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Identification of potent phenyl imidazoles as opioid receptor agonists

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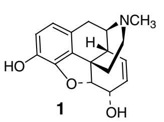
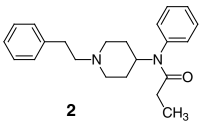
Abstract—Using previously reported opioid receptor (OR) agonist analogs **4a**–**c** as starting points, the structure–activity relationship (SAR) for their related series has been further refined. This SAR study has led to the identification of 2,6-di-Me-Tyr (DMT) analogs **4h** and **4j** as the most potent OR agonist within the series. In addition, it was discovered that 4-(aminocarbonyl)-2,6-dimethyl-Phe is a reasonable bioisostere surrogate for the DMT moiety, as supported by the OR activities of compounds **4x** and **4y**.

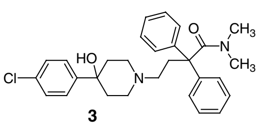
Compounds that modulate opioid receptors (ORs) are well recognized as being useful therapeutic agents for pain management (e.g., morphine, **1**, and fentanyl, **2**), and gastrointestinal (GI) motility regulation (e.g., loperamide, **3**).[1,2]



1
Morphine

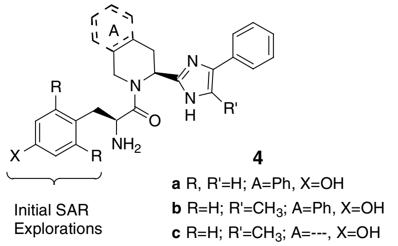


2
Fentanyl
(Duragesic)



3
Loperamide
(Imodium)

The OR has been well characterized, and has been divided into three major subclasses of receptors, δ, μ, and κ.[2] Recently, we reported our initial OR-related findings around a new series of compounds (e.g., **4a**, **4b**, and **4c**) that exhibit strong binding affinities at the δ and/or μ ORs, and also demonstrate significant OR functional activity as determined by GTPγS assays.[3] Compound **4a** from this series was further profiled for in vivo activity, and found to possess significant GI motility modulating effects.[3] Based on the initial promising biological results for compounds **4a**–**c**, we have further profiled the structure–activity relationship (SAR) for this new series. As a starting point, we chose to explore the identified phenyl moiety highlighted in generic structure **4**. The metrics for this follow-up SAR exploration included evaluating the minimal functional group requirements of the phenyl moiety, and also appraising whether the OR activities of our initial leads could be enhanced through related modifications. Following are described the associated experimental results for the preparation and activities for compounds **4**.



4
a R, R'=H; A=Ph, X=OH
b R=H; R'=CH$_3$; A=Ph, X=OH
c R=H; R'=CH$_3$; A=---, X=OH

The preparation of the initial set of phenyl modified products **4** proved rather straightforward, as commercially available synthetic Phe amino acids[4] were employed as starting materials (SMs) (Table 1 summarizes all products **4** prepared[5]). As outlined in Scheme 1, the diverse set of N-protected Phe amino acids were coupled under standard 1-[3-(dimethylami-

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Keywords: Opioid receptor ligands; Opioid receptor agonist; DMT; 2,6-Dimethyl-Tyr; 4-(Aminocarbonyl)-2,6-dimethyl-Phe; 2,6-Di-Me-4-carboxamido-Phe; Phenyl imidazole.

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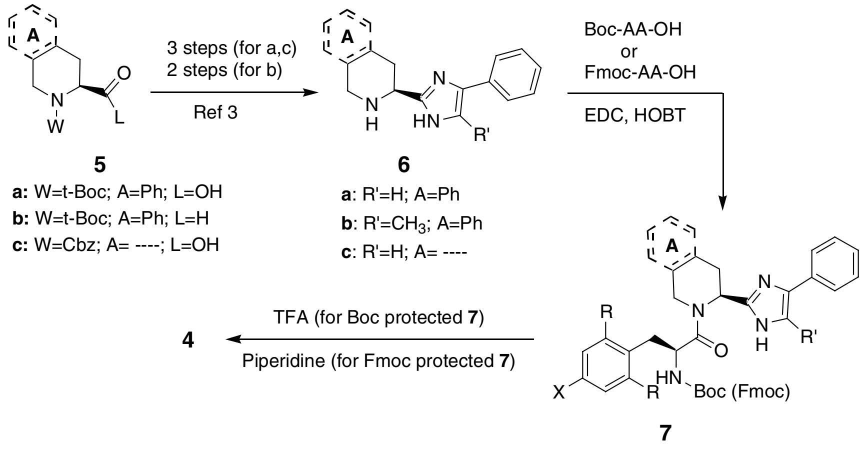
Table 1. Final compounds **4** and related δ and μ OR in vitro binding affinities

