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Benzylimidazolines as h5-$HT_{1B/1D}$ Serotonin Receptor Ligands: A Structure−Affinity Investigation

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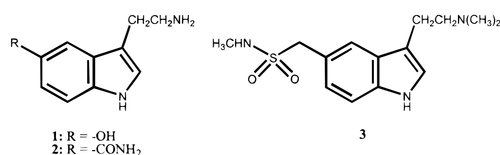
Benzylimidazolines may represent a class of 5-HT_{1D} ligands that has yet to be exploited. On the basis of a previous report that the 2-(substituted-benzyl)imidazoline α-adrenergic agonist oxymetazoline (**8**) binds with high affinity at calf brain 5-HT_{1D} receptors, we explored the structure−affinity relationships of a series of related derivatives. Each of the aromatic substituents was removed and then reinstated in a systematic manner to determine the influence of the individual substituents on binding. It was found that all of the aromatic substituents of **8** act in concert to impart high affinity. However, although the 3-hydroxy group could be removed without significantly reducing affinity for h5-HT_{1D} (i.e., human 5-$HT_{1D\alpha}$) receptors, this modification reduced h5-HT_{1B} (i.e., human 5-$HT_{1D\beta}$) receptor affinity by nearly 50-fold. The 2,6-dimethyl groups also contribute to binding but seem to play a greater role for h5-HT_{1B} binding than h5-HT_{1D} binding. With the appropriate structural modifications, several compounds were identified that display 20- to >100-fold selectivity for h5-HT_{1D} versus h5-HT_{1B} receptors. Preliminary functional data suggest that these compounds behave as agonists. Given that 5-HT_{1D} agonists are currently being explored for their antimigraine action and that activation of h5-HT_{1B} receptors might be associated with cardiovascular side effects, h5-HT_{1D}-selective agents may offer a new lead for the development of therapeutically efficacious agents.

At least 15 different populations of serotonin (5-HT) receptors have been identified.[1−6] These receptor types belong to 5-HT receptor families 5-HT_1−5-HT_7, and several of the families are composed of subpopulations. For example, 5-HT_1 receptors are a family of 5-HT receptors that are negatively coupled to adenylate cyclase and consist of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F} subtypes.[1−6] 5-HT_{1C} receptors are absent from this list because they have been recently renamed 5-HT_{2C} receptors (i.e., they share greater sequence homology and second-messenger coupling character with members of the 5-HT_2 family than with the other members of the 5-HT_1 family).[3,5] Of particular interest to the present investigation are the 5-HT_{1D} receptors. Certain rodent species (including rat and mouse) possess 5-HT_{1B} receptors that serve primarily as terminal autoreceptors.[1−6] In humans, the corresponding receptors that function in a similar manner were initially termed 5-HT_{1D} receptors.[1−6] 5-HT_{1D} receptors control neurotransmitter release and may modulate vascular tone.[2] There is some evidence, or at least speculation, that 5-HT_{1D} receptors may be involved in migraine, depression, anxiety, and agression.[2,7] Two distinct intraspecies subtypes of human 5-HT_{1D} receptors have been identified: 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$.[8−12] There is 77% homology between the two subtypes. Rat 5-HT_{1B} receptors and human 5-$HT_{1D\beta}$ receptors are considered species homologues, and there is >90% transmembrane sequence homology between them.[8] It has been recommended that 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors be termed h5-HT_{1D} and h5-HT_{1B} receptors, respectively.[13] [Herein, the term "5-HT_{1D}" will be reserved for reference to binding studies that have used brain homogenates or to refer to the family of 5-$HT_{1B/1D}$ receptors when it is difficult to make a distinction between the two.] Most agents that bind at rat 5-HT_{1B} (r5-HT_{1B}) receptors also bind at human 5-HT_{1D} (brain homogenate) and/or cloned h5-HT_{1B} receptors.[1,2,5−7]



Many agents that bind at 5-HT_{1D} receptors with high affinity are derivatives of 5-HT (**1**) or tryptamine. For example, 5-carbamoyltryptamine ("5-carboxamidotryptamine", **2**) is a widely used but nonselective 5-HT_{1D} agonist.[2] The more selective sumatriptan (**3**), an agent currently employed for the treatment of migraine, is perhaps the most commonly used 5-HT_{1D} agonist.[7] A number of newer agents are currently being explored in clinical or preclinical trials, but these, too, are tryptamine derivatives or are closely related in structure to sumatriptan (reviewed in ref 14). Some novel agents that diverge somewhat from a tryptamine structure include alniditan (**4**)[14,15] and MDL-74721 (**5**).[14] GR 55562 (**6**) and related agents also lack a tryptamine structure; these compounds, however, function as 5-HT_{1D} antagonists.[16]

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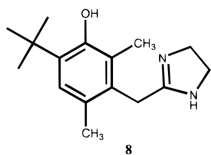
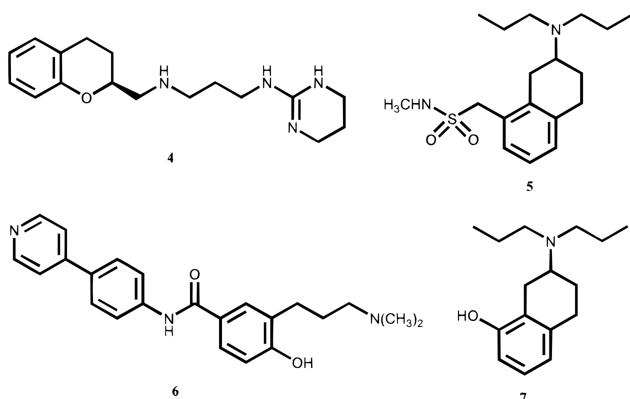


With the exception of **4** and **5**, there are relatively few new structural leads that might serve as templates for the development of novel 5-HT_{1D} agonists. Even the partial ergoline **5** bears some resemblance to sumatriptan; furthermore, **5**-related aminotetralins with affinity for 5-HT_{1D} receptors typically bind with yet higher affinity at 5-HT_{1A} receptors[17] due to their close structural analogy to the prototypical 5-HT_{1A} agonist 2-(*N*,*N*-di-*n*-propylamino)tetralin (**7**). Clearly, new lead structures would be useful for the continued development of novel 5-HT_{1D} ligands. Oxymetazoline (**8**) may represent



such a lead. Oxymetazoline, an aryl-substituted benzylimidazoline, is an α-adrenergic agonist and an important tool in adrenergic receptor research.[18,19] In 1991, Schoeffter and Hoyer found that oxymetazoline (**8**) binds at calf caudate 5-HT_{1D} receptors with high affinity (K_i = 5 nM)[20] and with an affinity comparable to that displayed by oxymetazoline for various subpopulations of α-adrenergic receptors; **8** was also shown to function as a 5-HT_{1D} agonist.[20] Essentially nothing else is known about the binding of related benzylimidazoline derivatives at 5-HT_{1D} receptors; in fact, the binding of **8** at human 5-HT_{1D} receptors has not been reported. Although oxymetazoline binds with high affinity at α-adrenergic receptors,[18,21] if it binds at human 5-HT_{1D} receptors it offers a new structural lead that might be exploited for the development of novel 5-HT_{1D} ligands. Theoretically, if the structural requirements for the binding of oxymetazoline (**8**) can be identified, and if these requirements are different than those necessary for α-adrenergic binding,[19] it should be possible to use the benzylimidazoline moiety as a template to design new 5-HT_{1D} agents. Thus, the purpose of the present investigation was to initiate such a study in order to determine exactly what features of oxymetazoline are important for 5-HT_{1D} binding. Specifically, we began our investigations with calf brain 5-HT_{1D} receptors and then focused on binding at human h5-HT_{1B} and h5-HT_{1D} receptors.

