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Research paper

Design, synthesis and biological evaluation of novel coumarin-based benzamides as potent histone deacetylase inhibitors and anticancer agents

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ABSTRACT

Histone deacetylases (HDACs) are attractive therapeutic targets for the treatment of cancer and other diseases. It has four classes (I-IV), among them especially class I isozyme are involved in promoting tumor cells proliferation, angiogenesis, differentiation, invasion and metastasis and also viable targets for cancer therapeutics. A novel series of coumarin-based benzamides was designed and synthesized as HDAC inhibitors. The cytotoxic activity of the synthesized compounds (**8a**-**u**) was evaluated against six human cancer cell lines including HCT116, A2780, MCF7, PC3, HL60 and A549 and a single normal cell line (Huvec). We evaluated their inhibitory activities against pan HDAC and HDAC1 isoform. Four compounds (**8f**, **8q**, **8r** and **8u**) showed significant cytotoxicity with IC_{50} in the range of 0.53–57.59 μM on cancer cells and potent pan-HDAC inhibitory activity (consists of HDAC isoenzymes) (IC_{50} = 0.80 −14.81 μM) and HDAC1 inhibitory activity (IC_{50} = 0.47–0.87 μM and also, had no effect on Huvec (human normal cell line) viability (IC_{50} > 100 μM). Among them, **8u** displayed a higher potency for HDAC1 inhibition with IC_{50} value of 0.47 ± 0.02 μM near equal to the reference drug Entinostat (IC_{50} = 0.41 ± 0.06 μM). Molecular docking studies and Molecular dynamics simulation of compound **8a** displayed possible mode of interaction between this compound and HDAC1enzyme.

1. Introduction

Cancer, the uncontrolled, rapid and pathological proliferation of abnormal cells, is one of the most life-threating diseases and cause of death worldwide. Cancer causes about 550,000 deaths a year and is second leading cause of death in the world next to heart diseases [1,2]. The numerous drugs have been used for the cancer treatment but have severe side effects. Consequently; increasing interest has been devoted to the design and discovery of more effective anticancer agents with promising activity and high therapeutic index in current medicinal chemistry. Over the recent years, a great many of important targets such as the histone deacetylase (HDAC) has been considered for anticancer therapies. HDACs and histone acetyl transferases (HATs) are crucial post-translational modification and play a pivotal role in the epigenetic regulation of gene expression through chromatin modification [3–6]. Histone acetyl transferase

Abbreviations: HDAC, Histone Deacetylases; HATs, Histone Acetyl Transferases; HSP 90, Heat Shock Protein 90; SAHA, suberoylanilide hydroxamic acid; TSA, Trichostatin A; ZBG, Zinc Binding Group; CU, Connect Unit; MD, Molecular Dynamics; CDI, *N*, *N*'-Carbonyldiimidazole.

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(HATs) catalyzes acetylation of positive charged lysine residues and leads to the relaxation of chromatin and activates transcription [7]. Inversely, histone deacetylase (HDAC) catalyze the removal of acetyl groups of the ε-amino of lysine residues on core histone and other cellular proteins (e.g. HSP 90, tubulin), resulting in chromatin condensation and transcriptional repression [8,9].

HDACs have an important effect on gene transcription, the cell cycle, differentiation, apoptosis and tumourigenesis. Therefore, HDACs have been become as prominent therapeutic target for a broad range of human disorder such as cancer [10]. There are 18 isoforms of human HDAC, divided into four main classes based on their homology to yeast models, subcellular localization and enzymatic activities [11].

HDACI (HDAC 1, 2, 3 and 8), class IIa (HDAC 4, 5, 7 and 9), class IIb (HDAC 6 and 10) and class IV (HDAC11) are zinc-dependent enzymes, whereas class III HDACs (sirtuins 1–7) are NAD^+ dependent enzymes [12–14]. Class I HDACs are homologous to yeast Rpd3 and are predominantly located in the nucleus (350–500 amino acids in length) and class II HDACs have sequence homology to yeast Hda2 and shuttle between the cytoplasm and nucleus (about 1000 amino acids in length) [15–17]. Class III are homologues of the yeast Sir2 and class IV shows the characteristics of both class I and II HDACs [18].

Zn^{2+}-dependent HDACs, especially class I isozymes are involved in promoting tumor cells proliferation, angiogenesis, differentiation, invasion and metastasis and also viable targets for cancer therapeutics [19]. Among the class I HDAC isoforms, HDAC1 has a key role in cancer [20] and overexpressed in prostate, ovarian, breast, colon, leukemia and pancreas cancers [21].

HDAC inhibitors (HDACIs) are categorized into four classes according to their chemical structure in clinical studies: hydroxamic acids (Trichostatine A (TSA)) [22], (Vorinostat (SAHA)) [23], (Panobinostat (LBH-589)) [24], (Belinostat (PXD-101)) [25], benzamides ((Entinostat (MS-275)) [26], (Mocetinostat (MGCD-0103)) [27,28], (Chidamide (CS-055)) [29], (Tacedinaline (CI-994)) [30], short-chain fatty acids (valproic acid) [31,32] and depsipeptides (Romidepsin (FK-228)) [33]. Up to now, five HDACIs Vorinostat, Romidepsin, Belinostat, Chidamide and Panobinostat approved by FDA for the treatment of lymphoma or myeloma [34]. The structures of several approved and clinical HDACI are shown in Fig. 1.

