Bioorganic & Medicinal Chemistry Letters 24 (2014) 2578–2581

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

The discovery of diazepinone-based 5-HT$_3$ receptor partial agonists

David D. Manning [a,*], Cheng Guo [a], Zhenjun Zhang [a], Kristen N. Ryan [a], Jennifer Naginskaya [a], Sok Hui Choo [a], Liaqat Masih [a], William G. Earley [a], Jonathan D. Wierschke [a], Amy S. Newman [b], Catherine A. Brady [b], Nicholas M. Barnes [b,c], Peter R. Guzzo [a]

[a] Albany Molecular Research Inc. (AMRI), 26 Corporate Circle, Albany, NY 12212-5098, United States
[b] Celentyx Ltd, Birmingham Research Park, Vincent Drive, Edgbaston, Birmingham B15 2SQ, UK
[c] Clinical and Experimental Medicine, The Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

ARTICLE INFO

Article history:
Received 23 January 2014
Revised 19 March 2014
Accepted 24 March 2014
Available online 2 April 2014

Keywords:
5-HT$_3$ receptor partial agonist
Irritable bowel syndrome (IBS)
Ligand-gated ion channel
Ramosetron
Alosetron
Diazepinone

ABSTRACT

Serotonin type 3 (5-HT$_3$) receptor partial agonists have been targeted as potential new drugs for the symptomatic relief of irritable bowel syndrome (IBS). Multiple diazepinone-based compounds have been discovered, which exhibit nanomolar binding affinity for the *h*5-HT$_3$A receptor and display a range of intrinsic activities (IA = 7–87% of 5-HT E_{max}) in HEK cells heterologously expressing the *h*5-HT$_3$A receptor. Favorable physicochemical properties and in vitro ADME profile coupled with oral activity in the murine von Bezold–Jarisch reflex model demonstrates the series has promise for producing low to moderate IA partial agonists suitable for an IBS indication.

Irritable bowel syndrome (IBS) is a functional disorder of the gastrointestinal (GI) tract that affects as many as 10–20% of the US adult population. Symptoms range from constipation to diarrhea or a combination of the two, coupled with severe abdominal pain and discomfort.[1] The common symptoms of abdominal pain and altered bowel habits suggest a dysfunction of neural pathways involved in sensory and/or motor pathways of the GI tract may be operating for many IBS patients.[2]

Serotonin (5-HT) is a key monoamine neurotransmitter that plays a central role in normal gut function. Ninety-five percent of all 5-HT in the body is found in enterochromaffin cells in the GI tract.[3] Enterochromaffin cells release serotonin in response to various luminal stimuli (e.g., by mechanical pressure, nutrients or toxins) consequently activating nerve endings bearing serotonin receptor subtypes including the 5-HT$_3$ receptor.[3] The extracellular level of released 5-HT is controlled through action of the serotonin transporter (SERT), which provides re-uptake of the neurotransmitter. Aberrant 5-HT signaling is associated with several GI disorders including IBS.[4]

5-HT$_3$ receptor antagonists, which block the action of 5-HT, are exceptional pharmaceuticals well known to be effective in treating acute chemotherapy induced nausea and vomiting (CINV). Antagonism of the 5-HT$_3$ receptor also represents one of the few clinically validated and effective strategies for the symptomatic relief of diarrhea predominant IBS (IBS-D).[5] Broad use of 5-HT$_3$ receptor antagonists in IBS therapy has been hampered due to severe constipation and rare occurrences of ischemic colitis associated with alosetron, the earliest pharmaceutical product introduced for this indication.[6] In contrast, ramosetron hydrochloride, a generic 5-HT$_3$ receptor antagonist originally developed and sold for the treatment of acute CINV, was recently repurposed for an IBS-D indication. Since its launch in 2008, we are unaware of any reports of ischemic colitis associated with ramosetron demonstrating that safer 5-HT$_3$ receptor modulators can be achieved.[7]

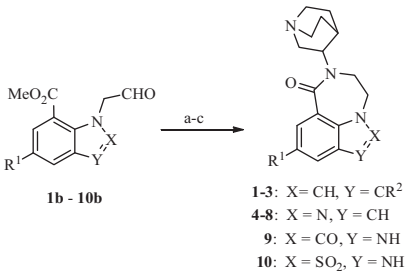
Based upon the principle that a partial agonist can attenuate the action of the endogenous agonist without fully blocking receptor function, we envision that a low to moderate intrinsic activity 5-HT$_3$ receptor agonist can normalize GI function in IBS patients.[8] Pharmacological retention of a modicum of 5-HT$_3$ receptor function is predicted to reduce the risk of constipation and other GI side effects associated with full receptor inhibition in IBS-D patients.

