Development of daunomycin-peptide conjugates for pancreatic tumor targeting

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INTRODUCTION

The Pancreatic Ductal Adenocarcinoma (PDAC) is a type of the most dangerous cancerous diseases of the pancreas leading to high mortality [1]. Here we report peptide – drug conjugates, in which daunomycin as anticancer agent is linked *via* oxime bond to different types of homing peptides.

In the literature several peptides are mentioned that can recognize pancreatic cancer cells with high affinity. For this study three of them were selected. One of them is the 8-13 fragment of neurotensin (R^8RPYIL^{13}) which binds to neurotensin receptors overexpressed on several type of tumors [2]. The second one that was identified by phage display technique is CKAAKN that was efficiently used to prepare PDAC-cell targeting nanoparticles [3]. Finally, we selected the KTLLPTP sequence, which is able to recognize plectin, a protein that is overexpressed by PDAC-cells [4].

Based on this background we designed:

- two plectin targeting peptide based,
- five neurotensin$^{8\text{-}13}$ based ($K^6P^7R^8R^9P^{10}Y^{11}I^{12}L^{13}$),
- four CKAAKN based peptide daunomycin conjugates,
  (In four cases the cysteine was replaced by serine.)
- two daunomycin-conjugate, which combines the previous two sequences by a tioeter bond between the cysteine of *Ac*-CKAAKN-*OH* and the chloroacetyl group which was built into the side chain amino group of lysine, which can be found in the neurotensin$^{8\text{-}13}$ based sequences.

In case of seven conjugates GFLG spacer was built into the sequences between the homing peptide and the aminooxy moiety. This spacer is cleavable by the lysosomal enzyme Cathepsin B [5], therefore it is suitable to enhance the intracellular degradation and the release of active metabolite (*Dau=Aoa*-Gly-*OH*)..

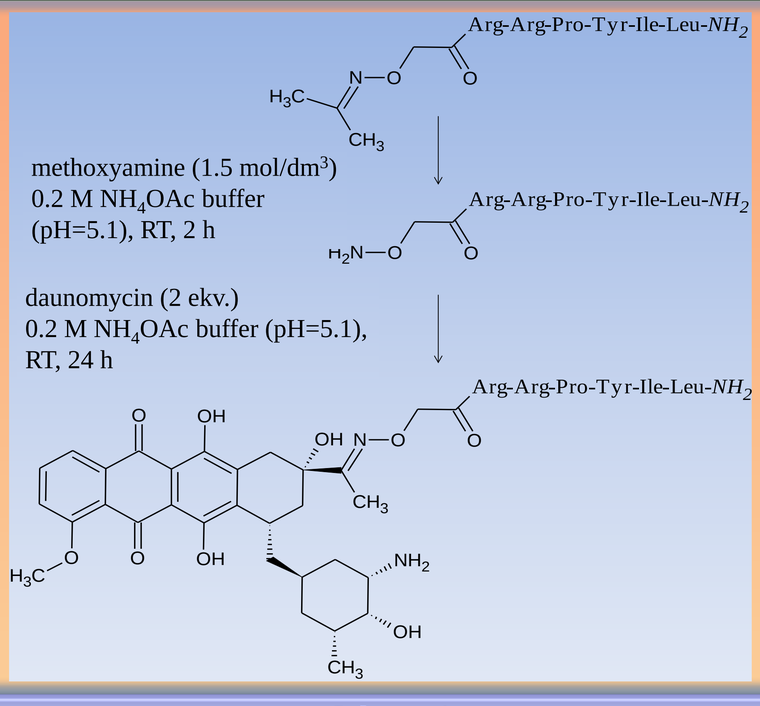
AIMS

- Design and synthesis of effective PDAC-cell targeting anti-cancer drug-conjugates, based on various homing peptides
- Characterization of their cytotoxicity effect on PDAC and other cancerous cell types.
- Laying down of structure − activity relationships (SAR)
- Design and synthesis of more effective conjugates, based on the structure − activity relationships

RESULTS

I. Synthesis

The peptides were synthesised by solid phase peptide synthesis using manual Fmoc/^{t}Bu strategy.



