

Assessing the translation of AI-prioritized genome-derived peptide fragments into validated antimicrobial candidates

Supplementary Information

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Name	Sequence	L	Q	HR	HM	AI	II	pI
PIAM29-7	WVEMQHGRIRLGRLQKVSKAVRRLGGLHR	29	7.07	0.34	0.23	100.69	32.87	12.41
PIAM23-3	AASLTFFKARTLRTVAGLASNH	23	3.03	0.48	0.26	85.22	8.40	12.13
PIAM23-4	TRVKLDKVDHLLRVHVKLHRVVH	23	4.16	0.43	0.45	143.48	-5.87	11.39
PIAM21-4	KHGVGMCERRGNAYGNRVHRL	21	3.92	0.29	0.45	50.95	11.65	11.11
PIAM21-6	RLTKCRGAKGGWCKCKPRQIS	21	6.53	0.29	0.12	41.90	11.14	11.03
PIAM18-5	IGQGLVKVKCKPVGTKKG	18	4.83	0.33	0.18	91.67	-6.11	10.86
PIAM11-4	RRGTPNGIRKS	11	3.99	0.09	0.56	35.45	24.35	12.41

Table 1: Amino acid sequences and physicochemical properties of the seven candidate peptides identified through the AI-guided genome-mining pipeline. L, length (aa); Q, net charge; HR, hydrophobic ratio; HM, hydrophobic moment; AI, aliphatic index; II, instability index; pI, isoelectric point. Four of these (PIAM11-4, PIAM21-4, PIAM23-4, and PIAM29-7) were selected for synthesis and experimental validation, hereafter referred to in the main text as PIAM11, PIAM21, PIAM23, and PIAM29, respectively.

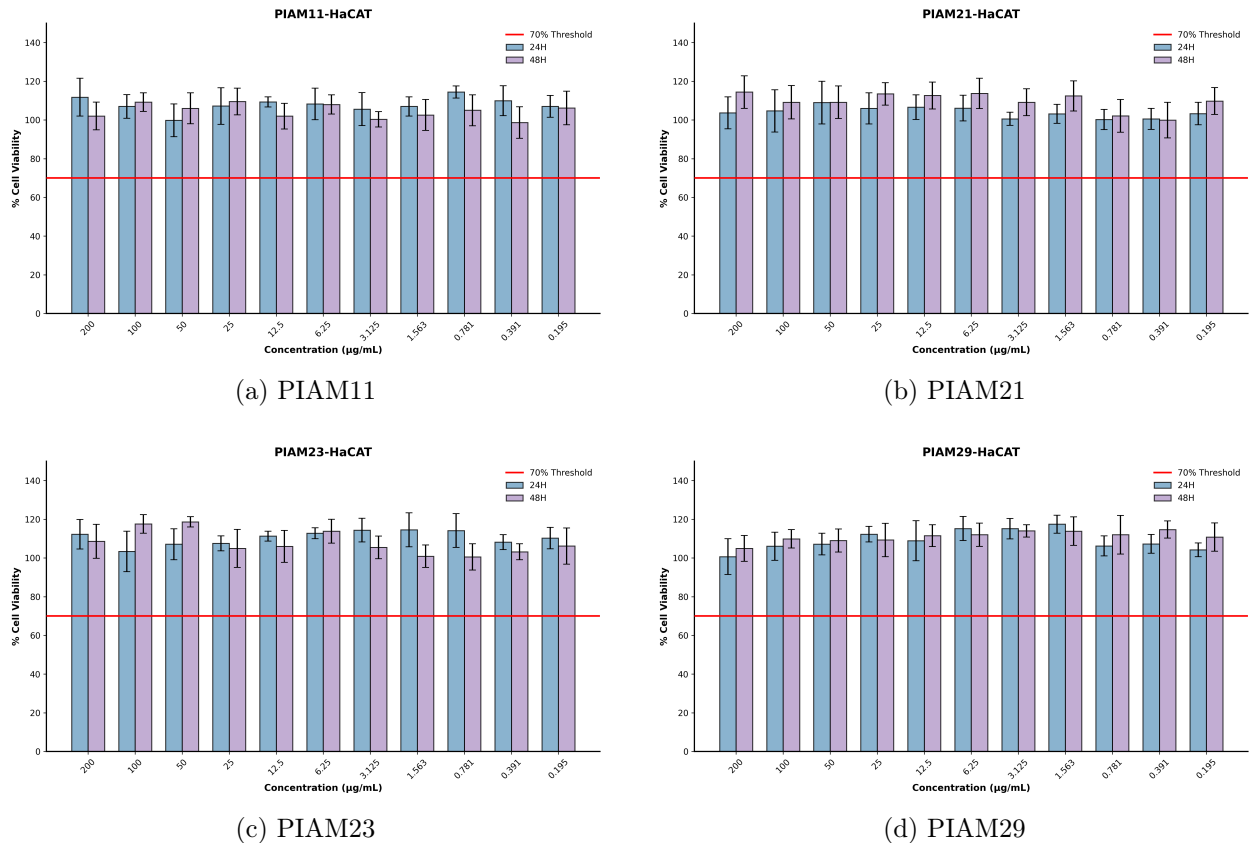


Figure 1: Representative microscopy images of HaCaT human keratinocytes following exposure to the four PIAM candidates. Images are shown for PIAM11, PIAM21, PIAM23, and PIAM29 and provide morphological evidence complementary to the quantitative cell-viability measurements obtained using the MTT assay.

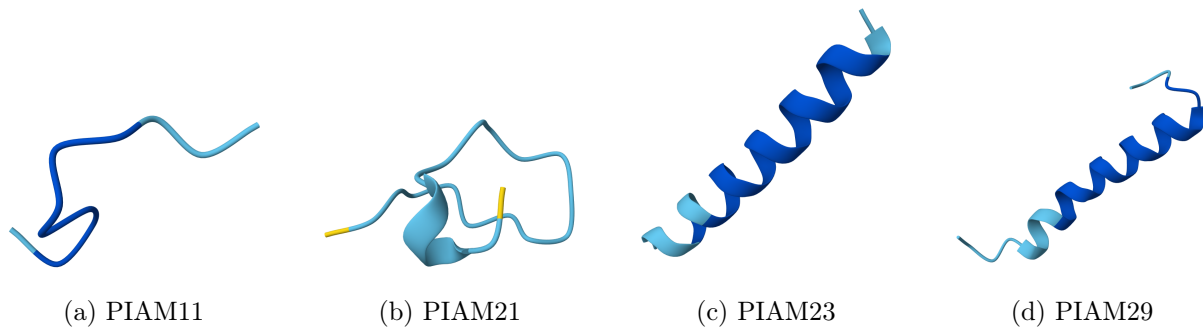


Figure 2: AlphaFold-predicted three-dimensional structures of the four peptide candidates, shown here for comparison only. Under these predictions, PIAM23 and PIAM29 appeared as well-defined amphipathic helices, whereas PIAM11 and PIAM21 lacked defined secondary structure. However, PEP-FOLD3 predictions generated under solution conditions approximating physiological pH and ionic strength (Figure 2, main text), corroborated by FT-IR secondary-structure analysis, indicate that all four peptides predominantly adopt disordered, random-coil conformations. The AlphaFold predictions shown here are therefore not considered representative of the peptides' behavior under the conditions used in this study.