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2-(Anilino)imidazolines and 2-(benzyl)imidazoline derivatives as *h*5-HT$_{1D}$ serotonin receptor ligands

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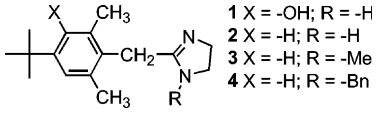
Abstract—2-(Anilino)imidazolines were identified as novel human 5-HT$_{1D}$ receptor ligands, but offered no particular advantage over previously reported 2-(benzyl)imidazolines. Pharmacokinetic and functional data were obtained for selected 2-(benzyl)imidazoline derivatives.

The 'triptans' are a class of agents that are generally well tolerated and that appear to be effective in the treatment of migraine.[1,2] Sumatriptan, the first and perhaps best known member of this class, possesses some therapeutic limitations including poor oral bioavailability, short half-life, and reduced ability to penetrate the blood–brain barrier.[3] Newer triptans have been developed in efforts to overcome these shortcomings.[4] The triptans are thought to act primarily by stimulating 5-HT$_{1B}$ and 5-HT$_{1D}$ serotonin receptors. Another potential problem with certain triptans is that they can, on occasion, produce coronary artery constriction by activation of 5-HT$_{1B}$ receptors.[5,6] Agents selective for 5-HT$_{1D}$ versus 5-HT$_{1B}$ receptors might, then, possess some clinical advantage over the less selective agents. Furthermore, such agents should be useful for continued investigation of 5-HT$_{1D}$ receptor pharmacology.

We previously demonstrated that variously substituted 2-(benzyl)imidazoline analogs bind at 5-HT$_{1D}$ receptors and, with appropriate structural modification, display reasonable selectivity for human 5-HT$_{1D}$ (*h*5-HT$_{1D}$) over human 5-HT$_{1B}$ (*h*5-HT$_{1B}$) receptors.[7–9] The benzylimidazoline oxymetazoline (**1**), an α-adrenergic/5-HT$_{1D}$ agonist, binds at *h*5-HT$_{1D}$ receptors (K_i = 0.4 nM) with twenty times the affinity of sumatriptan, but binds equally well at *h*5-HT$_{1B}$ receptors (K_i = 0.3 nM).[7] Lack of selectivity seems to rest, in large part, on the presence of the phenolic hydroxyl group and its removal leads to analogs with enhanced *h*5-HT$_{1D}$ selectivity.[7] Replacement of the phenolic hydroxyl group by hydrogen halved affinity for the former population of receptors but decreased affinity at *h*5-HT$_{1B}$ receptors by about 40-fold (**2**; *h*5-HT$_{1D}$ K_i = 0.7 nM, *h*5-HT$_{1B}$ K_i = 14 nM) resulting in 20-fold 5-HT$_{1D}$ selectivity.[7] Compound **2** behaved as a 5-HT$_{1D}$ agonist in an adenylate cyclase assay, and in the rabbit saphenous vein (RSV) assay—an isolated tissue preparation useful for examination of such agents.



1 X = -OH; R = -H
2 X = -H; R = -H
3 X = -H; R = -Me
4 X = -H; R = -Bn

Compound **2** while retaining affinity for α$_1$-adrenergic receptors (K_i = 91 nM) still displayed >100-fold selectivity.[7] In contrast, compound **5** binds with lower affinity at *h*5-HT$_{1D}$ (K_i = 105 nM) and at α$_1$-adrenergic receptors (K_i = 2625 nM), but failed to produce a contractile response in the RSV preparation.[7] This implicates a binding and/or functional role for at least one of the *ortho*-methyl groups and suggests that at least one such group be retained in subsequent compounds. In general, *N*-alkylation of the imidazoline ring tends to reduce 5-HT$_{1B}$, 5-HT$_{1D}$, and α-adrenergic affinity, but does so in such

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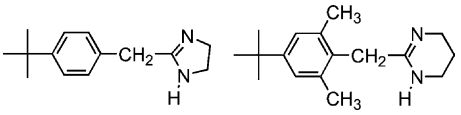
a fashion that it results in overall enhanced 5-HT_{1D} selectivity.[7] For example, compounds **3** and **4** (*h*5-HT_{1D} K_i = 86 and 30 nM, respectively) displayed 50- to 60-fold selectivity for *h*5-HT_{1D} receptors over *h*5-HT_{1B} receptors, and compound **3** was a full agonist in the RSV assay.[7,9]

Another strategy explored to enhance selectivity was to enlarge the size of the imidazoline ring. Compound **6**, for example, possesses high affinity for *h*5-HT_{1D} receptors (K_i = 27 nM) with 160-fold selectivity over *h*5-HT_{1B} receptor binding.[8]



5 **6**

The purpose of the present investigation was twofold. First, we examined several 2-(anilino)imidazolines (i.e., ring-substituted analogs of **2** where the benzylic methylene group was replaced by an NH moiety) to determine if they might bind with higher affinity and/or selectivity than the 2-(benzyl)imidazolines, and to obtain some preliminary structure–affinity data. Next, our intent was to select either one of the new analogs, or compound **6** if the anilinoimidazolines offered no apparent advantage, for comparison with representative benzylimidazolines in several pharmacological and pharmacokinetic assays.

