Journal Pre-proofs

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Discovery of a lead series of potent benzodiazepine 5-HT_{2C} receptor agonists with high selectivity in functional and binding assays

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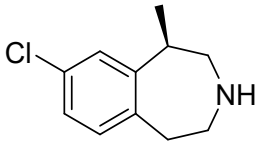
Abstract

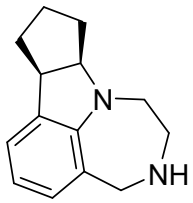
A series of potential new 5-HT_2 receptor scaffolds based on a simplification of the clinically studied, 5-HT_{2C}R agonist vabicaserin, were designed. An *in vivo* feeding assay early in our screening process played an instrumental part in the lead identification process, leading us to focus on a 6,5,7-tricyclic scaffold. A subsequent early SAR investigation provided potent agonists of the 5-HT_{2C} receptor that were highly selective in both functional and binding assays, had good rat PK properties and that significantly reduced acute food intake in the rat.

The 5-HT_{2C} receptor (5-HT_{2C}R) has been proven to be an important mediator of food intake. The receptor is primarily expressed in brain, including the hypothalamus, a region known to be involved in the regulation of appetite and feeding.[1] In rodents, classical 5-HT_{2C}R agonists including mCPP, Ro 60-0175 and fenfluramine (via its active metabolite, norfenfluramine), reduce food intake and body weight.[2] These effects are reversed upon pre-administration of a selective 5-HT_{2C}R antagonist. In addition, 5-HT_{2C} receptor null mice are hyperphagic and mildly obese, and the hypophagic effects of even nonselective 5-HT_{2C} agonists are significantly attenuated in such animals.[3] Non-selective 5-HT_2 receptor agonists including fenfluramine and dexfenfluramine also induced weight loss in the clinical setting, and were widely prescribed anorectic agents in the 1990s, particularly when used in combination with phentermine (the combination known as fen-phen). Retrospective safety analyses of these patients however, showed a strong association with heart valve disease as well as a connection to pulmonary hypertension,[4] resulting in their withdrawal from the market in the US in 1997. This valvulopathy finding was later connected to the activation of the 5-HT_{2B}R in cardiac valvular interstitial cells.[5] It has also been proposed that activation

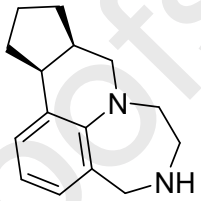
of the central 5-HT_{2A}R could be the cause of some of the adverse CNS effects observed including changes in perception and even hallucination.[6] The challenge in bringing new 5-HT_{2C}R compounds to the market then, can be largely defined by the need to rigorously address receptor selectivity to deal with these potential side effect issues. This approach eventually resulted in the discovery and FDA approval of the selective 5-HT_{2C}R agonist lorcaserin (**1**, BELVIQ®, Figure 1).[7] In addition to obesity, the 5-HT_{2C}R has been demonstrated to be involved in other diseases.



1, **Lorcaserin**
5-HT_{2C}R; K_i = 15 nM
selectivity >7x vs 5-HT_{2A}R
and >11x vs 5-HT_{2B}R [7b]



2, **WAY-163909**
5-HT_{2C}R; K_i = 10.5 nM
selectivity: >20x vs 5-HT_{2A}R;
and >40x vs 5-HT_{2B}R[12]



3, **Vabicaserin**
5-HT_{2C}R; K_i = 3 nM
selectivity >50x vs 5-HT_{2A}R
and >4x vs 5-HT_{2B}R[13]

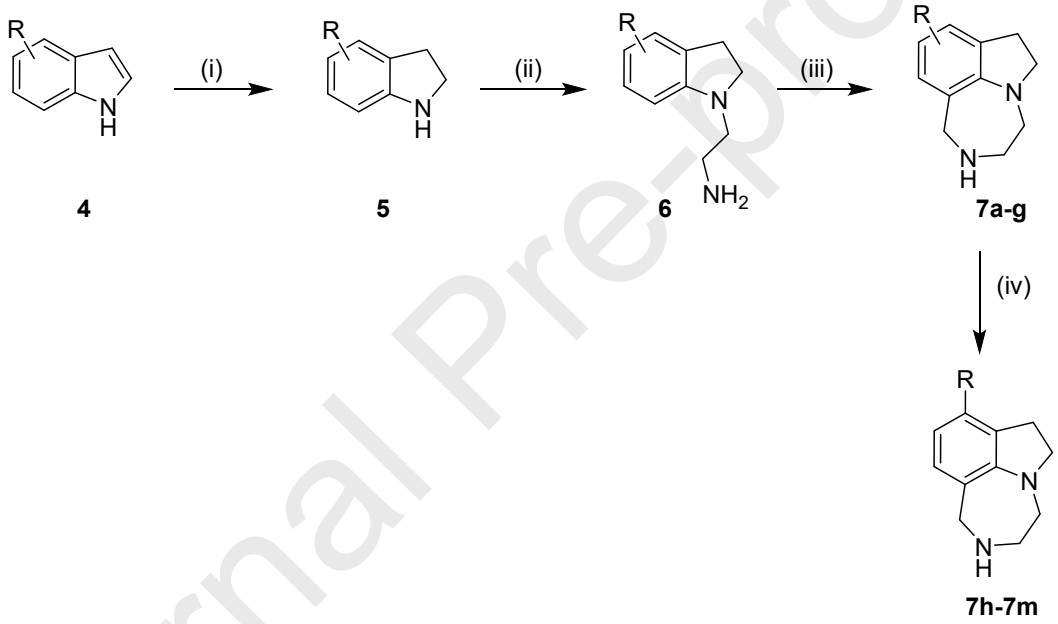
Figure 1. Structures of historical selective 5-HT_{2C}R agonists with their literature binding affinity data

