STUCTURE –ACTIVITY RELATIONSHIP OF PANC-1 SPECIFIC SMALLPEPTIDE BASED DRUG DELIVERY SYSTEMS

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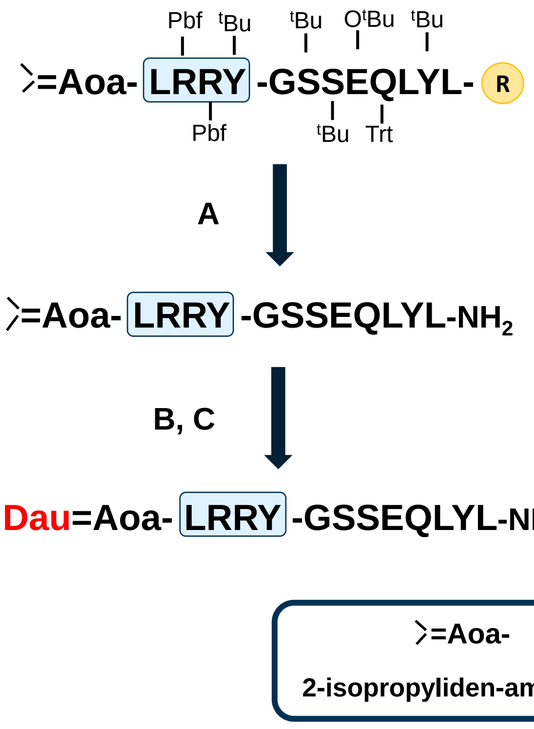
Introduction

Pancreatic ductal adenocarcinoma (PDAC) carries an extremely poor prognosis, generally shows high metastatic activity and exhibits profound resistance to existing therapies. Thus, development and identification of selective and targeted biomarkers as new therapeutic agents for PDAC is of importance. [1] Therefore, our aim was to design a peptide-based conjugate, specific for PDAC. For this reason, as a targeting molecule, we have choosen a peptide discovered by phage display. The targeting peptide sequence (GSSEQLYL) previously showed high selectivity for PANC-1 cell line*, in vivo* [2].

In our study we aimed to optimize the structure of the homing peptide and thereby enhance the anti-tumor activity of the formed conjugate. Through our work, we have modified the conjugate, by changing the position of the spacer, or by using Ala-scan to identify a position in which amino acid substitution can be performed for better biological activity.

Synthesis

Conjugates were synthetised manually on solid phase, using Fmoc/ ^{t}Bu technique following in solution drug conjugation

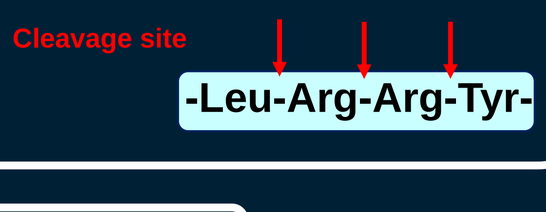


=Aoa-
2-isopropyliden-aminooxy-

a. Cleavage from the resin: 2.5% TIS/ 2.5% H_2O/ 95% TFA
b. Deprotection of Aoa: 0.2 M NH_4OAc buffer/ 1M methoxylamine
c. Daunomycin conjugation: 0.2 M NH_4OAc buffer (pH 5.0)

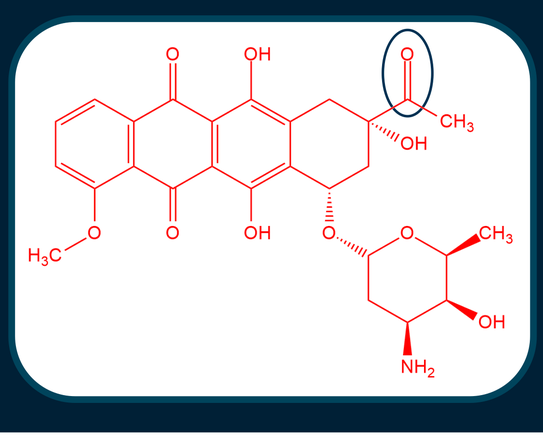
Spacer

We have incorporated a Chatepsyn B enzyme labile spacers into our conjugates to ensure selective drug realese in .



Conjugated drug

Daunomycin (Dau) is a pontent anti-cancer drug, that belonges to the anthracyclin family and has high flurescence activity. It can be effectively conjugated through oxime ligation in the C13 position.



