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Synthesis and opioid receptor binding properties of a highly potent 4-hydroxy analogue of naltrexone

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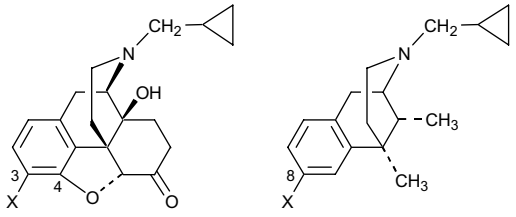
Abstract—Very high affinity for opioid receptors (e.g., K_i = 0.052 nM for μ) has been observed in the rationally designed naltrexone analogue **5**. SAR and physical data supports the hypothesis that the 4-OH group of **5** stabilizes the 3-carboxamido group in the putative bioactive conformation.

As part of our broad goal to identify long-acting opioid receptor interactive agents useful in the treatment of cocaine and heroin addiction in humans, we recently reported the synthesis and opioid receptor binding data of 3-desoxy-3-carboxamidonaltrexone **1**.[1] This novel analogue of naltrexone (**2**) where its phenolic OH group, typical of opioid receptor interactive agents, was replaced with a carboxamido group displayed high affinity for the μ opioid receptor. Relative to naltrexone, carboxamide **1** had approximately one order of magnitude reduced affinity for μ. This SAR was not in synch with that noted for 2,6-methano-3-benzazocines [e.g., cyclazocine (**4**)] where comparable or enhanced affinity was seen for the 8-carboxamides [e.g., 8-carboxamidocyclazocine (8-CAC, **3**)] relative to the corresponding phenolic OH parent (Fig. 1).[2]

To explain this divergent SAR, we hypothesized that the ether oxygen of the furan ring in the 4,5α-epoxymorphinan derivative **1** stabilizes a conformation (**1a** in Fig. 2) that is different than the putative bioactive conformation **1b** and that an energy penalty would be required for the more stable (unbound state) conformer **1a** to attain the bioactive conformation resulting in reduced binding affinity. Evidence supporting **1b** as the bioactive conformation was derived, in part, from comparative



1: X = $CONH_2$
2: X = OH
3: X = $CONH_2$
4: X = OH

Figure 1. Structures of lead compounds for this study.

proton NMR data for **1** and **3** in $CDCl_3$. For **1**, we observed a strong intramolecular H-bond that bridges the carboxamido group to the neighbouring ether oxygen of the furan ring (NH protons appear as singlets at δ 5.77 and 7.16 that do not coalesce upon warming to 45 °C). These NMR data contrast that of **3** where the two carboxamide NH's appear as broad singlets (δ 5.63 and 6.04) and coalesce into a very broad singlet (δ 5.80) between 30 and 40 °C. 8-CAC (**3**) has no neighbouring furan ring with which the carboxamide can intramolecularly H-bond, which results in little or no preference/barrier for/between conformations **3a/b** in the unbound state. This knowledge, coupled with the divergent SAR noted above, led us to reason that the μ opioid receptor can bind **3b**, the putative bioactive conformation, with no conformational energy penalty.

