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Design, synthesis, and characterization of 6β-naltrexol analogs, and their selectivity for in vitro opioid receptor subtypes

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ABSTRACT

Since the mu opioid receptor (MOR) is known to be involved in the therapeutically relevant pathways leading to the manifestation of pain and addiction, we are currently studying the specific structural characteristics that promote antagonism at the MOR. The opiates 6β-naltrexol and 6β-naltrexamide function as neutral antagonists in in vitro and in vivo systems previously exposed to morphine, and are under investigation as improved treatments for narcotic dependence. In this research, we synthesized and characterized carbamate and sulfonate ester derivates of 6β-naltrexol that do not contain a protic group at C_6, and evaluated these compounds for opioid receptor affinity. In vitro receptor subtype (μ, κ, and δ opioid receptors) binding data of the carbamate and sulfonate derivatives is reported. All four compounds synthesized exhibited affinity for the MOR better than the standard 6β-naltrexol HCl. Based on K_i data, the order of MOR affinity is as follows: **9** > **13** > **14** > **10** > 6β-naltrexol HCl. Carbamate **9** and tosylate **13** displayed subnanomolar affinity for the MOR, while **10** was the most μ-selective compound synthesized. In conclusion, our data indicate that the absence of a hydrogen-bond donor on the C_6 oxygen enhances rather than impedes the in vitro affinity of naltrexol derivatives for the MOR. Additionally, data also suggest that increasing the bulk around C_6 may allow control of subtype selectivity within these compound series.

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Of the three primary opioid receptor subtypes (μ[MOR], κ[KOR], and δ[DOR]), the μ opioid receptor remains a clinically-significant therapeutic target for the treatment of pain and addiction. High efficacy opioids like the μ-agonist morphine are indispensable for acute and chronic pain management (Fig. 1).[1,2] However, the clinical use of morphine for pain control is complicated by serious side effects including gastrointestinal distress (e.g., constipation, nausea and vomiting), itching, respiratory depression and addiction liability.[2a,3] To manage these side effects, treat opioid overdose, and address opioid abuse/addiction, peripherally selective MOR antagonists like naloxone (NLO) and naltrexone (NTX) are often administered (Fig. 1).[4]

The medicinal use of the prototypical opioid antagonists NLO and NTX is limited in part by their pharmacokinetic and pharmacodynamic properties. Both NLO and NTX are antagonists but exert inverse agonist effects in the opioid dependent state. These inverse agonism (IA) properties increase the severity of the withdrawal syndrome because the basal or constitutive signaling of the MOR is suppressed. Presence of the IA response may subsequently effect compliance with long-term medication use.[5]

Sadée and co-workers have identified several NTX analogs that differ in terms of their intrinsic efficacy at the cloned opioid receptors.[5a] These compounds, 6β-naltrexol (6β-NTXol) and 6β-naltrexamide (6β-NTXam), are devoid of a ketone at C_6 and do not affect basal signaling levels of the μ and δ opioid receptors in the opioid naïve and dependent states (Fig. 1). Functionally, 6β-NTXol and 6β-NTXam act as neutral antagonists (NAs). Because 6β-naltrexol and 6β-naltrexamide are NAs in the opioid dependent state, they are being explored as possible treatments for opioid overdose, opioid addiction, and as therapies that may decrease side effects commonly associated with opioid analgesics.[6]

The demonstrated clinical utility of 6β-NTXol and the therapeutic potential of 6β-NTXam have motivated us to further explore the efficacy, potency, and receptor subtype selectivity for related derivatives. Specifically, we are interested in further understanding the structural requirements that promote MOR selectivity and neutral antagonism (in vitro and in vivo). We believe that the nature and identity of the C_6 substituent can be altered to yield optimum compound properties. Also, we hypothesize that differences in opioid ligand structure may give rise to

