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Preliminary Pharmacological Evaluation of Enantiomeric Morphinans

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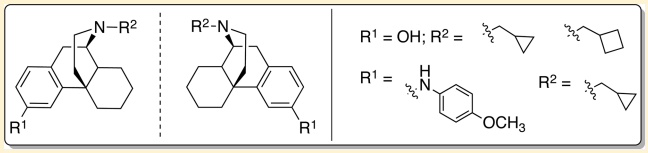
ABSTRACT: A series of levo- and dextromorphinan pairs have been synthesized and evaluated for their affinities to the mu, kappa, and delta opioid receptors, the *N*-methyl-D-aspartate (NMDA) channel, and sigma 1 and 2 receptors. It was found that levo isomers tended to have higher affinities at the opioid receptors and moderate to high affinities to the NMDA and sigma receptors, while dextro isomers tended to have lower affinities to the opioid receptors but comparatively higher affinities to the NMDA and sigma receptors. This series of compounds have interesting and complex pharmacological profiles, and merit further investigation as potential therapies for drug abuse treatment.



KEYWORDS: *Drug abuse, opioid receptor, sigma receptor, NMDA receptor, enantiomer, ICSS*

Opioids have been used as a therapeutic agent to manage and treat pain and a variety of other disorders. Naturally occurring opioids and their semisynthetic derivatives are stereospecific, levorotatory alkaloids with generally high affinities to the mu, kappa, and delta opioid receptors.[1] These include morphine, levorphanol, oxycodone, and hydrocodone among others (Figure 1). Agonist activity at the mu and delta opioid receptors (MOR and DOR) are largely responsible for their analgesic properties.[1] However, opioids also possess undesirable effects as well, including sedation, nausea, constipation, respiratory depression, and the development of tolerance and euphoria, the latter which can pave the way toward abuse and addiction.[1] Carefully tuning the activities at the different opioid receptors has led to the reduction of some of these undesirable effects. For example, the opioids buprenorphine, cyclorphan, naloxone, and naltrexone have been explored as treatments for alcoholism and for opioid and cocaine addiction. These compounds exhibit MOR and kappa opioid receptor (KOR) antagonist or partial agonist activity and act, at least in part, by attenuating reward-related effects of dopamine release in the nucleus accumbens, which is caused by MOR or DOR activation.[2] However, KOR activation is dysphoric, which can compromise patient compliance.[3]

Dextrorotatory opioids have very different pharmacological profiles than their levorotatory isomers. Unlike the levorotatory opioids, they generally have little or no affinity to the MOR, DOR, or KORs, and thus do not carry the same abuse and addiction potential as their levorotatory enantiomers. They typically act as weak to moderate noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists, and have affinity to the σ1 and nicotinic receptors.[4] Like the levorotatory opioids, the dextro isomers also have antitussive properties. For example, dextromethorphan (DXM) is commonly found in many over-the-counter cough preparations. DXM and its *O*-desmethyl analogue, dextrorphan (DX; the enantiomer of levorphanol) have been shown to possess anticonvulsive and neuroprotective effects, likely due to its noncompetitive NMDA antagonist and σ1 activities. MOR agonists catalyze an intracellular G-protein mediated cascade leading to analgesia as well as to an increase in NMDA-gated currents via a protein kinase C mediated mechanism which leads to increased tolerance.[5] Blocking the latter action by using noncompetitive NMDA antagonists such as DXM effectively inhibits development of tolerance without affecting analgesia.[6] Interestingly, this interaction is not coupled to activation of the KOR.[3] On the other hand, KOR activation modulates inhibitory afferents that synapse on neurons which express NMDA receptors in the medial prefrontal cortex (mPFC).[7] Glutamatergic transmission and dopamine release is attenuated by KOR activation, while NR1 (an NMDA subunit) stimulation facilitates dopamine release in the mPFC.[7] KOR and NR1 regulate some of the same terminals although they have opposing actions.[7]

It has been hypothesized that this combination of actions may be helpful in attenuating drug abuse.[8] Indeed, several reports in the literature support this hypothesis. Analogues of DXM were found to attenuate the stimulant and convulsive effects of high doses of cocaine in mice.[9] Both DXM and DX were found to be equipotent in decreasing methamphetamine self-administration and nicotine self-administration in rats, suggesting that these effects were mediated via a non-NMDA pathway, perhaps through blockade of $\alpha 3\beta 4$-nicotinic receptors.[4] Another dextrorotatory opioid, (+)-naloxone,[10] is also devoid of opioid receptor activity but has shown efficacy in treating drug addiction. Administration of (+)-naloxone has been shown to reduce cocaine- and amphetamine-induced

