Selective 5-HT_{2C} receptor agonists: Design and synthesis of pyridazine-fused azepines

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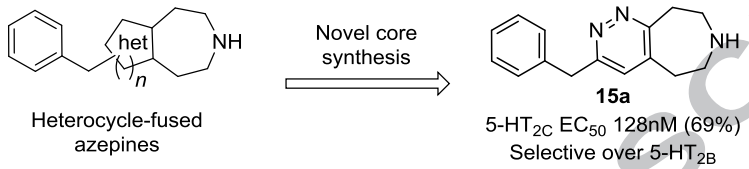

Heterocycle-fused azepines

15a

5-HT$_{2C}$ EC_{50} 128nM (69%)
Selective over 5-HT$_{2B}$

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Selective 5-HT$_{2C}$ receptor agonists: design and synthesis of pyridazine-fused azepines

Martin P. Green[a], Gordon McMurray[b], R. Ian Storer[a,*]

[a]*Worldwide Medicinal Chemistry,* [b]*Discovery Biology, Pfizer Global Research and Development, Sandwich Laboratories, Sandwich, Kent CT13 9NJ, UK.*

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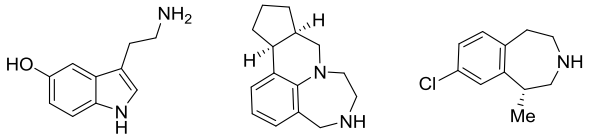
ABSTRACT

Heterocycle-fused azepines are discussed as potent 5-HT$_{2C}$ receptor agonists with excellent selectivity over 5-HT$_{2B}$ agonism. Synthesis and structure activity relationships are outlined for a series of bicyclic pyridazino[3,4-*d*]azepines. By comparison with earlier published work, *in vitro* assays predict a high probability for achieving CNS penetration for a potent and selective compound **15a**, a pre-requisite to achieve *in vivo* efficacy.

Serotonin (5-hydroxytryptamine, 5-HT **1**) acts as an agonist of at least 14 different receptors classified into seven major families, 5-HT$_{1-7}$. The 5-HT$_2$ class of GPCR receptors comprises three members 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$. Studies have identified agonism of 5-HT$_{2C}$ in the CNS to have potential for the treatment of a number of conditions including obesity, urinary incontinence, psychiatric disorders and sexual dysfunction.[1-3]

However, in order to develop safe treatments, it has been established that selectivity over agonism of the structurally related receptors 5-HT$_{2A}$ and 5-HT$_{2B}$ is of paramount importance. Previously, unselective serotonergic agonists have been linked to adverse events in humans. These include acute effects such as hallucinations and cardiovascular events due to 5-HT$_{2A}$ agonism.[4-7] However, arguably the greater concern, is chronic 5-HT$_{2B}$ agonism which has been established to lead to irreversible heart defects and pulmonary hypertension.[8-9] Notably the unselective anti-obesity treatment Fen-Phen was withdrawn from the market in 1997 for causing irreversible valvulopathy in a significant proportion of patients due to 5-HT$_{2B}$ agonism.

The search for potent and selective 5-HT$_{2C}$ agonists identified vabicaserin (**2**) (SCA-136; Pfizer, formerly Wyeth)[10] as a potential therapy for schizophrenia and lorcaserin (**3**) (APD-356; Arena) which gained limited approval in 2012 as Belviq® for treatment of obesity (Figure 1).[11-12] Furthermore, several other small molecule 5-HT$_{2C}$ agonists have also been reported to be in early clinical development or preclinical optimisation.[13-18]



1: serotonin (5-HT) **2**: vabicaserin (SCA-136) **3**: lorcaserin (Belviq(R), APD-356)

Figure 1. Serotonin and selected 5-HT$_{2C}$ clinical agonists.

