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New Opioid Designed Multiple Ligand from Dmt-Tic and Morphinan Pharmacophores

John L. Neumeyer[‡], Xuemei Peng[‡], Brian I. Knapp[∞], Jean M. Bidlack[∞], Lawrence H. Lazarus[§], Severo Salvadori[†], Claudio Trapella[†], and Gianfranco Balboni^{*,†,¶}

[‡]Alcohol and Drug Abuse Research Center, McLean Hospital, Harvard Medical School, 115 Mill Street, Belmont, Massachusetts 02478, USA

[∞]Department of Pharmacology and Physiology, School of Medicine and Dentistry, University of Rochester, Rochester, New York 14642, USA

[§]Medicinal Chemistry Group, Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA

[†]Department of Pharmaceutical Sciences and Biotechnology Center, University of Ferrara, I-44100 Ferrara, Italy.

^{*}Department of Toxicology, University of Cagliari, I-09124, Cagliari, Italy

Abstract

Here we report the facile synthesis of a designed multi-pharmacophore ligand derived from the linkage of a delta selective peptide antagonist (Dmt-Tic) and a *mu* / *kappa* morphinan agonist butorphan (MCL 101) through a two methylene spacer. The new compound MCL 450 maintains the same characteristics as the two reference compounds. MCL 450 represents a useful starting point for the synthesis of other multiple opioid ligands endowed with analgesic properties with low tolerance and dependence.

Introduction

For many years, clinicians have treated unresponsive patients by combining therapeutic mechanisms with a cocktail of drugs. The design of ligands that act on specific multiple targets (multiple ligands) is a more recent trend, as indicated by the substantial increase over the past few years in the number of publications describing such approaches. Numerous terms are currently used to describe ligands that have multiple activities: the words dual, binary, bivalent, dimeric, mixed, triple or balanced are used in conjunction with numerous suffixes, for example, ligand, inhibitor, agonist, antagonist, conjugate or blocker. To improve communication and awareness of this emerging field within the drug discovery community, some authors propose using the term “designed multiple” (DM) ligands as a generic phrase to describe compounds that are rationally designed to modulate multiple targets of relevance to a disease, with the overall goal of enhancing efficacy and / or improving safety.^{1, 2-4} Compared with drug combinations, there are some advantages associated with multiple ligands, such as the more predictable pharmacokinetic and pharmacodynamic relationship that is a consequence of the administration of a single drug, as well improved patient compliance. The molecular starting point for a multiple-ligand project is generated using one of two distinct approaches - either rational design by a combination of pharmacophores or the screening of compounds libraries of known drugs. The methodical combination of pharmacophores from selective ligands is

[¶]To whom Correspondence should be addressed. Tel.: +39-532-291-275; Fax: +39-532-291-296; E-mail: gbalboni@unica.it; bbg@unife.it.