Compound	R	R′	X	A	OR binding		Ratio μ/δ
					K_i δ (nM)	K_i μ (nM)	
4a	H	H	–OH	Fused Ph	0.9	55	63
4b	H	CH_3	–OH	Fused Ph	0.3	21	75
4c	H	CH_3	–OH	—	22	1.5	0.1
4d	H	H	OH	—	198	3.8	0.02
4e	H	H	H	Fused Ph	266	443	1.7
4f	H	CH_3	H	Fused Ph	236	1835	8
4g	H	H	H	—	1130	18	0.02
4h	CH_3	H	–OH	Fused Ph	0.1	0.3	3.2
4i	CH_3	CH_3	–OH	Fused Ph	0.1	0.3	3.2
4j	CH_3	H	–OH	—	1.9	0.05	0.03
4k	H	CH_3	–F	Fused Ph	687	12,800	19
4l	H	CH_3	–OCH_3	Fused Ph	28	179	6.4
4m	H	CH_3	–NH_2	Fused Ph	93	857	9
4n	H	CH_3	–NHAc	Fused Ph	34	207	6
4o	H	H	–Cl	Fused Ph	1130	5260	4.6
4p	H	H	–CN	Fused Ph	752	1335	1.8
4q	H	H	–$NHSO_2CH_3$	Fused Ph	342	356	1
4r	H	H	–CH_2OH	Fused Ph	466	912	2
4s	H	H	–$COCH_3$	Fused Ph	30	413	14
4t	H	H	–SO_2NH_2	Fused Ph	174	592	3.4
4u	H	H	–CO_2H	Fused Ph	5200	5800	1.1
4v	H	H	–$CONH_2$	Fused Ph	1.3	23	18.4
4w	H	H	–$CONH_2$	—	65	1.2	0.02
4x	CH_3	H	–$CONH_2$	Fused Ph	0.06	1.4	24
4y	CH_3	H	–$CONH_2$	—	14	0.13	0.01

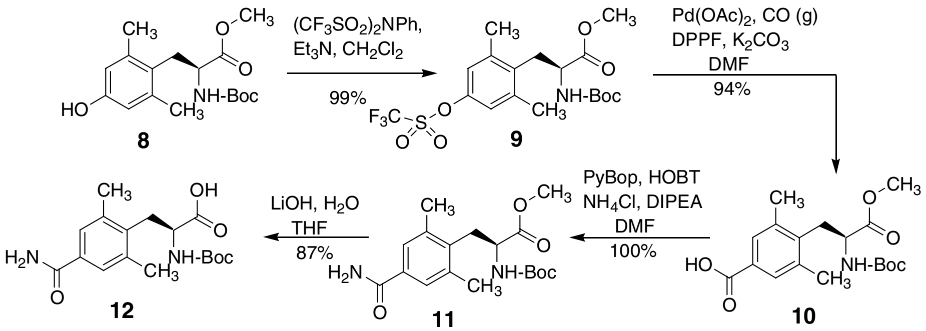


Scheme 1.

no)propyl]-3-ethylcarbodiimide/1-hydroxybenzotriazole (EDC/HOBT) amide forming reaction conditions with common intermediate amines **6a–c**, whose preparations and chemical characterizations have been previously described in detail.[3] For the resulting Boc-protected amides **7**, the Boc group was readily removed with trifluoroacetic acid (TFA) at 0 °C to yield their respective products **4**. For the Fmoc-protected adducts **7**, treatment with 20% piperidine in MeOH at 0 °C cleanly cleaved the Fmoc group to generate their respective targets **4**.

