Bioorganic & Medicinal Chemistry Letters 21 (2011) 34–37

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel 6,7,8,9-tetrahydro-5*H*-1,4,7,10a-tetraaza-cyclohepta[*f*]indene analogues as potent and selective 5-HT$_{2C}$ agonists for the treatment of metabolic disorders ☆

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ARTICLE INFO

Article history:
Received 21 October 2010
Revised 17 November 2010
Accepted 18 November 2010
Available online 24 November 2010

Keywords:
5HT2C agonist
Metabolic disease
hERG inhbiiton

ABSTRACT

The discovery of a novel series of 5-HT$_{2C}$ agonists based on a tricyclic pyrazolopyrimidine scaffold is described. Compounds with good levels of in vitro potency and moderate to good levels of selectivity with respect to the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors were identified. One of the analogues (**7g**) was found to be efficacious in a sub-chronic weight loss model. A key limitation of the series of compounds was that they were found to be potent inhibitors of the hERG ion channel. Some compounds, bearing polar side chains were identified which showed a much reduced hERG liability however these compounds were sub-optimal in terms of their in vitro potency or selectivity.

The serotonin 5-HT$_{2C}$ receptor is one of 14 serotonin receptor sub-types. The 5-HT$_2$ sub-family is comprised of 5-HT$_{2A}$, 5HT$_{2B}$ both expressed in the CNS and peripherally and 5-HT$_{2C}$ which is thought to be expressed solely in the CNS. The development of small molecule 5-HT$_{2C}$ receptor agonists has attracted a high level of interest in recent years offering the potential for the treatment of wide ranging conditions such as obesity, schizophrenia, sexual dysfunction, urinary incontinence and, more recently, diabetes. Efficacy in pre-clinical animal models has been reported for several compounds some of which have progressed into human clinical trials.[1]

One of the key issues for developing viable 5-HT$_{2C}$ receptor agonists is the requirement for high levels of selectivity over the other 5-HT$_2$ receptors. 5-HT$_{2A}$ agonism can result in CNS mediated hallucinogenic as well as cardiovascular side-effects.[2] 5HT$_{2B}$ agonists have recently been shown to be responsible for cardiac valvulopathy effects and are also implicated in pulmonary hypertension.[3]

Many of the 5-HT$_{2C}$ agonists described in the literature possess a fused arylazepine motif (Fig. 1).[1,4,5] Taking this feature as a starting point we focussed on the development of a novel tricyclic scaffold (**1**) containing a fused pyrazolopyrimidine as the aryl motif. Our primary objective was to develop potent (<100 nM EC$_{50}$) and selective (>100-fold based on EC$_{50}$ vs. 5-HT$_{2A}$/5-HT$_{2B}$ or no intrinsic agonist activity at either receptor) 5-HT$_{2C}$ agonists derived from this scaffold. An additional requirement was to identify compounds with acceptable PK profiles suitable for use in the treatment of metabolic disorders such as obesity or diabetes.

Synthesis of the scaffold (**5**) was achieved in four steps starting with the ring expansion of *N*-Boc piperidinone[6] to give the keto-ester (**2**) as a key cyclization precursor (Scheme 1). Reaction of (**2**) with 2-aminopyrazole under acidic conditions led to the formation of the tricyclic scaffold which was then chlorinated using $POCl_3$. The crude product was then re-protected using Boc_2O to furnish the building block (**5**) in good yield (41% over the 4 steps).

Derivatization of the scaffold (**5**) was readily achieved by reaction with primary or secondary amines in ethanol solvent at 80 °C followed by acid mediated de-protection to furnish the target compounds (**6–10**) as their TFA salts.

☆ Published in part: Heather Tye, Stephan G. Mueller, Juergen Prestle, Stefan Scheuerer, Marcus Schindler, Bernd Nosse, Natacha Prevost, Christopher J. Brown, Alexander Heifetz, Clemens Moeller, Anna Pedret-Dunn, Mark Whittaker, Abstracts of Papers, 239th ACS National Meeting, San Francisco, CA, United States, March 21–25, 2010 (2010), MEDI-213.

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doi:10.1016/j.bmcl.2010.11.089

