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14-*O*-Heterocyclic-substituted naltrexone derivatives as non-peptide mu opioid receptor selective antagonists: Design, synthesis, and biological studies

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ABSTRACT

Mu opioid receptor antagonists have clinical utility and are important research tools. To develop non-peptide and highly selective mu opioid receptor antagonist, a series of 14-*O*-heterocyclic-substituted naltrexone derivatives were designed, synthesized, and evaluated. These compounds showed subnanomolar-to-nanomolar binding affinity for the mu opioid receptor. Among them, compound **1** exhibited the highest selectivity for the mu opioid receptor over the delta and kappa receptors. These results implicated an alternative 'address' domain in the extracellular loops of the mu opioid receptor.

Opioid receptors were generally classified into three subtypes based on the pharmacological, behavioral, and biochemical studies.[1–3] Opioid antagonists have played very important roles in the study of opioid receptors. In fact, an agonist is characterized as opioid-receptor-mediated only if its effect is competitively inhibited by an opioid antagonist.[4,5] It is important to have receptor-selective opioid antagonists as tools to identify the receptor types related to the interaction with opioid agonists.[4–6] The mu opioid receptor (MOR) is the major type that mediates opioid analgesic effects of morphine, although all three opioid receptors can be involved in analgesia. The characterization of the MOR structure–function relationship is essential because it has been found that morphine's analgesic effect, addictive properties, and other major side effects are abolished in MOR knock-out mice.[7,8] Moreover, it has been demonstrated that the analgesic effects and the adverse side effects (including addiction and abuse liability) of morphine are primarily due to its interaction with the MOR.[4] In fact, naltrexone, an opioid antagonist with moderate selectivity for the MOR, has been shown to block relapse and curb drug craving in post-dependent opiate addicts.[9,10] Recent research results also indicate that MOR antagonists can be used in the treatment of obesity, psychosis and Parkinson's disease.[11] Furthermore, highly selective MOR antagonists can be used as probes to characterize the MOR-binding pocket. Yet the lack of a non-peptidyl, highly selective, and potent MOR antagonist limits our understanding of the structure–function relationship of the MOR, the interaction of non-peptidyl MOR agonists with the receptor, and more specifically, the activation mechanism of the receptor related to its role in drug abuse and addiction.

Schwyzer et al. proposed the 'message-address' concept in his analysis of the structure–activity relationship of ACTH, adrenocorticotropic hormone, and related hormones.[12] By applying the 'message-address' concept, highly selective non-peptide antagonists for the kappa opioid receptor (KOR) (e.g., norbinaltorphimine (norBNI) and 5′-guanidinonaltrindole (GNTI)),[13,14] and for the delta opioid receptor (DOR) (e.g., naltrindole (NTI))[15] were designed and synthesized several years ago (Fig. 1). Thus far no potent and highly selective antagonist derived from morphinan's structural skeleton has been developed for the MOR, although some moderately potent ligands, for example, cyprodime,[16] are available. Compared with the high selectivity of GNTI for the KOR (K_i value ratios are mu/kappa ≈ 120, delta/kappa ≈ 250)[14] and NTI for the DOR (K_i value ratios are mu/delta ≈ 152, kappa/delta ≈ 276),[15] cyprodime only has a moderate selectivity for the MOR over the DOR and KOR (K_i value ratios are kappa/mu ≈ 45, delta/mu ≈ 40).[16a] At the same time, β-funaltrexamine (β-FNA), clocinnamox, and other compounds, act as selective but irreversible antagonists for the MOR.[17] Therefore the development of a highly selective, non-peptidyl, and reversible MOR antagonist is highly desired.

It was reported that the extracellular loop (EL) domains of the MOR are critical for the binding of MOR-selective agonists, such as morphine, sufentanil, lofentanil, and DAMGO.[18] At the same time, site-directed mutagenesis studies have revealed that certain amino acid residues in this domain may be essential for ligand

