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Synthesis and binding affinity of novel mono- and bivalent morphinan ligands for κ, μ, and δ opioid receptors

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ABSTRACT

A novel series of homo- and heterodimeric ligands containing κ/μ agonist and μ agonist/antagonist pharmacophores joined by a 10-carbon ester linker chain were synthesized and evaluated for their in vitro binding affinity at κ, μ, and δ opioid receptors, and their functional activities were determined at κ and μ receptors in [^{35}S]GTPγS functional assays. Most of these compounds had high binding affinity at μ and κ receptors (K_i values less than 1 nM). Compound **15b**, which contains butorphan (**1**) at one end of linking chain and butorphanol (**5**) at the other end, was the most potent ligand in this series with binding affinity K_i values of 0.089 nM at the μ receptor and 0.073 nM at the κ receptor. All of the morphinan-derived ligands were found to be partial κ and μ agonists; ATPM-derived ligands **12** and **11** were found to be full κ agonists and partial μ agonists.

1. Introduction

Bivalent ligands have the potential for bridging vicinal receptors. Such bridging should be manifested by a substantial increase in potency due to the high local concentration of the free pharmacophore in the vicinity of the proximal recognition site when the bivalent ligand is bound in a monovalent mode.[1,2] Due to homo- and heterooligomerization among the opioid receptors,[3–5] a number of bivalent ligands containing opiate or peptide pharmacophores have been designed and synthesized, which proved to possess enhanced binding affinity and selectivity. For example, bivalent ligands containing oxymorphone or naltrexamine pharmacophores with specific spacer lengths have been reported to have enhanced opioid agonist or antagonist potency and selectivity. Portoghese et al.[2,6–10] has also reported a range of homo- and heterodimeric ligands with varying linker lengths designed to investigate pharmacodynamic and organizational features of opioid receptors. These reported heterodimeric ligands were demonstrated to possess significantly greater potency and selectivity compared to their monomer congeners, providing further evidence for the opioid receptor heterooligomerization phenomena.[10–12]

The development of bivalent ligands that bridge the gap between binding sites on dimerized receptors could lead to a new generation of analgesic drugs that may not cause physical dependence or tolerance with chronic use.[13] Behavioral studies suggested that κ opioid agonists with varying activity at the μ receptor effectively reduced cocaine self-administration in nonhuman primates with fewer undesirable side effects than the highly selective κ agonists.[14–16] These results encouraged us to further develop novel bivalent ligands targeting both κ and μ receptors. Hence, we investigated a new series of homo- and hetero- bivalent ligands which contain the κ agonist, μ partial agonist butorphanol (**5**)[26] and its analogue 14-methoxybutorphanol (**8**). The synthesis of a bivalent ligand (**11**) containing ATPM (**10**),[27] which is a full κ and partial μ agonist, has also been achieved. These ligands were evaluated in vitro for their binding affinity at κ, μ, and δ opioid receptors, and for their pharmacological properties in the [^{35}S]GTPγS binding assay.

We have previously reported that bivalent ligands incorporating two molecules of butorphan (**1**) ('homobivalent') or two distinct ('heterobivalent') morphinan ligands, when connected by a molecular spacer, can lead to compounds with improved binding affinities and selectivities.[17–21] Bivalent ligands **3** and **4**, which contain butorphan (**1**), a mixed κ/μ agonist[24] at both ends of the linking ester chain (Fig. 1), are the most potent ligands in this series, with κ agonist and partial μ agonist activity.[17,18] Ligand **2** (Fig. 1) derived from the linkage of a δ-selective peptide antagonist Dmt-Tic (2′,6′-dimethyl-L-tyrosine-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) and a κ/μ agonist butorphan (**1**) through a repeated 3-aminopropionyl spacer was found to maintain the same characteristics as the two parent compounds.[22] Thus, the binding profile of such bivalent ligands is dependent on the

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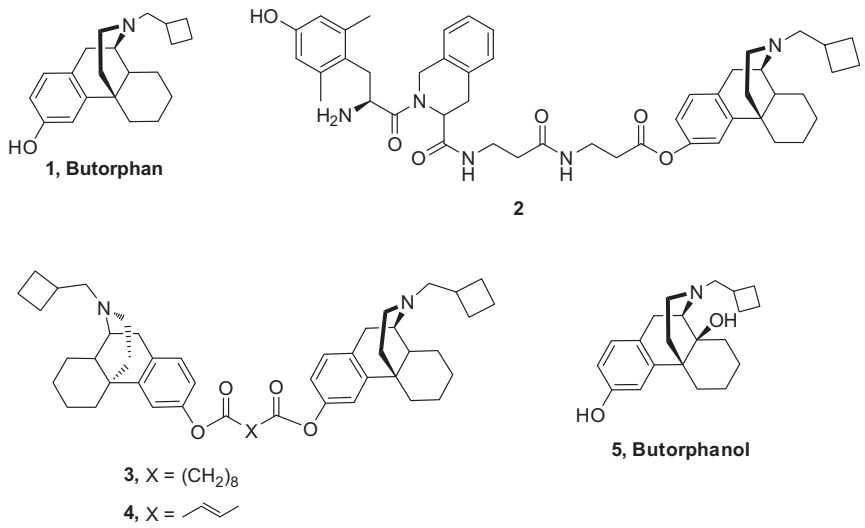


Figure 1. Structure of homo- and heterobivalent ligands.

molecular structure of the opioid pharmacophore, the character and length of the connecting spacer, and the connecting site of the two pharmacophores.[2,8,17–23,25]

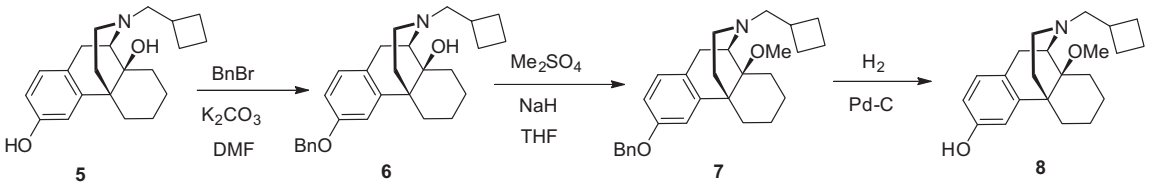
We reasoned that by varying the particular combination of morphinans in each dimer, we would be able to modulate the combination of selectivity, activity, and potency at κ, μ, and δ opioid receptors for a given ligand. Such dimers, with specifically chosen activity for each receptor subtype, could prove highly useful in studying the biological effects of receptor oligomerization and could also lead to a superior therapeutic agent for the treatment of cocaine abuse. We thus investigated a new series of homo- and hetero- bivalent ligands containing the κ agonist, μ partial agonist butorphanol (**5**)[26] and its analogue 14-methoxybutorphanol (**8**). The synthesis of the homobivalent ligand (**11**) which contains ATPM (**10**),[27] a full κ but partial μ agonist, has also been achieved. These ligands were evaluated in vitro for their binding affinity at κ, μ, and δ opioid receptors. These ligands retained or displayed better affinity at κ and μ receptors compared to their parent compounds.

