

Novel Peptides: From Synthesis and Characterization to Biomedical Applications

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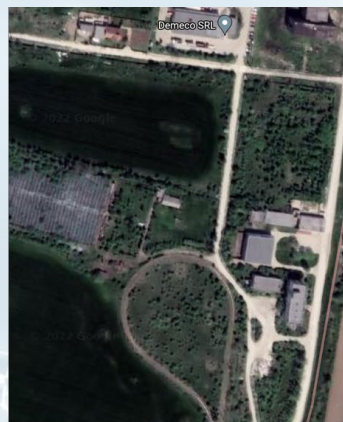
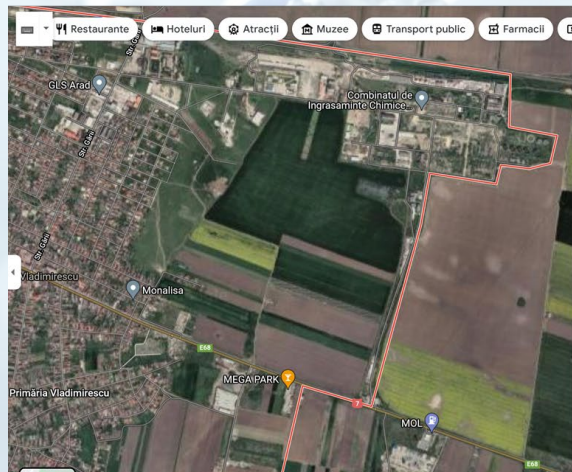


INTERNATIONAL MEDICAL CONFERENCE
Cancer Care without Borders - Innovation, Precision Medicine and
