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Probes for narcotic receptor mediated phenomena. 46.° *N*-Substituted-2,3,4,9,10,10a-hexahydro-1*H*-1,4a-(epiminoethano)phenanthren-6- and 8-ols - carbocyclic relatives of f-oxide bridged phenylmorphans

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Abstract

Oxide-bridged phenylmorphans were conceptualized as topologically distinct, structurally rigid ligands with 3-dimensional shapes that could not be appreciably modified on interaction with opioid receptors. An enantiomer of the *N*-phenethyl-substituted *ortho*-f isomer was found to have high affinity for the μ-receptor (*K*i = 7 nM) and was about four times more potent than naloxone as an antagonist. In order to examine the effect of introduction of a small amount of flexibility into these molecules, we have replaced the rigid 5-membered oxide ring with a more flexible 6-membered carbon ring. Synthesis of the new *N*-phenethyl-substituted tricyclic *N*-substituted-2,3,4,9,10,10a-hexahydro-1*H*-1,4a-(epiminoethano)phenanthren-6- and 8-ols resulted in a two carbon-bridged relative of the f-isomers, the dihydrofuran ring was replaced by a cyclohexene ring. The carbocyclic compounds had much higher affinity and greater selectivity for the μ-receptor than the f-oxide-bridged phenylmorphans. They were also much more potent μ-antagonists, with activities comparable to naltrexone in the [^{35}S]GTPγS assay.

Keywords

Synthesis; opioid receptor binding and efficacy; *N*-substituted-2,3,4,9,10,10a-hexahydro-1*H*-1,4a-(epiminoethano)phenanthren-6- and 8-ols; f-oxide-bridged phenylmorphans; opioid antagonist

°Probes 45 – see reference[1].

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1. Introduction

We have reported the synthesis of all of the possible a- through f-oxide-bridged phenylmorphans (**1**, Figure 1)[2-16]. The f- and the e-oxide bridged phenylmorphans were of interest because an enantiomer of the *N*-phenethyl-substituted *ortho*-f isomer had high affinity to the μ-receptor (*K*i = 7 nM) and was about 4 times more potent than naloxone as an antagonist (**2**, Fig. 1). In contrast, an enantiomer of the *para*-e isomer had morphine-like agonist activity. The *N*-phenethyl *ortho*-b isomer was a moderately selective kappa antagonist, and the *N*-phenethyl substituted *ortho*-c isomer had very high affinity for μ-receptors (*K*i = 1 nM), and was a very potent μ- and κ-antagonist (*K*e = 0.7 and 3 nM, respectively). Prior to the determination of the structures of the opioid receptors, we believed that a structurally rigid ligand that was selective and had high affinity to an opioid receptor would give us insight into the spatial requirements necessary for interaction with the amino acids in the receptor binding pocket. Since the oxide-bridged phenylmorphans were rigid ligands, an element of ambiguity introduced by the flexibility of most ligands would be removed. The recent determination of the crystal structures of all of the opioid receptors[17-20] will allow us to examine the ligand-receptor interaction differently in the future.

Having examined the affinity and activity of the rigid oxide-bridged phenylmorphans, we considered the possibility of allowing a small amount of determinable flexibility into some of these compounds to see whether that would alter their affinity or activity. It has been previously shown that the affinity and selectivity of cannabinoids for the CB_1 and CB_2 receptors could be very much altered, by enlarging a 5-membered ring to a 6-membered ring (**3**, Fig. 1)[21]. We thought it would be of interest to see whether this modification could also be applied to the f-isomers of the oxide-bridged phenylmorphans, where the 5-membered oxide ring would be changed to a 6-membered carbocyclic structure. The f-oxide-bridged phenylmorphans were initially chosen because they were pharmacologically interesting and synthetically more accessible than many of the other oxide-bridged phenylmorphans. Also, a path to the *N*-methyl substituted 2,3,4,9,10,10a-hexahydro-1*H*-1,4a-(epiminoethano)phenanthren-6-ols had been previously reported[22, 23].

2. Chemistry

The synthesis of the target compounds was straightforward and started from the known 9-oxomorphan **4** (5-(3-methoxyphenyl)-2-methyl-9-oxo-morphan), which was prepared in six steps using modified literature procedures (Scheme **1**)[12, 24-27]. The condensation of compound **4** and malononitrile proceeded normally to give the unsaturated dinitrile **5** in good yield[22]. Catalyzed hydrogenation of **5** with platinum oxide was not chemically selective and gave two diastereomers in a 7:1 ratio. Fortunately, the desired isomer was separable by flash chromatography and the stereochemically pure, saturated dinitrile **6** was obtained in good yield. Palladium on charcoal was unsuccessfully used to attempt to improve the ratio of the two isomers, at both atmosphere pressure and higher pressure (50 psi). Platinum oxide worked under both conditions, although the reaction was slower (40 h or longer) at atmospheric pressure. Under higher pressure (50 psi), and lengthy reaction times (> 4 h), a very polar uncharacterized by-product was obtained. Saturated dinitrile **6** was smoothly cyclized in refluxing hydrochloric acid (10 M) to ketophenanthrene-type structures, in good yield, aided by the electron-donating methoxy group. 3-*O*-Demethylation was achieved with refluxing hydrobromic acid, to afford cyclized regioisomers **7** and **8**, easily isolated by chromatography. The *ortho*-hydroxyphenyl isomer **7** was much less polar than the *para*-isomer **8**, possibly due to hydrogen bonding between the phenolic hydroxyl and the C9-ketone. *N*-Demethylation of **7** and **8** with ethyl chloroformate and subsequent

realkylation with phenethyl bromide afforded the corresponding *N*-phenenthyl analogues **9** (Scheme 2) and **18** (Scheme 3). Because the reduction of these ketones with $NaBH_4$ was very sluggish, a number of reducing agents and conditions were explored (Table 1).

We found that superhydride and $LiAlH_4$ (conditions 2 and 3 in Table 1) at a low temperature rapidly reduced **7** and **8** in the *N*-methyl series and **9** and **18** in the *N*-phenethyl series. Interestingly, the reduction of *ortho*-hydroxyphenyl **7** gave two stereoisomers, **10** with an 9α-OH group, and **11** with the 9β-OH (Scheme 2), while reduction of the *para*--hydroxyphenyl compound **8** under the same conditions only gave the single isomer **19** with a 9α-OH (Scheme 3). The low yields of **10** and **11** may be due to the repeated purification that was needed.

The difference in reactivity of the *ortho*- and *para*-hydroxyphenyl compounds might be attributed to intramolecular hydrogen bonding between the phenolic hydroxyl and the carbonyl group in **7**, the *ortho*-hydroxyphenyl compound, that is not possible in **8**. Compounds **12**, **13**, and **22** were obtained similarly. The relative configurations of compounds **12**, **19** and **22** were confirmed by X-ray crystallographic analyses (Figure 1), and the relative configurations of compounds **11** and **13** were confirmed by comparing the polarity and the chemical shift of the benzyl proton with those of compound **12**. Compounds **10** and **12** were less polar (by tlc) than corresponding isomers **11** and **13**. On the other hand, the chemical shifts of the benzyl proton in the proton NMR spectra of **10** and **12** with 9α-OH (δ 4.92 ppm and 4.99 ppm, respectively) were at higher field than those of compounds **11** and **13** with a 9β-OH (δ 5.02 ppm and 5.23 ppm, respectively), possibly because of the deshielding effect of the *ortho*-hydroxyphenyl group. These benzylic alcohols were very susceptible to dehydration under acidic conditions to give the corresponding phenanthrene-like compounds **14**, **16**, **20** and **23** in good to excellent yield. The double bond was saturated by catalytic hydrogenation to give the more flexible corresponding compounds **15**, **17**, **21** and **24**, in good yield (Schemes 2 and 3).

3. Results and discussion

The μ, δ, and κ-opioid receptor binding affinities of **7-25** were determined by an initial screen (see experimental, section 5.3.2). Ten of these compounds (**10**, **12-14**, **16-17**, **20**, **22-24**) were estimated to have a *K*i < 60 nM. These were examined in the full binding assay (Table 2). Only a few of the remaining compounds, **18** and **20,** had an estimated *K*i (μ) of < 100 nM (80 and 67 nM, respectively), and **9**, **11**, and **25** had an estimated *K*i (μ) of > 200 nM (estimated *K*i (μ) = 297, 258, and 227, respectively); these were not examined further, nor were the remaining compounds that were found to have an estimated *K*i > 1 μM.

In Table 2, only those compounds that were found to have *K*i < 50 nM in the full binding assay were examined for their efficacy in the [^{35}S]GTPγS assay. It is apparent in Table 2 that the *N*-methyl substituted compounds had less affinity than the comparable *N*-phenethyl substituted relatives. Compound **10**, the best of the *N*-methyl compounds, had, for example, half the affinity of the comparable *N*-phenethyl-substituted relative, compound **12**, and, unlike **12**, had almost no efficacy in the [^{35}S]GTPγS assay (μKe > 700 nM). Among the *N*-phenethyl substituted compounds, those with a C9-$\tilde{\beta}$ OH showed less μ-receptor affinity than the comparable C9-$\tilde{\alpha}$OH compounds. Compound **31**, for example, had less than a tenth of the affinity of **12**, a similar compound, but with a C9-$\tilde{\alpha}$OH moiety. All of the C9-OH substituted compounds in both the *ortho*-hydroxyphenyl series or in the *para*-hydroxyphenyl series, except for **12**, were generally seen to have less affinity and efficacy than comparable compounds lacking that C9-OH. At that C9-position in this new tricyclic carbocyclic opioid series, both the carbonyl and, usually, the hydroxyl group appear to have a deleterious effect. The effect of oxygen at C9 seems to become more damaging when it is present in the

para-hydroxyphenyl compounds (e.g., compound **22**). This observation has precedent in that introduction of a keto group at the benzyl position has been found to generally decrease the affinity of morphinans at μ-, δ-, and κ-opioid receptors[28]. Removal of the C9-$\tilde{\alpha}$OH moiety in the *ortho*-hydroxyphenyl series (**12**), to form the unsaturated compound **16**, or its saturated relative **17**, does not appreciably improve μ-affinity. Both **16** and **17** have about the same affinity as the C9-$\tilde{\alpha}$OH compound **21**. Indeed, both **12** and **17** also have about the same antagonist efficacy in the [^{35}S]GTPγS assay (μ*K*e = 6 or 7 nM). The unsaturated relative, the *ortho*-hydroxyphenyl compound **16**, however, was found to have surprisingly good efficacy (μ*K*e = 1.7 nM); efficacy was improved in that compound by removal of the C9-$\tilde{\alpha}$OH group.

