

CONFORMATIONALLY RESTRICTED TETRAHYDRO-1-BENZOXEPIN ANALOGS OF HALLUCINOGENIC PHENETHYLAMINES¹

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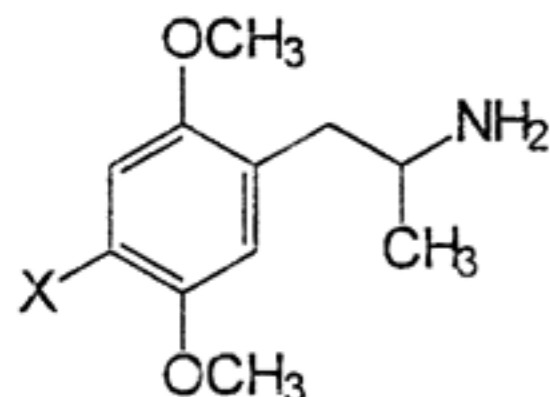
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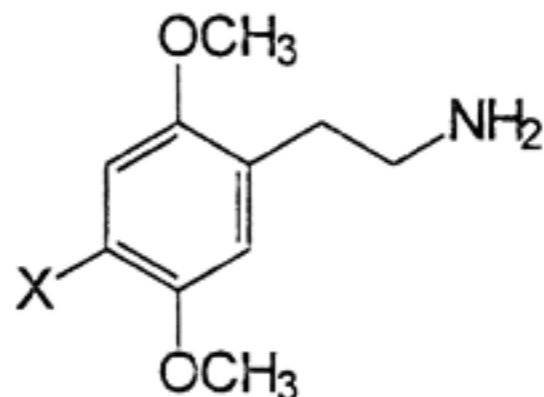
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Abstract: Tetrahydro-1-benzoxepin analogs of prototypical 4-substituted-2,5-dimethoxyphenylisopropylamines were prepared as agents having restricted conformational mobility in the 2-alkoxy group and alkylamine sidechain. The derivatives were evaluated for their ability to produce a discriminative stimulus in LSD-trained rats using the drug discrimination paradigm, for their affinity for radiolabeled serotonin 5-HT_{2A} and 5-HT_{1A} receptors, and for their ability to inhibit the accumulation of neurotransmitter monoamines in synaptosome preparations. None of the benzoxepins or substituted phenylisopropylamines had significant affinity for monoamine uptake sites. While both series of compounds had comparable low micromolar affinity for 5-HT_{1A} receptors, the benzoxepins had much lower affinity for 5-HT_{2A} receptors than the phenylisopropylamines (300-400 nM vs. ca. 20 nM). Probably reflecting this fact, only one of the benzoxepins fully substituted in the drug discrimination assay, with a potency about one-third that of the phenylisopropylamines tested.

Hallucinogenic phenylisopropylamines **1** and phenethylamines **2** currently represent some of the most potent and selective ligands for the agonist binding sites of serotonin 5-HT₂ receptors.²⁻⁷ For several years, we have been engaged in studies of the structure-activity relationships (SAR) of these agents and of hallucinogens in general to understand what unique structural features of this pharmacological class of molecules lead to their characteristic behavioral and psychological effects.^{8,9} Site-specific phenylalkylamines such as **1** and **2**, which primarily have high affinity for only 5-HT_{2A} and 5-HT_{2C} receptors,^{7,10,11} are of special interest to us as they can provide definite clues about the topography of the agonist binding sites of these membrane-bound proteins. As such, studies of these agents and their rationally-designed analogs may ultimately assist in our understanding of the basic molecular mechanisms of hallucinogenesis and of the role that serotonin and serotonergic neurons play in maintaining normal states of consciousness, as well as the pathophysiology of certain mental disorders.

