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Journal Name

ARTICLE

Design, Synthesis and Biological Evaluation of 4'-Demethyl-4-Deoxypodophyllotoxin Derivatives as Novel Tubulin and Histone Deacetylase Dual Inhibitors

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A new class of 4'-demethyl-4-deoxypodophyllotoxin derivatives have been designed and synthesized as tubulin–HDAC dual inhibitors. Biological evaluations of these hybrids included the inhibitory activity of HDAC, *in vitro* cell cycle analysis in HCT–116 cells as well as cytotoxicity against two cancer cell lines (A549 and HCT116). The distance and angle between HDAC capping group and zinc binding group were systematically varied. Compounds **14a** and **14c** showed most potent dual inhibitory activity and powerful antiproliferative activity on HCT116 and A549 cell lines.

Introduction

According to World Cancer Report 2014, there were 14 million new cancer cases in 2012 globally and the number is expected to continuously increase within the next two decades.[1] The development of cancer pharmacotherapy has a history of more than 70 years. Traditional anti–cancer drugs including cytotoxic agents and endocrine medications have been widely used in a variety of cancer chemotherapy treatments. But the usage of these drugs is always hindered by severe toxicity and other undesirable side effects. The development of targeted molecularly anti–cancer drugs has made significant achievement over the past decade. However, only part of the patients show positive response. In addition, the acquired drug resistance always limits the use of these agents. Diseases with linear pathways might be well treated with single target agents. Cancer is a disease with complex signaling networks. For this reason, it is difficult to treat cancer by using classical targeted drugs alone.[2]

Anti–tumor drug combination therapy can block several key signaling pathways and create synergistic antitumor effects.[3-11] This kind of therapeutic regimens can improve therapeutic efficacy and simultaneously reduce drug toxicity. The major defects of combination therapy are the complicated pharmacokinetics and the adverse effects associated with drug interactions.

Recently, the design of multi–target drugs has become a new strategy to overcome these limitations.[12-14] These agents can create synergistic anti–cancer effects and exhibit simpler pharmacokinetics. Histone deacetylase (HDAC) involved multi-target drug design is

one of the hotspots in this area. HDAC plays an important role in the regulation of gene expression. HDAC inhibitors can cause growth arrest, differentiation, and apoptosis in cancer cells.[15-18] Up to date, two HDAC inhibitors, vorinostat (SAHA) and romidepsin have been approved by FDA for the treatment of cutaneous T–cell lymphoma (CTCL).[19-21] In general, a HDAC inhibitor consists of a capping group, a zinc–binding group (ZBG) and an appropriate linker (Fig. 1). The simple SAR together with the effectiveness in oncotherapy have attracted many oncologists into the exploration of HDAC–involved multi–target agents. [22-28]
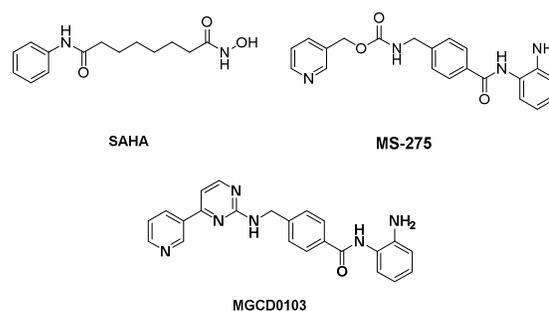
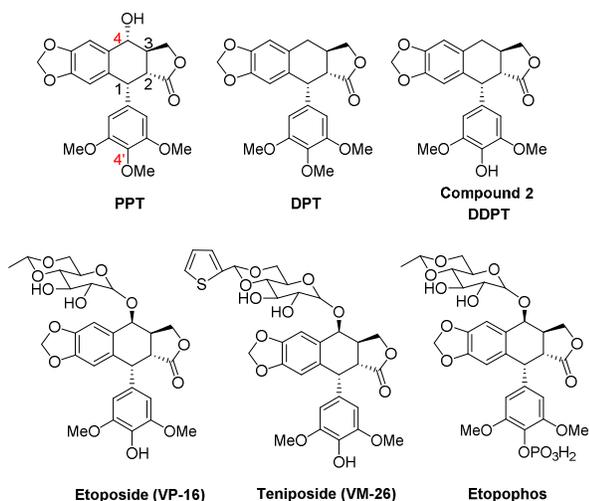**Figure 1.** Representative structures of HDAC inhibitors.

