H2020-ITN THERACAT (765497)

Work Package Number	WP4	**Task Number**	T4.1	**Deliverable Number**	D4.1	**Lead Beneficiary**	EDI
Deliverable Title	Library of anticancer prodrugs						
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Overview/Abstract

A library consisting of 10 different prodrugs of 9 distinct anticancer drugs (5FU, gemcitabine, floxuridine, vorinostat, doxorubicin, SN-38, panobinostat, paclitaxel and dasatinib PROTAC) has been prepared and is now available for use by the consortium members. The prodrugs were synthesized by hemisynthesis or total synthesis approaches.

Explanation for large delay in submitting deliverable

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1. Library of anticancer prodrugs

Introduction

Bioorthogonal reactions are purposely designed to take place in cells and organisms without interfering with biological functions. In the pursuit of exploiting such processes in cancer therapy, reactions and tools that were once exclusively used to synthesize drugs in chemistry labs have been recently adapted to perform such tasks in living systems. Chemotherapeutics as **5FU**,[1,2] **gemcitabine**,[3] **floxuridine**,[4] **vorinostat**,[5,6] **doxorubicin**[6,7] or **SN-38** (active metabolite of irinotecan)[8] can be "manufactured" from inactive precursors in biological environments through a variety of bio-independent processes, including bioorthogonal organometallic catalysis. In combination with a suitable cancer targeting strategy (e.g. intratumoral implantation), these highly selective reactions can facilitate the spatially controlled synthesis of one or more therapeutic agents to localize drug activity at the disease site. While such approaches are yet to demonstrate its utility in the clinic, they have the potential to reduce systemic side effects and enhance treatment efficacy by generating greater drug levels at the disease site than can be safely achieved by systemic pharmacotherapies.

Objectives

The aim of this deliverable was to synthesize a range of metal-activatable prodrugs, including prodrugs already validated at EDI[1-8] and new prodrugs from alternative therapeutic agents, to be tested by other THERACAT consortium partners.

Results and Discussion

The collection of prodrugs synthesized during this task is shown in Figure 1. Palladium/gold-activatable prodrugs **1-7** were synthesized as previously described (30 to 500 mg available),[1-8] while two new prodrugs were also prepared: **8**[9] and **9**[10], which have not yet been published.

Palladium-activatable prodrug **8** was synthesized following an unpublished protocol.[9] Given the essential role of the Zn-chelating hydroxamate group of **panobinostat** in its cytotoxic mode of action, the *O*-alkyl hydroxamate derivative **8** was designed to reduce its capacity to inhibit histone deacetylases (HDACs) while, at the same time, making it sensitive to Pd chemistry.

