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Substituted Hexahydrobenzodipyrans as 5-HT$_{2A/2C}$ Receptor Probes

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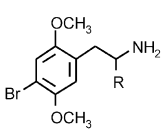
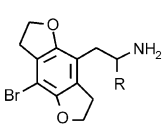
Abstract—A pair of substituted hexahydrobenzodipyrans was designed as molecular probes for determining the steric restrictions of the agonist binding site of serotonin 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. The rationale for the design of these receptor ligands, their chemical synthesis, rat behavioral pharmacology in the two-lever drug discrimination assay using LSD-trained rats, affinity for cloned rat 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors and agonist functional activities are reported.

Introduction

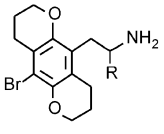
Efforts to model the ligand binding domain of the family of G-protein coupled receptors (GPCRs) have been somewhat hampered by a lack of X-ray crystallographic data for these proteins. Whereas there is little sequence homology between bacteriorhodopsin and the GPCRs,[1,2] past models of the more relevant rhodopsin have not yet provided the structural detail required to map accurately the ligand binding domain of the GPCRs. The recent publication of a 2.8 Å structure for bovine rhodopsin offers the exciting possibility of developing more precise models of GPCRs.[3] In the meantime, our structure–activity relationship (SAR) investigations of hallucinogens have sought to define precisely the topographical features of the agonist binding sites of serotonin 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, members of the larger family of GPCRs that are selectively activated by these hallucinogens.[4] In a series of SAR studies, we have applied a rigid analogue approach based on the 5-HT$_2$-specific, methoxylated phenethylamines, represented by **1a** and **1b**, to study these serotonin receptors.[5–8] Ultimately, we described the synthesis and pharmacological characterization of racemic **2a** and **2b**, which were found to have high affinity for the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor subtypes, with modest 5-HT$_{2C}$ subtype selectivity.[9] In that work, ligands **2a** and **2b** were shown to substitute fully for LSD in the rat, two-lever drug discrimination assay, and both had K_i values in the nanomolar and sub-nanomolar range in radioligand competition studies using both rat and human 5-HT$_2$ receptors, respectively. These results suggested that the tetrahydrobenzodifurans **2** define the optimum binding orientation of the arene alkoxy substituents for this entire class of hallucinogens. In a more recent investigation by Parker et al., the fully aromatized analogue of **2b** was found to have even higher affinity for 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, but showed no subtype selectivity.[10]



1a, R = H ('2C-B')
1b, R = CH_3('DOB')



2a, R = H
2b, R = CH_3



3a, R = H
3b, R = CH_3

In furthering our rigid analogue approach, the hexahydrobenzodipyrans **3a** and **3b** were designed as ring-expanded homologues of **2.** These compounds were constructed to probe the steric limitations of the 5-HT$_2$ agonist biding site in the regions surrounding the arene alkoxy substituents of this class of agents. As there are currently no agonists available that can differentiate between the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor subtypes,

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another goal of our investigations was to develop such an agent. Thus, we report here the synthesis of 10-bromo-5-(2-aminoethyl)-2,3,4,7,8,9-hexahydrobenzo[1,2*b*:4,5*b*′]dipyran **3a,** and 10-bromo-5-(2-aminopropyl)-2,3,4,7,8,9-hexahydro [1,2*b*:4,5*b*′]dipyran **3b**, as well as the results of our in vivo and in vitro pharmacological assays.

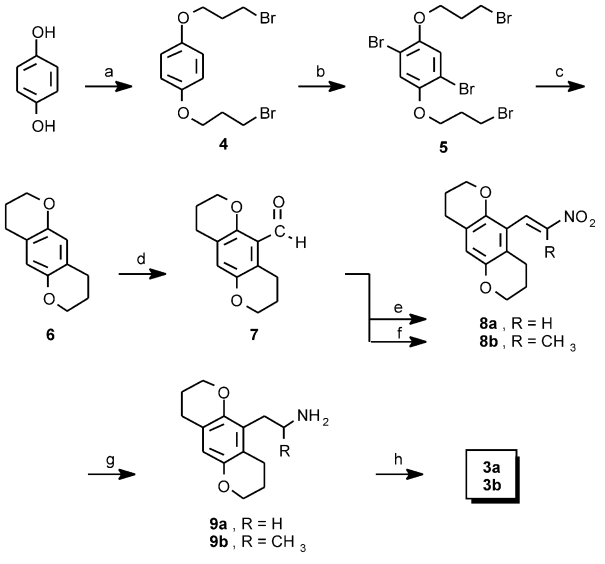
Results

Chemistry

The synthesis of target molecules **3a** and **3b** is outlined in Scheme 1. Briefly, commercially available hydroquinone was alkylated with 1,3-dibromopropane using K_2CO_3 in acetone, and the resulting diether **4** was brominated in methylene chloride with iron catalysis. Tandem cyclization of the tetrabromo compound **5** was accomplished by the addition of *n*-butyllithium at −78 °C, providing the key hexahydrobenzodipyran intermediate **6**. This dipyran (**6**) was then formylated by reaction with dichloromethyl methyl ether and tin(IV) chloride to provide the aldehyde **7**, which was condensed with either nitromethane or nitroethane to give the respective nitroalkenes, **8a** and **8b**. Lithium aluminum hydride was used to reduce the nitroalkenes to their corresponding amines, **9a** and **9b**, and these were brominated to yield the final racemic target compounds, **3a** and **3b**, respectively.

