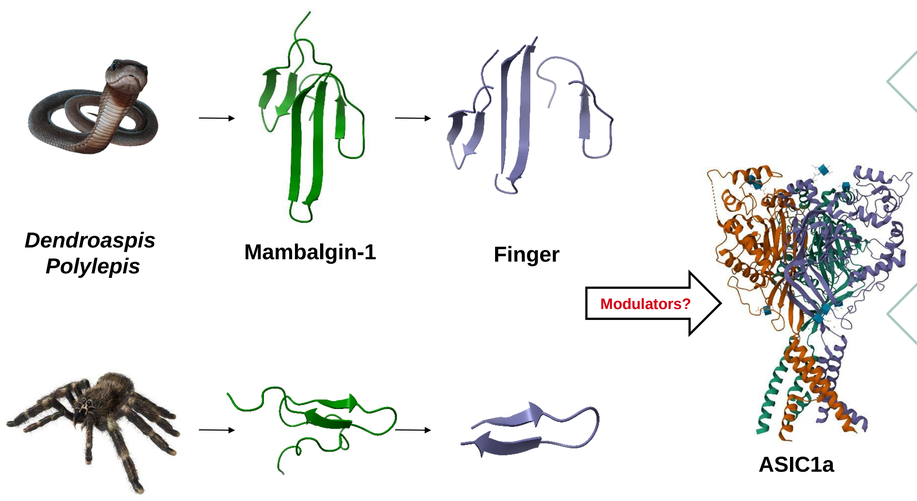
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Rational Design and Structure–Activity Relationship of Spider- and Snake-Venom-Derived Peptides Targeting Acid-Sensing Ion Channels

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Graphical abstract and Background



ASIC1a

- CNS/PNS-expressed, DEG/ENaC channel
- Homotrimeric, proton-activated channel
- Na^+/Ca^{2+} permeable; $pH_{50} \approx 6.4\text{-}6.7$
- Roles in pain, plasticity and neuronal injury

Mambalgin-1

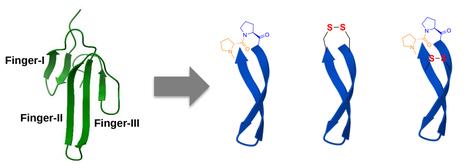
- Isolated from the venoms of *Dendroaspis polylepis*
- 57-mer, disulfide-rich
- Three-finger toxin fold
- Inhibits ASIC1-containing channels
- IC_{50} = 55 nM (rASIC1a)

Psalmotoxin-1

- Isolated from the venom of *Psalmopoeus cambridgei*
- Disulfide-rich, 40-mer
- Knottin fold
- Selective inhibitor of ASIC1a
- IC_{50} = 1,17 nM (rASIC1a)

Experimental design

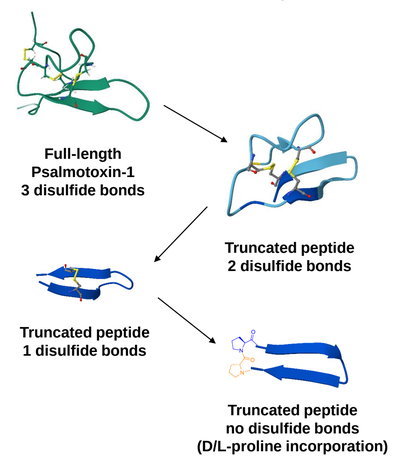
Mambalgin-1 library:



