

Article

Integrating Computational and Experimental Approaches for the Discovery of Multifunctional Peptides from the Marine Gastropod *Pisania pusio* with Antimicrobial and Anticancer Properties

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1. Supplementary Materials

Additional Figures and Tables

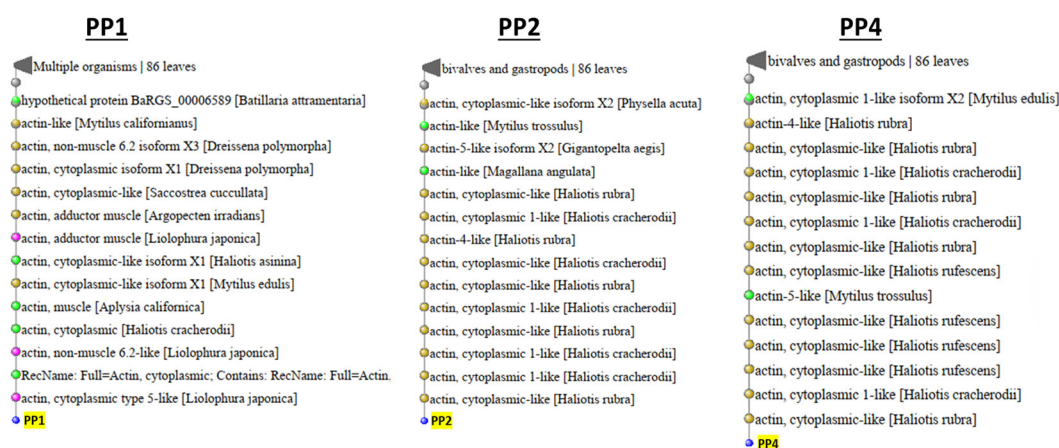


Figure S1. Phylogenetic trees showing the homology relationship of *Pisania pusio* peptides PP1, PP2 and PP4 with actin sequences from mollusks. The trees were constructed from an analysis of homologous sequences identified by BLAST against the NCBI database, restricted to the *Mollusca* taxon. The observed clustering confirms the high conservation of these peptide sequences within structural proteins of bivalves and gastropods. Peptides PP3 and PP5 are not shown as their short sequences produced ambiguous phylogenetic trees with homologies distributed across multiple unrelated protein families, lacking the clear evolutionary relationship observed for the actin-derived peptides.

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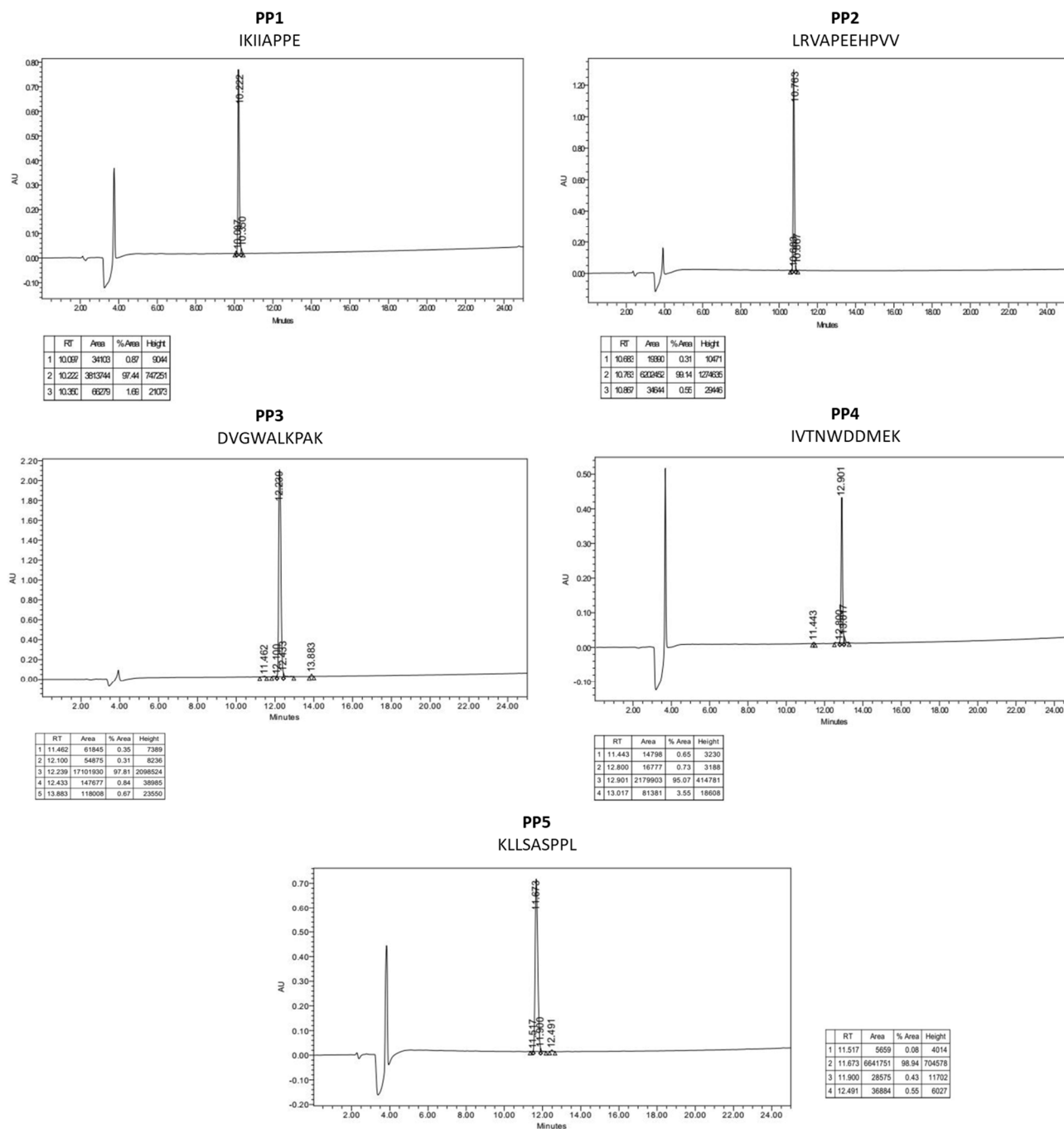