currently the predominant technique used for the generation of multiple ligands. The pharmacophores are joined together by a cleavable or non-cleavable linker (termed “conjugates”) or, more commonly, they are overlapped by taking advantage of structural commonalities (“overlapping pharmacophore”). The majority of reported examples of cleavable conjugates contain an ester linker that is cleaved by plasma esterases to release two individual drugs that then act independently. Although the pharmacokinetic-pharmacodynamic relationship could become complex after cleavage of the linker, at the same time of administration cleavable conjugates are a single molecule, which is one potential advantage that this approach has over drug cocktails.^{3, 4} The prevalence in the literature of “designing in” new activities, that is the synthesis of designed multiple (DM) ligands from selective ligands, indicates that this approach is certainly more popular, and probably more feasible than “designing out” activities from non-selective ligands. As an example, a mixed μ agonist / δ antagonist pseudopeptide was obtained linking tail to tail a selective δ antagonist (H-Tyr-TicΨ[CH₂-NH]Cha-Phe-OH) with a selective μ agonist (H-Dmt-D-Arg-Phe-Lys-NH₂).⁵ Recently, Neumeyer *et al.* reported the synthesis and pharmacological evaluation of “bivalent ligands” containing homo- and heterodimeric pharmacophores related to morphinans.⁶ Ligands having two pharmacophores connected by a spacer have the potential for binding vicinal receptors.⁷⁻⁹ Such bridging should be manifested by a substantial increase in potency due to the high concentration of the pharmacophore in the vicinity of the recognition site when the ligand is bound in a monovalent mode. Portoghese *et al.* reported a range of “homo-“ and “heterodimeric ligands” with varying linker lengths designed to investigate pharmacodynamic and organizational features of opioid receptors.¹⁰ For example, recently reported heterodimeric ligands containing δ antagonist (naltrindole) and κ_1 agonist (ICI-199,441) pharmacophores joined by variable length oligoglycyl-based linkers were demonstrated to possess significantly greater potency and selectivity compared to their monomer congeners, providing further evidence for the opioid receptor hetero-oligomerization phenomena.¹¹ Considering earlier data in this field, we report the synthesis and pharmacological evaluation of the first opioid designed multiple ligand obtained through the linkage of the δ -selective peptidic Dmt-Tic pharmacophore and the κ agonist / μ partial agonist morphinan compound MCL 101 (butorphan).

Chemistry

The synthesis of compound MCL 450 is reported in Scheme 1. Boc- β -ala-OH was condensed with MCL 101¹² via EDC/DMAP¹³ to yield crude MCL 438, which after solvent evaporation, was purified by column chromatography as reported in the experimental section. After N-terminal Boc deprotection with TFA / CH₂Cl₂,¹⁴ the product was condensed with Boc-Dmt-Tic-OH¹⁵ via EDC/HOBt. Boc deprotection with TFA / CH₂Cl₂ gave MCL 450. Condensation of Boc-Dmt-Tic-OH with HCl·H- β -ala-OMe via EDC/HOBt gave the corresponding protected tripeptide which was hydrolyzed at C-terminus methyl ester by NaOH and deprotected at Boc N-terminus with TFA to yield crude MCL 451. Final compounds were purified by reverse phase preparative HPLC.

Pharmacological Results and Discussion

Affinity and Selectivity of the Synthesized Ligands

MCL 450 and MCL 451 were evaluated for their affinity at and selectivity for μ , δ and κ opioid receptors with Chinese hamster ovary (CHO) cell membranes stably expressing the human opioid receptors. Data are summarized in Table 1. For comparison purposes, opioid binding affinity data for MCL 101 and Dmt-Tic are included. The designed multiple ligand MCL 450 derives from the ester bond formation between MCL 451 and MCL 101. As expected, its selectivity derives from a combination of affinity data from MCL 101 (essentially a κ / μ ligand) and MCL 451 (δ ligand). In fact, μ and κ affinity drop 3 and 3.5 fold from MCL 101 to MCL

450, respectively; while δ affinity increases 3.9 fold. Considering MCL 451 in comparison with MCL 450, μ affinity increases about 1600 fold and unexpectedly δ affinity increases 2.8 fold. Moreover, MCL 451 confirms once again the importance of the Dmt-Tic pharmacophore in the induction of δ affinity and selectivity, especially if a free carboxylic function is present at C-terminus.¹⁶ Starting from a quite selective κ/μ ligand (MCL 101) and a selective δ ligand (MCL 451) a nonselective MCL 450 was obtained.

Functional Activity

Table 2 shows the agonist and antagonist properties of MCL 450 and MCL 451 in stimulating [³⁵S]GTP γ S binding mediated by the μ , δ and κ opioid receptors. The binding assays (Table 1), MCL 450 summarizes binding properties of its constituents MCL 101 (μ partial agonist / κ agonist) and MCL 451 (δ antagonist). The data show a decrease of EC₅₀ for μ and κ receptors of 5.6 and 4.6 fold respectively, in comparison to MCL 101; at the same time the corresponding E_{max} (%) values increase of 1.58 and 1.63 fold (transforming it into an almost full μ agonist). On the other hand, MCL 450 is characterized by a δ antagonist activity furnished by MCL 451, moreover it is a δ antagonist more potent (2.6 fold) than the δ selective counterpart. As expected, MCL 451 results in a potent and selective δ antagonist, in line with all other δ antagonists containing the Dmt-Tic pharmacophore.^{15, 17-19}