Chemistry

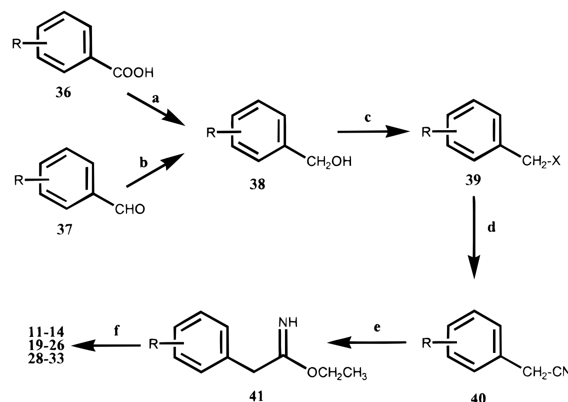
The general procedure used for the synthesis of the target benzylimidazolines in Table 1 is shown in Scheme 1. Nearly all of the requisite benzoic acid (**36**), benzyl alcohol (**38**), benzyl halide (**39**), and phenylacetonitrile (**40**) precursors were commercially available; the substituted benzaldehydes and benzoic acids necessary for the preparation of **17**–**19**, **30**, and **31** were synthesized according to literature methods.[22–25] In general, the intermediate benzyl alcohols **38** were prepared by reduction of the corresponding aldehydes **37** or acids **36** with $NaBH_4$ or $LiAlH_4$, respectively. The alcohols were converted to the benzyl halides **39** by standard methods, and the halides were treated with NaCN to afford nitriles **40**. Subsequent treatment of the nitriles with HCl gas in ethanol provided the imidates **41** which were cyclized by reaction with ethylenediamine to the desired benzylimidazolines **11**–**14**, **19**–**26**, and **28**–**33** (Scheme 1); compounds **10** and **32** were prepared by use of

Table 1. Physicochemical Data for Benzylimidazolines and Related Derivatives

compd	yield (%)	RS[a]	mp (°C)	empirical fomula[b]
10	18	P	87–89	$C_{11}H_{14}N_2{\cdot}C_2H_2O_4$[c]
11	90	C	232–234	$C_{14}H_{20}N_2{\cdot}HCl$
12	61	P	250–253[d]	
13	64	P	211–212[e]	$C_{10}H_{12}N_2O{\cdot}HCl$
14	92	P	195–197[f]	$C_{11}H_{14}N_2O{\cdot}HCl$
15	75	P	153–155	$C_8H_{10}N_2{\cdot}C_2H_2O_4$
16	71	ME	142–144[g]	
17	72	ME	246–247	$C_{12}H_{16}N_2O{\cdot}HBr$
18	86	P	260–261	$C_{12}H_{10}N_2O{\cdot}HCl$
19	84	ME	188–190	$C_{19}H_{22}N_2O{\cdot}HCl$
21	64	AE	150–152	$C_{12}H_{16}N_2{\cdot}HCl$[h]
22	61	ME	173–175	$C_{13}H_{18}N_2{\cdot}C_2H_2O_4$[h]
23	79	AE	176–178	$C_{13}H_{18}N_2{\cdot}HCl$
24	88	ME	143–145	$C_{14}H_{20}N_2{\cdot}HCl$
25	63	P	210–212	$C_{10}H_{11}BrN_2{\cdot}HCl$
26	73	A	255–257[i]	
27	89	ME	217–219[j]	
28	85	ME	192–194	$C_{16}H_{16}N_2{\cdot}HCl$
29	63	ME	273–275[k]	
30	70	ME	278–280	$C_{15}H_{20}BrN_2{\cdot}HCl$
31	85	ME	293–295	$C_{12}H_{15}BrN_2{\cdot}HCl$
32	73	AE	250–251	$C_{17}H_{26}N_2{\cdot}HCl$
33	34	AE	245–250	$C_{15}H_{22}N_2{\cdot}HCl$[h]

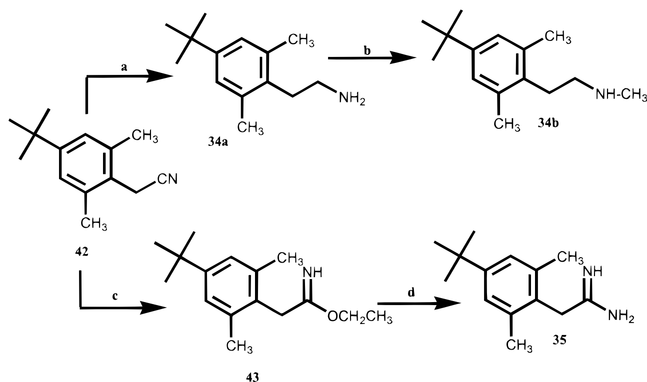
[a] Recrystallization solvents: A, absolute EtOH; M, MeOH; E, anhydrous Et_2O; P, *i*PrOH; C, MeCN. [b] All compounds analyzed correctly for C,H,N within 0.4% of theory. [c] Crystallized with 0.5 mol of H_2O. [d] Lit.[31] mp 253–255 °C. [e] Compound previously reported but not characterized.[32] [f] Compound previously reported but not characterized.[28] [g] Lit.[33] mp 142–144 °C. [h] Crystallized with 0.25 mol of H_2O. [i] Lit.[34] mp 244–246 °C. [j] Lit.[35] mp 217–218 °C. [k] Lit.[31] mp 273–276 °C.

Scheme 1[a]



[a] (a) $LiAlH_4$, THF; (b) $NaBH_4$, absolute EtOH; (c) $SOCl_2$/benzene or 48% HBr; (d) NaCN; (e) HCl(g), absolute EtOH; (f) H_2N-CH_2CH_2-NH_2.

Scheme 2[a]



[a] (a) H_2, 10% Pd/C, MeOH; (b) (1) ClCOOEt, (2) $LiAlH_4$; (c) HCl(g), absolute EtOH; (d) NH_3(g).

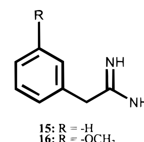
N-monomethylethylenediamine in place of ethylenediamine. A typical procedure is exemplified by the preparation of **11**. Compound **18** was obtained by debenzylation of **19** in methanol using hydrogen gas and 30% Pd/C. Reduction of the nitro group of **26** by catalytic hydrogenation gave **27**, and O-demethylation of 2-(2,6-dimethyl-3-methoxybenzyl)-2-imidazoline with excess boron tribromide gave **17**. Phenylacetonitrile and its 3-methoxy derivative were converted to the corresponding imidate **41** as shown in Scheme 1 but were then treated with ammonia gas at room temperature to obtain amidines **15** and **16**.

