Synthesis and Pharmacological Evaluation of 6,7-Indolo/Thiazolo-Morphinans−Further SAR of Levorphanol

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To further extend the structure−activity relationships of levorphanol, two series of novel morphinans were prepared by incorporation of an indole or aminothiazole fragment to the hexyl ring (ring C) in levorphanol. Such morphinans differed from previously reported ligands in that such indole- or aminothiazole-containing morphinans displayed enhanced binding affinity to the δ opioid receptor, while the affinity to κ and μ receptors was slightly reduced.

Introduction

Analogs of opioids, such as morphine (**1**), codeine (**2**), thebaine (**3**), and levorphanol (**4**), are of current interest due to their utilities as analgesics and as building blocks for potential therapeutic agents for the treatment of drug abuse.[1−3] With levorphanol (**4**) as the structural prototype, a number of morphinanoids have been developed (Figure 1)[4−6] by substitution of the N-methyl group in **4** resulting in **5** (cyclorphan) with high affinity at both κ and μ opioid receptors, while further changes at the 3-position of this compound with the aminothiazole moiety produced highly κ-selective morphinans, such as **6** (ATPM, K_i = 0.049 nM).[6] However, no structure−activity relationship (SAR) study has been conducted investigating the modifications of the hexyl ring (ring C) of levorphanol (**4**).

Extensive SAR studies on ring C of other opioids have been conducted resulting in numerous structures possessing various pharmacological properties. For example, starting from naltrexone, Portoghese et al.[7] developed the 6,7-indole-fused morphinoid **7** (naltrindole, NTI), a widely used δ opioid receptor antagonist. Similarly, Coop and co-workers[8] reported several series of 4-hydroxy-6,7-indole morphinoids with a 14-OH group (**8**) or lacking a 14-OH group (**9**) by opening the 4,5-furanyl ring. These compounds also displayed interesting pharmacological properties at opioid receptors. Thus, it would be of interest to develop indole analogs without the 4-OH, 14-OH, and 4,5-furanyl groups (Figure 1, compound **10**). These new compounds can be viewed as direct ring C-modified indole analogs of levorphanol (**4**). We report herein the synthesis and pharmacological investigation of the indole morphinans **10a**−**c**, as well as the aminothiazole morphinans **22a**−**c**.

Chemistry. The synthesis was initiated from codeine (**2**), which was treated with *n*-BuLi followed by hydrogenation to afford the dihydrothebainone **12**[9] (Scheme 1). This compound can also be prepared from dihydrocodone **11** by treating with zinc powder in concd HCl and HOAc.[8] However, using this method, 4-OH-morphinan **13** was also obtained as a side product. A modified Sawa's Ullmann coupling (Cu/Cs_2CO_3/Py) was used to convert the 4-OH of **12** to its phenyl ether **14**.[10−13] After protection of the 6-keto group, ketal **15** was subjected to Na/NH_3 (liquid) reduction (Birch reduction) at −78 °C to yield **16**, which was hydrolyzed in 1 N HCl to yield the key intermediate 6-oxo-morphinan **17**[14] (Scheme 1). *N*-Demethylation of **17** with ethyl chloroformate[15] and the subsequent realkylation with cyclopropylmethyl bromide (CPMBr) or cyclobutylmethyl bromide (CBMBr) afforded the corresponding N-substituted 6-oxo-morphinans **18** and **19**.

The Fisher indole cyclization strategy[16] was used to prepare the 6,7-indolo-morphinans **10a**−**c**. Thus, treating the ketones **17**−**19** with $PhNHNH_2$·HCl in refluxing EtOH followed by O-demethylation with BBr_3 afforded the target compounds **10a**−**c** in 40−60% yield (Scheme 2). Bromination[17] of the ketones **17**−**19** followed by treatment of the intermediates with thiourea yielded aminothiazoles **21a**−**c**. Similarly, O-demethylation of **21a**−**c** with BBr_3 afforded 6,7-aminothiazolo-morphinans **22a**−**c** in 30−60% yield.

Results and Discussion. All new compounds including indoles **10a**−**c**, the 3-methoxy-6,7-indolomorphinans **20a**−**c**, and aminothiazolomorphinans **22a**−**c** were evaluated for their binding affinity at all three opioid receptors (μ, δ, and κ; Table 1) using a previously reported procedure.[5,6] For comparison purposes, opioid binding affinity data for compounds **4**[6] (levorphanol), **5**[6] (cyclorphan), **6**[6] (ATPM), and indoles **7**−**9**[8] were also included.

