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Identification and optimisation of 5-amino-7-aryldihydro-1,4-diazepines as 5-HT$_{2A}$ ligands

Christopher J. Swain,[a,*] Ana Teran,[b] Marta Maroto[b] and Angeles Cabello[b]

[a]*The Neuroscience Research Centre, Merck Sharp and Dohme, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR, UK*
[b]*CIBE, Merck Sharp and Dohme, Josefa Valcarel 38, 28027 Madrid, Spain*

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Abstract—A several series of low molecular weight 5-HT$_{2A}$ leads were identified from an analysis of HTS data, the exploration of SAR and optimization of one series using parallel synthesis are described, affording compound **22** (5-HT$_{2A}$ IC$_{50}$ 1.1 nM).

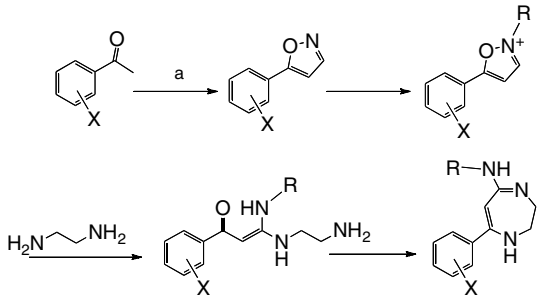
Serotonin (5-hydroxytryptamine, 5-HT) receptor ligands have proved beneficial in a wide variety of clinical indications,[1] and in particular 5-HT$_{2A}$ antagonists have potential utility in depression,[2] schizophrenia[3] and sleep disorders.[4] 5-HT was one of the first neurotransmitters associated with the regulation of the sleep-wake cycle.[5] Furthermore, in clinical trials, several agents that antagonize 5-HT$_{2A}$ receptor activity have been shown to be effective in the treatment of alcohol dependence,[6] panic disorders,[7] and in controlling agitation and delusions in most patients with Parkinson's disease and psychotic symptoms.[8,9]

As part of our ongoing efforts to identify new 5-HT$_{2A}$ antagonists[10] we undertook a high-throughput screening (HTS) campaign using a functional assay (FLIPR) to identify novel structural classes of antagonists as potential leads. This assay was also used to confirm the lack of efficacy for selected novel ligands. Whilst a conventional analysis of the screening results identified several interesting starting points we were also interested in identifying low molecular weight starting points since a number of studies have highlighted the correlation between molecular weight and the likelihood of success in reaching the marketplace.[11]

Our strategy was to try to identify low molecular weight scaffolds, which could then be optimized by appropriate substitution. However, we were also mindful that low molecular weight ligands might well have only modest to low affinity.

The strategy adopted involved selecting all compounds from the screening collection with molecular weight in the range 150–250, and then clustering the compounds using maximum common substructure.[12] The high-throughput screening data were then added and the 150 clusters were then selected that contained at least one example with >50% inhibition at 5 μM in the HTS. SMARTS queries[13] were then constructed to represent each of the active clusters and the individual SMARTS queries used to search a file of known 5-HT$_{2A}$ ligands constructed from both internal and literature sources. The SMARTS queries were also used to search against a file of known NK$_1$ receptor antagonists using iBabel.[14]

Many of the SMARTS queries identified structures in the file of known 5-HT$_{2A}$ ligands, whilst others were



Scheme 1. Reagents: (a) HCOOEt, NaH, NH_2OH; (b) ROH, $HClO_4$.

Keywords: 5HT2A; Antagonist; Library; High-throughput; Screening; Synthesis.
* Corresponding author at present address: Cambridge MedChem Consulting. Tel.: +44 1223 835121; e-mail: swain@mac.com

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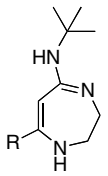
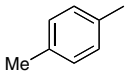
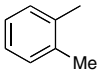
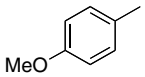
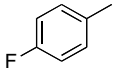
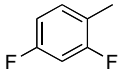
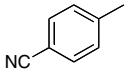
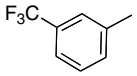
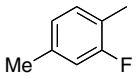
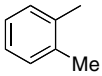
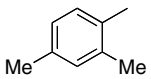
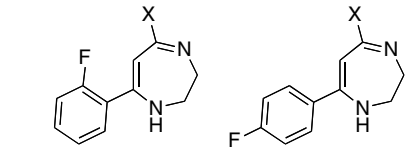
Table 1. 5-HT_{2A} binding affinity of 5-amino-7-aryldihydro-1,4-diazepine analogues **1**–**13**



Compound	R	h5-HT_{2A} IC_{50}[a] (nM)
1		35 (5-HT_{2C} 1050 nM)
2		11
3		112
4		534
5		18
6		21 (5-HT_{2C} 637 nM)
7		11
8		76
9		2800
10		514
11		14
12		112
13		71

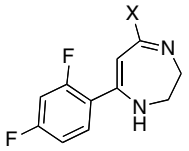
[a] 5-HT_{2A} affinity was determined as described in Ref. 15.