HDAC inhibitors can have a synergistic antitumor effect when combined with tubulin inhibitors.[29-31] Previously, we disclosed a new class of podophyllotoxin (PPT) derivatives as topoisomerase II (Topo II)–HDAC dual inhibitors.[32] Among this series of hybrids, compound **1** displayed the best anti–HDAC activity and was 10– to

20 fold more potent than SAHA, which suggested that the PPT moiety might be a suitable capping group for HDAC inhibitors.
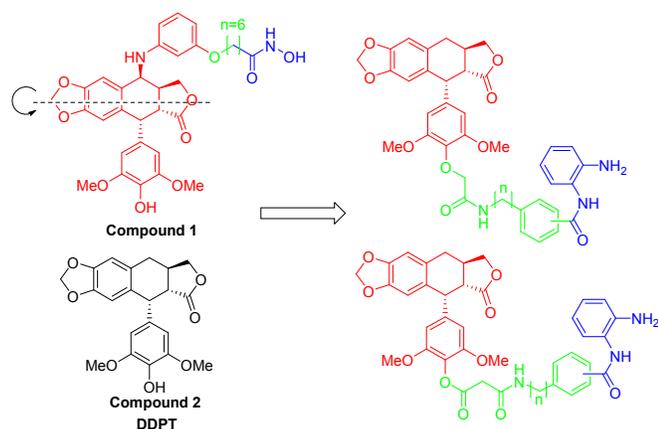
Figure 2. Design of tubulin–HDAC dual inhibitors.

The structure of PPT was first elucidated in 1932 and hundreds of derivatives have been reported. Three semi–synthetic analogues, etoposide (VP–16), teniposide and etopophos have been approved to use in chemotherapy (Fig. 3).[33, 34] Interestingly, these semi–synthetic derivatives exert their antiproliferative activity through the inhibition of Topo II, which is different from the parent compound PPT. 4-Deoxypodophyllotoxin (DPT) is a PPT analogue, which can inhibit tubulin polymerization and cause cell cycle arrest at G_2/M followed by apoptosis.[35-37] The structural modifications of DPT are focused on the 4'-position,[38-43] the representative compound 4'-demethyl-4–deoxypodophyllotoxin (DDPT) has a comparable antitumor activity with DPT.


Figure 3. Representative structures of PPT analogues.

As shown in Figure 2, compound **2** can be regarded as an axial symmetry structure. Therefore, we envisioned that the transplant of HDAC pharmacophore from the 4-position to the 4'-position can achieve a new series of tubulin–HDAC dual inhibitors. Aromatic capping group connection, linker length and ZBG connection were systematically varied to regulate the distance and angle between the cap and ZBG. Biological evaluation of these hybrids includes their

anti–HDAC activities, *in vitro* cell cycle analysis in HCT–116 cells and their antiproliferative activities in two cancer cell lines.

