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Design and synthesis of pyridazinone-based 5-HT$_{2C}$ agonists

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ABSTRACT

The SAR of a series of pyridazinone derived 5-HT$_{2C}$ agonists has been explored and resulted in identification of a compound with excellent levels of 5-HT$_{2C}$ functional agonism and selectivity over 5-HT$_{2A}$ and 5-HT$_{2B}$. This compound displayed good in vivo efficacy in pre-clinical models of stress urinary incontinence, despite having physiochemical properties commensurate with impaired CNS penetration.

Serotonin (5-HT) is a neurotransmitter which plays an important role in mood, sleep, emesis, sexuality and appetite.[1] 5-HT receptors mediate the effects of serotonin. There are many sub-types of 5-HT receptors and the processes they mediate are quite diverse. The 5-HT$_{2C}$ receptor is found primarily in the brain, and 5-HT$_{2C}$ agonists have the potential for treating a number of conditions including obesity, schizophrenia, sexual dysfunction and urinary incontinence.[2] Our aim was to identify potent 5-HT$_{2C}$ agonists, with selectivity over 5-HT$_{2A}$ and 5-HT$_{2B}$ and good CNS penetration, for potential use in the treatment of urinary incontinence and sexual dysfunction. The selective serotonin and noradrenaline reuptake inhibitor Duloxetine has shown efficacy in reducing episodes of stress urinary incontinence (SUI) in controlled clinical trials.[3] This effect has been hypothesized to be at least partly due to increases in serotonergic and noradrenergic stimulation of neurons within Onuf's nucleus, a key site within the sacral spinal cord which is known to be involved in contraction of the external urethral sphincter.[4] Interestingly pre-clinical studies have indicated a role for 5-HT$_{2C}$ receptors in these pathways. In particular 5-HT$_{2C}$ receptor agonism appears to increase urethral tone in multiple species, suggesting potential utility in the treatment of SUI.[5]

Similarly pre-clinical studies have highlighted a role for 5-HT$_{2C}$ agonism in the control of bladder function[6] and in sexual function in both males and females.[7] Achieving functional selectivity over the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptor sub-types was a key challenge for the project, and particularly important since 5-HT$_{2A}$ agonism is associated with hallucinogenic and cardiovascular effects, while 5-HT$_{2B}$ agonism has been linked to potentially fatal heart valvulopathy in man.[8]

Our SAR studies commenced with the pyrazine lead compound **1**, which originated from in-house work optimizing the known non-selective 5-HT$_{2C}$ agonist *meta*-chlorophenyl piperidine (*m*-CPP) (Fig. 1).[9] Compound **1** is a potent and CNS penetrant 5-HT$_{2C}$ agonist with moderate selectivity over the 5-HT$_{2A}$ sub-type but poor selectivity over 5-HT$_{2B}$. In addition, compound **1** was mutagenic in the in vitro AMES assay.[10]

SAR studies were commenced to look at a range of alternative templates with the aim of identifying a series with an improved in vitro functional selectivity profile over compound **1** and no mutagenic activity. The pyridazinone template as exemplified by compound **2** was identified as meeting the above requirements. Compound **2** is a partial 5-HT$_{2C}$ agonist with excellent functional selectivity over the 5-HT$_{2B}$ receptor sub-type, and displayed no mutagenic activity in the AMES assay. In addition the compound showed improved metabolic stability due to the reduced lipophilicity, but this also resulted in impaired CNS penetration (free plasma concentration:CSF 5:1). Hence SAR work commenced with the aim of maintaining the selectivity seen with compound **2**, while minimizing the potential dose required to achieve efficacy in man, by enhancing potency and preferably also improving CNS penetration. In addition the SAR studies were focused on determining the optimal lipophilicity range to enable good CNS penetration, while maintaining metabolic stability.

