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Epiminocyclohepta[*b*]indole analogs as 5-HT$_6$ antagonists

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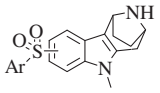
ABSTRACT

A new series of epiminocyclohepta[*b*]indoles with potent 5-HT$_6$ antagonist activity were discovered and optimized using in vitro protocols. One compound from this series was progressed to advanced pharmacokinetic (PK) studies followed by 5-HT$_6$ receptor occupancy studies. The compound was found to have excellent oral absorption, a highly favorable PK profile and demonstrated pharmacodynamic interaction with the 5-HT$_6$ receptor as shown by ex vivo autoradiography.

5-HT$_6$ is a serotonin receptor that is expressed in various regions of the brain, including the hypothalamus and pre-frontal cortex.[1] Due to the fact that these receptors are almost exclusively located within the CNS,[1] 5-HT$_6$ represents an attractive target as it is unlikely that modulation of the receptor will be associated with any of the peripheral side effects that often plague CNS targeted programs. Modulation of the 5-HT$_6$ receptor in pre-clinical animal models has been shown to induce promising efficacy in conditions associated with sleep,[2] anxiety and depression,[3] epilepsy[4] and pain.[5] However, most of the focus of 5-HT$_6$ research (particularly antagonists) has centered on weight loss and cognition. Modulation of the 5-HT$_6$ receptor has been shown to produce significant weight loss in rodent models of obesity,[6] defining the receptor as a promising new target for weight management. Furthermore, antagonism of the 5-HT$_6$ receptor has demonstrated beneficial effects in treating cognitive deficits associated with conditions such as Alzheimer's disease and schizophrenia.[7] A number of 5-HT$_6$ antagonists are currently undergoing clinical evaluation, with the most advanced compound SB-742457 in Phase II trials for treating cognitive deficits associated with Alzheimer's disease.[8]

Through a process of rational drug design, and the use of ligand–receptor pharmacophore models, we initiated a 5-HT$_6$ program to identify potent antagonists of the receptor which had an excellent in vitro profile coupled with favorable pharmacokinetic properties consistent with oral dosing. The aryl-substituted epiminocyclohepta[*b*]indole motif **1** was identified as a scaffold with the potential to interact with the 5-HT$_6$ receptor.



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In order to test this concept, three phenyl-substituted arylsulfones were synthesized with the arylsulfone located on various positions of the indole ring. All of the compounds were prepared as racemates. 5-HT$_6$ binding affinities were determined via a scintillation proximity assay using membranes expressing the human 5-HT$_6$ receptor.[9] A clear SAR trend developed, with the R^8 substituted phenylsulfone **4** having very good affinity for 5-HT$_6$, whilst the R^6 and R^7 substituted analogs (**2** and **3**, respectively) showed a much weaker interaction with the target. In order to fully characterize compound **4**, it was resolved into its constituent (−) and (+) enantiomers (**4a** and **4b**, respectively) via chiral chromatography. Both enantiomers had very similar 5-HT$_6$ binding affinities. Determination of functional activity through measurement of cAMP levels in a cell-based assay[10] showed that both enantiomers were 5-HT$_6$ antagonists, with the (+) enantiomer having slightly improved affinity for the receptor in this instance (Table 1).

Based on these data, SAR development around the 8-substituted aryl sulfones was undertaken. A general synthesis was developed

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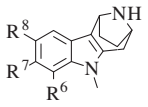
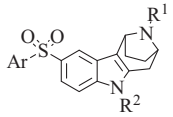
Table 1
SAR of phenylsulfonyl-substituted epiminocyclohepta[*b*]indoles



Compound	R^6	R^7	R^8	5-HT_6 K_i [a,b] (nM)	5-HT_6 K_b [b,c,d] (nM)
2	$-SO_2Ph$	H	H	814	
3	H	$-SO_2Ph$	H	131	
4	H	H	$-SO_2Ph$	8.2	
4a (−)	H	H	$-SO_2Ph$	15	11
4b (+)	H	H	$-SO_2Ph$	9.6	3.9

[a] Displacement of [^{3}H]LSD from human 5-HT_6 receptor membranes.[9]
[b] Values are means of at least two determinations where each determination is within ±50% of the mean value shown.
[c] Production of cAMP from CHO-1 cells expressing the human 5-HT_6 receptor.[10]
[d] All compounds were antagonists (<15% agonist activity).