Figure S2. Purity analysis of synthesized peptides by analytical HPLC. Chromatograms are shown for peptides PP1, PP2, PP3, PP4, and PP5. The retention time (RT, min) and the relative peak area (% Area) for the main product are indicated within each panel. The high purity of each peptide (>95%) is demonstrated by the dominance of the major peak and the minimal area contribution from minor impurities.

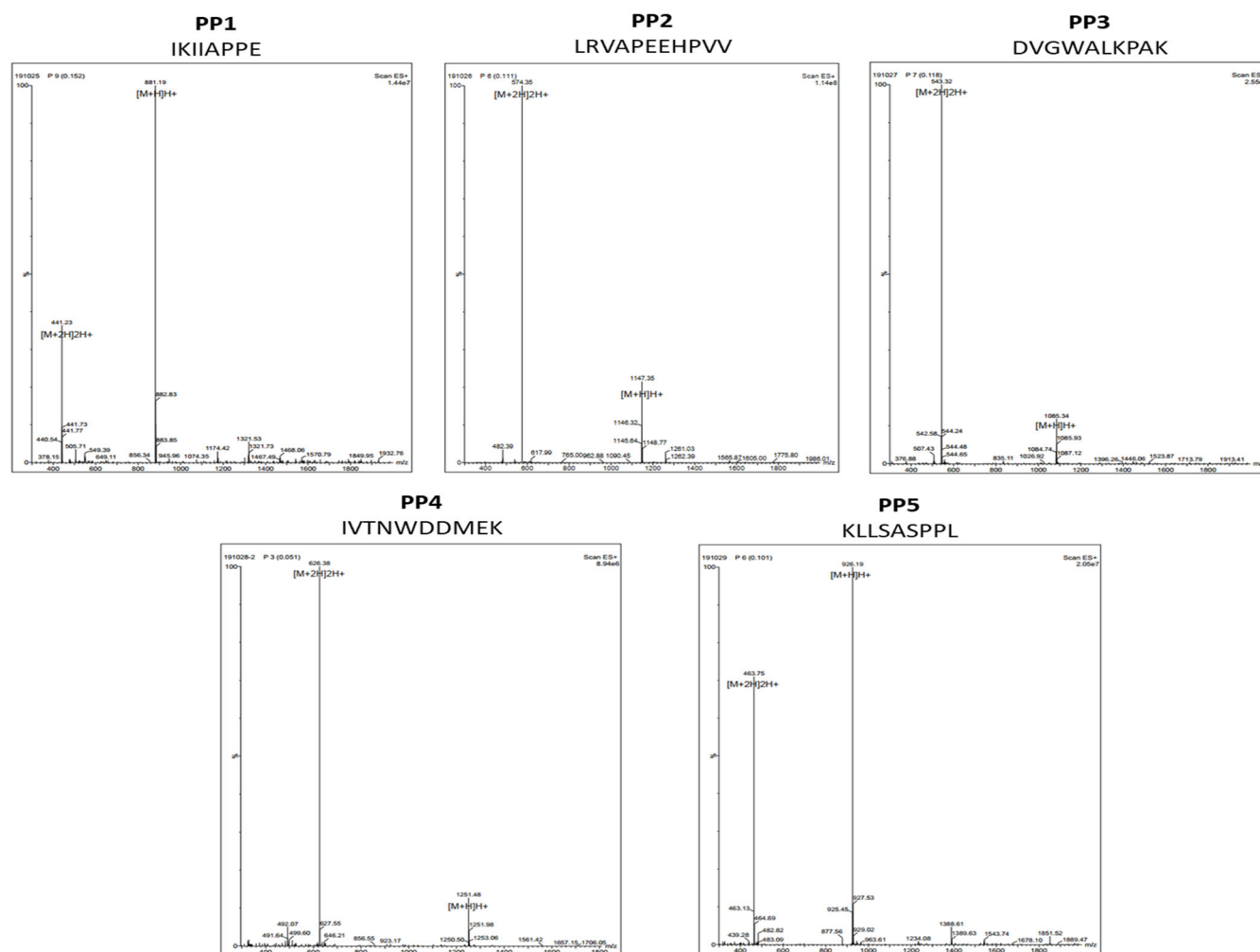


Figure S3. Mass spectrometry analysis of synthesized peptides. ESI mass spectra confirming the molecular weight of the synthesized and purified PP peptides. The observed monoisotopic masses $[M+H]^+$ correspond to the theoretical masses of the target peptides with C-terminal amidation.

Table S1. Biophysical parameters of *P. pusio* peptides

Peptide	Sequence	ΔG -Interface (kcal/mol)	Boman Index (kcal/mol)	Hydrophobic Moment (μH)
PP1	IKIAPPE	3.15	-0.52	0.326
PP2	LRVAPEEHPV	5.67	1.83	0.293
PP3	DVGWALKPAK	1.67	0.39	0.330
PP4	IVTNWDDMEK	3.71	2.53	0.483
