3-Deoxyclocinnamox: The First High-Affinity, Nonpeptide μ-Opioid Antagonist Lacking a Phenolic Hydroxyl Group

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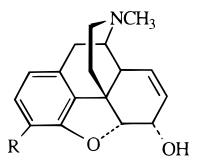
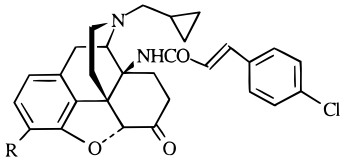
The C_3-substituent in morphinan opioids is of critical importance; the 3-OH group is usually associated with very much higher affinity for μ-receptors than H or −OMe. However in this series of 14β-cinnamoylamino derivatives the codeinones (e.g. methoclocinnamox, MC-CAM) had unexpectedly high μ-opioid receptor affinity, similar to that of the morphinone (clocinnamox, C-CAM). The current report relates to the synthesis and in vitro evaluation of deoxyclocinnamox (DOC-CAM) which acted as a high-affinity opioid antagonist similar to C-CAM but with greater μ selectivity. Thus it appears that the C_3-substituent does not play a major role in the binding of the 14β-cinnamoyl series and that the cinnamoyl group itself may in fact be the dominant binding feature.

Introduction

The phenolic hydroxyl group in morphine (**1**) and related series of opioids is recognized as one of the key structural features for binding to the μ-receptor and for in vivo opioid activity. The μ binding of equivalent methyl ethers is very much lower with codeine (**2**) having 200-fold lower affinity than morphine.[1] When the phenolic hydroxy was removed to give 3-deoxymorphine (**3**), binding was also markedly reduced (30-fold) though in vivo antinociceptive potency was much less affected (3−8-fold).[3] The relationship between morphinone (3-OH) and codeinone (3-OMe) in the 14β-cinnamoylamino series is unusual as exemplified by clocinnamox (C-CAM, **4**) and methoclocinnamox (MC-CAM, **5**).[4,5] The latter has very high affinity for μ-opioid receptors, very similar to that of C-CAM. Thus the cinnamoylamino group appeared to have a greater influence on opioid binding than the presence of a phenolic hydroxyl group. It could be active as a Michael acceptor in forming a covalent bond to a receptor thiol group, but in vitro investigation failed to demonstrate such activity.[2] Nevertheless C-CAM, MC-CAM, and their analogues display irreversible μ-antagonist actions in in vitro and in vivo assays of opioid function.[2,7,12−16] It was thus of interest to synthesize and evaluate deoxyclocinnamox (DOC-CAM, **6**) to determine whether high affinity for μ-receptors is maintained when there is no 3-oxygen function to form a hydrogen bond to the receptor. We here report the synthesis of DOC-CAM and its evaluation in displacement binding and in vitro functional assays.



1: R = OH
2: R = OCH_3
3: R = H



4: R = OH C-CAM
5: R = OCH_3 MC-CAM
6: R = H DOC-CAM

Synthesis

The procedure shown in Scheme 1 was followed. *N*-Cyclopropylmethyl-14β-amino-7,8-dihydronorcodeinone (**7**),[2] protected as the formylamino derivative **8**, was 3-O-demethylated with boron tribromide[10] to the morphinone **9** and converted to the phenyltetrazolyl ether **10**. Hydrogenolysis[11] afforded the 3-deoxy-14-formylaminomorphinone derivative **11** which was hydrolyzed and re-acylated to give DOC-CAM (**6**) in 13% yield over six steps. The oxalate salt of **6** was formed prior to pharmacological testing.