A considerable improvement in affinity (μ*K*i = ca. 2 nM), and a remarkable increase in efficacy (μ*K*e = 0.3 to 0.7 nM), was found in the unsaturated and saturated carbocyclic compounds **23** and **24**, in the *para*-hydroxyphenyl series. Thus, removal of the C9-OH group in the *para*-hydroxyphenyl series, where the presence of a C9-OH group is greatly disadvantageous, gave us μ-antagonists of great potency, comparable to naltrexone. Unfortunately, like all of the known μ-receptor antagonists, selectivity for the μ-opioid receptor was compromised in **23** and **24** in that they had some affinity (*K*i = 50 to 60 nM) for the κ-opioid receptor, and the μ/κ ratio was 20 to 27 (Table 2). Thus, the carbocyclic compounds had reasonable, but not excellent selectivity for the μ-receptor.

4. Conclusion

A tricyclic ring system was conceptualized to have greater flexibility than the f-isomers of the oxidebridged phenymorphans, and a few of the synthesized compounds were found to have high μ-opioid receptor affinity and exceptionally potent antagonist activity. These carbocyclic compounds had greater affinity for μ-opioid receptors and were much more potent as μ-opioid antagonists than the original f-oxide-bridged phenymorphans with a dihydrofuran ring.

It was initially thought that the slight relaxation of the rigid framework of the oxide-bridged phenylmorphan that was allowed by substituting a 6-membered carbocyclic ring for the 5-membered dihydrofuran ring in the oxide-bridged phenylmorphans would be sufficient to permit more extensive interaction with the amino acids in the binding site of the μ-opioid receptor; however, the fact that both the unsaturated compound **23** and its less flexible saturated relative **24** had similar affinity and efficacy did not lend weight to that hypothesis. Further exploration will be undertaken in the future to determine if the slightly different spatial positions of the heteroatoms in the carbocyclic molecules caused by increasing the size of the ring from a 5- to a 6-membered ring caused the observed increase in affinity and potency. Alternatively, alteration of the polarity and H-bonding characteristics of the original dihydrofuro compound by the substitution of two carbon atoms for an oxygen could also affect affinity and potency.

5. Experimental section

5.1. Synthetic chemistry

5.1.1. General—Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR, 400 or 500 MHz) and carbon nuclear magnetic resonance (^{13}C NMR, 100 or 125 MHz) spectra were recorded on a Bruker DMX500 wide-bore spectrometer in $CDCl_3$ (unless otherwise noted) with the values given in ppm and *J* (Hz) assignments of ^{1}H resonance coupling. For ^{1}H NMR spectra ($CDCl_3$), the residual solvent peak was used as the reference (7.26 ppm) while the central solvent peak was used as the ^{13}C NMR reference (77.0 ppm in $CDCl_3$). The

high-resolution electrospray ionization (ESI) mass spectra were obtained on a Waters LCT Premier time-of-flight (TOF) mass spectrometer. Thin-layer chromatography (TLC) was performed on 0.25 mm Analtech GHLF silica gel and used to determine the completion of the reaction (solvent system: chloroform/methanol/ammonia (19:0.9:0.1 or 9:0.9:0.1) depending on the polarity of the compounds. Flash column chromatography was performed with Bodman silica gel LC 60 A. Elemental analyses were performed by Micro-Analysis, Inc, Wilmington, DE, and were within 0.4% for C, H, and N. IR spectra were recorded on a PerkinElmer Spectrum One FT-IR spectrometer.

5.1.2. 2-(5-(3-methoxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-9-ylidene)malononitrile (5)—A mixture of 5-(3-methoxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-9-one hydrochloride **4** (2.96g, 10 mmol), malononitrile (0.92 g, 14 mmol), ammonium acetate (0.23 g, 3 mmol) and acetic acid (0.5 mL) in toluene was heated azeotropically for 2 h. After cooling to ambient temperature, the mixture was extracted with 2 N HCl. The aqueous layer was cooled in ice-bath, basified with 28% NH_4OH to pH 9 and extracted with $CHCl_3$. The combined extracts were washed with brine and dried over anhydrous Na_2SO_4. The crude product was purified by flash chromatography (silica, gradient: $CHCl_3$ to 3% MeOH/NH_4OH/$CHCl_3$) to afford the racemic 5-(3-methoxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-9-ylidenemalononitrile (**5**) in 73% yield (2.26 g) as a yellow oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.31 (t, *J* = 7.5 Hz, 1H), 6.98-6.88 (m, 3H), 4.06 (s, 1 H), 3.81 (s, 3H), 3.23-3.19 (m, 1H), 2.73-2.70 (m, 1H), 2.63-2.57 (m, 1H), 2.54 (s, 3H), 2.44-2.41 (m, 2H), 2.31-2.15 (m, 3H), 1.79-1.76 (m, 1H), 1.72-1.66 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) (mixture of two rotamers) δ 188.6, 188.5, 161.0, 160.0, 154.6, 154.5, 130.1, 129.8, 121.5, 119.1, 115.4, 114.2, 113.8, 113.4, 112.3, 109.4, 82.4, 64.5, 64.4, 55.44, 55.36, 50.1, 50.0, 47.9, 47.8, 43.9, 43.8, 41.6, 41.4, 40.4, 32.4, 32.1, 20.3, 20.2; IR (film) νmax = 3017, 2937, 2854, 2226, 1722, 1602, 1583, 1449, 1219, 1046, 1033, 772, 701 cm^{-1}; ESI-MS 308.2 (M^++1); HRMS (ES^+) calcd for $C_{19}H_{22}N_3O$ $(M + H)^+$ 308.1673; found 308.1671.

5.1.3.5-(3-Methoxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-9-yl)-malononitrile (6)—Compound **5** (6 g, 19.5 mmol) was dissolved in MeOH (100 mL) in a glass bomb and PtO_2 (0.44 g,1.95 mmol) was added. The bomb was placed in a Parr apparatus, evacuated and backfilled with H_2 three times, then filled with H_2 to 50 psi and shaken for 4 h. The reaction mixture was filtered through a pad of celite and the filtrate was concentrated. The residue was purified by flash chromatography (silica, gradient: $CHCl_3$ to 5% MeOH/0.5%NH_4OH/$CHCl_3$) to yield 5-(3-methoxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-9-ylmalononitrile (**6**) as a yellow foam (4.8 g, 80%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.31 (t, *J* = 8.0 Hz, 1H), 6.98 (d, *J* = 7.5 Hz, 1H), 6.94 (s, 1H), 6.83 (dd, *J* = 8.0, 2.0 Hz, 1H), 3.80 (s, 3 H), 3.66 (d, *J* = 6.5 Hz, 1H), 3.23 (s, 1H), 3.08 (td, *J* = 12.5, 5.0 Hz, 1H), 2.87-2.83 (m, 2H), 2.52 (s, 3H), 2.42-2.33 (m, 2H), 2.21 (dd, *J* = 16.5, 6.0 Hz, 1H), 2.06-1.92 (m, 3H), 1.79 (dd, *J* = 14.5, 4.5 Hz, 1H), 1.71-1.66 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 160.2, 147.2, 130.2, 118.1, 112.9, 112.7, 112.1, 112.0, 55.8, 55.4, 50.9, 48.1, 43.2, 41.6, 37.8, 28.0, 22.5, 21.0, 17.7; ESI-MS 310.2 (M^++1); HRMS (ES^+) calcd for $C_{19}H_{24}N_3O$ $(M + H)^+$ 310.1919; found 310.1922. The free base was converted into its HCl salt for analysis, mp 253.0~256.8 °C. IR (film) νmax = 3408, 2937, 2836, 2485, 2409, 1608, 1583, 1493, 1454, 1434, 1219, 1049, 771 cm^{-1}. Anal. calcd for ($C_{19}H_{23}N_3O$·HCl·0.3H_2O) C, 64.97; H, 7.08; N, 11.96; found C, 64.91; H, 7.38; N, 11.84.

5.1.4 8-Hydroxy-13-methyl-3,4,10,10a-tetrahydro-1H-1,4a-(epiminoethano)phenanthren-9(2H)-one (7) and 6-hydroxy-13-methyl-3,4,10,10a-tetrahydro-1H-1,4a-(epiminoethano)phenanthren-9(2H)-one (8)—The free base **6** (7.1 g, 22.9 mmol) was dissolved in 10 N HCl (80 mL) and the

resulting solution was heated to reflux under argon for 16 h. The solvent was removed and the residue was redissolved in 48% HBr (40 mL) and heated to reflux under argon for 24 h. After the solvent was removed, the residue was treated with crushed ice and basified with 28% NH_4OH to pH 9-9.5. The mixture was extracted with a mixed solvent of $CHCl_3$ and MeOH (20:1, v/v) several times. The combined extracts were washed with brine and dried over Na_2SO_4. After filtration and concentration, the crude product was purified by flash chromatography (silica, gradient: 5-10% MeOH/1% $NH_4OH/CHCl_3$) to give 8-hydroxy-13-methyl-3,4,10,10a-tetrahydro-1*H*-1,4a-(epiminoethano)phenanthren-9(2*H*)-one (**7**) as a brown oil (2.1 g, 34%) and 6-hydroxy-13-methyl-3,4,10,10a-tetrahydro-1*H*-1,4a-(epiminoethano)-phenanthren-9(2*H*)-one (**8**) as a brown foam (3.29 g, 53%).

7: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.7 (s, 1H, -OH), 7.37 (t, *J* = 8.0 Hz, 1H), 6.79-6.74 (m, 2H), 3.09 (td, *J* = 12.5, 5.0 Hz, 1H), 2.96-2.88 (m, 2H), 2.67 (d, *J* = 1.5 Hz, 1H), 2.48-2.33 (m, 3H), 2.42 (s, 3H), 2.03 (dd, *J* = 14.0, 4.0 Hz, 1H), 1.96-1.89 (m, 1H), 1.88-1.78 (m, 2H), 1.63-1.60 (m, 2H), 1.45-1.38 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 204.4, 163.1, 154.4, 136.8, 115.6, 115.4, 115.3, 57.0, 51.6, 42.9, 41.5, 38.6, 36.8, 34.5, 33.3, 21.7, 17.8; ESI-MS 272.2 (M^++1); HRMS (ES^+) calcd for $C_{17}H_{22}NO_2$ $(M + H)^+$ 272.1651; found, 272.1639. The free base was converted into its HCl salt for analysis, mp 205.2~207.6 °C. IR (film) νmax = 3706, 3681, 3474, 2967, 2938, 2475, 1635, 1615, 1453, 1345, 1230, 1055, 1033, 1012, 801, 749 cm^{-1}; Anal. calcd for ($C_{17}H_{22}NO_2 \cdot HCl \cdot 0.5H_2O$) C, 64.45; H, 7.32; N, 4.42; found C, 64.24; H, 7.44; N, 4.38.

