Bioorganic & Medicinal Chemistry Letters 21 (2011) 58–61

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Novel serotonin type 3 receptor partial agonists for the potential treatment of irritable bowel syndrome

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ARTICLE INFO

Article history:
Received 24 September 2010
Revised 12 November 2010
Accepted 16 November 2010
Available online 21 November 2010

Keywords:
5-HT_3 receptor partial agonist
Irritable bowel syndrome
IBS
Ligand-gated ion channel
Ramosetron
Alosetron
Indole
Indazole

ABSTRACT

Serotonin type 3 (5-HT_3) receptor partial agonists are being targeted as potential new drugs for the treatment of irritable bowel syndrome (IBS). Two new chemical series bearing indazole and indole cores have exhibited nanomolar binding affinity for the *h*5-HT_3A receptor. A range of partial agonist activities in HEK cells heterologously expressing the *h*5-HT_3A receptor were measured for the indazole series. Excellent 5-HT_3 receptor selectivity, favorable in vitro metabolic stability and CYP inhibition properties, and good oral in vivo potency in the murine von Bezold–Jarisch reflex model is exemplified thereby indicating the series to have potential utility as improved IBS agents.

Irritable bowel syndrome (IBS) affects as many as 10% of the US adult population, with symptoms ranging from constipation to diarrhea or a combination of the two, coupled with severe abdominal pain and discomfort.[1] Direct medical care costs in the US have been estimated to be as high as $8 billion per year though only 6% of the costs are attributed to medication, implying that there is significant unmet medical need in IBS therapy.[2] While the underlying cause of IBS is currently unknown, the typical symptoms of pain and altered bowel habits suggest a potential dysfunction of neural pathways involved in sensory and/or motor function processing.

Serotonin, a key neurotransmitter synthesized and stored in the GI tract, plays a central role in the normal function of the gut and has been reported in abnormal levels in IBS patients.[3] Acting at multiple receptor subtypes, serotonin activates both enteric and central neurons and gastrointestinal tract smooth muscle, which can ultimately lead to gut movement and/or the perception of pain and discomfort.[4] One type of serotonin receptor, the 5-HT_3 receptor, is located on vagal afferents within the intestinal wall and can be activated by serotonin release induced, for example, by stress or in response to intracolonic pressure.[5]

Among its pharmacological actions, the 5-HT_3 receptor has shown that it can affect intestinal transit, small bowel secretion and the perception of visceral pain.[6] In fact, antagonism of the 5-HT_3 receptor represents one of the few clinically validated and effective strategies for the symptomatic treatment of diarrhea predominant IBS (IBS-D). Unfortunately, broad utilization of 5-HT_3 receptor antagonists in IBS therapy has been hampered due to rare occurrences of severe constipation and ischemic colitis associated with alosetron, the earliest pharmaceutical product introduced in this class.[7] Ramosetron hydrochloride, a 5-HT_3 receptor antagonist launched in 2008 in Japan, has no reports of the same serious adverse events in IBS patients thereby demonstrating that safer 5-HT_3 receptor modulators can be achieved.[8]

Partial activation of ligand gated ion channels is an established drug discovery strategy and principally employed to improve side effect profiles of first generation ligands.[9] A high affinity 5-HT_3 receptor partial agonist is predicted to attenuate 5-HT_3 receptor function in the presence of excessive endogenous serotonin, yet maintain a basal level of receptor activity. The preservation of a

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0960-894X/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2010.11.080

Figure 1. Preferred functional activity of 5-HT_3 receptor ligands for IBS.

modicum of 5-HT_3 receptor function is predicted to reduce the risk of constipation and possible other side effects associated with full receptor inhibition in IBS-D patients. Further, by tuning the intrinsic activity of a partial agonist through chemical modification, it may be possible to identify compounds that will treat a range of IBS symptom classes (Fig. 1).[10]

Herein we report the discovery of two new series of heterocyclic compounds derived from indole and indazoles, which have shown early promise toward achieving our goal to identify a 5-HT_3 receptor partial agonist drug candidate suitable for the treatment of IBS (Fig. 2).

The general synthesis of indazole analogs, **5–12**, is shown in Scheme 1. The commercial 3-formyl indazoles **1** were either alkylated or arylated and then subjected to reductive amination conditions using either (*R*)- or (*S*)-3-aminoquinuclidine to provide aminomethyl indazoles **3a,b**. The methyl ester was hydrolyzed and then cyclized using HBTU to provide the desired indazoles **5–12**.

