Notes

2-(1-Naphthyloxy)ethylamines with Enhanced Affinity for Human 5-HT$_{1D\beta}$ (h5-HT$_{1B}$) Serotonin Receptors

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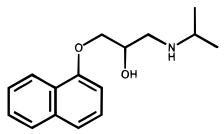
Although the β-adrenergic antagonist propranolol (**1**) binds at rodent 5-HT$_{1B}$ serotonin receptors, it displays low affinity (K_i > 10 000 nM) for its species homologue 5-HT$_{1D\beta}$ (i.e., h5-HT$_{1B}$) receptors. The structure of propranolol was systematically modified in an attempt to enhance its affinity for the latter population of receptors. Removal of the alkyl hydroxyl group, shortening of the *O*-alkyl chain from three to two methylene groups, and variation of the terminal amine substituent resulted in compounds, such as *N*-monomethyl-2-(1-naphthyloxy)-ethylamine (**11**; K_i = 26 nM), that display significantly higher h5-HT$_{1B}$ affinity than propranolol. Compound **11** was shown to bind equally well at human 5-HT$_{1D\alpha}$ (h5-HT$_{1D}$) receptors (K_i = 34 nM) and was further demonstrated to possess h5-HT$_{1B}$ agonist character in an adenylate cyclase assay. It would appear that such (aryloxy)alkylamines may represent a novel class of 5-HT$_{1D}$ receptor agonists.

Certain aryloxyalkylamines such as propranolol (**1**) and pindolol (**2**) are recognized as β-adrenergic antagonists. However, these and related agents are known to also bind with nanomolar affinity at certain populations of serotonin receptors, particularly 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors (e.g. 1−3). We[1] and others[4] have previously examined the SAR for the binding of such compounds at these receptors. Much less is known about the binding of these agents at other populations of 5-HT receptors.



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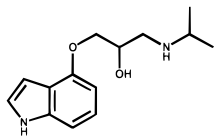

2

5-HT$_{1B}$ receptors represent serotonin autoreceptors in rodents; most other species including humans possess a species homologue of 5-HT$_{1B}$ receptors (i.e., 5-HT$_{1D}$ receptors) that seems to serve the same function.[5−9] Two distinct populations of human 5-HT$_{1D}$ receptors have been identified, 5-HT$_{1D\alpha}$ or h5-HT$_{1D}$ receptors and 5-HT$_{1D\beta}$ or h5-HT$_{1B}$ receptors (i.e., intraspecies subtypes). The h5-HT$_{1B}$ receptors are considered a species homologue of rat (r) 5-HT$_{1B}$ receptors,[10−15] and agents that bind at one population typically bind at the other. (Aryloxy)alkylamines are a curious exception. For example, propranolol (**1**) binds with high affinity ($K_i \approx$ 15−60 nM) at r5-HT$_{1B}$ receptors but displays low affinity (K_i > 5000 nM) at h5-HT$_{1B}$ receptors.[3] The difference in affinity has been related to the presence of a particular amino acid residue; that is, h5-HT$_{1B}$ receptors possess a threonine at position 355 whereas r5-HT$_{1B}$ receptors possess an asparagine moiety at the cognate position. The presence of the asparagine moiety, also found at a corresponding position in 5-HT$_{1A}$ receptors,[16] appears important for the binding of (aryloxy)alkylamines. Although there exist other structural differences between h5-HT$_{1B}$ and r5-HT$_{1B}$ receptors, mutant h5-HT$_{1B}$ receptors (i.e., a T355N mutant), in which threonine 355 has been replaced by an asparagine, display enhanced affinity for propranolol.[17−20] Indeed, mutation of this single amino acid enhances the binding of (−)-propranolol such that it is now comparable to the affinity of (−)-propranolol at r5-HT$_{1B}$ receptors.[17−20]

In the course of our work on the binding of **1** at 5-HT$_{1A}$, r5-HT$_{1B}$, and bovine 5-HT$_{1D}$ receptors, we found that the presence of the hydroxyl group of **1** is not critical for binding, and that replacement of the ether oxygen atom with a methylene group abolishes affinity.[1,2,21] The results of this preliminary study, coupled with increased interest in 5-HT$_{1B/1D}$ receptors,[2] prompted us to undertake further study of the interaction of propranolol and related (aryloxy)alkylamines at this latter population of receptors. Specifically, we wished to determine the role of the naphthalene ring, the importance of alkyl chain length, and particularly the effect of amine substitution, on h5-HT$_{1B}$ binding. Selected compounds were also examined at h5-HT$_{1D}$ receptors to determine if they possess any selectivity.

