Published in final edited form as:
J Med Chem. 2006 August 24; 49(17): 5333–5338.

Structural Determinants of Opioid Activity in Derivatives of 14-Aminomorphinones: Effect of Substitution in the Aromatic Ring of Cinnamoylaminomorphinones and codeinones

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Abstract

In recent years there has been substantial interest in the 14-aminodihydromorphinone derivatives methoclocinnamox (MC-CAM) and clocinnamox (C-CAM). In order to investigate the importance of the cinnamoyl ring substituent a series of analogues have been prepared with chloro, methyl and nitro-substituents in the 2'- and 4'-positions. Despite some discrepancies between the in vitro and in vivo data, a clear SAR could be observed where the 2'-chloro and 2'-methyl ligands consistently displayed higher efficacy than their 4'-substituted analogues. The new series also followed the well established SAR that 17-methyl ligands have greater efficacy at the mu opioid receptor than their 17-cyclopropylmethyl counterparts.

Introduction

The 14-substituted 7,8-dihydromorphinone and derived series of opioid ligands have provided a greater range of therapies, therapeutic opportunities and pharmacological tools than any other opioid chemical series. The oxymorphone derivatives (**1**) include the parent (**1a**) and its codeinone equivalent, oxycodone, which are important opiate analgesics and the prototype opioid antagonists naloxone (**1b**) and naltrexone (**1c**). The latter, though predominantly a mu opioid receptor (MOR) ligand, was the starting material for the first selective antagonist for delta opioid receptors (DOR), naltrindole (NTI, **2a**)[1] and the prototype selective antagonists for the kappa opioid receptor (KOR), norbinaltorphimine (norBNI, **3**)[2] and GNTI (**2b**).[3] The 14-O-phenylpropyl ethers (**1d**, **1e**) of naloxone (**1b**) and naltrexone (**1c**) have recently been shown to be high potency and high efficacy MOR agonists in vivo[4] whereas other 14-O ethers and esters of naloxone (**1b**) and naltrexone (**1c**) had previously been found to be antagonists themselves.[5]

Our own interest in this field has been primarily in the 14-amino analogues (**4**) of the oxymorphones, particularly the 14-cinnamoylaminodihydromorphinones C-CAM (**6b**)[6] and M-CAM (**6c**)[7] and the equivalent codeinones MC-CAM (**5b**)[8] and MM-CAM (**5c**).[9] **6b** and **6c** have been shown to be pseudo-irreversible MOR selective antagonists functionally equivalent to and superior to β-FNA respectively.[7] **5b** and **5c** are MOR-partial agonists with agonist and antagonist activity of long duration so that they could be regarded as similar to buprenorphine,[8] the MOR partial agonist used as a treatment for opioid dependence and as an

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analgesic. We here report extended structure-activity data in this series, particularly concerning the effect of substitution and orientation in the cinnamoyl aromatic ring. Some major effects have been discovered, particularly differences resulting from substitution in the ortho and para positions.

Synthesis

The starting material for the synthesis of codeinones (**5**) and morphinones (**6**) was 14-amino-17 (N)-cyclopropylmethyl-7,8-dihydronorcodeinone (**4a**), while for codeinones (**7**) and morphinones (**8**) it was 14-amino-7,8-dihydrocodeinone (**4b**).[10,11] Acylation of the 14β-amino group was achieved readily using freshly prepared acid chlorides. 3-O-demethylation of dihydrocodeinones (**5**) to dihydromorphinones (**6**) was carried out with boron tribromide.[12,13]

Results

Due to the poor ability of in vitro assays to predict the in vivo activity of ligands from these series[14,15] only a selection of the new ligands was evaluated in opioid receptor binding assays and in the [^{35}S]GTPγS stimulation functional assay,[16] in both cases using recombinant human MOR, DOR and KOR transfected into CHO cells.[17] All of the new ligands tested had subnanomolar MOR affinity in the binding assay and also high affinity for DOR and KOR so that no significant selectivity for any receptor type was apparent (Table 1).

