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Thiazoles and thiopyridines: novel series of high affinity h5HT$_7$ ligands

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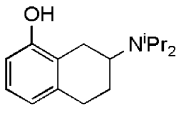
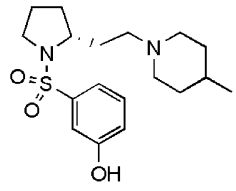
Abstract—A series of thiazole based 5HT$_7$ ligands has been identified from screening. Optimisation of the pendent aryl group and modification of the core gave a related series of high affinity, selective thiopyridine based 5HT$_7$ ligands, the most active of which behaves as a partial agonist.

Based on localisation and pharmacological studies mainly with non-selective ligands,[1] the human 5HT$_7$ receptor has been implicated in a variety of CNS functions. These include depression,[2,3] schizophrenia[4] and control of circadian rhythms.[5] New, high affinity and selective ligands for this receptor would greatly facilitate our understanding of the role of 5HT$_7$ receptors in the brain and potentially lead to new drug entities in multiple therapeutic areas.

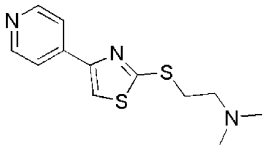


1



2

Current ligands in use include 8-OH-DPAT (**1**), an agonist at 5HT$_7$ which has varying selectivity over other 5HT receptors.[6] More recently selective antagonists have been developed, such as SB-269970[7] (**2**). We therefore set out to discover a novel series of high affinity and selective h5HT$_7$ ligands to further probe the role of this receptor.



3a

Compound **3a** was identified from in-house screening as having reasonable binding affinity at the cloned h5HT$_7$ receptor and some selectivity over other receptors in preliminary counter-screening (Table 1). All compounds of interest were routinely screened against a small selection of other common G-protein coupled receptors. These included 5HT$_{1A}$, as currently used h5HT$_7$ ligands such as 5-carboxamidotryptamine (5-CT) and 8-OH-DPAT show poor selectivity over this subtype,[6] and 5HT$_{2A}$ and D$_2$ receptors, as activity at these could potentially confound any subsequent behavioural studies.

Two areas of the lead molecule were initially selected for modification — the pyridyl ring and the central thiazole core. Chemistry to synthesise these analogues was conveniently carried out from commercially available chlorides or thiols as shown in Scheme 1.

Where neither of these starting materials was available, for example, in the synthesis of the *tert*-butylthiazole **4**, condensation of the relevant α-bromoketone with ammonium dithiocarbamate[8] (Scheme 2) yielded the

Keywords: Thiazoles; Thiopyridines; h5HT$_7$ ligands; Partial agonist.
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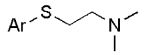
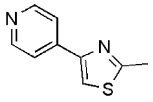
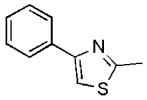
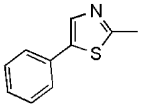
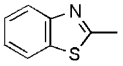
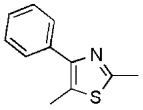
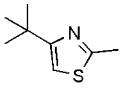
Table 1.



3

No.	Ar	h5HT$_7$ K_i/nM[a]	h5HT$_{1A}$ K_i/nM[b]	h5HT$_{2A}$ K_i/nM[c]	hD$_2$ K_i/nM[d]
3a		27	90[e]	>200[e]	130[e]
3b		6800			
3c		12	210[e]	640[e]	>1000[e]
3d		3200			
3e		84	830	1000[e]	>1000
3f		170[e]			
3g		22	2200	1200[e]	>1000

Values represent the geometric mean of 3–6 determinations unless specified.

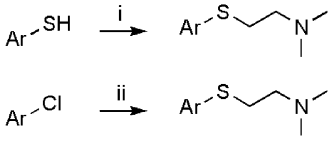
[a] Displacement of [^{3}H]-5HT from the cloned receptor expressed in CHO cells.[12]

[b] Displacement of [^{3}H]-5HT from the cloned receptor expressed in HeLa cells.[13]

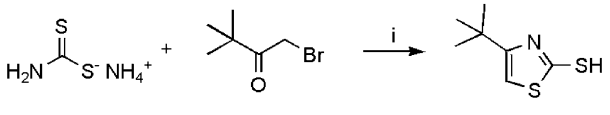
[c] Displacement of [^{3}H]-Ketanserin from the cloned receptor expressed in CHO cells.[14]

[d] Displacement of [^{3}H]-Spiperone from the cloned receptor expressed in CHO cells.[15]

[e] Geometric mean of 2 determinations.



