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In Vivo Characterization of (–)(–)MCL-144 and (+)(–)MCL-193: Isomeric, Bivalent Ligands with Mu/Kappa Agonist Properties

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Abstract

Once opioid receptor dimers were postulated, a goal has been to synthesize and screen novel opioids, with the hope of furthering our knowledge of the structure-activity relationship of opioid ligands with the opioid receptors. The aim of the current study was to address whether two isomeric bivalent ligands would have pharmacological differences after central administration, *in vivo*. The two compounds, (–) bis(N-cyclobutylmethyl-morphinan-3-yl) sebacoylate dihydrochloride (MCL-144) and 1-(+)-N-cyclobutylmethylmorphinan-3-yl-10-(–)-N-cyclobutylmethylmorphinan-3-yl sebacoylate (MCL-193) are each linked by a 10-carbon chain ester. The active (–) enantiomer for both ligands is 3-hydroxy-N-cyclobutylmethyl morphinan ((–)MCL-101), a N-cyclobutylmethyl analogue of cyclorphan (1). MCL-144 contains two active levo rotatory (–)(–) pharmacophores, while MCL-193 contains one active (–) and one inactive (+) pharmacophore of MCL-101. *In vitro* analysis demonstrated that all three compounds, (–)(–) MCL-144, (+)(–) MCL-193 and (–)MCL-101 were κ agonists and μ partial agonists. (–)(–)MCL-144 and (–)MCL-101 had much higher affinity for both the μ and κ opioid receptors compared to (+)(–)MCL-193. *In vivo*, (–)(–)MCL-144 and (+)(–)MCL-193 produced full dose-response curves, in the 55°C tail-flick test, with each compound having an ED₅₀ value of 3.0 nmol after intracerebroventricular (i.c.v.) administration. The analgesic properties of both compounds were antagonized by the μ -selective antagonist, β -funaltrexamine and the κ -selective antagonist nor-binaltorphimine. Concomitant, i.c.v., administration of either (–)(–) MCL-144 or (+)(–)MCL-193 with morphine, did not significantly antagonize morphine-induced antinociception at any dose tested. In antinociceptive tests, (–)(–)MCL-144 and (+)(–)MCL-193 had the same pharmacological properties, demonstrating that having two active pharmacophores separated by a 10-carbon spacer group did not increase the antinociceptive efficacy of the compound. Additionally, it was also of interest to compare (–)(–)MCL-145 and (–)(–)MCL-144, as the only difference between these bivalent ligands is the spacer region connecting the two pharmacophores, yet (–)(–)MCL-145 produced an ED₅₀ value 10-fold lower than (–)(–)MCL-144 (ED₅₀ values = 0.3 nmol and 3.0 nmol, respectively).

Keywords

opioid dimers; mu opioid; partial agonist; enantiomers

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Introduction

It has been the objective of medicinal chemists and pharmacologists to synthesize and screen novel opioids to find ligands which would retain the analgesic potency of currently available agonists, such as morphine, with a much improved side effect and abuse liability profile. However, such a compound has remained elusive. One approach has been to synthesize ligands which were designed to bridge two opioid receptors, or dimers (2,3).

The concept of G-protein coupled receptor (GPCR) dimerization has evolved tremendously, since its inception over 20 years ago (4). Studies have demonstrated that opioid receptor dimerization alters signaling (5–8), trafficking (5,9), and internalization (10). It also has been suggested that opioid receptor heterodimers could explain pharmacological subtypes (3), such as δ_1 and δ_2 which can be observed, in vivo, with the receptor-selective agonists such as [D-Pen²,D-Pen⁵]enkephalin (DPDPE) and ([D-Ala²]-Deltorphin II (Deltorphin II) respectively, yet, these same subtypes have not been found utilizing cloning methods.