Results and discussion

Chemistry
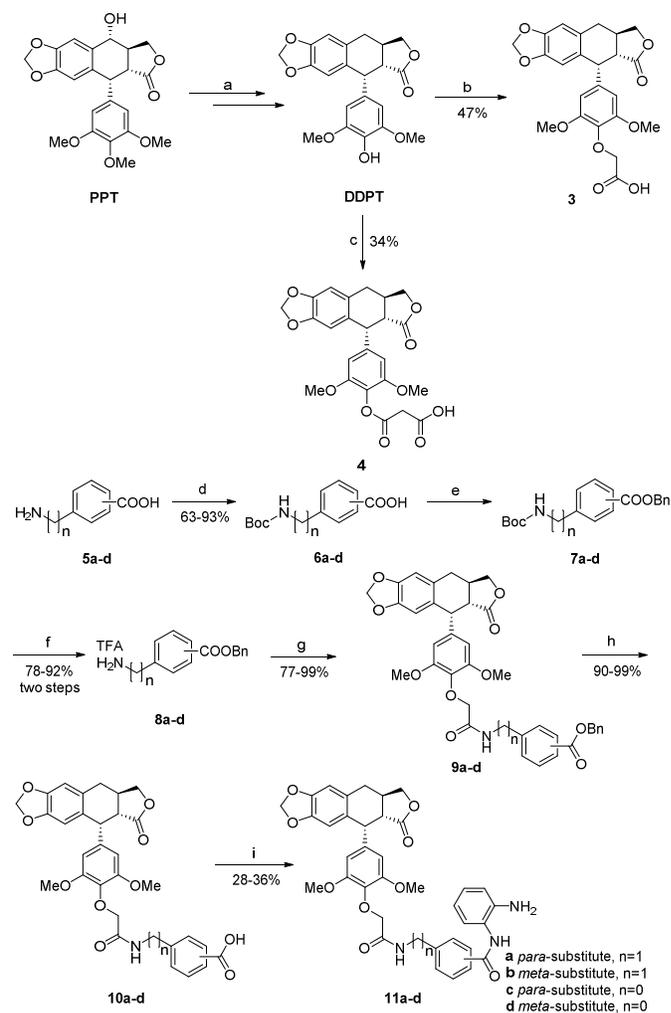
Reagents and conditions: (a) TMSI, DCM; BaCO$_3$, acetone, H$_2$O; Pd/C, H$_2$, AcOH, 95 ^{o}C; (b) tert-butyl 2-bromoacetate, K$_2$CO$_3$, KI; CF$_3$COOH, DCM; (c) 2,2-dimethyl-1,3-dioxane-4,6-dione, toluene, reflux; d) Boc$_2$O, NaHCO$_3$, dioxane, H$_2$O; e) BnBr, Cs$_2$CO$_3$, DMF; f) TFA, DCM; g) compound **3**, HATU, DIPEA, DCM; h) Pd(OH)$_2$, H$_2$, MeOH, ethyl acetate; i) HATU, DIPEA, DCM.

Scheme 1. Synthesis of ether series hybrids.

The general route used for the synthesis of the ether series is depicted in Scheme **1**. According to the reported literature, DDPT can be easily prepared from PPT in three steps.[43] Treatment of DDPT with tert-butyl 2-bromoacetate followed by removal of the protecting group gave the key intermediate **3** of ether series. Ester series intermediate **4** was prepared through treatment of DDPT with 2,2-dimethyl-1,3-dioxane-4,6–dione.
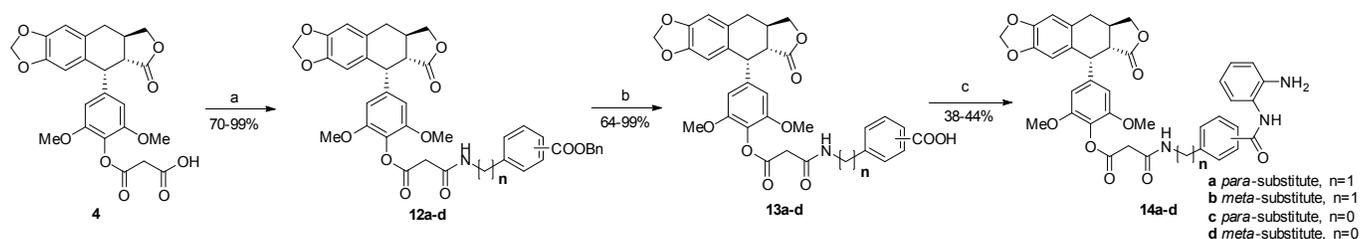
Reagents and conditions: a) HATU, DIPEA, DCM; b) Pd(OH)$_2$, H$_2$, MeOH, ethyl acetate; c) HATU, DIPEA, DCM.