The 5-HT_{2C}R agonist vabicaserin (**3**) shows good 5-HT_2R selectivity in functional assays and has been shown to be clinically effective in the treatment of the positive symptoms of schizophrenia[8] and the non-selective compound fenfluramine showed clinical efficacy as a treatment for the rare childhood epilepsy disorder Dravet's Syndrome.[9] It remains to be seen whether fenfluramine can be used safely for long-term treatment in this population in spite of its clear lack of selectivity against the 5-HT_{2B}R. Interestingly, a small trial with the much more selective 5-HT_{2C}R agonist lorcaserin in Dravet patients demonstrated similar and promising reductions in seizure frequency and severity.[10] Both lorcaserin and other selective 5HT_{2C}R agonists have also demonstrated significant potential for the treatment of stress incontinence in preclinical models.[11] With these additional potential indications, some of which remain to be fully validated in the clinic, a high safety bar will still be required so there remains a need to identify new 5-HT_{2C}R agonist compounds with improved receptor selectivity compared to lorcaserin.

During the lorcaserin approval process, we initiated a follow-on program to identify a second-generation compound to be able to investigate additional potential indications such as those outlined above. As discussed, our primary focus in this effort was on receptor selectivity as measured in both functional and binding assays. Our secondary objective was to expand structural diversity amongst the ligands we investigated compared to our original program.

In one such iteration, we were interested in investigating a series of analogues of the two related molecules WAY-163909 (**2**)[12] and vabicaserin (**3**, SCA-136).[13] In our hands, vabicaserin was somewhat less potent than lorcaserin but was highly selective in functional assays, with only a very weak partial agonist effect at the 5-HT_{2A}R (Table 1). It was, however, essentially devoid of selectivity between 5-HT_{2C}R and 5-HT_{2B}R in binding assays. We envisaged instead, an alternative simplified scaffold in which the fused cyclopentane ring would be absent. This reduction in complexity from 4 rings to 3 should provide two significant advantages. The first would be a simplification of the synthesis, partly by virtue of the removal of 2 chiral centres, with the second related advantage being easier access to substitution around the aromatic ring and potentially in other positions.