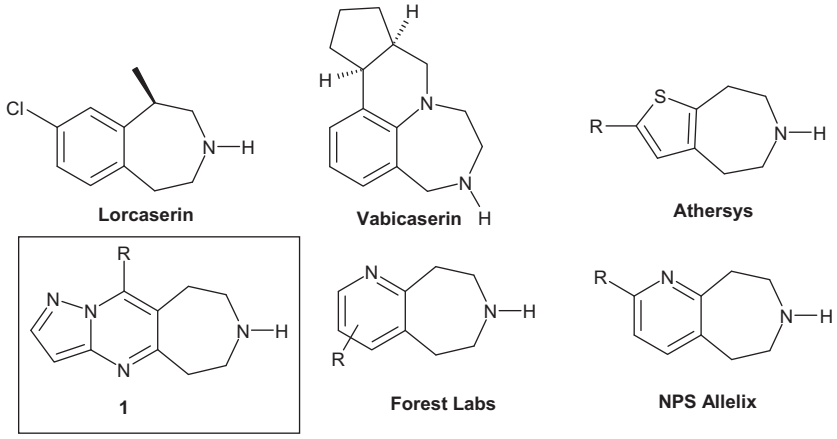
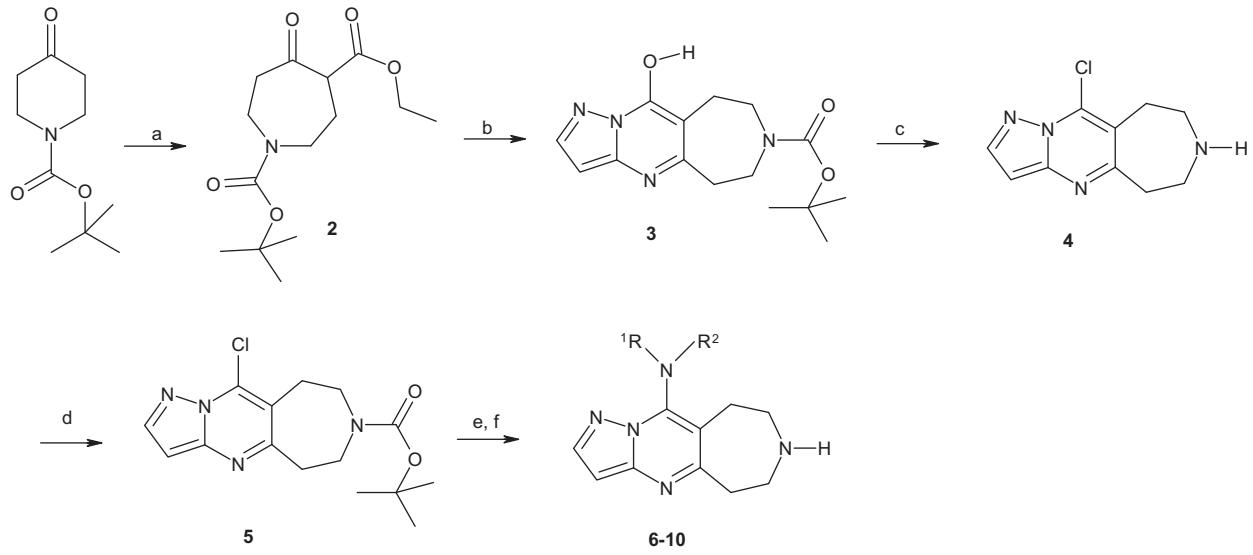


Figure 1. 5-HT_{2C} agonists containing a fused arylazepine motif.



Scheme 1. Synthesis of pyrazolopyrimidine azepine analogues. Reagents and conditions: (a) ethyl diazoacetate, $BF_3{\cdot}Et_2O$; (b) aminopyrazole, AcOH, 80 °C ; (c) $POCl_3$, iPr_2EtN, 90 °C; (d) Boc_2O, Et_3N, DCM; (e) R^1R^2NH, EtOH, iPr_2EtN, 80 °C; (f) TFA/DCM.

The agonist activity of target compounds was evaluated in vitro using a FLIPR based assay employing recombinant CHO cells expressing the human 5-HT_{2A}, 5-HT_{2B} or 5-HT_{2C} receptors.[7]

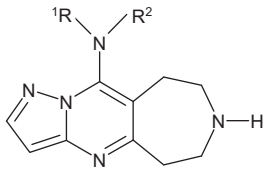
A preliminary exploration of simple amine analogues (Table 1) led to the identification of azetidine derivative (**6a**) as a potent 5-HT_{2C} agonist with acceptable selectivity over 5-HT_{2A} (>100-fold, partial agonist) but poor selectivity over 5-HT_{2B} (<20-fold, full agonist). Taking this compound as a starting point we explored a number of substituted azetidines which led to the discovery of phenyl ether (**7a**) which retained a good level of potency and 5-HT_{2A} selectivity and had a reduced level of activity at 5-HT_{2B} (partial agonist).

We then embarked on the synthesis of a library of aryl ether analogues. Data for a sub-set of compounds (**7a–g**) from the library is shown in Table 2. Aryl groups bearing electron withdrawing groups (e.g., F) tended to retain good potency whereas compounds possessing electron donating groups (e.g., OMe) tended to be less potent. Whilst all analogues retained a good level of 5-HT_{2A} selectivity the position of the substituent on the aryl ring had varying effects on the 5-HT_{2B} selectivity, substitution at the 3- or 4-position giving the best results.

