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# Discovery of a novel azepine series of potent and selective 5-HT$_{2C}$ agonists as potential treatments for urinary incontinence

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ABSTRACT

A range of heterocycle fused azepines were synthesized in order to find a CNS penetrant, selective 5-HT$_{2C}$ agonist for the treatment of incontinence. The pyridazo-azepines such as compound **11** were shown to be potent 5-HT$_{2C}$ agonists and have potential for CNS penetration and good in vitro ADME properties but lacked selectivity against 5-HT$_{2B}$. Fusing a further heterocycle gave the selective triazolopyrimido-azepines. An example of this series, compound **36**, was shown to be potent, selective, metabolically stable in vitro and efficacious in an in vivo model of stress urinary incontinence.



The neurotransmitter serotonin (5-HT) has been found to play a key role in mechanisms involved in micturition and continence.[1,2] Recent studies have additionally shown that central activation of the 5-HT$_{2C}$ receptor increases urethral muscle tone and inhibits micturition reflexes,[2] indicating that centrally acting agonists of 5-HT$_{2C}$ may be effective treatments of both stress urinary incontinence (SUI) and mixed urinary incontinence (MUI, combination of stress and urge incontinence).

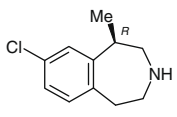
One of the major challenges in the area of 5-HT$_{2C}$ agonist discovery is selectivity over the closely related 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors. Activation of 5-HT$_{2A}$ receptors is implicated in a number of adverse events, including hallucination and cardiovascular effects.[3] In addition, peripheral 5-HT$_{2B}$ receptor stimulation is associated with cardiac valvulopathy and heart disease in humans.[4] Designing compounds with potent 5-HT$_{2C}$ agonist activity and excellent selectivity over 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors would form a critical part of our drug discovery program. Another key medicinal chemistry design criterion was good blood–brain barrier (BBB) penetration. A number of in silico and in vitro assays have been used to estimate the ability of a compound to cross the BBB.[5]

A widely accepted in vitro system involves measuring membrane permeability and efflux across MDCK cells which overexpress the MDR-1 gene encoding the efflux transporter P-glycoprotein (P-gp).[6]

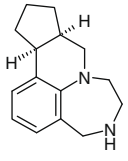
In addition to screening compounds in the MDCK MDR-1 assay we also employed an in silico model of MDCK MDR-1 efflux ratio (cMDR BA/AB), which proved to be a powerful tool to guide medicinal chemistry design, ensuring that the majority of targets synthesized had minimal P-gp mediated efflux.[7]

There have recently been a number of disclosures highlighting the discovery of selective 5-HT$_{2C}$ agonists, including the azepines lorcaserin **1**,[8] and vabicaserin **2**[9] (Fig. 1). We were attracted to the azepine class of 5-HT$_{2C}$ agonists for a number of reasons: (i) this class had demonstrated encouraging levels of 5-HT$_{2B}$ selectivity, (ii) this series had delivered molecules into the clinic, (iii) structural rigidity that restricts the number of readily available conformations may decrease metabolism and deliver low dose and high bioavailability,[10] (iv) we saw opportunities to further explore this series by introduction of heterocycles **3** via the key intermediate **4** (Fig. 2).[11] From this work a sub-series of tricyclic azole-azepines emerged with potent 5-HT$_{2C}$ agonist activity, and excellent 5-HT$_{2A}$ and 5-HT$_{2B}$ selectivity, along with encouraging efficacy in a dog model of SUI.[12]

Heterocycle-fused-azepines were prepared via the N-protected β-keto esters **4a–c**.[13] In the synthesis of pyridazino-azepines




