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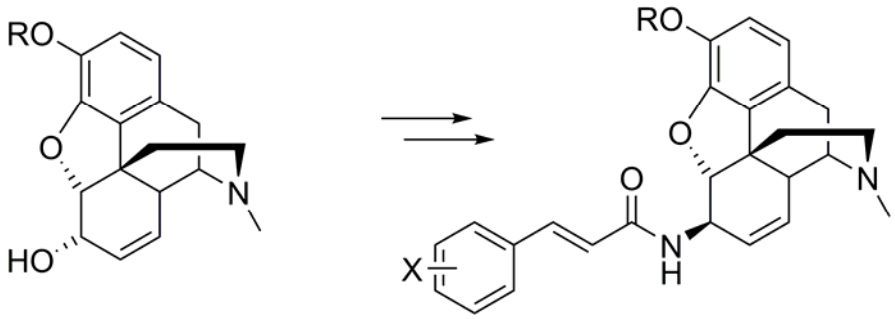

Novel 6β-acylaminomorphinans with analgesic activity

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Abstract:

Aminomorphinans are a relatively young class of opioid drugs among which substances of high in vitro efficacy and favorable in vivo action are found. We report the synthesis and pharmacological evaluation of novel 6β-acylaminomorphinans. 6β-Morphinamine and 6β-codeinamine were stereoselectively synthesized by Mitsunobu reaction. The aminomorphinans were subsequently acylated with diversely substituted cinnamic acids. In vitro binding studies on cinnamoyl morphinamines showed moderate affinity for all opiate receptors with some selectivity for mu opioid receptors, while cinnamoyl codeinamines only showed affinity for mu opioid receptors. In vivo analgesia studies showed significant analgesic activity of 6β-cinnamoylmorphinamine mediated by mu and delta receptors. The lead compound was found to be roughly equipotent to morphine (ED_{50} 3.13 ± 1.09 mg/kg) but devoid of the dangerous side-effect respiratory depression, a major issue associated with traditional opioid therapy.

Keywords:
aminomorphinan
MOR/DOR agonist
opioid
analgesia
respiratory depression
cinnamoyl morphinamine

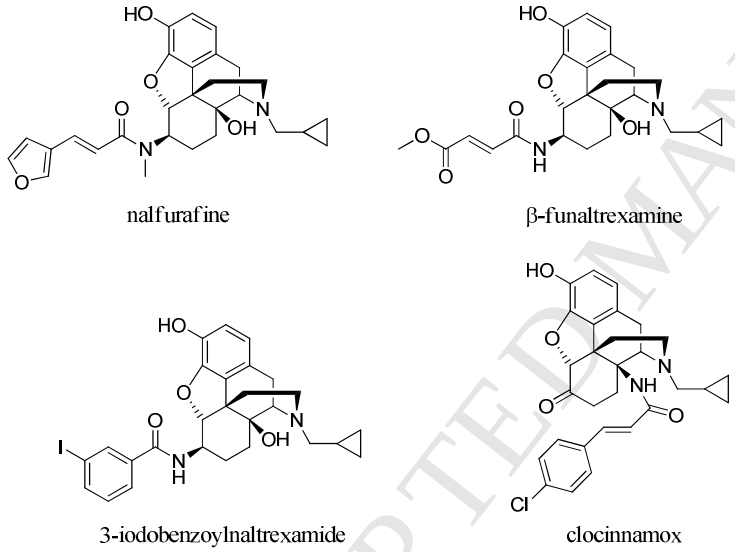
Morphine, the principal drug of the opioid family is among the most important agents used for the treatment of severe pain. [1] Clinically relevant effects, including various dangerous adverse effects are predominantly mediated by mu-opioid receptors (MOR-1). The structural features of the morphine skeleton have long been the basis of successful drug development and the development of novel morphine-like drugs with improved efficacy, receptor selectivity and side-effects. Aminomorphinans and their derivatives containing a nitrogen atom instead of oxygen in position C-6 of the morphinan ring system have been used in the development of a number of new opioids. Naloxamine and naltrexamine were the first two representatives of this family followed by an array of compounds generated by acylation and alkylation of the C-6 amino moiety, especially that of β-naltrexamine (β-NTA). [2] This group of compounds includes the novel kappa-opioid receptor (KOR-1) agonist nalfurafine (TRK-820) (Remitch® in Japan) used as a treatment for hemodialysis-related uremic pruritus [3,4] and β-funaltrexamine (β-FNA), a covalent MOR-1 antagonist affinity label with partial KOR-1 agonist activity has been widely used to investigate opioid receptor mechanisms. [5,6]

More recently iodobenzoylnaltrexamide (IBNtxA), a very potent analgesic with a novel pharmacological profile devoid of most side-effects characteristic of traditional opioid agonists due to its activity at a recently identified target (6TM/E11 sites) has been reported. [7,8] The naloxone analog, IBNalA, is more selective for these sites and has a similar side-effect profile. [9] _ENREF_7The equatorial β-acylamino moiety is a common structural feature of these molecules. The C-6 amino group has been acylated by a diverse range of substituted aliphatic and

aromatic carboxylic acids, including furanylacrylic acid (nalfurafine), 3-iodobenzoic acid (IBNtxA and IBNalA) and methylfumaric acid (β-FNA).

The cinnamoyl amides of β-NTA possess KOR-1 agonist and MOR-1 antagonist activity, similar to β-FNA. [10-12] C-14 cinnamoyl amides (e.g. clocinnamox) and esters of various morphinans have received significant attention for their varying MOR-1 activity (Figure 1). [13-15] 6-arylamido morphinans have been proposed as analogues of morphine-6-*O*-glucuronide. [16] Similarly, aryl naloxamides have been considered as alcohol cessation agents [17,18] and as potential MOR-1 antagonists. [19-22] *N*-Naphthoyl-naltrexamide (NNTA) reportedly targets MOR-KOR opioid dimers. [23]

Figure 1



To the best of our knowledge, C-6 acylamines of morphine and its congeners carrying Δ7-8 double bond and a methyl group on N-17 have not been reported. Herein, we present the synthesis of novel C-6 acylamines of morphine and codeine analogues acylated by a series of cinnamic acids. The synthesized compounds were evaluated for their binding affinities to mu (MOR-1), kappa$_1$ (KOR-1) and delta (DOR-1) opioid receptors and analgesia. Based on *in vivo* results, 6β-cinnamoylmorphinamine was subjected to more detailed studies.