Pharmacology

Compounds **3a** and **3b** were evaluated in the two-lever drug discrimination (DD) behavioral assay to assess their hallucinogen-like activity in LSD-trained rats (Table 1). The new ring-expanded compounds, **3a** and **3b**, were found to substitute completely for LSD, and the potencies of LSD, **2a**, and **2b** from our previous studies are presented in the table for comparison. Additionally, **3a** and **3b** were tested for their ability to compete for radioligand binding sites in cloned rat 5-HT_{2A} and 5-HT_{2C} serotonin receptor subtypes, with data for compounds **1b** and **2b** included for comparison (Table 2). The functional activities of **3a** and **3b** were measured by determining their ability to stimulate phosphoinositide accumulation in NIH 3T3 cells expressing the 5-HT_{2A} receptor. Using methods described previously,[17] the mean EC_{50} values (±SEM, $n=3$) and intrinsic activities as a percent of maximal 5-HT (10 μM) stimulation were 257 (±47) nM and 45 (±3.5)% for **3a** and 117 (±26) nM and 82 (±8.1)% for **3b**.



Scheme 1. (a) 1,3-Dibromopropane, K_2CO_3, acetone; (b) Br_2, CH_2Cl_2, Fe; (c) *n*-butyllithium, THF; (d) dichloromethyl methyl ether, $SnCl_4$, CH_2Cl_2; (e) nitromethane, NH_4OAc; (f) nitroethane, NH_4OAc; (g) $LiAlH_4$, THF; (h) Br_2, CH_2Cl_2.

Table 1. Results of the drug dscrimination tests in rats trained to discriminate LSD (0.08 mg/kg; 186 nmol/kg) from saline

	Dose				ED_{50} (95% CI)	
Drug	mg/kg	μmol/kg	*N*	%SDL	mg/kg	μmol/kg
LSD[a]	0.01	0.023	13	46	0.012	0.026
	0.02	0.047	14	79	(0.006–0.019)	(0.014–0.045)
	0.04	0.093	16	81		
	0.08	0.186	15	100		
2a[b]	0.16	0.5	9	63	0.098	0.31
	0.32	1	12	67	(0.03–0.36)	(0.08–0.114)
	0.64	2	8	83		
2b[b]	0.02	0.06	13	46		
	0.04	0.13	14	80	0.02	0.061
	0.08	0.25	15	82	(0.009–0.04)	(0.03–0.12)
	0.17	0.5	7	86		
	0.33	1	7	100		
3a	0.35	1	10	11	0.87	2.48
	0.7	2	12	25	(0.58–1.29)	(1.65–3.71)
	1.39	4	11	82		
3b	0.18	0.5	9	56		
	0.36	1	11	64	0.17	0.47
	0.73	2	11	78	(0.06–0.45)	(0.18–1.23)
	1.45	4	10	91		
	2.9	8	6	100		

[a]Values shown for comparison taken from Gerasimov et al.[15]
[b]Values shown for comparison taken from Monte et al.[9]

Discussion

From the results of both the behavioral and in vitro pharmacological assays, it is clear that steric expansion

Table 2. Results of the radioligand competition binding studies at [^{125}I]DOI-labeled, cloned rat 5-HT_{2A} and 5-HT_{2C} receptors

Drug	5-HT_{2A} receptor K_i (nM)±SEM	5-HT_{2C} receptor K_i (nM) SEM
1b[a,b]	2.16±0.33	2.82±0.68
2b[a,c]	0.48±0.03	0.30±0.02
3a	1.76±0.09	1.52±0.48
3b	3.87±0.95	1.85±0.51

[a]Racemic α-methyl and α-desmethyl compounds have been shown to possess similar affinities in these assays.[16]
[b]Values taken from Chambers et al. with [^{3}H]DOB used in the 5-HT_{2A} receptor assay.[17]
[c]Values taken from Monte et al. using cloned *human* 5-HT_{2A} and 5-HT_{2C} receptors.[9]

from the di*furan* ring system of **2** to the di*pyran* ring system of **3** leads to a slight decrease in activity. In the drug discrimination assay, both **3a** and **3b** did fully substitute for LSD, but were approximately 9 times less potent than the smaller, difuranyl homologues (**2a** and **2b**). Additionally, it is interesting to note that whereas the receptor affinities of **3a** and **3b** are similar (2-fold difference), as expected,[16] there is a greater difference in potency between these compounds in the in vivo assay (5-fold difference). This result is analogous to those published previously by Monte et al. for the smaller compounds, **2a** and **2b**, and was not unexpected.[9] In the functional assays, the isopropylamine **2b** was approximately 2 times more potent and had a 2-fold greater intrinsic activity than the phenethylamine **2a**, indicating that the α-methyl group increases agonist potency and intrinsic activity (in addition to increasing hydrophobicity and reducing in vivo metabolism).