**1**: lorcaserin




**2**: vabicaserin

**Figure 1.** Structures of selective 5-HT$_{2C}$ agonists in clinical development.

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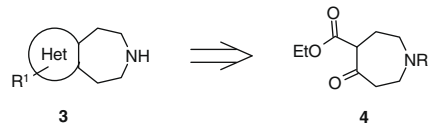
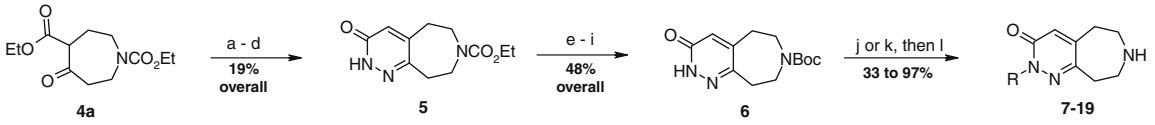



**Figure 2.** Design hypothesis and retrosynthesis of proposed 5-$HT_{2C}$ agonists.

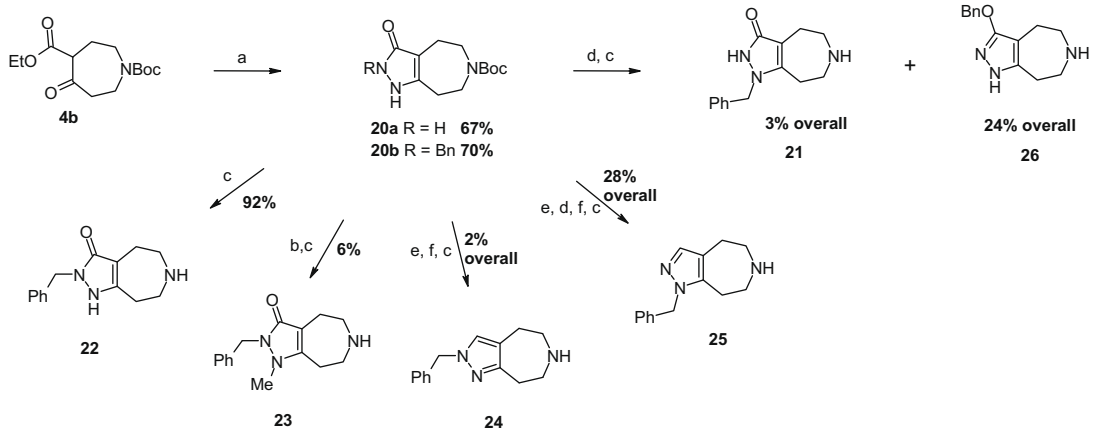
**7–19**, homologation of the ester in **4a** was achieved by alkylation with ethyl bromoacetate, followed by decarboxylation. Treatment of the γ-keto-ester with hydrazine followed by oxidation with bromine yielded pyridazinone **5**. The ethyl carbamate protecting group was replaced with Boc at this point for ease of removal at the end of the synthesis. Treating pyridazinone **6** with the appropriate alkyl/benzyl halides afforded compounds **7** and **9–19** after Boc-deprotection. Copper-catalyzed arylation, followed by Boc removal gave compound **8** (Scheme 1).[14]

The syntheses of pyrazolo-azepines **21–26** is summarized in Scheme 2. Treating **4b** with the appropriate hydrazine yielded pyrazolones **20a** and **20b**. Boc-deprotection of pyrazolone **20b** gave compound **22**. N-methylation of **20b** followed by Boc removal yielded compound **23**. Triflation, palladium-catalyzed reduction and deprotection of **20b** afforded pyrazole **24**. For compound **25**, pyrazolone **20a** was first triflated, followed by benzylation, which yielded exclusively the N1-benzylated isomer. Reduction of the triflate followed by deprotection afforded the desired compound. Benzylation of **20a** yielded a mixture of N1-benzylated and O-benzylated isomers, which after deprotection, furnished compounds **21** and **26**, respectively. Although many of the unoptimized yields of compounds **21–26** were low, the routes were sufficient to deliver enough material to test in the desired assays.