From the data shown in Table 1, the incorporation of an indole moiety into the morphinans (**4** and **5**) dramatically changed the opioid receptor selectivity. Compared to the κ and/or μ selectivity profiles of levorphanol (**4**) and cyclorphan (**5**),[4] all these compounds **20a**−**c** and **10a**−**c** displayed high affinity and selectivity at the δ receptor, although moderate to good affinities at κ and μ receptors were also observed. Compared to the 3-OH compounds, the corresponding 3-OCH_3 morphinans generally had reduced, but not abolished affinity at all three opioid receptors. All 3-OH indoles **10a**−**c** had similar affinity at the δ receptor, with K_i values of 0.17−0.35 nM. *N*-Methyl indolomorphinan **10a** displayed the highest selectivity for δ over κ (40-fold) and μ (63-fold) receptors. Compounds **10b** and **10c** also had good affinity at κ and μ, only slightly lower than that of δ receptors.

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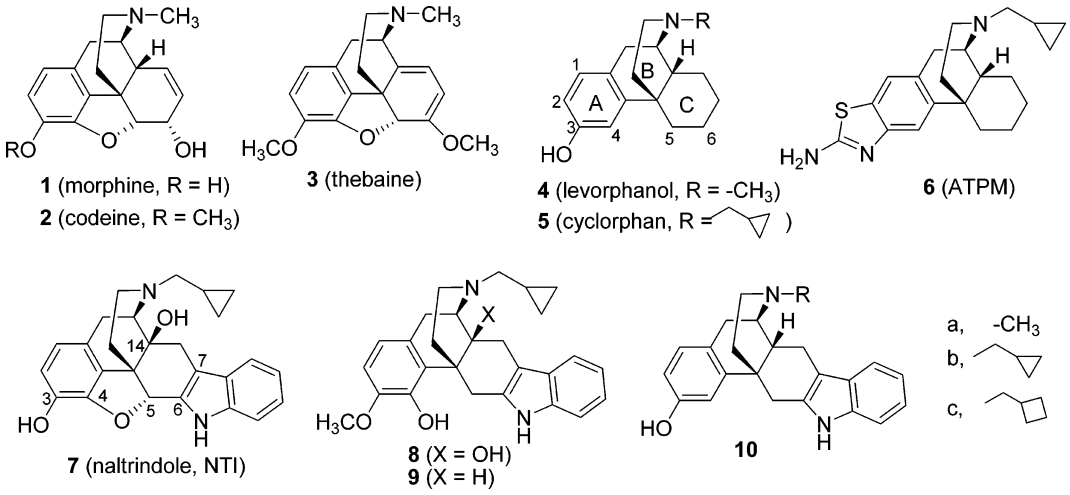
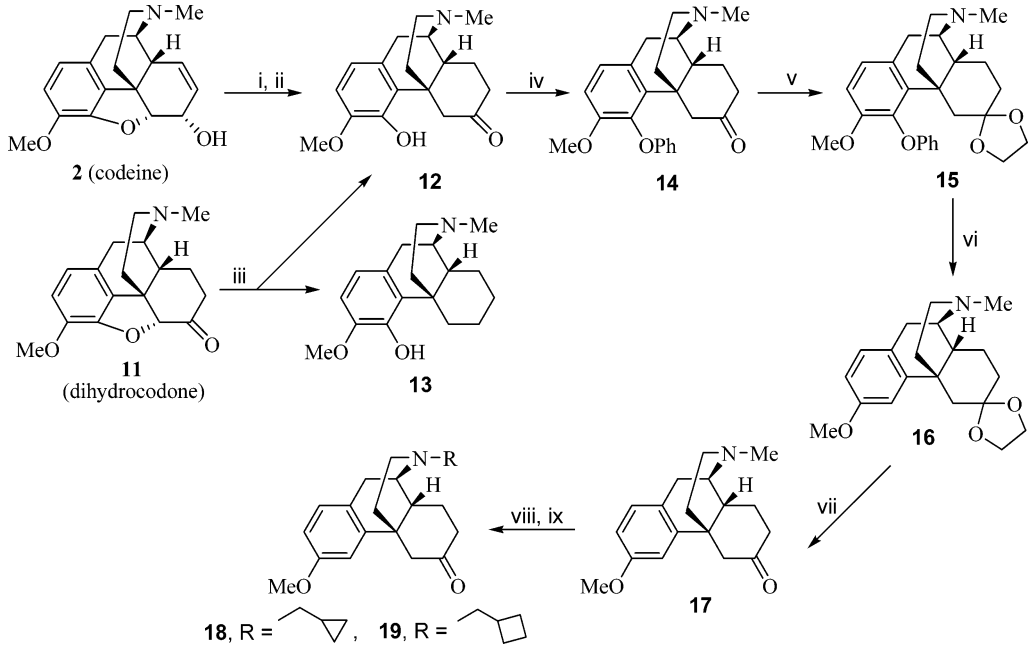