Although the receptor affinities of **3a** and **3b** are somewhat lower than those of **2a** and **2b** at both receptor subtypes, they are still in the low nanomolar range. This finding indicates that the six-membered rigid scaffold of the methoxy groups present in both **3a** and **3b** still does not present an unacceptable steric footprint to these receptors. Our initial goal was to test whether the 5-HT_{2A} and 5-HT_{2C} ligand binding domains could tolerate this ring expansion, and clearly they do. However, a considerable loss of functional activity is noted with expansion from the difuran to dipyran ring system. Chambers et al.[17] reported an EC_{50} value of 8.38 (±1.86) nM for *R*-**2b** in the phosphoinositide hydrolysis assay, an order of magnitude higher potency than the EC_{50} value for racemic **3b** found here.

In terms of mapping the 5-HT_2 agonist binding site, our findings are particularly interesting in light of Shulgin's earlier studies on the unconstrained ethyl homologues of 2,4,5-trimethoxyamphetamine (TMA-2).[18] In that work, it was shown that homologation of either the 2- or 5-methoxy substituents of TMA-2 to ethoxy groups led to a marked loss of pharmacological activity. In our present work, it is evident that this added steric bulk does not as greatly diminish activity when the oxygen substituents are constrained into the planar and semiplanar conformations of **2** and **3**, respectively. Furthermore, the structure of the ring system of **3** continues to support our model of the agonist binding site which indicates that the directionality of the 2-*O* and 5-*O* lone pair electrons is a crucial element in this chemical class of 5-HT agonists. Given the appropriate orientation of *O*-substituents, the receptor appears to have a fairly narrow range of tolerance of steric bulk, especially if this bulkiness can be projected far out of the plane of the aromatic ring. Thus, **2b**[9,17] and its aromatic analogue[10] still represent the best-fit models for the agonist binding sites of the 5-HT_{2A} and 5-HT_{2C} receptors, and these agents also possess the highest functional potencies. The dipyrans **3a** and **3b** may also be assumed to complement the agonist binding sites fairly well but show a notable reduction in functional activity. Furthermore, our model remains incomplete regarding the relative steric limitations about the 2-*O* versus the 5-*O* substituent. It now seems that it may be promising to explore how steric bulk around these ring substituents might be further manipulated to alter 5-HT_2 receptor affinity in general, as well as the 5-HT_{2A} versus 5-HT_{2C} subtype selectivity. These studies are currently underway in our laboratories.

Concerning receptor subtype selectivity, it is interesting to note here that both **3a** and **3b** demonstrated slightly increased affinity for the 5-HT_{2C} receptor over the 5-HT_{2A} subtype, in comparison with **1b**. In fact, both rigid heterocycles, **2** and **3**, demonstrated this increased affinity for the 5-HT_{2C} receptor, with a slightly greater 5-HT_{2C} selectivity being evident in the dipyranyl compound **3b**, versus its smaller difuranyl analogue **2b**. Although the observed differences in affinity between 5-HT_{2A} and 5-HT_{2C} receptors (2- to 5-fold) are not sufficient for **2** or **3** to be considered 'selective' 5-HT_{2C} agonists, they do emphasize the subtle differences in the topography of these two closely related receptor proteins. It is hoped that these subtle differences will ultimately enable the construction of rigid analogues related to **2** and **3** that might discriminate between these similar serotonin receptor subtypes.

Experimental

Chemistry

All reagents were commercially available and were used without further purification. Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded using a Bruker AC300 300 MHz NMR spectrometer, with chemical shifts reported in δ values (ppm) relative to an internal reference of tetramethylsilane (TMS). Coupling constants are reported in hertz (Hz), and abbreviations used are as follows: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, ArH = aromatic hydrogen. IR spectra were taken with a MIDAC Prospect IR, and are reported in cm^{-1}. Elemental analyses were performed by Quantitative Technologies, Inc. (QTI) of Whitehouse, NJ, USA, and all are within ±0.25% of the calculated values. Thin-layer chromatography was performed using silica gel IB2-F (2.5×7.5 cm) plastic-backed plates from J. T. Baker Inc. All reactions were carried out under an inert atmosphere of nitrogen unless otherwise noted.