2. Chemistry

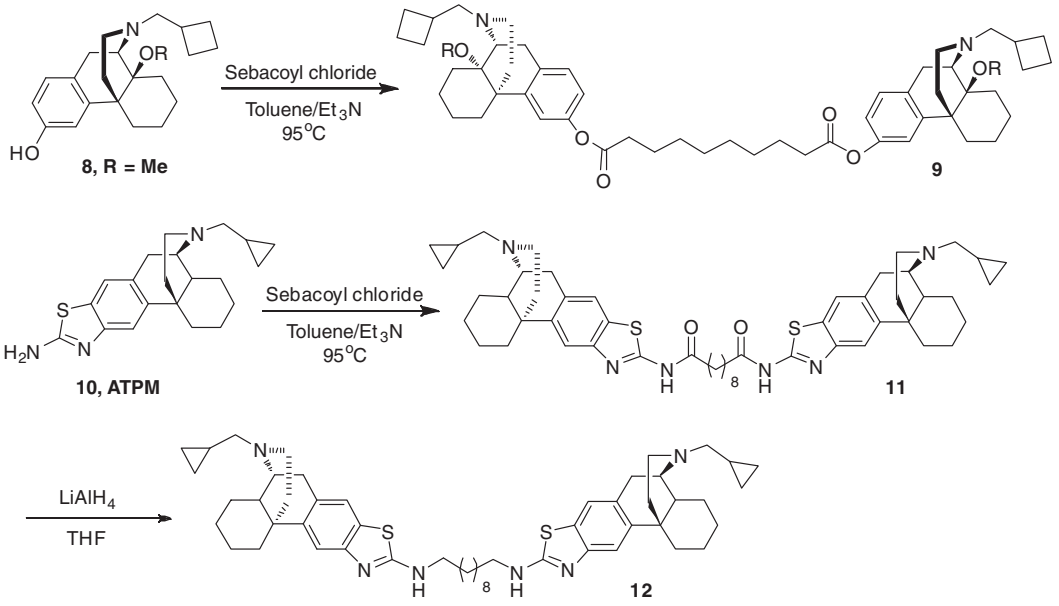
Butorphanol (**1**) free base was treated with $BnBr/K_2CO_3$ to give the corresponding benzyl ether. After treatment with NaH/Me_2SO_4, the benzyl group was removed to give 14-methoxybutorphanol (**8**) (Scheme 1) and were pharmacologically characterized at the κ opioid receptor and the μ opioid receptor using the [^{35}GTPγS] assay. Treatment of sebacic acid (1 equiv) with oxalyl chloride gave sebacoyl chloride, which was then coupled to the morphinans **8** and **10** (2 equiv) to afford the homobivalent compounds **9** and **11**, respectively (Scheme 2).[18] The heterodimeric ligands were prepared stepwise, by esterifying sebacoyl chloride monobenzyl ester with morphinan **5** or **8** to afford **13**, deprotecting the benzyl ether of the latter, and condensing the resulting free acid **14** with **5**, **8**, or naloxone in the presence of DCC and DMAP, as previously reported (Scheme 3).[18]

3. Results and discussion

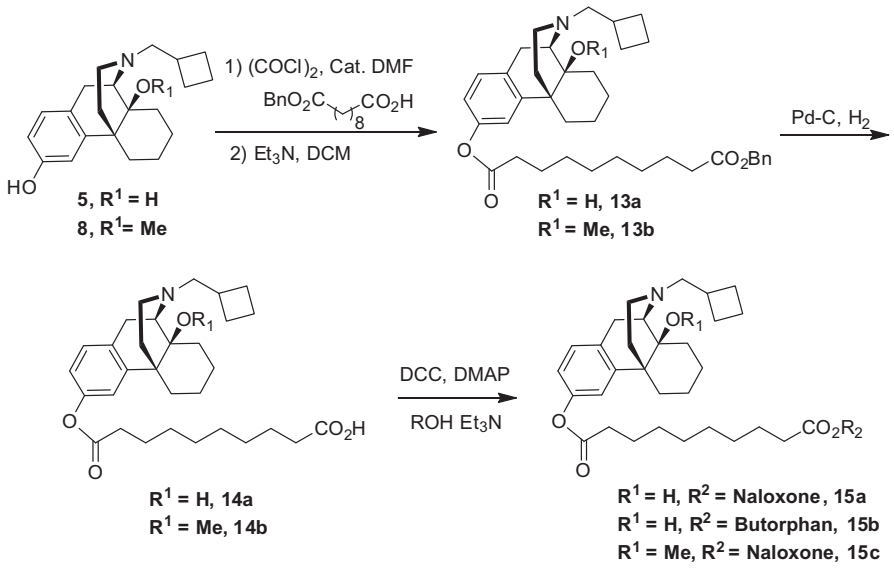
All the novel opioid ligands were evaluated for their binding affinities and selectivity for μ, κ, and δ opioid receptors with Chinese hamster ovary (CHO) membranes stably expressing one of the human opioid receptors. The data are summarized in Table 1. For comparison purposes, the data for naloxone, butorphan (**1**), butorphanol (**5**), ligands **3**, **4**, and **10** are also included in Table 1. Monovalent compound **13b** containing a 10-carbon linking chain, exhibits the same binding affinity at κ and μ receptors as the parent ligand **8** but shows slightly lower affinity at δ receptor. Similarly, **13b** shows slightly higher binding affinity at κ and μ receptors, and shows the same affinity at δ receptor compared to homobivalent ligand **9b**. Heterobivalent ligands **15c** and **15a** exhibit comparable binding affinity at μ receptor, but have increased binding affinity at both κ and δ receptors when compared to naloxone. In contrast, ligand **15a** shows a lower affinity at μ, κ, and δ receptors than butorphanol. Ligand **15c** also shows lower affinity at μ and δ receptors, but a slightly increased affinity at the κ receptor than monomeric parent ligand **8**. It is interesting to note that **15b** has enhanced binding affinity both at μ and κ receptors when compared to its parent compounds butorphan (**1**) and butorphanol (**5**). Since it has been previously demonstrated that ATPM, a full κ agonist with μ activity, effectively reduced cocaine self-administration in nonhuman primates with fewer undesirable side effects than the highly selective kappa agonists,[14–16] we analyzed homodimers of ATPM. It was found that ATPM-derived homodimeric **11**



Scheme 1.