Scheme 2. Synthesis of ester series hybrids.
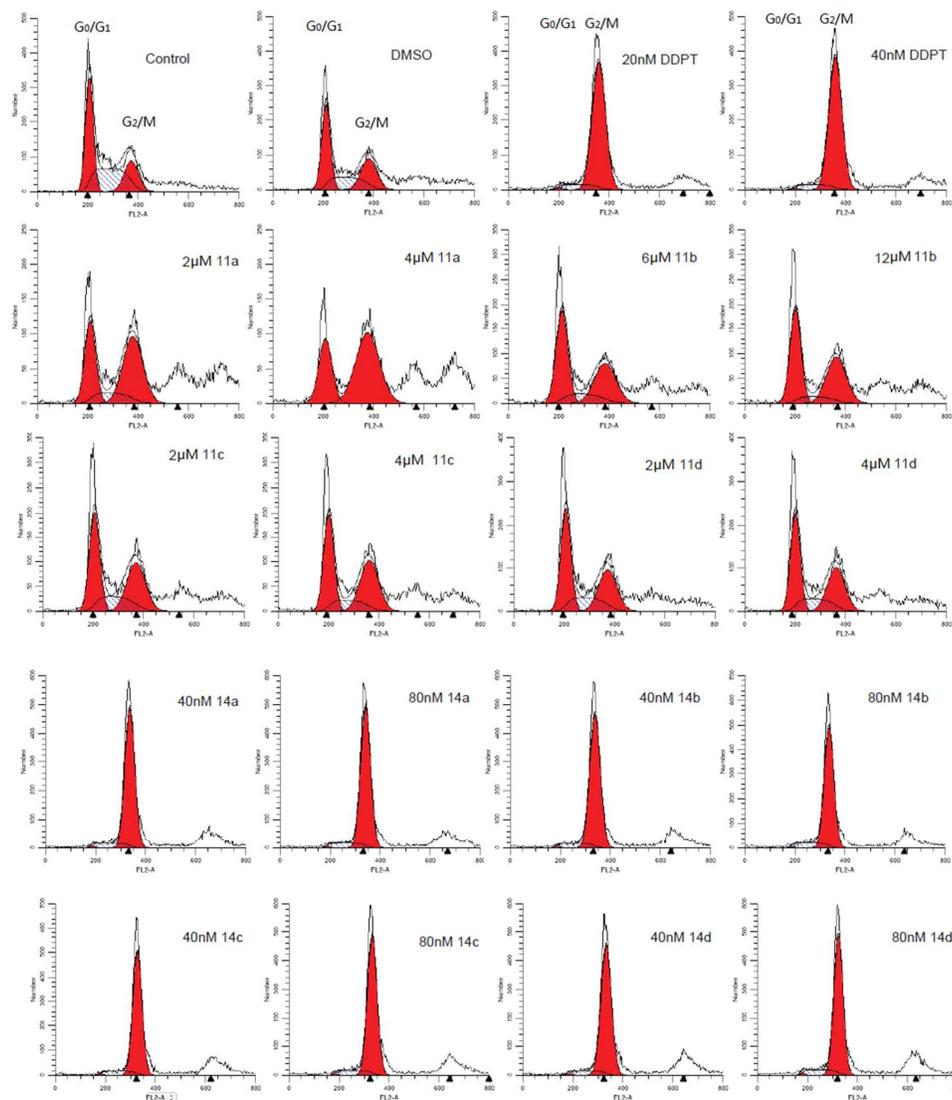
Figure 4. *In vitro* cell cycle analysis of **11a–d** and 1**4a–d** in HCT-116 cells

On the other hand, starting from amino acids **5a–d**, amines **8a–d** can be synthesized smoothly in three steps. Subsequent amidation of acid **3** with the corresponding amine afforded compounds **9a–d**. Hydrogenation of these compounds using Pd(OH)$_2$ under hydrogen atmosphere led to benzoic acids **10a–d**. Finally, the ether series hybrids **11a–d** were obtained after amidation.

Similarly, as shown in Scheme 2, preparation of ester series hybrids **14a–d** was started from the amidation of compound **4** and amines **8a–d**. Subsequently, esters **12a–d** were treated with Pd(OH)$_2$ under hydrogen atmosphere to afford acids **13a–d**. Amidation of these acids and 1,2-benzenediamine gave designed hybrids **14a–d**.

Biological Assays

In vitro HDAC Inhibition

Table 1. *In vitro* HDAC Inhibition [a]

Compound	IC$_{50}$ [μM] ± SD		
	HDAC–1	HDAC–2	HDAC–3
MGCD0103	0.95 ± 0.16	0.28 ± 0.02	1.67 ± 0.01
Etoposide	–[b]	–	–
DDPT	–	–	–
11a	15.10 ± 6.17	1.69 ± 0.11	6.87 ± 2.12
11b	–	35.43 ± 5.46	–
11c	–	7.51 ± 0.32	–
11d	–	–	–
14a	11.09 ± 0.90	1.73 ± 0.21	11.00 ± 0.80
14b	40.71 ± 6.49	18.58 ± 4.20	30.56 ± 11.99
14c	10.96 ± 3.45	0.75 ± 0.05	5.30 ± 1.15
14d	–	13.45 ± 1.71	44.11 ± 3.69