8: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.0 (s, 1H, -OH), 7.82 (d, *J* = 8.0 Hz, 1H), 6.56 (s, 1H), 6.52 (d, *J* = 8.5 Hz, 1H), 3.10 (s, 1H), 2.94 (s, 1H), 2.74-2.70 (m, 2H), 2.42 (s, 4H), 2.32 (d, *J* = 14.5 Hz, 1H), 2.19 (d, *J* = 9.0 Hz, 1H), 1.98-1.97 (m, 1H), 1.85 (s, 1H), 1.75 (s, 1H), 1.57-1.51 (m, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 195.9, 164.5, 155.4, 130.2, 122.2, 155.0, 111.8, 57.3, 51.4, 42.4, 41.1, 38.2, 35.9, 34.0, 32.7, 21.3, 17.5; ESI-MS 272.2 (M^++1); HRMS (ES^+) calcd for $C_{17}H_{22}NO_2$ $(M + H)^+$ 272.1651; found, 272.1645. The free base was converted into its HCl salt for analysis, mp 279.6~284.5 °C. IR (film) νmax = 3126, 2945, 2642, 1670, 1606, 1571, 1462, 1285, 1260, 1248, 1234, 1198, 1052, 855, 729 cm^{-1}. Anal. calcd for ($C_{17}H_{22}NO_2 \cdot HCl \cdot 0.2H_2O$) C, 65.57; H, 7.25; N, 4.50; found C, 65.29; H, 7.38; N, 4.40.

5.1.5. General procedure for synthesis of 8-hydroxy-13-phenethyl-3,4,10,10a-tetrahydro-1H-1,4a-(epiminoethano)phenanthren-9(2H)-one (9) and 6-hydroxy-13-phenethyl-3,4,10,10a-tetrahydro-1H-1,4a-(epiminoethano)phenanthren-9(2H)-one (18): represented by synthesis of 18—

Compound **8** (2.2 g, 8.1 mmol) was dissolved in 1,2-dichloroethane (50 mL). To the solution was added K_2CO_3 (7.85 g, 56.8 mmol) and ethyl chloroformate (5.28 g, 4.63 mL, 48.7 mmol). The reaction mixture was refluxed under argon for 24 h. Water (100 mL) was added to the reaction mixture after cooling to ambient temperature and the mixture was extracted with $CHCl_3$. The combined extracts were washed with brine and dried over anhydrous Na_2SO_4. After filtration and concentration, the crude product was purified by flash chromatography (silica, gradient: $CHCl_3$ to 3% $MeOH/NH_4OH/CHCl_3$) to afford the intermediate ethyl 6-((ethoxycarbonyl)oxy)-9-oxo-2,3,4,9,10,10a-hexahydro-1*H*-1,4a-(epiminoethano)phenanthrene-13-carboxylate in 94% yield as a brown oil (3.05 g). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.03 (d, *J* = 8.5 Hz, 1H), 7.15 (d, *J* = 6.5 Hz, 1H), 7.10 (d, *J* = 9.0 Hz, 1H), 4.28 (q, *J* = 7.0 Hz, 2H), 4.16-4.05 (m, 3H), 3.87-3.81 (m, 1H), 3.76-3.71 (m, 1H), 2.82 (td, *J* = 16.5, 3.0 Hz, 1H), 2.54-2.48 (m, 1H), 2.42-2.35 (m, 1H), 1.96-1.76 (m, 4H), 1.70-1.65 (m, 3H), 1.36 (t, *J* = 7.0 Hz, 3H), 1.23 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 195.6, 155.9, 155.4, 154.8 152.8, 129.4, 128.5, 119.6, 118.0, 65.2, 61.3, 50.5, 41.3,

39.5, 38.4, 35.6, 34.9, 33.8, 23.7, 19.8, 14.8, 14.2; ESI-MS 402.2 (M^++1); HRMS (ES^+) calcd for $C_{22}H_{28}NO_6$ (M + H)$^+$ 402.1917; found, 402.1901.

The above intermediate carbamate (0.15 g, 0.37 mmol) was dissolved in 33% HBr-AcOH (3 mL) at room temperature. The resulting mixture was heated to reflux (110 °C) under argon for 18 h. The solvent was removed and the residue was redissolved in MeOH and basified with a small amount of NH_4OH, and then evaporated to dryness. The product was purified by flash chromatography (silica, gradient: 10-40% MeOH/NH_4OH/ $CHCl_3$) to yield 6-hydroxy-3,4,10,10a-tetrahydro-1*H*-1,4a-(epiminoethano)phenanthren-9(2*H*)-one as a white solid (70 mg, 74%). ^{1}H NMR ($CDCl_3$+CD_3OD, 500 MHz) δ 7.59 (d, *J* = 8.5 Hz, 1H), 6.48-6.46 (m, 2H), 3.49 (td, *J* = 13.5, 5.5 Hz, 1H), 3.31 (s, 1H), 3.18-3.14 (m, 1H), 2.54-2.48 (m, 1H), 2.39-2.32 (m, 2H), 2.18 (dd, *J* = 17, 3.5 Hz, 1H), 1.87-1.54 (m, 7H); ESI-MS 258.1 (M^++1); HRMS (ES^+) calcd for $C_{16}H_{20}NO_2$ (M + H)$^+$ 258.1494; found, 258.1494.

A mixture of the secondary amine prepared above (70 mg, 0.27 mmol), 2-phenylethyl bromide (61 mg, 46 μL, 0.33 mmol) and triethylamine (33 mg, 46 μL, 0.33 mmol) in EtOH (5 mL) was refluxed under argon for 48 h. The solvent was removed and the residue was purified by flash chromatography (silica, gradient: $CHCl_3$ to 15% MeOH/1.5%NH_4OH/ $CHCl_3$) to afford 6-hydroxy-13-phenethyl-3,4,10,10a-tetrahydro-1H-1,4a-(epiminoethano)phenanthren-9(2*H*)-one (**18**) as a white solid (50 mg, 52%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.94 (d, *J* = 7.5 Hz, 1H), 7.28-726 (m, 2H), 7.21-7.16 (m, 3H), 6.71-6.69 (m, 2H), 3.16-3.15 (m, 2H), 2.98 (s, 1H), 2.91-2.79 (m, 6H), 2.55 (d, *J* = 13.5 Hz, 1H), 2.45 (d, *J* = 14 Hz, 1H), 2.28 (d, *J* = 12.5 Hz, 1H), 2.03-1.94 (m, 2H), 1.84 (s, 2H), 1.66-1.58 (m, 2H); ^{13}C NMR ($CDCl_3$+CD_3OD, 100 MHz) δ 197.2, 162.7, 156.1, 140.0, 130.2, 128.6 (2), 128.4 (2), 126.1, 123.3, 114.2, 111.3, 57.5, 55.3, 50.2, 41.5, 38.6, 36.4, 34.8, 33.9, 33.1, 21.7, 18.2; ESI-MS 362.2 (M^++1); HRMS (ES^+) calcd for $C_{24}H_{28}NO_2$ (M + H)$^+$ 362.2120; found, 362.2127; The free base was converted into its HCl salt for analysis, mp >300 °C (dec). IR (film) νmax = 3707, 3681, 3665, 2973, 2922, 2868, 1673, 1593, 1454, 1282, 1055, 1032, 1012, 870, 758, 703 cm^{-1}. Anal. calcd for ($C_{24}H_{27}NO_2$·HCl·0.3H_2O) C, 71.47; H, 7.15; N, 3.47; found C, 71.48; H, 7.23; N, 3.38.

9: white solid (90%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.80 (s, 1H, -OH), 7.47 (t, *J* = 8.0 Hz, 1H), 7.39-7.28 (m, 5H), 6.82 (t, *J* = 8.0 Hz, 2H), 3.21-3.15 (m, 2H), 3.03-2.85 (m, 6H), 2.58-2.50 (m, 2H), 2.45-2.40 (m, 1H), 2.12-2.01 (m, 2H), 1.97-1.88 (m, 2H), 1.78-1.72 (m, 2H), 1.56-1.49 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.4, 163.2, 154.4, 140.4, 136.8, 128.7 (2), 128.4 (2), 126.0, 115.6, 115.5, 115.4, 57.6, 55.6, 50.0, 41.1, 38.7, 36.8, 35.0, 34.3, 33.4, 21.7, 18.6; ESI-MS 308.2 (M^++1); HRMS (ES^+) calcd for $C_{19}H_{22}N_3O$, 308.1673 (M + H)$^+$ found 308.1671. The free base was converted into its HCl salt for analysis, mp 298~302 °C (dec). IR (film) νmax = 3706, 3681, 3664, 2981, 2973, 2938, 2923,2866, 1631, 1576, 1454, 1055, 1032, 1012, 796, 754 cm^{-1}. Anal. calcd for ($C_{24}H_{27}NO_2$·HCl·0.5H_2O) C, 70.83; H, 7.18; N, 3.44; found C, 70.88; H, 7.37; N, 3.43.

