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In-vitro investigation of oxazol and urea analogues of morphinan at opioid receptors

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Abstract—A series of 2-amino-oxazole (**7** and **8**) analogs and 2-one-oxazole analogs (**9** and **10**) were synthesized from cyclorphan (**1**) or butorphan (**2**) and evaluated in-vitro by their binding affinity at μ, δ, and κ opioid receptors and compared with their 2-aminothiozole analogs **5** and **6**. Ligands **7**–**10** showed decreased affinities at κ and μ receptors. Urea analogs (**11**–**14**) were also prepared from 2-aminocyclorphan (**3**) or 2-aminobutorphan (**4**) and evaluated in-vitro by their binding affinity at μ, δ, and κ opioid receptors. The urea derived opioids retained their affinities at μ receptors while showing increased affinities at δ receptors and decreased affinities at κ receptors. Functional activities of these compounds were measured in the [^{35}S]GTPγS binding assay, illustrating that all of these ligands were κ agonists. At the μ receptor, compounds **11** and **12** were μ agonist/antagonists.

1. Introduction

Opioid receptors (μ, κ, and δ) are distributed throughout the central and peripheral nervous system and are involved in a variety of physiological processes, especially analgesia.[1–3] Opioids bind to specific neuronally located proteins and initiate physiological responses. It is generally accepted that the phenol moiety is necessary for narcotic analgesic binding to its receptors.[4] The phenolic hydroxyl group is recognized as a requisite for the formation of a hydrogen bond with a dipolar site on the receptor and for good antinociceptive activity. Interest in investigating the 3-position was driven by our desire to identify replacements of the 3-OH group of morphinans, which like many opioid-receptor interactive agents is prone to metabolic inactivation resulting in low oral bioavailability and short duration of action. An approach to improving the pharmacological properties of analgesics such as morphine is to modify the phenolic hydroxy function.

Previous reports from our laboratories demonstrated the replacement of the 3-OH of cyclorphan (**1**) or butorphan (**2**) with 2-aminothiozole as in compounds **5** and **6**. Compound **5** or **6** resulted in sustained high affinity binding to opioid receptors (Fig. 1).[5,6] Our investigations extended these earlier observations by the replacement of the phenolic hydroxyl moiety with 2-aminooxazole (**7** and **8**) and 2-one-oxazole (**9** and **10**) incorporated into cyclorphan (**1**) and butorphan (**2**).

In continuing these studies on the development of effective analgesics, and the development of pharmaceutical agents for cocaine abuse, we focused our interests on the structural modification and pharmacological evaluation of analogs of cyclorphan and butorphan, which were known to possess mixed κ agonist and μ agonist/antagonist pharmacological profiles.[7,8] We also wish to examine the effect of substitution of the 3-OH group with a series of urea analogs (**11**–**14**). The urea group was introduced since the substitution of oxygen for nitrogen increases the ability to interact at opioid receptors by the formation of hydrogen bonds.[9] Ideally, these modified compounds would have a longer duration of action and better bioavailability.

2. Results and discussion

2.1. Chemistry

The leboratory morphinans (**1** and **2**) were prepared from commercially available (−)-3-hydroxy-*N*-methylmorphinan tartrate (levorphanol) which was

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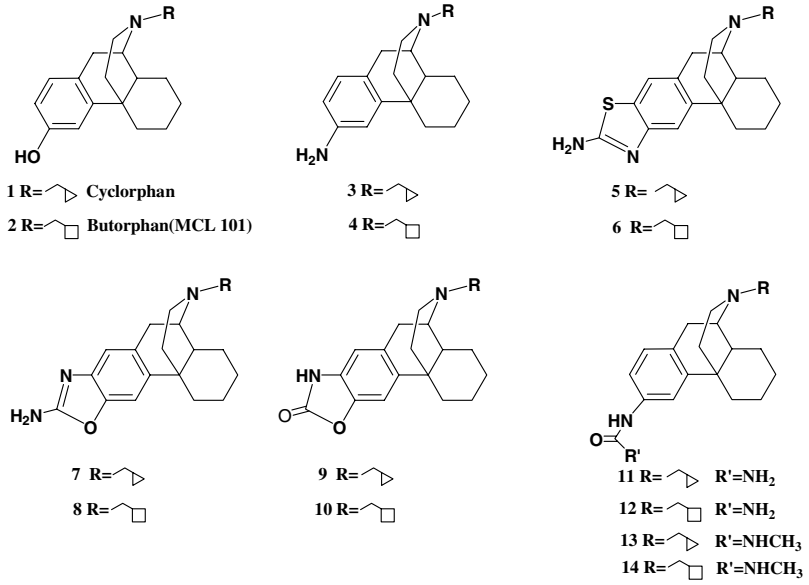