Keywords: Opiate; SAR.
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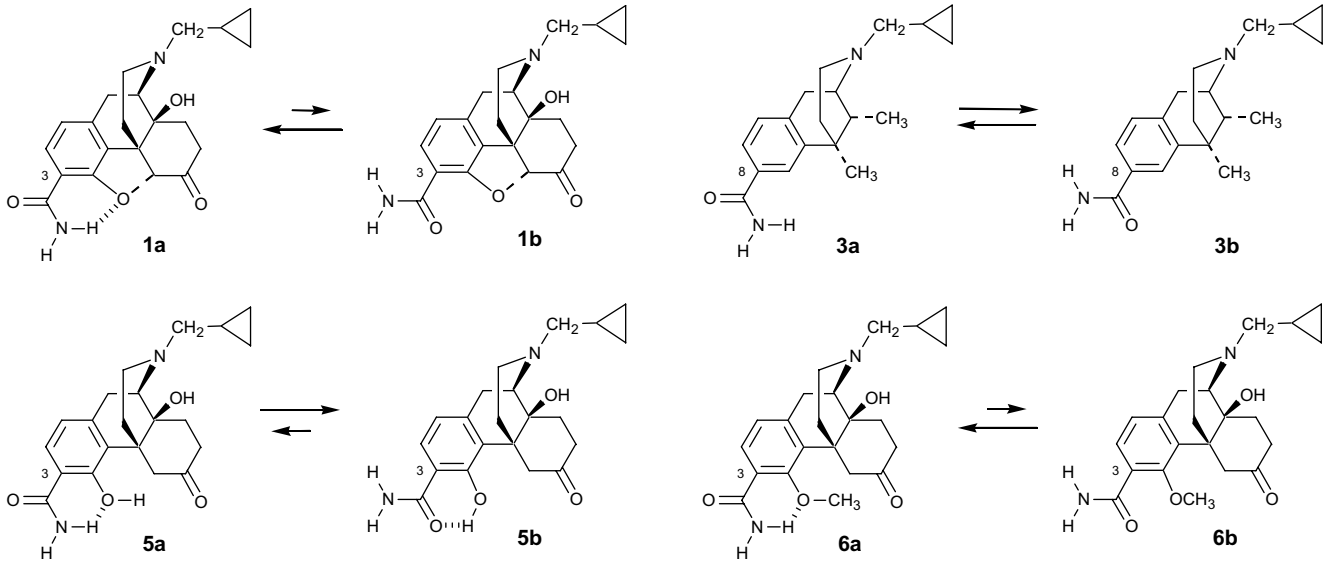
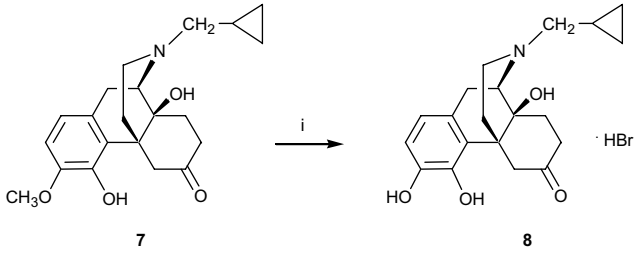


Figure 2. Conformational preferences of carboxamide groups.

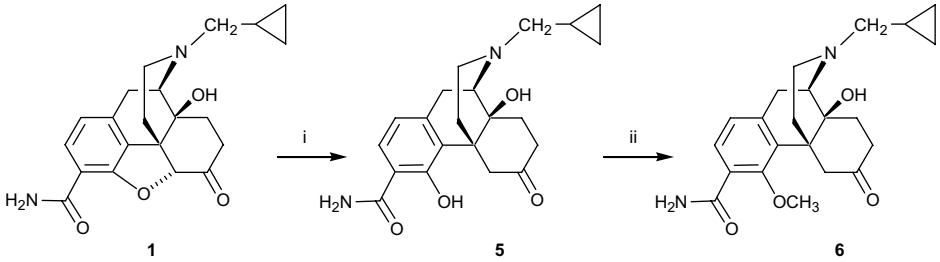
In hopes of gaining additional insight into the SAR of these carboxamido-containing opiates, we have designed and synthesized a series of analogues of **1** as probes to help further define its bioactive conformation. We reasoned that higher affinity would arise by rigidifying the carboxamido group of **1** into a conformational state similar to **1b**. This could be accomplished by, for example, incorporating the carboxamido group into a cyclic structure with an appropriate 4-substituent via covalent or non-covalent bonds. We now wish to report our results showing that such a rigidified naltrexone analogue **5** characterized by having a 4-OH group has very high affinity for μ and κ opioid receptors that is 14- and 48-fold, respectively, higher than **1**. We also made and evaluated novel naltrexone analogues **6** (3-$CONH_2$–4-OCH_3) and **8** (3,4-dihydroxy) as well as the *N*-cyclobutylmethyl derivative **13** to aid in the interpretation of these SAR data.

