Development and Characterization of a HER2-Targeting Antibody-Drug Conjugates Based on CPL410005 UBA1 inhibitor

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INTRODUCTION

HER2-overexpessing cancers, such as breast and ovarian tumors, often exhibit resistance to standard therapies, necessitating novel treatment strategies. Antibody-drug conjugates (ADCs) is a targeted approach by delivering cytotoxic agents selectively to tumor cells while sparing healthy tissues. From our previous research we demonstrated a potent UBA1 (E1) inhibitor CPL410005 (**1**) that was characterized by a strong anticancer activity, however a narrow therapeutical window and off-target effects were observed. Therefore, this study aims to design and synthesize novel group of antibody-drug conjugates in which CPL41005 inhibitor has a role of a toxic payload fragment, incorporated into self-immolative linkers based on structures of glucuronide and galactose that can be selectively hydrolysed by intracellular glycoside hydrolases.

MATERIALS AND METHODS

In vitro assays. Stability assays were conducted at physiological and tumor-like conditions to evaluate linker integrity and assessed using HPLC-MS/MS, HPLC-TOF-MS). Enzymatic cleavage of colorymetric substrates (X-gluc, B-Gal) were performed to confirm the controlled release of the payload. Antibody binding assay was performed with Biacore S200 SPR system (Cytiva) on a CM5 sensor chip in single-cycle mode. Cytotoxicity of antiHER2-CPL410005 was tested in HER2+ (SKOV-2, SKBR3) and HER2-(HEK292) cell lines.

Conjugation. CPL410005(**1**) was conjugated to antiHER2 antibodies via sugar-based covalent maleimide linkers **11** (β-galactose) and **12** (β-glucuronide). Conjugation efficiency was assessed using RP-HPLC, SEC-UV, and mass spectrometry (HPLC-MS/MS, HPLC-TOF-MS).

In vivo studies. PK studies were performed on SCID mice xenografted with HCT-116 cell line. The plasma and tumor concentrations were analyzed using Ultrahigh performance liquid chromatography coupled with mass spectrometry (UHPLC-MS) with electrospray (ESI) ionization detection, quadrupole.

RESULTS

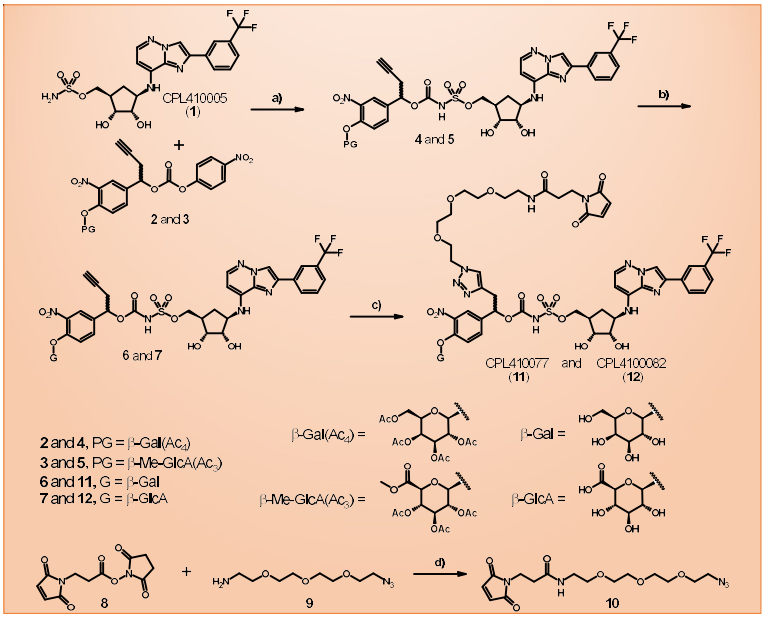


Figure 1. Synthesis of CPL410005-based cleavable linkers 11 and 12. Reaction conditions: a) DMAP, TEA, MS 4 Å, DMF, 60 °C, 26 h, 43%; b) MeONa in MeOH, MeOH, 3 h, 64%; c) **10**, CuI, DMF, rt, 20 h, 35% for **11** and $CuSO_4$ x 5 H_2O in H_2O, TBTA, sodium L-ascorbate, DMF, rt, 4 h, 46% for **12** d) DMF, 45min, in situ;

Synthesis discussion

In the first step, amino group from sulfamate **1** was attached to the linker by the carbamate formation with *p*-nitrophenyl carbonate in **2** and **3** giving corresponding carbonylsulfamates **4** and **5**. The following deprotection with sodium methoxide in case of galactoside or two-step path with additional hydroxide hydrolysis in case of glucuronide derivative led to the corresponding intermediates **6** and **7**. Maleimide group was introduced to the structure of the linker by the 1,3-dipolar cycloaddition in „click" reaction of maleimido-PEG-azide derivative **10** with acetylene function attached to the benzyl group. The reaction of galactoside derivative **6** was catalysed with copper (I) iodide wherein for the glucuronide derivative **7,** copper (II) sulfate with sodium L-ascorbate and TBTA additive was chosen. As a result of the "click" reaction the final galactoside **11** and glucuronide **12** derivatives were obtained respectively.

