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Development of Pancreatic Tumor Specific Daunomycin – Peptide Conjugates Using Homing Peptides Selected by Phage Display Technique

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Introduction

Pancreatic Ductal Adenocarcinoma (PDAC) is one of the most dangerous cancerous diseases leading to high mortality [1]. This tumor type is only 3-4% of all newly diagnosed cancer cases but the mortality of this disease is more than 80% [2]. Furthermore, the average 5-years survival rate is not more than 10% [3], because approximately 80% of the patients are diagnosed with metastatic or inoperable status. Therefore, new approaches for efficient treatment are necessary. Here we report the development of peptide - drug conjugates which contain daunomycin as anticancer drug attached to different homing peptides *via* oxime bound – a linkage that might be suitable for targeted tumor therapy. In the literature, several peptides can be found that were identified by phage-display technique and recognized PDAC cells with high affinity. However, the efficacy of drug targeting has not been compared. Four of them (**(I)** SYENFSA [4], **(II)** IVRGRVF [4], **(III)** PFWSGAV [5], **(IV**) TMAPSIK [6]) were selected for this study. Based on our previous results four-members conjugate families were designed. Linear and branched structures were synthesized using various enzyme labile spacers for enhanced drug release.

Results and Discussion

For targeting of PDAC cells, sixteen different daunomycin - peptide conjugates were designed and synthesized using the above mentioned four homing moieties (Table 1).

The synthesis of conjugates was carried out by solid phase peptide synthesis, using Fmoc/^{t}Bu technique. To the *N*-terminal α-amino group isopropylidene-protected aminooxyacetyl-group was attached as a linker between the homing part and the payload. The aminooxyacetyl peptide derivatives were cleaved from the resin and the crude products were purified by RP-HPLC. For identification of products ESI-MS was used in all cases. After the cleavage of the isopropylidene-protecting group with 0.2M NH_4OAc buffer solution (pH 5.0), daunomycin was coupled to the aminooxyacetyl moiety forming oxime bond (Figure 1). In the case of eight conjugates cathepsin B enzyme labile spacer was built into the sequences between the homing peptide and the aminooxy moiety that may enhance the intracellular (lysosomal) degradation and the release of the active drug-containing metabolites. In cases of apolar homing peptide sequences (**1**, **2**, **3**) LRRY segment was built in as spacer but the more polar **4** sequence encouraged the application of GFLG spacer [7].

The *in vitro* cytotoxic effect of conjugates was investigated on PANC-1 pancreatic cancer cells by an impedimetric technique, xCELLigence System. The effect of them was characterized also on further three cell lines as control (colon cancer (Colo-205); melanoma (A2058) and non-small cell lung cancer (EBC-1)) for characterization their selectivity using Alamar blue test.

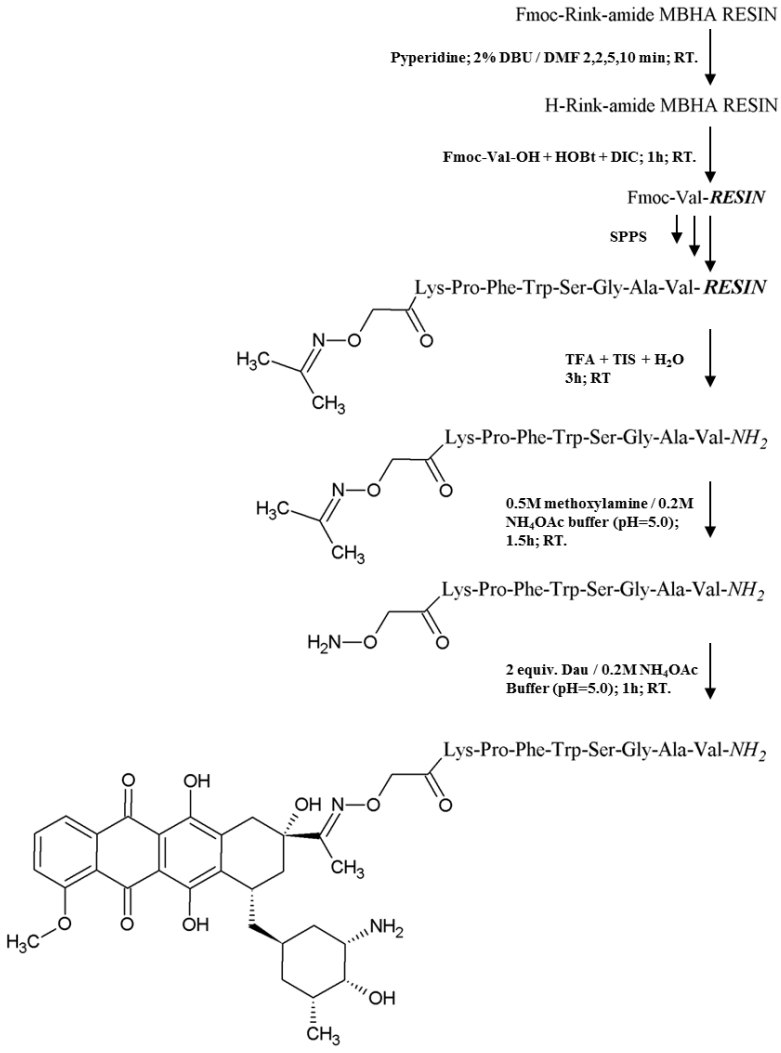

Fig. 1. Outline of synthesis of conjugates.

To investigate further structure-activity relationships Ala-scanning of conjugate **10** was carried out to study the possible positions for further development of more efficient compounds.

Ala-scanning showed that the alanine substitution resulted in the loss of efficiency in all cases except conjugate **22** which presented moderated antitumor effect (Table 2).