Table 2
SAR of 8-arylsulfonyl epiminocyclohepta[*b*]indoles

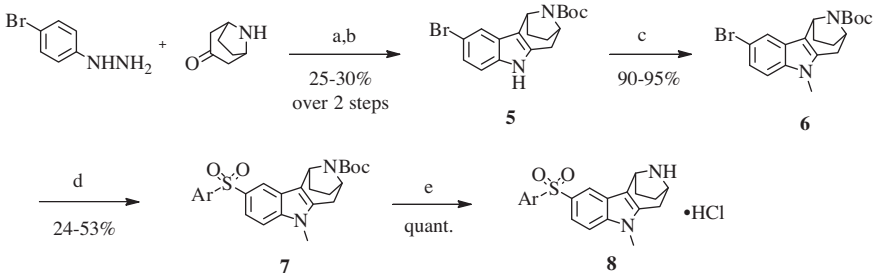


Compound	Ar	R^1	R^2	5-HT_6 K_i[a,b] (nM)
4	Phenyl	H	Me	8.2
9	Phenyl	Me	Me	7.8
10	Phenyl	Et	Me	91
11	Phenyl	H	H	54
12	Phenyl	H	Et	52
13	Phenyl	H	*i*-Pr	350
14	2-Fluorophenyl	H	Me	4.6
15	3-Fluorophenyl	H	Me	4.1
16	4-Fluorophenyl	H	Me	60
17	2-Chlorophenyl	H	Me	4.8
18	3-Chlorophenyl	H	Me	1.8
19	4-Chlorophenyl	H	Me	37
20	3-Trifluoromethylphenyl	H	Me	2.4
21	3-Trifluoromethoxyphenyl	H	Me	7.2
22	3-Aminophenyl	H	Me	6.3
23	3-Hydroxyphenyl	H	Me	11
24	3-Cyanophenyl	H	Me	35

[a] Displacement of [^{3}H]LSD from human 5-HT_6 receptor membranes.[9]
[b] Values are means of at least two determinations where each determination is within ±50% of the mean value shown.

for the rapid production of racemic 8-arylsulfone epiminocyclohepta[*b*]indoles (Scheme 1). Commercially available 4-bromophenylhydrazine and nortropinone were reacted together under Fisher-indole[11] conditions to generate the epiminocyclohepta[*b*]indole intermediate. This was isolated and Boc-protected without purification, providing the N-Boc intermediate **5**. The yield for this two-step transformation was low, due to a significant proportion of the hydrazone intermediate apparently failing to cyclize during the Fisher-Indole cyclization. Various attempts to optimize this step were unsuccessful. N-methylation of the indole was achieved under standard conditions to produce **6**, which was subjected to a palladium-catalyzed aryl sulfonylation[12] using arylsulfinic acid sodium salts (which could be prepared in a single step from the sulfonyl chloride[13]) generating **7**. The yields for this particular conversion were substrate-dependent, and although the reaction generally appeared to progress cleanly using standard monitoring techniques, high yields were never achieved. Removal of the Boc group under acidic conditions delivered the target racemates **8** as hydrochloride salts. These were further purified by chiral chromatography to give the constituent enantiomers if deemed necessary.

