Analogous peptides acting on different pathways: a comparative study of Bicarinalin and U_9-MYRTX-Tb1a from an ant venom

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Introduction

For decades, venoms have been emerging as a new source of therapeutic leads, bioinsecticides and pharmacological tools. To date, six venom-derived drugs and one venom-derived insecticide were approved and are now available on the market[1]. Ant venoms exhibit a complex molecular diversity and harbor a great potential for the discovery of new bioactive molecules, although they remain understudied in comparison with other animal venoms. Also, recent studies revealed a broad array of biological effects such as antimicrobial, anti-inflammatory, ion channel modulation[2,3]. Herein, we focused on M-MYRTX-Tb1a (i.e. Bicarinalin) and U_9-MYRTX-Tb1a, two venom peptides characterized from the venom of the ant *Tetramorium bicarinatum*[4], displaying low amino acid sequence identity but sharing similar physicochemical properties.

Previous functional investigations revealed that Bicarinalin is a an amphipathic α-helical peptide able to form pores in bacterial membranes[5]. In order to assess the potential of ant venoms for the discovery of new bioactive peptides, we investigated the biological activity of Bicarinalin and U_9 on a *D. melanogaster* cell line. Considering the physicochemical properties of both toxins, we hypothesized that they display similar biological effects and mode of action, even though their predicted three-dimensional structure were different. Our preliminary results confirmed that Bicarinalin is a membrane-active peptide and suggest that U_9 display a cytotoxic effect through a different pathway.

Materials and Methods

LC-MS analysis - The venom composition was analyzed by LC-MS (LCQ-Ion trap Advantage equipped with an ESI-LC system). Peptides were separated using a Luna-C_{18} column and eluted using a mobile phase composed of 0.1% aqueous formic acid and 0.1% formic acid in acetonitrile.

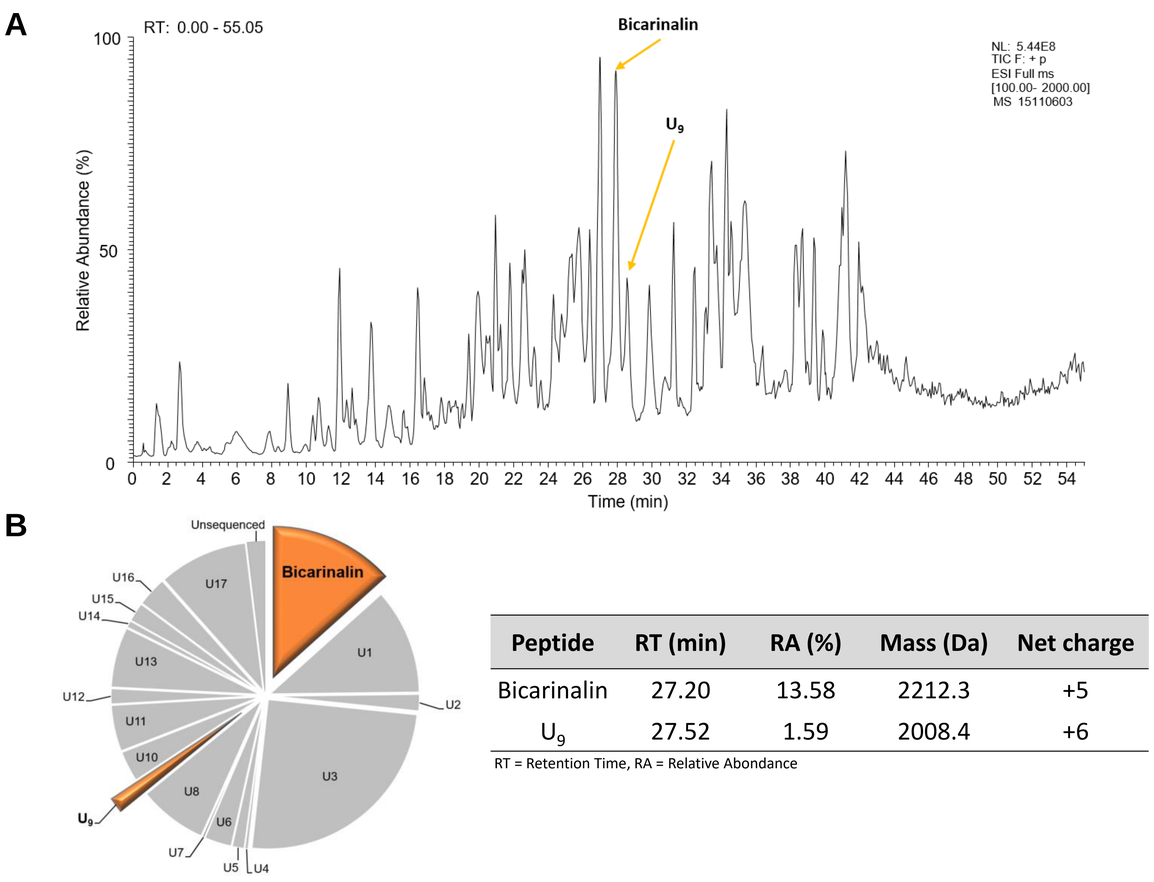
Cell culture - S2 cell line of *D. melanogaster* (ThermoFisher) was maintained in Schneider's insect medium (+ 10% Fetal Bovine Serum, + 1% Penicillin/Streptomycin) at 25°C without CO_2.