Conclusion

We have demonstrate the possibility of obtaining a designed multiple ligand in the opioid peptide field with the Dmt-Tic pharmacophore. Contrary to other researcher reports, in this case the distance between the two pharmacophores constituting the designed multiple ligand, seems not to be important. In fact, we selected a two methylene spacer simply because in a similar compound, synthesized by Schiller *et al.*, he reported the same spacer between a μ agonist and a δ antagonist (H-Dmt→D-Arg→Phe→Lys-NH-CH₂-CH₂-NH-Phe←Cha[NH-CH₂]ΨTic←Tyr-H), was reported the same spacer.⁵ We synthesized a very similar compound incorporating a δ antagonist and a μ agonist (H-Dmt→Tic-NH-CH₂-CH₂-NH-Phe←Phe←Pro←Tyr-H) and obtained very similar pharmacological results (personal data). On the other and, when the δ antagonist H-Dmt-Tic-Glu is linked to a fluorophore through a five methylene spacer, it maintains selectivity and δ antagonist properties similar to the reference tripeptide.^{20, 21} MCL 450 could represent a useful starting point in the synthesis of other designed multiple ligands from peptidic and morphinan pharmacophores; as an example some interesting compound could be obtained by esterification of δ antagonists (from the Dmt-Tic pharmacophore) and morphine. Such a compound, as for example diacetylmorphine (heroin), should be able to cross the blood brain barrier and then hydrolyzed by esterases to give morphine and a δ antagonist potentially useful to decreases or eliminate morphine induced side effects such as tolerance and dependence.²² Vice versa, MCL 101 or morphine could be esterified by the carboxylic function of arginine derivatives (NO synthase inhibitors) to prevent tolerance induced by morphinan μ - and κ -agonists.²³ It is interesting to note that cleavable conjugates, such as these reported here, do not need spacers because their individual activities can be affected just after hydrolysis.

Experimental Section

(-)-3-Hydroxy-N-cyclobutylmethylmorphinan (MCL 101)

(-)-3-Hydroxy-N-cyclobutylmethylmorphinan free base was made from commercially available levorphanol tartrate (Mallinckrodt Inc.) by a procedure previously reported.¹²

1-((-)-N-Cyclobutylmethylmorphinan-3-yl)-N-Boc- β -Alanine. (Boc- β -ala-O-MCL 101). (MCL 438)

MCL-101 (0.16 g, 0.5 mmol) and Boc- β -ala-OH (0.11 g, 0.6 mmol) were dissolved in anhydrous dichloromethane (10 mL) under nitrogen. A catalytic amount of 4-dimethylaminopyridine (0.006 g, 0.05 mmol) was added, followed by EDC (0.12 g, 0.6 mmol). The mixture was stirred at room temperature overnight, after solvent evaporation, the crude product was purified by column chromatography on silica gel (EtOAc/Et₃N, 100:1, v/v) to afford a colorless oil: yield 0.12 g (50%); *R*_f(B) 0.77; HPLC *K'* 8.68; oil; [α]_D²⁰ -22.6; MH⁺ 484; ¹H-NMR (CDCl₃) δ 1.05-2.83 (m, 35H), 3.02 (d, *J*=18.6 Hz, 1H), 3.47-3.53 (m, 2H), 6.85 (dd, *J*=8.1 Hz, 2.4 Hz, 1H), 6.94 (d, *J*=2.4 Hz, 1H), 7.11 (d, *J*=8.1 Hz, 1H).