Chemistry

Compounds **4**,[22] **5**−**10**,[1] and **16**−**20**[22] have been previously reported. Compounds **11**−**15**, **21, 22**, **24**, **26**, and

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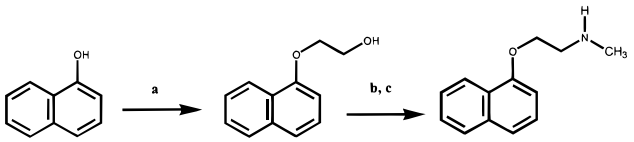
Table 1. Physicochemical Properties of (Aryloxy)alkylamines

$ArO(CH_2)_nNRR'$

	Ar	*n*	R	R′	mp (°C)	RS[a]	% yield	empirical formula[b]
11	1-naphthyl	2	H	Me	179−181	A	50	$C_{13}H_{15}NO\cdot HCl$[c]
12	1-naphthyl	2	H	Et	155−157	A	60	$C_{14}H_{17}NO\cdot HCl$
13	1-naphthyl	2	H	CH_2CH_2OH	198−200	B	72	$C_{14}H_{17}NO_2\cdot C_2H_2O_4$[d]
14	1-naphthyl	2	H	*n*-Pr	236−238	A	40	$C_{15}H_{19}NO\cdot C_2H_2O_4$[d,f]
15	1-naphthyl	2	H	*i*-Pr	228−230	A	40	$C_{15}H_{19}NO\cdot C_2H_2O_4$[d]
21	phenyl	2	H	Me	174−175[g]	C	40	
22	2,3-diMe Ph	2	H	Me	169−171	A	83	$C_{11}H_{17}NO\cdot HCl$
24	2-CPP[e]	3	Me	*n*-Pr	135−138	D	25	$C_{18}H_{29}NO\cdot C_2H_2O_4$[d]
25	2-CPP[e]	2	Me	*n*-Pr	193−195	E	40	$C_{17}H_{27}NO\cdot HCl$
26	4-indolyl	3	H	*i*-Pr	164−166	A	46	$C_{15}H_{20}NO\cdot C_2H_2O_4$[d]
27	4-indolyl	2	H	*i*-Pr	150−152	A	20	$C_{14}H_{18}NO\cdot C_2H_2O_4$[d]

[a] Recrystallization solvents: A = EtOH/Et_2O; B = MeOH; C = MeCN; D = EtOAc; E = 2-butanone; F = MeOH/Et_2O. [b] All compounds analyzed for C, H, N within 0.4% of theory. [c] Crystallized with 0.25 mol of H_2O. [d] Oxalate salt. [e] 2-CPP = 2-(cyclopentyl)phenyl. [f] Crystallized with 0.2 mol of H_2O. [g] Lit.[25] mp 175−176 °C.

Scheme 1[a]



11

[a] (a) $ClCH_2CH_2OH$, NaOH; (b) TsCl/pyridine, 0−10 °C; (c) NH_2CH_3, K_2CO_3, Δ.

27 were prepared in a simple three-step process as exemplified for **11** in Scheme 1. In general, the appropriate hydroxy compound (i.e., naphthol, phenol, or 4-hydroxyindole) was O-alkylated with 2-chloroethanol or 3-chloropropanol, and the resulting alcohol was converted to a tosylate and allowed to react with an amine (see Table 1); synthetic detail is provided for **11**. Compound **25** was prepared in a slightly different manner from the remainder of the series; 2-cyclopentylphenol was alkylated with 1,2-dibromoethane, and the resultant product was allowed to react with N-*n*-propyl-*N*-methylamine.

