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Identification of a dual δ OR antagonist/μ OR agonist as a potential therapeutic for diarrhea-predominant Irritable Bowel Syndrome (IBS-d)

Henry J. Breslin [†,*], Craig J. Diamond [†], Robert W. Kavash [†], Chaozhong Cai, Alexey B. Dyatkin [†], Tamara A. Miskowski [†], Sui-Po Zhang, Paul R. Wade [†], Pamela J. Hornby, Wei He [†]

Janssen Research and Development, L.L.C. (formerly known as Johnson and Johnson Pharmaceutical Research and Development, L.L.C.), Welsh and McKean Roads, PO Box 776, Spring House, PA 19477-0776, USA

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ABSTRACT

A small set of acyclic analogs **5** were prepared to explore their structure–activity relationships (SARs) relative to heterocyclic core, opioid receptor (OR) agonists **4**. Compound **5l** was found to have very favorable OR binding affinities at the δ and μ ORs (*r* K_i δ = 1.3 nM; *r* K_i μ = 0.9 nM; *h* K_i μ = 1.7 nM), with less affinity for the κ OR (gp K_i κ = 55 nM). The OR functional profile for **5l** varied from the previously described dual δ/μ OR agonists **4**, with **5l** being a potent, mixed dual δ OR antagonist/μ OR agonist [δ IC_{50} = 89 nM (HVD); μ EC_{50} = 1 nM (GPI); κ EC_{50} = 1.6 μM (GPC)]. Compound **5l** has progressed through a clinical Phase II Proof of Concept study on 800 patients suffering from diarrhea-predominant Irritable Bowel Syndrome (IBS-d). This Phase II study was recently completed successfully, with **5l** demonstrating statistically significant efficacy over placebo.

Compounds that modulate opioid receptors (ORs) have long been accepted as a standard of care for pain management (e.g., morphine, **1**, and fentanyl, **2**), and more recently have become appreciated as therapeutics for their gastrointestinal (GI) motility modulation (e.g., loperamide, **3**) (Fig. 1).[1,2] The ORs are categorized into three major subclasses, δ, μ, and κ, with their classifications based on well defined pharmacological profiles.[2]

We previously reported on a series of phenyl imidazoles **4** that demonstrated potent dual δ/μ OR agonist activities, both in vitro and in vivo (Fig. 2).[3,4] Initially described was the discovery of **4a**, a potent δ OR agonist whose OR activity was directly correlated with modulation of GI motility in vivo.[3] Subsequently reported was the improvement of **4a'**s overall OR activities by exploring variations of its Tyr moiety (arbitrarily labeled 'A' segment), ultimately yielding the more potent compounds **4b–e** at both the δ and μ ORs.[4]

Following are described secondary structure–activity relationship (SAR) endeavors evaluating OR activities relative to alterations of the central tetrahydroisoquinoline (Tic) and piperidine 'B' segments of analogs **4a–e**. Recognizing that this SAR exploration could prove laboriously slow if various core substituted heterocycles had to be synthesized in lieu of **4a–e**'s Tics and piperidines, we chose to prepare more readily accessible acyclic analogs akin to **5** (Fig. 3) with the hope they would prove equivalent, or hopefully better, as OR ligands. Key experimental findings are discussed below for some 'B' acyclic analogs **5**. These analogs **5** are summarized in Table 1, along with their respective δ and μ OR binding affinities.

An initial set of acyclic analogs (**5a–5d**) had modest OR binding affinities, considerably poorer than parent cyclic analogs **4a–c** (Δ K_i δ: >200-fold; Δ K_i μ: 20–250-fold). For analogs such as **5a–5d**, where the amide *R'* is H, it is well established that the expected stereochemical preference around the 2° amide bond is more predominately pseudo trans (Fig. 4) relative to analogous 3° amides. Conversely, there is a higher percentage of pseudo cis configuration expected for 3° amides such as **4**, which is the observed energy-minimized modeling configuration of **4a**.[3] This pseudo cis/trans amide conjecture for analogs **4** and **5** is a potential contributing factor for the noted relative loss of OR binding affinities for **5a–5d**. Further supportive evidence for this pseudo cis/trans amide supposition is that the additionally *R''* sterically encumbered **5c** (*R'* = *i*-Pr; K_i δ = 5198 nM; K_i μ = 121 nM) is considerably less active than **5b**

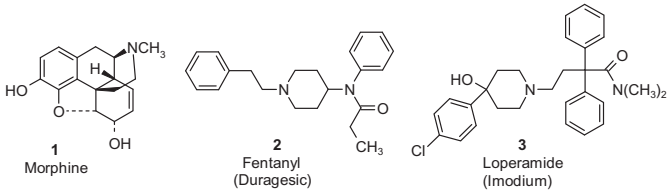

Figure 1.

