, d, J = 7.8 Hz, Ar-H), 7.09 (1H, t, J = 7.9 Hz, Ar-H). MS: m/z 216 (M$^+$ + 1), 200, 161, 134, 115. Anal. (C$_{14}$H$_{17}$ON·HCl·$^1/_4$H$_2$O): C, H, N.

***trans*-8-Methoxy-2-[*N*-[3′-(tributylstannyl)-2′-propenyl]amino]tetralin (4).** A mixture of **3** (200 mg, 0.93 mmol), HSnBu$_3$ (650 mg, 0.6 mL, 2.2 mmol, 2.5 equiv), and AIBN (32 mg, 0.2 mmol) in toluene (5 mL) was stirred at 105 °C under N$_2$ for 4 h. The solvent was removed and the residue was purified by flash chromatography (silica gel; eluent: hexene–EtOAc, 3:1) to give **4** as a yellow oil (402 mg) in 85% yield. IR (film, ν_{max}): 3000, 1620, 1600, 1450, 1250 cm^{-1}. ^{1}H NMR (CDCl$_3$, δ ppm): 0.88 (15H, t, J = 7.3Hz, CH$_3$, SnCH$_2$), 1.30 (6H, hex, J = 7.1 Hz, CH$_3$CH$_2$), 1.50 (6H, m, CH$_3$CH$_2$CH$_2$), 1.6 (2H, m, ArCH$_2$CH$_2$CHN), 2.05 (1H, m, NH), 2.36 (1H, dd, J = 16.7, 9.2 Hz, ArCH$_a$H$_b$CHN), 2.88 (2H, m, ArCH$_2$CH$_2$CHN), 2.98 (1H, m, NCH), 3.10 (1H, dd, J = 16.8, 5.1 Hz, ArCH$_a$H$_b$CHN), 3.46 (2H, d, J = 3.7 Hz, NHCH$_2$), 3.81 (3H, s, OCH$_3$), 6.09 (1H, dt, J = 18.9, 4.0 Hz, CH=CHSn), 6.16 (1H, d, J = 18.9 Hz, CH=CHSn), 6.66 (1H, d, J = 8.0 Hz, Ar-H), 6.72 (1H, d, J = 7.7 Hz, Ar-H), 7.09 (1H, t, J = 7.9 Hz, Ar-H). MS: m/z 508 (M$^+$ + 1), 450, 392. Anal. (C$_{26}$H$_{45}$ONSn): C, H, N.

***trans*-8-Methoxy-2-[*N*-(3′-iodo-2′-propenyl)amino]tetralin (5).** To a solution of **4** (260 mg, 0.51 mmol) in CHCl$_3$ (20 mL) was added iodine (0.1 M solution in CHCl$_3$) dropwise at room temperature until an iodine color persisted. The mixture was stirred at room temperature overnight. KF (6 mL, 1 M in MeOH) and KHSO$_3$ (6 mL, 5% aqueous solution) were added. The mixture was stirred at room temperature for 30 min and extracted with CH$_2$Cl$_2$. After condensation, the crude product was purified by flash chromatography (eluent: hexane–EtOAc, 3:1) to give the desired product (145 mg) in 82% yield, which was purified by preparative thin-layer chromatography (PTLC) to give trans and cis isomers in 2:1 ratio (silica gel G F; hexane–EtOAc, 3:1, as the developing solvent). IR (film, ν_{max}): 2950, 2850, 1620, 1600, 1450, 1420, 1300, 1250 cm^{-1}. ^{1}H NMR (CDCl$_3$, δ) trans isomer: 1.48, 1.52 (1H, dt, J = 10.1, 6.1 Hz, CH$_2$CH$_a$H$_b$CHN), 1.70 (1H, m, NH), 1.93 (1H, m, CH$_2$CH$_a$H$_b$CHN), 2.25 (1H, dd, J = 16.6, 8.9 Hz, ArCH$_a$H$_b$CHN), 2.76 (2H, m, ArCH$_2$CH$_2$CHN), 2.87 (1H, m, CHN), 2.98 (1H, dd, J = 16.8, 4.8 Hz, ArCH$_a$H$_b$-