4-Hydroxy-morphinans can be easily made via furan ring cleavage of a wide variety of 4,5α-epoxymorphinan substrates using reductive,[3–8] based-induced elimination,[4,9] photolytic,[10] and acid catalysis[11] methods. As shown in Scheme 1, we used one of these reductive cleavage methods[4] (Zn/HOAc/HCl) to prepare target **5** from **1** in 50% yield. The corresponding 4-methoxy target **6** was made in 72% yield by treating **5** with $(CH_3)_3SiCHN_2$, Et_3N in methanol/acetonitrile.[12] To determine the effect of 4-OH substitution on naltrexone itself, we made novel catechol **8** as the HBr salt in 73% yield by treating known compound **7**[3,4] with BBr_3 in chloroform (Scheme 2).

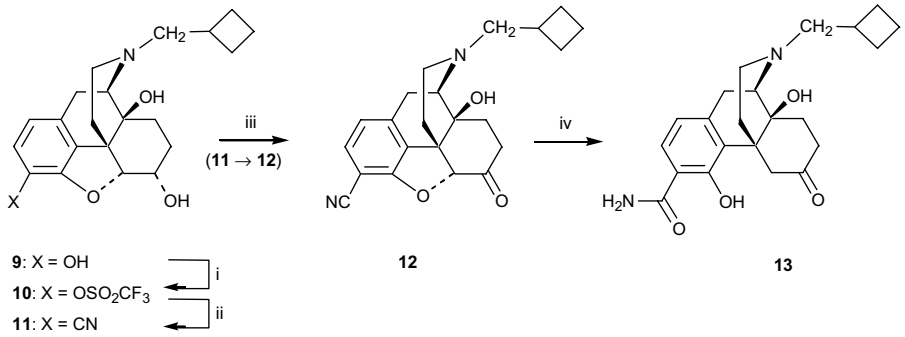
An additional 3-carboxamido-4-hydroxymorphinan analogue, **13**, was made to determine if changes (cyclopropylmethyl → cyclobutylmethyl) at the basic nitrogen would affect binding. Commercially available nalbuphine (**9**) was converted to its corresponding 3-triflate ester **10** in 98% yield using $PhN(Tf)_2$[13] in Et_3N/CH_2Cl_2. Treating **10** with $Zn(CN)_2/Pd(PPh)_4$[14] provided nitrile **11** in 83% yield, which was oxidized [$(COCl)_2$, Et_3N, DMSO] to ketone **12** in 92% yield. Target **13** was made



Scheme 2. Reagents and conditions: (i) BBr_3, $CHCl_3$, 25 °C, 30 min.



Scheme 1. Reagents and conditions: (i) Zn, 37% HCl, HOAc, 125 °C, 15 min; (ii) $(CH_3)_3SiCHN_2$, Et_3N, MeOH, MeCN, 25 °C, 24 h.

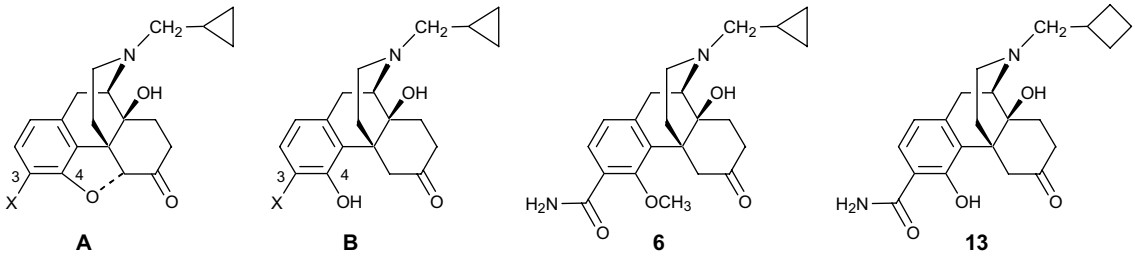


Scheme 3. Reagents and conditions: (i) $PhN(Tf)_2$, Et_3N, CH_2Cl_2, 25 °C, 16 h; (ii) $Zn(CN)_2$, $Pd(PPh)_4$, DMF, 135 °C, 24 h; (iii) $(COCl)_2$, Et_3N, DMSO, −78 °C, 20 min; (iv) Zn, 37% HCl, HOAc, 125 °C, 3 h.

in 71% yield from **12** via a reductive/hydrolytic process using Zn in $HCl/H_2O/HOAc$ (Scheme 3).