Results and Discussion

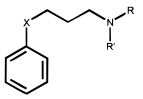
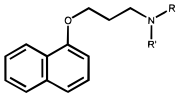
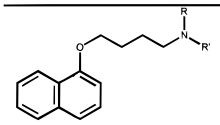
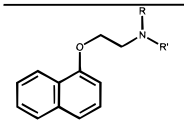
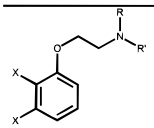
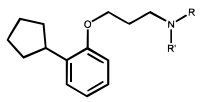
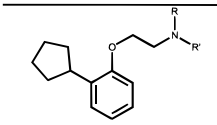
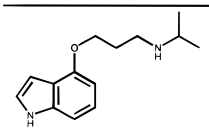
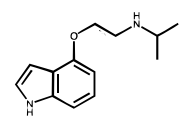
For purpose of comparison, we determined or redetermined the h5-HT_{1B} affinities of (±)-propranolol (**1**) (K_i > 10 000 nM), (−)-propranolol (K_i = 4100 nM), (±)-pindolol (**2**) (K_i > 10 000 nM), and (−)-pindolol (K_i = 2600 nM) (see Table 2). As previously reported, none of these agents displayed high affinity for h5-HT_{1B} receptors. We have already demonstrated that deshydroxypropranolol (**3**) (K_i = 7660 nM) binds with low affinity, but at least as well as racemic propranolol, and with an affinity comparable to (−)-propranolol at h5-HT_{1B} receptors.[23] Because the *N*-isopropyl substituent of **1** is considered an important feature for β-adrenergic binding, and because *N*-mono- and *N*,*N*-dimethyl substitution is tolerated by r5-HT_{1B} receptors,[1] we began our investigation with simpler *N*-alkyl substituents. Initially, we found that replacement of the *N*-isopropylamine of **3** with a primary amine or with *N*,*N*-dimethylamine resulted in enhanced affinity (i.e., **4** and **5**, K_i = 290 and 255 nM, respectively; Table 2). Removal of the unsubstituted benzene ring of **5** (i.e., **6**) abolished affinity. Replacement of the ether oxygen atom of **6** with a nitrogen atom (i.e., **7**) resulted in an inactive compound. However, monodemethylation of **6** (i.e., **8**) resulted in enhanced affinity, suggesting that secondary amines may bind with higher affinity than tertiary amines. Chain lengthening of **5** by one methylene unit (i.e., **9**, K_i = 470 nM) halved affinity, whereas chain shortening by a single methylene (i.e., **16**, K_i = 94 nM) doubled affinity. Consequently, we continued by examining the effect of various amine substituents in additional naphthalene-containing chain-shortened ether analogues (i.e., **10**−**20**).

The primary amine **10** (K_i = 100 nM) binds with much higher affinity than propranolol. The secondary amines **11**, **12**, and **14**, where the amine substituent is varied from methyl to ethyl to *n*-propyl (K_i = 26, 66, and 200 nM), reveal a small but consistent trend of reduction in affinity as substituent length is increased. The hydroxyethyl derivative **13** and isopropyl derivative **15** (K_i = 107 and 120 nM, respectively) bind with the same affinity as the primary amine. In general, the secondary amines bind with relatively little variation in affinity. N-Monomethylation of **11** and **14**, to give the tertiary amines **16** and **17**, results in a 4-fold reduction in affinity. The tertiary amines **16**−**20** bind, but there seems to be limited bulk tolerance; that is, apparently, only a certain amount of bulk is tolerated around the amine group, and the N-monomethyl derivative **11** was identified as the highest affinity agent in this series.

Having established that the shorter chain compounds (e.g. compare **4** with **10**, and **5** with **16**) bind with severalfold higher affinity than those with an *O*-alkyl substituent length corresponding to that found in propranolol, and that an *N*-monomethylamine is optimal, we reexamined the role of the unsubstituted benzene ring. The chain-shortened monocyclic derivative **21** (K_i = 5960 nM) binds, as expected, with low affinity; its affinity is >200-fold lower than that of **11**. Evidently, however, the intact naphthalene nucleus is not essential for binding; for example, the 2,3-dimethyl derivative (**22**; K_i = 316 nM) binds with enhanced affinity relative to **21**. Although the naphthyl ring still seems optimal, the methyl substituents of **22** may be sufficient to mimic, at least in part, the binding properties of the unsubstituted benzene ring of **11**. Nevertheless, **22** still binds with 10-fold lower affinity than **11**.

