3-[2-(Pyrrolidin-1-yl)ethyl]indoles and 3-[3-(Piperidin-1-yl)propyl]indoles: Agonists for the h5-HT$_{1D}$ Receptor with High Selectivity over the h5-HT$_{1B}$ Subtype

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The discovery of Sumatriptan (**1**, Figure 1),[1,2] a selective serotonin 5-HT$_{1D}$ receptor agonist, as an effective acute treatment for migraine headache has intensified research in this area, and over the past years several related compounds, including Merck's Rizatriptan (MK-462, **2**),[3] have entered late phases of clinical development. The mechanism of action of Sumatriptan is still a matter of much debate,[4] and both a direct vasoconstrictor effect on intracranial, extracerebral arteries and/or an inhibition of neuropeptide (calcitonin gene-related peptide, substance P, neurokinin A) release from perivascular trigeminal sensory nerves in the dura mater and large cerebral arteries have been suggested to play a significant role in headache relief.[5,6] Since the development of Sumatriptan and the other compounds in the "triptan" class, it has been found that two subtypes of the human 5-HT$_{1D}$ receptor exist, 5-HT$_{1D}$ (previously termed 5-HT$_{1D\alpha}$) and 5-HT$_{1B}$ (previously termed 5-HT$_{1D\beta}$).[7] The vasoconstrictor effects of Sumatriptan are thought to be mediated by activation of 5-HT$_{1B}$ receptors, due to the preponderance of mRNA coding for this subtype in human blood vessels (including cerebral microvessels).[8,9] The identity of the receptor-mediating inhibition of neuropeptide release is less clear as both 5-HT$_{1D}$ and 5-HT$_{1B}$ mRNAs have been shown to be present in human trigeminal ganglia by reverse transcriptase-polymerase chain reaction (RT-PCR).[9,10] Neither Sumatriptan nor the other "triptan" agents developed so far show much selectivity for either subtype of the 5-HT$_{1D}$ receptor.

Sumatriptan has been shown to produce coronary artery vasoconstriction possibly through activation of 5-HT$_{1B}$ receptors,[11] an effect that may or may not be related to the chest pains experienced by some of the patients.[12] Although coronary side effects are rare, care should be taken when the drug is given to patients with suspected or proven coronary diseases. Although Rizatriptan has been shown to be less effective than Sumatriptan in causing contraction of isolated human coronary artery segments,[13] the differential expression of 5-HT$_{1D}$ and 5-HT$_{1B}$ receptors in neural and vascular tissue prompted us to investigate the possibility of developing selective serotonin 5-HT$_{1D}$ receptor agonists as potential second generation antimigraine agents. In the present communication we report on the design, synthesis, and biological evaluation of agonists for this receptor with up to 200-fold selectivity over the 5-HT$_{1B}$ subtype.

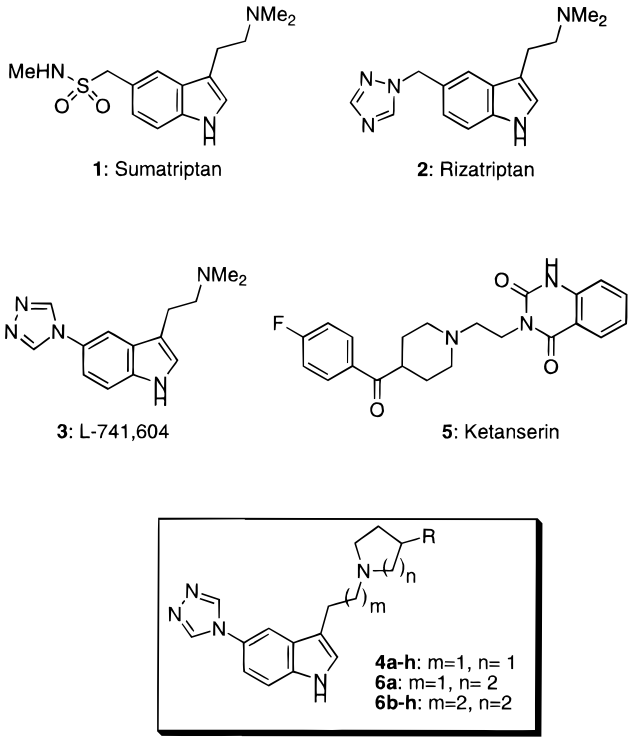

Figure 1.

