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Pyrimido[4,5-*d*]azepines as potent and selective 5-HT$_{2C}$ receptor agonists: Design, synthesis, and evaluation of PF-3246799 as a treatment for urinary incontinence

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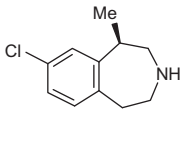
ABSTRACT

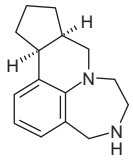
New pyrimido[4,5-*d*]azepines **7** are disclosed as potent 5-HT$_{2C}$ receptor agonists. A preferred example, **7b** had minimal activation at either the 5-HT$_{2A}$ or 5-HT$_{2B}$ receptors combined with robust efficacy in a preclinical canine model of stress urinary incontinence (SUI) and attractive pharmacokinetic and safety properties. Based on this profile, **7b** (PF-3246799) was identified as a candidate for clinical development for the treatment of SUI. In addition, it proved to be critical to build an understanding of the translation between recombinant cell-based systems, native tissue preparations and in vivo preclinical models. This was a significant undertaking and proved to be crucial in compound selection.

The neurotransmitter serotonin (5-HT) mediates its effects through at least 14 different receptor subtypes that have been classified into seven major families, 5-HT$_{1-7}$. The 5-HT$_2$ family has three members 2A, 2B, and 2C and, unlike 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors, the expression of 5-HT$_{2C}$ receptors appears to be restricted to the central nervous system (CNS). 5-HT$_{2C}$ receptor agonists have become attractive drug targets that have potential use in the treatment of a number of conditions including obesity, psychiatric disorders, sexual dysfunction, and urinary incontinence.[1] Selectivity over agonism at the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors would be a key objective because 5-HT$_{2A}$ agonists can potentially be hallucinogenic and have cardiovascular (CV) effects,[2] whereas 5-HT$_{2B}$ agonism has been associated with heart valvulopathy and pulmonary hypertension.[3]

The search for potent and selective 5-HT$_{2C}$ agonists has identified lorcaserin (**1**) (APD-356; Arena) which has completed phase 3 clinical trials for the treatment of obesity[4] and vabicaserin (**2**) (SCA-136; Pfizer) as a potential therapy for schizophrenia.[5] Furthermore, several small molecule 5-HT$_{2C}$ agonists have been reported to be in early clinical development or undergoing preclinical optimization and evaluation.[6]



1: APD-356
lorcaserin



2: SCA-136
vabicaserin

At Pfizer, we have disclosed several new templates as 5-HT$_{2C}$ receptor agonists[7–14] and some of these compounds have now progressed to clinical trials. As part of our research efforts to identify

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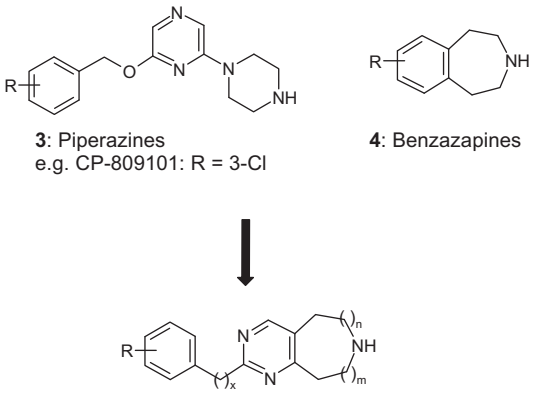
potential new 5-HT_{2C} agonist drug candidates, we adopted a strategy of exploring multiple chemical templates in order to increase our chances of having compounds survive to become advanced clinical candidates. In this Letter, we disclose pyrimido[4,5-*d*]-azepines (**7**) as potent and selective 5-HT_{2C} agonists.

Aromatic tethered heterocyclic piperazines **3** have been identified as potent 5-HT_{2C} agonists.[7a] However, some compounds in this series were shown to be mutagenic in the Ames assay and mechanistic studies proposed bioactivation of the piperazine ring as the likely cause.[7b] In order to try and eliminate the activity in the Ames assay, whilst retaining excellent 5-HT_{2C} agonist potency, we sought to remove the piperazine substituent and replace it with a fused six- or seven-membered ring containing a basic amine group (Fig. 1). Although benzazepines **4** were known to be active as 5-HT_{2C} ligands, we wished to retain a heterocyclic azine core in order to minimize the lipophilicity as it was anticipated that this would lead to improved drug-like properties. The tetrahydro-pyridopyrimidines **5**, **6** and tetrahydro-pyrimidoazepines **7** were then selected based on anticipated ease of synthesis.

Given the structural changes relative to the starting point **3** we were unsure what length of linker would be required between the pyrimidine and the pendant aromatic ring and so compounds were synthesised with a directly attached phenyl ring (**5–7**: $x = 0$) and with 1 to 3 methylene spacers (**5–7**: $x = 1$–3).

