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

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Here, we report the 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 those 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 terms dual, binary, bivalent, dimeric, mixed, triple, or balanced are used in conjunction with numerous suffixes, such as 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.[1−3] Compared with drug combinations, there are some advantages associated with multiple ligands, such as the more predictable pharmacokinetic and pharmacodynamic relationship, which is a consequence of the administration of a single drug, as well as improved patient compliance. The molecular starting point for a multiple-ligand project is generated by 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 currently the predominant technique used for the generation of multiple ligands. The pharmacophores are joined together by a cleavable or noncleavable 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 the cleavage of the linker, at the same time of administration, cleavable conjugates are single molecules, which is one potential advantage that this approach has over drug cocktails.[2,3] 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 nonselective ligands. For example, a mixed μ agonist/δ antagonist pseudopeptide was obtained by linking tail to tail a selective δ antagonist (H-Tyr-TicΨ[CH_2-NH]Cha-Phe-OH) with a selective μ agonist (H-Dmt-D-Arg-Phe-Lys-NH_2).[4] Recently, Neumeyer et al. reported the synthesis and pharmacological evaluation of bivalent ligands containing homo and heterodimeric pharmacophores related to morphinans.[5] Ligands having two pharmacophores connected by a spacer have the potential for binding vicinal receptors.[6−8] 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.[9] 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.[10] 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^a 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 101[11] via EDC/DMAP[12] 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/CH_2Cl_2,[13] the product was condensed with Boc-Dmt-Tic-OH[14] via EDC/HOBt. Boc deprotection with TFA/CH_2Cl_2 gave

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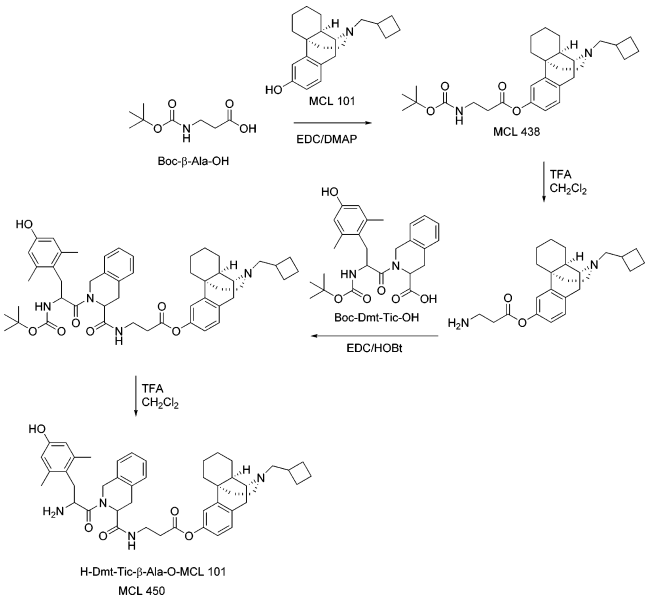
Scheme 1



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 the *C*-terminus methyl ester by NaOH and deprotected at the 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 opioid receptors. Data are summarized in Table 1. For comparison purposes, opioid binding affinity data for MCL 101and Dmt-Tic are included. The designed multiple ligand MCL 450 derives from the ester 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, whereas δ affinity increases 3.9-fold. A comparison of MCL 450 with MCL 451 shows that μ affinity increases about 1600-fold and δ affinity unexpectedly 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 the *C*-terminus.[15] Starting from a 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 [^{35}S]-GTPγS binding mediated by the μ, δ, and κ opioid receptors. Binding assays shown in Table 1 for MCL 450 confirm the pharmacological behaviors of its constituents MCL 101 (μ partial agonist/κ agonist) and MCL 451 (δ antagonist). The data show a decrease of EC_{50} for μ and κ receptors of 5.6 and 4.6-fold, respectively, in comparison to that of MCL 101; at the same time, the corresponding E_{max} (%) values increase 1.58 and 1.63-fold. However, MCL 450 is characterized by a δ antagonist activity contributed by MCL 451 and is slightly more potent (2.6-fold) than the δ selective counterpart MCL 451. As expected, MCL 451 results in a potent and selective δ antagonist, in line with all other δ antagonists containing the Dmt-Tic pharmacophore.[14,16−18]