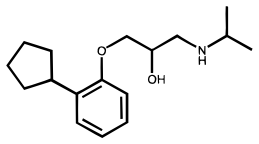
The naphthyl ring of propranolol (**1**) is known to be important for high affinity at 5-HT_{1A} and r5-HT_{1B} receptors; that is, replacement of the naphthyl group with an unsubstituted phenyl ring results in a dramatic decrease in affinity at these populations of receptors.[1] It is also known, however, that certain substituted-

Table 2. Results of Radioligand Binding Studies with (Aryloxy)alkylamines

		X	R	R'	h5-HT$_{1B}$ Affinity Ki, nM(± SEM)[a,b]	
(±)Propranolol **(1)**					> 10,000	
(-)Propranolol					4,100	(380)
(±)Pindolol **(2)**					> 10,000	
(-)Pindolol					2,600	(260)
6		O	Me	Me	>10,000	
7		NH	Me	Me	>10,000	
8		O	H	Me	3,450	(820)
3			H	*i*-Pr	7,660	[c]
4			H	H	290	(30)
5			Me	Me	255	(47)
9			Me	Me	470	(130)
10			H	H	100	(12)
11			H	Me	26	(6)
12			H	Et	66	(17)
13			H	CH_2CH_2OH	107	(23)
14			H	*n*-Pr	200	(45)
15			H	*i*-Pr	120	(15)
16			Me	Me	94	(2)
17			Me	*n*-Pr	750	(35)
18			Me	Benzyl	260	(47)
19			Et	*n*-Bu	1,225	(50)
20			*n*-Bu	*n*-Bu	2,675	(350)
21		H	H	Me	5,960	(2,350)
22		Me	H	Me	316	(10)
24			Me	*n*-Pr	1,205	(144)
25			Me	*n*-Pr	425	(82)
26					> 10,000	
27					1,120	(15)

[a] Standard error not determined where K_i > 10 000 nM. [b] Affinities for h5-HT$_{1D}$ (i.e., 5−HT$_{1D\alpha}$) receptors were determined for selected compounds for purpose of comparison; K_i values (±SEM) are as follows: **11**, 34(9); **13**, 68(7); **17**, 103(12); **18**, 590(162); **19**, 112(8); **21**, 1800(340); **24**, 143(20); **25**, 82(12) nM. [c] K_i value previously reported.[23]

phenyl derivatives retain affinity for 5-HT$_{1A/1B}$ receptors. For example, penbutolol (**23**) binds at both populations of receptors with 15−20 times the affinity of propranolol.[3,4] Accordingly, we examined the cyclopentyl derivatives **24** and **25**. Both compounds bind only with



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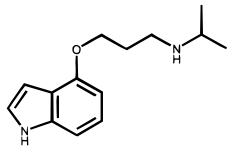

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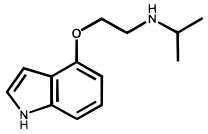

27

modest affinity at h5-HT$_{1B}$ receptors (Table 2), but **25** binds with an affinity comparable to that of naphthyl derivative **17**.

Finally, in an effort to determine if the affinity-enhancing effect of chain shortening was specific for the propranolol series or whether it could be generalized to other (aryloxy)alkylamines, the affinity of racemic pindolol (**2**; K_i > 10 000 nM) and deshydroxypindolol (**26**; K_i > 10 000 nM) was compared with that of compound **27**. Compound **27** (K_i = 1120 nM) was found to bind with enhanced affinity, but with 10-fold lower affinity than its corresponding propranolol analogue **15**. Apparently, the affinity-enhancing effect of chain shortening is not limited solely to derivatives of propranolol.

h5-HT$_{1D}$ (5-HT$_{1D\alpha}$) Binding. To determine if some of the present compounds display any selectivity for h5-HT$_{1B}$ versus h5-HT$_{1D}$ receptors, several were examined in a h5-HT$_{1D}$ binding assay (see footnote to Table 2). None of the compounds possessed appreciable selectivity.

