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Design, synthesis and biological evaluation of novel hydroxamic acid based histone deacetylase 6 selective inhibitors bearing phenylpyrazol scaffold as surface recognition motif

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ABSTRACT

In recent years, inhibition of HDAC6 became a promising therapeutic strategy for the treatment of cancer and HDAC6 inhibitors were considered to be potent anti-cancer agents. In this work, celecoxib showed moderate degree of HDAC6 inhibition activity and selectivity in preliminary enzyme inhibition activity assay. A series of hydroxamic acid derivatives bearing phenylpyrazol moiety were designed and synthesized as HDAC6 inhibitors. Most compounds showed potent HDAC6 inhibition activity. **11i** was the most selective compound against HDAC6 with IC_{50} values of 0.020 μM and selective factor of 101.1. Structure-activity relationship analysis indicated that locating the linker group at 1′ of pyrazol gave the most selectivity. The most compounds **11i** (GI_{50} = 3.63 μM) exhibited 6-fold more potent than vorinostat in HepG2 cells. Considering of the high selectivity against HDAC6 and anti-proliferation activity, such compounds have potential to be developed as anti-cancer agents.

1. Introduction

The acetylation level of histone regulated by histone acetyl transferases (HATs) and histone deacetylases (HDACs) plays an important role in the development of many diseases such as HIV, HCV, cancer *etc.*[1,2] The 18 isoforms of HDACs are grouped into four classes: class I (HDACs 1, 2, 3 and 8), class II (HDACs 4, 5, 6, 7, 9 and 10), class III (SIRT1-7) and class IV (HDAC 11). The HDACs can be classified into two categories based on their mechanisms: zinc-dependent deacetylases (class I, II and IV) and class III NAD^+-dependent (class III) deacetylases.[3] Inhibition of HDACs has been proved to be an effective therapeutic strategy for cancers.[4] There are five agents have been approved by FDA or CFDA (Vorinostat,[5] Romidepsin,[6] Panobinostat,[7] Belinostat[8] and Chidamide[9]) for the treatment of lymphoma and multiple myeloma. However, all the approved drugs are class I selective or pan-HDAC inhibitors which have multiple side-effects as reported.[10]

HDAC6, which is primarily located at cytoplasm, deacetylated lysine residues of many non-histone substrates, including Hsp90, α-tubulin and Ku70.[11,12] Recently, The dysregulated expression of HDAC6 was proved to be related to many diseases exemplified by HIV,[13] Alzheimer's disease,[14] inflammation[15] and cancer.[16] Development of HDAC6 selective inhibitors as novel anticancer agents seemed to be preferable than the pan-inhibitors, considering the fact that HDAC6 selective inhibitors showed fewer side-effects.[17] To date, Tubacin,[18] ACY-1215,[19] tubastatin A[20] and other HDAC6 selective inhibitors have been reported by many groups. Among them, ACY-1215 is the most promising molecule of which phase II clinical trial for treating multiple myeloma has been completed. All these achievements prompted us to develop potent HDAC6 inhibitors with high selectivity. Considering all the reported structures, a HDAC6 inhibitor can be divided into three pharmacophores: (1) zinc-binding group (ZBG), interacting with the Zn^{2+} at the bottom of the active site; (2) linker group, matching the hydrophobic tunnel of HDAC6; (3) surface recognition motif (SRM), covering the entrance of the active pocket (Fig. 1).

In our previous study, a small library of approved drugs was screened for inhibitory activity of HDAC6 using fluorescence assay. Celecoxib, an anti-inflammatory drug, which is also accounted as a carbonic anhydrase II inhibitor for its zinc binding capacity,[21] showed moderate degree of HDAC6 inhibition and selectivity (IC_{50} = 0.643 μM and SF = 1.8). In addition, celecoxib was well tolerated and had no clinically significant adverse effects with the total daily dosage of 400 mg in a six weeks trial.[22] Considering the activity, selectivity and security, we chose celecoxib as an outstanding lead compound for the development of HDAC6 selective inhibitors in this study. We designed and synthesized a series of phenylpyrazole derivatives based on celecoxib. Several

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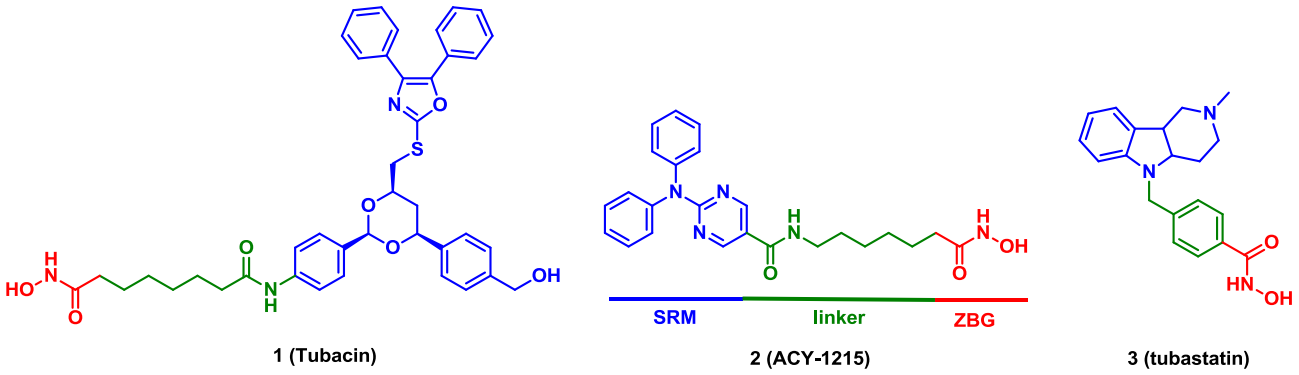