The 6β-amino derivatives of morphine and codeine were synthesized using the Mitsunobu-reaction. [24] Morphine (**1**) was selectively acetylated in the C-3 position and subsequently treated with phthalimide and diisopropyl azodicarboxylate (DIAD) in the presence of Ph_3P. Codeine (**5**) was reacted directly under the same conditions. The 6β-phthalimidoyl intermediates

(**3,6**) were isolated as HCl salts and then treated with hydrazine hydrate in ethanol to yield the desired 6β-amino derivatives (**4, 7**). For amidation reactions, the appropriate carboxylic acids were treated with thionyl chloride and reacted with the 6β-amino compounds in dichloromethane in the presence of Et_3N. In case of 6β-morphinamine (**4)** formation of 3-*O*-esters could also be detected; the amidation reaction mixture was evaporated to dryness and dissolved in methanolic aqueous sodium carbonate solution and left to stand overnight to ensure cleavage of C-3 phenolic esters. The crude products were purified by column chromatography using chloroform-methanol 9:1 isocratic eluent and crystallized from hexane (Scheme 1, Figure 2).

Scheme 1

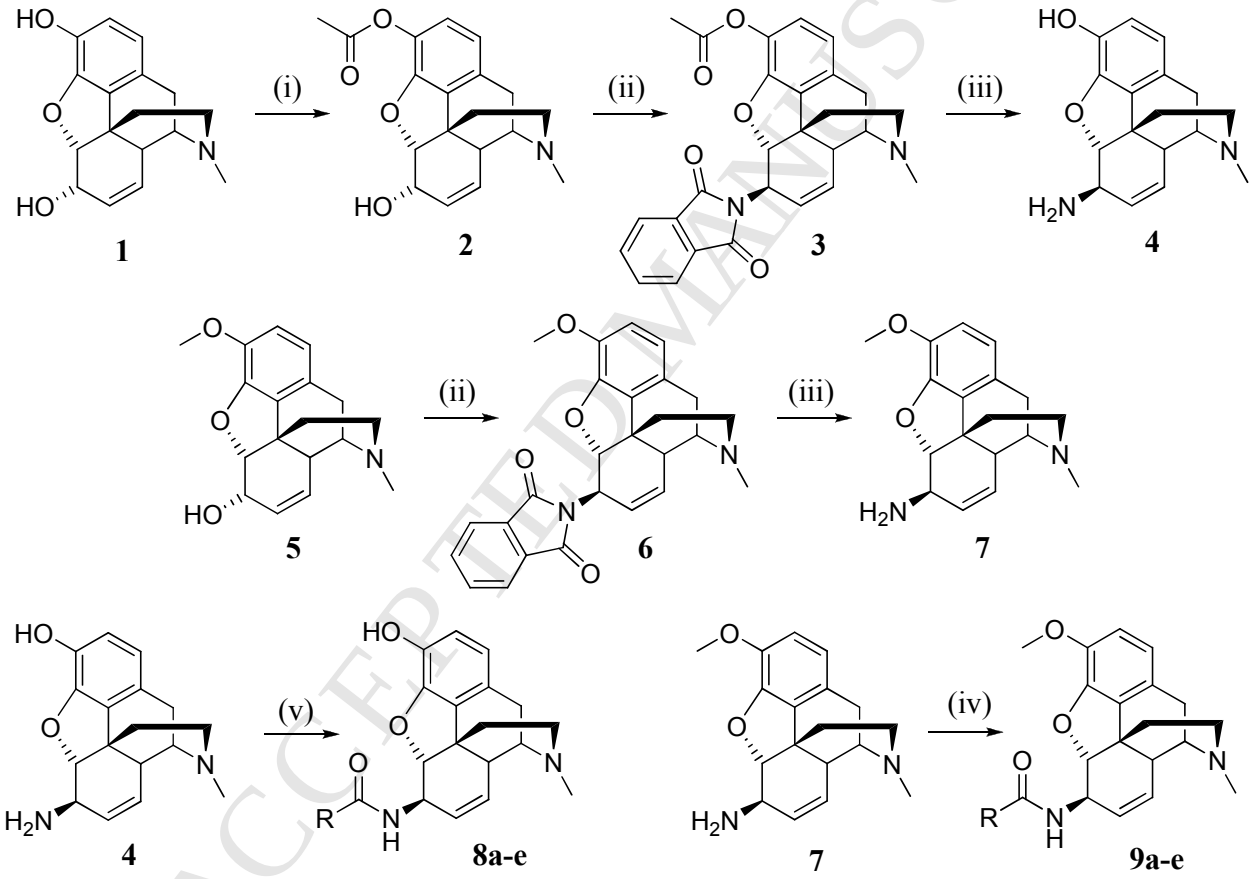
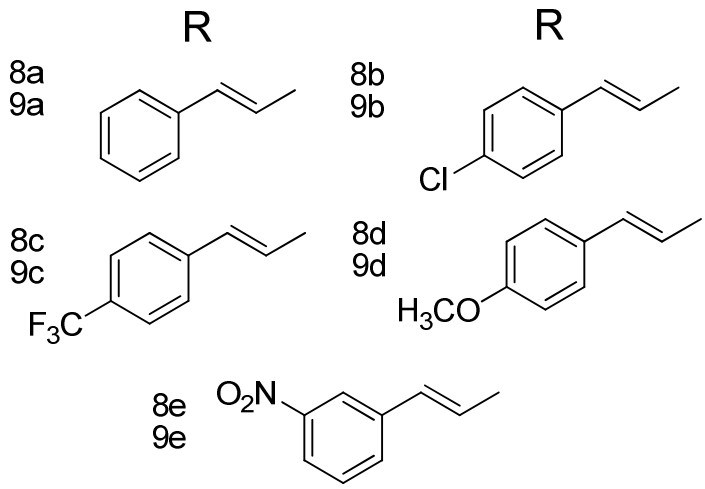


Figure 2



The compounds showed moderate to high affinity for MOR-1 with lower affinities for KOR-1 and DOR-1 (Table 1). Not surprisingly, affinities of codeinamine-derived amides (**9a-e**) were significantly lower than those of the amides of morphinamine (**8a-e**). Derivatives **8a-e** had very similar subnanomolar affinitites for MOR-1 irrespective of the aryl ring substituent of the acylamino moiety, while KOR-1 binding was approximately 10-fold lower, with highest KOR-1/MOR-1 K_i ratio for **8c** (55.4) and the lowest for **8e** (6.8). DOR-1 receptor affinity was roughly two magnitudes lower than MOR-1 affinity. DOR-1/MOR-1 K_i ratio for **8a** was 102.6. Cinnamoyl morphinamines in our series lacked the marked KOR-1 affinity seen with the previously reported acylated aminomorphinans. [10]

