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## Mixed Kappa/Mu Opioid Receptor Agonists: The 6 $\beta$ -Naltrexamines

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### Abstract

Ligands from the naltrexamine series have consistently demonstrated agonist activity at kappa opioid receptors (KOR), with varying activity at the mu opioid receptor (MOR). Various 6 $\beta$ -cinnamoylamino derivatives were made with the aim of generating ligands with a KOR agonist/MOR partial agonist profile, as ligands with this activity may be of interest as treatment agents for cocaine abuse. The ligands all displayed the desired high affinity, non-selective binding in vitro and in the functional assays were high efficacy KOR agonists with some partial agonist activity at MOR. Two of the new ligands (**12a**, **12b**) have been evaluated in vivo, with **12a** acting as a KOR agonist, and therefore somewhat similar to the previously evaluated analogues **3–6**, while **12b** displayed predominant MOR agonist activity.

### Introduction

Various studies have demonstrated that kappa opioid receptor (KOR) agonists can reduce, or even prevent, some of the behavioural effects caused by cocaine.<sup>1–4</sup> Selective KOR agonists are, however, unlikely to be seriously considered as treatment agents as they themselves cause dysphoria, both in drug naïve individuals and in those with a history of drug use.<sup>5</sup> Preclinical evidence suggests that a more effective approach may be to target KOR-agonists that also display partial agonist activity at the mu opioid receptor (MOR).<sup>4,6</sup> Compounds of this type, such as EKC, are more effective in reducing cocaine self-administration, and display fewer side effects, than their more selective counterparts such as enadoline and spiradoline.<sup>4</sup> Derivatives of  $\beta$ -naltrexamine ( $\beta$ -NTA) have consistently demonstrated KOR agonist properties. The most well known of this series is  $\beta$ -FNA (**1**), a ligand widely utilized for its irreversible MOR antagonist properties, but which also displays short-lived KOR agonist effects.<sup>7–9</sup> More recently, in a search for potent KOR agonists from the  $\beta$ -NTA series, TRK-820 (**2**) emerged as the lead compound identified from a comprehensive study of such derivatives.<sup>10</sup> However TRK-820 was the result of optimisation for high KOR selectivity and potency and therefore is not an ideal candidate for

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Supporting Information Available: <sup>1</sup>H NMR, <sup>13</sup>C NMR, mass spectra and microanalysis data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

further development against cocaine. Independently we found the cinnamoyl derivatives (**3–6**) to have KOR agonist activity *in vivo*,<sup>11,12</sup> but we did not seek to optimise for KOR selectivity and found that with no methyl group on the 6 $\beta$ -nitrogen (where a methyl group is found in TRK-820) the ligands display equal affinity at MOR and KOR and are therefore potentially more interesting. The effect of the NMe group replicates the importance of having an N-alkyl group (usually NMe), rather than NH in the arylacetamide series of KOR agonists.<sup>13</sup>

The cinnamoyl derivatives (**3–6**) have KOR agonist activity *in vivo*,<sup>11,12</sup> with **6** and **5** similar to  $\beta$ -FNA, having short-lived KOR agonist effects and then substantial MOR antagonist activity, while **3** and **4** were potent KOR agonists in both tail withdrawal (TW) and antiwrithing (AW) assays.<sup>11,12</sup> The aim of the current work was therefore to increase the MOR efficacy of this series to achieve the desired, mixed KOR/MOR profile.

In related series of 14-cinnamoylaminomorphinones,<sup>14</sup> substitution at the 2'-position of the cinnamoyl aryl ring produced ligands with significantly altered efficacy compared to their 4'-substituted homologues and so the 2'-substituted analogues of **3–6** were targeted to see if a similar effect was observed in the  $\beta$ -NTA series.

It has also become clear that introduction of substituents to the C<sub>14</sub>-hydroxyl group can lead to an increase in MOR affinity and efficacy. The effect is most robust when a three carbon chain, or substituted three carbon chain is used. The most dramatic example of this effect is provided by Schmidhammer and colleagues<sup>15</sup> who converted the MOR antagonist naltrexone (**7a**) into a powerful MOR agonist by substitution of a phenylpropyl group in the C<sub>14</sub>-hydroxyl (**7b**). In addition, the 14-phenylacetyloxy derivatives were targeted as introduction of the phenylacetyl group in 14-amino analogues of naltrindole (**8**) and in 14-aminodihydromorphinones and codeinones also raised MOR efficacy.<sup>16,26</sup>

## Chemistry

The synthesis of the target ligands relied on the efficient production of the key intermediate  $\beta$ -NTA **10** which can be achieved by reductive amination of naltrexone **7a** with dibenzylamine followed by hydrogenolysis of the benzyl groups (Scheme 1).<sup>17</sup> Although this synthetic approach is well established, in our hands removal of the benzyl group proved problematic and we opted for an alternative method. Portoghese and colleagues have reported a 9:1 mixture of  $\beta$ : $\alpha$ -diastereoisomers obtained from the reduction of naltrexone oxime (**11a**) with BH<sub>3</sub>-THF.<sup>18</sup> In our hands, using BH<sub>3</sub>-Me<sub>2</sub>S, the result was 7:1 and required rigorous silica gel chromatography to obtain the pure  $\beta$ -NTA **10**. It is proposed that in the course of the reaction the boron-complexed oxime mimics the bulky dibenzyliminium formed during the preparation of **9** and exists in a pseudochair conformation, hence exposing the  $\alpha$ -face to preferential reduction.<sup>17,18</sup> Therefore, we chose to add bulk to the oxime and synthesised ethers **11b**, **11c** by quantitative oximation of naltrexone with the relevant alkoxyamines. Upon reduction of the methyl oxime ether **11b** with ZrCl<sub>4</sub>/NaBH<sub>4</sub> (scheme 1) the stereoselectivity was improved to 13:1 in favour of the desired  $\beta$ -diastereoisomer **10**. Reducing the benzyl ether **11c** gave almost equal stereoselectivity (12:1). This new method, **via 11c**, was adopted for the present study and gram quantities of **10** could be reproducibly obtained in 2 steps with one chromatographic purification. The  $\beta$ -configuration of the 6-amino group was confirmed by comparison with previously reported data on this compound.<sup>17,18</sup> In particular, a coupling constant of 6.8 Hz was determined for H-5.

Synthesis of the target C-6- $\beta$  amides **3**, **12a–d** was achieved by acylation of  $\beta$ -NTA (**10**) with the relevant cinnamoyl chlorides (Scheme 2). The 14-*O*-acyl targets (**15**) could be

accessed from **3** by protection of the 3-phenol and then acylation of the C<sub>14</sub>-hydroxyl (Scheme 2).

Due to the low reactivity of the tertiary alcohol, alkylation of the C<sub>14</sub>-hydroxyl required forcing conditions and protection of the 3-phenol as the methyl ether (Scheme 3). Introduction of the 6 $\beta$ -amino group (**17**) was again accomplished using the method of asymmetric reduction of the ketoxime (**16**).

## Results

Binding affinities of the new compounds for MOR and KOR were determined by competition with [<sup>3</sup>H]-diprenorphine binding in C6-rat glioma cells expressing recombinant rat MOR and CHO cells expressing recombinant human KOR.<sup>19</sup> DOR activity of the new ligands was not determined as in no case has DOR activity proved relevant to the pharmacological profile of ligands with significant MOR and/or KOR efficacy. All of the ligands bound with high to very high affinity to both KOR and MOR, with little selectivity for either receptor (Table 1). Neither the nature, nor position of substituents on the phenyl ring of the cinnamoyl group substantially affected affinities and similarly, introduction of substituents at C14 also had relatively little effect.