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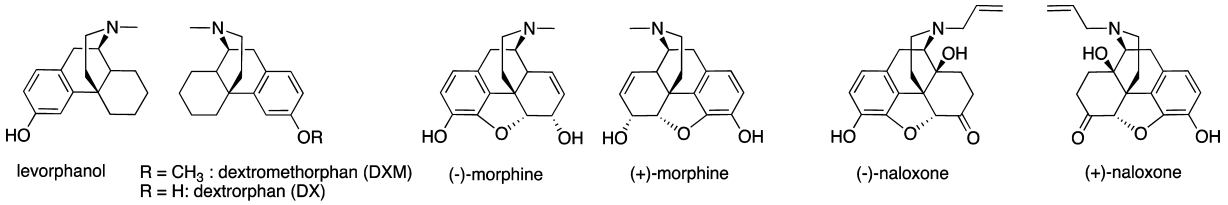
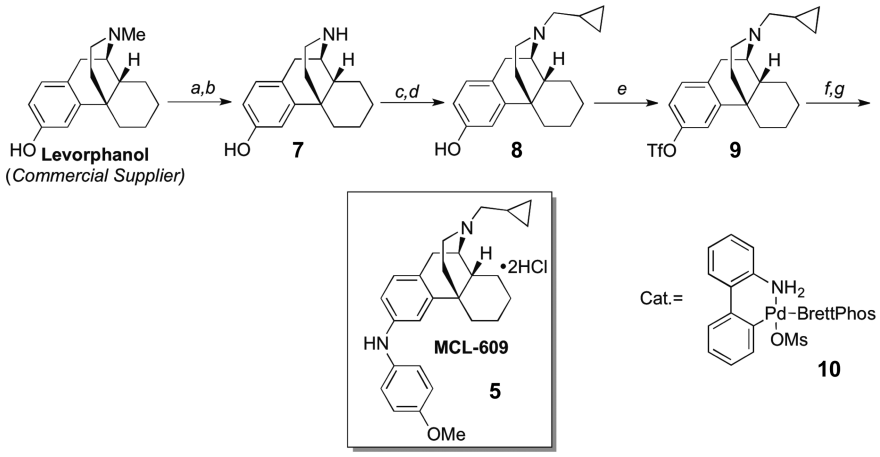


Figure 1. Representative dextrorotatory and levorotatory morphinans.

Scheme 1. Synthetic Route toward 5 and 6



Conditions: *a*) $ClCO_2Et$, K_2CO_3, $CHCl_3$, 70°C; *b*) 12 N HCl, AcOH, 90°C; *c*) Cyclopropanecarbonyl chloride, Et_3N, CH_2Cl_2, 0°C-RT; *d*) $LiAlH_4$, THF, 0°C-RT; *e*) $PhNTf_2$ Et_3N, CH_2Cl_2, 0°C-RT; *f*) *i. p*-anisidine, Cat., K_2CO_3, *t*-BuOH, 110°C; *g*) 1N HCl in Et_2O

hyperactivity.[11,12] It was found that (+)-morphine and (+)-naloxone attenuated (−)-morphine-induced antinociception through activation of the σ receptor.[13] It has been hypothesized that levorotatory opioids activate the toll-like receptor 4 (TLR-4) receptor and that this activation contributes to drug reinforcement; (+)-naloxone can block TLR-4 and thus inhibit reinforcement and development of tolerance.[14]

A focus of our program has been the development of opioid derivatives possessing mixed MOR and KOR activities as a treatment for cocaine abuse.[15] Encouraged by the reports described above, we selected four candidate levomorphinans with desirable activities at the MOR and KOR for further investigation as cocaine abuse medications, and we synthesized their dextro enantiomers and compared their *in vitro* binding affinities to the MOR, DOR, KOR, NMDA, and sigma receptors. The behavioral effects of one morphinan pair on sensitivity to cocaine reward were assessed using intracranial self-stimulation (ICSS) in rats.