Scheme 1. Reagents: (i) $ClCH_2CH_2NMe_2$·HCl, NaH, DMF; (ii) $HSCH_2CH_2NMe_2$·HCl, NaH, DMF.



4

Scheme 2. (i) EtOH.

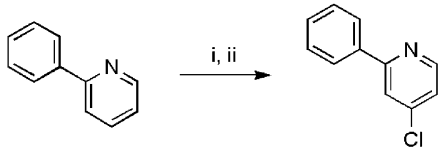
thiol which could then be alkylated as before. For examples **3d** and **3f**, condensation of ammonium dithiocarbamate with bromophenylacetaldehyde[9] and 2-bromopropiophenone gave the desired thiols.

In the case of pyridyl analogue **3j**, synthesis was carried out by rearrangement of the *N*-oxide of 2-phenylpyridine, followed by subsequent displacement of the resulting 4-pyridyl chloride by the sidechain thiol (Scheme 3). Isomer **5a** was formed from 6-phenyl-2-pyridone, which was converted to the thiopyridone, and subsequent alkylation occurred exclusively on sulfur (Scheme 4).

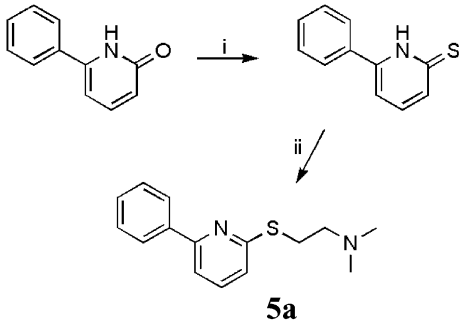
Synthesis of further pyridine analogues started from 2,6-dibromopyridine (Scheme 5). Displacement of one bromine could be carried out with the anions of benzene methanethiol, benzyl alcohol or 2-dimethyl aminoethanethiol. Displacement with benzene methanethiol directly introducing the sulphur gave compound **6**. This intermediate could be lithiated and quenched with ketones to give hydroxyl substituted compounds, but attempts to couple Grignard reagents using nickel catalysis[10] failed, possibly due to the presence of sulfur. The hydroxyalkyl intermediates were debenzylated to give the thiopyridones, which were alkylated on sulfur with the chloroethyl amine as before.

In the case of the benzyloxy compound **7**, substitution of the remaining bromine with Grignard reagents under nickel catalysis went smoothly, except for *tert*-butylmagnesium chloride where no substitution was observed. The protected pyridones could be debenzylated and converted to the thiopyridones with Lawesson's reagent. Again, alkylation on sulfur with the chloroethyl amine yielded the final compounds **5**.

Compound **5e** was prepared from **5b** using copper mediated addition[11] of *tert*-butylmagnesium chloride.

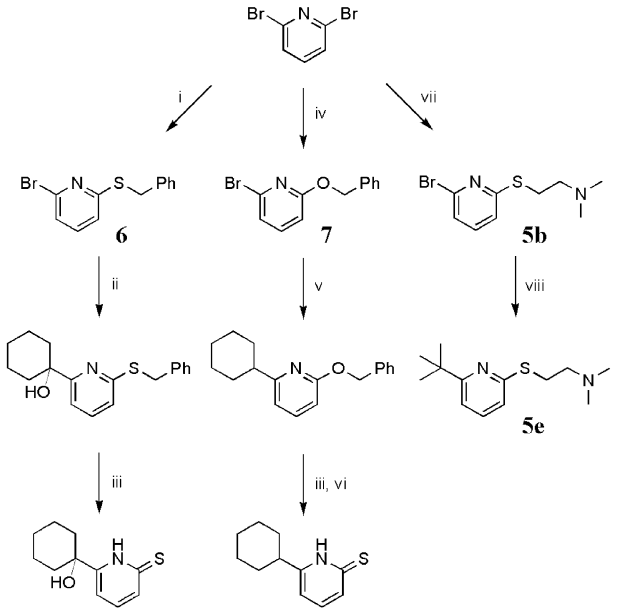


Scheme 3. Reagents: (i) H_2O_2, AcOH; (ii) $POCl_3$, $CHCl_3$.



