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3-Carboxamido Analogues of Morphine and Naltrexone: Synthesis and Opioid Receptor Binding Properties

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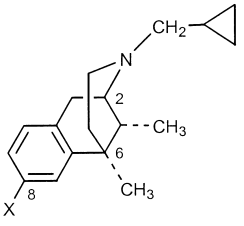
Abstract—In response to the unexpectedly high affinity for opioid receptors observed in a novel series of cyclazocine analogues where the prototypic 8-OH was replaced by a carboxamido group, we have prepared the corresponding 3-$CONH_2$ analogues of morphine and naltrexone. High affinity ($K_i = 34$ and 1.7 nM) for μ opioid receptors was seen, however, the new targets were 39- and 11-fold less potent than morphine and naltrexone, respectively.

We recently reported that unexpectedly high affinity for opioid receptors was resident in a novel series of cyclazocine (**1**) analogues where the prototypic 8-OH was replaced by a carboxamido group.[1] The observation that the primary carboxamido derivative **2** displayed K_i values of 0.41 and 0.53 nM versus μ and κ opioid receptors, respectively (corresponding cyclazocine K_i values = 0.32 and 0.18 nM), was not predicted nor rationalized by current knowledge of SARs of opioid-interactive agents.[2] For **2**, a high enantiopreference [(2*R*,6*R*,11*R*)-] for binding was observed and the compound also displayed potent antinociceptive activity in mice when administered icv. This study was part of a larger effort to identify bioisoteres of the 8-OH that would retain the essential H-bond donating properties of cyclazocine but have the potential for an improved ADME profile (e.g., reduced *O*-glucuronide formation). Prior to the publication of the 8-carboxamido analogues, we had shown that the 8-NH_2 cyclazocine analogue **3** had significant affinity for μ and κ opioid receptors, however, it was 30- and 23-fold less potent than cyclazocine, respectively.[3] Consistent with the long-standing teaching that 2,6-methano-3-benzazocines (a.k.a. benzopmorphans) and other μ opioid-receptor interactive agents (e.g., morphine) require H-bond donation at that site provided by the prototypic phenolic OH,[2,4,5] these studies showed that the 8-N of **3** had to have at least one hydrogen substituent for appreciable binding.



1: X = OH
2: X = $CONH_2$
3: X = NH_2

Our first examples of this novel carboxamido replacement for the phenolic OH of opiates were limited to 2,6-methano-3-benzazocine core structures, namely, cyclazocine, ethylketocyclazocine (EKC), and ketocyclazocine. For all three derivatives, we found a consistent pattern of bioisosterism, that is, the 8-carboxamido analogues had nearly comparable binding affinity to μ and κ opioid receptors as their 8-OH counterparts.[1,6] To determine if the benefits of the OH to $CONH_2$ conversion were transferable to other core opiate structures, we set out to prepare and evaluate the hitherto unknown 3-carboxamido analogues of morphine (**4**) and naltrexone (**8**), two important members of the 4,5α-epoxymorphinan core structural family. In searching the literature, we could not find any reports where the prototypic phenolic OH of these or opioid-receptor

