Selective, Orally Active 5-HT$_{1D}$ Receptor Agonists as Potential Antimigraine Agents

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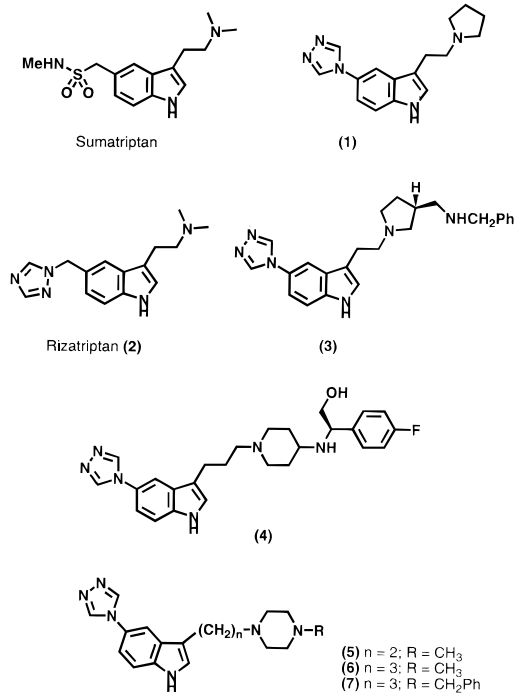
The serotonin (5-HT) receptor agonist Sumatriptan is the first of a new class of therapeutic agents for the acute treatment of migraine headaches.[1] Of the 13 currently identified human 5-HT receptors,[2] Sumatriptan exhibits binding selectivity for the 5-HT$_1$ subclass, in particular[3] for the 5-HT$_{1D}$ and 5-HT$_{1B}$ subtypes,[4] and it has been generally accepted that its clinical efficacy is mediated through action at these receptors.[5] Neither Sumatriptan nor the growing number of related compounds in late clinical trials (Zolmitriptan, Naratriptan, Rizatriptan, Eletriptan) distinguish significantly between these two subtypes in their binding affinities.[6] It has also been found that sumatriptan has high affinity for the recently discovered 5-HT$_{1F}$[7] receptor, leading to the suggestion[8] that some, or all, of its effects in migraine relief are through this receptor. LY-334370 has been developed[9] as a selective 5-HT$_{1F}$ agonist that may test this hypothesis.

While the 5-HT$_{1D/1B}$ class of compounds are effective in the treatment of migraine, some side effects may be experienced[10] such as chest discomfort, paraesthesia, and dizziness. There is also a potential[11] for coronary artery constriction with these drugs that precludes their use in patients with known heart disease. The possibility that clinical efficacy of these compounds may be mediated through either one or the other of the 5-HT$_{1D/B}$ receptors prompted us to consider seeking a compound that discriminates between these two receptors with a potentially lower side effect liability.

Neurogenic inflammation of the dura mater[12] and dilatation of cranial blood vessels[13] are considered the two major potential factors that cause the pain of migraine headache. The efficacy of Sumatriptan in aborting migraine headaches has been attributed to vasoconstriction of cerebral blood vessels or inhibition of inflammation caused by inappropriate stimulation of the trigeminal ganglion. It has also been proposed that some 5-HT agonists may have a centrally mediated contribution to their antimigraine effects.[14] Receptor-mapping studies[15] have shown that the 5-HT$_{1B}$ receptor is widely distributed in the CNS in neural and vascular tissues whereas the 5-HT$_{1D}$ receptor is restricted to neural tissues, including the trigeminal ganglion, suggesting that a ligand with 5-HT$_{1D}$ selectivity may lack the vasoconstrictor actions of unselective compounds like Sumatriptan. On the basis of these studies we have sought to identify a selective 5-HT$_{1D}$ agonist to examine whether such a compound would retain antimigraine activity. This communication describes the synthesis and biological evaluation of the first orally bioavailable 5-HT$_{1D}$ agonist.



The starting point in the exploration for selective agents was based on the triazolyltryptamine **1**, a close analogue of Rizatriptan (**2**), that had (Table 1) almost 1 order of magnitude higher binding affinity for 5-HT$_{1D}$ compared to 5-HT$_{1B}$. Elaboration of this pyrrolidine-based compound yielded[16] derivatives (**3**) with high binding affinity and >90-fold selectivity for 5-HT$_{1D}$. Homologation of the basic amine to the piperidinylpropylindole series yielded additional full agonists (**4**) with >200-fold binding selectivity. However, none of the selective compounds from these series had appreciable oral bioavailability in animals, and this prevented their further development.

In parallel with the studies on pyrrolidine- and piperidine-based compounds, it was found that the 4-methylpiperazine analogue (**5**) of **1** has relatively weak binding at 5-HT$_{1D}$ while the propylene homologue (**6**) is 4-fold higher in affinity than **5**. The addition of an aromatic ring to **6** to give the benzylpiperazine **7** led to a dramatic increase in activity at both receptors with a greater effect at 5-HT$_{1D}$, resulting in higher selectivity for this subtype. Of particular note with this compound was its oral bioavailability in rats (F = 21%), which encouraged further work in this series to optimize *in vitro* activity. Extension of the aryl ring to the phenethylpiperazine (**8**) reduced affinity at 5-HT$_{1D}$ (although still at the nanomolar level) but increased selectivity further to >100-fold. However, both **7** and **8** had submaximal intrinsic efficacy in a cell-based measure (stimulation of [^{35}S]GTPγS binding) of receptor activation.