Table 2. 5-HT_{2A} binding affinity of 5-amino-7-aryldihydro-1,4-diazepine analogues **5**–**29**

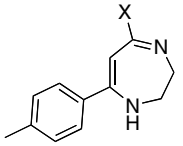


A

B



C



D

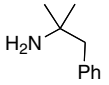
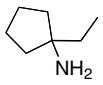
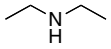
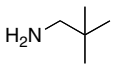
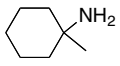
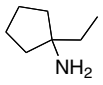
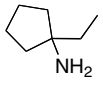
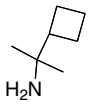
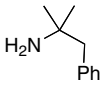
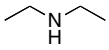
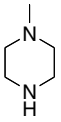
Compound	R	X	h5-HT_{2A} IC_{50}[a] (nM)
5	A		18
14	A		4.5
15	A		3.8
16	A		3.6
17	A		>1000
18	A		1515
19	B		1.6
20	B		1.5 (5-HT_{2C} 16 nM)
21	B		0.8
22	C		1.1 (5-HT_{2C} 27 nM)
23	C		1.4

Table 2 (*continued*)

Compound	R	X	h5-HT_{2A} IC_{50}[a] (nM)
24	C		3.7 (5-HT_{2C} 86 nM)
25	D		2.4
26	D		14.9 (5-HT_{2C} 75 nM)
27	D		>1000
28	D		>1000
29	D		>1000

[a] 5-HT_{2A} affinity was determined as described in Ref. 15.

found in both 5-HT_{2A} and NK_1 ligand files and possibly represent promiscuous motifs. For the purpose of this exercise the most interesting SMARTS queries are those representing the 17 clusters containing actives in the HTS that are not present in either known 5-HT_{2A} or NK_1 ligands. Based on this analysis seventeen compounds (50–70% inhibition at 5 μM in the HTS) were selected for titration[15] (one per cluster) and ten of the seventeen were subsequently shown to have a measured $IC_{50} < 200$ nM. The exploration and optimization of one of these leads (**1**) is described herein. The synthetic route used to prepare these analogues is shown in Scheme 1.[17] The aryl isoxazolines were prepared from the corresponding substituted acetophenones by reaction with ethyl formate and hydroxylamine in the presence of sodium hydride.[16] Subsequent reaction with an alkyl alcohol in the presence of perchloric acid, followed by reaction with diaminoethane and subsequent cyclisation, afforded the 1,4-diazepine ring. The nitrile of compound **9** was introduced via cuprous cyanide displacement of the corresponding bromide.

The chemistry proved eminently suitable for parallel synthesis and a scanning library was prepared using 13 amines and 20 substituted acetophenones. In this communication, we describe initial results from a limited subset of this library.

Initial SAR was developed to aid understanding of the influence of aromatic ring substitution (Table 1).

Small lipophilic substituents at the *para*-positions of the phenyl ring were beneficial (**2**, IC_{50} 11 nM, **6** IC_{50} 21 nM) affording a modest 2- to 3-fold increase in affinity, however polar substituents were not tolerated (**9**. IC_{50} 2800 nM). *ortho*-Substitution was more limited with only fluoro being tolerated (**5**, IC_{50} 18 nM). Combination of *ortho*- and *para*-substitution afforded a further modest increase in affinity (**7**, IC_{50} 11 nM). Introduction of a trifluoromethyl at the meta position (**10**) was not well tolerated.

Using the 4-methyl (**2**) and three fluoro-substituted analogues (**5**, **6** and **7**) as templates the influence of the alkyl amine was investigated (Table 2). Replacement of the *tert*-butyl amine by a secondary amines resulted in a dramatic loss in affinity (e.g., **17**, **27**, **28** and **29**, IC_{50} >1000 nM), perhaps indicating the requirement for a hydrogen bond donor. In contrast, reaction with a variety of larger primary amines gave a significant increase in affinity. The introduction of an additional two carbons gave a 5- to 10-fold increase in affinity; compare **5** with **14** (IC_{50} 4.5 nM), or **7** with **20** (IC_{50} 1.5 nM). Replacement of one of the methyl groups of the *t*-butyl by benzyl also gave a similar increase in affinity (**15**, IC_{50} 3.8 nM, **25**, IC_{50} 2.4 nM) but was less attractive due to the increase in molecular weight. Interestingly, introduction of the phenyl ring also gave the only examples of compounds with significant HERG activity (**15**, 270 nM, **25**, 300 nM) all other compounds being >1000 nM.