1,4-Bis-(3-bromopropoxy)benzene (4).[19] Hydroquinone (80 g, 0.7265 mol), 222 mL (3.0 equiv) of 1,3-dibromopropane, 425 g (4.5 equiv) of K_2CO_3, and 1300 mL of acetone were added to a 3-L, three-neck, round-bottom flask equipped with a condenser and mechanical stirrer and stirred at reflux for 24 h. The K_2CO_3 was removed by filtration through Celite, and the solvent was removed on the rotary evaporator. The resulting brown solid was taken up in methylene chloride and washed with H_2O (3×100 mL), 3 N NaOH (3×100 mL), 3 M HCl (3×100 mL), and brine (3×100 mL) and dried over $MgSO_4$. The solvent was removed on the rotary eva-

porator to yield a brown solid that was crystallized from ethyl acetate and hexane to yield 124.34 g (49%) of **4** as white crystals: mp 68–69 °C (lit. mp 71–72 °C); IR: Ar–O–R (1220, 1031). ^{1}H NMR ($CDCl_3$) δ 2.30 (p, 4, $ArOCH_2CH_2CH_2Br$, $J=6.0$ Hz), 3.52 (t, 4, $ArOCH_2CH_2CH_2Br$, $J=7.5$ Hz), 4.15 (t, 4, $ArOCH_2CH_2CH_2Br$, $J=7.5$ Hz).

1,4-Bis-(3-bromopropoxy)-2,5-dibromobenzene (5). A 2-L, three-neck round-bottom flask was equipped with an addition funnel, condenser, N_2 inlet, and magnetic stir bar, and 50.76 g (0.144 mol) of **4**, followed by 600 mL of methylene chloride and a catalytic amount of iron filings were added. A mixture of 15.8 mL (2.14 equiv) of Br_2 and 100 mL of methylene chloride was placed in the addition funnel and was introduced to the reaction vessel dropwise, with stirring, over 1 h. After stirring for an additional 24 h, the mixture was poured into a separatory funnel and washed with 5% $Na_2S_2O_3$ (3 75 mL), saturated $NaHCO_3$ (3×75 mL), and brine (3×75 mL). The organic phase was dried ($MgSO_4$), and the solvent was removed on the rotary evaporator. The resulting dark-brown oil solidified when dried under high vacuum, and the solid was recrystallized from ethyl acetate to yield 52.46 g (71%) of **5** as a white powder: mp 115–117 °C. IR Ar–O–R (1216, 1066), Ar–Br (1028). ^{1}H NMR ($CDCl_3$) δ 7.4 (s, 2, Ar*H*), 4.0 (t, 4, $ArOCH_2CH_2CH_2Br$), 3.6 (t, 4, $ArOCH_2CH_2CH_2Br$), 2.3 (p, 4, $ArOCH_2CH_2CH_2Br$). Anal. ($C_{12}H_{14}O_2Br_4$) C, H.

2,3,4,7,8,9-Hexahydrobenzo [1,2*b*:4,5*b*] dipyran (6).[20] The tetrabromo compound, **5**, (30.45 g, 60.00 mmol) was added to a 1-L, three-neck, round-bottom flask equipped with a N_2 inlet, and a magnetic stir bar. Approximately 450 mL of anhydrous tetrahydrofuran (THF) was then added, and the mixture was cooled to −100 °C. Next, 27.0 mL (4.5 equiv) of 10 M *n*-butyllithium in hexanes was added slowly so the solution did not warm above −80 °C. After holding the reaction mixture at −100 °C for 45 min, the solution was warmed to −78 °C, and stirred for an additional 4.5 h before being quenched with 75 mL of H_2O. The THF was removed on the rotary evaporator, and the remaining liquid was taken up in ether, placed in a separatory funnel, and washed with H_2O (3×50 mL). The organic phase was dried ($MgSO_4$), and the ether was removed to yield a light-yellow solid that was recrystallized from ethyl acetate to give 7.63 g (67%) of **6** as white needles: mp 104–106 °C (lit. mp 105–106 °C). IR Ar–O–R (1235). ^{1}H NMR ($CDCl_3$) δ 6.4 (s, 2, Ar*H*), 4.1 (t, 4, $ArOCH_2$), 2.7 (t, 4, $ArOCH_2CH_2CH_2$), 1.9 (p, 4, $ArOCH_2CH_2$). Anal. ($C_{12}H_{14}O_2$) C, H.