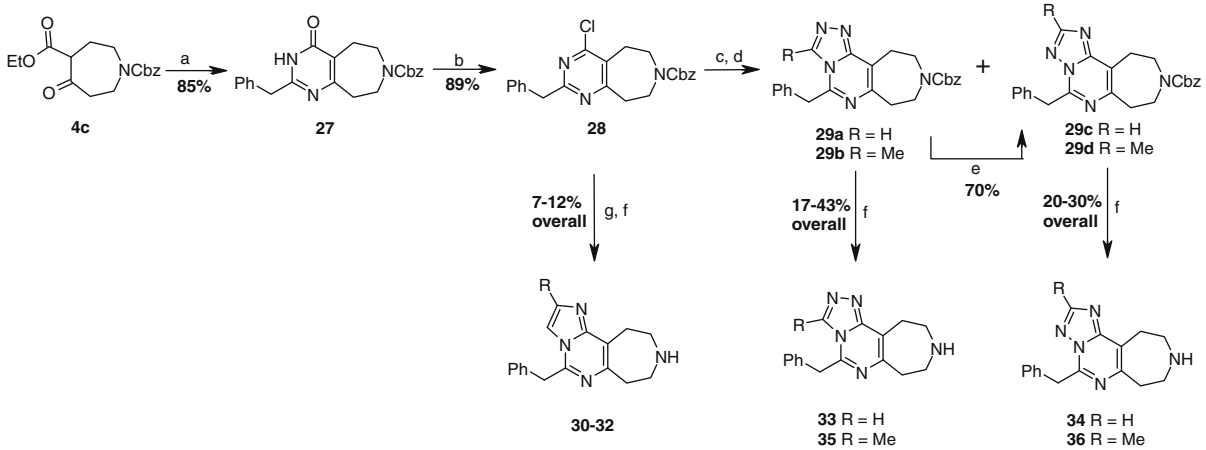
For the syntheses of tricyclic azole-azepines, chloropyrimidine **28** was the key intermediate (Scheme 3). Intermediate **28** was easily prepared from cyclization of β-keto ester **4c** with benzyl ami-




**Scheme 1.** Reagents and conditions: (a) NaOEt, ethyl bromoacetate; (b) (i) HCl reflux; (ii) ethylchloroformate, $K_2CO_3$; (c) $NH_2NH_2$, EtOH; (d) $Br_2$, $CHCl_3$; (e) $POCl_3$; (f) NaOMe, MeOH; (g) KOH, MeOH; (h) HBr, AcOH (i) $Boc_2O$, TEA, $CH_2Cl_2$; (j) R-Br, $K_2CO_3$; (k) CuI, PhI, *trans*-1,2-diaminocyclohexane; (l) HCl, dioxane.

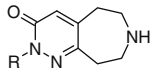



**Scheme 2.** Reagents and conditions: (a) $RNHNH_2$, EtOH; (b) MeI, $K_2CO_3$; (c) HCl; (d) BnBr, *t*BuOK; (e) $PhNTf_2$, py; (f) formic acid, $Pd(OAc)_2$, DMF.




**Scheme 3.** Reagents and conditions: (a) $BnC(NH)NH_2$, MeOH; (b) $POCl_3$; (c) hydrazine, EtOH; (d) $RC(OEt)_3$; (e) NaOEt, EtOH; (f) HBr; (g) (i) $NH_3$, MeOH; (ii) $RCOCH_2X$.