Multidisciplinary Approaches to Cancer Diagnosis, Treatment and Long-Term Care
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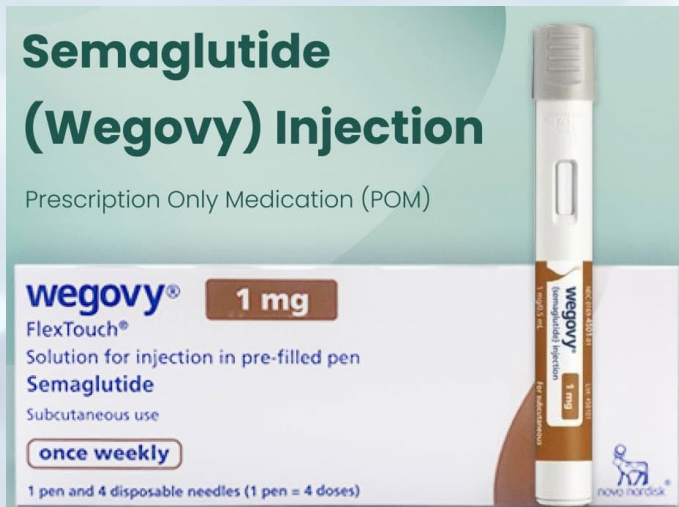
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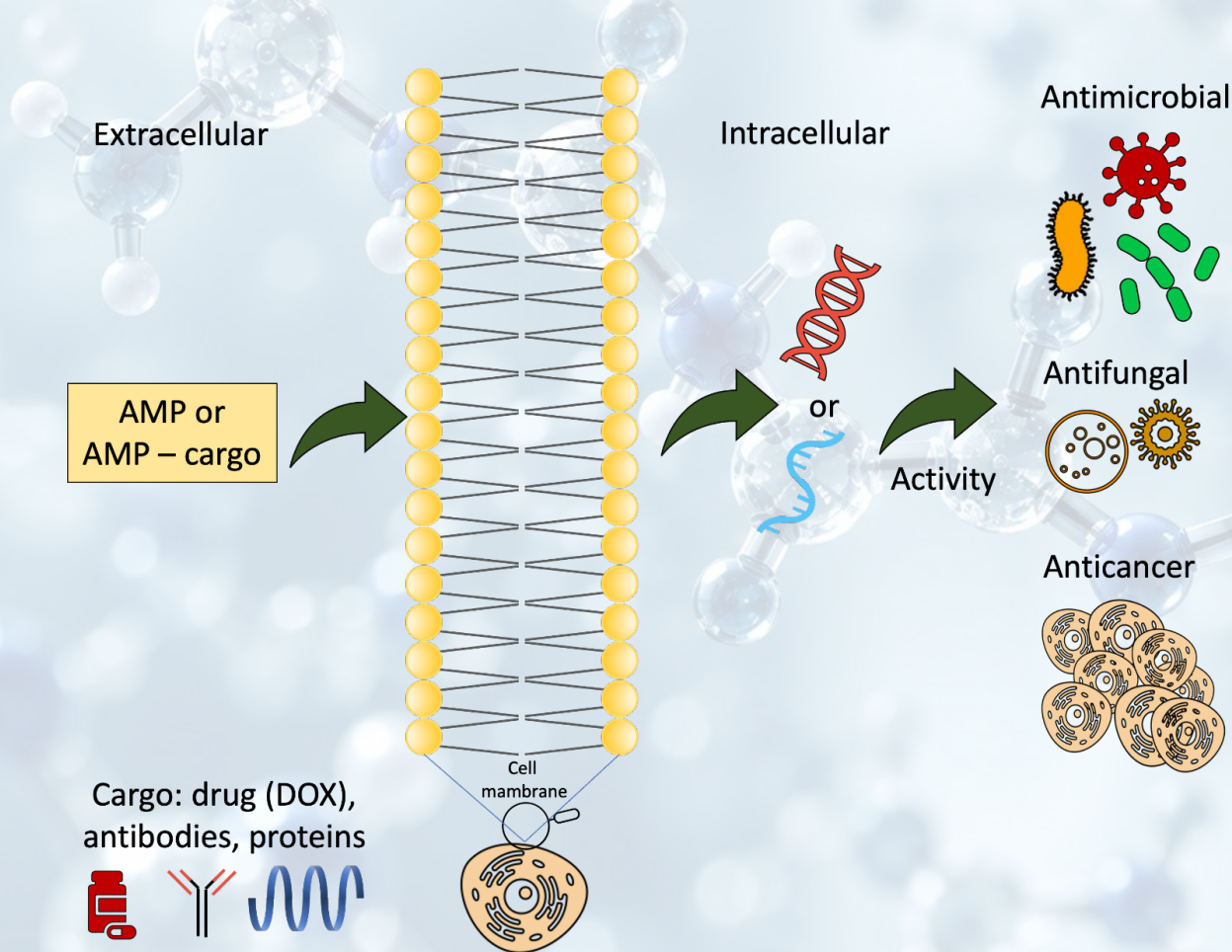
Introduction: Peptides

