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Original article

A novel class I histone deacetylase inhibitor, I-7ab, induces apoptosis and arrests cell cycle progression in human colorectal cancer cells

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ABSTRACT

Epigenetic mutations are closely associated with human diseases, especially cancers. Among them, dysregulations of histone deacetylases (HDACs) are commonly observed in human cancers. Recent years, HDAC inhibitors have been identified as promising anticancer agents; several HDAC inhibitors have been applied in clinical practice. In this study, we synthesized a novel N-hydroxyacrylamide-derived HDAC inhibitor, I-7ab, and examined its antitumor activity. Our investigations demonstrated that I-7ab exerted cytotoxicity toward and inhibited the growth of human cancer cell lines at micromolar concentrations. Among tested cells, HCT116 was the most sensitive one to the treatment of I-7ab. However, I-7ab displayed far less cytotoxicity in human normal cells. In HCT116 cells, I-7ab inhibited the expression of class I HDACs, especially that of HDAC3, and suppressed EGFR signaling pathway. With respect to the cytotoxic effect of I-7ab, it induced apoptosis via increasing the Bax/Bcl-2 ratio and suppressing the translocation of NF-κB. Other than inducing apoptosis, I-7ab inhibited the expression of cyclin B1 and thereby arrests cell cycle progression at G2/M phase. Further analyses revealed potential role of p53 and p21 in I-7ab-induced apoptosis and cell cycle arrest. According to our findings, I-7ab may serve as a lead compound for potential antitumor drugs.

1. Introduction

Epigenetic modification to chromatin is a critical regulatory mechanism of gene expression and thus has a strong impact on cellular events [1]. Whether the transcription of a specific gene is enhanced or repressed is affected by chromatin conformation and recruitment of transcription factors, both of which are controlled by the epigenetic modifications [2,3]. These modifications are dynamic and reversible, whose abnormalities are closely related with human diseases, especially cancers [4,5]. Therefore, much efforts have been paid on developing more effective and selective drugs that can reverse pathogenic "epimutations" [6,7].

Histone deacetylases (HDACs) are a family of enzymes that control the acetylation state of a certain chromatin locus, cooperating with their counterparts, histone acetyltransferases (HATs) [8]. HDACs exert their functions via removing acetyl groups from conserved lysines within the N-terminal tail of histones, leading to changes in chromatin conformation and transcriptional repression of a subset of genes involved in cell differentiation, proliferation, and apoptosis [9]. In addition, several non-histone proteins have been recognized as substrates of HDACs, which suggests that HDACs may regulate cellular events depending on their functions other than controlling chromatin structure [10,11].

It has been found that HDACs are fundamental to normal embryonic development and biological processes in cells including stress response and cell growth [12]. On the other hand, aberrant expression of HDACs is commonly observed in human cancers [13,14]. Several studies have reported that expression of class I HDACs is enhanced in various types of human tumors [15]. These observations suggest that class I HDACs are potential targets for antitumor drugs.

In 2006, Vorinostat (SAHA), the first histone inhibitor applied in clinical therapy for cancer, was approved by the U.S. Food and Drug Administration (FDA) for the treatment of cutaneous T-cell

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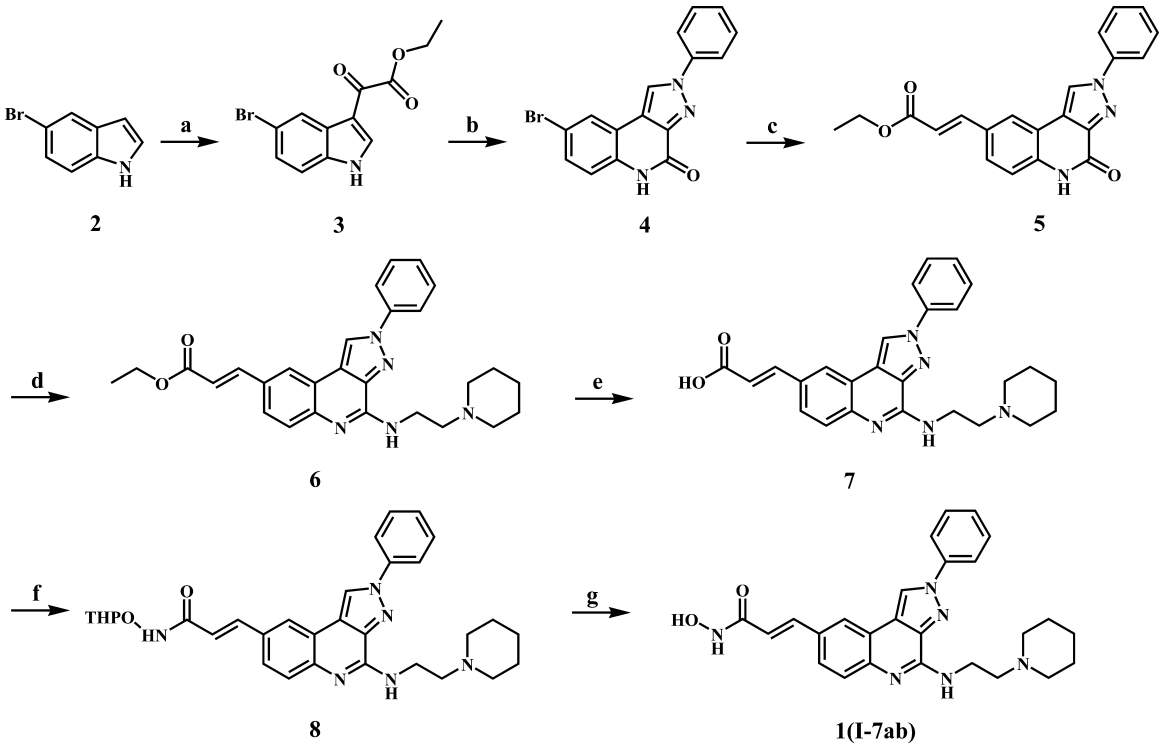