2TFA·H- β -ala-O-MCL 101

Boc- β -ala-O-MCL 101 (0.12 g, 0.25 mmol) was treated with 50% TFA in CH₂Cl₂ (2 mL) for 2 h at room temperature. After solvent evaporation, Et₂O/Pe (1:5, v/v) was added to the solution until the product precipitated: yield 0.14 g (90%); *R*_f(A) 0.68; HPLC *K'* 7.26; mp 121-123 °C; [α]_D²⁰ -15.1; MH⁺ 384.

Boc-Dmt-Tic- β -ala-O-MCL 101

To a solution of Boc-Dmt-Tic-OH (0.05 g, 0.11 mmol) and 2TFA·H- β -ala-O-MCL 101 (0.07 g, 0.11 mmol) in DMF (10 mL) at 0 °C, NMM (0.02 mL, 0.22 mmol), HOBT (0.02 g, 0.12 mmol), and EDC (0.02 g, 0.12 mmol) were added. The reaction mixture was stirred for 3 h at 0 °C and 24 h at room temperature. After DMF was evaporated, the residue was dissolved in EtOAc and washed with NaHCO₃ (5% in H₂O), and brine. The organic phase was dried (Na₂SO₄) and evaporated to dryness. The residue was precipitated from Et₂O/Pe (1:9, v/v): yield 0.07 g (83%); *R*_f(B) 0.75; HPLC *K'* 9.52; mp 112-114 °C; [α]_D²⁰ -15.6; MH⁺ 834; ¹H-NMR (DMSO-*d*₆) δ 1.05-3.17 (m, 46H), 3.47-4.92 (m, 6H), 6.29-7.02 (m, 9H).

TFA·H-Dmt-Tic- β -ala-O-MCL 101 (MCL 450)

Boc-Dmt-Tic- β -ala-O-MCL 101 (0.07 g, 0.08 mmol) was treated with 50% TFA in CH₂Cl₂ (2 mL) for 2 h at room temperature. After solvent evaporation, Et₂O/Pe (1:1, v/v) was added to the solution until the product precipitated: yield 0.07 g (92%); *R*_f(A) 0.65; HPLC *K'* 7.21; mp 128-130 °C; [α]_D²⁰ -17.9; MH⁺ 734; ¹H-NMR (DMSO-*d*₆) δ 1.05-3.17 (m, 37H), 3.47-4.92 (m, 6H), 6.29-7.02 (m, 9H).

Boc-Dmt-Tic- β -ala-OMe

To a solution of Boc-Dmt-Tic-OH (0.14 g, 0.31 mmol) and HCl·H- β -ala-OMe (0.04 g, 0.31 mmol) in DMF (10 mL) at 0 °C, NMM (0.03 mL, 0.31 mmol), HOBT (0.05 g, 0.34 mmol), and EDC (0.06 g, 0.34 mmol) were added. The reaction mixture was stirred for 3 h at 0 °C and 24 h at room temperature. After DMF was evaporated, the residue was dissolved in EtOAc and washed with citric acid (10% in H₂O), NaHCO₃ (5% in H₂O), and brine. The organic phase was dried (Na₂SO₄) and evaporated to dryness. The residue was precipitated from Et₂O/Pe (1:9, v/v): yield 0.15 g (88%); *R*_f(B) 0.62; HPLC *K'* 8.21; mp 124-126 °C; [α]_D²⁰ +7.2; MH⁺ 555; ¹H-NMR (DMSO-*d*₆) δ 1.40-3.17 (m, 21H), 3.59-4.92 (m, 9H), 6.29 (s, 2H), 6.96-7.02 (m, 4H).

Boc-Dmt-Tic- β -ala-OH

To a solution of Boc-Dmt-Tic- β -ala-OMe (0.15 g, 0.27 mmol) in MeOH (10 mL) was added 1N NaOH (0.32 mL). The reaction mixture was stirred for 24 h at room temperature. After solvent evaporation, the residue was dissolved in EtOAc and washed with citric acid (10% in H₂O) and brine. The organic phase was dried (Na₂SO₄) and evaporated to dryness. The residue

was precipitated from Et₂O/Pe (1:9, v/v): yield 0.12 g (82%); *R_f*(B) 0.58; HPLC *K'* 7.92; mp 138–140 °C; [α]_D²⁰ +9.1; MH⁺ 541.