Since there is no definitive animal assay for migraine,[17] it was considered essential that an optimal, selective compound should have efficacy at 5-HT$_{1D}$

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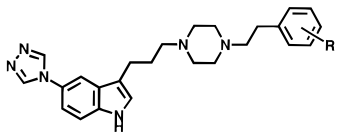
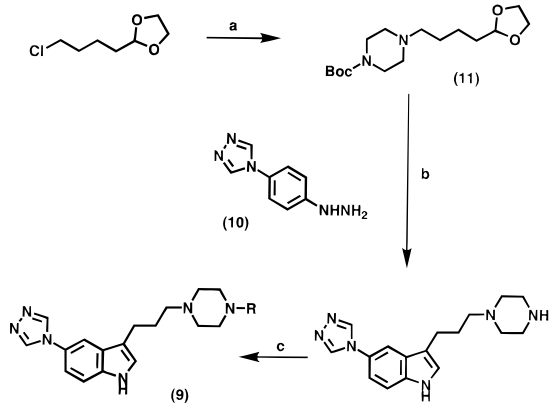
Table 1. 5-HT_{1D} Receptor Binding Selectivity and Functional Efficacy



compound	R	IC_{50} (nM) 5-HT_{1D}[a]	IC_{50} (nM) 5-HT_{1B}[a]	selectivity	EC_{50}(nM) 5-HT_{1D}[b]	efficacy[c] (% 5-HT)
Sumatriptan		5.0	16.0	3.2	16	100 ± 4.4
1		3.4	30.2	8.9	2.6	120
5		140	310	2.2	80	63
6		35	430	12	53	104
7		0.2	8.1	40	0.1	56
8	H	1.4	170	120	0.9	65
12	3-$NHCOCH_3$	1.8	170	94	1.6	96 ± 9.0
13	4-$NHCOCH_3$	1.3	251	190	1.9	85 ± 1.8
14	2-F	0.6	100	170	1.5	80
15	3-F	0.6	75	125	1.2	88 ± 2.2
16	4-F	0.8	120	150	1.5	81
17	3,5-di-F	0.7	47	67	0.6	86
18	3,4-di-F	1.0	98	98	1.9	86 ± 4.1

[a] Displacement of [^{3}H]5-HT binding from the cloned human 5-HT_{1D} and 5-HT_{1B} receptors stably expressed in CHO cells. All values are the means of at least two independent determinations performed in triplicate. In each case the radioligand concentration used was at the K_D for the receptor. The maximum variance from the mean of the log IC_{50} values was 3.2%. [b] EC_{50} for stimulation of [^{35}S]GTPγS binding in CHO cells expressing the 5-HT_{1D} receptor. [c] Maximum stimulation of [^{35}S]GTPγS binding expressed relative to the maximal effect produced by 5-HT. Values are the mean of at least two independent determinations; the standard deviation is given where $n \geq 3$.

Scheme 1. Synthesis of Triazolylindoles[a]



[a] Reagents: (a) *N*-Boc-piperazine, Na_2CO_3, NaI, 1,2-dimethoxyethane; (b) 4% sulfuric acid; (c) AcOH, sodium cyanoborohydride, CH_3OH, RCHO.

receptors similar to the earlier unselective, clinically effective compounds, in order to unambigously determine whether 5-HT_{1D} activity alone is sufficient to intervene in the migraine process. Since Sumatriptan behaves as a full agonist in the [^{35}S]GTPγS assay,[18] the efficacy of compounds **7** and **8** was considered too low, and further effort was directed to increasing intrinsic activity while retaining the affinity, selectivity, and bioavailability of these compounds. Derivatives with the general structure **9** were prepared (Scheme 1) by a Fischer reaction of triazolylphenylhydrazine (**10**)[19] with the acetal (**11**) derived from chloropentanal acetal and Boc-piperazine (with concommitant deprotection of the piperazine), and reductive amination with the appropriately substituted benzaldehyde or phenylacetaldehyde.

A variety of different substituents on the phenethyl moiety of **8** were investigated and it was found that both the 3′- and 4′-acetamido derivatives (**12** and **13**) retained high affinity and selectivity for 5-HT_{1D} but had efficacy at the cloned receptor significantly higher than that of the parent compound. However, neither of these compounds had appreciable bioavailability in rats. Substitution with fluorine at any position of the phenyl ring resulted in a significant increase in efficacy for compounds **14**-**16** with the greatest effect obtained by substitution at the *meta* position. The increased efficacy seen with each monofluorination was not additive since the 3,5- and 3,4-difluoro analogues **17** and **18** were not significantly higher in efficacy than **15** and, in fact, had lower binding selectivity.

Table 2. Comparison of 5-HT Receptor Affinities of **15** and Sumatriptan

receptor	IC_{50} (nM)[a] Sumatriptan	**15**
5-HT_{1A}	450	55
5-HT_{1D}	7.3	0.6
5-HT_{1B}	9.3	75
5-HT_{1E}	2400	>10000
5-HT_{1F}	27	3800
5-HT_{2A}	5500	370
5-HT_{2C}	>1000	9000
5-HT_3	>10000	>10000

[a] All values are the means of at least two independent determinations performed in triplicate.

The fluorinated phenylpiperazines **14**−**18** were further screened against the remaining 5-HT receptor subtypes and evaluated in pharmacokinetic assays. The *m*-fluoro analogue **15** was shown to have high selectivity for the 5-HT_{1D} subtype (Table 2) and >500-fold weaker activity in a broader range of receptor and enzyme assays.[20] In addition, **15** has appreciable oral bioavailability in rats (27%), dogs (25%), and rhesus monkeys (24%). On the basis of its overall *in vitro* and *in vivo* profile, compound **15** (L-775,606) was selected for further investigation as a potential development candidate.

Supporting Information Available: Experimental procedure for the synthesis of compound **15** (4 pages). Ordering information is given on any current masthead page.

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