CHN), 3.30 (2H, d, J = 6.4 Hz, NCH_2), 3.74 (3H, s, OCH_3), 6.22 (1H, dt, J = 14.5, 1.2 Hz, CH=C*H*I), 6.58 (dt, J = 14.5, 6.2 Hz, C*H*=CHI), 6.59 (1H, d, J = 8.1 Hz, Ar-*H*), 6.67 (1H, d, J = 7.4 Hz, Ar-*H*), 7.02 (1H, t, J = 7.9 Hz, Ar-*H*). Cis isomer: 1.51, 1.56 (1H, dt, J = 10.2, 5.9 Hz, $CH_2CH_aH_bCHN$), 2.02 (1H, m, $CH_2CH_aH_bCHN$), 2.19 (1H, m, N*H*), 2.25 (1H, dd, J = 16.7, 9.0 Hz, $ArCH_aH_bCHN$), 2.78 (2H, m, $ArCH_2CH_2CHN$), 2.88 (1H, m, C*H*N), 3.03 (1H, dd, J = 16.6, 5.0 Hz, $ArCH_aH_bCHN$), 3.43 (2H, d, J = 5.8 Hz, NCH_2), 3.73 (3H, s, OCH_3), 6.28 (1H, dt, J = 7.5, 1.4 Hz, CH=C*H*I), 6.33 (dt, J = 7.5, 5.7 Hz, C*H*=CHI), 6.58 (1H, d, J = 8.1 Hz, Ar-*H*), 6.63 (1H, d, J = 7.6 Hz, Ar-*H*), 7.01 (1H, t, J = 7.9 Hz, Ar-*H*). MS: m/z 344 ($M^+ + 1$), 216, 212, 161. Anal. ($C_{14}H_{18}ONI$·HCl): C, H, N.

8-Hydroxy-2-[*N*-(3′-iodo-2′-propenyl)amino]tetralin (6). To a solution of **5** (60 mg, 0.17 mmol) in CH_2Cl_2 (5 mL) was added BBr_3 (1 mL, 1M in CH_2Cl_2, 5 equiv) dropwise at −78 °C in a dry ice–acetone bath. After completion of the addition, the cold bath was removed and the mixture was stirred at room temperature overnight. Ice water (5 mL) was added. The mixture was stirred at room temperature for 30 min and extracted with CH_2Cl_2. The aqueous phase was filtered, and the pH of the filtrate was adjusted to 7–8 with NaOH (1 M) solution, a white solid precipitated, and the whole mixture was extracted with CH_2Cl_2–MeOH (96:4). The organic layer was dried and evaporated to give a crude product which was purified by PTLC (silica gel G F; CH_2Cl_2–MeOH, 96:4, as solvent) to give the product (23 mg) in 40% yield. IR (film, ν_{max}): 3300, 2900, 2850, 1620, 1600, 1450, 1350, 1250 cm^{-1}. ^{1}H NMR ($CDCl_3$, δ) trans isomer: 1.17 (1H, br, N*H*), 1.46, 1.50 (1H, dt, J = 10.2, 6.5 Hz, $CH_2CH_aH_bCHN$), 1.96 (1H, m, $CH_2CH_aH_bCHN$), 2.25 (1H, dd, J = 16.2, 9.4 Hz, $ArCH_aH_b$-CHN), 2.73 (2H, m, $ArCH_2CH_2CHN$), 2.90 (1H, m, C*H*N), 3.00 (1H, dd, J = 16.4, 5.0 Hz, $ArCH_aH_bCHN$), 3.28 (2H, d, J = 6.8 Hz, NCH_2), 6.32 (1H, d, J = 14.6 Hz, CH=C*H*I), 6.52 (2H, d, J = 7.7 Hz, Ar-*H*), 6.56 (1H, dt, J = 14.8, 6.5 Hz, C*H*=CHI), 6.87 (1H, t, J = 7.7 Hz, Ar-*H*). MS: m/z 330 ($M^+ + 1$), 202, 162, 146, 120. Anal. ($C_{13}H_{16}ONI$): C, H, N.