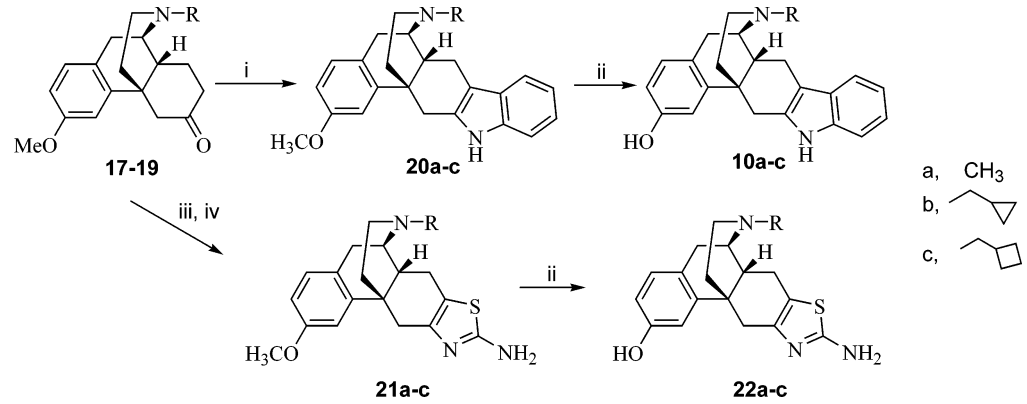
Figure 1. Representative opioids.

Scheme 1[a]



[a] Reagents and conditions: (i) BuLi, THF, 0 °C−rt; (ii) Pd/C, H_2, AcOH; (iii) Zn, HCl, AcOH; (iv) PhBr, Cu, Cs_2CO_3, Py, 110 °C; (v) ethylene glycol, TsOH, benzene, reflux: (vi) Na, NH_3 (liquid), -78 °C−rt; (vii) HCl (1 N); (viii) ClCOOEt, K_2CO_3, $CHCl_3$, reflux, then HCl, AcOH, reflux; (ix) CPMBr or CBMBr, $NaHCO_3$, EtOH, reflux.

Scheme 2[a]



[a] Reagents and conditions: (i) $PhNHNH_2$·HCl, TsOH, EtOH, reflux; (ii) BBr_3, CH_2Cl_2, −78 °C; (iii) Br_2, AcOH, 60 °C; (iv) NH_2CSNH_2, 110 °C.

The aminothiazolomorphinans **22a**−**c** all displayed good affinity at all three opioid receptors, with the selectivity remaining at the κ or μ receptors, which was similar to the prototypes **4** and **5**. *N*-Methyl aminothizaole **22a** had a K_i of 0.49 nM at the μ receptor and selectivity of 4- and 8-fold for κ and δ receptors, respectively. Compound **22b** was equipotent

Table 1. K_i Values Inhibition of δ, κ, and μ Opioid Binding to CHO Membranes by Novel Compounds[a]

cmpd	K_i (nM) ± SE			selectivity	
	[^{3}H]DAMGO (μ)	[^{3}H]naltrindole (δ)	[^{3}H]U69,593 (κ)	μ/κ	δ/κ
4 (levorphanol)[b]	0.21 ± 0.02	4.2 ± 2.3	2.3 ± 0.3	0.1	2
5 (cyclorphan)[b]	0.062 ± 0.003	1.9 ± 0.1	0.034 ± 0.002	2	56
6 (ATPM)[b]	1.5 ± 0.2	29 ± 2	0.049 ± 0.005	31	590
7 (NTI)[c]	27 ± 1	0.22 ± 0.13	30.4 ± 3.6	0.9	0.007
8[c]	1850 ± 380	22 ± 7	3160 ± 210	0.6	0.007
9[c]	13300 ± 700	7 ± 1	7900 ± 890	2	0.001
20a	340 ± 12	35 ± 5	390 ± 16	0.9	0.1
10a	22 ± 4	0.35 ± 0.05	14 ± 1	1.6	0.03
20b	5.9 ± 0.6	1.5 ± 0.1	1.4 ± 0.2	4	1
10b	1.1 ± 0.1	0.19 ± 0.02	0.64 ± 0.01	2	0.3
20c	330 ± 15	8.7 ± 0.5	89 ± 3	4	0.1
10c	3.9 ± 0.7	0.17 ± 0.03	1.0 ± 0.08	4	0.2
22a	0.49 ± 0.03	4.2 ± 1.1	2.2 ± 0.3	0.2	2
22b	0.12 ± 0.007	0.23 ± 0.01	0.07 ± 0.004	2	3
22c	0.37 ± 0.03	1.1 ± 0.2	0.14 ± 0.008	3	8

[a] CHO membranes, 0.5 mg of protein/sample, were incubated with 12 different concentrations of the compounds in the presence of receptor-specific radioligands at 25 °C, in a final volume of 1 mL of 50 mM Tris-HCl, pH 7.5. Nonspecific binding was determined using 10 uM naloxone. Data are the mean values ± SEM from three experiments, performed in triplicate. [b] Data for compounds **4** (levorphanol), **5** (cyclorphan), and **6** (ATPM) were obtained from reference 6. [c] Data for compounds **7** (naltrindole, NTI), **8**, and **9** were from the reference 8.