CHEMISTRY

Dextrorphan was prepared by demethylating the methoxy group of DXM.[16] (−)-Cyclorphan (**1**), (+)-MCL-190 (**2**), (−)-butorphan (**3**), and (+)-MCL-191 (**4**) were prepared as described previously.[15a,17] (−)-MCL-609 (**5**) was prepared analogously to the previously reported procedure,[15e] but using a more efficient palladium precatalyst, which allowed the cross coupling step to approach quantitative yields (Scheme 1). (+)-MCL-740 (**6**) was prepared analogously to (−)-MCL-609 (**5**) from DX.

The compounds were tested for binding affinities at MOR, KOR, DOR, NMDA, σ1, and σ2 receptors. The binding affinities are described in Table 1.

RESULTS AND DISCUSSION

As can be seen in Table 1, both dextrorotatory and levorotatory morphinans possess an unexpected combination of affinities to various receptor targets. All dextrorotatory morphinans exhibited lower affinities to MOR, KOR, and DOR than their levorotatory enantiomers, although there was no evident correlation between structure and affinity to the various opioid receptors. When comparing (+)-DX to (−)-levorphanol, both MOR and KOR affinities were about 3 orders of magnitude lower for (+)-DX, but (+)-DX essentially lost affinity to the DOR. Interestingly, both isomers exhibited similar affinity to the NMDA receptor. This trend is observed between (−)-butorphan (**3**, MCL-101), a KOR agonist and MOR partial agonist (Table 2), and (+)-MCL-191 (**4**). In this case, (+)-MCL-191 (**4**) had measurable affinity to MOR and KOR (50−60 nM) and no affinity to the DOR. Following the trend established for the (−)-levorphanol/(+)-DX pair, (+)-MCL-191 (**4**) has even lower affinity to the NMDA receptor by an order of magnitude compared to (−)-MCL-101 (**3**, butorphanol). Interestingly, both (−)-MCL-101 (**3**, butorphan) and (+)-MCL-191 (**4**) display high affinity to σ1 and poor affinity to σ2, whereas (+)-DXM and (+)-DX display poor affinity to σ1 and no affinity to σ2. (+)-MCL-190 (**2**) exhibits the largest loss of affinity and the lowest affinity to MOR (K_i = 1100 ± 69 nM). Its NMDA affinity compares to that of (−)-levorphanol, (+)-DX, and (+)-DXM, but unlike these three it also possesses high σ1 affinity. (−)-Cyclorphan (**1**) has a comparatively higher affinity to NMDA than either (−)-levorphanol or (−)-butor-

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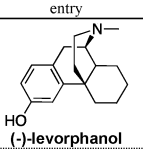
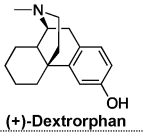
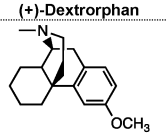
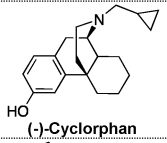
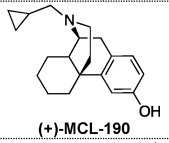
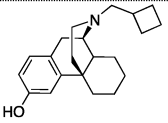
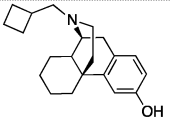
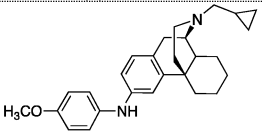
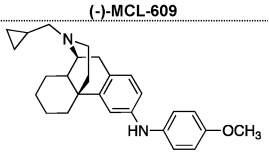
Table 1. Binding Affinities of Levorotatory and Dextrorotatory Morphinans

entry	Ki (nM) μ	κ	δ	NMDA[a]	σ1[a]	σ2[a]
 (-)-levorphanol	0.21 ± 0.017[b]	2.3 ± 0.26[b]	4.2 ± 0.45[b]	630 ± 126[c]	NA	NA
 (+)-Dextrorphan	520 ± 87	>1μM	>10μM	220 ± 30[e]	144 ± 37[f]	>10μM[f]
 (+)-Dextromethorphan	1,280[d]	7,000[d]	>10μM[d]	1,680 ± 220[e]	205 ± 42[f]	>10μM[f]
1  (-)-Cyclorphan	0.062 ± 0.003[b]	0.034 ± 0.002[b]	1.9 ± 0.1[b]	59 ± 6	344 ± 45	452 ±73
2  (+)-MCL-190	1,100 ± 69[g]	480 ± 13[g]	>10μM	453 ± 341	12 ± 2	908 ±139
3  Butorphan [(-)-MCL-101]	0.23 ± 0.017[b]	0.079 ± 0.003[b]	5.9 ± 0.55[b]	203 ± 113	19 ± 2.4	143 ± 19
4  (+)-MCL-191	60 ± 4.0[g]	25 ± 2.9[g]	>10μM	3300 ± 700	5.1 ± 0.4	332 ± 40
5  (-)-MCL-609	0.026 ± 0.0021[h]	0.030 ± 0.0037[h]	1.7 ± 0.10[h]	3000 ± 1400	109 ± 23	73 ± 18
6  (+)-MCL-740	48 ± 4	25 ± 0.74	>1μM	No affinity	33 ± 3	37 ± 6