Next, the substances were administered to mice subcutaneously to determine their *in vivo* analgesic activity (Table 1, Figure 3 *A*). Somewhat surprisingly only the unsubstituted cinnamoyl morphamine analog **8a** (ED_{50} 3.13 ± 1.09 mg/kg) was analgesic with potency similar to that of morphine (4.96 ± 0.97 mg/kg *s.c.*). [25] Despite similar affinities against MOR-1, KOR-1 and DOR-1 binding, substitution on the aromatic ring of the cinnamoyl acid eliminated the analgesic activity of analogs (**8b-e)** at the highest dose tested, i.e. 10 mg/kg. Thus, the *in vivo* active compound **8a** was selected as lead compound and further characterized. The analgesic activity of **8a** was partially reversed by both the MOR-1 selective antagonist β-FNA and the DOR-1 selective antagonist NTI but, interestingly, was insensitive to the KOR-1 selective antagonist norBNI (Table 1, Figure 3 *B*).

Table 1

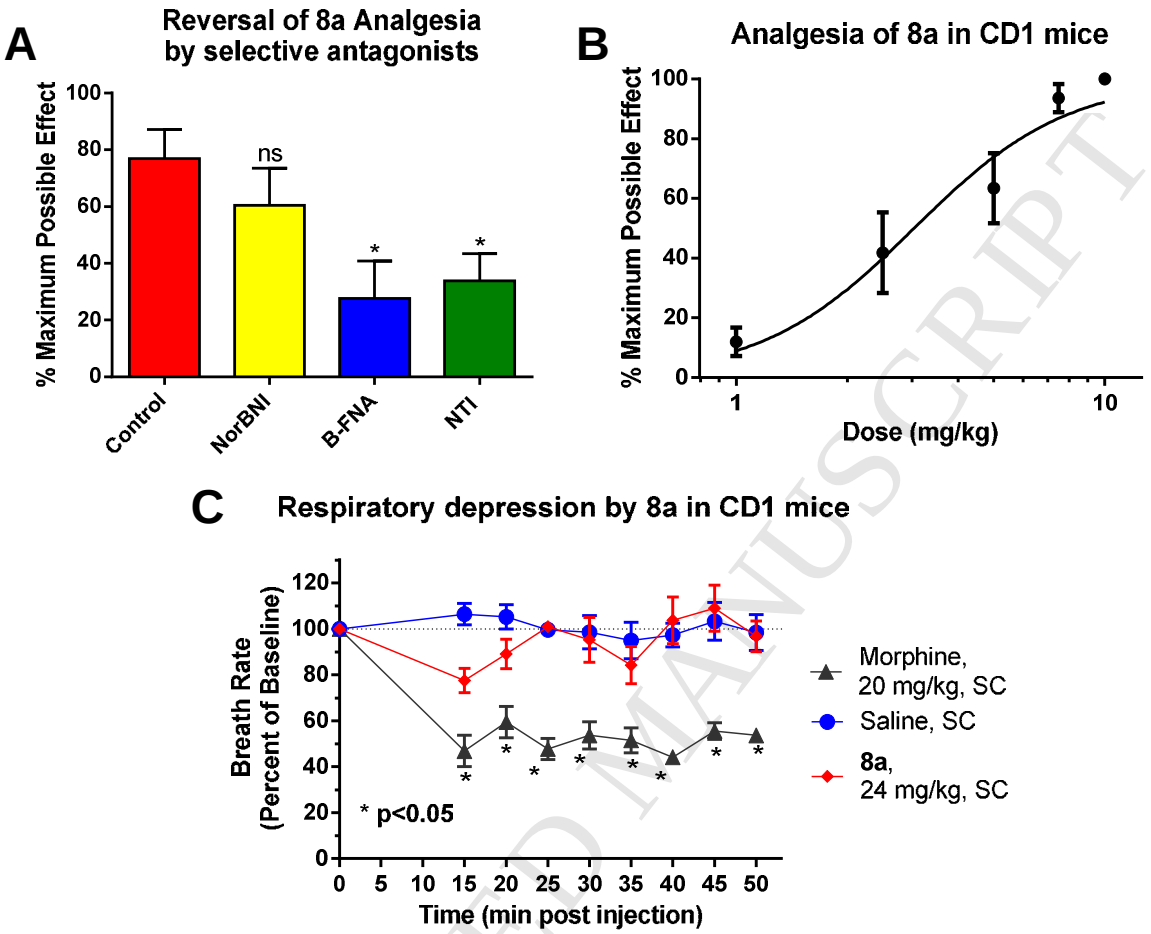
Table 1

Receptor binding and *in vivo* analgesia data of selected 6β-acylaminomorphinans[a]

Compound	Affinity (K_i, nM)			Analgesia (ED_{50}, mg/kg, *s.c.*)
	MOR-1	KOR-1	DOR-1	
8a	0.10 ± 0.02	2.90 ± 0.66	10.26 ± 6.76	3.13 ± 1.09
8b	0.15 ± 0.03	1.97 ± 0.01	9.38 ± 1.53	>10
8c	0.19 ± 0.09	10.52 ± 0.90	14.52 ± 6.82	>10
8d	0.74 ± 0.12	5.43 ± 1.38	15.26 ± 1.74	>10
8e	0.12 ± 0.006	0.81 ± 0.09	5.15 ± 0.75	>10
9a	4.73 ± 1.64	>100	>100	>10
9b	3.81 ± 0.15	>100	>100	>10
9c	5.44 ± 0.93	>100	>100	>10
9d	25.16 ± 13.02	>100	>100	>10
9e	3.74 ± 0.83	>100	>100	>10
morphine	4.60 ± 1.81[b]			4.96 ± 0.96[c]

[a] Competition studies were performed with the indicated compounds against ^{125}I-BNtxA (0.1 nM) in membranes from CHO cells stably expressing the indicated cloned mouse opioid receptors. K_i values were calculated from the IC_{50} values [26] and represent the means ± SEM of at least three independent replications. ^{125}IBNtxA K_D values for MOR-1, KOR-1, DOR-1 sites were 0.11, 0.03 and 0.24, respectively. [b] Values from the literature. [7] [c] Values from the literature. [25]

Figure 3



The [^{35}S]GTPγS-binding assay was used to characterize the functional activity of **8a** on *in vitro* opioid transfected cell lines (Table 2). **8a** produced near maximum stimulation in all cell lines when compared with the MOR-1 agonist DAMGO, DOR-1 agonist DPDPE and KOR-1 agonist U50,488H suggesting that it acts as a full agonist at all three opioid receptors. **8a** showed a 25-fold and 4-fold selective potency for MOR-1 and DOR-1 over the KOR-1 in this assay which partially explains why in spite of high, nanomolar affinity for KOR-1 it does not exert KOR-mediated agonism *in vivo*, as shown by the *in vivo* behavior assay in which **8a** analgesia could be antagonized by selective MOR-1 and DOR-1 antagonists but not by KOR-1 antagonist. Since *in*

vivo behavior is a better predictor of functional activity, it is safe to say that analgesia of **8a** is mediated by agonism at both MOR-1 and DOR-1.