- **Semaglutide** is a polypeptide that contains a linear sequence of 31 amino acids
- The mechanism of action of semaglutide is as a Glucagon-like Peptide-1 (GLP-1) Agonist
- Utilized to control type 2 diabetes in conjunction with lifestyle modifications, such as dietary limitations and enhanced physical exercise

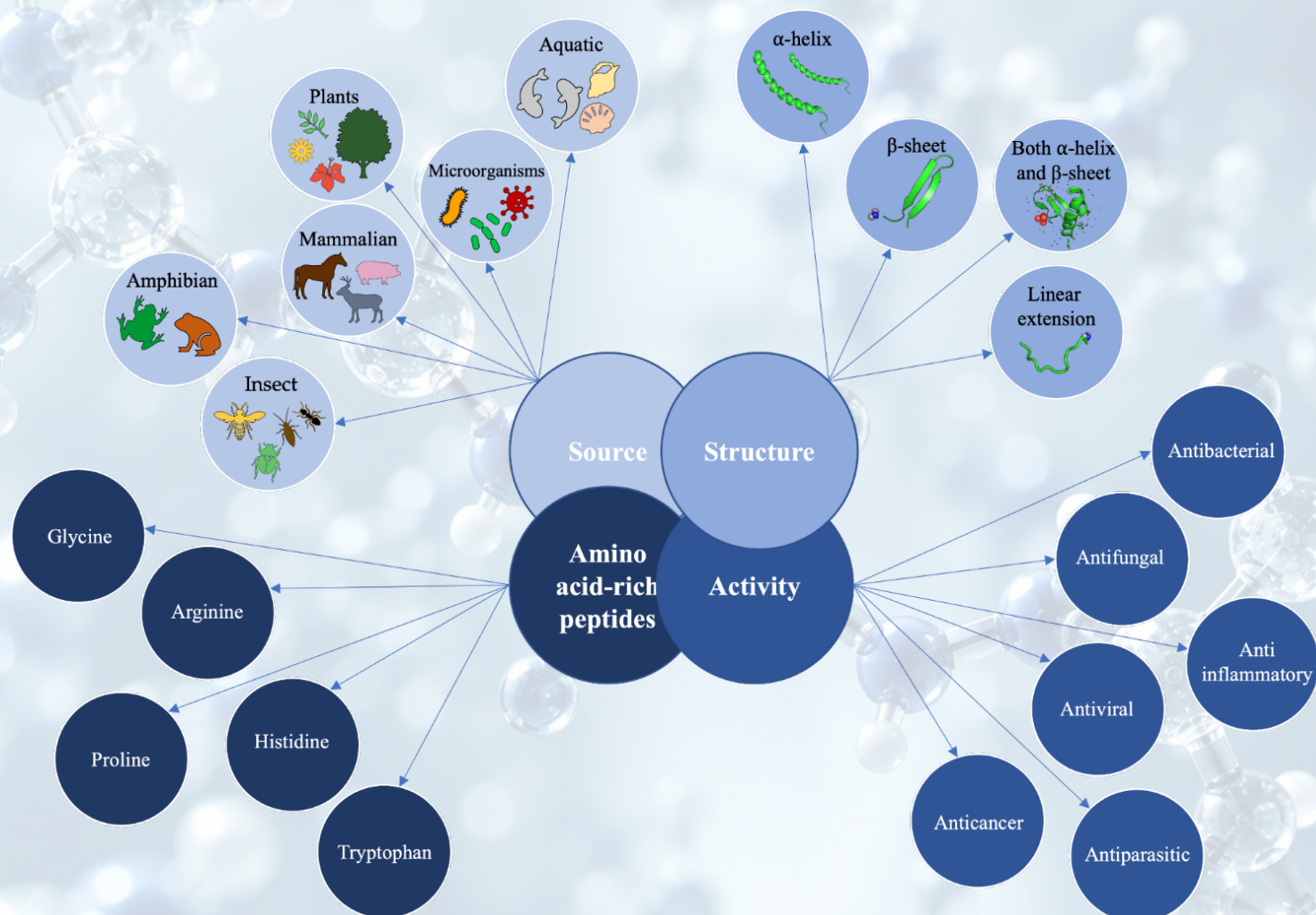


Introduction: Cell-Penetrating Peptides (CPPs) and Antimicrobial Peptides (AMPs)

Antimicrobial peptides (AMP)



Introduction: Peptides



<https://cpps-cancer.uav.ro/>



Introduction: Peptides in Clinical Practice

Peptide	Peptide structural characteristic	Clinical development / current status (2026)
Bacitracin	Natural cyclic non-ribosomal peptide antibiotic	Approved drug
Pexiganan (MSI-78)	Synthetic linear 22-residue cationic amphipathic α -helical peptide, derived from magainin	Reached Phase III , but the pivotal diabetic-foot studies are completed ; development did not result in approval. Therefore: Phase III – completed/unsuccessful . (ClinicalTrials.gov)
Omiganan (MBI-226; CLS001)	Synthetic linear 12-residue indolicidin-derived cationic peptide	Phase III development for papulopustular rosacea. (Canadian Clinical Trials Search Portal)
LL-37	Natural human 37-residue cathelicidin, cationic amphipathic α -helical peptide	Reached Phase IIb for hard-to-heal venous leg ulcers; that study has been completed and published. (PubMed Central (PMC))
LTX-109	Synthetic antimicrobial peptidomimetic, membrane-active	A new Phase IIa intranasal study within ECRAID-Prime enrolled 188 patients; recruitment closed in May 2026 and results are expected later in 2026. (Ecraid)
PLG0206 (WLBU2)	De novo engineered 24-residue cationic amphipathic α -helical peptide H-RRWVRRVRRVWRRVVRVRRWVRR-NH ₂	Phase II/III – recruiting , RETAIN trial for prosthetic joint infection after knee arthroplasty; started in 2026. (TrialScreen)
Peceliganan (PL-5; V681)	Synthetic 26-residue α -helical hybrid AMP, related to cecropin/melittin design	One Phase III wound-infection trial is completed and another Phase III study has been reported as ongoing in China; Phase II study for diabetic foot infection also exists.