Herein we report on diazepinone-based 5-HT$_3$ receptor partial agonists discovered from our IBS program.

Multiple diazepinone-based scaffolds were devised and synthesized (Scheme 1). The target compounds are an extension from an earlier series of six-membered lactams based on the idea that lactam ring expansion could enable access to several new scaffolds

* Corresponding author. Tel.: +1 518 512 2129.
E-mail address: David.Manning@amriglobal.com (D.D. Manning).

http://dx.doi.org/10.1016/j.bmcl.2014.03.074
0960-894X/



Scheme 1. Diazepinone series. Reagents and conditions: (a) (i) (*R*)- or (*S*)-3-aminoquinuclidine·2HCl, NaOMe, MeOH or NaH, CH_2Cl_2 or dioxane; (ii) 1% HOAc, (iii) $NaBH_3CN$ or $NaBH(OAc)_3$; (b) LiOH, 1:1 THF/H_2O, heat; (c) T3P, DIPEA, THF. Three-step yields: 18–65%.

with increased heterocyclic diversity and lead to a better understanding of the range of partial agonism at the 5-HT_3 receptor that could be derived from such compounds for a potential therapeutic agent.[9]

To prepare these compounds, we adopted a synthetic strategy to introduce the quinuclidine bicyclic amine late in the synthetic sequence using a general three step sequence. Principally, this approach overcomes several practical complications using the polar, highly nucleophilic quinuclidine at an early stage. Quinuclidine's unusual properties can dominate a chemical route. For example, the quinuclidine tertiary nitrogen is $\sim 10^4$ times more nucleophilic than triethylamine.[10] It can therefore preferentially undergo facile alkylation chemistry, even with CH_2Cl_2, which confounds the use of common normal phase flash chromatography eluents ordinarily effective for amine-bearing compounds (e.g., CH_2Cl_2/MeOH mixtures). Quinuclidine-bearing intermediates can adversely affect the efficiency of aqueous work-up strategies due to generally good water solubility or by their polar nature. Collectively, these considerations led us to introduce the quinuclidine moiety as late as feasible in the synthesis.

Accordingly, enantiomerically pure (*R*)- or (*S*)-3-aminoquinuclidine dihydrochloride, which can be readily purchased, was coupled with the requisite aldehyde using reductive amination conditions (Scheme 1).[11] It was important to first free base the amine hydrochloride salt. The seven-membered diazepinone ring was then generated by first unmasking the carboxylic acid through basic saponification of the ester followed by subsequent lactamization, usually with propylphosphonic anhydride (T3P®).[12] This sequence led to the synthesis of compounds **1**–**10** which encompass four heterocyclic scaffolds.

The aldehydes (**1b**–**10b**) necessary to prepare the corresponding target diazepinones (**1**–**10**) were prepared by several methods (Schemes 2–5). Methyl 1*H*-indole-7-carboxylate,[13] methyl 5-chloro-3-methyl-1*H*-indole-7-carboxylate,[14] methyl 5-fluoro-1*H*-indazole-7-carboxylate,[14] and methyl 5-chloro-1*H*-indazole-7-carboxylate,[14] were alkylated with 2-bromo-1,1-dimethoxyethane by in situ conversion of the bromide to the iodide (Scheme 2).

For indazole rings (X = N), the major product from the alkylation was the undesired N2 alkylated isomer. Despite this drawback, the shortness of the route enabled the rapid synthesis of several target compounds. The acetal group of the desired regioisomer was deprotected using aqueous hydrochloric acid to provide the aldehyde.

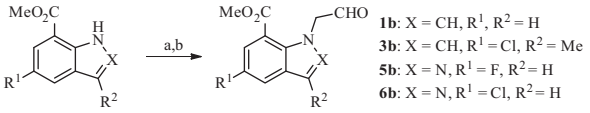
Indazole rings with 5-methyl and 5-methoxy substituents were accessed by using a diazotization approach (Scheme 3). Treatment of methyl 2-amino-5-bromo-3-methylbenzoate with isoamyl nitrite and potassium acetate provided the indazole nucleus in good yield. The product was subsequently alkylated with 2-bromo-1,1-dimethoxyethane to provide bromide **7a** following separation of the regioisomers. The methyl group was installed using trimethylboroxine under palladium catalysis to provide aldehyde **7b** following acetal removal. The 5-methoxy substituent of aldehyde **8b** was prepared following a three-step sequence with the key transformation involving the oxidative hydroxylation of an intermediary pinacol boronate ester.