Conclusion

We demonstrate the possibility of obtaining a multiple ligand in the opioid peptide field incorporating the Dmt-Tic pharmacophore. We selected a two methylene spacer because Schiller et al. reported the same spacer between a μ agonist and a δ antagonist (H-Dmt → D-Arg → Phe → Lys-NH-CH_2-CH_2-NH-Phe ← Cha[NH-CH_2]ΨTic ← Tyr-H).[5] We synthesized a similar compound incorporating a δ antagonist and a μ agonist (H-Dmt → Tic-NH-CH_2-CH_2-NH-Phe ← Phe ← Pro ← Tyr-H) and obtained similar pharmacological results (personal data). However, when the tripeptide H-Dmt-Tic-Glu-NH_2 containing the Dmt-Tic pharmacophore is linked to a fluorophore through a five methylene spacer (H-Dmt-Tic-Glu-NH-$(CH_2)_5$-NH-fluorophore), it maintains the selectivity and δ antagonist properties characteristic of this pharmacophore.[19,20] MCL 450 could represent a useful starting point in the synthesis of other designed multiple ligands from peptidic and morphinan opioid pharmacophores.

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]

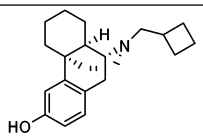
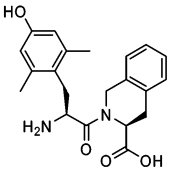
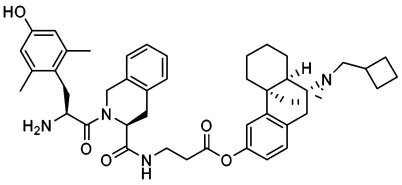
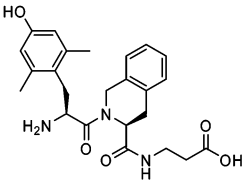
1-((−)*N*-Cyclobutylmethylmorphinan-3-yl)-*N*-Boc-β-Alanine. (Boc-β-Ala-O-MCL 101). (MCL 438). MCL-101 (0.16 g, 0.5 mmol) and Boc-β-Ala-OH (0.11 g, 0.6 mmol) were dissolved in anhydrous dichloromethane (10 mL) under nitrogen. A catalytic amount of 4-(dimethylamino)pyridine (0.006 g, 0.05 mmol) was added, 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/Et_3N, 100:1, v/v) to afford a colorless oil: yield 0.12 g (50%); *Rf*(B) 0.77; HPLC *K*′ 8.68; oil; $[\alpha]^{20}_D$ −22.6; MH^+ 484; ^{1}H NMR ($CDCl_3$) 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 CH_2Cl_2 (2 mL) for 2 h at room temperature. After solvent evaporation, Et_2O/Pe (1:5, v/v) was added to the solution until the product precipitated: yield 0.14 g (90%); *Rf*(A) 0.68; HPLC *K*′ 7.26; mp 121−123 °C; $[\alpha]^{20}_D$ −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 $NaHCO_3$ (5% in H_2O) and brine. The organic phase was dried (Na_2SO_4) and evaporated to dryness. The residue was precipitated from Et_2O/Pe (1:9, v/v): yield 0.07 g (83%); *Rf*(B) 0.75; HPLC *K*′ 9.52; mp 112−114 °C;

[a] Abbreviations: Boc, *tert*-butyloxycarbonyl; CHO, Chinese hamster ovary; DMAP, 4-(dimethylamino)pyridine; DAMGO, [D-Ala^2,N-Me-Phe^4,Gly ol^5]-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; Et_3N, triethylamine; Et_2O, 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. These abbreviations and symbols are used in addition to those by the IUPAC−IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1985**, *260*, 14−42).

