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Design and synthesis of novel small molecule N/OFQ receptor antagonists

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Abstract—Small molecule N/OFQ receptor antagonists were designed and synthesized to further investigate the therapeutic potential of N/OFQ receptor modulators. The resulting octahydrobenzimidazol-2-ones **14** and **23** show excellent antagonistic activity towards both N/OFQ and mu receptors with high affinity to the human N/OFQ receptor.
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1. Introduction

The discovery of the nociceptin/orphanin FQ (N/OFQ) receptor (NOP, previously named ORL-1) in 1994,[1] and its endogenous ligand N/OFQ[2] in 1995 has generated considerable interest within the scientific community due to the important roles of classical opioid receptors in the CNS. A number of in vivo experiments have demonstrated that N/OFQ and its peptide analogues modulate a variety of biological functions such as food intake, memory processes, cardiovascular functions, locomotor activity, and control of neurotransmitter release at peripheral and central sites.[3,4] In addition, N/OFQ modulates pain mechanisms at the level of the spinal cord and may also play a role in CNS disorders such as anxiety and drug abuse.[4,5] However, a clear understanding of the potential therapeutic value associated with the modulation of the N/OFQ receptor requires the development of selective non-peptide agonists and antagonists with better pharmaceutical profiles than the peptide analogues.[6,7]

Recently, several research groups have reported the discovery of small molecule N/OFQ receptor ligands (Fig. 1).[8–11] The discovery of highly potent and selective agonists (Ro 64-6198, Roche 5a) and antagonists (J-113397, JTC-801) has been instrumental in further illustrating the therapeutic potential of N/OFQ receptor modulators. For example, in vivo studies have shown that JTC-801 antagonizes N/OFQ-induced allodynia in mice and produces analgesic effects in the hot plate test in mice and the formalin test in rats.[9] In addition, Ro 64-6198 elicits dose-dependent anxiolytic effects in the elevated plus-maze, fear-potentiated startle and operant conflict tests.[12] The results of these in vivo experiments suggest that the identification of small molecule N/OFQ receptor agonists and antagonists with good pharmaceutical and pharmacological profiles could lead to new medications for pain and anxiety.[6] In this paper, we present our progress toward the design and synthesis of novel small molecule N/OFQ receptor antagonists.

2. Design of N/OFQ receptor antagonists

Our primary interest in a N/OFQ receptor antagonist is its therapeutic potential as a pain modulator. Our design of this series of N/OFQ receptor antagonists was based on the known features of N/OFQ receptor antagonists (Figs 1 and 2).[8–11] Compound **1** (J-113397) was reported to be a N/OFQ receptor antagonist with an IC_{50} of 2.3 nM.[8] We envisioned that replacing the benzimidazol-2-one of J-113397 with an octahydrobenzimidazol-2-one might retain potency and antagonistic activity. The hydrophobic cyclohexyl ring, lacking potential π electron interaction with N/OFQ receptor, might result in a pharmacological profile different than the J-113397 series. In addition, the *cis* and *trans* isomers of the disubstituted cyclohexane ring might offer potential handles for further manipulation of pharmacological selectivity. Based on these hypotheses, we chose compounds **5** and **6** as starting points for the identification of novel N/OFQ receptor antagonists.

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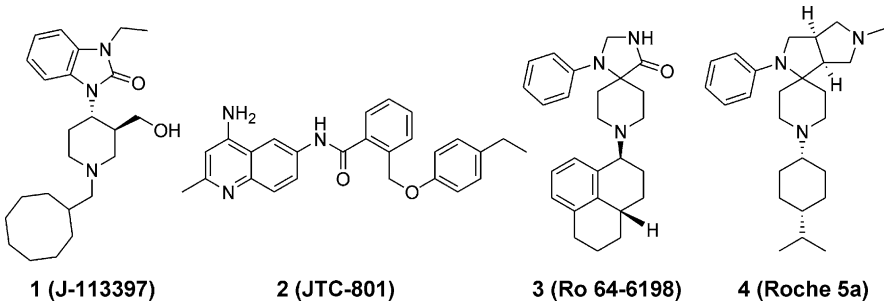


Figure 1. Representative N/OFQ receptor agonists and antagonists.

