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# Imidazoline-Modified Benzylimidazolines as h5-HT$_{1D/1B}$ Serotonergic Ligands

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**Abstract**—Sumatriptan, a h5-HT$_{1D}$ and h5-HT$_{1B}$ receptor agonist used clinically as a migraine-abortive, produces certain side effects thought to result from its affinity for h5-HT$_{1B}$ receptors. The present investigation extends our work with benzylimidazolines as novel non-tryptamine h5-HT$_{1D/1B}$ ligands. The effect of *N*-methylation, *N*-benzylation, ring-aromatization, and variation of the imidazoline ring on affinity both at h5-HT$_{1D}$ and h5-HT$_{1B}$ receptors was examined. Several compounds were identified with good affinity and enhanced (i.e., >100-fold) h5-HT$_{1D}$ versus h5-HT$_{1B}$ selectivity. 

## Introduction

Migraine is a major public health problem. It is estimated, for example, that the disorder affects 10–15% of the total US population[1–3] The mechanism or etiology of migraine has had a long association with serotonin (**1**; Fig. 1) dysfunction.[4] A number of serotonergic agents, such as methysergide and dihydroergotamine, can be effective in the treatment of migraine.[5,6] However, the use of these agents is limited due to their accompaniment by a variety of side-effects thought to result from their affinity for other receptor populations.[7] The development of the tryptaminergic serotonin (5-HT) receptor agonist sumatriptan has proven to be a significant advance in the treatment of acute migraine. Sumatriptan (**2**) exhibits selectivity for the 5-HT$_1$ family of receptors, particularly h5-HT$_{1D}$ and h5-HT$_{1B}$ receptors. However, it displays little selectivity between these two receptor subtypes.[6] It is generally accepted that the clinical efficacy of sumatriptan is probably mediated through its action at either one or both of these receptors.[8–11]

Despite its clinical effectiveness, sumatriptan suffers from problems of bioavailability and, in patients with heart disease, it can occasionally produce coronary artery constriction that is thought to result from its affinity for h5-HT$_{1B}$ receptors.[12–16] Although it is not yet clear which of the two populations (if either) should be targeted by migraine-abortive agents, nor is it certain which population is the major contributor to sumatriptan's side effects,[17–20] there exists a need to identify novel entities that display selectivity for h5-HT$_{1D}$ versus h5-HT$_{1B}$ receptors. Most current h5-HT$_{1D}$ ligands are tryptamine-based and, hence, are likely to suffer from related drawbacks including poor absorption and low bioavailability.[21] As a result, nontryptamine agents with selectivity for h5-HT$_{1D}$ over h5-HT$_{1B}$ receptors are attractive targets for development. Russell et al.,[20] for example, have recently reported a series chain-extended tryptamine derivatives, that is, 3-[3-(aminopropyl)]indoles, that show up to 240-fold selectivity for h5-HT$_{1D}$ over h5-HT$_{1B}$ receptors.

We have previously identified 2-benzylimidazolines as novel nontryptamine-based h5-HT$_{1B/1D}$ ligands.[22] Our original study examined the aryl portion of benzylimidazolines and its influence on affinity and selectivity. Oxymetazoline (**3**), for example, is a high affinity (h5-HT$_{1D}$ $K_i = 0.3$ nM; 5-HT$_{1B}$ $K_i = 0.4$ nM) but nonselective agent.[22] Modification of the aryl portion was found to result in retention or a slight decrease in affinity, but in an increase in selectivity. For example, in the course of our investigations xylometazoline (**4a**) was found as being 20-fold selective for h5-HT$_{1D}$ ($K_i = 0.7$ nM) versus h5-HT$_{1B}$ ($K_i = 14$ nM) receptors.[22] The present investigation further examines the binding of benzylimidazolines

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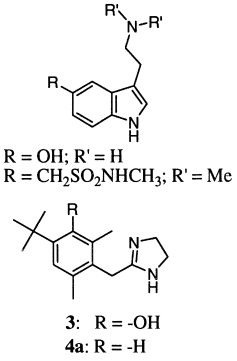


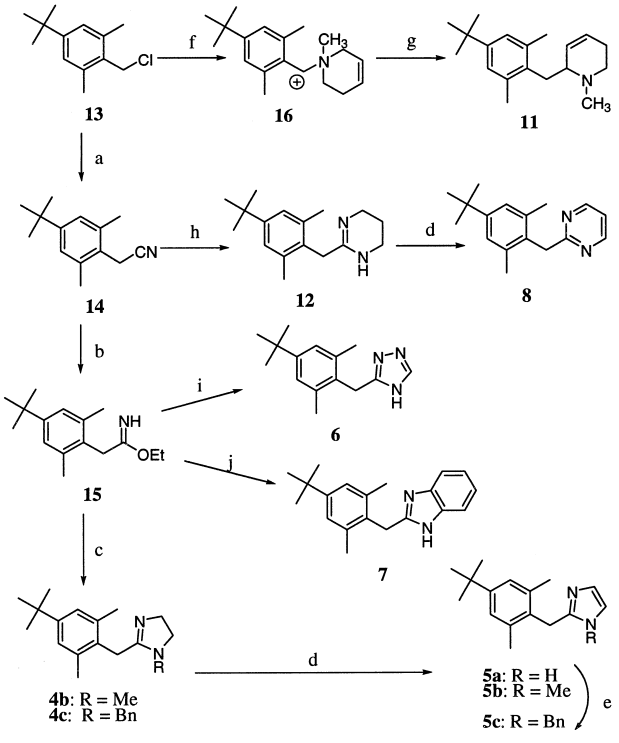

**Figure 1.** Structures of serotonin (**1**), sumatriptan (**2**), oxymetazoline (**3**), and xylometazoline (**4a**).

at h5-$HT_{1B/1D}$ receptors by focusing on the contribution of the imidazoline ring. Specifically, the imidazoline ring of **4a** was modified by (a) *N*-methylation, (b) *N*-benzylation, (c) aromatization, and (d) replacement with selected heterocycles.