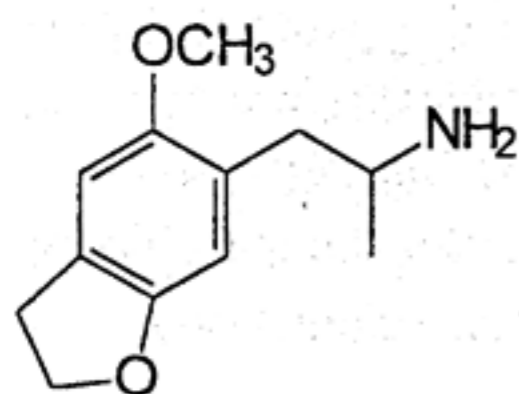


- 1a** X = CH₃, DOM
1b X = Br, DOB
1c X = I, DOI
1d X = CF₃, DOTFM

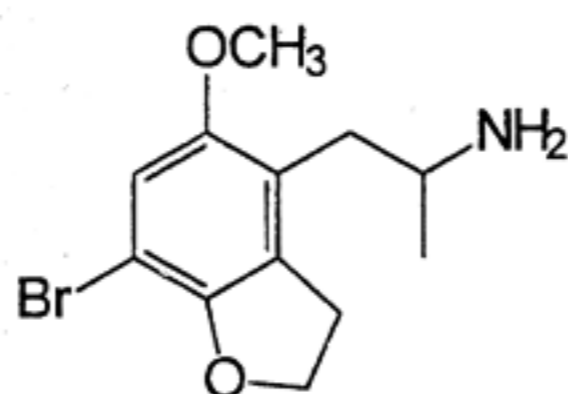


- 2a** X = Br, 2C-B
2b X = I, 2C-I
2c X = CF₃, 2C-TFM

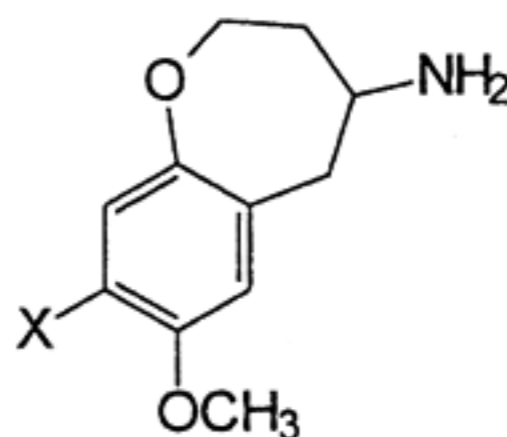
Given that the general orientation and nature of aromatic substituents seen in phenylalkylamines **1** and **2** (that is, the presence of the 2- and 5-methoxy groups coupled with a hydrophobic 4-substituent) seem to be required for optimum 5-HT_{2A/2C} receptor affinity,^{12,13} we recently began a series of investigations of the *conformations* of the aromatic methoxy groups in this class of compounds using a rigid analog approach. Thus, the rigid dihydrofuran derivatives **3** and **4**, agents in which the lone pair electrons on the heterocyclic oxygen atom are oriented either *syn* or *anti*, respectively, with respect to the sidechain, were synthesized and evaluated for their hallucinogen-like pharmacological activity.^{14,15} In these studies, the rigid DOM (**1a**) analog **3** was found to be nearly inactive in an *in vivo* behavioral assay for hallucinogen-like activity in rats,¹⁴ whereas the rigid DOB (**1b**) analog **4** was equipotent to the untethered parent compound, **1b**, in both *in vitro* and *in vivo* assays for hallucinogen-like pharmacological activity.¹⁵ Thus, it was evident from this work that the orientation of the aromatic methoxy groups strongly influenced receptor affinity and *in vivo* activity, and that this rigid analog approach could provide definite clues about the topography of the 5-HT₂ agonist binding site such as the relative locations of putative H-bond donors within the receptor.



3



4



- 5a** X = Br
5b X = I
5c X = CF₃

As an extension of those previous studies, the present work utilizes the tetrahydro-1-benzoxepin nucleus as a rigid analog of phenylalkylamines such as **1** in which the 2-methoxy group is tethered into the alkylamine sidechain such that the O2 lone pair electrons are oriented *anti* to, or away from, the sidechain. Thus, the racemic benzoxepin analogs **5a** and **5b** of racemic DOB (**1b**) and DOI (**1c**), respectively, were prepared and evaluated in a series of pharmacological assays for hallucinogen-like pharmacology. Since Nichols *et al.*⁷ recently reported that the 4-trifluoromethyl compounds **1d** and **2c** were even more potent 5-HT₂ agonists *in vivo* and *in vitro* than the bromo or iodo analogs, the 8-trifluoromethyl-1-benzoxepin **5c** was also prepared and evaluated in this work.