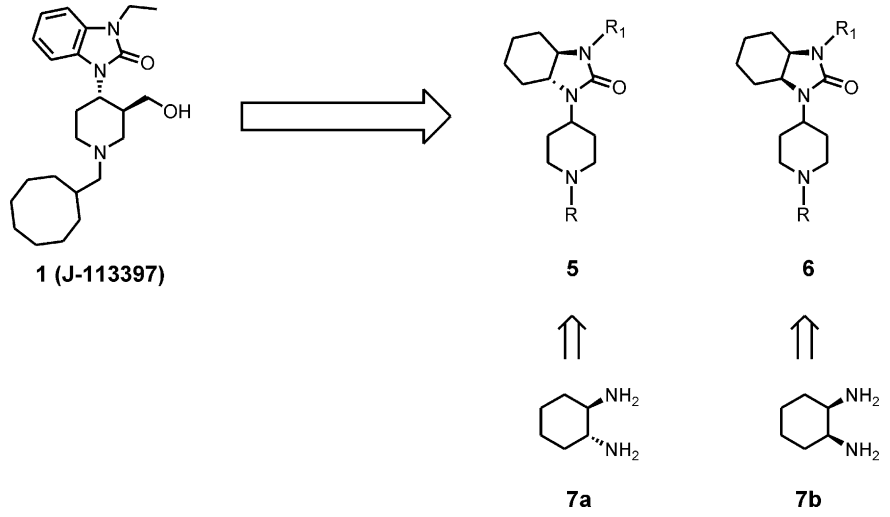
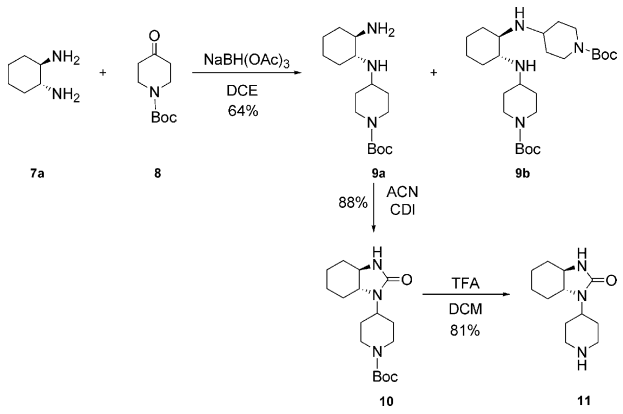


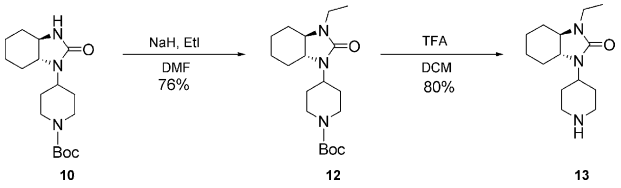
Figure 2. Design of N/OFQ receptor antagonists.