AMPs and Peptide Mimetics in Clinical Trials

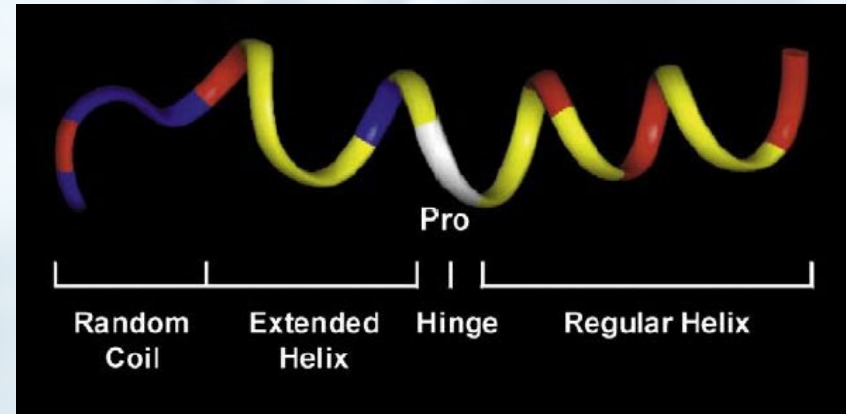
Peptide	Current clinical phase / status	Clinical indication	Peptide structure
Gosteganan (PL-18; HPRP-A1/PL-18)	Clinical development continuing; Phase Ib/II programme reported after the first-in-human Phase I study	Bacterial vaginosis / vulvovaginal infection, including vulvovaginal candidiasis programmes	15-residue, all-D, amphipathic α -helical cationic peptide, with N-terminal acetylation and C-terminal amidation
Melimine / Mel4	Reported in Phase III clinical development	Prevention of microbial keratitis associated with contact lenses	Melimine: linear cationic peptide, TLISWIKNKRKQRPRVSRRRRRRGRRRR. Mel4: shorter 17-residue derivative, KNKRKRRRRRRGRRRR
Brilacidin (PMX-30063)	Completed Phase II trials for acute bacterial skin and skin-structure infections; it has also been studied for oral mucositis and COVID-19.	Perturbs microbial membranes; also exhibits immunomodulatory and anti-inflammatory properties	Synthetic host-defense-peptide mimetic. It is a non-peptidic small-molecule/arylamide-type mimetic designed to reproduce defensin-like amphiphilicity

Aim of the Study

To obtain new peptides with antimicrobial properties with potential for developing innovative therapeutic strategies to address infections, cancer, and challenges in drug administration

Development of new peptides

Peptide	Amino acid sequence
Buforin I	AGRGKQGGKVRAKAKTRSSRAGLQFPV GRVHRLLRKGNV
Buforin II	TRSSRAGLQFPVGRVHRLLRK
Buforin IIb	RAGLQFPVGRLLRLLRLLR
Histonin	AGLQFPVGKLLKKLLKRLKR



Ribbon-model representation of the backbone structure of buforin II in 50% TFE.

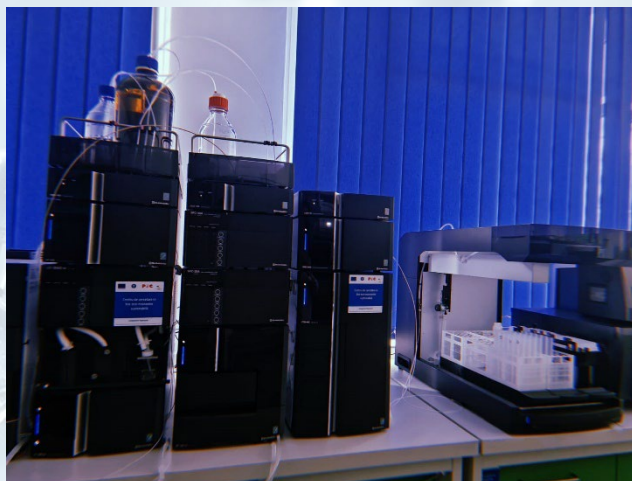
For buforin II derivatives the proline hinge region (QFPVG, residues 5-9) is a crucial structural determinant for the cell-penetrating property and the cell-penetrating efficiency of the peptides and the antibacterial potency.