To address the limitations of the N1 alkylation approach used for the indazole scaffold in Scheme 2, a regio-controlled synthesis was developed for indazole aldehyde **4b** (Scheme 4). Bromination of methyl 2-fluoro-3-methylbenzoate followed by DMSO oxidation of the benzylic bromide gave intermediate aldehyde **4a**. Compound **4a** was treated with 2-hydrazinylethanol at room temperature in methanol for 1 hour to promote hydrazone formation. The solution of the putative hydrazone was subsequently heated in a microwave to efficiently close the ring. Complex mixtures resulted if the room temperature condensation step was omitted, presumably due in part to competing S_NAr displacement of the doubly activated aryl fluoride. Aldehyde **4b** was obtained after Swern oxidation of the intermediate alcohol.

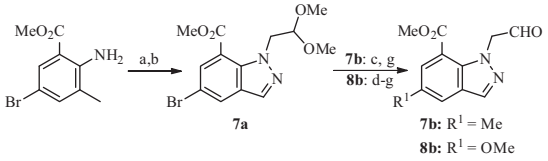
Aldehyde **9b** was accessed in a four step sequence (Scheme 5). A high yielding displacement of the chloride was effected by treatment of methyl 2-chloro-3-nitrobenzoate with 2,2-dimethoxyethanamine and triethylamine in THF under reflux. The nitro group was subsequently reduced with hydrogen and palladium on carbon to provide diamine **9a**. The benzimidazolidinone ring was achieved by treatment of **9a** with carbonyl diimidazole. Aldehyde **9b** was then generated by deprotection of the acetal protecting group with wet TFA in methylene chloride.

Aldehyde **10b** was prepared from common intermediate **9a** (Scheme 5). Diamine **9a** was treated with sulfuric diamide in refluxing diglyme to give the sulfonamide heterocycle. The acetal was deprotected with TFA in water to provide aldehyde **10b**.

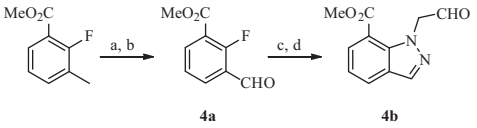
The new diazepinone series are potent *h*5-HT_3A receptor inhibitors. K_i values for **3**, **6**, **7** are comparable to alosetron (Table 1).[14] There is an affinity preference for (*S*)-enantiomer (compare **1** and **2**). Four heterocyclic ring systems were explored (e.g., indole, indazole, imidazolidinone and 1,3-dihydrobenzo[*c*][1,2,5]thiadiazole 2,2-dioxide). Single digit nanomolar K_i values were observed for the unsubstituted parent compounds (**2**, **4** and **9**) excepting sulfonamide **10**. For optimization efforts, the flexibility to use different heterocyclic cores was attractive, in part, because the in vitro agonist responses as measured using HEK293 cells heterologously expressing the *h*5-HT_3A receptor covered a wide range of starting intrinsic activities for these same compounds.[14]



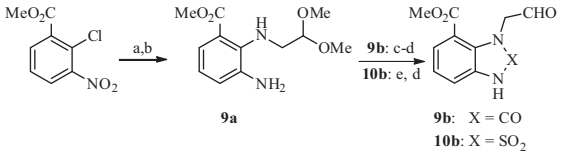
Scheme 2. Synthesis of aldehydes **1b**, **3b**, **5b** and **6b**. Reagents and conditions: (a) **1b** and **3b**: NaH, KI, 2-bromo-1,1-dimethoxyethane, DMF, 80 °C, 59–68% or for **5b**, **6b**: DBU, KI, 2-bromo-1,1-dimethoxyethane, DMSO, 80 °C, 11–15%; (b) 1–2 N HCl, THF, 60 °C, 2 h, 76–89%.



Scheme 3. Synthesis of aldehyde **7b** and **8b**. Reagents and conditions: (a) (i) Ac_2O, $CHCl_3$; (ii) KOAc, isoamyl nitrite, reflux, 88%; (b) KI, 2-bromo-1,1-dimethoxyethane, DBU, DMSO, 80 °C, 20%; (c) $Pd(PPh_3)_4$, K_2CO_3, trimethylboroxine, 100 °C, 51%; (d) KOAc, bis(pinacolato)diboron, $Pd(dppf)Cl_2·CH_2Cl_2$, DMSO, 80 °C, 50%; (e) 30% H_2O_2, MeOH, 85%; (f) Cs_2CO_3, MeI, DMF, 96%; (g) 2 N HCl, dioxane, 75 °C.