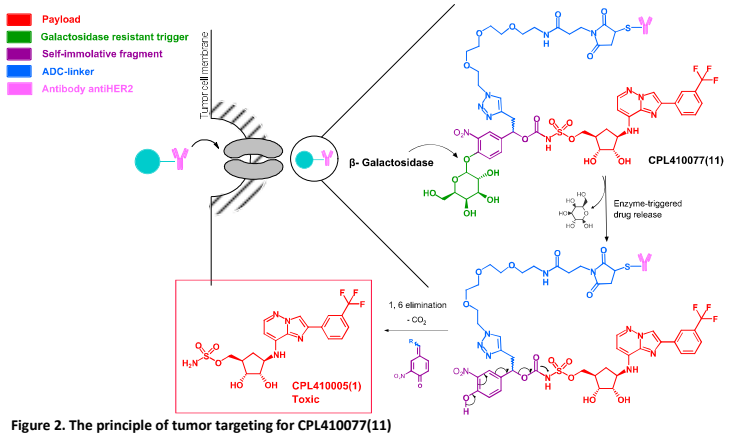
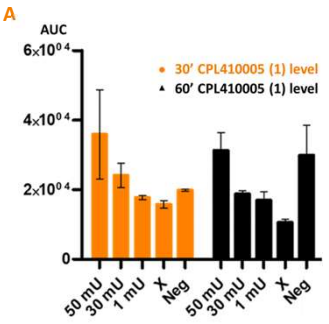


Figure 2. The principle of tumor targeting for CPL410077(11)
Step1: ADC-receptor recognition; Step2: Endocytosis; Step3: β-Galactosidase-catalyzed drug release mechanism

RESULTS



B

CPL410077 (11)

pH	T0 [%]	2 h [%]	4 h [%]	7 h [%]	24 h [%]
4,50	89,16	89,92	89,48	90,93	87,61
6,80	89,16	82,64	74,14	68,07	26,97
7,40	89,16	71,94	52,16	40,31	6,3
8,00	89,16	25,15	6,17	2,28	0,37

CPL410082 (12)

pH	T0 [%]	2 h [%]	4 h [%]	7 h [%]	24 h [%]
4,50	92,52	93,1	93,53	-	90,35
6,80	92,52	88,72	77,47	-	40,72
7,40	92,52	74,58	56,81	-	9,01
8,00	92,52	29,45	8,63	-	0,26

Figure 3. ***In vitro*** **studies of cleavable linkers based on CPL410005 (1). (A)** Enzymatic cleavage confirms *in vitro* **CPL410082 (12)** linker cleavage by β-glucuronidase and **CPL410005 (1)** payload release; payload release in negative control was observed after 60 minutes of incubation. **(B)** 92% of **CPL410005 (1)** was released within 24 hours under tumor-mimicking conditions with application of glycosyl hydrolases (pH 7.4; 37ºC).

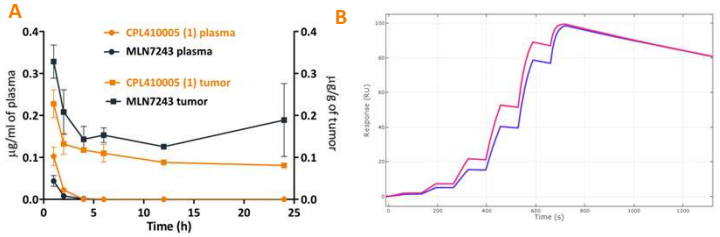


Figure 4. Pharmacokinetics of CPL410005 and ADC binding analysis ***in vitro*****. (A)** Pharmacokinetic analysis in SCID Mice Model showed half-time for the free **CPL410005 (1)** in plasma at $t_{1/2}$ =2.9 h, with much longer retention time in the HCT116 (colon cancer cell-line) tumor. **(B) antiHER2-CPL410077 (11)** ADC retains excellent binding parameters of the antibody (purple) after conjugation with cleavable linkers (pink), with fast association and long dissociation time.

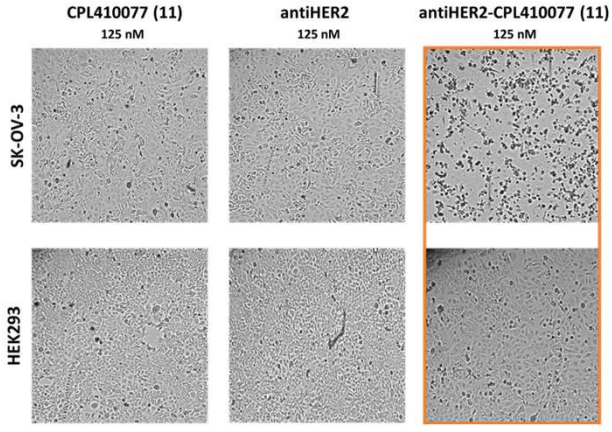


Figure 5. ADC greatly improves CPL410005 (1) selectivity towards cancer cells and reduces toxicity on normal cel-lines. CPL410005 (1) is a potent inhibitor of cell viability of human ovarian adenocarcinoma cell line (SK-OV-3) and the human breast adenocarcinoma cell line (SK-BR-3) at low nanomolar IC50 (17 nM and 40 nM, respectively), human embryonic kidney cell line (HEK293), a normal non-cancerous cell line, IC50 (118 nM). ADC antiHER2-**CPL410077 (11)** effectively kills cancer cells (SK-OV-3) at 125 nM, where unconjugated cleavable linker **CPL410077 (11)** and antiHER2 antibody show no cytotoxicity. HEK293 control cell-line is not affected by the ADC at 125 nM.

CONCLUSIONS AND PERSPECTIVES

We have successfully designed and developed the conjugation of **CPL410005 (1)** to an antiHER2 scaffold that effectively improved tumor targeting and reduced systemic toxicity, while maintaining potent anticancer activity. However, challenges remain in linker stability, requiring further refinement to optimize therapeutic effectiveness and minimize premature drug release. These results support the potential of HER2-targeted UBA1 inhibitors as a promising approach for HER2-overexpressing cancers therapeutic strategy.

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