Pfizer has previously disclosed several 5-HT$_{2C}$ receptor agonist templates.[19-31] In particular, a variety of aromatic heterocyclic piperazines **4** have been reported to be potent and selective 5-HT$_{2C}$ agonists. However, these series proved challenging, requiring high lipophilicity to deliver sufficient potency leading to suboptimal drug properties. In addition, a number of advanced compounds showed unacceptable levels of toxicity including mutagenicity in the Ames test. Subsequent mechanistic studies proposed bioactivation of the piperazine ring, a key part of the pharmacophore, to be the likely cause of this toxicity.[25] In order to eliminate this inherent mutagenicity, it was hypothesised based on molecular overlays that the piperazine could be replaced with an aromatic-fused azepine ring (Figure 2). Despite benzazepines **5** being established as 5-HT$_{2C}$ agonists,[27, 32] introducing a heterocyclic core in order to minimise the lipophilicity was desired as it was anticipated that this would lead to improved ADME and safety properties.

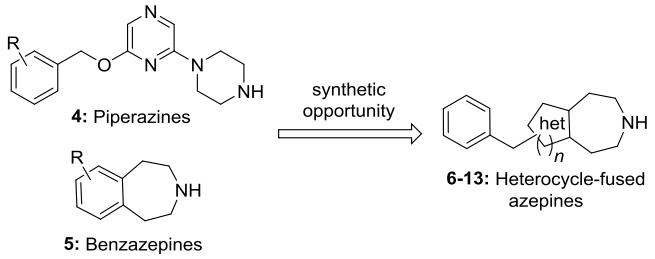
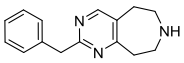
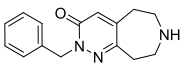
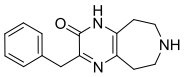
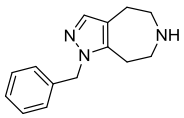
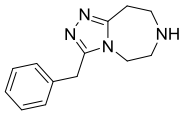
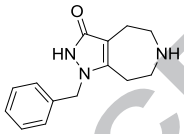
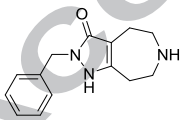
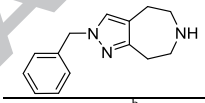


Figure 2. Design of heterocycle-fused azepines.

To explore this hypothesis in the absence of structure-based data necessitated building SAR for various fused heterocyclic systems. A range of fused heterocycles were explored. In the first instance these were limited to those where there was a suitable, synthetically enabled and potentially scalable synthetic route available. As a result, a variety of azepine-fused azines and azoles were synthesized (Table 1). Pharmacology was tested in an established FLIPR functional agonism assay system measuring the ability to induce a fluorescent based calcium mobilization in recombinant CHO-K1 cells expressing the human 5-HT_{2C} receptor.[21] Selected compounds were also tested for their ability to inhibit binding of [^{3}H]-meselurgine at the human 5-HT_{2C} receptor utilizing SPA technology and cellular membrane preparations generated from recombinant Swiss 3T3 cells.[21] The selectivity of compounds for 5-HT_{2C} over 5-HT_{2B} was also determined using an analogous FLIPR assay for 5-HT_{2B} functional agonism (Table 1).[21]

Table 1. Primary pharmacology, selectivity, physicochemistry and in vitro ADME for fused-heterocycle azepines.

Compound		logD	5-HT_{2C} EC_{50}[b] (μM)	5-HT_{2C} E_{max}[a]	5-HT_{2C} K_i[b] (μM)	5-HT_{2B} EC_{50}[b] (μM)	5-HT_{2B} E_{max}[a]	HLM Cl_{int} (mL min^{-1} mg^{-1})	RRCK (x10^{-6} cm/s)	MDCK-MDR1 ER (BA/AB)
	**6**	0.1	0.19	75%	0.18	-	33-50%	<8	20	1.1
	**7**	0.3	0.03	79%	0.01	0.36	48%	<7	13	1.0
	**8**	-0.1	0.07	90%	0.11	-	24%	<8	NT	3.0
	**9**	0.2	>10	0%	NT	-	13%	<7	14	1.0
	**10**	-0.3	>10	0%	>10	-	0%	<8	2	1.3
	**11**	-0.1	>10	0%	NT	-	0%	<7	<1	1.2
	**12**	-0.5	>10	0%	NT	-	0%	13	<1	NT
	**13**	0.1	4.98	57	2.70	-	3%	<7	13	1.0

[a] % activation at 10 μM.; [b]Mean of at least two replicates; NT denotes not tested; "-" denotes no defined EC_{50} curve obtained

