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14β-*O*-Cinnamoylnaltrexone and related dihydrocodeinones are mu opioid receptor partial agonists with predominant antagonist activity

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Abstract

14-*O*-cinnamoyl esters of naltrexone (**6**) were synthesised and evaluated in isolated tissue assays in vitro and in vivo in mouse antinociceptive assays. Their predominant opioid receptor activity was mu receptor (MOR) antagonism but the unsubstituted cinnamoyl derivative (**6a**) and the *p*-methylcinnamoyl derivative (**6c**) had partial MOR agonist activity in vitro and in vivo. When compared to the equivalent 14-cinnamoylaminomorphinones (**5**) the cinnamoyloxy morphinones (**6**) as MOR antagonists had shorter duration of action and were less effective as pseudoirreversible antagonists. The antinociceptive activity of the cinnamoyloxycodeinones (**7**) was not significantly greater than that of the morphinones (**6**) but they showed no evidence of any pseudoirreversible MOR antagonism. In both respects these profiles differed from those of the equivalent 14-cinnamoylaminocodeinones (**4**).

Introduction

Naloxone (**1b**) and naltrexone (**1a**) are prototype opioid antagonists having some limited selectivity for mu opioid receptors (MOR). They have found clinical utility respectively as a treatment for opiate[1] and alcohol dependence[2], and to reverse narcotic overdosage.[3] 14-*O*-alkyl ethers (**2**) of naltrexone retain predominant MOR antagonist activity[4,5] but the 14-*O*-3-phenylpropyl ether (**2a**) has recently been shown to have high efficacy and high potency MOR agonist activity in antinociceptive assays.[6] We have made extensive studies of derivatives (**3**) of 14β-amino-7,8-dihydromorphinone with a major focus on the cinnamoylamino- derivatives (**4, 5**) of which the MOR-selective irreversible antagonists clocinnamox (C-CAM; **5b**) and methcinnamox (M-CAM; **5c**) are the most studied examples.[7-10] We here report preparation and evaluation of 14-*O*-cinnamoyl esters of naltrexone (**6**) and the equivalent codeinones (**7**) for comparison with the 14-amino derivatives (**4, 5**) and with the 14-*O*--phenylpropyl ether of naltrexone (**2a**).

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Supporting Information Available: Full experimental details and spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR, mass spectra and microanalysis data), including biological assay methods. This method is available free of charge via the internet at http://pubs.acs.org.

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Synthesis

Acylation of naltrexone 3-*O*-methyl ether (**8a**) to give **7a-7c** was achieved using the appropriate anhydrides (Scheme 1), themselves prepared from their equivalent acid chlorides by the method of Armesto et al.[11] Ligands **6a-6c** were similarly prepared from 3-*O*-(tert-butyldimethylsilyl)naltrexone (**8b**)[12] by acylation and then removal of the protecting group with potassium fluoride to yield the morphinones (Scheme 1).

Results

The new ligands (**6, 7**) in opioid receptor binding assays[13,14] displayed high affinity for MOR and significantly lower affinity for delta opioid receptors (DOR) and kappa opioid receptors (KOR) (Table 1). Affinity of the morphinones (**6**) was generally higher than that of the codeinones (**7**) with DOR affinity showing greater disparity than MOR and KOR affinity. The unsubstituted cinnamoylmorphinone (**6a**) had the highest affinity for all three receptors, comparable to naltrexone and for MOR, higher than C-CAM (**5b**).

The morphinones (**6**) were evaluated for opioid receptor functional activity in the mouse *vas deferens* (MVD) and guinea pig *ileum* (GPI) isolated tissue assays.[13] In MVD the morphinones displayed little agonist activity, but were very potent opioid receptor antagonists of the standard selective opioid receptor agonists DAMGO (MOR), DPDPE (DOR) and U69593 (KOR), having subnanomolar Ke values for all three opioid receptors (Table 2). Although MOR antagonist potency was higher than DOR and KOR potency, there was no appreciable selectivity for MOR over DOR and KOR. In GPI morphinones **6** partially inhibited the electrically-stimulated contractions of the tissue. This opioid receptor partial agonist effect was not reversed by the selective antagonists CTAP (MOR) or norBNI (KOR) indicating very slow receptor offset such as was observed with the similarly lipophilic opioid ligands buprenorphine (**10**)[15] and C-CAM (**5b**).[16]

Opioid receptor functional activity of the codeinones (**7**) was investigated by their effects on stimulating [^{35}S]GTPγS binding in recombinant human opioid receptor transfected into CHO cells (Tables 3 and 4).[14] The unsubstituted cinnamoyl ester (**7a**) showed partial agonist activity of modest potency for all three opioid receptors (Table 4), whereas the *p*-chloro analogue (**7b**) had insignificant agonist activity for any opioid receptor but was an antagonist of the standard agonists DAMGO (MOR), DPDPE (DOR) and U69593 (KOR) (Table 3) which were also used as the standards against which the agonist stimulation of **7a** was measured. **7c** like **7b** had only antagonist activity for MOR (Table 3) but was a KOR partial agonist (Table 4).