In the [^{35}S]GTPγS assays the 17-methyl dihydrocodeinones and dihydromorphinones (**7**, **8**) showed agonist or partial agonist activity for MOR, DOR and KOR (Table 2). MOR potency was higher than DOR or KOR potency, but only in the case of the dihydrocodeinone **7c** was there substantial selectivity for MOR. Dihydromorphinones **8e** and **8f** had exceptionally high MOR efficacy and potency; they also had high DOR and KOR agonist potency. The equivalent dihydrocodeinones (**7e**, **7f**) had efficacy for MOR, DOR and KOR similar to that of the morphinones but potency was an order of magnitude reduced, though still high. The only 17-methyl-14-cinnamoylamino derivatives tested which lacked MOR agonist efficacy were **8c**[18] and **8d**,[10] but **4c**[18] with the dihydrocinnamoylamino side chain also lacked MOR efficacy. Both **8c** and **4c** were MOR antagonists of subnanomolar potency and moderately potent KOR antagonists, but whereas **8c** was also a DOR antagonist, **4c** was a DOR partial agonist (Table 3). **8d** was almost identical in profile to **8c** in this assay. The 17-cyclopropylmethyl ligands with 2'-chloro- or 2'-methyl substitution were MOR antagonists (dihydromorphinones **6e**, **6f**; Table 3) or low efficacy MOR partial agonists (dihydrocodeinones **5e**, **5f**; Table 2). Dihydromorphinones (**6e**, **6f**) were also DOR and KOR antagonists whereas dihydrocodeinones (**5e**, **5f**) were partial DOR and KOR agonists.

The new ligands were investigated in several in vivo antinociceptive assays. Two thermal assays, in which heat is applied to the tails of groups of mice, were used. In one, the tail flick assay an infrared beam is used[19] whilst in the other, the tail withdrawal assay, the source is warm water that is maintained at either 50°C or 55°C.[7] In both assays the time to removal of the tail from the heat is a measure of antinociceptive effect. Activity in these assays is indicative of relatively high efficacy opioid agonist activity, most likely mediated by MOR agonist effects.[20]

In tail flick a number of the new ligands were very potent antinociceptive agents. In this category were found the 4'-chloro and 4'-methyl dihydrocodeinones (**7b**, **7c**), the 2'-chloro and 2'-methyl dihydromorphinones (**8e**, **8f**) and the 17-cyclopropylmethyl-2'-methyldihydrocodeinone (**5f**) (Table 4). Other than **5f** the 17-cyclopropylmethyl ligands tested were inactive as agonists in the tail flick but in this assay they showed potent ability to antagonise the antinociceptive actions of morphine. None of the new MOR antagonists were

as potent as C-CAM (**6b**) and M-CAM (**6c**), but the 17-methyl-4'-methyldihydromorphinone (**8c**) had no agonist activity in tail flick and as a MOR antagonist in this assay was only 3-fold less potent than its N-cyclopropylmethyl congener **6c** (Table 4). However the equivalent dihydrocinnamoyl derivative (**4c**) in tail flick was a potent agonist without MOR antagonist activity. The 17-methyldihydromorphinone (**8b**)[18] was inactive as an agonist and was a moderately potent MOR antagonist whereas the equivalent morphinone (**9**)[21] was a potent antinociceptive agent in tail flick with no MOR antagonist activity. The ligands with MOR antagonist activity but no antinociceptive activity in tail flick, including the unsubstituted cinnamoylaminodihydromorphinone (**6a**),[22] with only one exception, **6c** had some antinociceptive activity in the phenylquinone-induced writhing assay in which the chemical nociceptor provides less intensive nociceptive stimulus than the thermal stimulus in tail flick (Table 4). Thus only **6c** in this series is a MOR antagonist without opioid agonist activity, comparable to naloxone and naltrexone in this respect.[23]