Peptide synthesis - Peptides were chemically synthetized by GenScript® with C-terminal amidation.

Cytotoxicity assays - Cells were counted by using trypan blue dye exclusion. They were then seeded in 96-well plates at a density of 2.10^5 and 6.10^5 cells/mL and incubated at 25°C for 24h before being treated with Bicarinalin or U_9 for 24h. In order to assess their effect on cell viability, the absorbance was measured after performing of CCK-8 (450nm) and LDH (490 nm) assays at 2.10^5 and 6.10^5 cells/mL densities, respectively.

Image kinetic of peptide effect - Cells were treated with 50 µM of peptides and photos were acquired every second for 5 min (Bicarinalin) and every 5 seconds for 60 min (U_9). Absorbance measurements and photos were acquired with the Biotek Cytation 1 (Biotek®).

Molecular features of Bicarinalin and U_9 from *T. bicarinatum* venom



Peptide	RT (min)	RA (%)	Mass (Da)	Net charge
Bicarinalin	27.20	13.58	2212.3	+5
U_9	27.52	1.59	2008.4	+6

RT = Retention Time, RA = Relative Abondance

FIGURE 1. Peptide composition of *Tetramorium bicarinatum* venom
A) Total ion chromatogram of crude *T. bicarinatum* venom**.** Bicarinalin and U_9 elute with quite similar retention time indicating close physicochemical properties. **B)** Relative abundance of each group of peptides in the venom. Bicarinalin is seven times as present as U_9 in *T. bicarinatum* venom and possess close molecular weight and positive net charge.