Results and Discussion

The affinity and selectivity of DOC-CAM for the three types of opioid receptor were determined in a displacement binding assay in monkey brain membranes in which the displaced radioligands were [^{3}H]DAMGO (μ), [^{3}H]DPDPE (δ), and [^{3}H]U69593 (κ).[6] DOC-CAM bound with very high affinity at μ-receptors (K_i 0.54 nM) with lower affinity for δ (K_i 36.8 nM) and κ (K_i 11.9 nM) receptors (Table 1). Though data in monkey brain binding assays are not available for C-CAM and MC-CAM, comparable data for opioid binding in a mouse brain membrane preparation have been reported.[7] The K_i values for μ-receptor binding were 0.25 and 0.46 nM for C-CAM and MC-CAM, respectively. It is thus clear that DOC-CAM, as well as MC-CAM, has unusually high μ affinity. The functional activity of DOC-CAM at μ-receptors was determined for stimulation of [^{35}S]-GTPγS binding in cloned human μ-opioid receptors transfected into a rat glioma cell line.[8] DOC-CAM failed to stimulate the binding of [^{35}S]GTPγS up to a concentration of 1 μM. A similar result was found with C-CAM,

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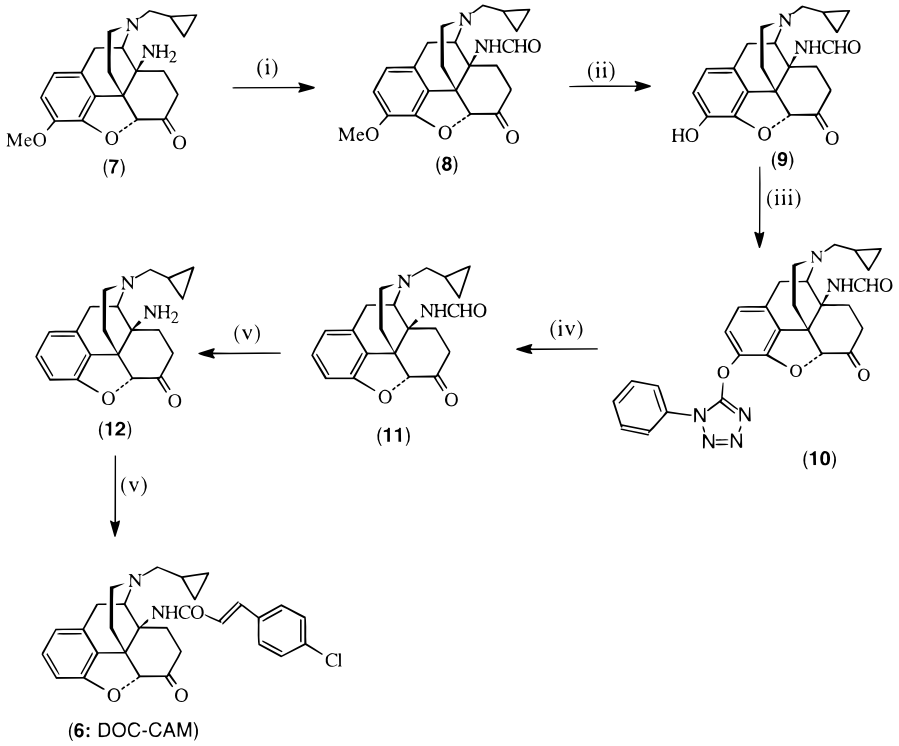
Scheme 1[a]



[a] (i) HCO_2H, acetic anhydride; (ii) BBr_3, DCM; (iii) 5-Cl-1-phenyltetrazole, K_2CO_3, DMF; (iv) 10% Pd/C, AcOH, 45 psi H_2; (v) 4 N HCl, reflux; (vi) *p*-Cl-cinnamoyl chloride, NEt_3, CH_2Cl_2.

Table 1. Binding of DOC-CAM, C-CAM, and MC-CAM to Opioid Receptors

	K_i (nM)		
compound	[^{3}H]DAMGO (μ)	[^{3}H]DPDPE (δ)	[^{3}H]U69593 (κ)
DOC-CAM, **6**	0.54 (0.32−0.91)	36.8 (34.0−43.2)	11.9 (9.7−14.2)
C-CAM, **4**[a]	0.25	1.6	7.2[b]
MC-CAM, **5**[a]	0.46	4.5	29[b]

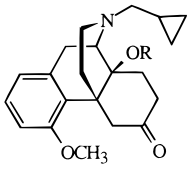
[a] Values taken from ref 7 (no confidence limits given). [b] Displacement of [^{3}H]bremazocine in the presence of 1 μM DAMGO and 1 μM DPDPE.