Scheme 1.
Outline of a typical synthesis of daunomycin peptide-conjugates with oxime bond

The *in vitro* cytotoxic effect of conjugates was investigated either by an impedimetric technique, xCELLigence System (PANC-1) or AlamarBlue assay (Colo-205, A2058, EBC-1).
The effect of the conjugates were characterized on PANC-1 (pancreatic ductal adenocarcinoma); Colo-205 (colon adenocarcinoma); A2058 (amelanotic melanoma); EBC-1 (squamous cell lung carcinoma) cell lines.

II. Effect on PANC-1 cell-line

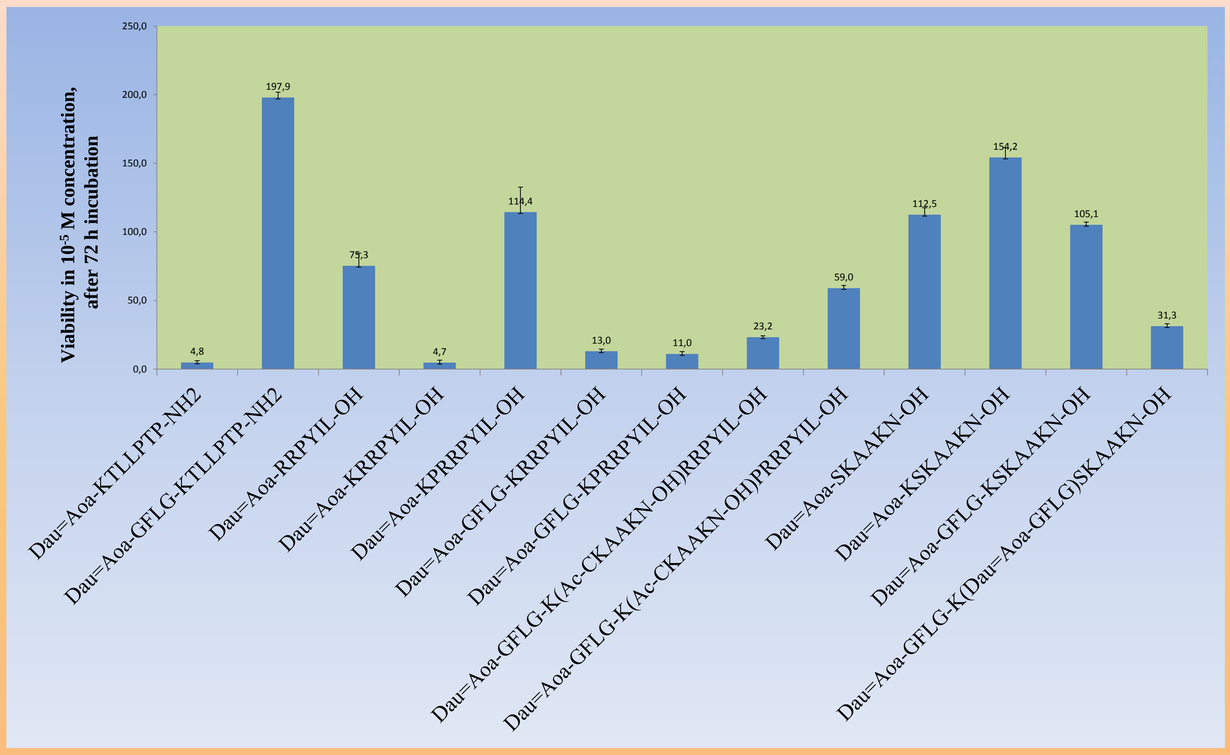


Figure 1.
The cytotoxic effect of various daunomycin – peptide conjugates on PANC-1 cells

