The discovery of diazepinone-based 5-HT₃ receptor partial agonists

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

aAlbany Molecular Research Inc. (AMRI), 26 Corporate Circle, Albany, NY 12212-5098, United States
bCelentyx Ltd, Birmingham Research Park, Vincent Drive, Edgbaston, Birmingham B15 2SQ, UK
cClinical and Experimental Medicine, The Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

ABSTRACT

Serotonin type 3 (5-HT₃) 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 5-HT₃A receptor and display a range of intrinsic activities (IA = 7–87% of 5-HTmax) in HEK cells heterologously expressing the 5-HT₃A 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.

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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. 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-HT₃ receptor. 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-HT₃ 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-HT₃ 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-HT₃ 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-HT₃ 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-HT₃ 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-HT₃ receptor agonist can normalize GI function in IBS patients.8 Pharmacological retention of a modicum of 5-HT₃ 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-HT₃ 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...
with increased heterocyclic diversity and lead to a better understanding of the range of partial agonism at the 5-HT3 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 highly nucleophilic quinuclidine tertiary nitrogen is ineffective for amine-bearing compounds (e.g., CH2=CH2.10 It can therefore preferentially undergo facile alkylation chemistry, even with CH2=CH2.10

Accordingly, enantiomerically pure (R)- or (S)-3-aminquinuclidine dihydrochloride, which can be readily purchased, was coupled to introduce the quinuclidine bicyclic amine late in the synthetic sequence early. Quinuclidine’s unusual properties can dominate a chemical route. For example, the quinuclidine tertiary nitrogen is ~10^4 times more nucleophilic than triethylamine.10 It can therefore preferentially undergo facile alkylation chemistry, even with CH2=CH2, which confounds the use of common normal phase flash chromatography eluents ordinarily effective for amine-bearing compounds (e.g., CH2=CH2:MeOH mixtures). Quinuclidine-alkylating 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, the new diazepinone series are potent h5-HT3A 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-dihydropyrazol-5-yl) using nitro, 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 h5-HT3A receptor covered a wide range of starting intrinsic activities for these same compounds.14

Scheme 1. Diazepinone series. Reagents and conditions: (a) (i) (R)- or (S)-3-aminquinuclidine 2HCl, NaOMe, MeOH or NaH, CH2Cl2, or dichloro; (ii) Ti. HOAc, (iii) NaBH2CN or NaBH(OAc)2; (b) LiOH, 1:1 THF/H2O, heat; (c) T3P, DIPEA, THF. Three-step yields: 18–65%.

Scheme 2. Synthesis of aldehydes 1b, 3b, 5b and 6b. Reagents and conditions: (a) 1b and 3b: NaH, Kl, 2-bromo-1,1-dimethoxyethane, DMF, 80 °C, 59–68% or for 5b, 6b: DBU, Kl, 2-bromo-1,1-dimethoxyethane, DMSO, 80 °C, 11–15%; (b) 1–2 N HCl, THF, 60 °C, 2 h, 76–85%.

Scheme 3. Synthesis of aldehydes 7b and 8b. Reagents and conditions: (a) (i) ArCO, CHCl3; (ii) KOAc, isopropyl nitrite, reflux, 88%; (b) Kl, 2-bromo-1,1-dimethoxyethane, DBU, DMSO, 80 °C, 20%; (c) Pd(PPh3)2Cl2, K2CO3, trimethylboroxine, 100 °C, 51%; (d) KOAc, bis(pinacolato)diboron, Pd(dppf)Cl2, CH2Cl2, DMSO, 80 °C, 50%; (e) 30% H2O, MeOH, 85%; (f) C6H5ONa, Me, DMF, 96%; (g) 2 N HCl, dioxane, 75 °C.

Scheme 5). A high yielding displacement of the chloride was effected by treatment of methyl 2-fluoro-3-methylbenzoate followed by DMSO oxidation of the benzylic bromide gave intermediate aldehyde 4a. Compound 4a was treated with 2-hydradzinylenethanol at room temperature in methanol for 1 hour to promote hydrazine formation. The solution of the putative hydrazine 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 S2Ar 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-dimethoxyethane 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 h5-HT3A 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-dihydropyrazol-5-yl) using nitro, 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 h5-HT3A receptor covered a wide range of starting intrinsic activities for these same compounds.14
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-HT3A receptor (m5-HT3A). 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 P450 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-HT3 receptor antagonist.

The von Bezold–Jarisch reflex bradycardia model\textsuperscript{15} has been used to characterize all commercial 5-HT3 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-HT3 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 h5-HT3A 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.\textsuperscript{16} Mechanistically, these compounds represent a departure from the classic 5-HT3 receptor antagonist class. In particular, compound 4 showcases that a favorable profile can be achieved as it

### Table 1
In vitro 5-HT3 receptor binding and functional data

<table>
<thead>
<tr>
<th>R\textsuperscript{1}</th>
<th>R\textsuperscript{2}</th>
<th>Q\textsuperscript{2}</th>
<th>h5-HT3A K\textsubscript{h} (nM)</th>
<th>HEK293 h5-HT3A\textsuperscript{c} (%)</th>
<th>HEK293 m5-HT3A\textsuperscript{c} (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>H</td>
<td>(R)</td>
<td>67.6 ± 11.0</td>
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<tr>
<td>2</td>
<td>H</td>
<td>H</td>
<td>(S)</td>
<td>3.1 ± 0.9</td>
<td>7</td>
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<tr>
<td>3</td>
<td>Cl</td>
<td>Me</td>
<td>(S)</td>
<td>0.2 ± 0.03</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>H</td>
<td>(S)</td>
<td>8.1 ± 0.7</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>H</td>
<td>(S)</td>
<td>6.4 ± 0.9</td>
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<tr>
<td>6</td>
<td>Cl</td>
<td>H</td>
<td>(S)</td>
<td>0.5 ± 0.02</td>
<td>28</td>
</tr>
<tr>
<td>7</td>
<td>Me</td>
<td>H</td>
<td>(S)</td>
<td>0.8 ± 0.03</td>
<td>87</td>
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<tr>
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<td>OMe</td>
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<td>(S)</td>
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<td>87</td>
</tr>
<tr>
<td>9</td>
<td>H</td>
<td>H</td>
<td>(S)</td>
<td>5.7 ± 0.8</td>
<td>83</td>
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<tr>
<td>10</td>
<td>H</td>
<td>H</td>
<td>(S)</td>
<td>701\textsuperscript{d}</td>
<td>NR\textsuperscript{e}</td>
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<tr>
<td>Alosetron</td>
<td>H</td>
<td>H</td>
<td>(S)</td>
<td>0.50 ± 0.05</td>
<td>NR\textsuperscript{e}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Q represents quinuclidine stereochemistry.

\textsuperscript{b} Mean K\textsubscript{h} ± SE, n ≥ 3.

\textsuperscript{c} Mean response of at least 3 wells reported (n = 1) relative to maximum 5-HT response (EC\textsubscript{50} = 178 ± 20 nM). Compounds tested at 1 μM.

\textsuperscript{d} n = 1 value.

\textsuperscript{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

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