Molecular Docking, ADMET Study, Synthesis, Characterization, and Preliminary Antiproliferative Activity of Potential Histone Deacetylase Inhibitors with Isoxazole as New Zinc Binding Group *

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*2nd Scientific Conference for Postgraduate Students Researches.
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Abstract

Histone acetylation is a highly interesting epigenetic target for drug therapy. Histone deacetylases (HDACs) are overexpressed in several diseases, including cancers. Most of the clinically used HDAC inhibitors involve hydroxamate group as a zinc-binding group (ZBG). Hydroxamates have a poor pharmacokinetic properties and high toxicity profile. Therefore, developing non-hydroximate HDAC inhibitors is a promising strategy for potency and selectivity enhancement. In this work, we designed new HDAC inhibitors with isoxazole moiety as ZBG using Ligand Designer from Glide (Schrodinger LLC). The cap group and the linker were optimized through trying various aliphatic and aromatic residues. The potential inhibition over HDAC8 for the optimally designed products was virtually evaluated using licensed Schrodinger modelling software. The results showed that the isoxazole has a potential bidentate interaction with HDAC8 active site zinc ion with acceptable fitness. ADMET study performed to predict the pharmacokinetic properties for the final compounds. The final compounds showed a decent estimated drug-like properties. The intermediates and final compounds were successfully synthesized and purified using column chromatography. The chemical structure for intermediates and final compounds were characterized by IR and NMR spectroscopy. Compounds Va and Vb revealed an encouraging antitumor activity in colon cancer cells (LS-174T) with IC50 of 0.8 μM and 0.88 μM, respectively, which is comparable to vorinostat inhibition activity IC50 of 0.6 μM.

Keywords: Histone Deacetylase, Molecular Docking, ADMET, Cancer, Vorinostat.

Introduction

Uncontrolled epigenetic modifications are associated with human diseases including cancer, Huntington’s, Alzheimer’s, and Parkinson’s diseases, spinocerebellar ataxia, and amyotrophic lateral sclerosis (1). Cancer is one of the major causes of death, and it gained the interest of many scientists to develop a new drug that can stop cancer development (2). A strong correlation between cancer and abnormal epigenetic modifications was recorded (1). Histone acetylation is a known epigenetic modification in the development of cancers and other diseases. Histone acetylation is a dynamic process that controlled by two enzymes of histone acetylate (HAT) and histone deacetylate (HDAC). The importance of targeting HDACs is illustrated by the fact that they are overexpressed in many diseases, including cancers. Histone deacetylases (HDACs) are enzymes that remove acetyl groups from histone tails, leading to chromatin condensation and gene silencing. This activity is crucial in various cellular processes, including gene expression, cell proliferation, and differentiation. Overexpression of HDACs has been associated with various diseases, such as cancer, where it leads to chromatin condensation and gene silencing, leading to tumorigenesis. Therefore, developing HDAC inhibitors has become an attractive strategy for the treatment of cancer and other diseases. HDAC inhibitors, also known as HDACi, are a class of drugs that inhibit HDACs, leading to the activation of gene expression and the induction of apoptosis in cancer cells. HDAC inhibitors are considered promising drugs for the treatment of various diseases, including cancer. They are currently being evaluated in clinical trials as potential therapeutic agents for the treatment of various types of cancer, such as breast, prostate, and colorectal cancer. The most well-known HDAC inhibitor is vorinostat (1). Vorinostat was approved by the US Food and Drug Administration (FDA) in 2006 for the treatment of cutaneous T-cell lymphoma and Kaposi’s sarcoma. Since then, numerous HDAC inhibitors have been developed and evaluated in preclinical and clinical studies. However, the discovery of new HDAC inhibitors with improved potency, selectivity, and pharmacokinetic properties remains an important challenge in the field of oncology and other diseases. New HDAC inhibitors with improved properties are needed to overcome the limitations of the currently available drugs, such as high toxicity, poor pharmacokinetic properties, and lack of selectivity. In this context, the development of new HDAC inhibitors with improved properties is an ongoing effort in the field of drug discovery. The development of new HDAC inhibitors requires a comprehensive understanding of the structure-activity relationships, the pharmacokinetic properties, and the potential side effects of the new compounds. The identification of new HDAC inhibitors is a complex process that involves the design, synthesis, and evaluation of the new compounds. The success of this process depends on the availability of tools and technologies that allow for the rapid evaluation of the new compounds. The current work aimed to design new HDAC inhibitors with improved properties, using a computational approach. The design process involved the selection of a suitable ZBG, the optimization of the linker and cap group, and the evaluation of the potential inhibition over HDAC8. The results showed that the isoxazole has a potential bidentate interaction with HDAC8 active site zinc ion with acceptable fitness. ADMET study performed to predict the pharmacokinetic properties for the final compounds. The final compounds showed a decent estimated drug-like properties. The intermediates and final compounds were successfully synthesized and purified using column chromatography. The chemical structure for intermediates and final compounds were characterized by IR and NMR spectroscopy. Compounds Va and Vb revealed an encouraging antitumor activity in colon cancer cells (LS-174T) with IC50 of 0.8 μM and 0.88 μM, respectively, which is comparable to vorinostat inhibition activity IC50 of 0.6 μM.
Inhibition of HDAC enzyme is promising anticancer therapy strategy. HDAC inhibitors of vorinostat, romidepsin, panobinostat, and belinostat are FDA approved for treating cancer as monotherapy or in combination with other anticancer medications. Three of the clinically used HDAC inhibitors are involving hydroxamate moiety as zinc binding group (ZBG). Hydroxamate claimed to have high toxicity and poor pharmacokinetics properties. Ligand Designer to design a new zinc binding group focusing on heterocyclic ligand generated using Ligand Designer to design a new zinc binding group (ZBG). Virtual compounds were generated by Ligand Designer to design a new zinc binding group. The designed compounds were completed by adding cap and linker using the general pharmacophoric properties based on vorinostat. The protein was chosen from homosapiens to simulate compound that may work on humans with vorinostat as co-crystallized ligands. Crystal structure of HDAC8 (1T69) is obtained from protein data bank (PDB). The protein was prepared using protein preparation wizard including preprocessing of the protein to assign bond order, adding hydrogen, adding terminal oxygen to the chain, deleting water beyond 3 Å, water beyond 3Å from the ligand forming less than 3 hydrogen bond with ligand or amino acid had less probability to affect the docking, from active site, and generating het stat with EPiK Optimizing H-bond assignments was performed using default setting and clean up the structure using OPLS (2005) force field built in with the Schrodinger software 13.1 (1-2023). Receptor grid was generated using the cocrystallized ligand that interacted with the protein using the default setting limiting the size of the grid to 15Å*15Å*15Å. Using ligprep, identify and prepare the set of ligands to be docked, ligprep goes beyond simple 2D and 3D structure conversion by including tautomeric, stereochemical and ionization state as well as energy minimization using OPLS (2005) force field. The prepared ligands were docked against HDAC8 (1T69) using the default setting of XP docking limiting out to 10 poses. The generated poses were visualized by observing the fitness of the ligand into the active site.