Screening of the Merck collection of 5-substituted tryptamines, synthesized during the discovery of Rizatriptan, mainly revealed compounds with marginal 5-HT$_{1D}$ over 5-HT$_{1B}$ selectivity (3−5-fold), despite exploring different electronic and steric properties at the indole 5-position. The pyrrolidine analogue **4a**, however, showed promising levels of selectivity (9-fold; Table 1), and this was utilized as a lead for further medicinal chemistry elaboration. The *N*,*N*-dimethylethylamine analogue of **4a**, L-741,604 (**3**),[17] has 10-fold higher affinity for 5-HT$_{1D}$ receptors but arguably lower receptor subtype selectivity (Table 1). This, combined with the knowledge that ketanserin (**5**) was a moderately selective 5-HT$_{1D}$ receptor ligand (1B/1D, 25), which incorporates a more elaborate amine side chain functionality, suggested that improvements in 5-HT$_{1D}$ subtype selectivity might be gained by substituting the pyrrolidine ring of **4a.**[14] This would help to define the steric requirements for binding to the 5-HT$_{1D}$ and 5-HT$_{1B}$ receptors in the region of the aspartic acid binding domain.[15] In addition, a series of 4-substituted piperidines (**6c**−**h**) was explored with the aim of simplifying the chemistry by removing the chiral center present in the substituted pyrrolidines.

The 3-substituted pyrrolidines **4b**−**h** and 4-substituted piperidines **6d**−**h** were prepared as shown in Schemes 1−3. Thus, hydrogenation of the enantiomerically pure benzylpyrrolidines **7a,b** and **8a,b**[16] in the presence of (BOC)$_2$O, followed by alkylation with benzyl bromide, gave the benzyl ethers **9** and **10** (Scheme 1). The pyrrolidines resulting from removal of the BOC protecting group and alkylation with 4-chlorobutanal dimethyl acetal were subjected to Fischer indolisation with 4-(1,2,4-triazol-4-yl)phenylhydrazine[17] to give the

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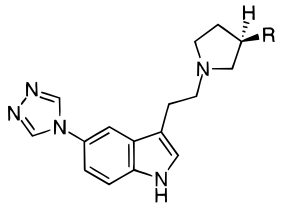
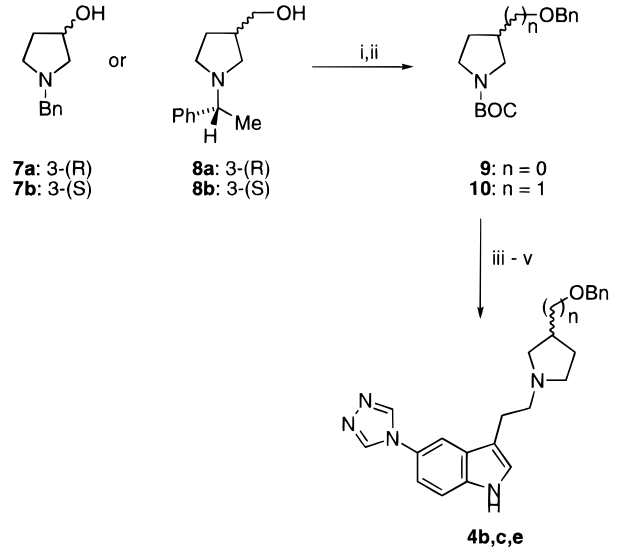
Table 1. Binding of Standard Compounds and Substituted Pyrrolidines to Cloned Human 5-HT_{1D} Receptors



compd[a]	R	IC_{50} (nM)[b] h5-HT_{1D}	IC_{50} (nM)[b] h5-HT_{1B}	1B/1D[c]	EC_{50}(nM,[d] % 5-HT[e]) h5-HT_{1D}
Sumatriptan (**1**)		5.0	16.0	3.2	16 (100)
Rizatriptan (**2**)		11	41	3.7	8.4 (90)
L-741,604 (**3**)		0.30	1.4	4.7	0.45 (117)
ketanserin (**5**)		260	6500	25	
4a	H	3.4	30.2	9.0	2.6 (120)
4b	OBn	6.3	146	23	15 (81)
4c	OBn[f]	23	280	12	33 (84)
4d	NMeBn	38	210	5.5	
4e	CH_2OBn	2.3	88	38	2.1 (68)
4f	CH_2NMeBn	0.70	33	47	1.6 (102)
4g	CH_2NHBn	0.50	47	94	0.93 (100)
4h	CH_2NHBn[f]	1.6	50	31	4.1 (102)