[a]NMDA, σ1, and σ2 binding assays were carried out at the NIMH Psychoactive Drug Screening Program (PDSP) from a minimum of three determinations. Cloned rat NMDA receptor, [^{3}H]-MK-801 as radioligand, and MK-801 as control; clone rat σ1 receptor, [^{3}H]-(+)-pentazocine and haloperidol as control; cloned rat σ2 receptor, [^{3}H]-DTG as radioligand and haloperidol as control. [b]See ref 15d. [c]See ref 18. [d]See ref 19. [e]See ref 20. [f]See ref 21. [g]See ref 17. [h]See ref 15e.

Table 2. Stimulation/Inhibition of [^{35}S]GTPγS Binding Mediated by the MOR and KOR

	MOR				KOR			
compd	E_{max} (%)	EC_{50} (nM)	I_{max} (%)	IC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	I_{max} (%)	IC_{50} (nM)
1 (cyclorphan)[a]	40 ± 2.9	0.80 ± 0.6	50 ± 1	1.7 ± 0.4	90 ± 10	0.19 ± 0.04	NI	NA
3 (butorphan)[a]	50 ± 2.5	1.6 ± 0.2	50 ± 3	20 ± 3	80 ± 6.8	1.3 ± 0.4	NI	NA
5 (MCL-609)[b]	93 ± 9.9	20 ± 3.2	28 ± 3.6 at 10 μM	NA	140 ± 7.4	53 ± 4.9	NI	NA

[a]Membranes from CHO cells that stably expressed the human MOR were incubated with varying concentrations of the compounds in the presence of 0.08 nM [^{35}S]GTPγS. Data are the mean values ± SEM from three experiments, performed in triplicate. NI = No inhibition. NA = Not applicable. [b]See ref 15d.

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phan (**3**) and is close to that found for PCP (K_i = 23 nM).[20] Cyclorphan (**1**) also has poor affinity to σ1 and σ2 receptors. (+)-MCL-190 (**2**) also shows increased affinity to the σ1 receptor compared to its enantiomer cyclorphan (**1**) (12 vs 344 nM), but affinity to σ2 is comparatively lower for both. (−)-MCL-609 (**5**) is a levorotatory morphinan derivative with very high affinity to MOR and KOR (0.026 and 0.030 nM, respectively) and agonist at both KOR and MOR (Table 2). It has low affinity for the NMDA receptor. As expected, its dextrorotatory enantiomer, (+)-MCL-740 (**6**), had lower affinities to MOR and KOR by 3 orders of magnitude, but unlike the other compounds in this series it had no affinity to the NMDA receptor whatsoever. Unlike the other morphinans, however, (−)-MCL-609 (**5**) and (+)-MCL-740 (**6**) have high affinity to σ2 (73 and 37 nM, respectively).