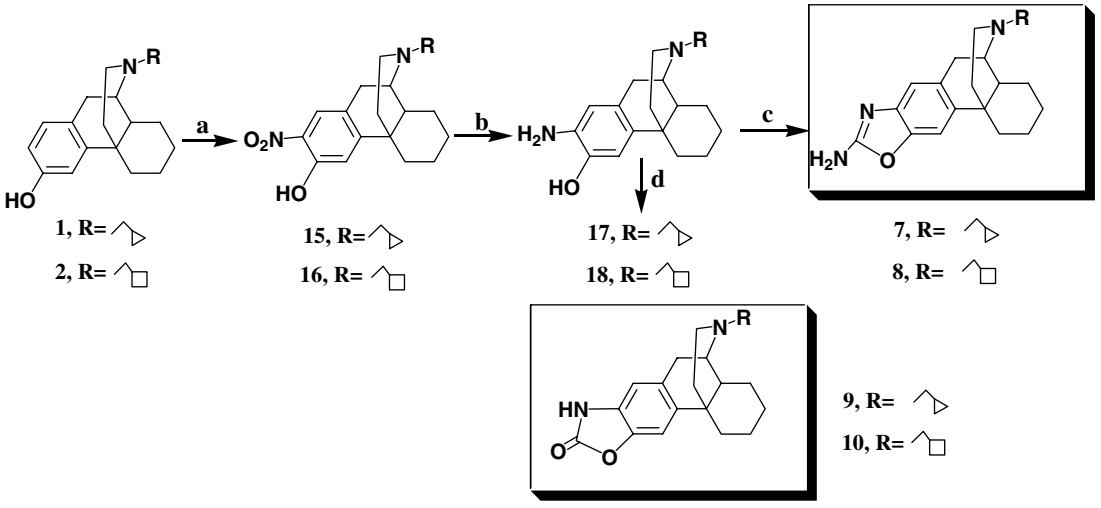
Figure 1.

converted to the free base, and *N*-demethylated[10] to yield the normorphinan, followed by alkylation with cyclobutylmethyl (or cyclopropylmethyl) bromide and characterized as their crystalline mandelate salts (**1** and **2**), respectively. As shown in Scheme 1, the oxazole analogs of morphinans were made by first treating morphinans with HNO_3/CH_3COOH to give 2-nitro-3-hydroxymorphinans **15** and **16**. The nitriated morphinans were reduced (Pd/C, H_2) to give 2-amino-3-hydroxylmorphinans **17** and **18**, which without further purification were cyclized with di(imadazole-1-yl)methanimine[11] to give the oxazols **7** and **8**.[12] A similar strategy was used to prepare the oxazol-one derived morphinans **9** and **10**. Upon cyclization of **17** and **18** with 1,1′-carbonyl-diimidazole, the amino-oxazoles **9** and **10** were obtained in moderate yields.[13]

After triflation of the phenols (**1** and **2**), the corresponding triflates were subjected to palladium-catalyzed amination yielding the amines **3** and **4** in moderate yields.[5,6] The monosubstituted urea targets **11** and **12** were made in 59–63% yield by treating **3** and **4** with KNCO in acetic acid.[9] The *N*-methyl ureas **13** and **14** were made in yields ranging from 66% to 78% by treating **3** and **4** with the corresponding commercially available methyl isocyanate.

2.2. Pharmacological results and discussion

2.2.1. Affinity and selectivity of the synthesized ligands. All the novel morphinan ligands were evaluated for their affinity at and selectivity for μ, δ, and κ opioid receptors with Chinese hamster ovary (CHO) cell membranes stably expressing the human opioid receptors.



Scheme 1. Synthesis of Oxazol analogs. Reagents: (a) HNO_3/CH_3COOH; (b) Pd/C, H_2; (c) THF, Di(imidazole-1-yl)methanimine; (d) THF, 1,1′-Carbonyl-diimidazole.