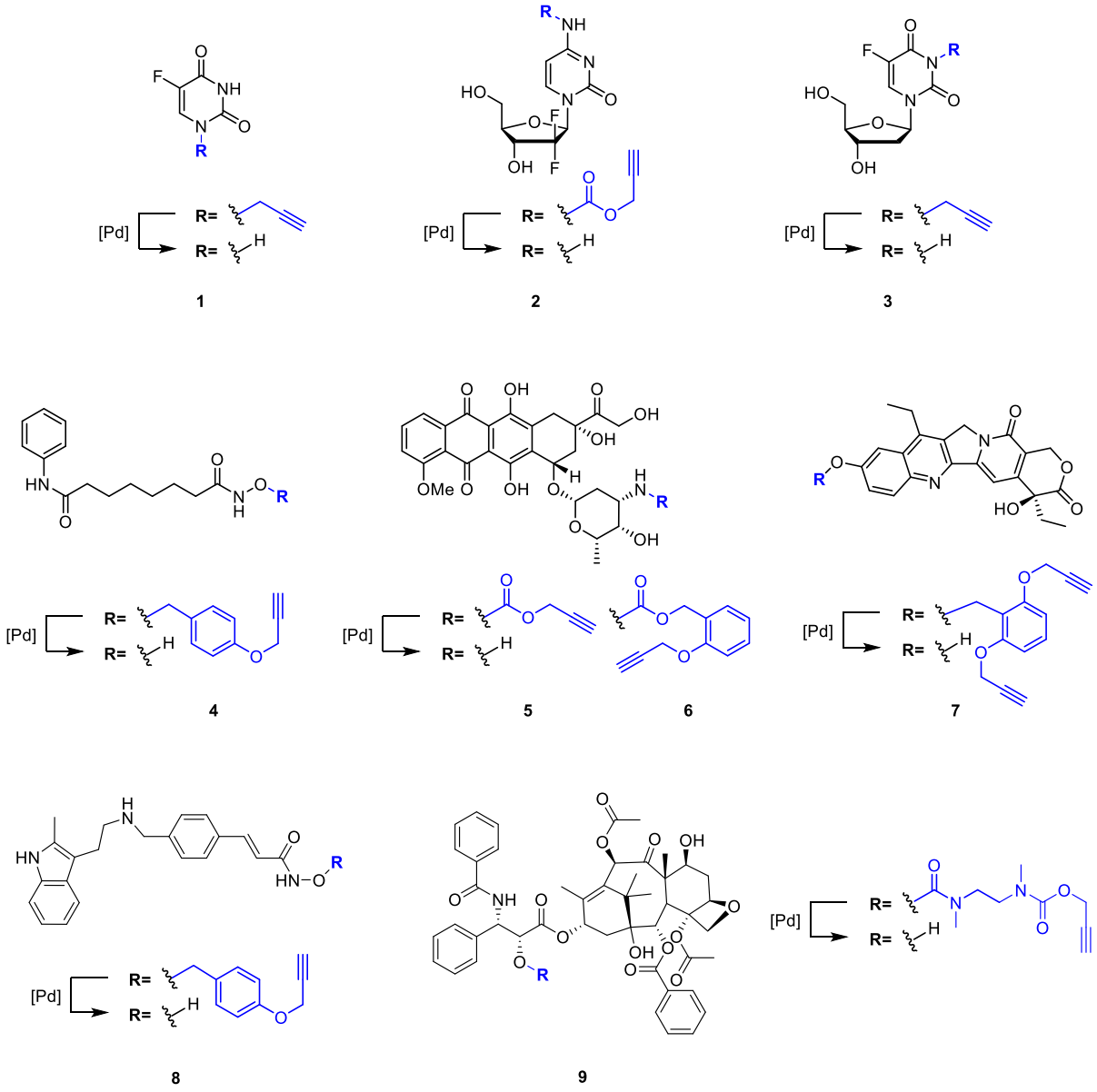

FIGURE 1. Structure of the bioorthogonally-activated prodrugs synthesized in task 4.1.

Direct attempts to alkylate the OH group of **panobinostat** with *p*-(propargyloxy)benzyl bromide were unsuccessful due the presence of various nucleophilic groups in the molecule, resulting in a mix of mono and bis-protected products at different positions, including the indole NH and the alkylamino group. Consequently, prodrug **8** was prepared by total synthesis (see synthesis route in Figure 2). Briefly, Reductive amination of 3-(4-formyl-phenyl)-acrylic acid methyl ester with 2-methyltryptamine followed by basic hydrolysis afforded the β-Substituted-acrylic derivative **E** that was coupled to *p*-(propargyloxy)benzyloxyamine **C** by carbodiimide-mediated coupling to generate prodrug **8** in moderate yield (42 %). Total quantity synthesized: 16 mg.

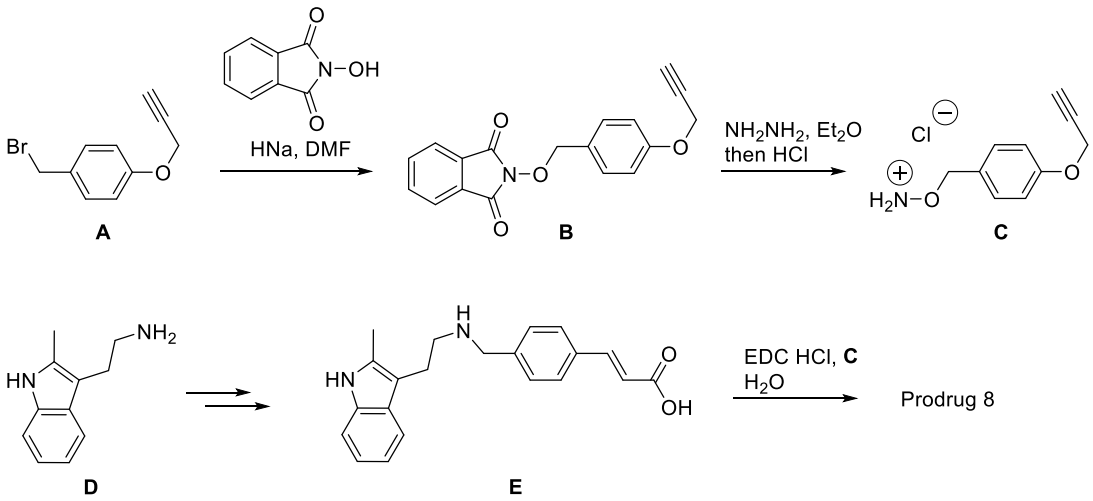

FIGURE 2. Total synthesis of **panobinostat** prodrug **8** in task 4.1.