***trans*-8-Methoxy-2-[*N*-propyl-*N*-(3′-iodo-2′-propenyl)amino]tetralin (7).** A mixture of *trans* **5** (145 mg, 0.42 mmol), 1-iodopropane (1 g, 0.6 mL, 5.9 mmol), and K_2CO_3 (0.3 g, 2.2 mmol) in EtOH (6 mL) was refluxed under N_2 for 40 h. The solvent was removed, and the residue was purified by flash chromatography (eluent: CH_2Cl_2–MeOH, 96:4) to give the product (130 mg) in 80% yield. IR (film, ν_{max}): 2900, 2800, 1620, 1600, 1450, 1400, 1250 cm^{-1}. ^{1}H NMR ($CDCl_3$, δ): 0.82 (3H, t, J = 7.4 Hz, CH_3), 1.39 (2H, hex, J = 7.4 Hz, CH_2CH_3), 1.50–2.78 (9H, m, CH_2, C*H*N), 3.12 (2H, d, J = 6.2 Hz, NCH_2), 3.75 (3H, s, OCH_3), 6.15 (1H, d, J = 14.4 Hz, CH=C*H*I), 6.53 (1H, dt, J = 14.3, 6.3 Hz, C*H*=CHI), 6.59 (1H, d, J = 8.4 Hz, Ar-*H*), 6.63 (1H, d, J = 7.7 Hz, Ar-*H*), 7.01 (1H, t, J = 7.9 Hz, Ar-*H*). MS: m/z 385 (M^+), 355, 258, 228, 161, 122. Anal. ($C_{17}H_{24}ONI$·HCl): C, H, N.

***trans*-8-Hydroxy-2-[*N*-propyl-*N*-(3′-iodo-2′-propenyl)amino]tetralin (8).** To a solution of **7** (120 mg, 0.31 mmol) in CH_2Cl_2 (10 mL) was added BBr_3 (1.3 mL, 1 M in CH_2Cl_2, 4 equiv) dropwise at −78 °C in a dry ice–acetone bath. The cold bath was removed, and the mixture was stirred at room temperature overnight. Ice water (10 mL) was added, and the mixture was stirred at room temperature for 30 min. The organic phase was separated, dried, and evaporated to give a crude product which was purified by PTLC (silica gel G F; CH_2Cl_2–MeOH, 96:4, as solvent) to give the product (88 mg) in 76% yield. IR (film, ν_{max}): 3400, 3100, 3000, 1620, 1600, 1450, 1300, 1250 cm^{-1}. ^{1}H NMR ($CDCl_3$, δ): 0.82 (3H, t, J = 7.4 Hz, CH_3), 1.41 (2H, hex, J = 7.4 Hz, CH_2CH_3), 1.48–2.98 (9H, m, CH_2, C*H*N), 3.15 (2H, d, J = 6.3 Hz, NCH_2), 6.19 (1H, d, J = 14.4 Hz, CH=C*H*I), 6.53 (1H, dt, J = 14.3, 5.3 Hz, C*H*=CHI), 6.54 (1H, d, J = 8.1 Hz, Ar-*H*), 6.61 (1H, d, J = 7.6 Hz, Ar-*H*), 6.92 (1H, t, J = 7.8 Hz, Ar-*H*). MS: m/z 372 ($M^+ + 1$), 341, 244, 147. Anal. ($C_{16}H_{22}ONI$·HCl): C, H, N.

