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Structural Determinants of Opioid Activity in Derivatives of 14-Aminomorphinones: Effects of Changes to the Chain Linking the C₁₄-Amino Group to the Aryl Ring

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Abstract

The 14-aminodihydromorphinone and codeinone series of opioid ligands have produced a number of ligands of substantial interest. In order to investigate the importance of the 14-substituent a series of analogues in which the side chain length is varied and the amide and alkene functions are reduced have been prepared. Binding affinity, particularly at the mu opioid receptor (MOR), was largely determined by the aromatic group of the side chain. In the [³⁵S]GTPγS functional assay, the ligands having a three carbon side chain were more potent antagonists than their longer chain counterparts, whilst shorter, 2 carbon chain analogues were of higher MOR efficacy, an effect that was confirmed in vivo. Wash-resistant binding was observed within this series and appeared to be unrelated to side chain length.

Introduction

Among the close structural analogues of the opium alkaloids morphine (**1a**) and codeine (**1b**), the dihydromorphinones (**2**) have provided the major opportunities for therapeutic analogues and the starting materials for series of opioid ligands selective for the individual types of opioid receptor.¹ Introduction of a hydroxyl at C₁₄ provided the therapeutic analgesics oxymorphone (**2a**) and oxycodone (**2b**), but importantly also in naloxone (**2c**) and naltrexone (**2d**), the first 'pure' opiate antagonists² which have become indispensable pharmacological tools with therapeutic utility in treating narcotic overdose (naloxone) and in maintenance therapy for opiate dependence and alcoholism (naltrexone).^{3,4}

For a number of years we have studied derivatives in the formally similar 14-aminodihydromorphinone series (**3**) and in particular the cinnamoylamino derivatives (**4**).⁵ The lead compounds from this series clocinnamox (C-CAM, **4a**)^{6,7} and methocinnamox (M-CAM, **3b**)⁸ are important pharmacological tools as selective irreversible antagonists for the μ-opioid receptor (MOR) having the advantage over the prototype MOR-irreversible antagonist β-FNA that they have no short-term agonist activity. The codeinone (MC-CAM,

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5a) equivalent of **4a** had long-duration potent antinociceptive activity and when this had waned, irreversible MOR antagonist activity similar to that of **4a**.⁹

This report relates to analogues of **4a** and **5a**, in which the side chain has been extended or shortened (**4f**, **k**, **5e**, **f**, **k**), together with the equivalent ligands in which the side chain amide function (-NHCO-) has been reduced to the equivalent amine (-NHCH₂-) (**4h**, **i**, **l**; **5h**, **i**, **l**) and those having the alkene moiety reduced (**4g**, **j**, **5g**, **j**). Also included are the close relatives of **4a** and **5a** with saturated side-chains (**4b**, **5b**) and with the amide carbonyl reduced (**4c**, **d**, **5c**, **d**) which were prepared earlier but not reported in any detail.¹⁰

Chemistry

N-Cyclopropylmethyl-14 β -amino-7,8-dihydronormorphinone (**7a**) and the equivalent codeinone (**7b**) were prepared via ketals (**6**) from thebaine using established procedures (Scheme 1).^{11,12} Acylated codeinone derivatives **5b**, **5f**, **5g** and **5k** were prepared from **7b** and the appropriate acid chloride. Their phenolic counterparts **4f**, **4g** and **4k** were prepared from **7a** by bis-acylation with subsequent hydrolysis of the C₃-phenolic ester or for **4b** by 3-O-demethylation of **5b** (Scheme 1).

Direct alkylation of **7a** and **7b** using the corresponding alkyl bromides gave target compounds **4h**, **5h**, **4l** and **5l** (Scheme 1). Direct alkylation of ethylene ketals **6a** and **6b** with subsequent deprotection of the carbonyl group under acidic conditions, gave the 14 β -alkylaminocodeinone/morphinone analogues **4i**, **5i**, **4j** and **5j**. In these latter cases this two-stage strategy gave superior results to the direct alkylation of **7a** and **7b** (Scheme 1). **6a** was also used in the preparation of **5d**. Acylation of **6a** to give **22** followed by LiAlH₄ reduction of the amide and deprotection of the C₆-carbonyl gave **5d** that could then be 3-O-demethylated to yield **4d**.

Having discovered that 4-(4'-chlorophenyl)-2-butenic acid (**11**) could not readily be isolated, owing to its preference to isomerise to **10**, an alternative strategy to the routine acylation approach was required for the preparation of **5e** (Scheme 2). The protocol selected involved the formation of the α,β -unsaturated amide via a Horner-Wadsworth-Emmons type reaction.¹³ Acylation of **7b** using diethoxyphosphorylacetyl chloride^{14,15} afforded **12**. Treatment of **17** with LDA at low temperature in the presence of 4-chlorophenylacetaldehyde afforded **5e** exclusively in the (*E*)-configuration.