Investigation of the in vivo activity of an extended series of 17-cyclopropylmethyl dihydrocodeinones (**5**) and dihydromorphinones (**6**) was undertaken in the tail withdrawal assay (Table 5). The potent agonist activity of the 2'-methyldihydrocodeinone (**5f**) in tail flick was confirmed and similar activity for the 2'-chloro (**5e**) and 4'-nitro (**5d**) dihydrocodeinones was found. Substantial but lesser agonist activity in tail withdrawal was also discovered for the unsubstituted cinnamoylaminodihydrocodeinone (**5a**), the 2'-nitrodihydrocodeinone (**5g**) and, as the only 17-cyclopropylmethyl-dihydromorphinone with substantial antinociceptive effect in tail withdrawal, the 4'-nitro derivative (**6d**). The ability of these N-cyclopropylmethyl ligands to demonstrate long-lasting (≥24h) pseudo-irreversible morphine antagonist activity was investigated in tail withdrawal. Such activity for **6b** and **6c** had previously been reported.[6,7] Equivalent activity was found for the 4'-nitro analogue (**6d**) and similar though somewhat less pronounced activity was also shown by the unsubstituted dihydrocodeinone and dihydromorphinone (**5a**, **6a**), 2'-chloro and 2'-methyldihydromorphinones (**6e**, **6f**), 4'-chloro and 4'-methyldihydrocodeinones (**5b**, **5c**) and the 4'-nitrodihydrocodeinone (**5d**). The 2'-chloro, 2'-methyl and 2'-nitrodihydrocodeinones (**5e**, **5f**, **5g**) which had substantial agonist activity in tail withdrawal showed no delayed morphine antagonism in this assay.

Structure-Activity Relationships (SAR) and Discussion

The main purpose of the present study was to establish SAR for the orientation of substitution in the cinnamoyl aromatic ring in analogues of **6b** and **6c**. The in vitro functional and in vivo data show very clear distinction between the effects of substitution in the 4'- and 2'-positions, but there were discrepancies for some of the new ligands between their in vitro and in vivo profiles. The 4'-chlorodihydrocodeinone (**7b**) was a low efficacy partial MOR agonist in the [^{35}S]GTPγS assay (Table 2) but in TF it was a very potent full agonist without morphine antagonist effect (Table 4). In the antinociceptive assay it showed the Straub tail effect characteristic of a high efficacy MOR agonist and in withdrawn morphine-dependent rhesus monkeys it fully suppressed withdrawal with potency 50 times greater than morphine.[24] Similarly the 17-cyclopropylmethyl-2'-methyldihydrocodeinone (**5f**) had very low efficacy MOR partial agonist activity in vitro (Table 2) yet was a potent full agonist in tail flick (Table 4) with Straub tail effect in mice and complete suppression of abstinence in withdrawn morphine-dependent rhesus monkeys.[24] Further light was thrown over these discrepancies by the report that **7b**, when administered i.c.v. in tail withdrawal, had no antinociceptive activity and had MOR antagonist activity which was delayed in onset, peaked at 24h and lasted beyond 48h.[25] The delay in onset of antagonism was dependent on the dose of **7b** with higher doses substantially reducing the delay time. The cinnamoylaminodihydrocodeinones behave as irreversible MOR antagonists but it is thought that they exert their receptor blockade effects i.c.v. only after binding to a significant portion of the receptor reserve; the delay could result from limitation of the number of receptors available for binding by the rate of receptor turnover.

[25] The predominant MOR agonism observed after peripheral administration would result from there being insufficient brain concentrations to effect any significant receptor blockade. In the in vitro situation of the [^{35}S]GTPγS assay for MOR efficacy, the test ligand is present in sufficient concentration to bind 'irreversibly' at a substantial rate so that its efficacy appears to be quite low.

Despite discrepancies between in vitro and in vivo activity in certain cases as described above, in both the [^{35}S]GTPγS and the antinociceptive assays the 2'-chloro and 2'-methyl-substituted dihydrocodeinones (**5e**, **5f**, **7e**, **7f**) and dihydromorphinones (**8e**, **8f**) consistently showed very much higher efficacy for MOR, DOR and KOR in the in vitro assay and for MOR in the antinociceptive assays than the 4'-substituted equivalents (**5b**,[8] **5c**,[9] **7b**, **7c**, **8b**,[18] **8c**[18]). Only in the 17-cyclopropylmethyl dihydromorphinones (**6e**, **6f**, **6b**, **6c**) including the unsubstituted parent (**6a**) was there less differentiation between the effects of 2'- and 4'-substitution. Nevertheless the evidence from the 17-cyclopropylmethyl series in vivo (Tables 4, 5) is that the 2'-substituted dihydrocodeinones (**5e**, **5f**) have higher antinociceptive efficacy than the unsubstituted cinnamoylaminodihydrocodeinone (**5a**) which in turn has higher efficacy than the 4'-substituted derivatives (**5b**, **5c**).