Scheme 4. Synthesis of aldehyde **4b**. Reagents and conditions: (a) NBS, benzoyl peroxide, CCl_4, reflux; 67%; (b) $NaHCO_3$, DMSO, 110 °C, 67%; (c) $NH_2NHCH_2CH_2OH$, MeOH room temp 1 h, then microwave 150 °C, 2-3 h, 95%; (d) oxalyl chloride, DIPEA, DMSO, DCM, −78 °C, 53%.



Scheme 5. Synthesis of aldehyde **9b** and **10b**. Reagents and conditions: (a) 2,2-dimethoxyethanamine, triethylamine, THF, reflux, 98%; (b) hydrogen (1 atm), 10% palladium on carbon, ethanol, quantitative; (c) CDI, THF, reflux, 60%; (d) TFA, H_2O, CH_2Cl_2, 60%; (e) sulfuric diamide, diglyme, reflux, 35 min, 27%.

We turned our attention to test whether the agonist responses could be tuned with substituents. One goal was to maintain the excellent physicochemical properties inherent with the parent compounds; therefore, small R1 and R2 substituents were explored. Using the indazole-derived scaffold, we found, that a wide range of intrinsic activities (19–87%) could be achieved (compounds **4**–**6** and **8**) while simultaneously maintaining excellent target affinity. Similar intrinsic activities were observed for the mouse 5-HT_3A receptor (*m*5-HT_3A). The combination of appropriate R1 and R2 substituents can greatly improve receptor binding to subnanomolar levels (compound **3**).

Several compounds were selected to advance to in vitro ADME profiling (Table 2). The compounds generally exhibited minimal inhibition of cytochrome P_{450} enzymes and had excellent (**2** and **4**) to acceptable stability in assay with human liver microsomes. The compounds compared favorably to alosetron, a marketed 5-HT_3 receptor antagonist.

Table 2
Selected drug properties

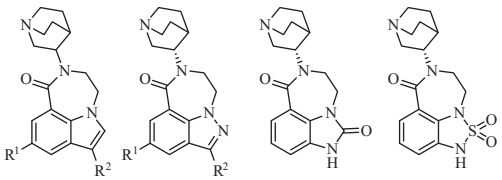
Compd	CYP inhibition IC_{50}[a] (μM)	Metabolic stability *h*-CL_{int}[b]	von Bezold–Jarisch mouse model[c] (%)
2	>50	<1.7	
3	8.8 (1A2)	2.3	
4	>50	<1.7	77
5	>50	8.8	
6	>50	3.1	
8	>50	1.8	
9	>50	8.4	
Alosetron	0.6 (3A4)	3	95

[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 1 mg/kg (alosetron) or 3 mg/kg (compound **4**) po to mice one hour prior to 0.1 mg/kg 5-HT challenge, $n = 5$ mice per dose. % Reversal of 5-HT induced bradycardia reported.

The von Bezold–Jarisch reflex bradycardia model[15] has been used to characterize all commercial 5-HT_3 receptor inhibitors irrespective of indication and was used as an inexpensive model to investigate initial oral activity (Table 2). 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. Diazepinone **4** and positive control alosetron both show significant inhibition of 5-HT induced bradycardia at 3 and 1 mg/kg po, respectively.

As part of our IBS discovery program, we have found several new diazepinones that exhibit low partial to nearly full agonist responses for the *h*5-HT_3A receptor. Given that the functional response could be altered by either changing the core heterocycle or by the addition of small substituents, it may be possible to identify multiple compounds from the series with low to moderate intrinsic activity to target the different IBS symptom classes.[16] Mechanistically, these compounds represent a departure from the classic 5-HT_3 receptor antagonist class. In particular, compound **4** showcases that a favorable profile can be achieved as it