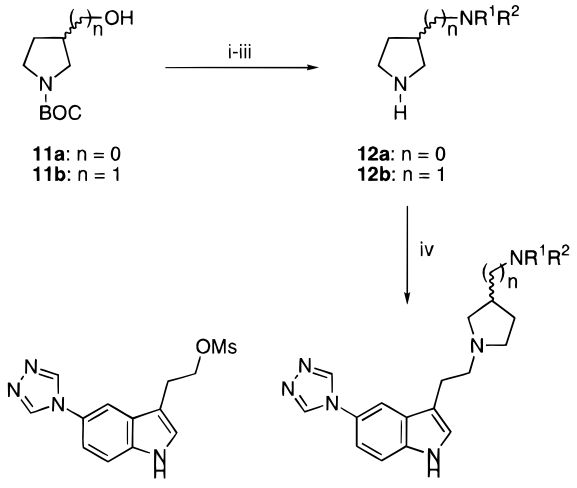
[a] Where applicable, all compounds are single enantiomers with absolute stereochemistry as drawn. [b] Displacement of [^{3}H]-5-HT binding to cloned 5-HT_{1D} and 5-HT_{1B} receptors in CHO cells. The figures are the mean of two independent determinations perfomed in triplicate. In each case the radioligand used was at the K_D for the receptor. The maximum variance from the mean of the log (IC_{50}) values was 3.2%. [c] Binding selectivity for 5-HT_{1D} receptors. [d] Measurement of agonist-induced [^{35}S]GTPγS binding in CHO cells stably transfected with 5-HT_{1D} receptors. [e] Efficacy relative to 5-HT. Values are the mean of two independent determinations. [f] These compounds are the enantiomers of **4b** and **4g**.

Scheme 1[a]



[a] Reagents: (i) $Pd(OH)_2$, H_2, $(BOC)_2O$,MeOH/H_2O; (ii) NaH, benzyl bromide, THF; (iii) 90% HCO_2H; (iv) 4-chlorobutanal dimethyl acetal, NaI, Na_2CO_3, DME; (v) 4% H_2SO_4, 4-(1,2,4-triazol-4-yl)phenylhydrazine, reflux.

Scheme 2[a]

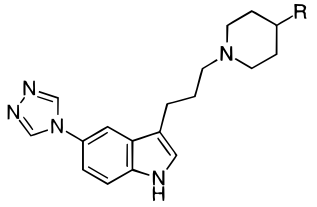


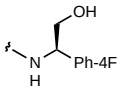
[a] Reagents: (i) $MeSO_2Cl$, Et_3N, CH_2Cl_2; (ii) R^1R^2NH, toluene, 90 °C; (iii) 90% HCO_2H; (iv) **13**, DME, NaI, Na_2CO_3, reflux.

indoles **4b,c,e.**[18] Mesylation of alcohols **11a,b** followed by reaction with the appropriate amine and removal of the BOC group gave the pyrrolidines **12a,b**.[19] These were N-alkylated with mesylate **13**[20] to give the required indoles **4d,f**−**h** in a single step (Scheme 2). 4-Substituted piperidines **6d**−**h** were prepared starting from 4-hydroxypiperidine, which was alkylated with 5-bromopentanal dimethyl acetal to afford alcohol **14** (Scheme 3). Fischer indolization, followed by Parick oxidation of the intermediate 4-piperidinol, gave the versatile ketone intermediate **15**, easily converted into piperidines **6d**−**h** by reductive amination with the appropriate amine. Compound **6c** was prepared from 4-[(*N*-benzyl-*N*-methylamino)methyl]piperidine by alkylation with 5-bromopentanal dimethyl acetal followed by Fischer reaction as above.