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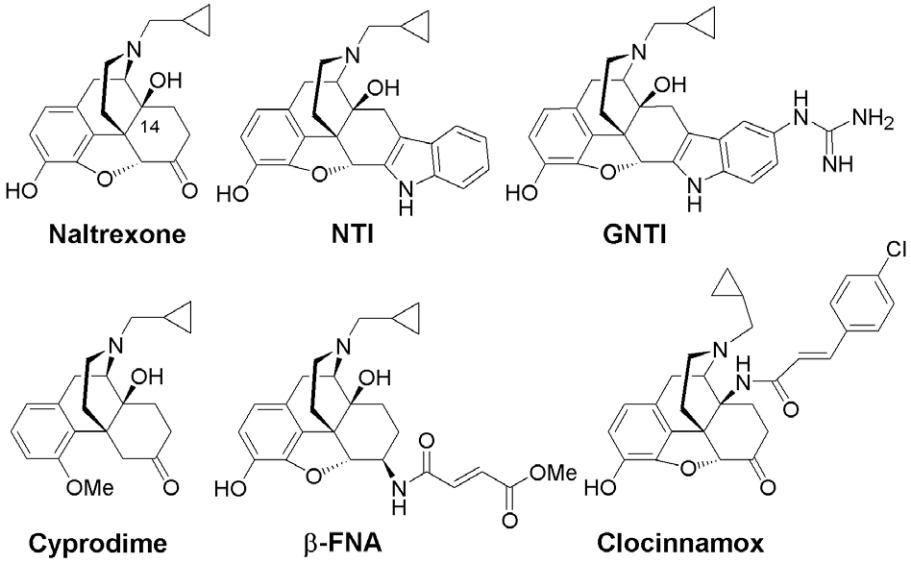


Figure 1. Morphinan derivatives as opioid selective antagonists.

(including agonist and antagonist) selectivity for the MOR over the other two opioid receptor types.[19] Therefore, a non-peptide ligand with potential interaction with the EL domains of the MOR, would be favorable for its selectivity for the MOR.

Due to the lack of the crystal structure of the MOR, so far most molecular design efforts directed toward development of selective opioid ligands have been based on structure–activity relationship studies. As a matter of fact, in the entire superfamily of GPCRs, only the X-ray crystal structures of bovine rhodopsin,[20–23] opsin,[24] and the human β2- [25–28] and β1-adrenergic receptors[29] have been successfully obtained with high resolution. Thus far, most of the molecular models of other GPCRs have been constructed using rhodopsin's structure as a template via homology modeling. Homology modeling of GPCRs has been successfully applied to further understand ligand–protein interactions, and to identify new and potent ligands. It is believed that with all the lessons learned from previous experience, GPCR homology modeling based on the bovine rhodopsin X-ray crystal structure can aid in structure-based drug design and virtual screening for therapeutic applications.[30–37] For example, a homology model of the Angiotensin II Type 1 (AT1) receptor was used to further explore the binding sites of several non-peptide AT1 receptor antagonists.[38] A homology model of the M1 muscarinic acetylcholine receptor was applied to understand the mechanism by which the agonist–receptor complex activates G proteins.[39]

Recently, we reported the construction of a MOR homology model based on the crystal structure of bovine rhodopsin.[40] This model contained not only the transmembrane helical domains, but also the extracellular and intracellular loops so that the model we obtained was integrated and complete. This model was further optimized in a membrane-aqueous system by molecular dynamics simulations. Similar homology models of the DOR and KOR were then constructed (see Supplementary information for details). Naltrexone is an ideal template for the design of selective MOR antagonists, because it has subnanomolar-to-nanomolar affinity for all three opioid receptor types and shows moderate selectivity for the MOR over the other two opioid receptor types. Figure 2 shows that in a representative binding mode of naltrexone in the MOR, the 14-hydroxyl group of naltrexone is pointing to the EL3 loop and the upper-level region of TM6/7. Compared to the amino acid residues in the corresponding domains of the KOR and DOR, some non-conserved residues, for example, Tyr212 and Trp320, in MOR could act as hydrogen bonding donor/acceptors. This unique feature in the MOR antagonist-binding locus might form an alternative 'address' domain to differentiate the antagonist binding mode of the MOR over the DOR and KOR. Therefore, a new compound containing specific structural features to interact with these amino acid residues might have increased selectivity for the MOR over the DOR and KOR.

Based on this hypothesis, a series of novel 14-*O*-substituted naltrexone derivatives (Fig. 3) have been designed and synthesized. The ester bond in these novel ligands was assumed to provide a flexible conformation for the whole side chain. The nitrogen atom in the hetero-aromatic moiety on the 14-*O*-position of naltrexone was introduced to provide an opportunity for hydrogen bonding and/or aromatic stacking interaction with the amino acid residues Tyr212 and Trp320 in the MOR binding pocket (compounds **1**–**3** and **5**–**7**). Compounds **4** and **8** were designed as control compounds to test this hypothesis. These ligands could also be considered as derivatives of clocinnamox without the Michael acceptor character.

Using naltrexone as the starting material, the syntheses of these 14-*O*-heterocyclic-substituted derivatives was straightforward (Scheme 1). To be noticed, in the second step of the synthesis route, K_2CO_3 aqueous solution was used to prepare the control compounds **4** and **8** instead of using the acidic condition. All the final compounds were obtained with reasonable yield and characterized with NMR, IR, MS, and HPLC (see Supplementary information).