Functional Assay. (Aryloxy)alkylamines such as propranolol (**1**) and pindolol (**2**) typically behave as 5-HT antagonists (or as very weak partial agonists).[24] It would seem likely that compound **11**, being an (aryloxy)-alkylamine, might also behave as a 5-HT$_{1D}$ antagonist. However, with a shorter side chain and different terminal amine substituent than that found in **1** and **2**, it would be difficult to predict the functional activity of this type of compound *a priori*. Thus, **11** was examined in a functional assay in order to determine if it is a 5-HT$_{1D}$ agonist or antagonist. Compound **11** did not function as an antagonist of forskolin-stimulated inhibition of adenylate cyclase at concentrations of up to 10^{-5} M. In contrast, a nearly full agonist effect was observed (Figure 1). Hence, **11** behaves as a h5-HT$_{1B}$ agonist (EC_{50} = 216 nM) relative to 5-HT itself (EC_{50} = 5.5 nM).

Summary. Human 5-HT$_{1D\beta}$ or h5-HT$_{1B}$ receptors represent a species homologue of rodent 5-HT$_{1B}$ receptors. The (aryloxy)alkylamines propranolol and pindolol are rather unique in that they bind with high affinity at r5-HT$_{1B}$ receptors but with low affinity at h5-HT$_{1B}$ receptors. On the basis of some preliminary investigations from our laboratory suggesting it should be possible to enhance the affinity of (aryloxy)alkylamines for h5-HT$_{1B}$ receptors if the appropriate structural changes are made, we undertook the present structure−affinity investigation. Relative to racemic propranolol (**1**; K_i > 10 000 nM), deshydroxy derivatives (a) with a two- rather than three-methylene spacer between the ether oxygen atom and the terminal amine, and (b) that possess a primary or secondary amine with small alkyl groups, bind at h5-HT$_{1B}$ receptors with affinities of ≤100

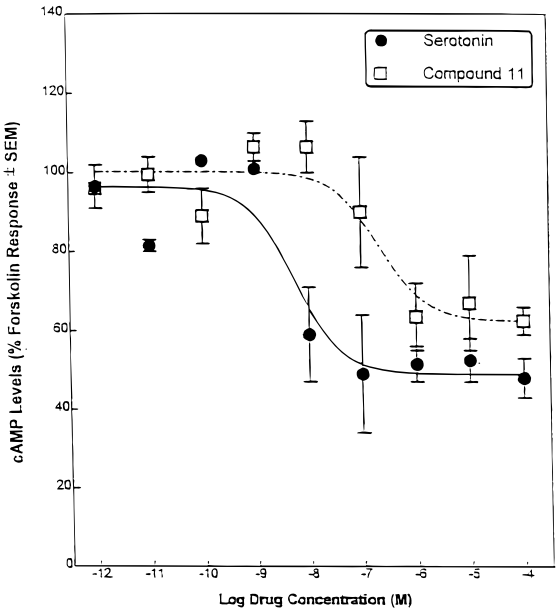

Figure 1. Agonist effect of compound **11** and 5-HT on cAMP levels in cells stably expressing human 5-$HT_{1D\beta}$ (h5-HT_{1B}) serotonin receptors.

nM. Although the naphthyl moiety appears optimal for binding, it can be replaced with certain substituted phenyl groups (e.g. **22**, **25**) or with a 4-indolyl group (e.g. **27**). Several compounds were examined at h5-HT_{1D} receptors but none showed much more than 10-fold selectivity for one population of 5-HT_{1D} receptors over the other. One compound in particular, **11**, was examined for its ability to act as a h5-HT_{1B} agonist or antagonist using an adenylate cyclase assay. Compound **11** was found to be devoid of antagonist character; in contrast, it behaved as an agonist. Additional work is obviously required on these types of compounds. Nevertheless, it would appear that 2-(1-aryloxy)ethylamines may represent a novel class of 5-HT_{1D} receptor ligands.