## Results and Discussion

### Chemistry

Compounds **4b**, **4c**, **5a**–**c**, **6**–**8**, **11** and **12** were prepared according to Scheme 1. Treatment of 4-*tert*-butyl-2,6-dimethylbenzyl chloride[23] (**13**) with NaCN afforded 4-*tert*-butyl-2,6-dimethylphenylacetonitrile[24] (**14**), which was subsequently treated with HCl gas in absolute ethanol to afford imidate **15**. The reaction of imidate **15** with the appropriate diamine or hydrazine afforded targets **4b**, **4c**, **6** and **7**. Imidazoles **5a** and **5b** were obtained by heating a toluene solution of imidazolines **4a** and **4b**, respectively in the presence of 10% Pd/C. Imidazole **5c** was obtained by treating **5a** with benzyl bromide under basic conditions. The fusion of **14** and the mono *p*-toluenesulfonic acid salt of 1,3-diaminopropane afforded tetrahydropyrimidine **12**. Pyrimidine **8** was obtained by heating a *m*-xylene solution of **12** in the presence of 10% Pd/C. The addition of **13** to 1-methyl-1,2,5,6-tetrahydropyridine afforded quaternary salt **16**; compound **16** was converted to tetrahydropyridine **11** via Stevens rearrangement by treatment with phenyl lithium at room temperature.

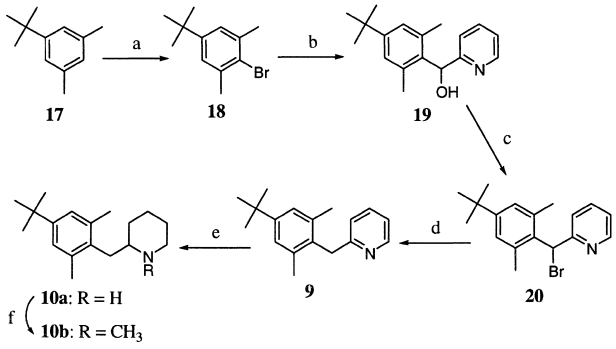



**Scheme 1.** Synthesis of targets **4b**, **4c**, **5a**–**5c**, **6**–**8**, **11** and **12**. Reagents: (a) NaCN; (b) HCl (g), absolute EtOH; (c) $RNH(CH_2)_2NH_2$, absolute EtOH; (d) 10% Pd/C, reflux; (e) benzyl bromide, $K_2CO_3$ DMF; (f) 1-methyl-1,4,5,6-tetrahydropyridine, acetone; (g) phenyl lithium; (h) *p*-toluenesulfonic acid, heat; (i) 1) $NH_2$-$NH_2$, 2) HCOOH; (j) *o*-phenylenediamine, absolute EtOH.

Compounds **9**, **10a** and **10b** were prepared according to the procedure in Scheme 2. The addition of bromine to a mixture of sodium acetate and **17** in glacial acetic acid gave **18**.[25,26] The addition of 2-pyridinecarboxaldehyde to the Grignard reagent prepared from **18** gave biaryl alcohol **19**. Treatment of **19** with $PBr_3$ in dry $CH_2Cl_2$ provided bromo derivative **20** which was subsequently reduced to 2-benzylpyridine **9** using hydrogen gas and 10% Pd/C in absolute EtOH. Further reduction of **9** using hydrogen gas and 10% Pd/C in a mixture of concentrated HCl and EtOH afforded 2-benzylpiperidine **10a**.[27] The methylated derivative **10b** was obtained by the reductive alkylation of **10a** using formaldehyde and sodium cyanoborohydride.[28]

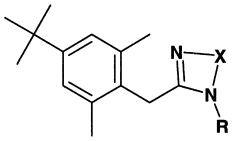
### Radioligand binding

The effect of *N*-methylation, *N*-benzylation, and aromatization of the imidazoline ring of **4a** (h5-$HT_{1D}$ $K_i = 0.7$ nM, h5-$HT_{1B}$ $K_i = 14$ nM; 20-fold selectivity) on 5-HT receptor affinity is shown in Table 1. *N*-Methylation of **4a** (i.e., **4b**) decreased affinity both at h5-$HT_{1D}$ ($K_i = 82$ nM) and h5-$HT_{1B}$ ($K_i = 2160$ nM) receptors by $>100$-fold but resulted in retention of h5-$HT_{1D}$ selectivity (26-fold selectivity). Replacement of the methyl group with a benzyl group had little effect on affinity (**4c**; h5-$HT_{1D}$ $K_i = 30$ nM, h5-$HT_{1B}$ $K_i = 1860$ nM) or selectivity (62-fold selectivity). Aromatization of **4a** to **5a** (h5-$HT_{1D}$ $K_i = 1.2$ nM) did not effect h5-$HT_{1D}$ affinity, but




**Scheme 2.** Synthesis of targets **9**, **10a** and **10b**. Reagents: (a) $Br_2$, NaOAc, AcOH; (b) (1) Mg, THF, (2) 2-pyridinecarboxaldehyde, THF; (c) $PBr_3$, $CH_2Cl_2$; (d) $H_2$, 10% Pd/C, absolute EtOH; (e) $H_2$, 10% Pd/C, concd HCl, EtOH; (f) HCHO, $NaBH_3CN$.