In vivo activity of the 14-*O*-acyl derivatives was investigated in mouse antinociceptive tests using assays with thermal (tail withdrawal from 50°C warm water (TW)) or chemical (acetic acid induced writhing (AW)) stimulation.[8] None of the naltrexone derivatives (**6,7**) showed any significant opioid receptor agonist activity in TW but all were effective antagonists of morphine in this assay. A high dose (32 mg/kg given 30 minutes before morphine) of each of the morphinones (**6**) flattened the morphine dose-response curve up to 320-1000 mg/kg of the agonist (illustrated for **6b** in Fig. 1). Pre-treatment (24 h) with the antagonist **6b** resulted in a 10-fold parallel rightward shift of the morphine dose-response curve, but the shift produced by **6a** and **6c** was negligible (Fig. 1). The codeinones (**7b, 7c**) were antagonists of morphine in TW but at 32 mg/kg only shifted the morphine dose-effect curve in the standard assay 3-4-fold to the right with no evidence of flattening and there was no antagonist effect with 24 h pre-treatment (data not shown).

The *p*-substituted cinnamoyloxymorphinone **6c** and the equivalent codeinone **7c** unimpressively inhibited the acetic acid induced writhing effect; whereas **6a** was

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substantially more potent and effective (Figure 2). The only opioid antagonists without any *in vivo* agonist effects were the *p*-chlorocinnamoyloxy derivatives (**6b, 7b**). This data confirms that the chemical nociceptor used in the AW assay presents a less intense challenge than the thermal stimulus in TW.

Agonist selectivity for the individual opioid receptor in AW was determined for **6a** by the use of selective antagonists for MOR, DOR and KOR. These were the antagonists β-FNA (MOR), naltrindole (DOR) and norBNI (KOR). β-FNA and norBNI were administered 24 h before **6a** to ensure a competitive (and selective) antagonist effect. The agonist effect of **6a** in AW was partially antagonized by β-FNA and by naltrindole but not by norBNI (Fig. 3) so that it appears that in AW the agonist effects of **6a** are primarily mediated by DOR and MOR. **6b** was evaluated as an antagonist versus the agonists morphine (MOR), BW373U86 (DOR) and bremazocine (KOR), only proving effective against morphine at 24h pretreatment (data not shown).

Discussion

Our prime interest in the activity of the naltrexone esters (**6**) was in comparison to the activity of the equivalent amides C-CAM (**5b**) and M-CAM (**5c**). The latter are highly effective and selective MOR antagonists with insignificant agonist effects *in vivo*.[7,8] They are more effective than β-FNA in flattening the dose-response curve of MOR agonists[8] but since they do not form covalent bonds *in vitro* by Michael addition of protein nucleophilic groups they have been termed pseudo-irreversible antagonists.[16,17] The very powerful binding to MOR *in vivo* seems very likely to involve the lipophilic cinnamoylamino group functioning in a manner similar to the *t*-butyl group in buprenorphine (**10**).[18] The present 14-*O*-acylmorphinones (**6**) fell short of C-CAM and M-CAM as pseudo-irreversible antagonists; though in TW they all flattened the morphine dose-response curve 30 minutes after their administration, their MOR antagonist effect was much reduced at 24h, whereas the amides C-CAM (**5b**) and M-CAM (**5c**) had very pronounced MOR antagonist effects at 24h and beyond.[8] **6b**, with *p*-chloro substitution in the cinnamoyl aromatic ring, was the most effective pseudo-irreversible antagonist among the esters; its MOR antagonist profile was comparable to that of β-FNA.[8] Since, together with the corresponding codeinone (**7b**), **6b** was the only ester to lack any demonstrable antinociceptive action; the profile of **6b** is therefore not dissimilar to that of C-CAM (**5b**). The unsubstituted morphinone ester **6a** *in vivo* was also basically similar to the equivalent amide **5a**.[9] This means it showed little agonist activity in TW but substantial activity in AW. Again the most significant difference between **6a** and **5a** is the duration of morphine antagonist activity in TW. **5a** with 24 h pre-treatment produced a 0.5 – 1 log unit shift of the morphine dose-response curve[9] whereas the shift from **6a** was barely significant.

