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N-METHYL AND N-CYCLOPROPYLMETHYL-14α,14'β-[DITHIOBIS[(2-OXO-2,1-ETHANEDIYL)IMINO]]BIS(7,8-DIHYDRO-5β-METHYL-MORPHINONE) MET-TAMO AND N-CPM-MET-TAMO: SYNTHESIS AND OPIOID BINDING PROPERTIES

Jean M. Bidlack,[a] Richard A. Kaplan,[a] Alice Sebastian,[b] Ahmad Seyed-Mozaffari,[b] Ian Hutchinson[b] and Sydney Archer[b*]

[a]*Department of Pharmacology, The University of Rochester, School of Medicine and Dentistry, Rochester, New York 14642-8711 and* [b]*Cogswell Laboratory, Department of Chemistry, Rensselaer Polytechnic Institute, Troy, New York, 12180-3590*

Abstract: MET-TAMO and N-CPM-MET-TAMO were prepared by the same procedure used for the corresponding 5-desmethyl compounds, TAMO and N-CPM-TAMO, except that a new procedure was employed to synthesize the intermediate, 14β-amino-7,8-dihydromorphinone. Both MET-TAMO and N-CPM-MET-TAMO produced wash-resistant inhibition of μ, δ and κ binding but were more potent at the μ receptor.

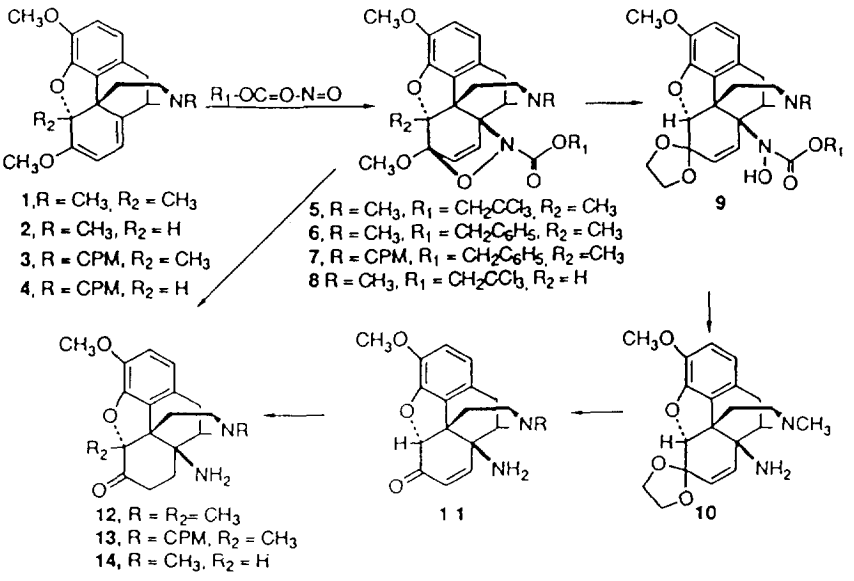
5β-Methyl-7,8-dihydromorphinone (metopon) was reported to be from three to ten times more active as an analgesic than morphine and more effective orally.[1,2] Later clinical studies showed that the drug was no more effective orally than morphine.[3] In a recent study, McLaughlin *et al.*[4] found that in the mouse tail-flick test for antinociception, morphine was as potent as metopon. On the other hand, Schmidhammer and his colleagues[5] reported that 14β-methoxymetopon was 150 times more potent than oxymorphone in the mouse writhing assay.

Recently, we reported[6] on the opioid binding properties of TAMO **20** and N-CPM-TAMO **21**. In this communication we report the synthesis and the relative opioid binding properties of the corresponding 5β-methyl derivatives, MET-TAMO **17** and N-CPM-MET-TAMO **19**.

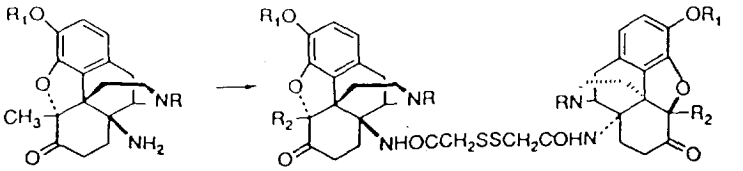
Kirby and McLean[7] reported a five-step synthesis of 14β-amino-7,8-dihydrocodeinone from thebaine as shown in Scheme 1. Thebaine was condensed with the nitroso compound, prepared *in situ* by periodate

oxidation of trichloroethyl N-hydroxycarbamate to give the adduct **8**. Treatment with ethylene glycol followed by reduction with zinc gave the ketal **9** which was hydrolyzed to furnish **10**. Catalytic reduction gave 14β-amino-7,8-dihydrocodeinone **14**. The overall yield was 67-70%. We had previously reported[8] that the two-step sequence **1** to **8** to **14** proceeded in approximately the same yield.