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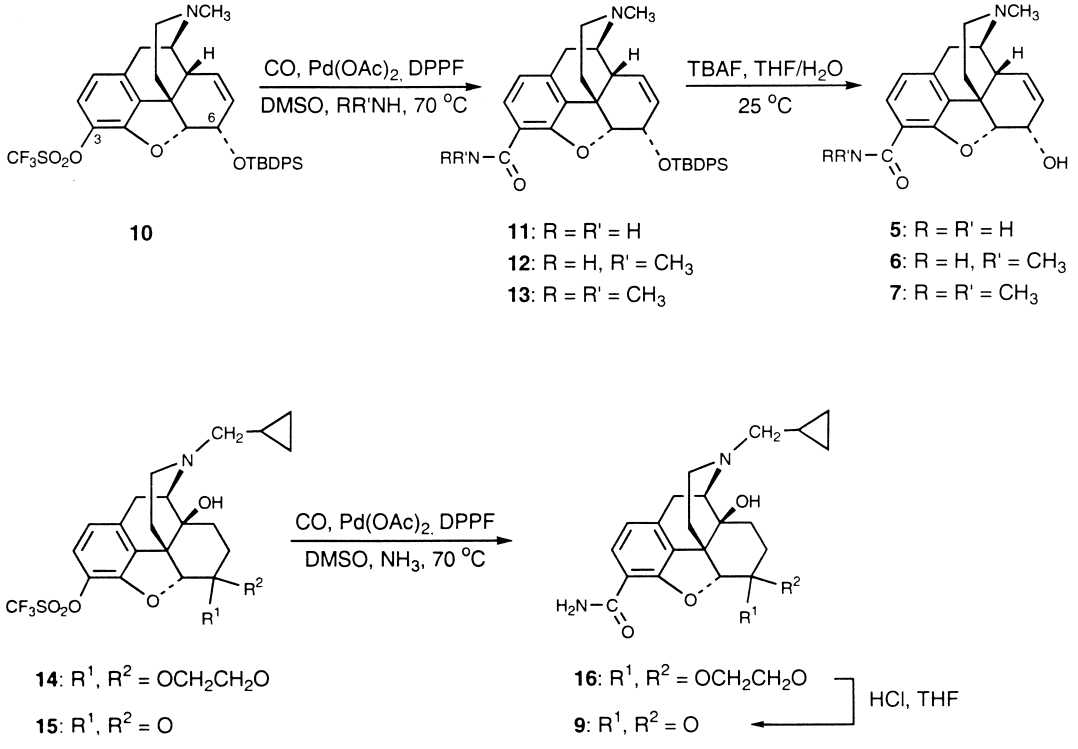
interactive agents was replaced by CONRR′. In fact, there are very few papers where any type of carbon attachment at that position is described. In two studies, the 3-OH group of morphine[7] and natrindole[8] was replaced by H, alkyl, acetyl, aryl, and/or heteroaryl groups; all targets had substantially diminished affinity for opioid receptor relative to their 3-OH counterparts. A recent report described the synthesis of the 3-carboxymethoxy analogues (i.e., 3-CO_2CH_3) of the 6-dioxolane (i.e., ketal protected) derivatives of naltrexone and oxymorphone.[9] These esters were used as intermediates to make the 3-sulfonamido analogues (i.e., 3-$NHSO_2CH_3$) of naltrexone and oxymorphone via Curtius rearrangements; no opioid binding data were reported for these ester intermediates. A recent report has described the irreversible binding of the 3-desOH analogue of C-CAM (a μ antagonist) to the μ receptor.[10]

We now wish to report the synthesis and opioid receptor binding properties of the 3-carboxamido analogues of morphine and naltrexone. We also made and evaluated the *N*-methyl and *N*,*N*-dimethyl analogues of the morphine derivative to gain insight as to the importance of the steric and H-bonding characteristics of the carboxamido group.

