A Novel Series of Piperidin-4-yl-1,3-Dihydroindol-2-ones as Agonist and Antagonist Ligands at the Nociceptin Receptor

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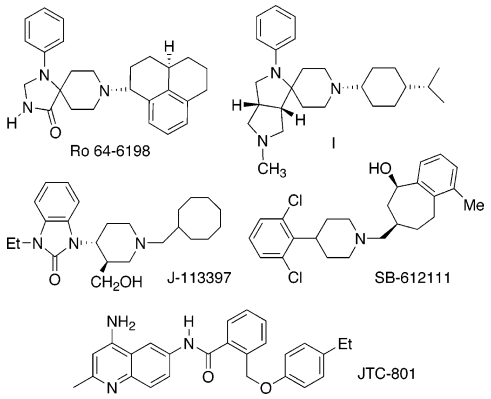
Received December 10, 2003

Abstract: A series of *N*-(4-piperidinyl)-2-indolinones were discovered as a new structural class of nociceptin receptor (NOP) ligands. Unlike other previously reported classes of NOP receptor ligands, modifications of the piperidine N substituents afforded both potent agonists and antagonists, with modest selectivities over other opioid receptors. The SAR revealed in this new series will provide important insights for the development of pharmacophores for agonist and antagonist actions at the NOP receptor.

The nociceptin receptor (NOP receptor, previously known as the opioid receptor-like receptor ORL1) was discovered in 1994 because of its homology to the opioid receptors.[1] Interestingly, this fourth member of the opioid receptor family did not bind classical opioids with appreciable affinity. Soon after, two groups independently identified its endogenous ligand, a heptadecapeptide named nociceptin[2] or orphanin FQ (N/OFQ).[3] Since then, the physiological role of the NOP receptor and its ligand N/OFQ has been the focus of intense research. Although both the NOP receptor and its ligand share significant homology with the classical opioid receptors and their ligands, none of the mature experience with the opioid pharmacology can be brought to bear on the study of the NOP−N/OFQ system because none of the known opioid ligands or synthetic opiates bind appreciably to the NOP receptor.

The NOP−N/OFQ system has been shown to be involved in pain and analgesia, anxiety, learning and memory, tolerance development, and reward pathways.[4] The ligand N/OFQ has been shown to have a potent anxiolytic effect in rodent models,[5] inhibits release of serotonin and dopamine in reward pathways,[6] and blocks conditioned place preference to morphine,[7] suggesting a role for NOP agonists as anxiolytics and drug abuse treatments. Studies in NOP −/− mice showed that they exhibited improved memory and attention[8a,b] and reduced tolerance development to morphine analgesia,[8c] suggesting that NOP antagonists could be useful for improving memory and modulating tolerance development when given in combination with potent analgesics such as morphine. Indeed, peptide and small-molecule antagonists of NOP have been shown to potentiate analgesia in tolerant mice.[9]

Chart 1. Structures of Reported NOP Agonist and Antagonist Ligands



Several peptide and small-molecule NOP ligands have now been reported.[10] Among the small-molecule ligands, Roche has reported an extensive series of NOP agonists based on the spirodecanone (e.g., Ro 64-6198) and hexahydropyrrolopyrrole (**I**) heterocyclic moieties (Chart 1).[11] NOP antagonists such as the benzimidazolone J113397,[12] the quinoline JTC-801,[13] and recently the phenylpiperidine SB-612111[9a] (Chart 1) have also been reported and pharmacologically characterized. Except for JTC-801, almost all small-molecule ligands contain a common central piperidine core, with a 4-position heterocyclic moiety and a lipophilic group on the piperidine nitrogen.[10] Despite the extensive series of agonists and antagonists studied, there is no clear understanding about the pharmacophoric features that confer NOP agonist or antagonist activity, or selectivity versus opioid receptors. Furthermore, among the different classes of ligands reported thus far, only agonists or only antagonists have been reported within the same structural class. These series therefore do not offer much insight for the rational design of specific NOP agonists or antagonists at the NOP receptor.

We report a new series of NOP ligands based on a 1,3-dihydroindol-2-one heterocyclic scaffold. This series is particularly interesting because subtle structural changes in the nature of the piperidine N-1 substituent resulted in converting potent antagonists into potent agonists with modest selectivities. Thus, unlike the previously reported series of NOP ligands, this new series of indolinone ligands produced both potent NOP agonists and antagonists within the same structural series.

Our discovery of this new class of ligands began with the random screening hit **1a** (Table 1), which had higher affinity for the μ and κ opioid receptors. Modification of the piperidine N-1 substituent of the lead compound resulted in potent NOP ligands, a trend that has been observed with other reported series of NOP ligands. We first replaced the N-1 benzyl group of **1a** with the cyclooctylmethyl group to give **1b**. This resulted in a dramatic improvement in the affinity at the NOP receptor and reduced the affinity for the opioid receptors, resulting in a 38-fold selectivity versus the κ

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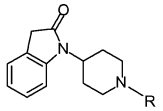
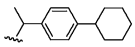
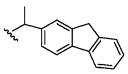
10.1021/jm034249d CCC: $27.50
Published on Web 05/04/2004

Table 1. Binding Affinities and Functional Activities of the New Dihydroindolinone Ligands



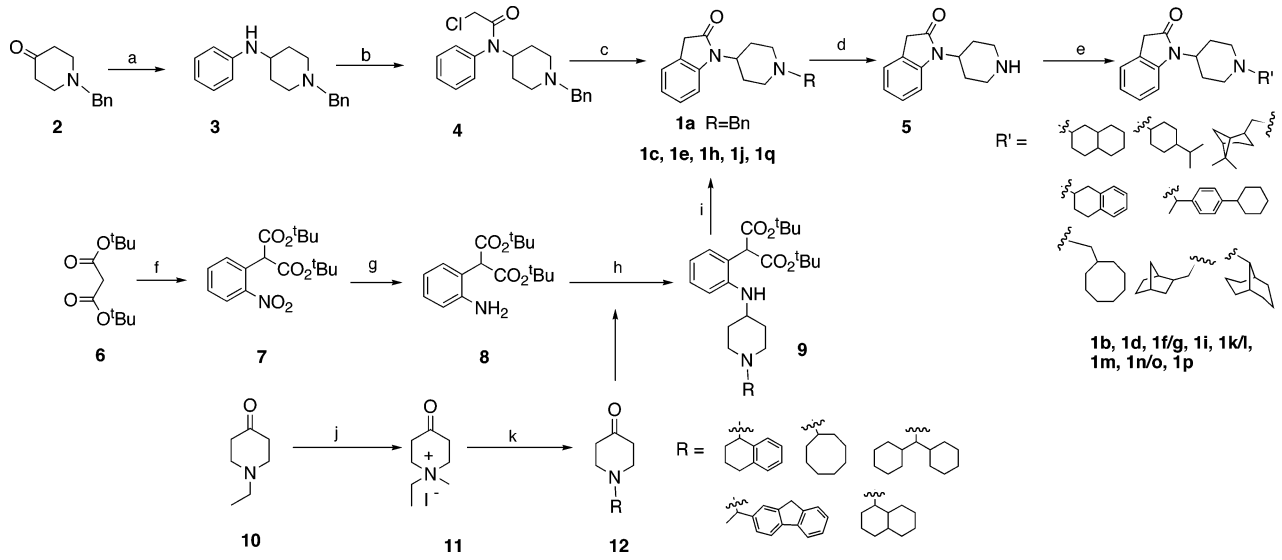
		Receptor Binding[a]				Functional Activity[a]		
		K_i (nM)				NOP [^{35}S] GTPγS		
Cmpd	R	NOP	μ	κ	δ	EC_{50} (nM)	% Stim	K_e[c] (nM)
1a		201 ± 51	91.1 ± 16	84.5 ± 0.8	ND[b]	174 ± 51	18 ± 2	
1b		6.04 ± 0.42	14.4 ± 1.1	229 ± 33	>10,000	>10,000	-	15.3 ± 1.6
1c		1.39 ± 0.42	29.9 ± 2.1	42.7 ± 1.0	ND	19.9 ± 3.4	59.1 ± 7.1[d]	
1d		29.3 ± 11.4	38.9 ± 9.9	55.1 ± 14.9	ND	92.5 ± 16	30.9 ± 3.1	
1e		11.7 ± 1.6	21.2 ± 2.6	21.5 ± 2	ND	ND		
1f		4.67 ± 1.96	16.4 ± 3.3	137 ± 2	ND	74.9 ± 2	78 ± 14	
1g		5.64 ± 2.89	10.6 ± 1.2	52.1 ± 31.5	ND	16 ± 3	62 ± 6	
1h		15.6 ± 1.8	36.7 ± 2.7	202 ± 18	ND	117 ± 43	40 ± 8	
1i		253 ± 19	210 ± 40	3869 ± 365	ND	>10,000		
1j		135 ± 50	20.2 ± 8.2	74.4 ± 9.1	ND	>10,000		
1k		22.0 ± 8.9	115 ± 0.9	372 ± 0.2	>10,000	>10,000		ND
1l		27.9 ± 8.7	118 ± 1.2	318 ± 27	>10,000	>10,000		ND
1m		7.49 ± 0.78	2.70 ± 0.5	31.7 ± 4.82	>10,000	28.7 ± 0.6	45 ± 5[d]	
1n		3.96 ± 1.55	8.0 ± 0.97	148 ± 9	ND	26.3 ± 8	100	
1o		71.2 ± 20.1	22.4 ± 6.5	369 ± 116	ND	117 ± 4	65	
1p		15.5 ± 7.1	27.8 ± 5.4	175 ± 3	ND	>10,000		67.1±7.8
1q		>10,000	227 ± 80	343 ± 92	ND	ND		

[a] Receptor binding and [^{35}S]GTPγS binding were conducted as described previously.[18,20] K_i values were derived from the equation $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] (the concentration of the radioligand) is approximately 0.2, 1.9, 1.1, and 1.2 nM for NOP, μ, κ, and δ binding, respectively. Each experiment was conducted at least twice in triplicate. An EC_{50} value of >10000 for [^{35}S]GTPγS binding could indicate either low affinity or lack of agonist activity. [b] ND not determined. These ligands appear to have very low affinity for the δ opioid receptor. [c] K_e = [A]/(dose ratio − 1); [A] = antagonist concentration. [d] Agonist activities were confirmed with the cAMP assay, as described in the Supporting Information.

receptor. When tested for functional activity in the [^{35}S]-GTPγS assay (described below), **1b** completely lacked agonist activity and was found to be an antagonist (K_e = 15 nM). However, when the N-1 substituent was replaced with a cyclooctyl (**1c**), it not only improved the binding affinity at the NOP receptor but, to our surprise, converted the compound into a potent agonist with an EC_{50} of 20 nM in the [^{35}S]GTPγS functional assay. **1c** also had a 21-fold and 30-fold selectivity versus μ and κ receptors, respectively. Encouraged by the effect of this subtle structural change in the functional activity of our new ligands, we examined a range of lipophilic N-1 substituents to gain an understanding of the predictability of the structural changes that could afford both potent NOP agonists and antagonists. The different N-1 substituents examined are shown in Table 1 (**1b**−**1q**).

The ligands were synthesized as shown in Scheme 1. Most ligands were synthesized by reductive amination of the appropriate aldehyde or ketone with the common intermediate *N*-1-(4-piperidinyl)-1,3-dihydroindol-2-one **5**, which was obtained from **1a** by debenzylation. Initially, **1a** was prepared by a literature method starting from *N*-benzylpiperidone **2**.[14] However, for larger scale preparations of **5**, we resorted to the more convenient route, starting from di-*tert*-butyl malonate **6**, also reported in the literature.[15] This route also worked well for those target compounds where the ketone synthons corresponding to the piperidine N

Scheme 1[a]



[a] Reagents and conditions: (a) aniline, $NaBH(OAc)_3$, AcOH, DCE, room temp, 3 h (90%); (b) Cl−CO−CH_2−Cl, DCM, room temp, 16 h (90%); (c) $AlCl_3$, 160 °C, 3 h, (70%); (d) Pd−C, HCOOH, MeOH, 60 °C, 4 h (78%); (e) ketone or aldehyde, $NaBH(OAc)_3$, AcOH, DCE, room temp (10−70%); (f) (1) NaH/DMF−THF, 0 °C, 30 min; (2) *o*-nitrofluorobenzene, room temp, 2 h (83%); (g) Pd−C/H_2, EtOAc, room temp, 3 h (100%); (h) N-1 substituted 4-piperidone, $NaBH(OAc)_3$, AcOH, DCE, room temp, 3−16 h (75−90%); (i) pTsOH, toluene, reflux, 1−4 h (70−90%); (j) MeI, acetone, room temp, 18 h (95%); (k) R−NH_2, K_2CO_3, EtOH−H_2O, reflux, 1 h (55−100%).

substitutent did not afford appreciable yields by direct reductive aminations of **5**. For **1c**, **1e**, **1h**, **1j**, and **1q**, the requisite ketones (corresponding to the R group on the piperidine nitrogen) were first converted to the amine using a standard amination procedure (NH_4OAc, $NaCNBH_3$).[16] The appropriate amine was then condensed with 1-ethyl-1-methyl-4-oxopiperidinium iodide via an elimination−addition sequence[17] to afford the substituted piperidone **12**, which was then condensed with **8**, followed by a decarboxylative cyclization to give the target compounds **1c**, **1e**, **1h**, **1j**, and **1q**. Most aldehydes and ketones used were commercially available or could be easily accessed by Swern oxidation of their hydroxyl precursors. In the cases of **1f**,**g** and **1n**,**o**, the cis and trans isomers were separated by chromatography and their structures were confirmed by X-ray crystallography. The endo and exo isomers **1k** and **1l** were also separated by chromatography and analyzed separately. The endo configuration of **1l** was also confirmed by X-ray crystallography.

These new indolinone-based compounds were tested for binding affinity at human NOP receptors transfected into Chinese hamster ovary (CHO) cells and at human μ, δ, and κ opioid receptors also transfected into CHO cells. Binding to NOP was conducted with [^{3}H]N/OFQ, as described previously.[18] Binding to the opioid receptors utilized the selective agonists [^{3}H]DAMGO, [^{3}H]Cl-DPDPE, and [^{3}H]U69593 for the μ, δ, and κ receptors, respectively.[19] Functional activity of these compounds was determined by stimulation of [^{35}S]GTPγS binding to cell membranes[19,20] and was confirmed with cAMP inhibition assays for selected compounds **1b**, **1c**, and **1m**.[21] Binding affinities and functional activity are shown in Table 1.

For the rational design of ligands for the NOP receptor, as tools or therapeutics, two issues need to be addressed: one of selectivity versus the opioid receptors and the other of agonist/antagonist activity at the NOP receptor. Although several small-molecule ligands of different structural classes have been reported for the NOP receptor, no pharmacophore models for selectivity or for agonist versus antagonist activity have been proposed. We have been interested in defining structural requirements not only for selectivity but also for efficacy at the NOP receptor. In our new series of dihydroindolinone ligands, we observed that a subtle structural change in the piperidine N-1 substituent converted the NOP antagonist **1b** to an agonist (**1c**). We therefore explored the effects of the piperidine N-1 substituents on this new heterocyclic series by synthesizing several analogues, **1b**−**q**, containing lipophilic piperidine N-1 substituents of different sizes.

In general, we find that saturated alicyclic or bicyclic N-1 substitution affords ligands of higher affinity than those containing aromatic character (**1f**,**g** versus **1d**,**i**,**j**). Among the saturated N-1 substituents, potent agonist ligands were obtained with the cyclooctyl (**1c**), decahydronaphthyl (**1f** and **1g**), bicyclooctyl (**1m**), and 4-isopropylhexyl (**1n**) groups. The tolerated size of the saturated cyclic group is also limited because the biscyclohexylmethyl-containing compound (**1q**) has no affinity for the NOP receptor. Interestingly, the decahydronaphthyl and 4-isopropylhexyl groups on the indolinone scaffold did not afford the same selectivity (~40-fold versus κ) versus the opioid receptors, as seen when the same N-1 substituents were on the hexahydropyrrolopyrrole scaffold (~1000-fold) reported by Roche (**I**, Chart 1).[11b] This indicates that the heterocyclic moiety at the 4-position of the piperidine-containing NOP ligands is an important determinant of selectivity of that structural class over the opioid receptors. On the other hand, our results show that the piperidine N-1 substituent plays a role in determining agonist versus antagonistic activity and that one can modulate efficacy at the NOP receptor by modifying the piperidyl N-1 substituent, within the same structural class. We find that when the saturated lipophilic substituent on the piperidyl N-1 is directly linked to the cyclic substituent

(as in **1c**, **1f**, **1d**, **1m**, **1n**), the ligand is an NOP agonist, whereas those ligands that are linked via a methylene (**1b**, **1k**, **1l**, **1p**) are antagonists at the NOP receptor. This effect of the change in the functional activity at the receptor with a change in the N-1 piperidyl substituent is reminiscent of the effect of the nitrogen substituent in morphine and oxymorphone-like μ ligands, where the change in the N substituent from a methyl (in morphine and oxymorphone) to an *N*-allyl or *N*-cyclopropylmethyl converts the μ agonists to potent antagonists nalorphine, naloxone, and naltrexone, respectively. It is interesting to note that **1m**, which can be viewed as a conformationally restricted analogue of the antagonist **1b** and has the cyclohexyl ring of its bicyclo structure still directly linked to the piperidyl N-1, has the same binding affinity as the antagonist **1b** but is an NOP agonist. We are currently expanding our SAR studies on the piperidyl N-1 substitution to establish the observation that the N-1 substituent plays a role in determining the agonist/antagonist activity at the NOP receptor in different structural classes of piperidine-containing NOP ligands. These studies will shed light on the pharmacophoric requirements for not only selectivity but also efficacy at the NOP receptor and will provide useful information for the rational design of potent NOP agonists and antagonists. We will report the results of our expanded SAR studies in the near future.

Acknowledgment. This work was supported by a grant from the National Institutes on Drug Abuse (Grant DA14026 to N.T.Z.).

Supporting Information Available: Experimental details, analytical and spectral data, and X-ray crystal structure data for final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) See Supporting Information.

JM034249D