Scheme 1 : General synthesis of the 6,5,7-ring series

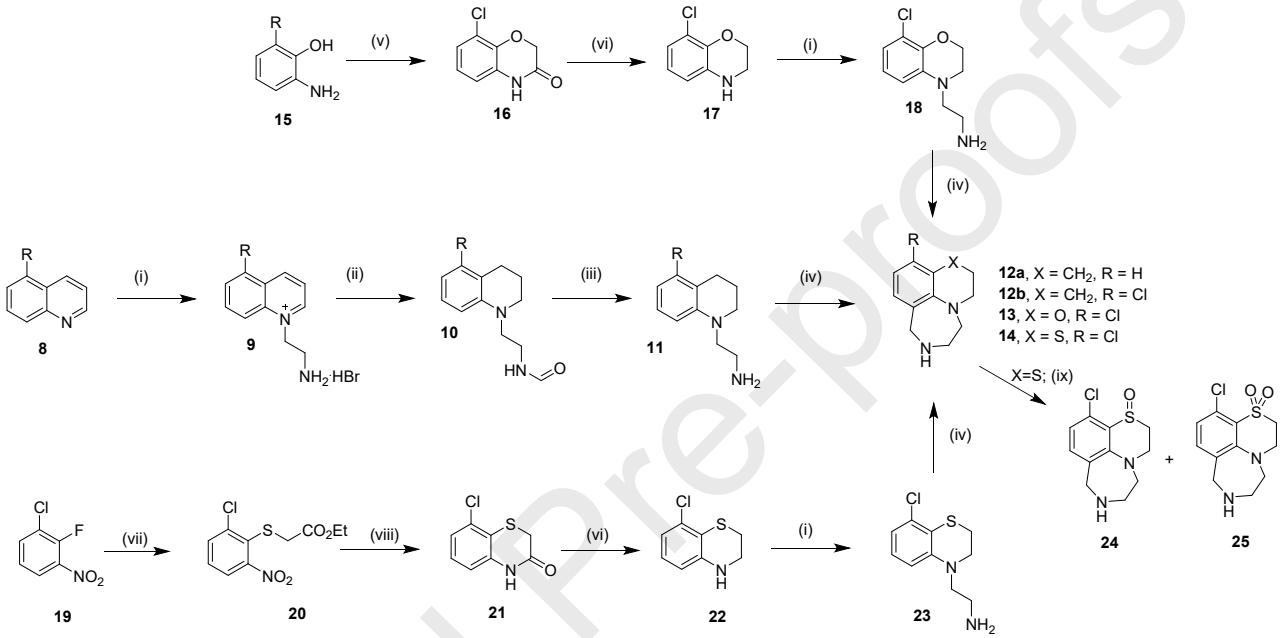


Reagents and Conditions i) Et_3SiH, TFA, r.t. ii) a) 2-hydroxy acetonitrile, 105°C b) $CoCl_2.6H_2O$, $NaBH_4$, r.t. c) HCl *or* iii) formaldehyde, MeOH, TFA, 80 °C iv) For R = 8-Br *either* $RB(OH)_2$, bis(di-t-butyl-p-dimethylaminophenylphosphino)palladium chloride, Na_2CO_3, dioxane, H_2O *or* RZnCl, dihydrogen di-μ-chlorodichlorobis(di-tert-butylphosphinito-κP)dipalladate (2-) ($POPd_2$), THF.

Our first iteration of this approach was to maintain the diazepine ring, based on the assumption that the secondary amine in that ring formed the key binding interaction with the conserved aspartate residue in TM3 in the 5-HT_2 receptor sub-family.[14] We thereafter investigated a range of ring sizes and hetero-atom insertions in the second partially saturated ring, as well as checking the effect of halo-substitution on the

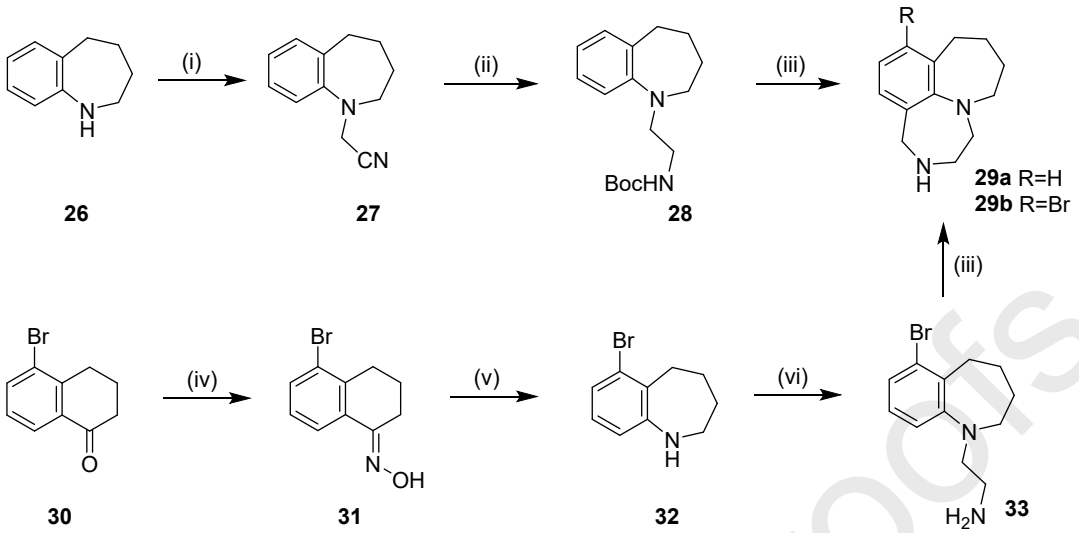
aromatic ring (Table 1). In general, the synthesis of compounds of this type involved the construction of the diazepine ring as the final step of the route via the addition of an amino ethyl moiety to the desired bicyclic system (indoline, tetrahydroquinoline, tetrahydro-1H-benzazepine etc.) followed by ring closure with a Pictet-Spengler type cyclization with formaldehyde. The general synthetic routes to such compounds are shown in Schemes 1-3.

Scheme 2: General synthesis of the 6,6,7-ring series



Reagents and Conditions i) 2-Bromoethanamine hydrobromide, neat, Δ; ii) $HCOOH$, $HCONH_2$, 120 °C; iii) 1.25M HCl in MeOH, 75 °C; iv) formaldehyde, MeOH, TFA, 80 °C; v) $ClCH_2COCl$, $NaHCO_3$, MeCN, 0 °C; vi) BH_3.THF, 100 °C; vii) Ethyl 2-mercaptoacetate, Et_3N, r.t.; viii) Fe (powder), AcOH, 90 °C; ix) mCPBA, DCM, r.t.