Paclitaxel-derived prodrug **9** was prepared by a semisynthetic approach by direct functionalization of **paclitaxel** (see synthetic route in Figure 3). The secondary OH group of the side chain of paclitaxel was masked as a carbamate by reaction with p-nitrophenyl chloroformate at -50 °C in pyridine:DCM followed by coupling with amine **10** in the presence of DIPEA and DMF (36% yield). Total quantity synthesized: 213 mg.

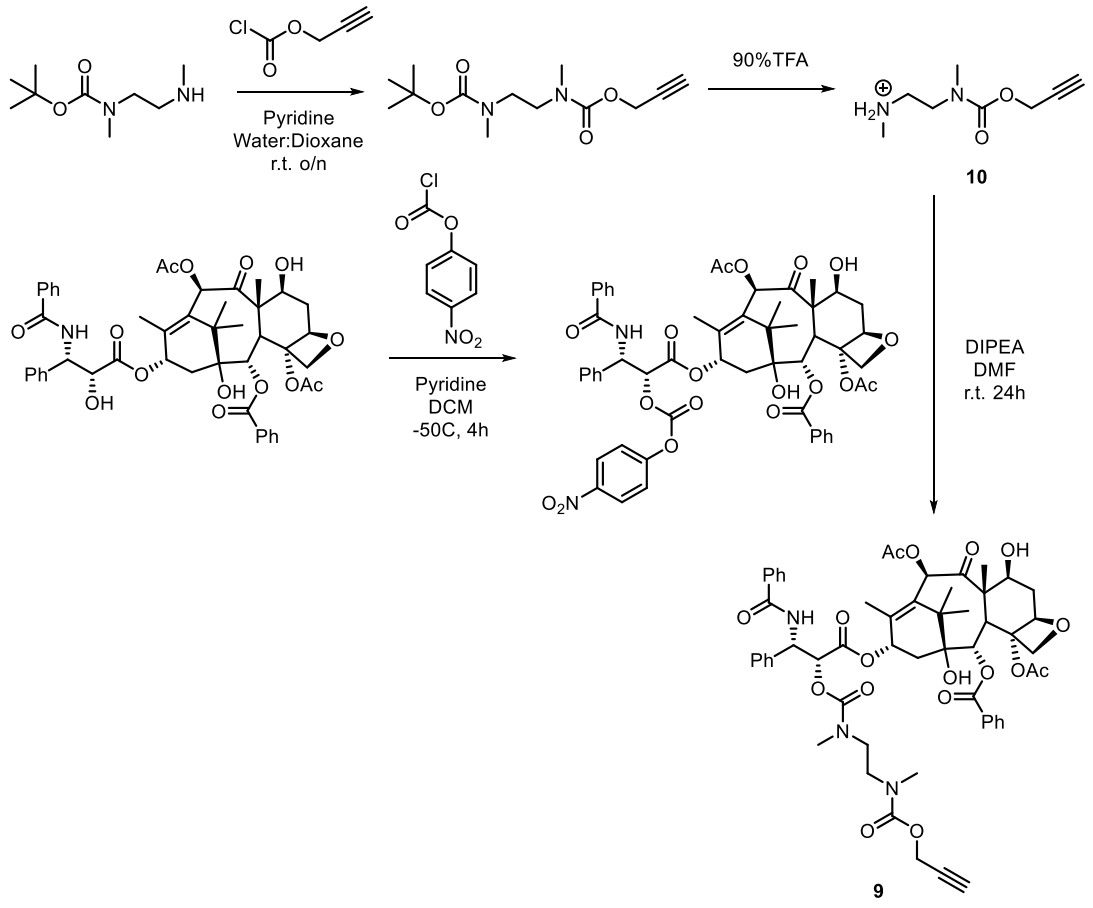

FIGURE 3. Hemi-synthesis of paclitaxel prodrug **9** in task 4.1.

Besides the synthesis of prodrugs **1-9**, the main body of research work of ESR5 have consisted on developing a new design for PROTACs (proteolysis targeting chimeras), an emerging class of cancer therapeutics with PK problems due to their large size and systemic toxicity. The novel design aims to improve PK properties by administering PROTAC precursors as two half-size components that can be assembled by a bioorthogonal copper-based catalytic device to generate the therapeutic PROTAC at the desired site. The new strategy required the synthesis of two precursor molecules functionalized with adequate bioorthogonal tags[11] that can be assembled into a triazole group by a Copper-catalyzed alkyne-azide cycloaddition (CuAAC) reaction (see Figure 4): an alkyne-tagged cereblon ligand (thalidomide ligand of E3 ligase to promote ubiquitination) and azide-functionalized dasatinib (a ligand of the kinases BCR-ABL and SRC, which are highly overexpressed in many cancers including chronic myeloid leukaemia and breast cancer, respectively).[11]

