Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 15, pp. 1825-1830, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00324-1

THE CHEMICAL EVOLUTION OF N,N-DIMETHYL-2-[5-(1,2,4-TRIAZOL-4-YL)-1H-INDOL-3-YL]ETHYLAMINE (L-741,604) AND ANALOGUES: POTENT AND SELECTIVE AGONISTS FOR 5-HT_{1D} RECEPTORS[1]

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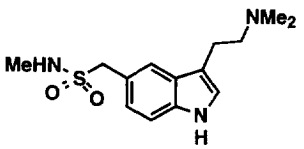
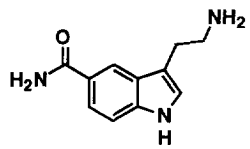
Abstract: Optimisation of a series of 5-(heterocyclyl)tryptamines led to the identification of the symmetrically substituted, N-4 linked 1,2,4-triazole as the best indole C-5 substituent for 5-HT_{1D} receptor affinity and selectivity. The triazole (**8**) is the most potent and selective, orally bioavailable, 5-HT_{1D} receptor agonist identified to date, showing an order of magnitude greater potency than the clinical compound sumatriptan with improved subtype selectivity.

The recent introduction of the selective 5-HT_{1D} receptor agonist sumatriptan (Imigran) (**1**, Figure 1) for the acute treatment of migraine[2] has prompted an intense research effort to discover more potent and selective 5-HT_{1D} receptor agonists with improved pharmacokinetic profiles. Sumatriptan selectively constricts intracranial vascular smooth muscle and inhibits neuropeptide release from perivascular trigeminal sensory neurones and both mechanisms have been proposed to be important in elicting its antimigraine action.[3,4]

Figure 1



1: Sumatriptan

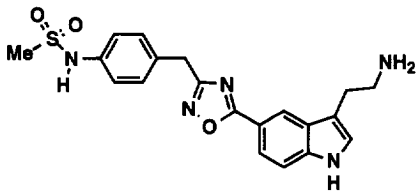


2: 5-CT

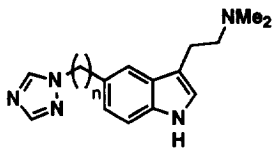
Our own initial strategy to designing novel 5-HT_{1D} receptor selective agonists was to identify heteroaromatic rings as replacements for the carboxamide group of the potent but unselective 5-HT_1 receptor agonist 5-CT (**2**). This work led to the discovery of the benzyloxadiazole (**3**) (L-694,247) as a highly potent 5-HT_{1D} receptor agonist with good selectivity for 5-HT_{1D} receptors but low oral bioavailability.[5,6] More recently, optimisation of the heterocycle for oral bioavailability led to the identification of our clinical candidate, the 1,2,4-triazole MK-462 (**4**).[7] MK-462 has a comparable *in vitro* and *in vivo* pharmacological profile to sumatriptan but has higher bioavailability and is rapidly absorbed following oral administration. Other groups have reported further compounds undergoing evaluation as antimigraine agents.[8,9]

In a continuation of the programme, compounds were sought which had improved 5-HT_{1D} receptor potency and selectivity. At the same time, a clear goal was to modify the pharmacokinetic profile to achieve a longer duration of action, whilst maintaining good oral bioavailability and low central nervous system (CNS) penetration (log D <-0.5) which is important for potential antimigraine agents of this class.[2]