Results and Discussion

Affinity for the individual types of opioid receptor (OR) was determined in displacement binding assays in recombinant human opioid receptors transfected into chinese hamster ovary (CHO) cells; the displaced selective radioligands were [³H]DAMGO (MOR), [³H]U69593 (κ ; KOR) and [³H]CI-DPDPE (δ ; DOR).¹⁶ All the morphinone and codeinone ligands had high affinity for MOR with the morphinones (**4**) having modestly higher ($\leq$ 6-fold) affinity than the equivalent codeinones (**5**) (Table 1). KOR affinity of the codeinones was also lower ($\leq$ 59-fold) than that of the equivalent morphinones as was DOR affinity ($\leq$ 71-fold). Thus the codeinones showed significant MOR over DOR selectivity ($\leq$ 64-fold). MOR over KOR selectivity was generally modest but the 4-carbon chain arylalkyl substituents in **5i** and **5j** conferred a MOR selective profile and the 3-carbon chain analogues **5c** and **5d** were even more selective for MOR (Table 1).

4a and **5a** with a 3-carbon chain C₁₄ substituent have similar MOR and DOR affinity (Table 1) but the KOR affinity of **5a** is 12-fold lower than that of **4a**. The equivalent new ligands with a four-carbon side-chain (**4f**, **5e**, **5f**) generally had similar binding affinities to **4a** and **5a** with the notable exception that the DOR affinity of **5e** was 50-fold lower and the DOR affinity of

5f more than 20-fold lower than that of **5a**. Among the new ligands there was relatively little effect on OR binding affinities resulting from differences of chain length, though the new three-carbon chain derivatives (**4b**, **4d**, **5b**, **5d**) generally showed higher affinity than their two- and four-carbon equivalents (**4g**, **4j**, **4k**, **4l**, **5g**, **5j**, **5k**, **5l**). There was a similar lack of substantial effect from introducing unsaturation into the side-chain and replacing NHCO in the side chain with NHCH₂. It can be concluded that the predominant entity in the side chain for OR binding affinity is the aromatic group. The affinities of naltrexone (**3d**) were 25-fold (DOR), 320-fold (KOR) and 430-fold (MOR) greater than those of its 3-O-methyl ether (**3e**) (Table 1). The big loss of KOR and MOR affinity resulting from 3-O-methylation of naltrexone is in sharp contrast to the modest differences in KOR and MOR affinity between the series of morphinones (**4**) and codeinones (**5**). That the presence of a free 3-OH group is not necessary for high MOR affinity in series **4** and **5**, but is necessary in naltrexone-related ligands confirms that the C₁₄ substituent in **4** and **5** is relatively a more important determinant of MOR affinity than the C₃ substituent.¹⁷

In vitro OR functional activity of the new ligands was determined in assays in which stimulation of [³⁵S]GTPγS binding is measured for recombinant human OR transfected into CHO cells.^{16,18} All the new ligands with 4-carbon chain C₁₄ substituents (**4f-4j**; **5e-5j**) were potent MOR antagonists (Table 2) with the codeinones having 4-7-fold lower potency than the morphinones. The only exception was **5f** which was equipotent as a MOR antagonist to **4f**. The morphinones (**4f-4j**) were also potent DOR antagonists whereas the codeinones were low potency DOR antagonists (**5g**, **5h**, **5j**) or low potency, low efficacy DOR partial agonists (**5f**, **5i**). Only the morphinones with saturated 4-carbon chains in the C₁₄ substituent (**4g**, **4j**) were KOR antagonists; those with unsaturated 4-carbon chains (**4f**, **4h**, **4i**) were KOR low efficacy partial agonists of moderate potency. The codeinones (**5e-5j**) were all KOR partial agonists of generally low potency. As was found in the binding assays, the *in vitro* functional profiles of the morphinones (**4f-4j**) and codeinones (**5e-5j**) were relatively insensitive to the change of C₁₄-amide (NHCO) to amine (NHCH₂) and to the introduction of unsaturation into the side chain.

While the location of the double bond, or degree of saturation did not appear to greatly influence the potency of the compounds having a 4-carbon chain, differences were seen within the series having a 3-carbon chain. All the ligands having a 3-carbon chain (**4a**, **4b**, **4d** and **5a**, **5b**, **5d**) were antagonists at each of the opioid receptors, with the more flexible, saturated analogues (**4b**, **4d** and **5b**, **5d**) being more potent than their cinnamoyl counterparts (**4a**, **5a**). This was most noticeable at MOR and KOR with antagonist potency at DOR least affected. Thus **4b**, **4d** and **5b** were more potent than **4a** and **5a** by around 20-40-fold at MOR and KOR and 10-fold at DOR. **5d** was a slight exception, being more potent than **5a** by around 5-fold at each receptor. As expected the morphinones (**4a**, **4b**, **4d**) were of higher antagonist potency than their codeinone counterparts (**5a**, **5b**, **5d**) at KOR and DOR, with little or no change seen at MOR. The most striking SAR is found in comparison of the new ligands with 3-carbon side chains (**4b**, **4d**, **5b**, **5d**) with their 4-carbon counterparts (**4g**, **4j**, **5g**, **5j**). At each of the three opioid receptors the 3-carbon chain analogues were very much more potent (20-150-fold) in the functional assay than their 4-carbon homologues, suggesting that it is at 3-carbons that optimal antagonist potency is reached. In fact the addition of the extra carbon in codeinones **5g** and **5j** results in the introduction of some efficacy at KOR.