As opioid receptor dimerization has become recognized as an important phenomenon, many ligands have been synthesized to directly target these complexed receptors, and are generally termed bivalent ligands. Several key considerations in the design of these bivalent ligands are: to choose a monomeric ligand with the desired pharmacological profile, which also has the necessary connecting sites; and to find an appropriate spacer with the correct length and character to allow for bridging of the receptors (11).

Previously, our laboratories have reported on a series of ligands for the μ and κ opioid receptors. One monovalent ligand, of particular interest, has been (–)MCL-101, a N-cyclobutylmethyl analogue of cyclorphan (1). In radioligand binding assays, (–)MCL-101 displayed high affinity for both the μ and κ receptors. (–)MCL-101 was also shown to be a full agonist at the κ receptor and displayed mixed agonist/antagonist properties at the μ receptor, as measured by the [³⁵S] GTP γ S assay (1,2) (Tables 1–3). In vivo, (–)MCL-101 acted as a κ agonist with μ agonist/antagonist properties (1).

The characterization of this compound was important, as it now serves as the pharmacologically active (–) pharmacophore for a series of novel bivalent ligands. Within the bivalent ligand series, we previously reported on (–)(–)MCL-145, a bivalent compound with two (–)MCL-101 pharmacophores bridged by a conformationally constrained fumaryl ester (2,12) (Fig. 1). In both radioligand binding and [³⁵S]GTP γ S assays, (–)(–)MCL-145 displayed virtually identical pharmacological properties to (–)MCL-101 (1,2) (Tables 1–3). In vivo, (–)(–)MCL-145 was a κ agonist and μ partial agonist, similar to (–)MCL-101 (1,12).

Further studies have yielded two isomeric, bivalent ligands, (–)(–)MCL-144 and (+)(–)MCL-193 (Fig. 1). Both compounds are linked by a 10-carbon chain ester, and the active enantiomer for both ligands is (–)MCL-101. In radioligand binding assays, (–)(–)MCL-144 displayed higher affinity for both the μ and κ receptors (K_i = 0.09 nM and 0.05 nM, respectively) compared to (+)(–)MCL-193 (K_i = 2.2 nM and 1.2 nM, respectively) (2,11) (Table 1). For comparison, the bivalent ligand, (+)(+)MCL-192 (11) (Fig. 1), was also studied in the radioligand binding assay, and displayed poor affinity for the μ , κ , and δ receptors, respectively (K_i = 130 nM, 700 nM, and 130 nM) (2) (Table 1). These data demonstrated that the compounds with (–) pharmacophores: (–)MCL-101, (–)(–)MCL-145, and (–)(–)MCL-144, had high affinities for the μ and κ receptors, and were all similar to each other. (+)(–)MCL-193, with only one active pharmacophore, had lower affinity than the others, but displayed markedly higher affinities than bis((+)N-cyclobutylmethylmorphinan-3-yl)sebacoylate (MCL-192), which contains the two (+)(+) isomers of (–)MCL-101. Structurally, (–)(–)MCL-145 and (–)(–)MCL-144 are similar, with two active enantiomers of (–)MCL-101. The only difference between these two compounds is the spacer length. (–)(–)MCL-145 is bridged by a

conformationally constrained fumaryl ester, while (-)(-)MCL-144 is bridged by a flexible 10-carbon chain.

The efficacy of (-)(-)MCL-144 and (+)(-)MCL-193 for stimulating [35 S]GTP γ S binding mediated by the κ opioid receptor was similar, as both produced maximal stimulation ($E_{\max}$) values of 60–70%. (-)(-)MCL-144 was more efficacious at the μ receptor for stimulating [35 S]GTP γ S binding producing an $E_{\max}$ value of 50%, than (+)(-)MCL-193 which produced an $E_{\max}$ value of 28% (Tables 2–3). Both compounds were μ antagonists, in this assay, with maximal inhibition ($I_{\max}$) values of 60–70% (2,11) (Tables 2–3). (+)(+)MCL-192 was not tested in the [35 S]GTP γ S assay due to its low binding affinities. In this functional assay, (-)MCL-101, (-)(-)MCL-145, (-)(-)MCL-144, and (+)(-)MCL-193 all acted as κ agonists, and μ agonist/antagonists. All produced similar $E_{\max}$ values at the κ receptor while (+)(-)MCL-193 displayed lower efficacy than the other three at the μ receptor.