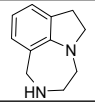
Scheme 3: Synthesis of the 6,7,7-ring analogues

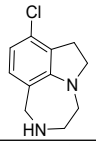
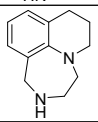
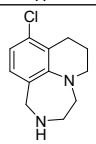
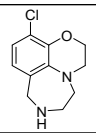
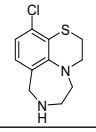
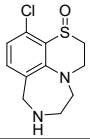
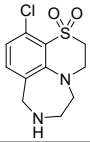
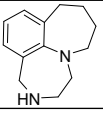
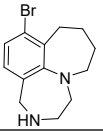


Reagents and Conditions i) 2-hydroxy acetonitrile, water, Δ; ii) a) Raney Ni, 7M NH_3 in MeOH, 70psi o/n b) Boc_2O, Et_3N, DCM, r.t.; iii) formaldehyde, MeOH, TFA, 80 °C; iv) $NH_2OH.HCl$, pyridine, r.t.; v) Dibal, -10 °C to r.t.; vi) 2-Bromoethanamine hydrobromide, neat, Δ

Our testing approach was to initially use the functional assay as a guide to potency and selectivity. However, there have historically been significant differences in the selectivity as measured by binding or functional assays, so as we were attempting to improve on the overall profiles of both lorcaserin and vabicaserin (data shown in the Tables is from this study, but is comparable to the published data for both lorcaserin[7b] and vabicaserin[13]),we were equally interested in the compounds' receptor profile in binding assays. Thus, for compounds with reasonable activity and selectivity in the functional assays, we then measured displacement of ^{125}I-labelled 1-(4-(125I)Iodanyl-2,5-dimethoxyphenyl)propan-2-amine (^{125}I-DOI) from all three 5-HT_2 receptors.

Table 1

		5-HT_{2C}R		**5-HT_{2B}R**		**5-HT_{2A}R**	
		EC_{50}, nM ($E_{max,}$ %)	^{125}I-DOI binding, K_i, nM	EC_{50}, nM ($E_{max,}$ %)	^{125}I-DOI binding, K_i, nM	EC_{50}, nM ($E_{max,}$ %)	^{125}I-DOI binding, K_i, nM
1	Lorcaserin	7.6 (95) n=12	15.2 (n=5)	970 (100) n=12	220 (n=3)	1920 (28) n=4	98.5 (n=3)
3	Vabicaserin	66 (101) n=7	10.6 (n=5)	>50000 n=6	13.8 (n=5)	1240 (7) n=6	149 (n=5)
7a		12600 n=3	>50000	>50000	>50000	>50000	>50000

7b		52 (88) n=5	30 (n=7)	5930 (58)	637 (n=6)	>50000	1140 (n=6)
12a		490 (87) n=3	250 (n=2)	>50000	>50000	>50000	>50000
12b		12 (85) n=8	11 (n=4)	2110 (35)	226 (n=5)	>50000	389 (n=5)
13		360 (60) n=2	192 (n=2)	>50000	3730 (n=1)	>50000	2430 (n=1)
14		37 (76) n=5	34 (n=2)	391 (6) n = 4	442 (n=2)	>50000	180 (n=2)
24		2650 (77) n=2	n.d.	>50000	n.d.	>50000	n.d.
25		1320 (78) n=2	905 (n=1)	>50000	>50000	>50000	>50000
29a		1400 (79) n=2	n.d.	500 (7)	n.d.	>50000	n.d.
29b		260 (48) n=5	51 (n=2)	>50000	360 (n=2)	>50000	59 (n=2)

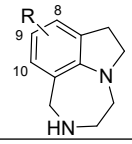
The data from the first, unsubstituted scaffolds - the direct analogues of vabicaserin and WAY-163909 with the fused cyclopentyl 4th ring removed - were not initially promising with **7a** having no measurable 5-HT_2 receptor activity at all, although **12a** did appear to have a selective, albeit weak, interaction with the 5-HT_{2C}R (Table 1). However, a chloro-substitution in the appropriate position on the aromatic ring resulted in an improvement in activity for both analogues, immediately providing compounds with potency and selectivity comparable to that seen for lorcaserin in both functional and binding assays (**7b**, **12b**). As a follow-up, we also prepared a number of other 6,6,7-fused heterocycles with a halo- substituent in the position equivalent to the 9-position in compound **12**, however neither the morpholino (**13**) nor thiomorpholino (**14**) analogues provided any further improvement in activity over **12b**, with **14** showing