3. Synthesis of N/OFQ receptor antagonists

We have developed a new synthetic method for the construction of *trans*- and *cis*-octahydrobenzimidazol-2-one ring systems that utilizes the stereochemistry of the readily available racemic *trans*- and *cis*-diamines **7a** and **7b** (Fig. 2). The synthesis starts with the preparation of head groups **11** and **13** (Schemes 1 and 2). The reductive amination of *trans*-diamine **7a** and ketone **8** using $NaBH(OAc)_3$ in 1,2-dichloroethane provided a mixture of mono- and dialkylated products **9a** and **9b**. This reaction was quickly optimized by varying the ratios of reactants. A 2:1:1.4 ratio of **7a**, **8** and $NaBH(OAc)_3$ was found to be essential for a good isolated yield of the desired product **9a** (64% after separation). Compound **9a** was further cyclized with 1,1′-carbonyldiimidazole (CDI) in acetonitrile to yield compound **10**. Removal of the Boc group under acidic conditions provided the head group **11**. This novel synthetic procedure for head group **11** is more versatile than the previously reported method since it has been applied to the *cis*-diamine series without any modification.[15,16] In contrast, the previously reported synthetic procedure via the 1,4-addition of 4-amino-1-benzylpiperidine to 1-nitrocyclohexene affords the *trans* isomer as the major product.[15] The synthesis of the *cis*-isomer via this previously reported route would likely be difficult. Finally, alkylation of compound **10** with ethyl iodide in DMF gave compound **12** in high yield (Scheme 2). Removal of the Boc group provided the head group **13**.



Scheme 1. Synthesis of head group **11**.

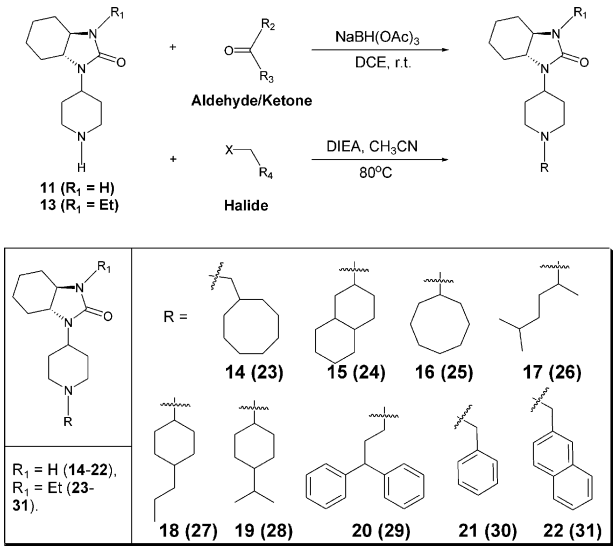


Scheme 2. Synthesis of head group **13**.

After the efficient synthesis of head groups **11** and **13** had been developed, we used parallel synthesis for the construction of our final N/OFQ receptor ligands. We utilized two high yielding reactions, namely alkylation and reductive amination, for the parallel syntheses. One aldehyde (cyclooctyl aldehyde) and five ketones were chosen for the reductive amination reaction while three halides were selected for the alkylation reaction (Scheme 3). These reagents were carefully selected to include alkyl, cycloalkyl and aromatic groups and also to include better N/OFQ receptor or mu selective R groups based on literature precedents.[8,13,14]

The six reductive amination reactions (products **14**–**19** and **23**–**28**) and three alkylation reactions (products **20**–**22** and **29**–**31**) were run in parallel fashion in 10 mL vials at 0.5 mM scale. All reactions were found to have a crude purity of 70–90% by LC/MS except for products **16** and **25** due to the high steric hindrance of the cyclooctanone starting material. The reactions were worked up by adding 1 N NaOH and ether in sequence. The ether layers were pipetted into a new set of vials and solvent was removed in a Speedvac. The residues were dissolved in a small amount of DCM and loaded into 20 mL tubes containing 5 g of silica gel. The tubes were eluted in parallel first by a mixture of EtOAc/hexanes (1:9), followed by a mixture of Et_3N/EtOAc/hexanes (5:25:70). The fractions were collected in 10 mL vials and analyzed by LC/MS. The solvents were removed in a Speedvac. The pure fractions (>97% pure by LC/MS and NMR) were collected and submitted for biological screening.[17] Compounds **15**, **17**, **18**, **19**, **24**, **26**, **27**, and **28** were screened as a mixture of isomers, no further separation has been done.

