Design, Synthesis, and Characterization of Analogs of the mu-Opioid Receptor Antagonist 6β–Naltrexol, and Their Evaluation in *In Vitro* Opioid Receptor Models

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ABSTRACT

The opiates 6ß-naltrexol and 6ß-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. We are currently studying the specific structural characteristics that differentiate inverse agonists from neutral antagonists at the mu opioid receptor. In this research, we synthesized carbamate and sulfonate derivates of 6ß-naltrexol that do not contain a protic group at C_6, and characterized these derivatives using ^1H and ^{13}C NMR, IR, and mass spectrometry. Data on *in vitro* receptor subtype selectivity (mu, kappa, and delta opioid receptors) of the carbamate and sulfonate derivatives is reported. In conclusion, the data obtained further directs the design of improved analogs for the treatment of narcotic dependence, and enhances our understanding of the relationship between structure and opioid subtype selectivity.

BACKGROUND & SIGNIFICANCE

- Although opioid abuse and addiction are responsible for serious health, economic, and social problems in the United States, high efficacy opioids (e.g., morphine) are commonly used for acute and chronic pain management
- The clinical use of opioids for pain management is complicated by various side-effects including gastrointestinal effects (e.g., constipation, nausea and vomiting), pruritis, respiratory depression and addiction liability
- Opioid antagonists have been used as adjunct agents for the treatment of opioid overdose, opioid abuse/addiction, and for management of opioid-mediated side-effects (e.g., peripherally selective antagonists)
- The use of the prototypic opioid antagonists naloxone and naltrexone (NTX) is limited in part by pharmacokinetic and pharmacodynamic properties of these drugs
  - Both compounds exert inverse agonist effects in the opioid dependent state which increases the severity of the withdrawal syndrome (Raehal et al., 2005; Wang et al., 2004, 2001; Bilsky et al., 1996)
  - In the case of naltrexone, the inverse agonist effects of the compound may affect compliance with long-term medication use
  - The short duration of action of naloxone can also place the patient at risk for reintoxication, especially in the case of an overdose with a long-acting opioid
  - In addition, naloxone and naltrexone both readily enter the central nervous system, which may not be desirable in some circumstances
- Our research collaborators have identified a number of naloxone and naltrexone analogs that differ in terms of their intrinsic efficacy at the cloned opioid receptors (see Sadée et al., 2005)
  - These compounds (e.g., 6ß-naltrexol and 6ß-naltrexamide) do not affect basal signaling levels of the mu and delta opioid receptors in the opioid naïve and opioid dependent states
  - Functionally, this class of compounds act as neutral antagonists
- Because 6β-naltrexol and 6β-naltrexamide are neutral antagonists in the opioid dependent state, they are being explored as possible treatments for opioid overdose, opioid addiction, and as medications that will decrease side-effects associated with opioid analgesics
- Due to the promise of the above agents, we are interested in modifying the 6-position on naltrexol to improve efficacy, potency, and receptor subtype selectivity. Ultimately we desire to improve our understanding of the molecular characteristics that promote neutral antagonism *in vivo*
  - Our target compounds, **(12)** and **(13)**, possess a carbamate group instead of a ketone or alcohol at C_6.
  - Our target compounds, **(16)** and **(17)**, possess a sulfonate ester instead of a ketone or alcohol at C_6.
  - The synthesis and biological characterization of derivatives **(12)**, **(13)**, **(16)**, and **(17)** may lead to improved treatments for pain and/or addiction. Furthermore, this research could also lead to a better understanding of the biochemical mechanism responsible for the addictions experienced by patients using narcotics such as morphine or heroin.

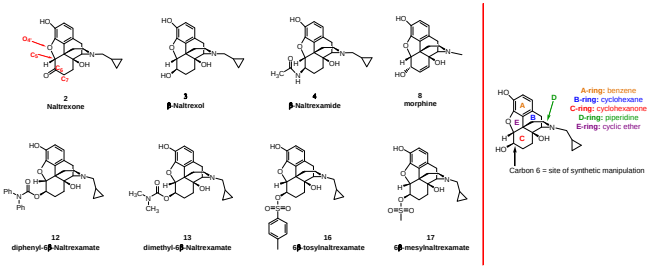


Figure 1. (left) Structures of opioids and target compound. (right) Ring nomenclature of 6β-naltrexol

HYPOTHESES & SPECIFIC AIMS

Hypotheses:

- We hypothesize that a protic functional group (-OH or -NH) may be required for naltrexol derivatives elaborated at position 6 to hydrogen bond in a favorable way with the mu opioid receptor and generate neutral antagonism effects
- We hypothesize that differences in opioid ligand structure give rise to different functionally-relevant conformations of the mu opioid receptor

Chemistry Research Goals:

