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Discovery of a potent and selective 5-ht$_{5A}$ receptor antagonist by high-throughput chemistry

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Abstract—High-throughput screening of an array of biphenylmethylamines synthesised by high-throughput solid-phase chemistry resulted in the identification of compounds with high-affinity for the 5-ht$_{5A}$ receptor. The structure–activity relationship within this series and further array synthesis led to the identification of the biphenylmethylamine derivative **11**, a potent and selective 5-ht$_{5A}$ receptor antagonist.
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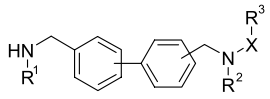
5-ht$_5$ Receptors are subdivided into 5-ht$_{5A}$ and 5-ht$_{5B}$ receptors representing distinct gene products. To date, the 5-ht$_{5A}$ receptor has been cloned from a number of species including human[1] and guinea pig.[2] In contrast, the 5-ht$_{5B}$ receptor is a pseudogene (not functionally expressed) in man.[3] mRNA and receptor immunolocalisation studies have shown the 5-ht$_{5A}$ receptor to be preferentially expressed in brain, including cortical and limbic areas[3,4] suggesting a potential role for the receptor in higher brain function. Although the human 5-ht$_{5A}$ receptor was cloned in 1994, the role of the 5-ht$_{5A}$ receptor in brain function is still very poorly understood due, at least in part, to the lack of selective ligands. This has provided the impetus to perform a high-throughput screen to identify novel ligands for the receptor that might serve as pharmacological tools for brain functional studies.



3,2-, 3,3-, 3,4-, 4,2-, 4,3-, 4,4-isomers

1; X =CO and CONH

High-throughput screening of the in-house compound collection using [^{3}H]LSD radioligand binding[2] resulted in the identification of a series of active biphenylmethylamine compounds. These screening hits originated from an array of compounds **1**, synthesised by high-throughput chemistry. As the biphenyl moiety is known to be a privileged motif for binding to 7-transmembrane (7-TM) receptors,[5] the array **1** was designed specifically to be cross-screened against a range of 7-TM receptor targets. It was synthesised by solid-phase chemistry according to Scheme 1 employing a carbamate linker and a Suzuki reaction–reductive amination–acylation sequence. The synthesis platform utilised IRORI™ microkans in a

Keywords: 5-ht$_{5A}$ Receptor; 5-ht$_{5A}$ Antagonist; High-throughput chemistry; Solid-phase synthesis.
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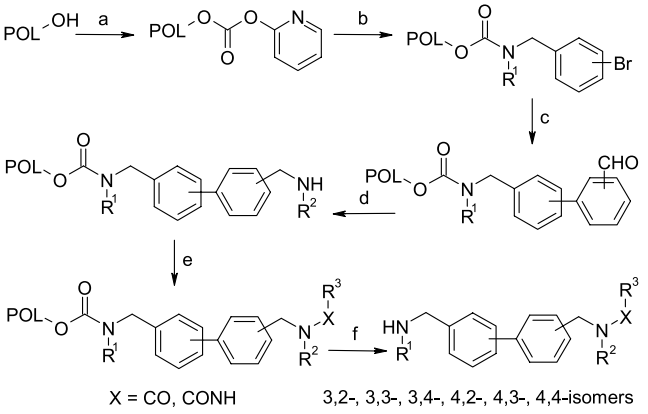


Scheme 1. Reagents and conditions: (a) Wang resin (1.7 mmol/g, 150–300 μm), di-2-pyridylcarbonate, Et_3N, CH_2Cl_2; (b) *N*-R^1-3- or 4-bromobenzylamine, CH_2Cl_2, rt, or 1,2-dichloroethane, 70 °C; (c) 2-, 3-, or 4-formylbenzeneboronic acid, $Pd(Ph_3P)_4$, Na_2CO_3, 1,2-dimethoxyethane–water (9:1), 80 °C under argon; (d) R^2NH_2, Na_2SO_4, AcOH, $NaBH(OAc)_3$, 1,2-dichloroethane; (e) R^3COCl, Et_3N, CH_2Cl_2, or R^3NCO, CH_2Cl_2; (f) 20% CF_3CO_2H, 80% CH_2Cl_2.

split-mix directed sorting combinatorial process[6] to afford the products as single compounds. After cleavage from the resin, the individual products were obtained as trifluoroacetate salts in quantities of up to 35 μmol. The robust nature of the solid-phase chemistry is demonstrated by the isolation of 6625 compounds in >80% LCMS purity from the planned 6912 component array. Only 45% of these products required purification, which was achieved using parallel preparative LC techniques.[7]

A significant advantage of the high-throughput chemistry approach is that analogues of screening hits are immediately available and SAR can be established quickly. Although the array comprised six different biphenyl isomer combinations, designed to allow the substituent groups to be presented in different orientations, the compounds with affinity for the 5-ht_{5A} receptor consisted almost exclusively of the 4,4-isomers. Additionally, it was clear that a basic group was required in the R^2 substituent and amides were generally much more active than ureas (data not shown). The most potent compound from the original array was derivative **2** (Table 1) with pK_i 7.6.