Table 2. E_{max}/EC_{50} and I_{max}/IC_{50} Values for the Stimulation and Inhibition of [^{35}S]GTPγS Binding by Novel Compounds[a]

cmpds	μ (mean ± S.E)		κ (mean ± S.E)		δ (mean ± S.E)	
	cmpd alone EC_{50} (nM)/E_{max}(%)	with DAMGO IC_{50}(nM)/I_{max}(%)	cmpd alone EC_{50} (nM)/E_{max}(%)	with U50,488 IC_{50}(nM)/I_{max}(%)	cmpd alone EC_{50} (nM)/E_{max}(%)	with SNC-80 IC_{50} (nM)/I_{max}(%)
10a	NT/NT	NT/NT	NT/NT	NT/NT	0.47 ± 0.09/23 ± 2	1.5 ± 0.7/45 ± 8
20b	1.7 ± 2.5/72 ± 6	440 ± 160/43 ± 3	24 ± 8/68 ± 7	NI/NI	1.5 ± 0.5/56 ± 1	NI/NI
10b	92 ± 10/47 ± 3	12 ± 6/84 ± 3	61 ± 7/67 ± 5	17 ± 6/49 ± 1	0.16 ± 0.06/22 ± 2	1.2 ± 0.4/73 ± 4
20c	NT/NT	NT/NT	NT/NT	NT/NT	23 ± 9/54 ± 6	NI/NI
10c	110 ± 10/68 ± 2	NA/23 ± 5	190 ± 22/87 ± 7	NI/NI	0.8 ± 0.4/37 ± 4	0.6 ± 0.2/55 ± 2
22a	140 ± 3/42 ± 6	NA/45 ± 7	21 ± 3/44 ± 2	NI/NI	8.4 ± 1.0/67 ± 2	NI/NI
22b	0.4 ± 0.02/34 ± 2	3 ± 1/64 ± 4	0.34 ± 0.08/37 ± 2	NI/NI	0.17 ± 0.04/49 ± 1	NI/NI
22c	2.6 ± 0.3/33 ± 4	26 ± 9/68 ± 1	2.6 ± 0.2/40 ± 5	93 ± 17/44 ± 4	2.5 ± 0.9/51 ± 3	NI/NI

[a] CHO membranes, expressing either the κ or μ receptor, were incubated with varying concentrations of the novel compounds in the presence of 0.8 nM [^{35}S]GTPγS. Data are the mean values ± SE from three experiments, performed in triplicate. NA = not available, no value could be determined; NI = no inhibition; NT = not tested, no experiments were done to determine value.

at all three receptors, with K_i values of 0.07, 0.1, and 0.2 nM at κ, μ, and δ receptors, respectively.

The stimulation and inhibition of [^{35}S]GTPγS binding studies were shown in Table 2. The indolomorphinans **10b** and **10c** displayed both agonist and antagonist activities at μ and δ receptors. At κ receptors, **10b** was a partial agonist/antagonist, whereas **10c** was a full agonist (E_{max} = 87%) without inhibition to the κ agonist, U50,488. It was of interest to note that **10b** was a κ and δ partial agonist, but its 3-methoxy analog **20b** was a κ and δ agonist. Similarly, **10c** was a δ partial agonist, but its 3-methoxy analog **20c** was a δ agonist. All aminothiazolomorphinans **22a**−**c** showed both agonist and antagonist actions at μ but only pure agonist properties at the δ receptor. At κ receptor, both **22a** and **22b** displayed pure agonist activity, but **22c** had both agonist and antagonist properties (Table 2).

