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Design, Synthesis and Biological Activity of Novel Dimethyl-{2-[6-substituted-indol-1-yl]-ethyl}-amine as Potent, Selective, and Orally-Bioavailable 5-HT_{1D} Agonists

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Abstract—A novel series of highly potent human 5-HT_{1D} agonists, dimethyl-{2-[6-substituted-indol-1-yl]-ethyl}-amine, was synthesized. Structure–activity relationship (SAR) investigation revealed 4-[1-(2-dimethylamino-ethyl)-1*H*-indol-6-yl]-tetrahydro-thiopyran-4-ol, **11b** (ALX-2732), as a potent ($K_i = 2.4$ nM) agonist at the human 5-HT_{1D} receptor with good selectivity over the other serotonin receptor subtypes. This compound demonstrated favorable in vitro metabolic stability in human and rat liver microsomes and was found to be orally bioavailable in rats ($F_{po} = 51\%$).

The 5-HT_{1D} receptor functions as a terminal autoreceptor regulating the synthesis and release of 5-hydroxytryptamine from serotonergic neurons, and it also acts as a presynaptic heteroreceptor regulating the release of other neurotransmitters (e.g., substance P and calcitonin gene related peptide, CGRP) from their respective systems.[1,2] This regulatory effect of the 5-HT_{1D} receptor subtype is of great importance for drug therapies in neuronal abnormalities occurring particularly in the area of migraine management.[3] Activation of the 5-HT1D receptor, which is negatively coupled to cyclic adenosine monophosphate (cAMP), has been shown to inhibit neuropeptide release thought to be involved in the onset of a migraine attack and chronic tension-type headache. Therefore, 5-HT_{1D} agonism should be of great importance in the treatment of migraine and tension-type headaches.[4,5]

Receptor mapping studies using polymerase chain reaction (PCR) amplification have shown that in the human trigeminal ganglia, mRNA's encoding for both h5-HT_{1D} and h5-HT_{1B} receptor subtypes (previously termed 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$, respectively) appear to be present. In human cerebral blood vessels, there is a preponderance of mRNA encoding for the h5-HT1B subtype only. Studies using h5-HT_{1D} and h5-HT_{1B} receptor-specific antibodies showed that only h5-HT_{1B} receptor protein was found on dural arteries, whereas only h5-HT_{1D} receptor protein was found on trigeminal sensory neurons both on the peripheral projections to dural blood vessels and on the central projections that terminate behind the blood–brain barrier where they synapse with neurons that convey pain impulses to higher brain centers. This suggests that 5-HT_{1D} receptors are responsible for blocking the release of CGRP in the peripheral meningeal arteries and also for inhibiting neurotransmitter release within the brainstem and interrupting central pain transmission, whereas 5-HT_{1B} receptors are involved in direct vasoconstriction (Fig. 1).[6]

Since the discovery of sumatriptan, a 5-$HT_{1B/1D}$ receptor agonist, as an effective treatment for migraine headache, intensive research in this area has lead to several

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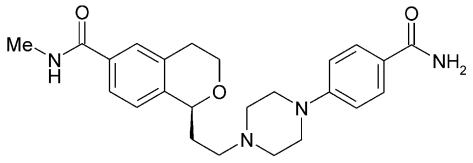
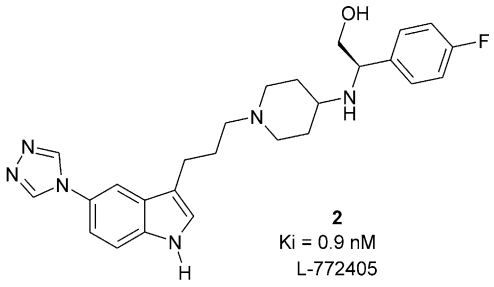


1
Ki = 4.6 nM
PNU-142633
(Pharmacia & Upjohn)



2
Ki = 0.9 nM
L-772405
(Merck)

Figure 1. Examples of selective 5-HT_{1D} selective agonists.

second-generation triptans such as naratriptan, zolmitriptan, rizatriptan, eletriptan, almotriptan and frovatriptan. Despite the favorable clinical efficacy of the 'triptans', there exists the potential for cardiovascular adverse events thought to be associated with the activity at the 5-HT_{1B} receptor.[6,7] Therefore, identification of selective h5-HT_{1D} receptor agonists is necessary in order to confirm the target tissue for antimigraine drugs and to develop an antimigraine agent with a potentially lower side effect liability.[7,8] Recently, PNU-142633 **1**, a selective peripherally acting ligand was reported to be clinically ineffective.[9] However, this ligand had weak efficacy in activating the 5-HT_{1D} receptors and a fully efficacious agonists may be a more successful strategy that requires testing clinically. L-772405 **2**, a potent and selective 5-HT_{1D} agonist was found to be fully efficacious in vitro, however this ligand exhibited low oral bioavailability due to poor absorption and gut wall metabolism.[10] In this paper, we wish to report on the discovery and SAR of a novel series of compounds that lead to the identification of a selective 5-HT_{1D} agonists demonstrating good bioavailability (Fig. 2).