Perhaps the most serious, potentially life-threatening side-effect of opiate therapy is respiratory depression. We tested the effect of **8a** on the respiratory rate of CD1 mice and found that at doses approximately four times the ED_{50} morphine (20 mg/kg) caused the expected marked respiratory depression, while after treatment with even a high, 8-times the ED_{50} dose of **8a** (24 mg/kg) no significant difference was found in respiratory rate compared with saline (Figure 3 *C*). Significantly reduced risk of respiratory depression has been reported in the literature for MOR-1/DOR-1 mixed opioid agonists, including DPI-3290 and the peptide MMP-2200. [27, 28]

Table 2

Table 2

Opioid receptor efficacy of **8a**[a]

Compound	EC_{50} (nM)			% stimulation		
	MOR-1	KOR-1	DOR-1	MOR-1	KOR-1	DOR-1
8a	1.38 ± 0.8	36.5 ± 19.2	9.1 ± 3.0	95.3 ± 0.8	106.3 ± 8.5	101.7 ± 2.8
DPDPE	nd[b]	nd[b]	2.05 ± 0.6			
DAMGO	5.6 ± 3	nd[b]	nd[b]			
U50,488H	nd[b]	21.7 ± 4.6	nd[b]			

[a] Efficacy data were obtained using agonist induced stimulation of [35S]GTPγS binding assay. Efficacy is represented as EC50 (nM) and percent maximal stimulation relative to standard agonist DAMGO (MOR-1), DPDPE (DOR-1), or U50,488H (KOR-1) at 100 nM. All values are expressed as the mean ± SEM of three separate assays performed in triplicate. [b] Not determined

In conclusion we have synthesized a series of novel 6β-cinnamoyl morphinamines carrying various cinnamoyl side chains. Characterization of compounds *in vitro* and *in vivo* revealed high affinity for MOR-1 receptors. Analgesic activity of 6β-cinnamoylmorphinamine was found to be comparable to morphine, but without causing respiratory depression, a major side-effect of commonly used opioids. Functional assays and reversal of analgesia by selective antagonists revealed an interesting receptor activity profile. The results suggest that new 6β-acylamino derivatives of morphine and its congeners are promising new opioids worthy of further investigation.

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Captions and titles

Figure 1. Structures of pharmacologically important aminomorphinans.

Figure 2. Acyl side chains of the synthesized 6β-cinnamoyl morphinamines and codeinamines.

Figure 3. Pharmacology of 6β-cinnamoylmorphinamine 8a. (A) Analgesia: Cumulative dose-response curves were carried out on groups of mice (n = 10) with **8a** at the indicated doses (s.c.) and analgesia tested 30 min later at peak effect. The ED_{50} value was 3.13 ± 1.09 mg/kg in CD1 mice by using the radiant heat tail-flick assay. Results were evaluated as %MPE [(observed latency − baseline latency)/(maximal latency − baseline latency)] and shown as the average of each group (n = 10). (B) Sensitivity of **8a** to opioid antagonists: Groups of mice (n = 6) received a fixed dose of **8a** (5 mg/kg, s.c.) alone or with NorBNI (10 mg/kg, s.c.), β-FNA (40 mg/kg, s.c.), NTI (20 mg/kg, s.c.). β-FNA and NorBNI were given 24h before **8a** while NTI was given 15 min before **8a**. Tail flick analgesia was measured 30 min after **8a**. Similar results were observed in two independent replications. **8a** analgesia is insensitive to NorBNI while analgesia is partially antagonized by both β-FNA and NTI (ANOVA followed by Bonferroni multiple comparison test ($p < 0.05$). (C) Respiratory rate. Animals were randomly assigned to receive saline (n = 3), **8a** (24 mg/kg, n = 3), or morphine (20 mg/kg, n = 3). Each animal's baseline average breath rate was measured every 5 min for 25 min before drug injection, and breath rates after drug injection are expressed as a percent of baseline. **8a** did not depress respiratory rate and was not significantly different from saline at any time point, whereas morphine decreased respiratory depression in comparison with both saline and **8a** ($p < 0.05$) as determined by repeated-measures ANOVA followed by Bonferroni multiple-comparison test.

Scheme 1. Synthesis of 6β-cinnamoyl morphinamines and codeinamines. (i) acetic anhydride, $NaHCO_3$, H_2O, r.t., 1 h. (ii) phthalimide, DIAD, Ph_3P, benzene, r.t., 2h. (iii) hydrazine hydrate, EtOH. (iv) 1.1 eq. acyl chloride, Et_3N, CH_2Cl_2, r.t., 2 h. (v) 1.1 eq. acyl chloride, Et_3N, CH_2Cl_2, r.t., 2 h. then Na_2CO_3, H_2O, MeOH, r.t., 12h.