8-Methoxy-2-(*N*-propyl-*N*-2′-propynylamino)tetralin (9). A mixture of **3** (500 mg, 2.33 mmol), 1-iodopropane (3.5 g, 2 mL, 20.5 mmol), and K_2CO_3 (1 g, 7.2 mmol) in EtOH (10 mL) was refluxed under N_2 for 18 h. The solvent was removed, and the residue was purified by flash chromatography (silica gel; eluent: hexene–EtOAc, 6:1) to give the product (520 mg) in 87% yield. IR (film, ν_{max}): 3300, 3000, 2800, 2200, 1620, 1600, 1450, 1420, 1300, 1250 cm^{-1}. ^{1}H NMR ($CDCl_3$, δ): 0.94 (3H, t, J = 7.4 Hz, CH_3), 1.57 (2H, m, CH_3CH_2), 1.60–2.16 (2H, m, CH_2CH_2CHN), 2.18 (1H, t, J = 2.3 Hz, propynyl), 2.47 (1H, dd, J = 16.6, 10.4 Hz, $ArCH_aH_bCHN$), 2.66 (2H, t, J = 7.5 Hz, $NCH_2CH_2CH_3$), 2.83–3.01 (3H, m, $ArCH_2CH_2CHN$, C*H*N), 3.08 (1H, dd, J = 16.6, 4.9 Hz, $ArCH_aH_bCHN$), 3.56 (2H, d, J = 2.3 Hz, NCH_2), 3.82 (3H, s, OCH_3), 6.66 (1H, d, J = 8.1 Hz, Ar-*H*), 6.71 (1H, d, J = 7.5 Hz, Ar-*H*), 7.09 (1H, t, J = 7.9 Hz, Ar-*H*). MS: m/z 257 (M^+), 242, 228, 161, 122. Anal. ($C_{17}H_{23}ON$·HCl·$^1/_4H_2O$): C, H, N.

8-Hydroxy-2-(*N*-propyl-*N*-2′-propynylamino)tetralin (10). To a solution of **9** (460 mg, 1.79 mmol) in CH_2Cl_2 (30 mL) was added BBr_3 (7.5 mL, 1 M in CH_2Cl_2, 4 equiv) dropwise at −78 °C in a dry ice–acetone bath under N_2. The cold bath was removed, and the mixture was stirred at room temperature overnight. Ice water (20 mL) was added, and the mixture was stirred at room temperature for 10 min. NaOH (1 M) solution was added to adjust the pH to 8, and the resulting mixture was extracted with CH_2Cl_2. Workup gave a crude product which was purified by flash chromatography (silica gel; eluent: CH_2Cl_2–MeOH, 96:4) to give the product (385 mg) in 89% yield. IR (film, ν_{max}): 3300, 3050, 2950, 2200, 1600, 1450, 1400, 1300, 1270 cm^{-1}. ^{1}H NMR ($CDCl_3$, δ): 0.93 (3H, t, J = 7.4 Hz, CH_3), 1.47–2.18 (4H, m, CH_2), 2.19 (1H, t, J = 2.3 Hz, propynyl), 2.49–3.08 (7H, m, CH_2, C*H*N), 3.57 (2H, d, J = 1.9 Hz, NCH_2), 6.59 (1H, d, J = 7.9 Hz, Ar-*H*), 6.68 (1H, d, J = 7.6 Hz, Ar-*H*), 6.99 (1H, t, J = 7.7 Hz, Ar-*H*). MS: m/z 243 (M^+), 228, 214, 147. Anal. ($C_{16}H_{21}ON$·HCl·$^1/_4H_2O$): C, H, N.

***trans*-8-Hydroxy-2-[*N*-propyl-*N*-[3′-(tributylstannyl)-2′-propenyl]amino]tetralin (11).** A mixture of **10** (90 mg, 0.37 mmol), $HSnBu_3$ (325 mg, 0.3 mL, 1.1 mmol, 3 equiv), and AIBN (24 mg, 0.15 mmol) in toluene (10 mL) was stirred at 105 °C under N_2 for 3 h. The solvent was removed after cooling, and the residue was purified by PTLC (silica gel G F; hexane–EtOAc, 1:3) to give an oil (95 mg) in 48% yield. IR (film, ν_{max}): 3400, 2970, 2920, 2850, 1600, 1350, 1300, 1250 cm^{-1}. ^{1}H NMR ($CDCl_3$, δ): 0.87 (9H, t, J = 7.2 Hz, CH_3), 0.84–0.92 (9H, m, $SnCH_2$, CH_3-CH_2), 1.21–2.86 (23 H, m, CH_2, C*H*N, CH_2N), 3.31 (2H, t, J = 4.3 Hz, NCH_2), 6.04 (1H, dt, J = 18.8, 4.8 Hz, CH_2CH=CHSn), 6.13 (1H, d, J = 18.9 Hz, CH=C*H*Sn), 6.60 (1H, d, J = 7.9 Hz, Ar-*H*), 6.67 (1H, d, J = 7.8 Hz, Ar-*H*), 6.98 (1H, t, J = 7.7 Hz, Ar-*H*). MS: m/z 536 ($M^+ + 1$), 478, 391, 332, 246. Anal. ($C_{28}H_{49}ONSn$): C, H, N.