Compounds from this first library were profiled in additional in vitro ADME-Tox assays including permeability (Caco-2) and hERG assays. Whilst all of the analogues tested showed good levels of Caco-2 permeability they exhibited strong levels of hERG inhibition (>80% at 10 μM). This finding was not entirely surprising considering the compounds have structural features which match quite well with established hERG pharmacophores[8] and their lipophilicity is in an undesirable range (*c* Log *P* >3). This is also consistent with data published recently by Fish et al. who reported high levels of hERG inhibition for compounds bearing a related azetidine aryl ether motif.[9]

In an attempt to address the hERG liability for this series a second library of compounds was prepared incorporating polar groups on the aryl ring or replacing the aryl ring with heteroaryls in order to reduce lipophilicity (Table 2). 2-Pyridyl analogue (**8a**) retained potency and showed a slight reduction in hERG inhibition, however the 3 and 4-pyridyl analogues (**8b, 8c**) gave a significant loss in potency. The best results were achieved with acetamide (**9**) and indolone (**10**) which showed much reduced hERG activity. Unfortunately these compounds exhibited a drop in potency or selectivity and, in the case of the acetamide (**9**), a significant drop

Table 1
5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} agonist activity for compounds **6a–e**

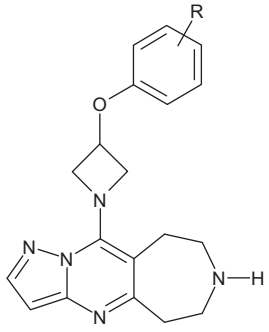


6a-e

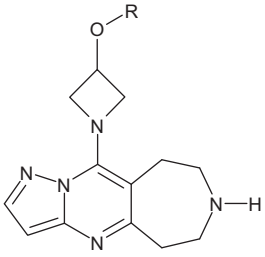
Compound	R^1	R^2	5-HT_{2C}[a]		5-HT_{2A}[a]		5-HT_{2B}[a]	
			EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
6a	Azetidine		5.4	103	2348	51	94	97
6b	Pyrrolidine		764	110	2310	15	2242	36
6c	Piperidine		4564	139	585	10	2848	28
6d	Me	Et	362	105	nd	nd	842	69
6e	H	Et	63	104	138	62	602	97

[a] Values reported as an average of $n = 2$ measurements (in triplicates) where SEM is typically below 20%.

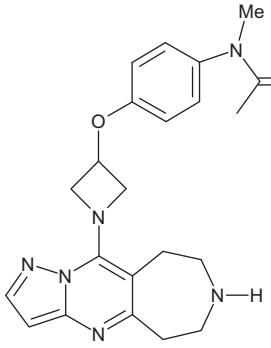
Table 2
5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} agonist activity and in vitro ADMET data for compounds **7–10**



7a-g



8a-c



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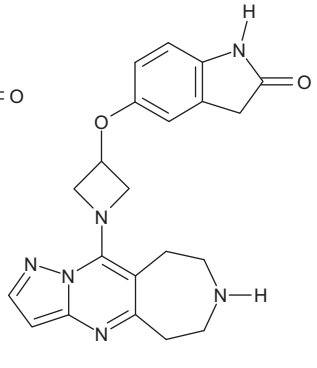

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Compound	R group	5-HT_{2C}[a]		5-HT_{2A}[a]		5-HT_{2B}[a]		hERG[b] (% inhib @ 10 μM)	*c* Log *P*[c]	Caco-2[d]	
		EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)			(A–B) × 10^{-6} cm/s	Efflux ratio
7a	H	18	95	7774	22	218	58	81 (5)	3.1	13.3	2.3
7b	2-F	5	85	648	15	94	69	nd	3.2	nd	nd
7c	3-F	25	78	32	5	13	23	93 (1)	3.4	7.9	1.6
7d	4-F	23	95	3	8	163	43	95 (2)	3.4	nd	nd
7e	2-MeO	84	84	2676	48	379	89	88 (1)	2.8	nd	nd
7f	3-MeO	65	88	40	9	215	43	97 (1)	3.2	10.1	2.1
7g	4-MeO	95	76	2634	21	503	31	93 (1)	3.2	10.4	1.3
8a	2-Pyridyl	37	93	5165	29	706	71	78 (4)	2.5	10.3	2.2
8b	3-Pyridyl	211	99	63	94	1478	73	nd	2.1	nd	nd
8c	4-Pyridyl	574	102	1227	27	1158	79	nd	2.1	nd	nd
9	—	188	96	2046	35	1184	60	5 (1)	2.2	0.62	6.9
10	—	8.4	99	762	86	73	82	11 (2)	1.8	nd	nd

[a] Values reported as an average of $n = 2$ measurements (in triplicates) where SEM is typically below 20%.
[b] Whole-cell potassium currents were investigated electrophysiologically by means of the patch-clamp technique in CHO cells using PatchXpress. Average leak- and run-down-corrected current inhibitions, measured upon application of the test compound at 10 μM, ($n = 2–4$, ±SD is given in brackets).
[c] *c* Log *P* values calculated using BioByte software.
[d] Caco-2 A–B flux values reported as an average of two measurements with SD typically in the range of ±30% or less. Efflux ratio expressed as a ratio of the B–A/A–B values.

