Inhibition of Human Prolyl Oligopeptidase Activity by the Cyclotide Psysol 2 Isolated from *Psychotria solitudinum*

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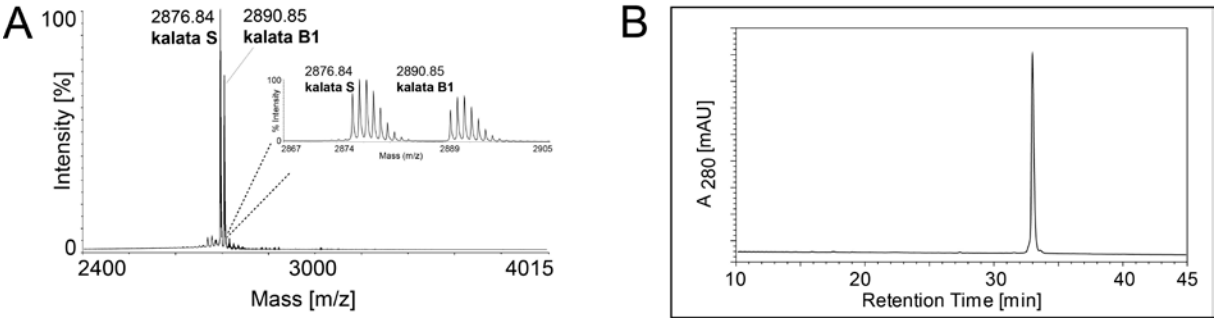
Supporting Information

Supplementary Table S1. Concentration-dependent POP inhibitory activity of *Psychotria solitudinum, P. capitata, P. poeppigiana* and *Viola tricolor* HPLC fractions

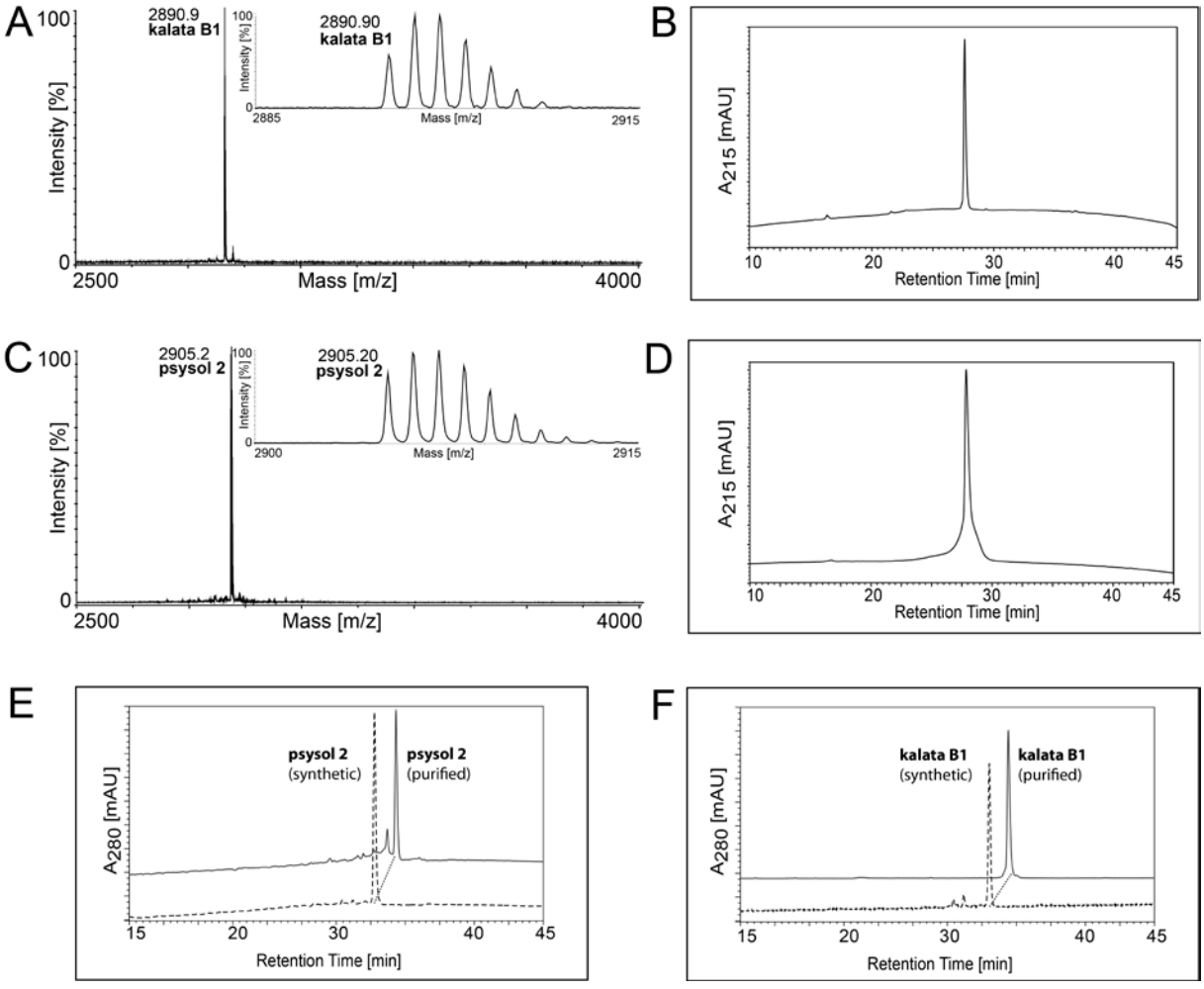
plant fraction	concentration [µg/mL]	POP inhibition [%][a]	plant fraction	concentration [µg/mL]	POP inhibition [%][a]
Psychotria solitudinum-F1	400	59	*Psychotria capitata*-F1	400	67 ± 4
	200	40		200	46 ± 2
	100	27		100	33 ± 1
Psysol-F2	400	64	Psycap-F2	400	50 ± 3
	200	40		200	37 ± 1
	100	30		100	17 ± 3
Psysol-F3	400	78	Psycap-F3	400	55 ± 5
	200	61		200	38 ± 3
	100	48		100	29 ± 4
Psychotria poeppigiana-F1	400	61	*Viola tricolor*-F1	400	75 ± 2
	200	32 ± 2		200	51 ± 7
	100	24 ± 1		100	36 ± 7
Psypoe-F2	400	51 ± 5	Vitri-F2	400	86 ± 1
	200	32 ± 6		200	72 ± 1
	100	21 ± 5		100	51 ± 0
Psypoe-F3	400	49 ± 1	Vitri-F3	400	76 ± 3
	200	29 ± 3		200	54 ± 4
	100	13 ± 5		100	37 ± 2
Psypoe-F4	400	83	Vitri-F4	400	55
	200	63		200	43
	100	47		100	26

