(*E*)- and (*Z*)-7-Arylidenenaltrexones: Synthesis and Opioid Receptor Radioligand Displacement Assays

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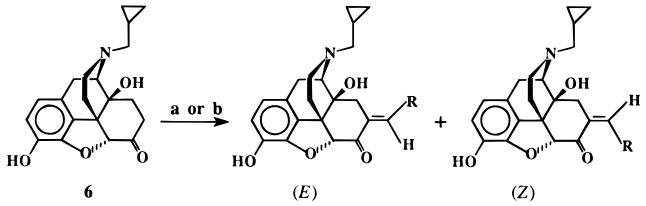
The *E*-isomer of 7-benzylidenenaltrexone (BNTX, **1a**) was reported by Portoghese[1,2] as a highly selective *δ*-opioid antagonist. The corresponding *Z*-isomer **1b** was not readily available through direct aldol condensation of naltrexone (**6**) with benzaldehyde. Using the photochemical methods employed by Lewis to isomerize cinnamamides,[3] we have obtained *Z*-isomer **1b** in good yield from *E*-isomer **1a**. A series of (*E*)- and (*Z*)-7-arylidenenaltrexone derivatives was prepared to study the effect of larger arylidene groups on opioid receptor affinity in this series. By aldol condensation of naltrexone (**6**) with benzaldehyde, 1-naphthaldehyde, 2-naphthaldehyde, 4-phenylbenzaldehyde, and 9-anthracaldehyde, the (*E*)-arylidenes were readily obtained. Photochemical isomerization afforded the corresponding *Z*-isomers. These compounds were evaluated *via* opioid receptor radioligand displacement assays. In these assays, the *Z*-isomers generally had higher affinity and were more *δ*-selective than the corresponding *E*-isomers. The (*Z*)-7-(1-naphthylidene)naltrexone (**3b**) showed the greatest selectivity (*δ*:*μ* ratio of 15) and highest affinity *δ*-binding (K_i = 0.7 nM). PM3 semiempirical geometry optimizations suggest a significant role for the orientation of the arylidene substituent in the binding affinity and *δ*-receptor selectivity. This work demonstrates that larger groups may be incorporated into the arylidene portion of the molecule with opioid receptor affinity being retained.

Naltrexone (**6**) is an important opioid antagonist that binds at all classes of opioid receptors but has a small preference for the *μ*- and *κ*-receptors over the *δ*-receptors.[4] In a search for receptor subtype specific antagonists, it has been found that naltrindole[5] and (*E*)-7-benzylidenenaltrexone (BNTX, **1a**) are agents with significant *δ*-receptor selectivity.[2] It has been postulated that the similar activities of these two compounds are due, at least in part, to sharing the same conformational space by their aromatic moieties in the ligand−receptor interaction.[2] Rigidly fixing the location of the aryl group, as through the olefin in **1a**, allows the isolation of that component of binding influenced by the conformational space occupied by the aryl group.

Though the influence of the direction of the aryl group with regard to the rest of the structure has been partially addressed,[2] its size limitations have not. Using BNTX (**1a**) as a starting point, a series of 10 7-arylidenenaltrexone derivatives was synthesized. Both the *E*- and *Z*-isomers of the 7-benzylidene compound (**1a**,**b**) were obtained *via* the standard aldol condensation of naltrexone and benzaldehyde; however, the *Z*-form was incompletely characterized due to the very low *Z*/*E* ratio of products (∼2/∼98).[1,2] This difficulty has now been overcome through the use of a photochemical isomerization process to convert *E*-olefins to the corresponding *Z*-olefins. Using the methods employed by Lewis to photoisomerize cinnamamides,[3] *Z*-isomer **1b** was readily obtained from *E*-isomer **1a**. Using the same approach, the *E*- and *Z*-isomers of the aldol condensation products of naltrexone with a series of aldehydes (1-naphthaldehyde, 2-naphthaldehyde, 4-phenylbenzaldehyde, and 9-anthracaldehyde) were prepared. This series is a useful battery of compounds to probe the spatial requirements at C-7 of *δ*-selective opioid receptor binding.

Scheme 1[a]



[a] Reagents: (a) R-CHO, NaOH, MeOH, 0 °C for 12 h; (b) R-CHO, 87% aq KOH, MeOH, reflux for 2−4 h.

