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BUFORIN-DERIVED SYNTHETIC PEPTIDES WITH POTENTIAL APPLICATIONS IN MEDICINE

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Introduction

Efforts to find efficient and specific treatments for cancer have prompted the investigation of novel approaches that utilize the distinctive characteristics of antimicrobial peptides. Bioconjugates containing peptides, including antimicrobial peptides and cell-penetrating peptides, have emerged as an intriguing and effective therapy for cancer [1,2,3]. They can be used to treat cancer, genetic disorders, cardiovascular diseases, infectious diseases, and inflammatory diseases.

Materials and Methods

Ten newly designed buforin derivatives were synthesized using solid-phase peptide synthesis (including microwave-assisted SPPS) by Fmoc chemistry strategy. The purified peptides were analyzed using MALDI-TOF-MS (Table 1 and Fig. 1). The antimicrobial activity of the new peptides was determined by broth microdilution method in several Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*) and Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis subsp. spizizenii*). Microscopy and Alamar Blue assays were used to measure cytotoxicity, cell proliferation, and cellular metabolic activity of the peptides in tumor cells: U266B1, Jurkat, MDA-MB-231, MCF-7 breast cancer cells, and in normal PBMC and mesenchymal stem cells (Fig. 2).

Results and discussions

Table 1. Peptides obtained and analyzed.

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

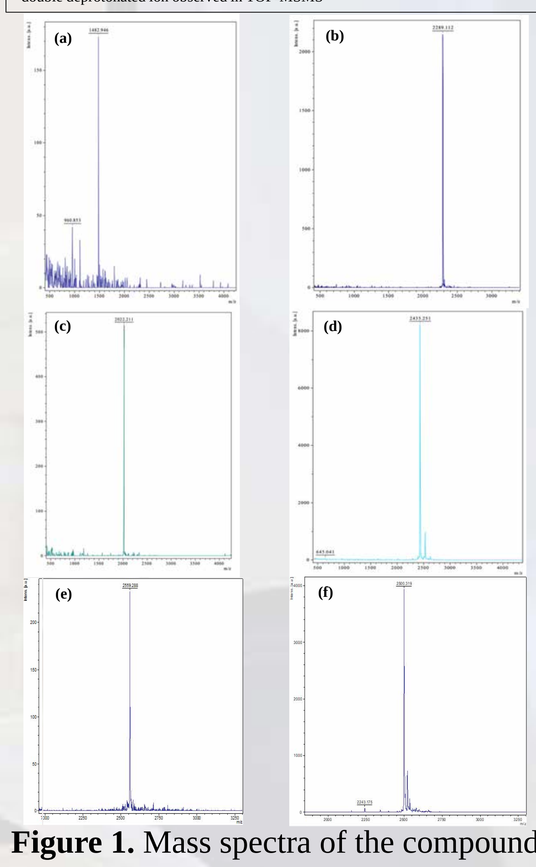

Figure 1. Mass spectra of the compounds: CPP-2 (a), CPP-3 (b), CPP-4 (c), CPP-6 (d), CPP-8 (e), CPP-10 (f).