Table 3
Rat PK data for compound **7g** (iv—male Wistar rats ($n = 2$), dose 10 mg/kg; po—fasted male Wistar rats ($n = 3$), dose 30 mg/kg)

Compound	Cl (ml/min/kg)	V_{ss} (L/kg)	MRT (iv, h)	MRT (po, h)	AUC dn (po, nMh)	CSF/Plu ratio[a]
7g	14	11	13	>24	720	0.15

[a] CSF and plasma levels measured at 2 h post dose after 30 mg/kg po administration. Plu is fraction unbound in plasma based on % unbound in rat plasma as determined by equilibrium dialysis method (data not shown).

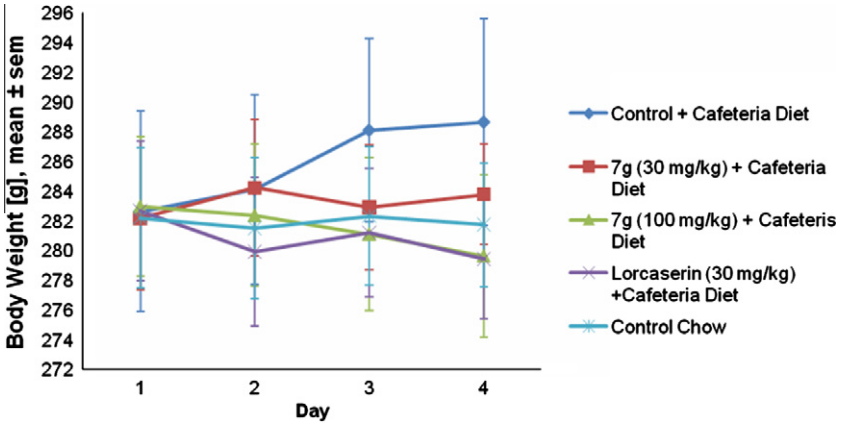

Figure 2. Data for compound (**7g**) and Lorcaserin in the 3 day cafeteria diet model.

in Caco-2 permeability compared to earlier analogues. Consequently these analogues were not considered to be suitable for further profiling.

At this stage we were looking to identify a suitable tool compound to establish whether compounds of this structural class would be efficacious in vivo. Despite its high level of hERG inhibition (0.4 μM in manual patch-clamp) 4-MeO analogue (**7g**) showed the best overall profile. It met the potency and selectivity criteria we had set based on its in vitro profile (<100 nM 5-HT_{2C}, weak partial agonism at 5-HT_{2A} and 5-HT_{2B}) and it was found to be neutral at 5-HT_{2A} and an antagonist at 5-HT_{2B} in ex vivo tissue assays.[10,11] In addition this compound exhibited an excellent rat PK profile (Table 3) having low clearance and long exposure after oral administration (MRT >24 h). Based on the good CSF/Plasma-unbound ratio of 0.15 we expected this compound to be brain penetrant and therefore effective in vivo.

Compound (**7g**) was found to efficacious in a 3 day weight loss experiment where the compound was administered orally to rats fed on a so-called 'cafeteria' chocolate based diet. Statistically significant weight loss of 3.3% ($P = 0.0014$) relative to the cafeteria fed control group was observed at a dose of 100 mg/kg which compared well to the 3.2% ($P = 0.06$) weight loss observed with Lorcaserin dosed at 30 mg/kg (Fig. 2).

Female Wistar rats (ex-breeder; $n = 6$ each group) were fed a cafeteria diet for three consecutive days. The diet consisted of different chocolate bars and cookies add libitum on top of standard chow diet. Animals were randomized to body weight at day one. Compound **7g**, Lorcaserin, and vehicle control (0.5% natrosol) was applied once daily (po application) and body weight was assessed every day. Control chow received standard chow diet only.

In summary we have identified a novel series of 5-HT_{2C} agonists based on a tricyclic pyrazolopyrimidine scaffold. Compounds with good levels of in vitro potency and moderate to good levels of selectivity with respect to the 5-HT_{2A} and 5-HT_{2B} receptors were identified. One of the analogues (**7g**) was found to be efficacious in a sub-chronic weight loss model. However a key limitation of the series of compounds was found to be their inhibition of the hERG ion channel. Whilst some compounds, bearing polar side chains did show a much reduced hERG liability this came at the expense of potency and permeability. Further research efforts are focussed on identifying compounds which are not limited by such off target activity issues.

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