5-Formyl-2,3,4,7,8,9-hexahydrobenzo[1,2*b*:4,5*b*′]dipyran (7). A 3.277 g (17.2 mmol) sample of **6** was placed in a 250-mL, three-neck, round-bottom flask equipped with a N_2 inlet and a magnetic stir bar. Approximately 100 mL of methylene chloride was added, and the solution was cooled on an ice bath. Tin(IV) chloride (3.00 mL, 1.5 equiv) was introduced to the stirred solution via syringe, whereupon the solution turned reddish-brown. Approximately 15 min later, 2.34 mL (1.5 equiv) of dichloromethyl methyl ether was added, changing the color of the solution to bright pink. After 45 min, the solution was poured into a separatory funnel containing approximately 25 mL of ice and water. The layers were separated and the organic phase was washed with 3 M HCl (2×20 mL), H_2O (2×20 mL), and brine (1×50 mL). The organic layer was dried ($MgSO_4$), and the solution was run through a short flash column of silica gel (100–200 mesh), eluting with methylene chloride. The solvent was then evaporated on the rotary evaporator, yielding a light-yellow solid that was recrystallized from ethyl acetate and hexane to yield 3.363 g (90%) of **7** as yellow needles: mp 79–80 °C. IR C=O (1681). ^{1}H NMR ($CDCl_3$) δ 10.5 (s, 1, ArCO*H*), 6.7 (s, 1, Ar*H*), 4.1 (t, 2, $ArOCH_2$, $J=5.1$ Hz), 4.05 (t, 2, $ArOCH_2$, $J=5.1$ Hz), 3.0 (t, 2, $ArOCH_2CH_2CH_2$, $J=6.6$ Hz), 2.7 (t, 2, $ArOCH_2CH_2CH_2$, $J=6.5$ Hz), 2.0 (m, 4, $ArOCH_2CH_2$). Anal. ($C_{13}H_{14}O_3$) C, H.

5-(2-Nitroethenyl)-2,3,4,7,8,9-hexahydrobenzo[1,2*b*:4,5*b*′] dipyran (8a). The aldehyde **7** (6.51 g, 29.80 mmol) was placed in a 100-mL, three-neck, round-bottom flask equipped with a N_2 inlet and a magnetic stir bar. Ammonium acetate (3.19 g, 1.39 equiv) was added to the flask, followed by 30.0 mL of nitromethane. The mixture was stirred at 80 °C for 4.5 h before the volatiles were removed under vacuum. The residue was taken up in methylene chloride and washed with 3 N HCl (3×25 mL), H_2O (2×25 mL), and brine (1×25 mL). The product was dried ($MgSO_4$), and the solvent was removed on the rotary evaporator. The crude product was recrystalized from methanol to yield 6.55 g (84%) of **8a** as orange needles: mp 139–140 °C. IR Ar–O–R (1247), R-NO_2 (1515). ^{1}H NMR ($CDCl_3$) δ 8.1 (d, 1, ArCH=C*H*, $J=13.4$ Hz), 8.0 (d, 1, ArC*H*=CH, $J=13.5$ Hz), 6.6 (s, 1, Ar*H*), 4.24 (t, 2, $ArOCH_2$, $J=5.2$ Hz), 4.0 (t, 2, $ArOCH_2$, $J=5.1$ Hz), 2.8 (t, 2, $ArOCH_2CH_2CH_2$, $J=6.7$ Hz), 2.7 (t, 2, $ArOCH_2CH_2CH_2$, $J=6.4$ Hz), 2.0 (m, 4, $ArOCH_2CH_2$). Anal. ($C_{14}H_{15}NO_4$) C, H, N.

5-(2-Nitro-1-propenyl)-2,3,4,7,8,9-hexahydrobenzo [1,2*b*:4,5*b*′]dipyran (8b). The aldehyde **7** (10.16 g, 46.50 mmol) was placed in a 100-mL, three-neck, round-bottom flask equipped with a magnetic stir bar and a N_2 inlet. Ammonium acetate (3.9 g, 1.08 equiv) was then added, followed by 40 mL of nitroethane. The mixture was stirred at 80 °C for 4.5 h before the nitroethane was removed on the rotary evaporator. The remaining residue was taken up in methylene chloride, placed in a separatory funnel, washed with 3 N HCl (2×25 mL), H_2O (2×25 mL), brine (1×25 mL), and dried ($MgSO_4$). After solvent removal under vacuum, the resulting orange solid was recrystallized from methanol to yield 10.68 g (83%) of **8b** as yellow crystals: mp 91–92 °C. IR Ar–O–R (1235), R-NO_2 (1524). ^{1}H NMR ($CDCl_3$) δ 7.8 (s, 1, ArC*H*=C), 6.5 (s, 1, Ar*H*), 4.1 (m, 4, $ArOCH_2CH_2CH_2$), 2.7 (t, 2, $ArOCH_2$, $J=6.5$ Hz), 2.5 (t, 2, $ArOCH_2$, $J=6.5$), 2.1 (s, 3, ArCH=CCH_3), 1.9 (m, 4, $ArOCH_2CH_2$). Anal. ($C_{15}H_{17}NO_4$) C, H, N.

