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High-affinity carbamate analogues of morphinan at opioid receptors

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Abstract—A series of carbamate analogues were synthesized from levorphanol (**1a**), cyclorphan (**2a**) or butorphan (**3a**) and evaluated in vitro for their binding affinity at μ, δ, and κ opioid receptors. Functional activities of these compounds were measured in the [^{35}S]GTPγS binding assay. Phenyl carbamate derivatives **2d** and **3d** showed the highest binding affinity for κ receptor (K_i = 0.046 and 0.051 nM) and for μ receptor (K_i = 0.11 and 0.12 nM). Compound **1c** showed the highest μ selectivity. 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 all of these ligands were κ agonists. At the μ receptor, compounds **1b**, **1c**, **2b**, and **3b** were agonists, while compounds **2c**–**e** and **3c**–**e** were μ agonists/antagonists.

Treatment of severe pain often requires use of opioid analgesics such as morphine, their use is limited by serious side-effects including tolerance, physical dependence, respiratory depression, and addiction liability. Numerous opiates have been synthesized in an effort to obtain analgesics that are free of such side effects.[1]

Opioids bind to specific neuronally located proteins which initiate biologic responses. Opioid receptors were first discovered in mammalian brain and belong to the rhodopsin subfamily in the superfamily of over 1000 G protein-coupled receptors.[2] Three opioid receptor types, *kappa* (κ), *delta* (δ), and *mu* (μ),[3–5] differ in their distribution and ligand binding, and were identified through pharmacological and physiological studies using selective ligands.

By the synthesis of a series of carbamate analogues of morphinans and evaluation of their binding affinities at opioid receptors, our objective is to identify long-acting opioid receptor ligands useful for the treatment of cocaine abuse. Earlier studies have shown that the basic nitrogen and a phenol moiety were necessary for narcotic analgesics to bind to its opioid receptors.[6] As is the case for morphine derivatives, the phenolic hydroxyl group in morphinan contributes to analgesic activity, which is also a potential site for metabolism, conjugation, and excretion via O-glucuronidation resulting in low oral bioavailability and short duration of action.[1] An approach to improving the pharmacological properties of analgesics such as morphine is to modify the phenolic hydroxy function.

To develop additional insight into the SAR surrounding the 3-position of morphinan, we wished to modify the 3-OH function of morphinan. The carbamate group was introduced because it is a structure of medium polarity, capable of forming hydrogen bonds as donor and acceptor.[7–9] Ideally, these modified compounds would have a longer duration of action and better bioavailability. We report a series of morphinans where the 3-OH was modified by incorporating the carbamate function at the 3-position of the morphinan template.

The levorotatory morphinans (**2a** and **3a**) were prepared from commercially available (−)-3-hydroxy-*N*-methylmorphinan tartrate (levorphanol) **1a** which was converted to the free base and N-demethylated[10–12] to yield the normorphinan. Alkylation with cyclopropylmethyl or cyclobutylmethyl bromide led to the (−)-N-substituted morphinan characterized as their crystalline mandelate salts (**2a** and **3a**), respectively.

A series of carbamates were prepared using morphinans (**1a**, **2a** and **3a**) as starting materials. The carbamates were prepared in yields ranging from 55% to 90% by treating the morphinans (**1a**, **2a** and **3a**) with the

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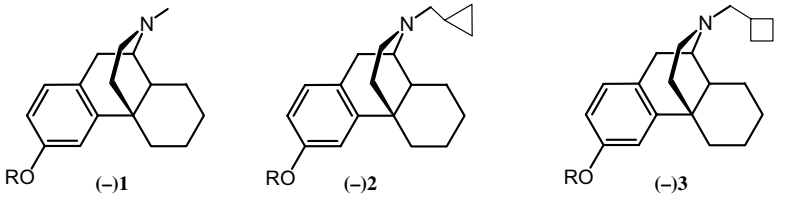

1a, R=H, levorphanol
1b, R=CONHCH$_3$
1c, R=CONHCH$_2$CH$_3$

2a, R=H, cyclorphan
2b, R=CONHCH$_3$
2c, R=CONHCH$_2$CH$_3$
2d, R=CONHC$_6$H$_5$
2e, R=CONHCH$_2$C$_6$H$_5$

3a, R=H, butorphan
3b, R=CONHCH$_3$
3c, R=CONHCH$_2$CH$_3$
3d, R=CONHC$_6$H$_5$
3e, R=CONHCH$_2$C$_6$H$_5$

Figure 1. Structures of morphinans levorphanol, cyclorphan, butorphan and carbamate analogues.

corresponding commercially available aryl or alkyl isocyanates (Fig. 1).

Synthesized 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 one type of the human opioid receptors. The data are summarized in Table 1. For comparison purposes, opioid-binding affinity data for levorphanol (**1a**), cyclorphan (**2a**), and butorphan (**3a**) are also included.[11]

From the data shown in Table 1, the binding affinities of the carbamate-derived opioids were generally lower than the binding affinities of the phenol precursors. It is noteworthy that the phenyl carbamate analogues (**2d** and **3d**) retained the same high affinity (K_i = 0.046 –0.051 nM) at the κ receptor, and a 2-fold decrease at μ for **2d** and a 2-fold increase at μ for **3d** were observed when compared with cyclorphan (**2a**) and butorphan (**3a**).