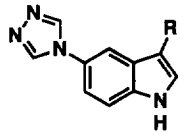
Figure 2



3: L-694,247



4: n=1 MK-462
13: n=0



5: R = ethylamine, cyclic amine

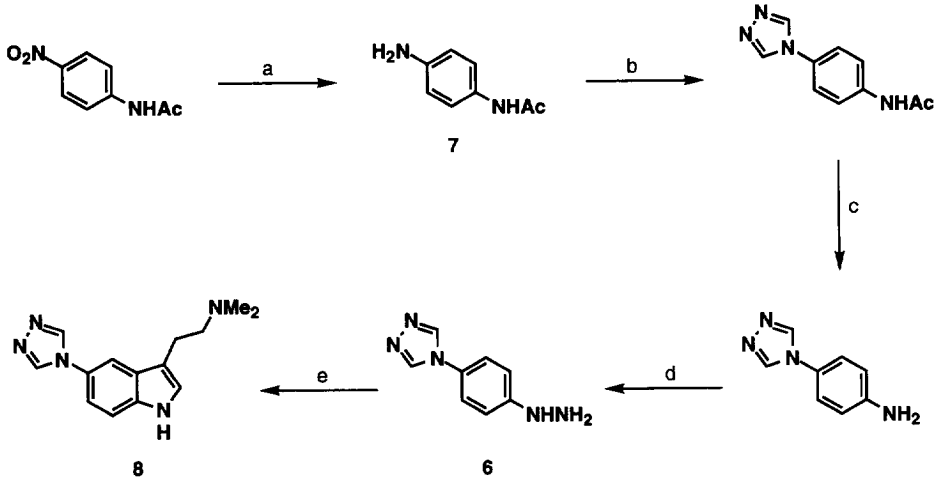
Our earlier work had established the importance of a hydrogen bond acceptor interaction between the heterocyclic ring and the 5-HT_{1D} receptor. In particular, a hydrogen bond acceptor interaction between a β-nitrogen in an azole ring and the 5-HT_{1D} receptor is required for high affinity. Accordingly, we were keen to explore the effect of two β-nitrogens. This work has led to the identification of the 5-(1,2,4-triazol-4-yl)indole series of compounds (**5**, Figure 2), and the discovery that *the symmetrically substituted, N-4 linked, 1,2,4-triazole, when directly attached to indole, represents the best indole 5-substituent for 5-HT_{1D} receptor affinity and selectivity identified to date.* Whilst the 1-substituted triazole ring has commonly been included in SAR studies across a range of medicinal chemistry programmes, use of the 4-linked triazole is rare. Moreover, this approach to improving receptor selectivity is complementary to the previously reported method of ethylamine side chain modification.[10,11] We describe in this paper the synthesis, the 5-HT receptor activity and pharmacokinetics of compounds in this series.

Compounds in this series were prepared by Fischer indole methodology using the hydrazine (**6**) as a common intermediate. Since simple alkylation of 1,2,4-triazole with, for example, 1-fluoro-4-nitrobenzene provided almost exclusively N-1 alkylation, rather than the required N-4 alkylation, hydrazine (**6**) was prepared as shown in Scheme 1. Construction of the required 4-substituted 1,2,4-triazole ring was achieved in excellent yield by reaction of 4'-aminoacetanilide (**7**) with *N,N*-dimethylformamide azine[12] in refluxing toluene with *p*-toluenesulphonic acid catalysis. Subsequent acid hydrolysis and diazotisation/reduction provided hydrazine (**6**). Fischer reaction of (**6**) with 4-(*N,N*-dimethylamino)butanal dimethyl acetal[13], in refluxing 4% sulphuric acid, gave the indole (**8**) (L-741,604) in 45% yield.[14,15]

Conformationally restricting the ethylamino chain of the "traditional" tryptamine 5-HT ligands can lead to improvements in receptor subtype selectivity.[10,11] Furthermore, modification of the metabolically soft tryptamine side chain should provide a tool to manipulate the pharmacokinetic profile of ligands. We chose to investigate the piperidine analogue of (**8**) (Scheme 2). Horner-Wittig olefination of *N*-methyl-4-piperidone (**9**) followed by

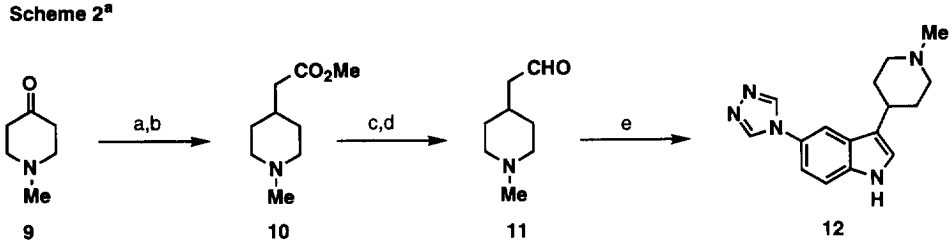
hydrogenation gave the homologated ester (**10**), which was processed to the aldehyde (**11**) in two steps. Reaction of (**11**) with hydrazine (**6**) gave the Fischer adduct (**12**) (L-741,519) in 54% yield.

Scheme 1[a]



[a]Reagents and conditions: (a) H_2, 10% Pd-C, EtOH/EtOAc, 2N HCl, 90%; (b) $(Me_2NCH{=}N)_2$, $TsOH.H_2O$ (cat) toluene, reflux,91%; (c) 5N HCl, reflux , 76%; (d) (i) $NaNO_2$, H_2O, conc. HCl, -10°C then (ii) $SnCl_2.2H_2O$, conc. HCl, -10°C→+25°C, 87%; (e) 4-(*N,N*-dimethylamino)butanal dimethyl acetal, 4% H_2SO_4, reflux, 45%.