TFA·H-Dmt-Tic-β-ala-OH (MCL 451)

Boc-Dmt-Tic-β-ala-OH (0.12 g, 0.22 mmol) was treated with TFA (1 mL) for 30 min. at room temperature. After solvent evaporation, Et₂O/Pe (1:1, v/v) was added to the solution until the product precipitated: yield 0.11 g (92%); *R_f*(A) 0.46; HPLC *K'* 6.12; mp 145–147 °C; [α]_D²⁰ +12.3; MH⁺ 441; ¹H-NMR (DMSO-*d*₆) δ 2.35 (s, 6H), 2.51–3.59 (m, 8H), 3.95–4.92 (m, 4H), 6.29 (s, 2H), 6.96–7.02 (m, 4H).

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Supporting Information Available. Chemistry general methods, biological general methods and elemental analysis. This material is available free of charge via the Internet at: <http://pubs.acs.org>.

References

- (1). J. Biol. Chem 1985;260:14–42. Abbreviations. In addition to the IUPAC-IUB Commission on Biochemical Nomenclature this paper uses the following additional symbols and abbreviations: Boc, *t*-butoxycarbonyl; CHO, Chinese hamster ovary; DMAP, 4-dimethylaminopyridine; DAMGO, [D-Ala², N-Me-Phe⁴, Gly-ol⁵]-enkephalin; DMF, N,N-dimethylformamide; DMSO-*d*₆, hexadeuteriodimethyl sulfoxide; Dmt, 2',6'-dimethyl-L-tyrosine; EDC, 1-ethyl-3-(3'-dimethyl) aminopropyl]-carbodiimide hydrochloride; EtOAc, ethyl acetate; Et₃N, triethyl amine; Et₂O, diethyl ether; HOBt, 1-hydroxybenzotriazole; HPLC, high performance liquid chromatography; MALDI-TOF, matrix assisted laser desorption ionization time-of-flight; MeOH, methanol; NMM, 4-methylmorpholine; Pe, petroleum ether; TFA, trifluoroacetic acid; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; TLC, thin-layer chromatography; U69,593, (5α,7α,8β)-(+)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide
- (2). Hopkins AL, Mason JS, Overington JP. Can we rationally design promiscuous drugs? Curr. Opin. Struct. Biol 2006;16:127–136. [PubMed: 16442279]
- (3). Morphy R, Rankovic Z. Designed multiple ligands. An emerging drug discovery paradigm. J. Med. Chem 2005;48:6523–65433. [PubMed: 16220969]
- (4). Morphy R, Kay C, Rankovic Z. From magic bullets to designed multiple ligands. Drug Discov Today 2004;9:641–651. [PubMed: 15279847]
- (5). Weltrowska G, Lemieux C, Chung NN, Schiller PW. A chimeric opioid peptide with mixed μ agonist / δ antagonist properties. J. Peptide Res 2004;63:63–68. [PubMed: 15009527]
- (6). Peng X, Knapp BI, Bidlack JM, Neumeyer JL. Synthesis and preliminary in vitro investigation of bivalent ligands containing homo- and heterodimeric pharmacophores at μ , δ , and κ opioid receptors. J. Med. Chem 2006;49:256–262. [PubMed: 16392810]
- (7). Jordan BA, Cvejic S, Devi LA. Opioid and their complicated receptor complexes. Neuropsychopharmacology 2000;23:S5–S18.
- (8). George SR, Fan T, Xie Z, Tse R, Tam V, Vargase G, O'Dowd F. Oligomerization of μ - and δ -opioid receptors. Generation of novel functional properties. J. Biol. Chem 2000;275:26128–26135. [PubMed: 10842167]
- (9). Portoghese PS, Nagase H, Takemori AE. Only one pharmacophore is required for the κ opioid antagonist selectivity of norbinaltorphimine. J. Med. Chem 1988;31:1344–1347. [PubMed: 2838632]
- (10). Portoghese PS. From models to molecules: Opioid receptor dimers, bivalent ligands, and selective opioid receptor probes. J. Med. Chem 2001;44:2259–2269. [PubMed: 11428919]