Table 1. K_i values for the inhibition of μ, δ, and κ opioid binding to chinese hamster ovary membrane by novel opioids

Compound	$K_i \pm$ SEM (nM)		
	[^{3}H]DAMGO (μ)	[^{3}H]U69,593 (κ)	[^{3}H]Naltrindole (δ)
1 (Cyclorphan)	0.062 ± 0.003	0.034 ± 0.002	1.9 ± 0.072
2 (Butorphan)	0.23 ± 0.01	0.079 ± 0.003	5.9 ± 0.55
3 (MCL-149)	1.3 ± 0.029	0.18 ± 0.003	150 ± 2.0
4 (MCL-182)	3.7 ± 0.26	1.8 ± 0.06	180 ± 85
5 (MCL-147) ATPM	1.5 ± 0.2	0.049 ± 0.005	29 ± 2
6 (MCL-183) ATBM	7.1 ± 0.5	0.79 ± 0.02	230 ± 21
7 (MCL-453)	150 ± 5.1	52 ± 1.0	18% inh at 10 μM
8 (MCL-454)	40 ± 1.7	6.6 ± 0.27	46% inh at 10 μM
9 (MCL-457)	160 ± 21	47 ± 1.6	44% inh at 10 μM
10 (MCL-458)	5.8 ± 0.56	1.9 ± 0.057	360 ± 10
11 (MCL-448)	1.4 ± 0.032	0.61 ± 0.015	23 ± 2.9
12 (MCL-447)	3.2 ± 0.17	8.9 ± 1.1	160 ± 19
13 (MCL-446)	12 ± 0.90	2.3 ± 0.045	50 ± 2.0
14 (MCL-445)	22 ± 1.0	20 ± 0.19	270 ± 7.4

The data are summarized in Table 1. For comparison purposes, opioid binding affinity data for cyclorphan (**1**), butorphan (**2**), 3-aminocyclorphan (**3**), 3-aminobutorphan (**4**), and 2-aminothiozole compounds (**5** and **6**) are also included.

Previous reports from our laboratories indicated that 2-aminothiazole-derived morphinans (**5** and **6**) possess high affinity and selectivity at the κ receptor. Our synthetic studies extended these earlier observations by incorporating 2-amino-oxazole and 2-one-oxazole into cyclorphan (**1**) and butorphan (**2**). Compared to the 2-aminothiozole analogs (**5** and **6**), it was unexpected to find that a remarkable decrease of affinity at μ and at κ receptors was observed in 2-amino-oxazole (**7** and **8**) and oxazole-2-one analogs (**9** and **10**), especially with *N*-cyclorpropylmethyl morphinan analogs (**7** and **9**). Compounds **7**, **8**, and **9** showed 18–46% inhibition at δ receptor at 10 nM. It is apparent that there is a binding pocket at the 3-hydroxy position for both μ and κ receptors. Although the 2-amino-oxazole moiety cannot be considered as a bioisosteric replacement of the phenol group, it can be viewed as an extension of the aromatic functionality of the molecule. And the 2-one oxazol analogs can be considered as a tautomeric form of 2-hydroxy oxazol (**19**, Fig. 2). We expected that the 2-amino and 2-hydroxy group may effectively substitute the phenolic hydroxyl group to form a presumed hydrogen bond on its receptor binding site, which may result in improved pharmacological properties.

The 3-D structures may explain the reason why compounds (**7**–**10**) possess low affinities at opioid receptors.

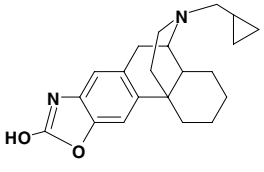


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Figure 2.

As shown in Figure 3, the 2-amino-oxazole ring of compound **7** (Fig. 3a) has a different orientation to the 2-aminothiozole compound (**5**, Fig. 3b). The orientation of the 2-amino group appears to be important for forming a hydrogen bond on its receptor binding site.

The receptor binding affinities of the urea derivatives (**11**–**14**) were evaluated. From the data shown in Table 1, it is noteworthy that the monosubstituted urea analogs **11** and **12** retained the same affinity at the μ receptor, and a 3- to 5-fold decrease at κ and a 6.5-fold increase at δ for **11** were observed when compared with 3-aminocyclorphan (**3**) and 3-aminobutorphan (**4**). The binding affinities of the methyl urea derived opioids (**13** and **14**) at all three receptors were generally lower than the binding affinities of the amino precursors (**3** and **4**).

2.2.2. Efficacy assay of selected ligands. To characterize the relative efficacy of these morphinan ligands, compounds cyclorphan (**1**) and butorphan (**2**) were selected for the [^{35}S]GTPγS assay. Table 2 shows the pharmacological properties of these ligands in stimulating [^{35}S]GTPγS binding mediated by the κ opioid receptor.