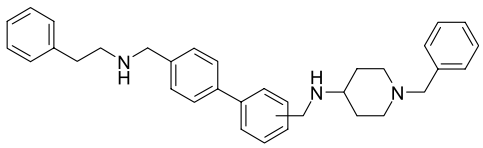
Affinity was confirmed in the [^{3}H]LSD radioligand-binding assay by re-preparation of the most potent compounds, and a new focused array (969 compounds) was synthesised based on the initial SAR, using the solid-phase chemistry in Scheme 1 in both cases. The two arrays were instrumental in probing further SAR and selectivity data for the 5-ht_{5A} receptor.

A selection of illustrative SAR data is summarised in Table 1. Compounds **2**–**4** exemplify the requirement for a basic group in the R^2 substituent for good affinity. Replacement of the *N*,*N*-dimethylaminoethyl group in **2** with the less basic 3-pyridylmethyl substituent in **3** resulted in comparable affinity, whereas compound **4** with a non-basic substituent was more than 10-fold less active. From a range of R^1 variables, the phenethyl substituent gave by far the most potent derivatives, as illustrated by the lower affinity of compounds **5** and **6**. Varying the lipophilicity of the R^3 substituent also had a dramatic effect on affinity. Replacement of cinnamoyl in R^3 by benzoyl while retaining optimum R^1 and R^2 substituents, as in compound **7**, resulted in reduced affinity. The phenyl urea **8** had comparable affinity to the benzamide **7**, although most ureas were much less active than amides. The piperonoyl derivative **9** retained moderate affinity while the *trans*-3-phenylcyclopropanoyl compound **10** was only slightly less active than the cinnamoyl analogue **2**. However, the 3-cyclopentylpropionyl analogue **11a** gave enhanced affinity at the 5-ht_{5A} receptor with a pK_i of 8.2.

Keeping the favourable R^3 3-cyclopentylpropionyl substituent constant, the effects of varying R^1 and R^2 were probed further. Analogues **12** and **13**, where R^1 is H and indanyl, respectively, were significantly less active, but the 2-(2-pyridyl)ethyl (**14**) and 2-phenoxyethyl (**15**) derivatives retained good affinity. Maintaining R^1 as phenethyl and R^3 as 3-cyclopentylpropionyl, a range of R^2 substituents containing basic groups afforded compounds which retained good affinity, e.g., derivative **16**, but none of these matched that of **11a**. The sulfonamide **17**, analogous to amide **2**, had reduced affinity with a pK_i of 6.6.

Receptor-binding affinities for a selection of the most potent compounds against a range of relevant receptors (5-HT, dopamine, and α-adrenoceptors) are shown in Table 2. The compounds examined possess generally good 5-ht_{5A} selectivity when compared with binding affinities at other 5-HT and dopamine receptor subtypes, showing a range between 10- and 1000-fold. The selectivity profile improves with increasing 5-ht_{5A} binding affinity as demonstrated by compound **11a**, which is $\gtrsim$100-fold selective against the majority of the other 5-HT receptor subtypes. Similarly, amongst the analogues tested, **11a** shows the highest selectivity against the adrenergic α_{1B} receptor (ca. 50-fold). It does, however, possess significant affinity for the human serotonin transporter.

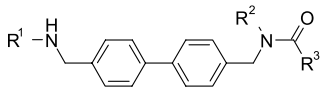
The requirement for lipophilic groups in both R^1 and R^3 limits the opportunity to increase the hydrophilicity of molecules in the active series. However, some compounds lacking the R^3 amide were found to possess moderate affinity, e.g., the *N*-benzylpiperidinyl derivative **18**. Interestingly, in this series the 4,3-isomer **19** maintained comparable affinity. However, amines of the types **18** and **19** lacked selectivity against other receptors in Table 2 (data not shown).