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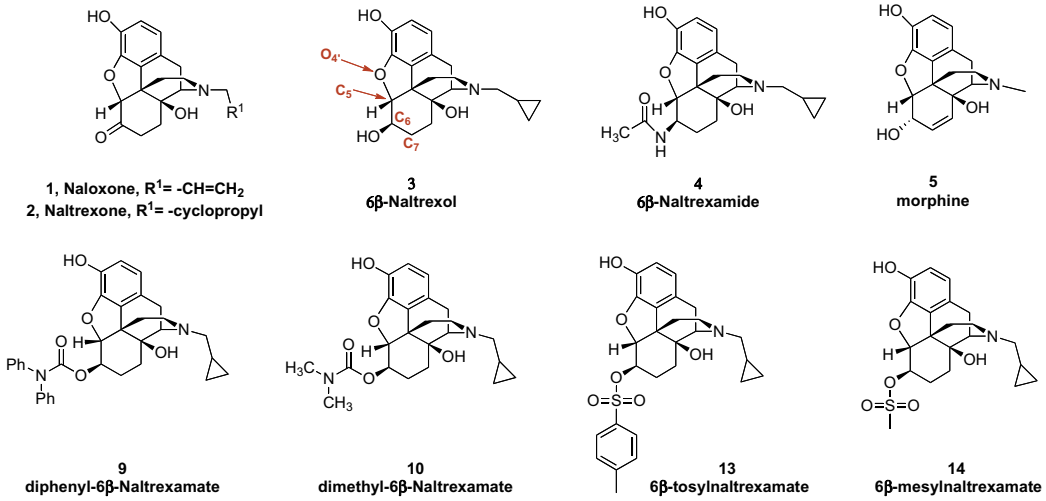

Figure 1. Structures of 6β-naltrexol and related derivatives, and synthetic targets **9** and **10**, and **13** and **14**.

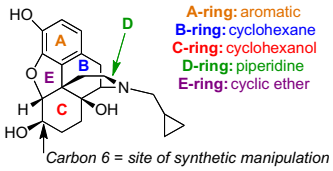

Figure 2. Key structural elements of the naltrexol skeleton.

different functionally-relevant conformations of the mu opioid receptor (Figs. 1 and 2).

In this preliminary study, we synthesized and characterized four derivatives of 6β-NTXol that contain a carbamate moiety (**9** and **10**) or sulfonate ester functional group (**13** and **14**) at C_6 (Fig. 3). We subsequently screened these compounds for in vitro binding to μ, κ, and δ-opioid receptors and compared their selectivities (δ/μ and κ/μ). Because the C_6 oxygen is devoid of a hydrogen atom, we are exploring whether a H-bond donor is necessary at the position. (Figs. 1 and 2) Furthermore, with this series of compounds we are investigating the steric and electronic preferences for the region surrounding C_6.

The synthesis of carbamates **9** and **10** and sulfonate esters **13** and **14**, respectively, was achieved in three steps using modified literature procedures (Fig. 3). To simplify purification of intermediates and provide increased flexibility for synthetic manipulation of intermediates in our ongoing research, we first protected the phenol on 6β-NTXol as a benzyl ether using benzyl bromide with K_2CO_3 in refluxing acetone in 92% yield.[7,8] Benzyl ether **6** was subsequently treated with either diphenyl- or dimethylcarbamoyl chloride in pyridine under reflux to yield C_6-carbamates **7** and **8** in 26% yield and 47% yield, respectively. We were not surprised that the carbamoylation yields were low due to the reduced electrophilicity of the carbonyl in the starting carbamoyl chlorides. Similarly, sulfonate esters **11** (56% yield) and **12** (40% yield) were easily synthesized by stirring **6** with either tosyl or mesyl chloride in room temperature pyridine.[9] Although sulfonate esters are typically good leaving groups and are commonly used in β-elimination and substitution reactions,[10] the sulfonate esters **11** and **12** (and their debenzylated derivatives **13** and **14**) are stable. We attribute