Effects of MCL-101 (3) and MCL-191 (4) on Sensitivity to Cocaine Reward in Rats. MCL-101 (**3**) and MCL-191 (**4**) were chosen for evaluation in ICSS studies because they have very different binding profiles at the opioid receptors. MCL-101 (**3**) has high affinity to MOR and KOR and acts as a KOR agonist and MOR partial agonist (Table 2), making it an ideal candidate as a cocaine abuse treatment, whereas MCL-191 (**4**) has much lower activity at the opioid receptors, and thus may show its effect at the σ1 receptor. In experiments examining the effects of MCL-101 (**3**) and MCL-191 (**4**) on the reward-potentiating effect of cocaine on ICSS, (Figure 2), there were significant interactions between treatment and time [MCL-101 (**3**), $F_{(10,75)}$ = 4.02, p < 0.001; MCL-191 (**4**), $F_{(10,115)}$ = 4.28, p < 0.001]. Cocaine decreased ICSS thresholds (Figure 2A, C), indicative of increased reward function. MCL-101 (**3**), but not MCL-191 (**4**), reduced the peak threshold-decreasing (reward-enhancing) effect of cocaine on ICSS thresholds observed in the first 15 min after cocaine injection (Figure 2A). MCL-101 (**3**), but not MCL-191 (**4**), interacted with cocaine to produce significant decreases in rates of responding [Interaction; $F_{(10,75)}$ = 2.93, p < 0.01; Figure 2B, C]. Previously, it has been shown that selective KOR agonists increase ICSS thresholds, have anxiogenic effects in the elevated plus maze, and produce conditioned place aversions.[22] These results are consistent with the neurochemical finding that KOR agonists profoundly suppress dopamine release in reward-related brain regions[23] and have anticocaine behavioral effects.[23]

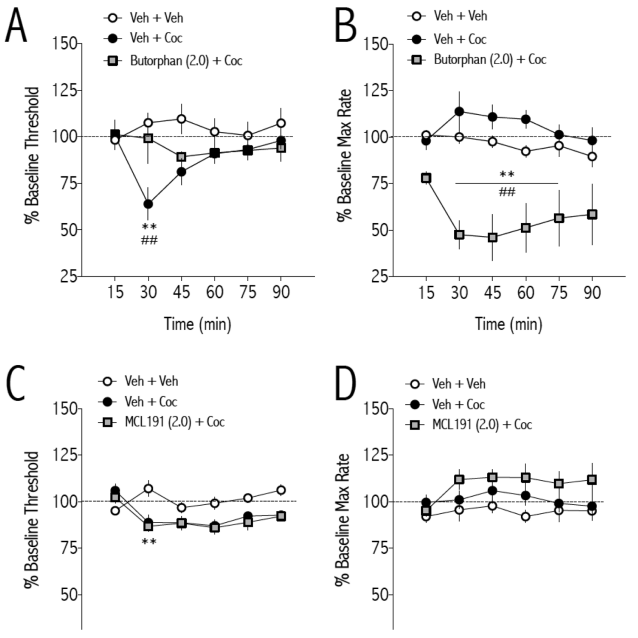

Figure 2. Effects of butorphan (3) and MCL-191 (4) on cocaine-potentiated reward function. Butorphan (**3**) or MCL-191 (**4**) was injected intraperitoneally to adult male rats, and ICSS thresholds (A, C) and maximum rates of responding (B, D) were measured for 15 min. Cocaine (5.0 mg/kg) was then injected i.p. and ICSS thresholds (A, C) and maximum rates of responding (B, D) were measured for an additional 75 min. Cocaine significantly decreased ICSS thresholds on its own, and butorphan (A) but not MCL-191 (C) blocked the initial reward-potentiating effects of cocaine. Data are expressed as 100 × post-treatment/pretreatment thresholds or max rates of responding +SEM. **p < 0.01 comparing Veh + Veh to Veh + Coc; $^{\#\#}p$ < 0.01 comparing Veh + Coc to MCL compound + Coc. N = 6−9 rats/treatment.

Overall, the levo- and dextrorotatory morphinans described in this series possess rather diverse pharmacological profiles. Surprisingly, several levo- and dextrorotatory morphinans described in Table 1 possessed affinities to NMDA receptors, some of which were close to that for PCP.[20] Among the dextrorotatory analogs, DX and DXM, and (+)-MCL-190 (**2**) have very weak affinities to MOR, KOR, DOR, and moderate affinity to NMDA; (+)-MCL-190 (**2**), however, possesses relatively high affinity to σ1. In comparison, (+)-MCL-191 (**4**) and (+)-MCL-740 (**6**) possess similar, moderate binding affinities to the MOR, KOR, and relatively high affinity to the σ1 receptor but differ markedly at NMDA: (+)-MCL-191 (**4**) has low affinity and (+)-MCL-740 (**6**) has no affinity. A comparison of the action of the dextrorotatory morphinans in animal models of drug abuse may elucidate the significance or action of each component or combination thereof (MOR, KOR, DOR, NMDA, or σ) on addiction treatment.