Scheme 2.



Scheme 3.

and **12** maintain high binding affinity at κ receptor (less than 1 nM), although they have lower affinity at all three receptors than the parent compound ATPM.

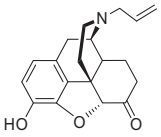
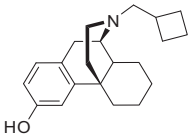
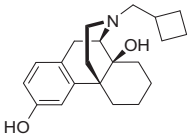
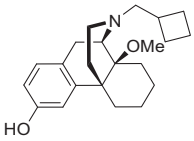
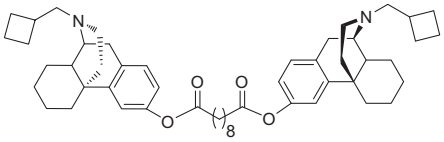
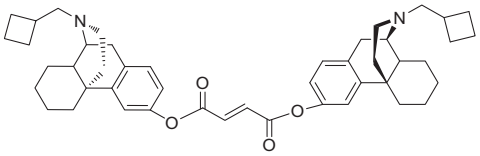
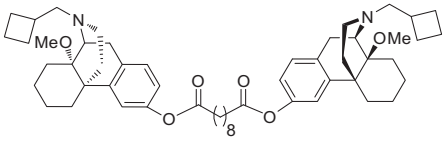
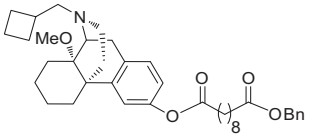
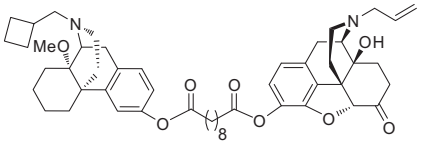
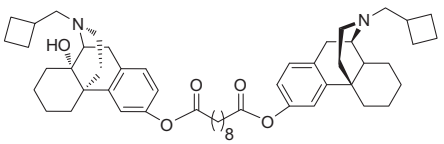
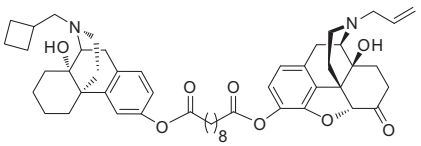
The activities of 14-methoxybutorphanol (**8**), univalent ligand **13b**, homobivalent ligands **9b**, **12**, **11**, and heterobivalent ligands **15c**, **15b**, and **15a** at both κ and μ receptors were assessed using the [^{35}S]GTPγS binding assay, and reported in Tables 2 and 3, respectively.

The results indicate that all 14-methoxybutorphanol (**8**) and butorphanol-derived univalent and bivalent ligands **8**, **9b**, **13b**, **15c**, **15b**, and **15a** acted as partial κ agonists, all having high κ agonist activity (E_{max} range between 74% and 130% maximal stimulation) and varying degrees of κ antagonist activity (I_{max} ranged from 23% to 43% maximal inhibition). In contrast, the ATPM-derived homobivalent ligands **11** and **12** were found to be full agonists. All compounds were found to be partial μ agonists in the [^{35}S]GTPγS binding assay (Table 3).

4. Conclusions

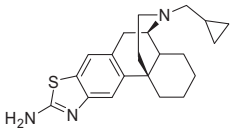
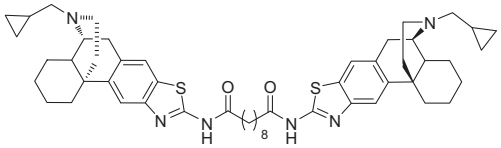
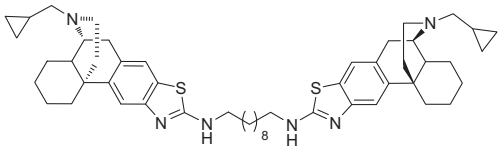
Homo- and heterobivalent ligands, which contain κ/μ agonist butorphanol (**5**), its analogue 14-methoxybutorphanol (**8**) and the aminothiazolomorphinan **10**[27] (ATPM), have been synthesized and their binding affinities evaluated at μ, κ, and δ opioid receptors. All of these ligands have been found to have high binding affinity at μ and/or κ opioid receptors. The heterobivalent ligand **15b** is more potent than either of its parent compounds butorphan (**1**) or butorphanol (**5**) at μ and κ receptors, and was found to be the most potent ligand in this series. With the exception of the two ATPM-derived ligands **11** and **12**, all other ligands which were

Table 1
K_i values for the inhibition of μ, κ, and δ opioid binding to CHO membranes[a]

Compound	Structure	K_i[a] (nM)			Selectivity
		[^{3}H]DAMGOμ	[^{3}H]U69,593κ	[^{3}H]Naltrindoleδ	μ:κ:δ
Naloxone[b]		0.23 ± 0.05	0.25 ± 0.02	38 ± 3	1:1:152
Butorphan[c] 1		0.23 ± 0.01	0.079 ± 0.003	5.9 ± 0.6	3:1:75
Butorphanol 5		0.22 ± 0.012	0.12 ± 0.0068	12 ± 1.1	2:1:100
MCL-691 **8**		0.14 ± 0.014	0.20 ± 0.020	8.1 ± 0.071	1:1:58
MCL-144 **3**[d]		0.090 ± 0.004	0.049 ± 0.001	4.2 ± 0.4	2:1:90
MCL-145 **4**[d]		0.2 ± 0.03	0.08 ± 0.01	9.4 ± 0.5	3:1:120
MCL-692 **9b**		0.26 ± 0.037	0.37 ± 0.040	13 ± 1.0	1:1:50
MCL-693 **13b**		0.15 ± 0.014	0.23 ± 0.011	14 ± 3.8	1:1:93
MCL-694 **15c**		0.55 ± 0.10	0.14 ± 0.019	39 ± 6.1	4:1:280
MCL-695 **15b**		0.089 ± 0.012	0.073 ± 0.001	8.0 ± 2.4	1:1:110
MCL-696 **15a**		0.82 ± 0.11	0.28 ± 0.014	35 ± 11	3:1:130