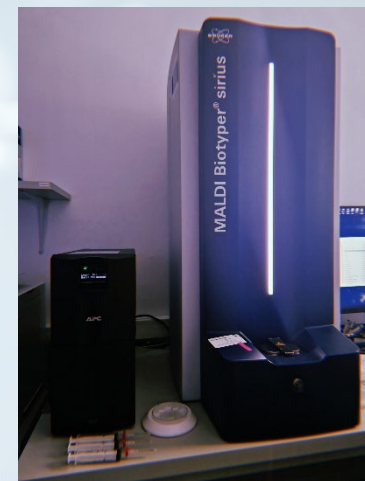
Methodology



Solid Phase-Peptide Synthesis



HPLC Purification



MALDI-TOF-MS analyses



Multimode Reader



Flow cytometer

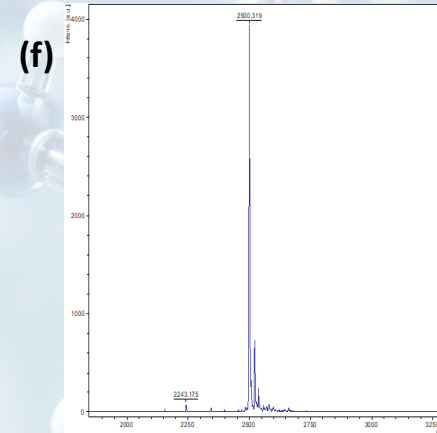
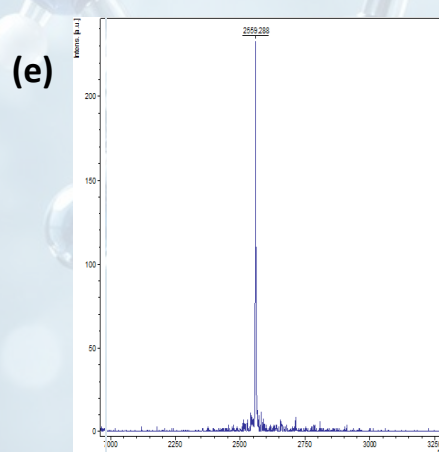
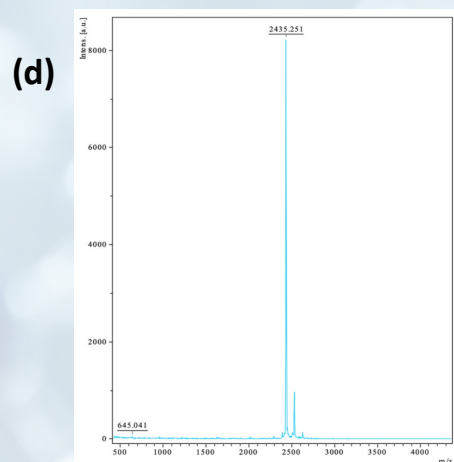
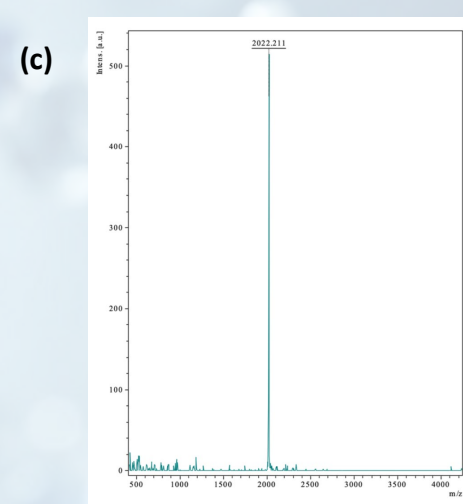
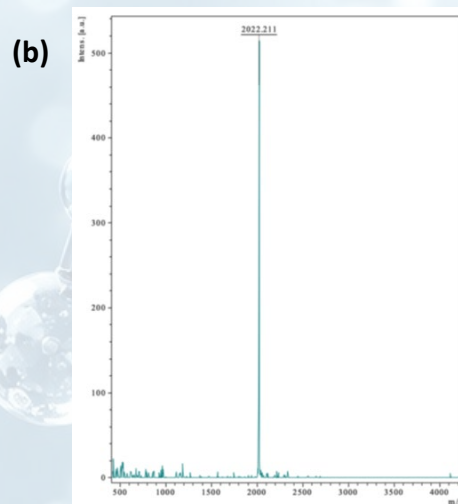
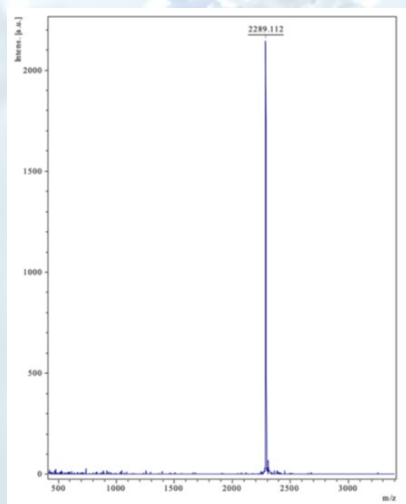


Development of new peptides

Name	Composition	M_w calc (Da)	M_w det (Da)
CPP-1	(C18-13 aa)	1752.36	878.81*
CPP-2	(13 aa)	1480.89	1482.94
CPP-3	(C18-17 aa)	2285.73	2289.11
CPP-4	(17 aa)	2019.27	2022.21
CPP-5	(C18-21 aa)	2700.33	2701.88
CPP-6	(21 aa)	2432.43	2435.25
CPP-8	(21 aa)	2559.20	2559.28
CPP-10	(21 aa)	2499.95	2500.31
CPP-14	(13 aa)	1547.85	1548.99
CPP-18	(17 aa)	2086.55	2086.97

*double deprotonated ion observed in TOF-MSMS

Development of new peptides

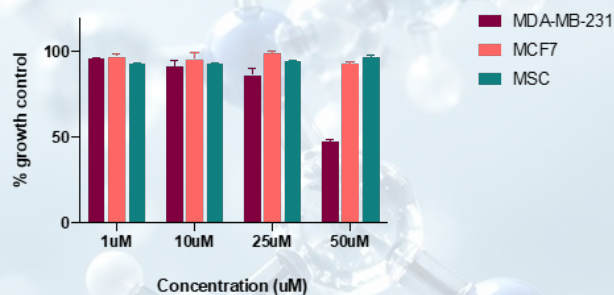


Mass spectra of the compounds: CPP-2 (a), CPP-3 (b), CPP-4 (c), CPP-6 (d), CPP-8 (e), CPP-10 (f).