The 5-HT_{2C} agonist activity of target compounds **5–7** (Tables 1 and 2) was evaluated by measuring the ability to induce a fluorescence based calcium mobilization signal in a FLIPR assay employing recombinant CHO K1 cells expressing the human 5-HT_{2C} receptor.[15] Midway through our research program it proved necessary to re-express this cell-line with lower 5-HT_{2C} receptor density to give better translation between in vitro and in vivo outcomes; these have been designated as 'high' and 'low' receptor expression cell-lines respectively. As would be anticipated, the high expression cell-line was more highly amplified and showed compounds to be more potent (EC_{50}) with higher intrinsic activity (E_{max}) agonists than the low expression cell-line. Agonist activity at the 5-HT_{2B} receptor was measured in a similar manner with recombinant cell-based systems expressing the human 5-HT_{2B} receptor. 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.[15]

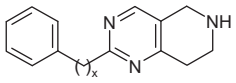
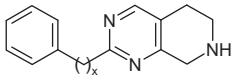
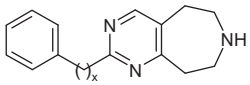
Initial results with the tetrahydro-pyridopyrimidines **5** and **6** were disappointing, with only **5d** and **6a** showing some modest



5: 5,6,7,8-tetrahydropyrido[4,3-d]pyrimidines, m = 1; n = 0
6: 5,6,7,8-tetrahydropyrido[3,4-d]pyrimidines, m = 0; n = 1
7: 6,7,8,9-tetrahydro-5H-pyrimido[4,5-d]azepines, m = n = 1

Figure 1. Design of pyrimidine targets **5–7**.

Table 1
5-HT_{2C} activity for compounds **5–7**[a,b,c]

	Compound	x	5-HT_{2C}	
			EC_{50} (nM)	E_{max} (%)
	**5a**	0	—	Inactive
	5b	1	—	64[d]
	5c	2	—	50[d]
	5d	3	1900	100
	**6a**	0	380	92
	6b	1	—	27[d]
	6c	2	—	Inactive
	6d	3	—	Inactive
	**7a**	0	—	Inactive
	7b	1	4.5	95
	7c	2	110	99

[a] See Ref. 15 for complete details of assay conditions.
[b] Values (EC_{50}, E_{max}) are geometric means of 2–4 experiments. Differences of <2-fold should not be considered significant.
[c] 5-HT_{2C} high receptor expression cell-line (see text).
[d] % Activation at 10 μM.

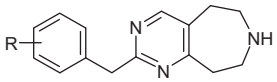
5-HT_{2C} potency (EC_{50} 1900 and 380 nM respectively) in the high expression cell-line (Table 1). However, this activity almost disappeared in the low expression cell-line with these compounds only showing weak activation of the 5-HT_{2C} receptor at 10 μM. Results with the pyrimidoazepines **7** were much more encouraging, with both benzyl (**7b**: $x = 1$, $EC_{50} = 4.5$ nM) and phenethyl substituents (**7c**: $x = 2$, $EC_{50} = 110$ nM) giving significant agonist activity in the high expression cell-line. Once again there was a drop-off in activity when **7b** and **7c** were screened in the low expression cell-line with **7c** becoming a weaker partial agonist (EC_{50} 4800 nM, E_{max} 40%). However, **7b** was found to retain both good potency and intrinsic activity (EC_{50} 190 nM, E_{max} 75%) which identified azepine **7b** as a preferred template for optimization from this set. Furthermore, **7b** was determined to be negative in a panel of in vitro genetic toxicology studies (Ames, micronucleus) either with or without metabolic activation.

At the outset of our work, there were very few reports of pyrimido[4,5-*d*]azepines **7** and these were restricted to the patent literature for targets other than 5-HT_{2C} receptor agonists.[16] Hence, we initiated a lead-to-candidate research program to explore the structure-activity relationships (SAR) of **7** with the objective of seeking potent 5-HT_{2C} agonists with minimal activity at either the 5-HT_{2A} or 5-HT_{2B} receptors. Furthermore, target compounds were designed to have drug-like properties consistent with CNS target space.[17]

Pyrimido[4,5-*d*]azepine target compounds **7** were prepared using a short 4-step synthesis as described in Scheme 1. Condensation of phenylacetamidines **8** with azepine ketoester **9**,[18] followed by cyclization, created the 4-pyrimidones **10** which then underwent chlorination to the corresponding 4-chloropyrimidines **11** by treatment with $POCl_3$. Dechlorination of **11** with zinc[19] gave pyrimidines **12** and finally deprotection of the azepine *N*-benzyl protecting group gave **7**. For some examples, it was possible to convert **11** directly to **7** by hydrogenolysis.