- (11). Daniels DJ, Kulkarni A, Xie Z, Bhushan RG, Portoghese PS. A bivalent ligand (KDAN-18) containing δ -antagonist and κ -agonist pharmacophores bridges δ_2 and κ_1 opioid receptor phenotypes. *J. Med. Chem* 2005;48:1713–1716. [PubMed: 15771416]
- (12). Neumeyer JL, Bidlack JM, Zong R, Bakthavachalam V, Gao P, Cohen DJ, Negus SS, Mello NK. Synthesis and opioid receptor affinity of morphinan and benzomorphan derivatives: mixed κ agonists and μ agonists / antagonists as potential pharmacotherapeutic for cocaine dependence. *J. Med. Chem* 2000;43:114–122. [PubMed: 10633042]
- (13). Ogawa T, Araki M, Miyamae T, Okayama T, Hagiwara M, Sakurada S, Morikawa T. Synthesis and antinociceptive activity of orally active opioid peptides: improvement of oral bioavailability by esterification. *Chem. Pharm. Bull* 2003;51:759–771. [PubMed: 12843580]
- (14). Baxter, AJG.; Robinson, DH.; Brown, RC. Pharmaceutically active compounds. 1990. EP0384636
- (15). Salvadori S, Guerrini R, Balboni G, Bianchi C, Bryant SD, Cooper PS, Lazarus LH. Further studies on the Dmt-Tic pharmacophore: hydrophobic substituents at the C-terminus endow δ antagonists to manifest μ agonism or μ antagonism. *J. Med. Chem* 1999;42:5010–5019. [PubMed: 10585210]
- (16). Balboni G, Salvadori S, Guerrini R, Negri L, Giannini E, Bryant SD, Jinsmaa Y, Lazarus LH. Direct influence of C-terminally substituted amino acids in the Dmt-Tic pharmacophore on δ -opioid receptor selectivity and antagonism. *J. Med. Chem* 2004;47:4066–4071. [PubMed: 15267245]
- (17). Salvadori S, Attila M, Balboni G, Bianchi C, Bryant SD, Crescenzi O, Guerrini R, Picone D, Tancredi T, Temussi PA, Lazarus LH. δ Opioidmimetic antagonists: prototypes for designing a new generation of ultrasensitive opioid peptides. *Mol. Med* 1995;1:678–689. [PubMed: 8529134]
- (18). Salvadori S, Balboni G, Guerrini R, Tomatis R, Bianchi C, Bryant SD, Cooper PS, Lazarus LH. Evolution of the Dmt-Tic pharmacophore: N-terminal methylated derivatives with extraordinary δ opioid antagonist activity. *J. Med. Chem* 1997;40:3100–3108. [PubMed: 9301674]
- (19). Balboni G, Guerrini R, Salvadori S, Negri L, Giannini E, Bryant SD, Jinsmaa Y, Lazarus LH. Conversion of the potent δ -opioid agonist H-Dmt-Tic-NH-CH₂-Bid into δ -opioid antagonists by N¹-benzimidazole alkylation. *J. Med. Chem* 2005;48:8112–8114. [PubMed: 16366592]
- (20). Balboni G, Salvadori S, Dal Piaz A, Bortolotti F, Argazzi R, Negri L, Lattanzi R, Bryant SD, Jinsmaa Y, Lazarus LH. Highly selective fluorescent analogue of the potent δ -opioid receptor antagonist Dmt-Tic. *J. Med. Chem* 2004;47:6541–6546. [PubMed: 15588089]
- (21). Vázquez ME, Blanco JB, Salvadori S, Trapella C, Argazzi R, Bryant SD, Jinsmaa Y, Lazarus LH, Negri L, Giannini E, Lattanzi R, Colucci M, Balboni G. 6-*N,N*-Dimethylamino-2,3-naphthalimide a new environment-sensitive fluorescent probe in δ -selective and μ -selective opioid peptides. *J. Med. Chem.* in press
- (22). Abdelhamid EE, Sultana M, Portoghese PS, Takemori AE. Selective blockage of delta opioid receptors prevents the development of morphine tolerance and dependence in mice. *J. Pharmacol. Exp. Ther* 1991;258:299–303. [PubMed: 1649297]
- (23). Bargava HN, Cao Y-J, Zhao G-M. Effect of 7-nitroindazole on tolerance to morphine, U-50,488H and [D-Pen², D-Pen⁵]enkephalin in mice. *Peptides* 1997;18:797–800. [PubMed: 9285927]