The phenylalkylamines **34a**,**b** and the amidine **35** were prepared from a common intermediate: **42** (Scheme 2). Catalytic reduction of **42** afforded **34a**; acylation of **34a** with ethyl chloroformate followed by reduction of the resulting carbamate with $LiAlH_4$ provided **34b**. Treatment of **42** with gaseous HCl gave the imidate **43**; reaction of **43** with NH_3 afforded **35**.

Results

We began our study by examining the affinity of oxymetazoline (**8**) for 5-HT_{1D} receptors of calf striatal membrane homogenates;[26] confirming the results of Schoeffter and Hoyer,[20] compound **8** ($K_i = 1.1 \pm 0.1$ nM) was found to bind with high affinity. Preliminary results were also obtained for **9** (K_i > 10 000 nM), **13** ($K_i = 700 \pm 40$ nM), and a number of other compounds (data not shown); however, after the discovery of h5-HT_{1B} and h5-HT_{1D} receptors, the binding studies were repeated, and data are shown in Table 2. The initial selection of compounds was based on calf membrane data. The following results will be discussed first in terms of h5-HT_{1B} and, subsequently, in terms of h5-HT_{1D} binding.

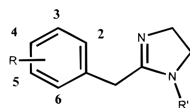
h5-HT_{1B} Binding. Compound **8** binds at human h5-HT_{1B} receptors ($K_i = 0.3$ nM; Table 2) with nearly 4-fold higher affinity than it displays at calf brain 5-HT_{1D} receptors. Compound **9** represents **8** with all the aromatic substituents removed; we also prepared the *N*-methyl analogue of **9** (i.e., **10**). Both **9** and **10** (K_i > 10 000 nM) lacked affinity for h5-HT_{1B} receptors indicating that one or more of the aryl substituents of **8** likely participate in binding. We rebuilt the structure of **8** in a stepwise manner. Compound **11** possesses only the 4-*t*Bu group of **8**, whereas **12** possesses only the 2,6-dimethyl groups; compound **11** binds only with modest affinity ($K_i = 6830$ nM), whereas **12** is inactive (K_i > 10 000 nM), suggesting that the 4-position substituent may contribute more to binding than the two methyl groups. A seemingly important structural feature, at least as indicated by calf membrane data, missing from **11** and **12** is the 3-hydroxy group. The monosubstituted 3-hydroxy derivative **13** ($K_i = 3735$ nM) was found to display rather modest affinity for h5-HT_{1B} receptors. Because hydroxy substitution may be more important than methoxy substitution for adrenergic activity,[19,27,28] it was of interest to examine the corresponding *O*-methyl derivative of **13**. Consequently, we prepared *O*-methyl ether **14**; compound **14** retained the affinity of **13** but, nevertheless, still displayed low affinity. Ring opening of **14** to amidine **16** (as well as of **9** to amidine **15**) resulted in inactive compounds (K_i > 10 000 nM in both cases) suggesting that an intact imidazoline ring may be necessary for binding. Reintroduction of the 2,6-dimethyl groups to **13** gives **17**, which may be viewed as oxymetazoline (**8**) minus the 4-*t*Bu group; **17** (K_i = 5785 nM) was found to bind with no higher affinity than the monohydroxy compound **13**, but with higher affinity than the 2,6-dimethyl derivative **12**.



Up to this point, the only derivatives with h5-HT_{1B} K_i values < 6000 nM were those bearing an oxygen substituent at the 3-position. We surmised that if a 3-hydroxy group contributes to binding, the difference in affinity between **8**, **13**, and **17** might be related to the nature of the −O−H bond. That is, in **8** the −O−H bond is forced out of the plane of the aromatic ring by the two adjacent substituents, whereas in the lower-affinity **13** the −O−H bond will probably be in the plane of the ring in order to achieve maximal π overlap. With the low-affinity **17**, the −O−H bond will likely lie in the plane of the ring with the hydrogen atom of the −OH group forced away from the 2-position methyl substituent. We contrived to force the hydrogen of the 3-hydroxy group out of the plane of the aromatic ring, attempting to mimic the presumed orientation of the 3-OH group in oxymetazoline (**8**), by flanking it with two small ortho substituents. Accordingly, we prepared and examined the 2,4-dimethyl-3-hydroxy derivative **18**. However, the affinity of compound **18** for h5-HT_{1B} receptors ($K_i = 1995$ nM) was not very different from that of the simpler monosubstituted 3-hydroxy compound **13** ($K_i = 3735$ nM). Compound **19**, the *O*-benzyl derivative of **18**, also displayed similarly low affinity.

Although there was some initial evidence that the 3-OH group might be involved in binding (as reflected by the binding of **13** relative to **9**), the difference in affinity between **8** and **13** was still about 10000-fold; thus, we questioned the importance of the hydroxyl group. Compound **20** is simply the deshydroxy derivative of oxymetazoline (i.e., xylometazoline); although it binds with reduced affinity ($K_i = 14$ nM) relative to oxymetazoline (**8**), it clearly shows that the presence of the hydroxy group is not an absolute requirement for binding. Nevertheless, the presence of this hydroxy

Table 2. Binding Properties of 2-(Substituted-benzyl)Imidazoline Derivatives



compd	R_2	R_3	R_4	R_6	R′	K_i, nM (SEM) h5-HT_{1B}	h5-HT_{1D}	h5-HT_{1D} selectivity[a]
8	Me	OH	tBu	Me	H	0.3 (0.1)	0.4 (0.01)	0.8
9	H	H	H	H	H	>10 000	>10 000	–
10	H	H	H	H	Me	>10 000	–[b]	–
11	H	H	*t*Bu	H	H	6 830 (600)	105 (12)	65
12	Me	H	H	Me	H	>10 000	2 210 (330)	>4
13	H	OH	H	H	H	3 735 (620)	>10 000	<1
14	H	OMe	H	H	H	1 630 (400)	–	–
17	Me	OH	H	Me	H	5 785 (660)	1 035 (420)	6
18	Me	OH	Me	H	H	1 995 (16)	1 900 (305)	1
19	Me	OBn	Me	H	H	3 865 (530)	1 095 (165)	4
20	Me	H	*t*Bu	Me	H	14 (2)	0.7 (0.2)	20
21	H	H	Et	H	H	>10 000	>10 000	–
22	H	H	*n*Pr	H	H	>10 000	2 375 (340)	>4
23	H	H	*i*Pr	H	H	2 780 (470)	340 (50)	8
24	H	H	*n*Bu	H	H	>10 000	2 520 (350)	>4
25	H	H	Br	H	H	>10 000	4 390 (0)	>2
26	H	H	NO_2	H	H	>10 000	>10 000	–
27	H	H	NH_2	H	H	>10 000	>10 000	–
28	H	H	Ph	H	H	>10 000	>10 000	–
29	Me	H	Me	Me	H	3 480 (1 690)	72 (12)	48
30	Me	H	*i*Pr	Me	H	37 (17)	1.1 (0.2)	34
31	Me	H	Br	Me	H	1 570 (10)	74 (27)	21
32	Me	H	*t*Bu	Me	Me	>5 000	86 (6)	>58
33	H	H	*t*Bu	Me	H	712 (130)	6.8 (0.9)	105

[a] Selectivity for h5-HT_{1D} binding (i.e., h5-HT_{1B} K_i value ÷ h5-HT_{1D} K_i value). [b] Value not determined.

group seems to account for the nearly 50-fold difference in affinity between the latter two compounds.