We used the same synthetic method as one we described in our cyclazocine study to prepare new target carboxamides.[1] As shown in Scheme 1, this method involves Pd-catalyzed carbonylation of an aryl triflate in the presence of ammonia (or other amines) and the Pd(0) ligand, DPPF ([1,1′-bis(diphenylphosphino)ferrocene]). This method represents a slight variation over known procedures[11–13] where we found that using DMSO rather than DMF gave superior yields. The morphine-based targets were made by treating 6-OTBDPS-protected morphine 3-triflate (**10**)[14] at 70 °C for 12 h with $CO/Pd(OAc)_2$/DPPF/DMSO and either ammonia (g), methylamine (2 M in THF), or dimethylamine (g) to provide intermediates **11**–**13** in yields of 54, 64, and 64%, respectively. Using a deprotection method we previously reported,[14] the *t*-butyldiphenylsilyl protecting groups of **11**–**13** were removed with 1 M TBAF in THF (some water present in the commercially available reagent) at 25 °C for 2 h to provide targets **5**–**7** in 84, 86, and 92% yield, respectively.[15] The 3-carboxamido derivative **9** of naltrexone **8** was made by two similar procedures from known triflate starting materials. In one method, the 3-triflate ester **15**[8] of naltrexone was directly converted to target **9** in 40% yield through treatment with $CO/Pd(OAc)_2$/DPPF/DMSO/NH_3 at 70 °C for 12 h. Alternatively, compound **14**,[9] the 3-triflate-6-dioxolane derivative of naltrexone, was converted to the carboxamido intermediate **16** in 55% yield using the conditions just described. Treating **16** with 6 N HCl/THF (1:2) at 25 °C for 5 h provided target **9** in 72% yield.

Opioid receptor binding data and a brief description of the receptor binding assays are found in Table 1. The two new carboxamido derivatives, **5** and **9**, have high affinity for μ opioid receptors (K_i values of 34 and 1.9 nM, respectively), however, they have 39- and 11-fold lower affinity than the corresponding 3-OH comparators, morphine (**4**) and naltrexone (**8**). Similar to morphine and naltrexone, compounds **5** and **9** have much lower affinity (56- and 58-fold, respectively) for δ receptors than μ. Against κ, compound **5** also has low affinity relative to μ in line with morphine, however, the naltrexone derivative **9** has much lower affinity for κ



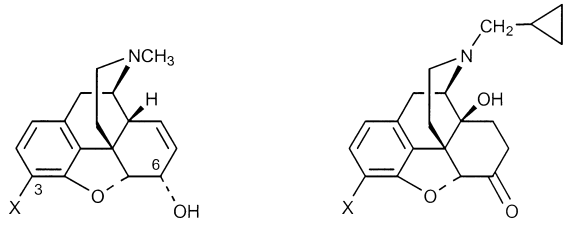
Scheme 1. Syntheses of 3-carboxamido-3-desOH-4,5α-epoxymorphinans.

(12-fold less than μ) than would be predicted by naltrexone's binding profile (i.e., 2-fold). With regard to methyl substitution of the carboxamide N of **5**, affinity for μ receptors declines precipitously (13- and 171-fold) as methyl groups are added and is abolished for δ and κ; this rank order was identical to that observed in our cyclazocine-carboxamide study.

For the 3-OH to 3-$CONH_2$ replacement in the morphine core structure, the results are somewhat different than that observed in the cyclazocine study where the two analogues had nearly comparable affinity for μ. Thus, it appears that $CONH_2$ is a very effective bioisostere of OH when appended to the 8-position of the cyclazocine core structure, however, when appended to the corresponding 3-position of the morphine, it is considerably less effective (39-fold lower affinity). In the naltrexone example, the isosteric replacement is still quite effective with difference in μ affinity being 11-fold. While all the members of the core 4,5α-epoxymorphinan and benzomorphan family share many similarities in structure, they also have a number of different structural features which accounts for stabilizing different conformations of the μ opioid G-protein coupled receptor. For example, cyclazocine, naltrexone and morphine share a similar trait in that they have high affinity for μ, however, a striking dissimilarity is that naltrexone and cyclazocine are antagonists at μ while morphine is an agonist. The nature of the *N*-substituent is largely responsible for this effect in that the antagonists naltrexone and cyclazocine have the prototypic antagonist group, *N*-cyclopropylmethyl, whereas morphine has the prototypic agonist appendage, a *N*-methyl group.[2,16,17] Thus, the resting conformation of the receptor is stabilized by naltrexone and cyclazocine binding and the activated form stabilized by morphine. If, as existing SAR data indicate, the *N*-substituent determines agonist/antagonist selectivity for a particular ligand, it is possible that the conformation stabilized by the antagonists naltrexone and cyclazocine may be more accommodating to the 3- or 8-$CONH_2$ group while the receptor conformation stabilized by the agonist morphine may be less accommodating to the new group. Functional data for **2**, **5**, and **9** will be secured to aid in the interpretation of our results since this rationalization assumes **2** and **9** to be μ antagonists and **5** a μ agonist.