The early discovery and optimization of 5-hetreroalkyltryptamine **3** and 5-aryltryptamine **4** led to potent human 5-HT_{1D} agonists with K_i of 0.15 and 0.56 nM, respectively.[11,12] These triptan analogues exhibited similar pharmacodynamic properties to sumatriptan, a potent 5-$HT_{1D/1B}$ agonist. Despite the enhanced lipophilicty conferred to these triptan analogues by the 5-alkyl and 5-aryl substituents, we hypothesized that the intrinsic hydrogen-bonding property of the indole NH renders these molecules more polar and hence less membrane penetrant. By transposing the dimethylaminoethyl side chain from the 3-position of the indole onto the indole nitrogen and simultaneously shifting the lipophilic group from the 5-position of the indole to the 6-position would provide molecules of structures **13c** and **10a**, respectively. The pharmacophores **13c** and **10a** were expected to retain h5-HT_{1D} affinity, however the decreased hydrophilicity should increase membrane penetrability thereby increasing intestinal absorption and blood–brain barrier penetration. Compounds **13c** and **10a** were found to possess good affinity for the human 5-HT_{1D} receptor with K_i's of 3.7 and 7.3 nM, respectively. With these two initial lead compounds in hand, further SAR exploration were performed with the goal of identifying a potent human 5-HT_{1D} receptor agonists (K_i <10 nM), with selectivity over

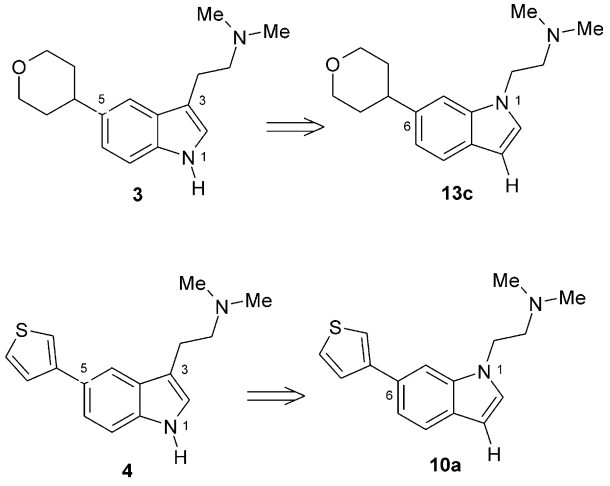
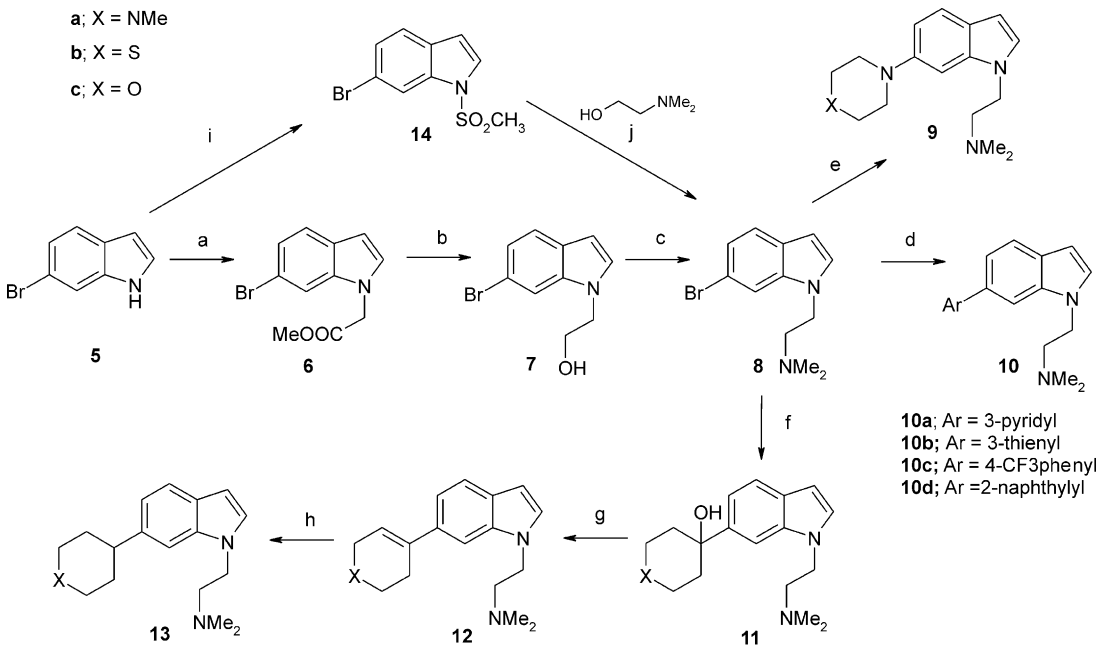


Figure 2. Transposition of substituent at the 3- and 5-position of indole to the 1- and 6-position.

human 5-HT_{1B} receptor (>50-fold) and also possessing favorable in vitro microsomal stability in rat and human microsomal fractions.

