POTENT ANTICOLORECTAL CANCER ACTIVITY OF ANALOG PEPTIDES DERIVED FROM PARASPORIN-2Aa1

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HIGHLIGHTS

The anticancer activity of analog peptides derived from Parasporin-2Aa1 (PS-2Aa1) was evaluated.

Activity was tested against the SW480 and SW620 cell lines, using 5-Fluoracil as positive control.

The selectivity of Lys-substituted peptide was better than that of Trp-peptide or His-peptide.

Lys-analog, P264-V268K, was positive for both annexin V-Cy3 and 6-CFDA, which is indicative of the early stage of apoptosis.

INTRODUCTION

The number of cases of colorectal cancer continues to increase. The drugs currently available for treatment produce adverse side effects to patients.

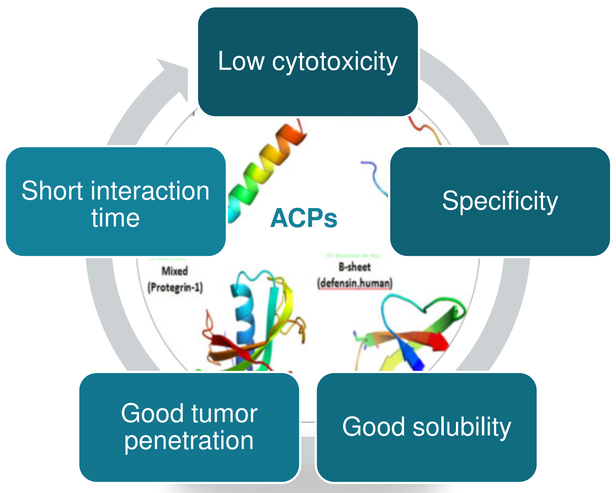


Figure 1. Properties of anticancer peptides (ACPs) [1].

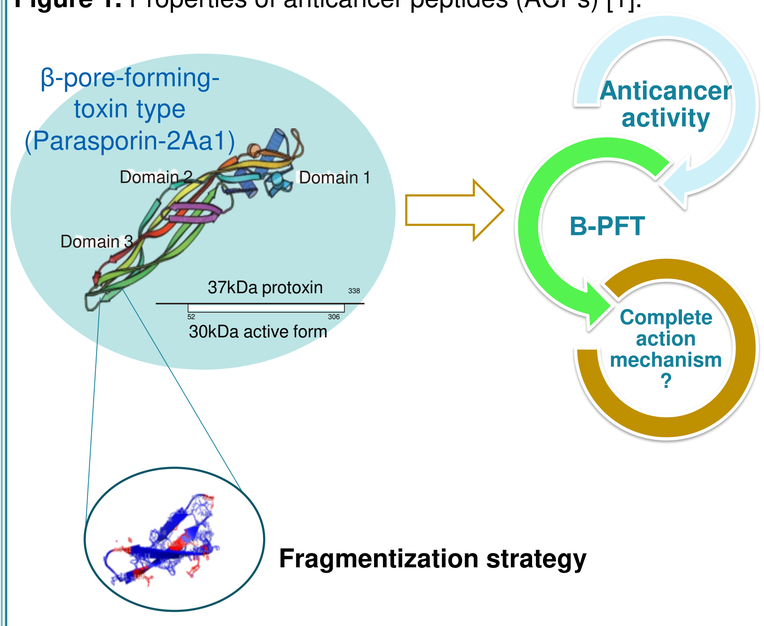


Figure 2. Structure of the PS2-Aa1 and characterist [2].