The *in vitro* assay used to determine opioid receptor functional activity was the [<sup>35</sup>S]GTP $\gamma$ S binding stimulation assay which, like the binding assays, was performed on recombinant opioid receptors transfected into C6-rat glioma cells (for MOR) and CHO cells (for KOR). Assays were performed as previously described by Traynor and Nahorski.<sup>20</sup> Agonist efficacy at these opioid receptors was determined in comparison to the standard selective agonists DAMGO (MOR), and U69593 (KOR) (Table 2). The ligands were, as expected, agonists at the KOR, efficacies mostly ranging from 76% to 120% relative to U69593, the exception being **15b** which had only low efficacy and potency KOR antagonist activity; otherwise potencies were in the low to sub-nanomolar range. The ring substituted analogues (**12a** – **12d**) had very similar efficacy at KOR, though at 81% stimulation **12a**, the *o*-nitro analogue, was somewhat lower than the rest. There was slightly greater variation in the KOR efficacy of the C14-substituted analogues, with the allyl substituted (**20b**) displaying the highest efficacy of the compounds in this study.

All of the new ligands were low efficacy partial agonists at MOR, with the 14-substituted series providing the highest efficacy compounds (**20b** and **15c**). Again **15b** stood out for having a substantially different profile to the other ligands, it did not stimulate MOR and was in fact a high potency MOR antagonist.

The unsubstituted 6 $\beta$ -cinnamoylnaltrexamine (**3**) has previously been evaluated *in vivo* in the warm water tail withdrawal and acetic acid induced abdominal stretch assays and found to be a potent and efficacious KOR agonist.<sup>12</sup> For comparison, two of the current series of ligands were chosen for preliminary evaluation in the similar paraphenylquinone (PPQ) induced abdominal stretch assay,<sup>21</sup> an assay in which MOR and KOR agonists of even relatively low efficacy would be expected to show activity. **12b** was chosen as it has a very similar profile to **3** *in vitro*, while the second choice, **12a**, had lower efficacy *in vitro* at both MOR and KOR. **12a** was found to be an agonist of quite low potency (ED<sub>50</sub> 18.5 (13.2 – 26.1) mg/kg, s.c.) and the effect of an ED80 concentration of **12a** could be blocked by norBNI (AD<sub>50</sub> 5.28 mg/kg, s.c.), a selective KOR antagonist but not by the MOR antagonist  $\beta$ -FNA (up to 30  $\mu$ g/kg icv) or the DOR antagonist naltrindole (NTI) up to 30 mg/kg sc. In contrast **12b** was significantly more potent (ED<sub>50</sub> 3.5 (1.6 – 7.7) mg/kg, s.c.) and the effect of an ED80 dose was blocked by  $\beta$ -FNA (AD<sub>50</sub> 7.1  $\mu$ g/kg icv), inconsistently by norBNI (38%

at 1, 13% at 3  $\mu\text{g}/\text{brain}$ ) and partially NTI (26% at 10, 23% at 30 mg/kg, s.c.) indicating a predominantly MOR mediated mode of action for **12b**.

## Discussion

The current series of 6 $\beta$ -naltrexamine derived ligands confirms our earlier finding that having a secondary amide at C6 results in compounds with no selectivity for any particular opioid receptor. This effect is in contrast to the tertiary amides such as TRK-820 (**2**), which are selective towards the KOR. The low nanomolar to subnanomolar affinities seen here are entirely in keeping with those observed with earlier members of the series.<sup>11,12</sup> The predominant activity in the [<sup>35</sup>S]GTP $\gamma$ S assay of these new ligands is of high efficacy agonist activity at the KOR. Again this is in keeping with our earlier work in which **3** and *p*-substituted analogues were KOR agonists in the electrically stimulated guinea pig ileal longitudinal muscle (GPI)<sup>12</sup> and the work of others.<sup>7,10</sup> Compared to the *p*-methyl and *p*-chloro derivatives, previously reported as partial agonists<sup>11</sup> the *o*-analogues (**12c**, **12d**) reported here seem to be of higher efficacy, certainly equivalent to the unsubstituted parent compound (**3**).

Our previous studies with **3** indicated that it had little efficacy at MOR in either the GPI (no reversal by the MOR antagonist CTAP) or *in vivo* (antinociceptive effect not reversed by  $\beta$ -FNA).<sup>12</sup> The new results in the [<sup>35</sup>S]GTP $\gamma$ S assay confirm its low MOR efficacy. Of the substituted analogues (**12a** – **12d**) the *o*-Cl, *o*-Me and *m*-NO<sub>2</sub> substituted ligands (**12b** – **12d**) all have similar low efficacy at MOR of around 20% of DAMGO's effect, whereas the MOR efficacy of **12a** (*o*-NO<sub>2</sub>) is even lower (7% of DAMGO). This is reminiscent of the effect of aryl substitution in the cinnamoyl derivatives of the 14-aminomorphinones where the effect of orientation with nitro substitution was different to that with chloro and methyl.<sup>14</sup>

Substituents were added to the C14-oxygen in expectation of increasing the MOR efficacy of the ligands. This approach has shown partial success with both the allyl ether (**20b**) and the phenylacetate (**15c**) having double the MOR efficacy of **3**. However the effect was not consistent, both the benzyl ether (**20a**) and the acetate (**15a**) displayed no change in efficacy compared to **3**, while the benzoate (**15b**) showed no stimulation of [<sup>35</sup>S]GTP $\gamma$ S binding at MOR. Certainly the effect of introducing a substituent at this position is much less dramatic than seen in the somewhat related series of substituted 14-amino analogues of oxymorbindole where significant changes in potency and efficacy could be seen.<sup>16</sup> The effect of benzyl substitution in this series can also be compared with the effect of 14-O benzylation of naltrexone.<sup>22</sup> With naltrexone, this modification lead to an increase in affinity and antagonist potency at KOR compared to naltrexone itself, which is in contrast to the current findings where **20a** has somewhat lower affinity and potency at KOR compared to **3**.

The activity of the two nitro-substituted cinnamoyl-6 $\beta$ -naltrexamines (**12a** and **12b**) *in vivo* is worthy of note. In the [<sup>35</sup>S]GTP $\gamma$ S binding assay both appeared to be high efficacy KOR agonists with some low level efficacy at MOR, with the *o*-substituted analogue (**12a**) having somewhat lower efficacy at both receptors compared to **12b**. In the PPQ induced abdominal stretch assay **12a** was found to be a KOR agonist, correlating well with the *in vitro* data, while **12b**, in the same assay, had predominant MOR activity which would not have been predicted from the *in vitro* data. Either the [<sup>35</sup>S]GTP $\gamma$ S binding assay has overestimated efficacy at KOR or underestimated efficacy at MOR. The latter has been observed and discussed previously in a related series of 14-cinnamoylaminomorphinones and may be related to the slower rate and extent of receptor occupation by the ligands *in vivo* compared to in the *in vitro* assays.<sup>14,23</sup>

## Experimental

Reagents and solvents were purchased from *Aldrich* or *Lancaster* and used as received. M.p.: *Gallenkamp MFB-595* melting point apparatus; uncorrected. NMR Spectra: *Jeol Delta-270-MHz* instrument:  $^1\text{H}$  at 270 MHz, and *Varian Mercury-400-MHz* instrument:  $^1\text{H}$  at 400 MHz,  $^{13}\text{C}$  at 100 MHz;  $\delta$  in ppm,  $J$  in Hz with TMS as an internal standard. ESIMS: microTOF (BRUKER). Microanalysis: *Perkin-Elmer 240C* analyser. Ligands were tested as their hydrochloride or oxalate salts, prepared by adding one equivalent of (5N hydrochloric acid in isopropanol) to an ether solution and one equivalent oxalic acid to an ethanolic solution of the compound. Synthetic methods are detailed for one example in each series. Full details for all experiments can be found in the Supporting Information.