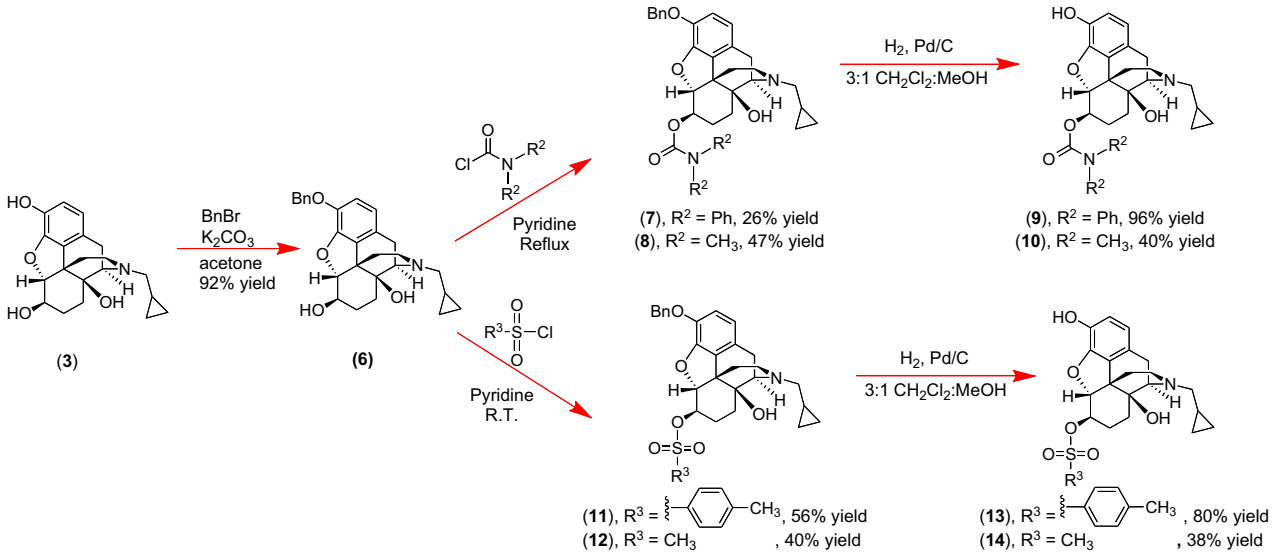

Figure 3. Synthesis of carbamate (**9** and **10**) and sulfonate ester (**13** and **14**) derivatives of 6β-naltrexol.

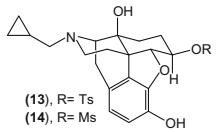

Figure 4. Proposed stable conformation of 6β-OTs and -OMs.

this stability to Portoguese's previous insight[9] that the preferred chair conformation of Ring C places the 6β-sulfonate ester in an unfavorable orientation to undergo an S_N2 or E2 reaction (Fig. 4).[11]

Once obtained, the resulting sulfonate ester (**11** and **12**) and carbamate (**7** and **8**) intermediates were debenzylated using standard hydrogenolysis conditions (H_2, Pd/C, 3:1:CH_2Cl_2:CH_3OH) to yield final compounds **9** and **10** and **13** and **14** in good yields (Fig. 3). All intermediate and final compounds were purified using silica gel chromatography and spectroscopically characterized using NMR (^{1}H, ^{13}C, and 2D NMR spectroscopy), high resolution mass spectrometry, and IR.[12] The isolated yields reported in this work are unoptimized.

The four compounds synthesized were next screened in in vitro opioid receptor binding assays according to procedures outlined by Fontana et al.[13] Data is provided in Table 1. All four compounds synthesized exhibited affinity for the MOR better than the standard, 6β-naltrexol HCl. Our most potent compounds for binding at the MOR, diphenylcarbamate **9** (K_i = 0.56 nM) and tosylate **13** (K_i = 0.79 nM), both contained one or more phenyl rings. Based on K_i data, the order of MOR affinity for the sulfonates and carbamates is as follows: **9** > **13** > **14** > **10** > 6β-naltrexol HCl. These data suggests that bulky, hydrophobic substituents protruding from the oxygen atom off of C_6 may create favorable interactions that could enhance the affinity of compounds for the MOR.

