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Original article

Design, synthesis and biological evaluation of colchicine derivatives as novel tubulin and histone deacetylase dual inhibitors

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ABSTRACT

A new class of colchicine derivatives were designed and synthesized as tubulin–HDAC dual inhibitors. Biological evaluations of these hybrids included the inhibitory activity of HDAC, tubulin polymerization analysis, *in vitro* cell cycle analysis in HCT-116 cells and cytotoxicity against different cancer cell lines. Hybrid **6d** behaved as potent HDAC–tubulin dual inhibitor and showed comparable cytotoxicity with colchicine. Compound **11a** exhibited powerful tubulin inhibitory activity, moderate anti-HDAC activity and the most potent cytotoxicity (IC_{50} = 2–105 nM).

1. Introduction

Though cancer pharmacotherapy has a long history of more than 70 years, it is still a great challenge to cure cancer. In 2012, there were more than 14 million new cancer cases around the world and the number is expected to continuously increase in the next 20 years [1]. Traditional antitumor drugs including cytotoxic agents have been widely used for years. However, the usage of these drugs is 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. But only part patients show positive response. In addition, the acquired drug resistance may limit the use of these agents. As a matter of fact, diseases with linear pathways might be well treated with single target agents. However, cancer is a disease with complex signaling networks, which means it is difficult to treat most cancers by using single classical targeted drug [2]. Combination chemotherapy can simultaneously block several key signaling pathways and create synergistic antitumor effects [3–11]. These regimens are widely used and can improve therapeutic efficacy and reduce drug toxicity.

Recently, multi-target drug design has become an active research field [12–14]. These agents can create synergistic anti-cancer effects. Compared with combination chemotherapy, they always 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]. In general, a HDAC inhibitor consists of a capping group, a zinc-binding group (ZBG) and an appropriate linker (Fig. 1). The effectiveness in oncotherapy together with the simple SAR has attracted many oncologists into the exploration of HDAC-involved multi-target agents [19–26].

Three HDAC-targeting drugs, vorinostat (SAHA), romidepsin and belinostat have been approved by FDA for the treatment of cutaneous T-cell lymphoma and peripheral T-cell lymphoma [27–30]. Over 12 HDAC inhibitors are currently in clinical trials for different cancers [31,32]. Zinc-binding groups play an important role in the binding efficiency between HDAC inhibitors and enzyme. Among

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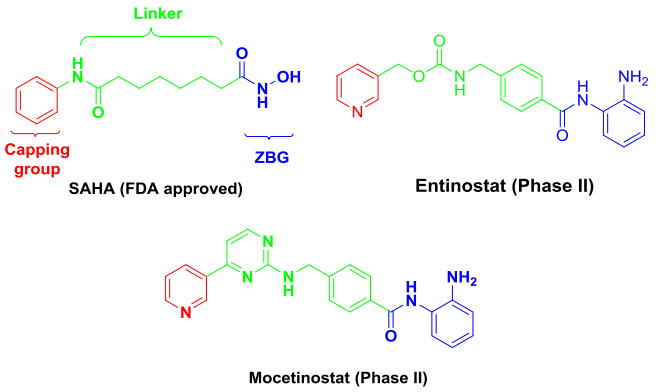

Fig. 1. Representative structures of HDAC inhibitors.

all types of ZBGs, hydroxamate and benzamide are most widely studied [23].

Previously, we reported a series of colchicine–SAHA hybrids as novel antitumor agents based on the synergistic antitumor effect between HDAC inhibitors and tubulin inhibitors [25,33–35]. Among these hybrids, compound **1** behaved as tubulin–HDAC dual inhibitor and displayed the best *in vitro* antiproliferative activity. This work suggested that the colchicine moiety is an appropriate capping group for HDAC inhibitors. For HDAC-involved dual inhibitors, ZBG might contribute to the binding activities of both targets. To further explore this kind of tubulin–HDAC dual inhibitors, we designed and synthesized a new series of colchicine derivatives with benzamide moiety as HDAC ZBG (Fig. 2). The biological evaluations of these molecules include the inhibitory activity of HDAC, tubulin polymerization analysis, *in vitro* cell cycle analysis in HCT-116 cells and cytotoxicity against different cancer cell lines.