From this initial study, it was concluded that the fused diazine systems **6-13** provided the better opportunities based on both agonist potency and lipophilic efficiency. As a result, further work was initiated, focusing on optimization of potency and selectivity over 5-HT_{2B} for both pyrimidine-fused **6**[19, 33] and pyridazinone-fused **7**[20] templates. In addition, data generated in a Ralph Russ Canine Kidney (RRCK) cell line highlighted that these compounds should exhibit good levels of passive membrane permeability.[34] Furthermore, good permeability and low levels of efflux in Madin-Darby Canine Kidney (MDCK) cells transfected with the MDR-1 gene encoding the P-glycoprotein (P-gp) efflux transporter, suggested these templates should also be readily CNS penetrant and therefore ideal for further optimization.[35]

As previously disclosed, optimization of the pyrimidine-fused template led to the discovery that 4-methylamino substitution **14** could offer enhancement to 5-HT_{2C} agonist potency and simultaneously offer beneficial improvements of selectivity over 5-HT_{2B} agonism.[30]

However, these structural changes also led to amino-substituted compounds becoming substrates for P-glycoprotein (P-gp) as shown by a large efflux ratio in the MDCK-MDR1 assay (Figure 3). As P-gp is highly expressed on the blood brain barrier it was anticipated that this would increase the risk that such compounds would display prohibitive levels of CNS restriction, thereby not accessing the desired site of action *in vivo* for effective modulation of 5-HT_{2C}.[35-38] This risk was confirmed by *in vivo* dog pharmacokinetic and pharmacology studies with compound **14**. The use of a clinically correlated dog peak urethral pressure (PUP) urology model of stress urinary incontinence (SUI) showed limited efficacy when treated with compound **14** relative to freely brain penetrant analogue **6** (PF-03246799) from the same template.[19, 30] Analysis of cerebral spinal fluid (CSF) samples from the study animals implied low levels of CNS exposure even at high blood plasma concentrations. Additionally, this model allowed for a projection in regard to therapeutically meaningful 5-HT_{2C} EC_{50} and E_{max} values, specifically a targeted profile of <200 nM and >30%, was deemed adequate with the assay utilised. Such an in vitro pharmacology profile for 5-HT_{2C} was also thought to be adequate for other relevant clinical indications (unpublished pre-clinical in vivo observations).

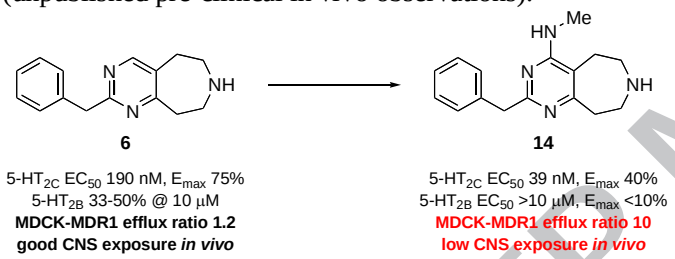


Figure 3. Aminopyrimidines show good selectivity but poor CNS exposure.

In an effort to retain the excellent 5-HT_{2C} potency and selectivity of compound **14** but with improved CNS penetration, compound designs were sought to provide reduced propensity for P-gp efflux. Literature pharmacophore models for P-gp have highlighted hydrogen bond Acc-Acc distances of ~2.5 Å and ~4.6 Å as P-gp recognition features. As shown in Figure 4, compound **14** modelling Acc-Acc distances of 2.4 Å, 4.1 Å and 4.6 Å indicated close similarity to this P-gp pharmacophore pattern of hydrogen bonding (Figure 4).[39]

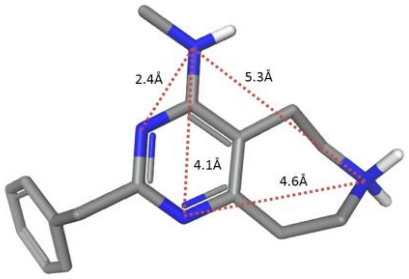


Figure 4. Distances between hydrogen bond acceptors on compound **14**. Modelled using Macromodel® with OPLS-2005 force field in conjunction with GB/SA solvation model.

