Abstract #1657

Structure-activity relationship studies and biological evaluation of novel maytansinoids, a class of highly selective tubulin inhibitors

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INTRODUCTION & RATIONALE

Maytansine and its analogs (e.g. DM1 and DM4) are potent microtubule-targeting compounds with a narrow therapeutic window.

So far, only T-DM1, an antibody-maytansinoid conjugate targeting the HER2 receptor, has been approved for the treatment of Herceptin®-resistant breast cancer.

The goal of this work is:

- The discovery of novel potent derivatives of maytansine for the development of maytansinoid-based drug delivery systems
- Elucidation of structure-activity as well as structure-stability relationships from a spectrum of 35 novel maytansinoids (screening in cell-based cytotoxicity assays and evaluation of their stability in plasma).

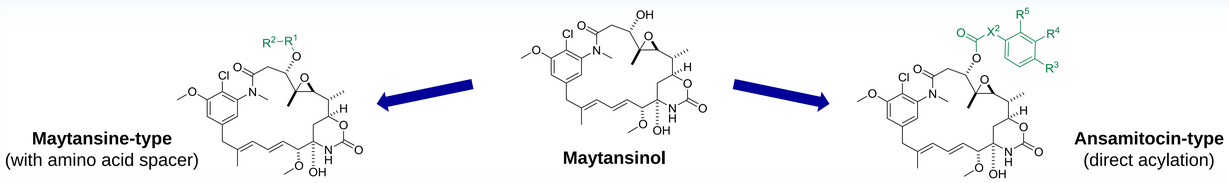
In summary, a library of 35 different maytansinoids was synthesized by esterification of maytansinol with various anchor molecules in order to introduce an attachment point (ketone) for conjugation chemistry.

The obtained compounds can be classified into maytansine-type derivatives (May) bearing an amino acid spacer and ansamitocin-type derivatives (Ansa or AnsC) which are obtained through direct acylation with the anchor molecule.

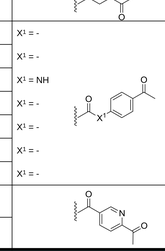
EXPERIMENTAL PROCEDURES

Cytotoxicity Assay: Cells are harvested from exponential phase cultures, counted and plated in 96-well flat-bottom microtiter plates at a cell density ranging from 4,000 to 60,000 cells. After a 24 hours recovery period, compounds are applied in half-log dilution steps at 10 concentrations in duplicate and cells are treated continuously for 96 h, followed by a CellTiter-Blue® based fluorescent read out. Sigmoidal concentration response curves are fitted to the data points (T/C values) obtained for each cell line using 4 parameter non-linear curve fit (Oncotest Warehouse Software). IC_{50} values are reported as relative IC_{50} values defined as the concentration of test compound that inhibit colony formation/viability half way between the top and bottom plateau of the sigmoidal concentration response curve.

Plasma Stability Assay: Blood plasma from different species is filtered with a 0.45 µm CA membrane and pre-incubated at 37 °C for 20 min. Then the compound stock solution is added (100 µM or 300 µM in DMSO) resulting in a 10 % solution in plasma and again pre-incubated for 20 min. Aliquots are taken at 0 h, 1 h, 3 h, 4 h, 21 h and 24 h, precipitated in the presence of the internal standard (5 µg/mL maytansine or ansamitocin P3) with an Impact Strata® precipitation plate. The supernatant is subsequently analyzed by LC-MS. The experiments were performed in triplicate.