The biggest difference between the 14-cinnamoyloxy morphinones and equivalent 14-cinnamoylamino morphinones was found in the *p*-methyl substituted derivatives (**6c** and **5c**). Whereas in the cinnamoylamino series M-CAM (**5c**) had no agonist activity in TW or AW and was a substantially more effective pseudo-irreversible MOR agonist than the *p*-chloro analogue C-CAM (**5b**),[8] the *p*-methylcinnamoyloxy derivative **6c** was a less effective MOR antagonist than the *p*-chloro congener **6b** and had measurable agonist activity in AW. However, the SAR established for the 14-cinnamoylamino- series (**5**) that the 4′-substituted derivatives (**5b, 5c**) *in vivo* had lower MOR efficacy than the unsubstituted parent (**5a**)[9] also applied to the present 14-cinnamoyloxy series (**6**).

The cinnamoyloxy codeinones (**7b, 7c**) in the antinociceptive assays had no agonist activity in TW and showed parallel rightward shifts of the morphine dose-response curve in this assay indicating a competitive MOR antagonist effect. In AW **7c** but not **7b** had a weak

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opioid receptor agonist effect. These profiles are not dissimilar to those of the equivalent morphinones (**6b, 6c**) in the antinociceptive assays, the main difference being the lack of any flattening of the morphine dose-response curve by the codeinones in the MOR antagonist assay in TW. The similarity of the *in vivo* agonist effects of the cinnamoyloxycodeinones and morphinones contrasts with the 14-cinnamoylamino series in which the codeinones (**4**) all had substantially higher MOR efficacy *in vivo* than the equivalent morphinones (**5**).[9] In the *in vitro* functional assays (Tables 2, 3), the cinnamoyloxymorphinones (**6b, 6c**) were very much more potent as MOR antagonists than the equivalent codeinones (**7b, 7c**). This contrasts with the very small difference in potency between the cinnamoylamino morphinone (C-CAM) and equivalent codeinone (MC-CAM) (Table 3).[10]

It is of interest to compare the activity of 14-cinnamoylnaltrexone (**6a**) with the phenylpropyl ether (**2a**) which is structurally similar in having a 3-carbon chain linking the side chain aromatic ring to the C_{14}-oxygen atom. The ether (**2a**) *in vivo* gave a full response in a battery of thermal antinociceptive assays with potency up to 400 times greater than morphine.[7] In comparison the cinnamoyl ester has much more modest *in vitro* and *in vivo* MOR agonist activity. It must be assumed that the relative conformational restraint of the α,β-unsaturated cinnamoyl ester prevents an optimum interaction with MOR in the preferred agonist conformation.

Conclusions

The 14-*O*-cinnamoyl esters of naltrexone have predominant opioid receptor antagonist activity both *in vitro* and *in vivo*. In this regard they are similar to the equivalent 14-*N*-cinnamoylamino derivatives, but the latter are more potent antagonists of longer duration. Additionally the naltrexone esters (**6**) have similar *in vivo* and *in vitro* MOR efficacy to the corresponding codeinones (**7**) whereas the codeinone amides (**4**) have substantially higher MOR efficacy than the morphinones (**5**). These differences are less significant than the difference between 14-cinnamoylnaltrexone (**6a**) and 14-*O*-phenylpropylnaltrexone (**2a**). The greater side chain conformational freedom of the latter allows it to display very high potency *in vivo* MOR agonist activity.

Experimental

Column chromatography was performed under gravity, over silica gel 60 (35-70μm) purchased from Merck. Analytical TLC was performed using aluminium-backed plates coated with Kieselgel 60 F_{254}, from Merck. The chromatograms were visualised using either UV light (UVGL-58, short wavelength), ninhydrin (acidic) or potassium permanganate (basic). Melting points were carried out using a Reichert-Jung Thermo Galen Kopfler block or a Gallenkamp MFB-595 melting point apparatus and are uncorrected. High and low resolution electron impact (EI) mass spectra were recorded using EI ionisation at 70eV, on a VG AutoSpec instrument, equipped with a Fisons autosampler. ^{1}H NMR and ^{13}C NMR spectra were recorded using a JEOL 270 (operating at 270 MHz for ^{1}H and 67.8 MHz for ^{13}C) spectrometer. Chemical shifts (δ) are measured in ppm. Spectra were referenced internally using TMS as the standard. Only diagnostic peaks have been quoted for proton NMR. Microanalysis was performed with a Perkin-Elmer 240C analyser. Chemicals and solvents were purchased from Aldrich chemical company. Compounds were submitted for testing as their oxalate salts, formed by adding one equivalent of oxalic acid to an ethanolic solution of the ligand.