There is an alternate explanation for these differences in binding affinities that relies on several physical observations. For both carboxamido analogues of morphine and naltrexone, we see a very strong intramolecular H-bond (**5a** and **9a** in Fig. 1) that bridges the carboxamido group to neighboring ether oxygen. This undoubtedly stabilizes these conformations relative to others involving rotation about the C–C or C–N bonds of the benzamide moiety, both of which are restricted due to resonance stabilization. This conformational preference contrasts that of the cyclazocine core, where it is hard to

Table 1. Opioid receptor binding data for 3-carboxamido-3-desOH-4,5α-epoxymorphinans



A B

Compd		mp, °C[b]	K_i (nM ± SE)[a] versus [3H]DAMGO (μ)	[3H]Naltrindole (δ)	[3H]U69,593 (κ)
4	**A**: X=OH (morphine)		0.88 ± 0.14	140 ± 18	24 ± 2.3
5	**A**: X=$CONH_2$[c]	264–266	34 ± 1.8	1900 ± 81	2000 ± 97
6	**A**: X=$CONHCH_3$[d]	128–130	440 ± 9.2	> 10,000	> 10,000
7	**A**: X=$CON(CH_3)_2$[e]	182–184	5800 ± 210	> 10,000	> 10,000
8	**B**: X=OH (naltrexone)		0.17 ± 0.03	11 ± 1.1	0.31 ± 0.03
9	**B**: X=$CONH_2$[f]	211–213	1.9 ± 0.21	110 ± 8.1	22 ± 0.85

[a]Binding assays used to screen compounds are similar to those previously reported (see ref 18). Guinea pig brain membranes, 500 μg of membrane protein, were incubated with 12 different concentrations of the compound in the presence of either 1 nM [3H]U69,593 (κ), 0.25 nM [3H]DAMGO (μ) or 0.2 nM [3H]naltrindole (δ) 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 [3H]U69,593 and [3H]DAMGO. Because of a slower association of [3H]naltrindole with the receptor, a 3 h incubation was used with this radioligand. Samples incubated with [3H]naltrindole also contained 10 mM $MgCl_2$ and 0.5 mM phenylmethylsulfonyl fluoride. Nonspecific binding was measured by inclusion of 10 μM naloxone. The binding was terminated by filtering the samples through Schleicher & Schuell No. 32 glass fiber 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 [3H]naltrindole and [3H]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 unlabeled compounds were calculated from the equation $K_i = (IC_{50})/1 + S$ where S = (concentration of radioligand)/(K_d of radioligand)—see ref 19. Data are the mean ± SE from at least three experiments performed in triplicate.

[b]See ref 15.

[c]$[\alpha]_D^{25}$ −95.3° (*c* 0.43, EtOH).

[d]$[\alpha]_D^{25}$ −87.8° (*c* 0.82, EtOH).

[e]$[\alpha]_D^{25}$ −174.5° (*c* 0.55, EtOH).

[f]$[\alpha]_D^{25}$ −188° (*c* 0.50, $CHCl_3$).