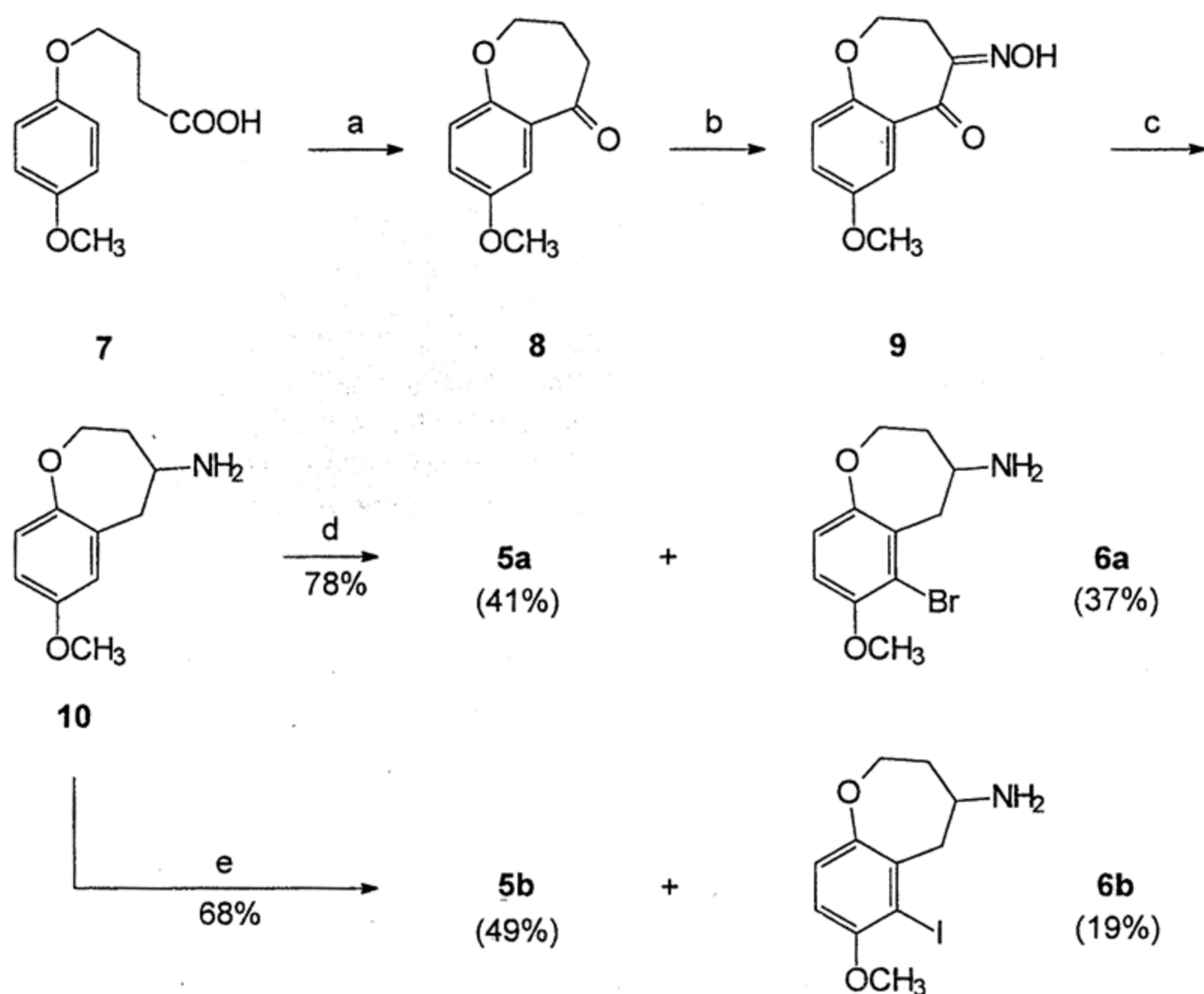
METHODS

Chemistry

Target compounds **5a** - **5c**, were synthesized in a relatively facile manner as depicted in Schemes 1 and 2. The known carboxylic acid **7** was formed by alkylation of *para*-methoxyphenol with ethyl 4-

bromobutyrate, followed by basic hydrolysis of the intermediate ester. Cyclodehydration was then accomplished by treatment with hot polyphosphoric acid as described by Khanna *et al.*¹⁶ to afford the tetrahydro-1-benzoxepin-5-one **8** in modest yield. Formation of the α -oxime **9**, followed by catalytic hydrogenation under acidic conditions using methods described previously,¹⁷ led to 4-amino-7-methoxy-2,3,4,5-tetrahydro-1-benzoxepin **10**. Bromination of **10** with elemental bromine in glacial acetic acid gave a mixture of 8-bromo **5a** and 6-bromo **6a** regioisomers that were easily separated by preparative centrifugal radial chromatography (Chromatotron) and identified by their characteristic aromatic proton coupling patterns. Alternatively, **10** was iodinated according to the method of Sy *et al.*^{18,19} using I_2 and Ag_2SO_4 in ethanol, to give a mixture of separable 8-iodo **5b** and 6-iodo **6b** regioisomers. This iodination method was found to be effective in producing the desired target compound **5b** as the major isomer (49% desired product vs. 19% undesired product).

Scheme 1^a

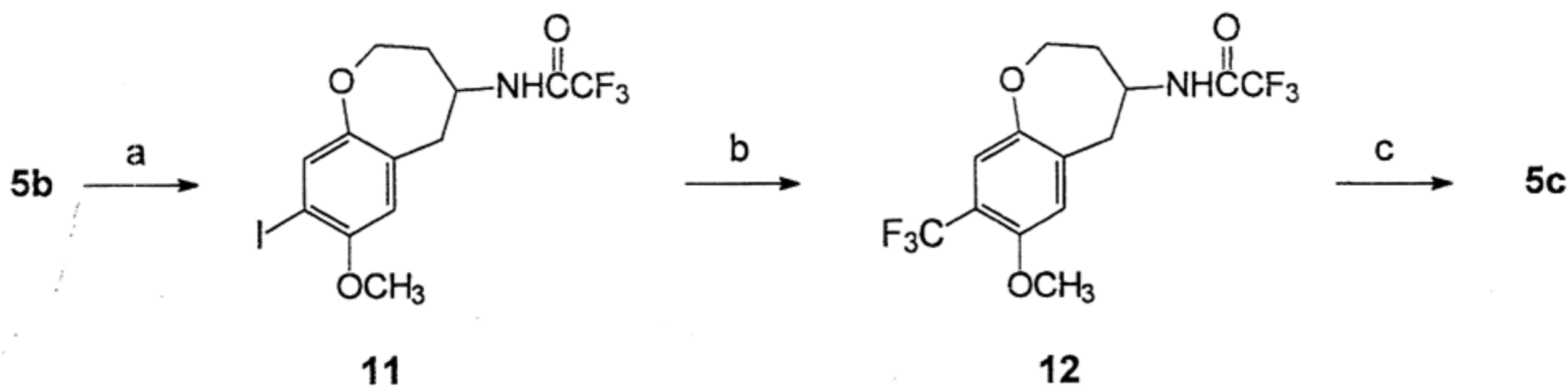


^a(a) polyphosphoric acid, 100 °C; (b) isoamyl nitrite, conc. HCl, CH₃OH, 50 °C; (c) 10% Pd/C, H₂, CH₃COOH, H₂SO₄; (d) Br₂, CH₃COOH; (e) I₂, Ag₂SO₄, ethanol.

The trifluoromethyl group was introduced into the benzoxepin nucleus as outlined in Scheme 2. Pure 8-iodo-1-benzoxepin **5b** was first *N*-protected to give **11**, and the iodine atom was replaced with the trifluoromethyl group using the procedure of Su *et al.*²⁰ The 8-trifluoromethyl-1-benzoxepin **12** was

then deprotected by holding at reflux in aqueous KOH and isopropanol for 5 h to afford pure **5c** in excellent overall yield.

Scheme 2^a



^a(a) $(CF_3CO)_2O$, CH_2Cl_2 , Et_3N ; (b) methyl-2-chloro-2,2-difluoroacetate, KF , CuI , DMF , $115\text{ }^\circ C$;
(c) $5N\text{ KOH}$, isopropyl alcohol, reflux.

Pharmacology

Following methods described in detail elsewhere,²¹ target compounds **5a** - **5c** were tested for their discriminative stimulus properties using the two-lever drug-discrimination (DD) paradigm in rats trained to discriminate LSD from saline. The data are shown in Table 1.