**Table 1**
5-$HT_2$ activity[a] and ADME properties of 4H-pyridazo[4,5-*d*]azepin-3-ones **7–19**



| Compd | R | $EC_{50}$ nM ($E_{max}$)[b] | | Log $D_{7.4}$ | HLM $Cl_{int}$ (μl/min/mg protein) | MDCK MDR-1 AB $P_{app}$ ($\times 10^{-6}$ cm/s) | MDCK MDR-1 BA/AB | cMDR1 BA/AB |
|---|---|---|---|---|---|---|---|---|
| | | 5-$HT_{2C}$ | 5-$HT_{2B}$ | | | | | |
| **5-HT** | — | 25 (98%) | 11 (91%) | NT[c] | NT | NT | NT | NT |
| **mCPP** | — | 170 (71%) | 125 (35%) | NT | NT | NT | NT | NT |
| **7** | Me | 338 (52%) | (32%)[d] | NT | NT | NT | NT | NT |
| **8** | Ph | >10,000 | >10,000 | −0.3 | NT | NT | NT | 1.0 |
| **9** | $PhO(CH_2)_2$ | >10,000 | >10,000 | 0.4 | <7 | NT | NT | 1.6 |
| **10** | Isopentyl | 62 (77%) | (65%) [d] | 0.6 | <7 | NT | NT | 1.0 |
| **11** | Bn | 31 (79%) | 358 (48%) | 0.3 | <7 | 25 | 1.0 | 1.2 |
| **12** | *o*-Cl–Bn | 33 (56%) | 94 (67%) | 0.9 | <7 | NT | NT | NT |
| **13** | *m*-Cl–Bn | 30 (70%) | 81 (73%) | 1.1 | <7 | 32 | 1.2 | 1.4 |
| **14** | *p*-Cl–Bn | 31 (40%) | 267 (73%) | 1.1 | <7 | NT | NT | NT |
| **15** | *p*-CN–Bn | 6630 (51%) | (42%) [d] | −0.1 | <7 | 11 | 1.7 | 1.7 |
| **16** | *p*-$CF_3$–Bn | 125 (47%) | 473 (59%) | 1.1 | 10 | NT | NT | 1.4 |
| **17** | *p*-$OCF_3$–Bn | 48 (66%) | >10,000 (58%) | 1.5 | <7 | NT | NT | 1.6 |
| **18** | *o*-$OCF_3$–Bn | 107 (57%) | 142 (86%) | 1.2 | <7 | NT | NT | 1.7 |
| **19** | *p*-cPr–Bn | 26 (51%) | 84 (34%) | 1.4 | <7 | NT | NT | 1.4 |

[a] See Ref. 11 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] NT denotes not tested.
[d] Activation at 10 μM.

dine, followed by chlorination of the resulting pyrimidinone **27** with $POCl_3$. The chlorine atom in **28** was displaced with hydrazine followed by a ring cyclization with triethyl formate to give a mixture of 1,3,4- and 1,2,4-triazoles (compounds **29a** and **29c**), which upon deprotection gave compounds **33** and **34**. Cyclization with triethyl orthoacetate yielded exclusively the 1,3,4-triazole **35** after deprotection. Base-induced rearrangement of compound **29b** afforded the 1,2,4-triazole **36** after CBz removal. Reaction of compound **28** with ammonia, followed by ring cyclization with chloroacetaldehyde, chloroacetone or bromobutan-2-one yielded the imidazoles **30–32** after CBz removal.

The 5-$HT_{2C}$ agonist activity of the target compounds was evaluated by measuring the ability to induce a fluorescent based calcium mobilization signal in a FLIPR assay employing recombinant CHO K1 cells expressing the human 5-$HT_{2C}$ receptor (Table 1).[11] Agonist activity at the 5-$HT_{2B}$ receptor was measured in recombinant cell-based systems expressing the human receptor. Agonist maximum efficacy ($E_{max}$) was calculated in relation to 5-HT. We deemed selectivity over the 5-$HT_{2B}$ receptor to be critical and would only consider developing a compound which had <10% effect at 10 μM. The known hallucinogen *meta*-chlorophenyl-piperazine (mCPP) was used to validate the FLIPR assays and was shown to be a full agonist on 5-$HT_{2C}$ and a partial agonist on 5-$HT_{2B}$ receptors. Relevant pharmacological evaluation for activity at the 5-$HT_{2A}$ receptor proved to be challenging. Recent disclosures by Fish et al.[15] showed that our FLIPR assay employing Swiss 3T3 cells expressing the recombinant human 5-$HT_{2A}$ receptor was a highly expressed/coupled cell-line which over estimated 5-$HT_{2A}$ activity. Consequently, 5-$HT_{2A}$ activity was not measured routinely, with compounds selective over the 5-$HT_{2B}$ receptor being investigated in 5-$HT_{2A}$ tissue assays, which were found to be a better predictor of in vivo outcomes.[16,17]