5.2.1. General procedure for synthesis of 13-methyl-2,3,4,9,10,10a-hexahydro-1H-1,4a-(epiminoethano)-phenanthrene-8,9α-diol (10), 13-methyl-2,3,4,9,10,10a-hexahydro-1H-1,4a-(epiminoethano)-phenanthrene-8,9β-diol (11), 13-phenethyl-2,3,4,9,10,10a-hexahydro-1H-1,4a-(epiminoethano)-phenanthrene-8,9α-diol (12) and 13-phenethyl-2,3,4,9,10,10a-hexahydro-1H-1,4a-(epiminoethano)phenanthrene-8,9β-diol (13), 13-methyl-2,3,4,9,10,10a-hexahydro-1H-1,4a-(epiminoethano)phenanthrene-6,9-diol (19), and 13-phenethyl-2,3,4,9,10,10a-hexahydro-1H-1,4a-(epiminoethano)-phenanthrene-6,9α-diol (22): represented by synthesis of 19

Compound **8** (0.49 g, 1.8 mmol) was dissolved in THF (10 mL) under argon. The solution was cooled to −78 °C in a dry ice-acetone bath. A solution of Superhydride (1 M, 2.72 mL,

2.72 mmol) was added dropwise and the reaction solution was stirred at −78 °C for 1 h and gradually warmed to room temperature overnight. The reaction was quenched with H_2O and the pH was adjusted to 9 with 28% NH_4OH. The aqueous layer was extracted several times with a mixed solvent of $CHCl_3$ and MeOH (20:1, v/v). The combined extracts were washed with brine and dried over anhydrous Na_2SO_4. After filtration and concentration, the crude product was purified by flash chromatography (silica, gradient: 10-20% MeOH/NH_4OH/$CHCl_3$) to afford a single product, 13-methyl-2,3,4,9,10,10a-hexahydro-1*H*-1,4a-(epiminoethano)phenanthrene-6,9-diol (**19**) as a white solid (0.21 g, 43%). ^{1}H NMR ($CDCl_3$+CD_3OD, 500 MHz) δ 7.39 (d, *J* = 7.5 Hz, 1H), 6.68 (d, *J* = 8.0 Hz, 1H), 6.62 (s, 1H), 4.80 (t, *J* = 7.5 Hz, 1H), 3.23-3.21 (m, 1H), 3.06 (s, 1H), 2.88 (s, 1H), 2.54 (s, 3H), 2.31 (d, *J* = 11.5 Hz, 1H), 2.11-1.80 (m, 7H), 1.72-1.61 (m, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 155.8, 146.8, 129.2, 128.2, 113.6, 111.1, 68.8, 59.0, 51.7, 41.8, 40.2, 36.6, 34.2, 33.8, 33.2, 21.3, 18.4; ESI-MS 274.2 (M^++1); HRMS (ES^+) calcd for $C_{17}H_{24}NO_2$ (M + H)$^+$ 274.1807; found, 274.1809. IR (film) νmax = 3185, 2942, 2910, 2873, 1618, 1579, 1219, 771 cm^{-1}. Anal. calcd for ($C_{17}H_{23}NO_2$·0.5H_2O) C, 72.31; H, 8.57; N, 4.96; found C, 72.42; H, 8.59; N, 4.78.

10: white solid (24%)—^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.93 (t, *J* = 7.9 Hz, 1H), 6.58 (d, *J* = 7.6 Hz, 1H), 6.50 (d, *J* = 7.9 Hz, 1H), 4.92 (m, 1H), 3.00 (td, *J* = 12.6, 5.0 Hz, 1H), 2.78 (dd, *J* = 12.0, 7.2 Hz, 1H), 2.59 (m, 1H), 2.30 (s, 3H), 2.17 (dd, *J* = 13.4, 4.2 Hz, 1H), 1.99-1.63 (m, 7H), 1.57-1.44 (m, 2H), 1.43-1.38 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.8, 148.1, 128.9, 123.4, 116.9, 113.9, 69.4, 58.7, 52.1, 42.6, 40.7, 37.9, 34.7, 34.3, 32.6, 22.2, 18.9; ESI-MS 274.2 (M^++1); HRMS (ES^+) calcd for $C_{17}H_{24}NO_2$ (M + H)$^+$ 274.1807; found, 274.1805. IR (film) νmax = 3055, 2933, 2866, 2628, 1612, 1578, 1452, 1267, 1229, 1146, 1054, 1007, 797, 763 cm^{-1}. Anal. calcd for ($C_{17}H_{23}NO_2$) C, 74.69; H, 8.48; N, 5.12; found C, 74.56; H, 8.61; N, 5.22.

11: pale yellow solid (20%)—^{1}H NMR ($CDCl_3$+CD_3OD, 500 MHz) δ 7.10 (t, *J* = 8.0 Hz, 1H), 6.73-6.70 (m, 2H), 5.02 (s, 1H), 3.18-3.14 (m, 1H), 2.91-2.88 (m, 1H), 2.82 (s, 1H), 2.50 (s, 3H), 2.34 (d, *J* = 13.5 Hz, 1H), 2.26 (t, *J* = 7.0 Hz, 1H), 2.14 (t, *J* = 12.5 Hz, 1H), 2.02 (d, *J* = 11.0 Hz, 1H), 1.80-1.62 (m, 6H), 1.54-1.51 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 155.6, 147.3, 128.4, 122.5, 115.9, 112.2, 61.9, 58.2, 51.4, 41.7, 36.6, 35.6, 33.6, 32.6, 30.8, 21.3, 18.0; ESI-MS 274.2 (M^++1); HRMS (ES^+) calcd for $C_{17}H_{24}NO_2$ (M + H)$^+$ 274.1807; found, 274.1804. IR (film) νmax = 3192, 2965, 2919, 2877, 2855, 1587, 1471, 1292, 1281, 1037, 931, 787, 747 cm^{-1}. Anal. calcd for ($C_{17}H_{23}NO_2$·*i*-PrOH) C,72.04; H, 9.37; N, 4.20; found C,72.10; H, 9.41; N, 4.26.

12: white solid (58%, crystallized from *i*-PrOH and Et_2O)—^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30 (t, *J* = 8.0 Hz, 2H), 7.23-7.21 (m, 3H), 7.05 (t, *J* = 8.0 Hz, 1H), 6.67 (d, *J* = 8.0 Hz, 2H), 4.99 (m, 1H), 3.25-3.11 (m, 2H), 3.02 (s, 1H), 2.95-2.89 (m, 4H), 2.31(dd, *J* = 13.6, 4.0 Hz, 1H), 2.19 (s, 1H), 2.08-2.03 (m, 2H), 1.99-1.83 (m, 4H), 1.81-1.72 (m, 1H), 1.70-1.55 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.7, 147.3, 139.2, 128.8, 128.6, 128.5, 126.5, 123.2, 116.7, 114.5, 69.4, 57.1, 56.9, 50.5, 39.6, 37.2, 34.6, 34.0, 33.1, 32.4, 21.7, 18.9; ESI-MS 364.2 (M^++1); HRMS (ES^+) calcd for $C_{24}H_{30}NO_2$ (M + H)$^+$ 364.2277; found, 364.2271. IR (film) νmax = 3062, 3027, 3006, 2939, 2904, 2872, 2846, 2819, 1579, 1454, 1441, 1269, 724, 697 cm^{-1}. Anal. calcd. for ($C_{24}H_{29}NO_2$) C, 79.30; H, 8.04; N, 3.85; found C, 79.36; H, 8.12; N, 3.73.

13: white solid (20%, crystallized from *i*-PrOH and Et_2O)—^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36 (t, *J* = 7.6 Hz, 2H), 7.29-7.25 (m, 3H), 7.10 (t, *J* = 8.0 Hz, 1H), 6.75 (d, *J* = 6.0 Hz, 1H), 6.57 (t, *J* = 7.2 Hz, 1H), 5.23 (m, 1H), 3.29-3.25 (m, 2H), 3.09 (s, 1H), 2.97-2.92 (m, 4H), 2.71 (t, *J* = 11.2 Hz, 1H), 2.37 (d, *J* = 10.0 Hz, 1H), 2.16 (t, *J* = 11.2 Hz, 1H), 2.07

(d, *J* = 12.0 Hz, 1H), 1.90-1.83 (m, 3H), 1.75-1.59 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.0, 147.9, 140.0, 128.8 (2), 128.7, 128.5 (2), 126.3, 124.2, 116.0, 112.3, 62.3, 57.6, 56.8, 50.8, 37.3, 35.5, 34.6, 33.6, 33.2, 30.9, 22.2, 19.1; ESI-MS 364.1 (M^++1); HRMS (ES^+) calcd for $C_{24}H_{30}NO_2$ $(M + H)^+$ 364.2277; found, 364.2262. IR (film) νmax = 2981, 2940, 2923, 1583, 1470, 1455, 1286, 1220, 1055, 1033, 772, 700 cm^{-1}. Anal. calcd for ($C_{24}H_{29}NO_2$·*i*-PrOH·0.9H_2O) C, 73.74; H, 8.89; N, 3.18; found C, 73.60; H, 8.57; N, 3.11.

22: white foam; yield 85.0%—^{1}H NMR ($CDCl_3$+CD_3OD, 400 MHz) δ 7.31 (d, *J* = 8.4 Hz, 1H), 7.22 (t, *J* = 7.6 Hz, 2H), 7.16-7.12 (m, 3H), 6.60 (d, *J* = 8.4 Hz, 1H), 6.55 (s, 1H), 4.74 (t, *J* = 9.6 Hz, 1H), 3.33-2.74 (m, 1H), 3.09 (d, *J* = 8.4 Hz, 1H), 2.92 (s, 1H), 2.80-2.76 (m, 4H), 2.22 (d, *J* = 13.6 Hz, 1H), 2.04 (d, *J* = 12.6 Hz, 1H), 1.96-1.72 (m, 5H), 1.68-1.45 (m, 3H); ^{13}C NMR ($CDCl_3$+CD_3OD, 100 MHz) δ 159.8, 151.4, 143.4, 133.3, 132.6, 132.4, 132.1, 130.2, 117.5, 115.2, 73.1, 61.1, 60.6, 54.5, 44.4, 41.1, 38.8, 38.1, 37.6, 37.3, 25.8, 23.0; ESI-MS 364.2 (M^++1); HRMS (ES^+) calcd for $C_{24}H_{30}NO_2$ $(M + H)^+$ 364.2277; found, 364.2266. IR (film) νmax = 3526, 3396, 3024, 2910, 2871, 2821, 1602, 1490, 1452, 1304, 1251, 1075, 834, 765, 709 cm^{-1}. Anal. calcd for ($C_{24}H_{29}NO_2$•H_2O) C, 75.56; H, 8.19; N, 3.67; found C, 75.34; H, 8.29; N, 3.61.