The more limited in vivo data on the 17-cyclopropylmethyl 2'-nitro and 4'-nitro derivatives (**5d**, **5g**, **6d**) suggest that the effect of orientation with nitro substitution is different from chloro- and methyl-substitution. Thus the 4'-nitrodihydrocodeinone (**5d**) had (MOR) antinociceptive activity in tail withdrawal substantially greater than that of the 2'-isomer (**5g**). It also had significant delayed antagonist activity comparable to that of the 4'-methyl analogue (**5c**) (Table 5). This profile is similar to that reported for **5d** by McLaughlin et al[25] using i.c.v. administration. In the present study the 4'-nitrodihydromorphinone (**6d**), though with no 2'-isomer for comparison, had higher efficacy in tail withdrawal than any other 17-cyclopropylmethyl-dihydromorphinone tested including the unsubstituted parent (**6a**) and the 2'-chloro and 2'-methyl derivatives (**6e**, **6f**). In addition to its impressive antinociceptive activity in tail withdrawal, **6d** was also an effective delayed MOR antagonist.

Since the SAR for methyl and chloro substitution in the cinnamoyl group is basically similar, it is unlikely that electronic factors are responsible for the difference between these substituents and nitro substitution. Though the nitro group is somewhat larger than chloro and methyl and this may have some effect it seems more likely that the main factor is lipophilicity since both the chloro and methyl groups have positive π values indicating substantial lipophilicity, whereas the nitro group has a negative π value to indicate more hydrophilicity in its character.[26]

Comparison of the 17-cyclopropylmethyl series (**5**, **6**) with the 17-methyl series (**7**, **8**) might be expected to comply with well established SAR in morphinan and epoxymorphinan series of opioids; when 17-methyl is replaced by 17-cyclopropylmethyl, MOR efficacy is very much reduced but KOR efficacy is relatively little affected so that in vivo KOR agonist effects usually predominate.[27,28] In the in vitro assays, the current series generally show greater loss of MOR efficacy than KOR efficacy when 17-methyl is replaced by 17-cyclopropylmethyl. In the antinociceptive assays in which relative MOR and KOR efficacy was not determined, there was no overt evidence of predominant KOR-agonist effects for any 17-cyclopropylmethyl ligand tested. However, in the thermal (tail flick, tail withdrawal) assays clear evidence of lower MOR efficacy for the 17-cyclopropylmethyl ligands when compared to their 17-methyl equivalents was observed. Thus in tail flick the only 17-cyclopropylmethyl ligand tested which showed antinociceptive activity was the 2'-methylcodeinone (**5f**). The equivalent activity of **5f** in tail withdrawal was fully reversed by naltrexone (data not shown) and in the acetic acid induced writhing assay, the antinociceptive effect of **5f** was fully reversed by the MOR-selective antagonist M-CAM (**6c**) but not by the KOR-selective antagonist norBNI nor by the

DOR selective antagonist naltrindole (Broadbear, personal communication). The overall conclusion can be drawn that the 14-cinnamoylamino group in this series predominantly affects MOR activity and that its' lipophilic nature, particularly with 4'-chloro and 4'-methyl substitution is associated with low MOR efficacy and irreversible binding.

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. Infrared spectroscopy was performed on either a Perkin-Elmer 782 Instrument. 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. Compound recrystalisation was from ethanol.