The *in vitro* functional profiles of the new ligands with two carbon chain C₁₄ substituents were notable in so far as they were the only ligands tested which showed significant MOR efficacy in the [³⁵S]GTPγS assay (Table 2). **4k**, **4l**, **5k** and **5l** were all potent MOR partial agonists with the phenylacetylaminomorphinone (**4k**) having greatest potency with EC₅₀ 0.26nM. The codeinones **5k**, **5l** had marginally higher MOR efficacy than the morphinones **4k**, **4l** but **4k** was over thirty times more potent than **5k**. The difference in potency between **4l** and **5l** was

only about two-fold, in keeping with the MOR affinities in the binding assays (Table 1). **4k** and **4l** were also potent DOR partial agonists and had potent, very low efficacy, or antagonist KOR functional activity. The efficacy of the phenylethylaminomorphinone (**4l**) for DOR and KOR was somewhat lower than that of the phenylacetylaminomorphinone (**4k**). The equivalent codeinones (**5k**, **5e**) had low potency DOR partial agonist activity but whereas **5l** was also a low potency KOR partial agonist, the phenylacetylaminocodeinone (**5k**) was a nearly full KOR agonist of significant potency (Table 2).

Three of the new 14-aminodihydromorphinones (**4f**, **4g** and **4k**) and **4a** (C-CAM) were investigated in membranes from C₆ cells expressing MOR to determine whether evidence could be found for irreversible binding to MOR. The test compound, or vehicle treated membranes were incubated in Tris-HCl buffer for 1h at 25°C, following which the membranes were collected by centrifugation, resuspended and incubated at 37° to promote dissociation of weakly bound ligand then collected by recentrifugation and twice further washed. This procedure was sufficient to cause complete washout of 10µM of the reversible antagonist naloxone (**2c**) whereas the test compounds (**4a**, **4f**, **4g**, **4k**) remained bound to the membranes. This was confirmed in experiments in which test compound or vehicle treated membranes (15µg) were incubated at 25°C for 1h with increasing concentrations of DAMGO in the presence of buffered [³⁵S]GTPγS to determine the effects of the ligand combinations on [³⁵S]GTPγS binding. The effects on DAMGO concentration-effect curves for incubation with the test ligands is shown in Figures 1 and 2. For **4a** a concentration of 10nM had a relatively small effect in suppressing the stimulation of [³⁵S]GTPγS binding produced by DAMGO (Fig. 1); a concentration of 100nM was fully effective (Fig. 1). The homologue (**4f**) had a similar effect to **4a** at 10nM concentration but the phenylbutylamido derivative (**4g**) and phenylacetyl amino derivative (**4k**) at 10nM concentration both produced a greater effect than **4a** (Fig. 2). The effect of **4g** at 1nM was at least as great as **4a** at 10nM (data not shown). The flattening of the dose-response curve of an agonist in the presence of an antagonist is an indication that the antagonist binds irreversibly to the receptor responsible for the agonist effect¹⁹ and in this series the magnitude of this effect was unrelated to side chain length.

The phenylacetylaminomorphinone (**4k**) was investigated *in vivo* in the mouse tail withdrawal assay with water at 50°C (Fig 3).⁸ **4k** was fully active in this assay at a dose of 1mg/kg, representing ten times greater potency than morphine. The antinociceptive effect of **4k** had gone by 24h and at that pretreatment time **4k** did not affect the antinociceptive dose-effect curve of morphine which would have indicated a delayed MOR-antagonist effect. The high efficacy agonist effect of **4k** *in vivo* is apparently at odds with its modest efficacy (29% of DAMGO) in the [³⁵S]GTPγS *in vitro* assay. However such disparity between activity *in vitro* and *in vivo* for lipophilic ligands in the 14-substituted morphinone series has been noted elsewhere.⁸ One explanation for this disparity is that the receptor reserve in the 50°C water antinociceptive assay is substantially greater than in the MOR [³⁵S]GTPγS assay. Differences are also noted for antagonist activity where, for example, **4k** was a powerful non-competitive antagonist of DAMGO in the [³⁵S]GTPγS assay but had no discernible delayed antagonism in the tail withdrawal assay. More work is needed to better understand these disparities and whether the antagonism seen *in vitro* provides any beneficial effects in the search for MOR agonists with reduced abuse potential.

Conclusion

The lack of any agonist effect and the exceptional non-competitive antagonism displayed *in vivo* by **4a** cannot be explained by covalent bond formation to the receptor^{20,21} but seems likely to involve dominant lipophilic binding by the cinnamoyl aromatic group. This could involve binding of the aromatic group outside the helical loops of the receptor to the lipid bilayer. In that case the interaction could be sensitive to the length of the C₁₄ side-chain. The

data presented here indicate that, of the new ligands which were more extensively studied *in vitro*, MOR profiles of **4f** and **4g**, with 4 carbon C₁₄-side chains, are similar to that of the 3 carbon chain analogues such as **4a** and **4b**, whereas the ligand (**4k**) with a 2-carbon chain is different in having substantial MOR-agonist activity that was confirmed *in vivo*. However, the *in vitro* data provide evidence that **4k** is a MOR partial agonist and shows non-competitive antagonist activity in suppressing the agonist effects of the selective MOR-agonist DAMGO. It is therefore unclear whether extra-helical binding is responsible for the profile of **4a** as a non-competitive antagonist.