The synthesis of a related indole analog is shown in Scheme 2. Mannich coupling to methyl 1-methyl-1*H*-indole-4-carboxylate (**13**) using formaldehyde and either (*R*)- or (*S*)-3-aminoquinuclidine provided aminomethyl indole **14a,b**. As in the synthesis of the indazoles, the methyl ester was hydrolyzed using lithium hydroxide, followed by cyclization to the desired indoles **16a,b**.

Novel indole and indazole-derived heterocycles have been identified and found to exhibit potent in vitro *h*5-HT_3A receptor binding affinity (Table 1).[11] On the indazole core, substituents at the R^2 position are well tolerated, with only a slight decrease in affinity observed when substituted phenyl substituents were incorporated at R^2 (**12a,b**). Fluorine was tolerated at R^1. Several pairs of enantiomers were examined and, in general, the binding affinities were equivalent between the two.

A number of analogs were selected for assessment of functional activity in HEK293 cells expressing the *h*5-HT3A receptor subunit. In order to confirm the agonist activity of compounds with small signals, compounds were tested for their ability to evoke 5-HT_3 receptor mediated responses with and without addition of the positive allosteric modulator, 5-chloroindole, which has been used to magnify partial agonist responses of the 5-HT_3 receptor, but has no effect on 5-HT_3 receptor antagonists.[12] The results for select analogs are shown in Table 2. Two pairs of enantiomers (**5a,b** and **6a,b**) suggest that stereochemistry plays a role in dictating the extent of partial agonist activity of the scaffold. While the (*R*)-enantiomer **5a** exhibited a strong partial agonist response both with and without 5-chloroindole, (*S*)-enantiomer (**5b**) only demonstrated a partial agonist response when potentiated with 5-chloroindole.

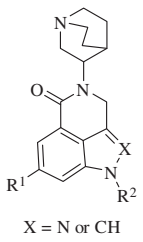
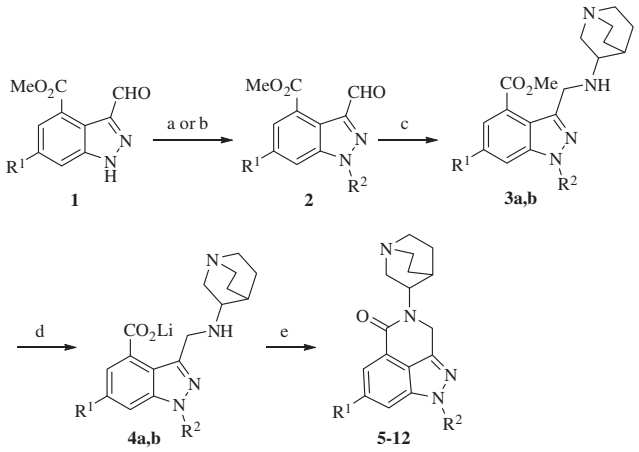
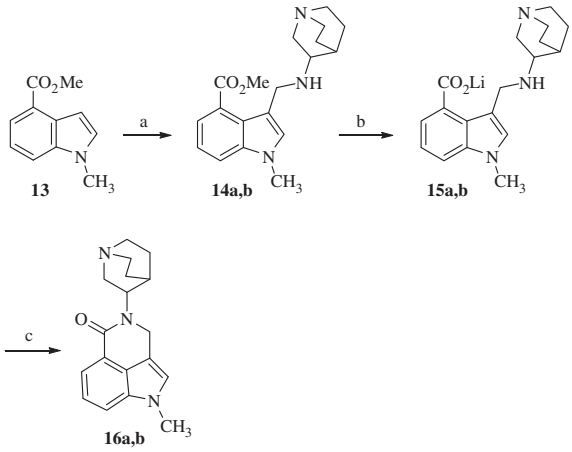


Figure 2. Indazole and indole derived chemical series.



Scheme 1. General synthesis of indazole series. Reagents and conditions: (a) Cs_2CO_3, R^2X, DMSO; (b) R^2 = Ar; $ArB(OH)_2$, $Cu(OAc)_2$, Et_3N, CH_2Cl_2; (c) 3-aminoquinuclidine dihydrochloride, $NaBH(OAc)_3$, 1% HOAc in CH_2Cl_2; (d) LiOH·H_2O, 1:1 THF/H_2O; (e) HBTU, DMF.