Initial SAR development around racemic 8-arylsulfonyl epiminocyclohepta[*b*]indoles is shown in Table 2. Early efforts were focused on substitution of the basic nitrogen (R^1) where it was found, through limited analoging, that methyl was tolerated whereas extending to ethyl had a detrimental effect on 5-HT_6 binding affinity. It was decided to halt exploration of basic nitrogen substituents at this stage, as it was apparent that no potency gains were likely, coupled with the fact that the *N*-alkyl analogs were predicted to suffer from poor metabolic stability. Next, our attention turned to the N-substituent of the indole (R^2). Once again, limited SAR exploration was undertaken here, as it was quickly determined that the methyl substituent was likely optimal based on the drop in potency for the unsubstituted (**11**), ethyl (**12**) and isopropyl (**13**) analogs. The main focus of our attention was on modifications of the pendant arylsulfone (Ar). Initial efforts centered on substituted phenyl rings. Monosubstitution with either fluoro or chloro in the 4-position led to a decrease in potency compared to the parent phenyl compound (**4** vs **16** and **19**, respectively). Substitution at the 2- and 3-position was well tolerated and a slight increase in potency was observed (**14**, **15**, **17** and **18**). In the case of the chloro-substituted analogs, there was a definite preference for *meta* substitution, with the 3-chlorophenyl analog **18** being approximately threefold more potent than the 2-substituted regioisomer **17**. Compound **18** was also approximately fivefold more potent than the parent compound **4** suggesting there was significant potential to improve the 5-HT_6 binding affinity through arylsulfone substitutions. Taking the slightly improved potency of **18** into account, as well as the added synthetic challenge associated with preparing 2-substituted analogs, the SAR focus was concentrated on 3-substituted arylsulfones. Introduction of lipophilic substituents such as trifluoromethyl (**20**, *c*log*P* = 4.0) and trifluoromethoxy (**21**, *c*log*P* = 4.1) were well tolerated, with **20** having similar potency to the 3-chlorophenyl analog **18**.



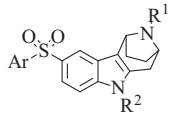
Scheme 1. Reagents and conditions: (a) EtOH, HCl, reflux; (b) Boc_2O, K_2CO_3, *i*-PrOH, H_2O, 0 °C to room temperature; (c) NaH, MeI, DMF, 0 °C to room temperature; (d) $ArSO_2Na$, $Pd_2(dba)_3$, xantphos, Cs_2CO_3, toluene, reflux; (e) HCl, Et_2O, MeOH, CH_2Cl_2, room temperature.

Somewhat surprisingly, polar substituents were also tolerated, with the 3-amino compound **22** ($c\log P = 2.1$) and 3-hydroxy analog **23** ($c\log P = 2.7$) having similar affinity to the parent phenyl **4**. This was encouraging, as it offered an opportunity to significantly alter the physicochemical properties of the scaffold whilst still retaining 5-HT_6 affinity. However, not all substituents were tolerated; the 3-cyano analog **24** ($c\log P = 2.6$) was approximately fourfold less potent for 5-HT_6 than the parent compound.

Attention was then switched to modifications of the phenyl ring itself by introducing heteroatoms into the structure (Table 3). All attempts at replacing the phenyl ring with an unsubstituted pyridine were unsuccessful, with significant drops in potency registered for all three regioisomers **25, 26** and **27**. Similarly, introduction of a thiophene (**28**), C-linked pyrazole (**29**) or N-linked pyrrole (**30**) resulted in reduced 5-HT_6 affinity, suggesting that heteroaromatic rings were not well tolerated as phenylsulfonyl replacements. However, this problem was somewhat circumvented by introduction of the N-linked indole **31** where a marked improvement in potency was achieved and sub-nM affinity was apparent for the first time. Interestingly, the indole ring was found to be a highly flexible substituent; switching the point of connectivity to the 3-position (**32**) or 5-position (**33**) was not detrimental to 5-HT_6 affinity highlighting that both C-linked and N-linked heteroaryl sulfones could be tolerated. Indeed, the 5-substituted indole **33** turned out to be extremely potent, having greater affinity for 5-HT_6 than all other compounds. Furthermore, replacement of the NH of the indole with a sulfur atom (benzthiophene **34**) imparted a significant increase in potency over the comparable indole **32** (approximately fourfold) suggesting the enhanced 5-HT_6 affinity was not due to any H-bonding interactions of the indole NH. This was confirmed by preparing the *N*-methyl indole **35**, which was extremely potent.