Our initial targets were designed to fuse a heterocycle to the azepine in order to create a polar core with opportunity for extension. When substituted with a benzyl group pyridazinone **11** was found to be a potent 5-$HT_{2C}$ agonist (Table 1). The compound was stable to human liver microsome (HLM) degradation and was predicted to have good CNS penetration due to symmetric flux in the MDCK MDR-1 assay. Based on the validation of our cMDR BA/AB model we restricted our design of compounds to those with predicted efflux ratio <2.5.[7] The results in Table 1 illustrate the power of the model to predict in vitro efflux in MDCK MDR-1 cells. Although 10-fold selective over 5-$HT_{2B}$, an $E_{max}$ of 48% was still considered too high against this key selectivity target. Substitution for a directly linked phenyl or phenoxyethyl group gave a dramatic loss of potency (compounds **8** and **9**). Truncating the benzyl group to methyl (compound **7**) gave a 10-fold drop in activity. Extending the methyl to longer alkyl chains lost potency except in the case of the isopentyl group; compound **10** was only twofold less potent than **11** but did not have improved 5-$HT_{2B}$ selectivity.

With benzyl determined to be the best substituent, we looked at further substitution in order to improve 5-$HT_{2B}$ selectivity while maintaining potency, metabolic stability and potential for CNS exposure. Substitution on the benzyl group with a chlorine at any aromatic position in compounds **12–14** maintained potency but did not improve 5-$HT_{2B}$ selectivity. Of a range of other substituents examined the most potent were compounds **15–19**, however none offered an advantage over the original unsubstituted benzyl group of compound **11**.

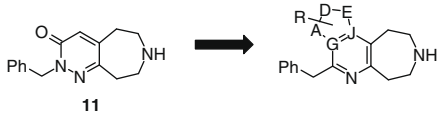
We then investigated alternative heterocycles in place of the pyridazinone in order to find a template with improved selectivity. The pyrazoles and pyrazolones of Table 2 showed that, in general, five-membered heterocycles fused to the azepine were poorly tolerated. The pyrazolones **21–23** were all inactive or weakly active. The only moderately potent compound of this set was alkoxypyrazole **26**. This compound was the undesired side product of alkylation of pyrazolone **20a**.

The pyridazinone and pyrazole derivatives examined so far had limited opportunity for further substitution off the heterocyclic ring. We postulated that a bicyclic heterocyclic system fused to the azepine would offer several positions to append substituents to explore increasing selectivity. In order to keep the key benzyl substituent in a similar orientation to that imposed in pyridazinone **11**, we proposed targets based on generic tricyclic ring

**Table 2**
5-$HT_2$ activity[a] and ADME properties of pyrazolo-azepines **21–26**

| Compd | $EC_{50}$ nM ($E_{max}$)[b] | | Log *D* | HLM $Cl_{int}$ (μl/min/mg protein) | MDCK MDR-1 AB $P_{app}$ ($\times 10^{-6}$ cm/s) | MDCK MDR-1 BA/AB | cMDR1 BA/AB |
|---|---|---|---|---|---|---|---|
| | 5-$HT_{2C}$ | 5-$HT_{2B}$ | | | | | |
| **21** | >10,000 | >10,000 | −0.1 | <7 | NT[c] | NT | 1.2 |
| **22** | >10,000 | >10,000 | −0.5 | 13 | 2 | 1.5 | 1.5 |
| **23** | 2930 (68%) | >10,000 | 0 | <7 | 13 | 1.1 | 1.2 |
| **24** | 4980 (57%) | >10,000 | 0.1 | <7 | 27 | 1.0 | 1.0 |
| **25** | >10,000 | >10,000 | 0.2 | <7 | 31 | 1.0 | 1.0 |
| **26** | 176 (65%) | 353 (56%) | 0.5 | <7 | 24 | 1.0 | 1.1 |

[a] See Ref. 11 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] NT denotes not tested.