Table 2. Antagonism of the μ-Opioid Agonist Fentanyl in the [^{35}S]GTPγS Assay

	DOC-CAM, **6**	C-CAM, **4**	MC-CAM, **5**
K_e (nM)	0.28 ± 0.13	0.37 ± 0.17	6.37 ± 2.7

but MC-CAM acted as a very weak partial agonist having a maximum stimulation of 12% relative to the full μ-agonist fentanyl. All the cinnamoylamino derivatives were able to antagonize the action of fentanyl in this preparation with K_e values of 0.28, 0.37, and 6.37 nM, respectively, for DOC-CAM, C-CAM, and MC-CAM.

Thus DOC-CAM in these in vitro binding and functional assays is a high-affinity μ-antagonist equivalent to C-CAM but more selective. The very similar μ-opioid receptor affinities displayed by all members of the C-CAM series suggest that the cinnamoyl side chain is now the dominant binding motif, certainly of more importance than the phenolic hydroxyl. The only other μ-antagonists lacking a 3-phenol group are cyprodime (**13**) and its 14-*O*-benzyl analogue (**14**). However the in vitro μ-antagonist potency of cyprodime is 200-fold lower and that of the *O*-benzyl analogue over 40-fold lower than that of DOC-CAM which is thus the first nonphenolic, high-affinity μ-antagonist to be reported.[9]



13: R = CH_3
14: R = CH_2Ph

Experimental Section

Infrared spectra were obtained on a Perkin-Elmer 881 spectrophotometer. The proton (300 MHz) and carbon-13 (75 MHz) NMR spectra were obtained on a JEOL JNM-GX FT 300 MHz spectrometer at 20 °C in $CDCl_3$ unless otherwise stated. Tetramethylsilane was used as the internal standard. Mass spectra were obtained on a Fisons Autosampler instrument with electron impact ionization (70 eV). Melting points were determined on a Reicher hot-stage microscope and are uncorrected. Elemental analyses were obtained on a Perkin-Elmer 240C analyzer. All reagents were used as supplied by Aldrich. Column chromatography employed flash silica gel (Fluka, Silica gel 60). Thin-layer chromatography was performed on aluminum sheets coated with silica gel F_{254}.

***N*-Cyclopropylmethyl-14-formamidocodeinone (8).** A solution of **7** (4.42 g, 12.5 mmol) in formic acid (97%, 45 mL) was stirred at 5 °C under nitrogen. Acetic anhydride (18 mL, 180 mmol) was added dropwise and the yellow solution stirred for a further 4 h before quenching with NH_4OH and extracting with DCM (3 × 100 mL). The combined organics were dried (Na_2SO_4) and the solvent was removed in vacuo to leave a cream foam. The product was purified by column chromatography (5−10% MeOH:DCM): yield 3.82 g (66%); R_f 0.35 (10% MeOH:DCM); EIMS *m*/*z* 382 (M^+, 70%); δ_H 8.37 (1 H, d, J = 2 Hz, NCHO), 6.81 (1 H, br, s, NHCO), 6.72 (1 H, d, J = 8 Hz, 2-H), 6.63 (1 H, d, J = 8 Hz, 1-H), 4.94 (1 H, s, 5-H), 3.88 (3 H, s, 3-OCH_3); δ_C 207.50, 162.10, 145.34, 142.96, 128.46, 125.07, 119.11, 114.97, 89.77, 60.19, 59.35, 56.83, 56.56, 48.53, 44.06, 37.00, 30.55, 29.98, 21.41, 9.43, 4.10, 3.66; HRMS calcd for $C_{22}H_{26}N_2O_4$ 382.1893, found 382.1885.

