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Communications to the Editor

Discovery of the First Potent and Selective Small Molecule Opioid Receptor-like (ORL1) Antagonist: 1-[(3*R*,4*R*)-1-Cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one (J-113397)

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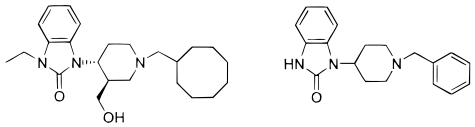
Introduction. Classical opioid receptors (μ, δ, and κ) have important physiological and pharmacological roles, especially in pain regulation. A fourth opioid receptor, opioid receptor-like 1 (ORL1), was identified in 1994 through cDNA expression cloning techniques.[1] ORL1 is a member of the G-protein coupled receptor superfamily with a high degree of amino acid sequence homology to the classical opioid receptors. Despite this homology, native opioid peptides and synthetic agonists selective for μ-, δ-, or κ-receptors do not show significant affinity to ORL1. As an endogenous ligand for ORL1, a peptide containing 17 amino acids, termed nociceptin as well as orphanin FQ (NC/OFQ), was isolated from brain and identified.[2] The amino acid sequence of NC/OFQ has notable homology to that of opioid peptide dynorphin A; however, this new peptide does not show significant affinity to the other opioid receptors. As for the classical opioid receptors, ORL1 is coupled to activation of inwardly rectifying K^+ (GIRK) channels and/or negatively coupled to adenylate cyclase.[3]

NC/OFQ and ORL1 are widely distributed in the central nervous system. Pharmacological studies using NC/OFQ and ORL1-deficient mice have shown that the NC/OFQ−ORL1 system may have important roles in the regulation of pain response,[4] morphine tolerance,[5] learning and memory,[6] food intake,[7] anxiety,[8] the cardiovascular system,[9] locomotor activity,[10] and so on.[11] Unfortunately, further pharmacological evaluation of the NC/OFQ−ORL1 system has been hampered due to the lack of selective ORL1 antagonists. To understand the physiological roles of the NC/OFQ−ORL1 system, development of potent and selective ORL1 antagonists has been desired. Recently, a mutant of NC/OFQ, [Phe$^1\psi$(CH_2-NH)Gly^2]OFQ/N(1−13)-NH_2, was reported to be a selective antagonist of ORL1 in guinea pig ileum and mouse vas deferens.[12] On the other hand, this pseudopeptide was described as an agonist in controlling cAMP levels in both CHO cells transfected with human ORL1 and mouse N1E-115 neuroblastoma.[13] Thus, it is still debatable whether this pseudopeptide acts as an antagonist or agonist on ORL1. Naloxonebenzoylhydrazone (NalBzOH) was shown to act as an ORL1 antagonist;[14] however, this compound was also reported to interact with κ3-opioid receptor subtypes.[15] Thus, pharmacological results using NalBzOH may be complicated.

Chart 1



1 J-113397 2

In this paper, we describe our studies resulting in the development of a potent and selective small molecule NC/OFQ antagonist. 1-[(3*R*,4*R*)-1-Cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one (J-113397, **1**) (Chart 1) binds to ORL1 at nanomolar concentration with a selectivity greater than 600-fold over μ-, κ-, and δ-receptors and inhibits ORL1 function. To the best of our knowledge, this is the first potent and selective small molecule ORL1 antagonist reported. We identified the lead compound 1-(1-benzyl-4-piperidyl)-1,3-dihydro-2*H*-benzimidazol-2-one (**2**) in our chemical library, as it showed high affinity for ORL1 with an IC_{50} of 200 nM but poor selectivity for ORL1 over μ- and κ-receptors.

Chemistry. Synthetic methods to derivatize 4-piperidyl-1,3-dihydro-2*H*-benzimidazol-2-one are summa-

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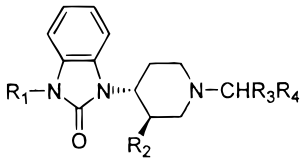
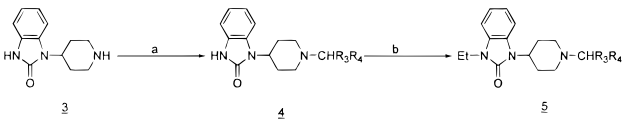
Table 1. Structure and Biological Properties of Benzimidazolidinone Derivatives



					binding IC_{50} (nM)				GTPγS	
no.	R_1	R_2	R_3	R_4	ORL_1[a]	μ[b]	κ[c]	δ[d]	antagonism IC_{50} (nM)[e]	agonism EC_{50} (nM)[f]
2	H	H	H	Ph	200	1700	110	>10000	>10000	6900
13	H	H	Me	2-Cl-Ph	5.9	43	32	1900	>10000	25
14	H	H	H	*c*-Oct	6.8	780	12	>10000	270	>10000
15	Et	H	H	*c*-Oct	6.8	950	440	>10000	20	>10000
J-113397 (3*R*,4*R*)	Et	CH_2OH	H	*c*-Oct	2.3	2200	1400	>10000	5.6	>10000
J-112444 (3*S*,4*S*)	Et	CH_2OH	H	*c*-Oct	820	3300	2600	>10000	>10000	>10000

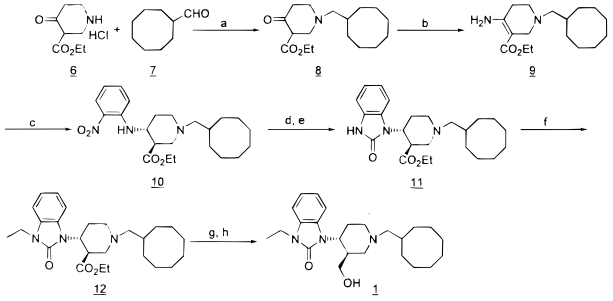
[a–d] Displacement of a [^{125}I]Tyr^{14}-nociceptin, [b][^{3}H]diprenorphin, [c][^{3}H]U-69593, [d][^{3}H][D-Ala^2,D-Leu^5]enkephalin binding from the CHO cells stably expressing cloned human ORL1, opioid μ-, opioid κ- and opioid δ-receptors, respectively. [e] IC_{50} values on nociceptin-produced [^{35}S]GTPγS binding to ORL1 expressed in CHO cells. [f] EC_{50} values relative to the maximal [^{35}S]GTPγS binding produced by nociceptin in ORL1 expressed CHO cells. All values are the means of three independent determinations performed in duplicate.