**Table 1.** Effect of ring aromatization and N-alkylation on h5-$HT_{1B/1D}$ receptor affinity



| Compound | R | X | h5-$HT_{1D}$ $K_i$ (nM)[a] | h5-$HT_{1B}$ $K_i$ (nM)[a] | Selectivity[b] |
|---|---|---|---|---|---|
| **4a** | H | $CH_2CH_2$ | 0.7[c] | 14[c] | 20 |
| **4b** | Me | $CH_2CH_2$ | 82 | 2160 | 26 |
| **4c** | Bn | $CH_2CH_2$ | 30 | 1860 | 62 |
| **5a** | H | CH=CH | 1.2 | 45 | 38 |
| **5b** | Me | CH=CH | 800 | > 10,000 | > 13 |
| **5c** | Bn | CH=CH | 8780 | > 30,000 | > 3 |

[a] $K_i$ values determined in triplicate and SEM typically < 20%.
[b] Selectivity for h5-$HT_{1D}$ binding (i.e., h5-$HT_{1B}$ $K_i$ value ÷ h5-$HT_{1D}$ $K_i$ value). For comparison, sumatriptan (h5-$HT_{1D}$ $K_i = 5.8 \pm 0.7$ nM; h5-$HT_{1B}$ $K_i = 51 \pm 2$ nM) binds with approximately 9-fold selectivity for h5-$HT_{1D}$ receptors under the assay conditions employed.
[c] $K_i$ values were previously reported and are included only for comparison.[22]

led to enhanced selectivity (38-fold) relative to **4a**. As with the imidazoline series, *N*-methylation and *N*-benzylation of **5a** (i.e., **5b** and **5c**, respectively) decreased affinity at both 5-HT receptor populations.

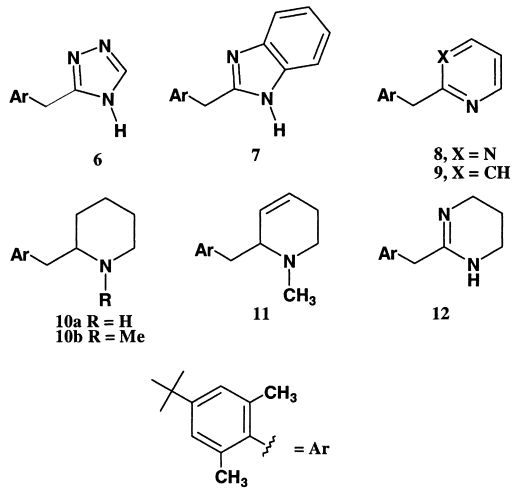
The results of more dramatic alteration of the imidazoline ring are shown in Table 2. Introduction of a third ring-nitrogen atom (i.e. conversion of imidazole **5a** to triazole **6**; $K_i = 15{,}780$ nM) dramatically decreased h5-$HT_{1D}$ affinity, as did benz-fusion of **5a** to the benzimidazole **7** ($K_i > 30{,}000$). Both compounds also displayed reduced affinity for h5-$HT_{1B}$ receptors.

The aromatic ring of **5a** was expanded to pyrimidine **8**, but **8** lacked affinity for h5-$HT_{1D}$ and 5-$HT_{1B}$ receptors ($K_i > 30{,}000$ nM). Removal of one of the ring nitrogen atoms, to afford the pyridine analogue **9**, led to increased h5-$HT_{1D}$ affinity ($K_i = 762$ nM), and in a compound that displayed substantially higher affinity than **8**. Reduction of the pyridine ring of **9** to the piperidine **10a** further enhanced h5-$HT_{1D}$ affinity ($K_i = 84$ nM) and selectivity (110-fold selectivity). As with **4a** and **5a**, *N*-methylation of **10a** to **10b** decreased affinity both at h5-$HT_{1D}$ and h5-$HT_{1B}$ receptors ($K_i > 5000$ nM).

One of the major differences between **10a** and **4a**/**5a**, apart from ring size, is the electronic character of the heterocyclic ring. Consequently, we examined tetrahydro-pyridine **11** and tetrahydropyrimidine **12**. Compound **11** ($K_i = 1470$ nM) was found to bind with relatively low, but slightly higher affinity than **10b** at h5-$HT_{1D}$ receptors. However, compound **12**, which might be viewed as a ring-expanded analogue of **4a**, binds with good affinity ($K_i = 27$ nM) and high (160-fold) selectivity.

The selectivity of compound **12** was further evaluated by examining its effect at a concentration of 1000 nM at various receptor populations. In some instances, a $K_i$ value was determined. Compound **12** was found to bind with $K_i > 1000$ nM at each of the following receptor

**Table 2.** Effect of imidazoline ring modification on h5-$HT_{1B/1D}$ receptor affinity




| Compound | h5-$HT_{1D}$ $K_i$ (nM)[a] | h5-$HT_{1B}$ $K_i$ (nM)[a] | Selectivity[b] |
|---|---|---|---|
| **5a** | 1.2 | 45 | 38 |
| **6** | 15,780 | > 30,000 | > 2 |
| **7** | > 30,000 | > 30,000 | — |
| **8** | > 30,000 | > 30,000 | — |
| **9** | 762 | 24,300 | 32 |
| **10a** | 84 | 9340 | 110 |
| **10b** | 5410 | > 30,000 | > 6 |
| **11** | 1470 | > 30,000 | > 20 |
| **12** | 27 | 4370 | 160 |