[a]Values are presented as mean (of three replicates) or mean ± STDEV (n=2)

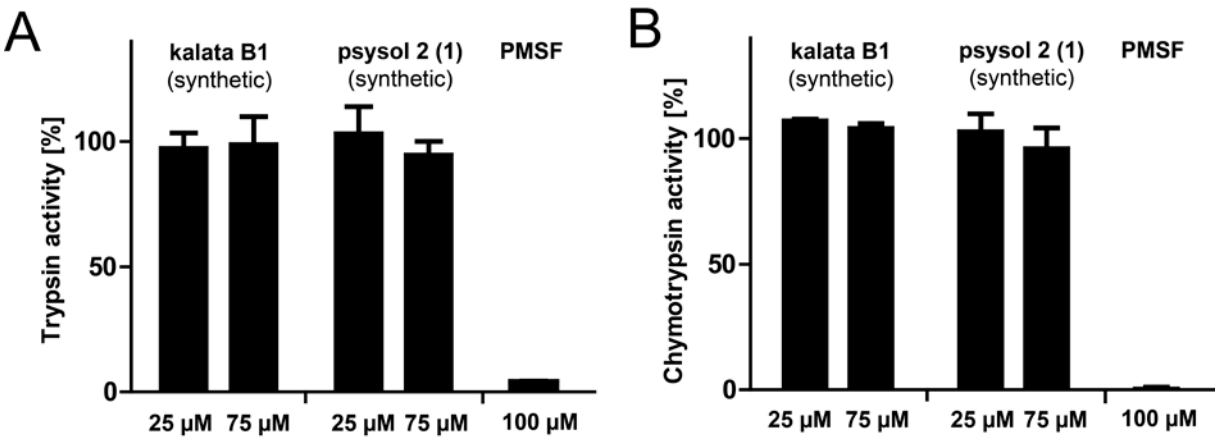
Supplementary Figure S1. Analysis of purified fraction Vitri-F3 from *Viola tricolor*. A subfraction of Vitri-F3 yielded a mixture of two main cyclotides, namely kalata B1 (2890.85 *m/z*) and kalata S (2876.84 *m/z*) as indicated by mass traces of a MALDI-TOF-MS spectrum (A). The two cyclotides were found to co-elute as indicated in the A_{280} RP-HPLC-UV spectrum (B).