* Corresponding author.
E-mail address: HBreslin60@gmail.com (H.J. Breslin).
† Not currently employed within the Janssen organization.

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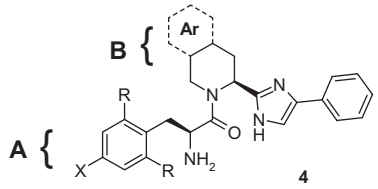


4

				OR Binding		OR Functional	
	Ar	R	X	r K_i δ (nM)	r K_i μ (nM)	δ EC_{50}	μ EC_{50}
4a	Fused Ph	H	OH	0.9	55	19	2445
4b	Fused Ph	CH_3	OH	0.1	0.3	0.9	27
4c	---	CH_3	OH	1.9	0.05	37	2
4d	Fused Ph	CH_3	$CONH_2$	0.06	1.4	22	161
4e	---	CH_3	$CONH_2$	14	0.13	135	9

Figure 2.

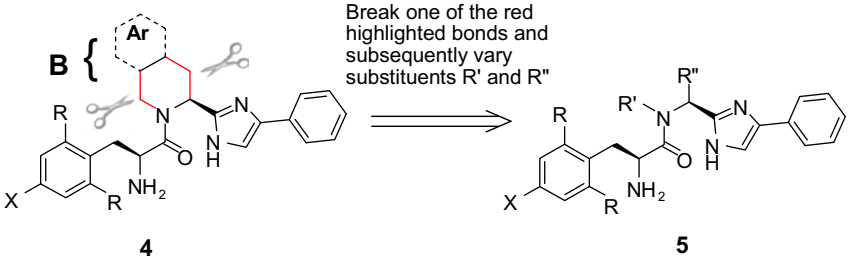
(*R*′ = Me; K_i δ = 708 nM; K_i μ = 17 nM) or **5d** (*R*′ = Bn; K_i δ = 255 nM; K_i μ = 13 nM). Not surprisingly, also observed from this preliminary set of analogs was improved OR binding affinities for the 2,6-di-Me-phenyl substituted Tyr (DMT) analog **5b** (*R* = Me; K_i δ = 708 nM; K_i μ = 17 nM) relative to Tyr analog **5a** (*R* = H; K_i δ = 5660 nM; K_i μ = 1260 nM). These improved OR affinity trends for DMT analogs are consistent with our previously reported work, [4] and further illustrate the utility of the DMT discovery by Lazarus and co-workers.[5]

Next evaluated was the SAR for varied *R*′ and *R*″ alkyl substitutions (**5e–i**), where R and X were held constant as Me and OH, respectively (i.e., with DMT as the 'A' substituent). Pleasingly, OR binding affinities for the R′ alkyl substituted acyclic analogs **5** were more reflective overall of the favorable OR binding affinities for parent cyclic structures **4b** and **4c** than had been observed for the *R*′ = H analogs. For example, relatively consistent binding affinities for the μ OR were seen for acyclic analog **5g** (*R*′ = Me; *R*″ = Me; K_i δ = 15 nM; K_i μ = 0.1 nM) and cyclic parent analog **4c** (K_i δ = 1.9 nM; K_i μ = 0.05 nM), although there was still a comparative loss (~7 fold) of binding affinity at the δ OR for **5g**. Weighing the relative activities of **5g** to **5e** (*R*′ = Me; *R*″ = H; K_i δ = 26 nM; K_i μ = 0.3 nM) suggested a slight enhancement in OR binding affinities by having a methyl group as *R*″. A more prominent example where the OR binding affinities were enhanced by the *R*″ equals Me substituent was observed when comparing **5h** (*R*′ = *i*-Pr; *R*″ = Me; K_i δ = 1.4 nM; K_i μ = 0.03 nM) with **5f** (*R*′ = *i*-Pr; *R*″ = H; K_i δ = 15 nM; K_i μ = 0.1 nM). Most encouraging about the binding affinities for acyclic analog **5h** was that they mirrored those of a cyclic relative, **4c** (K_i δ = 1.9 nM; K_i μ = 0.05 nM). The benzyl (Bn) analog **5i** also showed very favorable OR binding affinities (*R*′ = Bn; *R*″ = Me; K_i δ = 1.5 nM; K_i μ = 0.03 nM). Somewhat unexpected, the relative δ/μ OR binding affinities for benzyl analog **5i** did not closely resemble those for its more closely related cyclic Tic analogs **4a**, **4b**, and **4d**, whose relative δ/μ OR binding affinities generally favored better binding affinity at the δ OR.