Results and Discussion

Chemistry. Compounds **1a**,**b**−**5a**,**b** were prepared by aldol condensation of the appropriate aldehyde with naltrexone (**6**) (Scheme 1). Liquid aldehydes were converted to their 7-arylidene derivatives using a procedure analogous to that for the formation of 7-benzylidenenaltrexones **1a**,**b**.[1,2] Solid aldehydes were condensed with naltrexone using an adaptation of the procedure reported by Rice.[6] In all cases of these nonstereospecific aldol condensations, more of the sterically less-encumbered *E*-isomer was produced than the *Z*-isomer. *E*:*Z* isomeric ratios were on the order of >90:<10. The yield of aldol condensation product decreased with increasing size of the aromatic aldehyde, presumably due to steric effects and perhaps due to the solubility of the aldehyde in the reaction medium. The photoisomerizations (Scheme 2) of the *E*- to *Z*-compounds also showed a less-favorable *E*- to *Z*-photoequi-

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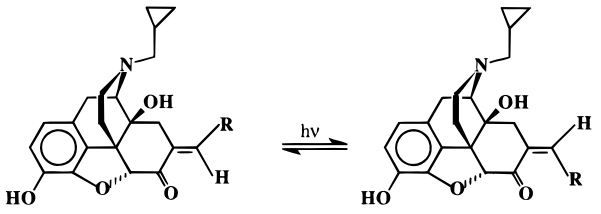
Scheme 2



librium with increasing size of the 7-arylidene group. The photoreactions went to photostationary states with the *E*:*Z* ratios being 60:40 to 25:75. In all cases, only trace amounts of the (*E*)-7-arylidenenaltrexone decomposed, allowing for reisolation of nonisomerized *E*-isomer.

Stereochemical characterization of the individual isomers was based on the chemical shifts of the signals of the vinyl protons in the NMR. In the ^{1}H NMR spectra of α,β-unsaturated carbonyl compounds, the chemical shift of a β-vinyl proton of a *Z*-isomer appears upfield of the signal for the corresponding *E*-isomer.[7] This difference was observed in these *E*/*Z* isomeric pairs in every case.

Opioid Receptor Affinity. The affinities of the 10 7-arylidene *E*/*Z* pairs of compounds (**1a**,**b**−**5a**,**b**) at the three opioid receptor sites (μ, δ, and κ) were determined in a crude membrane preparation from guinea pig brain. The radioligand displacement assays were determined using [^{3}H]bremazocine (total opioid sites), [^{3}H]DAMGO (μ-sites), [^{3}H]DPDPE (δ-sites), and [^{3}H]U69,593 (κ-sites). Naltrexone (**6**) was included as a standard in the assays. The K_D value for each of the radioligands determined in the guinea pig brain homogenate are reported (Table 1). Both Scatchard plots and saturation curves were derived and provided analogous results (not shown). An average value of these two determinations is reported as the K_D. The derived K_D's from guinea pig brain homogenate preparations were then used with the IC_{50}'s in the Cheng−Prusoff estimation[8] to derive K_i's for each of the test ligands (Table 1). Comparison of the results of these K_i determinations with those previously reported for naltrexone (**6**) shows consistent values.[9] A slight difference for the IC_{50} value for κ-sites was noted but may be attributed to the use of different radioligands (1.0 nM [^{3}H]U69,593 in this work versus 0.5 nM [^{3}H]bremazocine in the presence of 100 nM DAMGO and 100 nM DPDPE in the earlier experiment).

None of the new compounds had an overall receptor affinity as high as was observed for naltrexone (**6**). With the exceptions of (*E*)-biphenyl compound **2a** and (*E*)-2-naphthyl compound **4a**, all of the synthetic compounds showed the highest affinity at the δ-sites. This is in contrast to naltrexone (**6**) which showed lower affinity at the δ-receptors than at the other opioid receptor types. Though high δ-selectivity of the (*E*)-benzylidene compound **1a** in a guinea pig brain homogenate was previously reported,[1,2] we did not observe the same result in our guinea pig brain preparation. We observed

Table 1. K_i's of Synthetic 7-Arylidenenaltrexones in Radioligand Displacement Assays[a]

test ligand	total	K_i (nM) μ	δ	κ	selectivity for δ-sites (δ:μ ratio)
(*E*)-benzyl (**1a**)	100	26	6.2	48	4.0
(*Z*)-benzyl (**1b**)	120	33	3.7	34	8.6
(*E*)-biphenyl (**2a**)	210	50	75	190	0.6
(*Z*)-biphenyl (**2b**)	750	210	47	400	4.2
(*E*)-1-naphthyl (**3a**)	110	36	4.6	120	7.5
(*Z*)-1-naphthyl (**3b**)	22	11	0.7	37	15
(*E*)-2-naphthyl (**4a**)	138	23	39	74	0.6
(*Z*)-2-naphthyl (**4b**)	190	51	16	180	3.0
(*E*)-anthracyl (**5a**)	150	110	17	170	6.4
(*Z*)-anthracyl (**5b**)	50	34	2.4	100	14
naltrexone (**6**)	2.6	0.8	11	1.1	0.1