Conserved prepro-peptide and similar amphiphilic properties

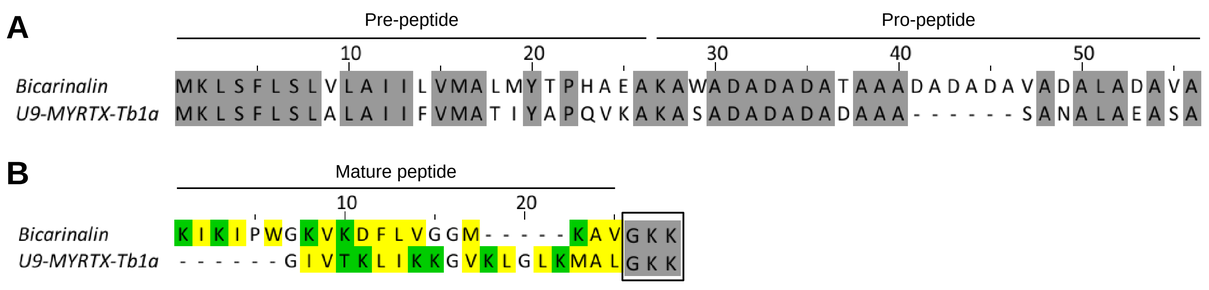


FIGURE 2. Alignment of prepro and mature peptides of Bicarinalin and U_9
A) Alignment of prepro-peptide sequences showing strictly conserved residues (grey). **B)** Alignment of mature peptide sequences with the hydrophobic residues (in yellow) and the hydrophilic residues (in green). The boxed region indicates the amidation signal.

Presence of a turn in predicted U_9 structure

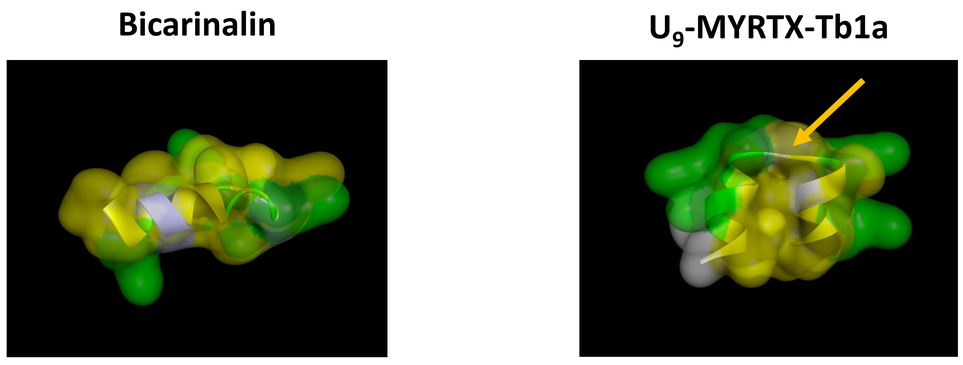


FIGURE 3. Structural analysis of Bicarinalin and U_9
The prediction of three-dimensional structure using *de novo* PepFold3 system. Secondary structures dominated by α-helices and amphipathic surfaces (hydrophilic surface in green and hydrophobic surface in yellow) were revealed. U_9 is predicted to have two α-helices separated by a turn (indicated by the arrow) while Bicarinalin contains one α-helix.