Preparation of [^{125}I]-*trans*-8-OH-PIPAT ([^{125}I]-8). No-carrier-added [^{125}I]-*trans*-8-OH-PIPAT, [^{125}I]-**8**, was prepared by an iododestannylation reaction similar to the procedure reported previously.[20-22] Hydrogen peroxide (50 μL, 3% w/v) was added to a mixture of 50 μL of tributyltin precursor, **11** (11 mg/mL EtOH), 50 μL of 1 N HCl and I-125 (2–3 mCi) in a sealed vial. The reaction was allowed to proceed for 20 min at room temperature and was then terminated by addition of 0.1 mL of saturated sodium bisulfite. The reaction mixture was extracted with ethyl acetate (3 × 1 mL) after neutralization with saturated $NaHCO_3$ solution. The extracted ethyl acetate layers were evaporated to dryness, and the remaining residue was dissolved in EtOH and purified by HPLC using a reverse-phase column (PRP-1 column, Hamilton Co., Reno, NV) eluted with an isocratic solvent of 80% acetonitrile–20% pH 7.0 buffer (5 mM 3,3′-dimethylglutaric acid); the retention time was 9 min (1 mL/min). The fractions containing the desired product were collected, condensed, and reextracted with ethyl acetate (3 × 1 mL). The final product of no-carrier-added (yield 70–80 %; purity >98%) was evaporated to dryness and redissolved in 100 μL of 50% EtOH with 100 μg of ascorbic acid added as an antioxidant. The final product [^{125}I]-*trans*-8-OH-PIPAT, [^{125}I]-**8** , was stored at −20 °C. The stability of the product was evaluated from three preparations and was found to be stable for at least 4 weeks (>95% pure, analyzed by HPLC).

5-HT1$_A$ Binding Assay. The measurements of 5-HT$_{1A}$ binding sites, with [^{3}H]-8-OH-DPAT (Du Pont, Boston, MA) and [^{125}I]-*trans*-8-OH-PIPAT, [^{125}I]-**8**, respectively, were carried out as described previously.[10] 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 and subsequently preincubated at 37 °C and recentrifugated 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 0.2 mL containing 50 μL of tissue preparations (40–60 μg of protein), various amounts of radioligands (for saturation experiments) or appropriate amounts of labeled ligand (0.2–0.5 nM) and different concentrations of inhibitors (for competition experiments). The tubes were incubated at 37 °C for 15 min and then terminated by vaccum filtration through glass filter filters (Schleicher & Schuell, No. 25, 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. Both Scatchard and competition experiments were analyzed using the iterative nonlinear least-square curve-fitting program LIGAND.[24]

Dopamine Receptor Binding Assay. Dopamine D_2 and D_3 receptors expressed in *Spodoptera frugiperda* (Sf9) cells were kindly provided by Drs. P. Molinoff and V. Boundy. The binding assays for D_2 and D_3 receptors, respectively, with [^{125}I]NCQ298 as the labeled ligands were carried out as described.[25] Compounds at concentrations up to 10^{-5} M were examined for their ability to inhibit [^{125}I]NCQ298 at a concentration of 0.1 nM binding to D_2 and D_3 receptors, respectively.