- To execute the semi-synthesis of **(12)**, **(13)**, **(16)**, and **(17)** from 6β-naltrexol
- To fully characterize **(12)**, **(13)**, **(16)**, and **(17)** and their intermediates using modern spectroscopic methods including ^1H and ^{13}C NMR, mass spec, and IR
- To develop a structure-activity profile for carbamate and sulfonate derivatives of 6β-naltrexol using *in vivo* and *in vitro* data, log P estimations, and conformational analysis/molecular modeling

Biochemical/Pharmacological Research Goals:

- To determine the opioid receptor subtype specificity of **(12)**, **(13)**, **(16)**, and **(17)** in comparison to 6ß naltrexol
- To determine whether **(12)**, **(13)**, **(16)**, and **(17)** function as antagonists or agonists in *in vitro* receptor models. If compounds are antagonists, we aim to further classify them as neutral antagonists or inverse agonists

SYNTHETIC METHODOLOGY: SEMISYNTHESIS OF CARBAMATE AND SULFONATE ESTER-BASED NALTREXOL DERIVATIVES

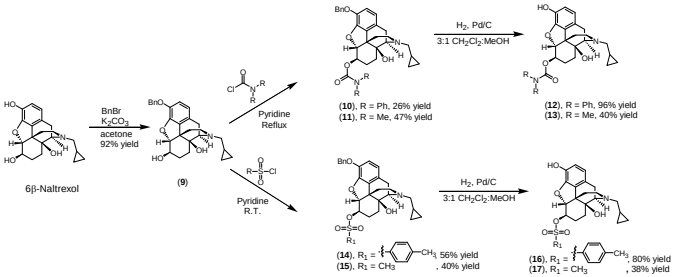


Figure 3. Semisynthesis of carbamate (12 & 13) and sulfonate ester (16 & 17) derivatives of 6β-naltrexol.

REPRESENTATIVE SPECTRAL DATA FOR DIPHENYL β-NALTREXAMATE, (12)

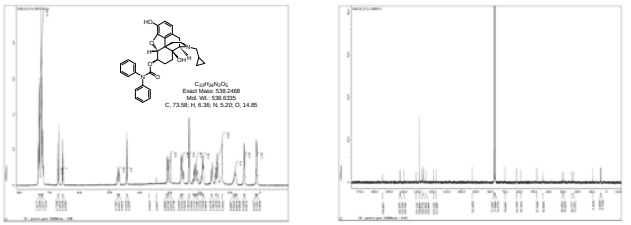


Figure 4. Proton (^1H, left) and carbon (^{13}C, right) NMR spectra of compound (12). NMR experiments were completed in $CDCl_3$ using a 300 MHz Jeol ECX300 NMR Spectrometer.

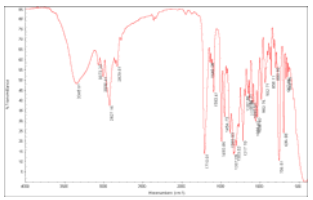


Figure 5. IR spectrum of (12) was acquired on a ThermoNicolet IR 300 Spectrometer. The sample was prepared by creating a thin film using CCl_4.

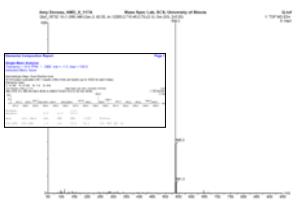


Figure 6. High Resolution mass spec of (12) showing elemental composition of $[M+H]^+$. The spectrum was acquired on a Micromass Quadrupole Time-of-Flight Ultima (Q-TOF) mass spectrometer using electrospray ionization (ESI) in positive ion mode.

DISCUSSION: MODELING AND CHEMISTRY EFFORTS

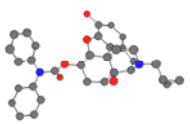
Synthesis:

- The benzyl protection of was completed in 92% yield using K_2CO_3, BnBr, and acetone to yield 3-OBn-6β-naltrexol. Benzyl protection increased the ease of purification in subsequent steps.
- Yields for carbamate formation were expectedly low because the carbamoyl chlorides are not good electrophiles.
- Visualization of compounds by thin layer chromatography (TLC) was achieved using UV-Vis, an iodine chamber, and or the appropriate TLC stain.
- The synthesis and purification of compounds related to **(13)** and **(17)** is currently being optimized
- Compounds are presently being scaled-up to meet *in vivo* screening needs.