Binding data for the anilinoimidazolines[10] (compounds **10**–**16**) are shown in Table 1. Comparing the *h*5-HT_{1D} affinities of the unsubstituted anilinoimidazoline **10** (K_i > 30,000 nM) and its 2,6-dimethyl counterpart **11** (K_i = 2075 nM) with that of **12** (K_i = 0.3 nM), it is clear that the 4-*tert*-Bu group makes a significant contribution to binding. We have previously demonstrated that increased lipophilicity of the aryl 4-position substituent results in enhanced *h*5-HT_{1D} receptor affinity in the benzylimidazoline series.[9] This seems to be the case with the anilinoimidazolines as well. In addition, we examined two anilinoimidazolines where the *ortho*-methyl groups were replaced by chloro groups: clonidine (**15**) and iodoclonidine (**16**); compound **16**, in particular, indicated that it is possible to replace the methyl groups with chloro groups with retention of affinity. However, iodoclonidine (**16**) is known to bind at α-adrenergic receptors. Comparing **16** with **15** again suggests an important role for a lipophilic 4-position substituent. The structure–affinity relationships of anilinoimidazolines remain to be further explored; nevertheless, although compound **12** seemed to possess slightly higher affinity and selectivity than its benzylimidazoline counterpart **2** (Table 1), there was no clear evidence that this series offered any particular advantage over the benzylimdazoline series. Hence, compound **6** was selected for subsequent comparison with **3** and **4**.

The binding affinity of **6** was re-determined[11] and found to be comparable (*h*5-HT_{1D} K_i = 33 nM, *h*5-HT_{1B} K_i = 4435 nM) to what we had previously reported.[8] A comparison of the binding profile of **4** and **6** in a panel of 28 different receptor populations (CEREP) indicated that compound **4** displayed some affinity (i.e., 68% inhibition at 1000 nM) for *h*m_4-muscarinic receptors. In contrast, compound **6** displayed no affinity for these receptors. In particular, compound **6** produced ⩽10% inhibition at α- and β-adrenergic, muscarinic, or opioid (μ, δ, κ) receptors.

Compounds **3** and **4**, *N*-substituted analogs of **2**, and the ring-expanded analog **6**, were evaluated in several functional studies of serotonergic activity. Compounds **4** and **6** (IC_{50} = 0.4 and 5.0 nmol/kg) were at least equivalent in potency to sumatriptan (IC_{50} = 3–7 nmol/kg), whereas compound **3** (IC_{50} = 10 nmol/kg) was somewhat less potent on dural protein extravasation induced by electrical stimulation in guinea-pigs (data not shown) in a neurogenic inflammation model.[12] Compound **4** was examined both as an agonist and an antagonist using an isolated rabbit saphenous vein preparation as a measure of functional activity.[11] Compound **4** showed agonist activity (EC_{50} = 0.78 μM, relative to 0.22 μM for

Table 1. *h*5-HT_{1B} and *h*5-HT_{1D} serotonin receptor binding data for representative compounds[a]

R_2	R_4	R_6		*h*5-HT_{1B} K_i (nM)[b]	*h*5-HT_{1D} K_i (nM)[b]	5-HT_{1D} Selectivity		*h*5-HT_{1B} K_i (nM)[c]	*h*5-HT_{1D} K_i (nM)[c]	*h*H5-HT_{1D} Selectivity
H	H	H	**7**	>10,000	>10,000	—	**10**	>30,000	>30,000	—
CH_3	H	CH_3	**8**	>10,000	2210	>4	**11**	>30,000	2075	>10
CH_3	*t*-Bu	CH_3	**2**	13	0.7	20	**12**	12	0.3	40
CH_3	Br	CH_3	**9**	1570	74	20	**13**	1430	54	26
CH_3	NO_2	CH_3		—	—	—	**14**	10,240	330	30
Cl	H	Cl		—	—	—	**15**	—[d]	—[d]	—
Cl	I	Cl		—	—	—	**16**	1980	50	40

[a] For comparison, sumatriptan binds at *h*5-HT_{1B} and *h*5-HT_{1D} receptors with K_i = 54 and 7.2 nM, respectively. Radioligand binding studies were performed as previously reported.[7]

[b] K_i values were previously reported[7] and are included only for comparison.