The aim of the current study was to explore whether the two isomeric bivalent ligands, (-)(-)MCL-144 and (+)(-)MCL-193 would have appreciable pharmacological differences after central administration, in vivo, utilizing mouse antinociceptive assays. Additionally, (-)(-)MCL-145 and (-)(-)MCL-144 were compared. These two compounds differ only in their spacer length, yet display distinct differences in their pharmacological properties. Preliminary metabolism studies were used to address some of these differences.

Materials and methods

Animals

Male, ICR mice (20–30 g) (Harlan Industries, Indianapolis, IN) were housed in groups of five with food and water available ad libitum before any procedures. Animals were maintained on a 12-hr light/dark cycle in a temperature-controlled animal colony. Studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health.

Chemicals

(-)MCL-101, (-)(-)MCL-144, (+)(-)MCL-193, (+)(+)MCL-192, and (-)(-)MCL-145 (Fig. 1) were synthesized at ADARC, McLean Hospital, as previously described (1,2,11). (-)(-)MCL-144, (+)(-)MCL-193, (+)(+)MCL-192 and (-)MCL-101 were initially solubilized in DMSO and all subsequent dilutions were in distilled water. Morphine sulfate was purchased from Mallinckrodt (Saint Louis, MO). ICI 174,864 (N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH (where Aib is α -aminoisobutyric acid), β -funaltrexamine (β -FNA), and nor-binaltorphimine (nor-BNI) were purchased from Sigma Chemical Co. (Saint Louis, MO).

Injections

Intracerebroventricular (i.c.v.) injections were made directly into the lateral ventricle according to the modified method of Haley and McCormick (13). Briefly, the mouse was lightly anesthetized with ether, an incision was made in the scalp, and the injection was made 2 mm lateral and 2 mm caudal to bregma at a depth of 3 mm using a 10- μ l Hamilton syringe. The volume of all i.c.v. injections was 5 μ l.

Analgesia Assay

Antinociception was assessed using the 55°C warm-water tail-flick test. For the tail-flick test, the latency to the first sign of a rapid tail-flick was taken as the behavioral endpoint (14). Each mouse was first tested for baseline latency by immersing its tail in the water and recording the time to response. Mice not responding within 5 sec were excluded from further testing. Mice were then administered the test compound and tested for antinociception 20 min after the

injection. A maximum score was assigned (100%) to animals not responding within 15 sec to avoid tissue damage. Antinociception was calculated by the following formula: % antinociception = $100 \times (\text{test latency} - \text{control latency}) / (15 - \text{control latency})$.

Agonist Effects of (-)(-)MCL-144 and (+)(-)MCL-193

To further determine which receptors were responsible for the analgesic activity of (-)(-)MCL-144 and (+)(-)MCL-193, mice were pretreated with a μ -(β -FNA, 20 nmol i.c.v., -24 hr), δ -(ICI-174,864, 4 nmol i.c.v., -20 min), or κ -(nor-BNI, 3 nmol i.c.v., -24 hr) selective antagonist. These times and doses have been demonstrated previously to produce antagonism at the μ , δ , and κ receptors, respectively (15–17). Control mice received a vehicle injection (5 μ l of vehicle, i.c.v., -24 hr or -20 min). Then, mice received either (-)(-)MCL-144 or (+)(-)MCL-193 (10 nmol, i.c.v.). Antinociception was measured 20 min after agonist injection in the 55°C warm-water tail-flick assay.

Antagonist Effects of (-)(-)MCL-144 and (+)(-)MCL-193

Mice were treated concomitantly with morphine (3 nmol, i.c.v.) and graded doses of (-)(-)MCL-144 or (+)(-)MCL-193 i.c.v. (0.1, 0.3, 1.0 nmol). Control mice received a vehicle injection (5 μ l of vehicle, i.c.v., -20 min). Antinociception was assessed 20 min after the injection in the 55°C tail-flick test.