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3-*O*-(tert-Butyldimethylsilyl)-14β-cinnamoyloxy-N-cyclopropylmethyl-7,8-dihydronormorphinone (9a)

A solution of **8b** (593 mg:1.3 mmol) and cinnamoyl anhydride (830 mg: 3.0 mmol) in dry toluene (12 mL) was heated to reflux for 3 h. After cooling, the reaction mixture was washed with sodium bicarbonate solution (2 × 5 mL) and water (5 mL), dried over magnesium sulphate and the solvent removed in vacuo. The residue was purified by silica gel chromatography (CH_2Cl_2:MeOH, 49:1) to give (**9a**) (269 mg: 44%); EIMS m/z 585 (M^+); HRMS (EI) m/z 585.2925 (M^+) $C_{35}H_{43}NO_5Si$ requires 585.2910; ^{1}H NMR 0.07 (2H, m), 0.19 (3H, s), 0.28 (3H, s), 0.44 (2H, m), 0.74 (1H, m), 1.00 (9H, s), 4.69 (1H, s), 6.57 (1H, d), 6.59 (1H, d), 6.66 (1H, d), 7.70 (1H, d); ^{13}C NMR δ −4.64, −4.48, 3.75, 4.00, 9.49, 18.29, 23.24, 25.75, 26.99, 30.45, 35.75, 43.97, 51.08, 55.46, 59.31, 82.77, 89.95, 119.31, 119.37, 122.55, 126.39, 128.20, 128.71, 130.39, 134.39, 138.01, 144.01, 146.80, 165.85, 207.22.

14β-Cinnamoyloxy-N-cyclopropylmethyl-7,8-dihydronormorphinone (6a)

A solution of (**9a**) 140 mg: 0.24 mmol) and potassium fluoride (35 mg: 0.60 mmol) in MeOH (11 mL) and CH_2Cl_2 (1 mL) was stirred for 1 h at ambient temperature. Solvent evaporation gave a residue that was purified by silica gel column chromatography (CH_2Cl_2:MeOH, 49:1) to give **6a** as a white foam (57%); EIMS m/z 505 (M^+); ^{1}H NMR δ 0.05 (2H, m), 0.43 (2H, m), 0.76 (1H, m), 4.83 (1H, s), 6.58 (1H, d), 6.62 (1H, d), 6.80 (1H, d), 7.38 (3H, m), 7.56 (2H, m), 7.72 (1H, d); ^{13}C NMR δ 3.71, 4.00, 9.46, 23.18, 27.11, 30.19, 35.78, 44.00, 51.43, 55.56, 59.31, 82.77, 90.26, 118.29, 119.28, 120.07, 125.18, 128.39, 128.93, 129.31, 130.39, 134.39, 138.90, 143.53, 144.74, 165.88, 209.22; Anal. ($C_{29}H_{29}NO_5$.$(CO_2H)_2$.$2H_2O$) CHN.

3-*O*-(tert-Butyldimethylsilyl)-14β-(4-chlorocinnamoyloxy)-N-cyclopropylmethyl-7,8-dihydronormorphinone (9b)

8b (429 mg: 0.94 mmol) and 4-chlorocinnamoyl anhydride (616 mg: 1.77 mmol) were treated as for (**9a**) to yield (**9b**) as a clear oil (228 mg: 21%); EIMS m/z 619 (M^+); HRMS (EI) m/z 619.2537 (M^+), $C_{35}H_{42}NO_5Si$ requires 619.2521; ^{1}H NMR δ 0.04 (2H, m), 0.19 (3H, s), 0.27 (3H, s), 0.44 (2H, m), 0.72 (1H, m), 1.02 (9H, s), 4.67 (1H, s), 6.55 (1H, d), 6.56 (1H, d), 6.66 ((1H, d), 7.28 (2H,d), 7.52 (2H, d), 7.65 (1H, d); ^{13}C NMR d −4.70, −4.54, 3.68, 3.94, 9.46, 18.25, 23.21, 25.68, 26.92, 30.45, 35.68, 43.94, 51.02, 55.43, 59.27, 82.96, 89.53, 119.34, 119.85, 122.55, 126.32, 128.61, 129.18, 129.31, 132.86, 136.23, 137.98, 143.12, 146.72, 165.53, 207.06.