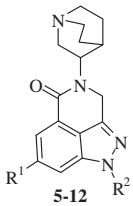
Scheme 2. Synthesis of indoles **16a**,**b**. Reagents and conditions: (a) 3-aminoquinuclidine dihydrochloride, CH_2O, HOAc, rt; (b) LiOH·H_2O, 1:1 THF/H_2O, reflux; (c) HBTU, DMF, 50 °C.

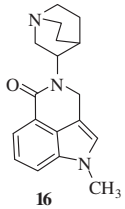
A different outcome was observed for the enantiomeric pair **6a,b**. In this case, the (*R*)-enantiomer **6a** proved to be a slightly weaker partial agonist than its non-alkylated parent **5a**, while the (*S*)-enantiomer **6b** proved to be strictly an antagonist both in the absence and presence of 5-chloroindole. Analogs from the series demonstrated a range of partial agonist activities, falling both below and above the value measured for DDP733, a reference 5-HT_3 receptor partial agonist.[13] In contrast, known 5-HT_3 receptor antagonists showed no effect in this assay, both with and without the potentiator 5-chloroindole (Table 2).

A control experiment was carried out using the 5-HT_3 receptor antagonist granisetron (Fig. 3) to confirm that the responses were mediated by the 5-HT_3 receptor. As shown, the serotonin-induced agonist response in the HEK293 cells is sensitive to the addition of granisetron (5-HT panel). Likewise, addition of granisetron completely abolished the partial agonist responses attributed to indazole **6a** both in the presence and absence of 5-chloroindole.

Compounds **5a** and **6a** which exhibited E_{max} values of 35% and 6%, respectively, were selected for further investigation. Subsequent in vitro ADME profiling (Table 3) with **5a** and **6a** demonstrated a low risk for inhibition of cytochrome P_{450} enzymes and a stability in human liver microsomes comparable to alosetron. In addition, no significant off-target activity (>50% inhibition at

Table 1
In vitro 5-HT_3 receptor binding data





Compound	R^1	R^2	Stereochemistry	$h5\text{-}HT_3A$ K_i[a] (nM)
5a	H	H	(*R*)	4.4 ± 0.5
5b	H	H	(*S*)	4.9 ± 1.5
6a	H	CH_3	(*R*)	1.7 ± 0.6
6b	H	CH_3	(*S*)	0.7 ± 0.1
7	F	CH_3	(*R*)	2.0 ± 0.2
8a	H	Ethyl	(*R*)	2.0 ± 0.7
8b	H	Ethyl	(*S*)	1.6 ± 0.9
9a	H	Isopropyl	(*R*)	3.6 ± 0.8
9b	H	Isopropyl	(*S*)	2.3 ± 1.0
10	H	Isobutyl	(*S*)	2.4 ± 0.6
11	H	Benzyl	(*R*)	7.1 ± 1.4
12a	H	4-Fluorophenyl	(*R*)	23.4 ± 4.8
12b	H	4-Fluorophenyl	(*S*)	7.9 ± 1.9
16a	H	CH_3	(*R*)	0.9 ± 0.6
16b	H	CH_3	(*S*)	0.7 ± 0.1
		Alosetron		0.5 ± 0.1
		Ramosetron		0.06 ± 0.01

[a] Mean K_i, $n \geqslant 3$.

Table 2
Comparison of the functional responses of select indazoles with selective 5-HT_3 receptor literature reference standards

Drug[a]	Drug alone[b]		Drug + 10 μM 5-chloroindole[c]	
	EC_{50} (nM)	% max response[d]	EC_{50} (nM)	% max response[d]
5-HT	178 ± 20	100 ± 4	141 ± 13	120 ± 7
DDP733	5.4 ± 1.4	22 ± 4	9.2 ± 1.0	71 ± 11
5a	14.5 ± 5.8	35 ± 3		97 ± 1[*]
5b		0 ± 1		84 ± 3[*]
6a	12.6 ± 6.1	6 ± 2	6.9 ± 1.5	23 ± 2
6b		−2 ± 1		−2 ± 2[**]
Alosetron		2 ± 3		−1 ± 2
Ramosetron		−1 ± 1		−1 ± 1
Palonosetron		−2 ± 1		−2 ± 2
Ondansetron		−1 ± 1		0 ± 1
Granisetron		−2 ± 1		0 ± 0

[a] 5-HT_3 reference antagonists tested @ 10 μM.
[b] Functional response in HEK293 cells expressing $h5\text{-}HT_{3A}$ (n = 4–6), compounds **5b** and **6b** tested at 1 and 10 μM, respectively.
[c] Functional response in HEK293 cells expressing $h5\text{-}HT_{3A}$ (n = 4–6).
[d] % max response is normalized to 5-HT at 3 μM.
[*] Compounds **5a** and **5b** tested at 1 μM with 100 μM 5-chloroindole.
[**] Compound **6b** tested at 10 μM.