[a] $K_i$ values determined in triplicate and SEM typically < 20%.
[b] Selectivity for h5-$HT_{1D}$ binding (i.e., h5-$HT_{1B}$ $K_i$ value ÷ h5-$HT_{1D}$ $K_i$ value). See also footnote b of Table 1.

populations (percent inhibition at 1000 nM, or $K_i$ value): 5-$HT_{1A}$ and 5-$HT_{2A}$ (< 50% inhibition), 5-$HT_{1E}$ (2%), 5-$HT_{1F}$ ($K_i = 4830$ nM), 5-$HT_6$ (4%), 5-$HT_7$ (23%), $\alpha_1$ ($K_i > 10{,}000$ nM), $\alpha_2$ ($K_i = 1320$ nM), $\alpha_{2A}$ (10%), $D_1$–$D_5$ (0–17%), $m_2$ (36%), $m_3$ (12%), $ET_A$ (30%). Thus, **12** is not only reasonably selective for h5-$HT_{1D}$ versus h5-$HT_{1B}$ receptors, it also possesses good selectivity over other receptor populations examined.

## Conclusions

The present investigation sought to identify structural features that might enhance affinity and/or selectivity of benzylimidazolines for h5-$HT_{1D}$ receptors. It was found that *N*-methylation and *N*-benzylation, while decreasing h5-$HT_{1B/1D}$ affinity, had little effect on selectivity for h5-$HT_{1D}$ over h5-$HT_{1B}$ receptors. Aromatization of **4a** to **5a** had little effect on affinity but doubled its selectivity. In addition, the imidazoline ring could be replaced by either a pyridine or piperidine ring with retention of h5-$HT_{1D}$ receptor affinity, but replacement with a triazole, benzimidazole, or a pyrimidine ring was not tolerated. In the course of our investigations, two ligands were identified with > 100-fold h5-$HT_{1D}$ versus h5-$HT_{1B}$ selectivity. In particular, tetrahydropyrimidine **12** binds with high affinity ($K_i = 27$ nM) and is one of the most h5-$HT_{1D}$-selective non-tryptaminergic ligands developed to

date. Compound **12**, which also displays good selectivity over a number of other receptor populations, might represent the first of a new generation of h5-$HT_{1D}$ ligands for the investigation of h5-$HT_{1D}$ pharmacology, and might possess antimigraine action.

## Experimental

Melting points, determined with a Thomas-Hoover melting point apparatus, are uncorrected. Proton magnetic resonance spectra were obtained with a GE QE-300 or Varian Gemini 300 spectrometer; tetramethylsilane was used as an internal standard, and *J* values are in Hz. Infra-red spectra were recorded on a Nicolet 5ZDX FT-IR spectrophotometer. Flash chromatography was performed on silica gel (Merck grade 60, 230–400 mesh, 60 Å). Thin-layer chromatography (TLC) was performed using silica-gel-coated GHIF plates (250 μ, 2.5×10 cm, Analtech Inc., Newark, DE). Dry THF, toluene, and benzene were obtained by distillation over sodium metal and benzophenone. Dry $CH_2Cl_2$ and ethyl acetate were obtained by distillation over phosphorus pentoxide ($P_2O_5$). Dry acetone was obtained by distillation over Drierite® ($CaSO_4$). Elemental analysis was performed by Atlantic Microlabs Inc. (Norcross, GA), and determined values are within 0.4% of theory.

**2-(4-*tert*-Butyl-2,6-dimethylbenzyl)-1-methylimidazoline oxalate (4b).** *N*-Methylethylenediamine (0.3 g, 3.6 mmol) was added to a solution **15**[22] (0.5 g, 1.8 mmol) in absolute EtOH (20 mL) in an ice–$H_2O$ bath. The reaction mixture was allowed to stir at 0 °C for 1 h followed by heating at reflux for 3 h. The solvent was removed under reduced pressure and the oily residue was suspended in $H_2O$ (5 mL) and then extracted with $CH_2Cl_2$ (3× 30 mL). The $CH_2Cl_2$ solution was dried ($MgSO_4$) and the solvent removed under reduced pressure. The crude oil was taken up in anhydrous $Et_2O$ and oxalic acid was added. The precipitate was collected and recrystallized from absolute MeOH/anhydrous $Et_2O$ to afford 0.3 g (45%) of **4b** as a white solid, mp 162–165 °C: $^1$H NMR (DMSO-$d_6$) δ 1.3 (s, 9H, –$C(CH_3)_3$), 2.2 (s, 6H, Ar–$CH_3$), 3.1 (s, 3H, –$NCH_3$), 3.6 (t, $J$ = 11 Hz, 2H, Ar-$CH_2$), 3.8 (d, $J$ = 15 Hz, 4H, –$CH_2$-), 7.1 (s, 2H, Ar–H), 9.3 (bs, NH). Anal. calcd for $C_{17}H_{26}N_2 \cdot C_2H_2O_4$: C, 65.49; H, 8.10; N, 8.04. Found: C, 65.21; H, 8.11; N, 7.96.