Fig. 1. Structures of some reported HDAC6 inhibitors.

modifications of celecoxib were performed to improve the potency and selectivity: (1) to increase HDAC6 binding affinity, the sulfanilamide group was replaced by hydroxamic acid in order to chelate Zn^{2+} by more coordination bond; (2) based on the conception that SRM structures strongly influence the selectivity of compounds against HDAC isotype,[23] a SAR study for the SRM by changing the location of linker group on pyrazole was proceed to optimize HDAC6 selectivity of the lead compound; (3) to deeply discuss the effect of linker group on the activity and selectivity against HDAC6, the phenyl was replaced by several linear alkyls of different lengths (Fig. 2).

2. Results and discussion

2.1. Chemistry

The synthetic route to compound **8** was illustrated as Scheme 1. A condensation reaction of *p*-methylacetophenone **5a** with trifluoroacetic acid ethyl ester in THF at room temperature was performed to obtain dione **6a**, which was coupled with sulfonamidophenylhydrazine hydrochloride in the next step. Intermediate **7** was reacted with 50% hydroxylamine to afford the designed hydroxamic acid **8**.

Compounds **11a**–**11p** were synthesized from substituted acetophenones **5a**–**5c** by the route displayed in Scheme 2. Compounds **6a**–**6g** were treated with hydrazine hydrate in acetic acid at 120 °C to give intermediates **9a**–**9g**. Intermediates **9a**–**9g** were reacted with methyl 4-(bromomethyl)benzoate or methyl 4-(2-bromoethyl)benzoate, then the ester groups of products were converted into the corresponding hydroxamic acids of **11a**–**11p**.

The synthetic route to compounds **16a**–**16f** was depicted in Scheme 3. Intermediate **14** was synthesized from 4-fluoroacetophenone through condensation, cyclization and hydrolysis successively. Intermediate **14** was coupled with appropriate amino acid methyl esters to afford the required amides **15a**–**15f**, which were treated with 50% hydroxylamine to produce **16a**–**16f**.

2.2. In vitro HDAC inhibition and selectivity

The HDAC inhibition activities of novel compounds against HeLa nuclear extracts and recombinant human HDAC1, 2, 3, 6, 8 enzymes were investigated, using vorinostat and Rolinostat as positive controls. As shown in Tables 1and 2, the ZBG motif had a significant influence on HDAC inhibition activity. Compound **8** (IC_{50} = 0.359 μM) with a hydroxamic acid group showed more potent activity against HDAC6 than celecoxib (IC_{50} = 0.643 μM). The substituent groups on benzene ring of SRM also have influence on the selectivity. When the linker is on 1′ of the pyrazol, the activity against HDAC6 follows the trends of **11e** (R^1 = Me, IC_{50} = 0.029 - μM), **11g** (R^1 = 3, 4-OCH_2O, IC_{50} = 0.036 μM) and **11i** (R^1 = F, IC_{50} = 0.020 μM), suggesting a bulk substituent is unfavorable for HDAC6 inhibition activity. For the R^2 group, the selectivity and activities decreased when the substituent getting larger (exemplified by **11i**, **11k** and **11m**). For the distance between pyridine and the phenyl in linker, carbon length with n = 1 (**11a** and **11b**) gave the superior activities with IC_{50}s of 0.027 μM and 0.016 μM against HDAC6, respectively. Systematic evaluation reflected that the position of linker on pyrazol largely influenced HDAC6 selectivity (1′ substituted > 2′-substituted > 3′-substituted). For example, compound **11i** is the most selective HDAC6 inhibitor with a SF of 101.1 which higher than **11j** (SF = 60.0) and **16a** (SF = 38.0). For the linker group, benzene ring is favorable for HDAC6 selectivity and the length with 5 or 6 carbon is favorable for HDAC inhibition activity (exemplified by **16a**, **16e** and **16f**).

2.3. Cell growth inhibition assay

The antiproliferative effects of novel compounds were tested against A549 (human lung cancer) and HpeG2 (human liver cancer) cell lines with Vorinostat as a positive control.

The results of the anti-proliferation assay of synthesized compounds are summarized in Table 3. Most hydroxamate analogues manifested significant anti-proliferative activities against two

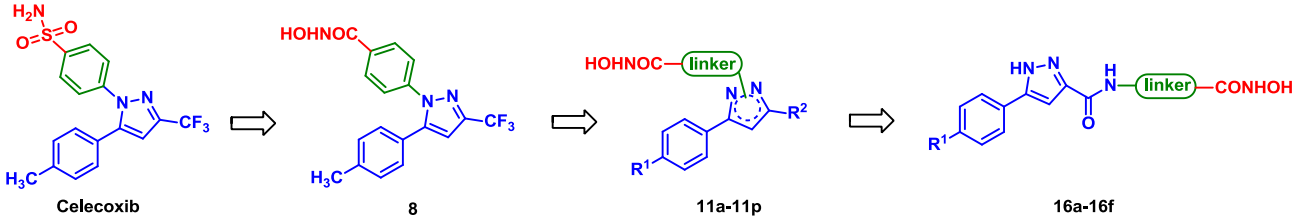

Fig. 2. Rational design of Celecoxib derivatives.