Statistical Analysis

Data from dose-response experiments were fitted using nonlinear regression analysis, and ED₅₀ values and 95% confidence limits (CL) were calculated (GraphPad Prism 4.03, San Diego, CA). All data points are the mean of 7–10 mice, with standard error of the mean represented by error bars. Statistical analysis of the antagonist data used the Student's *t* test. Statistical significance was set at $p < 0.05$.

HPLC Analysis and Preparation of Rat Brain Homogenate

HPLC analysis was performed on a Varian Prostar HPLC modular system operated by Star Chromatography Workstation software, Version 5. Chromatographic separations were performed on a Supelco Discovery C18 column (4.6 mm $\times$ 25 cm, 5 micron) operated at ambient temperature. The samples were injected using a Rheodyne 7725 manual sample injector equipped with a 20 μ l injection loop. The mobile phase of 0.1% TFA in acetonitrile and 0.1% TFA in water was operated at a gradient of 20–100% acetonitrile over 20 min at 1.0 ml/min. Detection was at 280 nm for (-)(-)MCL-145 and 265 nm was used to detect (-)(-)MCL-144. To prepare the brain homogenate, a 1.87 g frozen Sprague-Dawley rat brain was homogenized in 18 ml of ice cold 25 mM phosphate buffered saline (PBS) pH 7.3 by sonication for one min with a Polytron PCU-2-110 sonicator. The homogenate was then stored frozen in one ml aliquots.

Metabolism of (-)(-)MCL-145

To 1.0 mg (1.42 μ mol) of (-)(-)MCL-145 in 1 ml of ether was added 100 μ l (10 μ mol) of 0.1 M HCl in ether to give a white precipitate. The mixture was allowed to stir for 15 min and then concentrated in vacuo to give the white dihydrochloride salt of (-)(-)MCL-145. To the salt was added 1 ml of 25 mM PBS pH 7.3 and 100 μ l aliquots were removed and added to 100 μ l of 10% rat brain homogenate in 25 mM PBS pH 7.3, in duplicate. The tubes were incubated in a 37°C water bath and at appropriate times were removed, quenched with 100 μ l of acetonitrile, vortexed 30 sec, and centrifuged at 10,000 rpm for 5 min. The supernatant was injected directly on to the HPLC column for analysis. The rate of disappearance of (-)(-)MCL-145 was analyzed by HPLC at 280 nm. The relative percent peak areas of (-)(-)

MCL-145 (13.4 min) and (-)(-)MCL-101 (10 min) from the two tubes for each time point were averaged and plotted versus time.

Metabolism of (-)(-)MCL-144

To 3.4 mg (4.3 μ mol) of (-)(-)MCL-144 in 400 μ l of methanol was added 860 μ l (8.6 μ mol) of 0.01M L-tartaric acid in methanol to give a white precipitate. The mixture was allowed to stir for 15 min and then concentrated in vacuo to give the white ditartrate salt of (-)(-)MCL-144. To the salt was added 2 ml of 25 mM PBS pH 7.3, 50 μ l was removed and diluted with 50 μ l of PBS, in duplicate. The tubes were incubated in a 37°C water bath and at appropriate times removed and injected directly on to the HPLC column for analysis. To study the metabolism of (-)(-)MCL-144 in rat brain homogenate, 100 μ l aliquots of the 2 ml stock mixture above were removed and added to 100 μ l of 10% rat brain homogenate in 25 mM PBS pH 7.3 in duplicate. The tubes were incubated in a 37°C water bath and at appropriate times were removed, quenched with 100 μ l of acetonitrile, vortexed 30 sec, and centrifuged at 10,000 rpm for 5 min. The supernatant was injected directly on to the HPLC column for analysis. The rate of disappearance of (-)(-)MCL-144 was analyzed by HPLC at 265 nm. The relative percent peak areas of (-)(-)MCL-144 (13.4 min) and (-)MCL-101 (10 min) from the two tubes for each time point were averaged and plotted versus time.