Compounds **5a** - **5c** were also evaluated for their affinity at [³H]ketanserin-labeled 5-HT_{2A} and [³H]8-OH-DPAT-labeled 5-HT_{1A} serotonin receptor populations using procedures described previously.²¹ The K_i values for the target compounds and selected reference compounds at these sites are listed in Table 2.

Finally, **5a** - **5c** and their analogous, non-rigidified phenylisopropylamines **1b** - **1d** were assayed for their ability to inhibit the accumulation of monoamine neurotransmitters (5-HT, DA, NE) in crude synaptosome preparations. The IC_{50} values from this assay are presented in Table 3.

RESULTS AND DISCUSSION

Only the trifluoromethyl-1-benzoxepin **5c** fully substituted for the training drug, LSD, in the drug discrimination assays (Table 1). The bromo **5a** and iodo **5b** compounds only partially substituted for LSD, but the majority of rats responding did select the drug lever at the dosage levels indicated (6.8 and $5.8\text{ }\mu\text{mol/kg}$, respectively). The results of these *in vivo* behavioral assays correlate fairly well with the 5-HT_{2A} receptor affinities shown in Table 2. Thus, these data show that the rigid benzoxepins **5a** - **5c**, while having some affinity for 5-HT_{2A} sites, are approximately 15 times less potent than the untethered parent molecules, **1b** and **1c**. In agreement with our previous findings regarding the rank order of potency of the untethered compounds **1b** - **1d**,⁷ the trifluoromethyl substituted benzoxepin **5c** was likewise found to be the most potent agent in this series. As anticipated, none of the compounds had significant affinity for serotonin 5-HT_{1A} sites (Table 2).

Table 1. Results of the Drug Discrimination Studies in LSD-trained Rats

Compound	ED ₅₀ (μmol/kg)	95% CI
5a	67% @ 6.8	PS ^a
5b	71% @ 5.8	PS ^a
5c	3.2	(2.31 - 4.48)
LSD	0.04	(0.03 - 0.05)
1b	1.12	(0.86 - 1.46)
2a	1.13	(0.83 - 1.54)

^aPS = partial substitution

Table 2. Results of the Radioligand Competition Studies at Rat 5-HT_{2A} and 5-HT_{1A} Receptors (*K_i* values ± SEM)

Compound	[³ H]Ketanserin-labeled 5-HT _{2A} Sites (nM)	[³ H]8-OH-DPAT- labeled 5-HT _{1A} Sites (nM)
	5a	422 ± 16
5b	307 ± 45	1010 ± 40
5c	340 ± 38	1300 ± 20
6a	1100	NA ^a
LSD	4.4 ± 0.2	5.1 ± 0.4
1b	22 ± 3	610 ± 50
1c	21 ± 3	1300 ± 100

^aNA = data not available.

There are at least three possible explanations for the reduced activity of compounds **5a - c**, as compared with their untethered parent compounds. First of all, the active conformation of the side chain in **1a - d** is not known, and it may simply be that the rigid analogues do not allow the phenethylamine moiety to adopt the necessary orientation for receptor activation. Second, the tethered compounds **5a - c** may be viewed as phenethylamines having alkyl substituents larger than a methyl group alpha to the primary amine--a feature that typically leads to reduced hallucinogen-like activity in this class of compounds.^{9,13} The other structural feature of **5a - 5c** possibly leading to reduced potency could be the improper orientation of the heterocyclic oxygen lone pair electrons for optimal receptor complementarity. Just as dihydrobenzofuran **3** was inactive,¹⁴ while its analog possessing the "opposite" O5 alkoxy group conformation **4**¹⁵ was quite potent both *in vitro* and *in vivo*, the benzoxepins may represent molecules having an O2 alkoxy conformation that is not complementary to the receptor topography. The evaluation of analogs of **1** in which the 2-methoxy group is tethered into the 3-position of the aromatic ring would be most revealing in this regard, since the O2 lone pairs would be oriented in the opposite direction of those in **5**. Indeed, such investigations are now underway in our laboratory.²²

Because we are also interested in novel agents that interact with monoamine transport proteins,²³ the benzoxepins **5a** - **5c** and their untethered analogs **1b** - **1d** were evaluated for their ability to inhibit the accumulation of [³H]serotonin (5-HT), [³H]dopamine (DA), and [³H]norepinephrine (NE) into crude synaptosomes. As shown in Table 3, none of the test compounds significantly inhibited the uptake of DA or NE, although all compounds did inhibit 5-HT accumulation at concentrations of less than 25 μ M with slope (Hill) coefficients of approximately 1, indicating that the drugs interacted with a single site. In general, all of the benzoxepins and phenylisopropylamines weakly inhibited 5-HT uptake at similar concentrations and were not particularly potent in this assay when compared with other specific 5-HT releasing agents recently discovered in our laboratory.^{17,23-25}

Table 3. Results of the Monoamine Uptake Inhibition Studies (IC_{50} values μ M \pm SEM)

Compound	[³ H]5-HT	[³ H]DA	[³ H]NE
5a	10.3 \pm 1.2	>50	>25
5b	10.9 \pm 2.5	>50	>25
5c	9.9 \pm 1.4	>100	>50
1b	14 \pm 2	>100	>50
1c	7.60 \pm 0.04	>50	>50
1d	19.9 \pm 0.9	>100	>50

