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High Affinity of 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* by their binding affinity at μ , δ and κ opioid receptors. Functional activities of these compounds were measured in the [³⁵S]GTP γ S binding assay. Phenyl carbamate derivatives **2d** and **3d** showed the highest binding affinity for κ receptor (K_i = 0.046 nM and 0.051 nM) and for μ receptor (K_i = 0.11 nM and 0.12 nM). Compound **1c** showed the highest μ selectivity. The preliminary assay for agonist and antagonist properties of these ligands in stimulating [³⁵S]GTP γ S 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**, **2d**, **2e**, **3c**, **3d** and **3e** were μ agonists/antagonists.

Keywords

Morphinan; Carbamate; Opioid; Receptors

Treatment of severe pain often requires use of an opioid analgesic 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.

Our objective is to identify long-acting opioid receptor ligands useful for the treatment of cocaine abuse by the synthesis and evaluation of opioid receptor binding affinities of a series carbamate analogues of morphinans. 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

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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 levoratory morphinans (**2a**, **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**, **3a**) as starting materials. The carbamates were made in yields ranging from 55–90% by treating the morphinans (**1a**, **2a**, **3a**) with the corresponding commercially available aryl or alkyl isocyanates.

Affinity and selectivity of the 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 analogs (**2d** and **3d**) retained the same high affinity ($K_i = 0.046$ nM–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 isomer **2e** and **3e** displayed slightly lower affinity (4 fold) at κ , at 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 as 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**) were selected for the [³⁵S]GTP γ S assay. Table 2 shows the agonist and antagonist properties of these ligands in stimulating [³⁵S]GTP γ S binding mediated by the κ opioid receptor.

Ligands **2c**, **2d**, **2e** and **3e** produced high maximal stimulation of [³⁵S]GTP γ S binding (E_{max}) comparable to that of selective agonist U50,488, while ligands **1b**, **2b**, **3b** and **3d** produced

similar maximal stimulation comparable to that of the compounds **2a** and **3a**, but less than that of selective agonist U50,488.

The EC₅₀ values of these ligands are similar which substantially correlates with the K_i values obtained for the compounds in the binding assays with [³H]U69,593. Similar to cyclorphan (**2a**) and butorphan (**3a**), these ligands did not inhibit U50,488-stimulated [³⁵S]GTPγS, which suggested that most of these ligands were full κ agonists.

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

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

The preliminary assay for agonist and antagonist properties of these ligands in stimulating [³⁵S]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**, **2d**, **2e**, **3c**, **3d** and **3e** 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* by 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 [³⁵S]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**, **2d**, **2e**, **3c**, **3d** and **3e** were μ agonist/antagonist. Such carbamate derivatives may be useful for the development of longer-acting analgesics as well as medications for drug abuse.

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Fig. 1.

Table 1

K_i Values For The Inhibition of μ , δ and κ Opioid Binding to Chinese Hamster Ovary Membrane by Carbamate Opioids

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

Table 2

Agonist and Antagonist Properties of Compounds in Stimulating [35 S]GTP γ S 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 $\pm$ 2.0	46 $\pm$ 16	- - -
(-) 2a (cyclophane)	agonist	90 $\pm$ 10	0.19 $\pm$ 0.04	- - -
(-) 3a (butorphan)	agonist	80 $\pm$ 6.8	1.3 $\pm$ 0.4	- - -
(-) 1b (MCL-433)	agonist	96 $\pm$ 10	29 $\pm$ 5.7	No effect
(-) 2b (MCL-434)	agonist	78 $\pm$ 6.8	16 $\pm$ 0.19	No effect
(-) 2c (MCL-449)	agonist	140 $\pm$ 4.7	1.2 $\pm$ 0.41	No effect
(-) 2d (MCL-429)	agonist	120 $\pm$ 7.5	0.38 $\pm$ 0.10	No effect
(-) 2e (MCL-444)	agonist	130 $\pm$ 10	1.1 $\pm$ 0.12	No effect
(-) 3b (MCL-435)	agonist	88 $\pm$ 8.5	9.6 $\pm$ 0.4	No effect
(-) 3c (MCL-432)	agonist	61 $\pm$ 2.5	23 $\pm$ 3.6	No effect
(-) 3d (MCL-428)	agonist	74 $\pm$ 4.7	2.2 $\pm$ 0.91	No effect
(-) 3e (MCL-443)	agonist	110 $\pm$ 0.88	4.7 $\pm$ 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]GTP γ S binding was measured as described in previously¹³. 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 [³⁵S]GTPγS Binding Mediated by the μ Opioid Receptor^a

compound	Pharmacological Properties	<i>E</i> _{max} (% maximal stimulation)	EC ₅₀ (nM)	<i>I</i> _{max} (% maximal inhibition)	IC ₅₀ (nM)
DAMGO	agonist	120 ± 12	110 ± 9.0	---	---
(-)-2a (cyclophosphamide)	agonist/antagonist	40 ± 2.9	0.80 ± 0.06	50 ± 1	1.7 ± 0.4
(-)-3a (butorphanol)	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	32 ± 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

^aMembranes from CHO cells that stably expressed only the opioid receptor were incubated with varying concentrations of the compounds. The stimulation of [³⁵S]GTPγS binding was measured as described in previously¹³. EC₅₀ 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 EC₅₀ 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 IC₅₀ value is the concentration of compound needed to produce half maximal inhibition. Dashed lines indicate that the compound was not tested.