markedly poorer selectivity, although the 5-HT_{2B}R agonist efficacy was very low. Oxidation of the sulfur in the ring to either the (racemic) sulfoxide (**24**) or the sulfone (**25**) further weakened 5-HT_{2C}R activity. Increasing the ring size to provide a 6,7,7 system did provide a compound with excellent agonist functional selectivity, albeit with a bromo rather than chloro substituent, but in our binding assay **29b** was not selective for 5-HT_{2C}R over 5-HT_{2A}R. This lack of binding selectivity was also confirmed in triplicate using tritiated 5-HT as the radioligand. Thus, even though the clear binding to the 5-HT_{2A}R and 5-HT_{2B}R did not appear to translate into an agonist effect on the Gq pathway as measured with an IP3 assay *in vitro*, we did not pursue this scaffold further as we were concerned that alternative second messenger pathways that we were not capturing with the functional assay may still be activated by this compound - as could be the case with vabicaserin. For example, several 5-HT_{2A} agonists and partial agonists are known to have hallucinogenic properties in humans and there is still no general agreement on how hallucinogenic and non-hallucinogenic 5-HT_{2A} agonists may differ in their ability to activate receptor second messenger pathways to exert their effects.[15] Computational approaches have proposed that distinct IC2 loop conformations can be induced by different ligands suggesting that multiple second messenger pathways may be activated, but this has not been confirmed experimentally.[16] We therefore focused our attention on the 6,5,7 and 6,6,7 series that each appeared to offer more promise with respect to selectivity in both functional and, equally importantly, radioligand binding assays.

With significant experience in screening for compounds of interest in the 5-HT_{2C}R agonist class, we chose to position an in vivo pharmacodynamic measure (an acute re-feeding assay in the fasted rat) very early on in the testing scheme. Thus, compounds of appropriate selectivity in both binding and function for the 5-HT_{2C}R were assessed in this assay at a screening dose of 10mg/kg PO. Active compounds produced both a significant decrease in food intake over 1-2 hours and induced other well-characterized 5-HT_{2C}R mediated behaviors in the rat, such as penile grooming and decreased motor activity, providing some confidence that the effect was on target. A positive effect in this assay represented an early indicator that the compound was orally bioavailable, had reasonable metabolic stability and was CNS penetrant, allowing us to focus our efforts on the analogues of highest interest. Active compounds were then progressed into both pharmacokinetic studies and a dose-response determination in the food intake assay. Testing of **7b** and **12b** in this manner showed **7b** to be the more active of the pair *in vivo* at the 10mg/kg PO screening dose. Following up with short time frame brain/plasma PK studies for each of these compounds offered a clear explanation for this difference. We observed that the whole brain exposure for **7b** was around 3-fold higher at all 3 time points measured (0.5, 1 and 2 h) than for **12b** and perhaps as significant, the CSF concentration (a potential surrogate for the free brain concentration) of **7b** was also

5-10 fold higher than **12b** at each timepoint. As a result of this clear difference in CNS partitioning, we chose to focus the bulk of our further exploratory efforts on the 6,5,7 series. This also had the advantage that more substituted indoles were commercially available than substituted quinolines and the reduction of the indoles to the indolines to provide the starting material for the cyclisation was somewhat more consistent in yield than the quinoline to tetrahydroquinoline reduction. Interestingly, a 6,6,7 tricyclic series similar to the one we decided not to pursue, has been extensively studied by the group from Abbvie,[17] but no detailed SAR has been published.

Table 2

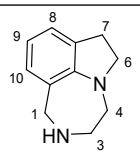
		**5-HT$_{2C}$R**		**5-HT$_{2B}$R**		**5-HT$_{2A}$R**	
	R	EC_{50}, nM ($E_{max,}$ %, n)	K_i,* nM (n)	EC_{50}, nM ($E_{max,}$ %, n)	K_i,* nM (n)	EC_{50}, nM ($E_{max,}$ %, n)	K_i,* nM (n)
7b	8-Cl	52 (88, 5)	30 (7)	5930 (58)	637 (6)	>50000	1140 (6)
7c	9-Cl	>50000	n.d.	>50000	n.d.	>50000	n.d.
7d	8-F	2500 (95, 4)	n.d.	>50000	n.d.	>50000	n.d.
7e	8-Br	84 (77, 6)	7.1 (3)	2150 (40, 9)	127 (2)	>50000	300 (3)
7f	8-CF_3	35 (88, 11)	6 (7)	1680 (57, 11)	105 (7)	>50000	168 (7)
7g	8-OMe	6300 (90, 2)	140 (3)	19000 (72, 5)	3500 (3)	>50000	>10000 (4)
7h	8-Me	250 (85, 4)	n.d.	>50000	n.d.	>50000	n.d.
7i	8-Et	190 (90, 4)	n.d.	1750 (70, 4)	n.d.	>50000	n.d.
7j	8-^{n}Pr	450 (105, 2)	n.d.	1220 (80, 2)	n.d.	>50000	n.d.
7k	8-^{i}Bu	780 (88, 3)	n.d.	320 (93, 3)	n.d.	>50000	n.d.
7l	8-^{c}Pr	72 (87, 2)	n.d.	260 (105, 2)	n.d.	>50000	n.d.
7m	8-CH_2Ph	295 (86, 2)	16 (3)	>50000	925 (3)	>50000	68 (3)