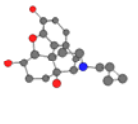
Molecular Modeling:

- The torsion angle controlling the disposition of the *C* ring differs only by 6.0° between **(12)** and **(1)**.
- We are interested in exploring whether there is a link between torsion angle, log P, and *in vitro* & *in vivo* potencies.
- Based on Log P values the rank order of lipophilicity for target compounds with respect to selected standards is: **(12)** > **(16)** > **(13)** > **(3)** > **(2)** > **(4)** ≈ **(17)**

MOLECULAR MODELING: UNDERSTANDING C-RING CONFORMATION & ESTIMATING LOG P FOR TARGET MOLECULES



Diphenyl β-naltrexamate,12
O_4-C_5-C_6-C_7 = 160.9 °



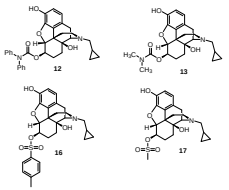
β-Naltrexol, 1
O_4-C_5-C_6-C_7 = 154.7 °

Compound #	Compound Name	Torsion angle	Calculated Log P
3	6β-naltrexol	154.9 °	1.17
2	naltrexone	79.9 °	0.82
4	naltrexamide	156.7 °	0.72
13	dimethyl-6β-naltrexamate	80.9 °	1.54
12	diphenyl-6β-naltrexamate	160.9 °	4.87
16	6β-tosylnaltrexamate	152.5 °	3.38
17	6β-mesylnaltrexamate	82.6 °	0.71

Figure 2. Energy minimized structures (MM2 Force Field, energy minimization; hydrogens are omitted for clarity) of (12) and (1), (left) and Log P estimations (right) using Chem3D. "Log P" is equal to the log of the octanol/water partition coefficient for a given compound.

CHARACTERIZATION OF OPIOID RECEPTOR SUBTYPE SPECIFICITY: RADIOLIGAND BINDING ASSAY

Compound	μ-Receptor (MOR) ^{3}H-DAMGO		δ-Receptor (DOR) ^{3}H-DADLE		κ-Receptor (KOR) ^{3}H-U69,593	
	K_i (nM)	N	K_i (nM)	N	K_i (nM)	N
12	0.56 ± 0.09	1.59 ± 0.34	74.60 ± 3.17	1.05 ± 0.04	14.73 ± 1.04	1.12 ± 0.08
13	1.85 ± 0.24	1.51 ± 0.26	333.58 ± 22.39	1.26 ± 0.11	28.18 ± 1.46	1.18 ± 0.07
16	0.79 ± 0.06	1.47 ± 0.15	5.16 ± 0.38	1.24 ± 0.10	1.66 ± 0.15	0.92 ± 0.07
17	1.11 ± 0.13	1.41 ± 0.21	93.06 ± 5.54	1.18 ± 0.09	6.75 ± 0.73	1.33 ± 0.17
6β-Naltrexol HCl	2.12 ± 0.29	1.46 ± 0.25	213.13 ± 17.94	1.19 ± 0.10	7.42 ± 0.45	1.04 ± 0.06



Binding Assay Methodology:

- Opioid receptor binding assays were conducted with minor modifications of published procedures (Rice et. al, 2007).
- Membranes were prepared from CHO cells expressing the cloned human mu opioid receptor (MOR), delta receptor (DOR) and kappa receptor (KOR).
- Radioligand binding assays used [^{3}H]DAMGO, [^{3}H][D-Ala2,D-Leu5]enkephalin or [^{3}H]U69,593 to label the MOR, DOR and KOR, respectively.
- Ki 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} and slope factor (N). The Ki value was calculated from the IC_{50} using standard equations.

DISCUSSION: UNDERSTANDING RECEPTOR SUBTYPE SELECTIVITY

- All four compounds synthesized exhibited affinity for the MOR better than the standard, 6β-Naltrexol HCl.
  - Diphenylcarbamate **(12)** and tosylate **(16)** displayed sub-nanomolar affinity for the MOR!
- Based on Ki data, the order of MOR affinity is as follows: **(12)** > **(16)** > **(17)** > **(13)** > 6β-Naltrexol HCl
  - Dimethylcarbamate **(13)** was our most selective compound synthesized. In general, **(12)** 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.
- Furthermore, the absence of a hydrogen-bond donor does not appear to influence *in vitro* affinity of naltrexol derivatives for the MOR. This will inform our future medicinal chemistry efforts.

FUTURE DIRECTIONS

- Complete *in vitro* functional binding assays studies ([^{35}S]GTP-γ-S) using in opioid receptor cell systems, analyze data, and integrate with subtype selectivity and *in vivo* data.
- Complete dose-response curves in *in vivo* dependence models to determine the potency and efficacy of compounds **(12)**, **(13)**, **(16)**, and **(17)** to precipitate withdrawal at various levels of physical dependence
- Calculate distribution coefficients (D) and compare to log P data to investigate the importance of lipophilicity in binding
- With data in hand, work toward developing a structure-activity profile for neutral antagonism versus inverse agonism.
- When our studies are completed, we should have an improved understanding of the structure-activity requirements that determines if a particular naltrexol/one analog will act as an inverse agonist or neutral antagonist

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