Supplementary Figure S2. Analytical characterization of synthesized psysol 2 and kalata B1. The quality of the synthetic cyclotides was characterized by MALDI-TOF MS and RP-HPLC. The molecular weight (monoisotopic, $[M+H]^+$) of synthesized cyclotides kalata B1 (A) and psysol 2 (C) was confirmed to be *m/z* 2890.9 and *m/z* 2905.2, respectively. Purity was >95% as determined by HPLC-UV for both kalata B1 (B) and psysol 2 (D). Furthermore, co-elution experiments indicated that both synthetic and plant-purified psysol 2 (E) as well as synthetic and purified kalata B1 (F) have identical retention times in RP-HPLC.

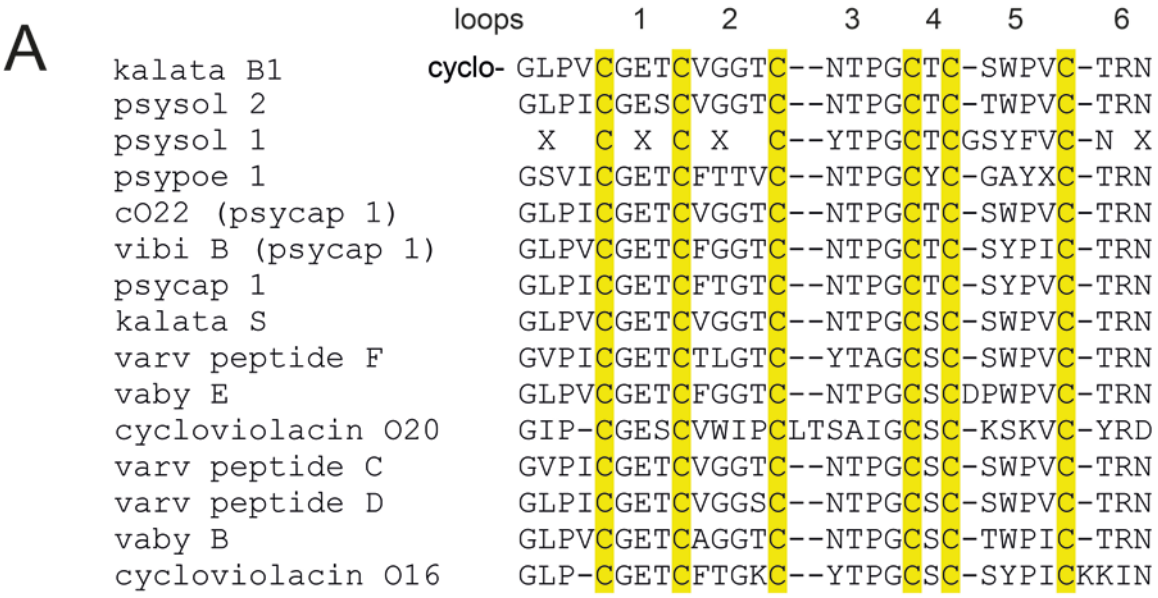


Supplementary Figure S3. Inhibitor specificity as determined by trypsin and chymotrypsin inhibition assays. To elucidate the specificity of the human POP inhibitory activity of cyclotides, putative inhibition of two common serine proteases, namely trypsin and chymotrypsin, was evaluated using psysol 2 and kalata B1. Protease activity of trypsin was not inhibited by the cyclotides kalata B1 or psysol 2 as shown in (A) at the tested concentrations of 25 µM and 75 µM, respectively. In contrast, the serine protease inhibitor PMSF (positive control) was able to inhibit trypsin activity at 100 µM (A). Similarly, kalata B1 and psysol 2 had no effect on the activity of chymotrypsin at concentrations of 25 µM and 75 µM, whereas PMSF inhibitor (100 µM) completely blocked chymotrypsin activity (B).



Supplementary Figure S4. Sequence alignment of cyclotides identified in *Psychotria* species and *Viola tricolor*. A cyclotide sequence alignment of identified cyclotides present in *Psychotria solitudinum, P. capitata, P. poeppigiana* and *Viola tricolor* is illustrated (A). The cyclotide loop number (1 to 6) is indicated above the alignment and the number of the conserved cysteine residues is shown by Roman characters below the alignment; X represents one or multiple unknown amino acids within the partial sequence of psysol 1. A sequence logo based on the sequences shown in A is presented in (B). The size of each individual amino acid (shown in one-letter code) is representative for its relative frequency in the sequence alignment. The conserved cysteine residues are indicated in yellow, and proline residues are highlighted in blue and are labelled with an asterisk (B).

A



B