14β-(4-Chlorocinnamoyloxy)-N-cyclopropylmethyl-7,8-dihydronormorphinone (6b)

(**9b**) was treated with KF as described for **6a** to yield **6b** as a white foam (77%); EIMS m/z 505 (M^+); ^{1}H NMR δ 0.04 (2H, m), 0.45 (2H, m), 0.74 (1H, m), 4.80 (1H, s), 6.57 ((1H, d), 6.62 (1H, d), 6.76 (1H, d), 7.36 (2H, d), 7.50 (2H, d), 7.64 (1H, d); ^{13}C NMR δ 3.68, 3.97, 9.43, 23.11, 27.08, 30.19, 35.75, 43.97, 51.40, 55.46, 59.27, 82.93, 90.19, 118.26, 119.82, 120.01, 125.15, 128.16, 129.18, 129.31, 132.86, 136.23, 138.83, 143.21, 143.50, 165.56, 209.09; Anal. ($C_{29}H_{28}NO_5$.$(CO_2H)_2$.$2.5H_2O$) CHN.

3-*O*-(tert-Butyldimethylsilyl)-14β-(4-methylcinnamoyloxy)-N-cyclopropylmethyl-7,8-dihydronormorphinone (9c)

8b (584 mg: 1.28 mmol) and 4-methylcinnamoyl anhydride (690 mg: 2.25 mmol) were treated as for **9a** to yield **9c** as a clear oil (424 mg: 55%); EIMS m/z 599 (M^+); HRMS (EI) m/z 599.3088, $C_{36}H_{45}NO_5Si$ requires 599.3067; ^{1}H NMR δ 0.05 (2H, m), 0.21 (3H, s), 0.29 (3H, s), 0.44 (2H, m), 0.75 (1H, m), 0.99 (9H, s), 2.38 (3H, s), 4.67 (1H, s), 6.53 (1H, d), 6.56 (1H, d), 6.65 (1H, d), 7.19 (2H,d), 7.48 (2H, d), 7.68 (1H, d); ^{13}C NMR δ −4.70,

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−4.51, 3.71, 4.48, 9.46, 18.25, 21.46, 23.24, 25.21, 30.70, 32.73, 35.68, 43.94, 51.05, 55.53, 59.30, 82.57, 89.56, 118.16, 119.31, 122.51, 126.36, 127.69, 128.61, 129.78, 137.98, 140.36, 143.34, 144.56, 146.77, 165.98, 207.09.

14β-(4-Methylcinnamoyloxy)-N-cyclopropylmethyl-7,8-dihydronormorphinone (6c)

9c was treated with KF as for **9a** to yield **6c** as an oil (99%); EIMS m/z 485 (M^+); ^{1}H NMR δ 0.04 (2H, m), 0.44 (2H, m), 0.72 (1H, m), 4.77 (1H, s), 6.53 ((1H, d), 6.60 (1H, d), 6.75 (1H, d), 7.15 (2H, d), 7.48 (2H, d), 7.70 (1H, d); ^{13}C NMR δ 3.62, 3.90, 9.49, 21.46, 24.70, 26.32, 30.99, 32.79, 43.97, 51.46, 55.59, 59.34, 82.61, 90.29, 118.23, 120.01, 125.24, 127.94, 128.23, 129.66, 131.66, 132.04, 138.90, 140.80, 143.53, 144.71, 166.61, 209.06; Anal. ($C_{30}H_{31}NO_5$.$(CO_2H)_2$.$2H_2O$) CHN.

N-Cyclopropylmethyl-14β-cinnamoyloxy-7,8-dihydrocodeinone (7a)

A solution of **8a** (500 mg, 1.41 mmol) in anhydrous toluene (80 ml) was treated with cinnamoyl anhydride (512 mg, 1.84 mmol) and the resulting mixture heated to reflux and stirred overnight. On cooling, the solution was washed with Na_2CO_3 solution (2 × 20 ml) and water (20 ml), dried over $MgSO_4$ and evaporated to dryness. Silica gel chromatography (CH_2Cl_2:MeOH:NH_3, 198:1:1) gave **7a** as a white solid (303 mg: 44%); ESMS m/z 486 (MH^+); HRMS (ES) m/z 486.2259 (MH^+), $C_{30}H_{32}NO_5$ requires 486.2275; ^{1}H NMR δ −0.01-0.08 (2H, m), 0.39-0.48 (2H, m), 0.69-0.77 (1H, m), 1.57-1.61 (1H, m), 1.69 (1H, dt), 2.13-2.20 (1H, m), 2.25-2.38 (3H, m), 2.53 (1H, dd), 2.60-2.75 (3H, m), 2.92-2.97 (1H, m), 3.11 (1H, d), 3.89 (3H, s), 4.58 (1H, d), 4.75 (1H, s), 6.57 (1H, d), 6.64 (1H, d), 6.71 (1H, d), 7.37-7.43 (3H, m), 7.56-7.60 (2H, m), 7.70 (1H, d); ^{13}C NMR δ 3.68, 3.90, 9.45, 23.06, 27.19, 30.26, 35.78, 43.93, 51.27, 55.43, 56.73, 59.29, 82.69, 90.13, 114.79, 119.22, 119.51, 125.88, 128.14, 128.64, 128.90, 130.35, 134.33, 142.92, 144.62, 144.88, 165.74, 207.54; m.p. (oxalate) 124-126°C; Anal. ($C_{30}H_{31}NO_5$.$(CO_2H)_2$.$0.5H_2O$) CHN.