### General procedure (A): Acylation of Naltrexone

To a stirring mixture of the appropriate cinnamic acid (2.7 mmol) in PhMe (10 mL), was added oxalyl chloride (14 mmol), followed by DMF (4 drop) at 0 °C. The reaction mixture was stirred for 1 h, and concentrated under reduced pressure to yield the appropriate cinnamoyl chloride. The crude cinnamoyl chloride (2.2 mmol), was added to a solution of 6- $\beta$ -naltrexamine (2.2 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (15 mL) at 25 °C.  $\text{Et}_3\text{N}$  (11.2 mmol) was added and the reaction mixture stirred for 1 h before diluting with  $\text{H}_2\text{O}$  (20 mL), and extraction with  $\text{CH}_2\text{Cl}_2$  ( $3 \times 20$  mL). The combined organic layers washed with (20 mL) brine, dried over  $\text{MgSO}_4$  and concentrated under reduced pressure. Flash-chromatography eluent (0.5/0.5/99 MeOH/ $\text{Et}_3\text{N}$ / $\text{CH}_2\text{Cl}_2$ ) afforded the corresponding cinnamoyl naltrexamine.

### General procedure (B): 14-O-acylation

To a stirred solution of 3-O- tetrabutyltrimethylsilyl-6- $\beta$ -cinnamoyl-naltrexamine (**13**) (0.25 mmol), in PhMe (14 mL), was added the appropriate anhydride. The reaction mixture was stirred at reflux for 24 h. After cooling to room temperature the suspension was diluted with (15mL), of saturated  $\text{NaHCO}_3$ , and extracted with  $\text{CH}_2\text{Cl}_2$  ( $3 \times 10$  mL). The combined organic extracts were dried over anhydrous  $\text{MgSO}_4$  filtered and concentrated under reduced pressure. Flash-chromatography (10/90 EtOAc/Hexane) gave the appropriate protected-6- $\beta$ -cinnamoyl-14-acyl naltrexamine (**14**).

### General procedure (C): TBDMS deprotection

To a stirred solution of the appropriate 3-O-tetrabutyltrimethylsilyl-6- $\beta$ -cinnamoyl-14-acyl-naltrexamine (**14**) (0.28mmol), in THF (3 mL), was added TBAF (0.18 mmol) and the suspension stirred for 2 h. Flash-chromatography (2/98 MeOH/ $\text{CH}_2\text{Cl}_2$ ) afforded the appropriate 6- $\beta$ -cinnamoyl-14-acyl-naltrexamine derivatives (**15**).

### General procedure (D): 14-O-alkylation

Sodium hydride (5.28 mg 0.22 mmol), was added to a solution of 3-O-methyl-6- $\beta$ -cinnamoyl naltrexamine (**18**) in DMF (anhydrous, 15mL) at 0 °C. The reaction mixture stirred for 5 min and then allowed to warm to r.t and stirred for a further 20 minutes before adding alkyl bromide (0.41 mmol) and stirring at r.t for 6 h. Excess NaH was destroyed carefully by addition of small pieces of ice and diluted with  $\text{H}_2\text{O}$  (50 mL), and the mixture extracted with  $\text{CH}_2\text{Cl}_2$  ( $3 \times 25$  mL). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure. Flash-chromatography (1/99 MeOH/ $\text{CH}_2\text{Cl}_2$ ) afforded the products (**19**).

### General procedure (E): 3-O-demethylation

To a solution of the appropriate (**19**) (0.019 mmol) in  $\text{CH}_2\text{Cl}_2$  (61  $\mu\text{L}$ ) was added a 1M solution of  $\text{BBr}_3$  in  $\text{CH}_2\text{Cl}_2$  (0.12 mL) over 10 min at  $-15$  °C. After 5 minutes ice (0.20 g)



and concentrated  $\text{NH}_3$  (94  $\mu\text{L}$ ) were added and the resulting mixture was stirred at 0 °C for 30 minutes. The organic layers were separated and the aqueous layers extracted with  $\text{CH}_2\text{Cl}_2$  (3  $\times$  10 mL). The combined organic layers were washed with brine (10 mL), dried over  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure. Flash-chromatography (5/95 MeOH/ $\text{CH}_2\text{Cl}_2$ ) afforded pure material.

### 17-Cyclopropylmethyl-3,14 $\beta$ -dihydroxy-4,5 $\alpha$ -epoxy-6 $\beta$ -

**(cinnamoylamino)morphinan (3)**—3 was prepared using the general method A described above, to obtain 675 mg (63%) of pale white solid.  $R_f$  0.40 (1/5/94  $\text{NH}_3/\text{MeOH}/\text{CH}_2\text{Cl}_2$ );  $^1\text{H-NMR}$  (270 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.59 (1H, d,  $J$  = 15.6 Hz,  $\text{CH}=\text{CH}$ ), 7.48 – 7.47 (2H, m,  $\text{CH}_{\text{Ar}}$ ), 7.34–7.32 (3H, m,  $\text{CH}_{\text{Ar}}$ ), 6.67 (1H, d,  $J$  = 8 Hz,  $\text{H}_1$ ), 6.55 (1H, d,  $J$  = 8 Hz,  $\text{H}_2$ ), 6.39 (1H, d,  $J$  = 15.6 Hz,  $\text{CH}=\text{CH}$ ), 5.43 (1H, brs, OH), 5.28 (1H, s, OH), 4.43 (1H, d,  $J$  = 6.8 Hz,  $\text{H}_5$ ), 4.10 – 4.10 (1H, brm,  $\text{H}_6$ ), 3.09 (1H, d,  $J$  = 5.6 Hz,  $\text{H}_9$ ), 3.00 (1H, d,  $J$  = 18.0 Hz  $\text{H}_{10\beta}$ ), 2.66 – 2.63 (2H, m), 2.33 (2H, d,  $J$  = 6.6 Hz, cyclopropylmethyl  $\text{CH}_2$ ), 1.83 – 1.79 (2H, m), 1.65 – 1.60 (2H, m), 1.50 – 1.47 (2H, m), 1.28 – 1.23 (1H, m), 0.84 – 0.82 (1H, m, cyclopropyl CH), 0.51 (2H, d,  $J$  = 8.0 Hz, cyclopropyl  $\text{CH}_2$  cis), 0.12 (2H, d,  $J$  = 8.0 Hz, cyclopropyl  $\text{CH}_2$  trans);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  166.5, 145.8, 141.6, 139.0, 135.3, 130.8, 128.6, 128.4, 127.6, 127.1, 120.9, 118.0, 77.2, 71.8, 71.0, 61.9, 60.5, 48.4, 46.4, 32.5, 27.1, 22.2, 19.4, 9.2, 3.8, 3.7; ESIMS  $m/z$ : 473  $[\text{M} + 1]^+$ ; Anal. ( $\text{C}_{29}\text{H}_{32}\text{N}_2\text{O}_4 \cdot \text{HCl} \cdot 2\text{H}_2\text{O}$ ) CHN.