The results showed that two conjugates out of four which were designed with homing moieties **I** and **II** did not give significant antitumor effect on PANC-1 cells (conjugates 1-8). However, these 8 conjugates have very poor antitumor effect (50-70 viability %) on one or more of the other control cell lines out of conjugate **6**.

Table 1. Effect of the conjugates on PANC-1 and other cell-lines as control.

CODE	CONJUGATES	***Viability % in 10^{-5} M concentration, after 72 h incubation***			
		PANC-1[a]	*Colo-205*[b]	*A2058*[b]	*EBC-1*[b]
1	*Dau=Aoa*-SYENFSA-NH_2	NOT SOLUBLE	66.0±1.9	58.3±1.0	40.1±0.6
2	*Dau=Aoa*-KSYENFSA-NH_2	117.1±3.5	62.6±3.2	68.9±1.9	83.1±4.6
3	*Dau=Aoa*-LRRYKSYENFSA-NH_2	107.4±4.4	82.1±2.4	65.6±3.0	75.8±2.0
4	*Dau=Aoa*-LRRYK(*Dau=Aoa*)SYENFSA-NH_2	128.0±13.5	56.3±7.6	55.8±2.4	62.8±5.9
5	*Dau=Aoa*-IVRGRVF-NH_2	89.7±12.0	69.6±1.1	58.0±3.1	50.0±0.5
6	*Dau=Aoa*-KIVRGRVF-NH_2	105.3±6.4	92.8±2.3	69.4±2.7	78.4±1.8
7	*Dau=Aoa*-LRRYKIVRGRVF-NH_2	125.0±7.4	63.7 ± 6.7	83.5±6.1	74.4±2.0
8	*Dau=Aoa*-LRRYK(*Dau=Aoa*)IVRGRVF-NH_2	122.6±9.0	63.4±6.7	56.0±2.5	70.7±3.7
9	*Dau=Aoa*-PFWSGAV-NH_2	23.2 ± 3.3	23.8 ± 0.7	38.6 ± 2.6	26.0 ± 0.6
10	*Dau=Aoa*-KPFWSGAV-NH_2	12.0 ± 2.5	19.6 ± 0.1	41.4 ± 5.0	24.8 ± 0.4
11	*Dau=Aoa*-LRRYKPFWSGAV-NH_2	85.7 ± 17.2	28.9 ± 0.3	54.7 ± 1.7	35.1 ± 2.7
12	*Dau=Aoa*-LRRYK(*Dau=Aoa*)PFWSGAV-NH_2	10.5 ± 0.5	19.4 ± 0.2	26.8 ± 1.7	30.2 ± 0.3
13	*Dau=Aoa*-TNleAPSIK-NH_2	31.5 ± 3.5	23.0 ± 0.5	44.0 ± 8.2	32.8 ± 0.8
14	*Dau=Aoa*-KTNleAPSIK-NH_2	120.3 ± 3.6	40.1 ± 2.2	82.7 ± 5.5	64.4 ± 3.3
15	*Dau=Aoa*-LRRYKTNleAPSIK-NH_2	37.5 ± 2.4	22.7 ± 0.3	43.5 ± 4.7	31.8 ± 1.1
16	*Dau=Aoa*-LRRYK(*Dau=Aoa*)TNleAPSIK-NH_2	49.8±1.5	79.3±7.3	26.9±5.8	48.8±2.6

[a]*x-Celligence System;*[b]*Alamar blue assay*

Table 2. Effect of the conjugates of Ala-scan on PANC-1 and further cell-lines as control.

CODE	CONJUGATES	***Viability % in 10^{-5} M concentration, after 72 h incubation***			
		PANC-1[a]	*Colo-205*[b]	*A2058*[b]	*EBC-1*[b]
17	*Dau=Aoa*-KPFWSGAA-NH_2	99.5±4.6	66.0±1.9	58.3±1.0	40.1±0.6
18	*Dau=Aoa*-KPFWSAAV-NH_2	101.7±12.1	63.2±1.4	53.1±1.9	33.9±0.5
19	*Dau=Aoa*-KPFWAGAV-NH_2	120.5±18.9	70.7±2.5	71.2±7.7	70.6±3.7
20	*Dau=Aoa*-KPFASGAV-NH_2	127.3±21.2	79.2±5.4	73.8±10.2	70.5±1.2
21	*Dau=Aoa*-KPAWSGAV-NH_2	133.0±7.4	73.3±6.4	73.5±3.7	63.0±2.9
22	*Dau=Aoa*-KAFWSGAV-NH_2	48.6±1.0	47.5±1.1	32.9±0.9	27.3±0.2
23	*Dau=Aoa*-APFWSGAV-NH_2	121.9±19.2	63.8±4.8	73.0±2.9	68.5±1.7

[a]*x-Celligence System;*[b]*Alamar blue assay*

In contrast, among the conjugates derived from the homing peptide family **III** or **IV** there were active compounds. Conjugates **10** and **12** showed significant antitumor effect on PANC-1 cells. Treatments with these conjugates at 10^{-5} M concentration resulted in lower than 15% viability of cells. Further four derivatives (conjugates **9**, **13**, **15** and **16** have moderate effect on PANC-1 cells. They generated lower than 50% viability.

Conjugates **10** and **12** have low, but significant selectivity to PANC-1 cells, while conjugates **9, 13, 15** and **16** showed approximately similar cytotoxic effect on all cell lines.

The presence of different enzyme labile spacer does not significantly enhance the effectivity of conjugates belong to the groups **III** and **IV**.

Ala-scanning of conjugate **10** showed that the alanine substitution resulted in the loss of efficiency in all cases except conjugate **22** which has moderated antitumor effect.

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