**1-Benzyl-2-(4-*tert*-butyl-2,6-dimethylbenzyl)imidazoline hydrochloride (4c).** The compound was prepared in a similar manner to **4b** using *N*-benzylethylenediamine to afford **4c** (68%) as a white solid, mp 216–218 °C: $^1$H NMR (DMSO-$d_6$) δ 1.3 (s, 9H, –$C(CH_3)_3$), 2.4 (s, 6H, Ar-$CH_3$), 3.3 (t, 2H, –$CH_2$), 3.6 (s, 2H, –$CH_2$), 3.9 (t, 2H,-$CH_2$), 4.4 (s, 2H, –$CH_2$), 7.3 (s, 2H, Ar–H), 7.6 (m, 5H, Ar–H), 9.5 (bs, NH). Anal. calcd for $C_{23}H_{30}N_2 \cdot HCl$: C, 74.47; H, 8.42; N, 7.55. Found: C, 74.35; H, 8.50; N, 7.54.

**2-(4-*tert*-Butyl-2,6-dimethylbenzyl)imidazole oxalate (5a).** A suspension of **4a** (0.1 g, 0.4 mmol) and 10% Pd/C (0.1 g) in toluene (10 mL) was heated at reflux under a nitrogen atmosphere for 3 days. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to give a crude solid. The solid was taken up in anhydrous $Et_2O$ and oxalic acid was added. The precipitate was collected by filtration and recrystallized from absolute MeOH/anhyd $Et_2O$ to afford 0.1 g (66%) of **5a** as a white solid, mp 154–156 °C. $^1$H NMR (DMSO-$d_6$) δ 1.3 (s, 9H, –$C(CH_3)_3$), 2.2 (s, 6H, Ar–$CH_3$), 4.2 (s, 2H, –$CH_2$), 7.1 (s, 2H, Ar–H), 7.3 (s, 2H, Ar-H). Anal. calcd for $C_{16}H_{22}N_2 \cdot C_2H_2O_4$: C, 65.04; H, 7.28; N, 8.43. Found: C, 64.94; H, 7.25; N, 8.39.

**2-(4-*tert*-Butyl-2,6-dimethylbenzyl)-1-methylimidazole hydrochloride (5b).** The compound was prepared in a similar manner using **4b** to afford **5b** (61%) as a white solid (absolute EtOH/anhydrous $Et_2O$), mp 231–233 °C. $^1$H NMR (DMSO-$d_6$) δ 1.4 (s, 9H, –$C(CH_3)_3$), 2.3 (s, 6H, Ar–$CH_3$), 3.6 (s, 3H, –$NCH_3$), 4.2 (s, 2H, –$CH_2$), 7.1 (s, 2H, Ar–H), 7.3 (s, 2H, Ar–H). Anal. calcd for $C_{17}H_{24}N_2 \cdot HCl$: C, 65.04; H, 7.28; N, 8.43. Found: C, 64.94; H, 7.25; N, 8.39.

**1-Benzyl-2-(4-*tert*-butyl-2,6-dimethylbenzyl)imidazole hydrochloride (5c).** A mixture of **5a** (0.20 g, 0.83 mmol), benzyl bromide (0.17 g, 0.99 mmol), and $K_2CO_3$ (0.11 g, 0.83 mmol) in DMF (50 mL) was stirred at 100 °C for 4 h. The solvent was removed under reduced pressure and the residue was taken up into $H_2O$ (20 mL). The aqueous mixture was extracted with EtOAc (2×25 mL). The EtOAc solution was dried ($MgSO_4$) and the solvent removed under reduced pressure. The crude oil was purified by column chromatography (silica gel, 20 g) using $CH_2Cl_2$:MeOH (99:1) to afford the free base. An anhydrous $Et_2O$ solution of the free base was cooled to 0 °C and saturated with dry HCl gas. The precipitate was collected by filtration and recrystallized from acetone to afford 0.18 g (60%) of **5c** as a white solid, mp 229–231 °C. $^1$H NMR (DMSO-$d_6$) δ 1.4 (s, 9H, –$C(CH_3)_3$), 2.1 (s, 6H, Ar–$CH_3$), 4.3 (s, 2H, –$CH_2$), 5.5 (s, 2H, –$CH_2$), 7.1 (s, 2H, Ar–H), 7.3 (s, 2H, Ar–H), 7.4 (m, 2H, Ar–H), 7.8 (s, 1H, Ar–H). Anal. calcd for $C_{23}H_{28}N_2 \cdot HCl$: C, 74.88; H, 7.92; N, 7.54. Found: C, 74.80; H, 7.88; N, 7.61.

**3-(4-*tert*-Butyl-2,6-dimethylbenzyl)-4*H*-1,2,4-triazole hydrochloride (6).** The compound was prepared in a manner similar to **4b** using hydrazine to afford **6** (26%) as a white solid (absolute EtOH/anhydrous $Et_2O$), mp 210–212 °C. $^1$H NMR ($CD_3OD$) δ 1.4 (s, 9H, –$C(CH_3)_3$), 2.3 (s, 6H, Ar–$CH_3$), 4.4 (s, 2H, –$CH_2$), 7.2 (s, 2H, Ar–H), 9.1 (s, 1H, Ar–H). Anal. calcd for $C_{15}H_{21}N_3 \cdot HCl$: C, 64.39; H, 7.92; N, 15.02. Found: C, 64.40; H, 7.90; N, 15.02.