As 4-aminocarbonyl-Phe products **4v** and **4w** proved biologically promising from this initial set of analogs prepared, follow-up analogs **4x** and **4y** were targeted for synthesis. To prepare **4x** and **4y**, the appropriate SM amino acid **12** had to be prepared, which was accomplished via a four-step sequence in good overall yield (Scheme 2) as we have recently described in detail.[6] The Boc-protected amino acid **12** was then sequentially carried on to respective intermediates **7** and subsequent final targets **4x** and **4y**, in a similar manner as outlined in Scheme 1.

As alluded to in the introduction, the initial follow-up SAR exploration around preliminary hits **4a–c** was to answer whether a functional group was needed on the highlighted phenyl moiety of **4** to maintain good OR



Scheme 2.

activity, and if so, which functional groups might prove most promising. The SAR strategy also encompassed trying to identify compounds with enhanced OR activities relative to our initial leads **4a**–**c**. The related δ and μ OR binding affinities[7] used as the initial biological metric for this SAR evaluation are summarized in Table 1. For the biologically most promising analogs, a secondary OR GTPγS functional screen[8] was run (Table 2).

The SAR starting point was to evaluate the OR binding affinity contributions of the phenol's OH moiety for **4a**–**c**. The Phe analogs **4e**, **4f**, and **4g** were all found to possess significantly lower relative binding affinities for both the δ (6- to 780-fold) and μ (10- to 90-fold) ORs relative to their sister Tyr analogs **4a**, **4b**, and **4d**, confirming that the phenolic OH group is a significant contributor to **4**'s OR binding affinities [**4a** (K_i δ = 0.9 nM; K_i μ = 55 nM) relative to **4e** (K_i δ = 266 nM; K_i μ = 443 nM); **4b** (K_i δ = 0.3 nM; K_i μ = 21 nM) relative to **4f** (K_i δ = 236 nM; K_i μ = 1835 nM); **4d** (K_i δ = 198 nM; K_i μ = 3.8 nM) relative to **4g** (K_i δ = 1130 nM; K_i μ = 18 nM)]. This finding is consistent with the generally accepted rule for OR ligands related to the endogenous opioids, that is, that the phenol OH moiety is critical for good OR binding.[2]

Finding the phenol OH moiety essential for maintaining significant OR binding, we prepared 2,6-di-Me-phenyl-substituted Tyr (DMT) derivatives **4h**, **4i**, and **4j**, again direct analogs of the Tyr derivatives **4a**, **4b**, and **4d**. We chose to prepare these di-Me-substituted analogs, as some other N-terminal Tyr-related endogenous opioids have been reported to show improved OR binding activities by substituting DMT for Tyr.[9] In the three direct comparisons for our series, the μ OR binding affinities were significantly enhanced (30- to 180-fold) for the DMT analogs. The δ OR binding affinities were affected to a lesser extent, with the DMT analogs demonstrating improved OR binding affinities between 3- and 100-fold over their sister Tyr analogs [**4a** (K_i δ = 0.9 nM; K_i μ = 55 nM) relative to **4h** (K_i δ = 0.1 nM; K_i μ = 0.3 nM); **4b** (K_i δ = 0.3 nM; K_i μ = 21 nM) relative to **4i** (K_i δ = 0.1 nM; K_i μ = 0.3 nM); **4d** (K_i δ = 198 nM; K_i μ = 3.8 nM) relative to **4j** (K_i δ = 1.9 nM; K_i μ = 0.05 nM)]. The associated OR functional GTPγS activities evaluated were reflective of the binding data, with the DMT analogs showing enhanced potency relative to the simple Tyr derivatives. Unfortunately we did not obtain functional data for analog **4d**, so the closest comparison for **4j** was **4c** [**4a** (EC_{50} δ = 19 nM; EC_{50} μ = 2445 nM) relative to **4h** (EC_{50} δ = 0.9 nM; EC_{50} μ = 27 nM); **4c** (EC_{50} δ = 500 nM; EC_{50} μ = 142 nM) relative to **4j** (EC_{50} δ = 37 nM; EC_{50} μ = 2 nM)].