Having identified **5i** with exceedingly desirable OR binding affinities (K_i δ = 1.5 nM; K_i μ = 0.03 nM), we subsequently replaced its DMT moiety with a DMT bioisostere, 4-(aminocarbonyl)-2,6-dimethyl-Phe,[4] to give compound **5j.** Analog **5j** (K_i δ = 12 nM; K_i μ = 0.3 nM) possessed about 10-fold weaker binding affinities relative to **5i** at both the δ and μ ORs. Tighter binding at the δ and μ ORs was revived by exploring various substitutions on the Bn group of **5i**, as exemplified by **5k** (K_i δ = 0.5 nM; K_i μ = 1.0 nM) and **5l** (K_i δ = 1.3 nM; K_i μ = 0.9 nM).

The compounds with low nanomolar binding affinities at the δ OR were next evaluated for δ OR functional agonist activities, as measured by a cell membrane-based [^{35}S]GTPγS assay[6] (Table 1). Compound **5h** (K_i δ = 1.4 nM; δ EC_{50} = 103 nM) showed modest δ OR agonist functional activity, despite very promising δ OR binding affinity. Compounds **5i** (K_i δ = 1.5 nM; δ EC_{50} = 20 nM) and **5j** (K_i δ = 12 nM; δ EC_{50} = 35 nM) had similar δ OR agonist functional activities, even though their OR binding affinities were ~10-fold different. The comparable OR functional results for **5i** and **5j** shares additional credence to the previously reported conclusion that the 4-(aminocarbonyl)-2,6-dimethyl-Phe group is a good bioisostere for the DMT moiety.[4] Compounds **5k** and **5l** exhibited no δ OR agonist functional activities at the maximum testing concentration of 10 μM, and actually inhibited [^{35}S]GTPγS binding stimulation of the δ OR agonist, SNC 80. Noteworthy, the inclusion of a meta carboxy moiety on the phenyl ring of R′ was the added structural commonality for both of these compounds that resulted in loss of all δ OR agonist functional activity, despite that both analogs maintained very favorable δ OR binding affinities. Based on the interesting δ OR functional profiles for **5k** and **5l**, both compounds were subsequently evaluated for μ OR functional activities. Compound **5l** (μ EC_{50} = 1 nM) proved ~60-fold more potent as a μ OR agonist relative to **5k** (μ EC_{50} = 61 nM), where the μ OR functional activity was also determined by a GTPγS assay.[6]

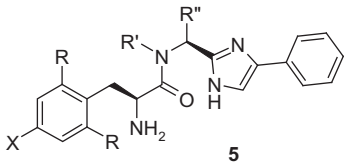
Because of **5l**'s interesting preliminary δ OR functional result, in conjunction with its promising μ OR functional activity, it was more extensively profiled biologically. Foremost, **5l** was found to have potent δ OR antagonist activity (δ IC_{50} = 89 nM), based on a hamster vas deferens (HVD) tissue assay.[7] Identifying a dual δ OR antagonist/μ OR agonist compound was viewed as a potentially favorable finding, based on the reports of attenuated dependence liability for such dual acting ligands[8] as well as possible analgesic advantages.[9] In contrast to the ~1 nM δ and μ OR binding affinities for **5l**, its affinity to the κ OR was lower (gp K_i κ = 55 nM) and in a guinea pig colon tissue assay was a rather weak agonist (EC_{50} = 1.6 μM).[10] Compound **5l**, as its dihydrochloride salt, also showed favorable pharmaceutical properties (Table 2 lists some key results), and had very positive outcomes in a battery of ex vivo and in vivo GI experiments, as recently determined.[11] Based on **5l**'s overall compelling in vitro, ex vivo, and in vivo OR



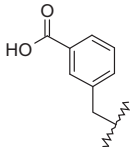
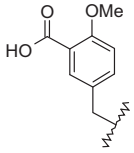
4 5

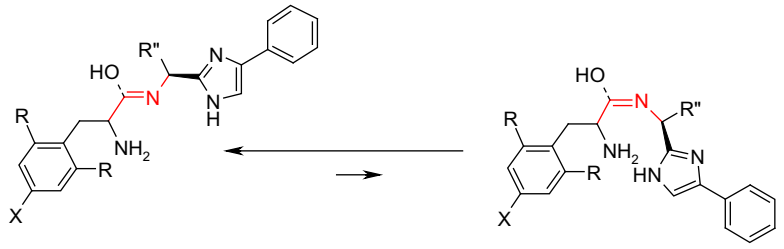
Figure 3.