N-Cyclopropylmethyl-14β-4′-chlorocinnamoyloxy-7,8-dihydrocodeinone (7b)

8a (310 mg: 0.87 mmol) in anhydrous toluene (50 mL) was added to 4-chlorocinnamoyl anhydride (400 mg: 1.16 mmol) as described for **7a** and give **7b** as a white solid (110 mg: 24%); EIMS m/z 519 (M^+); HRMS (EI) m/z 519.1822 (M^+), $C_{30}H_{30}NO_5Cl$ requires 519.1813; ^{1}H NMR δ 0.01-0.12 (2H, m), 0.37-0.52 (2H, m), 0.67-0.82 (1H, m), 2.54 (1H, dd), 3.13 (1H, d), 3.90 (3H, s), 4.58 (1H, d), 4.75 (1H, s), 6.56 (1H, d), 6.66 (1H, d), 6.73 (1H, d), 7.38 (2H, m), 7.52 (2H, m), 7.66 (1H, d); ^{13}C NMR δ 3.62, 3.88, 9.40, 23.04, 27.11, 30.23, 35.67, 43.89, 51.17, 55.39, 56.72, 59.22, 82.83, 90.02, 114.88, 119.49, 119.76, 125.78, 128.55, 129.12, 129.25, 132.79, 136.17, 142.88, 143.10, 144.84, 165.42, 207.33; m.p. (oxalate) 122-124°C; Anal. ($C_{30}H_{30}NO_5Cl$.$(CO_2H)_2$.$0.5H_2O$) CHN.

N-Cyclopropylmethyl-14β-4′-methylcinnamoyloxy-7,8-dihydrocodeinone (7c)

Using the same procedure as for **7b** but with 4-methylcinnamoyl anhydride gave **7c** (30%); EIMS m/z 499 (M^+); HRMS (EI) m/z 499.2361 (M^+), $C_{31}H_{33}NO_5$ requires 499.2359; ^{1}H NMR δ 0.06-0.13 (2H, m), 0.39-0.53 (2H, m), 0.69-0.82 (2H, m), 2.40 (3H, s), 2.55 (1H, dd), 3.13 (1H, d), 3.92 (3H, s), 4.60 (1H, d), 4.77 (1H, s), 6.55 (1H, d), 6.66 (1H, d), 6.74 (1H, d), 7.23 (2H, m), 7.49 (2H, m), 7.70 (1H, d); ^{13}C NMR δ 3.65, 3.85, 9.42, 21.43, 23.05, 27.16, 30.23, 35.73, 43.90, 51.22, 55.46, 56.73, 59.26, 82.50, 90.09, 114.86, 118.07, 119.47, 125.89, 128.09, 128.66, 129.58, 131.57, 140.74, 142.87, 144.57, 144.87, 165.89, 207.49; m.p. (oxalate) 126-128°C; Anal. ($C_{31}H_{33}NO_5$.$(CO_2H)_2$.$0.5H_2O$) CHN.

Supplementary Material

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Abbreviations

TW	tail withdrawal from warm water
AW	acetic acid induced writhing

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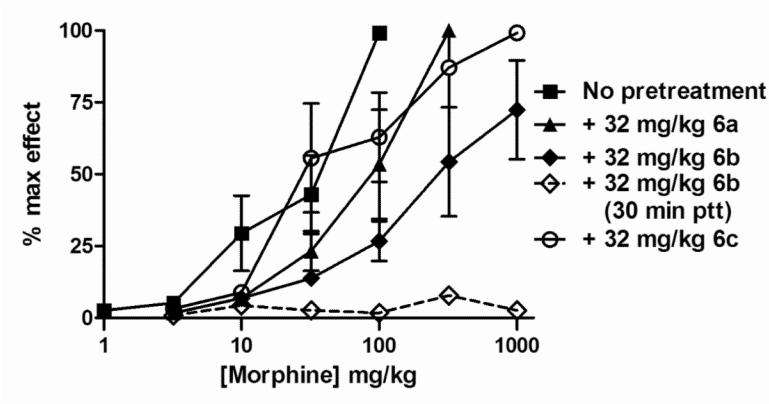

Figure 1.