Table 2. δ and μ OR GTPγS in vitro functional activities

Compound	EC_{50} (nM)	
	δ	μ
4a	19	2445
4c	500	142
4h	0.9	27
4j	37	2
4v	3	155
4w	730	100
4x	22	161
4y	135	9

Related to exploring potential alternate functional groups in place of the phenol OH moiety, we found that with one exception, replacing the phenol OH with either lipophilic or other polar functional groups significantly diminished both the δ and μ OR binding affinities. Direct comparisons where we replaced the phenol of **4b** (X = OH; K_i δ = 0.3 nM; K_i μ = 21 nM) with the other functionality included analogs **4k** (X = F; K_i δ = 687 nM; K_i μ = 12,800 nM), **4l** (X = OMe; K_i δ = 28 nM; K_i μ = 179 nM), **4m** (X = NH_2; K_i δ = 93 nM; K_i μ = 857 nM), and **4n** (NHAc; K_i δ = 34 nM; K_i μ = 207 nM). Other functional group analogs examined that also had disappointing OR binding affinities, when compared to alternate parent phenol **4a** (K_i δ = 0.9 nM; K_i μ = 55 nM), were **4o** (X = Cl; K_i δ = 1130 nM; K_i μ = 5260 nM), **4p** (X = CN; K_i δ = 752 nM; K_i μ = 1335 nM), **4q** (X = $NHSO_2CH_3$; K_i δ = 342 nM; K_i μ = 356 nM), **4r** (X = CH_2OH; K_i δ = 466 nM; K_i μ = 912 nM), **4s** (X = $COCH_3$; K_i δ = 30 nM; K_i μ = 413 nM), **4t** (X = SO_2NH_2; K_i δ = 174 nM; K_i μ = 592 nM) and **4u** (X = CO_2H; K_i δ = 5200 nM; K_i μ = 5800 nM). The sole promising functional group replacement found for the phenolic OH moiety from this exercise was the carboxamide group. The OR binding affinities for benzamide analogs **4v** (K_i δ = 1.3 nM; K_i μ = 23 nM) and **4w** (K_i δ = 65 nM; K_i μ = 1.2 nM) proved comparable to their respective parent phenol analogs **4a** (K_i δ = 0.9 nM; K_i μ = 55 nM) and **4d** (K_i δ = 198 nM; K_i μ = 3.8 nM). The associated OR functional GTPγS activity for benzamides **4v** (EC_{50} $\delta = 3$ nM; EC_{50} $\mu = 155$ nM) and **4w** (EC_{50} $\delta = 730$ nM; EC_{50} $\mu = 100$ nM) also maintained the OR agonist efficacy noted for respective phenols **4a** (EC_{50} $\delta = 19$ nM; EC_{50} $\mu = 2445$ nM) and **4c** (EC_{50} $\delta = 500$ nM; EC_{50} $\mu = 142$ nM), although slight variations were noted in terms of relative potencies. These biologically favorable OR results for our benzamides reflect the benzamide for phenol bioisostere correlation that Wentland et al. discovered for opiate-related OR ligands. [10] Our findings also further validate the more recently reported extension of the benzamide–phenol bioisostere equivalency for Tyr-related OR ligands,[11] which also complemented Wentland's initial opiate work.