This suggested that N-1 in combination with a 4-amino substituent could be instrumental to P-gp recognition. To test this hypothesis, it was proposed that pyridazine-fused azepines **15** would offer suitable CNS permeable profiles without P-gp efflux liability whilst maintaining ring polarity to promote both good levels of 5-HT_{2C} agonist potency and selectivity over 5-HT_{2B} (Figure 5).

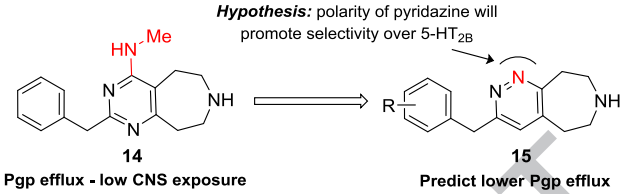
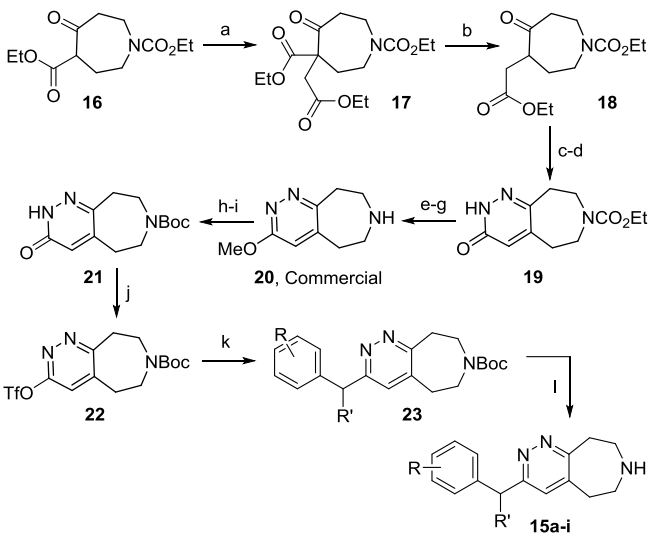


Figure 5. Design hypotheses for pyridazine-fused azepines.

These specific pyridazine-fused azepines had not previously been synthesized as part of the original heterocycle study (Table 1) due to lack of synthetic precedent. However, as a specific design rationale for their synthesis now existed, suitable chemistry was sought to access the substituted pyridazine-azepine core.

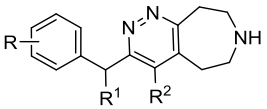
Considering the fused pyridazines **15a-i** it was anticipated that the synthesis of such poly-substituted pyridazines can present a challenge. However, it was recognized that pyridazinone intermediate **21**, which had previously been used in the synthesis of compound **7**, could potentially provide a convenient bulk precursor for the current synthesis, offering the option of a late-stage introduction of the benzylic group. As a result, this intermediate was intercepted to synthesise fused pyridazines **15a-i** in 3 steps (Scheme 1). This method proceeded smoothly via formation of the corresponding triflate **22**, followed by sp^2-sp^3 Negishi coupling with various benzyl zincates then Boc deprotection to yield the desired products **15a-i**.[20] In terms of access to the key intermediate **21**, this could be synthesised either from the commercially available methoxy compound **20** or synthesised on scale from 1,3-ketoester **16** by alkylation with ethyl bromoacetate to form diester **17**, followed by hydrolysis and decarboxylation to 1,4-keto-ester **18**. Subsequent treatment of the 1,4–keto-ester **18** with hydrazine, followed by oxidation with bromine formed pyridazinone **19**.



Scheme 1. General synthesis of pyridazines **15a-i**. Reagents: (a) NaOEt, ethyl bromoacetate; (b) (i) HCl reflux; (ii) ethylchloroformate, K_2CO_3; (c) NH_2NH_2, EtOH; (d) Br_2, $CHCl_3$ (19% yield over 4-steps); (e) $POCl_3$; (f) NaOMe, MeOH; (g) KOH, MeOH; (h) HBr, AcOH; (i) Boc_2O, TEA, CH_2Cl_2 (48% yield over 5-steps); (j) Tf_2O, 2,6-lutidine, CH_2Cl_2, 23 °C, 2 h (83% yield); (k) requisite zincate (RBnZnBr), $Pd(PPh_3)_4$, THF, rt for 15 mins then 60 °C for 3 h; (l) TFA, CH_2Cl_2, 23 °C, 2 h.