Affinities of target compounds for human μ, δ and κ opioid receptors stably expressed in CHO cells were assessed by generating K_i values using well-documented receptor binding assays.[15] These data are summarized in Table 1. Ring-opened target compound **5** displays very high affinity for μ and κ ($K_i = 0.052$ nM and 0.23 nM, respectively), and good affinity ($K_i = 2.6$ nM) for the δ receptor. Compared to **1**, the original lead having the furan ring intact, **5** has 14-, 212- and 48-fold higher affinity for μ, δ and κ, respectively. These results fit nicely with our pharmacophore hypothesis that the 4-OH (as H-bond donor) stabilizes the 'carboxamide-acceptor' putative bioactive conformation **5b**. Additional physical and SAR data were generated to preclude that (a) the 4-OH stabilizes the alternate 'carboxamide-donor' conformation **5a** and/or (b) the newly introduced 4-OH group enhances binding via direct interaction with receptor protein. Methyl ether **6**, an analogue close to **5** that is highly unlikely to adopt

Table 1. Opioid receptor binding data for 3-carboxamido-4-hydroxymorphinans and related compounds



Compd	X		K_i (nM ± S.E.)[a]		
			[^{3}H]DAMGO (μ)	[^{3}H]Naltrindole (δ)	[^{3}H]U69,593 (κ)
1[b,c]	**A**	$CONH_2$	0.71 ± 0.058	550 ± 40	11 ± 0.36
2 (Naltrexone)[c,d]	**A**	OH	0.11 ± 0.006	60 ± 3.2	0.19 ± 0.005
5[e]	**B**	$CONH_2$	0.052 ± 0.004	2.6 ± 0.26	0.23 ± 0.018
6[e]			11 ± 0.69	480 ± 35	23 ± 2.6
8[e]	**B**	OH	17 ± 4.0	130 ± 6.6	2.2 ± 0.16
13[e]			0.13 ± 0.0083	4.2 ± 0.36	0.27 ± 0.013

[a] Binding assays used to screen compounds are similar to those previously reported (see Ref. 15). Membrane protein from CHO cells that stably expressed one type of the human opioid receptor were incubated with 12 different concentrations of the compound in the presence of either 1 nM [^{3}H]U69,593 (μ), 0.25 nM [^{3}H]DAMGO (δ) or 0.2 nM [^{3}H]naltrindole (κ) in a final volume of 1 mL of 50 mM Tris–HCl, pH 7.5 at 25 °C. Incubation times of 60 min were used for [^{3}H]U69,593 and [^{3}H]DAMGO. Because of a slower association of [^{3}H]naltrindole with the receptor, a 3 h incubation was used with this radioligand. Samples incubated with [^{3}H]naltrindole also contained 10 mM $MgCl_2$ and 0.5 mM phenylmethylsulfonyl fluoride. Non-specific binding was measured by inclusion of 10 μM naloxone. The binding was terminated by filtering the samples through Schleicher & Schuell No 32 glass fibre filters using a Brandel 48-well cell harvester. The filters were subsequently washed three times with 3 mL of cold 50 mM Tris–HCl, pH 7.5, and were counted in 2 mL Ecoscint A scintillation fluid. For [^{3}H]naltrindole and [^{3}H]U69,593 binding, the filters were soaked in 0.1% polyethylenimine for at least 60 min before use. IC_{50} values will be calculated by least squares fit to a logarithm-probit analysis. K_i values of unlabelled compounds were calculated from the equation $K_i = (IC_{50})/1 + S$ where S = (concentration of radioligand)/(K_d of radioligand)—see Ref. 20. Data are the mean ± SEM from at least three experiments performed in triplicate.

[b] See Ref. 1.

