Discovery of six novel Kunitz-type peptides with differential Kv1.3 interacting and anti-Parkinsonism activities from the transcriptomes of two coral species

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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease. Potassium voltage-gated channel subfamily A member 3 (Kv 1.3) is a potential target for treatments of PD. To discover new novel blockers of Kv1.3, the transcriptomic data of two coral species (*Galaxea fascicularis* and *Favites acuticollis*) were analyzed. Thirty-three Kunitz-type peptides were chosen and annotated by Swiss-Prot and Pfam. After comparison, 13 peptides were selected since they showed characteristics of potassium ion channel blockers. The structures of the peptides were modeled and subjected to molecular dynamics (MD) simulation to verify their stability. Based partly on database annotations, the six peptides with the most significant structural stability were subjected to multiple sequence alignment and phylogenetic analysis. Molecular docking indicated that GfKuz1 (peptide from *G. fascicularis*) showed the highest potency to block Kv1.3 among the reference peptides. The MD simulation of the peptide-protein complexes showed that GfKuz1 interacted with Kv1.3, and was more compact and stable than the other peptides. The blocking effect was confirmed by potassium ion bioassay. Furthermore, GfKuz1 showed no toxicity to PC-12 cells or zebrafish at concentrations up to 100 μM. In addition, GfKuz1 increased the cell viability reduced by 6-hydroxydopamine hydrochloride (6-OHDA), and also down-regulated the level of reactive oxygen species and activated the Nrf2 pathway. In summary, GfKuz1 reversed PD symptoms and is a potential peptide drug prototype for PD treatment.

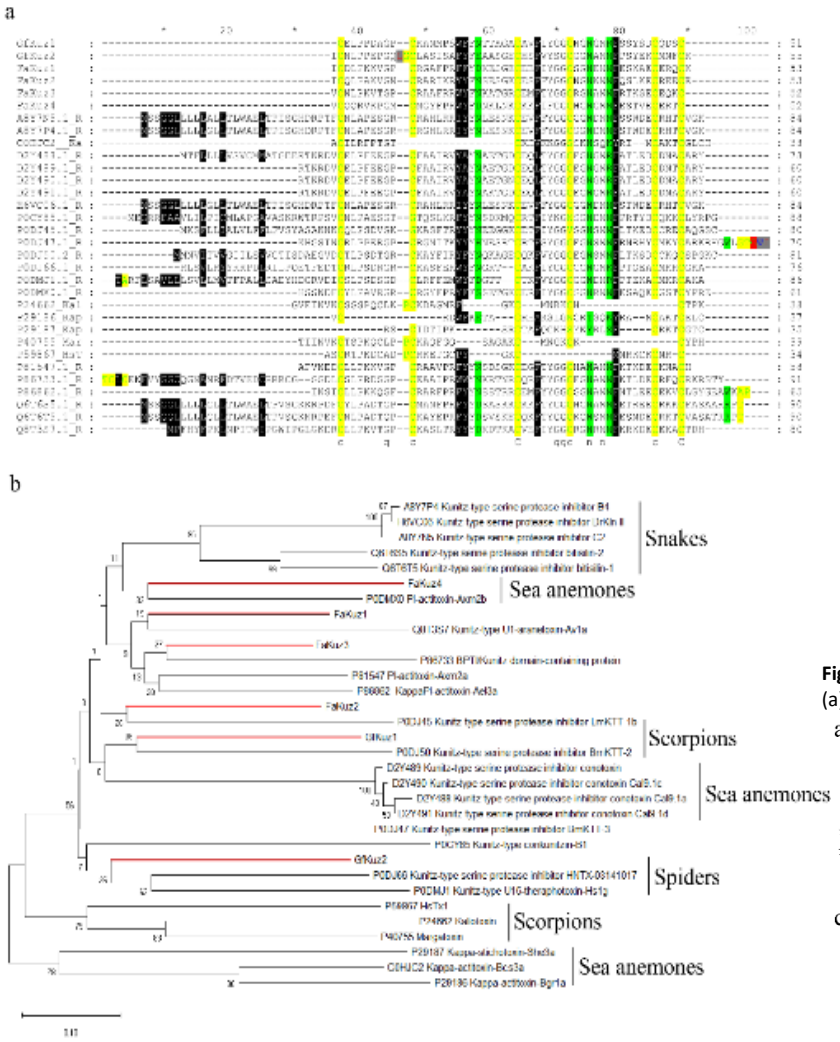

Fig. 1 (a) Multiple sequence alignment and (b) neighbor-joining tree for selectzed Kunitz-like peptides from *G. fascicularis* and *F. acuticollis.*

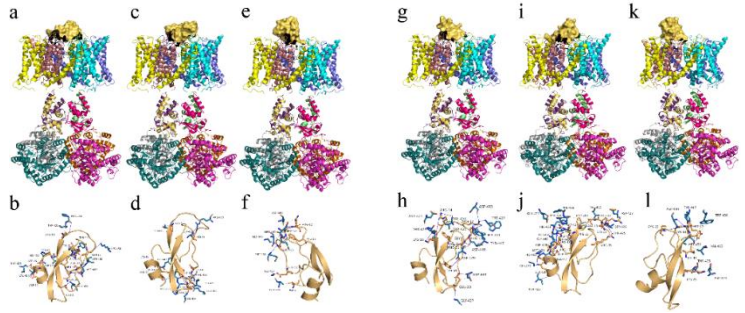

Fig. 2 Molecular docking conformation between Kv1.3 and Kunitz-like peptides from *G. fascicularis* and *F. acuticollis*.

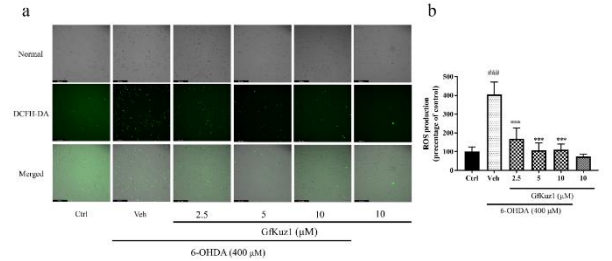

Fig. 3 Reduction of ROS production in PC-12 cells in response to GfKuz1 exposure.
(a) fluorescence images of ROS production on PC-12 cells. (b) ROS generation fluorescence ratio.

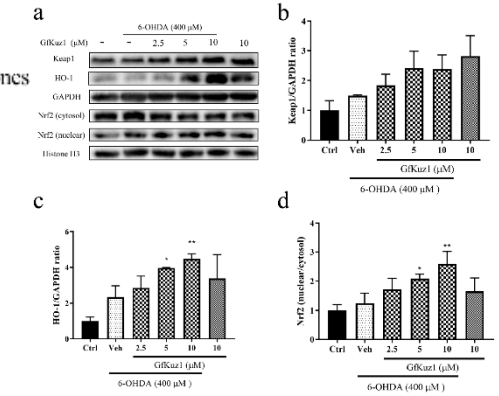

Fig. 4 Effect of GfKuz1 on the Nrf2 nuclear translocation and Nrf2/HO-1 signaling pathway. (a) Western blotting analysis. (b, c, d) Quantitative analysis.