Apart from the hydroxy group (i.e., see **13**), the 4-*t*Bu group (i.e., see **11**) seems to influence affinity. Accordingly, we prepared a series of 4-substituted derivatives (i.e., **21**–**28**) to determine the electronic/steric influence of the 4-position substituent on binding. However, with the exception of the 4-*i*Pr derivative **23** (K_i = 2780 nM), compounds **21**–**28** all lacked affinity for h5-HT_{1B} receptors (K_i > 10 000 nM; Table 2).

The chief structural difference between the higher-affinity **20** and the lower-affinity or inactive **21**–**28** is the presence of the 2,6-dimethyl groups. With the assumption that the structural relationship between the two rings, perhaps dictated by the two flanking methyl groups, may be contributing to binding, we prepared several 2,6-dimethyl-4-substituted derivatives. For example, compound **29** (K_i = 3480 nM), the 2,4,6-trimethyl derivative of 2-(benzyl)imidazoline, binds with nearly 250-fold lower affinity than **20**, but with higher affinity than the simpler 2,6-dimethyl derivative **12** (K_i > 10 000 nM). The corresponding 4-*i*Pr derivative **30** (K_i = 37 nM) binds with still higher affinity. Compound **31**, the 2,6-dimethyl-4-bromo derivative, binds with reduced affinity (K_i = 1570 nM) but with higher affinity than **25** (K_i > 10 000 nM) which lacks the 2,6-dimethyl substituents. Compound **32** (K_i > 5000 nM), the *N*-methyl derivative of **20**, binds with low affinity indicating that *N*-methyl substitution is not well-tolerated. Taken together, it would seem that the aromatic substituents of **8** act in concert to impart high affinity. Removal of any of the ring substituents results in reduced affinity for h5-HT_{1B} receptors.

h5-HT_{1D} Binding. Most compounds were evaluated at h5-HT_{1D} receptors (see Table 2) to determine if they would display any selectivity between the two 5-HT_{1D} subpopulations and also in order to identify any differences in structure–affinity relationships. What emerges from these studies is that oxymetazoline (**8**) is essentially a nonselective agent that does not discriminate between h5-HT_{1B} and h5-HT_{1D} receptors. The 4-*t*Bu derivative **11**, although binding only with modest affinity (K_i = 105 nM), displays 65-fold selectivity for h5-HT_{1D} receptors. Compound **11** represented the first h5-HT_{1D}- versus h5-HT_{1B}-selective agent identified in this investigation and was the first indication that the benzylimidazolines might represent a novel class of h5-HT_{1D}-selective agents. As with h5-HT_{1B} binding, the presence of the 2,6-dimethyl groups seems to enhance affinity; for example, the affinity of **20** (K_i = 0.7 nM) is significantly (150-fold) higher than that of **11**; however, the h5-HT_{1D} selectivity of **20** is only about a one-third that of **11**. The higher affinity and selectivity of **20** relative to **8** suggest that the 3-hydroxy group of **8** plays a more substantial role in binding at h5-HT_{1B} receptors than it does at h5-HT_{1D} receptors; that is, this hydroxy group does not seem as necessary for binding at h5-HT_{1D} receptors. Indeed, removal of this hydroxy group decreases the affinity of **8** (as compared with **20**) at h5-HT_{1B} receptors by nearly 50-fold whereas it decreases affinity at h5-HT_{1D} receptors by less than 2-fold. There also appears to be a decrease in affinity as the 4-position substituent is decreased in size; for example, comparing the 4-*t*Bu, 4-*i*Pr, and 4-Me derivatives (i.e., **20**, **30**, and **29**, respectively), affinity decreases from 0.7 to 1.1 to 72 nM, respectively. Nevertheless, compounds **29** and **30** retain or exceed the h5-HT_{1D} selectivity of **20**. Unlike what was seen with binding at h5-HT_{1B} receptors, N-methylation appears to be somewhat better tolerated at h5-HT_{1D} receptors; compound **32** (K_i = 86

Table 3. Adrenergic Receptor Binding Data and Functional Data for Selected Compounds[a]

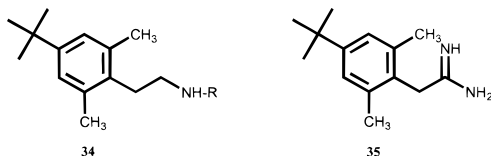
	adrenergic binding data (K_i, nM)		adenylate cyclase and rabbit saphenous vein (RSV) data: cAMP response as a % of forskolin response[b]				
			h5-HT$_{1D}$		h5-HT$_{1B}$		
compd	α_1	α_2	1 μM	10 μM	1 μM	10 μM	RSV[e] (EC$_{50}$, nM)
8[c]	42	>500	40	35	57	40	137
11[d]	2 625	1 120	69	65	99	86	>10 000
20	91	>500	38	41	51	64	369
29			23	49	49	52	150
30	65	>500	34	34	53	56	138
32	>10 000	1 320					
33	154	>500	51	50	63	58	1 670
3							220

[a] Standard errors were typically within 25%. [b] In separate assays, none of the compounds demonstrated any antagonist activity. [c] Compound **8**, oxymetazoline, included for comparison. [d] Compound **11** was found to bind with low affinity at human 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{2C}$, 5-HT$_6$, and 5-HT$_7$ receptors (<25% binding at 1000 nM). [e] None of the compounds demonstrated any antagonist activity with sumatriptan as agonist.

nM), although it binds with 120-fold lower affinity than **20** (K_i = 0.7 nM), is more selective than **20** for h5-HT$_{1D}$ receptors.

The 2,6-dimethyl substitution pattern found in **20**, relative to **11**, seems to have a greater impact on enhancing h5-HT$_{1B}$ affinity (i.e., 485-fold) than h5-HT$_{1D}$ affinity (150-fold); furthermore, the dimethyl derivative **20** displays 20-fold selectivity for h5-HT$_{1D}$ receptors, whereas **11** displays 65-fold selectivity. It was reasoned that the monodesmethyl analogue of **20** might possess somewhat lower affinity than **20** but display enhanced selectivity; indeed, compound **33** was prepared and found to bind at h5-HT$_{1D}$ receptors with high affinity (K_i = 6.8 nM) and 105-fold selectivity.

Ring-Opened Analogues. We previously examined amidines **15** and **16** and found them to bind with low affinity; however, their benzylimidazoline parents also displayed low affinity. Consequently, several ring-opened analogues of the higher-affinity **20** were examined. Phenylethylamines **34** (**34a**, R = H; **34b**, R = Me) failed to display appreciable affinity at h5-HT$_{1B}$ receptors (K_i > 10 000 nM) or h5-HT$_{1D}$ receptors (K_i = 1360 and >5000 nM, respectively). However, amidine **35** binds at both populations (K_i: h5-HT$_{1B}$ = 600 ± 140 nM; h5-HT$_{1D}$ = 13 ± 4 nM) indicating that the intact imidazoline ring is not an absolute requirement for binding. Nevertheless, although amidine **35** retains 45-fold selectivity for h5-HT$_{1D}$ receptors, its affinity is still 40- and 20-fold lower than that of **20** at h5-HT$_{1B}$ and h5-HT$_{1D}$ receptors, respectively. Thus, although the intact imidazoline ring may not be required for selectivity or for binding, its presence seems beneficial. Nevertheless, amidines would seem to represent a novel class of h5-HT$_{1D}$ ligands.