Scheme1



When benzyl N-hydroxycarbamate was substituted for the trichloroethyl ester the yield of the adduct **6** was 93%. Catalytic reduction to furnish **12** proceeded in 82% yield. The overall yield for the two-step procedure was 76%. This modification was employed to convert the N-cyclopropylmethyl compound **3** to **13** via the adduct **7**. Demethylation with BBr_3 of **12** gave the morphinone **15** and similar treatment of the N-CPM derivative **13** gave the corresponding morphinone which was treated with t-butyldimethylsilyl chloride to give the siliyl ether **16**. The morphinone **15** was condensed with acetylthioglycolyl chloride to give an ester amide which was hydrolyzed and then oxidized to afford the target compound the disulfide **17**, MET-TAMO. The silyl ether **16** was condensed with the same acid chloride and then oxidized to the disulfide **18**. Treatment with F^- gave the required N-CPM derivative **19**.



15, R = CH_3, R_1 = H
16, R = CPM, R_1 = t-Bu$(CH_3)_2$Si

17, R = CH_3, R_1 = H, R_2 = CH_3, **MET-TAMO**
18, R = CPM, R_1 = t-Bu$(CH_3)_2$Si, R_2 = CH_3
19, R = CPM, R_1 = H, R_2 = CH_3, **N- CPM-MET-TAMO**
20, R = CH_3, R_1 = R_2 = H, **TAMO**
21, R = CPM, R_1 = R_2 = H, **N-CPM-TAMO**

BIOLOGICAL RESULTS

The IC_{50} values for the inhibition of μ, δ and κ opioid binding to bovine striatal membranes by MET-TAMO, N-CPM-MET-TAMO, TAMO and N-CPM-TAMO are reported in Table 1. Binding to the μ opioid receptor was measured with the μ-selective peptide [^{3}H][D-Ala^2,(Me)Phe^4,Gly(ol)^5]enkephalin (DAMGO) at a final concentration of 0.25 nM. [^{3}H][D-Pen^2,p-Cl-Phe^4,D-Pen^5]enkephalin (pCl-DPDPE) at a final concentration of 0.2 nM and 1 nM [^{3}H]U69,593 were used to measure δ and κ binding respectively.[9]

Table 1. IC_{50} Values for the Inhibition of μ, δ and κ Opioid Binding to Bovine Striatal Membranes.

	IC_{50} (nM ± S.E.)		
Ligands	μ site	δ site	κ site
TAMO **20**	0.40 ± 0.06	11 ± 0.5	41 ± 0.2
N-CPM-TAMO **21**	3.9 ± 0.2	24 ± 2	10 ± 0.4
MET-TAMO **17**	2.9 ± 0.2	52 ± 13	89 ± 4
N-CPM-MET-TAMO **19**	1.4 ± 0.1	5.3 ± 0.6	7.7 ± 0.2

In the case of the N-methyl derivatives **20** and **17**, the former had high affinity for all three receptors and was more selective for the μ opioid receptor. On the other hand, the 5β-methyl N-CPM compound **19** had high affinity than **21** but neither was particularly selective.

Wash-resistant inhibition of opioid binding to bovine striatal membranes was measured as an indication of covalent binding of the compounds to the receptor.[6,9] The selectivity of the wash-resistant inhibition of the binding of 0.25 nM [^{3}H]DAMGO (μ), 0.2 nM [^{3}H]pCl-DPDPE (δ) and 1 nM [^{3}H]U69,593 (κ) by MET-TAMO **17** and N-CPM-MET-TAMO **19** is shown in Fig. 1. Membranes were preincubated with 100 μM N-tosyl-l-phenylalanyl chloromethyl ketone (TPCK),[10] a reagent that reacts with thiol groups but at this concentration does not interfere with opioid binding. TPCK reduced the concentration of MET-TAMO **17** and N-CPM-MET-TAMO **19** needed to inhibit binding in a wash-resistant manner and was included in the wash-resistant experiments.

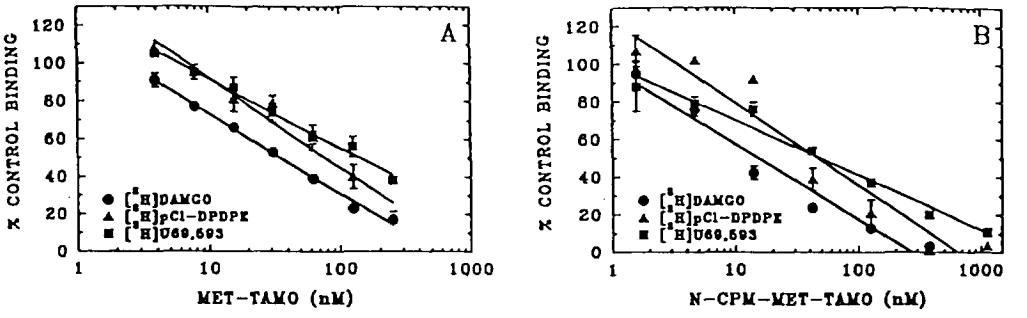


Figure 1A and B. Bovine striatal membranes, treated with 100 μM TPCK as described in Archer *et al.*[6] were incubated with different concentrations of MET-TAMO (A) and N-CPM-MET-TAMO (B) for 30 min at 25°C followed by four centrifugal washes. The binding of 0.25 nM [^{3}H]DAMGO, 0.2 nM [^{3}H]pCl-DPDPE and 1 nM [^{3}H]U69,593 to resuspended membranes was measured as described.[6] In the control binding experiments, membranes were treated under identical conditions except that the ligands were not added. Data are presented as the mean percentage of control binding $\pm$ S.E. from three experiments performed in triplicate.