EXPERIMENTAL SECTION

Chemistry. Melting points were determined using a Thomas-Hoover apparatus and are uncorrected. ¹H-NMR spectra were recorded using either a 500 MHz Varian VXR-500S or 300 MHz Bruker ARX-300 NMR spectrometer. Chemical shifts are reported in δ values (ppm) relative to tetramethylsilane (TMS) as an internal reference (0.03% v/v). Abbreviations used in NMR analyses are as follows: s = singlet, d = doublet, t = triplet, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets, q = quartet, p = pentet, m = multiplet, b = broad, Ar = aromatic, cp = cyclopropyl. Chemical ionization mass spectra (CIMS) using methane as the carrier gas were obtained with a Finnigan 4000 spectrometer. IR measurements were taken with a Perkin-Elmer 1600 Series FTIR spectrophotometer. Elemental analyses were performed by the Purdue University Microanalysis Laboratory and were within \pm 0.4% of the calculated values unless otherwise noted. Thin-layer chromatography (TLC) was typically performed using Baker-flex silica gel IB2-F, plastic-backed plates with fluorescent indicator (2.5 x 7.5 cm, J.T. Baker), eluting with CH₂Cl₂, and visualizing with UV light at 254 nm and/or I₂ vapor. Plates used for radial centrifugal chromatography ("Chromatotron," Harrison Research, Palo Alto, CA) were prepared from Silica Gel 60 PF2-54 containing gypsum.

7-Methoxy-2,3,4,5-tetrahydro-1-benzoxepin-5-one (8).¹⁶ A mixture of 61 g (0.290 mol) of the carboxylic acid **7** and 400 g of polyphosphoric acid was heated over a steam bath with vigorous stirring for 30 min. The mixture was cooled on an ice bath and 500 mL of ice cold water was added, followed

by 175 mL of diethyl ether. The layers were separated and the aqueous phase was extracted with ether (4 x 175 mL). The organic fractions were combined and washed with 0.5 N KOH (3 x 150 mL), water (3 x 500 mL), and brine (250 mL), dried (MgSO_4), and filtered through Celite. Removal of solvent on the rotary evaporator gave 33.5 g of a crude brown oil which was purified by Kugelrohr distillation (bp 110 °C at 0.5 mm Hg) to give 30.0 g (54%) of **8** as a colorless oil that darkened on prolonged exposure to air: ^1H NMR (acetone- d_6) δ 2.19 (p, 2, $\text{ArOCH}_2\text{CH}_2$, $J = 6.7$ Hz), 2.90 (t, 2, ArCOCH_2 , $J = 6.7$ Hz), 3.81 (s, 3, ArOCH_3), 4.20 (t, 2, $\text{ArOCH}_2\text{CH}_2$, $J = 6.6$ Hz) 6.80 (s, 1, ArH), 7.02 (s, 2, ArH); IR (neat) 1680 cm^{-1} (C=O); CIMS m/z 193 ($M + 1$).

7-Methoxy-3-oximino-2,3,4,5-tetrahydro-1-benzoxepin-5-one (9). To a solution of 28.5 g (0.148 mol) of the benzoxepin-one **8** in 350 mL of absolute methanol, warmed to 50 °C on an oil bath, was added 20.8 g (0.178 mol) of isoamyl nitrite, followed by 16 mL of concentrated HCl, added dropwise over a 15 min period. The mixture became dark yellow to reddish in color over the 2 h reaction time, after which it was cooled on an ice bath to induce precipitation of the product. The light yellow precipitate that formed was collected by suction filtration, rinsed with ether, and dried under high vacuum to give 15.2 g of product. The filtrate was concentrated on the rotary evaporator, and the residue was taken up in water and extracted with CH_2Cl_2 (3 x 100 mL). The organic fractions were combined, washed with 5% sodium bicarbonate solution (2 x 100 mL), water (100 mL), and brine, and dried over MgSO_4 . After filtration through Celite and removal of solvent on the rotary evaporator, the crude product was crystallized from hot methanol to give an additional 4.5 g of product as fluffy yellow crystals. Total yield of pure **9** was 19.7 g (60%): mp 170-171 °C; ^1H NMR (CDCl_3) δ 3.17 (t, 2, $\text{ArOCH}_2\text{CH}_2$, $J = 5.8$ Hz), 3.81 (s, 3, ArOCH_3), 4.38 (t, 2, $\text{ArOCH}_2\text{CH}_2$, $J = 5.8$ Hz), 7.00 (d, 1, ArH , $J = 9$ Hz) 7.09 (dd, 1, ArH , $J = 9$ Hz, 3 Hz), 7.40 (d, 1, ArH , $J = 3$ Hz); IR (KBr pellet) 1490 cm^{-1} (C=N), 1654 cm^{-1} (C=O), 3300 cm^{-1} (broad, N-OH); CIMS m/z 222 ($M + 1$), 206; Anal. ($\text{C}_{11}\text{H}_{11}\text{NO}_4$) C, H, N.

4-amino-7-methoxy-2,3,4,5-tetrahydro-1-benzoxepin hydrochloride (10). A 500 mL Parr hydrogenation flask was charged with 10.0 g (45.2 mmol) of the oximino-ketone **9**, 375 mL of glacial acetic acid, 6 mL of concentrated H_2SO_4 , and 1.0 g of 10% Pd on activated carbon. The mixture was shaken under 65 psi of H_2 until the theoretical amount of hydrogen had been taken up. The catalyst was removed by filtration through Celite, washing with CH_2Cl_2 and water, and the volatiles were removed on the rotary evaporator. The residue was taken up in water, washed with ether (2 x 50 mL), and basified with 5N NaOH while cooling over ice. The basic aqueous phase was extracted with CH_2Cl_2 (4 x 40mL) and the fractions were combined and dried over MgSO_4 and filtered through Celite. After removal of the solvent on the rotary evaporator, the free amine (7.85 g, 96%), a yellow oil, was taken up in ether and precipitated as its hydrochloride salt by the addition of an anhydrous solution of 1M HCl in ethanol. The volatiles were removed *in vacuo*, and the salt was recrystallized from ethanol-ethyl acetate to give 7.35 g (71%) of **10**·HCl as a white crystalline solid: mp 225-226 °C; ^1H NMR (free base in CDCl_3) δ 1.41 (bs, 2, NH_2), 1.90 (m, 1, $\text{ArOCH}_2\text{CH}_2$), 2.10 (m, 1, $\text{ArOCH}_2\text{CH}_2$), 2.80 - 2.90 (m, 2, ArCH_2CHN), 3.15 (m, 1, $\text{ArOCH}_2\text{CH}_2$), 3.78 (s, 3, ArOCH_3) superimposed on 3.79 (m, 1, $\text{ArOCH}_2\text{CH}_2$), 4.18 (m, 1, ArCH_2CHN), 6.66 (dd, 1, ArH , $J = 9$ Hz, 3 Hz), 6.70 (d, 1, ArH , $J = 3$ Hz), 6.91 (d, 1, ArH , $J = 9$ Hz); CIMS m/z 194 ($M + 1$), 177; Anal. ($\text{C}_{11}\text{H}_{15}\text{NO}_2\cdot\text{HCl}$) C, H, N.