Selected compounds from Tables 2 and 3 were chirally resolved to deliver their constituent enantiomers, which were subjected to more intensive in vitro profiling (Table 4). It was found that the sets of enantiomers had similar profiles with the (−) and (+) enantiomers having 5-HT_6 binding affinities within 1–3-fold of each other. A similar trend was noticed upon determination of K_b's, where it was also shown that all of the analogs were 5-HT_6 antagonists. In vitro determination of potential metabolic liability was assessed through percentage of compound remaining after one hour upon incubation with both rat and human liver microsomes. The majority of compounds tested were found to have good metabolic stability (>50% remaining after 1 h in both species). Higher significance was placed on compounds that exhibited >75% of parent remaining after 1 h with trifluoromethylphenyl analog **20b**, both enantiomers of the 1-indolyl (**31a** and **31b**) and 5-indolyl (**33a** and **33b**) and the benzthiophenyl analog **34b** having a particularly good profile. Further profiling in the bidirectional Caco2 flux assay highlighted potential permeability and efflux issues within the NH indole substituted compounds, with both enan-

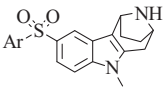
Table 3
SAR of 8-heteroarylsulfonyl epiminocyclohepta[*b*]indoles



Compound	Ar	R^1	R^2	5-HT_6 K_i[a,b] (nM)
25	2-Pyridyl	H	Me	192
26	3-Pyridyl	H	Me	156
27	4-Pyridyl	H	Me	>10000
28	3-Thiophenyl	H	Me	51
29	3-(1-Methyl)pyrazolyl	H	Me	1010
30	1-Pyrrolo	H	Me	32
31	1-Indolyl	H	Me	0.85
32	3-Indolyl	H	Me	1.3
33	5-Indolyl	H	Me	0.32
34	3-Benzthiophenyl	H	Me	0.33
35	3-(1-Methyl)indolyl	H	Me	0.81

[a] Displacement of [^{3}H]LSD from human 5-HT_6 receptor membranes.[9]
[b] Values are means of at least two determinations where each determination is within ±50% of the mean value shown.

Table 4
SAR of chirally pure 8-arylsulfonyl epiminocyclohepta[*b*]indoles



Compound	Ar	Optical rotation[a]	5-HT_6 K_i (nM)[b,c]	5-HT_6 K_b (nM)[c,d,e]	RLM% remaining at 1 h[f]	HLM% Remaining at 1 h[g]	$Caco_2$ Permeability A-B[h]	Efflux ratio[i]
18a	3-Chlorophenyl	(−)	2.7	3.5	63	76	7.49	6
18b	3-Chlorophenyl	(+)	1.9	4.0	72	93	8.55	4.4
20a	3-Trifluoromethylphenyl	(−)	3.7	15	65	78	16.5	2.8
20b	3-Trifluoromethylphenyl	(+)	1.5	12	79	100	24.8	1.8
31a	1-Indolyl	(−)	1.2	10	88	82	15	2.2
31b	1-Indolyl	(+)	0.68	9.7	86	96	12	1.9
32a	3-Indolyl	(−)	1.2	2.8	34	72	0.41	2.5
32b	3-Indolyl	(+)	0.74	2.6	57	86	1.05	8.5
33a	5-Indolyl	(−)	0.37	1.6	77	89	0.55	40
33b	5-Indolyl	(+)	0.28	1.5	91	99	ND	ND
34a	3-Benzthiophenyl	(−)	0.36	3.4	67	52	0.24	3.7
34b	3-Benzthiophenyl	(+)	0.28	2.5	80	78	15.7	2.8

ND, not determined.
[a] Optical rotation determined via rotation of polarized light using a polarimeter.
[b] Displacement of [^{3}H]LSD from human 5-HT_6 receptor membranes.[9]
[c] Values are means of at least two determinations where each determination is within ±50% of the mean value shown.
[d] Production of cAMP from CHO-1 cells expressing the human 5-HT_6 receptor.[10]
[e] All compounds were antagonists (<15% agonist activity).
[f] Rat liver microsomes.
[g] Human liver microsomes.
[h] Apical to basolateral flux ($\times 10^{-6}$ cm/s)
[i] Ratio of basolateral to apical flux/apical to basolateral flux.

Table 5
Oral exposure data in Sprague Dawley rats.