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References

(1) Frazer, A.; Maayani, S.; Wolfe, B. B. Subtypes of Receptors for Serotonin. *Annu. Rev. Pharmacol. Toxicol*. **1990**, *30*,307–348.
(2) Frazer, A.; Hensler, J. G. 5-HT$_{1A}$ Receptors and 5-HT$_{1A}$-mediated Responses: Effect of Treatments That Modify Serotonergic Neurotransmission. *Ann. N.Y. Acad. Sci*. **1990**, *600*,460–475.
(3) Eison, A. S. Azapirones: History of Development. *J. Clin. Psychopharmacol*. **1990**, *10*, 2S-5S.
(4) Ackland, M.; Dring, L.; Jones, J.; Gilon, N. Synthesis of R-(+)- and S-(-)-8-hydroxy-2-(N,N-dipropylamino)-[2-^{3}H]tetralin. HCl (8-OH-DPAT) a 5-HT$_{1A}$ Receptor Agonist. *J. Lab. Compds. Radiopharm.* **1992**, *29*,909–918.
(5) Arvidsson, L.; Hacksell, U.; Nilsson, J. L. G.; Hjorth, S.; Carlsson, A.; Lindberg, P.; Sanchez, D.; Wikstrom, H. 8-Hydroxy-2-(di-n-propylamino)tetralin, a New Centrally Acting 5- hydroxytryptamine Receptor Agonist. *J. Med. Chem.* **1981**, *24*,921–923.
(6) Hjorth, S.; Carlsson, A.; Lindbery, P.; Sanchez, D.; Wikstrom, H.; Arvidsson, L. E.; Hacksell, U.; Nilsson, J. L. G. 8-hydroxy-2-(di-n-propylamino)tetralin 8-OH-DPAT, a Potent and Selective Simplified Ergot Congener with Central 5-HT Receptor Stimulating Activity. *J. Neural. Transmission* **1982**, *55*,169–188.
(7) Hall, M. D.; Mestikawy, S. E. L.; Emerit, M. B.; Pichat, L.; Hamon, M.; Gozlan, H. [3H]8-hydroxy-2-(di-n-propylamino)tetralin Binding to Pre-and Postsynaptic 5- hydroxytryptamine Sites in Various Regions of the Rat Brain. *J. Neurochem.* **1985**, *44*,1685–1696.
(8) Karlson, A.; Pettersson, C.; Sundell, S.; Arvidsson, L.; Hacksell, U. Improved Preparation, Chromatographic Separation and X-ray Crystallographic Determination of the Absolute Configuration of the Enantiomers of 8-hydroxy-2-(dipropylamino)tetralin (8-OH-DPAT). *Acta Chim. Scand.* **1988**, *B42*,231–236.
(9) Hoyer, D.; Engel, G.; Kalkman, H. O. Molecular Pharmacology of 5-HT1 and 5-HT2 Recognition Sites in Rat and Pig Brain Membranes: Radioligand Binding Studies with [3H]5-HT, [3H]8-OH-DPAT, (-)[125I]iodocyanopindolol, [3H]mesulergine and [3H]-ketanserin. *Eur. J. Pharmacol.* **1985**, *118*,13–23.
(10) Chamberlain, J.; Offord, S. J.; Wolfe, B. B.; Tyau, L. S.; Wang, H.-L.; Frazer, A. Potency of 5-HT1A Agonists to Inhibit Adenyl Cyclase Activity is a Function of Affinity for the Low Affinity State of [3H]8-OH-DPAT Binding. *J. Pharm. Exp. Ther.*, in press.
(11) Mellin, C.; Vallgarda, J.; Nelson, D. L.; Bjork, L.; Yu, H.; Anden, N.-E.; Csoregh, I.; Arvidsson, L.-E.; Hacksell, U. A 3-D Model for 5-HT1A-Receptor Agonists Based on Stereoselective Methyl-substituted and Conformationally Restricted Analogues of 8-hydroxy-2-(dipropylamino)tetralin. *J. Med. Chem.* **1991**, *34*,497–510.
(12) Mellin, C.; Bjork, L.; Karlen, A.; Johnnson, A.; Sundell, S.; Kenne, L.; Nelson, D.; Anden, N.; Hacksell, U. Central Dopaminergic and 5-hydroxytryptaminergic Effects of C3-methylated Derivatives of 8-hydroxy-2-(di-n-propylamino)tetralin. *J. Med. Chem.* **1988**, *31*,1130–1140.