5a

Scheme 4. Reagents: (i) Lawesson's reagent, DME; (ii) $ClCH_2CH_2NMe_2$·HCl, NaH, DMF.



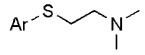
Scheme 5. Reagents: (i) $PhCH_2SH$, NaH, DMF; (ii) BuLi, cyclohexanone, THF; (iii) Pd/C, EtOH, H_2; (iv) $PhCH_2OH$, NaH, DMF; (v) *cyclo*-hexylmagnesium bromide, $NiCl_2$(dppe), Et_2O; (vi) Lawesson's reagent, DME; (vii) $HSCH_2CH_2NMe_2$·HCl, NaH, DMF; (viii) *tert*-butylmagnesium chloride, CuCN, THF.

Variation of the pyridyl group of **3a** showed some interesting structure–activity relationships (Table 1). To obtain good binding affinity at $h5HT_7$, substitution is necessary, but has to be at C4, with phenyl (**3c**) preferred over pyridyl. Benzofusion (**3e**) or 4,5-disubstitution (**3f**) are not tolerated. Both aromatic and *tert*-butyl groups are tolerated, but the *tert*-butyl group shows greater selectivity compared with aromatic substitutions.

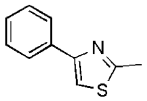
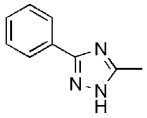
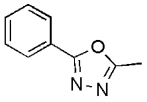
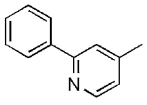
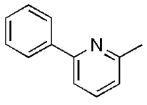
With the phenyl group in place of the pyridyl, variation of the core heterocycle was explored (Table 2). Two alternative phenyl substituted heterocycles were introduced (**3h** and **3i**) but were found to be detrimental. However, when the thiazole was replaced by 2,6-disubstituted pyridine (**5a**), the most potent compound at $h5HT_7$ from these series was found, with improved selectivity over $h5HT_{2A}$ and hD_2. The position of nitrogen is important, as 2,4-substituted pyridine (**3j**) does not show this high affinity binding.

Table 3 shows the effect of varying substituents at the 6-position of **5a**. A variety of lipophilic groups were tolerated giving high $h5HT_7$ binding. Larger groups seem to show an advantage in $h5HT_7$ binding over smaller or more polar substitution (**5d**). In the thiazole series, selectivity over the counter-screen receptors was increased by replacing phenyl with *tert*-butyl. However, in the pyridine series, aromatic and *tert*-butyl groups are very similar in profile. Pyridine **5a** still remains the highest affinity and most selective compound from the two series. This compound was therefore tested in a functional assay[16] and found to behave as a partial agonist, giving 80% of the response of the full agonist 5-CT in $h5HT_7$ receptors (Fig. 1).

Table 2.

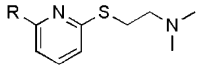


3

No.	Ar	$h5HT_7$ K_i/nM[a]	$h5HT_{1A}$ K_i/nM[b]	$h5HT_{2A}$ K_i/nM[c]	hD_2 K_i/nM[d]
3c		12	210[e]	640[e]	>1000[e]
3h		1200[e]			
3i		>4000			
3j		100	1200[e]		
5a		0.6	16	320[e]	450

[a,b,c,d,e] See Table 1 for details.

Table 3.



5

No.	R	$h5HT_7$ K_i/nM[a]	$h5HT_{1A}$ K_i/nM[b]	$h5HT_{2A}$ K_i/nM[c]	hD_2 K_i/nM[d]
5a		0.6	16	320[e]	450
5b	Br	4.1	37[e]	270[e]	870[e]
5c		0.7	8.7	230[e]	1300[e]
5d		7.2	41[e]	480[e]	>1000[e]
5e		1.3	17	100[e]	330

[a,b,c,d,e] See Table 1 for details.