The SAR within the pyridazinone series was limited, with the majority of substitutions resulting in a complete loss of 5-HT$_{2C}$

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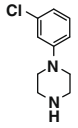
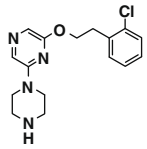


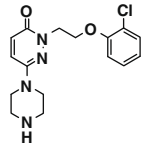
mCPP

5-HT_{2C} EC_{50} (Emax) 170 nM (71%)
5-HT_{2A} EC_{50} (Emax) 167 nM (58%)
5-HT_{2B} EC_{50} (Emax) 125 nM (35%)



1

5-HT_{2C} EC_{50} (Emax) 10 nM (69%)
5-HT_{2A} EC_{50} (Emax) 308 nM (56%)
5-HT_{2B} EC_{50} (Emax) 187 nM (42%)
$LogD_{7.4}$ = 2.2; HLM=94 μl/min/mg
MDCK efflux ratio = 2



2

5-HT_{2C} EC_{50}(Emax) 164 nM (58%)
5-HT_{2A} EC_{50}(Emax) 254 nM (41%)
5-HT_{2B} >10000 nM
$LogD_{7.4}$ = 0.6; HLM<7 μl/min/mg;
MDCK efflux ratio = 5; free plasma: CSF 5:1

Figure 1. Structures and activity data for lead compounds *m*-CPP, **1** and **2**.

agonism. Substitution off the pyridazinone ring with aliphatic groups, including formation of a bicyclic ring, resulted in a loss of 5-HT_{2C} functional agonism (Table 1). *R*-Me substitution was tolerated off the piperidine ring adjacent to the substituted nitrogen (**6**, Table 2), but homologation to an ethyl group resulted in a reduction of activity (**8**).

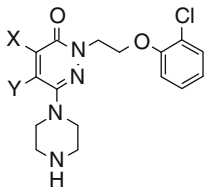
S-Me substitution off the ethyloxy side chain adjacent to the pyridazinone nitrogen resulted in good 5-HT_{2C} agonism (**12**, Table 3), while all other substitutions resulted in a loss of in vitro efficacy. *Ortho* substituents on the phenyl ring were found to be preferable for 5-HT_{2C} functional agonsim over *meta* and *para* substitution, although only a trifluromethyl group resulted in sub 100 nM potency (compound **21**, Table 4). Hence only compounds **6**, **12** and **21** displayed 5-HT_{2C} functional agonism worthy of further investigation.

The most encouraging compounds (compounds **6**, **12** and **21**, Table 5), all showed greater potency in the 5-HT_{2C} functional assay than the lead compound **2**, although they all displayed similar levels of partial intrinisic efficacy. In addition compound **24**, which combined the favorable *R*-Me piperazine substituent with the *ortho*-CF_3 phenyl substituent, showed the highest 5-HT_{2C} functional potency. The increases in functional potency seen with these compounds was achieved through an increase in binding affinity for the 5-HT_{2C} receptor, as demonstrated by the 5-HT_{2C} binding data (Table 5).

As stated earlier, achieving selectivity over the 5-HT_{2A} and 5-HT_{2B} sub-types was a key goal for the project in order to avoid the associated cardiovascular and hallucinogenic side effects. Compounds **6**, **12** and **24** (Table 5) showed low levels of selectivity vs 5-HT_{2A}, although benchmarking using an in vitro 5-HT_{2A} human platelet aggregation biomarker showed increased selectivity.[14] Compound **12** also displayed some functional agonist efficacy at the 5-HT_{2B} receptor which was considered to be a key risk for the compound, while compounds **6**, **21** and **24** showed excellent selectivity over this sub-type. This selectivity was further confirmed using a human colon in vitro biomarker.[15]

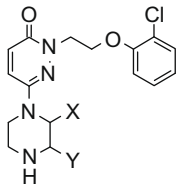
Due to their excellent potency and selectivity, compounds **6** and **24** were progressed. Both compounds displayed similar in vitro ADME data to the lead compound **2** (Table 6) indicating that the compounds should have low hepatic clearance in man, but that impaired CNS penetration would be expected. The project felt that it was unrealistic to look to increase CNS penetration through incorporating further lipophilicity since based on prior SAR this would compromise the excellent selectivity and metabolic stability profile of the compounds. Compound **24** was selected for further profiling since the project felt it had the best balance of potency, selectivity and ADME properties. It was screened for off-target pharmacology against a panel of receptors, enzymes and ion channels (CEREP, Bioprint™) and found to have sub 1 μM binding