Parent pyridazinone **15a** (EC_{50} 130 nM, E_{max} 69%, logD 0) proved to be a relatively potent and efficient 5-HT_{2C} lead, comparable to previous lead compound **6** (PF-03246799, EC_{50} 190 nM, E_{max} 75%, logD 0.1) which had shown excellent in vivo efficacy (Table 2).[19, 30] Importantly, there was no evidence of efflux by the P-glycoprotein (P-gp) transporter as measured in the MDCK-MDR1 cell-line (ER=1.0), in accordance with the original hypothesis. Additionally, compound **15a** caused no appreciable agonism of the 5-HT_{2B} receptor up to 10 μM.

Table 2. Primary pharmacology, selectivity, physicochemistry and in vitro ADME for pyridazine-azepines.



Cmpd	R	R^1	R^2	logD	5-HT_{2C}			5-HT_{2B}		HLM Cl_{int} ($\mu Lmin^{-1}$ mg^{-1})	RRCK ($x10^{-6}$ cm/s)	MDCK-MDR1 ER (BA/AB)
					EC_{50} (μM)	E_{max} [a]	K_i (μM)	EC_{50} (μM)	E_{max} [a]			
15a	H	H	H	0.0	0.13	69%	0.08	-	29%	<7	14	1.0
15b	p-Cl	H	H	0.8	0.31	32%	0.12	0.41	63%	<7	13	1.3
15c	o-Cl	H	H	0.7	0.31	71%	0.20	0.80	41%	<7	15	1.1
15d	m-CF_3	H	H	0.9	0.75	37%	0.25	0.45	57%	<7	13	1.7
15e	o-OMe	H	H	0.1	>10	14%	0.55	-	40%	9	16	1.9
15f	p-F	H	H	0.2	>10	16%	0.45	-	64%	<7	14	1.2
15g	H	Me ent1	H	0.4	0.19	63%	0.20	-	36%	<8	13	1.8
15h	H	Me ent2	H	0.4	0.11	75%	0.17	-	55%	<8	14	1.6
15i	H	H	Me	NT	0.22	80%	0.52	NT	NT	<8	14	1.9

[a] % activation at 10 μM.; [b]Mean of at least two replicates; NT denotes not tested; "-" denotes no defined EC_{50} curve obtained

However, attempts to improve the potency within this series by varying the ring and benzylic substituents (Table 2, compounds **15b-h**) plus addition of a 4-Me substituent (**15i**) were all unsuccessful. Furthermore, addition of lipophilic groups (compounds **15b-15d**), resulted in erosion of functional selectivity over 5-HT_{2B}. At this time, by analogy with other related templates,[20, 30-31] it was recognised that most potency and efficiency gains had been obtained by changes building off the two positions currently blocked by the pyridazine ring nitrogens. As a result, further optimisation of this template was not continued.

In summary, the rational design of a novel series of heterocyclic pyridazine-fused azepines with potent 5-HT_{2C} agonist activity and low P-gp efflux ratios has been described. This work delivered lead compound **15a** via the novel application of a late stage Negishi sp2-sp3 coupling of a pyridazine-2-triflate **22** with various benzyl zincates for exploration of optimal benzyl substitution.

Acknowledgements

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Supplementary data

Supplementary data is available: experimental procedures for the synthesis of compound **15a**. This material is available free of charge via the Internet.

Author Information

Corresponding Author:

Tel: +44 (0)1304 641854; E-mail: ian.storer@pfizer.com

Abbreviations

ADME, absorption, distribution, metabolism, and excretion; CHO, Chinese hamster ovary; CNS, central nervous system; ER, efflux ratio; FLIPR, fluorescence imaging plate reader; HLM, human liver microsomes; MDCK, Madin-Darby canine kidney cell line; MDR-1, multidrug resistance gene 1; P-gp, P-glycoprotein transporter; RRCK, Ralph Russ canine kidney cell line; SPA, scintillation proximity assay; TFA, trifluoroacetic acid.

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