Having found the carboxamide a good bioisostere replacement for the phenol OH for compounds within our series, we questioned whether the OR activities of the 4-aminocarbonyl-Phe derivatives **4v** and **4w** could be enhanced by preparing 4-(aminocarbonyl)-2,6-dimethyl-Phe derivatives **4x** and **4y**. This hypothesis was proposed with the hope of mirroring our earlier observation of improved OR activities for DMT derivatives relative to simple Tyr derivatives. The outcome of this exercise proved positive, with the 4-(aminocarbonyl)-2,6-dimethyl-Phe derivatives **4x** (K_i $\delta = 0.06$ nM; K_i $\mu = 1.4$ nM) and **4y** (K_i $\delta = 14$ nM; K_i $\mu = 0.13$ nM) showing improved OR binding affinities relative to the des-methyl Phe carboxamides **4v** (K_i $\delta = 1.3$ nM; K_i $\mu = 23$ nM) and **4w** (K_i $\delta = 65$ nM; K_i $\mu = 1.2$ nM). Consistent with our hypothesis, the OR binding affinities for **4x** and **4y** were more closely aligned with the OR binding affinities of their respective DMT analogs **4h** (K_i $\delta = 0.1$ nM; K_i $\mu = 0.3$ nM) and **4j** (K_i $\delta = 1.9$ nM; K_i $\mu = 0.05$ nM). Although benzamides **4x** (EC_{50} $\delta = 22$ nM; EC_{50} $\mu = 161$ nM) and **4y** (EC_{50} $\delta = 135$ nM; EC_{50} $\mu = 9$ nM) also both demonstrated significant δ and μ GTPγS OR agonist functional activities, their functional potencies did not prove quite as robust as for their sister DMT analogs **4h** (EC_{50} $\delta = 0.9$ nM; EC_{50} $\mu = 27$ nM) and **4j** (EC_{50} $\delta = 37$ nM; EC_{50} $\mu = 2$ nM).

In conclusion, we systematically evaluated the SAR for substitutions on the highlighted phenyl moiety of generic structure **4** relative to δ and μ OR activities. This exploration verified that for initial leads **4a**–**c** the phenolic OH moiety was a key functional group for both δ and μ OR binding and functional activities, as the related Phe analogs **4e**–**g** proved to be significantly less potent than **4a**, **4b**, and **4d**. In contrast, the OR-related activities for compounds **4a**, **4b**, and **4d** could be significantly enhanced by substituting 2,6-di-methyls on their phenolic moieties to generate DMT compounds **4h**–**j**, the most potent δ and μ OR analogs identified within this series. Relative to exploring various alternative aromatic functional groups to replace the phenolic OH moiety of Tyr derivatives **4a**–**c**, we found after a limited diversity search that the carboxamide was the sole functional group that could replace the phenolic OH group and maintain similar OR activities, as confirmed by comparing OR activities of benzamides **4v** and **4w** to phenols **4a** and **4d**. Recognizing that benzamides **4v** and **4w** were as promising biologically as phenols **4a** and **4d**, and that DMT phenols **4h** and **4j** proved extremely potent as OR ligands, led to the idea of hybridizing the two phenyl-substituted moieties to generate the first reported 4-(aminocarbonyl)-2,6-dimethyl-Phe OR ligands, **4x** and **4y**, specifically. Benzamides **4x** and **4y** maintained consistent δ and μ OR binding affinities as the DMT analogs **4h** and **4j**, although the benzamides proved a bit less potent in terms of OR functional activity. We are continuing to explore additional DMT-amide analogs, as well as further examining the remaining SAR for molecules akin to **4**. Related findings will be reported in subsequent publications.

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4. Starting materials containing the various functional groups X were commercially available as Boc-AA-OH to prepare final products **4e**–**4o**, **4q** and **4s**–**4t**, and commercially available as Fmoc-AA-OH to prepare final products **4p**, **4r**, **4v** and **4w**. The aniline intermediate **7** for compound **4m** was prepared by subjecting its respective Boc-protected 4-nitro-Phe coupled adduct **7** to a hydrazine/Raney Ni hydrogenation. The starting material for final product **4u** was a di-protected AA, that is, N4-bis[(1,1-dimethylethoxy)carbonyl]-L-Phe, with both protecting groups of its respective intermediate **7** being cleaved in the final TFA deprotection step. RSP Amino Acid Products supplied starting material **8**.
5. Final products **4** were analyzed for purity by HPLC (>98% purity at 214 and 254 nm; Hewlett-Packard Series 1050 HPLC with a 3 μm, 3.3 mm × 50 mm Supelco AZB+ C18 column using an acetonitrile/water/TFA gradient eluent system), and chemically characterized by MS (Finnigan 3300) and 300-MHz ^{1}H NMR (Bruker-Biospin, Inc. DPX-300).
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