The SARs were initially focused on exploring substituents on the phenyl ring of **7** with respect to activation of the 5-HT_{2C} and 5-HT_{2B} receptors (Table 2). A range of groups were tolerated at either the 2-, 3-, or 4-positions with only the 2-Cl (**7j**) and 2-CF_3 (**7m**) losing 5-HT_{2C} activity compared to **7b** (R = H). Although 5-HT_{2C} agonist activity could be retained with a number of groups, these substituents invariably introduced an unacceptable level of 5-HT_{2B} activation with some compounds becoming more active at the 5-HT_{2B} receptor (**7k**,**l**). Hence, the initial lead **7b** emerged as having a superior profile from this set.

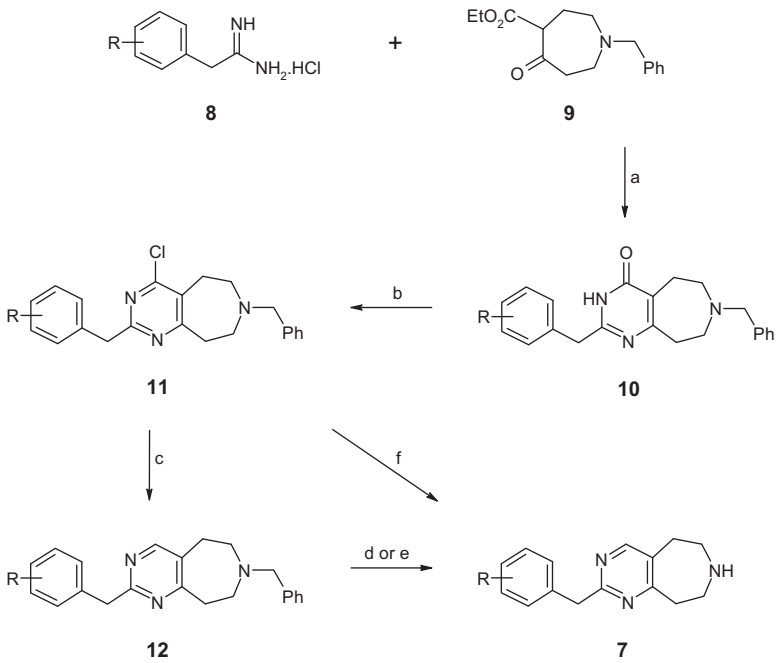
Table 2
5-HT_{2C} and 5-HT_{2B} activity for compounds **1** and **7**[a,b,c]



7

Compound	R	*c* log *P*	5-HT_{2C}		5-HT_{2C}	5-HT_{2B}	
			EC_{50} (nM)	E_{max} (%)	K_i (nM)	EC_{50} (nM)	E_{max} (%)
APD-356 (**1**)[d]	—	3.2	210	84	167	—	60–70[e]
7b	H	1.6	190	75	160	—	33–50[e]
7d	2-F	1.8	194	65	NT[f]	220	43
7e	3-F	1.8	125	67	NT	183	66
7f	4-F	1.8	310	60	470	610	90
7g	2-Me	2.1	330	70	305	1200	56
7h	3-Me	2.1	210	84	244	410	62
7i	4-Me	2.1	100	63	NT	164	80
7j	2-Cl	2.3	—	38[e]	1850	178	36
7k	3-Cl	2.3	180	56	84	30	50
7l	4-Cl	2.3	192	65	NT	60	62
7m	2-CF_3	2.5	—	15[e]	NT	—	15[e]
7n	3-CF_3	2.5	300	44	308	160	63

[a] See Ref. 15 for complete details of assay conditions.
[b] Values (EC_{50}, E_{max}, K_i) are geometric means of 2–4 experiments. Differences of <2-fold should not be considered significant.
[c] 5-HT_{2C} low receptor expression cell-line (see text).
[d] Data are presented for comparison in a common assay format. Literature values for APD-356 (**1**): h5-HT_{2C} EC_{50} 9 nM, E_{max} 100%, K_i 15 nM; h5-HT_{2B} EC_{50} 943 nM, E_{max} 100%. See Ref. 4b.
[e] % Activation at 10 μM.
[f] NT denotes not tested.



Scheme 1. General synthesis of pyrimidines **7**. Reagents and conditions: (a) NaOEt, EtOH, reflux, 18 h; (b) $POCl_3$, $Et_4N^+Cl^-$, $MeCH_2CN$, 100 °C, 18 h; (c) Zn metal, THF, concd $NH_{3\ (aq)}$, reflux, 6 h; (d) For R ≠ Cl: $NH_4^+HCO_2^-$, Pd/C, MeOH, reflux, 6 h; (e) for R = Cl: (i) 1-chloroethyl chloroformate, 1,8-bis(dimethylamino)naphthalene, CH_2Cl_2, rt, 18 h; (ii) MeOH, reflux, 2 h; (f) H_2 (50 psi), Pd/C, EtOH, 50 °C.