Table 1
SAR for compounds **3**–**5**, investigating substituents off the pyridazinone ring



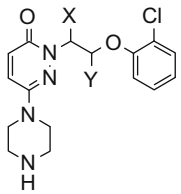
	X	Y	5-HT_{2C} EC_{50} (nM)[11] (E_{max}%)
3	Me	H	>10,000
4	–$(CH_2)_3$–		>10,000
5	H	Me	510 (31)

Table 2
SAR for compounds **6**–**10**, investigating substituents off the piperazine ring



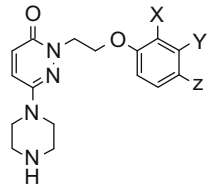
	X	Y	5-HT_{2C} EC_{50} nM[11] (E_{max}%)
6	*R*-Me	H	25 (51)
7	*S*-Me	H	>10,000
8	*R*-Et	H	407 (41)
9	C=O	H	>9980
10	H	*S*-Me	>10,000

Table 3
SAR generated via substituents off the alkyl side chain



	X	Y	5-HT_{2C} EC_{50} nM[11] (E_{max}%)
11	*R*-Me	H	>1920 (55)
12	*S*-Me	H	25 (77)
13	H	*R*-Me	858 (53)
14	H	*S*-Me	360 (87)

Table 4
SAR for compounds **2**, **15**–**23**, investigating substituents off the phenyl ring



	X	Y	Z	5-HT_{2C} EC_{50} nM[11] (E_{max}%)
2	Cl	H	H	164 (58)
15	H	H	Cl	>10,000
16	H	Cl	H	>10,000
17	H	H	H	>10,000
18	Cl	Cl	H	>2130
19	CN	H	H	114 (69)
20	Me	H	H	>4550 (36)
21	CF_3	H	H	47 (45)
22	OMe	H	H	>10,000
23	Br	H	H	101 (34)

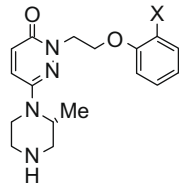
affinity for the human 5-HT_{1B}, 5-HT_{1D} 5-HT_6, B2 and $GABA_A$ receptors. On follow-up using in vitro functional assays, this pharmacology was found not to be significant. Additionally this compound was found to have low activity at the potassium hERG channel (IC_{50} = 3.7 μM). Initial studies in dogs (n = 3) showed a sustained (>20%) increase in peak urethral pressure during continuous i.v. infusion of compound **24**, at mean free plasma concentrations of 52 nM (±17 nM).[16] Further studies are required to fully elucidate the pharmacodynamic effects of this compound.

The main route used to synthesise the pyridazinones described in this paper is shown in Scheme 1. Mono halo displacement on the appropriate dichloro pyridazine template **25**[17] with benzyl or Boc protected piperazine **26** was followed by aqueous acetic acid mediated hydrolysis to give the pyridazinone core in high yield. These hydrolytic conditions also resulted in removal of the Boc protecting group, if present, which was then reintroduced by treatment with Boc anhydride. Incorporation of the side chain via alkylation, followed by deprotection led to the desired products in good yield. Compound **24** was made on multigram scale in an overall yield of 30% using the method outlined in Scheme 1.

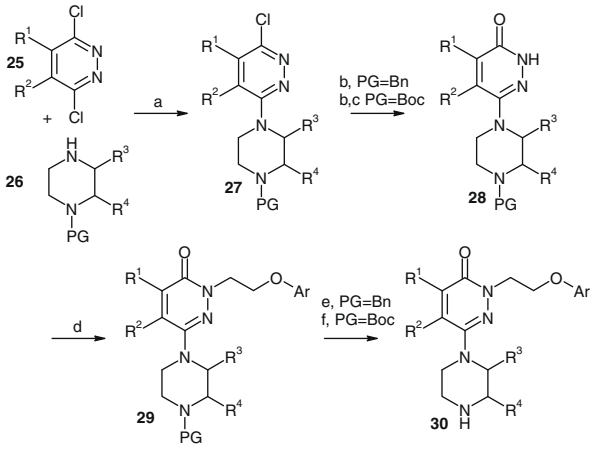
An alternative route was used to synthesise a range of pyridazinones in parallel (Scheme 2). Alkylation of the Boc protected pyridazinones **31** with ethylene carbonate gave the key alcohol intermediates **32**. Treatment of the alcohol **32** with phenols under modified Mitsunobu conditions (using polymer supported triphenylphosphine and ^{t}butyl azodicarboxylate), followed by treatment with HCl in dioxan then gave the desired pyridazinones.