In summary, we have extended the SARs of levorphanol (**4**) by incorporation of an indole or aminothiazole fragment to the ring C. Two series of novel morphinans **10a**−**c** and **22a**−**c** were prepared and their pharmacological properties at opioid receptors were evaluated. Compared to the high κ and/or μ and low δ affinity profiles of morphinans described in the previous reports,[4−6] these compounds were potent at the δ receptor. All 3-OH indoles **10a**−**c** had similar K_i values (0.17−0.35 nM) at the δ receptor. Compound **10a** displayed the highest selectivity for δ over κ (40-fold) and μ (63-fold) receptors. The aminothiazolomorphinan **22a** had a K_i of 0.49 nM at μ receptor and selectivities of 4- and 8-fold for κ and δ receptors, respectively. Compound **22b** can be considered as equipotent at all three receptors, with K_i values of 0.07, 0.1, and 0.2 nM at κ, μ, and δ receptors, respectively. These results further confirm Portoghese's and Coop's findings that the introduction of an indole function serves to reduce κ and μ receptor affinity and enhances the affinity at the δ receptor. However, the reduction in affinity at κ and μ receptors of compounds **10a**−**c** and **22a**−**c** was not significant. In comparison to the indole moiety, the aminothiazole component also improves δ receptor affinity, but the affinity at κ and μ receptors is not significantly affected.

Experimental Section

General. Melting points were determined on a Thomas−Hoover capillary tube apparatus and are reported uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker AC300 spectrometer using tetramethylsilane as an internal reference. Element analyses, performed by Atlantic Microlabs, Atlanta, GA, were within ±0.4% of theoretical values. Analytical thin-layer chromatography (TLC) was carried out on 0.2 mm Kieselgel 60F 254 silica gel plastic sheets (EM Science, Newark). Flash chromatography was used for the routine purification of reaction products. The column output was monitored with TLC.

General Procedure for Indolic Morphinans 20a−c and 10a−c: A solution of **17**−**19** (0.63 mmol), phenylhydrazine·HCl (100 mg, 0.69 mmol), and TsOH·H_2O (120 mg, 0.63 mmol) in EtOH (15 mL) was heated at reflux for 2 days. The cooled reaction mixture was evaporated, and the residue was diluted with water (20 mL). The solution was basified (pH 9) with NH_4OH and extracted with CH_2Cl_2. The extracts were combined, washed with brine, and dried over anhydrous Na_2SO_4. After removal of the solvent, the crude product was purified by column chromatography (EtOAc/Et_3N = 10/1), giving **20a**−**c** as a yellow solid.

The 3-methoxy indolic morphinans **20a**−**c** (0.3 mmol) were then dissolved in 5 mL of CH_2Cl_2, cooled to −78 °C, and dropped slowly into a solution of BBr_3 (1 M in CH_2Cl_2, 4 mL) in 4 mL of CH_2Cl_2. The mixture was stirred at −78 °C for 4 h and then at rt overnight. The solution was cooled to −78 °C again, MeOH (10 mL) was added dropwise, and the mixture was stirred at −78 °C to rt overnight. The solvent was evaporated and the residue was recrystallized from MeOH to give **10a**−**c** as HBr salts.

20a: yellow solid (57.4%). Mp 97−100 °C (dec); MS (EI) 358 (M^+); ^{1}H NMR ($CDCl_3$) δ 7.97 (s, 1H), 7.29 (d, J = 7.5 Hz, 1H), 7.23 (m, 1H), 7.01 (m, 3H), 6.80 (d, J = 2.4 Hz, 1H), 6.60 (dd, J = 2.7, 8.7 Hz, 1H), 3.66 (s, 3H), 3.48 (d, J = 16.5 Hz, 1H), 3.19 (m, 1H), 2.88 (m, 4H), 2.40 (s+m, 6H), 2.19 (m, 1H), 2.03 (m, 2H), 1.62 (m, 1H); Anal. ($C_{24}H_{26}N_2O\cdot0.5H_2O$) C, H, N.

10a: gray solid (57.6%). Mp > 250 °C; MS (EI) 344 (M^+ − HBr); ^{1}H NMR (CD_3OD) δ 7.20 (m, 2H), 6.99 (m, 2H), 6.88 (m, 1H), 6.82 (m, 1H), 6.61 (m, 1H), 3.93 (s, 1H), 3.63 (d, J = 16.8 Hz, 1H), 3.30 (m, 2H), 3.16 (m, 2H), 2.92 (s, 3H), 2.82 (m, 2H), 2.57 (m, 1H), 2.34 (m, 1H), 2.15 (m, 1H), 1.83 (m, 1H), 1.17 (m, 1H); Anal. ($C_{23}H_{25}N_2O\cdot HBr\cdot1.7H_2O$) C, H, N.