Compound **7b** was then evaluated in additional in vitro pharmacokinetic and pharmacology screens (Table 3). Azepine **7b** has good metabolic stability in both HLM and human hepatocytes consistent with low predicted clearance, weak CYP450 enzyme inhibition and good permeability. There was no evidence for recognition and efflux by the P-glycoprotein (P-gp) transporter as measured by transit performance in the MDCK-MDR1 cell-line. Compound **7b** had low ion channel activity as measured by binding to representative potassium, sodium and calcium channels.

Pharmacological evaluation for activity at the 5-HT_{2A} receptor was measured in a FLIPR assay employing Swiss 3T3 cells expressing the recombinant human 5-HT_{2A} receptor, in vitro tissue preparations with canine femoral artery, and in vivo models.[20] Screening of **7b** in the 5-HT_{2A} recombinant assay gave a significant response with an EC_{50} 68 nM and E_{max} 82% (Table 3). However, our experience has taught us that this was a highly expressed/coupled cell-line which over estimates 5-HT_{2A} activity and that the tissue preparation assay was a better predictor of in vivo outcomes.

Table 3
Physicochemical properties, in vitro activation of 5-HT_2 receptors, ADME profiles and ion channel binding affinities of **7b**[a]

	7b
Physicochemical properties	
mol wt	239
c log *P*	1.6
log $D_{7.4}$	0.2
TPSA (Å^2)	38
pK_a	8.5
5-HT_2 receptor activation[b]	
2C: EC_{50} (E_{max})	4.5 nM (95%)[c]
2C: EC_{50} (E_{max})	190 nM (75%)[d]
2C: K_i	160 nM
2A: EC_{50} (E_{max})	68 nM (82%)[c]
2B: EC_{50} (E_{max})	33–50% @ 10 μM[c]
ADME profile	
HLM, Cl_{int}[e] (μl/min/mg)	<7
H.hepatocytes, Cl_{int}[e] (μl/min/10^6 cells)	<5
CYP1A2 inhibition, IC_{50} (nM)	>30,000
CYP2C9 inhibition, IC_{50} (nM)	>30,000
CYP2C19 inhibition, IC_{50} (nM)	>30,000
CYP2D6 inhibition, IC_{50} (nM)	>30,000
CYP3A4 inhibition, IC_{50} (nM)	>30,000
PAMPA, $P_{app} \times 10^{-6}$ cm s^{-1}	28
MDCK-MDR1, AB/BA $P_{app} \times 10^{-6}$ cm s^{-1}	29/35
Ion channel binding affinities	
K^+ hERG, IC_{50} (nM)	>22,300
Na^+ site 2, IC_{50} (nM)[f]	>10,000
Ca^{2+} L-site (diltiazem), IC_{50} (nM)[f]	>10,000
Ca^{2+} L-site (verapamil), IC_{50} (nM)[f]	>10,000
Ca^{2+} L-site (DHP), IC_{50} (nM)[f]	>10,000

[a] See Ref. 28 for definition of terms.
[b] 5-HT_2 receptor activation EC_{50} and E_{max} values are geometric means of at least four experiments. Differences of <2-fold should not be considered significant.
[c] High receptor expression cell-line (see text).
[d] Low receptor expression cell-line (see text).
[e] Intrinsic clearance, Cl_{int}
[f] Data from Cerep, <20% I @ 10,000 nM.

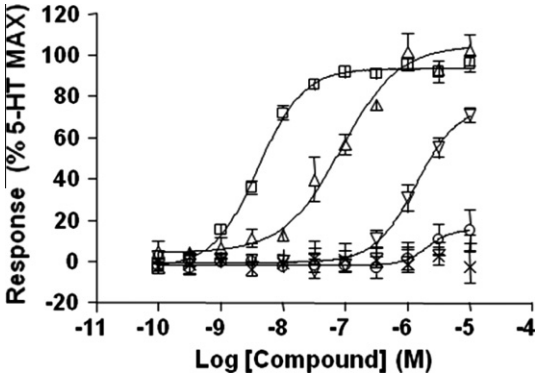


Figure 2. Effect of 5-HT (□, $n = 32$), norfenfluramine (Δ, $n = 6$), APD-356 (**1**)(▽, $n = 7$), PF-3246799 (**7b**)(○, $n = 12$) and DMSO (×, $n = 8$) in human colon as a measure of 5-HT_{2B} activity.

Table 4
Rat and dog pharmacokinetic data for **7b**

	Rat	Dog
Liver microsomes, Cl_{int} (μl/min/mg)	28	<7
Hepatocytes, Cl_{int} (μl/min/10^6 cells)	15	<5
Plasma protein binding (%)	0	0
Intravenous dose (mg/kg)	1.0[a]	0.030[c]
Elimination half-life, $T_{1/2}$ (h)	2.8	3.4
Plasma clearance, Cl (ml/min/kg)	51	11.6
Volume of distribution, V_d (l/kg)	8.9	3.5
Oral dose (mg/kg)	2[b]	–
C_{max} (ng/ml)	49	–
T_{max} (h)	0.25	–
$AUC_{0\rightarrow\infty}$ (ng/h/ml)	290	–
Bioavailability, F_o (%)	44	–

[a] Single intravenous bolus administration to male CD rat ($n = 1$).
[b] Single oral administration to male CD rat ($n = 2$).
[c] Single intravenous infusion over 1 h to female beagle dog ($n = 2$).