Dose response curves for morphine alone (■) and after pretreatment with a 32 mg/kg dose of the morphinones **6a, 6b** and **6c** in the mouse warm water tail withdrawal assay. Pretreatment times (ptt) were 24 h (**6a, 6b, 6c**) and 30 min (**6b**).

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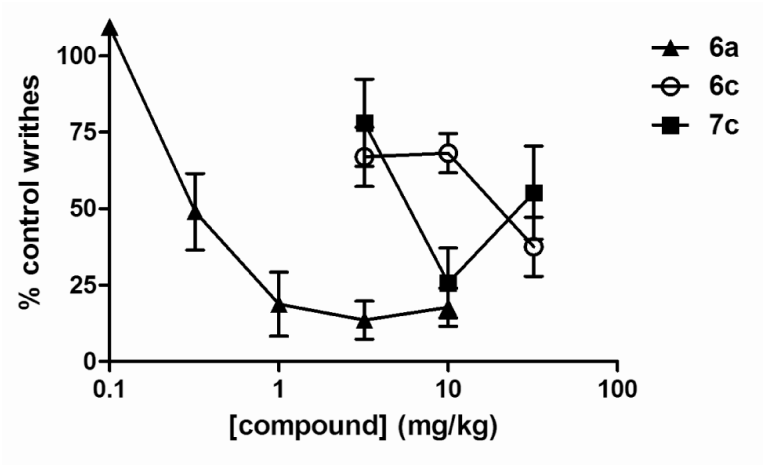

Figure 2.
Inhibition of acetic acid induced writhing by **6a, 6c and 7c** after s.c. administration.

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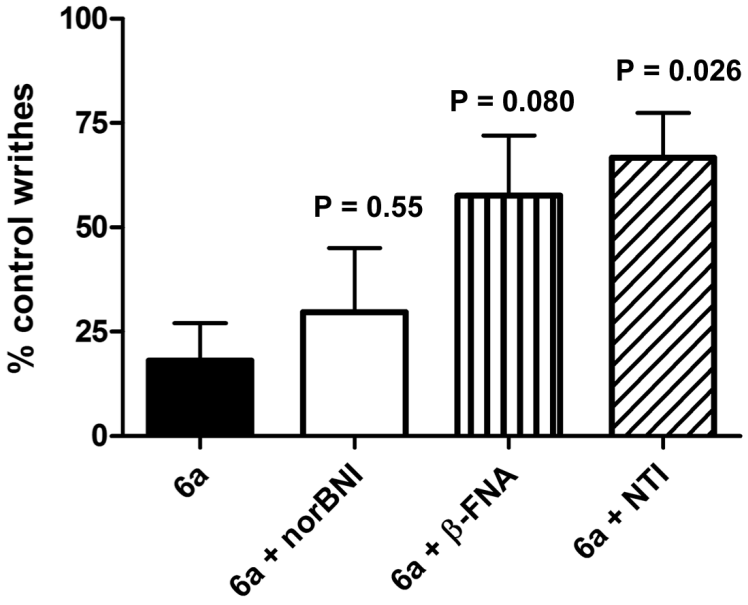
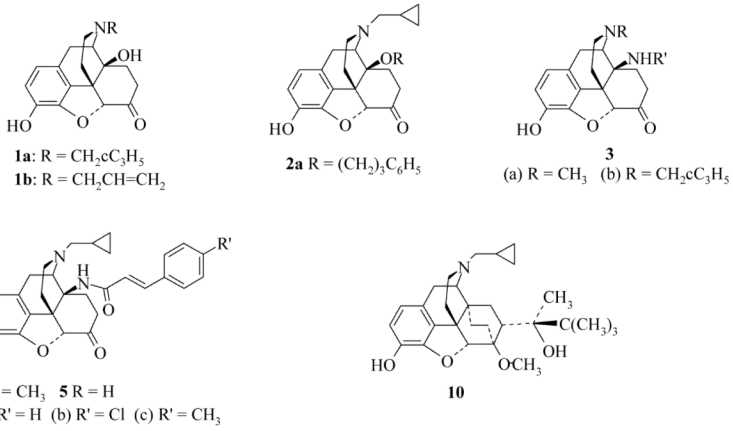


Figure 3.