Results

Antinociceptive Effects of (-)(-)MCL-144 and (+)(-)MCL-193 in the Mouse 55°C Warm-Water Tail-Flick Test

Administration of graded doses of (-)(-)MCL-144 (1, 3, 10 nmol) produced a full dose-response curve with an ED₅₀ value and 95% CL of 3.04 nmol (1.9 – 4.9 nmol) (Fig. 2, Table 4). (+)(-)MCL-193 produced a similar ED₅₀ value and 95% CL in the tail-flick test, 3.2 nmol (1.8 – 5.5 nmol) (Fig. 2, Table 4). The ED₅₀ values obtained for both of these bivalent compounds were 2-fold lower than the monovalent pharmacophore, (-)MCL-101 (Table 4). The bivalent ligand (+)(+)MCL-192, containing the inactive pharmacophore of (-)MCL-101, did not produce antinociception at a dose of up to 10 nmol, therefore the characterization of this compound was not continued.

Properties of (-)(-)MCL-144 and (+)(-)MCL-193 in the Presence of Receptor-Selective Antagonists

Pretreatment with the κ -selective antagonist, nor-BNI, significantly (** p < 0.01) inhibited the antinociceptive activity of both (-)(-)MCL-144 and (+)(-)MCL-193 (Fig. 3A, 3B). The antinociception produced by (-)(-)MCL-144 and (+)(-)MCL-193 was also significantly (** p < 0.01) inhibited by 24-hr pretreatment with the μ -selective antagonist, β -FNA (Fig. 3A, 3B). The δ -selective antagonist, ICI 174,864, had no effect on the antinociceptive properties of (-)(-)MCL-144 or (+)(-)MCL-193. (-)MCL-101-mediated antinociception, was also antagonized by β -FNA and nor-BNI (1).

(-)(-)MCL-144 and (+)(-)MCL-193 Did Not Antagonize Morphine-Mediated Antinociception

In vitro, both (-)(-)MCL-144 and (+)(-)MCL-193 inhibited [D-Ala², N-Me-Phe⁴, Gly⁵-ol]-enkephalin (DAMGO) stimulated [³⁵S]GTP γ S binding mediated by the μ opioid receptor (11) (Table 3). To assess if either compound would act as an antagonist in vivo a fixed dose of morphine (3 nmol) was administered concomitantly with increasing doses of either MCL compound (0.1, 0.3, 1.0 nmol). In vivo, (-)(-)MCL-144 and (+)(-)MCL-193 did not significantly antagonize morphine-mediated antinociception (Fig. 4), and did not act as μ antagonists at the doses tested.

(-)(-)MCL-145 and (-)(-)MCL-144 Displayed Different Half-Lives in Rat Brain Homogenate

The rate of metabolism for the bivalent ligands (-)(-)MCL-145 and (-)(-) MCL-144 were addressed using 5% rat brain homogenate in PBS at pH 7.3. The half-life for metabolism of (-)(-)MCL-145 to (-)MCL-101 was 38 min (Fig. 5A). For (-)(-)MCL-144, the half-life was 70 min (Fig. 5B). Studies with (+)(-) MCL-193 were not conducted, but would be expected to be similar to (-)(-)MCL-144, as both compounds have the same spacer region.

Discussion

With the recognition that opioid receptors were capable of dimerizing and forming novel signaling units, came the idea that targeting these complexed receptors with bivalent ligands may yield superior opioid analgesics and also new pharmacological tools for the scientist, in the continued study of these receptors.

Bivalent ligands have been synthesized to target many different GPCRs including the: serotonin (18), muscarinic (19), and adrenergic (20) receptors. Additionally, the design and synthesis of bivalent ligands has been successfully applied within the opioid field, yielding the important κ antagonist, nor-BNI (21) along with new pharmacological tools, such as KDN21, an antagonist ligand for the heterodimerized δ and κ receptors (22).