Fig. 1. Synthesis routine of I-7ab. Reagents and conditions were: (a) (i) $(COCl)_2$, Et_2O, 0 °C–r.t.; (ii) EtOH, r.t.; (b) $PhNHNH_2$·HCl, AcOH, *n*-BuOH, 110 °C; (c) Ethyl acrylate, $Pd(OAc)_2$, PPh_3, DIPEA, DMF, 110 °C; (d) (i) $POCl_3$, 90 °C; (ii) 2-(piperidin-1-yl)ethanamine, Et_3N, EtOH; (e) KOH/H_2O, EtOH; (f) $THPONH_2$, EDCI, HOBt, DMF, r.t.; (g) HCl/EtOAc, CH_2Cl_2/CH_3OH, r.t.

lymphoma (CTCL) [16]. Since then, several selective or pan HDAC inhibitors have been developed and are under clinical study [17,18]. These agents exert biological functions through different mechanisms and possessing varying efficiency and specificity [19]. Since functions of HDACs, even those of the same class, are not redundant [11], high specificity appears to be required in developing new HDAC inhibitors for cancer-specific therapy.

A close review of structures of HADC inhibitors that have been applied in clinical trials indicates the important role of hydroxamate. Previously, we designed and synthesized a compound possessing such structural feature. Its inhibitory effect on glioblastoma and neuroblastoma cell growth and underlying mechanism was investigated [20,21]. Based on the activity and structure of this compound, a new series of N-hydroxyacrylamide derivatives were designed and synthesized. Among them, (N-hydroxy-3-(2-phenyl-4-((2-(piperidin-1-yl)ethyl)amino)-*2H*-pyrazolo[3,4-*c*]quinolin-8-yl)acrylamide (hereinafter referred to as I-7ab) displayed the strongest potency against cancer cell lines. In this study, we investigated the inhibitory effect of I-7ab on the expression of class I HDACs and its ability to induce apoptosis and arrest cell cycle progression. In addition, we explored the possible molecular mechanism underlying I-7ab's antitumor activity. Our results indicated that I-7ab may serve as a lead compound for potent and safe antitumor drugs.

2. Materials and methods

2.1. Reagents and antibodies

I-7ab was synthesized according to the routine depicted in Fig. 1, purified to greater than 99%, and diluted in dimethyl sulfoxide (DMSO) to prepare a 20 mM stock solution, which was further diluted in culture media for all in vitro experiments. The control cells were treated with the same amount of vehicle alone.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Hoechst33258, propidium iodide (PI) and 5-bromo-4-chloro-3-indolyl-4-phosphate/nitrotetrazolium blue chloride (BCIP/NBT) were purchased from Sigma Chemical Co. (St. Louis, MO).

Antibodies included the following: Anti-p53, anti-EGFR, anti-HDAC1, anti-HDAC2, anti-HDAC3, HDAC-6, anti-Bcl-2, anti-β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA); Anti-NF-κB, anti-cyclin B1, anti-CDK1, anti-p21, and anti-Bax were purchased from Cell Signaling Technology (Beverly, MA).

2.2. Cell lines and cell culture

Human cell lines included: HeLa, MCF-7, HCT116, A549, MKN45, HEK293 and L-02. All cells were purchased from Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). HCT116, HeLa, MCF-7, A549 and MKN45 cells were grown in RPMI-1640 medium (Life Technologies, Grand Island, NY, USA) with 10% heat-inactivated calf serum, penicillin (100 U/ml) and streptomycin (100 U/ml). HEK293 and L-02 cells were cultured in RPMI 1640 medium, supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 U/ml). Cells were incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

2.3. Cytotoxicity assays and clonogenic assays

Cytotoxicity assays and clonogenic assays were performed as described in a previous study [22]. As for cytotoxicity assays, cells were plated in 96-well culture plates (1,00,000 cells/well) and were allowed to attach to the bottom of plates for 12 h before treatment of I-7ab. The optical density of control and treated cells were measured in an automated microplate reader (Multiskan Ex, Lab Systems, Finland). As for colony forming assays, cell suspension–agarose mixture (cell suspension:agarose = 3:1) were plated