The compounds in Tables 1 and 2 were evaluated for their affinity to cloned human 5-HT_{1D} and 5-HT_{1B} receptors stably expressed in CHO cells.[21] Their intrinsic efficacy, expressed as percent of the maximal 5-HT response, was measured in the same cell lines using agonist-induced [^{35}S]GTPγS binding.[22,23] It can be seen from the data in Table 1 that substitution at C-3 of the pyrrolidine ring of **4a** with a benzyloxy group improved 5-HT_{1D} over 5-HT_{1B} selectivity (**4b**: 1B/1D, 23). The same effect was observed when this group was attached through a methylene spacer (**4e**: 1B/1D, 38); in this case, however, 5-HT_{1D} affinity remained unchanged compared to **4a**. Both, **4b** and **4e** behaved as

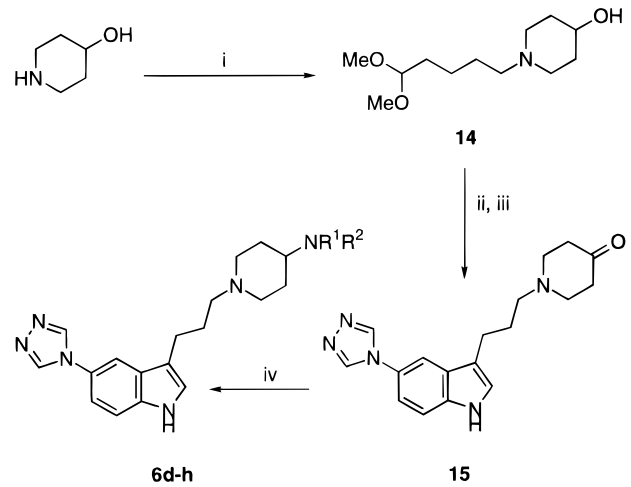
Table 2. Binding of Substituted Piperidines to Cloned Human 5-HT_{1D} Receptors



compd[a]	R	IC_{50} (nM)[b] h5-HT_{1D}	IC_{50} (nM)[b] h5-HT_{1B}	1B/1D[c]	EC_{50} (nM,[d] % 5-HT[e]) h5-HT_{1D}
6a		200	568	3	
6b	H	24	15	0.6	
6c	CH_2NMeBn	0.95	28	29	0.90 (93)
6d	NMeBn	2.2	145	66	1.6 (94)
6e	NHBn	4.8	125	26	8.0 (76)
6f		0.35	35	100	1.5 (96)
6g		1.3	114	88	1.8 (95)
6h		0.9	185	206	1.1 (104)

[a–e] See corresponding footnotes for Table 1.

Scheme 3[a]



[a] Reagents: (i) $(MeO)_2CH(CH_2)_4Br$, K_2CO_3, DMF, 90 °C; (ii) 4% H_2SO_4, 4-(1,2,4-triazol-4-yl)phenylhydrazine, reflux; (iii) pyridine·SO_3, DMSO, 25 °C; (iv) R^1R^2NH, $NaCNBH_3$, AcOH, MeOH.

partial agonists in the GTPγS binding assay when compared to serotonin. Replacement of the oxygen atom in **4b** and **4e** by a methylated nitrogen, to give the benzylamines **4d** and **4f**, respectively, appeared to be tolerated only in the latter case. This change, however, was found to be beneficial for improving the efficacy of the compounds, and benzylamine **4f** was as efficacious as 5-HT in the in vitro functional assay. A further 2-fold increase in 1B/1D binding selectivity was achieved by removal of the *N*-Me group on **4f** while retaining subnanomolar afffinity and high efficacy (**4g**, L-760,790; IC_{50}, 0.5 nM; 1B/1D, 94). The absolute stereochemistry of the pyrrolidine C-3 chiral center has an effect on both affinity and selectivity, which is illustrated in Table 1 by comparison of the benzyl ethers **4b** and **4c** and the benzylamines **4g** and **4h**. Although substitution at C-2 of the pyrrolidine was shown to tolerate a variety of groups without detrimental effect on affinity, it usually resulted in lower 1B/1D selectivity. By contrast with the pyrrolidine-derived 5-HT_{1D} receptor agonists **4**, which appeared to prefer a two-carbon linker between the indole nucleus and the pyrrolidine nitrogen, a trimethylene chain was optimal for the piperidine-based agonists **6** (e.g. **6a** vs **6b**, Table 2). In addition, direct attachment of the amino functionality to C-4 of the piperidine was preferred rather than through a methylene group (cf. **6c** and **6d**), and **6d** was a high-affinity, full agonist with good 1B/1D selectivity. Removal of the *N*-Me group of **6d** to give **6e** was slightly detrimental for 1B/1D selectivity, due to a 2-fold loss in 5-HT_{1D} affinity. Gratifyingly, this could be restored and improved by substitution at the benzylic position with methyl (**6f**) or hydroxymethyl (**6g**) groups, with retention of full agonist properties. Moreover, introduction of fluorine at the 4-position of the pendent phenyl ring of **6g** afforded a compound, **6h**, with excellent affinity and selectivity (1B/1D, 206) for 5-HT_{1D} receptors. Thus, piperidine **6h** (L-772,405) represents the most selective 5-HT_{1D} receptor full agonist reported to date. The pyrrolidines and piperidines also showed selectivity in the functional assay for the 5-HT_{1D} receptor. Thus both pyrrolidine **4g** (EC_{50}: 1D 0.93 nM; 1B 456 nM) and piperidine **6h** (EC_{50}: 1D 1.1 nM; 1B 1100 nM) have relatively low potency for the 5-HT_{1B} receptor in the GTPγS binding assay.