5-(2-Aminoethyl)-2,3,4,7,8,9-hexahydrobenzo [1,2*b*:4,5*b*′] dipyran hydrochloride (9a•HCl). A double tipped needle was used to transfer 250 mL of anhydrous THF to a 500 mL, three-neck, round-bottom flask equipped with

a magnetic stir bar, a water cooled condenser, and an addition funnel containing 4.90 g (18.8 mmol) of **8a** dissolved in 200 mL of anhydrous THF. An ice bath was used to cool the system before an excess of $LiAlH_4$ (1.5 g, 39.5 mmol) was introduced to the stirring THF. The nitropropene was then added dropwise over the next 1.5 h after which the reaction was heated to reflux for an additional 4.5 h. The reaction was then cooled to approximately room temperature, and quenched by the careful addition of 200 mL of 5 N NaOH. A small portion of Celite was added to the mixture, and the solids were filtered through Celite, rinsing the filter cake thoroughly with methylene chloride. The volatiles were removed in vacuo and the residue was taken up in ether and the amine was extracted into 3 M HCl (4×25 mL). The aqueous solution was then made strongly basic with the addition of 100 mL 5 M NaOH, and the free amine was extracted with CH_2Cl_2 (4×mL). The organics were washed with brine (2×20 mL), dried ($MgSO_4$), and the CH_2Cl_2 was removed on the rotary evaporator. The residue was taken up in ether, and the hydrochloride salt was precipitated with the addition of 1 equiv of a 1.0 M solution of HCl in EtOH. The salt was recrystallized from EtOH and ether to yield 1.172 g (23.12%) of **9a•HCl** as a white salt: mp 287–290 °C. ^{1}H NMR (free base in $CDCl_3$) δ 6.36 (s, 1, Ar*H*), 4.05 (m, 4, $ArOCH_2CH_2CH_2$), 2.79 (m, 2, $ArCH_2CH_2NH_2$) 2.69 (m, 6, $ArCH_2CH_2NH_2$ and $ArOCH_2CH_2CH_2$), 1.90 (m, 4, $ArOCH_2CH_2CH_2$), 1.65 (bs, 2, NH_2). Anal. ($C_{14}H_{19}NO_2$) C, H, N.

5-(2-Aminopropyl)-2,3,4,7,8,9-hexahydrobenzo [1,2*b*:4,5*b*′] dipyran hydrochloride (9b•HCl). Using a method identical to that for the preparation of **9a•HCl**, 5.006 g (18.2 mmol) of **8b** was reacted with $LiAlH_4$ (4.165 g, 110 mmol) in 400 mL of anhydrous THF. Following workup, the free amine was taken up in ether, and the hydrochloride salt was precipitated with the addition of 1 equiv of a 1.0 M solution of HCl in EtOH. The salt was recrystallized from ethanol and ether to yield 2.144 g (42%) of **9b•HCl** as a fine white solid: mp 274–276 °C. ^{1}H NMR (DMSO-d_6) δ 8.15 (bs, 3, NH_3), 6.35 (s, 1, ArH), 4.11 (m, 2, $ArOCH_2CH_2CH_2$), 3.95, (m, 2, $ArOCH_2CH_2CH_2$), 3.27 (m, 1, $ArCH_2CH$), 2.75 (m, 2, $ArCH_2CH$), 2.65 (t, 4, $ArOCH_2CH_2CH_2$), 1.80 (m, 4, $ArOCH_2CH_2CH_2$), 1.10 (d, 3, $ArCH_2CHCH_3$). Anal. ($C_{15}H_{22}ClNO_2$) C, H, N.

10-Bromo-5-(2-aminoethyl)-2,3,4,7,8,9-hexahydrobenzo [1,2*b*:4,5*b*′]dipyran hydrochloride (3a•HCl). A 0.536 g (1.99 mmol) sample of the hydrochloride salt **9a•HCl** was placed in a 100 mL round-bottom flask, followed by the addition of 40 mL glacial acetic acid. The mixture was stirred until the salt was completely dissolved, and 9.0 mL of a 0.266 M solution of bromine in acetic acid was introduced to the mixture dropwise. The bromine color slowly dissipated as a white precipitate formed over the next 4 h. The acetic acid was removed in vacuo, and the residue was taken up in 50 mL of 3 M HCl and washed with ether (2×50 mL). The aqueous layer was made strongly basic with the addition of 5 M NaOH, and the free amine was extracted into CH_2Cl_2 (5×20 mL). The organics were combined, washed with brine (2×20 mL), dried ($MgSO_4$), and filtered through Celite. The volatiles were removed on the rotary evaporator, and the free amine was taken up in anhydrous ether. The product was precipitated as the hydrochloride salt with the addition of one equivalent of a 1.0 M solution of HCl in EtOH. The salt was recrystallized from EtOH and ether to yield 0.20 g (28.8%) of **3a•HCl** as fine white crystals: mp 321–322 °C. ^{1}H NMR (DMSO-d_6) δ 8.15 (bs, 3H, NH_3), 4.24 (m, 4H, $ArOCH_2CH_2CH_2$), 2.95 (m, 4H, $ArOCH_2CH_2CH_2$), 2.87 (t, 2H, $ArCH_2CH_2$), 2.79 (t, 2H, $ArCH_2CH_2$), 2.08 (m, 4H, $ArOCH_2CH_2CH_2$), Anal. ($C_{14}H_{19}BrClNO_2$) C, H, N.