[a] Each value was reproduced in three experiments; [b] IC$_{50}$ > 20μg/mL

We first tested the HDAC inhibition activity of these hybrids against recombinant human HDAC–1, HDAC–2 and HDAC–3 enzymes, using MGCD0103 as the positive control compound (Table 1).[44] Overall, the majority of the designed hybrids maintained anti–HDAC–2 activity while some of them lost their HDAC–1 and/or HDAC–3 inhibitory activity. The aromatic capping group connection had an influence on anti–HDAC activity. The ester series hybrids **14a–d** exhibited stronger anti–HDAC activity than the ether series compounds **11a–d**. Besides, spatial orientation of ZBG also contributed to the HDAC inhibitory activity. A similar trend has been observed that the *para*–substituted hybrids showed better anti–HDAC activity than the *meta*–substitued one (**11a>11b**, **11c>11d**, **14a>14b**, **14c>14d**). Among all these hybrids, compound **14c** displayed the best HDAC inhibitory activity against HDAC–1 (IC$_{50}$ = 10.96 μM), HDAC–2 (IC$_{50}$ = 0.75 μM), HDAC–3 (IC$_{50}$ = 5.30 μM).

In vitro Cell Cycle Analysis in HCT-116 Cells

Tubulin inhibitors can induce mitotic arrest in the G$_2$/M phase of the cell cycle and result in apoptosis.[45] To probe the impact of the designed hybrids on tubulin polymerisation, *in vitro* cell cycle analysis using HCT-116 cells was performed. Figure 4 shows a significantly increased G$_2$/M peak after treatment of HCT-116 cells with DDPT at 20 nM or 40 nM for 24 h. Compared with the control group, all of the tested compounds showed an increasing trend of cell arrest in the G$_2$/M phase. Treatment with hybrids **14a-d** at a low concentration (40 or 80 nM) showed a striking G$_2$/M peak increase

while **11a-d** displayed a weaker response at a high concentration (2–12 μM).

In vitro Cell Growth Inhibition

Table 2. *In vitro* Cell Growth Inhibition

Compound	HCT116 IC$_{50}$ [μM] ± SD	A549 IC$_{50}$ [μM] ± SD
MGCD0103	1.572 ± 0.349	1.645 ± 0.235
Etoposide	0.873 ± 0.171	1.714 ± 0.165
DDPT	0.016 ± 0.000	0.011 ± 0.003
11a	1.583 ± 0.414	3.789 ± 0.409
11b	5.889 ± 0.254	2.692 ± 0.315
11c	1.600 ± 0.560	1.229 ± 0.030
11d	1.300 ± 0.770	1.033 ± 0.146
14a	0.037 ± 0.001	0.036 ± 0.001
14b	0.036 ± 0.001	0.019 ± 0.003
14c	0.040 ± 0.001	0.037 ± 0.001
14d	0.036 ± 0.002	0.040 ± 0.002

[a] Each value was reproduced in three experiments

The evaluation of the antiproliferative activity of these hybrids was performed using an MTT assay against A549 and HCT116 cells. As shown in Table 2, MGCD0103 and etoposide exhibited weaker cytotoxicity while DDPT showed strong antiproliferative activity. The ether series hybrids **11a–d** displayed moderate antiproliferative activity at micromolar what was close to MGCD0103. In contrast, the ester series compounds **14a–d** exhibited more potent antiproliferative activity against HCT116 and A549 cells (IC$_{50}$ = 19 ~ 40 nM) which was similar to reference compound DDPT.

Conclusions

A new class of DDPT derivatives was designed and synthesized as tubulin–HDAC dual inhibitors. Overall, the ester series hybrids showed better HDAC inhibitory activity and tubulin activity than the ether series. ZBG connection had an influence on HDAC inhibitory activity, the *para*–substituted molecules exhibited better anti–HDAC activity than the *meta*–substituted one. On the other hand, hybrids connected with ester bond displayed better anti-tubulin activity than the than the ether series hybrids. Compounds **14a** and **14c** acted as potent tubulin-HDAC dual inhibitors and showed good antiproliferative activity against A549 and HCT116 cell lines. The present encouraging results give a promising future optimization of the series.

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Notes and references

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