Experimental Section

Synthesis. Chemicals were purchased from Aldrich Chemicals (Milwaukee, WI). Elemental analyses was performed by Atlantic Microlab, Inc. (Norcross, GA); results are within 0.4% of theory. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Proton NMR spectra were recorded on a GE QE−300 FT NMR; chemical shifts are reported as ppm. Tetramethylsilane was used as an internal standard. Thin-layer chromatography was performed on precoated silica gel (60F254, Merck) glass plates.

***N*-Methyl-2-(1-naphthyloxy)ethylamine Hydrochloride (11).** A mixture of 1-naphthol (2.88 g, 20 mmol) and 2-chloroethanol (1.82 g, 20 mmol) in 2 N NaOH (0.80 g in 10 mL H_2O) was heated at reflux for 2 h and then allowed to stir at room temperature overnight. The reaction mixture was diluted with H_2O (2 mL) and extracted with CH_2Cl_2 (3 × 30 mL), washed with NaOH 10% (50 mL) and with H_2O (3 × 30 mL), dried ($MgSO_4$), and evaporated to give 3.00 g (80%) of 2-(1-naphthyloxy)ethanol as an oil. *p*-Toluenesulfonyl chloride (0.99 g, 5 mmol) was added in a portionwise manner to a solution of 2-(1-naphthyloxy)ethanol (0.94 g, 5 mmol) in pyridine (10 mL) at 0 °C. The reaction mixture was kept in a refrigerator for 2 days, after which time the reaction mixture was poured into ice−water (60 mL) and extracted with Et_2O (3 × 25 mL); the Et_2O extract was washed with 5% HCl (30 mL) and H_2O (30 mL). The Et_2O extract was dried ($MgSO_4$) and solvent evaporated. The solid residue was crystallized from Et_2O/petroleum ether (bp 60−80 °C) to give 1.50 g of the 2-(1-naphthyloxy)ethanol-*p*-toluenesulfonate; mp 103−105 °C.

A mixture of 2-(1-naphthyloxy)ethanol-*p*-toluenesulfonate (0.34 g, 1 mmol), an ethanolic solution of *N*-methylamine (5%, 2.0 mL), and anhydrous K_2CO_3 (0.14 g, 1 mmol) in dioxane (20 mL) was heated at reflux for 3 h. The solvent was evaporated under reduced pressure, and the residue was treated with 10% NaOH solution (10 mL) and extracted with Et_2O (3 × 20 mL). The combined Et_2O extract was washed with H_2O (3 × 20 mL) and dried ($MgSO_4$), and the solvent was evaporated. The product was separated by column chromotography using silica gel with $CHCl_3$/MeOH (9:1) as eluent. The HCl salt was prepared and recrystallized from absolute EtOH/Et_2O to give 0.12 g (50%) of white crystals: mp 179−181 °C; ^{1}H NMR (DMSO-d_6) δ 2.6 (s, 3H, CH_3), 3.4 (t, 2H, CH_2), 4.4 (t, 2H, CH_2), 7.0 (dd, 1H, Ar-H), 7.5 (m, 4H, Ar-H), 7.8 (m, 1H, Ar-H), 8.4 (m, 1H, Ar-H), 9.2 (bs, N^+H_2). Anal. ($C_{13}H_{15}NO\cdot HCl\cdot 0.25H_2O$) C, H, N.