[c] In our original work (Ref. 1), binding affinities for compounds **1** and **2** were measured for opioid receptors from guinea pig brain membranes. Relative affinities for **1** and **2** using human or guinea pig receptors were very similar.

[d] Obtained from commercial sources.

[e] Proton NMR, IR and MS were consistent with the assigned structures of all new compounds. C, H and N elemental analyses were obtained for all new targets and most intermediates and were within ±0.4% of theoretical values.

the 'carboxamide-acceptor' conformation **6b** due to incompatibility of the oxygen's lone pairs, has substantially lower (212-, 185- and 100-fold) affinity than **5** for μ, δ and κ, respectively.[16] Proton NMR data in $CDCl_3$ for **5** and **6** lend additional support of our pharmacophore hypothesis. Chemical shift difference (ca. 0.5 ppm) and the broad line shape for the two NHs of **5** is very different than seen with **1** but very close to that observed for **3**. Also, the proton of the phenolic hydroxyl of **5** is highly deshielded (δ 13.3) and appears as a sharp singlet, properties characteristic of participation in an intramolecular H-bond as donor (i.e., 'carboxamide-acceptor' conformation **5b**).[17] Results from NMR dilution experiments with **5** also confirms the relative stability of conformer **5b**. At five concentrations (100, 50, 10, 5 and 1 mM) of **5** in $CDCl_3$, no change was noted in the chemical shift or line shape of the phenolic H whereas the amide H's (broad singlets at δ 6.2 and 6.7 at 100 mM) coalesced into a broad singlet at δ 5.8 at 1.0 mM; both observations indicate that conformer **5b** ('carboxamide-acceptor') is highly stabilized relative to **5a**.[18] The NH's of the poorly active methyl ether **6** have magnetic environments very similar to those of **1** signifying the higher stability of conformer **6a** ('carboxamide-donor') relative to **6b**. Like naltrexone, compounds **1** and **5** were pure antagonists at the μ receptor, as measured by its inhibition of DAMGO-stimulated [^{35}S]GTPγS binding (unpublished results).

We also made catechol derivative **8**, the ring-opened version of naltrexone, to directly assess the contribution of the 4-OH to binding affinity in the 3-OH subseries and indirectly gauge if the 4-OH of **5** contributes to high affinity binding via intramolecular effects (as we hypothesized) or intermolecular interaction(s) with receptor. Our hypothesis was corroborated when we observed that compared to naltrexone (**2**), there is a substantial reduction (155-fold) in binding affinity for μ upon cleavage of the furan ring to give **8**. This result greatly contrasts the corresponding 3-$CONH_2$ pair, **1** and **5**, where the ring-cleaved analogue is 14-fold *more* potent. Compound **8** had a 2- and 12-fold lower affinity than **2** for δ and κ receptors, respectively. Lastly, the cyclobutylmethyl analogue **13**, which has the same 3-$CONH_2$, 4-OH motif and proton NMR pattern as **5**, has similarly high affinity (within 2-fold) for all three receptors.

Our results show that 4-OH substitution on morphinans significantly enhances binding affinity when the 3-substituent is $CONH_2$ but greatly decreases affinity with a 3-OH group, the prototypic substituent of opiates. There are hundreds of 4-hydroxy-morphinans in the literature with some, for example thebainone,[19] dating as far back as the 1920s. To our knowledge, however, none have a 3-$CONH_2$ or similar group and none have reached the therapeutic prominence of the corresponding 4,5α-epoxymorphinans. We have also presented SAR and proton NMR data showing the benefits of the 4-OH group with a 3-$CONH_2$ substituent is possibly a consequence of stabilizing an intramolecular H-bonded bioactive conformation where carboxamide is acceptor and 4-OH is donor. As these NMR studies were conducted in $CDCl_3$ rather than in an aqueous environment more relevant to the actual biological system, research aimed at the design, synthesis and evaluation of novel carboxamido-containing opiates where the $CONH_2$ (or isosteric group) is rigidified in the putative bioactive conformation via covalent attachments is underway in our laboratories.

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