5.2.2. General procedure for synthesis of 13-methyl-2,3,4,10a-tetrahydro-1H-1,4a-(epiminoethano)phenanthren-8-ol (14), 13-phenethyl-2,3,4,10a-tetrahydro-1H-1,4a-(epiminoethano)phenanthren-8-ol (16), 13-methyl-2,3,4,10a-tetrahydro-1H-1,4a-(epiminoethano)phenanthren-6-ol (20), and 13-phenethyl-2,3,4,10a-tetrahydro-1H-1,4a-(epiminoethano)phenanthren-6-ol (23): represented by synthesis of 16

To a solution of mixture of **12** and **13** (0.3 g, 0.82 mmol) in MeOH (10 mL) was added 2 M HCl (2 mL). The resulting solution was stirred at room temperature for 1 h and the solvent was removed. The residue was basified with 28% NH_4OH and extracted with $CHCl_3$ (3 × 20 mL). The combined extracts were washed with brine and dried over anhydrous Na_2SO_4. After filtration and evaporation, the crude product was purified by flash chromatography (silica, gradient: 5% MeOH/NH_4OH/$CHCl_3$) to afford **16** (234 mg, 82%) as a yellow foam. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.32 (t, *J* = 7.6 Hz, 2H), 7.27-7.22 (m, 3H), 7.04 (t, *J* = 8.0 Hz, 1H), 6.88 (dd, *J* = 9.6, 3.2 Hz, 1H), 6.75 (d, *J* = 8.0 Hz, 1H), 6.67 (d, *J* = 8.0 Hz, 1H), 5.62 (dd, *J* = 9.6, 2.0 Hz, 1H), 3.27-3.14 (m, 3H), 2.96-2.86 (m, 4H), 2.65 (s, 1H), 2.35 (d, *J* = 11.2 Hz, 1H), 2.13-2.05 (m, 2H), 1.97-1.89 (m, 1H), 1.80-1.69 (m, 1H), 1.65-1.60 (m, 1H), 1.57-1.48 (m, 1H), 1.36-1.32 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.2, 146.2, 140.2, 128.9(2), 128.8(2), 128.4, 128.1, 126.1, 121.4, 121.2, 115.6, 114.9, 57.5, 55.4, 42.9, 34.4, 33.9, 31.4, 21.0, 20.3; ; ESI-MS 346.2 (M^++1); HRMS (ES^+) calcd for $C_{24}H_{28}NO$ $(M + H)^+$ 346.2171; found, 346.2172. IR (film) νmax = 3707, 3681, 3665, 3016, 2982, 2967, 2939, 2923, 2866, 2845, 2570, 1578, 1464, 1456, 1283, 1055, 1033, 1016, 763, 698 cm^{-1}. Anal. calcd for ($C_{24}H_{27}NO$·HCl·0.8HCl) C, 72.73; H, 7.53; N, 3.53; found C, 72.66; H, 7.64; N, 3.41.

14: yellow powder (87)—^{1}H NMR ($CDCl_3$+CD_3OD, 400 MHz) δ 6.78 (t, *J* = 8.0 Hz, 1H), 6.66 (dd, *J* = 9.6, 2.8 Hz, 1H), 6.48 (d, *J* = 7.6 Hz, 1H), 6.42 (d, *J* = 8.0 Hz, 1H), 5.40 (d, *J* = 9.6 Hz, 1H), 2.95 (td, *J* = 12.6, 5.2 Hz, 1H), 2.75 (dd, *J* = 12.0, 7.6 Hz, 1H), 2.69 (d, *J* = 2.0 Hz, 1H), 2.41 (m, 1H), 2.23 (s, 3H), 2.14 (dd, *J* = 13.6, 5.2 Hz, 1H), 1.86 (m, 2H), 1.68 (m, 1H), 1.51 (m, 1H), 1.38 (m, 1H),1.26 (m, 1H), 1.08 (dd, *J* = 13.6, 5.6 Hz, 1H); ^{13}C NMR ($CDCl_3$+CD_3OD, 100 MHz) δ 156.6, 149.7, 132.1, 131.6, 125.3, 123.7, 118.4, 117.2, 61.5, 55.7, 46.8, 46.1, 38.7, 37.9, 35.2, 24.7, 23.5; ESI-MS, 256.2 (M^++1); HRMS calcd for $C_{17}H_{22}NO$ $(M + H)^+$ 256.1701; found 256.1701. IR (film) νmax = 3135, 2956, 2928, 2890, 2681, 2606, 2549, 1577, 1465, 1288, 1272, 1013, 768, 701 cm^{-1}. Anal. calcd for ($C_{17}H_{21}NO$·HCl· 0.2H_2O) C 69.12, H 7.64, N 4.74; Found C 69.13, H 7.78, N 4.84.

20: yellow solid (65%)—^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.87 (d, *J* = 8.0 Hz, 1H), 6.68 (s, 1H), 6.56 (d, *J* = 8.0 Hz, 1H), 6.40 (dd, *J* = 9.4, 2.0 Hz, 1H), 5.47 (d, *J* = 9.4 Hz, 1H), 3.20-3.12 (m, 1H), 3.07-3.04 (m, 2H), 2.79 (s, 1H), 2.51 (s, 3H), 2.24-2.04 (m, 1H), 1.88-1.83 (m, 1H), 1.75-1.60 (m, 2H), 1.53-1.50 (m, 1H), 1.28-1.20 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.9, 146.1, 128.4, 127.2, 126.4, 124.7, 113.2, 111.3, 57.6, 51.7, 42.8, 42.5, 34.5, 34.0, 31.6, 22.6, 20.9, 19.8; ESI-MS 256.2 (M^++1); HRMS (ES^+) calcd for $C_{17}H_{22}NO$ $(M + H)^+$ 256.1701; found, 256.1707. IR (film) νmax = 3134, 2948, 2930, 2855, 2493, 1602, 1441, 1247, 848 cm^{-1}. Anal. calcd for ($C_{17}H_{21}NO$•HCl•$0.9H_2O$) C, 66.29; H, 7.79; N, 4.55; found C, 66.12; H, 7.94; N, 4.39.

23: white foam (95%)—^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.31 (br s, 1H), 7.32 (t, *J* = 7.6 Hz, 2H), 7.25-7.23 (m, 3H), 6.92 (dd, *J* = 9.6, 3.2 Hz, 1H), 6.72 (d, *J* = 4.8 Hz, 1H), 6.61 (d, *J* = 6.4 Hz, 1H), 6.44 (d, *J* = 9.6 Hz, 1H), 5.55 (d, *J* = 10.0 Hz, 1H), 3.22-3.13 (m, 3H), 2.92 (m, 4H), 2.80 (s, 1H), 2.28-2.15 (m, 2H), 2.07 (d, *J* = 11.2 Hz, 1H), 1.92-1.87 (m, 1H), 1.75-1.62 (m, 2H), 1.54-1.50 (m, 1H), 1.31-1.27 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.8, 146.3, 140.0, 128.8 (2), 128.5 (2), 128.4, 127.2, 126.7, 126.2, 124.9, 113.5, 111.6, 57.4, 55.4, 50.5, 42.7, 34.6, 34.5, 33.7, 31.9, 21.0, 20.3; ESI-MS 346.2 (M^++1); HRMS (ES^+) calcd for $C_{24}H_{28}NO$ $(M + H)^+$ 346.2171; found 346.2184. The free base was converted into its HCl salt for analysis. IR (film) νmax = 3128, 2948, 2929, 2915, 2851, 2838, 2538, 1603, 1569, 1492, 1442, 1292, 1249, 1232, 1178, 825, 752, 701 cm^{-1}. Anal. calcd for ($C_{24}H_{27}NO$·HCl·$0.3H_2O$) C, 74.42; H, 7.44; N, 3.62; found C, 74.26; H, 7.43; N, 3.48.

5.2.3. General procedure for synthesis of 13-methyl-2,3,4,9,10,10a-hexahydro-1H-1,4a-(epiminoethano)phenanthren-8-ol (15) and 13-phenethyl-2,3,4,9,10,10a-hexahydro-1H-1,4a-(epiminoethano)phenanthrene-8-ol (17), 13-methyl-2,3,4,9,10,10a-hexahydro-1*H*-1,4a-(epiminoethano)phenanthren-6-ol (21) and 13-phenethyl-2,3,4,9,10,10a-hexahydro-1*H*-1,4a-(epiminoethano)phenanthren-6-ol (24): represented by synthesis of 21

Compound **19** (0.16 g, 0.58 mmol) was dissolved into EtOH (15 mL) in a glass bomb and to the solution was added 1.5 mL of water, 0.2 mL of conc. HCl and catalyst 5% Pd/C. The bomb was placed in a Parr apparatus, evacuated and backfilled with H_2 for three times, filled with H_2 to 60 psi and shaken for 16 h. The reaction mixture was filtered on a pad of celite and washed with MeOH. The filtrate was concentrated and the residue was basified with NH_4OH and extracted with $CHCl_3$. The combined extracts were washed with brine and dried over anhydrous Na_2SO_4. After filtration and concentration, the residue was purified by flash chromatography (silica, gradient, $CHCl_3$ to 10% MeOH/1%NH_4OH/$CHCl_3$) to give **21** as a white solid (0.13 g, 87%).

21—^{1}H NMR ($CDCl_3$+CD_3OD, 500 MHz) δ 6.88 (d, *J* = 8.5 Hz, 1 H), 6.68 (s, 1 H), 6.59 (d, *J* = 8.0 Hz, 1 H), 3.18-3.16 (m, 1 H), 2.97-2.94 (m, 1 H), 2.83-2.80 (m, 3 H), 2.47 (s, 3 H), 2.33-2.30 (m, 1 H), 2.03-1.94 (m, 3 H), 1.89-1.78 (m, 3 H), 1.68-1.55 (m, 4H); ^{13}C NMR ($CDCl_3$+CD_3OD, 125 MHz) δ 154.4, 147.1, 129.8, 112.8, 111.4, 58.6, 51.6, 42.8, 41.8, 37.2, 34.1, 34.0, 29.1, 23.3, 21.7, 18.5; ESI-MS 258.2 (M^++1); HRMS (ES^+) calcd for $C_{17}H_{24}NO$, 258.1858; found, 258.1857; The free base was converted into its HCl salt for analysis. HCl salt: mp 241.4~247.6 °C. IR (film) νmax = 3707, 3681, 3665, 2981, 2973, 2967, 2938, 2922, 2844, 1055, 1032, 1014 cm^{-1}. Anal. calcd for ($C_{17}H_{24}NO$·HCl·$0.7H_2O$) C, 66.63; H, 8.35; N, 4.57; found C, 66.49; H, 8.52; N, 4.50.