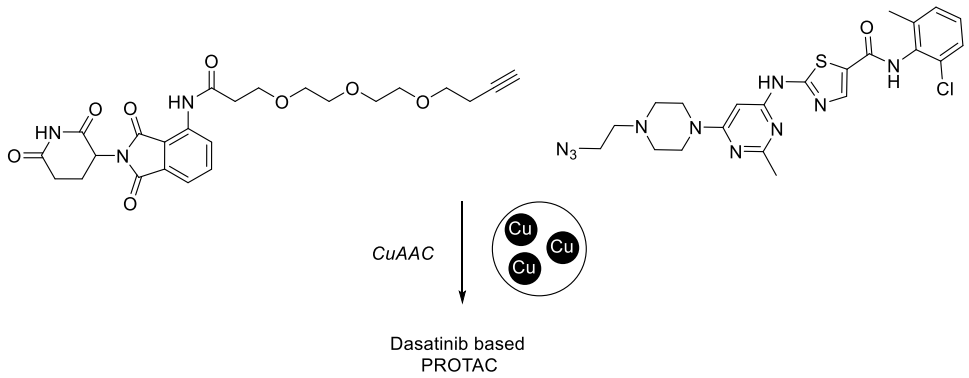

FIGURE 4. Alkyne-tagged thalidomide and azide-functionalized dasatinib (PROTAC precursors) synthesized in task 4.1. Once assembled, the PROTAC will bring together E3 ligases into the proximity of dasatinib's targets, thus inducing protein degradation and treating the disease locally.

Azide-functionalized **dasatinib** was synthesized in one-pot by reaction with methane sulfonyl chloride in dry DMF, followed by addition of sodium azide (63 % yield). The alkyne-tagged cereblon ligand was prepared by carbodiimide-mediated coupling of aminothalidomide and three PEG-carboxylic acid of different lengths in moderate yield (43-55 %).

These compounds are also available for partners to test them with their copper catalysts.

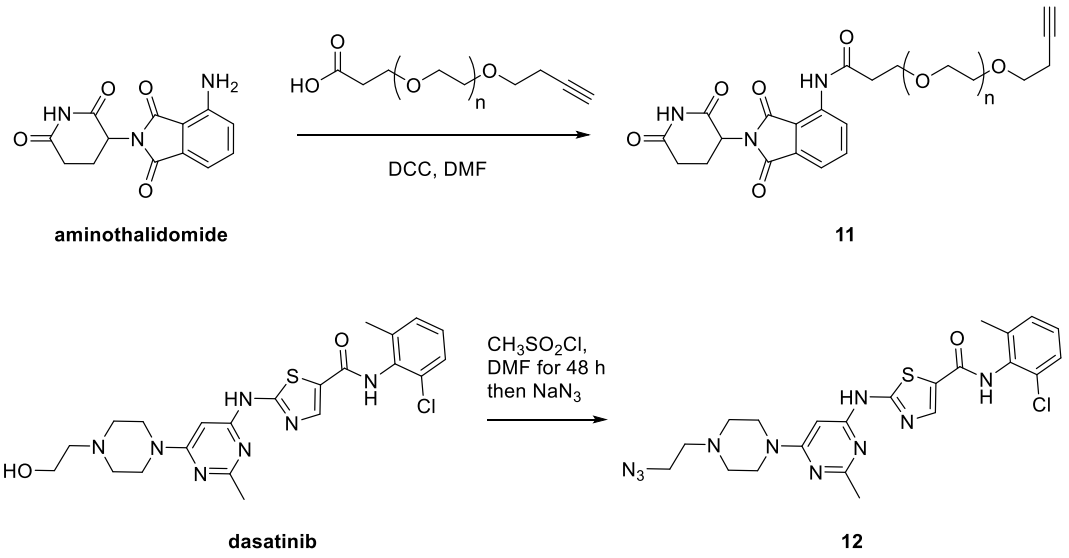

FIGURE 5. Synthesis of PROTAC precursors: alkyne-tagged thalidomides **11** (n= 2, 3 and 4) and azide-functionalized dasatinib **12**.

Conclusions

Nine prodrugs (including two unpublished ones) that can be activated by bioorthogonal Palladium or Gold catalysis, and four PROTAC precursors, that can be assembled by bioorthogonal CuAAC reactions, have been developed during T4.1, thereby completed D4.1. This prodrug library is accessible for the consortium partners at request to carry out their research work.

2. References

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[9] Manuscript submitted to *Nat. Catalysis* (under review).

[10] Manuscript in preparation, expected to be submitted to *Chem. Sci.*

[11] New PROTAC precursors made by Stephen Croke (ESR5).