PP5	KLLSASPPL	0.64	-0.46	0.310

¹ Calculated biophysical parameters of *Pisania pusio*-derived peptides. ΔG -Interface (Wimley-White interfacial hydrophobicity) and the Boman index (protein-binding potential) were predicted using the APD3 database. The hydrophobic moment (μH) was calculated for an ideal α -helix (100° rotation/residue) using the EMBOSS hmoment tool with the Eisenberg scale.

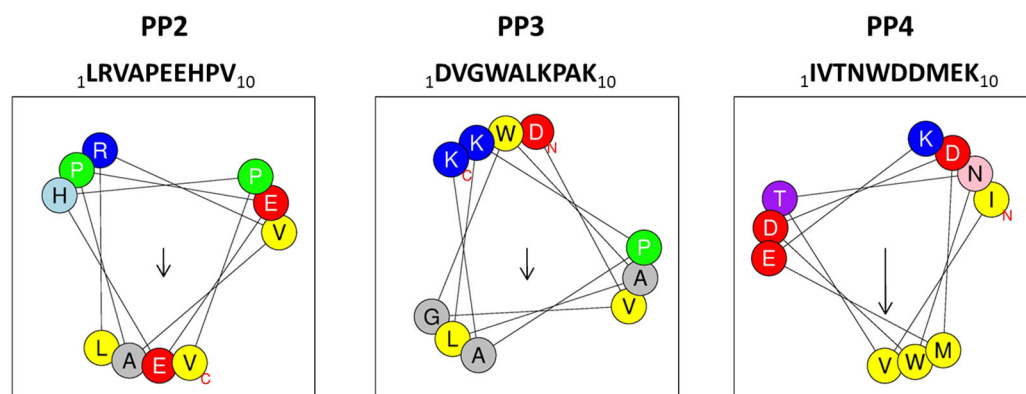


Figure S4. Helical wheel projections of *Pisania pusio*-derived peptides PP2, PP3, and PP4. Projections were generated using the HeliQuest server, assuming an ideal α -helical conformation (100° rotation per residue). The color code of residues as displayed by the server is as follows: hydrophobic (yellow), polar (cyan), positively charged (blue), negatively charged (red), glycine/proline/special (green), and other/neutral (gray). These visualizations highlight the spatial segregation of residues, contributing to the peptides' amphipathic character.