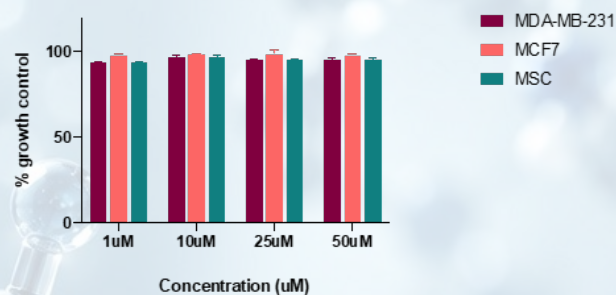


Development of new peptides

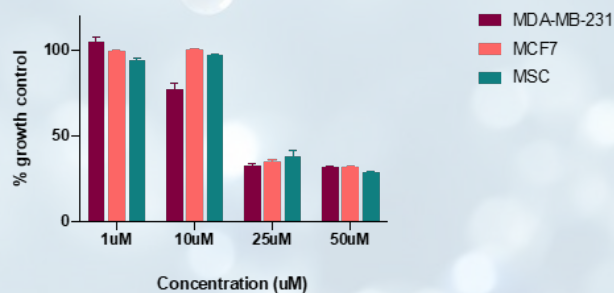
CPP-1



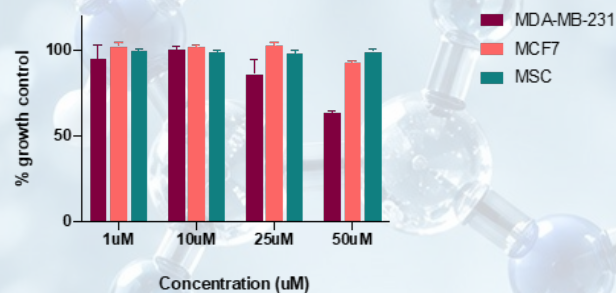
CPP-2



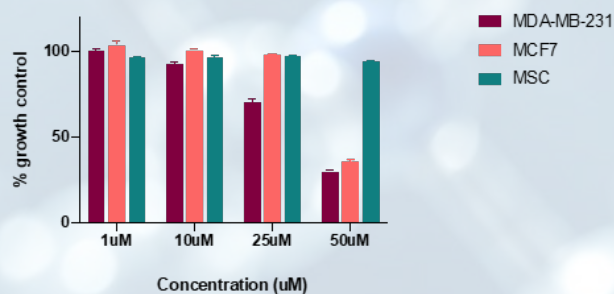
CPP-3



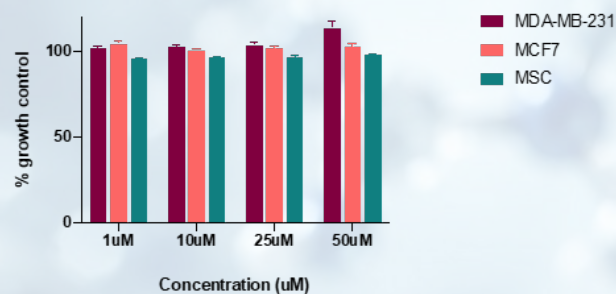
CPP-4



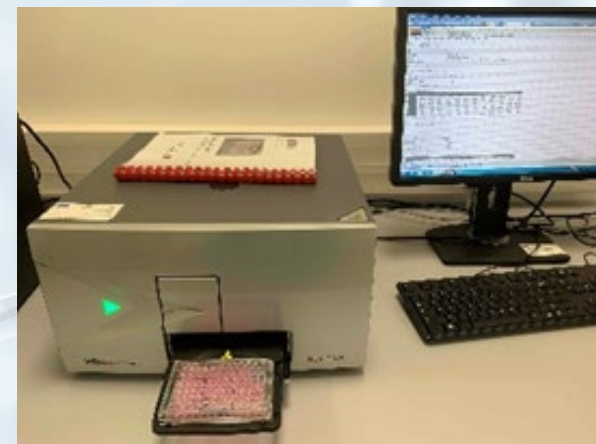
CPP-5



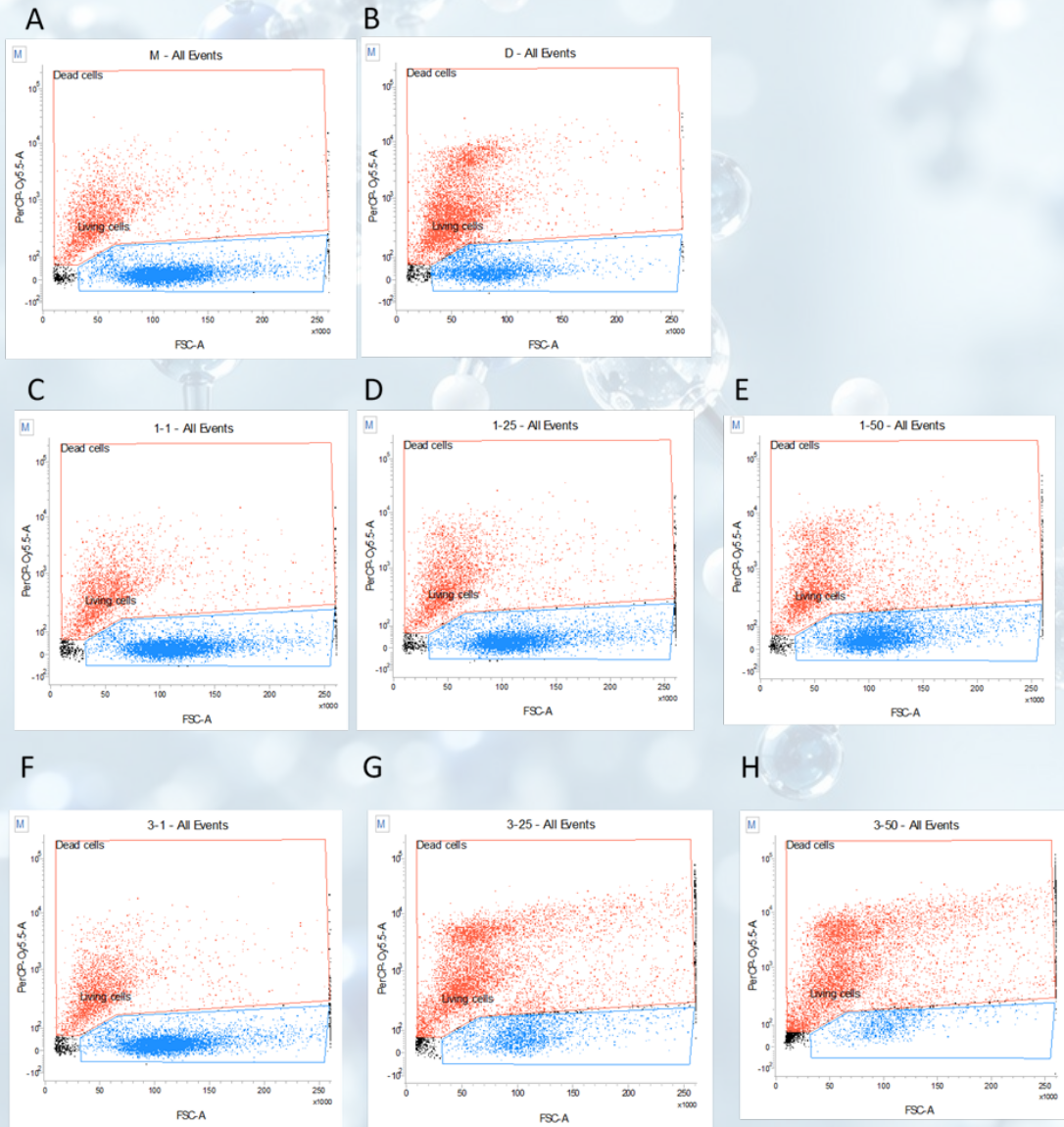
CPP-6



***In vitro* toxicity:**
cell proliferation assays in
MDA-MB-231, MCF-7 and
MSC cells
(Alamar blue assay).



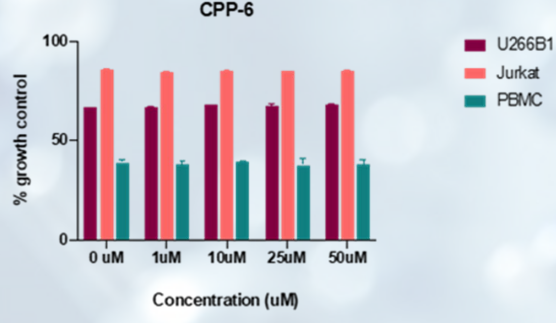
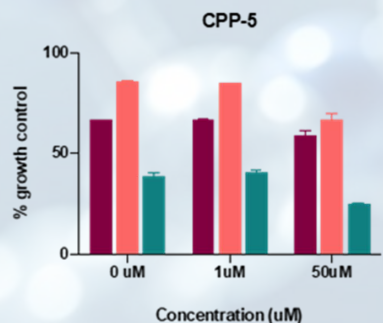
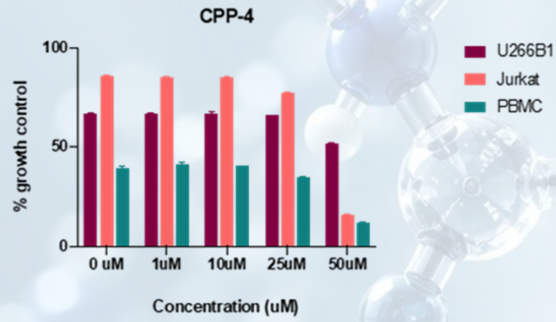
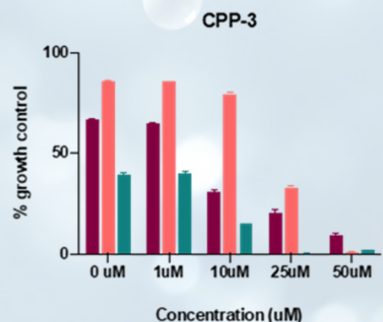
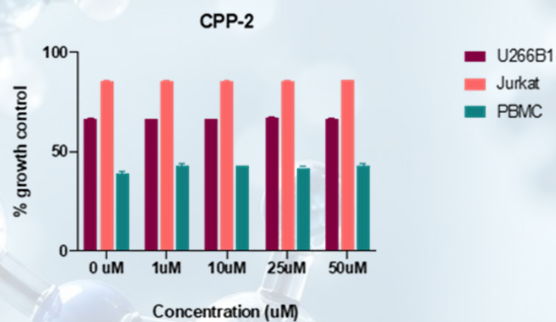
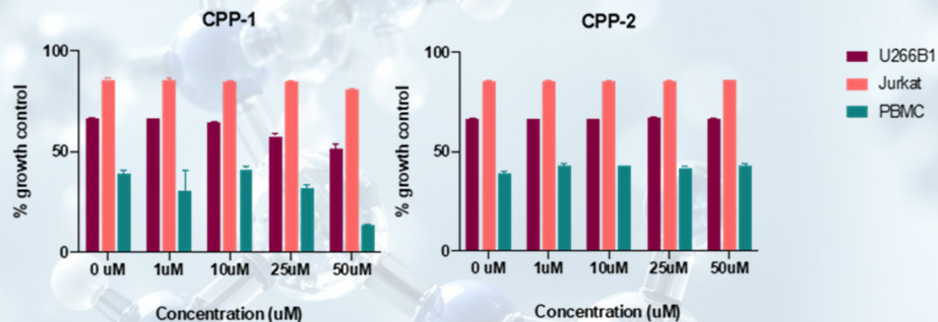
Development of new peptides



Flow cytometry analyses