CONCLUSION

In contrast, KOR antagonists have anxiolytic and anti-depressant-like effects,[22] and the reward-related effects of cocaine are prolonged in rats treated with the selective KOR antagonist norBNI.[24] Thus, the ability of MCL-101 (**3**) to block the effects of cocaine in ICSS without having aversive effects on its own is likely due to its KOR agonist activity combined with its MOR partial agonist activity. In comparison to butorphan (**3**), MCL-191 (**4**) does not possess appreciable KOR or MOR activity at the dose used in this study, but does have high affinity for σ1 receptors. Sigma 1 ligands have been investigated as potential treatments for substance abuse.[9,21] Both butorphan (**3**) and MCL-191 (**4**) have high affinity to the σ1 receptor (Table 1). It can be concluded form the ICSS data that KOR agonist activity or the combination of KOR agonism/MOR partial agonism is a significant component to the efficacy of these compounds in blocking the rewarding effects of cocaine, and that σ1 activity by itself at the doses tested does not have a measurable effect.

METHODS

General Synthetic Methods. ^{1}H (and ^{13}C NMR) spectra were recorded at 300 MHz (75 MHz) on a Varian Mercury 300 spectrometer. Chemical shifts are given as δ value (ppm) downfield from tetramethylsilane as an internal reference. Melting points were determined on a Thomas-Hoover capillary tube apparatus and are reported uncorrected. 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 μm Kieselgel 60F-254 silica gel aluminum sheets (EM Science,

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Newark, NJ). Flash chromatography was used for the routine purification of reaction products. Eluent systems are described for the individual compounds.

*MCL-609 (**5**).* To a dry 22 mL vial with a stir bar was added *p*-anisidine (137.8 mg, 1.12 mmol), palladium precatalyst (**10**, 8.45 mg, 9.32 μmol), and K_2CO_3 (179 mg, 1.30 mmol). The tube was sealed with a septum, evacuated, and backfilled with N_2 three times. Under an atmosphere of nitrogen, **9** (400 mg, 0.932 mmol) was added in *t*-BuOH (2 mL). The solution was stirred vigorously at 110 °C for 1.5 h. The reaction was then cooled to room temperature and filtered through a pad of silica gel washing with EtOAc/hexanes/Et_3N (10:10:1). The filtrate was then concentrated and purified on a silica gel column, eluting first with CH_2Cl_2/MeOH (50:1) and then EtOAc/hexanes/Et_3N (10:10:1) to obtain the free base of MCL-609 (**5**) (367.3 mg, 98% yield) as a brown foam. The free base was converted to the hydrochloride salt by dissolving the free base in a minimum amount of methanol and adding 1N HCl in Et_2O to the solution. Mp (HCl salt) = 162 °C (decomposes). ^{1}H NMR (300 MHz, CDCl3) δ 7.06−6.97 (m, 2H), 6.94 (d, *J* = 8.2 Hz, 1H), 6.89−6.80 (m, 3H), 6.74 (dd, *J* = 2.4, 8.1 Hz, 1H), 5.41 (s, 1H), 3.79 (s, 3H), 3.17−3.00 (m, 1H), 2.87 (d, *J* = 18.3 Hz, 1H), 2.71 (dd, *J* = 3.0, 11.9 Hz, 1H), 2.65−2.43 (m, 2H), 2.42−2.20 (m, 2H), 2.19−1.98 (m, 1H), 1.94−1.60 (m, 3H), 1.57−1.10 (m, 8H), 0.99−0.76 (m, 1H), 0.58−0.43 (m, 2H), 0.22−0.05 (m, 2H). ^{13}C NMR (75 MHz, CDCl3) δ 154.7, 142.9, 141.7, 137.1, 129.9, 128.5, 120.6, 114.8, 114.6, 113.9, 60.2, 56.1, 55.8, 46.0, 45.4, 42.1, 38.0, 36.8, 27.1, 26.8, 24.2, 22.5, 9.6, 4.3, 3.8. The spectra are in accordance with the literature data.[15e]

*MCL-740 (**6**).* Prepared analogously to MCL-609; 89% yield. Mp (HCl salt) = 166 °C (decomposes).