**2-(4-*tert*-Butyl-2,6-dimethylbenzyl)benzimidazole oxalate (7).** The compound was prepared in a manner similar to **4b** using *o*-phenylenediamine to afford **7** (71%) as a white solid (absolute EtOH/anhyd $Et_2O$), mp 245–247 °C: $^1$H NMR (DMSO-$d_6$) δ 1.3 (s, 9H, –$C(CH_3)_3$), 2.4 (s, 6H, Ar–$CH_3$), 4.1 (s, 2H, –$CH_2$), 7.1 (s, 2H, Ar–H), 7.4–7.5 (m, 4H, Ar–H). Anal. calcd for $C_{20}H_{24}N_2 \cdot HCl$: C, 73.04; H, 7.66; N, 8.52. Found: C, 73.11; H, 7.70; N, 8.50.

**2-(4-*tert*-Butyl-2,6-dimethylbenzyl)pyrimidine hydrochloride (8).** The compound was prepared in a manner similar to **5a** using *m*-xylene as solvent and **12** to afford **8** (90%) as a white solid (absolute MeOH/anhydrous

$Et_2O$), mp 177–179 °C: $^1H$ NMR ($CD_3OD$): δ 1.3 (s, 9H, –$C(CH_3)_3$), 2.4 (s, 6H, Ar–$CH_3$), 4.3 (s, 2H, –$CH_2$), 7.1 (s, 2H, Ar–H), 7.3 (s, 1H, Ar–H), 8.6 (m, 2H, Ar–H). Anal. calcd for $C_{17}H_{23}N_2$·HCl: C, 69.96; H, 8.29; N, 9.60. Found: C, 70.02; H, 8.01; N, 9.59.

**2-(4-*tert*-Butyl-2,6-dimethylbenzyl)pyridine hydrochloride (9).** A suspension of **20** (4.3 g, 13.0 mmol) and 10% Pd/C (0.3 g) in absolute EtOH (90 mL) was hydrogenated in a Parr apparatus at 40 psi for 4 h. The catalyst was removed by filtration and the solvent was removed under reduced pressure. The residue was suspended in $H_2O$ (100 mL) and made alkaline (pH 13) with 50% NaOH and extracted with EtOAc (3×100 mL). The EtOAc solution was dried ($MgSO_4$), and the solvent removed under reduced pressure affording 3.0 g (92%) of 2-(4-*tert*-butyl-2,6-dimethylbenzyl)pyridine as a brown oil. HCl(g)/saturated anhydrous $Et_2O$ was added to a solution of the oil (0.5 g) in anhydrous $Et_2O$. The precipitate was collected and recrystallized from absolute EtOH/anhydrous $Et_2O$ to give 0.5 g (90%) of **9**, mp 184-187 °C. $^1H$ NMR ($CDCl_3$) δ 1.3 (s, 9H, –$C(CH_3)_3$), 1.5 (bs, $H_2O$), 2.2 (s, 6H, Ar–$CH_3$), 4.7 (s, 2H, $Ar_2CH_2$), 7.1 (m, 2H, Ar–H), 7.1 (m, 1H, Ar–H), 7.8 (m, 1H, Ar–H), 8.2 (m, 1H, Ar–H), 8.8 (m, 1H, Ar-H). Anal. calcd for $C_{18}H_{23}N$·HCl·0.25$H_2O$: C, 73.45; H, 8.39; N, 4.76. Found: C, 73.78; H, 8.31; N, 4.74.

**2-(4-*tert*-Butyl-2,6-dimethylbenzyl)piperidine hydrochloride (10a).** A suspension of **9** (1.0 g, 4.0 mmol) and $PtO_2$ (0.1 g, 0.4 mmol) in a mixture of absolute EtOH (25 mL) and concentrated HCl (1 mL) was hydrogenated in a Parr apparatus at 25 psi for 2 h. The catalyst was removed by filtration and the solvent was removed under reduced pressure. The crude solid was recrystallized from absolute EtOH/anhydrous $Et_2O$ to afford 1.0 g (85%) of **10a** as a white solid, mp 274–277 °C. $^1H$ NMR (DMSO-$d_6$) δ 1.2 (s, 9H,-$C(CH_3)_3$), 1.3–1.7 (m, 6H, –$(CH_2)_3$), 2.3 (s, 6H, Ar–$CH_3$), 2.8–3.3 (m, 5H, –CH, Ar–$CH_2$, $NHCH_2$), 3.3 (bs, $H_2O$), 7.0 (s, 2H, Ar–H), 9.2–9.5 (m, 2H). Anal. calcd for $C_{18}H_{29}N$·HCl·0.25$H_2O$: C, 71.97; H, 10.23; N, 4.66. Found: C, 72.25; H, 10.06; N, 4.69.

**2-(4-*tert*-Butyl-2,6-dimethylbenzyl)-1-methylpiperidine hydrochloride (10b).** Sodium cyanoborohydride (0.1 g, 2.0 mmol) was added to a mixture of **10a** (0.3 g, 1.0 mmol) and 37% formaldehyde (0.4 mL, 5.5 mmol) in MeCN (10 mL) at rt. Glacial acetic acid was added to maintain the pH at 7. The reaction mixture was allowed to stir at rt for 12 h. The solvent was removed under reduced pressure and 2 N KOH (50 mL) was added to the residue. The aqueous mixture was extracted with $Et_2O$ (3×50 mL). The $Et_2O$ solution was dried ($MgSO_4$) and the solvent was removed under reduced pressure. The crude oil was purified by flash chromatography (silica gel, 30 g) using $CH_2Cl_2$:MeOH (9:1) to afford 0.2 g (73%) of 2-(4-*tert*-butyl-2,6-dimethylbenzyl)-1-methylpiperdine as a clear oil. Excess HCl(g)/saturated anhydrous $Et_2O$ was added to a solution of the oil (0.2 g) in anhydrous $Et_2O$, and the precipitate was collected and dried to afford 0.2 g (90%) of **10b**, mp 262–265 °C. $^1H$ NMR (DMSO-$d_6$) δ 1.2 (s, 9H, –$C(CH_3)_3$), 1.2–1.9 (m, 6H, –$(CH_2)_3$), 2.3 (s, 6H, Ar–$CH_3$), 2.8 (d, $J$ = 4.4 Hz, 3H, $NCH_3$), 3.0-3.3 (m, 5H, –CH, Ar–$CH_2$, $N(CH_3)CH_2$), 3.3 (bs, $H_2O$), 7.0 (s, 2H, Ar–H), 11.1 (bs, 1H). Anal. calcd for $C_{19}H_{31}N$·HCl·0.25$H_2O$: C, 72.58; H, 10.42; N, 4.54. Found: C, 72.51; H, 10.27; N, 4.61.