Compound	Dose (mg/kg)	C_{max} (0–6 h) (ng/mL)	T_{max} (0–6 h)
20b	30 (po)	732 ± 176	6
31b	30 (po)	41 ± 3.7	6

tiomers of the 3-indolyl (**32a** and **32b**) and the (−) enantiomer of the 5-indolyl (**33a**) substituents having poor permeability coupled with significant efflux. The (−) enantiomer of the 5-indolyl substituent **33b** was not tested in this assay, but was anticipated to have a similar profile to **33a**. Trifluoromethylphenyl analog **20b** and 1-indolyl analog **31b** were classified as being highly permeable with non-significant efflux. These two compounds were selected for in vivo assessment based on their attractive profile.

Compounds **20b** and **31b** were orally dosed to Sprague Dawley rats at 30 mg/kg, and plasma samples were taken over the following 6 h to obtain a preliminary indication of oral exposure (Table 5).

Compound **20b** was found to have an excellent PK profile; plasma levels increased steadily up to the terminal point (6 h). **31b** had significantly reduced oral exposure compared to **20b**, although, once again, the C_{max} over the 6 h timecourse occurred upon termination. Based on these findings, **20b** was selected for detailed pharmacokinetic and pharmacodynamic assessment.

Upon oral and iv dosing to Sprague Dawley rats, **20b** had a robust PK profile. At a 10 mg/kg oral dose, **20b** had a long half life and excellent bioavailability. This was driven in part by its low clearance and moderate volume of distribution. The compound also exhibited a favorable brain to plasma ratio which was important as it indicated preferential partitioning of the drug in the CNS over the periphery (Table 6).

Compound **20b** was then subjected to pharmacodynamic assessment through determination of 5-HT_6 receptor occupancy. Occupancy was measured using ex vivo methodology, with autoradiography techniques employed to determine the level of occupancy[14] at three different doses (Fig. 1). Two timepoints were chosen (6 and 24 h) to obtain an indication of the duration of action of compound **20b**. It was found that **20b** caused a dose-related increase in 5-HT_6 receptor occupancy after 6 h, although there was a plateau apparent between the 30 and 90 mg/kg doses, where the occupancy was found to be in the 75–80% range. Interestingly, 5-HT_6 receptor occupancy was sustained over 24 h at all doses, with very little change between the 6 and 24 h timepoints. This was fully anticipated, as the previously determined half life of the compound suggested it should have a long duration of action.

In summary, SAR development around the parent 8-arylsulfonyl epiminocyclohepta[*b*]indole identified a series of potent 5-HT_6 antagonists. In vitro assays were used to triage compounds for progression into in vivo studies. Through this process, compound **20b** was identified as a 5-HT_6 antagonist with excellent oral exposure, a favorable PK profile and high levels of sustained 5-HT_6 receptor occupancy upon acute dosing. This profile suggests **20b** would be suitable for once-daily dosing.

Acknowledgments

We would like to thank Sharon Cheetham and Jean Viggers of RenaSci Consultancy for generating receptor occupancy data. We would also like to thank AMRI's DMPK group for provision of bioanalysis data.

Table 6
Detailed PK data for **20b**

Compound	C_{max}[a] (ng/mL)	T_{max}[a] (h)	$t_{1/2}$[a] (h)	$AUC_{0\text{-inf}}$[a] (h ng/mL)	CL[b] (mL/min/kg)	V_{ss}[b] (L/kg)	F[a,b]	Brain/plasma ratio[a] (6 h)
20b	916	4	10	26687	5.7	4.7	91%	1.3

[a] Obtained from 10 mg/kg po dose.
[b] Obtained from 2 mg/kg iv dose.

