Supporting information

Design, Synthesis and Functional Analysis of Cyclic Opioid Peptides with Dmt-Tic Pharmacophore

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Figure S1. Analytical HPLC chromatogram of peptide Dmt-Tic-c[D-Lys-Phe-Phe-Asp]NH₂ (1).
Figure S2. Analytical HPLC chromatogram of peptide Dmt-Tic-c[D-Lys-Phe-D-2NaL-Asp]NH₂ (2).

Figure S3. Analytical HPLC chromatogram of peptide Dmt-Tic-c[D-Lys-Phe-2,4F₂-Phe-Asp]NH₂ (3).

Figure S4. Analytical HPLC chromatogram of peptide Dmt-Tic-c[D-Dap-Phe-Phe-Asp]NH₂ (4).
Figure S5. Analytical HPLC chromatogram of peptide Dmt-Tic-c[D-Lys-Phe-Asp]NH₂ (5).

Figure S6. Analytical HPLC chromatogram of peptide Dmt-Tic-c[D-Lys-Phe-Asp]-Tic-Dmt-NH₂ (6).

Table S1. Physicochemical data of analogs 1-6.

| No. | Sequence | Formula   | m/z [M + H]⁺⁺ | HPLC tᵣ [min] |
|-----|----------|-----------|---------------|--------------|
|     |          |           | Calcd | Obsd         |              |
| 1   | Dmt-Tic-c[D-Lys-Phe-Asp]NH₂ | C₄₀H₅₉N₃₀O₈ | 887.4450 | 887.4418 | 17.04 |
| 2   | Dmt-Tic-c[D-Lys-Phe-D-2Nal-Asp]NH₂ | C₅₃H₆₀N₄₀O₂ | 937.4606 | 937.4567 | 18.16 |
| 3   | Dmt-Tic-c[D-Lys-Phe-2,4F₂-Phe-Asp]NH₂ | C₄₉H₅₆F₂N₈O₈ | 923.4262 | 923.4244 | 17.21 |
| 4   | Dmt-Tic-c[D-Dap-Phe-Phe-Asp]NH₂ | C₄₆H₅₂N₃₀O₂ | 845.3981 | 845.3997 | 17.55 |
| 5   | Dmt-Tic-c[D-Lys-Phe-Asp]NH₂ | C₄₀H₅₀N₀O₂ | 740.3766 | 740.3760 | 16.87 |
Dmt-Tic-[D-Lys-Phe-Asp]-Tic-Dmt-NH₂  C₆₅H₇₁N₉O₁₀  1090.5396  1090.5345  17.86

ₐ Observed by ESI MS⁺ ionization.

ₜ Retention time on a Vydac C₁₈ column (4.6 x 250 mm, 5 μm,) using the solvent system of 0.1% TFA in water (A) and 80% acetonitrile in water containing 0.1% TFA (B) and a linear gradient of 0–100% solvent B over 50 min, with a flow rate of 1 mL/min.

High resolution mass spectra were recorded using Shimadzu IT-TOF (ion trap – time-of-flight) mass spectrometer equipped with standard ESI source (Shimadzu, Japan). For CID (collision-induced dissociation) experiments, the singly protonated precursor ions [M+H]⁺ were selected. The collision energy was adjusted to obtain the optimal fragmentation pattern. Argon was used as a collision gas. The obtained fragments were registered as an MS/MS (tandem mass spectrometry) spectrum.
**Figure S7.** Top panel: High resolution MS spectrum of peptide Dmt-Tic-c[D-Lys-Phe-Phe-Asp]NH₂ (1). Bottom panel: High resolution MS/MS spectrum for the [M+H]+ ion. In inset, the fragmentation scheme corresponding to MS/MS spectrum is proposed.
Figure S8. Top panel: High resolution MS spectrum of peptide Dmt-Tic-c[D-Lys-Phe-D-2Nal-Asp]NH₂ (2). Bottom panel: High resolution MS/MS spectrum for the [M+H]+ ion. In inset, the fragmentation scheme corresponding to MS/MS spectrum is proposed.
Figure S9. Top panel: High resolution MS spectrum of peptide Dmt-Tic-[D-Lys-Phe-2,4F₂Phe-Asp]NH₂ (3). Bottom panel: High resolution MS/MS spectrum for the [M+H]+ ion. In inset, the fragmentation scheme corresponding to MS/MS spectrum is proposed.
Figure S10. Top panel: High resolution MS spectrum of peptide Dmt-Tic-c[D-Dap-Phe-Phe-Asp]NH2Dmt-(4). Bottom panel: High resolution MS/MS spectrum for the [M+H]+ ion. In inset, the fragmentation scheme corresponding to MS/MS spectrum is proposed.
**Figure S11.** Top panel: High resolution MS spectrum of peptide Dmt-Tic-c[D-Lys-Phe-Asp]NH₂ (5). Bottom panel: High resolution MS/MS spectrum for the [M+H]+ ion. In inset, the fragmentation scheme corresponding to MS/MS spectrum is proposed.
Figure S12. Top panel: High resolution MS spectrum of peptide Dmt-Tic-c[D-Lys-Phe-Asp]-Tic-Dmt-NH₂ (6). Bottom panel: High resolution MS/MS spectrum for the [M+H]+ ion. In inset, the fragmentation scheme corresponding to MS/MS spectrum is proposed.