1 μM) was observed in a 65-membered selectivity panel or in an 11-membered serotonin receptor subtype panel.[14] In addition, solubility of compound **6a** in phosphate buffered saline (pH 7.4) proved to be quite high (>500 μM).

The von Bezold–Jarisch reflex bradycardia model[15] in mice has been used to characterize all commercial 5-HT_3 receptor inhibitors irrespective of indication and was used to investigate the in vivo oral activity of **5a** and **6a**, as compared to DDP733 and alosetron. Transient bradycardia induced by iv administration of 5-HT can be blocked by oral pre-treatment with both 5-HT_3 receptor antagonists and partial agonists. As shown in Table 3, compounds **5a** and **6a** demonstrated potency similar to DDP733 in this model.

In an effort to develop a new therapeutic treatment for IBS, we have discovered new indole and indazole-derived heterocycles and evaluated the compounds for activity as 5-HT_3 receptor partial

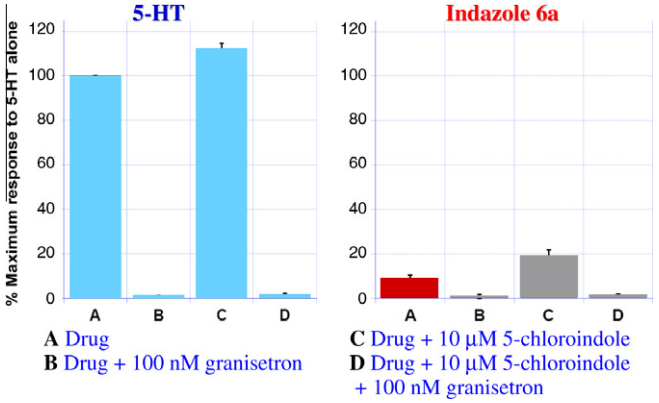


Figure 3. Ability of the selective 5-HT_3 receptor antagonist granisetron to block agonist effects of 5-HT or indazole **6a** in the absence and presence of 5-chloroindole induced increase in $[Ca^{2+}]_i$ in HEK293 cells expressing $h5\text{-}HT_{3A}$ receptor.

Table 3
Selected drug properties

Drug	CYP inhibition IC_{50}[a] (μM)	Human CL_{int}[b]	Bezold–Jarisch p.o. ED_{50}[c] (mpk)
5a	>50	7.3	0.96
6a	>50	3.8	0.64
DDP733	9.3 (3A4)	24	0.25
Alosetron	0.6 (3A4)	3	0.02

[a] Human CYP inhibition IC_{50} (μM), six CYP isoforms tested: 1A2, 2B6, 2C9, 2C19, 2D6, 3A4; CYP isoforms showing IC_{50} <10 μM are specified.
[b] Compounds were incubated with human liver microsomes; data reported in μL/min/mg.
[c] Compounds were dosed p.o. to mice 1 h prior to 0.1 mg/kg 5-HT challenge. Approximate ED_{50} determined by 4-point dose–response, n = 5 mice per dose.

agonists. Given that the series exhibited a range of functional activities, it may be possible to target a number of different IBS symptom classes. 5-HT_3 receptor partial agonists may avoid the unwanted side effects associated with the first 5-HT_3 receptor antagonist alosetron. Compounds **5a** and **6a** exhibited favorable in vitro functional activity, in vitro metabolic stability and CYP inhibition profiles, target receptor selectivity,[14] and in vivo activity in the von Bezold–Jarisch model, thereby well positioning this series of compounds toward the achievement of our goal. Further chemistry efforts and biological studies will be reported in due course.

Acknowledgments

The authors gratefully acknowledge Yuri Khmelnitsky, Ph.D. and the AMRI Metabolism and Biotransformation Department.

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