Evaluation of **7b** in the canine femoral artery gave a much weaker response of EC_{50} 3400 nM and E_{max} 51%. Screening of **7b** in the rat head-twitch model[21] at 10 mg/kg (po, $n = 8$) gave no response and **7b** had no significant effect on blood pressure or heart rate during a CV assessment in an anaesthetized dog model up to 0.5 mg/kg (iv infusion over 60 min, $n = 4$); hence, we concluded that **7b** had no significant 5-HT_{2A} activity.

Compounds with weak activity in the 5-HT_{2B} recombinant cell-based assay were then screened for 5-HT_{2B} activity in in vitro tissue preparations with human colon.[22–24] Compound **7b** was screened alongside APD-356 (**1**) with 5-HT and norfenfluramine acting as positive controls (Fig. 2). Compound **7b** showed minimal activation of the 5-HT_{2B} receptor compared to 5-HT and norfenfluramine and was significantly less active than APD-356 (**1**) in this model. On average, **7b** demonstrated <20% activation at 10 μM; hence, we concluded that **7b** had minimal 5-HT_{2B} activity.

Pharmacokinetic data for **7b** was generated in vivo in rat and dog (Table 4). Following single intravenous administration of **7b** to rat, plasma clearance was moderate relative to liver blood flow and volume of distribution was moderate resulting in an elimination half-life of 2.8 h. There was also a renal component to clearance with approx. 10% of parent being excreted unchanged in urine. Following single oral administration to rat, **7b** was rapidly absorbed and showed moderate oral bioavailability (40%) suggesting complete absorption from the gut, based on blood clearance of 51 ml/min/kg and an assumed liver blood flow of 70 ml/min/kg. Good CNS exposure was confirmed in rat (10 mg/kg po, $t = 30$ min, $n = 8$) with free drug levels in brain similar to free drug levels in plasma ($[brain]_{free}/[plasma]_{free} = 0.6$). Following single intravenous administration to dog, plasma clearance of **7b** was low relative to liver blood flow resulting in an elimination half-life of 3.4 h. These results established that **7b** had pharmacokinetic properties compatible with evaluation in rodent and canine models of disease.

The metabolic fate of **7b** was investigated in HLM. The major metabolic routes were identified as N-hydroxylation of the azepine group to give hydroxylamine **13** and *p*-hydroxylation of the phenyl ring to give **14** (Fig. 3). Several minor metabolites were due to mono-oxidation of the phenyl ring and benzylic methylene group. The metabolism of **7b** was investigated in individual CYP450 recombinant enzymes which identified that CYPs 2D6, 2B6, 2C8, and 2C19 are likely to be the major CYP450 enzymes contributing to the CYP-mediated clearance of **7b**.

Compound **7b** was screened for off-target pharmacology against a panel of 110 receptors, enzymes and ion channels (CEREP, Bioprint™) and was found to have binding affinities of IC_{50} >2.5 μM except for the muscarinic ($M_{1/3-5}$ 0.3–1.2 μM), 5-HT_3 (2.3 μM) and 5-HT_6 (1.8 μM) receptors. Further evaluation in functional assays showed no agonist activation of the $hM_{1/4/5}$ receptor at concentrations up to 10 μM and only weak antagonism at the hM_4 receptor with $IC_{50} = 6.8$ μM. CV risk through direct interaction with cardiac ion channels was viewed to be minimal from in vitro experiments where **7b** had no significant effect on either the hERG channel as measured by functional blockade in patch-clamp experiments (IC_{50} >100 μM) or the cardiac sodium channel (Nav 1.5) as assessed through functional blockade in the isolated rabbit cardiac myocyte (up to 100 μM).

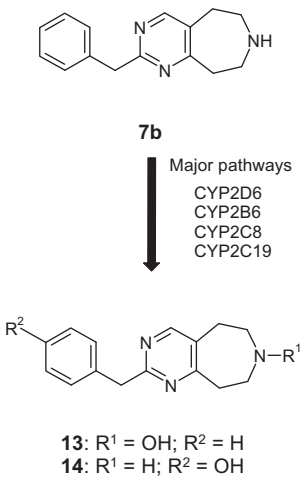


Figure 3. In vitro metabolism of **7b** in HLM.