(*continued on next page*)

Table 1 (*continued*)

Compound	Structure	K_i[a] (nM)			Selectivity
		[^{3}H]DAMGOμ	[^{3}H]U69,593κ	[^{3}H]Naltrindoleδ	μ:κ:δ
10[e] MCL-147 ATPM		1.5 ± 0.21	0.049 ± 0.0046	29 ± 2	31:1:592
MCL-715 **11**		8.8 ± 0.66	0.37 ± 0.024	170 ± 7.1	24:1:460
MCL-714 **12**		2.5 ± 0.19	0.27 ± 0.0023	39 ± 4.8	9:1:140

[a] Membranes from Chinese hamster ovary (CHO) cells, stably expressing either the human κ, μ, or δ opioid receptors, 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 μM naloxone. Data are the mean values ± SEM from three experiments, performed in triplicate.
[b] Ref. 19.
[c] Ref. 23.
[d] Ref. 17.
[e] Ref. 27.

evaluated in this study were partial agonists at the κ receptor (Table 2) and partial agonists at the μ receptor (Table 3). In contrast, the two ATPM-derived ligands **11** and **12** were full agonists at the κ receptor, but like the other ligands in this study, were also partial agonists at the μ receptor. These results suggest that appropriate design of bivalent ligands may enhance the binding affinity and selectivity of opioid ligands, and also provide evidence for opioid receptor oligomerization phenomena. These bivalent ligands may be used as chemical and pharmacological tools to elucidate the pharmacodynamic and organizational features of opioid receptors.

5. Experimental section

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

5.1.1. (−)-3-Benzyloxybutorphanol (6)

To a stirring suspension of butorphanol free base (**5**, 7.52 g, 23.0 mmol) and K_2CO_3 (9.52 g, 69.0 mmol) in DMF (100 mL) was added BnBr (4.13 g, 24.15 mmol), and the reaction mixture was stirred overnight at room temperature. The next day, EtOAc (200 mL) was added, and the organic layer was washed with water (100 mL × 3) and brine (50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was then purified over silica gel (EtOAc/hexane 1:3) to afford a colorless oil (9.04 g, 94%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (m, 5H), 7.01 (d, J = 8.4, 1H), 6.93–6.62 (m, 2H), 5.02 (s, 2H), 4.82–3.80 (br, 1H), 3.04 (d, J = 18.2, 1H), 2.75 (dd, J = 6.2, 18.2, 1H), 2.63 (d, J = 6.0, 2H), 2.54–1.37 (m, 19H), 0.99 (d, J = 12.4, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.4, 142.8, 137.1, 128.7, 128.5, 128.2, 127.9, 127.6, 112.1, 111.9, 70.0, 69.5, 61.3, 60.5, 44.7, 41.5, 37.0, 33.9, 31.7, 30.2, 27.0, 26.8, 24.9, 21.7, 21.6, 18.7.

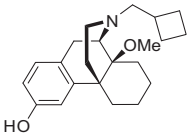
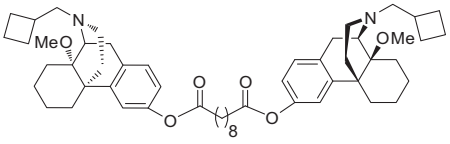
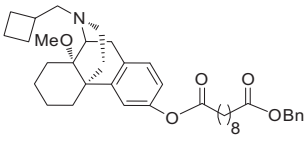
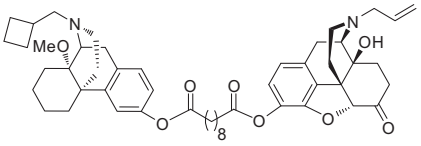
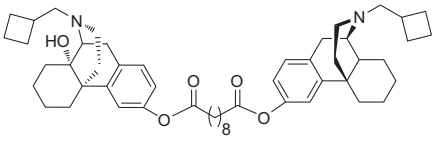
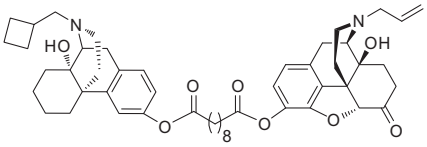
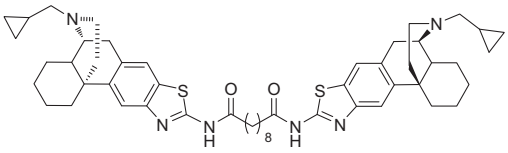
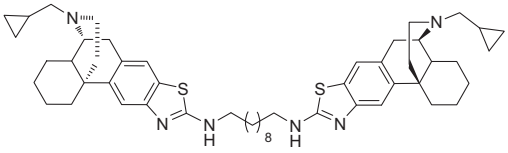
5.1.2. (−)-3-Benzyloxy-14-methoxybutorphanol (7)

To a solution of 3-benzyloxybutorphanol (**6**, 3.8 g, 9.11 mmol) in anhydrous DMF (50 mL) was added NaH (60% in mineral oil, 3.64 g, 91.1 mmol) at room temperature and stirred for 15 min. Next, Me_2SO_4 (2.6 mL, 27 mmol) was added to the suspension, and the reaction mixture was stirred at room temperature for 1 h. The reaction was quenched carefully by addition of water at 0 °C. Next, EtOAc (200 mL) was added, and the organic layer was washed with water (50 mL × 2) and brine, and dried over Na_2SO_4. After the solvent was removed under reduced pressure, the crude product was purified on silica gel (gradient: EtOAc/hexane 1:20 to EtOAc/hexane 1:3) to give a colorless oil (3.88 g, 98%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51–7.29 (m, 5H), 7.01 (d, J = 8.4, 1H), 6.87 (d, J = 2.5, 1H), 6.77 (dd, J = 2.4, 8.3, 1H), 5.02 (s, 2H), 3.22 (s, 3H), 3.13 (d, J = 18.2, 1H), 3.00 (d, J = 4.8, 1H), 2.62–2.35 (m, 5H), 2.27–1.16 (m, 16H), 0.89 (d, J = 12.0, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.3, 143.3, 137.1, 129.5, 128.5, 128.0, 127.8, 127.6, 111.9, 111.7, 74.2, 70.0, 61.3, 52.8, 46.9, 45.8, 42.1, 35.8, 34.4, 29.2, 28.1, 27.2, 25.5, 24.7, 21.5, 20.8, 18.8. Anal. Calcd for $C_{29}H_{37}NO_2 \cdot HCl\ 0.5H_2O$: C, 73.01; H, 8.24; N, 2.94. Found: C, 72.78; H, 7.97; N, 2.86.