Table 1
All final compounds **5** with respective δ and μ rat OR in vitro binding affinities. The binding assays may be associated with a margin of error between 10–20%. Compounds **5** identified with δ and μ OR functional activities were screened in an OR [^{35}S]GTPγS in vitro functional assay; NT = not tested



5

	R	X	R′	R″	OR binding		OR functional	
					r K_i δ (nM)	*r* K_i μ (nM)	δ EC_{50} (nM)	μ EC_{50} (nM)
5a	H	OH	H	Me	5660	1260	NT	NT
5b	CH_3	OH	H	Me	708	17	NT	NT
5c	CH_3	OH	H	*i*-Pr	5198	121	NT	NT
5d	CH_3	OH	H	Bn	255	13	NT	NT
5e	CH_3	OH	Me	H	26	0.3	NT	NT
5f	CH_3	OH	*i*-Pr	H	15	0.1	NT	NT
5g	CH_3	OH	Me	Me	15	0.1	NT	NT
5h	CH_3	OH	*i*-Pr	Me	1.4	0.03	103	NT
5i	CH_3	OH	Bn	Me	1.5	0.03	20	NT
5j	CH_3	$CONH_2$	Bn	Me	12	0.3	35	NT
5k	CH_3	$CONH_2$		Me	0.5	1.0	>10,000	61
5l	CH_3	$CONH_2$		Me	1.3	0.9	>10,000	1.0



Psuedo trans tautomer more preferred for secondary amides relative to tertiary amides

Pseudo cis tautomer less preferred for secondary amides relative to tertiary amides

Figure 4.

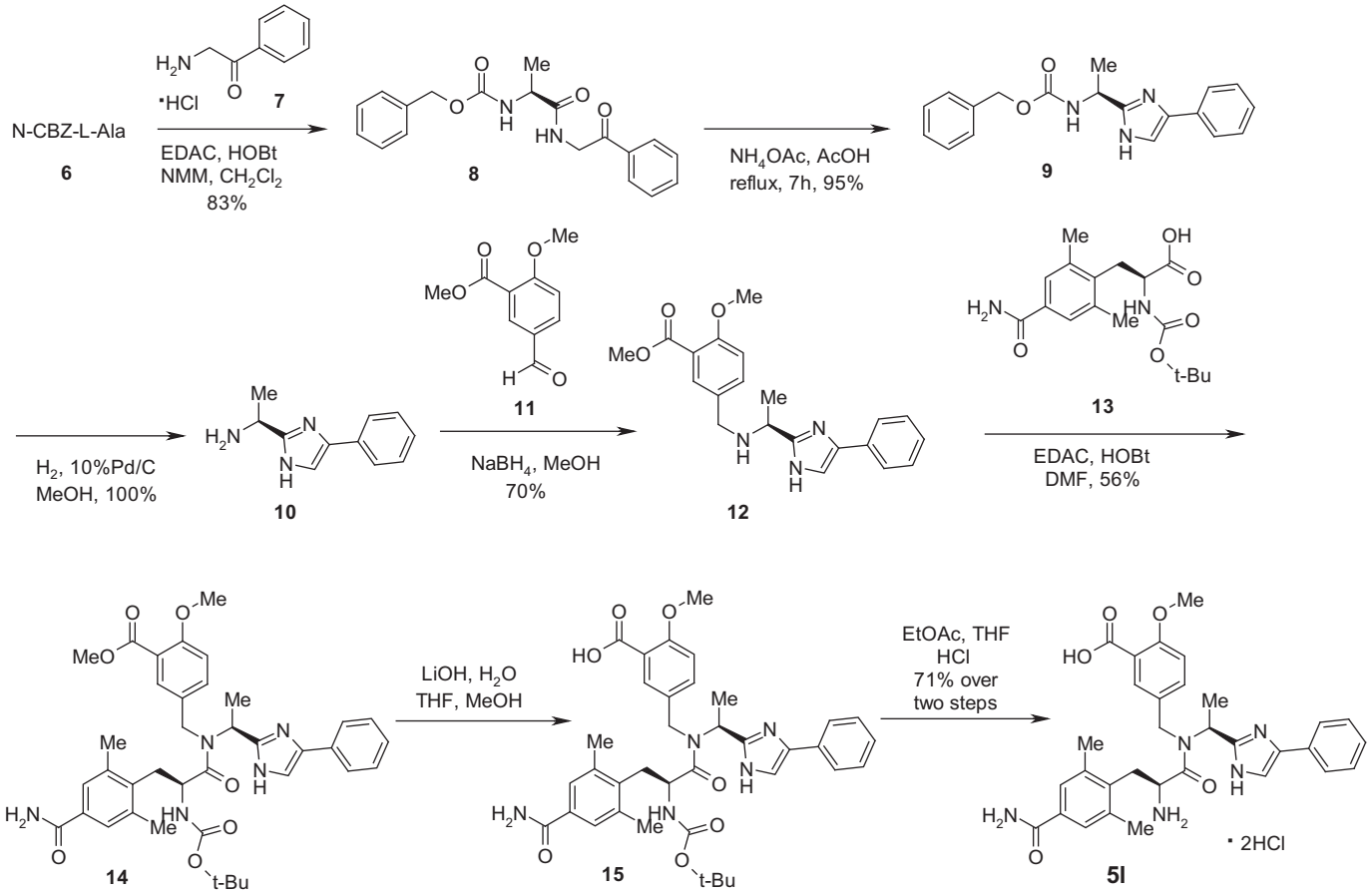
results, in addition to the local activity of **5l** in the GI tract and its low overall oral bioavailability,[11] this compound was further advanced through drug development and recently completed a Phase II Proof of Concept clinical trial successfully in over 800 patients with diarrhea-predominant Irritable Bowel Syndrome (IBS-d).[12]