The evaluation of **7b** in a preclinical in vivo canine model of stress urinary incontinence (SUI) showed a robust dose-dependant response in improving urethral tone that was superior to APD-356 (**1**) at similar free plasma concentrations of drug (Fig. 4).[25,26] Azepine **7b** increased peak urethral pressure (PUP) by 20–30% at a free plasma concentration of 20–45 nM which compared favorably with other 5-HT_{2C} agonists[10–12] and drugs that have been evaluated in this model, for example, duloxetine (SNRI);[27] PF-184298 (SNRI);[28] PF-3774076 (α_{1A}-adrenergic partial agonist).[25]

Finally, **7b** produced no dose-limiting toxicity in oral acute and sub-chronic rat (4-day, up to 30 mg/kg) and dog (7-day, up to 10 mg/kg) exploratory toxicology studies and salt screening with **7b** identified the hemi-fumarate salt as a crystalline, non-hygroscopic solid form (mp 210 °C) with excellent aqueous solubility (>10 mg/mL at pH 1.5–11.7) and stability (pH 1–12).

In summary, we have identified new pyrimido[4,5-*d*]azepines **7** as potent 5-HT_{2C} receptor agonists. A preferred example **7b** had minimal activation at either the 5-HT_{2A} or 5-HT_{2B} receptors, combined with robust efficacy in a preclinical canine model of SUI and attractive pharmacokinetic and safety properties. Based on this profile, **7b** (PF-3246799)[15] was identified as a candidate for clinical development for the treatment of SUI. In addition, it proved to be critical to build an understanding of the translation between recombinant cell-based systems, native tissue preparations and in vivo preclinical models. This was a significant undertaking and proved to be crucial in compound selection. Second generation pyrimido[4,5-*d*]azepines have been identified with improved agonist activity at the 5-HT_{2C} receptor combined with no measurable activation of the 5-HT_{2B} receptor.[15] These studies will be the subject of future publications.

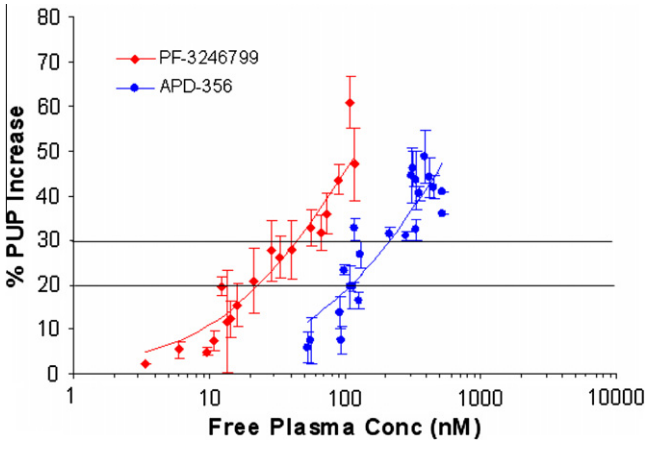


Figure 4. The effect of APD-356 (**1**) and PF-3246799 (**7b**) on peak urethral pressure (PUP) as percent change relative to control in the female anaesthetised dog ($n \geqslant 4$; iv infusion). Each bar represents the mean response ± SEM versus mean free plasma concentration. Dog 5-HT_{2C} functional potency, APD-356 (**1**): EC_{50} = 16.2 nM (82%) (n = 3); PF-3246799 (**7b**): EC_{50} = 10.8 nM (81%) (n = 3).