[c] K_i values were determined in triplicate and SEM. is within ±10%.

[d] Screening indicated ⩽5% displacement at 1000 nM.

Table 2. Radioligand binding and rat pharmacokinetic data for compounds **3**, **4**, and **6**[a]

	$h5\text{-}HT_{1B}$ K_i (nM)	$h5\text{-}HT_{1D}$ K_i (nM)	Dose (mg/kg)	$t_{1/2}$ (min)	Total Cl (mL/min/kg)	V_D (mL/kg)	F (%)[d]
3	>5000[b]	86[b]	5	57.0	66.4	3974	42.1
4	1862[b]	30[b]	5	98.5	57.6	7278	9.3
6	4435[c]	33[c]	5	61.8	28.9	1240	9.9

[a] The values are given as the mean PK values for three individual rats.
[b] Binding data previously reported and included only for comparison.
[c] K_i values were determined in triplicate and SEM. is within ±10%.
[d] Data presented are for an intravenous dose, except for the calculation of F.

sumatriptan) with no demonstrated antagonist activity (data not shown).

The rat pharmacokinetic (PK) properties were also investigated for compounds **3**, **4**, and **6** (Table 2). Compound **3** was found to have good PK properties as characterized by its oral bioavailability (F_{po} = 42.1%); the bioavailability of **4** and **6** were poorer (F_{po} = 9.3%, and 9.9%, respectively, for **4** and **6**). Intravenous dosing to rats at 5 mg/kg showed **3** and **4** to be well distributed in body tissue (V_D = 3974 and 7278 mL/kg, respectively) resulting in a plasma half-life of 57 and 98.5 min, respectively (Table 2). The compounds were also found to significantly penetrate into the brain following intravenous administration with brain/plasma ratios after 3 h of >3.5 and >6, respectively, for **3** and **4**. This could be relevant in order for these compounds to possess a centrally mediated component to their potential effectiveness as antimigraine agents.

In conclusion, several 2-(anilino)imidazolines (e.g., **12**, **13**, **16**) were found to bind at $h5\text{-}HT_{1D}$ receptors with 20- to 40-fold selectivity over $h5\text{-}HT_{1B}$ receptors and, as such, represent a novel class of $h5\text{-}HT_{1D}$ ligands. As in the benzylimidazoline series, the presence of a lipophilic 4-position substituent seems to be a major contributor to binding. However, the anilinoimidazolines did not seem to offer any apparent advantage over the benzylimidazoline series. Subsequent studies showed that benzylimidazolines **3** and **4**, and 2-benzyltetrahydropyrimidine **6**, represent novel, potent, and reasonably selective human $5\text{-}HT_{1D}$ receptor agonists with >60-fold selectivity over the human $5\text{-}HT_{1B}$ serotonin receptors. This effort also demonstrated that **4** and **6** show good selectivity over a range of other serotonergic and nonserotonergic receptors, and might be useful tools with which to further examine the role of $5\text{-}HT_{1D}$ receptors in migraine and other $5\text{-}HT_{1D}$-mediated disorders. In particular, compounds **4** and **6** will undergo further pharmacological evaluation and also serve as templates for development of additional $h5\text{-}HT_{1D}$ ligands. Another finding of the study is that the α-adrenergic agonist clonidine (**15**), unlike oxymetazoline (**1**), lacks affinity for $h5\text{-}HT_{1D}$ receptors, but that iodoclonidine (**16**) binds with reasonably high affinity. Future studies with iodoclonidine will need to take this latter observation into account, and the actions of this agent cannot be assumed to reflect simply its interaction with adrenergic receptors.

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10. The anilinoimidazolines, which were previously reported with the exception of **12**, were examined as their HCl salts and were generally prepared by the method of Yamato et al.[13] Melting points of compounds **10** (mp 215–220 °C) and **11** (mp 200–203 °C) were consistent with literature values (mp 218–220 °C and 203–205 °C, respectively).[14] Compound **12** (free base from MeOH, mp 187–189 °C; HCl salt from EtOH/Et_2O, mp 239–241 °C, analyzed within 0.4% of theory for C, H, and N) was prepared in a similar manner, and **13**[15] and **14**[16] were obtained by literature methods. Clonidine (**15**) and iodoclonidine (**16**) were purchased as their HCl salts from RBI (Natick, MA).
11. The radioligand binding assays and functional assays were conducted as previously reported[7].
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