METHODS

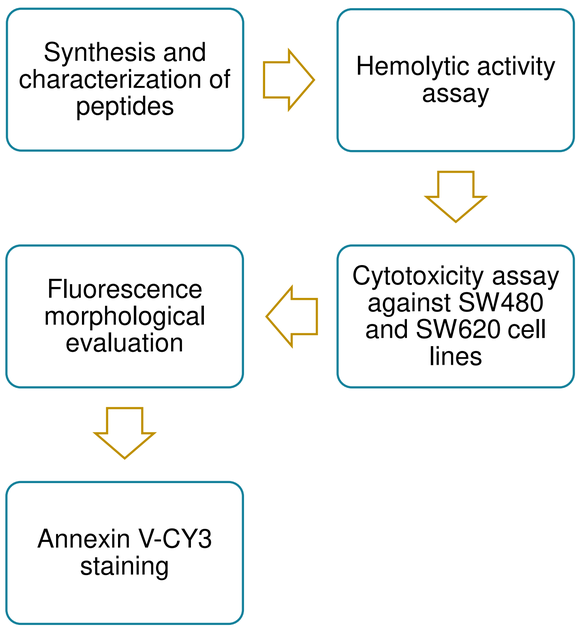


Figure 3. General scheme of the methodology [3-5]**.**

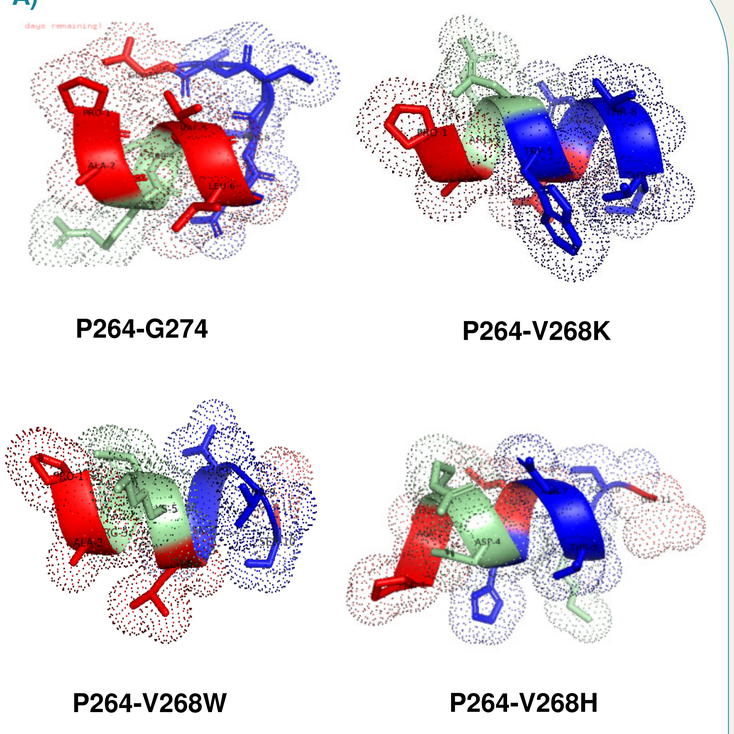
RESULTS

Table 1. Sequence and characteristics of native and analogs peptides from PS2-Aa1.

Peptide	Sequence	N° of residues	z	MW (Da)	
				Theoretical	observed
P264-G274	**PARDVL**TSG**-NH_2	11	+1	1129.23	1130.3
P264-V268K	PARD**K**L**TSG-NH_2	11	+2	1158.27	1160.4
P264-V268W	PARD**W**L**TSG-NH_2	11	+1	1216.3	1217.7
P264-V268H	PARD**H**L****K**SG-NH_2	11	+2.1	1211.38	1213.4

*The asterisks may correspond to the N and T amino acids.

z (net charge)
(https://www.bachem.com/service-support/peptide-calculator/)



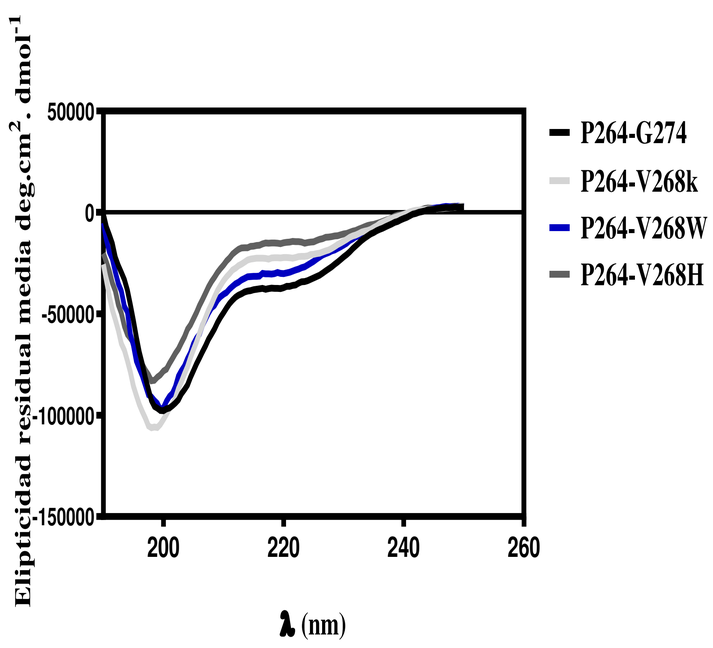


Figure 4. A) ***In silico*** **secondary structure of the peptides and B) circular dichroism spectra of peptides.**