Ligands **11**, **12**, and **13** produced maximal stimulation of [^{35}S]GTPγS binding (E_{max}) comparable to that of the monomers **1** and **2**, but less than that of selective agonist U50,488. The EC_{50} values of these ligands are higher than those of monomers, similar to U50,488. Similar to the parent compounds cyclorphan (**1**) and butorphan (**2**), all of these ligands did not inhibit U50,488-stimulated [^{35}S]GTPγS, which suggested that all of these ligands were full κ selective agonists.

The agonist and antagonist properties of these ligands in stimulating [^{35}S]GTPγS binding mediated by the μ opioid receptor are shown in Table 3.

Ligands **11** and **12** produced low maximal stimulation of [^{35}S]GTPγS binding mediated by μ receptor comparable to that of the parent compounds cyclorphan (**1**) and

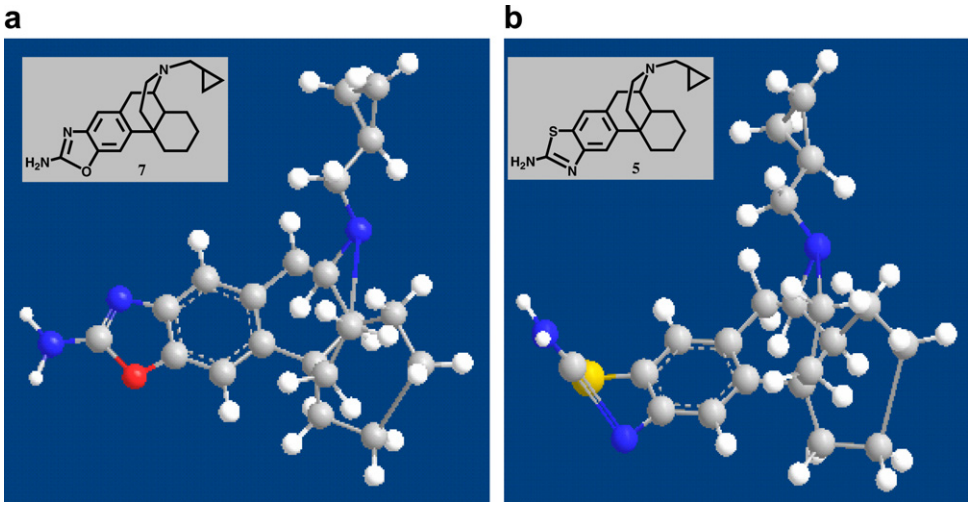

Figure 3. 3-D structures of compounds **7** and **5**.

Table 2. Agonist and antagonist properties of compounds in stimulating [^{35}S]GTPγS binding mediated by the κ opioid receptor[a]

Compound	Pharmacological properties	E_{max} (%maximal stimulation)	EC_{50} (nM)	I_{max} (% maximal inhibition)
(−)-U50,488	Agonist	110 ± 2.0	46 ± 16	—
1 (Cyclorphan)	Agonist	90 ± 10	0.19 ± 0.04	—
2 (Butorphan)	Agonist	80 ± 6.8	1.3 ± 0.4	—
5[b] (MCL-147)ATPM	Agonist	80 ± 6	2.4 ± 0.6	—
6[b] (MCL-183)ATBM	Agonist	80 ± 1	29 ± 4	—
13 (MCL-446)	Agonist	67 ± 5.5	84 ± 8.1	No effect
12 (MCL-447)	Agonist	64 ± 2.0	87 ± 10	No effect
11 (MCL-448)	Agonist	84 ± 7.6	33 ± 2.3	No effect

[a] Membranes from CHO cells that stably expressed only the κ opioid receptor were incubated with varying concentrations of the compounds. The stimulation of [^{35}S]GTPγS binding was measured as described in Section 4. To determine the antagonist properties of a compound, membranes were incubated with 100 nM of the κ agonist U50,488 in the presence of varying concentrations of the compound. The I_{max} value is the maximal percent inhibition obtained with the compound. Dashed lines indicate that the compound was not tested for antagonist properties because of its high E_{max} value.
[b] Data from Ref. 5.