Scheme 2[a]



[a]Reagents and conditions: (a) $MeO_2CCH_2P(O)(OEt)_2$, NaH, THF, 60°C, 30%; (b) H_2, 10% Pd-C, MeOH/ H_2O, 5N HCl, 89%; (c) DIBAL-H, toluene, -35°C→+25°C, 82%; (d) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -70°C, 69%; (e) **6**, 4% H_2SO_4, reflux, 54%.

The 5-HT receptor affinities of (**8**) and (**12**) were measured[6] and the data is presented in Table 1, together with that for *N,N*-dimethyl-2-[5-(1,2,4-triazol-1-yl)-1H-indol-3-yl]ethylamine, (**13**, Figure 2), the N-1 linked analogue of (**8**).[5] The *N,N*-(dimethylamino)ethylindole (**8**) has greater than 10-fold higher affinity for 5-HT_{1D} receptors than both sumatriptan and MK-462, and is one of the most selective 5-HT_{1D} receptor agonists reported to date, with 25-fold selectivity over 5-HT_{1A} receptors and greater than 1000-fold selectivity over 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptors. Significantly, it also has 10-fold higher affinity for 5-HT_{1D} receptors than the analogous N-1 linked triazole (**13**) and greatly improved selectivity over the 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} recognition sites. The log D for (**8**) (-1.0) is considerably lower than that for (**13**) (-0.34), predictive of low

CNS penetration (*cf* sumatriptan log D = -1.2). The *N*-methylpiperidine (**12**) (log D = -0.60) similarly shows greater affinity for the 5-HT_{1D} recognition site than MK-462 and sumatriptan and has excellent selectivity over 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptors. The binding profiles of both (**8**) and (**12**) at 5-HT receptors in human brain cortex mirror the animal tissue data.[16]

Table 1. Selectivity of Triazoles in Binding to 5-HT Receptors in Animal Tissue

	$IC_{50} \pm SEM$[a]					
compound	5-HT_{1D}[b]	5-HT_{1D}[c]	5-HT_{1A}[d]	5-HT_{2A}[e]	5-HT_{2C}[f]	5-HT_3[g]
Sumatriptan	7.7 ± 0.7	19 ± 2	540 ± 140	>10,000	7900	>10,000
MK-462	16 ± 4	49 ± 10	320 ± 50	7200 ± 1100	7300 ± 260	4100 ± 400
8	0.43	2	52	>10,000	3100	>10,000
12	1.2 ± 0.3	8	95 ± 19	>10,000	>10,000	2700 ± 430
13	-	20 ± 1	25	10,000	500	10,000

[a]SEM = standard error of the mean from n ≥ 3. Where SEM is not quoted the figures are the mean of two independent determinations typically with individual values within ± 10-15% of the mean. [b]Displacement of [^{125}I]-GTI binding to 5-HT_{1D} recognition sites in pig caudate membranes.[c]Displacement of [^{3}H]-5-HT binding to 5-HT_{1D} recognition sites in pig caudate membranes. [d]Displacement of [^{3}H]-8-OH-DPAT from pig cortex. [e]Displacement of [^{3}H]-DOB from rat cortex homogenates. [f]Displacement of [^{3}H]-mesulergine from pig cortex. [g]Displacement of [^{3}H]-Q-ICS 205-930 from rat cortex homogenates.

The *in vitro* functional activity of the compounds for 5-HT_{1D} receptors was assessed on the New Zealand white rabbit saphenous vein preparation[17]. In this model, contractions evoked by agonists are considered to be mediated by 5-HT_1-like receptors. Agonist potencies were calculated as EC_{50} values from plots of percentage 5-HT (1μM) response against concentration of the agonist. Triazoles (**8**) (EC_{50} 32nM) and (**12**) (EC_{50} 39nM) are full agonists in this assay and are equipotent with 5-HT (44nM) and greater than 10-fold more potent than sumatriptan (442nM). The increase in affinity observed on replacing the N-1 linked triazole ring in (**13**) (EC_{50} 62nM) with the N-4 linked triazole is reflected in the functional assay.

In vivo functional activity was assessed in two assays considered to be predictive of anti-migraine efficacy. In anaesthetised dogs, intravenous doses of 0.1 - 100 μg/kg of triazole (**8**) or (**12**) produced selective dose-related reductions in carotid arterial blood flow with no meaningful effects on coronary arterial blood flow or other cardiovascular variables.[18] The maximum falls in carotid flow were -65% and -67% respectively and are similar to those produced by sumatriptan (-67%). The plasma concentrations producing half of the maximal fall in carotid flow (IC_{50}) were 3.1nM (**8**) and 6.9nM (**12**), which is approximately 10-fold lower than the IC_{50} for sumatriptan (48.6nM). In anaesthetised rats, both (**8**) and (**12**) inhibited plasma extravasation produced in the dura mater by electrical stimulation of the trigeminal ganglion[19] (IC_{50} 13nM and 69nM, respectively).