The methyl carbamate analogues (**1b**, **2b** and **3b**), compared to the phenolic precursors, showed 1.4–100-fold decrease in affinity at κ, 3–22-fold decrease at δ and 5–85-fold decrease at the μ receptor and compound **3b** displayed a good selectivity for κ versus δ receptors. Similarly, appreciable decreases in affinity were observed in the ethyl carbamate analogues (**1c**, **2c** and **3c**) compared to the parent compounds. Compared to phenol **2a** and **3a**, the benzyl carbamate a **2e** and **3e** displayed slightly lower affinity (4-fold) at κ, μ, and δ receptors. All *N*-cyclopropylmethyl derivatives retained κ/μ selectivity, while all *N*-cyclobutylmethyl derivatives showed higher μ selectivity over κ receptors compared to butorphan (**3a**).

It is interesting to note that compound **2e** (benzyl carbamate of cyclorphan **2a**) displayed almost identical affinities at all three opioid receptors to the analogue ethyl carbamate of cyclorphan **2c**, while the benzyl carbamate of butorphan **3e** displayed higher affinities than the ethyl carbamate of butorphan **3c** at all three receptors.

The relative efficacy of these morphinan ligands, **1b**, **1c**, **2b**–**2e** and **3b**–**3e**, compared with levorphanol (**1a**), cyclorphan (**2a**), and butorphan (**3a**) was selected for the [^{35}S]GTPγS assay. Table 2 shows the agonist and antagonist properties of these ligands in stimulating [^{35}S]GTPγS binding mediated by the κ opioid receptor.

Ligands **2c**–**e** and **3e** produced high maximal stimulation of [^{35}S]GTPγS binding (E_{max}) comparable to that of selective agonist U50,488, while ligands **1b**, **2b**, **3b** and **3d** produced similar maximal stimulation to that of the compounds **2a** and **3a**, but less than that of selective agonist U50,488.

The EC_{50} values of these ligands are similar which substantially correlate with the K_i values obtained for the

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

Compound	K_i ± SEM (nM)			Selectivity	
	[^{3}H]DAMGO (μ)	[^{3}H]U69,593 (κ)	[^{3}H]Naltrindole (δ)	μ/κ	δ/κ
(−)**1a** (levorphanol)	0.21 ± 0.02	2.3 ± 0.3	4.2 ± 2.3	0.09	2
(−)**1b** (MCL-433)	1.4 ± 0.0087	3.3 ± 0.40	53 ± 4.5	0.4	16
(−)**1c** (MCL-431)	4.8 ± 0.20	36 ± 8.5	78 ± 4.6	0.1	2
(−)**2a** (cyclorphan)	0.062 ± 0.003	0.034 ± 0.002	1.9 ± 0.072	2	60
(−)**2b** (MCL-434)	5.3 ± 0.62	3.4 ± 0.63	42 ± 6.3	1.6	12
(−)**2c** (MCL-449)	0.29 ± 0.025	0.14 ± 0.015	2.8 ± 0.30	2	20
(−)**2d** (MCL-429)	0.11 ± 0.022	0.046 ± 0.005	1.6 ± 0.15	2.4	35
(−)**2e** (MCL-444)	0.30 ± 0.030	0.14 ± 0.017	2.7 ± 0.27	2	20
(−)**3a** (butorphan)	0.23 ± 0.01	0.079 ± 0.003	5.9 ± 0.55	3	70
(−)**3b** (MCL-435)	1.2 ± 0.12	0.81 ± 0.066	30 ± 0.67	1.5	37
(−)**3c** (MCL-432)	3.7 ± 0.69	4.3 ± 0.30	27 ± 2.5	0.9	6
(−)**3d** (MCL-428)	0.12 ± 0.01	0.051 ± 0.002	3.9 ± 0.26	2	76
(−)**3e** (MCL-443)	0.70 ± 0.063	0.30 ± 0.045	15 ± 1.5	0.4	50

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	—
(−)**2a** (cyclorphan)	Agonist	90 ± 10	0.19 ± 0.04	—
(−)**3a** (butorphan)	Agonist	80 ± 6.8	1.3 ± 0.4	—
(−)**1b** (MCL-433)	Agonist	96 ±10	29 ± 5.7	No effect
(−)**2b** (MCL-434)	Agonist	78 ± 6.8	16 ± 0.19	No effect
(−)**2c** (MCL-449)	Agonist	140 ± 4.7	1.2 ± 0.41	No effect
(−)**2d** (MCL-429)	Agonist	120 ± 7.5	0.38 ± 0.10	No effect
(−)**2e** (MCL-444)	Agonist	130 ± 10	1.1 ± 0.12	No effect
(−)**3b** (MCL-435)	Agonist	88 ± 8.5	9.6 ± 0.4	No effect
(−)**3c** (MCL-432)	Agonist	61 ± 2.5	23 ± 3.6	No effect
(−)**3d** (MCL-428)	Agonist	74 ± 4.7	2.2 ± 0.91	No effect
(−)**3e** (MCL-443)	Agonist	110 ± 0.88	4.7 ± 0.49	No effect