Experimental

Reagents and solvents were purchased from *Aldrich* or *Lancaster* and used as received. M.p.: *Gallenkamp MFB-595* melting point apparatus; uncorrected. IR Spectra: *Perkin-Elmer 881* instrument; in cm⁻¹. NMR Spectra: *Jeol Lambda-270-MHz* instrument: ¹H at 270 MHz, ¹³C at 67.5 MHz; δ in ppm, *J* in Hz with TMS as an internal standard. EIMS: *V.G.-Autospec* instrument equipped with a *Fisons* autosampler; EI at 70 eV; *m/z* (rel.%). Microanalysis: *Perkin-Elmer 240C* analyser. Ligands were tested as their oxalate salts, prepared by adding one equivalent of oxalic acid to an ethanolic solution of the compound.

General Procedure A: Preparation of acid chlorides and the *in situ* acylation of N-cyclopropylmethyl-14 β -amino-7,8-dihydronorcodeinones/morphinones

A suspension of oxalyl chloride (8.8 eqv) and the corresponding carboxylic acid (1.1 eqv) in anhydrous toluene was heated at reflux for 1 h. The resulting solution was allowed to cool and the solvent removed *in vacuo*. The residue was dissolved in anhydrous dichloromethane and added dropwise to a solution of N-cyclopropylmethyl-14 β -amino-7,8-dihydronorcodeinone (1 eqv) and triethylamine (1.1 eqv) in anhydrous dichloromethane, and the mixture stirred at room temperature overnight. The solvent was removed *in vacuo* and the crude residue purified by column chromatography (5% CH₃OH in CH₂Cl₂). In the acylation of N-cyclopropylmethyl-14 β -amino-7,8-dihydronormorphinone, a second equivalent (total: 2 eqv) of the corresponding acid chloride was used to afford the bis-acylated derivative. The crude residue was dissolved in methanol/water (9:1) before adding potassium carbonate (5 eqv), and the mixture stirred at room temperature overnight. The solvent was removed *in vacuo* and the crude residue purified by column chromatography (5% CH₃OH in CH₂Cl₂).

General Procedure B: Alkylation of N-cyclopropylmethyl-14 β -amino-7,8-dihydronorcodeinones/ morphinones and their ethylene glycol protected derivatives

A stirring suspension of N-cyclopropylmethyl-14 β -amino-7,8-dihydronorcodeinone/ morphinone or its ethylene ketal protected derivative (1 eqv), potassium carbonate (5 eqv) and the corresponding alkyl bromide (1.1 eqv) in dimethylformamide was heated at 90 °C for 3-12 h. The solvent was removed *in vacuo* and the crude residue purified by column chromatography (5% CH₃OH in CH₂Cl₂).

General Procedure C: Acid catalysed deprotection of N-cyclopropylmethyl-14 β -amino-7,8-dihydronorcodeinone/morphinone ethylene ketals

A solution of N-cyclopropylmethyl-14 β -amino-7,8-dihydronorcodeinone/morphinone ethylene ketal in methanol and hydrochloric acid (6N) was heated at reflux for 4 h. The solvent was removed *in vacuo*, the crude residue basified with concentrated ammonia and extracted with dichloromethane. The combined organic extracts were washed with water and dried over anhydrous magnesium sulfate. The solvent was removed *in vacuo* and the crude residue purified by column chromatography (5% CH₃OH in CH₂Cl₂).

N-Cyclopropylmethyl-14β-[3'-(4''-chlorophenyl)propanamido]-7,8-dihydronorcodeinone (5b)

7b (1.57g; 4.42mmol) was treated with 3-(4-chlorophenyl)propanoyl chloride (912 mg; 4.90 mmol) as described in general procedure A to afford **5b** as a white foam (1.75g, 3.37 mmol, 76%). Anal. (oxalate salt) (C₃₂H₃₅N₂ClO₈) C, H, N.

N-Cyclopropylmethyl-14β-[3'-(4''-chlorophenyl)propanamido]-7,8-dihydronormorphinone (4b)

A solution of BBr₃ (17 mL, 1M: 17 mmol) in CH₂Cl₂ was added at -78°C under N₂ to a solution of **5b** (1.48g; 2.8 mmol) in CH₂Cl₂ (10 mL). The mixture was allowed to warm to -20°C and stirred for 1h before again cooling to -78°C and adding MeOH (30 mL). The mixture was then basified using 2M NaOH (to pH 12) and then neutralized with dilute HCl. Extraction with CH₂Cl₂:MeOH (9:1, 3×20 mL), drying (MgSO₄) and evaporation gave a residue that was purified using column chromatography (CH₂Cl₂:MeOH, 19:1) to yield **4b** as a white solid (990 mg, 1.98 mmol, 69%). Anal. (free base) (C₂₉H₃₁N₂ClO₄.CH₃OH) C, H, N.