Our current study was designed to address whether two isomeric bivalent ligands would have appreciable pharmacological differences after i.c.v. administration in mice. In vitro, (-)(-) MCL-144 displayed similar pharmacological properties, both in affinity and efficacy, to its monomeric parent compound, (-)MCL-101 (Tables 1–3). In vitro, (+)(-)MCL-193, displayed weaker binding affinities than the previous two compounds, but similar efficacy in stimulating GTP γ S binding mediated by both the μ and κ receptors (Tables 1–3).

The analgesic properties of (-)(-)MCL-144 and (+)(-)MCL-193 were significantly attenuated by the receptor-selective antagonists, nor-BNI and β -FNA, similar to the parent compound, (-)MCL-101. These data would support the conclusion that both (-)(-) MCL-144 and (+)(-) MCL-193 are capable of binding to both the μ and κ receptors, in vivo.

We had hypothesized that (-)(-)MCL-144 would be more efficacious, either in binding a dimer pair or binding to single receptors, as only the (-) stereoisomer is effective in producing analgesia (23). After i.c.v. administration in mice, both (-)(-)MCL-144 and (+)(-)MCL-193 produced similar ED₅₀ values, which were 2-fold lower than that for (-)MCL-101. It was not surprising that a compound with two active (-)MCL-101 pharmacophores, (-)(-)MCL-144, would have an ED₅₀ value half that of a compound with only one, (-)MCL-101. What was surprising was that (+)(-)MCL-193 produced the same ED₅₀ value as (-)(-)MCL-144. These data would strongly suggest that these compounds are not binding to dimerized receptors, but are in fact, binding to single receptors. If the bivalent ligands were bridging dimerized receptors, it would seem, (-)(-)MCL-144 would be more efficacious and this would be reflected in a lower ED₅₀ value, relative to (+)(-) MCL-193. Another possibility is that the bivalent ligands are bridging recognition sites on a single receptor (24) and the (+) pharmacophore is interacting non-specifically with either a vicinal opioid site or another membrane site (25). However, it can not be completely ruled out that (-)(-)MCL-144 and (+) (-)MCL-193 are in fact binding to dimerized receptors. If this were the case, these data would suggest that within the dimer pair, only one receptor needs to be activated by ligand and the other receptor is just accessory, and does not need to be activated in its own right.

It is also of interest that (-)(-)MCL-145 produced an ED₅₀ value 10-fold lower than (-)(-) MCL-144 (ED₅₀ values = 0.3 nmol and 3.0 nmol, respectively). The only difference between these two ligands is the spacer region connecting the two pharmacophores. In (-)(-)MCL-145,

this spacer is a conformationally constrained fumaryl ester, while in (-)(-) MCL-144 the spacer is a 10-carbon chain. It is possible that (-)(-)MCL-145, due to the shorter length of the spacer region, is interacting with the receptors in a manner which is different from (-)(-)MCL-144. It is suggested that (-)(-)MCL-145 may not be bridging between two receptors in a dimer since previous studies indicated a linker length of 8–20 atoms was optimal for binding affinity (2,3). Preliminary metabolism studies conducted in the rat brain homogenate indicated that (-)(-)MCL-145 was rapidly metabolized to (-)MCL-101, with a half-life of 38 min, while the half-life for (-)(-)MCL-144 was 70 min. It is also plausible that the intermediates from metabolism of (-)(-)MCL-145 and (-)(-)MCL-144 are different. It is easy to imagine a metabolic intermediate which consisted of an (-)MCL-101 monomer and a portion of the spacer region. The in vivo pharmacology of this intermediate may contribute to the differences in ED₅₀ values between (-)(-)MCL-145 and (-)(-)MCL-144. Future studies will address specific details concerning metabolism of the bivalent ligands to monomers.