Pyridazinone **5** was synthesised in two steps from chloropyridazinone **34**[18] via N-alkylation with *o*-chlorophenoxyethyl bromide, followed by reaction with piperazine in *tert*-butanol at 120 °C (Scheme 3).

Table 6
In vitro ADME data on compounds **2**, **6** and **24**

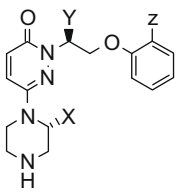


	X	5-HT_{2C} EC_{50} nM[11] (E_{max}%)	MDCK efflux ratio	Log $D_{7.4}$	HLM (μl/min/mg)
2	Cl	341 (55)	5	0.6	<7
6	Cl	25 (51)	6	0.9	9.8
24	CF_3	47 (45)	3	1.4	15.3

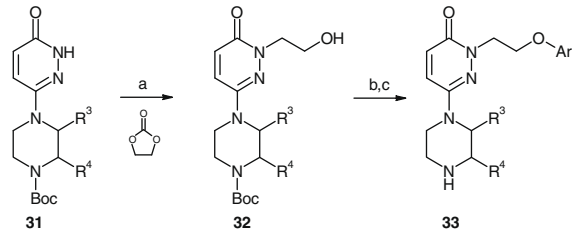


Scheme 1. General synthesis of pyridazinones. PG = BOC or Bn. Reagents and conditions: (a) toluene, Et_3N, reflux (46–83%); (b) AcOH, reflux (42–80%); (c) Boc_2O, CH_2Cl_2 (90–95%); (d) NaHMDS, LiBr, aryloxyethyl bromide or chloride, DMF, 60 °C (50–86%); (e) ACE–Cl, iPr_2NEt, DCM then MeOH, reflux (58–85%); (f) 4 M HCl in dioxan (85–98%).

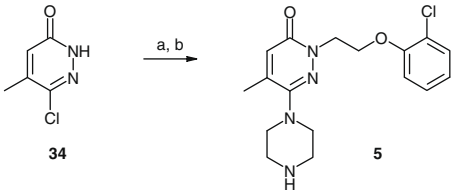
Table 5
Selectivity and binding data for compounds **2**, **6**, **12**, **21** and **24**



	X	Y	Z	5-HT_{2C} EC_{50} nM[11] (E_{max}%)	5-HT_{2C} K_i nM[12]	5-HT_{2A} EC_{50} nM[13] (E_{max}%)	5-HT_{2B} EC_{50} nM[13] (E_{max}%)
2	H	H	Cl	164 (58)	1600	254 (41)	>10,000
6	Me	H	Cl	25 (51)	325	114 (42)	>10,000
12	H	Me	Cl	25 (77)	728	65 (69)	506 (30)
21	H	H	CF_3	47 (45)	424	1090 (49)	>10,000
24	Me	H	CF_3	11 (51)	89	41 (61)	>2610



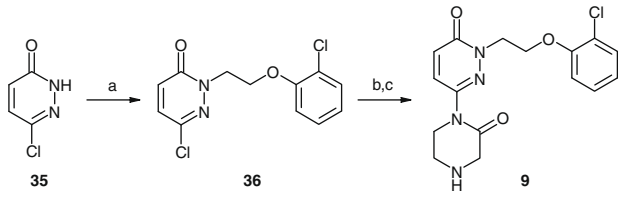
Scheme 2. Alternative pyridazinone synthesis. Reagents and conditions: (a) KOH, DMF, 110 °C (59–70%); (b) phenol, polymer supported PPh_3, ^{t}butyl azodicarboxylate, DCM; (c) 4 M HCl in dioxan (40–75% over two steps).