5.1.3. (−)-14-Methoxybutorphanol (8)

Pd/C (20 mg) was added to a stirring solution of 3-benzyloxy-11-methoxybutorphanol (230 mg, 0.533 mmol) in MeOH (5 mL), and the suspension was hydrogenated at room temperature over night. The next day, the suspension was filtered off and solvent was removed under reduced pressure to give a colorless oil (170 mg, 93%) which was used directly without purification; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.56 (d, J = 8.2, 1H), 6.42 (s, 1H), 6.30 (d, J = 8.1, 1H), 6.23–5.85 (br, 1H), 2.85 (s, 3H), 2.83–2.60 (m, 2H),

Table 2
Pharmacological characterization of morphinan monovalent and bivalent ligands at the kappa opioid receptor using the [^{35}S]GTPγS binding assay

Compound	Structure	Kappa (AgonMCL-692, MCL-693, ist)		Kappa (antagonist)	
		EC_{50} (nM)	E_{max} (% maximal stimulation over basal)	IC_{50} (nM)	I_{max} (% maximal inhibition)
MCL-691 **8**		2.8 ± 0.90	90 ± 3.6	22 ± 3.7	37 ± 3.0
MCL-692 **9b**		2.4 ± 0.57	83 ± 0.95	17 ± 2.9	43 ± 4.3
MCL-693 **13b**		2.1 ± 0.076	90 ± 6.2	6.4 ± 2.7	30 ± 4.7
MCL-694 **15c**		18 ± 4.5	80 ± 4.8	7.7 ± 3.3	33 ± 3.8
MCL-695 **15b**		0.84 ± 0.089	100 ± 0.95	3.7 ± 1.2	23 ± 2.9
MCL-696 **15a**		12 ± 4.9	74 ± 16	49 ± 13	35 ± 2.2
MCL-715 **11**		5.5 ± 0.31	130 ± 3.3	NI[a]	NI[a]
MCL-714 **12**		7.3 ± 0.81	130 ± 12	NI[a]	NI[a]

Membranes from CHO cells that expressed the human κ opioid receptor were incubated with 12 concentrations of the compound in the presence of 0.08 nM [^{35}S]GTPγS for 60 min at 30 °C. Nonspecific binding was measured by the inclusion of 10 μM GTPγS. For the inhibition experiments, [^{35}S]GTPγS binding was stimulated by the addition of 100 nM U50,488. Data are the mean ± SEM from three experiments performed in triplicate.

[a] NI = no inhibition.

2.30–0.83 (m, 21H), 0.50 (d, J = 11.9, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.8, 143.1, 128.2, 128.1, 113.3, 112.0, 74.5, 61.0, 52.8, 47.0, 45.9, 41.9, 35.5, 34.2, 29.2, 28.2, 27.3, 25.7, 24.8, 21.6, 20.9, 18.8. Mp (HCl salt): 195 °C (dec). Anal. Calcd for $C_{22}H_{31}NO_2$·HCl H_2O: C, 66.73; H, 8.65; N, 3.54. Found: C, 66.40, H, 8.27; N, 3.54.

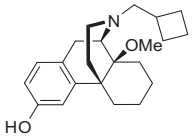
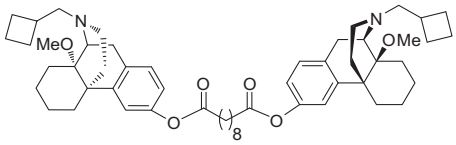
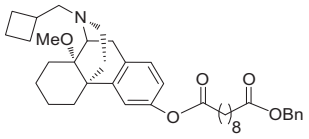
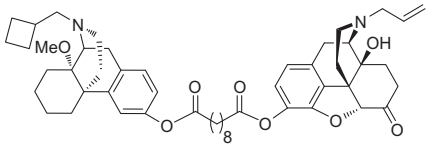
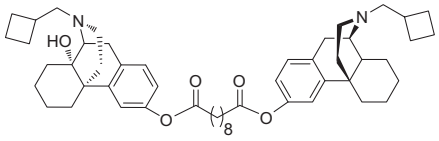
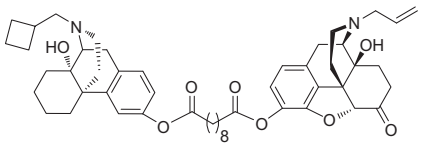
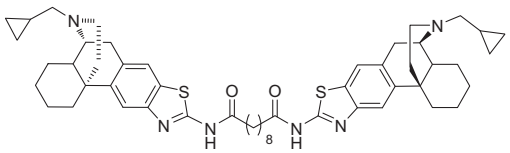
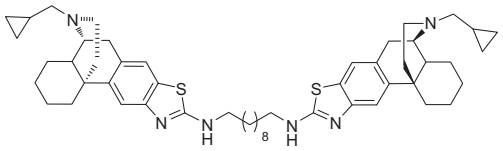
5.2. General procedure for converting morphinan ligands to the HCl salts

To a solution of the free base in a minimal amount of ethyl acetate was added excess ethereal 1 N HCl. A precipitate formed, and the resulting solid was filtered, washed with two portions of ether, and dried under vacuum to give the corresponding HCl salt.