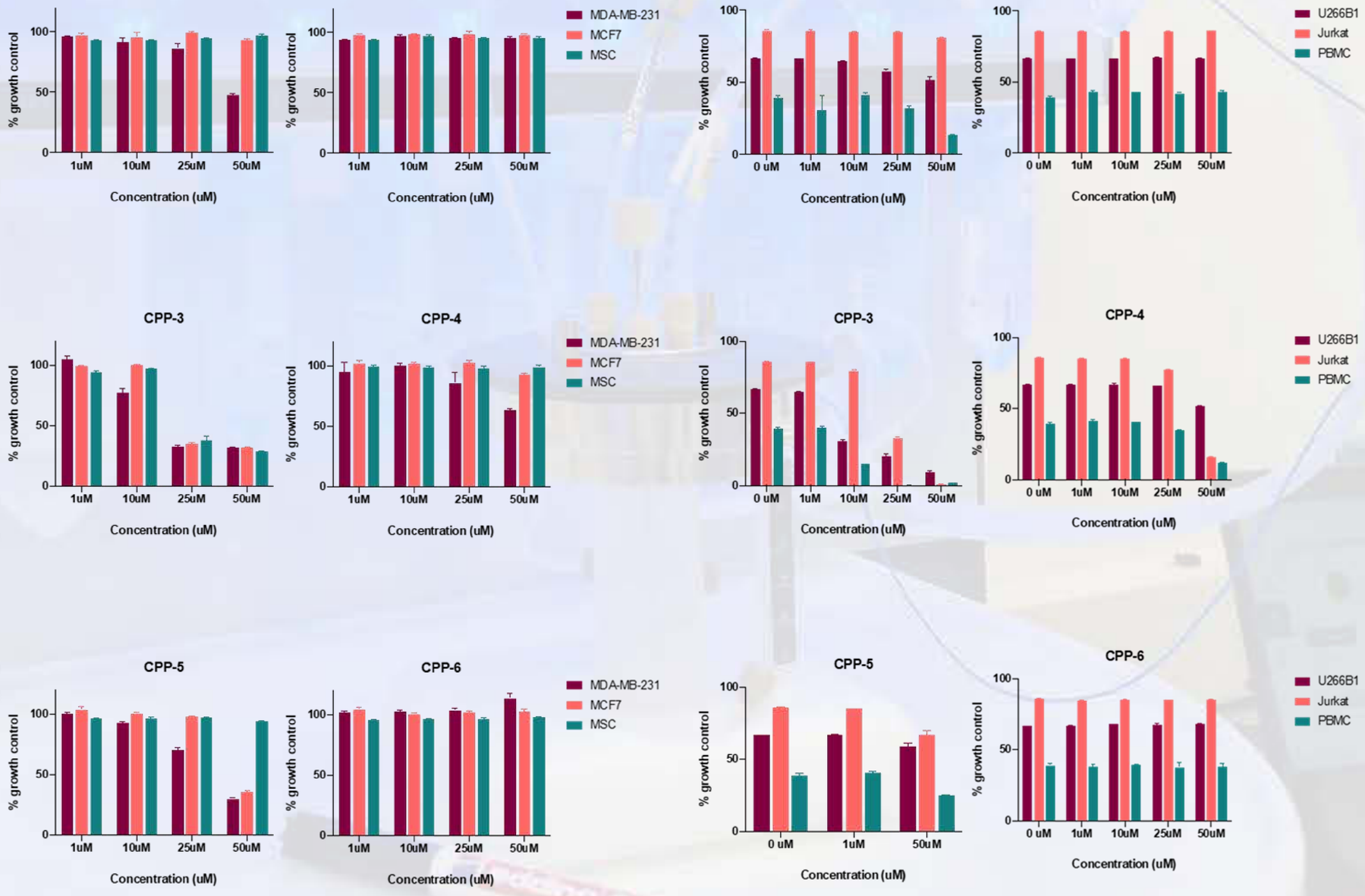

Figure 2. *In vitro* toxicity: cell proliferation assays in (a) breast cancer cells (MDA-MB-231, MCF-7), mesenchymal stem cells, and (b) multiple myeloma cells (U266B1 cells), Jurkat, and human peripheral blood mononuclear cells (PBMC) for CPP-1 – CPP-6.

Conclusions

To accelerate the progress of peptide-based medication systems, it is crucial to create peptides that can cross cell membranes or biological barriers, have an extended duration in the bloodstream, and are non-toxic and non-immunogenic to humans. The peptides presented selective toxicity in the tested cell lines in a dose-dependent manner. CPP-1, 2, 4, 6 are not toxic to breast cancer and normal cells (MSC) and could be further investigated as delivery vectors of biomolecules. CPP-3 and CPP-5 are toxic in tested breast cancer cells at min. 25 µM.

References

1. A. Gostaviceanu, S. Gavrilas, L. Copolovici, D. M. Copolovici, *Pharmaceutics*, 2023, 15(8), 2091.
2. D. M.Copolovici, K. Langel, E. Eriste, Ü. Langel. 2014, *ACS Nano*, 8(3): 1972.
3. A. M.Tolos (Vasii), C. Moisa, M. Dochia, C. Popa, L. Copolovici, D.M. Copolovici, *Polymers*, 2024, 16, 728.

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