The preparation of analog **5l** (Scheme 1) exemplifies a standard route used to synthesize acyclic compounds **5**. The commercially available starting materials Cbz-N-protected L-alanine (**6**) and aminobenzophenone (**7**) were coupled via a standard amidation reaction to give **8** in reasonable yield. Intermediate **8** was then treated with ammonium acetate under cyclization/dehydration conditions to cleanly generate imidazole **9**. The Cbz protecting group of **9** was

Table 2
Pharmaceutical properties for **5l**

Compound **5l**	
Solubility (pH 7.4)[a]	>1 mg/mL
Metabolic stability (HLM)[a]	$t_{1/2}$ = 150 min
hERG IC_{50}[b]	>10 μM
P-450 (3A4) IC_{50}[c]	>20 μM

[a] Assays performed at Absorption Systems following their standard protocols, HLM = human liver microsomes.[13]
[b] Inhibition by **5l** of [^{3}H]-astemizole binding to the hERG-encoded K^+ channel.[14]
[c] P450 (isoform 3A4) inhibition in HLM by **5l**.[15]



Scheme 1.

readily removed by Pd catalyzed hydrogenolysis to quantitatively give primary amine **10**. A sodium borohydride reductive amination of benzaldehyde **11** with **10** gave secondary amine **12**, which was subsequently coupled with acid **13**[16] providing a 56% isolated return of **14**. The ester moiety of **14** was easily hydrolyzed with lithium hydroxide to give acid **15**, which was then treated with hydrogen chloride to remove the Boc protecting group with simultaneous precipitation of desired final product **5l** straightaway as its dihydrochloride salt.

In summary, appropriately substituted acyclic analogs **5**, derived by opening the heterocyclic cores of parent structures **4** (Fig. 3), had favorable δ and μ OR binding affinities and were consistent with the binding affinities previously reported for compounds **4**. However, dependent on the R′ substituents of **5**, the compounds varied as δ OR agonists (E.g., **5h**, **5i**, **5j**; Table 1) or δ OR antagonists (E.g., **5k**, **5l**). From the set of acyclic analogs **5** evaluated, compound **5l** was identified as a compound of particular interest, demonstrating potent dual δ OR antagonist/μ OR agonist activities as well as possessing a favorable overall biological profile. Compound **5l** is currently advancing through clinical studies, recently completing a large Phase II Proof of Concept study successfully where it showed statistically significant efficacy for IBS-d patients.

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