20b: yellow foam (42.4%). Mp 147−150 °C (dec); MS (EI) 398 (M^+); ^{1}H NMR ($CDCl_3$) δ 7.97 (s, 1H), 7.29 (d, J = 7.5 Hz, 1H), 7.23 (m, 1H), 7.01 (m, 3H), 6.80 (d, J = 2.4 Hz, 1H), 6.60 (dd, J = 2.7, 8.7 Hz, 1H), 3.66 (s, 3H), 3.48 (d, J = 16.5 Hz, 1H), 3.19 (m, 1H), 2.88 (m, 4H), 2.69 (m, 1H), 2.47 (m, 4H), 2.13 (m, 3H), 1.63 (m, 1H), 0.95 (m, 1H), 0.57 (m, 2H), 0.18 (m, 2H).

10b: this compound was prepared as its HCl salt (68.7%). MS (EI) 384 (M^+); ^{1}H NMR ($CDCl_3$) δ 8.47 (s, 1H), 7.73 (d, J = 6.6 Hz, 1H), 7.63 (d, J = 7.2 Hz, 1H), 7.46 (m, 2H), 7.35 (d, J = 8.7 Hz, 1H), 7.25 (m, 1H), 7.00 (d, J = 8.7 Hz, 1H), 3.97 (s, 1H), 3.77 (d, J = 15.9 Hz, 1H), 3.11 (m, 8H), 2.63 (t, J = 11.4 Hz, 1H), 2.41 (m, 1H), 1.96 (m, J = 11.4 Hz, 1H), 1.74 (m, 2H), 1.36 (m, 1H), 0.99 (d, J = 7.2 Hz, 2H), 0.60 (m, 2H); Anal. ($C_{26}H_{29}ClN_2O\cdot1.3H_2O$) C, H, N.

20c: yellow foam (51.2%). Mp 95 °C (soften); MS (EI) 412 (M^+); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.71 (s, 1H), 7.29 (d, J = 7.5 Hz, 1H), 7.25 (d, J = 7.8 Hz, 1H), 7.02 (m, 3H), 6.78 (d, J = 2.7 Hz, 1H), 6.59 (dd, J = 2.4, 8.1 Hz, 1H), 3.67 (s, 3H), 3.45 (d, J = 16.8 Hz, 1H), 3.18 (d, J = 6.0 Hz, 1H), 3.06 (d, J = 18.3 Hz, 1H), 2.85 (m, 2H), 2.60 (m, 5H), 2.3 7 (m, 2H), 2.17−1.56 (m, 7H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 157.9, 140.5, 135.6, 132.0, 129.7, 128.5, 127.6, 120.8, 119.0, 117.5, 110.8, 110.4, 110.3, 109.0, 61.5, 55.3, 55.0, 45.2, 41.7, 41.2, 37.7, 35.0, 34.1, 27.9, 27.8, 24.0, 22.5, 18.8; Anal. ($C_{28}H_{32}N_2O\cdot0.3H_2O$) C, H, N.

10c: gray powder (43.1%). Mp > 270 °C; MS (EI) 398 (M^+ − HBr); ^{1}H NMR (CD_3OD) δ 7.44 (m, 1H), 7.28 (d, J = 7.8 Hz, 1H), 7.23 (d, J = 7.8 Hz, 1H), 6.99 (m, 2H), 6.80 (m, 1H), 6.62 (m, 1H), 3.84 (s, 1H), 3.58 (d, J = 15.9 Hz, 1H), 3.36 (m, 2H), 3.21 (m, 3H), 3.07 (m, 1H), 2.93 (m, 1H), 2.73 (m, 2H), 2.27 (m, 3H), 1.94 (m, 7H); ^{13}C NMR (CD_3OD, 75 MHz) δ 156.7, 138.1, 136.2, 131.6, 129.4, 127.2, 123.3, 121.0, 118.9, 117.4, 114.9, 111.6, 110.9, 106.4, 59.4, 57.6, 46.9, 38.6, 36.2, 32.7, 31.2, 27.9, 27.5, 23.9, 22.3, 18.8; Anal. ($C_{27}H_{30}N_2O\cdot1.5HBr$) C, H, N.

General Procedure for Aminothiazolic Morphinans 22a−c: To a solution of ketone **17**−**19** (0.31 mmol) in a mixture of 1 mL of AcOH and 2 drops of HBr (48%) was added Br_2 (50 mg, 16 μL, 0.31 mmol) slowly. The solution was heated at 60 °C for 1 h. Thiourea (48 mg, 0.63 mmol) was added and the mixture was refluxed overnight. The reaction mixture was cooled to rt, poured into ice/water, basified with NH_4OH, and extracted with $CHCl_3$. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatograph (EtOAc/Et_3N = 10/1 to 4/1) to give **21a**−**c** as a yellow oil.