General Procedure A

A suspension of the appropriate carboxylic acid (1.1 molar equiv.) in anhydrous toluene was refluxed with oxalyl chloride (8.8 molar equiv.) for 1h. Solvent was removed in vacuo and the residue taken up in CH_2Cl_2 before adding to a solution of codeinone (**4a** or **4b**: 1 molar equiv.), NEt_3 (1.1 molar equiv.) in anhydrous CH_2Cl_2 which was stirred under N_2 for 18h. Removal of the solvent in vacuo, silica gel column chromatography and recrystallization from methanol yielded the codeinones.

General Procedure B

To a solution of codeinone (1 molar equiv.) in anhydrous CH_2Cl_2 at −30°C under N_2 was slowly added BBr_3 (6 molar equiv.: 1M in CH_2Cl_2) and the solution warmed to RT over 1h. A 1:1 mixture of ice:conc. ammonia was added and the organic layer isolated. The aqueous layer was extracted with $CHCl_3$:MeOH (3:1, 3x), the organic layer washed with brine and dried (Na_2SO_4). Removal of the solvent in vacuo and silica gel chromatography yielded the morphinones.

N-Cyclopropylmethyl-14β-cinnamoylamino-7,8-dihydronorcodeinone (5a)

10 (0.24g: 0.67 mmol) was treated as in general procedure A to give **5a** as a clear oil (0.29g: 88%); Anal. ($C_{30}H_{32}N_2O_4$.oxalate.$2H_2O$) CHN.

N-Cyclopropylmethyl-14β-(4'-nitrocinnamoylamino)-7,8-dihydronorcodeinone (5d)

10 (0.85g: 2.4 mmol) was treated as in general procedure A to give **5d** as a white solid (0.69g: 54%); Anal. ($C_{30}H_{31}N_3O_6$.oxalate.$2H_2O$) CHN.

N-Cyclopropylmethyl-14β-(2'-chlorocinnamoylamino)-7,8-dihydronorcodeinone (5e)

10 (0.35g: 1.0 mmol) was treated as in general procedure A to give **5e** as a white solid (0.35g: 69%); Anal. ($C_{30}H_{31}N_2O_4Cl$.oxalate.$1.5H_2O$) CHN.

N-Cyclopropylmethyl-14β-(2'-methylcinnamoylamino)-7,8-dihydronorcodeinone (5f)

10 (0.33g: 0.90 mmol) was treated as in general procedure A to give **5f** as a white solid (0.23g: 50%); Anal. ($C_{31}H_{34}N_2O_4$.oxalate.H_2O) CHN.

N-Cyclopropylmethyl-14β-(2'-nitrocinnamoylamino)-7,8-dihydronorcodeinone (5g)

10 (0.92g: 2.9 mmol) was treated as in general procedure A to give **5g** as a white solid (0.39g: 28%); Anal. ($C_{30}H_{31}N_3O_6$.oxalate.1.5H_2O) CHN.

N-Cyclopropylmethyl-14β-(4'-nitrocinnamoylamino)-7,8-dihydronormorphinone (6d)

5d (0.69g: 1.30 mmol) was treated as in general procedure B to give **6d** as a pale yellow solid (0.38g: 57%); Anal. ($C_{29}H_{29}N_3O_6$.oxalate.H_2O) CHN.

N-Cyclopropylmethyl-14β-(2'-chlorocinnamoylamino)-7,8-dihydronormorphinone (6e)

5e (0.18g: 0.34 mmol) was treated as in general procedure B to give **6e** as a white solid (0.089g: 52%); Anal. ($C_{29}H_{29}N_2O_4Cl$.oxalate.EtOH) CHN.

N-Cyclopropylmethyl-14β-(2'-methylcinnamoylamino)-7,8-dihydronormorphinone (6f)

5f (0.40g: 0.8 mmol) was treated as in general procedure B to give **6f** as a white powder (0.12g: 30%); Anal. ($C_{30}H_{32}N_2O_4$.oxalate) CHN.

14β-(4'-Chlorocinnamoylamino)-7,8-dihydrocodeinone (7b)

11 (0.70g: 2.2 mmol) was treated as in general procedure A to give **7b** as a white solid (0.42g: 39%); Anal. ($C_{27}H_{27}N_2O_4Cl$ .oxalate.0.5H_2O) CHN.