- We designed and synthesized the 6β-acylamino derivatives of morphine and codeine
- The synthesis of 10 novel potentially analgesic compounds is reported
- The compounds were tested for opioid receptor binding and *in vivo* analgesia
- 6β-Cinnamoylmorphinamine were shown to possess MOR-1/DOR-1 analgesia
- 6β-Cinnamoylmorphinamine did not cause respiratory depression

Table 1

Receptor binding and *in vivo* analgesia data of selected 6β-acylaminomorphinans[a]

Compound	Affinity (K_i, nM)			Analgesia (ED_{50}, mg/kg, *s.c.*)
	MOR-1	KOR-1	DOR-1	
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8b	0.15 ± 0.03	1.97 ± 0.01	9.38 ± 1.53	>10
8c	0.19 ± 0.09	10.52 ± 0.90	14.52 ± 6.82	>10
8d	0.74 ± 0.12	5.43 ± 1.38	15.26 ± 1.74	>10
8e	0.12 ± 0.006	0.81 ± 0.09	5.15 ± 0.75	>10
9a	4.73 ± 1.64	>100	>100	>10
9b	3.81 ± 0.15	>100	>100	>10
9c	5.44 ± 0.93	>100	>100	>10
9d	25.16 ± 13.02	>100	>100	>10
9e	3.74 ± 0.83	>100	>100	>10
morphine	4.60 ± 1.81[b]			4.96 ± 0.96[c]

[a]Competition studies were performed with the indicated compounds against ^{125}I-BNtxA (0.1 nM) in membranes from CHO cells stably expressing the indicated cloned mouse opioid receptors. K_i values were calculated from the IC_{50} values [26] and represent the means ± SEM of at least three independent replications. ^{125}IBNtxA K_D values for MOR-1, KOR-1, DOR-1 sites were 0.11, 0.03 and 0.24, respectively. [b]Values from the literature. [7] [c]Values from the literature. [25]

Table 2

Opioid receptor efficacy of **8a**[a]

Compound	EC_{50} (nM)			% stimulation		
	MOR-1	KOR-1	DOR-1	MOR-1	KOR-1	DOR-1
8a	1.38 ± 0.8	36.5 ± 19.2	9.1 ± 3.0	95.3 ± 0.8	106.3 ± 8.5	101.7 ± 2.8
DPDPE	nd[b]	nd[b]	2.05 ± 0.6			
DAMGO	5.6 ± 3	nd[b]	nd[b]			
U50,488H	nd[b]	21.7 ± 4.6	nd[b]			

[a]Efficacy data were obtained using agonist induced stimulation of [35S]GTPγS binding assay. Efficacy is represented as EC50 (nM) and percent maximal stimulation relative to standard agonist DAMGO (MOR-1), DPDPE (DOR-1), or U50,488H (KOR-1) at 100 nM. All values are expressed as the mean ± SEM of three separate assays performed in triplicate. [b] Not determined

Supplementary material for

Novel 6β-acylaminomorphinans with analgesic activity

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1. Experimental procedures

1.1. Materials and methods

All chemicals were purchased from Sigma-Aldrich Chemicals (Darmstadt, Germany) and Alfa Aesar (Ward Hill, MA) and were used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VNMRS spectrometer (600 MHz for ^{1}H, 150.9 MHz for ^{13}C) equipped with a dual 5-mm inverse-detection gradient (IDPFG) probehead. Spectra were recorded in DMSO-d_6 or $CDCl_3$ at 25.0±0.1 °C and referenced to internal standard Me_4Si. NMR spectra were processed with VNMRJ 2.2C and MestReNova software (ver. 6.1.1.). The high resolution accurate masses (HRMS) were determined with an Agilent 6230 time-of-flight mass spectrometer. Samples were introduced by Agilent 1260 Infinity LC system, the mass spectrometer was operated in conjunction with a Jet Stream electrospray ion source in positive ion mode. Reference masses of m/z 121.050873 and 922.009798 were used to calibrate the mass axis during analysis. Mass spectra were processed using Agilent MassHunter B.02.00 software. Biological data were analyzed by Graphpad Prism (Graphpad Software, La Jolla, CA).

1.2. Chemical synthesis

1.2.1. Synthesis of 3-*O*-acetylmorphine (**2**):

The compound was synthesized according to the literature method.[1] Morphine hydrochloride (**1**) (0.90 g, 2.8 mmol) was dissolved in the mixture of water (100 mL) and sodium hydrogen carbonate (15 g) and acetic anhydride (4 x 1.5 mL) was added in 10 minute intervals and stirred for further 15 min at room temperature. The solution was then extracted with chloroform. The organic phase was separated, dried over anhydrous Na_2SO_4 and evaporated to dryness to yield 0.90 g of **2** (98% yield). Pale yellow oil.

1.2.2. General procedure for the synthesis of 6β-aminomorphinans (**4, 7**):

The compounds were synthesized according to the literature method.[2] Morphine derivative (**2** or **5**) (0.90 g) was dissolved in benzene (20 mL), Ph_3P (1.50 g), phthalimide (0.90 g) and diisopropyl azodicarboxylate (DIAD) (1.05 mL) were added and stirred for 1 hour at room temperature. The mixture was evaporated under reduced pressure and a solution of tartaric acid (5 g) and water (25 mL) was added and the resulting slurry was extracted with diethyl ether. The aqueous phase was basified (pH = 9) with 10% aq. NH_3 and extracted with chloroform. The organic phase was dried and evaporated to dryness. The resulting pale yellow oil (crude **4**, **7**) was dissolved in ethanol (30 mL) and 36% aq. HCl was added (0.1 ml). The white precipitate was filtered and washed with ethanol to yield white crystalline HCl salts of phthalimide derivatives **3**

and **6**. The HCl salt of phthalimide (**3** or **6**) derivative was dissolved in ethanol (15 mL) and hydrazine hydrate was added (0.5 mL) and the solution refluxed for 2 hrs. The solution was subsequently poured into 20% aq. acetic acid solution after cooling (20 mL) and the white precipitate was filtered. Ethanol was evaporated from the filtrate and basified (pH = 9) with 10% aq. NH_3 and extracted with chloroform. The organic phase was dried and evaporated to dryness to yield the crude 6β-aminomorphinans **4** and **7** (45-50 % yields).

1.2.3. General procedure for acylation of morphinamine derivatives (**8a-e**):

The appropriate carboxylic acid (1.2 molar equivalents) was mixed with benzene (5 mL) and thionyl chloride (0.5 mL) and refluxed for 1 h. The solution was evaporated to dryness under reduced pressure and then dissolved in dichloromethane (10 mL). 6β-morphinamine (**4**) (1.0 molar equivalent) and triethylamine (0.4 mL) were added and stirred for 2 hrs at room temperature. The solvent was evaporated under reduced pressure, methanol (10 mL) and Na_2CO_3 (0.5 g) were added and the mixture stirred overnight. The resulting slurry was neutralized with 20 % aq. acetic acid and extracted with chloroform. The organic phase was dried and evaporated to dryness. The crude products were purified by column chromatography using chloroform-methanol 9:1 isocratic eluent and crystallized from hexane to yield crystalline pale yellow products **8a-e** (20-60% yields).