[a] Results are calculated based on the Cheng−Prusoff estimation[8] and the experimental determinations of K_D reported herein. IC_{50}'s were calculated from duplicate samples (±10−15%) at nine concentrations of 1.0−1000 nM test ligand. The displacing radioligands were as follows: (−)-[9-^{3}H]bremazocine (0.5 nM) (K_D = 1.1 nM), total sites; [^{3}H]DAMGO or [^{3}H][D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (1.0 nM) (K_D = 2.7 nM), μ-sites; [^{3}H]DPDPE or [^{3}H][D-Pen^2,D-Pen^5]enkephalin (1.0 nM) (K_D = 2.3 nM), δ-sites; and [^{3}H]U69,593 or [^{3}H]-(5α,7α,8β)-(−)-*N*-methyl-*N*-(1-pyrrolidinyl-1-oxaspiro[4.5]dec-8-yl)benzeneacetamide (1.0 nM) (K_D = 7.0 nM), κ-sites. The slight differences in the K_D's reported herein and those reported by Schultz[16] ([^{3}H]DAMGO = 1 nM, [^{3}H]DPDPE = 4.7 nM, [^{3}H]U69,593 = 1.35 nM) are attributed to the differences in membrane preparations from guinea pig brain homogenate versus calf frontal cortex or calf caudate tissue.

very similar affinity profiles between **1a** and **1b,** with *Z*-isomer **1b** showing slightly greater δ-affinity and δ-selectivity.

The most δ-selective affinity profile was exhibited by (*Z*)-1-naphthyl compound **3b**. This compound provided the lowest K_i's of all four receptor affinities assayed. In the total sites (bremazocine) and μ-sites (DAMGO) assays, **3b** has a 2−20-fold higher affinity than the other nine test compounds. However, its affinity at these sites is about 10−15 times less (total and μ-sites, respectively) than that of naltrexone (**6**). In the assay for κ-sites (U69,593), **3b** has approximately equal affinity as the 7-benzylidene compounds **1a**,**b** and 10 times higher affinity than the least potent κ-ligand, the (*Z*)-4-biphenyl compound **2b**. At δ-receptors, **3b** is bound with greater affinity (3-fold) relative to the next closest compound, (*Z*)-9-anthracylidene **5b**, and with more than 100 times the affinity of the least potent δ-ligand, (*E*)-4-biphenyl compound **2a**. The δ-receptor affinity of **3b** is also 15 times higher than that of naltrexone (**6**) and 5−9 times higher than the (*Z*)- and (*E*)-benzylidene compounds **1b**,**a**, respectively.

The δ:μ selectivity demonstrated by the synthetic compounds is greater by 2−7-fold for each of the *Z*-isomers versus its corresponding *E*-isomer in all five cases (Table 1). The (*Z*)-1-naphthyl analog **3b** shows the highest selectivity of the series with a δ:μ K_i ratio of about 15. Benzylidene analogs **1a**,**b** exhibit only 25−50% the δ:μ selectivity of **3b**. Compound **3a**, the (*E*)-1-naphthylidene analog of **3b**, shows only 50% of the δ:μ selectivity of **3b**. Interestingly, the analog with the largest substituent, (*Z*)-anthracyl **5b**, is nearly as δ-selective as **3b**, and its *E*-isomer (**5a**) also shows roughly 50% of its δ:μ selectivity.

The δ:κ selectivity ratios for all of the ligands were generally higher than the δ:μ ratios (by almost 3-fold) because all of the ligands showed less affinity for κ-receptors than for μ-receptors, usually by about 3-fold.

One exception was *Z*-isomer **1b**, which showed a δ:κ ratio approximately equal to its δ:μ ratio.

Computational Chemistry. To gain some insight about the expected conformations of the aryl groups with respect to the olefin−carbonyl system in these compounds, geometry optimization studies were performed. In a previous study of **1a**,**b**, a molecular mechanics force field method was used.[2] We report the use of a semiempirical computational approach that includes *a priori* examination of electronic effects of the conjugated system. The geometries of naltrexone and the 10 arylidene derivatives were optimized with the PM3 semiempirical method[10] using SPARTAN 4.0 (Wavefunction, Inc., Irvine, CA). Comparison of the resulting structures for relative conformational space sampling was then accomplished by overlaying the rigid epoxymorphone moieties and examining that space sampled by the aromatic groups at C-7. The minimized energies of the *Z*-isomers were higher than those of their corresponding *E*-counterparts by 2.8−5.6 kcal/mol. Although the magnitude of the error associated with these calculations (±∼1 kcal/mol) precluded analysis of specific energy differences, a clear trend that larger aryl groups tend to have larger *E*/*Z* energy differences is observed.