The synthesis of this series of dimethylaminoethylindoles of general structures **9**, **10**, **11**, **12**, and **13** is shown in Scheme 1. *N*-Alkylation of 6-bromoindoles **5** with ethylbromoacetate afforded compound **6**. Subsequent reduction of the ester function with DIBAL-H gave the corresponding alcohol **7**. Treatment of **7** with methanesulphonylchloride followed by displacement with the various *N*,*N*-dimethylamine delivered the intermediate structure **8**. An alternative synthesis of **8** involved mesylation of the indole nitrogen of **5** followed by treatment with *N*,*N*-dimethylaminoethylalcohol in the presence of sodium hydride, potassium carbonate in toluene.

Compounds of structure **9** were synthesized as shown in Scheme 1 via the conditions of metal-catalyzed aryl amination of **8**.[13] The corresponding compounds **11**, **12**, and **13** were synthesized according to Scheme 1. The indole intermediate **8** was coupled to the cyclic ketones to give the carbinol **11**. Carbinol **11** was then treated with trifluoroacetic acid to effect the elimination to alkenes **12**. Alkenes **12** were subjected to palladium-catalyzed hydrogenation to give compounds of structure **13**.



Scheme 1. Reagents and conditions: (a) NaH, DMF, $BrCH_2CO_2C_2H_5$, 0 °C; (b) 2 equiv DIBAL-H, THF, rt; (c) (i) MsCl, Et_3N, CH_2Cl_2, rt; (ii) $HNMe_2$, Et_3N, THF, 70 °C; (d) $ArB(OH)_2$, 2 N Na_2CO_3, DME 110 °C.; (e) Amine, NaO*t*Bu, $Pd(OAc)_2$, P(*t*-Bu)$_3$, Xylene 120 °C; (f) KH, *t*-BuLi, ketone, THF, −78 °C; (g) TFA, THF; (h) H_2, Pd/C, MeOH; (i) MsCl, NaH, DMF 0 °C∼rt.; (j) NaH, K_2CO_3, toluene, 110 °C.

The 6-aryl substituted analogues **10** were prepared by subjecting the versatile intermediate **8** to the Suzuki cross-coupling conditions with commercially available arylboronic acids.

The synthesized compounds were primarily evaluated for their binding affinities to the human 5-HT_{1D} and 5-HT_{1B} receptors in vitro. The assay protocol includes the incubation of membranes prepared from Chinese hamster ovary cells (CHO cells) expressing these receptors with ^{3}H-serotonin, using sumatriptan as a standard.

Various concentrations of the test compound were incubated with the radioligand (^{3}H-5HT) and the receptor affinity (K_i in nM, or % inhibition @1 μM) was determined (Table 1).

The compounds were initially screened at 1 μM and if the binding at the human 5-HT_{1D} was found to be greater than ninety percent, the corresponding K_i's at the human 5-HT_{1D} and 5-HT_{1B} receptors were determined. The 6-amino analogues **9b** and **9c** were generally found to be less potent than their corresponding carbon analogues **13b** and **13c**. Compound **13b** ($K_i = 5.6$ nM) and **13c** ($K_i = 3.7$ nM) were found to possess relatively good selectivity over the human 5-HT_{1B} receptor (48- and 57-fold, respectively). The related alkene analogues **12b** and **12c** resulted in a significant loss in potency (K_i of 22 and 32 nM, respectively) at the 5-HT_{1D} receptors and a dramatic decrease in 1D/1B selectivity. The *N*-methylpiperidine derivatives **11a**, **12a**, and **13a** demonstrated considerably lower potency at the 5-HT_{1D} receptor compared to the analogous pyran (**11c**, **12c**, and **13c**) and thiopyran (**11b**, **12b**, and **13b**) derivatives. The thiopyran carbinol **11b** exhibit excellent human 5-HT_{1D} potency ($K_i = 2.0$ nM) and remarkably good selectivity over the 5-HT_{1B} receptor (57-fold). Despite the high potency displayed by the 6-pyridyl and 6-thienyl derivatives **10a** and **10b** ($K_i = 1.4$ and 7.3 nM for the human 5-HT_{1D} receptor, their selectivity over the 5-HT_{1B} receptor subtype were well below the desired 50-fold selectivity. In addition, these 6-aryl substituted analogues typically showed poor selectivity over the closely related 5-HT_{1A} receptor therefore making them considerably less attractive as selective 5-HT_{1D} ligands.