***N*-Cyclopropylmethyl-14-formamidomorphinone (9).** A solution of **8** (4.53 g, 11.86 mmol) in dry DCM (100 mL) was cooled to −30 °C under an atmosphere of nitrogen. Boron tribromide solution (100 mL, 1 M, 100 mmol) was added dropwise and the reaction stirred at this temperature for 4 h before allowing to come to room temperature over 0.5 h. The solution was poured onto a 1:1 mixture of ice and concentrated ammonium hydroxide (200 mL) and stirred for 15 min. After extraction with 3:1 DCM:MeOH (3 × 250 mL) the combined organics were dried (Na_2SO_4) and the solvent was removed under reduced pressure to yield the crude product which was purified by column chromatography (2−5% MeOH:DCM): yield 3.30 g (76%); mp 220 °C dec; R_f 0.23 (10% MeOH:DCM); EIMS *m*/*z* 368 (M^+, 100%); δ_H complex due to presence of rotomers; δ_C 208.14, 161.09, 143.48, 139.29, 128.39, 124.19, 119.05, 117.23, 88.54, 58.94, 56.76, 48.38, 43.56, 36.23, 29.05, 28.15, 21.22, 9.27, 3.74, 3.53; HRMS calcd for $C_{21}H_{24}N_2O_4$ 368.1736, found 368.1734.

***N*-Cyclopropylmethyl-14-formamido-3-(1-phenyl-1*H*-5-tetrazolyl)morphinone (10).** A mixture of **9** (3.14 g, 8.53 mmol), 5-chloro-1-phenyl-1*H*-tetrazole (1.85 g, 10.2 mmol) and anhydrous potasium carbonate (2.40 g, 17.07 mmol) in anhydrous DMF (35 mL) was stirred at room temperature for 22 h. Water (150 mL) was added and the mixture extracted with 3:1 $CHCl_3$:MeOH (3 × 200 mL). The combined organics were combined and dried (Na_2SO_4) and the solvent was removed in vacuo. The crude product was recrystallized (×2) from MeOH to yield off-white crystals: yield 3.19 g (73%); mp 137−139 °C; R_f 0.25 (5% MeOH:DCM); ν_{max} ($CHCl_3$) 3333 (NHCHO), 1721 (CO), 1693 (NHCHO) cm^{-1}; EIMS *m*/*z* 512 (M^+, 10%); δ_H 8.38 (1 H, d, J = 2 Hz, NCHO), 7.50−7.90 (5 H, m, N−Ph), 7.22 (1 H, d, J = 8 Hz, 2-H), 6.7822 (1 H, d, J = 8 Hz, 1-H), 5.03 (1 H, s, 5-H), 3.48 (1 H, d, J = 4 Hz, NHCO); δ_C 205.67, 162.21, 159.29, 146.97, 135.61, 133.00, 131.54, 130.18, 129.70, 129.67, 129.38, 122.37, 122.31, 121.57, 119.61, 90.43, 59.79, 59.25, 56.02, 48.46, 43.86, 36.52, 30.17, 29.89, 21.72, 9.18, 4.17, 3.60; HRMS calcd for $C_{28}H_{28}N_6O_4$ 512.2172, found 512.2179.