Mambalgin-1 — Strategies of cyclization for Finger I and II

No.	Sequence	linear	Back-bone / SH	Back-bone + S-S	S-S	Ac-NH + S-S
	Finger I					
Md-1	PKCYQHGKVVTCHRDp	1.1a			1d	
	AcNH-PKCYQHGKVVTCHRDp	1.2a				1e
	KCYQHGKVVTCHRDpP	1.3a				
	KVVTCHRDpPKCYQHG	1.4a	1b	1c		
Md-2	LKCYQHGVVTCHRDM	2.1a			2d	
	AcNH-LKCYQHGVVTCHRDM	2.2a				2e
	Finger II					
Md-3	LQGpPYHNTGMPFRNLKLI	3a	3b			
Md-4	LQpPHNTGMPFRNLKLI	4a	4b			
Md-5	LpPNTGMPFRNLKLI	5a	5b			
Md-6	QpPNTGMPFRNLKLI	6a	6b			
Md-7	AcNH-CYHNTGMPFRNLKLILQGC	7a				7e
Md-8	AcNH-CHNTGMPFRNLKLILQC	8a				8e
Md-9	AcNH-CNTGMPFRNLKLILC	9a				9e
Md-10	pPFCYHNTG-Nle-PFRNLKLILQGCA	_		10c		
Md-11	pPFVYHNTG-Nle-PFRNLKLILQGLA	_	11b			
Md-12	pPYHNTG-Nle-PFRNLKLILQG	_	12b			
	Finger III					
Md-13	HSSCSETENNKCCSTDRCNK	_			13d	

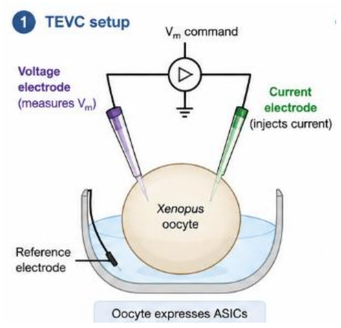
Psalmotoxin-1 library:



No.	Sequence	linear	Back-bone	S-S, $CONH_2$
Pd-1	LECWKRRRSFEVCVP	1a		1c
Pd-2	LpPVWKRRRSFEV	2a	2b	
Pd-3	pPLEAWKRRRSFEVAV	3a	3b	
Pd-4	KWKGCVNRHGD**(Acm)**CSEGLE CWKRRRSFEV**(Acm)**CV	4.1a		4.1c
	KWKGCVNRHGDCSEGLECWKR RRSFEVCV	4.2a		4.2c
	Full Psalmotoxin-1:			
PcTx-1	SEDCIPKWKGCVNRHGDCCEGL ECWKRRRSFEVCVPKTPKT	Lin PcTx-1		PcTx-1

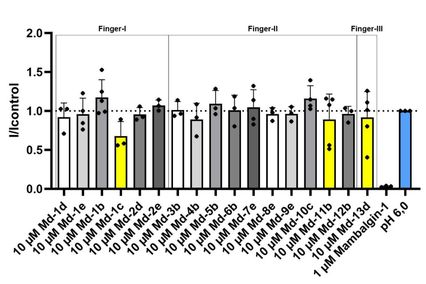
Key findings

TEVC



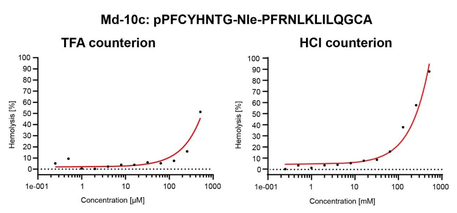
- The libraries were screened by manual two-electrode voltage clamp (TEVC) at 10 µM using *Xenopus oocytes* expressing human ASIC1a.
- Native Mambalgin-1 (Alomone Labs) and synthetic Psalmotoxin-1 were used as controls.

Screening of Mambalgin-1 library



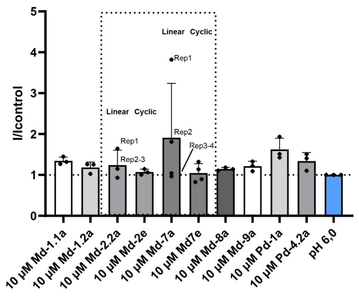
- Finger I: only the bicyclic variant **Md-1c** showed inhibitory activity under the conditions tested.
- Finger II: peptide **Md-11b** showed inconsistent responses between replicates, potentially due to lytic activity (to be confirmed).
- Finger III: **Md-13d** showed inconsistent responses across five replicates, potentially due to lytic activity (to be confirmed).