In the minimized structures, as expected, none of the aryl rings is coplanar with the olefin−carbonyl system. A nearly perpendicular relationship between the aryl group and the olefin−carbonyl system is found in each case. The aryl ring is forced out of planarity with the double bond and the carbonyl group by unfavorable steric interactions. The degree of nonplanarity of the 7-arylidene group from the olefin−carbonyl plane was quantitated in the minimized structures by measuring the dihedral angle formed by C-7-benzyl carbon−first aromatic carbon−second aromatic carbon. Planarity is defined by angles of 0° or ±180°, while the largest difference is at ±90°. This difference from planarity for the *E*-compounds **1a**−**5a** ranges from 68° in the case of (*E*)-4-biphenyl compound **2a** to 80° for (*E*)-9-anthracyl compound **5a**. The planarity deviations for the *Z*-isomers **1b**−**5b** ranged from −33° for (*Z*)-2-naphthyl compound **4b** to −81° for (*Z*)-9-anthracyl compound **5b**, the sign of the angle reflecting the *E* versus *Z* disposition of the 7-arylidene group. In the case of the 9-anthracylidene compounds, the differences are virtually identical: *Z*-isomer **5b**, −81°, and *E*-isomer **5a**, −80°. In all other cases, the *Z*-isomer has a smaller deviation from planarity. It is also noteworthy that the deviations are essentially identical in the benzyl series **1a**,**b** and the 4-biphenyl series **2a**,**b**. Values of 69° and 68° were obtained for *E*-isomers **1a** and **2a**, respectively, and −55° and −54° for *Z*-isomers **1b** and **2b**, respectively. Deviations of 71° and −33° were obtained for the (*E*)- (**4a**) and (*Z*)-2-naphthylidenes (**4b**), respectively, while deviations of 77° and −68° were obtained for the (*E*)- (**3a**) and (*Z*)-1-naphthylidenes (**3b**), respectively.

The (*E*)-1-naphthyl (**3a**) and (*Z*)-9-anthracyl (**5b**) compounds show the spatial extremes in the minimized structures. The aryl group in *Z*-isomer **5b** extends above and away from the epoxymorphinone nucleus, while in *E*-isomer **3a**, the aryl ring is in an orientation forming a "pocket" with the epoxymorphinone (Figure 1). The *Z*-isomer of the 1-naphthyl series (**3b**) occupies a similar area of conformational space as the (*Z*)-9-anthracyl compound **5b**. That these two compounds are similar in conformation and also possess the highest binding affinity and greatest δ-selectivity suggest that an aryl group fixed in this position is significant. The difference in binding affinities and δ-selectivities between the benzyl (**1a**,**b**) and extended 4-biphenyl (**4a**,**b**) compounds suggests that additional substitution on the distal portion of the benzyl ring is not tolerated, despite strong structural similarity.

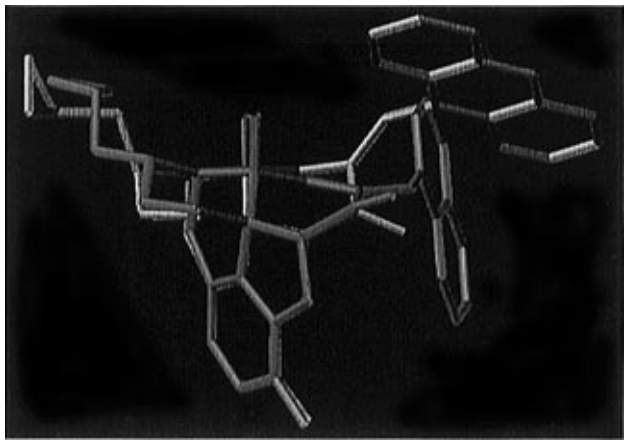


Figure 1. PM3-minimized structures of **3a** (green) and **5b** (yellow) showing the different dispositions of the 7-arylidene rings. The conformation exhibited by compound **5b** appears to be preferred for binding affinity and δ-selectivity.

