Supporting Information

Effect of Lysine at C-Terminus of the Dmt-Tic Opioid Pharmacophore

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Chemistry. General Methods. Crude peptides and pseudopeptides were purified by preparative reversed-phase HPLC [Waters Delta Prep 4000 system with Waters Prep LC 40 mm Assembly column C18 (30 cm x 4 cm, 15 μm particle)] and eluted at a flow rate of 25 mL/min with mobile phase solvent A (10% acetonitrile + 0.1% TFA in H_2O, v/v), and a linear gradient from 25 to 75% B (60%, acetonitrile + 0.1% TFA in H_2O, v/v) in 25 min. Analytical HPLC analyses were performed with a Beckman System Gold (Beckman ultrasphere ODS column, 250 mm x 4.6 mm, 5 μm particle). Analytical determinations and capacity factor (K') of the products used HPLC in solvents A and B programmed at flow rate of 1 mL/min with linear gradients from 0 to 100% B in 25 min. Analogues had less than 1% impurities at 220 and 254 nm.

TLC was performed on precoated plates of silica gel F254 (Merck, Darmstadt, Germany): (A) 1-butanol/AcOH/H_2O (3:1:1, v/v/v); (B) CH_2Cl_2/toluene/methanol (17:1:2). Ninhydrin (1% ethanol, Merck), fluorescamine (Hoffman-La Roche) and chlorine spray reagents. Melting points were determined on a Kofler apparatus and are uncorrected. Optical rotations were assessed at 10 mg/mL in methanol with a Perkin-Elmer 241 polarimeter in a 10 cm water-jacketed cell. Molecular weights of the compounds were determined by a MALDI-TOF analysis (Hewlett Packard G2025A LD-TOF system mass spectrometer) and α-cyano-4-hydroxycinnamic acid as a matrix. ^{1}H NMR (δ) spectra were measured, when not specified, in DMSO-d_6 solution using a Bruker AC-200 spectrometer, and peak positions are given in parts per million downfield from tetramethylsilane as internal standard. Elemental analysis is reported in Supporting Information.

Pharmacology. Competitive Binding Assays. Opioid receptor affinity were determined under equilibrium conditions [2.5 h at room temperature (23 °C)] in a competition assay using brain P_2 synaptosomal membranes prepared from Sprague-Dawley rats.[36, 37] Synaptosomes were

preincubated to remove endogenous opioid peptides and stored at –80 °C in buffered 20% glycerol.[36, 38] Each analogue was analyzed in duplicate assays using five to eight dosages and three to five independent repetitions with different synaptosomal preparations (*n* values are listed in Table 1 in parenthesis and results are mean ± SE). Unlabeled peptide (2 μM) was used to determine non-specific binding in the presence of 1.9 nM [^{3}H]deltorphin II (45.0 Ci/mmol, Perkin Elmer, Boston, MA; K_D = 1.4 nM) for δ-opioid receptors and 3.5 nM [^{3}H]DAMGO (50.0 Ci/mmol), Amersham Bioscience, Buckinghamshire, U. K.; K_D = 1.5 nM) for μ-opioid receptors. Glass fibre filters (Whatman GFC) were soaked in 0.1% polyethylenimine in order to enhance the signal-to-noise ratio of the bound radiolabeled-synaptosome complex, and the filters were washed thrice in ice-cold buffered BSA.[36] The affinity constants (K_i) were calculated according to Cheng and Prusoff.[39]

Biological Activity in Isolated Tissue Preparation. The myenteric plexus longitudinal muscle preparations (2-3 cm segments) from the small intestine of male Hartley strain guinea pigs (GPI) measured μ-opioid receptor agonism, and a single mouse vas deferens (MVD) was used to determine δ-opioid receptor agonism as described previously.[40] The isolated tissues were suspended in organ baths containing balanced salt solutions in a physiological buffer, pH 7.5. Agonists were tested for the inhibition of electrically evoked contraction and expressed as IC_{50} (nM) obtained from the dose-response curves. The IC_{50} values represent the mean ± SE of five or six separate assays. δ-antagonist potencies in the MVD assay were determined against the δ-agonist deltorphin-II; μ-antagonism in the GPI assay used the μ-agonist endomorphin-2 and both are expressed as pA_2 determined using the Schild Plot.[41]

Table 2. Elemental analysis of compounds **1**-**10**. [a]

Comp.	Formula	MH^+, *m/z*		C	H	N
1	$C_{44}H_{50}F_3N_5O_8$	721	Calc	63.37	6.04	8.40
			Found	63.21	5.97	8.23
2	$C_{38}H_{46}F_3N_5O_7$	629	Calc	61.53	6.25	9.44
			Found	61.82	6.41	9.12
3	$C_{38}H_{45}F_6N_5O_8$	587	Calc	56.08	5.57	8.61
			Found	56.34	5.71	8.73
4	$C_{43}H_{48}F_3N_5O_8$	707	Calc	62.99	5.90	8.54
			Found	62.86	5.72	8.40
5	$C_{37}H_{44}F_3N_5O_7$	615	Calc	61.06	6.09	9.62
			Found	60.90	6.02	9.45
6	$C_{37}H_{43}F_6N_5O_8$	573	Calc	55.57	5.42	8.76
			Found	55.86	5.58	8.44
7	$C_{45}H_{48}F_6N_6O_9$	704	Calc	58.06	5.20	9.03
			Found	57.90	5.13	8.86
8	$C_{45}H_{48}F_6N_6O_9$	704	Calc	58.06	5.20	9.03
			Found	58.35	5.36	8.71
9	$C_{39}H_{44}F_6N_6O_8$	612	Calc	55.84	5.29	10.02
			Found	56.10	5.43	10.14
10	$C_{39}H_{43}F_9N_6O_9$	570	Calc	51.43	4.76	9.23
			Found	51.30	4.58	9.09

[a] Only the analysis of the new compounds, detailed in the **Experimental Section**, are included.

References

(1) Abbreviations. In addition to the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1985**, *260*, 14-42), this paper uses the following additional symbols and abbreviations: Ac, acetyl; Bid, 1*H*-benzimidazole-2-yl; Boc, *tert*-butyloxycarbonyl; DAMGO, [D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]enkephalin; DEL C, deltorphin II (H-Tyr-D-Ala-Phe-

Asp-Val-Val-Gly-NH_2); DMF, *N*,*N*-dimethylformamide; DMSO-d_6, hexadeuteriodimethyl sulfoxide; Dmt, 2′,6′-dimethyl-L-tyrosine; GPI, guinea-pig ileum; HOBt, 1-hydroxybenzotriazole; HPLC, high performance liquid chromatography; MALDI-TOF, matrix assisted laser desorption ionization time-of-flight. MVD, mouse vas deferens; NMM, 4-methylmorpholine; pA_2, negative log of the molar concentration required to double the agonist concentration to achieve the original response; TFA, trifluoroacetic acid; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; TIP(P), H.-Tyr-Tic-Phe-(Phe)-OH.; TLC, thin-layer chromatography; WSC, 1-ethyl-3-[3′-dimethyl)aminopropyl]-carbodiimide hydrochloride; Z, benzyloxycarbonyl;

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