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Design, virtual screening, molecular docking and molecular dynamics studies of novel urushiol derivatives as potential HDAC2 selective inhibitors

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ABSTRACT

Three series of novel urushiol derivatives were designed by introducing a hydroxamic acid moiety into the tail of an alkyl side chain and substituents with differing electronic properties or steric bulk onto the benzene ring and alkyl side chain. The binding affinity toward HDAC2 of the compounds was screened by Glide docking. The best scoring compounds were processed further with molecular docking, MD simulations and binding free energy studies to analyze the binding modes and mechanisms. Six compounds, **21**, **23**, **10**, **19**, **9** and **30**, gave Glide scores of − 7.9 to − 8.5, which revealed that introducing −F, −Cl, triazole, benzamido, formamido, hydroxyl or nitro substituents onto the benzene ring could increase binding affinity significantly. Molecular docking studies revealed that zinc ion coordination, hydrogen bonding and hydrophobic interactions contributed to the high calculated binding affinities of these compounds toward HDAC2 and that His145, His146, Gly154, Glu103, His183, Asp104, Tyr308 and Phe155 contributed favorably to the binding. MD simulations and binding free energy studies showed that all complexes possessed good stability as characterized by low RMSDs; low RMSFs of residues, moderate hydrogen bonding and zinc ion coordination; and low values of binding free energies. van der Waals and electrostatic interactions provided major contributions to the stability of these complexes. These results show the promising potential of urushiol derivatives as potent HDAC2 binding lead compounds.

1. Introduction

Histone deacetylases (HDACs) are a group of zinc metalloenzymes that regulate chromatin remodeling and gene transcription by catalyzing the removal of an acetyl group moiety from the Ɛ-amino groups of lysine residues on the amino terminal tails of the core histones (Ning et al., 2013). HDACs play a pivotal role in the regulation of gene expression, cell growth, and proliferation (Hildmann et al., 2007). Overexpression of HDACs has been linked to the development of cancers in humans (Drummond et al., 2005). Thus, HDAC has become an important target enzyme for anticancer therapies.

To date, 18 HDACs isoforms have been found in humans. Based on their homology to yeast HDACs, they can be divided into four classes: class I (HDAC1–3, and 8), class II (HDAC4–7, 9 and 10), class III (SIRT 1–7), and class IV (HDAC11) (Johnstone, 2002). Classes I, II, and IV are Zn^{2+}-dependent metallohydrolases, whereas class III enzymes are NAD^{+}-dependent Sir2-like deacetylases (de Ruijter et al., 2003). Of note, HDAC2 belongs to the class I isoforms, which is highly conserved. It is overexpressed in most solid and hematological tumors and is highly correlating with a worse prognosis, but it is not found in resting endothelial cells and normal organs (Rikimaru et al., 2007). Therefore, selective targeting of the HDAC2 isoform by directly inhibiting its functions has become a major focus in cancer chemotherapy (Rikimaru et al., 2007).

HDAC inhibitors (HDACIs) have been recognized as a new class of anticancer agents. HDACIs enhance the level of histone acetylation and induce cell cycle arrest, differentiation and apoptosis (Hess-Stumpp, 2005). Many HDACIs have been developed and exhibit therapeutic efficacy in both preclinical and clinical trials. Trichostatin A (TSA) is an effective natural hydroxamate HDACI, and suberoylanilide hydroxamic acid (Vorinostat, SAHA) is the first HDACI approved by the FDA for the treatment of advanced cutaneous T-cell lymphoma (CTCL) in 2006. Additionally, many other small molecule agents, such as PXD-101, LBH-589, MM-232 and CUDC-101, are in different phases of clinical trials for the treatment of different cancers (Fig. 1) (Abdel-Atty et al., 2014). Most of these HDACIs are, however, pan-inhibitors, and they show side effects that might limit their further clinical application, such as diarrhea, electrolyte changes, and cardiac arrhythmias, in clinical trials

Abbreviations: HDAC, histone deacetylases; HDACIs, histone deacetylases inhibitors; HDLP, histone deacetylases - like protein; MDS, molecular dynamics simulation; MD, molecular dynamics; RMSD, root mean square deviations; RMSF, root mean squared fluctuation