4-Amino-8-bromo-7-methoxy-2,3,4,5-tetrahydro-1-benzoxepin hydrochloride (5a). To a stirred solution of 0.75 g (3.26 mmol) of the free amine **10** in 7 mL of glacial acetic acid was added a solution of 0.52 g (3.26 mmol) of elemental bromine in 1 mL of glacial acetic acid, dropwise over 30 min. After 6 h of stirring, the HBr salt of the product had formed, and dry ether was added to the reaction mixture to precipitate more salt. The salt was collected by suction filtration and rinsed with ether on the filter. The collected salt was dissolved in water, made strongly basic by the addition of 5N KOH, and the free base was extracted into CH₂Cl₂ (4 x 15 mL). The filtrate was concentrated on the rotary evaporator, taken up in 5N NaOH, and likewise extracted with CH₂Cl₂. The organic fractions were combined, washed with brine, dried over MgSO₄, filtered through Celite, and concentrated on the rotary evaporator. After drying under high vacuum, the crude product weighed 0.72 g and contained the 6- and 8-bromo regioisomers as evidenced by TLC analysis. These isomers were separated using centrifugal radial chromatography ("Chromatotron," 4 mm silica plate, CH₂Cl₂, N₂/NH₃ atmosphere), to give, in order of elution and after solvent removal, 0.33 g (37%) of the 6-bromo isomer **6a** and 0.36 g (41%) of the desired 8-bromo isomer **5a** as pale yellow oils. The regioisomers were differentiated on the basis of their aromatic proton ¹H NMR coupling constants. The hydrochloride salt was formed for each of the isomers by dissolving the free base in anhydrous ether and adding an equivalent amount of anhydrous HCl in absolute ethanol. After rotary evaporation of the volatiles, the salts were recrystallized from ethanol-ethyl acetate to give the pure compounds as white crystalline solids. For the title compound, **5a**·HCl: mp 228-229 °C; ¹H NMR (free base in CDCl₃) δ 1.30 (bs, 2, NH₂), 1.90 (m, 1, ArOCH₂CH₂), 2.10 (m, 1, ArOCH₂CH₂), 2.80 - 2.90 (m, 2, ArCH₂), 3.10 (m, 1, ArOCH₂CH₂), 3.78 - 3.85 (m, 1, ArOCH₂CH₂), 3.90 (s, 3, ArOCH₃), 4.19 (m, 1, ArCH₂CHN), 6.68 (s, 1, ArH), 7.20 (s, 1, ArH); CIMS *m/z* 272, 274 (M + 1), 255, 257, 194; Anal. (C₁₁H₁₅NO₂BrCl) C, H, N.

The undesired isomer, 4-amino-6-bromo-7-methoxy-2,3,4,5-tetrahydro-1-benzoxepin **6a**, was identified by the *ortho* coupling constants between the two aromatic protons. For the hydrochloride: mp 281-282 °C dec.; ¹H NMR (CD₃OD) δ 2.18 (m, 2), 3.18 (dd, 1), 3.38 (m, 1), 3.60 (d, 1), 3.71 (t, 1), 3.85 (s, 3), 4.37 (dt, 1), 6.90 (d, 1, *J* = 8.9 Hz), 7.00 (d, 1, *J* = 8.9 Hz).

4-Amino-8-iodo-7-methoxy-2,3,4,5-tetrahydro-1-benzoxepin hydrochloride (5b). Following the iodination procedure of Sy,^{18,19} 3.31 g (26.1 mmol) of I₂ was added to a stirred mixture of 1.68 g (8.7 mmol) of the free amine **10** and 8.14 g (26.1 mmol) of Ag₂SO₄ in 150 mL of absolute ethanol. The mixture was stirred under N₂ at room temperature for 42 h, filtered through Celite, and concentrated on the rotary evaporator. The residue was taken up into CHCl₃, the solution was washed with 3 x 100 mL of 1 N KOH and 2 x 100 mL of brine and filtered through Celite. The solvent was removed under reduced pressure to give 2.1 g of a brown oil that contained the 6- and 8-iodo regioisomers, as evidenced by TLC analysis. The isomers were separated on the Chromatotron (4 mm silica plate, CH₂Cl₂, N₂/NH₃), and the oily bases were converted to their hydrochloride salts. The minor 6-iodo regioisomer **6b** eluted from the Chromatotron first, and its hydrochloride salt was recrystallized from ethanol-ethyl acetate. The second band to elute from the Chromatotron contained the desired title compound **5b**. Its hydrochloride was recrystallized from methanol-ethyl acetate-ether to give 1.53 g (49%) of **5b**·HCl as fluffy, white crystals: mp 247 °C; ¹H NMR (hydrochloride in CD₃OD) δ 2.10 - 2.25 (m, 2,

ArOCH₂CH₂), 2.95 (d, 1, ArCH₂, *J* = 14 Hz), 3.10 (dd, 1, ArCH₂, *J* = 14 Hz, 10 Hz), 3.40 (m, 1, ArOCH₂CH₂), 3.67 (m, 1, ArOCH₂CH₂), 3.82 (s, 3, ArOCH₃), 4.29 (m, 1, ArCH₂CHNH₂), 6.79 (s, 1, ArH), 7.39 (s, 1, ArH); CIMS *m/z* 320 (*M* + 1), 303; Anal. (C₁₁H₁₄NO₂I·HCl) C, H, N.