15: white solid (67%)—^{1}H NMR ($CDCl_3$+CD_3OD, 500 MHz) δ 6.70 (t, *J* = 8.0 Hz, 1 H), 6.46 (d, *J* = 8.0 Hz, 1 H), 6.33 (d, *J* = 8.0 Hz, 1 H), 2.95 (td, *J* = 12.5, 5.0 Hz, 1 H), 2.73-2.66 (m, 2 H), 2.59 (s, 1 H), 2.31-2.20 (m, 4 H), 2.11 (dd, *J* = 13.5, 4.5 Hz, 1 H), 1.76-1.68 (m, 3 H), 1.61-1.53 (m, 3 H), 1.44 (dd, J = 12.5, 5.0 Hz, 1 H), 1.38-1.30 (m, 3 H); ^{13}C NMR

($CDCl_3$+CD_3OD, 125 MHz) δ 154.1, 146.7, 125.8, 121.6, 116.0, 111.3, 59.2, 51.5, 41.7, 41.4, 36.8, 33.6, 23.3, 22.1, 21.1, 18.1; ESI-MS 258.2 (M^++1); HRMS (ES^+) calcd for $C_{17}H_{24}NO$, 258.1858; found, 258.1853; The free base was converted into its HCl salt for analysis, mp >300 °C (dec). IR (film) νmax = 3707, 3681, 3218, 2981, 2938, 2923, 2675, 1582, 1464, 1338, 1054, 1012, 1000, 789, 726 cm^{-1}. Anal. calcd for ($C_{17}H_{24}NO{\cdot}HCl{\cdot}0.25H_2O$) C, 68.44; H, 8.28; N, 4.69; found C, 68.43; H, 8.48; N, 4.58.

17: white foam (76%)—^1H NMR ($CDCl_3$+CD_3OD, 400 MHz) δ 7.21-7.11 (m, 5H), 6.86 (t, *J* = 8.0 Hz, 1H), 6.53 (t, *J* = 6.0 Hz, 2H), 3.51 (s, 1H), 3.33 (s, 1H), 3.21-3.05 (m, 5H), 2.83 (dd, J = 17.6, 5.2 Hz, 1H), 2.53-2.44 (m, 1H), 2.38-2.34 (m, 2H), 2.04-1.99 (m, 1H), 1.88-1.65 (m, 8H); ^{13}C NMR ($CDCl_3$+CD_3OD, 100 MHz) δ 155.2, 145.4, 136.7, 129.5 (2), 129.2 (2), 127.8, 127.1, 122.2, 116.6, 112.8, 59.2, 56.2, 51.6, 40.2, 36.0, 34.1, 33.6, 31.0, 23.7, 22.3, 20.9, 18.1; ESI-MS 348.3 (M^++1); HRMS (ES^+) calcd for $C_{24}H_{30}NO$ $(M + H)^+$ 348.2327; found, 348.2318. IR (film) νmax = 3237, 2927, 2871, 1581, 1466, 1268, 764, 762 cm^{-1}. Anal. calcd for ($C_{24}H_{29}NO{\bullet}HCl{\bullet}0.7H_2O$) C, 72.69; H, 7.98; N, 3.53; found C, 72.74; H, 7.96; N, 3.46.

24: white solid; yield 43%—^1H NMR ($CDCl_3$+CD_3OD, 400 MHz) δ 7.18 (t, *J* = 6.4 Hz, 2H), 7.13-7.08 (m, 3H), 6.78 (d, *J* = 8.0 Hz, 1H), 6.59 (s, 1H), 6.48 (d, *J* = 8.0 Hz, 1H), 3.03-3.00 (m, 2H), 2.85 (s, 1H), 2.73-2.66 (m, 6H), 2.22 (d, *J* = 13.0 Hz, 1H), 1.93-1.68 (m, 6H), 1.57-1.42 (m, 4H); ^{13}C NMR ($CDCl_3$+CD_3OD, 100 MHz) δ 155.2, 148.2, 140.7, 130.7, 129.2 (2), 129.0 (2), 126.7, 126.5, 113.7, 112.4, 57.9, 57.3, 51.3, 43.3, 38.1, 35.3, 35.1, 34.3, 30.1, 24.2, 22.7, 19.8; ESI-MS 348.2 (M^++1); HRMS (ES^+) calcd for $C_{24}H_{30}NO$ $(M + H)^+$ 348.2327; found, 348.2332. IR (film) νmax = 3130, 2918, 2878, 2838, 2539, 1491, 1454, 1437, 1294, 1230, 754, 699 cm^{-1}. Anal. calcd for ($C_{24}H_{29}NO{\bullet}HCl{\bullet}0.4H_2O$) C, 73.69; H, 7.94; N, 3.58; found C, 73.61; H, 7.95; N, 3.52.

5.2.4. 13-Methyl-2,3,4,9,10,10a-hexahydro-1H-1,4a-(epiminoethano)phenanthrene-6,9β-diol (25): A round flask charged with compound **8** (0.6 g) was evacuated and backfilled with argon. Potassium *t*-butoxide (1.2 M in THF, 2.65 mL), *t*-butanol (0.21 mL) and THF (10 mL) were added successively and the solution was cooled to −78 °C (dry ice-acetone bath) and NH_3 (g) was bubbled into the mixture. Approximately 20 mL of NH_3 (l) was condensed. Lithium metal was added in small pieces until a blue color appeared which persisted for 2 h whereupon the reaction was quenched with solid NH_4Cl. The reaction was warmed to ambient temperature and H_2O was added. The solution was extracted with $CHCl_3$/MeOH (20/1) and the combined extracts were washed with brine and dried with Na_2SO_4. The crude product was purified by flash chromatography (silica, gradient: 10% to 15% MeOH/NH_4OH/$CHCl_3$) to give compound **10** (0.35 g, 58%) as a white solid and **25** (0.1 g, 17%) as yellow crystals (that were recrystallized in MeOH).

25: white solid: ^1H NMR (CD_3OD, 400 MHz) δ 7.16 (d, *J* = 8.4 Hz, 1H), 6.70 (d, *J* = 2.0 Hz, 1H), 6.65 (dd, *J* = 8.4, 2.0 Hz, 1H), 4.71 (m, 1H), 3.26 (td, *J* = 12.8, 5.2 Hz, 1H), 2.98 (dd, *J* = 12.0, 7.2Hz, 1H), 2.82 (m, 1H), 2.51 (s, 3H), 2.43 (d, *J* = 14.0 Hz, 1H), 2.38 (dd, *J* = 13.6, 4.8 Hz, 1H), 2.22 (td, *J* = 14.0, 4.0 Hz, 1H), 2.07 (m, 1H), 1.90 (m, 2H), 1.64 (m, 5H); ^{13}C NMR (CD_3OD, 100 MHz) δ157.0, 147.8, 132.0, 126.8, 113.3, 110.9, 66.6, 58.9, 51.6, 41.3, 36.7, 35.8, 34.0, 33.2, 31.9, 21.4, 18.3; ESI-MS, 274.2 (M^++1); HRMS calcd for ($C_{17}H_{24}NO_2$) 274.1807 $(M + H)^+$; found 274.1804. IR (film) νmax = 3202, 2942, 2868, 2685, 1606, 1436, 1293, 1242, 1196, 1030, 945, 866, 704 cm^{-1}. Anal.calcd for ($C_{17}H_{23}NO_2{\cdot}CH_3OH{\cdot}0.6H_2O$) C 68.37, H 8.99, N 4.43; found C 68.40, H 8.99, N 4.47.

5.3. Opioid binding assays

As previously described[29], the recombinant CHO cells (hMOR-CHO, hDOR-CHO and hKOR-CHO) were produced by stable transfection with the respective human opioid receptor cDNA, and provided by Dr. Larry Toll (SRI International, CA). The cells were grown in plastic flasks in DMEM (90%) (hDOR-CHO and hKOR-CHO) or DMEM/ F-12 (45%/ 45%) medium (hMOR-CHO) containing 5% FBS (Invitrogen), 5% FetalClone II (HyClone), and Geneticin (G-418: 0.10-0.2 mg/ml) (Invitrogen) under 95% air/5% CO_2 at 37° C. Cell monolayers were harvested and frozen in −80 °C. The hKOR-CHO, hMOR-CHO and hDOR-CHO cells are used for opioid binding experiments. For the [^{35}S]-GTP-γ-S binding experiments, we use hKOR-CHO and hMOR-CHO cells for assaying KOR and MOR receptor function. Currently, we use the NG108-15 neuroblastoma×glioma cell for the DOR [^{35}S]-GTP-γ-S binding assay, and obtain an excellent signal-to-noise ratio. In summary, we use the hDOR-CHO cells for DOR binding assays, and the NG108-15 cells for the DOR [^{35}S]-GTP-γ-S binding assay.

As noted formerly[30], [^{3}H][D-Ala^2-MePhe^4,Gly-ol^5]enkephalin ([^{3}H]DAMGO, SA=44-48 Ci/mmol) was used to label MOR, [^{3}H][D-Ala^2,D-Leu^5]enkephalin ([^{3}H]DADLE, SA=40-50 Ci/mmol) to label DOR and [^{3}H](-)-U69,593 (SA=50 Ci/mmol) to label KOR binding sites. On the day of the assay, cell pellets were thawed on ice for 15 min then homogenized with a polytron in 10 mL/pellet of ice-cold 10 mM Tris-HCl, pH 7.4. Membranes were then centrifuged at 30,000 × g for 10 min, resuspended in 10 ml/pellet ice-cold 10mM Tris-HCl, pH 7.4 and again centrifuged 30,000 × g for 10 min. Membranes were then resuspended in 25 °C 50 mM Tris-HCl, pH 7.4 (~100 mL/pellet hMOR-CHO, 50 mL/ pellet hDOR-CHO and 120 mL/pellet hKOR-CHO). All assays took place in 50 mM Tris-HCl, pH 7.4, with a protease inhibitor cocktail [bacitracin (100 μg/mL), bestatin (10 μg/ mL), leupeptin (4 μg/mL) and chymostatin (2 μg/mL)], in a final assay volume of 1.0 mL. All drug dilution curves were made up with buffer containing 1 mg/mL BSA. Nonspecific binding was determined using 20 μM levallorphan ([^{3}H]DAMGO and [^{3}H]DADLE) and 1 μM (-)-U69,593 (for [^{3}H]U69,593 binding). [^{3}H]Radioligands were used at ~ 2 nM concentrations. Triplicate samples were filtered with Brandel Cell Harvesters (Biomedical Research & Development Inc., Gaithersburg, MD), over Whatman GF/B filters, after a 2 h incubation at 25 °C. The filters were punched into 24-well plates to which was added 0.6 mL of LSC-cocktail (Cytoscint). Samples were counted, after an overnight extraction, in a Trilux liquid scintillation counter at 44% efficiency. Opioid binding assays had ~30 μg protein per assay tube. Inhibition curves were generated by displacing a single concentration of radioligand by 10 concentrations of drug. The pooled data of three experiments (typically 30 data points) were fit to the two-parameter logistic equation for the best-fit estimates of the IC_{50} and N values: $Y=100/(1+([INHIBITOR]/IC50)^N)$, where "Y" is the percent of control value. K_i values for test drugs are calculated according to the standard equation: $K_i = IC_{50}/(1+[radioligand]/K_d)$. For the [^{3}H]radioligands, the following K_d values (nM ± SD, n = 3) were used in the K_i calculation: [^{3}H]DAMGO (0.93 ± 0.04), [^{3}H]DADLE (1.9 ± 0.3) and [^{3}H](-)-U69,593 (11 ± 0.6).