Development of selective isotope HDACIs is a significant way for prevention of the side effects of HDACIs. Many hydroxamate HDACIs do not exert excellent selectivity toward a specific isoform, while 2-aminobenzamides exert some isoform selectivity such as Entinostat and Mocetinostat are relatively unique in inhibiting HDAC 1, 2 and 3 [35].

Despite the huge structural diversity, the HDACIs generally have a general pharmacophore model [36]: zinc binding group (ZBG) such as hydroxamic acid and 2-amino benzamide which interacting to Zn^{2+} at the bottom of active site and a requirement for the possession of HDAC inhibitory activity, a hydrophobic linker occupying the narrow tunnel of HDACs and connect the ZBG and the cap group, a polar connect unit (CU) that connecting cap group and linker, a surface recognition group (cap), a hydrophobic and aromatic or heteroaromatic group, is essential for recognizing and interacting with residues on the rim of active site of HDACs [37]. A cap group determines the potency, stability, bioavailability and efficacy of the compounds [11,38]. Coumarins (2H-1-benzopyran-2-one or 2H-chromen-2-one), an important bicyclic heterocycle as a part of flavonoid group of plant metabolite, are a wide class of natural and synthetic compounds that show anticancer [39], *anti*-HIV [40], anti-Alzheimer [41],antimicrobial [42],antioxidant [43] and antiviral [44] activities. Coumarin and its derivatives have rare nephrotoxicity, hepatotoxicity, cardio toxicity, dermal toxicity and other side effect [45].

In the present study some new coumarin-based benzamides have been designed and synthesized as HDAC inhibitors, as an attempt to check if the replacement of the benzyl carbamate moiety of Entinostat or (E)-3-(pyridin-3-yl)acrylamide moiety of Chidamide with the coumarin carboxamide are bioisosteric. The rationale for the design of these compounds was depicted in Fig. 2. We selected Entinostat and chidamide as the lead compounds and our design strategy for novel HDAC inhibitors was based on the common pharmacophore model of HDACs.

The synthesized compounds were evaluated for their cytotoxic activity against six different cancer cell lines including HCT116 (human colon cancer cells), A2780 (human ovarian cancer cells), MCF7 (human breast cancer cells), PC3 (human prostate cancer cells), HL60 (Human promyelocytic leukemia cells) and A549 (adenocarcinoma human alveolar basal epithelial cells) and normal Huvec cell line (Human Umbilical Vein Endothelial Cells). We evaluated their inhibitory activities of pan HDAC and HDAC1 isoform. The structure activity relationships (SAR) study performed on the cap moiety by investigating the electronic effects of the alkoxy or benzyloxy ring with various substituents. Docking and molecular dynamics (MD) simulation were performed for further investigating interaction of compounds with HDAC1.

2. Results and discussion

2.1. Chemistry

The synthetic routes to target compounds containing the coumarin ring were illustrated in Schemes 1–3. Appropriately, ethyl coumarin-3-carboxylates **3a-e** were synthesized through the knoevenagel condensation of salicylaldehydes **1a-e** with diethyl malonate in the presence of catalytic amount of piperidine in ethanol. Then, O-alkylation or *O*-benzylation of the 7-hydroxy derivative **3e** with alkyl halides or benzyl halides was done in DMF in the presence of potassium carbonate. The ethyl esters **3a-v** were hydrolyzed with aqueous solution of sodium hydroxide to the corresponding coumarin-3-carboxylic acids **4a-u** (Scheme 1) [46].

The condensation of the carboxylic acids **4a-u** with 4-(aminomethyl) benzoic acid using *N*, *N*'-carbonyldiimidazole (CDI) and trifluoroacetic acid (TFA) in dry THF at room temperature led to the formation of compounds **6a-u** (Scheme 2) [30].

Finally, compounds **6a-u** were converted into imidazole intermediate with CDI at 55–60 °C in dry THF, then reacted in situ with *o*-phenylene diamine in the presence of TFA at room temperature to obtain the target benzamide analogs **8a-u** as depicted in Scheme 3. The chemical structures of final compounds were characterized by ^1H NMR, ^{13}C NMR, elemental analysis, IR and MS spectroscopy.

2.2. Biological evaluation

2.2.1. In vitro anticancer activity

As depicted in Tables 1–3, the antiproliferative activity of the target compounds (**8a-u**) was evaluated against seven human cell lines including HCT116, A2780, MCF7, A549, PC3, HL60 and Huvec by 3-(4,5-dimethylthylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay using Entinostat as the positive control. Most of the target compounds showed significant anti proliferative activity with the IC_{50} values in micromolar range (0.27–80 μM) over all cell lines. Compounds **8k** was the most potent compound with IC_{50} values of 8.48, 10.14 and 16.6 μM in MCF7, A2780 and PC3, respectively. This compound also showed superior activity against HCT116, A549 and HL60 cells (IC_{50} = 0.27, 1.69 and 3.14 μM) compared with the reference drug Entinostat (IC_{50} = 2.03, 3.11 and 4.53 μM). Overall, the majority of the synthesized compounds were more potent against cell proliferation in HCT116 and A2780 cell,