Compound **11b** was further evaluated for metabolic stability in the presence of rat and human liver microsomes. These results are summarized in Table 2. Compound **11b** demonstrated favorable metabolic stability (>50%) in both human and rat microsomes with slightly greater stability in the human pooled micosomal system. Additional binding affinity studies at other serotonin receptors, dopamine receptors, muscarinic receptors and α-adrenergic receptor showed that **11b** possesses very good selectivity over the battery of receptors examined (Table 3).

The ligand **11b** was then examined for its ability to inhibit foskolin-stimulated adenylate cyclase activity in cell lines [Chinese hamster ovary (CHO) stable cell lines] expressing the 5-HT_{1D} receptors. **11b** was found to be a fully efficacious and functionally potent agonist at the human 5-HT_{1D} receptor with an EC_{50} if 0.3 nM compared to sumatriptan with an EC_{50} of 5.3 nM.

The rat pharmacokinetic (PK) properties were then evaluated for **11b** (Table 4). This compound was found to have good PK properties characterized by its good oral bioavailability ($F_{po} = 51\%$). Intravenous dosing to rats at 3 mg/kg showed **11b** to be present in good plasma concentrations ($C_{max} = 538$ ng/mL) and well distributed into body tissue ($V_D = 8.6$ L/kg) resulting in a plasma half-life of 1.5 h. The compound was also

Table 1. In vitro affinity of *N*,*N*-dimethyl-{2-[6-substituted-indol-1-yl]-ethyl}-amine at the human 5-HT_{1D} receptor with selectivity over the human 5-HT_{1B} receptor

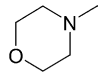
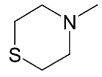
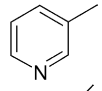
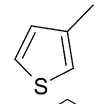
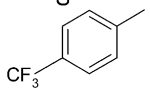
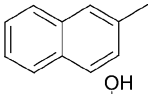
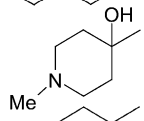
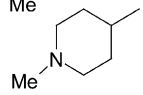
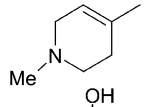
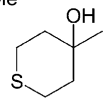
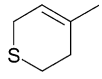
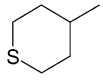
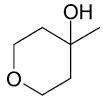
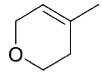
Compound	Indole-6-substituent	% h5-HT_{1D} binding @ 1 μM	h5-HT_{1D} affinity K_i (nM)	Selectivity K_i (1B)/K_i (1D)
9c		85	—	—
9b		88	—	—
10a		98	1.4	17
10b		99	7.3	22
10c		86	—	—
10d		79	57	5.5
11a		44	—	—
13a		74	—	—
12a		84	—	—
11b		99	2	57
12b		95	22	7.4
13b		99	5.6	48
11c		—	3.4	48
12c		—	32	28

Table 2. In vitro human and rat metabolic stability of compound **11b** after 30 min incubation

Microsome	% Remaining after 30 min incubation
Human liver microsome	70
Rat liver microsome	79

found to significantly penetrate the blood–brain barrier following intravenous administration with brain/plasma ratios at 4 h greater than 6. This could be relevant in order for **11b** to possess the centrally- mediated component to it potential effectiveness as an antimigraine agent.

Table 3. Receptor binding profile of **11b**

Receptor	Affinity (% inhibition @ 100 nM)
5-HT_{1D}	96
5-HT_{1B}	46
5-HT_{1A}	27
5-HT_{2A}	0
5-HT_{2C}	4
5-HT_6	0
5-HT_7	5
$M_1 + M_2$	0
α_1	1
D_1	0
D_2	0
D_3	0
D_4	2
D_5	1

Table 4. Rat pharmacokinetic data for compound **11b**, 3 mg per kg 1.v dose

iv (Intravenous) $t_{1/2}$(h)	C_{max} (ng/mL)	V_D (L/kg)	Cl (plasma) (mL/min/kg)	AUC PO (ng.mL/kg)	F (%)
1.5	538	8.613	74	21,265	51.1

In conclusion, a series of novel, potent and selective human 5-HT_{1D} receptor agonists have been developed which have up to 57-fold selectivity over the human 5-HT_{1B} subtype. This effort has led to the identification of compound **11b**, which showed good selectivity over a range of other serotonergic and non-serotonergic receptors. This compound also possesses good oral bioavailability and would therefore be a valuable tool to further delineate the role of 5-HT_{1D} receptors in migraine and other diseases.

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