N-Cyclopropylmethyl-14β-[3'-(4''-chlorophenyl)propanamido]-7,8-dihydronorcodeinone ethylene glycol ketal (22)

6a (1.65g; 4.15 mmol), 3-(4-chlorophenyl)propanoyl chloride (0.97g; 12.4 mmol) and NEt₃ (0.58 mL) in dry CH₂Cl₂ (10 mL) were stirred under N₂ for 3 h before evaporation to dryness and purification by silica gel column chromatography (CH₂Cl₂: MeOH, 19:1) to yield **22** as a white foam (1.92g, 3.40 mmol, 82%).

N-Cyclopropylmethyl-14β-[3'-(4''-chlorophenyl)propylamino]-7,8-dihydronorcodeinone (5d)

A solution of **22** (2.87g; 5.08 mmol) in dry THF (12 mL) was added to a suspension of LiAlH₄ (500 mg; 13.0 mmol) in dry THF (33 mL) under N₂. The mixture was refluxed for 24h, cooled and the reaction quenched by the addition of Rochelle's salt. The THF was removed in vacuo, CH₂Cl₂ and H₂O added with the organic layer being collected. The aqueous layer was further extracted with CH₂Cl₂:MeOH (9:1, 3×20 mL), the combined extracts were dried (MgSO₄) and evaporated in vacuo to give a white foam that was immediately dissolved in MeOH (25 mL) and 1M HCl added (15 mL). After refluxing for 5h the solution was cooled, neutralized with Na₂CO₃ and the MeOH evaporated. Extraction with CH₂Cl₂:MeOH (9:1, 3×15 mL), evaporation and purification by silica gel column chromatography (CH₂Cl₂:MeOH, 19:1) gave **5d** as a white foam (1.37g, 2.71 mol, 53%). Anal. (oxalate) (C₃₂H₃₇N₂ClO₇.0.5H₂O) C, H, N.

N-Cyclopropylmethyl-14β-[3'-(4''-chlorophenyl)propylamino]-7,8-dihydronormorphinone (4d)

5d (1.1g; 2.2 mmol) was treated with BBr₃ (13 mL, 1M, 13 mmol) as described for **4b**. Column chromatography (CH₂Cl₂: MeOH, 19:1) yielded **4d** as a white solid. Anal. (oxalate) (C₃₁H₃₅N₂ClO₇.0.5H₂O) C, H, N.

N-Cyclopropylmethyl-14β-[4'-(4''-chlorophenyl)-3'-butenamido]-7,8-dihydronorcodeinone (5f)

7b was treated with 4-(4'-chlorophenyl)-3-butenoyl chloride as in general procedure A to afford **5f** as a pale yellow solid (68 mg, 0.13 mmol, 64%). Anal. (oxalate) (C₃₃H₃₅N₂ClO₈.0.5H₂O) C, H, N.

N-Cyclopropylmethyl-14β-[4'-(4''-chlorophenyl)-3'-butenamido]-7,8-dihydronormorphinone (4f)

7a was treated with 4-(4'-chlorophenyl)-3-butenoyl chloride as in general procedure A to afford **4f** as a white solid (50 mg, 0.10 mmol, 48%). Anal. (oxalate) ($C_{32}H_{33}N_2ClO_8 \cdot 0.75H_2O$) C, H, N.

N-Cyclopropylmethyl-14β-[4'-(4''-chlorophenyl)-butanamido]-7,8-dihydronorcodeinone (5g)

7b was treated with 4-(4'-chlorophenyl)butanoyl chloride as in general procedure A to afford **5g** as a yellow solid (76 mg, 0.14 mmol, 71%). Anal. (oxalate) ($C_{33}H_{37}N_2ClO_8 \cdot 1H_2O$) C, H, N.

N-Cyclopropylmethyl-14β-[4'-(4''-chlorophenyl)-butanamido]-7,8-dihydronormorphinone (4g)

7a was treated with 4-(4'-chlorophenyl)butanoyl chloride as in general procedure A to afford **4g** as a white solid (55 mg, 0.11 mmol, 53%). Anal. (oxalate) ($C_{32}H_{35}N_2ClO_8 \cdot 0.75H_2O$) C, H, N.

N-Cyclopropylmethyl-14β-[4'-(4''-chlorophenyl)-2'-butenamido]-7,8-dihydronorcodeinone (5e)

To a solution of lithium diisopropylamide [butyl lithium 2.5 M in hexanes (100 μl, 0.24 mmol) and diisopropylamine (35 μl, 0.24 mmol)] in tetrahydrofuran (1 ml) at -78 °C was added **12** (0.11 g, 0.20 mmol) in tetrahydrofuran (1 ml) and the mixture stirred for 0.5 h maintaining this temperature.⁷ A solution of 4-chlorophenylacetaldehyde (**9**) (0.04 g, 0.26 mmol) in tetrahydrofuran (1 ml) was added dropwise at -78 °C and the resulting mixture stirred at room temperature for 1 h. Water was added and the aqueous layer extracted with diethyl ether, then dichloromethane. The combined organic extracts were washed with water, dried over anhydrous magnesium sulfate and the solvent removed *in vacuo*. Purification by column chromatography (5% CH₃OH in CH₂Cl₂) afforded **5e** as a white solid (30 mg, 0.06 mmol, 28%). Anal. (oxalate) ($C_{33}H_{35}N_2ClO_8$) C, H, N.

