"Supporting Information"

Replacement of the N-terminal Tyrosine Residue in Opioid Peptides with 3-(2,6-Dimethyl-4-carbamoylphenyl)propanoic Acid (Dcp) Results in Novel Opioid Antagonists

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Experimental Section

General Methods. Molecular masses of the compounds were determined by electrospray mass spectrometry on a Hybrid Q-Tof mass spectrometer interfaced to a MassLynx 4.0 data system or on a Finnigan/MAT 95XL-T spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 400 spectrometer or a Bruker Model *Avance* 300 MHz or DPX-300 NMR spectrometer, and referenced with respect to the residual signals of the solvent. The following abbreviations were used in reporting spectra: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets. Compounds **5**, **8**, **9** and **10** were purified by flash chromatography on silica gel (40 μm; Baker). Peptides were purified on a Vydac 218-TP1022 column (22 x 250 mm) with a linear gradient of 20-80% MeOH in 0.1% TFA over 30 min at a flow rate of 12 mL/min (peptides **1** and **2**) or with a linear gradient of 20-65% MeOH in 0.1% TFA over 50 in at a flow rate of 12 mL/min (peptide **3**). Analytical reversed-phase HPLC was performed on a Vydac 218-TP54 column (5 x 250 mm) under isocratic conditions (35% MeOH in 0.1% TFA) at a flow rate of 1 mL/min. The same column was also used for the determination of the capacity factors K' under the same conditions. Precoated plates (silica gel 60 F_{254}, 250 μm; Merck, Darmstadt, Germany) were used for ascending TLC in the following solvent systems (all v/v): (I) *n*-BuOH/AcOH/H_2O (4:1:1), (II) *n*-BuOH/pyridine/AcOH/H_2O (15:10:3:12).

In Vitro Bioassays and Receptor Binding Assays. The GPI[16] and MVD[17] bioassays were carried out as reported in detail elsewhere.[18,19] K_e values for antagonists were determined from the ratio of IC_{50} values obtained with an agonist in the presence and absence of a fixed antagonist concentration.[20] μ antagonist K_e values of compounds were determined in the GPI assay against the μ agonist TAPP[21] using antagonist concentrations ranging from 10 to 500 nM. κ antagonist K_e values of compounds were

also measured in the GPI assay against the κ agonist U50,488, using antagonist concentrations ranging from 50 to 200 nM. δ antagonist K_e values of compounds were determined in the MVD assay against the δ agonist DPDPE using antagonist concentrations ranging from 200 to 600 nM.

Opioid receptor binding studies were performed as described in detail elsewhere.[18] Binding affinities for μ and δ receptors were determined by displacing, respectively, [^{3}H]DAMGO (Multiple Peptide Systems, San Diego, CA) and [^{3}H]DSLET (Multiple Peptide Systems) from rat brain membrane binding sites, and κ opioid receptor affinities were measured by displacement of [^{3}H]U69,593 (Amersham) from guinea pig brain membrane binding sites. Incubations were performed for 2 h at 0 °C with [^{3}H]DAMGO, [^{3}H]DSLET, and [^{3}H]U69,593 at respective concentrations of 0.72, 0.78, and 0.80 nM. IC_{50} values were determined from log-dose displacement curves, and K_i values were calculated from the obtained IC_{50} values by means of the equation of Cheng and Prusoff,[22] using values of 1.3, 2.6, and 2.9 nM for the dissociation constants of [^{3}H]DAMGO, [^{3}H]DSLET, and [^{3}H]U69,593, respectively.

References

(16) Paton, W.D.M. The Action of Morphine and Related Substances on Contraction and on Acetylcholine Output of Coaxially Stimulated Guinea Pig Ileum. *Br. J. Pharmacol.* **1957**, *12*, 119-127.

(17) Henderson, G.; Hughes, J.; Kosterlitz, H.W. A New Example of a Morphine Sensitive Neuroeffector Junction. *Br. J. Pharmacol.* **1972**, *46*, 764-766.

(18) Schiller, P.W.; Lipton, A.; Horrobin, D.F.; Bodanszky, M. Unsulfated C-terminal 7-Peptide of Cholecystokinin: a New Ligand of the Opiate Receptor. *Biochem. Biophys. Res. Commun.* **1978**, *85*, 1332-1338.

(19) DiMaio, J.; Nguyen, T.M.-D.; Lemieux, C.; Schiller, P.W. Synthesis and Pharmacological Characterization In Vitro of Cyclic Enkephalin Analogues: Effects of Conformational Constraints on Opiate Receptor Selectivity. *J. Med. Chem.* **1982**, *25*, 1432-1438.

(20) Kosterlitz, H.W.; Watt, A.J. Kinetic Parameters of Narcotic Agonists and Antagonists with Particular Reference to N-Allylnoroxymorphone (Naloxone). *Br. J. Pharmacol.* **1968**, *33*, 266-276.

(21) Schiller, P.W.; Nguyen, T.M.-D.; Chung, N.N.; Lemieux, C. Dermorphin Analogues Carrying an Increased Positive Net Charge in Their "Message Domain" Display Extremely High μ Opioid Receptor Selectivity. *J. Med. Chem.* **1989,** *32*, 698-703.

(22) Cheng, Y.C.; Prusoff, W.H. Relationship Between the Inhibition Constant (K_I) and the Concentration of Inhibitor which Causes 50 Percent Inhibition (I_{50}) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099-3102.