To a solution of 3-methoxy aminothiazole **21a**−**c** (0.21 mmol) in 5 mL of CH_2Cl_2, cooled to −78 °C, was dropped slowly a solution of BBr_3 (1 M in CH_2Cl_2, 5 mL) in 5 mL of CH_2Cl_2. The mixture was stirred at −78 °C for 2 h and then at rt overnight. After cooling to −78 °C again, a solution of MeOH (10 mL) was dropped slowly. The mixture was stirred at rt for 2 h, and the solvents were evaporated. The residue was dissolved in 10 mL of anhyd MeOH and then evaporated. The residue was dried in vacuo and recrystallized from the mixture of MeOH and Et_2O to give the 3-hydroxy aminothiazolic morphinans **22a**−**c**.

21a: yellow oil (66.7%). MS m/z (%) 342 (MH^+); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.00 (d, J = 8.4 Hz, 1H), 6.84 (d, J = 2.1 Hz, 2H), 6.66 (dd, J = 2.1, 8.4 Hz, 1H), 5.11 (s, 2H), 3.70 (s, 3H), 3.56 (m, 1H), 3.38 (m, 2H), 3.05 (m, 2H), 2.45−1.26 (m, 10H).

22a: This compound was prepared as a free base and purified by column chromatography (EtOAc/ MeOH/Et_3N = 10/4/2) to give a yellow solid (43.5%). Mp > 250 °C; MS m/z (%) 328 (MH^+); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.96 (d, J = 8.4 Hz, 1H), 6.73 (d, J = 2.1 Hz, 2H), 6.68 (dd, J = 2.1, 8.4 Hz, 1H), 4.36 (s, 2H), 3.76 (m, 1H), 3.35 (m, 3H), 2.52 (m, 6H), 1.98 (m, 1H), 1.80 (m, 1H), 1.62 (m, 3H). The free base was converted to its acetic acid salt. Anal. ($C_{18}H_{21}N_3OS\cdot CH_3COOH$) C, H, N.

21b: yellow oil (58.2%). MS m/z (%) 382 (MH^+); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.85 (d, J = 8.4 Hz, 1H), 6.71 (d, J = 2.7 Hz, 2H), 6.53 (dd, J = 2.4, 8.4 Hz, 1H), 4.77 (s, 2H), 3.57 (s, 3H), 3.27 (d, J = 16.8 Hz, 1H), 3.23 (m, 1H), 2.66 (d, J = 6.6 Hz, 1H), 2.61 (m, 1H), 2.41(m, 6H), 1.89 (m, 3H), 1.20 (m, 2H), 0.77 (m, 2H), 0.39 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.1, 158.2, 143.6, 140.3, 129.0, 128.5, 116.8, 111.7, 110.3, 59.8, 55.1, 54.6, 44.9, 41.3, 41.2, 37.4, 37.2, 24.5, 23.9, 9.3, 3.8, 3.7.

22b: yellow solid (61.7%). Mp > 270 °C; MS m/z (%) 368 (M^+ − HBr); ^{1}H NMR (CD_3OD, 300 MHz) δ 7.10 (d, J = 8.4 Hz, 1H), 6.78 (d, J = 2.7 Hz, 2H), 6.71 (dd, J = 2.4, 8.4 Hz, 1H), 4.26 (s, 2H), 3.40 (m, 9H), 2.80 (m, 4H), 2.29 (m, 2H), 1.91 (d, J = 14.4 Hz, 1H), 1.17 (m, 1H), 0.79 (m, 2H), 0.52 (m, 2H); ^{13}C NMR (CD_3OD, 75 MHz) δ 171.1, 158.2, 137.5, 132.4, 131.0, 124.9, 116.2, 114.9, 111.8; Anal. ($C_{21}H_{25}N_3OS\cdot2.1HBr\cdot2H_2O$) C, H, N.

21c: yellow oil (30.7%). MS m/z (%) 396 (MH^+); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.93 (d, J = 8.4 Hz, 1H), 6.75 (d, J = 2.4 Hz, 1H), 6.59 (dd, J = 2.4, 8.1 Hz, 1H), 4.92 (s, 2H), 3.62 (s, 3H), 3.33 (d, J = 17.1 Hz, 1H), 2.95 (m, 2H), 2.50 (m, 5H), 1.98 (m, 3H), 1.79 (m, 5H), 1.44 (d, J = 12.6 Hz, 1H), 1.24 (m, 2H), 0.82 (m, 2H); ^{13}C NMR (CD_3OD, 75 MHz) δ 165.0, 158.0, 143.5, 140.2, 135.0, 128.5, 116.8, 111.5, 110.2, 61.3, 55.0, 54.7, 45.0, 41.2, 41.1, 37.2, 37.0, 34.7, 27.8, 27.7, 24.5, 23.9, 18.8.