Conformational Considerations. Comparing the affinity of the 4-substituted derivative **11** (h5-HT$_{1B}$ K_i = 6830 nM) with its higher-affinity 2,6-dimethyl counterpart **20** (K_i = 14 nM), it is evident that the methyl groups contribute to binding. A similar phenomenon is seen with h5-HT$_{1D}$ binding. The possibility exists that the two methyl groups influence conformation and that different conformations of **11** and **20** might account for the differences in 5-HT$_{1D}$ affinity. Previous studies with 2-(2,6-dimethylbenzyl)imidazolines indicate that the two rings are essentially perpendicular to one another. For example, in the crystal structure[29] of xylometazoline hydrochloride (**20**) τ_1 (defined as $C_2-C_1-C_{benzylic}-C_{imidazoline}$) = 75.6° and τ_2 (defined as $C_2-C_{benzylic}-C_{imidazoline}-N_{imidazoline}$) = −142.9°. Using this crystal structure as input, the calculated τ_1 and τ_2 are identical to the observed values. Energy minimization of the crystal structure results in a small change in torsion angles (τ_1 = 86.0° and τ_2 = −148.9°). The angles calculated for the desmethyl derivative **11** are quite similar (τ_1 = 87.2° and τ_2 = −145.0°). In fact, alteration of the τ_1 and τ_2 values of **11** by several degrees (including fixing the values to those obtained from the lowest-energy conformation of **20**) has almost no effect on energy (Δ kcal/mol < 0.1). It would seem then that the desmethyl compound **11** can, at least in theory, readily adopt the lowest-energy conformation of **20** and that the contribution of the two methyl groups to binding may be unrelated to their effect on conformation. Alternative roles for the methyl groups may be that they decrease overall flexibility or that one or both interact in a productive manner with some receptor feature. This remains to be explored.

α-Adrenoceptor Binding and Functional Studies. The binding profiles of the various benzylimidazolines have not yet been examined in detail. However, several compounds were selected simply to determine if they retain high affinity for α-adrenergic receptors (Table 3). It was found that compounds **11** (α_1-adrenergic and α_2-adrenergic K_i = 2625 and 1120 nM, respectively) and **32** (α_1-adrenergic and α_2-adrenergic K_i = >10 000 and 1320 nM, respectively) bind with reduced affinity relative to oxymetazoline (**8**); compounds **20**, **30**, and **33** bind with about one-half to one-third the affinity of **8**. There is no reason to believe that the SAR for α-adrenergic binding and h5-HT$_{1D}$ binding of the benzylimidazolines will be inextricably linked. However, continued work will be necessary to verify this.

Oxymetazoline (**8**) has been reported to be a 5-HT$_{1D}$ agonist.[20] To obtain a preliminary indication of whether any of the agents in Table 2 retain agonist character, several were evaluated for their ability to inhibit cAMP

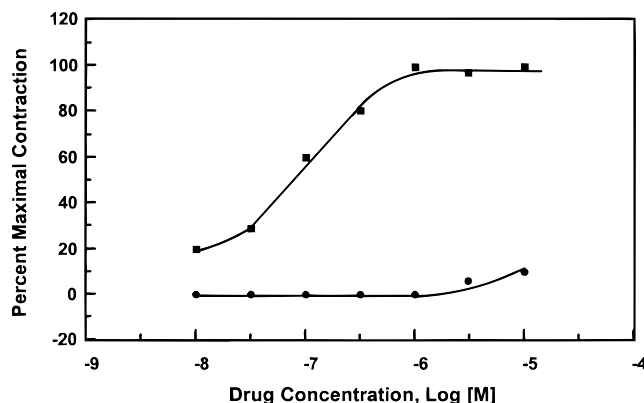


Figure 1. Contractions of rabbit saphenous vein produced in a representative experiment by compounds **29** (upper tracing) and **11** (lower tracing). Response is reported as a percent of the maximal contraction produced by 80 mM KCl.

production in cells transfected with h5-HT_{1D} or h5-HT_{1B} receptors (Table 3). Five compounds were evaluated and compared with oxymetazoline. None of the agents demonstrated antagonist properties up to concentrations of 10^{-5} M. In contrast, agonist effects were indicated by inhibition of adenylate cyclase activity. At 1 or 10 μM concentrations, all compounds inhibited cyclase activity to an extent comparable to that of oxymetazoline (Table 3). Due to the preliminary nature of the studies, quantitative comparisons were not made; nevertheless, the results in Table 3 suggest that each of the compounds possesses both h5-HT_{1D} and h5-HT_{1B} agonist properties. The same five compounds were examined both as antagonists and as agonists in the rabbit saphenous vein preparation as another measure of 5-HT_{1D} functional activity. None of the compounds demonstrated antagonist activity. All behaved as agonists, and EC_{50} values are reported for four of these relative to oxymetazoline (**8**) and sumatriptan (**3**) in Table 1. Representative dose−response curves are shown in Figure 1 for **11** and **29**. Compound **11** showed only weak agonist activity; however, **29** (EC_{50} = 150 ± 15 nM) produced 95% of the maximal effect with a potency similar to that of sumatriptan control (EC_{50} = 220 nM). As previously reported by Schoeffter and Hoyer[20] for oxymetazoline (**8**), compounds **20**, **29**, **30**, and **33** display 5-HT_{1D} agonist character and are devoid of antagonist properties.

Discussion

Oxymetazoline (**8**) binds at human h5-HT_{1B} receptors with high affinity (K_i = 0.3 nM). It would appear that the presence of the 4-position *t*Bu group and the 2,6-dimethyl groups is important for h5-HT_{1B} binding; the 3-hydroxy group also contributes to binding, but its presence is not a critical requirement. It seems unlikely, at this time, that the two aromatic methyl groups serve primarily to maintain the perpendicularity of the two rings; they do however contribute to binding. It seems that all features of **8** examined in the present investigation act in concert to impart high affinity for h5-HT_{1B} receptors. No structural modification resulted in a compound with higher h5-HT_{1B} affinity than **8**. Oxymetazoline (**8**) also binds at h5-HT_{1D} receptors with high affinity (K_i = 0.4 nM), and similar comments may be made about h5-HT_{1D} binding except that the 3-hydroxy group seems to play a less prominent role. That is, removal of the 3-hydroxy group has a more deleterious effect on h5-HT_{1B} affinity than on h5-HT_{1D} affinity, and compounds lacking this hydroxy group display 20- to >100-fold selectivity for h5-HT_{1D} versus h5-HT_{1B} receptors. As such, these compounds are among some of the first to discriminate between the two 5-HT_{1D} subpopulations. Preliminary results also indicate that benzylimidazolines possess 5-HT_{1D} agonist character.

