



Supplementary Part

Improved In Vivo Anti-Tumor and Anti-Metastatic Effect of GnRH-III-Daunorubicin Analogs on Colorectal and Breast Carcinoma Bearing Mice.

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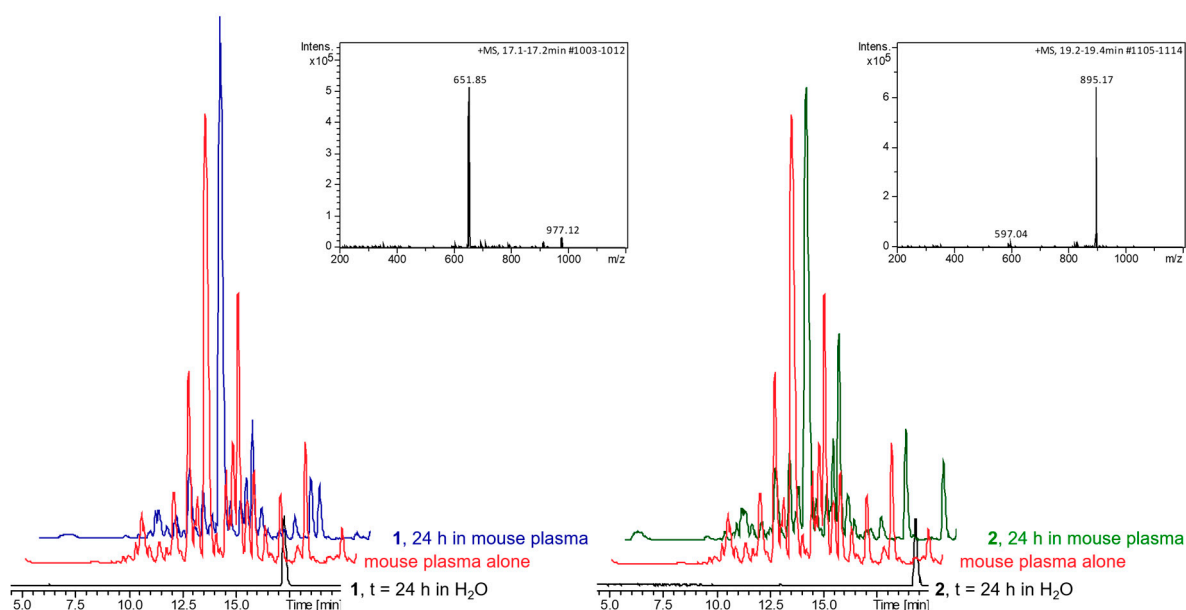
Stability of GnRH-III-Dau conjugates in mice plasma

Figure S1. Stability of the GnRH-III-Dau conjugates 1 and 2 in mouse plasma. LC-MS chromatogram of mouse plasma and the conjugates after 24 hours incubation at 37 °C in ddH₂O or in human plasma plus the corresponding MS compound spectra.

GnRH-R mRNA, absolute protein and cell surface expression level compared to MRC-5 and cellular uptake rates of conjugates 1 and 2

Table S1. GnRH-R mRNA, absolute protein and cell surface expression level compared to MRC-5. Cellular uptake of conjugates 1 and 2 (40 μ M), after 6 h treatment of the cell lines. Values from 2 independent experiments are shown.

	MRC-5	MDA-MB-231	HT-29	A2780	PANC-1	U87MG	4T1
mRNA	1.0	5.1	3.3	1.6	1.4	0.5	n.d.
	1.0	9.5	5.9	5.3	2.2	1.1	n.d.
Protein	1.0	1.5	2.7	5.8	0.9	4.5	4.3
	1.0	1.0	0.6	2.0	0.5	2.6	2.5
Cell surface	1.0	6.3	2.2	6.5	4.7	3.1	4.2
	1.0	5.0	1.8	4.7	3.4	4.3	3.4
Uptake Conjugate 1	8.1	12.0	12.4	12.2	5.8	12.9	13.0
	8.3	18.2	22.4	22.3	6.4	14.8	21.1
Uptake Conjugate 2	14.9	19.9	21.0	26.3	15.4	22.3	26.8
	15.3	34.0*	38.3*	42.8**	17.9*	26.8*	38.8**

n.d. = not determined. ¹ Relative GnRH-R mRNA expression normalized to β -actin and GAPDH. ² Relative GnRH-R protein expression normalized to β -actin. ³ Relative GnRH-R Geo MFI. ⁴ Relative Geo MFI normalized to auto fluorescence. Statistical analysis for uptake studies of conjugates 1 and 2 was performed by 2way ANOVA test. * and ** mean significant at $p < 0.05$ and $p < 0.01$, respectively.

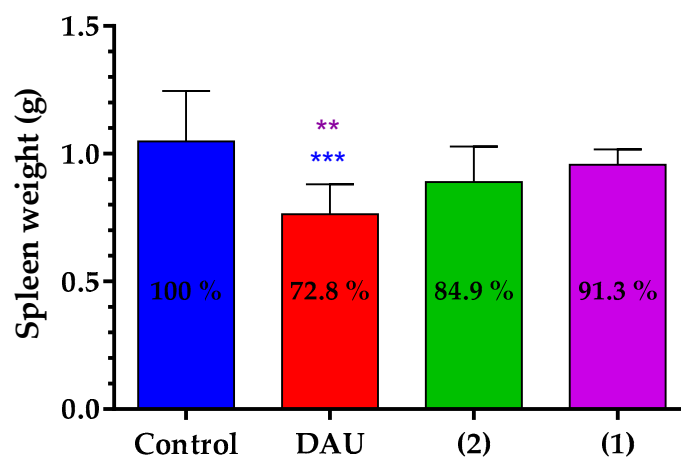
Spleen weight in 4T1 tumor bearing mice

Figure S2. Spleen weight in 4T1 tumor bearing mice (grams, average \pm SD) after termination of experiment, 28 days subsequent to cells inoculation, 7 animals per group, statistical analysis was performed by Mann-Whitney test. ** and *** mean significant at $p < 0.01$, and $p < 0.001$, respectively. * blue and purple mean significant difference compared to control and 1 groups respectively.



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