

Supplementary Information

Cytotoxic potency of small macrocyclic knot proteins: Structure-activity and mechanistic studies of native and chemically modified cyclotides

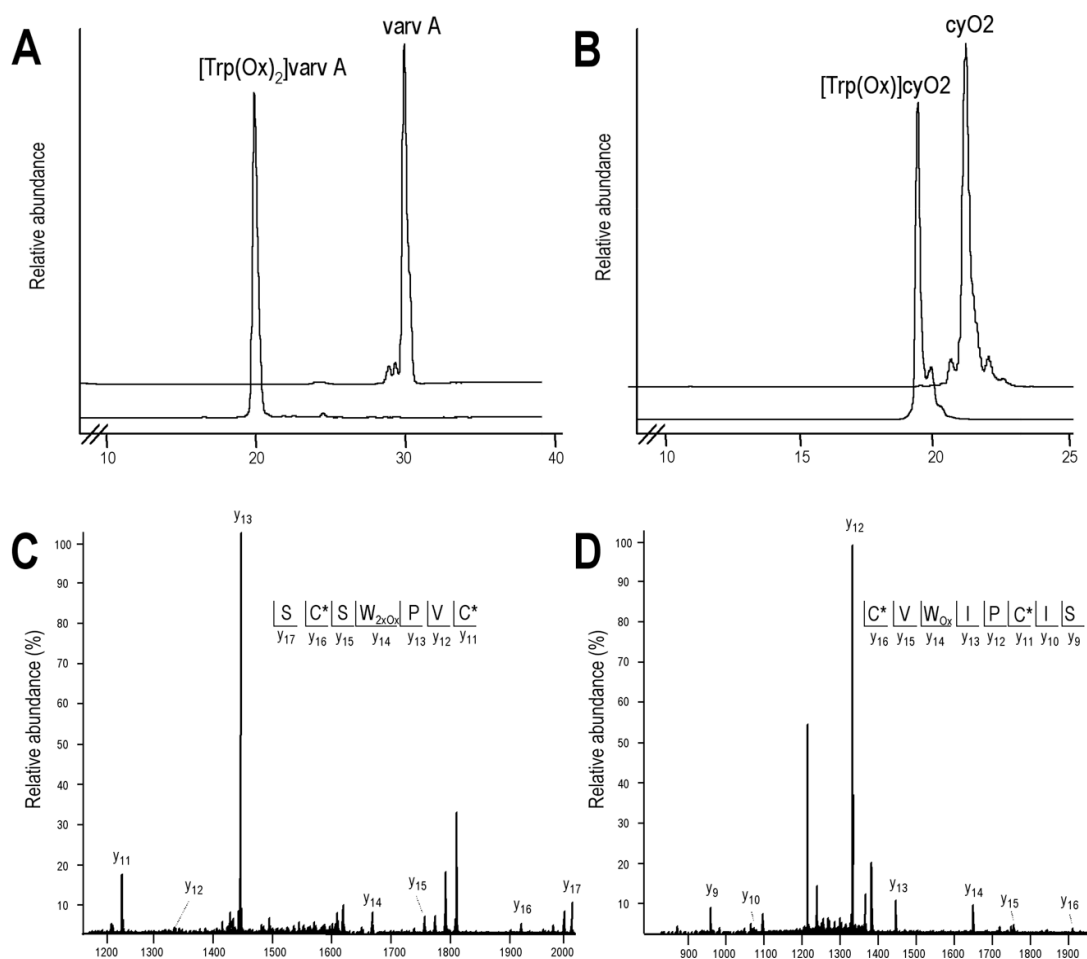
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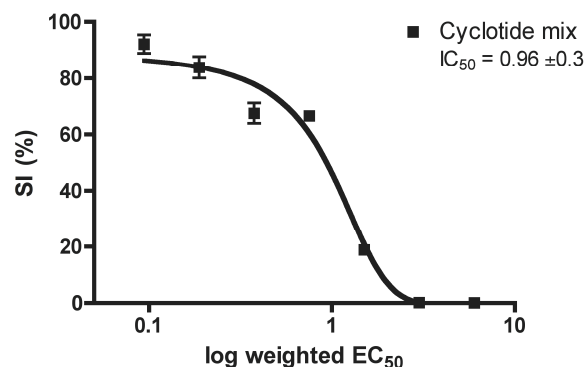
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Suppl. Fig. 1 Difference in retention time upon double oxidation of varv A (A) and single oxidation of cycloviolacin O2 (B) oxidation. The retention time of the derivative with a singly oxidized Trp is slightly lower than that of the native peptide, whereas the difference is much greater for the doubly hydroxylated product. The MS-MS spectra of [Trp(OH)₂]varvA (C) and [Trp(OH)]cyO2 (D) show that oxidation occurred specifically at the Trp residue. MS-MS was done on a single linear endoproteinase GluC fragment of varv A (TCVGGTCNTPGCSCSWPVCTRNGLPVCGE), and a tryptic fragment of cyO2 (NGIPCGESCWIPCISSAIGCSCK). Y-ion fragment series were predominant in the spectra. C* stands for S-carbamidomethylated Cys residues.



Suppl. Fig. 2 Mixture of three different cyclotides shows additive cytotoxic effects. Stock solutions of cycloviolacin O2, kalata B1 and B2 were made at concentrations equal to 60 times their IC₅₀ values and mixed together in a 1:1:1 ratio. A dilution series of that mixture was then prepared and tested for cytotoxicity at a 1:1 ratio. Because test substances are diluted 10 times in the assay, the highest concentration tested were 6 times the IC₅₀, followed by 3, 1.5, 0.75, 0.375, 0.188, 0.094 times the IC₅₀.

For convenience, the response was measured in relative terms towards the IC₅₀ of the mixture. That means that an additive effect would give a relative IC₅₀ of 1, whereas subadditive or superadditive effects would have relative IC₅₀:s of >1 and <1, respectively. The result was 0.96 ± 0.3, which unambiguously demonstrates that the more complicated cyclotide mixture containing three cyclotides has additive effects.