**2-(4-*tert*-Butyl-2,6-dimethylbenzyl)-1-methyl-1,2,5,6-tetrahydropyridine hydrochloride (11).** A solution of phenyl lithium (1.8 M in cyclohexane:anhydrous $Et_2O$ (7:3) (3.5 mL, 6.4 mmol) was added rapidly to **16** (1.0 g, 3.2 mmol) at 25 °C. The reaction mixture was stirred vigorously at rt for 2 h and then poured into an ice (50 g)- $H_2O$ (50 mL) mixture. The aqueous mixture was extracted with $Et_2O$ (2×100 mL). The combined $Et_2O$ portion was extracted with 5% HCl (3×50 mL). The combined acid extracts were made alkaline (pH 13) by the addition of 50% NaOH. The aqueous mixture was extracted with $Et_2O$ (3×75 mL). The $Et_2O$ solution was dried ($MgSO_4$) and the solvent was removed under reduced pressure. The crude oil was purified by flash chromatography using petroleum ether:acetone (1:1) to afford 0.7 g (81%) of a yellow oil. Excess HCl(g)/saturated anhydrous $Et_2O$ was added to a solution of the oil (0.2 g) in anhyd $Et_2O$, the precipitate was collected and recrystallized from absolute MeOH/anhyd $Et_2O$ to afford 0.2 g (90%) of **11** as a white solid, mp 230–234 °C. $^1H$ NMR (DMSO-$d_6$) δ 1.2 (s, 9H, –$C(CH_3)_3$), 2.3 (s, 8H, $ArCH_2$, Ar–$CH_3$), 2.6–3.0 (m, 3H, $NCH_3$), 3.1–3.4 (m, 2H), 3.35 (s, $H_2O$), 3.5–3.7 (m, 2H), 3.9–4.0 (bs, 1H), 5.0–5.2 (m, 1H, C=CH), 5.8–6.0 (m, 1H, C = CH), 7.0 (s, 2H, Ar–H), 11.4 (s, 1H, HCl); $^{13}C$ NMR (DMSO-$d_6$) δ 20.9, 21.3, 30.4, 31.5, 34.2, 40.3, 49.1, 59.9, 123.3, 125.6, 129.8, 136.8, 149.2. Anal. calcd for $C_{19}H_{29}N$·HCl·0.25$H_2O$: C, 73.05; H, 9.84; N, 4.48. Found: C, 73.23; H, 9.74; N, 4.53.

**2-(4-*tert*-Butyl-2,6-dimethylbenzyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (12).** A mixture of **14**[24] (2.0 g, 10 mmol) and trimethylenediammonium bis-*p*-toluenesulfonate[29] (2.1 g, 5 mmol) and 1,3-diaminopropane (0.4 g, 5 mmol) was heated in an oil bath at 200 °C for 1 h with the evolution of ammonia gas. After cooling the residue to rt, $H_2O$ (50 mL) was added. The mixture was made alkaline (pH 13) by the addition of 15% NaOH (~50 mL). The aqueous mixture was extracted with $CH_2Cl_2$ (3×50 mL). The $CH_2Cl_2$ solution was washed with saturated NaCl (100 mL), dried ($MgSO_4$), and the solvent was removed under reduced pressure affording a crude solid. Excess HCl(g)/saturated anhydrous $Et_2O$ was added to a solution of the solid in anhydrous $Et_2O$, and the precipitate was collected by filtration and recrystallized from absolute EtOH/anhyd $Et_2O$ to afford 1.6 g (54%) of **12** as a white solid, mp 282–284 °C. $^1H$ NMR (DMSO-$d_6$) δ 1.3 (s, 9H, $C(CH_3)_3$), 1.8 (m, 2H, $CH_2CH_2CH_2$), 2.3 (s, 6H, Ar–$CH_3$), 3.3 (m, 4H, –$NHCH_2$), 3.9 (s, 2H, $ArCH_2$), 7.1 (s, 2H, Ar–H), 9.5 (m, 2H, –NH). Anal. calcd for $C_{17}H_{26}N_2$·HCl: C, 69.25; H, 9.23; N, 9.50. Found: C, 69.33; H, 9.25; N, 9.55.