Table S2. Peptide sequences and their respective proteins of origin

Peptide	Accession	Found By
IVTNWDDMEK	Q964E1 ACTC_BIOOB:Q964E2 ACTC_BIOPF:Q964E3 ACTC_BIOAL:Q26065 ACT_PLAMG:Q964D9 ACTC_PL ATR:P17304 ACTM_APLCA:Q964E0 ACTC_BIOTE:O17320 ACT_CRAGI:P92179 ACTC_BIOGL	DB Search
GI- VTNWDDMEK	Q964E1 ACTC_BIOOB:Q964E2 ACTC_BIOPF:Q964E3 ACTC_BIOAL:Q26065 ACT_PLAMG:Q964D9 ACTC_PL ATR:P17304 ACTM_APLCA:Q964E0 ACTC_BIOTE:O17320 ACT_CRAGI:P92179 ACTC_BIOGL	DB Search
LRVAPEEHPV	Q964E1 ACTC_BIOOB:Q964E2 ACTC_BIOPF:Q964E3 ACTC_BIOAL:Q26065 ACT_PLAMG:Q92193 ACT_CRA VI:Q964D9 ACTC_PLATR:Q964E0 ACTC_BIOTE:O17320 ACT_CRAGI:P92179 ACTC_BIOGL	DB Search
IVTNWDDM(+1 5.99)EK	Q964E1 ACTC_BIOOB:Q964E2 ACTC_BIOPF:Q964E3 ACTC_BIOAL:Q26065 ACT_PLAMG:Q964D9 ACTC_PL ATR:P17304 ACTM_APLCA:Q964E0 ACTC_BIOTE:O17320 ACT_CRAGI:P92179 ACTC_BIOGL	DB Search
GI- VTNWDDM(+1 5.99)EK	Q964E1 ACTC_BIOOB:Q964E2 ACTC_BIOPF:Q964E3 ACTC_BIOAL:Q26065 ACT_PLAMG:Q964D9 ACTC_PL ATR:P17304 ACTM_APLCA:Q964E0 ACTC_BIOTE:O17320 ACT_CRAGI:P92179 ACTC_BIOGL	DB Search
	P13543 MLR_AR-	
LKEAPGPLN	GIR:P05963 MLR_CHLNI:P04113 MLRA_MIZYE:P04112 MLRB_MIZYE:P05944 MLRC_MIZYE:P02613 MLR_ PATSP	DB Search
	P13543 MLR_AR-	
MLKEAPGPLNF	GIR:P05963 MLR_CHLNI:P04113 MLRA_MIZYE:P04112 MLRB_MIZYE:P05944 MLRC_MIZYE:P02613 ML R_PATSP	DB Search
DVGWALKPAK		DeepNovo
LVVDNGSGM	Q964E1 ACTC_BIOOB:Q964E2 ACTC_BIOPF:Q964E3 ACTC_BIOAL:Q26065 ACT_PLAMG:Q964D9 ACTC_ PLATR:P17304 ACTM_APLCA:Q964E0 ACTC_BIOTE:O17320 ACT_CRAGI:P92179 ACTC_BIOGL	DB Search
KLLSASPPL		DeepNovo
IKIAPPE	Q964E1 ACTC_BIOOB:Q964E2 ACTC_BIOPF:Q964E3 ACTC_BIOAL:Q26065 ACT_PLAMG:Q964D9 ACTC_ PLATR:P17304 ACTM_APLCA:Q964E0 ACTC_BIOTE:P92179 ACTC_BIOGL	DB Search

² **Unique peptide sequences identified from *Pisania pusio* peptidomic analysis and their potential protein precursors.** For each peptide, the corresponding UniProtKB accession numbers (where available) of homologous proteins identified by BLAST search against the *Mollusca* taxon are listed, separated by a vertical bar.