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INTRINSIC ACTIVITY

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MEETING ABSTRACT

A6.1

Cyclotides as novel inhibitors of human prolyl oligopeptidase

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Background: Cyclotides are plant-derived mini-proteins. Cyclotides have been discovered in various flowering plant families in particular plants of the violet (Violaceae) and coffee (Rubiaceae) family [1]. Their characteristic circular cystine-knot motif confers them structural stability. They are expressed as natural peptide libraries essentially with great molecular diversity and hence these peptides are interesting starting points for drug discovery. For instance, native circular cystine-knot peptides are potent and selective inhibitors of serine-type proteases.

Methods: Here we present the discovery of the first cyclotide from the tropical plant *Psychotria solitudinum* as a specific inhibitor of the human prolyl oligopeptidase (POP) using a bioassay-guided fractionation approach combined with target-based pharmacology. Additionally, we biochemically and pharmacologically characterize the inhibition of other proline-specific endo- and exopeptidases for the reported and novel identified cyclotide inhibitors.

Results: Plant extracts of four species of the *Psychotria* and one *Viola* species were characterized for inhibition of human POP *in vitro* at concentrations of 100–400 µg/ml. The most promising *P. solitudinum* extract submitted to a pharmacology-guided isolation resulted in the novel cyclotide psysol 2 (IC_{50}: ~ 25 µM) as the most abundant compound in this plant peptide library. The molecular structure and amino acid sequence of psysol 2 was characterized by manual *de novo* sequencing using tandem mass spectrometry. The specificity for POP inhibition was determined by comparison of the inhibitory activity towards other serine proteases, namely trypsin and chymotrypsin, which both appeared unaffected by psysol 2 up to 100 µM. Preliminary structure–activity studies suggested that proline residues might by important for the observed POP inhibition since kalata B1, a cyclotide with high sequence homology to psysol 2, also inhibited POP activity with an IC_{50} of 5.6 µM [2].

Discussion: The enzyme POP is well known for its role in memory and learning processes, and it is currently being considered as a promising therapeutic target for cognitive deficits and neurodegenerative diseases, such as schizophrenia and Parkinson's disease. In the context of discovery and development of future POP inhibitors for therapeutic applications, cyclotides may be suitable candidates considering that small-molecule POP inhibitors fail to provide enough selectivity for the enzyme class of post-prolyl-cleaving endopeptidases.

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