(13) Wikstrom, H.; Andersson, B.; Elebring, T.; Lagerkvist, S.; Hallnemo, G.; Pettersson, I.; Jovall, P. A.; Svensson, K.; Ekman, A.; Carlsson, A. 6-Hydroxy-3-n-propyl-2,3,4,5-tetrahydro-1H-3-benzazepine d Analogs: New Centrally Acting 5-HT1A Receptor Agonists. *J. Med. Chem.* **1992**, *35*,3984–90.
(14) Naiman, N.; Lyon, R. A.; Bullock, A. E.; Tydelek, L. T.; Titeler, M.; Glennon, R. A. 2-(Alkylamino)tetralin Derivatives: Interaction with 5-HT1A Serotonin Binding Sites. *J. Med. Chem.* **1988**, *32*,253–256.
(15) Arvidsson, L.-E.; Hacksell, U.; Wilsson, J. L. G.; Hjorth, S.; Carlsson, A.; Lindberg, P.; Sanchez, D.; Wilkström, H. 8-Hydroxy-2-(di-n-propylamino)tetralin, A New Centrally Acting 5-Hydroxytryptamine Receptor Agonist. *J. Med. Chem.* **1981**, *24*, 921.
(16) Norman, A. B.; Battaglia, G.; Creese, I. [3H]WB4101 Labels the 5-HT$_{1A}$ Serotonin Receptor Subtype in Rat Brain: Guanine Nucleotide and Divalent Cation Sensitivity. *Mol. Pharmacol.* **1985**, *28*, 487–494.
(17) Dompert, W. U.; Glaser, T.; Traber, J. [3H]-TVX Q 7821: Identification of 5-HT$_1$ Binding Sites as Target For a Novel Putative Anxiolytic. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1985**, *328*, 467–470.
(18) Ransom, R. W.; Asarch, K. B.; Shih, J. C. [3H]-[2-(4-amino-phenyl)-ethyl]-4-(3-trifluoromethylphenyl) piperazine: A Selective Radioligand for 5-HT$_{1A}$ Receptors in Rat Brain. *J. Neurochem.* **1986**, *46*, 68–75.
(19) Ames, D. E.; Evans, D.; Grey, T.; Islop, P.; Richards, K. The Synthesis of Alkoxy-1,2,3,4-tetrahydronaphthalene Derivatives. Part I. 2-amino, alkylamino and dialkylamino Derivatives. *J. Chem. Soc.* **1965**, 2636.
(20) Kung, M.; Kung, H.; Chumpradit, S.; Foulon, C. In Vitro Binding of A Novel Dopamine D$_3$ Receptor Ligand: [^{125}I]*trans*-7-OH-PIPAT-A. *Eur. J. Pharm.* **1993**, *235*, 165–166.
(21) Chumpradit, S.; Kung, M.-P.; Billings, J. J.; Kung, H. F. Synthesis and Resolution of (+)-7-chloro-8-hydroxy-1-(3′-iodophenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzaepine (TISCH): A High Affinity and Selective Ligand for CNS D1 Dopamine Receptor. *J. Med. Chem.* **1991**, *34*,877–883.
(22) Kung, M.-P.; Kung, H. F.; Billings, J.; Yang, Y.; Murphy, R. A.; Alavi, A. The Characterization of IBF as a New Selective Dopamine D-2 Receptor Imaging Agent. *J. Nucl. Med.* **1990**, *31*,648–654.
(23) Foulon, C.; Kung, M.; Kung, H. Synthesis of (R,S)-2′-*trans*-7-hydroxy-2-[N-(3′-iodo-2′-propenyl)-N-n-propyl]aminotetralin (*trans*-7-OH-PIPAT): A new D3 Dopamine Receptor Ligand. *J. Med. Chem.* **1993**, *32*, 3114–3124.
(24) Munson, P.; Rodbard, D. LIGAND: A Versatile Computerized Approach for Characterization of Ligand-Binding Systems. *Anal. Biochem.* **1980**, *107*,220–239.
(25) Boundy, V. A.; Luedtke, R. R.; Gallitano, A. L.; Smith, J. E.; Filtz, T. M.; Kallen, R. G.; Molinoff, P. B. Expression and Characterization of the Rat D3 Dopamine Receptor: Pharmacologic Properties and Development of Antibodies. *J. Pharm. Exp. Ther.* **1993**, *264*,2001–2011.