**11**

**Figure 3.** Generic structure of proposed tricyclic target molecules.

system shown in Figure 3. Of the many possibilities, the imidazo- and triazolopyrimidines were selected for a mixture of predicted physical chemical properties (*c* log *P*, *c* p$K_a$), MW and synthetic accessibility. The unsubstituted fused imidazole **30** lost all measurable activity on 5-$HT_{2C}$ (Table 3). Extending from the 4-position of the imidazole moiety with methyl and then ethyl to give compounds **31** and **32** gave some potency without bringing in 5-$HT_{2B}$ activity but the compounds were still less potent than required. However, we were pleased to find that the unsubstituted triazolopyrimidines showed decreased 5-$HT_{2B}$ efficacy, close to our requirements of $\leqslant$ 10% $E_{max}$ at 10 μM, without sacrificing 5-$HT_{2C}$ affinity. It was not surprising that the two unsubstituted triazole isomers **33** and **34** had identical pharmacological and physical properties due to their almost identical size and shape. When either isomer was extended by a methyl group to give compounds **35** and **36** we observed only a slight drop in potency and complete selectivity over 5-$HT_{2B}$ with $E_{max}$'s within error of 0% at 10 μM. The methyl-substituted triazoles had good HLM stability and were not P-gp substrates. Both isomers were progressed to a dog bladder strip in vitro assay to examine 5-$HT_{2A}$ activity and were shown to be weak partial agonists.

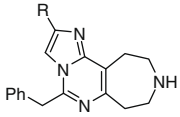
**Table 4**
Profile of compound **36**

| In vivo efficacy ($n$ = 2) | hERG $K_i$ (μM) | Wide ligand selectivity ($K_i$ μM) | CYP inhibition (at 3 μM) |
|---|---|---|---|
| 20% Increase in PUP at 85–109 nM free plasma | >22 | 5-$HT_3$ 3.7<br>$\beta_2$ 7.6<br>$M_3$ 8.2 | <10% vs CYP2D6, 3A4, 2C9, 1A2 |

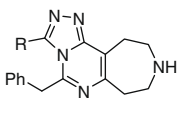
The slightly more potent and selective isomer **36** was tested in a dog peak urethral pressure model (PUP) of stress incontinence (Table 4).[12] When **36** was dosed by iv infusion (150 μg/kg over 60 min), a 20% increase in peak urethral pressure was obtained at a free plasma concentration of 89–105 nM (~2 × $EC_{50}$). Efficacy at low multiples of the in vitro $EC_{50}$ was a good indication that the compound was able to cross the BBB in vivo. An increase in PUP of 20% is a clinically relevant change, as mechanisms which are known to be efficacious against SUI in human (serotonin/noradrenaline reuptake inhibition, $\alpha_{1A}$ agonism) elicited a similar response at clinically relevant drug concentrations.[12]

With an encouraging profile, compound **36** was assessed further (Table 4). In vitro measures of displacement of labeled dofetilide from the hERG channel showed low risk of affecting the key cardiac ion channel. Screening against a panel of 70 drug targets

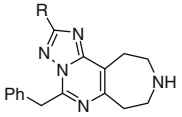
**Table 3**
5-$HT_2$ activity[a] and ADME properties of tricyclic azepines **30–36**