1.2.4. General procedure for acylation of codeinamine derivatives (**9a-e**):

The method is identical to the synthesis of **8a-e**, except that the hydrolysis with methanolic Na_2CO_3 is left out. Pale yellow crystals (29-50% yields).

1.3. Receptor-Binding Assays:

Competition-binding assays in CHO cells stably expressing MOR-1 (mu), DOR-1 (delta) or KOR-1 (kappa) were performed at 25°C in potassium phosphate buffer (50 mM; pH 7.4), with the inclusion of $MgSO_4$ (5 mM) in the MOR-1 assays. All competition assays were carried out using ^{125}I-BNtxA as described.[3] Specific binding was defined as the difference between total binding and nonspecific binding, determined in the presence of levallorphan (8 μM). Protein concentrations were between 30-40 μg/mL and incubation times were 90 minutes. Specific binding was defined as the difference between total binding and nonspecific binding, determined in the presence of levallorphan (8 μM). Protein concentration was determined as described by Lowry *et al.* [4] using bovine serum albumin as the standard.

1.4. Tail Flick Analgesia Assays:

Male CD-1 mice (25-35 g; Charles River Breeding Laboratories, Wilmington, MA) were maintained on a 12-hr light/dark cycle with Purina rodent chow and water available ad libitum. Mice were housed in groups of five until testing. All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of Memorial Sloan-Kettering Cancer Center. Analgesia was determined using the radiant heat tail-flick technique [5] using a

machine from (Ugo Basile; model number 37360). The intensity was set to achieve a baseline between 2-3 sec. The latency to withdraw the tail from a focused light stimulus was measured electronically using a photocell. Baseline latencies (2.0-3.0 sec) were determined before experimental treatments for all animals as the mean of two trials. Post-treatment tail-flick latencies were determined as indicated for each experiment, and a maximal latency of 10 sec for tail-flick was used to minimize tissue damage. Results were evaluated as %MPE [(observed latency − baseline latency)/(maximal latency − baseline latency)] and shown as the average of each group (n = 10). Drugs were given subcutaneously and cumulative dose-response experiments carried out with two independent assays with each group (n=10). The combined results presented as the ED_{50} with 95% confidence limits (n=20) presented. Analgesia was defined quantally as a doubling, or greater, of the baseline latency. Similar results were obtained analyzing the data in a graded response manner. Analgesic ED_{50} values and confidence limits were determined using non-linear regression analysis GraphPad Prism.

1.5. [^{35}S]GTPγS-Binding Assay:

[^{35}S]GTPγS binding was performed on membranes prepared from transfected cells in the presence and absence of the indicated opioid for 60 min at 30°C in the assay buffer (50 mM Tris-HCl, pH 7.4, 3 mM MgCl2, 0.2 mM EGTA, and 10 mM NaCl) containing 0.05nM [^{35}S]GTPγS and 30MGDP, as previously reported.[6,7] After the incubation, the reaction was filtered through glass-fiber filters (Whatman Schleicher & Schuell, Keene, NH) and washed three times with 3 ml of ice-cold 50 mM Tris-HCl, pH 7.4, on a semiautomatic cell harvester. Filters were transferred into vials with 5 ml of Liquiscent (National Diagnostics, Atlanta, GA), and the radioactivity in vials was determined by scintillation spectroscopy in a Tri-Carb 2900TR counter (PerkinElmer Life and Analytical Sciences). Basal binding was determined in the presence of GDP and the absence of drug.

1.6. Respiratory Depression Assay

Respiratory rate was assessed in awake, freely moving, adult male CD1 mice with the MouseOx pulse oximeter system (Starr Life Sciences), as previously reported.[8] Each animal was habituated to the device for 30 min and then tested. A 5-s average breath rate was assessed at 5-min intervals. A baseline for each animal was obtained over a 25-min period before drug injection, and testing began at 15 min post-injection and continued for a period of 35 min. Groups of mice (n = 3) were treated subcutaneously with either saline or morphine (20 mg/kg) or **8a** (24 mg/kg). Morphine was given at doses approximately four times its analgesic ED_{50}, while **8a** was administered at 8 times its ED_{50}. Groups were compared with repeated-measures ANOVA followed by Bonferroni multiple-comparison test.

2. Compound data

2.1. 6β-Cinnamoylmorphinamine (**8a**, $C_{26}H_{26}N_2O_3$)

Yield: 21%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.61 (d, J = 15.7 Hz, 1H), 7.40 (m, 2H), 7.30 (m, 2H), 6.69 (d, J = 7.6 Hz, 1H), 6.50 (m, 3H), 5.78 (d, J = 9.3 Hz, 1H), 5.59 (d, J = 9.3 Hz, 1H), 4.83 (m, 1H), 4.57 (m, 1H), 2.51 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.05, 144.56, 141.63, 138.96, 134.49, 131.89, 129.78, 129.55, 128.75, 127.90, 124.85, 120.02, 119.45, 117.36, 92.70, 59.27, 50.16, 47.17, 43.70, 42.66, 39.28, 34.92, 20.34. HRMS $(M+H)^+$ calcd for $C_{26}H_{26}N_2O_3$ 415.2022, found 415.2031.

2.2. 6β-(4-chlorocinnamoyl)-morphinamine (**8b**, $C_{26}H_{25}ClN_2O_3$)

Yield: 38%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.56 (d, J = 16.0 Hz, 1H), 7.47 (d, J = 7.8 Hz, 2H), 7.31 (d, J = 7.8 Hz, 2H), 6.68 (d, J = 8.1 Hz, 1H), 6.59 (d, J = 16.0 Hz, 1H), 6.52 (d, J = 8.1 Hz, 1H), 5.89 – 5.82 (m, 1H), 5.64 (d, J = 9.9 Hz, 1H), 4.71 (d, J = 9.0 Hz, 4H), 4.52 (d, J = 5.3 Hz, 1H), 2.71 (d, J = 9.5 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.32, 144.39, 139.62, 139.26, 135.29, 133.22, 129.75, 128.96, 128.81, 128.79, 128.68, 128.61, 122.77, 120.70, 119.40, 117.47, 92.20, 59.64, 49.51, 47.11, 42.91, 41.32, 37.63, 33.54, 22.45, 20.71. HRMS $(M+H)^+$ calcd for $C_{26}H_{25}ClN_2O_3$ 449.1626, found 449.1630.