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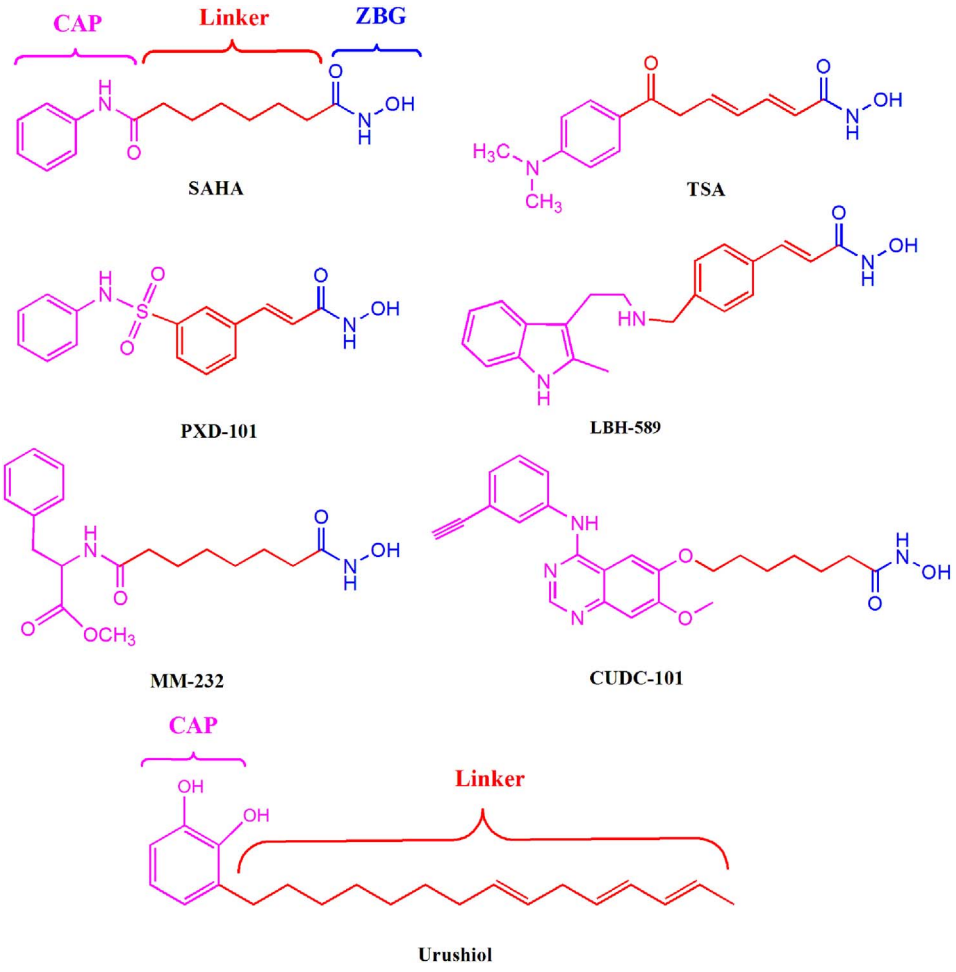

Fig. 1. Structures of some hydroxamate HDACIs and urushiol.

(Yao et al., 2014). Therefore, development of isoform- and class-selective HDACIs to reduce undesirable side effects from off-target activity is highly in demand (Yao et al., 2014).

X-ray crystallographic analysis of HDAC-like protein (HDLP) bound to SAHA revealed the active site of HDAC exists in a hydrophobic pocket, which comprises of a tube-like 11 Å internal channel, a Zn^{2+} center at the bottom of the internal channel, and a 14 Å internal cavity located near this channel (Wang, 2009). It is also known that the structural requirements of a typical HDAC inhibitor should include three distinct domains: metal binding (ZBG), linker and surface recognition (CAP) domains (Cai et al., 2015). The metal binding domain coordinates Zn^{2+} in the bottom of the active pocket of HDACs, and it appears to be a precondition for HDAC-inhibitory activity. Hydrophobicity is important for the linker domain. The surface recognition domain is essential for recognizing and binding to the rim of active pocket of enzymes (Huang et al., 2012b). All of the above provides useful structural information for the design and discovery of novel potent HDACIs.

Urushiol is a natural compound that exists in the sap of the lacquer tree (*Rhus verniciflua* Stokes, Anacardiaceae), which is widely cultivated in northeastern Asian countries, including China, Korea, and Japan (Honda et al., 2008). Urushiol consists of *o*-dihydroxybenzene (catechol) coupled to a unsaturated alkyl side chain of 15 or 17 carbons (Honda et al., 2008). Studies show that urushiol has potent anticancer activities, showing excellent inhibitory effects on many kinds of tumor cells by inducing cell cycle arrest and apoptosis, inhibiting nuclear transcription factors and inducing angiogenesis of tumor cells (Hong et al., 1999). However, urushiol can cause hypersensitivity reactions, and it is prone to oxidation and polymerization, negatively affecting its activity (Ma et al., 2012). As a result, the usefulness of urushiol as a potential anticancer agent has been limited. It is thought that the catechol group of urushiol is the main cause of allergic reactions and polymerization (Xia et al., 2004); therefore, many studies on chemical modifications of this moiety have been carried out to prepare structurally stable urushiol derivatives with no sensitization properties. In a previous study, we reported the replacement of the catechol group with a methylene acetal fragment to the synthesis of urushiol derivatives. These displayed excellent cytotoxicity activities against many types of tumor cells (Wang et al., 2015). Furthermore, some literature reports have shown that the catechol fragment can be changed to a phenylmethyl ether, methylsilyl ether or acetic ester structure to prepare urushiol derivatives, but their antitumor activities were not studied (Markiewitz and Dawson, 1965; Draper et al., 2002; Yamauchi et al., 1980). A recent investigation has shown that urushiol possesses significant HDACI activity (Ryckewaert et al., 2014). The structure of urushiol is similar to that of SAHA (Fig. 1), suggesting urushiol to be a promising lead compound for development of novel HDAC inhibitors. Compared to the classical structure of HDACI, urushiol has the linker and surface recognition (CAP) groups but has no metal binding (ZBG) group; therefore, it is necessary to introduce this key group of HDAC inhibition into its structure. Until now, there has been no report about the design and activity study of urushiol derivatives as potential HDACIs.

Molecular docking and molecular dynamics have proven their pivotal role in the design and virtual screening of new bioactive molecules for drug discovery. Molecular docking has helped in designing and synthesizing inhibitors against enzymes with high efficiency and specificity; molecular dynamics simulation (MDS) provides virtual