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References and notes

1. (a) Dutton, A. C.; Barnes, N. M. *Drug Discovery Today: Ther. Strateg.* **2006**, *3*, 577; (b) Smith, B. M.; Thomsen, W. J.; Grottick, A. J. *Expert Opin. Invest. Drugs* **2006**, *15*, 257; (c) Rosenzweig-Lipson, S.; Dunlop, J.; Marquis, K. L. *Drug News Perspect.* **2007**, *20*, 565; (d) Wacker, D. A.; Miller, K. J. *Curr. Opin. Drug Discov. Devel.* **2008**, *11*, 438; (e) Mbaki, Y.; Rammage, A. G. *Br. J. Pharmacol.* **2008**, *155*, 343.
2. (a) Nichols, D. E. *Pharmacol. Ther.* **2004**, *101*, 131; (b) Villalon, C. M.; Centurion, D. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2007**, *376*, 45.
3. Roth, B. L. *N. Eng. J. Med.* **2007**, *356*, 6.
4. (a) Smith, B. M.; Smith, J. M.; Tsai, J. H.; Schultz, J. A.; Gilson, C. A.; Estrada, S. A.; Chen, R. R.; Park, D. M.; Prieto, E. B.; Gallardo, C. S.; Sengupta, D.; Dosa, P. I.; Covel, J. A.; Ren, A.; Webb, R. R.; Beeley, N. R. A.; Martin, M.; Morgan, M.; Espitia, S.; Saldana, H. R.; Bjenning, C.; Whelan, K. T.; Grottick, A. J.; Menzaghi, F.; Thomsen, W. J. *J. Med. Chem.* **2008**, *51*, 305; (b) Thomsen, W. J.; Grottick, A. J.; Menzaghi, F.; Reyes-Saldana, H.; Espitia, S.; Yuskin, D.; Whelan, K.; Martin, M.; Morgan, M.; Chen, W.; Al-Sham, H.; Smith, B.; Chalmers, D.; Behan, D. *J. Pharmacol. Exp. Ther.* **2008**, *325*, 577; (c) Smith, S. R.; Prosser, W. A.; Donahue, D. J.; Moran, M. E.; Anderson, C. M.; Shanahan, W. R. *Obesity* **2008**, *17*, 494; (d) Smith, S. R.; Weissman, N. J.; Anderson, C. M.; Sanchez, M.; Chuang, E.; Stubbe, S.; Bays, H.; Shanahan, W. R. *N. Eng. J. Med.* **2010**, *363*, 245.
5. (a) Ramamoorthy, P. S.; Beyer, C.; Brennan, J.; Dunlop, J.; Gove, S.; Grauer, S.; Harrison, B. L.; Lin, Q.; Malberg, J.; Marquis, K.; Mazandarani, H.; Piesla, M.; Pulicicchio, C.; Rosenzweig-Lipson, S.; Sabb, A.-M.; Schechter, L.; Stack, G.; Zhang, J. *Abstracts of Papers*, 231st ACS National Meeting, Atlanta, GA, United States, March 26–30, 2006; MEDI-021.; (b) Pfizer Pipeline as of 27th January, 2010, see: www.pfizer.com.
6. For reviews of small molecule 5-HT_{2C} agonists, see: (a) Bishop, M. J.; Nilsson, B. M. *Expert Opin. Ther. Patents* **2003**, *13*, 1691; (b) Lacivita, E.; Leopoldo, M. *Curr. Top. Med. Chem.* **2006**, *6*, 1927; (c) Nilsson, B. M. *J. Med. Chem.* **2006**, *49*, 4023; (d) Monck, N. J. T.; Kennett, G. A. *Prog. Med. Chem.* **2008**, *46*, 281; For more recent disclosures of 5-HT_{2C} agonists, see: (e) Ahmad, S.; Ngu, K.; Miller, K. J.; Wu, G.; Hung, C.-P.; Malmstrom, S.; Zhang, G.; O'Tanyi, E.; Keim, W. J.; Cullen, M. J.; Rohrbach, K. W.; Thomas, M.; Ung, T.; Qu, Q.; Gan, J.; Narayanan, R.; Pelleymounter, M. A.; Robl, J. A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1128; (f) Cho, S. J.; Jensen, N. H.; Kurome, T.; Kadari, S.; Manzano, M. L.; Malberg, J. E.; Caldarone, B.; Roth, B. L.; Kozikowski, A. P. *J. Med. Chem.* **2009**, *52*, 1885; (g) Shimada, I.; Maeno, K.; Kondoh, Y.; Kaku, H.; Sugasawa, K.; Kimura, Y.; Hatanaka, K.-I.; Naitou, Y.; Wanibuchi, F.; Sakamoto, S.; Tsukamoto, S.-I. *Bioorg. Med. Chem.* **2008**, *16*, 3309; (h) Shimada, I.; Maeno, K.; Kazuta, K.-I.; Kubota, H.; Kimizuka, T.; Kimura, Y.; Hatanaka, K.-I.; Naitou, Y.; Wanibuchi, F.; Sakamoto, S.; Tsukamoto, S.-I. *Bioorg. Med. Chem.* **2008**, *16*, 1966.
7. (a) Siuciak, J. A.; Chapin, D. S.; McCarthy, S. A.; Guanowsky, V.; Brown, J.; Chiang, P.; Marala, R.; Patterson, T.; Seymour, P. A.; Swick, A.; Iredale, P. A. *Neuropharmacology* **2007**, *52*, 279; (b) Kalgutkar, A. S.; Dalvie, D. K.; Aubrecht, J.; Smith, E. B.; Coffing, S. L.; Cheung, J. R.; Vage, C.; Lame, M. E.; Chiang, P.; McClure, K. F.; Maurer, T. S.; Coelho, R. V., Jr.; Soliman, V. F.; Schildknegt, K. *Drug Metab. Dispos.* **2007**, *35*, 848.
8. Kalgutkar, A. S.; Bauman, J. N.; McClure, K. F.; Aubrecht, J.; Cortina, S. R.; Paralkar, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1559.
9. Fish, P. V.; Brown, A. D.; Evrard, E.; Roberts, L. R. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1871.
10. Brennan, P. E.; Whitlock, G. A.; Ho, D. K. H.; Conlon, K.; McMurray, G. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4999.
11. Andrews, M. D.; Green, M. P.; Allerton, C. M. N.; Batchelor, D. V.; Blagg, J.; Brown, A. D.; Gordon, D. W.; McMurray, G.; Millns, D. J.; Nichols, C. L.; Watson, L. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5346.