N-Cyclopropylmethyl-14β-[2'-(4''-chlorophenyl)-ethanamido]-7,8-dihydronorcodeinone (5k)

7b was treated with 2-(4'-chlorophenyl)acetyl chloride as in general procedure A to afford **5k** as a white solid (70 mg, 0.14 mmol, 69%). Anal. ($C_{31}H_{33}N_2ClO_8 \cdot 1.5H_2O$) C, H, N.

N-Cyclopropylmethyl-14β-[2'-(4''-chlorophenyl)-ethanamido]-7,8-dihydronormorphinone (4k)

7a was treated with 2-(4'-chlorophenyl)acetyl chloride as in general procedure A to afford **4k** as a white solid (50 mg, 0.10 mmol, 51%). Anal. (oxalate) ($C_{30}H_{31}N_2ClO_8 \cdot 0.5H_2O$) C, H, N.

N-Cyclopropylmethyl-14β-[2'-(4''-chlorophenyl)-ethanamino]-7,8-dihydronorcodeinone (5l)

7b was treated with 2-(4'-chlorophenyl)ethyl iodide as in general procedure B to afford **5l** as a pale brown solid (47 mg, 0.10 mmol, 48%). Anal. (oxalate) ($C_{31}H_{35}N_2ClO_7 \cdot 0.5CH_2Cl_2$) C, H, N.

N-Cyclopropylmethyl-14β-[2'-(4''-chlorophenyl)-ethanamino]-7,8-dihydronormorphinone (4l)

7a was treated with 2-(4'-chlorophenyl)ethyl iodide as in general procedure B to afford **4l** as a white solid (35 mg, 0.07 mmol, 37%). Anal. (oxalate) ($C_{30}H_{33}N_2ClO_7 \cdot 0.5CH_2Cl_2$) C, H, N.

N-Cyclopropylmethyl-14 β -[4'-(4''-chlorophenyl)-3'-butenamino]-7,8-dihydronorcodeinone (5i)

6a was treated with 4-(4'-chlorophenyl)-3-butenyl bromide as in general procedure B to afford N-Cyclopropylmethyl-14 β -[4'-(4''-chlorophenyl)-3'-butenamino]-7,8-dihydronorcodeinone ethylene ketal as a colourless oil (39 mg, 0.07 mmol, 50%).

This was treated as in general procedure C to afford **5i** as a pale yellow solid (21 mg, 0.04 mmol, 65%). Anal. (oxalate) (C₃₃H₃₇N₂ClO₇·1.5H₂O) C, H, N.

N-Cyclopropylmethyl-14 β -[4'-(4''-chlorophenyl)-3'-butenamino]-7,8-dihydronormorphinone (4i)

6b was treated with 4-(4'-chlorophenyl)-3-butenyl bromide as in general procedure B to afford N-Cyclopropylmethyl-14 β -[4'-(4''-chlorophenyl)-3'-butenamino]-7,8-dihydronormorphinone ethylene ketal as a colourless oil (77 mg, 0.14 mmol, 44%).

This was treated as in general procedure C to afford **4i** as a pale yellow solid (36 mg, 0.07 mmol, 54%). Anal. (oxalate) (C₃₂H₃₅N₂ClO₇·1H₂O) C, H, N.

N-Cyclopropylmethyl-14 β -[4'-(4''-chlorophenyl)-2'-butenamino]-7,8-dihydronorcodeinone (5h)

7b was treated with 4-(4'-chlorophenyl)-2-butenyl bromide as in general procedure B to afford **5h** as a yellow solid (82 mg, 0.16 mmol, 79%). Anal. (oxalate) (C₃₃H₃₇N₂ClO₇·1.5H₂O) C, H, N.

N-Cyclopropylmethyl-14 β -[4'-(4''-chlorophenyl)-2'-butenamino]-7,8-dihydronormorphinone (4h)

7a was treated with 4-(4'-chlorophenyl)-2-butenyl bromide as in general procedure B to afford **4h** as a yellow solid (70 mg, 0.14 mmol, 69%). Anal. (oxalate) (C₃₂H₃₅N₂ClO₇·1.25H₂O) C, H, N.

N-Cyclopropylmethyl-14 β -[4'-(4''-chlorophenyl)-butanamino]-7,8-dihydronorcodeinone (5j)

6a was treated with 4-(4'-chlorophenyl)butyl bromide as in general procedure B to afford N-Cyclopropylmethyl-14 β -[4'-(4''-chlorophenyl)-butanamino]-7,8-dihydronorcodeinone ethylene ketal as a colourless oil (51 mg, 0.09 mmol, 58%).

This was treated as in general procedure C to afford **5j** as a pale yellow solid (25 mg, 0.05 mmol, 63%). Anal. (oxalate) (C₃₃H₃₉N₂ClO₇·1.25H₂O) C, H, N.