2.3. 6β-(4-(trifluoromethyl)cinnamoyl)-morphinamine (**8c**, $C_{27}H_{25}F_3N_2O_3$)

Yield: 39%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 (d, J = 15.7 Hz, 1H), 7.50 (d, J = 8.3 Hz, 2H), 7.47 (d, J = 8.2 Hz, 2H), 6.70 (d, J = 8.1 Hz, 1H), 6.59 (d, J = 15.7 Hz, 1H), 6.51 (d, J = 8.1 Hz, 1H), 5.79 (dd, J = 6.4, 3.0 Hz, 1H), 5.60 (d, J = 9.9 Hz, 1H), 4.82 (s, 1H), 4.58 (t, J = 6.4 Hz, 1H) 2.54 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.70, 144.87, 139.97, 139.29, 138.24, 131.82, 131.58, 131.37, 129.66, 129.05, 128.18, 125.93, 124.81, 123.07, 119.79, 117.84, 92.86, 59.73, 50.42, 47.51, 43.90, 42.76, 39.31, 34.97, 20.73. HRMS $(M+H)^+$ calcd for $C_{27}H_{25}F_3N_2O_3$ 483.189, found 483.1873.

2.4. 6β-(4-(methoxy)cinnamoylamino)-morphinamine (**8d**, $C_{27}H_{28}N_2O_4$)

Yield: 41%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.51 (d, J = 15.5 Hz, 1H), 7.29 (d, J = 8.3 Hz, 2H), 6.72 (d, J = 8.3 Hz, 2H), 6.66 (d, J = 8.0 Hz, 1H), 6.47 (d, J = 8.0 Hz, 1H), 6.37 (d, J = 15.5 Hz, 1H), 5.72 (s, 1H), 5.53 (d, J = 9.7 Hz, 1H), 4.81 (s, 1H), 4.55 (t, J = 6.1 Hz, 1H), 3.74 (s, 3H), 2.48 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.67, 161.00, 144.99, 141.20, 139.49, 131.65, 129.91, 129.67, 129.16, 127.59, 124.88, 119.58, 118.21, 117.80, 114.30, 92.89, 59.45, 55.46, 50.34, 47.40, 43.90, 42.71, 39.27, 35.01, 20.72. HRMS $(M+H)^+$ calcd for $C_{27}H_{28}N_2O_4$ 445.2122, found 445.2138.

2.5. 6β-(3-nitrocinnamoyl)-morphinamine (**8e**, $C_{26}H_{25}N_3O_5$)

Yield: 21%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.38 (d, J = 1.8 Hz, 1H), 8.19 (dd, J = 8.2, 1.4 Hz, 1H), 7.92 (d, J = 7.7 Hz, 1H), 7.67 (t, J = 7.9 Hz, 1H), 7.58 (d, J = 15.8 Hz, 1H), 6.91 (d, J = 15.8 Hz, 1H), 6.53 (d, J = 8.0 Hz, 1H), 6.42 (d, J = 8.1 Hz, 1H), 5.81 – 5.74 (m, 1H), 5.64 (dd, J = 9.8, 1.6 Hz, 1H), 4.66 (s, 1H), 4.40 (t, J = 6.7 Hz, 1H), 2.44 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.90, 148.02, 144.22, 138.65, 136.65, 136.23, 133.64, 129.90, 129.70, 128.05, 124.63, 123.18,

120.89, 118.50, 116.41, 91.86, 58.43, 48.93, 46.42, 43.32, 42.37, 35.16, 28.91, 19.76. HRMS $(M+H)^+$ calcd for $C_{26}H_{25}N_3O_5$ 460.1867, found 460.1857.

2.6. 6β-Cinnamoylcodeinamine (**9a**, $C_{27}H_{28}N_2O_3$)

Yield: 32%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.62 (d, J = 15.7 Hz, 1H), 7.45 (m, 2H), 7.32 – 7.27 (m, 3H), 6.67 (d, J = 8.2 Hz, 1H), 6.55 (d, J = 8.2 Hz, 1H), 6.48 (d, J = 15.7 Hz, 1H), 5.88 (ddd, J = 9.1, 5.9, 3.0 Hz, 1H), 5.59 (dd, J = 9.8, 1.5 Hz, 1H), 4.86 (s, 1H), 4.61 (t, J = 6.7 Hz, 1H), 3.84 (s, 3H), 2.50 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.64, 146.06, 142.43, 141.26, 134.80, 131.62, 130.07, 129.61, 129.48, 129.25, 128.75, 128.67, 127.80, 127.72, 126.00, 120.45, 119.01, 114.04, 92.51, 77.29, 77.07, 76.86, 59.18, 58.15, 56.71, 49.54, 46.93, 43.72, 42.47, 39.20, 35.06, 20.58, 18.42. HRMS $(M+H)^+$ calcd for $C_{27}H_{28}N_2O_3$ 429.2173, found 429.2176.

2.7. 6β-(4-chlorocinnamoyl)-codeinamine (**9b**, $C_{27}H_{27}ClN_2O_3$)

Yield: 32%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.56 (d, J = 15.6 Hz, 1H), 7.37 (d, J = 8.4 Hz, 2H), 7.29 (d, J = 8.4 Hz, 2H), 6.66 (d, J = 8.2 Hz, 1H), 6.55 (d, J = 8.2 Hz, 1H), 6.39 (d, J = 15.6 Hz, 1H), 5.85 (ddd, J = 9.1, 5.8, 3.0 Hz, 1H), 5.62 (dd, J = 9.7, 1.1 Hz, 1H), 4.85 (s, 1H), 4.56 (t, J = 6.5 Hz, 1H), 3.83 (s, 3H), 2.43 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.48, 146.14, 142.45, 140.18, 135.66, 133.42, 132.72, 130.49, 129.21, 129.14, 127.00, 121.05, 119.11, 113.94, 92.63, 59.25, 56.84, 50.07, 47.11, 44.14, 43.12, 40.27, 35.96, 20.42, 18.61. HRMS $(M+H)^+$ calcd for $C_{27}H_{27}ClN_2O_3$ 463.1783, found 463.1782.

2.8. 6β-(4-(Trifluoromethyl)cinnamoyl)-codeinamine (**9c**, $C_{28}H_{27}F_3N_2O_4$)

Yield: 50%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.67 (d, J = 15.7 Hz, 1H), 7.61 (s, 4H), 6.74 (d, J = 8.2 Hz, 1H), 6.63 (d, J = 15.7 Hz, 1H), 6.61 (d, J = 8.2 Hz, 1H), 5.97 (m, 1H), 5.58 (d, J = 10.8 Hz, 1H), 4.90 (s, 1H), 4.69 (s, 1H), 3.88 (s, 3H), 2.81 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.27, 146.27, 143.18, 139.80, 138.48, 130.49, 130.02, 129.36, 128.21, 125.97, 125.95, 124.25, 123.23, 119.51, 114.77, 92.38, 60.33, 56.96, 49.65, 47.74, 43.41, 42.28, 38.48, 34.20, 21.33. HRMS $(M+H)^+$ calcd for $C_{28}H_{27}F_3N_2O_4$ 497.2047, found 497.2058.