**6 $\beta$ -naltrexamine (10)**—To  $\text{ZrCl}_4$  (392 mg, 1.6 mmol) in dry THF (15 mL) was added portion wise  $\text{NaBH}_4$  (212 mg, 5.6 mmol) at 0 °C. After the mixture had been stirred for 30 minutes naltrexene O-benzyl oxime (**11c**) (500 mg, 1.1 mmol) was added portion wise. The reaction mixture was warmed to r.t and then refluxed for 24 h under  $\text{N}_2$ . The suspension was concentrated to dryness, before adding 2M HCl at 0 °C (slowly), and then heated at 80 °C for at least 1 hour. The aqueous layer was basified with  $\text{Na}_2\text{CO}_3$  and extracted with EtOAc (3  $\times$  30 mL), washed with brine (30 mL), dried with  $\text{MgSO}_4$ , and concentrated under reduced pressure. Flash-chromatography eluent (5/95 MeOH/ $\text{CH}_2\text{Cl}_2$ ) afforded **10** as a white solid 161 mg, (42%).  $R_f$  0.24 (1/5/94  $\text{NH}_3/\text{MeOH}/\text{CH}_2\text{Cl}_2$ );  $^1\text{H-NMR}$  (270 MHz,  $\text{CDCl}_3$ ):  $\delta$  6.60 (1H, d,  $J$  = 8.0 Hz,  $\text{H}_1$ ), 6.51 (1H, d,  $J$  = 8.0 Hz,  $\text{H}_2$ ), 5.21 (3H brs, OH,  $\text{NH}_2$ ), 4.23 (1H, d,  $J$  = 6.8 Hz,  $\text{H}_5$ ), 3.04 (1H, d,  $J$  = 5.5 Hz,  $\text{H}_9$ ), 2.96 (1H, d,  $J$  = 18.0 Hz  $\text{H}_{10\beta}$ ), 2.60 – 2.56 (4H, m), 2.33 (2H, d,  $J$  = 6.6 Hz, cyclopropylmethyl  $\text{CH}_2$ ), 2.22 – 2.08 (2H, m), 1.80 – 1.72 (2H, m), 1.65 – 1.35 (2H, m), 0.80 – 0.79 (1H, m, cyclopropyl CH), 0.48 (2H, d,  $J$  = 8.0 Hz, cyclopropyl  $\text{CH}_2$  cis), 0.10 (2H, d,  $J$  = 8.0 Hz, cyclopropyl  $\text{CH}_2$  trans); ESIMS  $m/z$ : 343  $[\text{M} + 1]^+$ .

**Naltrexene O-benzyl oxime (11c)**—To a solution of naltrexene benzoate (3.02 g, 6.5 mmol), and sodium acetate (1.58 g, 19.2 mmol) in EtOH/ $\text{H}_2\text{O}$  (80 mL:5 mL), was added O-benzyl hydroxylamine hydrochloride (1.57 g, 9.8 mmol). The suspension of white solid was heated to reflux for 24 h. After evaporation to dryness the residue was diluted with  $\text{H}_2\text{O}$  (20 mL), basified with  $\text{Na}_2\text{CO}_3$  (pH 10), extracted into  $\text{CHCl}_3$  (3  $\times$  20 mL), washed with brine (20 mL), dried over  $\text{Na}_2\text{SO}_4$ , and concentrated under reduced pressure. Flash-chromatography eluent (5/95 MeOH/ $\text{CH}_2\text{Cl}_2$ ) afforded **11c** as a pale cream foam (2.6 g, 89%);  $R_f$  0.72;  $^1\text{H-NMR}$  (270 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.55 – 7.28 (5H, m,  $\text{CH}_{\text{Ar}}$ ), 6.60 (1H, d,  $J$  = 8.0 Hz,  $\text{H}_1$ ), 6.51 (1H, d,  $J$  = 8.0 Hz,  $\text{H}_2$ ), 5.35 (2H s, Ph- $\text{CH}_2$ -O), 5.00 (1H, brs, OH $_{14}$ ), 4.68 (1H, s,  $\text{H}_5$ ), 3.09 (1H, d,  $J$  = 5.5 Hz,  $\text{H}_9$ ), 3.05 (1H, d,  $J$  = 18.0 Hz  $\text{H}_{10\beta}$ ), 2.58 – 2.24 (6H, m), 1.87 (2H, d,  $J$  = 6.6 Hz, cyclopropylmethyl  $\text{CH}_2$ ), 1.59 – 1.58 (2H, m), 1.24 – 1.20 (1H, m), 0.86 – 0.83 (1H, m, cyclopropyl CH), 0.54 (2H, d,  $J$  = 8.0 Hz, cyclopropyl  $\text{CH}_2$  cis), 0.13 (2H, d,  $J$  = 8.0 Hz, cyclopropyl  $\text{CH}_2$  trans); ESIMS  $m/z$ : 447  $[\text{M} + 1]^+$ .

**3-*t*-Butyldimethylsilyloxy-17-cyclopropylmethyl-14 $\beta$ -hydroxy-4,5 $\alpha$ -epoxy-6 $\beta$ -(cinnamoylamino)morphinan (13)**—To a stirred solution of **3** (0.25 mmol), in  $\text{CH}_2\text{Cl}_2$

(5 mL) imidazole (0.67 mmol), and *t*-butyldimethylsilylchloride (0.25 mmol) were added at 0 °C. The suspension was stirred at r.t for 24 h. The reaction mixture then was diluted with H<sub>2</sub>O (10 mL), and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 10 mL). The combined organic extracts were washed with H<sub>2</sub>O, (20 mL) brine, (20 mL) and then dried over anhydrous MgSO<sub>4</sub> filtered and concentrated under reduced pressure. Flash-chromatography (10/90 EtOAc/Hexane) gave **13** (206 mg, 86%) of white powder. *R*<sub>f</sub> 0.86 (5/95 MeOH/CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H-NMR (270 MHz, CDCl<sub>3</sub>): δ 8.03 (1H, brs, NH), 7.55 (1H, d, *J* = 15.6 Hz, CH = CH), 7.48 – 7.47 (2H, m, CH<sub>Ar</sub>), 7.33–7.32 (3H, m, CH<sub>Ar</sub>), 6.68 (1H, d, *J* = 8 Hz, H<sub>1</sub>), 6.57 (1H, d, *J* = 8 Hz, H<sub>2</sub>), 6.43 (1H, d, *J* = 15.6 Hz, CH = CH), 4.40 (1H, d, *J* = 6.8 Hz, H<sub>5</sub>), 4.07 – 4.03 (1H, m, H<sub>6</sub>), 3.10 (1H, d, *J* = 5.5 Hz, H<sub>9</sub>), 3.03 (1H, d, *J* = 18.0 Hz H<sub>10β</sub>), 2.65 – 2.60 (2H, m), 2.35 (2H, d, *J* = 6.6 Hz, cyclopropylmethyl CH<sub>2</sub>), 2.34 – 2.18 (2H, m), 1.72 – 1.46 (5H, m), 0.92 (9H, s, Si-(CH<sub>3</sub>)<sub>3</sub>) 0.92 – 0.89 (1H, m, cyclopropyl CH), 0.51 (2H, d, *J* = 8.0 Hz, cyclopropyl CH<sub>2</sub> cis), 0.14 (6H, s, Si-(CH<sub>3</sub>)<sub>2</sub>), 0.12 (2H, d, *J* = 8.0 Hz, cyclopropyl CH<sub>2</sub> trans); ESIMS *m/z*: 587 [M + 1]<sup>+</sup>.