***N*-Cyclopropylmethyl-14-formamido-3-deoxymorphinone (11).** A mixture of **10** (2.68 g, 5.23 mmol) and 10% Pd/C in glacial acetic acid was hydrogentaed at room temperature at 45 psi for 72 h. The Pd/C was removed by filtration and the acetic acid then removed in vacuo. The solid was stirred with 35% NH_4OH solution, the organics were extracted with 3:1 $CHCl_3$:MeOH and dried (Na_2SO_4), and the solvent was then removed in vacuo to yield an off-white foam (column chromatography, 2.5−5% MeOH:DCM): yield 1.63 g (89%); mp 171−173 °C; R_f 0.25 (5% MeOH:DCM); ν_{max} ($CHCl_3$) 3336 (NHCHO) 1716 (CO), 1692 (NHCHO) cm^{-1}; EIMS *m*/*z* 352 (M^+, 80%); δ_H 8.38 (1 H, d, J = 2 Hz, NCHO), 6.93−7.18 (2 H, m, 3-H and NHCO), 6.69−6.73 (2 H, m, 2-H and 1-H), 4.91 (1 H, s, 5-H); δ_C 207.95, 162.05, 157.26, 133.37, 129.23, 126.73, 118.44, 107.71, 89.00, 59.60, 59.19, 56.34, 47.58, 43.76, 36.62, 30.03, 29.82, 21.93, 9.19, 3.91, 3.55; HRMS calcd for $C_{21}H_{24}N_2O_3$ 352.1787, found 352.1794.

***N*-Cyclopropylmethyl-14-amino-3-deoxymorphinone (12).** A solution of **11** (1.04 g, 2.95 mmol) in 4 N HCl (60 mL) was refluxed for 2 h. The solution was allowed to cool to room temperature and made basic with 35% NH_4OH solution; before extracting with DCM (3 × 125 mL), the combined organics were dried (Na_2SO_4) and the solvent was removed in vacuo to yield an off-white foam. This was purified by column chromatography (10% MeOH:DCM): yield 0.59 g (62%); mp 135−137 °C; R_f 0.11 (5% MeOH:DCM); ν_{max} 3671 (NH), 1723 (CO) cm^{-1}; EIMS *m*/*z* 324 (M^+, 32%); δ_H 7.05 (1 H, m, 3-H), 6.73 (1 H, d, J = 8 Hz, 2-H), 6.67 (1 H, d, J = 8 Hz, 1-H), 4.65 (1 H, s, 5-H); δ_C 209.26, 157.32, 133.79, 128.69, 128.17, 118.53, 107.74, 89.53, 63.75, 59.47, 52.77, 49.32, 43.86, 36.48, 32.01, 29.93, 22.88, 9.46, 3.77, 3.73; HRMS calcd for $C_{20}H_{24}N_2O_2$ 324.1838, found 324.1836.

***N*-Cyclopropylmethyl-14-(4-chlorocinnamoylamido)-3-deoxymorphinone (6, DOC-CAM).** To a stirred solution of **12** (0.74 g, 2.29 mmol) in DCM (15 mL) was added NEt_3 (1.6 mL, 11.5 mmol) followed by 4-chlorocinnamoyl chloride (0.92 g, 4.6 mmol) dropwise, as a solution in DCM (5 mL). After 3.5 h of vigorous stirring the solvent was removed in vacuo. The crude product was purified by column chromatography (2.5% MeOH:DCM): yield 0.69 g (62%); mp 117−119 °C; R_f 0.53 (5% MeOH:DCM); ν_{max} 3321 (NHCO), 1718 (CO), 1673 (NHCO) cm^{-1}; EIMS *m*/*z* 488 (M^+, 60%); δ_H 7.58 (1 H, d, J = 16 Hz, C═CH), 7.46 (2 H, m, Ph), 7.38(1 H, br, s, NHCO), 7.34 (2 H, m, Ph), 7.10 (1 H, m, 3-H), 6.72 (2 H, m, 2-H and 1-H), 6.58 (1 H, d, J = 16 Hz, C═CH), 4.95 (1 H, s, 5-H); δ_C 207.18, 166.58, 157.57, 139.99, 135.63, 133.64, 133.36, 129.53, 129.18, 129.16, 127.36, 121.79, 118.64, 107.92, 89.29, 60.29, 59.36, 56.20, 47.83, 44.04, 36.80, 30.49, 30.20, 22.21, 9.67, 4.27, 3.91; HRMS calcd for $C_{29}H_{29}N_2O_3Cl$ 488.1867, found 488.1874. Anal. ($C_{29}H_{29}N_2O_3Cl$·$(CO_2H)_2$·1.5H_2O) C, H, N.

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