The primary biological studies of these ligands included competitive radioligand-binding assays using mono-cloned opioid receptors expressed in CHO cell lines. [^{3}H] DAMGO, [^{3}H] NTI and [^{3}H] norBNI were used to label the MOR, DOR, and KOR, respectively. The binding affinities of these ligands for the MOR, DOR, and KOR, and comparative selectivities were summarized in Table 1. These compounds showed binding affinities in the subnanomolar-to-nanomolar range for the MOR.

Also as shown above, all of these compounds exhibited different levels of selectivity for the MOR over the KOR and DOR. Among these, compound **1** had approximately 800-fold selectivity for the MOR over the DOR and nearly 200-fold selectivity over the KOR. Compound **5** also showed over 100-fold selectivity for the MOR over the other two receptor types, although its binding affinity for the MOR was significantly lower than compound **1**. In addition, all of these compounds acted as MOR antagonists in ^{35}[S]GTPγS

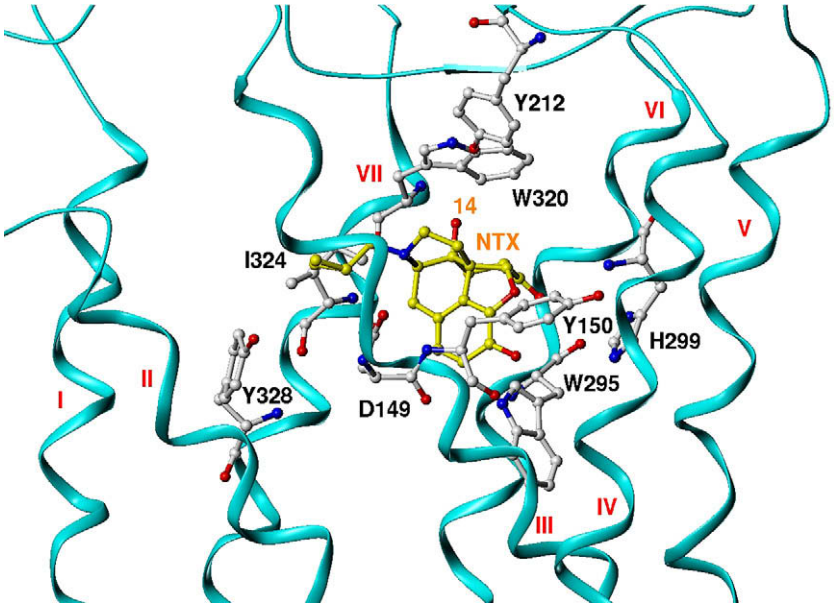


Figure 2. Naltrexone in MOR-binding pocket: mu opioid receptor model: ribbon and in cyan color; the residues in mu opioid receptor: ball and stick and in atom color; naltrexone molecular: ball and stick and carbon in yellow color, and oxygen red, nitrogen blue.

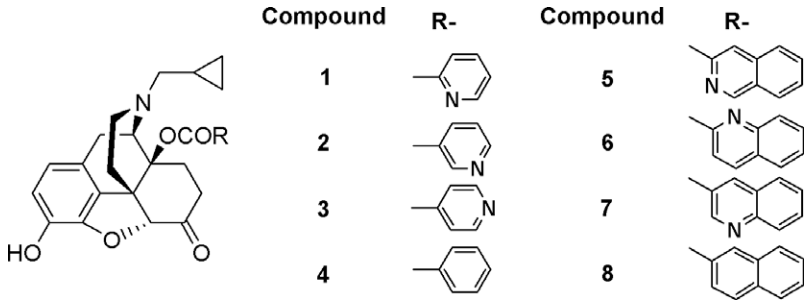
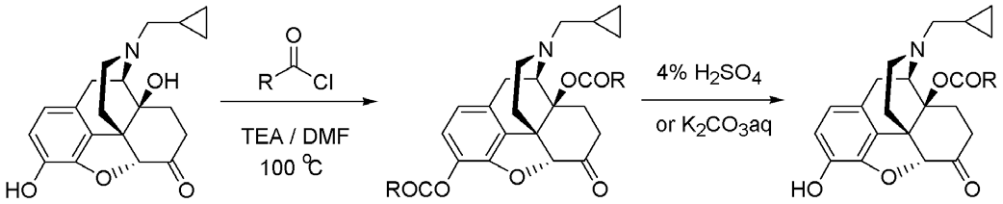


Figure 3. The designed ligand for primary study.



Scheme 1. The synthetic route for the 14-*O*-subsituted naltrexone derivatives.

functional assays except for compound **7**, which was a partial agonist.