Peptide counterion affects hemolytic activity



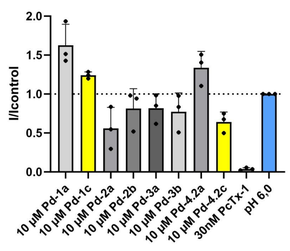
- A possible shielding effect of arginine residues was considered, given the high affinity of guanidinium groups for TFA.
- To minimize potential confounding effects of the counterion on oocyte viability and peptide activity, subsequent experiments were performed using the HCl salt forms.

Free cysteines: time-dependent ASIC1a potentiation



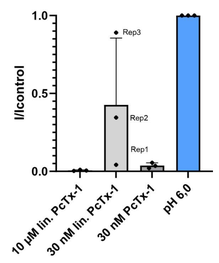
- Fresh linear peptides with free cysteines potentiated ASIC1a.
- Potentiation decreased with sample age, with later replicates increasingly resembling the corresponding cyclic peptides.
- Physiological pH and exposure to air may promote gradual cysteine oxidation and disulfide-bond formation.

Screening of Psalmotoxin-1 library



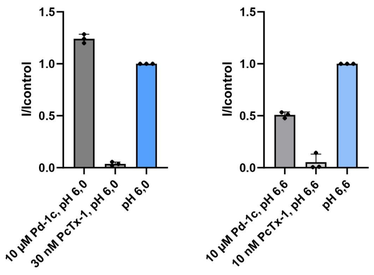
- **Pd-1c** was the only cyclic peptide showing potentiation (~25%).
- **Pd-4.2c** showed a consistent tendency toward inhibition. However, the native-like folding of the peptide remains to be confirmed.
- **Pd-3b** (30 µM) caused loss of oocyte viability. This concentration corresponds to a range previously associated with >30% hemolysis in the project.

Linear PcTx-1 showed unexpected behavior related to folding



- Linear PcTx-1 unexpectedly inhibited ASIC1a at 10 µM, consistent with formation of an active conformation in ND96.
- At 30 nM, inhibition declined from near-complete to ~20% across replicates, potentially reflecting time-dependent loss of the active conformation.

pH-dependent modulation of ASIC1a by Pd-1c



- **Pd-1c** modulates ASIC1a in a pH-dependent manner, switching from potentiation to inhibition at pH 6.6 (~50% inhibition).
- Similar pH-dependent modulation has also been reported for Mambalgin-1 and Psalmotoxin-1. However, further studies are required to elucidate the underlying mechanism and enable a proper interpretation of the observed effects.

Conclusions and outlook

Conclusions

- Both peptide libraries were successfully synthesized, characterized, and screened against ASIC1a by TEVC.
- Potential lead compounds were identified.
- Peptides containing free cysteine residues induced time-dependent ASIC1a potentiation, consistent with disulfide bond formation in ND96.
- Linear PcTx-1 exhibited ASIC1a activity consistent with formation of an active conformation in ND96.
- Counterion-dependent differences in hemolytic activity were observed.

Outlook

- Determine the hemolytic activity of lead compounds in the HCl salt form and of the native toxins.
- Characterize **Pd-4.1c** and assess its ASIC1a activity to validate the activity observed for **Pd-4.2c**.
- Determine the concentration and pH dependence of lead-compounds activity at ASIC1a.

Screening of the library by TEVC was done in collaboration with Prof. Jan Tytgat at KU Leuven

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Image / Structure Sources:
- Black mamba image: PNGAll, Black Mamba PNG
- Psalmopoeus cambridgei image: RenderHub, Jaka Ardian
- TEVC setup illustration: ChatGPT 5.0
- Graphics: GraphPad Prism 9.0

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