10-Bromo-5-(2-aminopropyl)-2,3,4,7,8,9-hexahydro benzo[1,2*b*:4,5*b*′]dipyran hydrochloride (3b•HCl). Using a method identical to that for the preparation of **3a•HCl**, 1.033 g (3.64 mmol) of **9b•HCl** was dissolved in 20 mL of glacial acetic acid and treated with 7.2 mL of a 0.716 M solution of bromine in acetic acid. Following workup, the free base was precipitated as its hydrochloride salt with the addition of one equivalent of a 1.0 M solution of HCl in anhydrous EtOH. The salt was recrystallized from EtOH and ether to yield 1.034 g (78%) of **3b•HCl** as a white crystalline solid: mp 295–296 °C. ^{1}H NMR (DMSO-d_6) δ 8.25 (bs, 3, NH_3), 4.18 (m, 4, $ArOCH_2CH_2CH_2$), 3.39 (m, 1, $ArCH_2CH$), 2.90 (m, 2, $ArCH_2CH$), 2.6–2.8 (dt, 4, $ArOCH_2CH_2CH_2$), 1.98 (m, 4, $ArOCH_2CH_2CH_2$), 1.20 (d, 3, $ArCH_2CHCH_3$). Anal. ($C_{15}H_{21}BrClNO_2$) C, H, N.

Pharmacology

Drug discrimination assay in LSD-trained rats. The methods used in the drug discrimination assay are detailed extensively elsewhere.[9,11–15] Briefly, all drugs were dissolved in 0.9% saline and were injected intraperitoneally in a volume of 1 mL/kg, 30 min before the training or test sessions. Data were scored in a quantal fashion, and the lever on which the rat first emitted 50 presses was scored as the 'selected' lever. Potencies were measured using ED_{50} values with 95% confidence intervals (95% CI). The degree of substitution was determined by noting the maximum percentage of rats selecting the drug lever (% SDL) for all doses of the test drug. For drugs that completely substituted for the training drug (at least one dose resulting in a % SDL of 80% or greater), the ED_{50} values and 95% CIs intervals were determined using the method of Litchfield and Wilcoxon.[21]

Radioligand competition assays in NIH3T3 cells. Materials. [^{125}I]DOI (2200 Ci/mmol) was purchased from New England Nuclear (Boston, MA, USA). Mianserin was purchased from Sigma Chemical Corp. (St. Louis, MO, USA) and cinanserin was purchased from Research Biochemicals Incorporated (Natick, MA, USA). *Cell culture*: NIH3T3 fibroblast cells stably transfected with either the 5-HT_{2A} or 5-HT_{2C} receptor were a gift from Dr. David Julius. They were maintained in minimum essential medium containing 10% dialyzed fetal bovine serum (Gibco BRL) and supplemented with L-glutamine, Pen/Strep, and Geneticin.

The cells were cultured at 37 °C in a water-saturated atmosphere of 95% air and 5% CO_2. For radioligand competition assays, cells were split into 100 mm^2 culture dishes when they reached 90% confluence. Upon reaching 100% confluency in the culture dishes, the cells were washed with sterile filtered phosphate–buffer solution and left to incubate in serum-free Opti-MEM (Gibco-BRL) for 5 h. After this incubation, the cells were harvested by centrifugation (15,000×*g*, 20 min) and placed immediately in the −80 °C freezer until the assay was performed. *Radioligand competition assays*: Nonspecific binding was defined as that measured in the presence of 10 μM cinanserin (5-HT_{2A} expressing cells) or 10 μM mianserin (5-HT_{2C} expressing cells). Competition binding experiments were carried out in a total volume of 500 μL with 0.20 nM [^{125}I]DOI. Previously harvested cells were resuspended and added to each well containing assay buffer (50 mM Tris, 0.5 mM EDTA, 10 mM $MgCl_2$; pH 7.4), radioligand, and unknown compound (or in the case of the saturation isotherm assays, cinanserin or mianserin). The incubation was carried out at 25°C for 60 min and terminated by rapid filtration using a pre-chilled Packard 96-well harvester with GF/B Uni-filters (Packard Instrument Corp.) that had been incubating for 30 min in 0.3% polyethylenimine. The filters were rinsed using chilled wash buffer (10 mM Tris, 154 mM NaCl) and left to dry overnight. The following day, Microscint-O (Packard Instrument Corp.) was added and radioactivity was determined using a TopCount (Packard) scintillation counter. GraphPad Prism was used to analyze the saturation and competition binding curves.