Biological studies

- **Viability of human pancreas PANC1 cell line was tested using impedimetry (xCELLigence SP System)**
- **For control, three different cell lines were used: Colo205- colon adenocarcinoma; A2058- human melanoma; EBC-1- human lung squamous cell carcinoma**

	Compound	Viability, 10µM, 72 h			
		PANC1	Colo-205	A2058	EBC-1
I.	H-GSSEQLYLK-NH_2 Dau=Aoa-LRRY	125.9± 1.8 %	72.2± 0.7 %	87.8± 1.6%	93.1± 4.9 %
	Dau=Aoa-LRRY-GSSEQLYL-NH_2	8.6± 0.3 %	63.1± 0.5 %	64.5± 2.2 %	39.0± 2.2%
	Compound	**PANC1**	**Colo-205**	**A2058**	**EBC-1**
II.	Dau=Aoa-LRRY-GSSEQLY**A**-NH_2	98.7± 0.5%	79.6± 0.9%	91.1± 1.6%	86.3± 1.0 %
	Dau=Aoa-LRRY-GSSEQL**A**L-NH_2	102.8± 1.7%	83.4± 1.2%	85.1± 3.0%	89.3± 1.6%
	Dau=Aoa-LRRY-GSSEQ**A**YL-NH_2	14.9± 0.2%	69.2± 1.1%	65.2± 0.5%	36.8± 1.0%
	Dau=Aoa-LRRY-GSSE**A**LYL-NH_2	47.1± 0.9%	85.7± 2.1%	84.3± 1.1%	71.9± 0.7%
	Dau=Aoa-LRRY-GSS**A**QLYL-NH_2	93.0± 1.3%	86.4± 0.8 %	85.3± 2.3%	88.1± 2.2%
	Dau=Aoa-LRRY-GS**A**EQLYL-NH_2	35.9± 0.2%	84.4± 1.1%	54.7± 0.7%	57.5± 1.0%
	Dau=Aoa-LRRY-G**A**SEQLYL-NH_2	92.9± 2.3%	93.5± 2.6%	99.7± 1.4%	90.0±3.2%
	Dau=Aoa-LRRY-**A**SSEQLYL-NH_2	53.7 ± 3.0%	101.9± 1.0%	102.7± 4.7 %	90.8± 5.0%
	Compound	**PANC1**	**Colo-205**	**A2058**	**EBC-1**
III.	Dau=Aoa-LRRY-GSSEQ**N**YL-NH_2	101.1± 3.1%	55.5± 0.3%	65.7± 3.2 %	76.2± 3.0%
	Dau=Aoa-LRRY-GSSEQ**P**YL-NH_2	97.0± 2.7%	67.5± 0.6%	71.7± 4.0%	76.0± 2.1%
	Dau=Aoa-LRRY-GSSEQ**S**YL-NH_2	96.1± 3.1%	68.6± 5.7%	83.1± 3.2%	89.7± 1.3%
	Dau=Aoa-LRRY-GSSEQ**W**YL-NH_2	86.0± 1.7%	36.9± 1.0%	52.8± 1.8%	45.2± 1.1%
	Dau=Aoa-LRRY-GSSEQ**I**YL-NH_2	15.0± 0.5%	68.7± %	83.6± 0.5%	78.1± 0.4%
	Dau=Aoa-LRRY-GSSEQ**Nle**YL-NH_2	70.8± 1.1%	47.1± 3.2%	63.1± 5.1%	57.0± 4.3%
	Dau=Aoa-LRRY-GSSEQ**F**YL-NH_2	57.3± 4.1%	56.4± 1.0%	35.4± 0.9%	51.6± 2.1%
	Dau=Aoa-LRRY–GSSEQ**F(4Cl)**YL-NH_2	9.7± 0.3%	25.8± 0.9%	12.5 ±0.7%	43.7± 0.7%

Conclusions

- **The results indicated that Leu^6 can be replaced by Ala, thus further replacements were done in this position.**
- **Substitution of Leu^6 with Ile or Nle resulted in worse biological activity compared to the original sequence.**
- **4-chloro-phenylalanine in position 6 showed similar activity as the original sequence on PANC1 cell line. However, this conjugate also showed significant activity on A2058 cells.**

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References

[1] M. Hyvönen, P. Laakkonen, *Methods Mol Biol.* **2015**. 1324, 205-22. [2] D. Bedi, J. W. Gillespie, V. A. Petrenko. *Protein Eng Des Sel.* **2014**. 27, 235–243.