5.2.1. Bis((−)-14-methoxy-17-*N*-cyclobutylmethyl)morphinan-3-yl) decanedioate (9)

Representative procedure: To a solution of sebacic acid (0.0981 mmol, 30.2 mg; 1 equiv) in anhydrous CH_2Cl_2 (2 mL) was added oxalyl chloride (1.962 mmol, 0.173 mL; 20 equiv) and two drops of DMF. Gas evolution was observed and the solution was stirred overnight. The next day, the reaction mixture was concentrated under reduced pressure. The resulting yellow oil was

Table 3
Pharmacological characterization of morphinan monovalent and bivalent at the mu opioid receptor using the [^{35}S]GTPγS binding assay

Compound	Structure	Mu (agonist)		Mu (antagonist)	
		EC_{50} (nM)	E_{max} (% maximal stimulation over basal)	IC_{50} (nM)	I_{max} (% maximal inhibition)
MCL-691 **8**		3.6 ± 1.8	50 ± 2.7	9.3 ± 3.1	50 ± 3.4
MCL-692 **9b**		1.8 ± 0.36	42 ± 2.1	260 ± 92	44 ± 11
MCL-693 **13b**		1.7 ± 0.39	54 ± 7.0	7.6 ± 2.3	28 ± 2.2
MCL-694 **15c**		10 ± 3.0	62 ± 3.4	19 ± 3.0	45 ± 2.8
MCL-695 **15b**		1.2 ± 0.23	46 ± 4.1	3.4 ± 0.63	50 ± 5.7
MCL-696 **15a**		2.0 ± 0.42	19 ± 3.5	34 ± 4.5	73 ± 7.7
MCL-715 **11**		130 ± 42	25 ± 1.8	NA[a]	74 at 10 μM
MCL-714 **12**		22 ± 5.3	38 ± 3.7	390 ± 100	71 ± 5.3

Membranes from CHO cells that expressed the human μ opioid receptor were incubated with 12 concentrations of the compound in the presence of 0.08 nM [^{35}S]GTPγS for 60 min at 30 °C. Nonspecific binding was measured by the inclusion of 10 μM GTPγS. For the inhibition experiments, [^{35}S]GTPγS binding was stimulated by the addition of 200 nM DAMGO. Data are the mean ± SEM from three experiments performed in triplicate.

[a] An IC_{50} value could not be determined because the inhibition curve had not reached a plateau at 10 μM of the compound.

redissolved in anhydrous CH_2Cl_2 (5 mL), 14-methoxybutorphanol (**8**) (100 mg, 0.294 mmol; 3 equiv) and Et_3N (40 mg, 0.392 mmol; 4 equiv) was added to the solution, and the mixture was stirred at room temperature overnight. The organic layer was washed with $NaHCO_3$ solution, dried over Na_2SO_4. The solvent was removed and the result crude product was purified on silica gel (EtOAc/Et_3N/MeOH 200:1:1) to give the pure compound as colorless oil (70 mg, 84%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08 (d, J = 8.3, 2H), 6.92 (d, J = 2.2, 2H), 6.84 (dd, J = 2.3, 8.2, 2H), 3.21 (s, 6H), 3.16 (d, J = 18.8, 2H), 3.01 (d, J = 5.4, 2H), 2.62–1.17 (m, 54H), 0.88 (d, J = 10.9, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 149.3, 143.4, 134.4, 128.1, 118.6, 118.1, 74.1, 61.3, 52.8, 46.9, 45.6, 42.1, 35.8, 34.4, 29.2, 29.05, 29.02, 28.1, 27.2, 25.5, 25.1, 24.8, 21.4, 20.8, 18.8; mp (HCl salt): 154 °C (dec). Anal. Calcd for $C_{54}H_{76}N_2O_6{\cdot}2HCl\ H_2O$: C, 68.99; H, 8.58; N, 2.98. Found: C, 69.03, H, 8.58; N, 2.96.

5.2.2. Bis((−)-3-aminothiazolo-*N*-cyclopropylmethylmorphinan)sebacamide hydrochloride (11)

A mixture of compound **10** (88 mg, 0.25 mmol), sebacoyl chloride (30 μL, 0.14 mmol) and triethyl amine (45 μL, 0.30 mmol) in 2 mL toluene was refluxed overnight. After cooling to room temperature, the reaction mixture was directly purified on silica gel (EtOAc/MeOH/Et_3N = 60:1:1). A slightly yellow foam (52 mg, 54%)

as product was obtained; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (s, 2H), 7.55 (s, 2H), 3.10 (m, 4H), 2.77 (m, 4H), 2.43 (m, 10H), 1.92 (m, 4H), 1.37 (m, 30H), 0.88 (m, 2H), 0.51 (d, J = 8.0, 4H), 0.11 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.98, 159.00, 146.92, 139.88, 134.44, 129.24, 119.91, 116.88, 59.96, 55.71, 45.59, 45.02, 42.57, 38.06, 36.87, 36.33, 28.80, 28.74, 26.90, 26.58, 24.95, 24.86, 22.18, 9.40, 4.10, 3.63. Mp (HCl salt): 270 °C (dec). Anal. Calcd for $C_{52}H_{68}N_6O_2S_2{\cdot}2HCl\ 3.5H_2O$: C, 61.88; H, 7.69; N, 8.33. Found: C, 61.75; H, 7.51; N, 7.94. HPLC analysis indicates a single compound (100%).

5.2.3. (−)-3,3′-(Decane-1,10-diaminothiazolo)bis(cyclopropylmethyl)morphinan hydrochloride (12)

To the solution of amide (52 mg, 0.06 mmol) in 3 mL anhydrous THF was added $LiAlH_4$ (10 mg, 0.24 mmol) at room temperature. The mixture was stirred at room temperature for 4 h, and then three drops water was added to quench the reaction, which was then directly purified on silica gel (EtOAc/MeOH/Et_3N = 60:1:1) to give slightly yellow foam (33 mg product, 66%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (s, 2H), 7.32 (s, 2H), 5.92 (s, 2H), 3.74–3.65 (m, 6H), 3.00 (m, 6H), 2.66 (m, 4H), 2.45 (m, 2H), 2.22 (m, 4H), 1.40 (m, 36H), 0.63 (d, J = 7.9, 4H), 0.29 (d, J = 10.8, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.10, 152.11, 137.55, 128.38, 119.46, 115.23, 62.56, 58.99, 56.16, 45.90, 45.40, 37.32, 36.15, 30.03, 29.10, 26.47, 26.03, 24.89, 21.87, 4.30, 4.27. Mp (HCl salt): 252 °C (dec). Anal. Calcd for $C_{52}H_{72}N_6S_2{\cdot}4HCl\ 2.2H_2O$: C, 60.17; H, 7.88; N, 8.10. Found: C, 60.27; H, 7.91; N, 7.92.