Compared to the control compounds **4** and **8**, the MOR selectivity over DOR and KOR had been enhanced greatly in all of the other compounds. This result suggested that the 14-*O*-substitutions introduced onto the naltrexone skeleton might interact with the proposed alternative 'address' domain in the MOR, and the nitrogen atom in the heterocyclic ring might act as a hydrogen bond acceptor and play an important role for the selectivity. Among all of these ligands, compound **1** showed the highest selectivity, which suggested that it had the most favorable orientation of its side chain towards this plausible 'address'-binding domain in the MOR. For compound **5**, its side chain might confer selectivity for the MOR, whereas the bulkiness of its side chain also might have reduced its binding affinity for the MOR.

To further characterize compound **1** as the lead for our next generation molecular design, its antagonism was evaluated against DAMGO in ^{35}[S]GTPγS functional assay. The concentration of compound **1** was 1.5 nM while DAMGO was in the range of 10–10,000 nM. The Ke value of compound was 0.20 ± 0.04 nM and apparent pA2 value was 9.72 ± 0.10. This observation was consistent with the binding affinity results and further verified that compound **1** could be used as the lead for future molecular design.

It has been reported by Schmidhammer et al., that 14-alkoxymorphinans showed very high opioid receptor affinity. These compounds exhibited significantly increased binding affinities at all opioid receptors without any specific preference for any one receptor type.[41–43] Recently, Husbands et al., investigated the SAR of the analogs of clocinnamox, 14-aminodihydromorphinones, and 14-aminodihydrocodeinones, to explore the effect of changing the

Table 1
Binding affinity and functional assay results for the 14-*O*-substituted naltrexone derivatives

Compound	K_i ± SEM (nM)			Selectivity		Percent Max of DAMGO
	[^{3}H]DAMGO (μ)	[^{3}H] NTI (δ)	[^{3}H] norBNI (κ)	δ/μ	κ/μ	
Naltrexone	0.26 ± 0.02	117.00 ± 8.90	5.15 ± 0.26	450	20	0.00
β-FNA	0.41 ± 0.04	27.78 ± 4.60	0.94 ± 0.05	68	2	0.00
CTAP	2.02 ± 0.71	1441.00 ± 106.10	1012.70 ± 174.80	713	501	0.00
1	0.14 ± 0.03	117.38 ± 17.97	25.50 ± 6.50	838	182	0.00
2	1.59 ± 0.61	170.30 ± 12.64	47.81 ± 8.48	107	30	0.00
3	5.58 ± 1.34	405.32 ± 234.68	49.21 ± 20.37	73	9	0.00
4	123.23 ± 38.23	>10,000.00	586.42 ± 32.39	>81	5	0.00
5	68.40 ± 6.04	>10,000.00	>10,000.00	>146	>146	0.00
6	1.44 ± 0.32	22.81 ± 19.52	67.15 ± 36.72	16	47	0.00
7	2.69 ± 0.72	818.43 ± 507.23	148.23 ± 55.53	304	55	22.00 ± 10.30
8	225.27 ± 46.6	907.18 ± 192.99	46.57 ± 13.53	4	<1	0.00

The K_i values for the mu, delta and kappa opioid receptors were $n = 3$. The averages were reported along with their standard error of the means, SEM, for each compound. The comparison to percent stimulation of DAMGO was the E_{max} of the compound compared to the E_{max} of DAMGO (normalized to 100%). The DAMGO EC_{50} value was 45.1 ± 6.63 nM and its E_{max} value was 366 ± 23% stimulation over basal using a [^{35}S]GTPγS functional assay. Naltrexone, β-FNA and CTAP were tested along as positive controls under the same conditions.

chain linking and substitution in the aromatic ring of cinnamoylaminomorphinones and codeinones.[44–46] These authors found that a modest selectivity for the MOR over the DOR and KOR was achieved when the side chain on the 14 positions was comparably rotatable in these 14-aminiodihydromorphinone compounds.

Comparing to the compounds reported by Schmidhammer and Husbands, the compounds reported here showed similar affinity for the MOR, but much higher selectivity over the DOR and KOR. One possible explanation might be that the introduction of a shorter side chain and a more flexible ester bond in our compounds might lead to a more favorable conformation and orientation of the side chain to target the 'address' locus and thereby improve selectivity for the MOR. Certainly this 'address' locus needs to be further verified, for example, by site-directed mutagenesis, in future studies.

In summary, a series of 14-*O*-heterocyclic-substituted naltrexone derivatives were designed, synthesized, and evaluated as selective MOR antagonists. Most of these novel ligands exhibited subnanomolar-to-nanomolar binding affinity for the MOR, with compound **1** showing the highest selectivity for the MOR over the DOR and KOR. These results implicated a plausible 'address' domain in the extracellular loops of the MOR. The knowledge gained from these studies will enrich the 'message-address' concept that has been applied successfully in opioid research and may lead to the identification of potent MOR-selective non-peptide antagonists.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.12.093.

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