* = ^{125}I-DOI ligand

Exploring first the effect of substitution on the aromatic ring (Table 2), we showed that moving the chloro-substituent from the 8-position to the 9-position (**7c**) abolished all 5-HT$_2$ receptor activity. Surveying the effect of alternative substitutions at the 8-position provided several other active substituents, such as halo-, trifluoromethyl- and small alkyl- groups that maintained 5-HT$_{2C}$R functional activity comparable to that for lorcaserin or **7b**. All the compounds had excellent functional selectivity for the 5-HT$_{2C}$R over 5-HT$_{2A}$R, however, as was seen previously several compounds had significantly lower selectivity in the ^{125}I-DOI binding assay. Selectivity over the 5-HT$_{2B}$R was more variable, but the 8-Me analogue (**7h**) while only a moderately potent agonist of 5-HT$_{2C}$R showed some promise in this regard whereas increasing the size of this alkyl group (**7i-l**) reduced selectivity over 5-HT$_{2B}$R in functional assays. Testing further compounds

in our *in vivo* screening assay confirmed that the *in vitro* functional effect remained in the appropriate range to see a significant decrease in food intake at 10 mg/kg, with **7f** being equipotent with **7b**. In addition, the abbreviated CNS assay showed that this compound could achieve high brain concentrations (Table 4), albeit around half of those seen for **7b**. With these data in hand we turned our attention to substitutions on the 5-membered ring.

Table 3

		**5-HT$_{2C}$R**		**5-HT$_{2B}$R**		**5-HT$_{2A}$R**	
	Substitution	EC_{50}, nM (% $E_{max,}$, n)	K_i,* nM (n)	EC_{50}, nM ($E_{max,}$ %, n)	K_i,* nM (n)	EC_{50}, nM ($E_{max,}$ %, n)	K_i,* nM (n)
7n	7-Me	390 (88, 3)	94 (3)	>50000	4350 (2)	>50000	3700 (2)
7o	6-Me	19000 (80, 2)	n.d.	>50000	n.d.	>50000	n.d.
7p	cis-6-Me, 7-Me	370 (89, 2)	n.d.	6000 (21, 2)	n.d.	>50000	n.d.
7q	*trans*-6-Me, 7-Me	700 (91,2)	n.d.	>50000	n.d.	>50000	n.d.
7r	7-CH_2CF_3	44 (95, 3)	19 (3)	3400 (63, 4)	395 (3)	4000 (22, 4)	290 (3)
7s	7,7-Me_2	175 (100, 53)	52 (6)	>50000	1800 (6)	>50000	2000 (8)
7t	7,7-Et_2	780 (92, 2)	n.d.	>50000	n.d.	>50000	n.d.
7u	7,7-(spiro)^{c}Bu	17 (100, 21)	14 (14)	6420 (73, 16)	550 (12)	4500 (40, 17)	265 (12)
7v	7,7-(spiro)-4-THP	7850 (100, 3)	n.d.	>50000	n.d.	>50000	n.d.
7w	7-Me, 8-Me	0.8 (90, 3)	2.4 (3)	175 (80, 3)	22 (3)	693 (21, 3)	44 (3)
7x	7-Me, 8-Cl	2.5 (91, 3)	0.8 (3)	325 (76, 4)	46 (3)	1220 (10, 4)	44 (3)
7y	7-Me, 8-Br	1.4 (95, 6)	0.6 (6)	160 (75, 5)	21 (6)	750 (19, 4)	28 (6)
7z	7-CH_2CF_3, 8-Br	2 (98, 7)	0.8 (3)	125 (96, 7)	18 (2)	250 (47, 6)	13 (2)
7aa	7-Me, 9-Cl	12000 (80, 2)	n.d.	>50000	n.d.	>50000	n.d.
7ab	7-Me, 8,9-Cl_2	30 (88, 3)	2.8 (3)	800 (37, 3)	168 (3)	1120 (38, 3)	58 (3)
7ac	7-Me, 8-Cl, 9-Me	30 (93, 3)	8 (3)	300 (22, 4)	150 (3)	1380 (38, 4)	96 (3)
7ad	7,7-Me_2, 1-Me	>50000	n.d.	>50000	n.d.	>50000	n.d.
7ae	7,7-Me_2, 3-Me	>50000	n.d.	>50000	n.d.	>50000	n.d.
7af	7,7-Me_2, 4-Me	400 (100, 2)	n.d.	>50000	n.d.	>50000	n.d.
7ag	7,7-Me_2, 8-Br	5 (88, 6)	0.9 (3)	200 (103, 3)	28 (3)	>50000	36 (3)
7ah	7,7-Me_2, 8-Cl	6.5 (88, 6)	1.4 (4)	320 (86, 6)	18 (3)	1340 (13, 7)	60 (4)
7ai	7,7-Me_2, 8-F	51 (90, 7)	11 (6)	10600 (70, 7)	320 (6)	>50000	350 (6)
7aj	7,7-Me_2, 8-Me	8 (100, 2)	4.2 (4)	215 (93, 3)	24 (4)	649 (17, 3)	70 (4)