2.9. 6β-(4-(Methoxy)cinnamoyl)-codeinamine (**9d**, $C_{28}H_{30}N_2O_4$)

Yield: 50%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58 (d, J = 15.5 Hz, 1H), 7.41 (d, J = 8.0 Hz, 2H), 6.85 (d, J = 8.0 Hz, 2H), 6.66 (d, J = 8.0 Hz, 1H), 6.54 (d, J = 8.0 Hz, 1H), 6.27 (d, J = 15.5 Hz, 1H), 5.90 (m, 1H), 5.62 (d, J = 9.4 Hz, 1H), 4.86 (s, 1H), 4.56 (s, 1H), 3.85 (s, 3H), 3.80 (s, 3H), 2.44 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.90, 160.88, 146.05, 142.26, 141.01, 132.37, 130.36, 129.36, 129.27, 127.44, 126.78, 118.85, 117.83, 114.22, 113.86, 92.49, 59.09, 58.23, 56.73, 55.31, 49.75, 46.94, 43.94, 42.93, 40.07, 35.77, 20.28, 18.42. HRMS $(M+H)^+$ calcd for $C_{28}H_{30}N_2O_4$ 459.2278, found 459.2280.

2.10. 6β-(3-Nitrocinnamoyl)-codeinamine (**9e**, $C_{27}H_{27}N_3O_5$)

Yield: 44%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.36 (s, 1H), 8.20 (d, J = 8.1 Hz, 1H), 7.77 (d, J = 7.8 Hz, 1H), 7.70 (d, J = 15.6 Hz, 1H), 7.56 (t, J = 7.9 Hz, 1H), 6.69 (d, J = 8.2 Hz, 1H), 6.57 (d, J = 8.2 Hz, 1H), 6.54 (d, J = 15.6 Hz, 1H), 5.90 (s, 1H), 5.67 (d, J = 9.7 Hz, 1H), 4.88 (s, 1H), 4.59 (t, J = 6.4 Hz, 1H), 3.87 (s, 3H), 2.50 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 164.72, 148.87, 146.23, 142.64, 139.08, 136.76, 134.14, 132.81, 130.34, 130.15, 129.27, 126.71, 124.27, 123.48, 121.97, 119.24, 114.14, 92.45, 59.48, 56.95, 50.23, 47.25, 44.14, 43.14, 40.28, 35.92, 20.59. HRMS $(M+H)^+$ calcd for $C_{27}H_{27}N_3O_5$ 474.2023, found 474.2026.

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Figure S1. ^{1}H NMR of 8a.

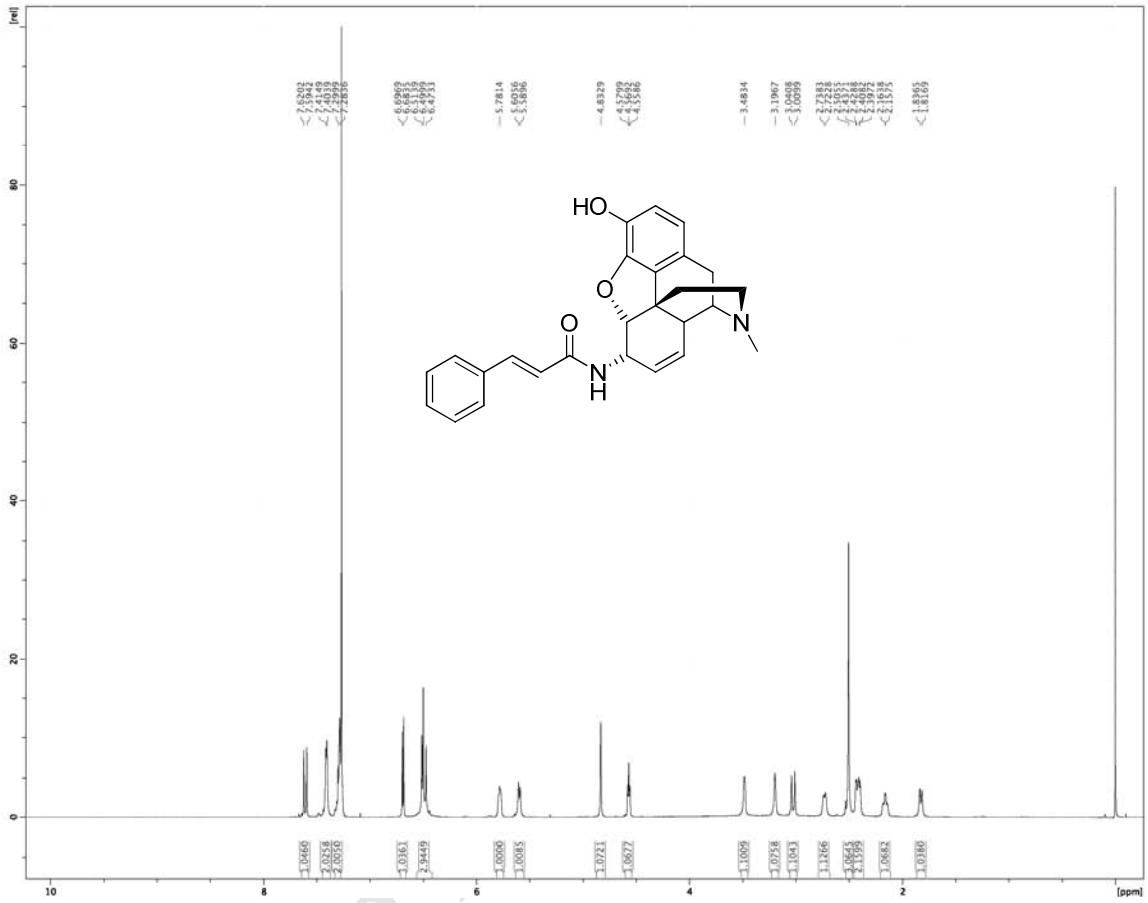

Figure S2. ^{13}C NMR of 8a.