5.3.1. [^{35}S]GTP-γ-S binding assays—As noted formerly[30], the assays were conducted with minor modifications of published methods[31]. In this description, buffer "A" is 50 mM Tris-HCl, pH 7.4, containing 100 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA and buffer "B" is buffer A plus 1.67 mM DTT and 0.15% BSA. On the day of the assay, cells were thawed on ice for 15 min and homogenized using a polytron in 50 mM Tris-HCl, pH 7.4, containing 4 μg/mL leupeptin, 2 μg/mL chymostatin, 10 μg/mL bestatin and 100 μg/mL bacitracin. The homogenate was centrifuged at 30,000 × g for 10 min at 4 °C, and the supernatant discarded. The membrane pellets were resuspended in buffer B and used for [^{35}S]GTP-γ-S binding assays. Test tubes received the following additions: 50 μL buffer A

plus 0.1% BSA, 50 μL GDP in buffer A/0.1% BSA (final concentration = 40 μM), 50 μL drug in buffer A/0.1% BSA, 50 μL [^{35}S]-GTP-γ-S in buffer A/0.1% BSA (final concentration = 50 pM), and 300 μL of cell membranes (50 μg of protein) in buffer B. The final concentrations of reagents in the [^{35}S]GTP-γ-S binding assays were: 50 mM Tris-HCl, pH 7.4, containing 100 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 40 μM GDP and 0.1% BSA. Incubations proceeded for 3 h at 25 °C. Nonspecific binding was determined using GTP-γ-S (40 μM). Bound and free [^{35}S]GTP-γ-S were separated by vacuum filtration (Brandel) through GF/B filters. The filters were punched into 24-well plates to which was added 0.6 mL LSC-cocktail (Cytoscint). Samples were counted, after an overnight extraction, in a Trilux liquid scintillation counter at 27% efficiency.

For the [^{35}S]GTP-γ-S binding experiments, K_e values were determined using the "shift" experimental design, agonist (DAMGO) dose-response curves were generated, using hMOR-CHO cells, in the absence and presence (ten points/curve) of a test compound. The data of several experiments, 3 or more, were pooled, and the K_e values were calculated according to the equation: [Test Drug]/($EC_{50\text{-}2}/EC_{50\text{-}1} - 1$), where $EC_{50\text{-}2}$ is the EC_{50} value in the presence of the test drug and $EC_{50\text{-}1}$ is the value in the absence of the test drug.

5.3.2 Data analysis of estimated Ki in opioid binding experiments—Formerly, initial dose-ranging experiments (n=1) were conducted using 1 nM, 10 nM, 100 nM, 1000 nM and 10000 nM test drug to facilitate the selection of an appropriate concentration range to be used in the final inhibition curves. We have now adopted a modified approach that we term the "sextuplet" method. The binding inhibition produced by 1 μM of a test compound was determined using six test tubes, rather than three, in order to increase the precision of the result. The "total" and nonspecific binding conditions were also determined as sextuplets. Given the known concentration of radioligand and its K_d value, an estimated K_i value was calculated according to the following equation, which was derived from standard binding equations:

$$K_i = [I] / ((([L] \times (1 - F_c)) / (F_c \times K_d)) + (1/F_e)) - 1$$

Where "K_d" = the K_d value of the radioligand at the receptor of interest; "[I]" = the concentration of the inhibitor "I" used in the binding assay; "[L]" = the concentration of the radioligand used in the assay; "total binding" = the specific binding of the radioligand in the absence of the inhibitor"; "inhibited binding" = the specific binding of the radioligand in the presence of the inhibitor, I; "F_c" = the fraction of control = "inhibited binding"/"total binding"; "A" = $([L] \times (1\text{-}F_c))/(F_c \times K_d)$; "B" = A + $(1/F_c) - 1$. The K_i value is the calculated as follows: $K_i = [I]/B$.

In general, full dose-response curves were generated only with compounds that had estimated K_i values indicative of high to moderate potency. In this case, the estimated K_i was then used to select the concentration range to be used in the final inhibition curves. In the event, full inhibition curves were not run, the estimated K_i values might be reported. The reported estimated SD was calculated as follows: estimated $K_I \times$ CV, where CV is the coefficient of variation of the % of control observed with the 1 μM test compound. When full inhibition curves were generated, the pooled data of three experiments (typically 30 data points) were fit to the two-parameter logistic equation for the best-fit estimates of the IC_{50} and N values: $Y=100/(1+([INHIBITOR]/IC50)^N)$, where "Y" is the percent of control value. K_i values for test drugs are calculated according to the standard equation: $K_i = IC_{50}/(1+$ [radioligand]/Kd])[32, 33]. For the [^{3}H]radioligands, the following Kd values (nM ± SD, n = 3) were used in the Ki calculation: [^{3}H]DAMGO (0.93±0.04), [^{3}H]DADLE (1.9±0.3) and [^{3}H](−)-U69,593 (11 ± 0.6). The corresponding Bmax values were (fmol/mg protein ± SD, n

= 3): [^{3}H]DAMGO (1912 ± 68), [^{3}H]DADLE (3655 ± 391) and [^{3}H](−)-U69,593 (3320 ± 364)[32, 33].

5.4. X-ray crystal data on compounds 12, 19, and 22

Single-crystal X-ray diffraction data on compounds **12** and **22** were collected using MoKα radiation and a Bruker APEX-2 CCD area detector. Single-crystal X-ray diffraction data on compound **19** were collected using CuKα radiation and a Bruker Platinum 135 CCD area detector. Crystals were prepared for data collection by coating with high viscosity microscope oil. The oil-coated crystal was mounted on a micro-mesh mount (Mitergen, Inc.) and transferred to the diffractometer. The structures were solved by direct methods and refined by full-matrix least squares on F2 values using the programs found in the SHELXTL suite (Bruker, SHELXTL v6.10, 2000, Bruker AXS Inc., Madison, WI). Corrections were applied for Lorentz, polarization, and absorption effects. Parameters refined included atomic coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms on carbons were included using a riding model [coordinate shifts of C applied to H atoms] with C-H distance set at 0.96 Å. Complete information on data collection and refinement is available in the supplemental material.

For compound **12** a 0.612 × 0.141 × 0.071 mm^3 crystal was prepared for data collection and a data set collected at 100°K. The crystal was triclinic in space group P -1, with unit cell dimensions a = 10.3229(5), b = 10.6517(5), c = 10.8196(5) Å, α = 76.573(2), β = 83.215(2), and γ = 77.843(2). Data was 98.5% complete to 68.26° θ (~ 0.73 Å) with an average redundancy of 2.13. The final anisotropic full matrix least-squares refinement on F^2 with 288 variables converged at R1 = 4.46%, for the observed data and wR2 = 12.00% for all data.

For compound **19** a 0.583 × 0.188 × 0.145 mm^3 crystal was prepared for data collection and a data set collected at room temperature. The crystal was orthorhombic in space group $P2_12_12_1$, with unit cell dimensions a = 7.4350(2), b = 12.9968(5), and c = 14.6395(6) Å. Data was 95.5% complete to 69.49° θ (~ 0.83 Å) with an average redundancy of 5.45. The final anisotropic full matrix least-squares refinement on F^2 with 187 variables converged at R1 = 2.75%, for the observed data and wR2 = 7.49% for all data.

For compound **22** a 0.453 × 0.101 × 0.054 mm^3 crystal was prepared for data collection and a data set collected at 100 °K. The crystal was triclinic in space group P -1, with unit cell dimensions a = 10.4069(6), b = 10.5010(6), c = 10.5882(7) Å, α = 81.084(2), β = 68.581(2), and γ = 72.607(2). Data was 97.4% complete to 29.16° θ (~ 0.73 Å) with an average redundancy of 2.16. The final anisotropic full matrix least-squares refinement on F^2 with 265 variables converged at R1 = 4.34%, for the observed data and wR2 = 10.19% for all data.

Atomic coordinates for **12**, **19**, and **22** have been deposited with the Cambridge Crystallographic Data Centre (deposition numbers CCDC 897094, CCDC 897095, and CCDC 897096, respectively). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)-1223-336033 or deposit@ccdc.cam.ac.uk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Synthesis of an *N*-phenethyl substituted carbocyclic tricyclic system with high affinity for μ-opioid receptors.
- Potent (*K*e < 1 nM) and reasonably μ-selective (μ/κ ratio 20 to 27) opioid antagonists.
- Oxygen atoms (carbonyl and OH) on the carbon bridge of the inner ring do not improve affinity.