Table 1
In vitro 5-HT_3 receptor binding and functional data



	R^1	R^2	Q[a]	*h*5-HT_3A K_i[b] (nM)	HEK293 *h*5-HT_3A[c] (%)	HEK293 *m*5-HT_3A[c] (%)
1	H	H	(*R*)	67.6 ± 11.0		
2	H	H	(*S*)	3.1 ± 0.9	7	
3	Cl	Me	(*S*)	0.2 ± 0.03		
4	H	H	(*S*)	8.1 ± 0.7	19	16
5	F	H	(*S*)	6.4 ± 0.9	30	31
6	Cl	H	(*S*)	0.5 ± 0.02	28	27
7	Me	H	(*S*)	0.8 ± 0.03		
8	OMe	H	(*S*)	13.5 ± 1.1	87	81
9	H	H	(*S*)	5.7 ± 0.8	83	
10	H	H	(*S*)	701[d]		
Alosetron				0.50 ± 0.05	NR[e]	NR[e]

[a] Q represents quinuclidine stereochemistry.
[b] Mean K_i ± SE, $n \geqslant 3$.
[c] Mean response of at least 3 wells reported ($n = 1$) relative to maximum 5-HT response (EC_{50} = 178 ± 20 nM). Compounds tested at 1 μM.
[d] $n = 1$ value.
[e] NR = no response, antagonist.

possesses acceptable on-target functional activity, excellent in vitro ADME profile and promising oral activity in the murine von Bezold–Jarisch model. Further discoveries from the program will be reported in due course.

Acknowledgments

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

References and notes

1. World Gastroenterology Organisation (WGO) Global Guidelines 2009, 1–20.
2. Gershon, M. D.; Tack, J. *Gastroenterology* **2007**, *132*, 397.
3. Hansen, M. B. *Pharmacol. Toxicol.* **2003**, *92*, 105.
4. Spiller, R. *Neuropharmacology* **2008**, *55*, 1072.
5. Brandt, L. J.; Chey, W. D.; Foxx-Orenstein, A. E.; Schiller, L. R.; Schoenfeld, P. S.; Spiegel, B. M.; Talley, N. J.; Quigley, E. M. M.; Moayyedi, P. *Am. J. Gastroenterol.* **2009**, *104*, S1.
6. (a) Clayton, N. M.; Sargent, R.; Butler, A.; Gale, J.; Maxwell, M. P.; Hunt, A. A. E.; Barrett, V. J.; Cambridge, D.; Bountra, C.; Humphrey, P. P. A. *Neurogastroenterol. Motility* **1999**, *11*, 207; (b) Mayer, E. A.; Bradesi, S. *Exp. Opin. Pharm.* **2003**, *4*, 2089.
7. Matsueda, K.; Harasawa, S.; Hongo, M.; Hiwatashi, N.; Sasaki, D. *Scand. J. Gastroenterol.* **2008**, *43*, 1202.
8. Moore, N. A.; Sargent, B. J.; Manning, D. D.; Guzzo, P. R. *ACS Chem. Neurosci.* **2013**, *4*, 43.
9. Manning, D. D.; Cioffi, C. L.; Usyatinsky, A.; Fitzpatrick, K.; Masih, L.; Guo, C.; Zhang, Z.; Choo, S. H.; Sikkander, M. I.; Ryan, K. N.; Naginskaya, J.; Hassler, C.; Dobritsa, S.; Wierschke, J. D.; Earley, W. G.; Butler, A. S.; Brady, C. A.; Barnes, N. M.; Cohen, M. L.; Guzzo, P. R. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 58.
10. Baidya, M. M. Ph.D. Thesis, Ludwig Maximilians Universität München, 2009.
11. Commercial amines were confirmed to be single enantiomers using Marfey's reagent. Bhushan, R.; Bruckner, H. *Amino Acids* **2004**, *27*, 231.
12. Basavaprabhu, V. T. M.; Panguluri, N. R.; Sureshbabu, V. V. *Synthesis* **2013**, *45*, 1569.
13. Obtained commerically.
14. For synthetic procedures, characterization data, and in vitro and in vivo pharmacology methods see: Guzzo, P. R.; Manning, D. D.; Earley, W. WO 2011008572.
15. Animal data was obtained at MDS Pharma according to: Saxena, P. R.; Lawang, A. *Arch. Int. Pharmacodyn. Ther.* **1985**, *277*, 235.
16. IBS patients are categorized into a number of subgroups based on the predominant symptom: diarrhea (IBS-D), constipation (IBS-C), mixed or alternating bowel patterns (IBS-M or IBS-A). Yao, X.; Yang, Y. S.; Cui, L. H.; Zhao, K. B.; Zhang, Z. H.; Peng, L. H.; Guo, X.; Sun, G.; Shang, J.; Wang, W. F.; Feng, J.; Qiyang, H. *J. Gastroenterol. Hepatol.* **2012**, *27*, 760.