**14β-Acetoxyloxy-3-*t*-butyldimethylsilyloxy-17-cyclopropylmethyl-4,5α-epoxy-6β-(cinnamoylamino)morphinan (14a)**—**14a** was prepared using the general method **B** described above, to obtain 90 mg (56%) of white solid. *R*<sub>f</sub> 0.33 (5/95 MeOH/CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): δ 7.56 (1H, d, *J* = 15.6 Hz, CH = CH), 7.49 – 7.46 (2H, m, CH<sub>Ar</sub>), 7.36–7.34 (3H, m, CH<sub>Ar</sub>), 6.62 (1H, d, *J* = 8 Hz, H<sub>1</sub>), 6.53 (1H, d, *J* = 8 Hz, H<sub>2</sub>), 6.36 (1H, d, *J* = 15.6 Hz, CH = CH), 4.46 (1H, d, *J* = 6.8 Hz, H<sub>5</sub>), 4.33. (1H, d, *J* = 5.5 Hz, H<sub>9</sub>), 3.78 – 3.66 (1H, m, H<sub>6</sub>), 3.02 (1H, d, *J* = 18.0 Hz H<sub>10β</sub>), 2.70 – 2.40 (2H, m), 2.36 – 2.23 (4H, m), 2.11 (3H, s CO-CH<sub>3</sub>), 2.06 – 1.36 (5H, m), 0.92 (9H, s, Si-(CH<sub>3</sub>)<sub>3</sub>) 0.75 – 0.68 (1H, m, cyclopropyl CH), 0.43 (2H, d, *J* = 8.0 Hz, cyclopropyl CH<sub>2</sub> cis), 0.12 (6H, s, Si-(CH<sub>3</sub>)<sub>2</sub>), 0.05 – 0.04 (2H, m, cyclopropyl CH<sub>2</sub> trans); <sup>13</sup>C-NMR (CDCl<sub>3</sub>): δ 169.9, 140.9, 129.6, 128.8, 127.7, 122.2, 120.6, 118.8, 91.3, 83.0, 77.3, 77.0, 76.6, 59.3, 55.7, 52.3, 48.1, 44.1, 30.0, 25.6, 24.1, 23.1, 22.3, 9.6, 3.82, 3.5, –4.5, –4.6; ESIMS *m/z*: 629 [M + 1]<sup>+</sup>.

**14β-Acetoxyloxy-17-cyclopropylmethyl-4,5α-epoxy-3-hydroxy-6β-(cinnamoylamino)morphinan (15a)**—**15a** was prepared using the general method **C** described above, to obtain 60 mg (93%) of white solid. *R*<sub>f</sub> 0.27 (5/95 MeOH/CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): δ 8.03 (1H, brs, NH), 7.56 (1H, d, *J* = 15.6 Hz, CH = CH), 7.49 – 7.46 (2H, m, CH<sub>Ar</sub>), 7.36–7.34 (3H, m, CH<sub>Ar</sub>), 6.62 (1H, d, *J* = 8 Hz, H<sub>1</sub>), 6.53 (1H, d, *J* = 8 Hz, H<sub>2</sub>), 6.36 (1H, d, *J* = 15.6 Hz, CH = CH), 4.77 (1H, d, *J* = 6.8 Hz, H<sub>5</sub>), 4.25 (1H, d, *J* = 4.2 Hz, H<sub>9</sub>), 3.62 – 4.03 (1H, m, H<sub>6</sub>), 2.93 (1H, d, *J* = 18.0 Hz H<sub>10β</sub>), 2.62 – 2.61 (1H, m), 2.51 – 2.44 (1H m), 2.38 – 2.23 (3H, m), 2.107 (3H, s, CO-CH<sub>3</sub>) 2.07 – 1.86 (2H, m), 1.78 – 1.72 (1H, m), 1.28–1.18 (2H, m), 0.92 (1H, t, *J* = 5.0 Hz), 0.76–0.68 (1H, m, cyclopropyl CH), 0.44 (2H, d, *J* = 8.0 Hz, cyclopropyl CH<sub>2</sub> cis), 0.6 – 0.2 (2H, m, cyclopropyl CH<sub>2</sub> trans); <sup>13</sup>C-NMR (CDCl<sub>3</sub>): δ 170.8, 166.4, 141.2, 140.9, 139.9, 134.4, 129.7, 128.6, 127.7, 120.7, 119.6, 82.7, 59.3, 55.7, 52.3, 48.1, 44.1, 29.7, 26.1, 24.0, 22.9, 22.2, 9.5, 3.8, 3.5; ESIMS *m/z*: 515 [M + 1]<sup>+</sup>; Anal. (C<sub>31</sub>H<sub>34</sub>N<sub>2</sub>O<sub>5</sub>.HCl) CHN.

**β-Naltrexamine-3-*O*-methyl ether (17)**—**17** was prepared from 3-*O*-methylnaltrexone following the same procedure for formation of 6β-naltrexamine **10**, to obtain 2.41 g (69%) of white solid. *R*<sub>f</sub> 0.31 (1/5/94 NH<sub>3</sub>/MeOH/CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): δ 6.73 (1H, d, *J* = 8.0 Hz, H<sub>1</sub>), 6.60 (1H, d, *J* = 8.0 Hz, H<sub>2</sub>), 5.56 (2H brs, NH<sub>2</sub>), 4.48 (1H, d, *J* = 6.8 Hz, H<sub>5</sub>), 3.88 (3H, s, OCH<sub>3</sub>), 3.49 (1H, d, *J* = 5.5 Hz, H<sub>9</sub>), 3.23–3.20 (1H, m, H<sub>6</sub>), 3.02 (1H, d, *J* = 18.0 Hz H<sub>10β</sub>), 2.77 – 1.38 (11H, m), 0.86–0.83 (1H, m, cyclopropyl CH), 0.54 (2H, d, *J* = 8.0 Hz, cyclopropyl CH<sub>2</sub> cis), 0.19 (2H, d, *J* = 8.0 Hz, cyclopropyl CH<sub>2</sub> trans); ESIMS *m/z*: 357 [M + 1]<sup>+</sup>.



**17-Cyclopropylmethyl-4,5 $\alpha$ -epoxy-14 $\beta$ -hydroxy-3-methoxy-6 $\beta$ -(cinnamoylamino)morphinan (18)—18**

was prepared using the general method A described above, to obtain 31 mg (18%) of white powder.  $R_f$  0.66 (1/5/94  $\text{NH}_3/\text{MeOH}/\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.03 (1H, brs,  $\text{NH}$ ), 7.41 (1H, d,  $J = 15.6$  Hz,  $\text{CH} = \text{CH}$ ), 7.47 – 7.35 (2H, m,  $\text{CH}_{\text{Ar}}$ ), 7.34–7.33 (3H, m,  $\text{CH}_{\text{Ar}}$ ), 6.70 (1H, d,  $J = 8$  Hz,  $\text{H}_1$ ), 6.60 (1H, d,  $J = 8$  Hz,  $\text{H}_2$ ), 6.41 (1H, d,  $J = 15.6$  Hz,  $\text{CH} = \text{CH}$ ), 5.29 (1H, brs,  $\text{OH}_{14}$ ), 4.45 (1H, d,  $J = 6.8$  Hz,  $\text{H}_5$ ), 4.14 – 4.09 (1H, m,  $\text{H}_6$ ), 3.83 (3H, s,  $\text{OCH}_3$ ), 3.11 (1H, d,  $J = 5.5$  Hz,  $\text{H}_9$ ), 3.00 (1H, d,  $J = 18.0$  Hz  $\text{H}_{10\beta}$ ), 2.68 – 2.61 (2H, m), 2.35 (2H, d,  $J = 6.6$  Hz, cyclopropylmethyl  $\text{CH}_2$ ), 2.19 – 2.03 (2H, m), 1.73 – 1.24 (5H, m), 0.85 – 0.83 (1H, m, cyclopropyl  $\text{CH}$ ), 0.52 (2H, d,  $J = 8.0$  Hz, cyclopropyl  $\text{CH}_2$  cis), 0.12 (2H, d,  $J = 8.0$  Hz, cyclopropyl  $\text{CH}_2$  trans); ESIMS  $m/z$ : 487  $[\text{M} + 1]^+$ .