In conclusion, the bivalent ligands, (-)(-)MCL-144 and (+)(-)MCL-193, are μ/κ agonists, in vivo. Though it is unlikely either compound is bridging dimerized receptors, both provided important information concerning the structure-activity relationship of the opioid receptor with isomeric bivalent ligands. Importantly, it was demonstrated, that after central administration, in mice, both compounds were potent analgesics. Comparison of (-)(-)MCL-145 and (-)(-)MCL-144 revealed the importance of the spacer region, in vivo. Future studies will continue to address the metabolism of these bivalent ligands, as well as the biological activity of the intermediates produced.

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Fig. 1. Chemical structures of the monovalent pharmacophore, (-)MCL-101, (+)MCL-101 and the bivalent ligands, (-)(-)MCL-145, (-)(-)MCL-144, (+)(-)MCL-193 and (+)(+)MCL-192.

Fig. 2.

Dose-response curves for (-)(-)MCL-144 and (+)(-)MCL-193. Mice were injected with (-)(-)MCL-144 or (+)(-)MCL-193, i.c.v., and antinociception was assessed 20 min after the injection in the 55°C warm-water tail-flick test. All data points are the mean of 7–10 mice, with standard error of the mean represented by error bars.

Fig. 3.

To determine the agonist profile of (-)(-)MCL-144 (A) and (+)(-)MCL-193 (B) in vivo, mice were injected with the receptor-selective antagonists, nor-BNI(κ) (3 nmol, i.c.v., -24 hr); β-FNA (μ) (20 nmol, i.c.v., -24 hr) or ICI 174,864 (δ) (4 nmol, i.c.v., -20 min). Twenty min after agonist injection mice were tested for antinociception in the 55°C warm-water tail-flick test. The antinociceptive properties of both (-)(-)MCL-144 and (+)(-)MCL-193 were significantly antagonized by the μ- and κ-selective antagonists, β-FNA and nor-BNI, respectively (**p<0.01).

Fig. 4.

(-)(-)MCL-144 and (+)(-)MCL-193 did not display μ antagonist properties, in vivo. Graded doses of either (-)(-)MCL-144 (A) or (+)(-)MCL-193 (B) (0.1 – 3.0 nmol, i.c.v.) were administered concomitantly with a fixed dose of morphine (3.0 nmol, i.c.v.). Antinociception was measured in the 55°C tail-flick assay, 20 min after the co-injection. At the doses tested, neither compound antagonized morphine antinociception.

Fig. 5. Metabolism studies conducted in rat brain homogenate at pH 7.3, in PBS buffer, indicate that both (-)(-)MCL-145 (A) and (-)(-)MCL-144 (B) are metabolized to the monomeric compound (-)MCL-101, but with different time courses. The half-life of (-)(-) MCL-145 in rat brain homogenate was 38 min (A), while for (-)(-)MCL-144 the half-life was 70 min (B).

Table 1

K_i Inhibition values of μ, δ, and κ opioid binding to CHO membranes by MCL-101, (-)(-)MCL-145, (-)(-)MCL-144, (+)(-)MCL-193, and (+)(+)MCL-192

Compound	K _i (nM) ± S.E.		Selectivity	
	³ H[DAMGO (μ)	³ H[NaItrindole (δ)]	³ H[U69,593 (κ)]	κ/μ
MCL-101	0.23 ± 0.01	5.9 ± 0.55	0.08 ± 0.003	3
(-)(-)MCL-145	0.20 ± 0.032	9.4 ± 0.54	0.08 ± 0.01	3
(-)(-)MCL-144	0.09 ± 0.004	4.2 ± 0.44	0.05 ± 0.001	2
(+)(-)MCL-193	2.2 ± 0.1	23 ± 1.2	1.2 ± 0.32	2
(+)(+)MCL-192	130 ± 7.9	700 ± 54	130 ± 10	1

Membranes were incubated with varying concentrations of the compounds in the presence of either 0.25 nM [³H]DAMGO, 0.2 nM [³H]naltrindole, or 1 nM [³H]U69,593 to measure binding to μ-, δ-, κ-sites, respectively. After equilibrium binding was reached, membranes were filtered onto glass fiber filters. Data are expressed as the mean K_i value ± S.E. for three experiments performed in triplicate (Modified from References 2 and 11).