N-Cyclopropylmethyl-14 β -[4'-(4''-chlorophenyl)-butanamino]-7,8-dihydronormorphinone (4j)

6b was treated with 4-(4'-chlorophenyl)butyl bromide as in general procedure B to afford N-Cyclopropylmethyl-14 β -[4'-(4''-chlorophenyl)-butanamino]-7,8-dihydronormorphinone ethylene ketal as a colourless oil (94 mg, 0.17 mmol, 49%).

This was treated as in general procedure C to afford **4j** as a pale yellow solid (40 mg, 0.08 mmol, 51%). Anal. (oxalate) (C₃₂H₃₇N₂ClO₇·1.5H₂O) C, H, N.

N-Cyclopropylmethyl-14 β -(2-diethoxyphosphoryl-1-oxoethyl)-7,8-dihydronorcodeinone (12)

To a stirring solution of N-cyclopropylmethyl-14 β -amino-7,8-dihydronorcodeinone (**7b**) (140 mg, 0.40 mmol) in tetrahydrofuran (3 ml) at -20 °C was added diethoxyphosphorylacetyl chloride (94 mg, 0.44 mmol) in tetrahydrofuran (1 ml). The mixture was allowed to warm and

stirred at room temperature for 0.5 h. Water was added and the mixture extracted with diethyl ether, then dichloromethane. The combined organic extracts were washed with water, dried over anhydrous magnesium sulfate and the solvent removed *in vacuo*. Purification by column chromatography (5% CH₃OH in CH₂Cl₂) afforded **17** as a colourless oil (128 mg, 0.24 mmol, 60%).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Effect of pre-incubation with CCAM (**4a**) followed by extensive washing on the concentration effect curve for DAMGO in C6 cells expressing a mu-opioid receptor.

Figure 2.
Effect of pre-incubation with CCAM analogues (**4f**, **4g**, **4k**) followed by extensive washing on the concentration effect curve for DAMGO in C6 cells expressing a mu-opioid receptor.

Figure 3.
Agonist effect of **4k** compared to morphine in the 50°C mouse tail-withdrawal test.

Scheme 1.

(i). BBr₃, DCM, -30 °C to r.t., 0.5 h, 40% (**6a** to **6b**) or 40% (**7b** to **7a**); (ii). HCl (6N), MeOH, reflux, 5 h, 50%; (iii). a). Alkyl bromide, K₂CO₃, DMF, 90 °C, 24 h, b). HCl (6N), MeOH, reflux, 5 h, (iv). Acid chloride, NEt₃, DCM, r.t., overnight; (v). K₂CO₃, MeOH, r.t., overnight; (vi). Alkyl bromide, K₂CO₃, DMF, 90 °C, 3 h; (vii). LiAlH₄, THF, reflux overnight then HCl (1N), MeOH, reflux, 5h, 53%.

Scheme 2.

(i). $(\text{EtO})_2\text{P}(\text{O})\text{CH}_2\text{COCl}$, THF, $-20\text{ }^\circ\text{C}$ to r.t., 0.5 h, 60%; (ii). a). LDA, THF, $-78\text{ }^\circ\text{C}$, 0.5 h, b). 4-Chlorophenylacetaldehyde, THF, $-78\text{ }^\circ\text{C}$ to r.t., 1 h, 28%.

Structures

Table 1
Binding affinities of ligands to human opioid receptor transfected into CHO cells.

Comp'd	R	X	DOR	KOR	MOR	DOR/MOR	KOR/MOR
5a	Me	COCH=CH	4.79±0.73	16.4±2.54	4.78±0.58	1.0	3.4
5b	Me	CO(CH ₂) ₂	5.10±0.14 ¹	6.50±0.26 ¹	0.24±0.05 ¹	21.2	27.1
5c	Me	CH ₂ CH=CH	44.5±4.6 ¹	53.6±0.95 ¹	0.70±0.10 ¹	63.6	76.6
5d	Me	(CH ₂) ₃	31.3±1.8 ¹	78.6±32.8 ¹	0.59±0.00 ¹	53.0	133
5e	Me	COCH=CHCH ₂	242±50.0	25.2±4.14	8.07±1.84	30.0	3.1
5f	Me	COCH ₂ CH=CH	104±21.7	21.5±4.19	8.19±0.94	12.7	2.6
5g	Me	CO(CH ₂) ₃	89.4±20.0	7.51±0.43	3.88±1.07	23.0	1.9
5h	Me	CH ₂ CH=CHCH ₂	94.9±21.2	25.9±5.9	6.76±2.39	14.0	3.8
5i	Me	(CH ₂) ₄	24.8±5.15	33.5±4.15	1.15±0.27	21.6	29.1
5j	Me	(CH ₂) ₄	55.9±1.67	41.5±7.79	2.23±0.80	25.1	18.6
5k	Me	COCH ₂	51.9±15.6	2.59±0.22	2.64±0.91	19.7	1.0
5l	Me	(CH ₂) ₂	33.8±1.40	13.1±2.29	1.33±0.24	25.4	9.8
4a	H	COCH=CH	2.69±0.23	1.41±0.52	2.98±0.22	0.9	0.5
4b	H	CO(CH ₂) ₂	0.60±0.00 ¹	0.65±0.07 ¹	0.04±0.00 ¹	15.0	16.2
4c	H	CH ₂ CH=CH	0.63±0.08 ²	0.91±0.12 ²	0.32±0.03 ²	2.0	2.8
4d	H	(CH ₂) ₃	1.10±0.00 ¹	1.90±0.85 ¹	0.13±0.08 ¹	8.5	14.6
4f	H	COCH ₂ CH=CH	3.25±0.28	4.94±1.75	1.87±0.45	1.7	2.6
4g	H	CO(CH ₂) ₃	3.85±0.25	2.95±0.16	1.34±0.05	2.9	2.2
4h	H	CH ₂ CH=CHCH ₂	6.65±0.20	3.72±0.05	1.89±0.01	3.5	2.0
4i	H	(CH ₂) ₂ CH=CH	2.10±0.79	3.69±1.31	1.00±0.28	2.1	3.7
4j	H	(CH ₂) ₄	2.18±0.30	5.27±2.06	0.96±0.35	2.3	5.5
4k	H	COCH ₂	1.51±0.17	1.00±0.14	0.73±0.00	2.0	1.4
4l	H	(CH ₂) ₂	1.91±0.17	1.53±0.05	0.86±0.20	2.2	1.8
3d	H		10.8±3.0	0.4±0.1	0.2±0.0	54.0	2.0
3e	H		6.5±1.3 ¹	0.6±0.1 ¹	0.4±0.05 ¹	16.2	1.5
3e	Me		272±72	128±23	85.6±0.22	3.2	1.5