Table 3. Agonist and antagonist properties of urea compounds in stimulating [^{35}S]GTPγS binding mediated by the μ opioid receptor[a]

Compound	Pharmacological properties	E_{max} (% maximal stimulation)	EC_{50} (nM)	I_{max} (% maximal inhibition)	IC_{50} (nM)
DAMGO	Agonist	120 ± 12	110 ± 9.0	—	—
1 (cyclorphan)	Partial agonist	40 ± 2.9	0.80 ± 0.06	50 ± 1	1.7 ± 0.4
2 (butorphan)	Partial agonist	50 ± 2.5	1.6 ± 0.2	50 ± 3	20 ± 3
12 (MCL-447)	Partial agonist	33 ± 1.5	63 ± 4.6	35 ± 1.6	1500 ± 300
11 (MCL-448)	Partial agonist	21 ± 0.70	31 ± 9.2	52 ± 3.6	380 ± 86

[a] Membranes from CHO cells that stably expressed the μ opioid receptor were incubated with varying concentrations of the compounds. The stimulation of [^{35}S]GTPγS binding was measured as described in Section 4. EC_{50} values were the concentration of compound needed to produce 50% of the E_{max} value. To determine the antagonist properties of a compound, membranes were incubated with 200 nM of the μ agonist DAMGO in the presence of varying concentrations of the compound. The I_{max} value is the maximal percent inhibition obtained with the compound. The IC_{50} value is the concentration of compound needed to produce half maximal inhibition. Dashed lines indicate that the compound was not tested.

butorphan (**2**). They also produced the highest maximal inhibition (I_{max}) of the DAMGO stimulated [^{35}S]GTPγS binding indicating that ligands **11** and **12** are μ antagonists/agonists.

The preliminary assay for agonist and antagonist properties of these ligands in stimulating [^{35}S]GTPγS binding mediated by the κ opioid receptor illustrated that compounds **11**, **12**, and **13** were κ agonists, however compounds **11** and **12** were μ agonist/antagonist.

3. Conclusions

A series of 2-amino-oxazole analogs (**7** and **8**) and 2-one-oxazole analogs (**9** and **10**) were synthesized from cyclorphan (**1**) or butorphan (**2**) and evaluated in vitro by their binding affinity at μ, δ, and κ opioid receptors. These series of opioids showed decreased affinities at κ and μ receptors. Compounds **7**, **8**, and **9** showed inhibition at δ receptors. The orientation of the 2′-amino group appears to be important criterion for forming hydrogen bonds on its receptor binding site.

A series of urea analogs were synthesized from 2-aminocyclorphan (**3**) or 2-aminobutorphan (**4**) and evaluated in vitro by their binding affinity at μ, δ, and κ opioid receptors. The urea derived opioids retained their affinities at μ receptors while showing increased affinities at δ receptors and decreased affinities at κ receptors. Such urea derivatives may be useful for the potential development of longer-acting analgesics as well as medications for drug abuse. Functional activities of these compounds were measured in the [^{35}S]GTPγS binding assay, indicating that ligands **11**, **12**, and **13** were κ agonists, while compounds **11** and **12** were μ agonist/antagonists.

4. Experimental

4.1. Chemical syntheses

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 Bruker AC300 spectrometer using tetramethylsilane as an internal reference. Elemental 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 by TLC.

4.1.1. 2-Nitro-3-hydroxyl-*N*-cyclopropylmethylmorphinan (15). To a solution of cyclorphan **1** (300 mg) in 5 mL formic acid was added a solution of 0.2 mL nitric acid in 2 mL of formic acid slowly. The mixture was stirred at room temperature overnight. It was basified with saturated sodium bicarbonate and extracted with methylene chloride. The combined organic extracts were washed with brine and concentrated. The residue was chromatographed eluting with EtOAc/Et_3N = 100:1 to give 190 mg (62.3%) of compound **15** as yellow solid. ^{1}H NMR (300 Hz, $CDCl_3$): 7.83 (s, 1H), 7.06 (s, 1H), 3.14–3.12 (m, 1H), 3.00 (d, J = 18.0 Hz, 1H), 2.77–2.72 (m, 1H), 2.66 (dd, J = 18.0, 5.7 Hz, 1H), 2.52 (dd, J = 6.3, 12.6 Hz, 1H), 2.37–2.28 (m, 2H), 2.06–0.83 (m, 13H), 0.53–0.50 (m, 2H), 0.12–0.09 (m, 2H). ^{13}C NMR (75 Hz, $CDCl_3$): 153.9, 153.3, 131.4, 130.6, 123.0, 116.3, 59.9, 55.2, 45.3, 44.5, 41.8, 38.9, 36.6, 27.1, 26.3, 23.7, 22.2, 9.3, 4.0, 3.6.