* = ^{125}I-DOI ligand

Substitution with a methyl group at the 7-position of the scaffold was tolerated with **7n** showing promising 5-HT_{2C}R activity and selectivity, again demonstrating that substitutions on the bare scaffold could alter potency compared to the parent **7a** (Table 3), even on the non-aromatic ring. Substitution in the 6-position, however, gave **7o** which was less potent. Combining these two substitutions to provide either *cis*- or *trans*-6,7-dimethyl analogues **7p** and **7q** provided compounds with potency comparable to **7n**, but all these analogues were less potent than the cyclized analogue vabicaserin (**2**). Adding a second substituent to the 7-position (**7s**) or elongating that substituent to a trifluoroethyl group (**7r**) gave compounds with a modest improvement in activity at the 5-HT_{2C}R. In addition, **7s** showed promise in terms of selectivity. There was no measurable functional effect at either the 5-HT_{2A}R or 5-HT_{2B}R and it had a 5-HT_{2C}R K_i of 52 nM and a >30-fold selectivity in binding over both 5-HT_{2A}R and 5-HT_{2B}R, making it therefore a compound of interest for further investigation. This compound showed good efficacy in the food intake assay at 10 mg/kg which triggered a dose-response. Reductions in food intake were observed as low as 2.5 mg/kg, with an ED_{50} of 6.3 mg/kg at 60 min post-dose (Table 4). As a result of these favorable properties, we prepared a handful of other 7,7-disubstituted compounds. The diethyl analogue **7t** was less potent than **7s** but reducing the volume of the substituents by the use of a spirocyclic group (**7u**) resulted in a compound that was an approximately 10-fold more potent agonist of the 5-HT_{2C}R than **7s** with comparable selectivity in both functional and binding assays. Expanding the size of this spiro-ring did not further improve potency (*e.g.* **7v**). When tested in vivo, **7u** showed excellent efficacy and as a result of its improved receptor potency provided full inhibition of food intake at 5 mg/kg and maintained an effect even at 2 mg/kg PO.

Table 4: In vivo pharmacodynamic and pharmacokinetic data

Compound	Inhibition of Food Intake at 1h (%)		Abbreviated CNS PK study (10mg/kg PO) Tissue concentrations at 1h		
	10 mg/kg PO screen	Additional doses (mg/kg PO)	Plasma (ng/mL)	Brain (ng/g)	CSF (ng/mL)
7b	97	-	332 ± 105	3940 ± 55	59.7 ± 1.2
7e	48	-	-	-	-
7f	94	28 (3)	78.2 ± 43.6	1710 ± 717	9.31†
7s	87	45 (5); 20 (2.5)	679 ± 178	5330 ± 597	423 ± 103
7u	99	97 (5); 48 (2); 30 (1)	634 ± 122	7380 ± 1850	229 ± 45
7ag	89	-	-	-	-
7ai	98	-	608 ± 55	8860 ± 2020	170 ± 59
12b	35	-	12.7 ± 3.3	957 ± 449	9.06†

Mean data (± SEM) for n=6 (food intake) or n=3 (PK study) animals per timepoint except † : n=2

To complete this portion of the SAR study we prepared a series of compounds that incorporated substituents on both the indoline ring as well as either the aromatic ring or the 7-membered ring. In our hands, methyl substitution on any of the 3 available carbon atoms in the 7-membered ring in combination with a 7,7-dimethyl substitution on the indoline ring resulted in a loss of activity compared to **7s**. In contrast, analogues with either 1 or 2 methyl groups on the indoline and a methyl or halogen substituent in the 8-position of the aromatic ring provided some of the most potent 5-HT_{2C}R agonists we had seen in this series with some examples having either sub-nanomolar EC_{50}s in the functional assay or K_i values of less than 1 nM in binding (Table 3). Interestingly, when we separated the enantiomers of **7w** by chiral HPLC, we observed that all the 5-HT_{2C}R activity resided in one isomer. However, while these compounds showed good potency, unlike **7s** and **7u** their selectivity was not superior to Lorcaserin when taking both binding and function into account. **7ai**, which had the best selectivity profile amongst the group was, as expected, a potent inhibitor of food intake *in vivo* with good CNS exposure (Table 4).