The minor isomer, 4-amino-6-iodo-7-methoxy-2,3,4,5-tetrahydro-1-benzoxepin hydrochloride **6b**·HCl, was obtained as a white, crystalline solid: mp 228-230 °C; ¹H NMR (hydrochloride in CD₃OD) δ 2.15-2.25 (m, 2), 3.34 (m, 2), 3.58 (d, 1), 3.72 (t, 1), 3.81 (s, 3), 4.39 (dt, 1), 6.80 (d, 1, *J* = 8.7 Hz), 7.02 (d, 1, *J* = 8.7 Hz).

***N*-(Trifluoroacetyl)-4-amino-8-iodo-7-methoxy-2,3,4,5-tetrahydro-1-benzoxepin (11)**. To an ice bath-cooled solution of 0.89 g (2.81 mmol) of free amine **5b** in 30 mL of dry CH₂Cl₂ was added 0.39 mL (2.81 mmol) of triethylamine and 0.79 mL (5.62 mmol) of trifluoroacetic anhydride *via* syringe through a septum. The mixture was stirred for 15 min, the ice bath was then removed, and the mixture was allowed to warm to room temperature. After 5 h, the volatiles were removed on the rotary evaporator, and the residue was taken up in ether. The ether phase was washed with 3 x 30 mL of 1 N HCl, 2 x 30 mL of 5% NaHCO₃, 30 mL of H₂O, and 30 mL of brine, dried (MgSO₄), and filtered through Celite. Removal of solvent under reduced pressure gave a white solid that was recrystallized from ethyl acetate-hexane to afford 0.98 g (84%) of **11** as small white needles: mp 148-149 °C; ¹H NMR (CDCl₃) δ 2.10 - 2.24 (m, 2, ArOCH₂CH₂), 3.02 (m, 2, ArCH₂), 3.83 (s, 3, ArOCH₃), 3.9 - 4.1 (m, 2, ArOCH₂CH₂), 4.25 (m, 1, ArCH₂CHN), 6.20 (bs, 1, NH), 6.58 (s, 1, ArH), 7.44 (s, 1, ArH); CIMS *m/z* 416 (*M* + 1), 302, 289; Anal. (C₁₃H₁₃NO₃F₃I) C, H, N.

***N*-(Trifluoroacetyl)-4-amino-7-methoxy-8-trifluoromethyl-2,3,4,5-tetrahydro-1-benzoxepin (12)**. Following a modification of the procedure by Su *et al.*²⁰ a mixture of the *N*-protected iodo-benzoxepin **11** (0.75 g, 1.81 mmol), 0.602 g (3.16 mmol) of CuI, 0.21 g (3.61 mmol) of KF, and 4 mL of dry DMF was heated on a 125 °C oil bath while stirring under N₂. After stirring for 30 min, a solution of 0.65 g (4.5 mmol) of methyl 2-chloro-2,2-difluoroacetate in 1 mL of DMF was introduced into the mixture portionwise over a 6 h period. (*Very slow addition of this reagent was crucial for the success of this reaction.*) During the addition, the reaction mixture changed in appearance from a dark orange color to light yellow. After 21 h the mixture was dark orange in color; it was cooled to room temperature, diluted with 40 mL of ether, and filtered through Celite to remove the pink precipitate. The ethereal filtrate was washed with 3 x 50 mL of water and 2 x 50 mL of brine, dried over MgSO₄, and filtered through Celite. Removal of solvent under reduced pressure gave an orange solid that was recrystallized from ethyl acetate-hexane to afford 521 mg (80 %) of **12** as a white crystalline solid: mp 140 °C; ¹H NMR (CDCl₃) δ 2.20 - 2.27 (m, 2, ArOCH₂CH₂), 3.10 (d, 2, ArCH₂), 3.84 (s, 3, ArOCH₃), 3.98 (m, 1, ArOCH₂CH₂), 4.20 (m, 1, ArOCH₂CH₂), 4.28 (m, 1, ArCH₂CHN), 6.23 (bs, 1, NH), 6.77 (s, 1, ArH), 7.24 (s, 1, ArH); CIMS *m/z* 358 (*M* + 1), 289; Anal. (C₁₄H₁₃NO₃F₆) C, H, N.

4-Amino-7-methoxy-8-trifluoromethyl-2,3,4,5-tetrahydro-1-benzoxepin hydrochloride (5c). The *N*-trifluoroacetyl benzoxepin **12** (420 mg, 1.18 mmol) was deprotected by reflux in 5 mL of 5 N KOH and 25 mL of 2-propanol for 5 h. The alcohol was removed on the rotary evaporator, and the residue was diluted by the addition of 30 mL of 6 N HCl. The aqueous phase was washed with CH₂Cl₂ (3 x 15 mL) and then made strongly basic by the addition of 5 N KOH. The aqueous phase was

extracted with 4 x 20 mL of CH₂Cl₂, and the fractions were combined and washed with brine. After drying (MgSO₄) and solvent removal *in vacuo*, 260 mg (85%) of the base **5c** was obtained as a white, crystalline solid. The amine was precipitated as its hydrochloride salt by dissolving it in ether and adding 1 N anhydrous HCl in ethanol. Recrystallization from ethanol-ethyl acetate gave 284 mg (81%) of pure **5c**·HCl as fine, white crystals: mp 229-231 °C; ¹H NMR (free amine in CDCl₃) δ 1.55 (bs, 2, NH₂), 1.90 - 2.10 (m, 2, ArOCH₂CH₂), 2.85 - 3.00 (m, 2, ArCH₂), 3.82 (m, 2, ArOCH₂CH₂), 3.85 (s, 3, ArOCH₃), 4.20 (m, 1, ArCH₂CHN), 6.78 (s, 1, ArH), 7.20 (s, 1, ArH); CIMS 262 (M + 1), 242; Anal. (C₁₂H₁₄NO₂F₃·HCl) C, H, N.