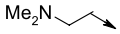
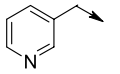
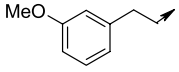
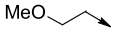
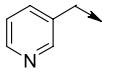
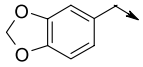
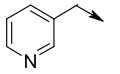
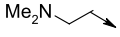
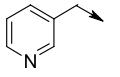
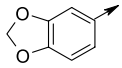
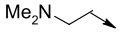
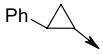
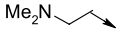
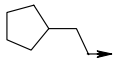
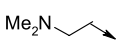
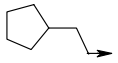
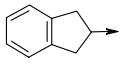
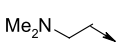
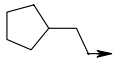
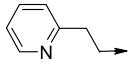
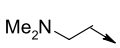
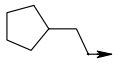
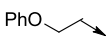
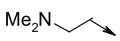
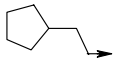


18; 4-isomer; pK_i 6.9
19; 3-isomer; pK_i 6.7

In another approach to reduce lipophilicity, analogues **20**–**23**, in which each phenyl ring in turn is replaced by a pyridyl moiety, were synthesised using variations of

Table 1. Affinities in 5-ht$_{5A}$ radioligand-binding assay



Compound[a]	R^1	R^2	R^3	pK_i
2				7.6
3				7.3
4				6.3
5				5.2
6				6.0
7			Ph	6.2
8			NHPh	6.4
9				6.7
10				7.1
11a				8.2
12	H			5.5
13				6.6
14				7.5
15				7.1
16				7.6

17; pK_i 6.6

[a] All compounds are trifluoroacetate salts.

the chemistry in Schemes 1 and 2. Here, SAR differed (Table 3) where the cinnamoyl compounds **20** and **22** were slightly more potent than their 3-cyclopentylpropionyl counterparts. Although excellent affinity was retained, especially in compound **20**, selectivity was noticeably poorer than in the biphenyl series, particularly against the α_{1B} receptor (**20**, pK_i 7.8).

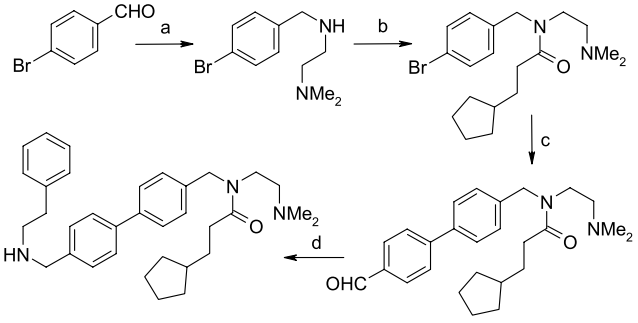
In summary, the compound identified from this series with the greatest affinity and selectivity for the 5-ht$_{5A}$ receptor is derivative **11a** (di-trifluoroacetate salt after array synthesis). It was re-synthesised using solution-phase chemistry by the route shown in Scheme 2, and converted to its dihydrochloride salt **11b.**

Table 2. Receptor-binding profiles of selected compounds

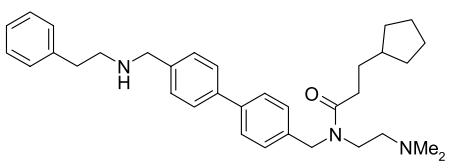
Receptor[a]	Affinity (pK_i)		
	2	**3**	**11a**
5-HT_{1A}	6.1	6	6.3
5-HT_{1B}	6.2	6.2	6.5
5-HT_{1D}	6.8	5.9	6.4
5-HT_{1E}	<5.0	<5.0	<5.0
5-HT_{1F}	<5.0	<6.0	<5.0
5-HT_{2A}	6.4	6.1	6.1
5-HT_{2B}	5.9	5.7	6.0
5-HT_{2C}	6.2	6.0	6.4
5-ht_{5A}	**7.6 ± 0.07**	**7.3 ± 0.14**	**8.2 ± 0.05**
5-HT_6	<5.1	<5.7	5.4
5-HT_7	6.0	5.9	5.4
Dopaminergic D_2	6.1	5.9	5.9
Dopaminergic D_3	6.0	5.8	6.2
Dopaminergic D_4	5.9	5.8	6.1
α_{1B}	6.7	6.5	6.5
SerT	—	—	7.6[b]

[a] Receptors and radioligands used in the binding assays: 5-HT_{1A} (human cloned receptors [HCR] in HEK 293 cells, [^{3}H]-8-OH-DPAT); 5-HT_{1B} and 5-HT_{1D} (HCR in CHO cells, [^{3}H]-5-HT); 5-HT_{1E}, 5-HT_{1F}, and 5-HT_{2B} (HCR in HEK 293 cells, [^{3}H]-5-HT); 5-HT_{2A} (HCR in HEK 293 cells, [^{3}H]ketanserin); 5-HT_{2C} (HCR in HEK 293 cells, [^{3}H]mesulergine); 5-ht_{5A} (HCR in CHO cells, [^{3}H]LSD); 5-HT_6 (HCR in HeLa cells, [^{3}H]LSD); 5-HT_7 (HCR in HEK 293 cells, [^{3}H]-5-CT); D_2, D_3, and D_4 (HCR in CHO cells, [^{125}I]iodosulpride); α_{1B} (HCR in CHO cells, [^{3}H]prazosin); serotonin transporter (SerT) in LLCPK cells, [^{3}H]citalopram.