5.2.4. Benzyl ((−)-14-hydroxy-17-*N*-cyclobutylmethyl) morphinan-3-yl) decanedioate (13a)

Method B: See the synthesis of **13b**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (s, 5H), 7.08 (d, J = 8.2, 1H), 6.91 (s, 1H), 6.84 (d, J = 8.2, 1H), 5.10 (s, 2H), 3.07 (d, J = 18.5, 1H), 2.78 (m, 1H), 2.62 (d, J = 5.9, 1H), 2.44 (m, 8H), 1.77 (m, 29H), 0.99 (d, J = 11.9, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6, 172.4, 149.3, 142.9, 136.0, 133.7, 128.5, 128.2, 128.1, 118.7, 118.3, 69.3, 66.0, 61.1, 60.5, 44.5, 41.5, 36.9, 34.3, 34.2, 33.7, 31.5, 30.1, 28.99, 28.96, 26.9, 26.7, 25.2, 24.83, 24.78, 21.6, 18.7. Mp (HCl salt): 87–92 °C. Anal. Calcd for $C_{38}H_{51}NO_5{\cdot}HCl\ 0.8H_2O$: C, 69.93; H, 8.28; N, 2.15. Found: C, 70.04; H, 8.21; N, 2.19.

5.2.5. Benzyl (−)-14-methoxy-butorphanol-3-yl decanedioate (13b)

Method A: To the solution of sebacic acid monobenzyl ester (1.08 g, 3.53 mmol) in anhydrous CH_2Cl_2 (15 mL) was added oxalyl chloride (0.62 mL, 7.04 mmol) and two drops of DMF. Gas evolution could be observed and the solution was stirred overnight. Next, CH_2Cl_2 and excess oxalyl chloride was removed under reduced pressure. The yellow oil was redissolved in anhydrous CH_2Cl_2. 14-Methoxy-butorphanol (**8**) (1.00 g, 2.93 mmol) and Et_3N (2.0 mL, 14.1 mmol) was added to the solution and the mixture was stirred overnight. The organic layer was washed with $NaHCO_3$ solution and dried over Na_2SO_4. After solvent was removed under reduced pressure, the crude product was purified on silica gel (EtOAc/hexanes 1:4 and EtOAc/hexanes 1:1) to give a yellow oil (460 mg, 25%).

Method B: To the solution of sebacic acid monobenzyl ester (206 mg, 0.676 mmol) and 14-methoxy-butorphanol (192 mg, 0.563 mmol) in anhydrous CH_2Cl_2 (6 mL) was added DCC (139 mg, 0.676 mmol), and DMAP (7 mg, 0.0563 mmol), and the reaction mixture was stirred overnight. The next day, after solvent was removed under reduced pressure, the residue was redissolved in an equal volume of ethyl acetate and the white solid was filtered off. The organic layer was washed with saturated $NaHCO_3$ solution and brine, and dried over Na_2SO_4. The crude product was purified on silica gel (EtOAc/hexanes 1:1) to a give yellow oil (110 mg, 31%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 5H), 7.09 (d, J = 8.3, 1H), 6.92 (s, 1H), 6.85 (dd, J = 2.3, 8.2, 1H), 5.12 (s, 2H), 3.22 (s, 3H), 3.16 (d, J = 18.5, 1H), 3.02 (d, J = 5.4, 1H), 2.60–1.20 (m, 37H), 0.89 (d, J = 11.1, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.5, 172.3, 149.3, 143.3, 136.0, 134.2, 128.4, 128.1, 118.5, 118.1, 74.0, 66.0, 61.2, 52.8, 46.9, 45.6, 42.0, 35.6, 34.3, 34.24, 34.19, 29.1, 29.0, 28.9, 28.0, 27.1, 25.4, 25.0, 24.81, 24.78, 21.4, 20.8, 18.7. Mp (HCl salt): 165–168 °C. Anal. Calcd for $C_{39}H_{53}NO_5{\cdot}HCl\ 2.5H_2O$: C, 67.17; H, 8.53; N, 2.01. Found: C, 67.21; H, 8.35; N, 2.22.

5.3. General procedure for the preparation of morphinans 14a and 14b

Pd/C (42 mg) was added to a solution of benzyl ester **4** (418 mg) in MeOH (20 mL). The reaction mixture was hydrogenated at room temperature overnight. The next day, the suspension was filtered and MeOH was removed under reduced pressure to give the product, which was used directly without purification.

5.3.1. Decanedioic acid (−)-butorphanol-3-yl ester (14a)

Colorless oil (205 mg, 97%); ^{1}H NMR (300 MHz, CD_3OD) δ 6.09 (d, J = 8.1, 1H), 5.86 (d, J = 2.1, 1H), 5.77 (dd, J = 2.1, 8.3, 1H), 2.87–0.14 (m, 41H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.6, 171.1, 148.8, 138.6, 128.8, 127.4, 118.5, 117.0, 67.1, 59.8, 55.8, 45.5, 38.3, 31.9, 31.8, 29.2, 29.1, 27.24, 27.20, 27.14, 27.07, 27.00, 25.3, 23.6, 23.0, 22.8, 19.1, 18.5, 16.4.

5.3.2. Decanedioic acid (−)-14-methoxyl-butorphanol-3-yl ester (14b)

Yellow solid (342 mg, 96%); ^{1}H NMR (300 MHz, CD_3OD) δ 7.31 (d, J = 8.4, 1H), 7.08 (d, J = 2.1, 1H), 6.99 (dd, J = 2.1, 8.3, 1H), 3.83 (d, J = 5.8, 1H), 3.55–3.09 (m, 4H), 3.37 (s, 3H), 3.04 (d, J = 8.6, 1H), 2.87–1.14 (m, 34H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.5, 172.3, 149.6, 142.5, 132.6, 128.2, 119.2, 118.2, 77.2, 73.8, 60.0, 52.9, 47.5, 45.3, 41.6, 35.8, 34.3, 34.2, 32.9, 29.05, 29.00, 28.2, 27.4, 25.5, 24.8, 21.1, 20.6, 18.7.

5.4. General procedure for the preparation of morphinans 15a, 15b, and 15c

Representative procedure: To a solution of morphinan (0.162 mmol; 1 equiv) and sebacic acid monomorphinan ester (0.162 mol; 1 equiv) in anhydrous CH_2Cl_2 (2 mL) was added DCC (0195 mmol; 1.2 equiv) and DMAP (0.0162 mmol; 0.1 equiv) and the reaction mixture was stirred overnight at room temperature. CH_2Cl_2 was removed and an equal volume of ethyl acetate was added. The resulting white solid was removed by filtration, and the organic layer was washed with saturated $NaHCO_3$ solution and brine, and dried over Na_2SO_4. Ethyl acetate was removed to give the crude product, which was purified on silica gel (EtOAc/Et_3N 100:1) to give pure product.