The viability of U266B1 multiple myeloma cells after 24h contact with different concentrations of peptides. Red - dead cells, [propidium iodide (PI) - PE and PerCP-Cy5.5 channels. Blue - live cells with intact membranes, into which PI does not penetrate. A positive control, B 10% DMSO negative control, C-D-E treatment with CPP-1 peptide at concentrations of 1, 25, and 50 μ M, respectively, F-G-H treatment with CPP-3 peptide at concentrations of 1, 25, and 50 μ M, respectively.

Development of new peptides



In vitro toxicity (flow cytometry)

Cell proliferation assays were performed in multiple myeloma cells (U266B1 cells), Jurkat cells, and human peripheral blood mononuclear cells (PBMCs) for CPP-1 to CPP-6.

Conclusions

Ten new CPPs have been designed and synthesized using a solid-phase peptide synthesis strategy with Fmoc chemistry.

- ▶ CPP-1, 2, 4, 6 are not toxic to cancer and normal cells and could be further investigated as delivery vectors of biomolecules.
- ▶ CPP-5 is toxic only in the tested breast cancer cells at a minimum of 25 μM .
- ▶ More studies need to be undertaken, especially *in vivo* experiments, to reveal the potential of the CPPs in pre-clinical and clinical studies in future gene therapy, cancer applications, antimicrobials, and personalized medicine.
- ▶ More AMPs may be used to test their potential as CPP and their possible application in biotechnology and medicine.





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Thank you!

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