III. Effect on further cancer cell lines

	Conjugates	Viability in 10^{-5} M concentration, after 72 h incubation			
		PANC-1	Colo-205	A2058	EBC-1
1.	*Dau=Aoa*-KTLLPTP-NH_2	4.8 ± 1.25 %	31.8 ± 1.82 %	27.4 ± 0.85 %	61.2 ± 2.54 %
2.	*Dau=Aoa*-GFLG-KTLLPTP-NH_2	197.9 ± 3.91 %	42.1 ± 1.74 %	60.4 ± 2.30 %	85.2 ± 3.28 %
3.	*Dau=Aoa*-RRPYIL-*OH*	75.3 ± 9.08 %	50.6 ± 3.57 %	56.4 ± 4.59 %	44.1 ± 3.11 %
4.	*Dau=Aoa*-KRRPYIL-*OH*	4.7 ± 1.78 %	32.4 ± 0.45 %	19.5 ± 0.64 %	62.7 ± 1.02 %
5.	*Dau=Aoa*-KPRRPYIL-*OH*	114.8 ± 18.16 %	NO DATA	NO DATA	NO DATA
6.	*Dau=Aoa*-GFLG-KRRPYIL-*OH*	13.0 ± 1.47 %	31.3 ± 0.55 %	27.2 ± 2.30 %	37.0 ± 1.26 %
7.	*Dau=Aoa*-GFLG-KPRRPYIL-*OH*	11.0 ± 1.70 %	30.4 ± 0.85 %	28.7 ± 2.49 %	36.9 ± 0.63 %
8.	*Dau=Aoa*-GFLG-K(*Ac*-CKAAKN-*OH*)RRPYIL-*OH*	23.2 ± 1.14 %	38.4 ± 1.83 %	40.1 ± 5.49 %	48.1 ± 0.87 %
9.	*Dau=Aoa*-GFLG-K(*Ac*-CKAAKN-*OH*)PRRPYIL-*OH*	59.0 ± 1.97 %	46.6 ± 1.48 %	50.6 ± 6.31 %	48.9 ± 3.2 %
10.	*Dau=Aoa*-SKAAKN-*OH*	112.5 ± 5.06 %	50.7 ± 1.63 %	61.5 ± 2.68 %	77.8 ± 1.03 %
11.	*Dau=Aoa*-KSKAAKN-*OH*	154.2 ± 7.12 %	51.0 ± 2.47 %	63.5 ± 4.64 %	90.2 ± 3.04 %
12.	*Dau=Aoa*-GFLG-KSKAAKN-*OH*	105.1 ± 1.93 %	59.3 ± 3.59 %	79.4 ± 17.57 %	76.2 ± 13.73 %
13.	*Dau=Aoa*-GFLG-K(*Dau=Aoa*-GFLG)SKAAKN-*OH*	31.3 ± 1.84 %	45.8 ± 2.63 %	61.9 ± 11.64 %	57.6 ± 11.12 %

viability < 20% | 20% < viability < 50% | 50% < viability < 80% | 80% < viability < 100%

Table 1.
Cytotoxic effects of conjugates on various tumor cell lines

CONCLUSIONS

- Four conjugates (**1, 4, 6, 7**) have significant cytotoxic effect at 10^{-5} M concentration. In the case of two of them (**1, 4**) the viability was lower, then 10%.
- Conjugates **1** and **4** have significant selectivity to PANC-1 cells, while **6** and **7** give approximately similar cytotoxic effect on all cell lines.
- The absence of proline^7 causes similar (**6** vs. **7**) or better (**4** vs. **5**) cytotoxic effect of neurotensin based conjugates.
- The plectin targeting sequence based daunomycin conjugate (**1**) has high cytotoxic effect, but the incorporation of GFLG spacer abolishes the effect on PDAC-cells
- The combination of **6** and **7** conjugates with *Ac*-CKAAKN-*OH* peptide (**8** and **9**) decreases the effect.
- From the SKAAKN based conjugates only one peptide derivative (**13**) (which contains *Dau=Aoa*-GFLG moiety on both α- and ε-amino group of lysine) has significant cytotoxic effect.

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ACKNOWLEDGEMENTS

This study was supported by grant from: National Research, Development and Innovation Office (NKFIH K119552 and NVKP_16-1-2016-0036). L.E. Dókus acknowledges the support for Foundation for the Hungarian Peptide and Protein Research, Budapest, Hungary and Pázmány Eötvös Foundation, Budapest, Hungary.