Cytotoxicity of peptides on S2 drosophila cells

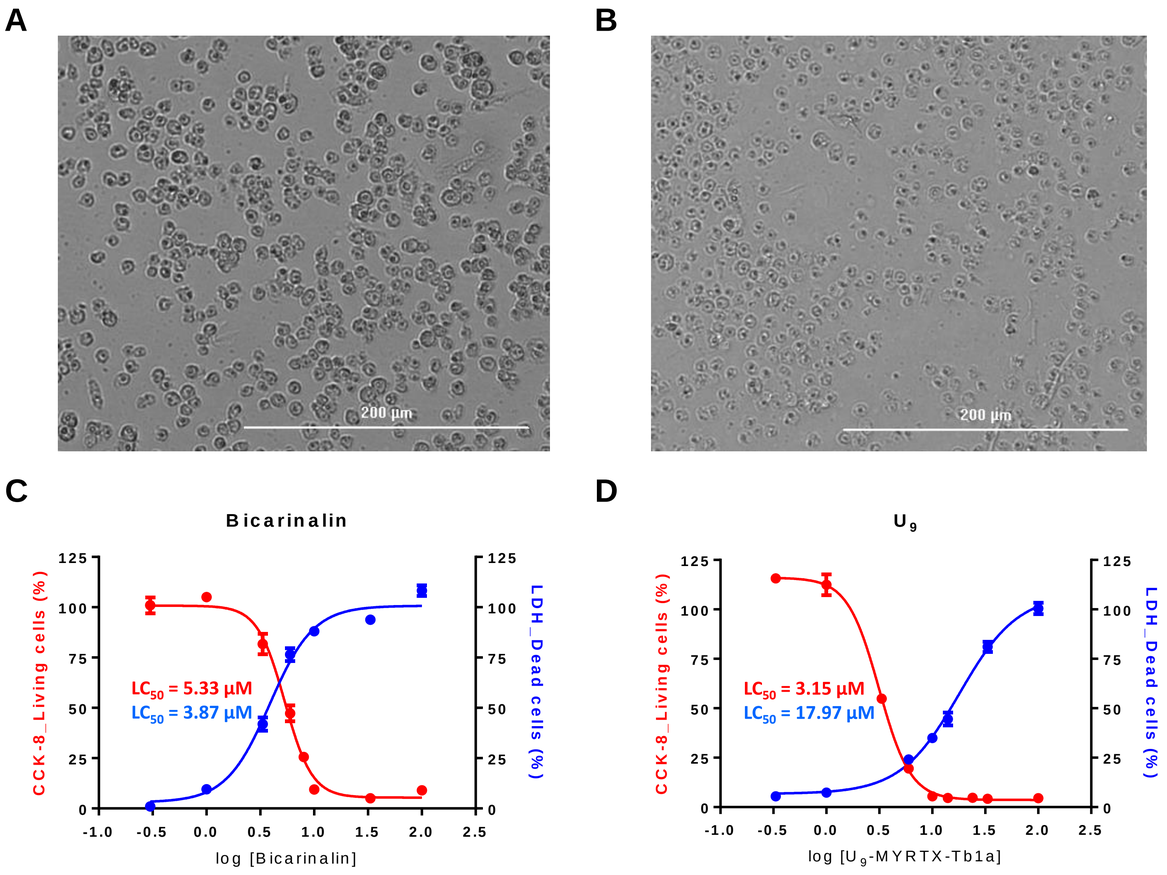


FIGURE 4. Effects of Bicarinalin and U_9 on the S2 cells
A-B) S2 cells after incubation with 100 µM of **(A)** Bicarinalin or **(B)** U_9 during 24 h. **C-D)** Concentration-response curves of **(C)** Bicarinalin (n= 3-4) and **D)** U_9 (n= 2-8)**.** For U_9, there is an offset around 1 log of LC_{50} between CCK-8 and LDH assays. This may indicate a cytotoxic activity at low concentration besides a cellular lysis being undetectable by LDH assays. The data were normalized by control and represented with ± SEM.