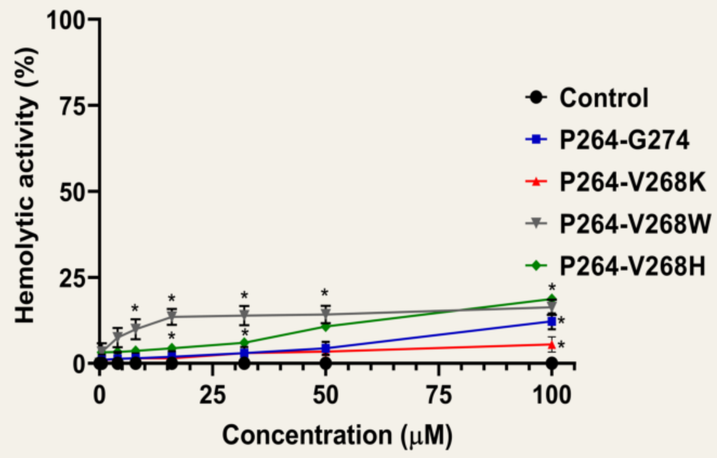


Figure 5. Hemolytic activity of peptides against human red blood cells at different peptide concentrations (0.5-100 μM), in Hank's glucose at 37ºC for 4h of exposure. Experiments were performed in three independent replicates. Dosages that caused a statically significant decrease in cell growth compared to the untreated control at each time point were indicated by asterisks (*p< 0.05; one-way ANOVA followed by Tukey's test).

Table 2. EC_{50} value of analogs and native bioactive peptides.

Peptide	EC_{50} (μM)		
	SW480	SW620	CHO-K1
P264-G274	90.98±0.75	11.28±0.52	> 150
P264-V268K	14.3 ± 0.9	6.3 ± 0.5	> 150
P264-V268W	19.3 ± 1.1	111.6 ± 0.8	>150
P264-V268H	49.2 ± 1.5	76.2 ± 1.2	>150
5-FU	24.38±0.82	11.92±1.20	>150

Annexin V-CY3 staining: When the cells were treated with the most active peptide, P264-V264K, for each of the cell lines after of 48h, both annexin V-Cy3 and 6-CFDA were positive which clearly indicates the early stage of apoptosis.

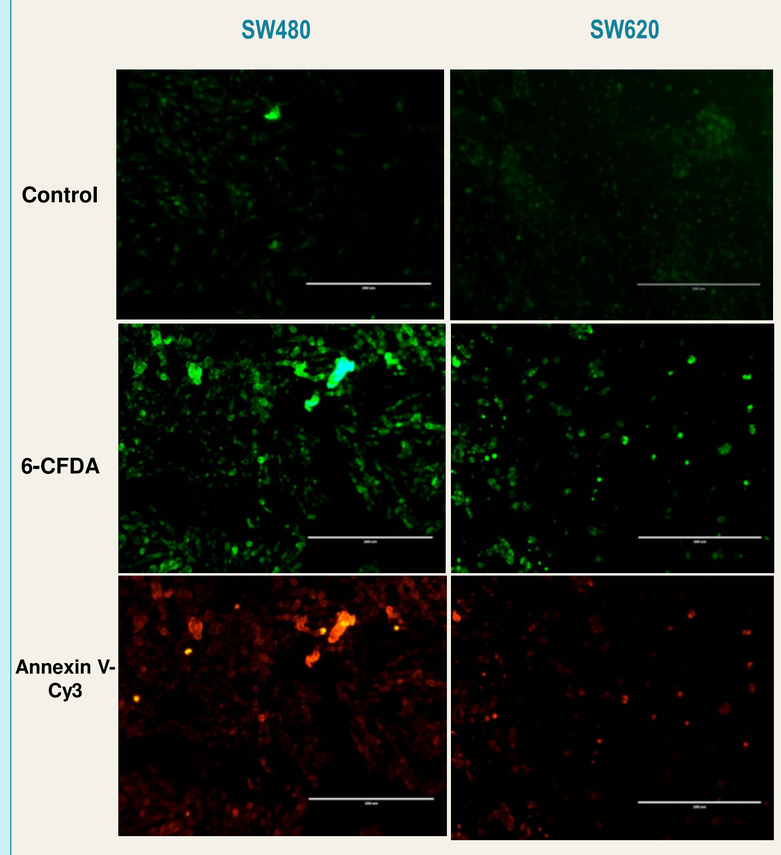


Figure 6. Photomicrographs of control and Annexin V-Cy3/6-CFDA stained SW480 and SW620 colon cancer cells treated with the P264-V268K peptide for 48 h.

CONCLUSIONS

Peptides exerted anticancer effects in all models colorectal cancer cells tested in the present study. Additionally, P264-V268K peptide exhibited stronger anticancer activity against SW480 and SW620 respectively and demonstrated high effectiveness and selectivity. This compound is proposed as a possible alternative as therapeutic agents for the treatment of colon cancer.

Likewise, the increase in the positive charge on the analogs with respect to the increase in hydrophobicity represented an increase in the activity.

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