14β-(4'-Methylcinnamoylamino)-7,8-dihydrocodeinone (7c)

11 (0.70g: 2.2 mmol) was treated as in general procedure A to give **7c** as a white solid (0.30g, 30%); Anal. ($C_{28}H_{30}N_2O_4$.oxalate.0.25H_2O) CHN.

14β-(2'-Chlorocinnamoylamino)-7,8-dihydrocodeinone (7e)

11 (1.0g: 3.0 mmol) was treated as in general procedure A to give **7e** as a white solid (1.04g, 69%); Anal. ($C_{27}H_{27}N_2O_4$.oxalate) CHN.

14β-(2'-Methylcinnamoylamino)-7,8-dihydrocodeinone (7f)

11 (0.60g: 1.9 mmol) was treated as in general procedure A to give **7f** as a white solid (0.62g, 71%); Anal. ($C_{28}H_{30}N_2O_4$.oxalate) CHN.

14β-(2'-Chlorocinnamoylamino)-7,8-dihydromorphinone (8e)

7e (0.80g: 1.7 mmol) was treated as in general procedure B to give **8e** as a white solid (0.50g: 64%); Anal. ($C_{26}H_{25}N_2O_4Cl$.oxalate) CHN.

14β-(2'-Methylcinnamoylamino)-7,8-dihydromorphinone (8f)

7f (0.62g: 1.4 mmol) was treated as in general procedure B to give **8f** as a white solid (0.34g: 57%); Anal. ($C_{27}H_{28}N_2O_4$.oxalate) CHN.

Supporting Information

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was funded through NIDA grants DA00254 and DA07315 and the in vitro characterisation of compounds carried out through the NIDA Abuse Treatment Discovery Program (ATDP). TF, PPQ and TF v M in vivo assays were performed by the Drug Evaluation Committee (DEC) of the College on Problems of Drug Dependence (CPDD).

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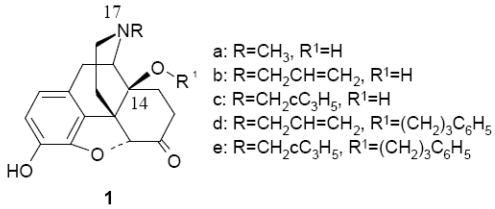
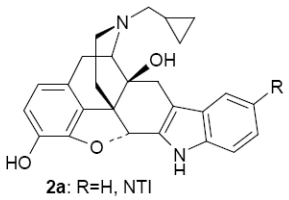
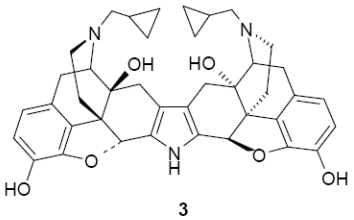


a: R=CH_3, R^1=H
b: R=$CH_2CH=CH_2$, R^1=H
c: R=$CH_2cC_3H_5$, R^1=H
d: R=$CH_2CH=CH_2$, $R^1=(CH_2)_3C_6H_5$
e: R=$CH_2cC_3H_5$, $R^1=(CH_2)_3C_6H_5$

1



2a: R=H, NTI
2b: R=$NHC(=NH)NH_2$, GNTI



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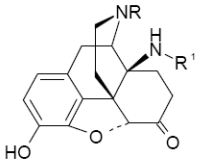
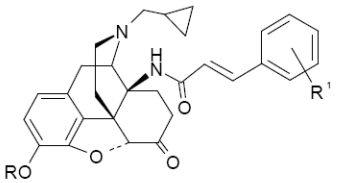


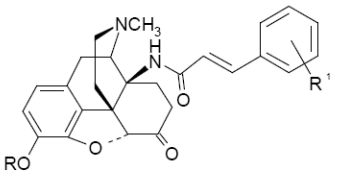
a: R=$CH_2cC_3H_5$, R^1=H
b: R=CH_3, R^1=H
c: R=CH_3, $R^1=CO(CH_2)_2C_6H_5$-4'-CH_3