In conclusion, two novel, related series of $h5HT_7$ ligands have been identified by elaboration of a screening lead, resulting in compound **5a** which shows high affinity and partial agonism for $h5HT_7$, and selectivity over counter-screen receptors. Affinity for $h5HT_7$, and selectivity over hD_2, $h5HT_{1A}$ and $h5HT_{2A}$ receptors can be manipulated in both series by modification of the pendent alkyl or aromatic groups.

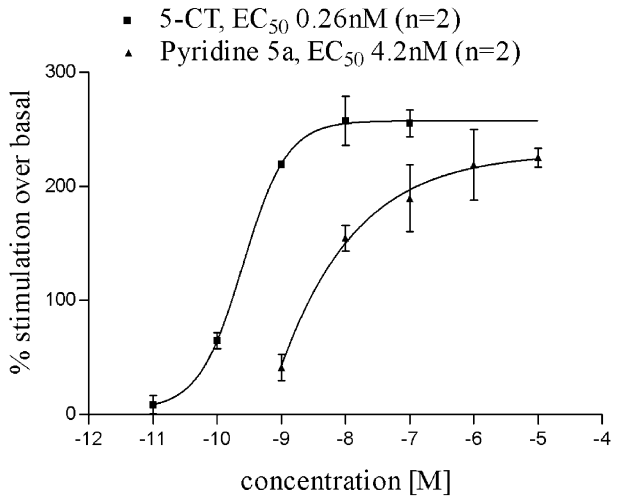


Figure 1. Stimulation of β-lactamase activity by 5-CT and compound **5a**, in reporter cells stably expressing $h5HT_7$ receptors.

Acknowledgements

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12. Binding assays were performed in 96-well microtitre plates in a total volume of 500 μL, containing 1 nM [^{3}H] 5-HT in assay buffer (50 mm Tris–HCl, pH 7.6 rt, containing 0.05% ascorbic acid and 10μM pargyline), with or without test drugs. The assay was initiated with the addition of $h5HT_7$-CHO membranes (15 mg wet weight/well) for 30 min at 24 °C, followed by termination by rapid filtration onto Unifilter GF/C filters (presoaked in water) using a Packard Filtermate 196 harvester and 2×1 mL washes with ice-cold 50 mM Tris–HCl (pH 7.6 at rt). Non-specific binding was determined using 10μM 5-HT.
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16. Functional responses were assessed with a β-lactamase reporter gene assay using Aurora 3, HEK293/CRE β-lactamase reporter cells stably transfected with the $h5HT_7$ receptor. The reporter system is based on cAMP response element (CRE) regulated transcription of β-lactamase enzyme following changes in cAMP production by receptor activation. β-Lactamase is detected with the use of a membrane permeable green fluorescent substrate, CCF4. Following cleavage of CCF4 by β-lactamase, an intracellular blue fluorescent product is detected by using a Cytofluor fluorescence spectrophotometer (excitation wavelength 409 nm, emission wavelengths of 460 nm for blue spectrum and 530 nm for green spectrum readings). Briefly, cells were plated out in Eagle's modified essential media (EMEM) + 10% dialysed foetal bovine serum (FBS) at a density of 35,000 cells/well/100 μL in 96-well microtitre plates. 24 h later, cells were washed in phosphate buffered saline (PBS). 100 μL of assay media (Dulbecco's modified essential media (DMEM) with 25 mM HEPES and 0.1% bovine serum albumin) containing (a) no test compound to measure basal β-lactamase activity, (b) 5-CT (0.01–100 nM) or (c) compound **5a** (1–10,000 nM) was added to the cells. Plates were incubated at 37 °C in a CO_2 incubator for 4 h. The resulting β-lactamase was detected with the addition of 20 μL CCF4 substrate. After 2 h, plates were read using a Cytofluor fluorescence spectrophotometer at the wavelengths specified above.