The compounds synthesized above were tested in N/OFQ receptor binding and GTPγS functional assays using membrane preparations from recombinant HEK-293 cells as previously reported.[18] Recombinant human mu, kappa and delta opioid receptor membranes were purchased from Perkin Elmer Life Sciences (Boston, MA). Dose displacement binding assays using [^{3}H]-diprenophine, [^{3}H]-U69,593 and [^{3}H]-naltrindole, respectively, were conducted according to the product inserts. The mu functional GTPγS assay was conducted using the commercially available membrane preparation under conditions previously described for the N/OFQ receptor.[18] The activity of the mu agonist DAMGO was used for data normalization (maximal effect elicited by 10 μM DAMGO = 100%; background GTPγS binding in the absence of agonist = 0%).



Scheme 3. Parallel synthesis of N/OFQ receptor antagonists.

Table 1 shows the results from N/OFQ receptor and mu receptor binding and functional assays for the compounds described above. In general, these compounds show good affinity towards the N/OFQ receptor ranging from 11 nM to 4.4 μM. The cyclooctylmethyl group was found to impart the highest affinity in both series (R_1 = H and ethyl). In addition, the octahydronaphthyl, cyclooctyl and 4-isopropylcyclohexyl groups are better groups for high N/OFQ receptor affinity (i.e., **15**, **16**, **19**, **24**, **25**, and **28**) while the 3,3-diphenylpropyl group tends to enhance affinity for the mu receptor (i.e., **20**). It is interesting that the octahydrobenzimidazol-2-one series are either antagonists or weak partial agonists throughout the entire series with the exception of compound **20** which is a full agonist. This is in contrast to the original J-113397 series in which 1-(1-benzyl-4-piperidyl)-1,3-dihydro-2*H*-benzimidazol-2-one, a direct analogue of compound **21**, is a N/OFQ receptor agonist.[8]

Although the ethyl substitution on the urea position (R_1 = ethyl) does not increase the resulting ligands affinity towards the N/OFQ receptor in general, it does

Table 1. N/OFQ and mu receptors binding and functional assays

Compd	NOP K_i (nM)[a]	NOP functional	Mu K_i (nM)[a]	Mu functional
14	16 (±4)	ANT[c]	1071(±327)	ANT [c]
15	51 (±18)	PA[d]	208 (±62)	ANT
16	125 (±31)	ANT	1402 (±208)	ANT
17	414 (±123)	PA	426 (±239)	ANT
18	634 (±117)	PA	435 (±61)	ANT
19	39 (±13)	PA	959 (±92)	ANT
20	437 (±46)	Agonist[b]	27(±9)	PA
21	1054 (±432)	ANT	ND	ND
22	1259 (±117)	ND	1237 (±437)	ND
23	11 (±3)	ANT	448 (±42)	ANT
24	184 (±94)	ANT	279(±15)	ANT
25	111 (±33)	ANT	909 (±205)	ANT
26	410 (±61)	ANT	395 (±39)	PA
27	1239 (±173)	ND	2165 (±786)	ND
28	89 (±26)	ANT	421 (±84)	ANT
29	4358 (±908)	ND	267 (±63)	ND
30	692 (±125)	ANT	716 (±202)	PA
31	824 (±100)	ANT	199(±47)	ND

[a] Values are means of at least three experiments, standard deviation is given in parentheses.
[b] A compound was considered to be a full agonist when its ability to stimulate GTPγS binding was >75% in comparison to N/OFQ (NOP) or DAMGO (mu).
[c] An antagonist (ANT) stimulated GTPγS binding with efficacy ≤10% in comparison to N/OFQ (NOP) or DAMGO (mu).
[d] A partial agonist (PA) stimulated GTPγS binding with efficacy >10% but <75% in comparison to N/OFQ (NOP) or DAMGO (mu). ND = not determined.

enhance the ligands antagonistic activity at the N/OFQ receptor. Several partial agonists in the unsubstituted urea series ($R_1 = H$, compounds **15**, **17** and **19**) are converted into antagonists by the ethyl substitution ($R_1 = Et$, compounds **24**, **26** and **28**). In addition, both series show general antagonism against the mu receptor with the exception of compounds **20**, **26**, and **30** which are either partial or weak partial mu agonists. For example, compound **23** shows complete antagonistic activity at both N/OFQ receptor and mu receptors with 40-fold selectivity over the mu receptor.