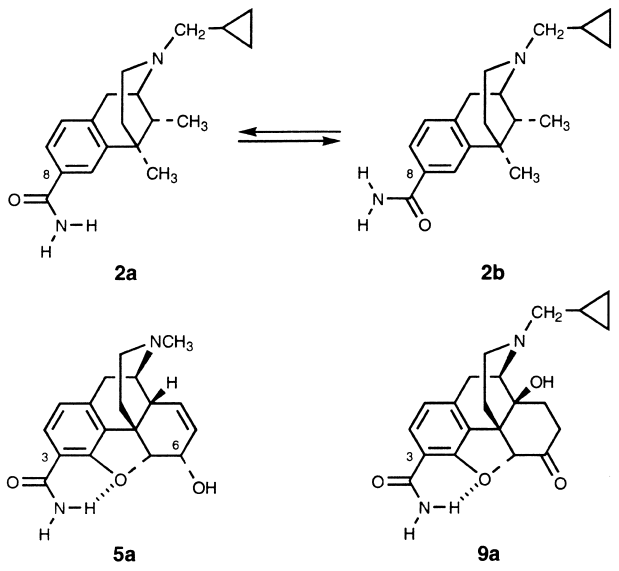


Figure 1. Conformational preferences of the carboxamide groups of compounds **2**, **5**, and **9**.

envision any significant difference in stability between the corresponding conformer **2a** and other stable conformations such as **2b**. The argument follows that if, for example, the bioactive conformation of carboxamido-containing ligands is similar to that depicted by **2b**, then a significant energy penalty would be required for **5a** and/or **9a** to attain that bioactive conformation resulting in reduced binding affinity. The effect of intramolecular H-bonds on the bioactive conformation has been studied by us and others.[20]

The physical data that support this hypothesis are as follows. At room temperature, the ^{1}H NMR spectrum ($CDCl_3$) of **2** shows the two exchangeable NH's appearing as broad singlets having chemical shifts at δ 5.63 and 6.04. When the temperature is raised to 30 °C, these broad singlets broaden further and between 30–40 °C, they coalesce into a very broad singlet at δ 5.80. At room temperature, the resonances for two NH protons in **5** and **9** appear as singlets at δ 5.77, 7.16 and δ 5.65, 7.46, respectively, and there is basically no change (i.e, no coalescence) upon warming either $CDCl_3$ solution to 45 °C. These data indicate that one of the NH's in both **5** and **9** has a significantly different magnetic environment that either NH in **2**. We believe these data consistent with the existence of a strong intramolecular H-bond in both 4,5α-epoxymorphinan structures. We do not know, however, the existence or stability of such an H-bond in the aqueous medium that the binding assays were performed in.

In conclusion, we have begun to redefine the relationship of the structure of opiates, specifically the phenolic OH position, to μ-opioid receptor binding affinity. We have capitalized on our recent benzomorphan studies by efficiently making and evaluating several novel 3-carboxamido-3-desOH-4,5α-epoxymorphinans. While affinity for the morphine and naltrexone 3-carboxamido analogues **5** and **9** for the μ-opioid receptor was somewhat less than the parent 3-OH compound, we believe that additional SAR and modeling studies will generate derivatives as good as or better than the parent structures. For example, we speculated that the ether oxygen of **5** and **9** may hinder the compounds from adopting the bioactive conformation. Crucial SAR information could therefore be obtained by making a 3-carboxamido-morphinan analogue (e.g., butorphanol), which lacks the ether bridge. Additional SAR studies are also planned to study the effect of the piperidine *N*-substituent on binding of 3- (or 8-)carboxamido-containing opiates. The role of the primary carboxamide group in receptor binding also needs to be further studied. For example, is it similar to that of the phenolic OH (or isosteric NH_2)[3,14] of typical opiates, namely, H-bond donation to the same (or different) complimentary acceptor site on the opioid receptor? Our initial studies with methyl substitution indicate that an N–H is very important for μ binding in that the *N*,*N*-dimethyl analogue **7** has very low affinity. Similar observations were noted in the cyclazocine studies.[1] However, the reduced binding affinity for the methylated derivatives may well be a consequence of negative steric interactions of the methyl groups with the receptor. Since our previous data for the 8-amino cyclazocine analogues indicated that binding affinity was enhanced by adding certain bulky substituents (e.g., **3**; X = NHPh),[3] the steric requirement(s) of the 3- or 8-substituent for opioid receptor binding remains to be determined and is the subject of further exploration in our laboratories.

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