**14 $\beta$ -Allyloxy-17-cyclopropylmethyl-4,5 $\alpha$ -epoxy-3-methoxy-6 $\beta$ -(cinnamoylamino)morphinan (19b)—19b**

was prepared using the general method D described above, to obtain 2.19 g (83%) of white powder.  $R_f$  0.46 (1/5/94  $\text{NH}_3/\text{MeOH}/\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$ -NMR (270 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.16 (1H, brs,  $\text{NH}$ ), 7.66 (1H, d,  $J = 15.6$  Hz,  $\text{CH} = \text{CH}$ ), 7.45 – 7.31 (5H, m,  $\text{CH}_{\text{Ar}}$ ), 6.77 – 6.67 (3H, m,  $\text{H}_1$ ,  $\text{H}_2$ ,  $\text{CH} = \text{CH}$ ), 6.04 – 5.92 (1H, m, allyl  $\text{CH} = \text{CH}_2$ ) 5.44 – 5.13 (1H, m, allyl  $\text{CH}_2$  cis), 4.79 – 4.65 (1H, m, allyl  $\text{CH}_2$  trans), 4.24 – 4.20 (2H, q,  $\text{H}_5$   $\text{H}_6$ ), 3.85 (3H, s,  $\text{OCH}_3$ ), 3.70 – 3.68 (2H, s,  $\text{CH}_2\text{-CH=CH}_2$ ), 3.09 – 3.05 (2H, m,  $\text{H}_9$   $\text{H}_{10\beta}$ ), 2.65 – 2.58 (2H, m, cyclopropylmethyl  $\text{CH}_2$ ), 2.37 – 2.22 (3H, m), 1.63 – 1.42 (5H, m), 1.27 – 1.26 (1H, m), 0.87 - 0.83 (1H, m, cyclopropyl  $\text{CH}$ ), 0.51 (2H, d,  $J = 8.0$  Hz, cyclopropyl  $\text{CH}_2$  cis), 0.14 (2H, d,  $J = 8.0$  Hz, cyclopropyl  $\text{CH}_2$  trans); ESIMS  $m/z$ : 527  $[\text{M} + 1]^+$ .

**14 $\beta$ -Allyloxy-17-cyclopropylmethyl-4,5 $\alpha$ -epoxy-3-hydroxy-6 $\beta$ -(cinnamoylamino)morphinan (20b)—20b**

was prepared using the general method E described above, to obtain 76 mg (39%) of white powder.  $R_f$  0.61 (1/5/94  $\text{NH}_3/\text{MeOH}/\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$ -NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.08 (1H, brs,  $\text{NH}$ ), 7.57 (1H, d,  $J = 15.6$  Hz,  $\text{CH} = \text{CH}$ ), 7.57 – 7.54 (1H, m,  $\text{CH}_{\text{Ar}}$ ), 7.45–7.43 (1H, m,  $\text{CH}_{\text{Ar}}$ ), 7.41 – 7.32 (3H, m,  $\text{CH}_{\text{Ar}}$ ), 6.95 (1H, d,  $J = 15.6$  Hz,  $\text{CH} = \text{CH}$ ), 6.75 (1H, d,  $J = 8$  Hz,  $\text{H}_1$ ), 6.65 (1H, d,  $J = 8$  Hz,  $\text{H}_2$ ), 6.09 – 5.94 (1H, m, allyl  $\text{CH} = \text{CH}_2$ ) 5.41 (1H, d,  $J = 17.0$  Hz, allyl  $\text{CH}_2$  cis), 5.32 – 5.14 (1H, m, allyl  $\text{CH}_2$  trans), 4.68 (1H, d,  $J = 7$  Hz,  $\text{H}_5$ ), 4.32 – 4.00 (3H, m,  $\text{H}_6$ ,  $\text{CH}_2\text{-CH=CH}_2$ ), 3.14 (1H, d,  $J = 5.5$  Hz,  $\text{H}_9$ ), 3.08 (1H, d,  $J = 18.7$  Hz  $\text{H}_{10\beta}$ ), 2.72 – 2.62 (2H, m), 2.42 (2H, d,  $J = 6.6$  Hz, cyclopropylmethyl  $\text{CH}_2$ ), 2.31 – 2.12 (2H, m), 1.65 – 1.24 (5H, m), 0.93 - 0.88 (1H, m, cyclopropyl  $\text{CH}$ ), 0.54 (2H, d,  $J = 8.0$  Hz, cyclopropyl  $\text{CH}_2$  cis), 0.16 (2H, d,  $J = 8.0$  Hz, cyclopropyl  $\text{CH}_2$  trans);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ ):  $\delta$  168.2, 143.4, 143.0, 142.5, 140.3, 139.9, 135.1, 134.8, 129.7, 129.3, 128.8, 127.9, 127.8, 119.0, 118.7, 118.4, 117.2, 70.0, 62.3, 62.1, 59.1, 47.8, 47.7, 43.9, 43.8, 30.6, 30.3, 22.7, 22.6, 9.4, 3.8, 3.7; ESIMS  $m/z$ : 513  $[\text{M} + 1]^+$ ; Anal. ( $\text{C}_{31}\text{H}_{35}\text{N}_2\text{O}_4 \cdot \text{HCl} \cdot \text{H}_2\text{O}$ ) CHN.

## Biology

**Materials**— $[\text{}^3\text{H}]$ diprenorphine and  $[\text{}^{35}\text{S}]\text{GTP}\gamma\text{S}$  were from Perkin Elmer Life and Analytical Sciences (Boston, MA). BCA protein assay reagents were from Pierce (Rockford, IL). Cell culture reagents were from Invitrogen (Carlsbad, CA). Sigma Cote, U69593, DAMGO and GDP were from Sigma-Aldrich (St. Louis, MO). EcoLume scintillation cocktail was from ICN (Aurora, OH). Whatman GF/C glass-fiber filtermats were from Brandel (Gaithersburg, MD).

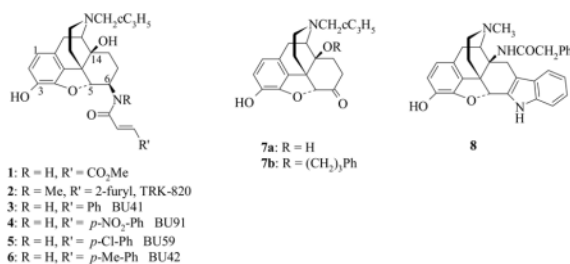
**Cell Culture**—C6 glioma cells stably expressing the rat mu-opioid receptor ( $\text{C6}\mu$ )<sup>25</sup> were grown in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 90 units/ml penicillin, 90  $\mu\text{g}/\text{ml}$  streptomycin, and 0.5 mg/ml geneticin. Chinese hamster ovary cells stably expressing the human kappa-opioid receptor ( $\text{CHO}\kappa$ )<sup>24</sup>

were maintained similarly, except that DMEM-F12 was used. Both cell lines were grown under 5% CO<sub>2</sub> at 37 °C.

**Preparation of membranes from C6<sub>3</sub>H or CHO<sub>k</sub> cells:** Cells were washed three times with 1X PBS and detached from plates using 37 °C harvesting buffer (20 mM HEPES, pH 7.4, 150 mM NaCl, and 0.68 mM EDTA). After centrifugation at 200 × g for 3 min, the cell pellet was resuspended in 50 mM Tris-HCl, pH 7.4 and homogenized with a Tissue Tearor (Biospec Products, Inc.) 10 times at setting 4. The homogenate was pelleted at 20,000 × g for 20 min at 4 °C and the pellet was resuspended in 50 mM Tris-HCl, pH 7.4 and homogenized with a Tissue Tearor 5 times at setting 2. The final pellet was resuspended in 50 mM Tris-HCl, pH 7.4 and frozen in aliquots at -80 °C. The protein concentrations of the aliquots were determined using the BCA protein assay.