Having focused heavily on receptor selectivity from the start of this project, we further profiled compounds **7s** and **7u** based on their excellent selectivity profiles even though they were not the most potent agonists at the 5-HT_{2C}R. A broader selectivity panel of 20 monoamine related receptors, transporters and ion channels showed that in addition to the 5-HT_2 receptor subfamily, both compounds showed some moderate binding to the human 5-HT_7R (~85% at 10 μM) and **7u** had some additional weak interactions with the 5-HT_{1A}R and 5-HT_{5A}R as well as dopamine D3 (all 55-66% inhibition of binding at 10 μM) but they were otherwise highly selective. Neither compound showed any significant inhibition of either the hERG channel (patch clamp; **7s** < 12% inhibition at 10 μM; **7u** < 30% inhibition at 3 μM) or the major CYP enzymes (IC_{50} >50 μM for CYP3A4, 2C8, 2C9, 2C19, 1A2, 2D6 for both compounds).

High metabolic stability for the two compounds was demonstrated *in vitro* ($T_{½}$> 45 mins for both compounds in human, rat, dog, cynomolgus monkey and mouse microsomes). As expected, based on the potent inhibition of food intake observed in the rat, both **7s** and **7u** had excellent exposure in the brain after oral administration of a 10 mg/kg dose (Table 4) in an abbreviated CNS/plasma PK study. Each compound also had high CSF concentrations, suggestive of a significant free fraction in the brain. We measured plasma protein binding for **7u** and as might be predicted for small, non-lipophilic basic compounds, these measurements confirmed the likelihood of high unbound tissue fractions (PPB: human 79.1%, rat 63.6%, mouse 51%). A full PK evaluation for each compound showed similar parameters for half-life (**7s** = 1.88h, **7u** =1.4h) and oral bioavailability (~100%) whereas clearance of **7u** was twice that of

7s and greater than hepatic blood flow in the rat (**7s** 2.28 L/hr/kg; **7u** 5.09 L/hr/kg). Notably the renal contribution to clearance was close to 10% of the total clearance for both compounds.

Based on its lower clearance in the rat and lower volume of distribution we selected **7s** to be tested in PK studies in higher species. However, the oral bioavailability was greatly reduced in dog (12%) and cynomolgus monkey (10%) and the half-lives were not longer than in the rat ($T_{½}$ dog = 2.3h, monkey 1.85h). We hypothesized that the high renal clearance observed in these species, likely accompanied by poorer than expected adsorption, were detrimental to the overall PK profile. As a result, we decided not to pursue this compound further but rather to prepare other analogues to further optimize the series.

In summary, we have shown that from a range of newly designed tricyclic diazepine compounds, the 6,5,7-series described herein showed excellent overall profiles in functional and binding assays for the 5-HT_2 receptors. Compounds from this series consistently showed good PK properties in the rat, including high plasma exposure after oral administration, good CNS partitioning and low protein binding. This core therefore became our main series in our subsequent lead optimization, with the focus on further improving selectivity and identifying a compound with an adequate PK profile in higher species - particularly with respect to renal clearance. The results of these studies, including the selection of a clinical candidate molecule, will be described in due course.

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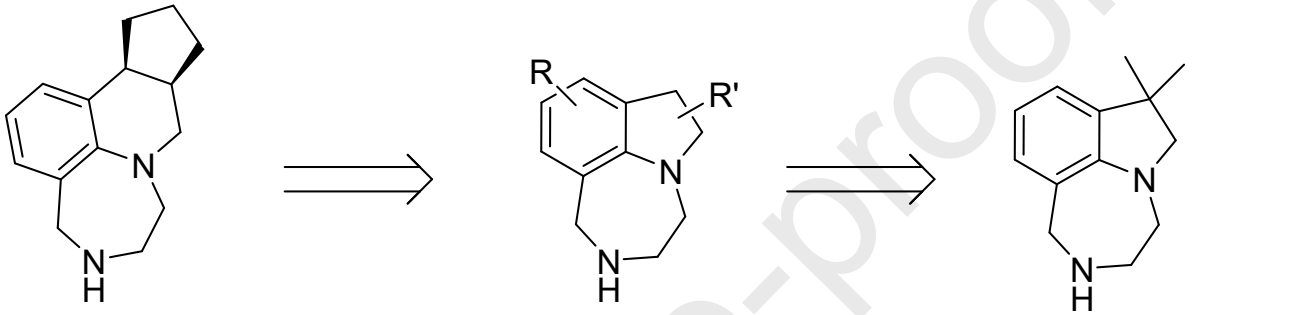

Vabicaserin **7a-7aj** **7s**

5-HT_{2C}R; K_i = 52 nM
selectivity >30x vs 5-HT_{2A}R
and 5-HT_{2B}R in both binding and functional assays

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: