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Small-Molecule Agonists and Antagonists of the Opioid Receptor-Like Receptor (ORL1, NOP): Ligand-Based Analysis of Structural Factors Influencing Intrinsic Activity at NOP

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ABSTRACT

The recently discovered fourth member of the opioid receptor family, the nociceptin receptor (NOP) and its endogenous ligand, the heptadecapeptide nociceptin, are involved in several central nervous system pathways, such as nociception, reward, tolerance, and feeding. The discovery of small-molecule ligands for NOP is being actively pursued for several therapeutic applications. This review presents a brief overview of the several recently reported NOP ligands, classified as NOP agonists and antagonists, with an emphasis on the analysis of the structural features that may be important for modulating the agonist/antagonist profile (intrinsic activity) of these ligands. Structure-activity relationships in our own series of dihydroindolinone-based NOP ligands and those of the various reported ligands indicate that the lipophilic substituent on the common basic nitrogen present in all NOP ligands plays a role in determining the agonist/antagonist profile of the NOP ligand. This analysis provides a basis for the rational drug design of NOP ligands of desired intrinsic activity and provides a framework for developing pharmacophore models for high affinity binding and intrinsic activity at the NOP receptor. Since NOP agonists and antagonists both have therapeutic value, rational approaches for obtaining both within a high-affinity binding class of compounds are very useful for designing potent and selective NOP ligands with the desired profile of intrinsic efficacy.

KEYWORDS: nociceptin, opioid receptor-like, ORL1, NOP, agonist, antagonist, intrinsic activity

INTRODUCTION

Since its discovery in 1994, the opioid receptor-like receptor (ORL1, NOP), the fourth member of the opioid receptor family, has been shown to be widely distributed in the brain and periphery.[1,2] The endogenous ligand for this receptor is a 17–amino acid peptide, nociceptin or orphanin FQ (N/OFQ). The functional roles of the NOP-N/OFQ system are still under active investigation, and the system's involvement in pain, tolerance and withdrawal, and other pathways is still not completely understood. It is clear, though, that this new member of the opioid receptor family plays a significant role in pathways of pain, anxiety, learning and memory, reward and tolerance, feeding, renal systems and circadian rhythms (see Mogil and Pasternak and Calo et al for excellent reviews on the pharmacology of the NOP-N/OFQ system).[3,4]

Despite significant sequence homology of this G-protein-coupled receptor with the classical opioid receptors, opioids do not bind the NOP receptor. However, several known small-molecule central nervous system (CNS)–active drugs show appreciable affinity for the NOP receptor. Neuroleptics such as pimozide and spiroxatrine and clinically used opiates such as buprenorphine have significant affinity for NOP,[5] which perhaps plays a role in the overall systemic effects of these drugs. Indeed, Lutfy et al[6] recently showed that the ceiling effect of the antinociceptive action of the mu partial agonist buprenorphine is in fact due to its activation of the ORL1 receptor. Determining structural features of small-molecule drugs that may lead to recognition at the NOP receptor is important for understanding the profiles of the systemic action of CNS drugs. On the other hand, small-molecule nonpeptide ligands for the NOP receptor are valuable as tools and broaden the armamentarium of CNS therapeutics that can be employed to treat several neurological disorders.

Several small-molecule NOP ligands have now been reported both in public and patent literature.[7,8] NOP agonists are being pursued as potential therapeutics for anxiety, analgesia, cough, and drug abuse. NOP antagonists have been considered for their utility in treating obesity and learning deficits.

Although many different classes of NOP ligands have been reported, no pharmacophore models have been defined for NOP binding, selectivity compared with opioid receptors, or intrinsic activity (agonist vs antagonist activity) at the receptor. In this review, we describe a preliminary 2-D

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pharmacophore that can be used as a starting point for understanding the structural parameters that play a role in determining the binding and selectivity as well as the intrinsic activity of small-molecule NOP ligands. This analysis is based on the structure-activity relationships (SAR) observed in our own series of NOP ligands[9] and is confirmed by activities reported for other classes of NOP ligands. Our analysis provides a basis for the rational drug design of NOP ligands and will be particularly useful for designing NOP agonists or antagonists as desired.

Factors that need to be considered while designing high-affinity NOP ligands are (1) binding affinity for the NOP receptor, (2) selectivity for NOP vs opioid and other receptors, and (3) intrinsic activity (agonist/antagonist activity). From the analysis presented below for NOP ligands, it appears that the first 2 factors, binding affinity and selectivity, can usually be modulated together, whereas intrinsic activity can be modulated through a distinct set of structural features.

With a few notable exceptions, almost all reported NOP ligands, agonists as well as antagonists, exhibit similar structural features that fall into a 2-D pharmacophoric pattern. Most ligands have a central alicyclic core ring containing the protonatable basic nitrogen, a heterocyclic moiety distal to the protonatable nitrogen by at least 3 carbons, and a lipophilic moiety on the basic nitrogen. This pharmacophore is shown in Figure 1. For the ease of discussion, we name the 3 pharmacophoric elements: (1) the heterocyclic A moiety, (2) the basic nitrogen-containing B moiety, and (3) the lipophilic C moiety on the basic nitrogen. From the results of our own studies and observations with other reported classes, we propose the hypothesis that the heterocyclic A portion is an important determinant of binding affinity and selectivity vs the opioid receptors, whereas the lipophilic C moiety plays a role in the intrinsic activity of the ligand at the receptor. From our analysis presented below, we propose that within the same class of NOP ligands, classified according to the heterocyclic A scaffold, it is possible to modulate the intrinsic activity of the ligand, to generate agonists and antagonists as desired, by the appropriate choice of the lipophilic C moiety. This analysis provides a rational approach to the design of potent and selective NOP agonists and antagonists, which can be further exploited for their therapeutic potential. Needless to say, optimization of all 3 pharmacophoric features will lead to an increase in binding affinity and selectivity, but only modifications in the C moiety appear to predict agonist-antagonist activity. This is similar to the effect of the nitrogen substituent in opiate ligands, where a change from N-Me in the agonists morphine and oxymorphone, to an N-allyl or N-cyclopropylmethyl, results in switching intrinsic activity to that of the antagonists nalorphine and naloxone, respectively.

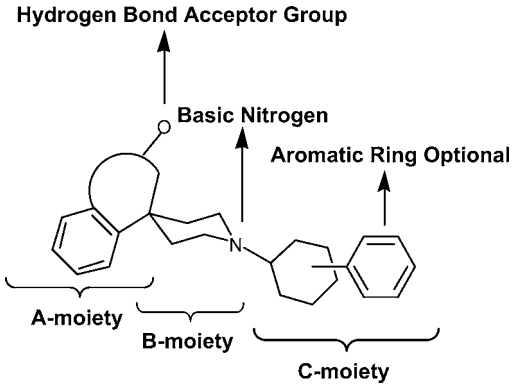


Figure 1. Preliminary 2-D pharmacophore depicting the 3 common elements present in most NOP ligands reported thus far.

NOP Receptor Agonists

The nonpeptide NOP agonist ligand that has been studied most extensively is the triazaspirodecanone **Ro 64–6198**, reported by the Roche group (Figure 2).[10] The Roche series of spiropiperidines were obtained by optimization of a high-throughput screening lead, **1**, containing the triazaspirodecanone (comprising the A and B moiety of the proposed pharmacophore) and a substituted 2-tetralinyl moiety as the lipophilic C moiety directly linked to the basic piperidine nitrogen.[11] This lead compound, which lacked selectivity over the other opioid receptors and was an agonist at NOP, was optimized to improve selectivity, mainly through modifications of the lipophilic C moiety of the piperidine nitrogen. Most of the C moieties examined contained some aromatic character, eg, **2**, **3**, **Ro 65–6570**, and **Ro 64–6198** (Figure 2). Modest 10-fold selectivity was obtained with **3** and **Ro 65–6570** (Table 1), and notably, all these compounds were still agonists in functional assays at NOP. The Roche group also examined alicyclic C moieties at the piperidine nitrogen on the same triazaspirodecanone A-moiety scaffold, to improve selectivity.[12] Substituents such as cyclooctyl (**4**), cyclononyl (**5**), and 4-isopropylcyclohexyl (**6**) afforded a 10-fold increase in binding affinity and a 40-fold selectivity vs mu opioid receptors (Table 1). All these ligands were agonists at NOP. Pfizer also patented a series of triazaspirodecanones exemplified by **7**, as agonists at the NOP receptor.[13]

In an effort to further improve selectivity, the Roche group reported a series of hexahydropyrrolopyrroles as highly selective NOP agonists.[14] The most potent and selective compound resulting from this series was **8**, containing the cis-4-isopropylcyclohexyl as the lipophilic C moiety. This compound had a binding affinity K_i of 0.49 nM, and more important, was over 1000-fold more selective at NOP than the opioid receptors. It is worth noting that the same lipophilic C substituent, cis-4-isopropylcyclohexyl, on the triazaspirodecanone A moiety as in **6** afforded selectivities of only 10-fold (Figure 1). This indicates that the A moiety plays a greater role in the selectivity for NOP vs the other

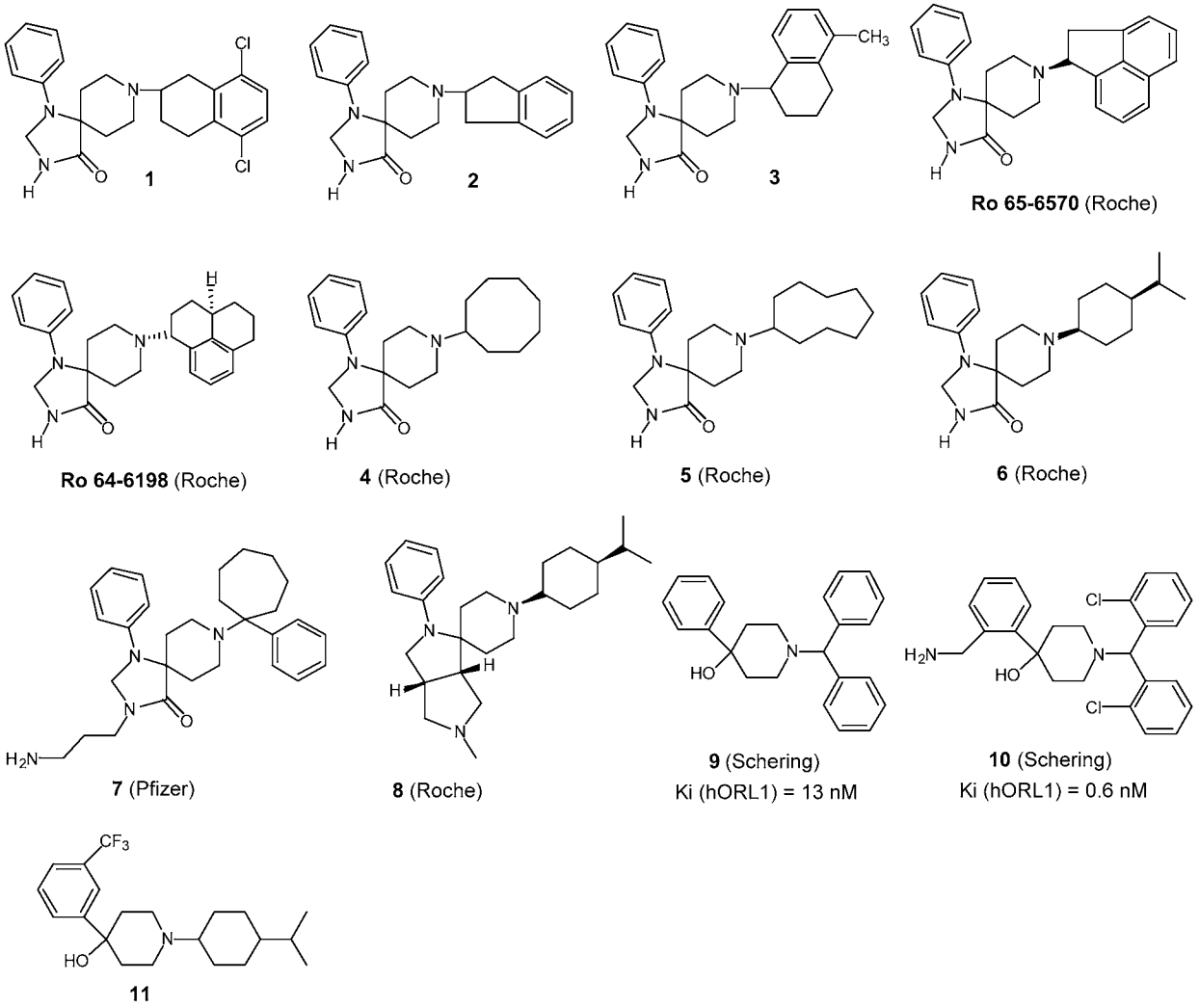


Figure 2. Structures of reported NOP agonists. NOP, nociceptin.

opioid receptors. This trend is further confirmed by our own results with the indolinone series of NOP ligands (see below).

Schering reported a series of phenylpiperidines (eg, **9** and **10**) as NOP agonists in a broad patent.[15] The phenylpiperidine class of NOP ligands represents a deviation from the usual A-moiety pharmacophore, in which most A moieties of reported ligands are heterocyclic rings. Nevertheless, the phenylpiperidines do possess the aromatic ring that is present in all A moieties. Notable, however, are the C moieties reported for the Schering agonists **9** and **10**. These agonists contain the bulky diphenylmethane as the C moiety. Although the selectivities of these compounds vs the opioid receptors were not reported, these C moieties were a departure from the usual alicyclic or cycloaromatic C moieties in the Roche and Pfizer agonists. Recently, another research group from Purdue Pharma also reported a series of phenylpiperidines, among which compound **11**, containing the 4-isopropylcyclohexyl ring as the C moiety directly linked to the basic piperidine nitrogen, was the most potent agonist but with only 3-fold selectivity vs mu receptors.[16] This further confirms the trend that the A moiety plays a significant role in the binding affinity and selectivity vs the opioid receptors.

Analysis of the C moieties of all the agonists from different classes of NOP ligands indicates that the C portion (Figure 1) of piperidine-based agonists can either contain significant aromatic character, as in **Ro 65–6570**, **Ro 64–6198**, and phenylpiperidines **9/10**, or contain lipophilic alicyclic rings like the cyclononyl and isopropylcyclohexyl. Assuming that these ligands bind at the same site on the NOP receptor, with the protonated basic piperidine nitrogen as an anchoring point, the binding site must be able to accommodate both these types of C moieties, such that binding of these ligands will result in transduction of a signaling event, leading to agonist action (intrinsic activity).

NOP Receptor Antagonists

The first nonpeptide NOP antagonist to be reported was **J-113397**, belonging to a class of benzimidazolinones,

Table 1. Binding Affinities of Reported NOP Ligands From Figure 2 at the NOP and Opioid Receptors*

	Receptor Binding				Functional Activity	
	K_i (nM)				GTPγS	
	ORL	μ	κ	δ	EC_{50} (nM)	% Stim
1	5.6	7.2	44.2	680	1500	100
2	2.5	26.0	161	710		
3	1.4	31.7	44	460		
Ro 65–6570	0.52	5.9	26	250	40	100
Ro 64–6198	0.39	46	89	1380	38	100
4	1.9	13	9.1	>200		
5	0.24	3.2	3.9	>200		
6	0.079	3.2	26	242		
7	NA					
8	0.49	537	309	2138		100
9	13					
10	0.6					
11	12	36	4153	5674		

*The binding affinity values in the above table have been taken from the publications describing the respective ligands and are for discussion only. These represent values from different experiments conducted in different laboratories and, as such, cannot to be used to compare ligands reported by different research groups.

patented by Banyu Pharmaceutical Co.[17,18]**J-113397** was obtained through optimization of a high-throughput screening lead, **12** (Figure 3), which contained an N-benzyl C moiety and was a nonselective NOP agonist. Selectivity increased 30-fold when an α-methyl substituent was introduced onto the N-benzyl group and a 2-chloro substituent was added on the pendant phenyl ring as in **13** (Figure 3), likely because of restrictive conformational freedom of the substituted N-benzyl group. However, this compound was still an agonist at NOP. When the piperidine nitrogen substituent (C moiety) was changed to cyclooctylmethyl, the resulting compound **14** retained the affinity and selectivity but was now an antagonist at NOP. Further optimization of binding affinity was achieved through modifications of the benzimidazolinone A moiety and the piperidine ring, resulting in the potent antagonist **J-113397**.

Another series of antagonists reported by Banyu were the isomeric triazaspirodecanones **15** and **16**, identical in the A and B moieties to the Roche and Pfizer agonist ligands **2** to **7** but containing lipophilic C moieties attached to the piperidine nitrogen by a 1-carbon linker, similar to the cyclooctylmethyl substituent of **J-113397**.[19] This indicates that with the same heterocyclic A moiety, the triazaspirodecanone, it was possible to obtain antagonists by a subtle 1-carbon homologation of the lipophilic C moiety on the piperidine nitrogen. This trend was also observed in our series of indolinones, as described in detail below.

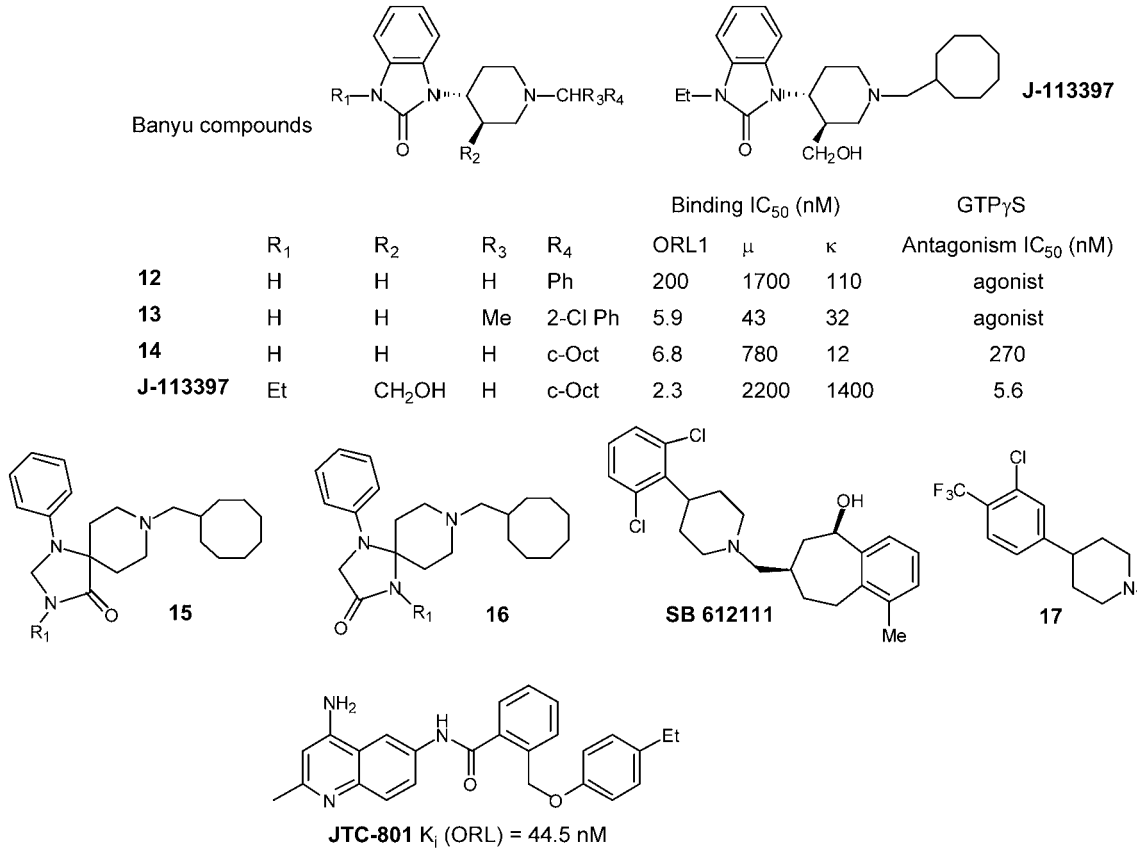
Among the phenylpiperidine class of compounds, **SB-612111** (Figure 3) was recently reported as an NOP antagonist that had a 174-fold selectivity over the mu opioid receptor.[20] It contains a cycloaromatic C moiety attached to the piperidine nitrogen by a 1-carbon linker, a feature that is commonly noted with NOP antagonists. Phenylpiperidines such as **17** reported by Purdue Pharma,[16] containing the same cyclooctylmethyl C moiety as **J-113397**, were also NOP antagonists. These observations and the results from our SAR on the indolinone series of NOP ligands, described below, indicate that the cyclooctylmethyl group could be a preferred C moiety for obtaining NOP antagonists in any heterocyclic class of NOP ligands.

One notable exception to the preliminary pharmacophore described in Figure 1 is the NOP antagonist, JTC-801, a 4-aminoquinoline, reported by Japan Tobacco Inc.[21] Furthermore, JTC-801 is the only small-molecule NOP antagonist that has demonstrated analgesic activity in vivo, in models of both acute and neuropathic pain, not reversed by naloxone.[22-24] The flat planar structure of JTC-801 is clearly distinct from that of almost all reported NOP ligands. It is likely that JTC-801 binds to the NOP receptor at a site partially overlapping the active site where most piperidine-based ligands anchor, via the electrostatic interaction of the protonated basic nitrogen of the ligand, and the Asp-130 at the active site.[25] Studies with photoaffinity ligands could confirm these observations.

As discussed above, although several extensive series of NOP ligands have been reported, only agonists or antagonists have been reported within the same structural class. The Banyu series of benzimidazolinones that resulted in the antagonist **J-113397** did contain agonists such as **13** (Figure 3); however, no rational design of NOP agonists or antagonists within the same structural class had been reported until we reported our series of indolinone-based NOP ligands, discussing the modulation of agonist/antagonist activity by subtle changes in the C moiety in this structural class.[9] Our results, discussed below, provide new insights into the rational design of selective ligands for the NOP receptor and shed light on the pharmacophoric requirements for intrinsic activity at the receptor.

NOP Receptor Agonists and Antagonists From the Dihydroindol-2-one Structural Class Give Insights Into the Modulation of Intrinsic Activity by Structural Manipulation of the C Moiety

We recently reported a new structural class of NOP ligands based on the dihydroindol-2-one A moiety.[9] This series was particularly interesting because, unlike with previously reported classes of NOP ligands, modifications of the



					Binding IC_{50} (nM)			GTPγS
	R_1	R_2	R_3	R_4	ORL1	μ	κ	Antagonism IC_{50} (nM)
12	H	H	H	Ph	200	1700	110	agonist
13	H	H	Me	2-Cl Ph	5.9	43	32	agonist
14	H	H	H	c-Oct	6.8	780	12	270
J-113397	Et	CH_2OH	H	c-Oct	2.3	2200	1400	5.6

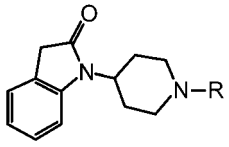
Figure 3. Structures of reported NOP antagonists. NOP, nociceptin.

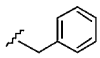
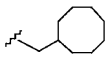
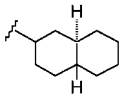
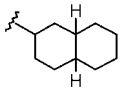
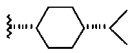
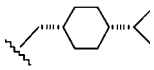
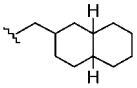
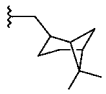
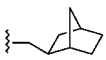
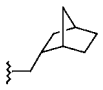
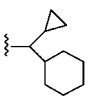
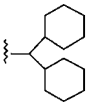
piperidine N-substituent (C moiety) afforded potent agonists as well as antagonists. Our SAR studies show that there are specific structural characteristics of the C moiety (size, shape, lipophilic volume, distance from the protonated nitrogen) that are responsible for transduction of intrinsic activity at the NOP receptor. This information can be used for designing potent agonists or antagonists of any class of NOP ligands containing appropriately selective A moieties.

We obtained this indolinone class of NOP ligands through a random screening hit, **18**, containing the N-benzyl group as the C moiety (Table 2). Replacement of the C moiety with a cyclooctylmethyl group, as in **19** (Table 2), provided a significant increase in binding affinity at NOP and reduced affinity at the opioid receptors, resulting in a ligand that was 38-fold more selective than κ receptors. In GTPγS assays for functional activity, **19** was found to be an antagonist (K_e = 15 nM). We continued our structural modifications of the C moiety and introduced other alicyclic lipophilic groups based on our results with the cyclooctylmethyl group. When the C moiety was a cyclooctyl group, compound **20**, it improved binding affinity and selectivity for the receptor, but importantly, converted the compound into an agonist, with an EC_{50} of 20 nM. This subtle structural change in the C moiety, therefore, changed the intrinsic activity of the molecule without affecting the binding affinity. Our results with different C moieties showed several interesting trends. C moieties such as the decahydronaphthyl (**21, 22**) and 4-isopropylcyclohexyl (**23**) gave potent agonist ligands with good binding affinities. Although the decahydronaphthyl and 4-isopropylcyclohexyl groups appear to be optimally sized lipophilic groups for the C moiety and have afforded potent ligands on various scaffolds, discussed earlier, these groups on the indolinone scaffold did not afford the same selectivity (only 40-fold vs kappa) vs the opioid receptors as seen when the same C moieties were on the hexahydropyrrolopyrrole scaffold (~1000-fold) reported by Roche (**8**, Figure 2). This finding indicates that the heterocyclic A moiety at the 4-position of the piperidine ring of NOP ligands is an important determinant of selectivity of that structural class over the opioid receptors.

However, when these lipophilic substituents of the C moiety were placed 1 methylene carbon away from the piperidine nitrogen (compounds **24** and **25**), it resulted not only in decreased binding but also in complete loss of agonist activity. There appears to be a volume constraint of the size of the lipophilic group around the piperidine nitrogen. Compounds **26** to **28**, which contain more compacted bicyclic lipophilic groups and are 1 carbon removed from the basic nitrogen, have higher affinity and are antagonists at the receptor. This finding is also illustrated by the increase in binding affinity observed when the extended 4-isopropyl

Table 2. Binding Affinities and Functional Activities of the New Piperidin-4-yl-1,3-dihydroindol-2-ones at the NOP and Opioid Receptors*



		Receptor Binding[a]				Functional Activity[a]		
		K_i (nM)				NOP [^{35}S] GTPγS		
	R	NOP	μ	κ	δ	EC_{50} (nM)	% Stim	K_e[b] (nM)
18		201 ± 51	91.1 ± 16	84.5 ± 0.8	ND[c]	174 ± 51	18 ± 2	
19		6.04 ± 0.42	14.4 ± 1.1	229 ± 33	>10K	>10K	–	15.3 ± 1.6
20		1.39 ± 0.42	29.9 ± 2.1	42.7 ± 1.0	ND	19.9 ± 3.4	59.1 ± 7.1	
21		4.67 ± 1.96	16.4 ± 3.3	137 ± 2	ND	74.9 ± 2	78 ± 14	
22		5.64 ± 2.89	10.6 ± 1.2	52.1 ± 31.5	ND	16 ± 3	62 ± 6	
23		3.96 ± 1.55	8.0 ± 0.97	148 ± 9	ND	26.3 ± 8	100	
24		183 ± 29	117 ± 4	1146 ± 392	ND	>10K		
25		366 ± 100	1190 ± 514	454 ± 127	ND	>10K		
26		15.5 ± 7.1	27.8 ± 5.4	175 ± 3	ND	>10K		67.1 ± 7.8
27		22.0 ± 8.9	115 ± 0.9	372 ± 0.2	>10K	>10K		ND
28		27.9 ± 8.7	118 ± 1.2	318 ± 27	>10K	>10K		ND
29		7.49 ± 0.78	2.70 ± 0.5	31.7 ± 4.82	>10K	28.7 ± 0.6	45 ± 5	
30		9.98 ± 2.8	3.44 ± 0.46	43.9 ± 9.2	ND	82.3 ± 16	60 ± 10	
31		27.8 ± 4.7	14.2 ± 1.4	61.4 ± 21.2	ND	63 ± 15	74 ± 14	
32		71.1 ± 25	81 ± 13	10.96 ± 2	ND	406 ± 2	56 ± 1	
33		>10K	227 ± 80	343 ± 92	>10K	>10K		

*NOP indicates nociceptin; GTP - guanidine triphosphate; EC_{50} - Concentration at which 50% effect is observed; Stim - Stimulation; ND - Not Determined. Receptor binding and [^{35}S]GTPγS binding were conducted as described previously, in Zaveri et al.[9]

[a]-Receptor binding and [^{35}S] GTPgammaS binding were conducted as described previously.

[b]-K_e = [A]/(dose ration-1); [A] = antagonist concentration

[c]-Not Determined

group of **24** is conformationally constrained into the bicyclo ring of **26**, resulting in a significant increase in binding affinity but not intrinsic activity. Our results show that when the piperidyl N-1 is directly linked to the cyclic C moiety (as in **20**–**23** and **29**), the compounds are potent NOP agonists, whereas those ligands that are linked via a methylene (**19**, **22**–**24**) are antagonists at the NOP receptor. However, when alkyl groups are added back on the methylene linker, as in **30** to **32**, agonist activity is regained. The complete loss of binding affinity of the biscyclohexylmethyl-containing compound **33** confirms that there is a size constraint on the volume of the lipophilic region around the basic piperidine nitrogen.

Our results suggest that (1) there is a lipophilic binding site in the vicinity of the protonated nitrogen binding site, which must be occupied (by the C moiety) for optimum binding affinity, for both agonist as well as antagonist ligands; (2) there may be a specific binding area of limited size, close to where the protonated nitrogen binds, that triggers an agonist response when the lipophilic binding site is occupied by an appropriately sized and placed C moiety; and (3) the 1-carbon homologation of the C moiety allows binding to the lipophilic site for good affinity but does not allow binding to the agonist trigger site. Such compounds do not trigger an agonist response and are antagonists. This analysis can be visualized in Figure 4, in which the agonists and antagonists from the dihydroindolinone class are superimposed in their low-energy conformations. The agonists (**20**, **23**, and **30**) and antagonists (**19** and **26**) possess C moieties that occupy the lipophilic binding pocket proximal to the protonated nitrogen binding site. In addition, the agonist groups interact with the putative agonist trigger site. The 1-carbon homologation of the lipophilic group in the antagonist ligands places

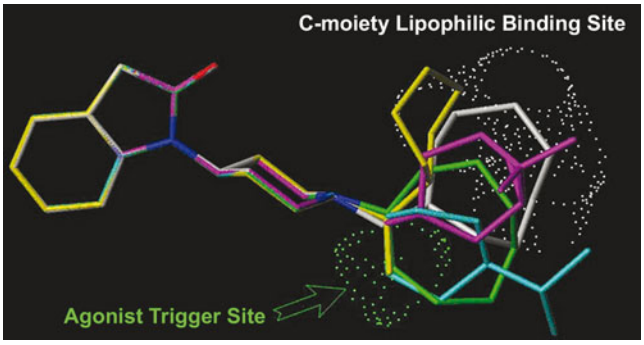


Figure 4. Overlay of the dihydroindolinone-based agonists **20** (green), **23** (cyan), and **30** (yellow) with the antagonists **19** (white) and **26** (magenta). The lipophilic site that must be occupied by the C-moiety for good binding affinity is depicted by the white van der Waals surface. The green van der Waals surface represents the agonist trigger site, which when occupied by the agonists (eg **20**, **23**, **30**), triggers signal transduction leading to intrinsic activity. Antagonists **19** and **20** bind to the lipophilic site and have good affinity but do not interact with the agonist trigger site and therefore lack intrinsic activity.

the C moiety in the lipophilic binding pocket but does not allow interaction with the agonist trigger site (Figure 4). These analyses can be further confirmed with site-directed mutagenesis studies of residues lining the NOP active site close to where the protonated nitrogen binds. An alternate explanation for these observations is that the optimally sized lipophilic C moieties directly linked to the protonated nitrogen bind to and stabilize an agonist receptor conformation that can transduce the agonist signal. C moieties 1 carbon away can still bind the receptor with high affinity but do not induce the agonist conformational change and therefore lack intrinsic activity. It appears very likely that the shape and volume of the lipophilic C moiety play a role in the transduction of intrinsic activity at the receptor, as illustrated by comparing the lead antagonist **19** and compound **29** (Table 2). Compound **29**, in which the cyclooctyl ring is linked to the methylene carbon linker of the cyclooctylmethyl group, can be considered a conformationally restricted analog of antagonist **19**. This structural modification retains the binding affinity in **29** but converts the antagonist profile of **19** to a partial agonist profile of **29**, suggesting that the extended nature of the cyclooctylmethyl and other groups linked via a methylene linker stabilize an antagonist conformation or, conversely, that compacted lipophilic groups stabilize an agonist conformation or interact with an agonist trigger site.

Conclusions

The ligand-based analysis presented can be confirmed by complementary studies with the 3-D model of the NOP receptor as well as site-directed mutagenesis of the NOP receptor. Our analysis provides the first basis for the rational design of high-affinity NOP agonists and antagonists and for understanding the structural factors that influence intrinsic activity at the NOP receptor. Future studies will address the issue of selectivity vs the opioid and other receptors. Such analyses are important for effective drug design of NOP ligands that can be developed as therapeutics to harness the potential of this receptor in pain, tolerance, withdrawal, and other important CNS pathways.

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