The amino acid sequence of N/OFQ receptor shares some homology to the classical opioid receptors (about 47% identity to the mu, delta and kappa receptors).[1] Hence the selectivity of our new N/OFQ receptor ligands over mu, kappa and delta receptors was also monitored. Table 2 shows the results for several selected compounds in the N/OFQ receptor, mu, kappa and delta binding assays. Most compounds in Table 2 show good to excellent selectivity against kappa and delta receptors. For example, compounds **23**, **24** and **28** are inactive in the kappa assay and compounds **15**, **19**, **23** and **28** are inactive in the delta assay. Overall, compounds **14** and **23** show the best selectivity against the three classic opioid receptors with >40-fold selectivity over mu, >106-fold over kappa and >408-fold over delta receptors. These compounds may have potential as pharmaceutical tools for further studying the role of the N/OFQ receptor in pain, drug abuse and other CNS disorders.

4. Conclusion

In conclusion, based on the reported N/OFQ receptor antagonist J-113397, we designed novel N/OFQ receptor antagonists by replacing the benzene ring with a *trans*-cyclohexane ring. A new synthetic method for the construction of *trans*-octahydrobenzimidazol-2-one ring system has been developed in the process of synthesizing the designed compounds. The resulting *trans*-octahydrobenzimidazol-2-one series shows excellent antagonistic activity towards both N/OFQ receptor and mu receptor while retaining high potency at N/OFQ receptor. Our work demonstrates that a non-aromatic hydrophobic group replacement of the benzene ring in J-113397 is sufficient to maintain effective interaction with N/OFQ receptor. In addition, the non-aromatic hydrophobic group results in a different pharmacological profile in terms of mu antagonistic activity. Future work will be focused on enhancing the selectivity and improving the pharmaceutical profile of this novel series.

Table 2. Selected data for NOP, mu, kappa and delta receptor binding assays

Compd	NOP K_i (nM)	Mu K_i (nM)	Kappa K_i (nM)	Delta K_i (nM)
14	16 (±4)	1071 (±327)	1701 (±657)	6532 (±2377)
15	51 (±18)	208 (±62)	1394 (±136)	>10,000
19	39 (±13)	959 (±92)	6212 (±2354)	>10,000
23	11 (±3)	448 (±42)	>10,000	>10,000
24	184 (±94)	279 (±15)	>10,000	ND
28	89 (±26)	421 (±84)	>10,000	>10,000

Values are means of at least three experiments, standard deviation is given in parentheses (>10,000 = not active).

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16. The synthesis of the *cis*-octahydrobenzoimidazol-2-one series was carried out in similar fashion to the preparation of compounds **11** and **13**, and the biological data will be reported at a later date.
17. Analytical data for **14**: ^{1}H NMR ($CDCl_3$) δ 1.20 (m, 2H), 1.30–1.70 (m, 19H), 1.78 (m, 4H), 1.95 (m, 3H), 2.05 (m, 2H), 2.30 (m, 1H), 2.90 (m, 2H), 3.00 (m, 2H), 3.75 (m, 1H), 4.40 (b, 1H). HR-LC/MS: 347.29286 (Exact Mass: 347.29366). Analytical data for **23**: ^{1}H NMR ($CDCl_3$) δ 1.05 (t, 3H), 1.10–2.10 (m, 30H), 2.27 (m, 1H), 2.74 (m, 1H), 2.82–2.87 (m, 3H), 3.16–3.24 (m, 2H), 3.75 (m, 1H). HR-LC/MS: 375.32225 (Exact Mass: 375.32496).
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