Agonist selectivity of **6a** (10 mg/kg, s.c.) in the writhing assay.
NorBNI (KOR), 32 mg/kg (24 hr pretreatment); β-FNA (MOR), 32 mg/kg (24 hr pretreatment); Naltrindole (DOR), 10 mg/kg (24h pretreatment) t-test P-values



Structures.

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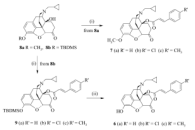

Scheme 1.
(i) $(RCO)_2O$, toluene, reflux (ii) KF, MeOH, CH_2Cl_2

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Table 1

Binding affinities of new ligands to opioid receptors

				Ki (nM)[d]	
	R	R'	MOR	DOR	KOR
6a [a]	H	H	0.40±0.05	3.4±0.8	3.6±2
6b [a]	H	Cl	1.3±0.45	15±6	8.3±0.2
6c [a]	H	CH_3	3.3±0.1	19±0.6	8.1±0.7
7a [b]	CH_3	H	2.5±0.7	180±58	4.3±0.7
7b [b]	CH_3	Cl	3.5±1.0	110±20	52±12
7c [b]	CH_3	CH_3	4.2±1.5	270±80	36±8.0
Naltrexone[a] (**1**)			0.40±0.05	6.5±1	0.6±0.1
Naltrexone[b] (**1**)			0.20±0.0	11±3	0.4±0.1
MC-CAM[b] (**4b**)			4.8±0.6	4.8±0.7	16±2.5
C-CAM[b] (**5b**)			3.0±0.2	2.7±0.2	1.4±0.5
PPN (**2a**)[b,c]			0.34±0.06	0.48±0.05	0.41±0.09

[a] binding to guinea pig brain membranes (method in ref. [13])

[b] binding to cloned human opioid receptors transfected into CHO cells (method in ref. [14])

[c] figures from ref [6].

[d] Values are the average from two experiments each carried out in duplicate. Tritiated ligands were [^{3}H]DAMGO (MOR), [^{3}H]Cl-DPDPE (DOR) and [^{3}H]U69593 (KOR)

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Table 2

Antagonist activity of new ligands in the mouse *vas deferens*

	R	R'	MOR	Ke (nM) DOR	KOR
6a	H	H	0.020±0.007	0.25±0.06	0.19±0.06
6b	H	Cl	0.060±0.03	0.060±0.01	1.3±0.2
6c	H	CH_3	0.12±0.02	0.66±0.2	0.54±0.07
Naltrexone (**1**)			0.44±0.09	7.2±0.3	8.0±0.6

Ke (nM) versus the standard selective agonists DAMGO (MOR), DPDPE (DOR) and U69593 (KOR). Values are the average of at least four experiments.

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Table 3

Inhibition of agonist stimulated [^{35}S]GTPγS binding in recombinant human opioid receptors

	R	R'	MOR Ke/nM[a]	DOR Ke/nM[b]	KOR Ke/nM[c]
7a	CH_3	H	Agonist[d]	Agonist[d]	Agonist[d]
7b	CH_3	Cl	8.2±0.34	57±2.2	96±14
7c	CH_3	CH_3	6.4±0.35	NT	Agonist[d]
MC-CAM (**4b**)			0.97±0.15	7.2±0.57	9.8±0.88
Naltrexone (**1**)			0.59±0.04	5.4±0.75	1.9±0.16
C-CAM (**5b**)			0.53±0.12	0.19±0.02	0.10±0.006

[a]Ke (nM) vs DAMGO.

[b]Ke (nM) vs DPDPE.

[c]Ke (nM) vs U69593.

[d]Agonist activity of these compounds is reported in Table 4.

NT = not tested.

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Table 4

Opioid agonist stimulation of [^{35}S]GTPγS binding in recombinant human opioid receptors.

			EC_{50} (nM); % stimulation		
	R	R'	MOR	DOR	KOR
7a	CH_3	H	34.4±5.0; 35	430±110; 64	26±4.9; 76
7c	CH_3	CH_3	ANT	NT	157±3.4; 37
MC-CAM (**4b**)[a]			17.8±11; 8	NT	ANT
Morphine			15.6±0.5; 93	316±4.9; 103	484±213; 62

Percent maximal stimulation with respect to the standard agonists DAMGO (mu), U69593 (kappa) and DPDPE (delta). ANT indicates antagonist activity (Ke values reported in Table 3). Values are the mean of 5 or 6 experiments. N.D. indicates not determined. Data supplied by NIDA Addiction Treatment Discovery Program.

[a]Data from Spagnolo et al, 2008[19]