Pharmacokinetic parameters for (**8**) and (**12**) are compared with sumatriptan and MK-462 in Table 2. Both (**8**) and (**12**) are orally bioavailable in rats and have reduced plasma clearance compared with sumatriptan and MK-462. The longer half-life of the piperidine (**12**), which may reflect improved metabolic stability over the N,N-dimethyltryptamines, holds promise for a reduction in breakthrough headache in the latter stages of a migraine attack.

Table 2. Pharmacokinetic Parameters for Triazoles and Sumatriptan in Rat[a]

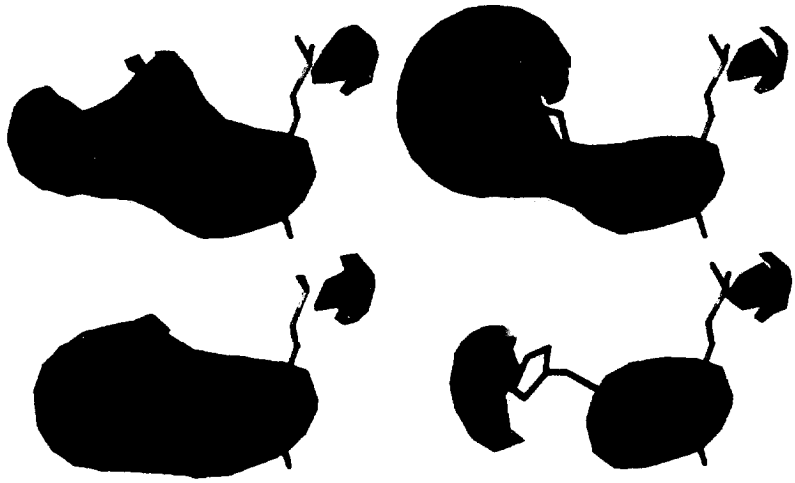
parameter	sumatriptan	MK-462	**8**	**12**
$T_{1/2}$ (h)	1.1	0.8	1.3	2.0
Cl_p (ml/min/kg)	69	79	37	48
C_{max} (ng/ml)	74	271	83	53
T_{max} (h)	1.75-4.0	0.75	1.0	3.0
F (%)	44	76	27	20

All compounds were dosed as aqueous solutions of the acid salt at 3mg/kg. Analysis of plasma samples was by HPLC with UV detection.

Since the N4-linked triazole (**8**) has 10-fold higher affinity for 5-HT_{1D} receptors than the essentially isosteric N1-linked triazole (**13**), the molecular electrostatic potentials were calculated for (**8**) and (**13**) and, for reference, 5-CT (**2**) and MK-462 (**4**) in order to understand this remarkable increase in affinity. The calculations were performed using MOPAC with AM1 on the (PRECISE convergence) optimised geometries of each compound (starting from the same sidechain orientation) and the electrostatic potentials were contoured directly from the quantum-mechanically derived grid at the –10 kcal/mol level. Figure 3 shows the molecular electrostatic potentials for the compounds with the part of the electrostatic potential above the plane of the indole cut away for clarity. The Figure highlights the dramatic difference in electrostatic potentials of (**8**) and (**13**), despite their very similar shapes. The significant difference in binding affinity of these two molecules may therefore be due to an interaction between the indole 5-substituent and a zone of positive electrostatic potential on the receptor (*eg* hydrogen-bond donor sites). The reference compounds (**2**) and (**4**) (the latter with a large negative out-of plane lobe) show electrostatic potentials consistent with this hypothesis, although shape differences may also play a role in determining their activity.

In conclusion, by further modification of the indole C-5 heterocycle to incorporate two equivalent β-nitrogens as potential hydrogen bond acceptors, we have identified a novel series of highly potent and selective 5-HT_{1D} receptor agonists with the required level of hydrophilicity. The triazole (**8**) is one of the most potent and selective, orally bioavailable 5-HT_{1D} receptor agonists reported to date. Replacement of the ethylamino side chain by piperidine is well-tolerated at the 5-HT_{1D} recognition site and enables manipulation of the metabolic profile to deliver a significant increase in half-life.

Figure 3. Stereoviews of 5-CT (bottom left), MK-462 (bottom right), (**13**) (top left) and (**8**) (top right) with electrostatic potentials. See text for details.



References and Notes

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(*Received in Belgium* 20 *May* 1996; *accepted* 4 *July* 1996)