Sumatriptan (**3**), an agent currently used for the treatment of migraine, binds equally well at both subpopulations of 5-HT_{1D} receptors. Likewise, there is no evidence of subpopulation selectivity among the other 5-HT_{1D} agonists being investigated as potential antimigraine agents.[14] Intravenous and subcutaneous administration of sumatriptan has been reported to produce cardiovascular side effects (e.g., see ref 30). Given that activation of 5-HT_1-like receptors, resembling h5-HT_{1B} more than h5-HT_{1D} receptors, may be responsible for coronary artery contraction and spasm,[30] agents displaying selectivity for h5-HT_{1D} receptors could offer a therapeutic advantage due to their reduced propensity to produce cardiovascular side effects. Studies are currently in progress with the benzylimidazoline template to identify compounds with even greater selectivity.

Experimental Section

Synthesis. Melting points, determined with a Thomas-Hoover melting point apparatus, are uncorrected. Proton magnetic resonance spectra were obtained with a JEOL FX90Q, GE QE-300, or Varian Gemini 300 spectrometer; tetramethylsilane was used as an internal standard. Infrared spectra were recorded on a Nicolet 5ZDX FT-IR. Elemental analysis was performed by Atlantic Microlab Inc., and determined values are within 0.4% of theory. Flash chromatography was performed on silica gel (Merck grade 60, 230−400 mesh 60 Å). Compounds **8** and **20** were purchased as their HCl salts from Sigma (St. Louis, MO).

2-(4-*tert*-Butylbenzyl)-2-imidazoline Hydrochloride (11). A mixture of NaCN (1.1 g, 22 mmol) and 4-*tert*-butylbenzyl bromide (5.0 g, 22 mmol) in EtOH−H_2O (70 mL, 6:1) was allowed to stir under reflux conditions for 4 h. After the mixture cooled to room temperature, solvent was removed under reduced pressure to afford a yellow oil; the oil was suspended in H_2O (25 mL) and extracted with Et_2O (3 × 25 mL). The combined extracts were evaporated to dryness under reduced pressure, and the resulting white oil was purified by Kugelrohr distillation (bp 120−125 °C, 0.2 mmHg) to afford 3.4 g (89%) of (4-*tert*-butylphenyl)acetonitrile as a colorless oil. HCl gas was bubbled through a solution of 3.0 g (17.8 mmol) of the oil in anhydrous Et_2O (50 mL) and absolute EtOH (0.7 g, 17.3 mmol) with cooling on an ice bath. The reaction mixture was allowed to stir at 0 °C for 1.5 h and then overnight at room temperature. The white solid was collected by filtration and washed with anhydrous Et_2O (3 × 15 mL) to give 3.7 g (83%) of ethyl 2-(4-*tert*-butylphenyl))acetimidate as white crystals: mp 119−120 °C.

A solution of ethylenediamine (1.6 g, 27.2 mmol) in absolute EtOH (5 mL) was added to a stirred solution of the above imidate (i.e., **41**, R = 4-*t*Bu) (3.5 g, 13.6 mmol) in absolute EtOH (20 mL) at ice-bath temperature. After stirring at 0 °C for 1 h, the reaction mixture was heated at reflux for 20 min; the solvent was evaporated, the oily residue was washed with H_2O (2 × 3 mL) and extracted with CH_2Cl_2 (3 × 25 mL), and the combined organic extracts were dried ($MgSO_4$) and evaporated to dryness to give 2.7 g (90%) of the product as its free base. HCl gas was bubbled through a solution of the base in anhydrous Et_2O to give the HCl salt as white crystals after recrystallization from MeCN: mp 232−234 °C; see Table 1; IR (KBr) 1610 cm^{-1}; ^1H NMR (D_2O) δ 1.45 (s, 9H, *t*BuH), 3.95

(br signal, 6H, 3 $-CH_2-$), 7.35 (d, 2H, Ar$-$H), 7.61 (d, 2H, Ar$-$H).

2-Phenylacetamidine Oxalate (15). A solution of ethyl phenylimidate (1 g, 0.5 mmol) in absolute EtOH (3.5 mL) was saturated with dry NH_3 gas for 30 min. The reaction mixture was allowed to stir at room temperature overnight. The solid material was removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. The residue was suspended in 5 N NaOH (2 mL) and extracted with $CHCl_3$ (2 × 10 mL). The combined $CHCl_3$ portions were dried ($MgSO_4$), and the solvent was removed under reduced pressure to afford 0.6 g of **15** (free base) as a white solid: mp 108−110 °C. An Et_2O solution of the amidine was treated with an Et_2O solution of oxalic acid to afford the crude salt. Recrystallization from 2-PrOH gave 0.8 g (75%) of **15** as white crystals: mp 153−155 °C. Anal. ($C_8H_{10}N_2 \cdot C_2H_2O_4$) C,H,N.

2-(2,6-Dimethyl-3-hydroxybenzyl)-2-imidazoline Hydrobromide (17). An excess of dry HCl gas was passed through a solution of 2,6-dimethyl-3-methoxyphenylacetonitrile[24] (50 mg, 0.29 mmol) in anhydrous Et_2O (10 mL) and absolute EtOH (12 mL) at ice-bath temperature. The resulting solution was stirred at 0 °C for 2 h and then overnight at room temperature. The white precipitate was collected by filtration, washed with Et_2O (2 × 10 mL), and dried to afford 47 mg (63%) of 2,6-dimethyl-3-methoxyphenylacetimidate as a white solid: mp 128−130 °C.

A solution of ethylenediamine (21 mg, 0.35 mmol) in absolute EtOH (0.5 mL) was added to a solution of the above (47 mg, 0.17 mmol) in absolute EtOH (2.5 mL) at ice-bath temperature. After stirring at 0 °C for 1 h, the solution was heated at reflux for 20 min. The solvent was evaporated, and the oily residue was washed with H_2O (0.2 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The solvent was dried ($MgSO_4$) and evaporated under reduced pressure to give 25 mg (65%) of 2-(2,6-dimethyl-3-methoxybenzyl)-2-imidazoline as a white solid: mp 118−120 °C.

Boron tribromide in CH_2Cl_2 (1 mL of a 1 M solution) was added in a dropwise manner to a stirred solution of the free base of 2-(2,6-dimethyl-3-methoxybenzyl)-2-imidazoline (25 mg, 0.11 mmol) in CH_2Cl_2 (1 mL) at −78 °C under a N_2 atmosphere; stirring was allowed to continue for 24 h. The solution was cooled at 0 °C, and MeOH (1 mL) was added. The solvent was removed under reduced pressure, and the solid was washed with excess Et_2O. The salt was recrystallized from a MeOH−anhydrous Et_2O mixture to give 22 mg (72%) of **17** as a white solid: mp 246−248 °C. Anal. ($C_{12}H_{16}N_2O \cdot HBr$) C,H,N.

2-(3-Hydroxy-2,4-dimethylbenzyl)-2-imidazoline Hydrochloride (18). Catalyst (30% Pd/C; 0.04 g) was added to a solution of **19** (0.4 g, 1.2 mmol) in absolute EtOH (25 mL). The mixture was hydrogenated at 50 psi for 2 h and filtered over a Celite pad; the solvent was evaporated under reduced pressure to afford a white solid. Recrystallization from 2-PrOH gave 0.25 g (86%) of **18** as a white solid: mp 260−261 °C. Anal. ($C_{12}H_{16}N_2O \cdot HCl$) C,H,N.