**1-(4-*tert*-Butyl-2,6-dimethylbenzyl)-1-methyl-1,2,5,6-tetrahydropyridinium chloride (16).** A solution of 1-methyl-1,2,5,6-tetrahydropyridine (1.0 g, 10.0 mmol) and **13**[23] (2.5 g, 12.0 mmol) in dry acetone (10 mL) was allowed to stir at rt for 18 h. The reaction mixture was

cooled to 0 °C and left overnight. The mixture was filtered and the crude solid was washed with $Et_2O$ (100 mL). The solid was dried under reduced pressure to afford 2.4 g (78%) of **16**, mp 205–210 °C. $^1$H NMR (DMSO-$d_6$) δ 1.3 (s, 9H, –C$(CH_3)_3$), 2.5 (s, 8H, Ar$CH_2$, Ar–$CH_3$), 2.9 (s, 3H, N$CH_3$), 3.5 (m, 2H), 3.8–4.0 (m, 2H), 4.7 (m, 2H), 5.7 (m, 2H, C=CH), 5.9 (m, 2H, C=CH), 7.2 (s, 2H, Ar–H); $^{13}$C NMR (DMSO-$d_6$) δ 21.3, 22.0, 31.2, 34.5, 45.8, 56.3, 57.5, 62.1, 106.6, 120.1, 122.8, 124.5, 126.7, 152.4. IR (KBr) 674, 873, 941, 1009, 1196, 1445, 1476, 1606, 2968 cm$^{-1}$. This compound was used without further characterization in the synthesis of **11**.

**4-*tert*-Butyl-1-bromo-2,6-dimethylbenzene (18).** A solution of bromine (6.4 g, 40.0 mmol) in glacial acetic acid (75 mL) was added dropwise to a solution of **17** (6.5 g, 40.0 mmol) and sodium acetate (5.0 g, 61.0 mmol) in glacial acetic acid (175 mL) at 25 °C. The reaction mixture was allowed to stir at 25 °C overnight. The solvent was removed under reduced pressure. $H_2O$ (150 mL) was added to the residue and the aqueous solution was extracted with EtOAc (3×100 mL). The EtOAc solution was washed with 5% $NaHCO_3$ (3×100 mL), dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residue was recrystallized from absolute EtOH to afford 8.5 g (88%) of **18** as a white solid, mp 48–50 °C (lit.[25] 48–50 °C).

**1-(4-*tert*-Butyl-2,6-dimethylphenyl)-1-(2-pyridyl)methanol (19).** A crystal of iodine was added to a suspension of Mg (0.6 g, 25.0 mmol) in dry THF (5 mL). A solution (1 mL) of **18** (5.0 g, 20.7 mmol) in dry THF (5 mL) was added to the suspension. The mixture was heated to begin the Grignard reaction. The remainder of the solution was added in a dropwise manner while maintaining a gentle reflux. After the addition was complete, the reaction mixture was heated at reflux for 2 h and then cooled to 0 °C. A solution of 2-pyridinecarboxaldehyde (2.0 g, 19.2 mmol) in dry THF (5 mL) was added in a dropwise manner and the reaction mixture was allowed to warm to rt overnight. 2 N HCl (100 mL) was added in a dropwise manner and the reaction mixture was allowed to stir at rt for an additional 2 h. The aqueous solution was washed with $Et_2O$ (2×100 mL), made alkaline (pH 13) with 50% NaOH, and extracted with EtOAc (3×100 mL). The EtOAc solution was washed with saturated NaCl solution (100 mL), dried ($MgSO_4$), and the solvent was removed under reduced pressure. The crude oil was purified by flash chromatography (silica gel, 200 g) using petroleum ether:ethyl acetate (8:2) affording 3.8 g (73%) of **19** as a tan oil. $^1$H NMR ($CDCl_3$) δ 1.3 (s, 9H, –C$(CH_3)_3$), 2.2 (s, 6H, AR-$CH_3$), 5.3 (bs, –OH), 6.2 (m, 1H, $Ar_2$-CH), 6.9 (m, 1H, Ar–H), 7.0 (m, 2H, Ar-H), 7.2 (m, 1H, Ar-H), 7.6 (m, 1H, Ar–H), 8.6 (m, 1H, Ar-H). The compound was used without further characterization in the synthesis of **20**.

**2-(α-Bromo-4-*tert*-butyl-2,6-dimethylbenzyl)pyridine (20).** A solution of $PBr_3$ (5.7 g, 21.0 mmol) in dry $CH_2Cl_2$ (20 mL) was added in a dropwise manner to a solution of **19** (3.8 g, 14.0 mmol) in dry $CH_2Cl_2$ (30 mL). The solution was allowed to stir at rt overnight. The solvent was removed under reduced pressure and $H_2O$ (100 mL) was added to the residue. The mixture was made alkaline (pH 13) with 50% NaOH and the aqueous solution was extracted with EtOAc (3×100 mL). The EtOAc solution was dried ($MgSO_4$), and the solvent removed under reduced pressure affording 4.3 g (92%) of **20** as a brown oil: $^1$H NMR ($CDCl_3$) δ 1.3 (s, 9H, –C$(CH_3)_3$), 2.2 (s, 6H, Ar–$CH_3$), 6.2 (m, 1H, $Ar_2$–CH), 6.9 (m, 1H, Ar–H), 7.0 (m, 2H, Ar–H), 7.2 (m, 1H, Ar–H), 7.6 (m, 1H, Ar–H), 8.6 (m, 1H, Ar–H). The product was used without further characterization in the synthesis of **9**.

### Radioligand binding assay[22]

Radioligand binding studies were performed in triplicate using 96-well polypropylene microtiter plates in a reaction volume of 500 μL. The test compound was initially assayed at 3, 1, and 0.1 μM and then followed by a $K_i$ determination where 3 μM of the test compound produced > 50% inhibition of binding. 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/well); the plates were vortexed for 20 s and then incubated at rt 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. Each $K_i$ determination represents a minimum of three replications.

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