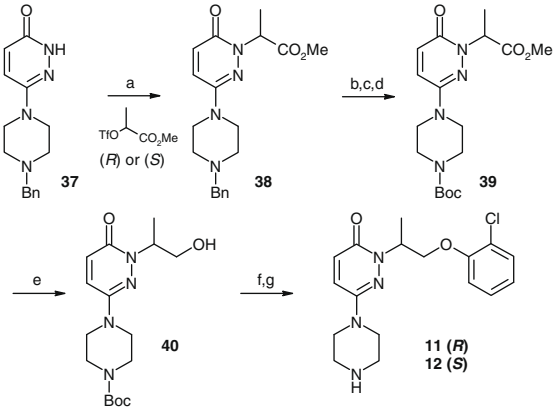
Scheme 3. Synthesis of pyridazinone **5**. Reagents and conditions: (a) NaHMDS, LiBr, *o*-chlorophenoxyethyl bromide, 60 °C (70–80%); (b) piperazine, ^{t}BuOH, 120 °C (reactivial), 3 days (10–20%).

Pyridazinone **9** was synthesised from chloropyridazinone **35** by alkylation with *o*-chlorophenoxyethyl bromide followed by Buchwald–Hartwig amidation and Boc deprotection, albeit in low overall yield (Scheme 4).

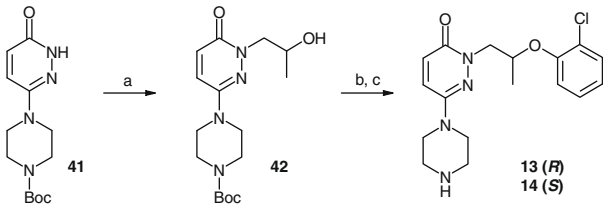
Pyridazinones **11** and **12** were synthesized from **37** by alkylation with the appropriate enantiomer of the triflate of methyl lactate (Scheme 5). Exchange of the nitrogen protecting group from benzyl to Boc was then followed by reduction of the methyl ester



Scheme 4. Synthesis of pyridazinone **9**. Reagents and conditions: (a) NaHMDS, LiBr, *o*-chlorophenoxyethyl bromide, 60 °C (53%); (b) 3-oxo-piperazine-1-carboxylic acid tert-butyl ester, $Pd(OAc)_2$, Cs_2CO_3, Xantphos, toluene, reflux; (c) 4 M HCl in dioxan (10% over two steps).



Scheme 5. Synthesis of pyridazinones **11** and **12**. Reagents: (a) NaH, THF, −40 °C (78%); (b) ACE–Cl, proton sponge, DCM; (c) MeOH (d) Boc_2O, iPr_2NEt CH_2Cl_2 (96% over three steps); (e) DIBAL, toluene, THF (11%); (f) phenol, polymer supported PPh_3, ^{t}butyl azodicarboxylate, DCM; (c) 4 M HCl in dioxan (22% over two steps).



Scheme 6. Synthesis of pyridazinones **13** and **14**. Reagents: (a) (*R*) or (*S*)-propylene oxide, DCM, water, benzyltriethylammonium chloride (70–80%); (b) polymer supported PPh_3, ^{t}butyl azodicarboxylate, phenol, DCM (20–40%); (c) 2 M HCl in MeOH (70–90%).

with DIBAL to give the alcohol **40**. Mitsunobu reaction followed by Boc deprotection then gave **11** or **12**.

Compounds **13** and **14** were synthesised from **41** by reaction with the appropriate enantiomer of propylene oxide under phase transfer conditions to give alcohol **42**, followed by Mitsunobu reaction and Boc deprotections (Scheme 6).

In summary, extensive SAR exploration of the pyridazinone template resulted in identification of compound **24**, as a potent, partial 5-HT_{2C} functional agonist with excellent selectivity over 5-HT_{2B} and other aminergic GPCRs. This compound resulted from optimization of the lead compound 2, through designing partial agonist compounds with increased binding affinity at the 5-HT_{2C} receptor. However, compound **24** did not have physiochemical properties commensurate with enhanced brain penetration compared to compound **2**. Despite this, compound **24** demonstrated good in vivo efficacy in pre-clinical models of stress urinary urge incontinence as a result of its excellent potency. In addition, compound **24** was clean in the AMES assay. Compound **24** was progressed for further studies, the results of which will be published in due course.

Acknowledgments

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