Constraining the alkyl groups into either a 5- or a 6-membered ring gave a further small increase in affinity and afforded compounds with single figure nanomolar affinity (**16**, **19**, **21** and **22**). Compound **22** IC_{50} 1.1 nM, M_W 319 was subsequently evaluated in variety of ion channels, GPCR and enzyme assays and shown to be >1000-fold selective except for modest activity at the 5-HT_{2C} receptor (IC_{50} 27 nM). Importantly, with respect to possible in vivo evaluation compound **22** also displayed excellent affinity for the rat 5-HT_{2A} receptor (IC_{50} 2.3 nM).

In conclusion, we have described the discovery and optimization of a novel class of 5-HT_{2A} antagonists developed from a low molecular weight hit (**1**) identified from high-throughput screening, by an analysis intended to identify selective low-molecular weight ligands.

Acknowledgment

We thank Ellen Crawford of the outsourcing group for arranging the synthetic work to be completed by WuXi PharmaTech Co., Ltd.

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15. Human 5-HT_{2A} receptor binding assay. Membranes (1 mg) obtained from confluent cultures of CHO K1 cells tansfected with the human 5-HT_{2A} receptor (licenced from Euroscreen, Belgium) were incubated with 1 mM [^{3}H]ketanserin (76 Ci/mmol, Perkin-Elmer, Cat. No. NET-791) in buffer containing 50 mM Tris[hydroxylmethylaminomethane], pH 7.7, 0.1% ascorbate, 10 mM pargyline, 10 mM $MgSO_4$ and 0.5 mM EDTA, in a final volume of 1 mL, for 15 min at 37 °C and at 180 rpm. The reaction was terminated by filtration onto GF/B filter plates. The filters were washed with 50 mM Tris[hydroxylmethylaminomethane], pH 7.7, using a Packard Filtermate Harvester. Then, the filter plates were dried and counted for radioactivity in a Topcount NXT (Packard) after addition of 50 mL Microscint 20 (Perkin-Elmer). Non-specific binding was assessed in the presence of 10 mM mianserin. Five-point log titrations of the compounds were tested for affinity determination. Percent inhibition of specific radioligand binding was calculated and data analyzed using a four-parameter curve fitting to determine IC_{50} and K_i.
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17. In a typical procedure, to a stirred mixture of sodium hydride (60%, 14.6 g, 385.4 mmol), ethyl formate (43.8 g, 730.8 mmol) and tetrahydrofuran (300 mL) was added substituted acetophenone (182.7 mmol) in tetrahydrofuran (100 mL) at 0 °C. The reaction mixture was stirred for 2.5 h at room temperature, and then diluted with water (200 mL) and washed with ethyl acetate. The aqueous layer was separated and hydroxylamine hydrochloride (12.7 g, 182.7 mmol) was added. The mixture was stirred for 17 h at room temperature, and then diluted with 1 N HCl and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate and concentrated to give a yellow solid.
A mixture of the isoxazoline (57.5 mmol) and *tert*-butyl alcohol (4.5 g, 60 mmol) was stirred in an ice bath, and 0.30 mole of 71% perchloric acid was added dropwise. A white precipitate was formed during addition. After addition, the precipitate gradually thickened as stirring was continued. About 100 mL of water was then added. The suspension was stirred until homogeneous, and the solid was filtered. Washing with water, air drying, and washing with dichloromethane left 12 g (90%) of the crude perchlorate salt.
Ethylenediamine 16 mL (240 mmol) was dissolved in methylene chloride (200 mL). The perchlorate salt (48 mmol) was added portionwise with stirring over 20 min. The temperature of the exothermic reaction was maintained at 20–30 °C by cooling. Stirring was continued for 1 h. The reaction mixture was diluted by addition of sufficient methylene chloride to bring the volume to 500 mL. The reaction mixture was then extracted (2× 300 mL) with water and the organic layer dried over anhydrous magnesium sulfate. The methylene chloride was evaporated at reduced pressure to obtain an oil. The oil was dissolved in 300 mL of anhydrous ether and filtered free of white solids. The white solids were washed with a small amount of anhydrous ether and the washings were combined with the filtrate. Evaporation at a reduced pressure gave the product as a colourless oil. This was then dissolved in absolute ethanol (5 mL). The solution was acidified to pH 1 with methanesulfonic acid, refluxed under nitrogen for 1 h and cooled to room temperature. Volatiles were evaporated and the residue was purified by preparative thin-layer chromatography to give target compound.