Conclusion

We have shown that the *E*-isomers of 7-arylidenenaltrexone derivatives can be readily converted photochemically to their corresponding *Z*-isomers without degradation. Furthermore, the results obtained from the opioid receptor binding assays demonstrate that the *Z*-isomers of the 7-arylidene series are generally more δ-selective than are the *E*-isomers in the radioligand displacement profile in guinea pig brain homogenate receptors. PM3 semiempirical geometry calculations suggest a singificant role for the orientation of the 7-arylidene substituent in the binding of these ligands. The results also suggest that the spatial requirements of the receptor will allow an extension to the lateral portion of the benzylidene moiety (as in a naphthyl or anthracyl group) to bind without difficulty. However, the placement of an additional phenyl substituent on the benzylidene in the position distal to the olefin, as in a 4-biphenyl compound, is not tolerated. Overall, the highest affinity and greatest δ-selectivity was observed in (*Z*)-7-(1-naphthylidene)naltrexone (**3b**).

Experimental Section

General Methods. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded as thin film depositions on NaCl plates with a Perkin-Elmer 1600 series FTIR. Absorptions are expressed in wavenumbers (cm^{-1}). ^1H NMR spectra were recorded at 300 MHz on a Varian VXR-300 or Bruker AMX-300 spectrometer. Chemical shifts are expressed in parts per million (δ) downfield from $Si(CH_3)_4$. Spectral assignments were supported by proton decoupling. Mass spectra were obtained on VG-7070 and VG-70SEQ mass spectrometers by direct-insertion probe. Analytical thin-layer chromatography (TLC) was performed on Analtech silica gel HLF TLC plates (0.25 mm thickness), and compounds were visualized using a short wave UV lamp. TLC eluent was 95:5:0.5 CH_2Cl_2:MeOH:NH_4OH, unless otherwise indicated. Merck silica gel 60 (230−400 mesh) was used for preparative flash

chromatography. All reactions were performed under an argon atmosphere unless otherwise noted.

Synthetic Procedures: Aldol Condensations. All liquid aryl aldehydes were condensed with naltrexone using adaptations of a previously reported method (procedure a, Scheme 1).[1,2] Solid aldehydes were condensed with naltrexone using the procedure outlined below (procedure b, Scheme 1).[6]

To 340 mg (1.0 mmol) of naltrexone (**6**) free base in 30 mL of methanol were added 456 mg (8.0 mmol) of KOH and 6.0 mmol of the appropriate aldehyde. The mixture was heated to reflux under an argon atmosphere for 2−4 h, during which time the solution turned a deep orange. The solution was poured into 100 mL of water, extracted with EtOAc (3 × 50 mL), and washed with brine (1 × 75 mL). The organic layers were combined and dried over Na_2SO_4, and the solvent was evaporated. Purification of the arylidene isomeric mixture was effected by column chromatography (SiO_2; CH_2Cl_2:MeOH:NH_4-OH, 95:5:0.5). The final separation of the *E*- and *Z*-isomers was accomplished by using multiple elutions on preparative TLC plates (SiO_2, 2.0 mm thickness) in the same solvent system. Yields for the aldol condensation ranged 36−75% with the *Z*-isomer present only in trace (<10%) amounts. Reported yields for the *E*-isomers represent yields from the aldol condensations; reported yields for the *Z*-isomers represent yields from the photoisomerization reactions (with the balance of the yield being unconverted *E*-isomer).

Photoisomerization. In a Pyrex test tube (20 mm × 150 mm) 100 mg of the appropriate (*E*)-arylidene ketone was dissolved in 25 mL of reagent grade CH_2Cl_2. The test tube was sealed with a latex septum and purged with dry nitrogen for 3−5 min. The test tube was then irradiated (Hanovia Hg Arc lamp) for 12−14 h with cooling such that the reaction temperature did not exceed 30 °C. The solvent was evaporated and the resultant brown oil chromatographed (SiO_2; CH_2Cl_2:MeOH:NH_4OH, 95:5:0.5). Separation of the *E*- and *Z*-isomers of the 7-arylidene product was accomplished by using multiple elutions on preparative TLC plates (SiO_2, 2.0 mm thickness) in the same solvent system. Photoequilibrium ratios were 40:60 to 25:75, *E:Z* (except in the case of the 9-anthracyl compound **5b**, where a ratio of *ca.* 60:40 *E:Z* was observed).