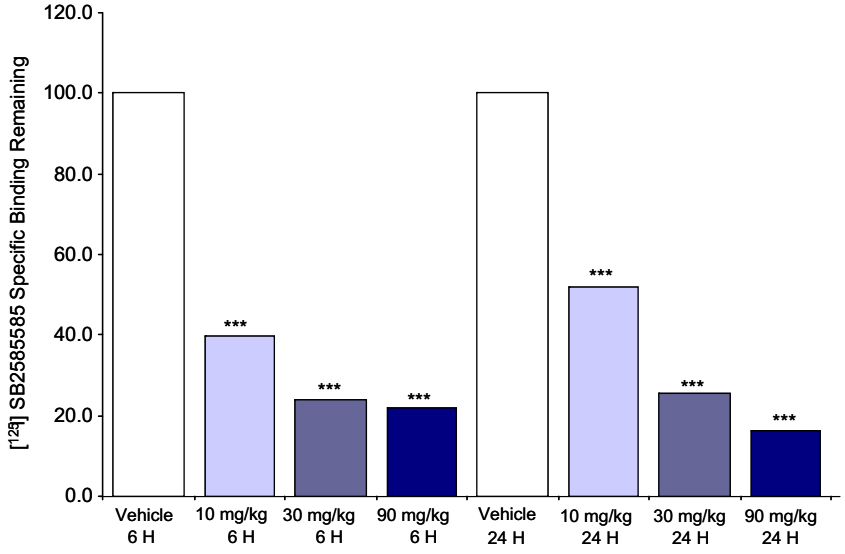


Fig. 1. Ex vivo 5-HT_6 receptor occupancy data determined in Sprague Dawley rats.[14] All values had $p < 0.0001$. Receptor occupancy values compared to vehicle (0%) at 6 h were 60%, 76% and 78% at the 10, 30 and 90 mg/kg doses respectively. Receptor occupancy values compared to vehicle (0%) at 24 h were 48%, 74% and 84% at the 10, 30 and 90 mg/kg doses respectively.

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9. Human 5-HT6 binding affinities were measured using a scintillation proximity assay (SPA) format. Samples were incubated in 50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 1 mM EDTA (4% DMSO final) with 10 nM [*N*-methyl-^{3}H]-LSD (Perkin–Elmer), 2.5 μg of human 5-HT_6 receptor membranes (Millipore) and 50 μg SPA beads (PVT-PEI-WGA, GE Healthcare) per well in a final volume of 0.2 mL. Total binding control contained compound dilution buffer only; nonspecific binding was determined in the presence of 100 μM 5-hydroxytryptamine. Specific binding was determined by subtracting nonspecific binding from total binding. All experiments were performed in duplicate using ten concentrations of competing ligand. IC_{50} values were determined from specific binding data using XLfit4.1 curve fitting software from IDBS Ltd. The inhibition constant (K_i) was calculated using the Cheng–Prusoff equation: ($K_i = IC_{50}/(1+(L/K_D))$, where L = concentration of radioligand in the assay, and K_D = affinity of the radioligand for the receptor.
10. Human 5-HT_6 functional activities were determined by measuring the production of cAMP from CHO-1 cells expressing the human 5-HT_6 receptor. Antagonist activity was determined via a 5-hydroxytryptamine challenge at the EC_{80} (30 nM 5-HT) and expressed as the ratio of cAMP produced in a test well to the average cAMP produced in the EC_{80} challenge control wells. Agonist activity was determined using 10 μM 5-HT as the E_{max} control and was expressed as the ratio of cAMP produced in a test well to the average cAMP produced in the E_{max} control wells. All cell-based experiments were performed in duplicate using 10 concentrations of test compound. IC_{50}/EC_{50} values were determined using XLfit4.1 curve fitting software from IDBS Ltd. For antagonists, the apparent dissociation constant (K_b) was calculated using a modified Cheng–Prusoff equation: $K_b = IC_{50}/(1 + (A/EC_{50A}))$, where A = concentration of reference agonist in the assay and $EC_{50A} = EC_{50}$ value of the reference agonist. An antagonist was defined as having an E_{max} <15% in the agonist mode.
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14. 5-HT_6 receptor occupancy was determined in the caudate putamen. Brain sections were incubated using [^{125}I]SB258585 (total binding) or [^{125}I]SB258585 and methiothepin (non-specific binding). Radioactivity was measured in a β-imager. Two determinations were made per animal, and 5 animals were used per dose group. Data was presented as mean specific binding and as a percentage of the vehicle-treated control which was taken as 100%.