12. Allerton, C. M. N.; Andrews, M. D.; Blagg, J.; Ellis, D.; Evrard, E.; Green, M. P.; Liu, K. K.-C.; McMurray, G.; Ralph, M.; Sanderson, V.; Ward, R.; Watson, L. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5791.
13. Liu, K. K.-C.; Cornelius, P.; Patterson, T. A.; Zeng, Y.; Santucci, S.; Tomlinson, E.; Gibbons, C.; Maurer, T. S.; Marala, R.; Brown, J.; Kong, J. X.; Eunsun, L.; Werner, W.; Wenzel, Z.; Vage, C. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 266.
14. Liu, K. K.-C.; Lefker, B. A.; Dombroski, M. A.; Chiang, P.; Cornelius, P.; Patterson, T. A.; Zeng, Y.; Santucci, S.; Tomlinson, E.; Gibbons, C. P.; Marala, R.; Brown, J. A.; Kong, J. X.; Lee, E.; Werner, W.; Wenzel, Z.; Giragossian, C.; Chen, H.; Coffey, S. B. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2365.
15. Andrews, M. D.; Blagg, J.; Brennan, P.; Fish, P. V.; Roberts, L.; Storer, R. I.; Whitlock, G. A. WO patent application 117169, 2008.
16. (a) Binggeli, A.; Maerki, H.-P.; Mutel, V.; Wostl, W.; Wilhelm, M. WO patent application 006288, 2002.; (b) Pitts, W. J.; Barbosa, J.; Guo, J. U.S. patent application 0191143, 2003.
17. Hitchcock, S. A.; Pennington, L. D. *J. Med. Chem.* **2006**, *49*, 1.
18. Moriya, T.; Oki, T.; Yamaguchi, S.; Morosawa, S.; Yokoo, A. *Bull. Chem. Soc. Jpn* **1968**, *41*, 230.
19. Mai, A.; Artico, M.; Sbardella, G.; Quartarone, S.; Massa, S.; Loi, A. G.; De Montis, A.; Scintu, F.; Putzolu, M.; La Colla, P. *J. Med. Chem.* **1997**, *40*, 1447.
20. Davey, D.; Davidson, M.; af Forselles, K.; McMurray, G. *Proc. Br. Pharmacol. Soc.* **2006**, *4*, 095; available at http://www.pa2online.org.
21. Fantegrossi, W. E.; Reissig, C. J.; Katz, E. B.; Yarosh, H. L.; Rice, K. C.; Winter, J. C. *Pharmacol. Biochem. Behav.* **2008**, *88*, 358. and references therein.
22. Borman, R. A.; Tilford, N. S.; Harmer, D. W.; Day, N.; Elis, E. S.; Sheldrick, R. L. G.; Carey, J.; Coleman, R. A.; Baxter, G. S. *Br. J. Pharmacol.* **2002**, *135*, 1144.
23. Rat stomach fundus has commonly been used to assess 5-HT_{2B} activation, see: Baxter, G. S.; Murphy, O. E.; Blackburn, T. P. *Br. J. Pharmacol.* **1994**, *112*, 323.
24. For alternative 5-HT_{2B} agonist endpoints, see: Huang, X.-P.; Setola, V.; Yadav, P. N.; Allen, J. A.; Rogan, S. C.; Hanson, B. J.; Revankar, C.; Robers, M.; Doucette, C.; Roth, B. L. *Mol. Pharmacol.* **2009**, *76*, 710.
25. The evaluation of test compounds in a preclinical in vivo canine model of SUI has been reported previously, see: Conlon, K.; Christy, C.; Westbrook, S.; Whitlock, G.; Roberts, L.; Stobie, A.; McMurray, G. *J. Pharmacol. Exp. Ther.* **2009**, *330*, 892.
26. PF-3246799 (**7b**) also showed a robust dose-dependant response in a rat spontaneous feeding model with a >78% reduction in food intake (1–10 mg/kg; $n = 4–5$; $t = 0–2$ h). These results will be published separately.
27. Fish, P. V.; Harrison, A. C.; Wakenhut, F.; Whitlock, G. A. In *Accounts in Drug Discovery: Case Studies in Medicinal Chemistry*; Barrish, J., Carter, P., Zahler, R., Eds.; RSC publishing: Cambridge, UK, 2010; pp 267–285. Chapter 12.
28. Wakenhut, F.; Allan, G. A.; Fish, P. V.; Fray, M. J.; Harrison, A. C.; McCoy, R.; Phillips, S. C.; Ryckmans, T.; Stobie, A.; Westbrook, D.; Westbrook, S. L.; Whitlock, G. A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5078.