17-(Cyclopropylmethyl)-4,5-α-epoxy-7(*E*)-benzylidene-3,14-dihydroxymorphinan (1a): ^{1}H NMR ($CDCl_3$) δ 7.68 (s, 1 H, vinyl H), 6.74 (d, J = 8.2 Hz, 1 H, C-2 H), 6.64 (d, J = 8.2 Hz, 1 H, C-1 H), 4.70 (s, 1 H, C-5 H), 1.67 (d, J = 11.9 Hz, 1 H, C-15′ H), 0.84 (m, 1 H, C-18 H), 0.55 (d, J = 8.3 Hz, 2 H, C-19/20 H), 0.13 (d, J = 4.9 Hz, 2 H, C-19′/20′ H); yield 75% as a yellow solid, mp 115−118 °C; FTIR (neat) 1685 (C═O), 1593 (C═C) cm^{-1}; HRFABMS calcd for $C_{27}H_{28}O_4N$ (M + H) 430.2018, obsd 430.2012. Anal. ($C_{27}H_{27}O_4N$) C, H, N.

17-(Cyclopropylmethyl)-4,5-α-epoxy-7(*Z*)-benzylidene-3,14-dihydroxymorphinan (1b): ^{1}H NMR ($CDCl_3$) δ 7.43 (m, 2 H), 7.28 (m, 3 H), 6.73 (d, J = 8.3 Hz, 1 H, C-2 H), 6.66 (s, 1 H, vinyl H), 6.62 (d, J = 8.3 Hz, 1 H, C-1 H), 4.78 (s, 1 H, C-5 H), 1.61 (d, J = 12.6 Hz, 1 H, C-15′ H), 0.88 (m, 1 H, C-18 H), 0.57 (d, J = 8.3 Hz, 2 H, C-19/20 H), 0.16 (d, J = 4.2 Hz, 2 H, C-19′/20′ H); yield 75% as a yellow solid, mp 171−172 °C; FTIR (neat) 1692 (C═O), 1614 (C═C) cm^{-1}; HRFABMS calcd for $C_{27}H_{28}O_4N$ (M + H) 430.2018, obsd 430.2014. Anal. ($C_{27}H_{27}O_4N$) C, H, N.

17-(Cyclopropylmethyl)-4,5-α-epoxy-7(*E*)-(4-phenylbenzylidene)-3,14-dihydroxymorphinan (2a): ^{1}H NMR ($CDCl_3$) δ 7.70 (s, 1 H, vinyl H), 7.58 (m, 4 H), 7.42 (m, 5 H), 6.76 (d, J = 7.5 Hz, 1 H, C-2 H), 6.64 (d, J = 7.7 Hz, 1 H, C-1 H), 4.72 (s, 1 H, C-5 H), 1.66 (d, J = 11.5 Hz, 1 H, C-15′ H), 0.84 (m, 1 H, C-18 H), 0.53 (d, J = 7.7 Hz, 2 H, C-19/20 H), 0.13 (d, J = 4.2 Hz, 2 H, C-19′/20′ H); yield 70% as a yellow solid, mp 133−136 °C; FTIR (neat) 1686 (C═O), 1639 (C═C) cm^{-1}; HRFABMS calcd for $C_{33}H_{32}O_4N$ (M + H) 506.2331, obsd 506.2317. Anal. ($C_{33}H_{31}O_4N$) C, H, N.

17-(Cyclopropylmethyl)-4,5-α-epoxy-7(*Z*)-(4-phenylbenzylidene)-3,14-dihydroxymorphinan (2b): ^{1}H NMR ($CDCl_3$) δ 7.58 (d, J = 8.3 Hz, 2 H), 7.53 (s, 4 H), 7.43 (m, 2 H), 7.35 (m, 1 H), 6.73 (d, J = 8.3 Hz, 1 H, C-2 H), 6.68 (s, 1 H, vinyl H), 6.62 (d, J = 8.3 Hz, 1 H, C-1 H), 4.80 (s, 1 H, C-5 H), 1.60 (d, J = 11.2 Hz, 1 H, C-15′ H), 0.88 (m, 1 H, C-18 H), 0.57 (d, J = 8.1 Hz, 2 H, C-19/20 H), 0.17 (d, J = 4.9 Hz, 2 H, C-19′/20′ H); yield 64% as a yellow solid, mp 179−181 °C; FTIR (neat) 1692 (C═O), 1620 (C═C) cm^{-1}; HRFABMS calcd for $C_{33}H_{32}O_4N$ (M + H) 506.2331, obsd 506.2313. Anal. ($C_{33}H_{31}O_4N$) C, H, N.