Table 1. K_i Values of the Inhibition of μ, δ, and κ Opioid Binding to CHO Membranes

Compound	Structure	K_i (nM) ± SE [^{3}H]DAMGO (μ)	[^{3}H]Naltrindole (δ)	[^{3}H]U69,593 (κ)	Selectivity K_i^{δ}/K_i^{μ}	$K_i^{\delta}/K_i^{\kappa}$	K_i^{μ}/K_i^{κ}
MCL 101[a]		*0.23*	*5.9*	*0.079*	*26*	*75*	*2.9*
Dmt-Tic[b]		*894*	*1.6*	*37500*	*559*[c]	*23438*[d]	*42*[e]
MCL 450		0.69±0.04	1.5±0.03	0.28±0.03	2.2	5.4	2.5
MCL 451		1100±33	4.2±0.58	62±0.82%[f]	262[c]	-	-

[a] Data from Peng, X., et al. *J. Med. Chem.* **2006**, *49*, 256−262. [b] Data from Pagé, D., et al. *J. Med. Chem.* **2001**, *41*, 2387−2390. [c] Selectivity K_i^{μ}/K_i^{δ}. [d] Selectivity $K_i^{\kappa}/K_i^{\delta}$. [e] Selectivity K_i^{κ}/K_i^{μ}. [f] Maximum inhibition of [^{3}H]U69,593 binding in the presence of 10 μM MCL 451.

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	NA	0.95 ± 2.2	NT	NT

[^{35}S]GTP-γ-S Binding (Antagonism)

	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 [^{35}S]GTPγS binding was measured as described in the Experimental Section. The E_{max} value is the maximal percent stimulation obtained with the compound. The EC_{50} 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 EC_{50} value. To determine the antagonistic properties of a compound, the membranes were incubated with the 100 nM κ agonist U50,488 or 200 nM μ agonist DAMGO or 100 nM δ agonist SNC-80 in the presence of varying concentrations of the conpound. 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. NT, not tested; NA, not active. [b] Data from Peng, X. et al. *J. Med. Chem.* **2006**, *49*, 256−262.

$[\alpha]^{20}_D$ −15.6; MH^+ 834; ^{1}H 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 CH_2Cl_2 (2 mL) for 2 h at room temperature. After solvent evaporation, Et_2O/Pe (1:1, v/v) was added to the solution until the product precipitated: yield 0.07 g (92%); *Rf*(A) 0.65; HPLC *K*′ 7.21; mp 128−130 °C; $[\alpha]^{20}_D$ −17.9; MH^+ 734; ^{1}H 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, and the residue was dissolved in EtOAc and washed with citric acid (10% in H_2O), $NaHCO_3$ (5% in H_2O), and brine. The organic phase was dried (Na_2SO_4) and evaporated to dryness. The residue was precipitated from Et_2O/Pe (1:9, v/v): yield 0.15 g (88%); *Rf*(B) 0.62; HPLC *K*′ 8.21; mp 124−126 °C; $[\alpha]^{20}_D$ +7.2; MH^+ 555; ^{1}H 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 1 N 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 H_2O) and brine. The organic phase was dried (Na_2SO_4) and evaporated to dryness.

The residue was precipitated from Et_2O/Pe (1:9, v/v): yield 0.12 g (82%); *Rf*(B) 0.58; HPLC *K′* 7.92; mp 138−140 °C; $[\alpha]^{20}_D$ +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, Et_2O/Pe (1:1, v/v) was added to the solution until the product precipitated: yield 0.11 g (92%); *Rf*(A) 0.46; HPLC *K′* 6.12; mp 145−147 °C; $[\alpha]^{20}_D$ +12.3; MH^+ 441; ^{1}H 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.

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