[a] Membranes from CHO cells that stably expressed only one type of the opioid receptor were incubated with varying concentrations of the compounds. The stimulation of [^{35}S]GTPγS binding was measured as described previously.[13] 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. The IC_{50} value is the concentration of compound needed to produce half-maximal inhibition. Dashed lines indicate that the compound was not tested for antagonist properties because of its high E_{max} value.

Table 3. Agonist and antagonist properties of 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	—	—
(−)**2a** (cyclorphan)	Agonist/antagonist	40 ± 2.9	0.80 ± 0.06	50 ± 1	1.7 ± 0.4
(−)**3a** (butorphan)	Agonist/antagonist	50 ± 2.5	1.6 ± 0.2	50 ± 3	20 ± 3
(−)**1b** (MCL-433)	Agonist	71 ± 4.1	50 ± 4.0	No effect	No effect
(−)**2b** (MCL-434)	Agonist	44 ± 2.9	14 ± 2.3	No effect	No effect
(−)**2c** (MCL-449)	Agonist/antagonist	40 ± 2.5	2.4 ± 0.24	68 ± 2.7	10 ± 1.9
(−)**2d** (MCL-429)	Agonist/antagonist	39 ± 3.0	0.44 ± 0.19	70 ± 3.5	6.4 ± 2.0
(−)**2e** (MCL-444)	Agonist/antagonist	39 ± 1.9	2.2 ± 0.18	71 ± 1.8	16 ± 2.9
(−)**3b** (MCL-435)	Agonist	60 ± 5.8	11 ± 1.1	No effect	No effect
(−)**3c** (MCL-432)	Agonist/antagonist	3 2± 0.88	39 ± 7.5	33 ± 3.1	NA
(−)**3d** (MCL-428)	Agonist/antagonist	80 ± 10	1.3 ± 0.16	37 ± 1.2	22 ± 1.9
(−)**3e** (MCL-443)	Agonist/antagonist	54 ± 1.2	7.1 ± 0.48	50 ± 2.5	260 ± 88

[a] Membranes from CHO cells that stably expressed only the opioid receptor were incubated with varying concentrations of the compounds. The stimulation of [^{35}S]GTPγS binding was measured as described previously.[13] EC_{50} values were the concentration of compound needed to produce 50% of the E_{max} value. When the E_{max} value was 30% or lower, it was not possible to calculate an EC_{50} 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.

compounds in the binding assays with [^{3}H]U69,593. Similar to cyclorphan (**2a**) and butorphan (**3a**), these ligands did not inhibit U50,488-stimulated [^{35}S]GTPγS, which suggested that all of these ligands were full κ agonists.

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

Ligands **1b**, **2b** and **3b** produced maximal stimulation of [^{35}S]GTPγS binding mediated by μ receptor comparable to that of the parent compound cyclorphan (**2a**) and butorphan (**3a**) and no inhibition of DAMGO-stimulated [^{35}S]GTPγS binding. These data indicated that **1b**, **2b** and **3b** were μ agonists. Ligands **2c**–**e** had the lowest maximal stimulation of [^{35}S]GTPγS binding and the highest maximal inhibition (I_{max}) of the DAMGO-stimulated [^{35}S]GTPγS binding indicating that ligands **2c**–**e** are μ antagonists with weak agonistic activities at the μ receptors. Compounds **3c**–**e** produced lower maximal inhibition (I_{max}) of the DAMGO-stimulated [^{35}S]GTPγS binding than the carbamates of the cyclorphan (ligands **2c**–**e**). These data indicated that ligands **3c**–**e** are weak μ agonists and antagonists.

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 all of these ligands were κ agonists, however compounds **1b**, **2b** and **3b** were agonists at the μ receptor, whereas compounds **2c**–**e** and **3c**–**e** were both agonists and antagonists at the μ receptor.

A series of carbamate analogues were synthesized from levorphanol (**1a**), cyclorphan (**2a**) or butorphan (**3a**) and evaluated in vitro for their binding affinity at μ, δ, and κ opioid receptors. The binding affinities of the carbamate-derived opioids were generally lower than the binding affinities of the phenol precursors. Phenyl carbamate derivatives **2d** and **3d** showed the highest binding affinity for κ receptor and increased affinity at μ for **3d**.

Functional activities of these compounds were measured in the [^{35}S]GTPγS binding assay, indicating that all of these ligands were κ agonists while, compounds **1b**, **1c**, **2b** and **3b** were μ agonists, and compounds **2c**–**e** and **3c**–**e** were μ agonists/antagonists. Such carbamate derivatives may be useful for the development of longer-acting analgesics as well as medications for drug abuse.

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