17-(Cyclopropylmethyl)-4,5-α-epoxy-7(*E*)-(1-naphthylidene)-3,14-dihydroxymorphinan (3a): ^{1}H NMR ($CDCl_3$) δ 8.18 (s, 1 H, vinyl H), 7.92 (m, 1 H), 7.84 (m, 2 H), 7.50 (m, 2 H), 7.44 (d, J = 8.2 Hz, 1 H), 7.34 (d, J = 7.2 Hz, 1 H), 6.76 (d, J = 8.2 Hz, 1 H, C-2 H), 6.63 (d, J = 8.3 Hz, 1 H, C-1 H), 4.77 (s, 1 H, C-5 H), 1.67 (d, J = 13.4 Hz, 1 H, C-15′ H), 0.79 (m, 1 H, C-18 H), 0.50 (d, J = 7.7 Hz, 2 H, C-19/20 H), 0.09 (d, J = 4.8 Hz, 2 H, C-19′/20′ H); yield 64% as a yellow solid, mp 208−210 °C; FTIR (neat) 1686 (C═O), 1611 (C═C) cm^{-1}; HRFABMS calcd for $C_{31}H_{30}O_4N$ (M + H) 480.2175, obsd 480.2151. Anal. ($C_{31}H_{29}O_4N$) C, H, N.

17-(Cyclopropylmethyl)-4,5-α-epoxy-7(*Z*)-(1-naphthylidene)-3,14-dihydroxymorphinan (3b): ^{1}H NMR ($CDCl_3$) δ 7.99 (m, 1 H), 7.79 (m, 2 H), 7.44 (m, 5 H), 7.19 (s, 1 H, vinyl H), 6.70 (d, J = 8.0 Hz, 1 H, C-2 H), 6.61 (d, J = 8.0 Hz, 1 H, C-1 H), 4.62 (s, 1 H, C-5 H), 1.56 (d, J = 10.9 Hz, 1 H, C-15′ H), 0.88 (m, 1 H, C-18 H), 0.57 (d, J = 7.6 Hz, 2 H, C-19/20 H), 0.17 (d, J = 4.4 Hz, 2 H, C-19′/20′ H); yield 68% as a yellow solid, mp 135−138 °C; FTIR (neat) 1704 (C═O), 1620 (C═C) cm^{-1}; HRFABMS calcd for $C_{31}H_{30}O_4N$ (M + H) 480.2175, obsd 480.2161. Anal. ($C_{31}H_{29}O_4N$) C, H, N.

17-(Cyclopropylmethyl)-4,5-α-epoxy-7(*E*)-(2-naphthylidene)-3,14-dihydroxymorphinan (4a): ^{1}H NMR ($CDCl_3$) δ 7.83 (s, 1 H, vinyl H), 7.81 (d, J = 8.2 Hz, 4 H), 7.42−7.52 (m, 3 H), 6.76 (d, J = 8.3 Hz, 1 H, C-2 H), 6.66 (d, J = 7.9 Hz, 1 H, C-1 H), 4.73 (s, 1 H, C-5 H), 1.67 (d, J = 11.1 Hz, 1 H, C-15′ H), 0.83 (m, 1 H, C-18 H), 0.53 (d, J = 8.1 Hz, 2 H, C-19/20 H), 0.12 (d, J = 4.9 Hz, 2 H, C-19′/20′ H); yield 57% as a yellow solid, mp 271−273 °C; FTIR (neat) 1682 (C═O), 1587 (C═C) cm^{-1}; HRFABMS calcd for $C_{31}H_{30}O_4N$ (M + H) 480.2175, obsd 480.2162. Anal. ($C_{31}H_{29}O_4N$) C, H, N.

17-(Cyclopropylmethyl)-4,5-α-epoxy-7(*Z*)-(2-naphthylidene)-3,14-dihydroxymorphinan (4b): ^{1}H NMR ($CDCl_3$) δ 7.92 (s, 1 H), 7.77 (m, 3 H), 7.46 (m, 3 H), 6.82 (s, 1 H, vinyl H), 6.73 (d, J = 7.8 Hz, 1 H, C-2 H), 6.62 (d, J = 7.8 Hz, 1 H, C-1 H), 4.83 (s, 1 H, C-5 H), 1.61 (d, J = 11.8 Hz, 1 H, C-15′ H), 0.87 (m, 1 H, C-18 H), 0.56 (d, J = 7.3 Hz, 2 H, C-19/20 H), 0.15 (d, J = 3.6 Hz, 2 H, C-19′/20′ H); yield 70% as a yellow solid, mp 163−165 °C; FTIR (neat) 1693 (C═O), 1614 (C═C) cm^{-1}; HRFABMS calcd for $C_{31}H_{30}O_4N$ (M + H) 480.2175, obsd 480.2167. Anal. ($C_{31}H_{29}O_4N$) C, H, N.