5.4.1. (5α-17-Allyl-14-hydroxy-6-oxo-4,5-epoxymorphinan-3-yl-17-((−)-*N*-cyclobutylmethyl-14-hydroxyl-mophinan-3-yl) decanedioate (15a)

Yellow oil (68 mg, 51%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09 (d, J = 8.3, 1H), 6.87 (ddd, J = 3.3, 8.2, 11.9, 3H), 6.69 (d, J = 8.2, 1H), 5.82 (ddt, J = 6.4, 10.1, 16.5, 1H), 5.21 (m, 2H), 4.69 (s, 1H), 3.25–1.20 (m, 54H), 0.91 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 207.6, 172.4, 171.3, 149.3, 147.7, 142.8, 133.6, 132.5, 129.9, 128.2, 122.9, 119.3, 118.8, 118.2, 90.5, 69.3, 62.0, 61.1, 60.5, 57.6, 50.5, 41.4, 34.3, 29.6, 29.0, 26.7, 22.9, 21.6, 14.1. Mp (HCl salt): 170–174 °C. Anal. Calcd for $C_{50}H_{64}N_2O_8{\cdot}2HCl\ 2.4H_2O$: C, 64.08; H, 7.61; N, 2.99. Found: C, 63.85; H, 7.28; N, 2.98.

5.4.2. ((−)-17-*N*-Cyclobutylmethyl)morphinan-3-yl-((−)-14-hydroxyl-17-*N*-cyclobutylmethyl) morphinan-3-yl) decanedioate (15b)

Colorless oil (52 mg, 42%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09 (d, J = 8.3, 2H), 6.87 (m, 4H), 3.04 (dd, J = 18.4, 28.6, 1H), 2.82 (d, J = 5.8, 1H), 2.65–0.98 (m, 64H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 149.3, 149.2, 142.9, 135.1, 133.7, 128.4, 128.2, 118.7, 118.4, 118.3, 118.0, 69.3, 61.4, 60.5, 55.7, 49.0, 45.6, 44.8, 44.5, 41.6, 41.5, 37.7, 36.9, 36.5, 34.8, 34.3, 33.9, 33.7, 31.5, 30.1, 29.02, 28.98, 27.8, 26.9, 26.7, 26.4, 25.6, 25.2, 24.9, 24.8, 24.3, 22.0, 21.6, 18.8, 18.7. Mp (HCl salt): 142–145 °C. Anal. Calcd for $C_{52}H_{72}N_2O_5{\cdot}2HCl\ 1.8H_2O$: C, 68.60; H, 8.59; N, 3.08. Found: C, 68.77; H, 8.42; N, 3.34.

5.4.3. (5α)-17-Allyl-14-hydroxy-6-oxo-4,5-epoxymorphinan-3-yl-((−)-14-methoxyl-17-*N*-cyclobutylmethyl)morphinan-3-yl) decanedioate (15c)

Yellow oil (36 mg, 51%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09 (d, J = 8.4, 1H), 6.92 (s, 1H), 6.88–6.78 (m, 2H), 6.68 (d, J = 8.2, 1H), 5.91–5.70 (m, 1H), 5.33–5.10 (m, 3H), 4.69 (s, 1H), 3.29–2.88 (m, 12H), 2.66–1.16 (m, 42H), 0.89 (d, J = 12.3, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 207.7, 172.4, 171.3, 149.3, 147.7, 143.4, 135.0, 134.3, 132.5, 130.0, 129.9, 128.1, 122.9, 119.3, 118.6, 118.2, 118.1, 90.5, 74.1, 70.0, 62.0, 61.2, 57.6, 52.7, 50.5, 46.9, 45.6, 43.1, 42.0, 36.0, 35.7, 34.4, 33.9, 31.1, 30.5, 29.2, 29.0, 28.9, 28.1, 27.2, 25.4, 25.0, 24.8, 24.7, 22.9, 21.4, 20.8, 18.8. Mp (HCl salt): 175 °C (dec). Anal. Calcd for $C_{51}H_{66}N_2O_8{\cdot}2HCl\ 2H_2O$: C, 64.89; H, 7.69; N, 2.97. Found: C, 65.07; H, 7.61; N, 3.06.

5.5. Opioid binding to the human μ, κ, and δ opioid receptors

Chinese hamster ovary (CHO) cells stably transfected with the human κ opioid receptor, δ opioid receptor were obtained from Dr. L.-Y. Liu-Chen (Temple University, Philadelphia, PA) and Dr. L. Toll (SRI International, Palo Alto, CA), respectively. The μ opioid receptors were obtained from Dr. G. Uhl (NIDA Intramural Program, Baltimore, MD). The cells were grown in 100-mm dishes in Dulbecco's modified Eagle's media supplemented with 10% fetal bovine serum 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. Nonspecific binding was measured by the inclusion of 10 μM naloxone. Samples were filtered through GF/B glass fiber filters, which were washed three times with 3-mL cold 50 mM Tris–HCl, pH 7.5. The filters were counted in 2 mL of ScintiSafe 30% scintillation fluid. IC_{50} values were determined by log-probit analysis and were converted to K_i values by the equation of Cheng and Prusoff.[28]

5.6. [^{35}S]GTPγS binding assay to measure pharmacological properties mediated by the kappa and mu opioid receptors

To determine the agonist properties of the compounds at the κ and μ opioid receptors, membranes expressing either the κ or μ receptor were incubated with 12 different concentrations of the compound in 0.5 mL of buffer containing 50 mM Tris–HCl, pH 7.4, 3 μM GDP, 3 mM $MgCl_2$, 0.2 mM EGTA, and 100 mM NaCl. [^{35}S]GTPγS was added at a final concentration of 0.08 nM. Nonspecific binding was measured by the inclusion of 10 μM GTPgS. After a 60-min incubation at 30 °C, samples were filtered through GF/B glass fiber filters, which were washed three times with 3-mL of cold 50 mM Tris–HCl, pH 7.5. The filters were counted in 2 mL of ScintSafe 30% scintillation fluid. The EC_{50} value and E_{max} values were calculated using SIGMAPLOT software.

To determine if the compound had antagonistic properties at the κ opioid receptor, membranes were incubated as described above. To stimulate [^{35}S]GTPγS binding, 100 nM U50,488 was added. Twelve different concentrations of the compound were added. To determine if a compound was an antagonist at the μ receptor, membranes expressing the μ opioid receptor were incubated with 12 concentrations of the compound and 200 nM DAMGO to stimulate [^{35}S]GTPγS binding. IC_{50} and I_{max} values were calculated with SIGMAPLOT software.

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