**[<sup>3</sup>H]diprenorphine competitive binding assay**—Due to the stickiness of the compounds plasticware was precoated with Sigma Cote. Membranes (20 µg) were incubated in 50 mM Tris-HCl, pH 7.4 with 0.2 nM [<sup>3</sup>H]diprenorphine in the absence or presence of varying concentrations of unlabeled compound for 60 min in a shaking water bath at 25 °C. Nonspecific binding was measured using 10 µM naloxone. Samples were filtered through GF/C glass-fiber filtermats mounted on a Brandel cell harvester and rinsed four times with 4 °C 50 mM Tris-HCl, pH 7.4 buffer. Filtermats were dried and 0.1 ml EcoLume scintillation cocktail was added to each sample area to soak the filter. Each filtermat was placed in a polyethylene bag, heat sealed, and radioactivity retained in each sample area was counted in a Wallac 1450 MicroBeta Liquid Scintillation and Luminescence Counter. *K<sub>i</sub>* values were determined using GraphPad Prism.

**[<sup>35</sup>S]GTPγS Binding Assay**—Due to the stickiness of the compounds plasticware was precoated with Sigma Cote. As described previously,<sup>20</sup> membranes (20 µg) were incubated in 20 mM Tris-HCl, pH 7.4, 5 mM MgCl<sub>2</sub>, 100 mM NaCl, 2.2 mM dithiothreitol (prepared freshly), 30 µM GDP, 0.1 nM [<sup>35</sup>S]GTPγS, and 10 µM compound or Super Q H<sub>2</sub>O. The membranes were incubated for 60 min in a shaking water bath at 25 °C. Samples were filtered through GF/C glass-fiber filtermats mounted on a Brandel cell harvester and rinsed four times with 4 °C 50 mM Tris-HCl, pH 7.4, 5 mM MgCl<sub>2</sub>, and 100 mM NaCl. Filtermats were then processed as described above. EC<sub>50</sub> and relative efficacy values were calculated using GraphPad Prism.



## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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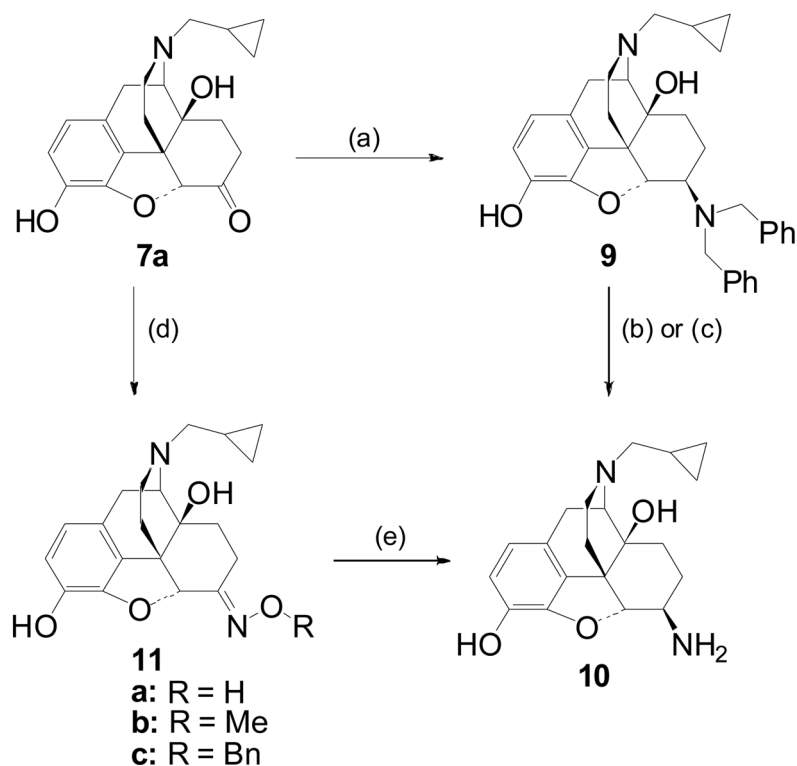
## Abbreviations

|              |                         |
|--------------|-------------------------|
| $\beta$ -FNA | $\beta$ -funaltrexamine |
| $\beta$ -NTA | $\beta$ -naltrexamine   |

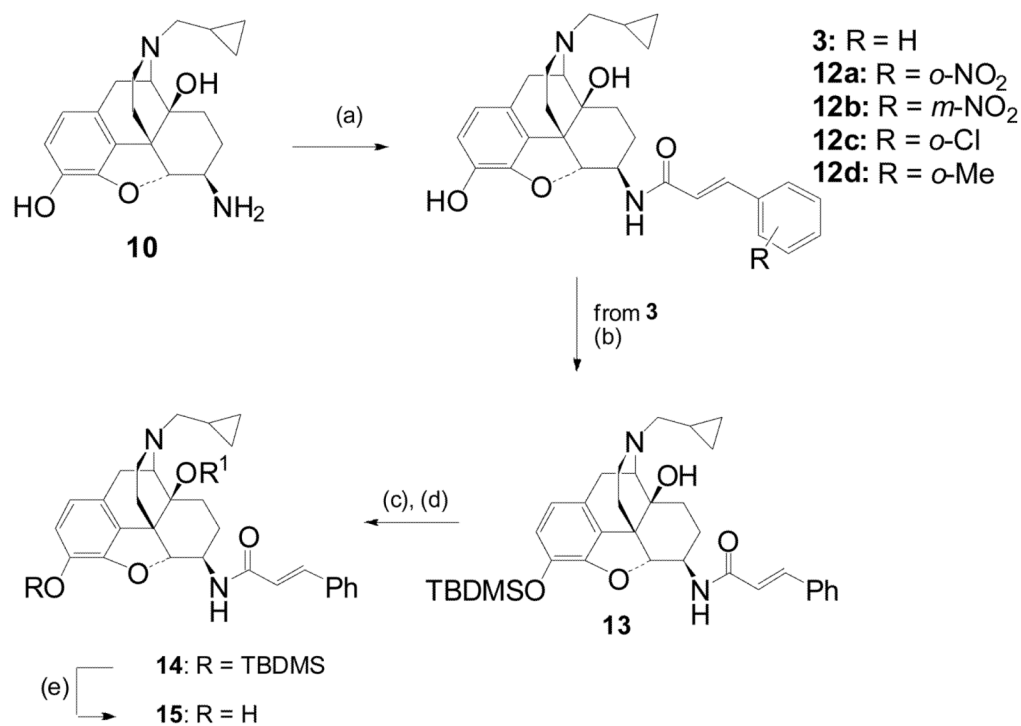
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**Scheme 1.**

*Reagents and conditions:* (a)  $\text{HNBn}_2$ ,  $\text{PhCOOH}$ ,  $\text{TsOH}$  then  $\text{NaCNBH}_3$ ,  $\text{EtOH}$ ,  $\Delta$ , 20 h, (69%); (b) 10%  $\text{Pd/C}$ , 40 PSI,  $\text{H}_2$ ,  $\text{MeOH/HCl}$ , 3 days; c) 10%  $\text{Pd/C}$ , 1:1 Cyclohexene/ $\text{MeOH}$ ,  $\Delta$ , 24 h; (d) *o*-Benzylhydroxylamine,  $\text{NaOAc}$ ,  $\text{EtOH/H}_2\text{O}$ ,  $\Delta$ , 20h; (e)  $\text{ZrCl}_4/\text{NaBH}_4$ ,  $\text{THF}$ ,  $\Delta$ , 24h 42% (for R = Bn).