2-(3-Benzyloxy-2,4-dimethylbenzyl)-2-imidazoline Hydrochloride (19). A solution of KOH (1.9 g, 34 mmol) in H_2O (6 mL) and MeOH (6 mL) was added in a dropwise manner to a warm (70 °C) solution of 3-hydroxy-2,4-dimethylbenzaldehyde[25] (5.1 g, 34 mmol) and benzyl bromide (5.8 g, 34 mmol) in absolute EtOH (45 mL). After the addition was complete, the reaction mixture was allowed to stir at 70 °C for 2 h. The solid material was removed by filtration, and the filtrate was evaporated under reduced pressure to give an orange oil. The residue was washed successively with 2 N NaOH (15 mL) and H_2O (15 mL) and extracted with Et_2O (3 × 30 mL). The solvent was dried ($MgSO_4$) and evaporated under pressure to give an orange oil which was purified by distillation (Kugelrohr, bp 160−165 °C/0.1 mmHg) to give 5.1 g (63%) of 3-benzyloxy-2,4-dimethylbenzaldehyde as a colorless oil.

A solution of $NaBH_4$ (0.3 g, 7 mmol) in 90% EtOH (5 mL) was added in a dropwise manner to a solution of the above 3-benzyloxy-2,4-dimethylbenzaldehyde (5 g, 20.8 mmol) in absolute EtOH (42 mL). The reaction mixture was allowed to stir at room temperature for 30 min and then heated at 60 °C for 30 min. The solution was cooled to 0 °C, and unreacted borohydride was decomposed by the addition of a few drops of 3 N HCl. The solvent was removed under reduced pressure to give a white solid which was suspended in H_2O (15 mL) and extracted with Et_2O (3 × 20 mL). The solvent was removed under reduced pressure to afford 4.9 g (98%) of 3-benzyloxy-2,4-dimethylbenzyl alcohol as a white solid: mp 95−97 °C.

Thionyl chloride (2.9 mL, 40.4 mmol) was added to a solution of 3-benzyloxy-2,4-dimethylbenzyl alcohol (4.9 g, 20.2 mmol) in dry benzene (40 mL). The reaction mixture was allowed to stir at room temperature for 2 h. The solvent was removed under reduced pressure to give a white solid. Recrystallization from 50% EtOH gave 4.7 g (88%) of 3-benzyloxy-2,4-dimethylbenzyl chloride as a white solid: mp 58−60 °C. Sodium cyanide (0.8 g, 17.7 mmol) was added to a stirred solution of the benzyl chloride (4.6 g, 17.7 mmol) in H_2O/EtOH (6:1) (90 mL). The reaction mixture was allowed to stir under reflux conditions for 4 h. After the reaction mixture had reached room temperature, the solvent was evaporated under reduced pressure to afford a yellow oil. The oil was suspended in H_2O (30 mL) and extracted with Et_2O (3 × 50 mL). The solvent was removed under reduced pressure to give a yellow oil which was purified by distillation (Kugelrohr, bp 160−165 °C/0.05 mmHg) to give 3.7 g (84%) of 3-benzyloxy-2,4-dimethylphenylacetonitrile as a clear liquid. An excess of dry HCl gas was passed through a solution of 3-benzyloxy-2,4-dimethylphenylacetonitrile (2.0 g, 7.9 mmol) in anhydrous Et_2O (50 mL) and absolute EtOH (0.3 g) at ice-bath temperature. The resulting solution was allowed to stir at 0 °C for 1.5 h and at room temperature overnight. The white solid was collected by filtration, washed with Et_2O (3 × 15 mL), and dried to give 2.1 g (80%) of ethyl (3-benzyloxy-2,4-dimethylphenyl)acetimidate hydrochloride as white crystals: mp 108−110 °C.

A solution of ethylenediamine (1.2 g, 3.6 mmol) in absolute EtOH (10 mL) was added to a solution of the imino ester hydrochloride (1.19 g, 3.56 mmol) in absolute EtOH (15 mL) at ice-bath temperature. After stirring at 0 °C for 1 h, the solution was heated at reflux for 20 min. The solvent was evaporated, and the oily residue was washed with H_2O (2 × 5 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The solvent was dried ($MgSO_4$) and evaporated under reduced pressure to give a white solid. Dry HCl gas was bubbled through a solution of the free base in anhydrous Et_2O; the crude salt was collected and recrystallized from MeOH/Et_2O to afford 1.0 g (84%) of **19** as a white solid: mp 188−190 °C. Anal. ($C_{19}H_{22}N_2O \cdot HCl$) C,H,N.

2-(4-*tert*-Butyl-2-methylbenzyl)imidazoline Hydrochloride (33). A solution of 4-*tert*-butyl-2-methylbenzoic acid[36] (0.9 g, 4.7 mmol) in dry THF (5 mL) was added in a dropwise manner to a well-stirred suspension of $LiAlH_4$ (0.6 g, 16 mmol) in THF (40 mL) at 0 °C. The reaction mixture was allowed to stir for 2 h at 0 °C and was then heated at reflux for 30 min. Excess $LiAlH_4$ was decomposed by the dropwise addition of H_2O (1 mL) at 0 °C. The reaction mixture was filtered, and the filtrate was evaporated to dryness; Et_2O (70 mL) and 2 M H_2SO_4 (27 mL) were added. The aqueous portion was extracted with Et_2O (3 × 50 mL). The combined ethereal fraction was washed with 15% NaOH (3 × 25 mL) and dried ($MgSO_4$), and solvent was removed under reduced pressure. The crude product was purified by column chromatography (silica gel, 60 mesh, 40 g) with petroleum ether/EtOAc (9:1) as eluent to afford 0.7 g (79%) of the benzyl alcohol as a clear colorless oil: IR (film) 3377 cm^{-1}.

Thionyl chloride (1.0 g, 8.2 mmol) was added to a solution of the above alcohol (0.7 g, 4.1 mmol) in dry benzene (10 mL), and the reaction mixture was allowed to stir at room temperature for 2 h. The solvent was removed under reduced pressure, and the crude product in a 6:1 mixture of EtOH/H_2O was heated overnight with NaCN (0.2 g, 4.1 mmol). Solvent was removed under reduced pressure, and the brown oil in H_2O (10 mL) was extracted with CH_2Cl_2 (3 × 25 mL). The organic portion was dried ($MgSO_4$) and evaporated to

dryness. The crude product was purified by column chromatography (silica gel, 60 mesh, 30 g) with petroleum ether/EtOAc (9:1) as eluent to afford 0.4 g (62%) of the corresponding nitrile as a yellow oil. The nitrile (1.7 g, 9.4 mmol) was treated with HCl gas as described below for the preparation of **35** to afford the crude imino ether. The imino ether was allowed to react with ethylenediamine and EtOH as described for **17** to afford 0.9 g (34%) of **33** after recrystallization from an absolute $EtOH/Et_2O$ mixture: mp 245−250 °C; see Table 1.