Different mode of action between U_9 and Bicarinalin

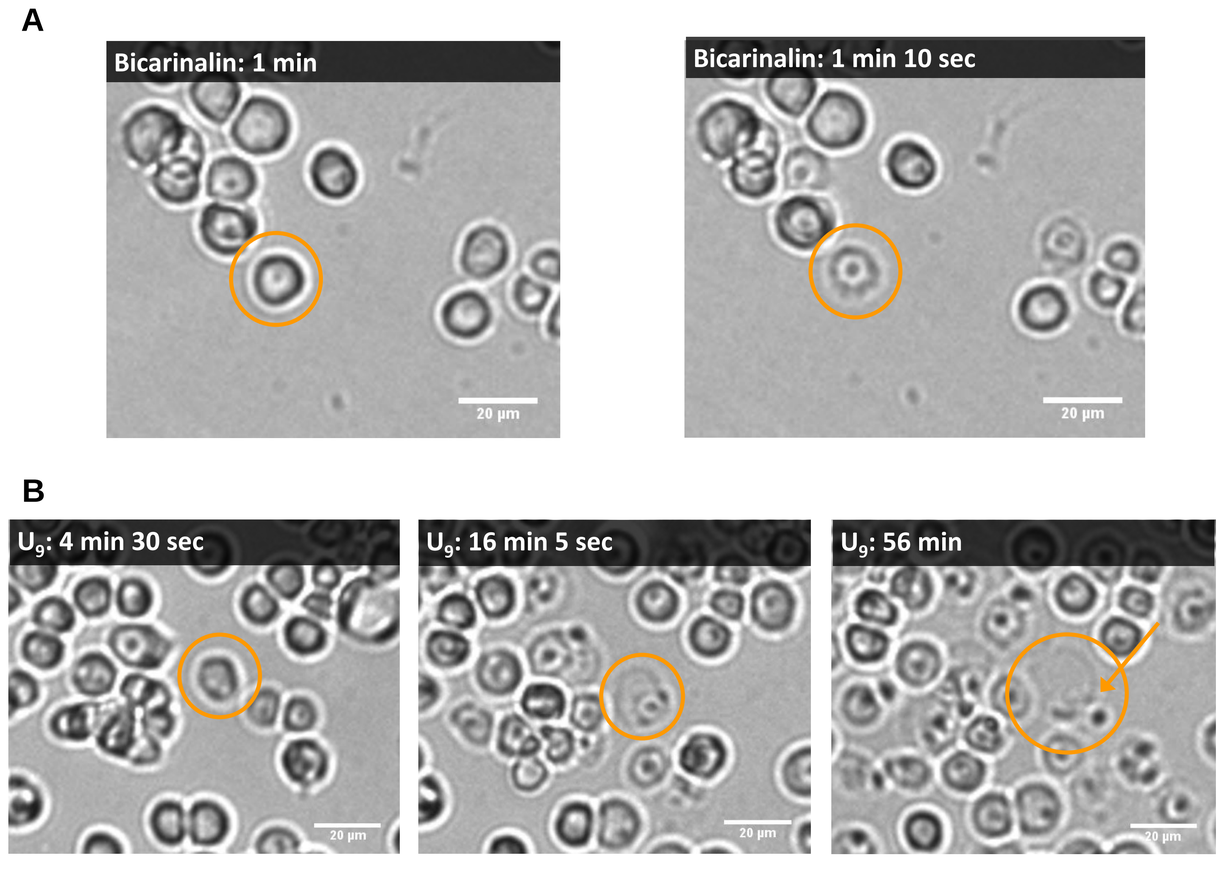


FIGURE 5. Morphological analysis of S2 cells after incubation with Bicarinalin and U_9
A-B) Effect of **(A)** Bicarinalin and **(B)** U_9 at 50 µM for different incubation times, showing fast and immediate lytic effect on treated cells for Bicarinalin. In contrast, a low and progressive cytotoxic effect on treated cells was observed for U_9, which is characterized by an increase of cell volume, a condensation and ejection of nucleus (arrowed). This observation could match to several pathways such as autophagy or apoptosis.

Conclusion

- Conserved prepropeptide sequences, similar physicochemical features of mature regions, and predicted 3D structures dominated by α-helices with the presence of a turn for U_9 were observed.
- Two distinct LC_{50} were observed for U_9, suggesting of cytotoxic effect at low concentration without membrane disruption.
- U_9 induced an increase in S2 cell volume, a condensation of cell nucleus followed by its expulsion from the cell.
- Several pathways including autophagy and apoptosis will be investigated to further explain this unusual observation.

References

[1] Herzig V, et al. Animal toxins — Nature's evolutionary-refined toolkit for basic research and drug discovery. *Biochem Pharmacol*. June 2020:114096.

[2] Touchard A, et al. The biochemical toxin arsenal from ant venoms. *Toxins (Basel)*. 2016;8(1).

[3] Walker AA, et al. Entomo-venomics: The evolution, biology and biochemistry of insect venoms. *Toxicon*. 2018;154:15-27.

[4] Touchard A, et al. Deciphering the Molecular Diversity of an Ant Venom Peptidome through a Venomics Approach. J Proteome Res. 2018;17(10):3503-3516.

[5] Téné N, al. Biochemical and biophysical combined study of bicarinalin, an ant venom antimicrobial peptide. *Peptides*. 2016;79:103-113.

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