4.1.2. 2-Amino-3-hydroxyl-*N*-cyclopropylmethylmorphinan (17). A mixture of (200 mg, 0.66 mmol, **15**) of 2-nitro-3-hydroxyl-*N*-cyclopropylmethylmorphinan (420 mg, 6.6 mmol), of ammonium formate, and (200 mg) of Pd/C (10% w/w) in 25 mL of methanol was stirred at room temperature overnight. The catalyst was filtered of and the filtrate was concentrated to yield 160 mg of the desired compound **16**, which was used for the next step without further purification. ^{1}H NMR (300 Hz, CD_3OD): 6.54 (s, 1H), 6.46 (s, 1H), 3.18 (s, 1H), 2.82–0.91 (m, 18H), 0.48 (d, J = 8.1 Hz, 2H), 0.12 (d, J = 4.8 Hz, 2H). ^{13}C NMR (75 Hz, CD_3OD): 144.2, 132.9, 130.1, 127.6, 115.0, 111.9, 59.2, 55.8, 50.2, 45.9, 43.7, 40.9, 36.6, 36.3, 26.5, 26.3, 23.9, 22.0, 8.1, 4.0, 3.9.

4.1.3. 2′-Amino oxazole[5,4-b]-*N*-cyclopropylmethylmorphinan (7). A solution containing di(imidazole-1-yl) methanimine (91.2 mg) and *N*-2-amino-3-hydroxyl-cyclo-rpropylmethylmorphinan **17** (47.1 mg) in anhydrous THF (10 mL) was allowed to reflux under nitrogen overnight. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate (20 mL) and washed successively with water, saturated aqueous NH_4Cl solution, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to afford the crude product. Further purification by silica gel chromatography gave the desired product as a yellow solid **7** (yield 37.5%). Mp 125–128 °C. ^{1}H NMR (300 Hz, $CDCl_3$): 7.16 (s, 1H), 7.06 (s, 1H), 5.36 (s, 2H), 3.11–2.98 (m, 2H), 2.75–0.84 (m, 17H), 0.52 (dd, J = 8.1, 1.2 Hz, 2H), 0.13–0.07 (m, 2H). ^{13}C NMR (75 Hz, $CDCl_3$): 161.9, 148.0, 140.3, 134.0, 133.5, 114.8, 105.3, 60.0, 55.8, 45.7, 45.2, 42.3, 37.9, 37.1, 26.8, 26.6, 25.0, 22.1, 9.4, 4.0, 3.6. Anal. Calcd ($C_{21}H_{27}N_3O{\cdot}0.8H_2O$): C, 71.68; H, 8.19; N, 11.94. Found: C, 71.69; H, 8.08; N, 11.46.

4.1.4. 2-Nitro-3-hydroxyl-*N*-cyclobutylmethylmorphinan (16). This reaction was carried out with butorphan. The compound was obtained as a yellow solid (yield: 25.1%).

^{1}H NMR (300 Hz, $CDCl_3$): 7.84 (s, 1H), 7.05 (s, 1H), 3.06–2.99 (m, 1H), 2.87–2.84 (m, 22H), 2.61–0.99 (m, 2H). ^{13}C NMR (75 Hz, $CDCl_3$): 154.0, 153.3, 131.4, 130.8, 123.0, 116.2, 61.4, 55.4, 45.4, 44.6, 41.9, 38.9, 36.6, 34.7, 27.7, 27.1, 26.3, 23.9, 22.2, 18.8.

4.1.5. 2-Amino-3-hydroxy-*N*-cyclobutylmethylmorphinan (18). The compound was obtained as light-brown solid (yield 94.0%). ^{1}H NMR (300 Hz, $CDCl_3$): 6.50 (d, J = 6.6 Hz, 2H), 3.47 (s, 1H), 2.57 (s, 2H), 2.53–2.18 (m, 5H), 2.11–1.87 (m, 4H), 1.84–1.57 (m, 7H), 1.38–1.15 (m, 6H). ^{13}C NMR (75 Hz, $CDCl_3$): 144.0, 132.9, 130.5, 128.5, 115.2, 112.0, 61.2, 55.8, 46.1, 44.2, 41.4, 36.6, 34.3, 28.2, 28.1, 26.7, 26.5, 24.0, 22.1, 18.8.