Even though dimethylcarbamate **10** did not possess the greatest affinity for the MOR over other compounds studied, **10** was the most selective compound for binding to the MOR subtype. Compound **10** was 180 times more selective for the MOR than the DOR, and 15 times more selective for the MOR versus the KOR. Since the MOR is known to be involved in the therapeutically relevant pathways leading to the manifestation of pain and addiction, we are encouraged by the subtype specificity and affinity trends observed with **10** and other compounds in the sulfonate and carbamate series. Our data suggest that the absence of a hydrogen-bond donor group on the oxygen atom connected to C_6 does not appear to negatively influence in vitro affinity of naltrexol derivatives for the MOR.

Although we were seeking μ-selective compounds as potential treatments for addiction in this research, we are also interested in the receptor subtype selectivity profiles for the sulfonate ester and carbamate derivatives synthesized. Overall, we found the selectivity rank for MOR over DOR to be: **10** > **9** > 6β-naltrexol HCl > **14** > **13**, while the preference for MOR versus KOR binding follows: **9** > **10** > **14** > 6β-naltrexol HCl > **13**. Both sulfonate esters and carbamates alike generally displayed a bias for the MOR. All compounds also bound to the KOR with nanomolar affinity ranging from approximately 1–28 nM. Conversely, tosylate **13** was the only compound of those synthesized that demonstrated low nanomolar binding (K_i = 5.16 nM) to the DOR.

The receptor binding data for **13** are particularly interesting. Due to **13**'s sub- to low-nanomolar affinity for all opioid receptors investigated, including a slight preference for the MOR (K_i = 0.79 nM), **13** may make a non-selective in vitro probe for general opioid receptor function (Table 1). Furthermore, other investigators are interested in opioid ligands with mixed receptor binding and functional profiles for varied applications in pain and addiction therapies. For example, opioid ligands possessing μ-agonist/δ-antagonist profiles are known to produce analgesia with less tolerance and dependence.[14] Clearly, present insights from this research into the relationship between structure and receptor binding for the sulfonate ester and carbamate derivatives will be further informed by functional binding experiments using the [^{35}S]GTP-γ-S assay.[15] Functional binding assay data and in vivo efficacy data on selected compounds from the sulfonate esters and carbamates will be disclosed in due course.

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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.2009.03.095.

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Table 1
Data from radioligand displacement assays with μ, δ, or κ-opioid receptors and subtype selectivity ratios for compounds **9**, **10**, **13**, and **14**.

Compound	μ opioid receptor[a] (MOR) [^{3}H]-DAMGO K_i (nM) ± SD	δ opioid receptor[a] (DOR) [^{3}H]-DADLE K_i (nM) ± SD	κ opioid receptor[a] (KOR) [^{3}H]-U69,593 K_i (nM) ± SD	Opioid Receptor Subtype Selectivity	
				δ/μ	κ/μ
9	0.56 ± 0.09	74.6 ± 3.2	14.7 ± 1.0	133	26.3
10	1.85 ± 0.24	334 ± 22	28.2 ± 1.5	180	15.2
13	0.79 ± 0.06	5.16 ± 0.38	1.66 ± 0.15	6.5	2.1
14	1.11 ± 0.13	93.1 ± 5.5	6.75 ± 0.73	83.8	6.1
6β-naltrexol HCl	2.12 ± 0.29	213 ± 18	7.42 ± 0.45	100	3.5

[a] Radioligand-based binding assays were performed using Chinese hamster ovary (CHO) cells that were stably transfected with either human μ, δ, or κ DNA and expressed the human μ, δ, or κ-opioid receptor, respectively.[10] All results are ± standard deviation (SD) with n = 3. K_i values were determined by fitting the pooled data of three curves (30 data points) to the two parameter logistic equation for the best-fit estimates of the IC_{50}. The K_i value was calculated from the IC_{50} using standard equations. Assays were conducted as described in Fontana et al.[13]

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