Scheme 1[a]



[a] Reagents: (a) R_3-CHO, $NaB(OAc)_3H$, THF or R_3R_4CH-X, K_2CO_3, DMF; (b) EtI, NaH, DMF.

Scheme 2[a]



[a] Reagents: (a) $NaB(OAc)_3H$, THF, 64%; (b) $AcONH_4$, MeOH; (c) 2-fluoronitrobenzene, $NaBH_3CN$, Na_2CO_3, *n*-BuOH, reflux, 37% (2 steps); (d) H_2, Pd−C, MeOH; (e) CDI, $CHCl_3$, 84% (2 steps); (f) EtI, NaH, DMF, 90%; (g) $LiAlH_4$, THF, 89%; (h) optical resolution by CHIRALPAK AD, hexane/2-propanol/Et_2NH = 800/200/1.

rized in Schemes 1 and 2. Reductive alkylation of commercially available **3** with alkyl aldehydes or *N*-alkylation with alkyl halides in the presence of K_2CO_3 afforded **4**. The synthesis of *N*-ethylbenzimidazolidinones **5** was achieved by treating **4** with NaH followed by ethyl iodide. J-113397 was synthesized as shown in Scheme 2. Commercially available keto ester **6** was converted to **8** by reductive alkylation with cyclooctyl aldehyde (**7**) in the presence of $NaB(OAc)_3H$ in THF. Treatment of **8** with ammonium acetate in MeOH gave stable enamine **9**, and reduction with $NaBH_3CN$ followed by coupling reaction of **9** with 2-fluoronitrobenzene in *n*-BuOH at reflux temperature afforded **10** as a mixture of *trans*- and *cis*-isomers. After separation of the isomers by silica gel column chromatography, the *trans*-isomer was hydrogenated in the presence of Pd−C and the resultant phenylenediamine was cyclized with carbonyldiimidazole (CDI) to give **11**. Treatment of **11** with NaH and ethyl iodide followed by reduction with $LiAlH_4$ gave **1** as a racemate. Optical resolution was achieved by using a chiral column (CHIRALPAK AD), and the resultant free base was converted to a water-soluble hydrochloride powder for use in biological studies. The absolute stereochemistry of the active enantiomer (J-113397) was determined by X-ray crystallography of its D-tartaric acid salt.

Results and Discussion. The compounds described here were tested for their inhibitory effects on ligand binding to the human ORL1 receptor and also on GTPγS binding to proteins using membrane fractions of CHO cells expressing ORL1. Binding affinities for the ORL1 were determined by displacement of [^{125}I]Tyr^{14}-NC/OFQ, and agonist/antagonist activities were measured by the [^{35}S]GTPγS binding method.[16] Affinities for human μ-, κ-, and δ-receptors were assayed similarly to ORL1 using membrane fractions of CHO cells expressed in each receptor.

Because the amino acid sequences of ORL1 and the classical opioid receptors are highly homologous and the selectivity of the lead compound **2** for ORL1 over μ- and κ-receptors was poor, the most important issue in identifying selective ORL1 antagonists was to determine the structural requirements of the ligands to show potent affinity for each receptor. In addition, since **2** showed weak agonistic activity for ORL1, identification of the structural requirements to show full antagonistic activity was also critical.

We studied structure−activity relationships around the lead compound **2**, and Table 1 highlights the results. The introduction of substituents into the benzyl group improved binding affinity; *o*-chlorophenethyl analogue **13** showed nanomolar affinity for ORL1. Unfortunately, this compound lacked selectivity over μ- and κ-receptors and antagonistic activity. Efforts to identify fully antagonistic compounds by changing the benzyl part with a variety of groups resulted in the discovery of the cyclooctyl analogue **14**. **14** showed full antagonistic activity in the GTPγS assay, although the IC_{50} value was rather large (IC_{50} 270 nM). To improve selectivity and antagonistic activity, we introduced a variety of substituents into the benzimidazolidinone nitrogen (R_1) and found that an ethyl analogue **15** dramatically improved not only the antagonistic activity but also the selectivity (>50-fold). Improvement of selectivity was

accomplished by introducing a hydroxymethyl group into the piperidine ring; J-113397 having (*R*,*R*)-stereochemistry showed excellent potency (IC_{50} 2.3 nM), antagonistic activity (IC_{50} 5.6 nM), and selectivity over μ, κ, and δ (>600-fold). The affinity for ORL1 of the less active enantioisomer (J-112444) was approximately 400-fold weaker than that of J-113397. This compound was confirmed to be active in vivo via testing antagonism against hyperalgesia produced by NC/OFQ (preliminary observation).

In conclusion, we discovered the first potent and selective small molecule ORL1 antagonist, **1** (J-113397), through chemical modifications of the lead compound **2** found in our chemical library. J-113397 should be a useful pharmacological tool to elucidate the physiological roles of the NC/OFQ−ORL1 system as well as the therapeutic potential of ORL1 antagonists and/or agonists.

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