4

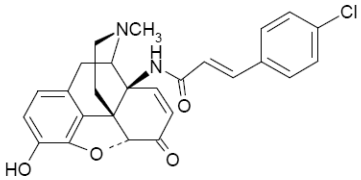


5: R=CH_3
6: R=H

a: R^1=H
b: R^1=4'-Cl
c: R^1=4'-CH_3
d: R^1=4'-NO_2
e: R^1=2'-Cl
f: R^1=2'-CH_3
g: R^1=2'-NO_2



7: R=CH_3
8: R=H



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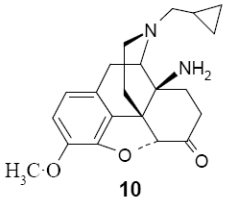

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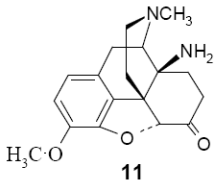

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1. .

Table 1

Binding affinities (Ki (nM)±SEM) to opioid receptors[a]

	[^{3}H]DAMGO MOR	[^{3}H]Cl-DPDPE DOR	[^{3}H]U69,593 KOR
4c	0.35±0.04	1.25±0.04	0.57±0.08
5e[b]	0.60±0.05	7.2±2.7	2.4±0.55
5f[b]	0.20±0.01	2.3±1.0	2.1±0.2
6e	0.71±0.02	1.3±0.19	2.4±0.53
6f	0.72±0.02	1.25±0.21	1.50±0.34
7b	0.60±0.01	1.0±0.04	6.4±2.0
7c	0.49±0.09	2.9±0.45	9.9±1.8
7e	0.68±0.11	0.58±0.08	2.5±0.32
7f	0.40±0.01	0.42±0.05	2.3±0.41
8c	0.34±0.03	4.0±1.1	1.5±0.05
8d	0.20±0.03	0.54±0.10	5.5±0.96
8e	0.15±0.005	0.08±0.02	0.54±0.20
8f	0.21±0.05	0.11±0.07	0.23±0.03
MC-CAM, 5b	4.78±0.58	4.8±0.73	16.4±2.5
C-CAM, 6b	2.98±0.22	2.7±0.23	1.4±0.52
Morphine	1.1±0.05	140±1.5	46.9±14.5
Naltrexone	0.20±0.0	10.8±3.0	0.40±0.1

[a] Binding to cloned human opioid receptors transfected into Chinese hamster ovary (CHO) cells.

[b] Binding to guinea pig brain membranes. Data are the average from two experiments, each carried out in triplicate.

All data provided through NIDA Abuse Treatment Discovery Program (ATDP).

Table 2

Stimulation of [^{35}S]GTPγS binding, in recombinant human opioid receptors, by test ligands.

	MOR		DOR		KOR	
Cpd	**EC_{50} (nM)**	**% stim[a]**	**EC_{50} (nM)**	**% stim[b]**	**EC_{50} (nM)**	**% stim[c]**
5e	3.80±0.31	24	17.5±5.9	36	9.0±1.48	55
5f	2.60±0.09	17	20.9±7.8	39	1.8±0.78	47
7b[e]	0.90±0.45	28	5.0±1.2	50	35±11	26
7c	2.5±1.5	68	76±21	86	84±4.7	51
7e[e]	0.50±0.20	108	2.2±0.95	110	8.5±1.6	78
7f	0.50±0.30	96.5	4.6±0.95	108	5.6±1.1	83
8e[e]	0.04±0.005	126	0.10±0.005	115	0.1±0.03	59
8f	0.04±0.005	111	0.40±0.22	71	0.2±0.03	81
morphine	15.6±0.5	93	316±4.9	103	484±213	62

Data provided through NIDA (ATDP).

[a] compared to DAMGO;

[b] compared to DPDPE;

[c] compared to U69593.

Values are means from 5 or 6 experiments

Table 3

Reversal of the stimulation of [^{35}S]GTPγS binding, in recombinant human opioid receptors transfected into CHO cells, by test ligands.