Pharmacology. The methods used in the drug-discrimination and radioligand binding assays have been described in detail elsewhere.²¹ Briefly, the drug discrimination (DD) paradigm was employed with rats trained to discriminate LSD tartrate (0.08 mg/kg) from saline. Rats were trained on a fixed ratio (FR50) schedule of reinforcement. ED₅₀ values from the DD assay were determined from dose-response curves using 4-6 doses per test compound with 8-12 rats tested at each dose. When full substitution did not occur, the maximum percentage of animals responding on the drug appropriate lever is reported, with the dose producing that response.

As described previously,²¹ affinity for the 5-HT_{2A} receptor was measured by competition with 0.75 nM [³H]ketanserin in rat frontal cortex homogenate at 37 °C, with a 15 min equilibration, followed by rapid filtration through GF/C filters and washing with ice-cold buffer. Specific binding was determined using 10 μM cinanserin. Affinity measurements at the 5-HT_{1A} receptor were measured by competition with 0.75 nM [³H]8-OH-DPAT in rat hippocampal homogenate at 37 °C, with a 10 min equilibration, followed by filtration through GF/C filters and washing. Specific binding was defined using 10 μM 5-HT. Filters were air-dried, placed in scintillation vials containing 10 mL of Ecolite scintillation cocktail, and allowed to sit overnight before counting. All experiments were performed in triplicate and the values from three to four separate experiments were combined. Data were analyzed using the computer programs EBDA and LIGAND.²⁶

The *in vitro* [³H]neurotransmitter uptake studies using crude synaptosomes were carried out as follows: For each experiment, three rats (260 - 305 g) were decapitated and their brains removed rapidly over ice. The cerebellums were removed and discarded, and the remaining brain tissue (4.37 ± 0.06 g wet weight) was pooled, diced, and homogenized in 5 volumes of ice-cold isotonic sucrose. Homogenization was done in a prechilled glass mortar with a motor-driven Teflon pestle at 0 °C for two periods of 1 min each, 6 strokes/min, with a 15 s interval between periods. The tissue homogenate was centrifuged (Beckman J2-21 with JA-20 rotor, 4 °C) at 1090 x g for 10 min. The P1 pellet was discarded and the supernatant was centrifuged at 17,400 x g for 30 min. The P2 pellet was resuspended with a polytron (Kinematica, setting 5, 20 s) in 45 mL of ice-cold, aerated (5% CO₂ in O₂) modified Krebs-Ringer bicarbonate (KR) buffer containing (mM): NaCl, 124.3; KCl, 2.95; MgSO₄, 1.30; KH₂PO₄, 1.25; NaHCO₃, 26.0; CaCl₂, 2.41; d-glucose, 10.4; Na ascorbate, 0.06; pH 7.4 - 7.6.

A 200 μL aliquot of the synaptosomal suspension was added to test tubes containing 1.65 mL of ice-cold KR buffer, 50 μL of test drug, or deionized water (for total and nonspecific determinations), and 50 μL of pargyline HCl solution (final concentration, 100 μM). Test drugs were dissolved in deionized

water, except **1d**, which required a small amount of methanol to effect dissolution. The test tubes were preincubated in an aerated (5% CO₂ in O₂, 15 psi), 37 °C, shaking water bath for 5 min. The tubes were then returned to the ice bath and chilled for 10 - 15 min. Tritiated neurotransmitter was added (50 μL; final concentration, 10 nM), giving a final incubation volume of 2 mL. All tubes except nonspecific tubes were returned to the aerated 37 °C shaking water bath for 5 min to initiate neurotransmitter uptake. Uptake was terminated by chilling the test tubes in an ice bath, then rapidly filtering the solution through glass fiber filters (Whatman GF/C) using a 24-well cell harvester (Brandel). Filters were washed with 2 x 3 mL of ice-cold KR buffer, allowed to air dry for 10 min, and then placed in plastic liquid scintillation vials. Scintillation cocktail (Ecolite, ICN Biomedicals, 10 mL) was added, and the vials were sealed, vortexed, and allowed to stand overnight. Radioactivity was measured using liquid scintillation spectroscopy (Packard 4430, 40% efficiency). Specific uptake was defined as uptake at 37 °C minus uptake at 0 °C in the absence of drugs. Specific uptake represented > 74% of the total uptake of 5-HT, > 91% of the total uptake of DA, and > 85% of the total uptake of NE under these conditions.

Drugs were screened at concentrations of 25, 50, or 100 μM. If a compound exhibited more than 50% inhibition of specific uptake at 25 μM concentration, the IC₅₀ ± SEM was determined from displacement curves from three experiments using eight drug concentrations, each run in triplicate. Data were transformed from dpm to % inhibition of specific uptake and fit to a four parameter logistic curve using the computer program EBDA,²⁶ from which the IC₅₀ value was calculated. Multiple IC₅₀ comparisons were done using ANOVA, followed by a Student-Newman-Keuls t-test.

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