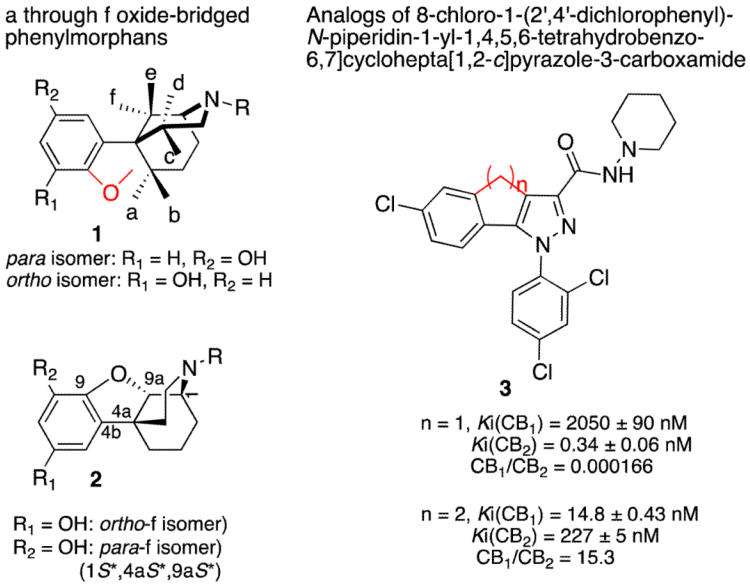


Fig. 1.
Structures of oxide-bridged phenylmorphans and cannabinoid receptor ligands

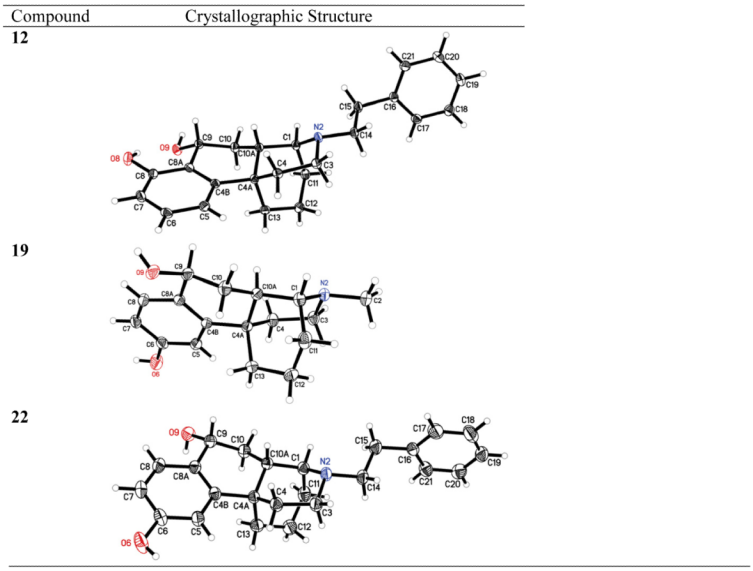
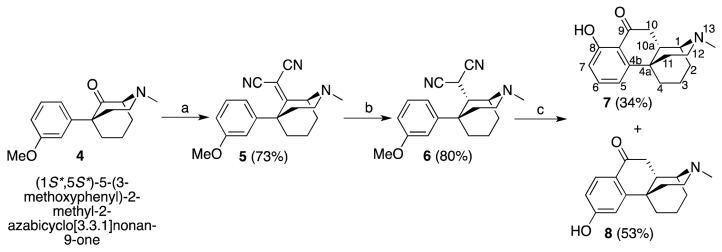


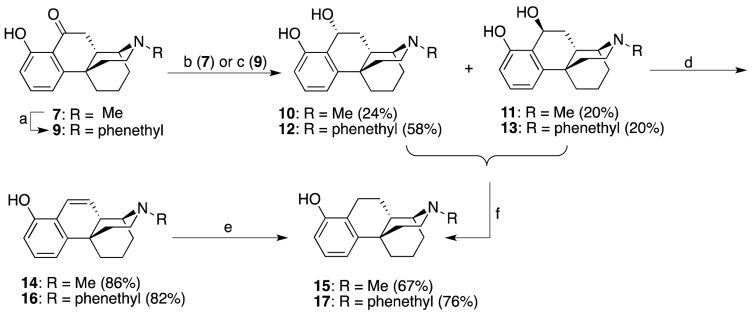
Figure 2.

X-ray crystallographic structure of (1*S**,4a*R**,9*R**,10a*S**)-13-phenethyl-2,3,4,9,10,10a-hexahydro-1*H*-1,4a-(epiminoethano)phenanthrene-8,9-diol (**12**), (1*R*,4a*S*,9*S*,10a*R*)-13-methyl-2,3,4,9,10,10a-hexahydro-1*H*-1,4a-(epiminoethano)phenanthrene-6,9-diol (**19**) and (1*S**,4a*R**,9*R**,10a*S**)-13-phenethyl-2,3,4,9,10,10a-hexahydro-1*H*-1,4a-(epiminoethano)phenanthrene 6,9-diol (**22**). Displacement ellipsoids are shown at the 30% level for compounds **19** and **22** and at the 50% level for compound **12**.



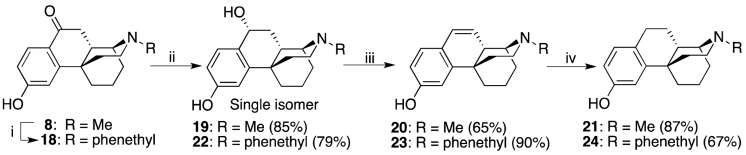
Scheme 1.

Synthesis of *ortho*- and *para*-hydroxyphenylketones **7** and **8**. Reagents and conditions: a) malononitrile, NH_4OAc, AcOH, toluene, reflux, 2 h; b) H_2 (60 psi) PtO_2, EtOH, 4 h; c) (1) 10 M HCl,reflux, overnight; (2) 48% HBr, reflux, 48 h.



Scheme 2.

Synthesis of *ortho*-hydroxyphenyl 9α- and 9β-hydroxy compounds and the saturated and unsaturated carbocyclics. Reagents and conditions: a) (1) $ClCO_2Et$, K_2CO_3, $ClCH_2CH_2Cl$, reflux, overnight; (2) 48% HBr, reflux, overnight; (3) phenethyl bromide, Et_3N, EtOH, reflux, 48 h; b) superhydride, THF, −78 °C to rt, overnight; c) LiAlH , THF, 0 °C, 1 h; d) 2 M HCl, MeOH, rt; e) H_2 (60psi), 5% Pd/C, EtOH, overnight; f) H_2 (60 psi), 5% Pd/C, 2 M HCl, EtOH.

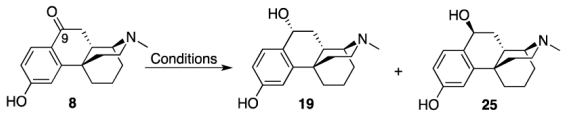


Scheme 3.

Preparation of the C9α-OH compound, and the unsaturated and saturated carbocyclic compounds in the *para*-hydroxyphenyl series. Reagents and conditions: i) (1) $ClCO_2Et$, K_2CO_3, $ClCH_2CH_2Cl$, reflux, overnight; (2) 48% HBr, reflux, overnight; (3) phenethyl bromide, Et_3N, EtOH, reflux, 48 h; ii) LiAlH , THF, 0 °C, 1 h; iii) 2 M HCl, MeOH, rt; iv) H_2 (60 psi), 5% Pd/C, EtOH,overnight.

Table 1

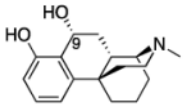
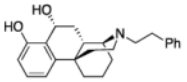
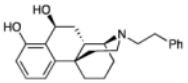
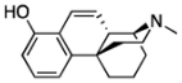
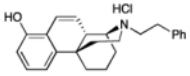
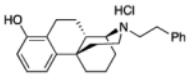
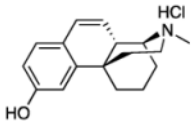
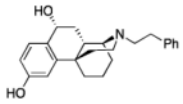
Attempts to introduce a C9β-OH in the *para*-hydroxyphenyl series

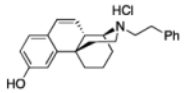
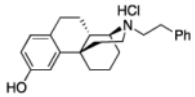


Conditions	Results
1) $NaBH_4$, MeOH, rt	**19** (25%, ~57% SM recovered)
2) Superhydride, −78 °C to rt, overnight	**19** (43%)
3) $LiAlH_4$, THF, O °C, 2h	**19** (93%)
4) Formamidinesulfinic acid, aq NaOH	SM, no reaction
5) K-selectride, KH, THF, rt, overnight	Decomposed
6) PtO_2, H_2	2h: **19** + SM; 48h: **20** + SM
7) $Al(O\textit{i}\text{-}Pr)_3$, *i*-PrOH, toluene, 50 °C	**20** (67%)
8) Li, NH_3 (liquid), *t*-BuOH, THF, −78 °C	**19** (58%) + **25** (17%)

Table 2

Opioid binding affinity (*K*i, nM) and efficacy ([^{35}S]GTPγS, *K*e, nM) of selected antagonists

#		K_i μ[a]	K_i δ[b]	$\tilde{\delta}$ μ	K_i κ[c]	κ/μ	K_e μ
10		30 ± 6	3220 ± 332	107	2020 ± 169	24	706 ± 135
12		14 ± 2	>10,000	714	525 ± 39	38	6 ± 1
13		179 ± 14	>10,000	56	923 ± 32	5	ND
14		67 ± 7	>10,000	149	3890 ± 441	58	ND
16		19 ± 4	1840 ±212	97	369 ± 26	19	1.7 ± 0.26
17		21 ± 5	2830 ± 223	135	873 ± 101	42	7 ± 1.8
20		57 ± 6	>10,000	175	4040 ± 363	71	ND
22		72 ± 10	>10,000	139	1810 ± 95	25	ND

#		*K*i μ[a]	*K*i δ[b]	$\tilde{\delta}$ μ	*K*i κ[c]	κ/μ	K_e μ
23		2.6 ± 0.35	127 ± 31	49	51 ± 4	20	0.34 ± 0.07
24		2.3 ± 0.2	186 ± 25	81	61 ± 6	27	0.71 ± 0.13

Opiate receptor binding assays were performed as indicated in section *5.3.2* using CHO hMOR, CHO hDOR and CHO hKOR cells. The data of three experiments were pooled (N=30 data points) and fit to the two-parameter logistic equation for the best-fit estimates (±SD) of the IC_{50}, the value of which was then used to calculate the *K*i value, as described in section *5.3.2*; the *K*e values from the [^{35}S]GTPgS assay was carried out as indicated in section *5.3.3*. ND = not done. In these assays, the μ*K*i of morphine = 2.6 ± 0.01 nM, DAMGO μ*K*i = 1.2 ± 0.1, and the μ*K*e of naloxone = 2.3 ± 0.3 nM.

[a] [^{3}H]-DAMGO

[b] [^{3}H]-DADLE

[c] [^{3}H]-U69,593

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