Data are the average from two experiments, each carried out in triplicate. Tritiated ligands were [³H]DAMGO(μ), [³H]Cl-DPPE(δ), [³H]U69593(κ).

¹ Binding to guinea pig brain homogenates.

² Displacement of 3H-diprenorphine from membranes of C6mu cells, C6delta cells or CHO kappa cells

Table 2
Agonist and Antagonist Effects of Ligands at Opioid Receptors Measured by the [³⁵S]GTPγS Binding Assay

Comp'd	R	X	DOR	KOR	MOR
5a	Me	COCH=CH	7.16±0.57 ANT	9.81±0.88 ANT	0.97±0.15 ANT
5b	Me	COCH ₂ CH ₂	0.76±0.11 ANT	0.29±0.07 ANT	0.03±0.003 ANT
5d	Me	(CH ₂) ₃	1.66±0.17 ANT	1.87±1.00 ANT	0.12±0.01 ANT
5e	Me	COCH=CHCH ₂	NT	26.2±0.62 23	12.1±0.38 ANT
5f	Me	COCH ₂ CH=CH	118±21.0 32	106±28.0 41	1.27±0.24 ANT
5g	Me	CO(CH ₂) ₃	79.9±6.19 ANT	20.2±8.3 30	4.91±0.54 ANT
5h	Me	CH ₂ CH=CHCH ₂	96.8±9.27 ANT	387±47.0 31	4.98±0.59 ANT
5i	Me	(CH ₂) ₂ CH=CH	148±47.0 29	208±66.0 43	1.64±0.28 ANT
5j	Me	(CH ₂) ₄	61.1±6.71 ANT	202±47.7 49	1.45±0.22 ANT
5k	Me	COCH ₂	88.7±29.6 32	9.07±2.53 89	8.84±0.22 39
5l	Me	(CH ₂) ₂	63.0±16.0 44	43.4±11.5 31	3.73±0.91 39
4a	H	COCH=CH	0.19±0.02 ANT	0.10±0.006 ANT	0.53±0.13 ANT
4b	H	CO(CH ₂) ₂	0.03±0.006 ANT	0.003±0.001 ANT	0.028±0.005 ANT
4d	H	(CH ₂) ₃	0.02±0.002 ANT	0.006±0.002 ANT	0.0125±0.002 ANT
4f	H	COCH ₂ CH=CH	1.70±0.45 ANT	13.8±3.09 33	1.84±0.32 ANT
4g	H	CO(CH ₂) ₃	1.37±0.21 ANT	0.59±0.08 ANT	0.87±0.10 ANT
4h	H	CH ₂ CH=CHCH ₂	3.35±0.42 ANT	3.45±0.41 24	1.21±0.06 ANT
4i	H	(CH ₂) ₂ CH=CH	1.17±0.14 ANT	16.7±2.34 44	0.24±0.04 ANT
4j	H	(CH ₂) ₄	0.67±0.04 ANT	0.44±0.06 ANT	0.29±0.01 ANT
4k	H	COCH ₂	2.41±0.85 50	1.12±0.29 23	0.26±0.07 29
4l	H	(CH ₂) ₂	1.59±0.01 27	0.34±0.05 ANT	1.56±0.57 34
3d	H		5.44±0.75 ANT	1.86±0.16 ANT	0.59±0.04 ANT
3e	Me		1000±52 ANT	410±104 ANT	96.3±18 ANT

Values are means from 5 or 6 experiments. NT: not tested.

Efficacy is measured against the standards DPDPE (δ), U69593 (κ) and DAMGO (μ), antagonist potency recorded as K_e values versus the same standards.