4.1.6. 2′-Amino oxazole[5,4-b]-*N*-cyclobutylmethylmorphinan (8). Yellow solid (yield 38.6%). Mp 165–166 °C. ^{1}H NMR (300 Hz, $CDCl_3$): 7.15 (s, 1H), 7.08 (s, 1H), 5.49 (s, 2H), 3.10 (d, J = 18 Hz, 1H), 2.83–0.83 (m, 24H). ^{13}C NMR (75 Hz, $CDCl_3$): 161.7, 148.1, 140.4, 134.2, 133.7, 115.0, 105.3, 61.5, 56.0, 45.8, 45.2, 42.4, 37.8, 37.1, 35.0, 31.6, 27.9, 26.8, 26.6, 25.2, 22.6, 18.8. Anal. Calcd ($C_{22}H_{29}N_3O{\cdot}0.4H_2O$): C, 73.67; H, 8.37; N, 11.71. Found: C, 73.83; H, 8.33; N, 11.32.

4.1.7. Oxazole-2-one-*N*-cyclopropylmethylmorphinan (9). A solution containing 1,1′-carbonyl-diimidazole (113.6 mg, 0.70 mmol) and *N*-2-amino-3-hydroxyl-cyclorpropyl-methylmorphinan (0.58 mmol) in anhydrous THF (10 mL) was allowed to reflux under nitrogen overnight. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate (20 mL) and washed successively with water, saturated aqueous NH_4Cl solution and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to afford the crude product. Further purification by silica gel gave the desired product

as a light yellow foam-solid **9** (yield 50.6%). Mp 125–128 °C. ^{1}H NMR (300 Hz, $CDCl_3$): 7.09 (s, 1H), 6.79 (s, 1H), 3.22 (s, 1H), 3.01 (d, J = 18.3Hz, 1H), 2.83–0.88 (m, 17H), 0.54 (d, J = 8.1Hz, 2H), 0.15–0.13 (m, 2H). ^{13}C NMR (75 Hz, $CDCl_3$): 156.6, 143.5, 134.4, 132.9, 128.4, 108.5, 106.4, 59.5, 55.4, 45.4, 44.3, 41.4, 37.7, 36.7, 26.6, 26.4, 25.0, 21.9, 8.8, 4.0, 3.7. Anal. Calcd ($C_{21}H_{26}N_2O_2$·0.2H_2O): C, 73.74; H, 7.78; N, 8.19. Found: C, 73.85; H, 7.79; N 7.94.

4.1.8. Oxazole-2-one-*N*-cyclobutylmethylmorphinan (10). Light yellow foam-solid (yield 50.6%). ^{1}H NMR (300 Hz, $CDCl_3$): 7.16 (s, 1H), 7.06 (s, 1H), 5.36 (s, 2H), 3.11–2.98 (m, 2H), 2.75–0.84 (m, 21H). Anal. Calcd ($C_{22}H_{28}N_2O_2$·0.2H_2O): C, 74.21; H, 8.04; N, 7.87. Found: C, 73.92; H, 7.82; N, 7.60.

4.1.9. *N*-cyclopropylmethylmorphinan-3-amino-urea (11). A solution of 3-amino-*N*-cyclopropylmethylmorphinan **3** (100 mg) in acetic acid (1 mL) was treated with a solution of potassium cyanate (40 mg) in water (1 mL). After brief heating on the oil bath, the resulting mixture was evaporated and the residue was dissolved in 5% NaOH, extracted with EtOAc. The extract was combined and washed with brine, evaporated to yield a yellow oil. The crude product was purified by column chromatography (SiO_2, EtOAc/Hexane/Et_3N = 50:50:1) to yield a product as white foam **5** (yield 59.0%). Mp 112–115 °C. ^{1}H NMR (300 Hz, $CDCl_3$): 7.7 (s, 1H), 7.16 (d, J = 2.1 Hz, 1H), 7.10 (dd, J = 8.1, 1.8 Hz, 1H), 6.98 (d, J = 8.1 Hz, 1H), 5.16 (s, 2H), 3.08–3.06 (m, 1H), 2.90–0.83 (m, 18H), 0.52–0.48 (m, 2H), 0.097–0.081 (m, 2H). ^{13}C NMR (75 Hz, $CDCl_3$): 157.5, 141.4, 136.7, 133.5, 128.2, 118.8, 118.1, 59.8, 55.6, 45.6, 44.9, 41.7, 37.7, 36.4, 26.7, 26.5, 24.1, 22.1, 9.2, 4.0, 3.6. Anal. Calcd ($C_{21}H_{29}N_3O$·0.25H_2O): C, 73.33; H, 8.64; N, 12.22. Found: C, 73.34; H, 8.66; N, 11.78.