[b] SerT data is for salt **11b**. Data represent the mean of at least three separate experiments except where mean pK_i values are quoted as <6.0 (n = 2).



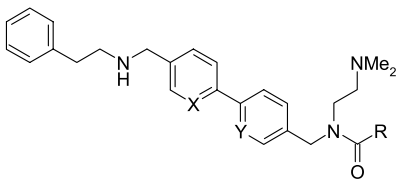
Scheme 2. (a) *N*1,*N*1-dimethylethylene-1,2-diamine, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, 44%; (b) 3-cyclopentylpropionyl chloride, Et_3N, CH_2Cl_2, quant.; (c) 4-formylbenzeneboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, H_2O/DME 1:9, 76%; (d) phenylethylamine, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, 57%.

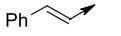
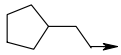
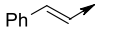
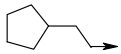


11a, di-CF_3CO_2H salt
11b, di-HCl salt

Compound **11b** was further evaluated in a human 5-ht_{5A} receptor functional assay utilising 5-carboxamidotryptamine (5-CT)-stimulated [^{35}S]GTPγS binding to cell membranes prepared from human embryonic kidney cells transiently expressing the human receptor. At a concentration of 1 μM it produced a parallel rightward-shift of the 5-CT concentration–response curve (Fig. 1) giving an apparent pK_B (mean ± SEM, n = 3) of 8.0 ± 0.04, comparable to the pK_i determined from [^{3}H]LSD binding (Table 2).

Table 3. 5-ht_{5A} Affinities of pyridyl analogues



Compound	X	Y	R	pK_i (5-ht_{5A})
20	N	CH		8.3
21	N	CH		7.9
22	CH	N		7.6
23	CH	N		7.5

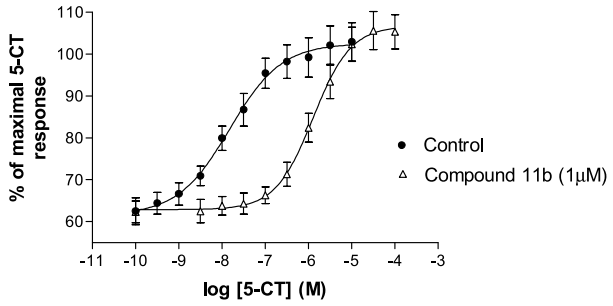


Figure 1. 5-CT-induced stimulation of [^{35}S]GTPγS binding to human 5-ht_{5A}/HEK 293 cell membranes in the absence and presence of compound **11b** (1 μM). Data points represent means ± SEM of at least three separate experiments. Results are expressed as percentage of the maximal 5-CT response.

The biphenyl derivative **11b** therefore shows a profile consistent with competitive antagonism at the human 5-ht_{5A} receptor.

A summary of the guinea pig pharmacokinetic profile for compound **11b** is shown in Table 4.[8] After intravenous dosing, compound **11b** has a moderate blood clearance, a large volume of distribution and a long half-life. Oral bioavailability is low at 4% but bioavailability is improved via the subcutaneous route (63%). CNS penetration was achieved via each dose route investigated (data not shown). Further in vivo pharmacological studies into the functional role of brain 5-ht_{5A} receptors using the potent and selective antagonist **11b** as a tool compound are ongoing and will be reported in a future publication.

Table 4. Guinea pig pharmacokinetic parameters for compound **11b**[8]

Parameter	Route of administration		
	iv	po	sc
Dose (mg/kg)	1	3	3
C_{max} (ng/mL blood)	—	20	388
$t_{1/2}$ (h)	3.5	3.9	2.2
CL_b (mL/min/kg)	22	—	—
Vd_{ss} (L/kg)	6.1	—	—
F (%)	—	4	63

References and notes

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8. The pharmacokinetics and brain penetration of 11b were determined in the male Dunkin Hartley guinea pig. Intravenous bolus dose administered at a target dose of 1 mg free base/kg; oral and subcutaneous doses administered at a target dose of 3 mg free base/kg. Blood and brain homogenate samples were extracted by protein precipitation and the resulting extracts analysed by LC-MSMS.