2. Results and discussion

2.1. Chemistry

The general route for the synthesis of colchicine derivatives with amide linkage is depicted in Scheme 1. The benzoic acid **2** or **3** was treated with $SOCl_2$ followed by the corresponding amines in the presence of triethylamine to afford compounds **4a**–**d**. Hydrolysis of these esters with LiOH gave the corresponding carboxylic acids. Subsequent amidation of these intermediates with de-Ac-colchicine [36,37] afforded compounds **5a**–**d**. Finally, treatment of these compounds with TFA gave the target molecules **6a**–**d**.

As shown in Scheme 2, preparation of dual inhibitors with amine linkage started from hydroxymethyl benzoic acids **7** or **8**. These acids were treated with $SOCl_2$ followed by the corresponding amines in the presence of triethylamine to give compounds **9a**–**d** in good yields. Subsequently, these molecules were treated with de-Ac-colchicine to afford compounds **10a**–**d**. Target hybrids **11a**–**d** were obtained after the removal of protecting groups.

2.2. Functional assay

2.2.1. In vitro HDAC inhibitory activity

We first tested the HDAC inhibition activity of the hybrids against recombinant human HDAC1, HDAC2 and HDAC3 enzymes, using mocetinostat as the positive control compound. As shown in Table 1, all of the hybrids exhibited inhibitory activity against HDAC1, HDAC2 and HDAC3 except compound **11c**. The ZBG type had a great influence on anti-HDAC activity. Compared with hybrids equipped with unsubstituted benzamide ZBG, their analogs with bis(aryl)-type ZBG showed better potency against HDAC1-3, which was consistent with the reported literature (**6a** vs **6b**, **6c** vs **6d**, **11a** vs **11b** and **11c** vs **11d**) [38]. Among these conjugates, compound **6d** showed the most potent anti-HDAC activity against HDAC1 (IC_{50} = 1.50 μM), HDAC2 (IC_{50} = 0.19 μM) and HDAC3 (IC_{50} = 1.49 μM).

2.2.2. Effect of hybrids on the tubulin polymerization

To probe the impact of these hybrids on tubulin polymerization dynamics, we followed the kinetics of tubulin polymerization in the presence of colchicine and hybrids. As shown in Fig. 3, colchicine can block the polymerization of tubulin into microtubules while mocetinostat had very little effect [39]. Most of the hybrids caused inhibition on tubulin polymerization when compared to control group. Among these tested molecules, compounds **6d**, **11a** and **11c** displayed comparable tubulin inhibitory activity with the positive control colchicine.

2.2.3. Effect of compounds *6d* and *11a* on cell cycle

Cell cycle analysis of the best HDAC inhibitor **6d** and the most powerful tubulin polymerization inhibitor **11a** was investigated. Fig. 4 shows a significantly increased G_2/M peak after treatment of HCT-116 cells with colchicine at 20 nM. In consistence with the tubulin polymerization inhibition effects, **6d** and **11a** have also exhibited the cell cycle arrest effects. Compared with colchicine, cells treated with **6d** or **11a** at 10 nM has already showed a striking G_2/M phase arrest.

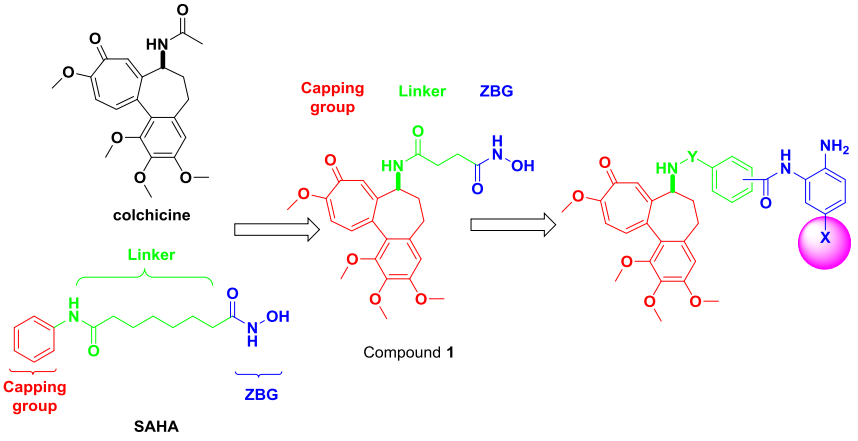

Fig. 2. Design of novel tubulin-HDAC dual inhibitors.