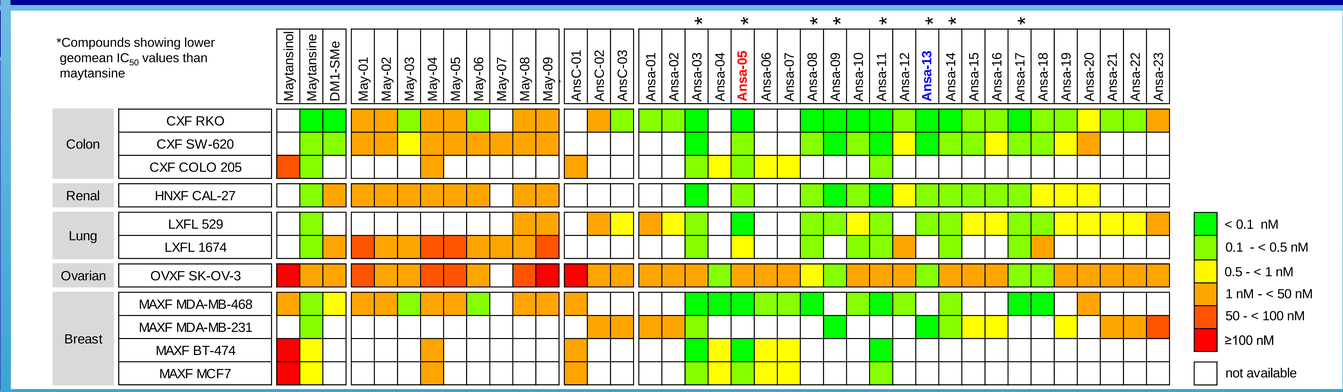
SYNTHESIZED COMPOUNDS & STABILITY IN BLOOD PLASMA

Name	R^1	R^2	Remaining in murine plasma after 4 h (24 h) [%]
Maytansinol	**H**	**-**	97 (83)
Maytansine	-*N*-Me-Ala-	 (Ac)	68 (68)
DM1-SMe	-*N*-Me-Ala-		5 (2)
May-01	-*N*-Me-Ala-		91 (91)
May-02	-*N*-Me-Ala-		85 (73)
May-03	-Pro-		69 (68)
May-04	-Pro-		89 (32)
May-05	-β-Ala-		93 (72)
May-06	-Gly-		56 (7)
May-07	-*N*-Et-Gly-		94 (91)
May-08	-*N*-Me-Gly-		93 (75)
May-09	-*N*-Me-Gly-		82 (81)
Ansa-23	-		14 (<1)

	X^2	R^3	R^4	R^5	Remaining in murine plasma after 4 h (24 h) [%]
AnsC-01	-NH-	Ac	H	H	94 (48)
AnsC-02	-NH-CH_2-	Ac	H	H	94 (53)
AnsC-03	-O-CH_2-	Ac	H	H	97 (56)

Name	X^2	R^3	R^4	R^5	Remaining in murine plasma after 4 h (24 h) [%]
Ansa-01	-	Ac	H	H	41 (2)
Ansa-02	-	H	Ac	H	96 (67)
Ansa-03	-CH_2-	Ac	H	H	92 (67)
Ansa-04	-CH_2-	Ac	F	H	60 (58)
Ansa-05	-CH_2-	Ac	H	F	88 (68)
Ansa-06	-CH_2-	Ac	NO_2	H	92 (85)
Ansa-07	-CH_2-	Ac	H	NO_2	99 (88)
Ansa-08	-CH_2-CH_2-	Ac	H	H	89 (69)
Ansa-09	-CH_2-CH_2-CH_2-	Ac	H	H	96 (78)
Ansa-10	-CH_2-N(CH_3)-	Ac	H	H	90 (61)
Ansa-11	-CH_2-O-	Ac	H	H	78 (33)
Ansa-12	-C(CH_3)$_2$-O-	Ac	H	H	87 (<1)
Ansa-13	-CH_2-O-	H	Ac	H	87 (50)
Ansa-14	-CH_2-S-	Ac	H	H	66 (36)
Ansa-15	-CH_2-S-	Ac	H	OCH_3	86 (68)
Ansa-16	-CH_2-S-	Ac	H	CH_3	89 (72)
Ansa-17	-CH_2-S-	Ac	H	Cl	90 (86)
Ansa-18	-CH_2-S-	Ac	H	Br	(n/a) (91)
Ansa-19	-CH_2-S-	Ac	H	CF_3	89 (74)
Ansa-20	-CH_2-S-	Ac	H	NO_2	93 (69)
Ansa-21	-	Ac	F	H	26 (<1)
Ansa-22	-	Ac	NO_2	H	24 (<1)

EVALUATION IN TUMOR CELL LINES



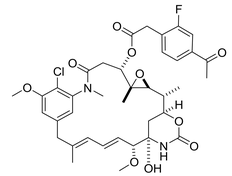
CONCLUSIONS

A total of 35 maytansinoids were synthesized and evaluated regarding cytotoxicity and stability *in vitro*:

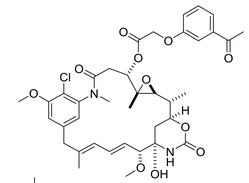
In the case of maytansine-type derivatives an *N*-substituted amino acid spacer is vital for stability in plasma. Ansamitocin-type derivatives with a substituent at R^4 or R^5 tend to be more stable in plasma. Moreover, the modification of the X^2 position influences the stability of these derivatives in murine plasma.

In cell-based cytotoxicity assays, eight compounds were identified that are equally or even more active than maytansine (mean IC_{50} values < 222 pM). Compounds of the ansamitocin-type generally tend to be more active than those of the maytansine-type.

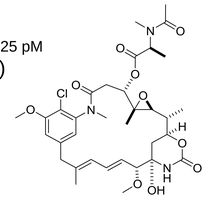
Based on their cytotoxicity and stability profile, several of these derivatives were evaluated as albumin-binding drugs *in vivo*, and two of them, the highly potent ansamitocin-type derivatives **Ansa-05** and **Ansa-13**, were selected for further development (see Poster **#2661**):



Ansa-05
Geomean IC_{50} = 125 pM
(10 cell lines)



Ansa-13
Geomean IC_{50} = 95 pM
(6 cell lines)



Maytansine
Geomean IC_{50} = 222 pM
(11 cell lines)

Overview of the stability in blood plasma of maytansine and the selected hit compounds **Ansa-05** and **Ansa-13**

	Remaining in Plasma after 4 h (24 h) [%]		
	Maytansine	**Ansa-05**	**Ansa-13**
Mouse	68 (68)	88 (68)	87 (50)
Rat	95 (n/a)	96 (78)	88 (43)
Beagle	96 (95)	98 (80)	93 (75)
Human	96 (93)	92 (75)	94 (42)