Pharmacological Assays. *Opioid Binding to the Human κ, δ, and μ Opioid Receptors.* Chinese hamster ovary (CHO) cells stably transfected with the human KOR (Dr. Lee-Yuan Liu-Chen, Temple University, Philadelphia, PA), the human DOR (Dr. Larry Toll, Torrey Pines Institute for Molecular Studies, Port St. Lucie, FL), and the human MOR (Dr. George Uhl, NIDA Intramural Program, Baltimore, MD) were used in the experiments. 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 at a final concentration of 0.25 nM, and the κ-selective ligand [^{3}H]U69,593 at a concentration of 0.4 nM nM. A 3-h incubation was used with the δ-selective ligand [^{3}H]naltrindole at a concentration of 0.2 nM.

[^{35}S]GTPγS Binding Studies to Measure Receptor Coupling to G Proteins. Membranes from CHO cells stably expressing either the human KOR or MOR 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 KOR (15 μg of protein per tube) or MOR (7.5 μg of protein per tube) were incubated with 12 different concentrations of the compounds 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 ScintiSafe 30% 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 MOR, CHO membranes expressing the MOR were incubated with the compound in the presence of 200 nM of the agonist DAMGO. To determine antagonist activity of a compound at the KOR, CHO membranes expressing the KOR were incubated with the compound in the presence of 30 nM of the κ agonist U50,488.

Intracranial Self-Stimulation (ICSS). Male Sprague−Dawley rats (*n* = 15) were implanted with stainless steel monopolar electrodes (0.25 mm diameter; Plastics One, Roanoke, VA) aimed at the medial forebrain bundle at the level of the lateral hypothalamus (2.8 mm posterior to bregma, 1.7 mm lateral to midline, 7.8 mm below dura). After 1 week of recovery from surgery, rats were trained to respond for brain stimulation using a continuous reinforcement schedule (FR1) at 141 Hz, where each lever press earned a 500 ms train of square wave cathodal pulses (100 ms per pulse). Stimulation current was adjusted (final range: 140−210 μA) for each rat to the lowest value that would sustain a reliable rate of responding (average of 40 responses per 50 s). After the minimal effective current was found for each rat, it was kept constant throughout the remainder of training and testing. Rats were trained using the rate-frequency method, which allows for calculation of the threshold frequency (Hz) at which the stimulation first sustains responding on the operant lever. These procedures have been described in detail:[25] Intracranial self-stimulation (ICSS) in rodents to study the neurobiology of motivation. Rats were trained until mean stimulation thresholds remained stable (±10% for 4 consecutive days).

One cohort of rats (*n* = 6) was used to test the effects of MCL-101 (**3**) (2.0 mg/kg, i.p.) on cocaine (5.0 mg/kg, i.p.) induced decreases in ICSS thresholds. A second cohort (*n* = 9) was used to test the effects of MCL-191 (**4**) on cocaine (5.0 mg/kg, i.p.) induced decreases in ICSS thresholds. This dose of MCL-101 (**3**) was chosen because we previously showed it to decrease the peak rewarding effects of cocaine without having significant effects on ICSS behavior on its own.[26] We chose to use 2.0 mg/kg MCL-191 (**4**) to match that of MCL-101 (**3**). For each experiment, three rate frequency functions (3 × 15 min) were determined for each rat immediately before the initial drug injection. ICSS thresholds and maximum rates of responding for the second and third functions were averaged and served as baseline parameters. Rats then received an injection of MCL-101 (**3**) or 191 (**4**) and were placed back in the ICSS chambers for one 15 min rate frequency trial. Rats were then injected with cocaine and placed back in the ICSS chambers for 5 more 15 min rate frequency trials. Data are expressed as a percent of predrug baseline threshold values. Drug treatment days were separated by 2−3 nondrug days during which six 15 min rate frequency trials were performed to maintain baseline ICSS thresholds. The time course of drug effects on ICSS thresholds and maximum rates were analyzed using two-way (drug dose × time) ANOVAs with repeated measures on time. All significant effects and interactions were analyzed further using Bonferroni's multiple comparison tests.

AUTHOR INFORMATION

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Notes

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■ ABBREVIATIONS

DOR, delta opioid receptor; KOR, kappa opioid receptor; MOR, mu opioid receptor; NMDA, *N*-methyl-D-aspartate receptor; σ, sigma; ICSS, intracranial self-stimulation; DX, dextrorphan; DXM, dextromethorphan; PCP, phencyclidine; mPFC, medial prefrontal cortex; NR1, subunit of NMDA receptor; TLR, toll-like receptor

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