1. .

Table 1
K_i values Inhibition of μ , δ , and κ Opioid Binding to CHO Membranes

Compound	Structure	K _i (nM) $\pm$ SE		Selectivity		
		[³ H]DAMGO (μ)	[³ H]Naltrindole (δ)	[³ H]U69,593 (κ)	K _i ^{δ} /K _i ^{μ}	K _i ^{δ} /K _i ^{κ}
MCL 101 ^a		0.23	5.9	0.079	26	75
Dmt-Tic ^b		894	1.6	37500	559 ^c	23438 ^d
MCL 450		0.69 $\pm$ 0.04	1.5 $\pm$ 0.03	0.28 $\pm$ 0.03	2.2	5.4
MCL 451		1100 $\pm$ 33	4.2 $\pm$ 0.58	62 $\pm$ 0.82%*	262 ^c	-

* Maximum inhibition of [³H]U69,593 binding in the presence of 10 μ M MCL 451.

^aData from Peng, X. et al. *J. Med. Chem.* 2006, 49, 256-262.

^bData from Pagé, D. et al. *J. Med. Chem.* 2001, 41, 2387-2390.

^cSelectivity K_i ^{μ} /K_i ^{δ} .

^dSelectivity K_i ^{κ} /K_i ^{δ} .

^e Selectivity $K_i/k/K_{ij}$

Table 2

Functional Activity at the μ , δ and κ Receptors^a

	³⁵ S[GTP- γ -S binding (Agonism)					
	μ		δ		κ	
	EC ₅₀ (nM)	E _{max} (%)	EC ₅₀ (nM)	E _{max} (%)	EC ₅₀ (nM)	E _{max} (%)
MCL 101 ^b	1.6±0.2	50±2.5	NT	NT	1.3±0.44	80±6.8
MCL 450	8.9±0.76	79±2.3	2.8±0.75	18±1.7	6.0±1.0	130±5.1
MCL 451	NT	NT	NT	NT	NA	0.95±2.2
	³⁵ S[GTP- γ -S binding (Agonism)					
	μ		δ		κ	
	IC ₅₀ (nM)	I _{max} (%)	IC ₅₀ (nM)	I _{max} (%)	IC ₅₀ (nM)	I _{max} (%)
MCL 101 ^b	20±3	50±3	NT	NT	NT	NT
MCL 450	97±21	47±1.7	3.9±0.34	84±5.2	No Inhibition	No Inhibition
MCL 451	NT	NT	10±1.5	93±1.0	NT	NT

^a Membranes from CHO that stably expressed only the μ , δ , or κ receptor were incubated with various concentrations of the compounds. The stimulation of [³⁵S]GTP- γ -S binding was measured as described in the Experimental Section. The E_{max} value is the maximal percent stimulation obtained with the compound. The EC₅₀ value is 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 antagonistic properties of a compound, membranes were incubated with 100 nM of the κ agonist U50,488 or 200 nM of the μ agonist DAMGO or 100 nM of the δ agonist SNC-80 in the presence of varying concentration 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. NT, Not Tested; NA, Not Active.

^b Data from Peng, X. et al. *J. Med. Chem.* **2006**, 49g, 256-262.