***N*-Methyl-*N*-*n*-propyl-2-(2-cyclopentylphenoxy)ethylamine Hydrochloride (25).** NaOMe in MeOH (25% solution, 0.7 g, 3.2 mmol) was added to 2-cyclopentylphenol (0.4 g, 2.5 mmol) in MeOH (5 mL), and the reaction mixture was allowed to stand at room temperature for 0.5 h. After removal of the solvent in vacuo, CH_3CN was added to the solid. The sodium 2-cyclopentylphenolate in CH_3CN was added in a dropwise manner to 1,2-dibromoethane (2.3 g, 12.0 mmol) in a screw-top test tube; the tube was sealed and heated at 100 °C for 12 h and then at 130 °C for an additional 12 h. The solvent was removed in vacuo, and petroleum ether (bp 60−90 °C) (5 mL) and aqueous NaOH (2 N, 3 mL) were added to the residue. The aqueous layer was removed, and the organic portion was extracted once more with aqueous NaOH (3 mL). The petroleum ether portion was dried ($MgSO_4$) and the solvent was removed in vacuo to yield an oil. Kugelrhor distillation of the oil (bp 52−70 °C, 0.05 mmHg) yielded 0.23 g (35%) of 1-bromo-2-(2-cyclopentylphenoxy)ethane as a clear oil. A solution of the above bromide (0.23 g, 0.85 mmol) and *N*-methylpropylamine (1.0 g, 14.0 mmol) were combined in CH_3CN (1 mL) and heated in a sealed tube at 60 °C for 24 h. The reaction mixture was allowed to cool, and the *N*-methylpropylamine and CH_3CN were recovered by distillation. An ethereal solution of the resulting oil was washed with 1 N NaOH (3 × 1 mL) and then extracted with HCl (3 × 10 mL). The combined acidic fractions were basified (to pH 12) with a saturated solution of KOH and extracted with Et_2O (3 × 15 mL). The combined Et_2O fractions were dried ($MgSO_4$), and the solvent was evaporated in vacuo. An ethereal solution of the free base was treated with HCl in Et_2O until salt formation ceased. The salt was collected by filtration and recrystallized from 2-butanone to yield 0.1 g (40%) of a white solid: mp 193−195 °C; ^{1}H NMR ($CDCl_3$, free base) δ 0.9 (t, 3H, CH_3), 1.0−2.5 (m, 12H), 2.8 (t, 2H, CH_2N, J = 3 Hz), 3.0−3.5 (m, 1H, CH), 4.1 (t, 2H, CH_2O, J = 3 Hz), 6.8−7.2 (m, 4H, ArH). Anal. ($C_{17}H_{27}NO\cdot HCl$) C, H, N.

Radioligand Binding Assays.[24] Binding studies were performed in triplicate using 96-well polypropylene microtiter plates with a reaction volume of 500 μL. Test compounds were initially assayed at 1 and 0.1 μM concentrations. Compounds showing <50% inhibition at 1 μM are reported as binding with K_i > 10 000 nM. K_i values were determined for those compounds showing >50% inhibition at 1 μM. [^{3}H]-5-HT trifluoroacetate (100 Ci/mmol, Amersham) was used as radioligand, 2.5 nM final concentration; nonspecific binding was defined using 20 μM 5-HT creatinine sulfate (Research Biochemicals Inc.). The incubation buffer was composed of 50 mM Tris, 10 mM $MgSO_4$, 0.5 mM EDTA, 10 μM pargyline, and 0.1% (w/V) ascorbic acid, pH 7.4 at 22 °C. Incubation was started by the addition of membrane homogenate (0.1 mg of protein per well); plates were vortexed for 20 s and then incubated at room temperature for 60 min. The binding reaction was stopped by filtration with the use of a Packard harvester under vacuum over GF/B Unifilters. Each reaction plate was washed six times with 1 mL of cold Tris buffer. Scintillant (Microscint 0, 35 μL) was added to the dried Unifilters, and the sealed plates were counted by liquid scintillation spectrometry (Packard TopCount). Binding dpm in the presence of test compounds were expressed as a percent of binding dpm in the absence of compounds. A percent-binding versus concentration curve was constructed from

which the IC_{50} value (concentration giving rise to 50% inhibition) was determined. K_i values were then calculated from the IC_{50} value using the Cheng and Prusoff transformation.

Adenylate Cyclase Assay. A CHO Pro 5 cell line was stably tranfected with human recombinant 5-$HT_{1D\beta}$ receptors. Drugs were screened for agonist activity on the basis of their ability to inhibit the cAMP production by this cell line in the presence of 10 μM forskolin and 0.5 mM IBMX. Test compound was added to the culture media, and the cells were incubated for 30 min at 37 °C. Following incubation, the reaction mixture was terminated by the addition of cold ethanolic 5 mM EDTA (2:1 v/v) to extract the cAMP. The amounts of cAMP were determined by the Enzyme Immuno Assay kit from Amersham. Serotonin was used for comparison. To test for antagonist activity, test compound was examined in the presence of 10 μM forskolin, 0.5 mM IBMX, and 10^{-5} M serotonin. Results are expressed as mean ± SEM from three or more experiments.

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