Cpd	MOR Ke (nM)[a]	DOR Ke (nM)[b]	KOR Ke (nM)[c]
4c	0.32±0.02	d	4.9±0.29
5b[e]	0.97±0.15	7.2±0.57	9.8±0.88
6b[e]	0.53±0.13	0.19±0.02	0.10±0.006
6e	0.45±0.01	0.67±0.07	0.38±0.01
6f	0.29±0.01	0.40±0.05	0.16±0.01
8c	0.30±0.01	2.2±0.14	4.10±0.75
8d	0.13±0.04	1.4±0.37	3.3±0.73
naltrexone	0.59±0.04	5.4±0.75	1.9±0.16

Data provided through NIDA (ATDP).

[a] vs DAMGO;

[b] vs DPDPE;

[c] vs U69593;

[d] partial agonist, EC_{50} 4.97nM, 45% stimulation compared to DPDPE;

[e] data from ref [29].

Values are means from 5 or 6 experiments

Table 4

Agonist and antagonist activity of the ligands in mouse antinociception tests.

Cpd	R	R^1	R^2	TF[a]	PPQ[b]	TF v M[c]
4c	H	4'-CH_3*	CH_3	0.5	0.09	>30
5b	CH_3	4'-Cl	$CH_2cC_3H_5$	>30	0.2	>6.0
5c	CH_3	4'- CH_3	$CH_2cC_3H_5$	>30	0.1	5.7
5f	CH_3	2'- CH_3	$CH_2cC_3H_5$	0.03	0.03	>30
6a	H	H	$CH_2cC_3H_5$	>30	66% @ 0.3	1.3
6b	H	4'-Cl	$CH_2cC_3H_5$	>30	69% @ 30	0.12
6c	H	4'-CH_3	$CH_2cC_3H_5$	>30	>30	0.2
6f	H	2'-CH_3	$CH_2cC_3H_5$	>30	3.17	50% @ 3
7b	CH_3	4'-Cl	CH_3	0.04	0.02	>30
7c	CH_3	4'-CH_3	CH_3	0.06	0.40	>30
8b	H	4'-Cl	CH_3	>30	1.2	3.8
8c	H	4'-CH_3	CH_3	>30	57% @ 1	0.6
8e	H	2'-Cl	CH_3	0.12	0.04	>30
8f	H	2'-CH_3	CH_3	0.08	0.03	>30
9	H	4'-Cl	CH_3	0.5	-	>30
morphine[d]	-	-	-	1.92	0.4	Inactive
naloxone[d]	-	-	-	>10	No activity	0.04
naltrexone[d]	-	-	-	>10	No activity	0.007

Data provided through DEC.

*dihydrocinnamoyl

Agonist activity determinations in [a]tail-flick (TF) and [b]phenylquinone abdominal stretching (PPQ) assays. Values are ED_{50}, mg/kg s.c. or % change.

[c]Antagonist activity determinations in tail-flick versus morphine (TF vs M). Values are AD_{50}, mg/kg s.c. or percentage change.

[d]Data from ref 23.

Table 5

Agonist and antagonist activity of the ligands in the mouse hot water tail-withdrawal (TW) assay.

Cpd	R	R^1	TW agonist activity[a]	TW morphine antagonism[a]
5a	CH_3	H	++	+++
5b	CH_3	4'-Cl	0	+++
5c	CH_3	4'- CH_3	+	++
5d	CH_3	4'-NO_2	++++[b]	++
5e	CH_3	2'-Cl	++++[b]	+
5f	CH_3	2'- CH_3	++++[c]	+
5g	CH_3	2'-NO_2	++	-
6a	H	H	0	++
6b	H	4'-Cl	0	++++
6c	H	4'- CH_3	0	++++
6d	H	4'-NO_2	++	++++
6e	H	2'-Cl	0	+++
6f	H	2'- CH_3	0	+++

[a] Effect as % of maximum possible effect at a dose of 32mg/kg s.c.. 0-10%, 0; 10-25%, +; 40-55%, ++; 65-85%, +++; >90%, ++++

[b] @ 10mg/kg;

[c] @ 1mg/kg