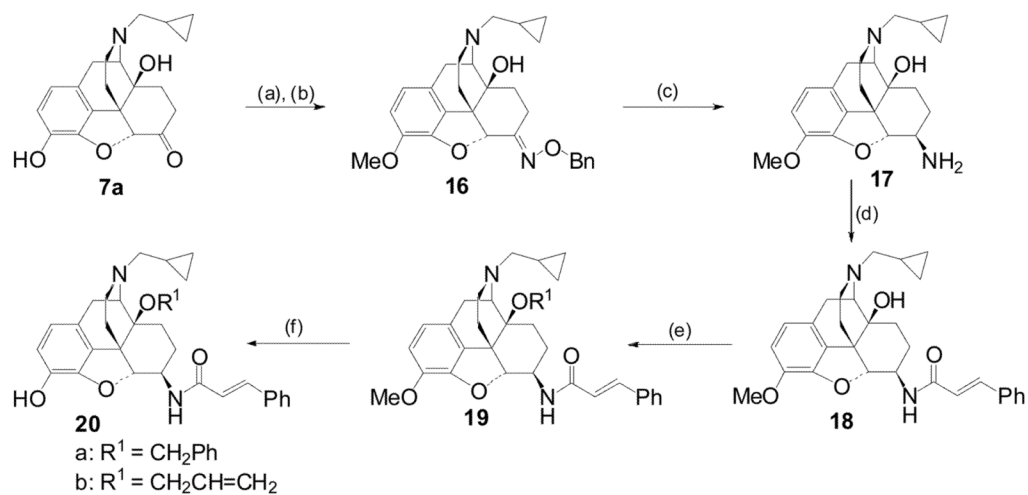


a: R<sup>1</sup> = COCH<sub>3</sub>, b: R<sup>1</sup> = COPh, c: R<sup>1</sup> = COCH<sub>2</sub>Ph

**Scheme 2.**

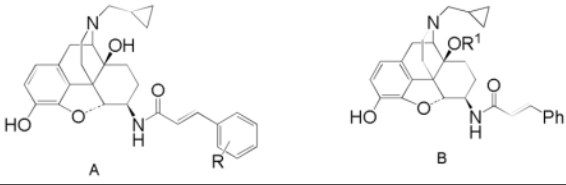
*Reagents and conditions:* (a) RC<sub>6</sub>H<sub>4</sub>CH=CHCOCl, NEt<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 25°C, 0.5h (b) TBDMSCl, imidazole, DCM, (73%); (c) anhydride (R'OR'), toluene, reflux, 24h (d) TBAF, THF, RT.



**Scheme 3.**

*Reagents and conditions:* a) MeI,  $\text{K}_2\text{CO}_3$ , MeCOMe/ $\text{H}_2\text{O}$ , (73 %); b) *o*-Benzylhydroxylamine, NaOAc, EtOH/ $\text{H}_2\text{O}$ ; (c)  $\text{ZrCl}_4/\text{NaBH}_4$ , THF,  $\Delta$ , 24h, (69%); (d) Cinnamoyl chloride,  $\text{Et}_3\text{N}$ , DCM, RT, (83%); (e)  $\text{R}^1\text{Br}$ , NaH, DMF; (f)  $\text{BBr}_3$ , DCM, 0 °C.

**Table 1**Binding affinities determined by displacement of [<sup>3</sup>H]diprenorphine binding to C6-μ and CHO-κ membranes

|  |   |               |                |
|---|---|---------------|----------------|
| Comp'd  | Type & Substituent  | Ki/nM         |                |
|   |   | Mu            | Kappa          |
| <b>3</b>  | A: R = H  | 0.17 ± 0.091  | 0.043 ± 0.0022 |
| <b>12a</b>  | A: R = <i>o</i> -NO <sub>2</sub>                                    | 0.35 ± 0.13   | 0.30 ± 0.094   |
| <b>12b</b>  | A: R = <i>m</i> -NO <sub>2</sub>                                    | 0.11 ± 0.036  | 0.36 ± 0.32    |
| <b>12c</b>  | A: R = <i>o</i> -Cl   | 0.079 ± 0.027 | 0.27 ± 0.062   |
| <b>12d</b>  | A: R = <i>o</i> -Me   | 0.085 ± 0.033 | 0.34 ± 0.16    |
| <b>20a</b>  | B: R <sup>1</sup> = CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>   | 0.16 ± 0.010  | 0.86 ± 0.22    |
| <b>20b</b>  | B: R <sup>1</sup> = CH <sub>2</sub> CH=CH <sub>2</sub>              | 0.25 ± 0.074  | 0.12 ± 0.014   |
| <b>15a</b>  | B: R <sup>1</sup> = COCH <sub>3</sub>                               | 0.21 ± 0.059  | 0.59 ± 0.069   |
| <b>15b</b>  | B: R <sup>1</sup> = COC <sub>6</sub> H <sub>5</sub>                 | 0.42 ± 0.19   | 1.1 ± 0.097    |
| <b>15c</b>  | B: R <sup>1</sup> = COCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub> | 0.28 ± 0.055  | 0.26 ± 0.0098  |

Values are an average ± SEM from three separate experiments

Table 2

Stimulation of [<sup>35</sup>S]GTPγS binding to C6-μ and CHO-κ membranes

| Comp'd     | Type & Substituent  | Mu           | Kappa             |                      |           |
|------------|---|--------------|-------------------|----------------------|-----------|
|            |   |              | %DAMGO            | EC <sub>50</sub> /nM | %U69593   |
| <b>3</b>   | A: R = H  | 1.8 ± 0.79   | 21 ± 1.5          | 0.12 ± 0.037         | 100 ± 4.5 |
| <b>12a</b> | A: R = <i>o</i> -NO <sub>2</sub>                                    | 0.69 ± 0.31  | 6.7 ± 0.73        | 1.9 ± 0.055          | 81 ± 2.4  |
| <b>12b</b> | A: R = <i>m</i> -NO <sub>2</sub>                                    | 0.18 ± 0.062 | 20 ± 3.4          | 0.12 ± 0.039         | 100 ± 3.3 |
| <b>12c</b> | A: R = <i>o</i> -Cl   | 12 ± 8.7     | 17 ± 1.6          | 0.40 ± 0.16          | 97 ± 3.1  |
| <b>12d</b> | A: R = <i>o</i> -Me   | 2.1 ± 2.0    | 21 ± 1.4          | 0.95 ± 0.69          | 105 ± 6.0 |
| <b>20a</b> | B: R <sup>1</sup> = CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>   | 2.0 ± 0.38   | 19 ± 2.1          | 0.78 ± 0.16          | 97 ± 6.2  |
| <b>20b</b> | B: R <sup>1</sup> = CH <sub>2</sub> CH=CH <sub>2</sub>              | 0.60 ± 0.10  | 39 ± 1.8          | 0.079 ± 0.012        | 120 ± 3.0 |
| <b>15a</b> | B: R <sup>1</sup> = COCH <sub>3</sub>                               | 1.0 ± 0.15   | 19 ± 0.86         | 2.4 ± 0.99           | 97 ± 7.1  |
| <b>15b</b> | B: R <sup>1</sup> = COC <sub>6</sub> H <sub>5</sub>                 | -            | N.S. <sup>a</sup> | 9.8 ± 5.3            | 26 ± 6.0  |
| <b>15c</b> | B: R <sup>1</sup> = COCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub> | 1.3 ± 0.21   | 41 ± 3.9          | 0.49 ± 0.053         | 76 ± 2.1  |

Values are an average ± SEM from three separate experiments

<sup>a</sup>N.S. = No stimulation; **15b** was evaluated as an antagonist at MOR, Ke (vs DAMGO) 0.41 ± 0.12 nM