The 5-HT_{1D} and 5-HT_{1B} receptors can therefore be differentiated by appropriate substitution of the ligand in the region which binds to the aspartic acid. Whereas relatively bulky substituents are tolerated by the 5-HT_{1D} receptor and indeed, in many cases, affinity is improved by this substitution, the 5-HT_{1B} receptor only tolerates dimethylamino substitution of the tryptamine and suggests that the binding pocket of the 5-HT_{1D} receptor which accommodates benzylamine substitution is not present for the 5-HT_{1B} receptor.[15] The selectivity

of pyrrolidine **4g** and piperidine **6h** vs other cloned serotonin receptors was also explored using radioligand binding techniques. Thus, **4g** and **6h** showed the following affinities (IC_{50}, nM) for h5-HT_{1A} (6.4 and 105, respectively), h5-HT_{1E} (>10 000), h5-HT_{1F} (>10 000), r5-HT_{2A} (>4000), r5-HT_{5A} (>1500), r5-HT_6 (>5000), and r5-HT_7 (3800 and 318, respectively). The selectivity observed for **6h** over 5-HT_{1A} receptors (115-fold) is noteworthy as this has been difficult to achieve with other 5-HT_{1D} receptor agonists. Additionally, both **4g** and **6h** had >1 μM affinity at over 100 other GPCRs, ion channels, and proteins.

In summary, two series of high-affinity 5-HT_{1D} receptor full agonists, with up to 200-fold selectivity over the 5-HT_{1B} subtype, have been identified. The pyrrolidine **4g** and piperidine **6h** show very good selectivities over a range of other serotonin and non-serotonin receptors and, therefore, constitute new useful tools to delineate the role of 5-HT_{1D} receptors in migraine and other diseases.

Acknowledgment. We thank Mr. Roy Pengilley for technical assistance.

Supporting Information Available: Synthetic procedures for pyrrolidine **4g** and piperidine **6h** (8 pages). Ordering information is given on any current masthead page.

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(14) Although the binding mode of ketanserin might be totally different to that of **4a**, we hypothesized that perhaps the benzoyleneurea or the 4-fluorobenzoyl moieties of the former might mimic the indole portion of the latter. If this was to be the case, the 5-HT_{1D} selectivity could arise from differential interaction of both receptors with the other appended piperidine substituent, and therefore exploration of space around the pyrrolidine ring of **4a** was investigated.
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(19) Note that in the case of **11a**, this operation results in inversion of the pyrrolidine C_3-chiral center.
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(22) In this assay, interaction of the agonist-occupied receptor with the G-protein results in dissociation of GDP from the G-protein α-subunit and the binding of a molecule of GTP. Under normal conditions the α-subunit dissociates from the $\beta\gamma$-subunits and, following modulation of its effector, intrinsic α-subunit GTPase hydrolyzes the GTP to GDP. Because [^{35}S]GTPγS is resistant to this GTPase, it accumulates in the membrane and it can be measured by virtue of its radiolabel (see for example ref 22).
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