Supplementary Material

Elemental analysis data

Compd	Molecular formula	Calculated (% C, H, N)	Found (% C, H, N)	Δ (% C, H, N)
5	$C_{12}H_{14}O_2Br_4$	28.27, 2.77, —	28.50, 2.49, —	0.23, 0.28, —
6	$C_{12}H_{14}O_2$	75.75, 7.43, —	75.91, 7.38, —	0.16, 0.05, —
7	$C_{13}H_{14}O_3$	71.53, 6.48, —	71.48, 6.45, —	0.05, 0.03, —
8a	$C_{14}H_{15}NO_4$	64.35, 5.80, 5.36	64.46, 5.76, 5.25	0.11, 0.04, 0.11
8b	$C_{15}H_{17}NO_4$	65.43, 6.24, 5.09	65.64, 6.20, 5.03	0.21, 0.04, 0.06
9a	$C_{14}H_{20}ClNO_2$	62.33, 7.47, 5.19	62.48, 7.56, 5.03	0.15, 0.09, 0.16
9b	$C_{15}H_{22}ClNO_2$	63.47, 7.83, 4.94	63.65, 7.84, 4.85	0.18, 0.01, 0.09
3a	$C_{14}H_{19}BrClNO_2$	48.23, 5.49, 4.02	48.40, 5.42, 3.85	0.17, 0.07, 0.19
3b	$C_{15}H_{21}BrClNO_2$	49.67, 5.85, 3.86	49.86, 5.92, 3.83	0.19, 0.07, 0.03

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References and Notes

1. Grigorieff, N.; Ceska, T. A.; Downing, K. H.; Baldwin, J.; Henderson, R. *J. Mol. Biol.* **1996**, *259*, 393.
2. Hulme, E. C.; Curtis, C. A.; Wheatley, M.; Harris, A. C.; Aitken, A. *Trends Pharmacol. Sci.* **1989**, *10*, 22.
3. Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Le Trong, I.; Teller, D. C.; Okada, T.; Stenkamp, T. E.; Yamamoto, M.; Miyano, M. *Science* **2000**, *289*, 739.
4. Trumpp-Kallmeyer, S.; Hoflack, J.; Bruinvels, A.; Hibert, M. *J. Med. Chem.* **1992**, *35*, 3448.
5. Nichols, D. E.; Hoffman, A. J.; Oberlender, R. A.; Riggs, R. M. *J. Med. Chem.* **1986**, *29*, 302.
6. Nichols, D. E.; Snyder, S. E.; Oberlender, R.; Johnson, M. P.; Huang, X. *J. Med. Chem.* **1991**, *34*, 276.
7. Monte, A. P.; Marona-Lewicka, D.; Cozzi, N. V.; Nelson, D. L.; Nichols, D. E. *Med. Chem. Res.* **1995**, *5*, 651.
8. Monte, A. P.; Waldman, S. R.; Marona-Lewicka, D.; Mayleben, M.; Mailman, R. B.; Wainscott, B.; Nelson, D. L.; Nichols, D. E. *J. Med. Chem.* **1997**, *40*, 2997.
9. Monte, A. P.; Marona-Lewicka, D.; Parker, M. A.; Mayleben, M.; Mailman, R. B.; Wainscott, B.; Nelson, D. L.; Nichols, D. E. *J. Med. Chem.* **1996**, *39*, 2953.
10. Parker, M. A.; Marona-Lewicka, D.; Lucaites, V. L.; Nelson, D. L.; Nichols, D. E. *J. Med. Chem.* **1998**, *41*, 5148.
11. Oberlender, R.; Nichols, D. E. *Psychopharmacology* **1988**, *95*, 71.
12. Monte, A. P.; Marona-Lewicka, D.; Cozzi, N. V.; Nichols, D. E. *J. Med. Chem.* **1993**, *36*, 3700.
13. Nichols, D. E.; Frescas, S.; Marona-Lewicka, D.; Huang, X.; Roth, B. L.; Gudelsky, G. A.; Nash, J. F. *J. Med. Chem.* **1994**, *37*, 4346.
14. Monte, A. P.; Marona-Lewicka, D.; Kanthasamy, A.; Sanders-Bush, E.; Nichols, D. E. *J. Med. Chem.* **1995**, *38*, 958.
15. Gerasimov, M.; Marona-Lewicka, D.; Kurrasch-Orbaugh, D. M.; Qandil, A. M.; Nichols, D. E. *J. Med. Chem.* **1999**, *42*, 4257.
16. Johnson, M. P.; Mathis, C. A.; Shulgin, A. T.; Hoffman, A. J.; Nichols, D. E. *Pharmacol. Biochem. Behav.* **1990**, *35*, 211.
17. Chambers, J. J.; Kurrasch-Orbaugh, D. M.; Parker, M. A.; Nichols, D. E. *J. Med. Chem.* **2001**, *44*, 1003.
18. Shulgin, A. T. *J. Med. Chem.* **1968**, *11*, 186.
19. Adams, R.; Whitehill, L. N. *J. Am. Chem. Soc.* **1941**, *63*, 2073.
20. Schill, G. *Ann. Chem.* **1966**, *64*, 17465.
21. Litchfield, J. T.; Wilcoxon, F. A. A. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99.