**30-32**



**33, 35**



**34, 36**

| Compd | R | $EC_{50}$ nM ($E_{max}$) [b] | | 5-$HT_{2B}$[d] | Log D | HLM | MDCK MDR-1 AB | MDCK MDR-1 BA/AB | cMDR1 BA/AB |
|---|---|---|---|---|---|---|---|---|---|
| | | 5-$HT_{2C}$ | 5-$HT_{2A}$[e] | | | | | | |
| **30** | H | >10,000 (0%) | NT[c] | 2% | NT | NT | NT | NT | 1.4 |
| **31** | Me | 2360 (35%) | NT | 6% | 1 | <8 | NT | NT | 1.7 |
| **32** | Et | 969 (91%) | NT | 21% | 0.5 | <8 | NT | NT | 1.1 |
| **33** | H | 40 (83%) | NT | 36% | 0.5 | <7 | 27 | 1 | 1 |
| **34** | H | 41 (80%) | NT | 30% | 0.4 | <7 | 30 | 0.9 | 0.9 |
| **35** | Me | 71 (83%) | 1600 (36%) | 2% | 0.7 | <7 | 30 | 1.1 | 1.1 |
| **36** | Me | 52 (82%) | 1800 (40%) | 3% | 0.6 | <7 | 29 | 1.1 | 1.1 |

[a] See Ref. 12 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] NT denotes not tested.
[d] Activation at 10 μM.
[e] 5-$HT_{2A}$ activity in bladder prep.

(receptors, ion channels and enzymes) showed a clean profile with only weak hits at 5-$HT_3$, $\beta_2$ and muscarinic receptors. Compound **36** was not an inhibitor of common CYP's when screened at 3 μM.

In summary, a novel series of heterocyclic fused azepines with potent 5-$HT_{2C}$ agonist activity has been described. SAR of the pyridazinone-fused-azepines was used to optimize the side chain for activity and drug-like properties, and a benzyl group was identified as the best substituent (compound **11**). Other heterocycles were examined to improve selectivity and the 5,6,7-triazole-pyrimidine-azepine system delivered compound **36**, a potent and selective 5-$HT_{2C}$ agonist which also showed efficacy in vivo in a model of SUI. Compound **36** possessed good metabolic stability, good wide ligand selectivity and CNS drug-like properties. The use of an in silico model of MDCK MDR-1 efflux ratio was a useful and reliable predictor of this parameter. This ensured most synthesized analogues possessed good membrane permeability with no P-pg mediated efflux.

## Acknowledgments

We thank Nick Edmunds (Drug Safety), Kerry af Forselles (GU-Biology) and the Primary Pharmacology Group for screening data, Peter Bungay (PDM) for additional studies, Hau Gao for the cMDR efflux model and Alan Jessiman (GU-Chemistry) for the synthesis of compounds **30–32**.

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16. Canine detrusor smooth muscle strips ($2 \times 2 \times 10$ mm) were dissected and placed under 4 g tension in a 5 ml organ bath containing Krebs' solution (with 2.5 mM $CaCl_2$, 1 μM cocaine, 100 nM corticosterone and 1 μM naproxen) at 37 °C. After 1 h equilibration, during which tissues were re-tensioned three times, tissues were twice challenged with 80 mM KCl and then washed. Subsequently cumulative concentration response curves were constructed for agonists over the range 1 nM to 30 μM. Responses were measured isometrically as the mean tension over 30 s using Notocord data capture software. The agonist responses were expressed as a percentage of the second 80 mM KCl maximum response. The agonist concentration–effect curves were analyzed using an in house Excel add-in which fits the data directly with a logistic function, providing the $EC_{50}$ value (the concentration required for an agonist to produce a half-maximal response), the maximum response ($E_{max}$), and Hill coefficient for the curve. The $EC_{50}$ and $E_{max}$ were reported as geometric mean and arithmetic mean, respectively.
17. Several compounds from different chemical series have been evaluated to assess the correlation of performance in our cell-based screen with established in vitro and in vivo models of 5-$HT_{2A}$ agonist activity. For example, compound **37** (structure not shown) gave a response in a recombinant 5-$HT_{2A}$ agonist assay ($EC_{50}$ 68 nM; $E_{max}$ 82%) but a much weaker response in canine bladder ($EC_{50}$ 765 nM; $E_{max}$ 46%). Evaluation of **37** in the rat head-twitch model[18] at 10 mg/kg (po, $n = 8$) gave no response and **37** 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, $n = 4$).
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