Agonist and antagonist properties of MCL-101, (-)(-)MCL-145, (-)(-) MCL-144, (+)(-) MCL-193, and (+)(+)MCL-192 in stimulating [³⁵S]GTPγS binding mediated by the κ opioid receptor

Compound	Pharmacological Properties	E _{max} (%)(% maximal stimulation)	EC ₅₀ (nM)	I _{max} (%)(% maximal inhibition)	IC ₅₀ (nM)
U50,488	agonist	110 ± 2.0	46 ± 16	-	
MCL-101	agonist	80 ± 6.8	1.3 ± 0.4	-	
(-)(-)MCL-145	agonist/antagonist	90 ± 6.0	2.3 ± 0.34	100	2400 ± 75
(-)(-)MCL-144	agonist	60 ± 0.68	0.85 ± 0.053	No inhibition	
(+)(-)MCL-193	agonist	77 ± 2.4	7.9 ± 0.3	No Inhibition	
(+)(+)MCL-192	-	-	-	-	-

Membranes from CHO cells that stably expressed only the κ opioid receptor were incubated with varying concentrations of the compounds. The stimulations of [³⁵S]GTPγS binding was measured as described previously (2,11). 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 Emax value is the maximal percent stimulation obtained with the compound. The EC50 value is the concentration of the compound needed to produce 50% of the Emax value. The Imax 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. (Modified from References 2 and 11.)

Agonist and antagonist properties of MCL-101, (-)(-)MCL-145, (-)(-)MCL-144, (+)(-)MCL-193, and (+)(+)MCL-192 in stimulating [³⁵S]GTPγS binding mediated by the μ opioid receptor

Compound	Pharmacological Properties	E _{max} (% maximal stimulation)	EC ₅₀ (nM)	I _{max} (% maximal inhibition)	IC ₅₀ (nM)
DAMGO	agonist	120 ± 12	110 ± 9.0	-	-
MCL-101	agonist/antagonist	50 ± 2.5	1.6 ± 0.15	50 ± 2.6	20 ± 2.7
(-)(-)MCL-145	agonist/antagonist	50 ± 2.5	3.1 ± 0.3	100 ± 0	605 ± 71
(-)(-)MCL-144	agonist/antagonist	50 ± 6.6	1.3 ± 0.15	60 ± 5.2	16 ± 3.0
(+)(-)MCL-193	agonist/antagonist	28	NA	71 ± 2.0	460 ± 43
(+)(+)MCL-192	-	-	-	-	-

Membranes from CHO cells that stably expressed only the μ opioid receptor were incubated with varying concentrations of the compounds. The stimulation of [³⁵S]GTPγS binding was measured as described previously (2,11). The E_{max} value is the maximal percent stimulation obtained with the compound. The EC₅₀ value is the concentration of the 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. (Modified from References 2 and 11.)

Table 4

Comparison of ED₅₀ values obtained for (–)MCL-101, (–)(–)MCL-145, (–)(–) MCL-144, (+)(–)MCL-193, and (+)(+)MCL-192, in vivo.

Compound	ED ₅₀ Value (nmol, i.c.v.)	95% CL
(–)MCL-101	7.3	5.7 – 9.4
(–)(–)MCL-145	0.27	0.13 – 0.58
(–)(–)MCL-144	3.0	1.9 – 4.9
(+)(–)MCL-193	3.2	1.8 – 5.5
(+)(+)MCL-192	No antinociception at doses up to 10 nmol.	

Mice were administered graded doses of compound. Antinociception was measured 20 min after agonist injection in the 55°C warm-water tail-flick test. Twenty min after an i.c.v. injection of the compound was the time needed to obtain maximal antinociception.