22c: yellow solid (42%). Mp > 270 °C; MS m/z (%) 381 (M^+ − HBr); ^{1}H NMR (CD_3OD, 300 MHz) δ 7.11 (d, J = 8.4 Hz, 1H), 6.77 (m, 1H), 6.72 (d, J = 8.4 Hz, 1H), 3.97 (s, 2H), 3.47 (m, 2H), 3.30 (m, 5H), 2.77 (m, 5H), 2.25 (m, 4H), 1.96 (m, 5H); ^{13}C NMR (CD_3OD, 75 MHz) δ 171.1, 158.2, 137.4, 132.4, 131.0, 124.9, 116.2, 114.9, 111.8, 60.3, 57.9, 39.4, 38.8, 36.4, 33.0, 32.3, 28.2, 28.0, 24.2, 23.9, 19.4; Anal. ($C_{22}H_{27}N_3OS\cdot2HBr\cdot2H_2O$) C, H, N.

General Procedure for Opioid Binding to the Human μ, δ, and κ Opioid Receptors. Chinese hamster ovary (CHO) cells stably transfected with the human κ opioid receptor (hKOR-CHO), δ opioid receptor (hDOR-CHO), and the μ opioid receptor (hMOR-CHO) were obtained from Drs. Larry Toll (SRI International, Palo Alto, CA) and George Uhl (NIDA Intramural Program, Bethesda, MD), respectively. The cells were grown in 100 mm dishes in Dulbecco's modified Eagle's media (DMEM), supplemented with 10% fetal bovine serum (FBS) and penicillin-streptomycin (10 000 units/mL) at 37 °C in a 5% CO_2 atmosphere. The affinity and selectivity of the compounds for the multiple opioid receptors were determined by incubating the membranes with radiolabeled ligands and 12 different concentrations of the compounds at 25 °C in a final volume of 1 mL of 50 mM Tris-HCl, pH 7.5. Incubation times of 60 min were used for the μ-selective peptide [^{3}H]DAMGO and the κ-selective ligand [^{3}H]U69,593. A 3 h incubation was used with the δ-selective antagonist [^{3}H]naltrindole.

General Procedure for [^{35}S]GTPγS Binding Studies To Measure Coupling to G Proteins. Membranes from CHO cells stably expressing either the human κ or μ opioid receptor were used in the experiments. Cells were scraped from tissue culture plates and then centrifuged at 1000 g for 10 min at 4 °C. The cells were resuspended in phosphate-buffered saline, pH 7.4, containing 0.04% EDTA. After centrifugation at 1000 g for 10 min at 4 °C, the cell pellet was resuspended in membrane buffer, which consisted of 50 mM Tris-HCl, 3 mM $MgCl_2$, and 1 mM EGTA, pH 7.4. The

membranes were homogenized by with a Dounce homogenizer, followed by centrifugation at 40 000 *g* for 20 min at 4 °C. The membrane pellet was resuspended in membrane buffer, and the centrifugation step was repeated. The membranes were then resuspended in assay buffer, which consisted of 50 mM Tris-HCl, 3 mM $MgCl_2$, 100 mM NaCl, and 0.2 mM EGTA, pH 7.4. The protein concentration was determined by the Bradford assay using bovine serum albumin as the standard. The membranes were frozen at −80 °C until use.

CHO cell membranes expressing either the human κ opioid receptor (15 μg of protein per tube) or μ opioid receptor (7.5 μg of protein per tube) were incubated with 12 different concentrations of the agonist in assay buffer for 60 min at 30 °C in a final volume of 0.5 mL. The reaction mixture contained 3 μM GDP and 80 pmol of [^{35}S]GTPγS. Basal activity was determined in the presence of 3 μM GDP and in the absence of an agonist, and nonspecific binding was determined in the presence of 10 μM unlabeled GTPγS. Then the membranes were filtered onto glass fiber filters by vacuum filtration, followed by three washes with 3 mL of ice-cold 50 mM Tris-HCl, pH 7.5. Samples were counted in 2 mL of Ecoscint A scintillation fluid. Data represent the percent of agonist stimulation [^{35}S]GTPγS binding over the basal activity, defined as [(specific binding/basal binding) × 100] − 100. All experiments were repeated at least three times and were performed in triplicate. To determine antagonist activity of a compound at the μ opioid receptors, CHO membranes expressing the μ opioid receptor were incubated with the compound in the presence of 200 nM of the agonist DAMGO. To determine antagonist activity of a compound at the κ opioid receptors, CHO membranes expressing the κ opioid receptor were incubated with the compound in the presence of 100 nM of the κ agonist U50,488.

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Supporting Information Available: Procedure for the preparation of morphinans 17−19 and results from the elemental analysis of the final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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