In a similar study with TAMO and N-CPM-TAMO, it was found that pretreatment of membranes with 80 nM TAMO inhibited [^{3}H]DAMGO binding in a wash-resistant manner by 80% but had no effect on δ or κ binding.[6] The N-cyclopropylmethyl analog strongly inhibited μ binding, moderately inhibited κ binding and weakly inhibited δ binding. At a concentration of 80 nM, MET-TAMO like TAMO inhibited [^{3}H]DAMGO binding by approximately 80% in a wash-resistant manner. However, 80 nM MET-TAMO also inhibited δ and κ binding. N-CPM-MET-TAMO behaved similarly. Thus, TAMO was the most selective of the four ligands and N-CPM-TAMO appeared to be the next most selective.

If the ligands formed disulfide bonds with the μ receptor to produce wash-resistant inhibition of binding, addition of dithiothreitol (DTT) should result in the reversal of covalent binding to the μ receptor. The effect of DTT on the wash-resistant inhibition of [^{3}H]DAMGO binding by MET-TAMO **17** and N-CPM-MET-TAMO **19** is shown in Table 2.

Table 2. Effect of DTT on Wash-Resistant Inhibition of [^{3}H]DAMGO Binding by MET-TAMO and N-CPM-MET-TAMO.

Condition	[^{3}H]DAMGO Binding
	% of Control
25 nM MET-TAMO	48 ± 0.7
25 nM MET-TAMO, followed by 40 mM DTT	70 ± 4
10 nM N-CPM-MET-TAMO	54 ± 2
10 nM N-CPM-MET-TAMO, followed by 40 mM DTT	58 ± 2
40 mM DTT alone	107 ± 0.9

TPCK-treated membranes were incubated with either MET-TAMO or N-CPM-MET-TAMO at 25 °C for 30 min, followed by a 10-min incubation with 40 mM DTT at 4 °C in a final volume of 2 mL. After four centrifugal washes, the binding of 0.25 nM [^{3}H]DAMGO to membranes was measured as described in Archer *et al.*[6] Control binding was to membranes treated in the same manner except for the omission of the affinity ligands and DTT. Significantly different from binding obtained with MET-TAMO (*$P \leq 0.05$).

Similarly to MET-TAMO, 10 nM of TAMO resulted in 50 ± 3% reduction in [^{3}H]DAMGO binding, which was restored to 78 ± 3% of control binding by the addition of 40 mM DTT.[6] In contrast to the results obtained with N-CPM-MET-TAMO, 80 nM of N-CPM-TAMO reduced [^{3}H]DAMGO binding to 63 ± 5% of control binding, which was elevated to 87 ± 4% by the addition of the same amount of DTT. Both differences were statistically significant at the $P \leq 0.05$ level. In the 5β-methyl series, only the inhibition of binding by MET-TAMO was significantly reversed by DTT.

Since the wash-resistant inhibition of μ binding obtained with N-CPM-MET-TAMO was not reversed by the addition of DTT, [^{3}H]DAMGO saturation binding experiments were performed on membranes that had been pretreated with N-CPM-MET-TAMO. A change in the K_d value suggests that the compound is producing a change in the affinity of the receptor for [^{3}H]DAMGO, indicative of a competitive interaction, whereas, a

decrease in the B_{max} value suggests that the inhibition of [^{3}H]DAMGO binding is occurring through a noncompetitive manner, suggesting covalent binding of the compound to the μ receptor. Table 3 shows that pretreatment of membranes with 20 nM N-CPM-MET-TAMO reduced the B_{max} value for [^{3}H]DAMGO binding by 60%, while the K_d value was not significantly changed. These results support the view that N-CPM-MET-TAMO binds irreversibly to the μ opioid receptor.

Table 3. Effect of Pretreating Membranes with N-CPM-MET-TAMO on the K_d and B_{max} Values for [^{3}H]DAMGO Binding.

Condition	K_d (nM)	B_{max} (fmol/mg of protein)
Control	0.28 ± 0.004	91 ± 13
N-CPM-MET-TAMO	0.40 ± 0.045	38 ± 4

TPCK-treated membranes were incubated with 20 nM N-CPM-MET-TAMO at 25 °C for 30 min in a final volume of 2 mL. After four centrifugal washes, [^{3}H]DAMGO binding at concentrations ranging from 0.025 to 3.2 nM was measured as described in Archer *et al.*[6] Control binding was to membranes treated in the same manner except for the omission N-CPM-MET-TAMO. Saturation binding data were analyzed by the nonlinear curve-fitting program LIGAND.[11]

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