17-(Cyclopropylmethyl)-4,5-α-epoxy-7(*E*)-(9-anthracylidene)-3,14-dihydroxymorphinan (5a): ^{1}H NMR ($CDCl_3$) δ 8.45 (s, 1 H, vinyl H), 8.27 (s, 1 H), 8.15 (m, 1 H), 7.99 (m, 2 H), 7.52 (m, 3 H), 7.43 (m, 1 H), 7.33 (m, 1 H), 6.78 (d, J = 8.3 Hz, 1 H, C-2 H), 6.58 (d, J = 8.3 Hz, 1 H, C-1 H), 4.83 (s, 1 H, C-5 H), 1.66 (d, J = 12.0 Hz, 1 H, C-15′ H), 0.89 (m, 1 H, C-18 H), 0.42 (d, J = 7.3 Hz, 2 H, C-19/20 H), 0.22 (d, J = 4.8 Hz, 1 H, C-19′/20′ H); yield 36% as an orange solid, mp 200−203 °C; FTIR (neat) 1690 (C═O), 1613 (C═C) cm^{-1}; HRFABMS calcd for $C_{35}H_{32}O_4N$ (M + H) 530.2331, obsd 530.2337. Anal. ($C_{35}H_{31}O_4N$) C, H, N.

17-(Cyclopropylmethyl)-4,5-α-epoxy-7(*Z*)-(9-anthracylidene)-3,14-dihydroxymorphinan (5b): ^{1}H NMR ($CDCl_3$) δ 8.40 (d, J = 8.3 Hz, 1 H), 8.35 (s, 1 H), 7.95 (d, J = 7.5 Hz, 2 H), 7.35 (m, 5 H), 6.63 (d, J = 8.1 Hz, 1 H, C-2 H), 6.61 (s, 1 H, vinyl H), 6.56 (d, J = 8.1 Hz, 1 H, C-1 H), 4.43 (s, 1 H, C-5 H), 1.76 (d, J = 12.4 Hz, 1 H, C-15′ H), 0.85 (m, 1 H, C-18 H), 0.60 (d, J = 8.2 Hz, 1 H, C-19/20 H), 0.19 (d, J = 4.3 Hz, 1 H, C-19′/20′ H); yield 40% as a brown solid, mp 195−197 °C; FTIR (neat) 1690 (C═O), 1620 (C═C) cm^{-1}; HRFABMS calcd for $C_{35}H_{32}O_4N$ (M + H) 530.2331, obsd 530.2328. Anal. ($C_{35}H_{31}O_4N$) C, H, N.

Biological Testing. A Brandel harvestor and FP-100 Whatman GF/B fired filter paper were used for protein filtration. The filter paper for κ-receptor binding was pretreated with aqueous 0.1% poly(ethylenimine) to coat the glass fibers.[12] All glassware used in the affinity assay was silanized with Prosil-28. Polypropylene culture tubes and scintillation vials were used in all binding assays.

The binding assays were carried out with slight modifications to the procedures described by Lin and Simon[13] and

Werling.[14] Hartley-VAF Plus guinea pigs (300−350 g) were sacrificed by decapitation. The brain, less cerebellum, was removed and homogenized in 6 vol of 0.05 M Trizma buffer (pH 7.4) with a Virtishear homogenizer at a control setting of 70, for three 5-s intervals. The homogenate was centrifuged at 25000*g* at 4 °C for 20 min. The pellet was resuspended in 6 vol of aqueous 0.32 M sucrose and stored at −70 °C until needed. Frozen homogenate was thawed at room temperature and diluted with 0.05 M Trizma buffer (pH 7.4) to give a final dilution ratio of 1:60 (initial brain weight:total solution volume). This corresponded to a final protein concentration of 0.8−1.5 mg of protein/mL of homogenate as determined according to the method of Ohnishi and Barr[15] by use of a Sigma Diagnostics Micro Protein Determination Kit.

Radioligands used were [^{3}H]bremazocine (0.5 nM) for total ligand binding, [^{3}H]DAMGO (1.0 nM) for μ-receptor binding, [^{3}H]DPDPE (1.0 nM) for δ-receptor binding, and [^{3}H]U69,593 (1.0 nM) for κ-receptor binding. Synthetic ligands were tested in duplicate at nine concentrations between 1.0 and 1000 nM. Nonspecific binding was measured in the presence of 10 μM naloxone. The samples were incubated for 60 min at 25 °C. Samples were filtered, rinsed with ice-cold buffer (3 × 2 mL), and eluted with 10 mL aliquots of Aquasol, and the radioactivity was counted. Specific binding for each concentration was calculated, and the data were analyzed by probit transformation and linear regression to obtain the IC_{50}. The IC_{50}'s were then transformed into the reported K_i's using the Cheng−Prusoff equation.[8]

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