2-(2,6-Dimethyl-4-*tert*-butylphenyl)-1-aminoethane Hydrochloride (34a). A solution of **42**[37] (2.1 g, 10.4 mmol) in MeOH (60 mL) and 10% Pd/C was hydrogenated in a Parr apparatus at 40 psi overnight. Catalyst was removed by filtration, and the solvent was removed by evaporation under reduced pressure. The crude product was purified by column chromatography (silica gel, 60 mesh, 70 g) with petroleum ether/EtOAc (9:1) as eluent to yield 1.6 g (76%) of **34a** as the free base: IR (film) 3421 cm^{-1}. The hydrochloride salt was prepared and recrystallized from MeOH: mp >350 °C (lit.[38] mp >350 °C).

***N*-Monomethyl-2-(2,6-dimethyl-4-*tert*-butylphenyl)-1-aminoethane Hydrochloride (34b).** Ethyl chloroformate (0.3 g, 2.4 mmol) in dry THF (10 mL) was added in a dropwise manner to a stirred solution of **34a** (free base; 0.5 g, 2.4 mmol) and Et_3N (0.5 g, 4.8 mmol) in dry THF (20 mL) at 0 °C. After the reaction mixture had been allowed to stir at room temperature overnight, it was filtered, and the filtrate was evaporated to dryness under reduced pressure. The crude material was purified by column chromatography (silica gel, 60 mesh, 40 g) with petroleum ether/EtOAc (9:1) as eluent to give the carbamate (0.6 g) which was used without further characterization. The carbamate (0.6 g) in dry THF (30 mL) was added to a suspension of $LiAlH_4$ (0.5 g, 12.0 mmol) in THF (10 mL), and the reaction mixture was heated at reflux for 4 h. Excess hydride was decomposed by the dropwise addition of H_2O (1 mL) at 0 °C. The mixture was filtered, solvent was removed from the filtrate by evaporation under reduced pressure, and 15% NaOH (20 mL) was added. The aqueous solution was extracted with Et_2O (3 × 30 mL); the combined ether portion was dried ($MgSO_4$) and evaporated to dryness. The hydrochloride salt was prepared and recrystallized from MeOH to afford 0.3 g (49%) of **34b** as a white solid: mp 255−260 °C (lit.[39] mp 252−254 °C).

2-(2,6-Dimethyl-3-*tert*-butylphenyl)acetamidine Hydrochloride (35). An excess of dry HCl gas was bubbled through a solution of 2-(2,6-dimethyl-4-*tert*-butylphenyl)acetonitrile[37] (**42**) (7.0 g, 34.8 mmol) in anhydrous Et_2O (100 mL) and absolute EtOH (1.6 g, 34.8 mmol) at ice-bath temperature. The reaction mixture was allowed to stir for 2 h at 0 °C and was then stored in a refrigerator for 4 days. The white precipitate was collected by filtration, washed with Et_2O (2 × 10 mL), and allowed to air-dry to afford 5.5 g (56%) of **43**: mp 147−149 °C. A solution of **43** (0.1 g, 0.35 mmol) in absolute EtOH (2 mL) was saturated with dry ammonia gas for 30 min. The reaction mixture was allowed to stir at room temperature overnight; the solid material was removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. The residue was suspended in 5 N NaOH (1 mL) and extracted with $CHCl_3$ (2 × 10 mL). The combined organic portion was dried ($MgSO_4$), and the solvent was removed under reduced pressure to afford crude **35** as the free base. Dry HCl gas was bubbled through a solution of the crude product in anhydrous Et_2O to give 0.8 g (83%) of **35** as white crystals after recrystallization from an absolute $EtOH/Et_2O$ mixture: mp 232−234 °C. Anal. ($C_{14}H_{22}N_2$·HCl) C,H,N.

Molecular Modeling. The structure of xylometazoline (**20**) was constructed from its X-ray coordinates[29] using the CRYSTAL interface of the SYBYL (version 6.3) molecular modeling program (SYBYL Molecular Modeling Package, Version 6.3 with Update #2 (1997); Tripos Inc., St. Louis, MO). The structure of **20** was manually abbreviated to **11** by removal of the methyl groups. After molecular mechanics minimization (MINIMIZE), charges were calculated by the Gasteiger−Huckel algorithm and torsion angles were measured by applying MEASURE TORSION.

Radioligand Binding Studies.[40,41] Receptor binding studies were performed in triplicate in 96-well polypropylene microtiter plates using a reaction volume of 500 μL. The test compound was initially assyaed at 1 and 0.1 μM and then followed by a K_i determination. The radioligand employed was [^{3}H]-5-HT trifluoroacetate (100 Ci/mmol; Amersham), 2.5 nM final concentration; nonspecific binding was determined 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% ascorbic acid, pH 7.4 at 22 °C. Incubation was started by the addition of membrane homogenate (0.1 mg of protein/well); the plates were vortexed for 20 s and then incubated at room temperature for 60 min. The binding reaction was terminated 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 Top-Count). Binding dpm in the presence of test drugs is expressed as a percent of binding dpm in the absence of drug. A percent binding-versus-concentration curve was then constructed from which the IC_{50} value (drug concentration resulting in 50% inhibition) was determined. K_i values were calculated from the IC_{50} values using the Cheng−Prusoff transformation.

Functional Studies.[41−43] A CHO Pro 5 cell line was stably transfected with human recombinant h5-HT_{1D} or h5-HT_{1B} receptors. Drugs were assayed for agonist activity on the basis of their ability to inhibit cAMP production by this cell line in the presence of 10 μM forskolin and 0.5 mM IBMX. Test compounds were added to the culture media, and the cells were incubated for 30 min at 37 °C. Following incubation, the reaction 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 immunoassay kit from Amersham. Serotonin was used for comparison. To test for antagonist activity, drugs were evaluated 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.

Evaluation of compounds for 5-HT_{1D}-like receptor-mediated vascular effect in rabbit saphenous vein used tissues obtained from New Zealand white rabbit (≈3−4 kg, male) sacrificed by pentobarbital overdose. The saphenous veins from both sides were placed in Krebs solution containing NaCl (118 mM), glucose (25 mM), $NaHCO_3$ (25 mM), KCl (4.7 mM), $CaCl_2$ dihydrate (2.5 mM), KH_2PO_4 (1.2 mM), and $MgSO_4·7H_2O$ (1.2 mM). Ring segments (4−5 mm) of the vein were obtained, and the endothelium was gently removed. The rings were mounted for isometric tension recording in 10-mL organ baths containing Krebs buffer aerated constantly with 95% O_2−5% CO_2 and maintained at 37 °C, pH 7.4. A resting tension of 2.5 g was applied, and the tissues were allowed to equilibrate for 90 min with washing every 15−20 min. After the equilibration period the rings were depolarized with 80 mM KCl. The tissues were then exposed to prazosin (1 μM), idazoxan (1 μM), and indomethacin (10 μM) for 30 min. Cumulative concentration−effect curves were then constructed to sumatriptan and compounds being evaluated. Responses were calculated as a percentage of the maximal contraction evoked by 80 mM KCl.

Note Added in Proof. While our manuscript was under review, Merck Sharp and Dohme reported the synthesis and evaluation of a series of tryptamine and homotryptamine derivatives (*J. Med. Chem.* **1997**, *40*, 3497-3500), examples of which bind with up to 200-fold selectivity at h5-HT_{1D} over h5-HT_{1B} receptors. These agents were demonstrated to behave as 5-HT_{1D} agonists.

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