4.1.10. *N*-Cyclobutylmethylmorphinan-3-amino-urea (12). White foam-solid (yield 63.0%). Mp 115–117 °C. ^{1}H NMR (300 Hz, $CDCl_3$): 7.14–7.02 (m, 4H), 4.95 (s, 2H), 2.98 (d, J = 18.3 Hz, 1H), 2.80 (d, J = 3.0 Hz, 1H), 2.60–1.03 (m, 23H). ^{13}C NMR (75 Hz, $CDCl_3$): 157.3, 141.8, 136.4, 134.3, 128.5, 119.5, 118.9, 61.4, 55.8, 45.7, 44.9, 41.8, 37.6, 36.5, 34.8, 27.8, 26.8, 26.5, 24.4, 22.2, 18.8, 14.1. Anal. Calcd ($C_{22}H_{31}N_3O$·0.2H_2O): C, 73.99; H, 8.86; N, 11.77. Found: C, 73.96; H, 8.85; N, 11.67.

4.1.11. 3-Methylurea-*N*-cyclobutylmethylmorphinan (13). 3-Amino-*N*-cyclobutylmethylmorphinan **4** (90.5 mg, 0.34 mmol) was dissolved in anhydrous THF (10 mL), and a piece of Na metal was added. The mixture was stirred at room temperature for 1 min and then the methyl isocyanate (37.5 mg) was added. The reaction mixture was continuously stirred at room temperature and monitored by TLC. When 3-amino-*N*-cyclobutylmethylmorphinan had virtually disappeared, 1 mL H_2O was added to destroy any remaining trace of unreacted isocyanate. The reaction mixture was diluted into 20 mL of Et_2O and washed with NaOH aqueous solution (approximately 5% concentration) followed by brine. The ether solution was dried over $MgSO_4$, with stirring, and then filtered to obtain a clear ether solution of the product. Evaporation of ether provided the crude compound which was purified by column chromatography (SiO_2, EtOAc/Et_3N = 100:1) to get the product as light-yellow solid (yield 78.6%). Mp 207–209 °C.

^{1}H NMR (300 Hz, CD_3OD): 7.20 (d, J = 2.1 Hz, 1H), 7.05 (dd, J = 8.4, 2.1 Hz, 1H), 6.94 (d, J = 8.1 Hz, 1H), 3.23–3.21 (m, 1H), 2.95 (d, J = 18.6 Hz, 1H), 2.82–2.79 (m, 1H), 2.66 (s, 3H), 2.60–1.01 (m, 24H). ^{13}C NMR (75 Hz, CD_3OD): 159.3, 141.5, 139.4, 132.5, 129.1, 118.6, 117.5, 62.2, 57.2, 47.1, 45.7, 42.3, 38.6, 37.6, 35.6, 29.1, 28.9, 28.0, 27.7, 26.9, 25.1, 23.3, 19.6. Anal. Calcd ($C_{23}H_{33}N_3O$·0.2H_2O): C, 74.43; H, 9.07; N, 11.32. Found: C, 74.12; H, 9.07; N, 11.32.

4.1.12. 3-Methylurea-*N*-cyclopropylmethylmorphinan (14). Light-yellow solid (yield 66.5%). Mp 212–215 °C. ^{1}H NMR (300 Hz, CD_3OD): 7.16 (s, 1H), 7.03 (s, 1H), 6.66 (s, 1H), 4.99–4.98 (d, J = 4.8 Hz, 1H), 3.10–3.07 (m, 1H), 2.93 (d, J = 18.6 Hz, 1H), 2.80 (s, 3H), 2.72–0.84 (m, 18H), 0.53–0.49 (m, 2H), 0.12–0.11 (m, 2H). ^{13}C NMR (75 Hz, CD_3OD): 157.1, 141.8, 136.5, 134.0, 128.4, 119.5, 119.0, 60.0, 55.7, 45.7, 45.0, 41.9, 37.8, 36.5, 27.0, 26.8, 26.6, 24.2, 22.2, 9.41, 4.0, 3.6. Anal. Calcd ($C_{22}H_{31}N_3O$·0.2H_2O): C, 73.99; H, 8.86; N, 11.77. Found: C, 74.17; H, 8.75; N, 11.66.

4.2. 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) were obtained from Dr. Larry Toll (SRI International, Palo Alto, CA), and the μ-opioid receptor (hMOR-CHO) were obtained from Dr. George Uhl (NIDA Intramural Program, Baltimore, MD). 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 U/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.

4.3. [^{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 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 those transfected with 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 used.

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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