ADMET studies

The prepared ligands undergo ligand base ADMET prediction, to assess drug likeness of designed compounds. QIKProp setting the software to identify the 5 most similar drug molecules, the output data checked for drugability of the designed compounds.

Chemical synthesis

Materials

Starting materials, reagents and catalysts were purchased from commercial suppliers (Sigma Aldrich, Glentham, Fluorochem, Macklin, Liyan, Thomas Beaker, Merck). All solvents used were dried using molecular sieves 3Å. Thin-layer chromatography (TLC) was performed using TLC silica gel 60 F254 sheet of 20*20 cm from Merck KGaA and detected under UV light of 254 nm. FT-IR spectroscopy carried out using Shimadzu IRAffinity-1 Spectrometer (Shimadzu, Japan) at the University of Baghdad-College of Pharmacy.
NMR and 13C-NMR analyses were performed at 400 MHz and 100 MHz respectively (d6-DMSO as the solvent) using Bruker Avance III, 400 MHz spectrometer at University of Basra.

**General procedure for the synthesis of 4-(((tert-butyldimethylsilyl)oxy)methyl)aniline (compound I)**

To a round bottom flask of 50 ml equipped magnetic stirrer, 1.5 g of TBDMSCI (10 mmol), 1.23 g of 4-aminobenzyl alcohol (10 mmol), 25 mmol imidazole added to 30 ml of acetonitrile and the reaction was stirred at room temperature overnight. The reaction was monitored with TLC (3 hexane: 1 ethyl acetate). Solvent removed and residue was mixed with hexane to remove unreacted imidazole. The reaction was separated using column chromatography using silica eluted with ethyl acetate / hexane to yield 1 g (43%) of product as colourless liquid (17). IR: 3363, 2954, 2927, 2854, 1253 cm⁻¹.

**General procedure for the synthesis compound II**

To a round bottom flask equipped with magnetic stirrer was added 3 mmol of compound I (711 mg), 3 mmol of substituted benzoic acid, 3 mmol of EDC.HCl (573 mg), 0.3 mmol of HOBt (40 mg), 3 mmol of DMAP (366 mg) and 2 equivalent of DIPEA, and 12 ml DCM. The reaction was monitored by TLC (1 ethyl acetate: 3 hexane) and stirred at room temperature for 18 hours. The resultant residue was washed with 10% NaHCO₃ and 5% HCl. Further purification was performed using column chromatography on neutral silica gel using ethyl acetate / hexane to yield 1 g (43%) of product as white powder (18).

Compound IIa yield is (80%). IR: 3363, 3055, 2951, 2931, 2897, 2854, 1658, 1597, 1523. 1H NMR (400 MHz, DMSO) δ 10.18 (s, 1H), 7.91 – 7.84 (m, 2H), 7.70 – 7.63 (m, 2H), 7.56 – 7.41 (m, 3H), 7.21 (d, J = 8.2 Hz, 2H), 4.60 (s, 2H), 0.82 (d, J = 5.7 Hz, 6H), 0.82 (s, 9H).

Compound IIb yield is (50%). IR: 3363, 3055, 2952, 2927, 2893, 2864, 1658, 1597, 1523. 1H NMR (400 MHz, DMSO) δ 10.10 (s, 1H), 7.84 – 7.77 (m, 2H), 7.69 – 7.62 (m, 2H), 7.50 – 7.43 (m, 2H), 7.20 (d, J = 8.3 Hz, 2H), 4.60 (s, 2H), 1.24 (s, 9H), 0.82 (d, J = 5.4 Hz, 1H), 0.82 (s, 6H).

**General procedure for synthesis of compound III**

To a 20 ml beaker, 1 mmol of compound II and 3 ml of dry methanol were added. At 0°C, 0.25 mmol of acetyl chloride (20 mg) was added, the reaction mixture is monitored by TLC (4 ethyl acetate in 10 hexane) for consumption of the starting material after 15-60 minutes. The solvent was evaporated, and the residue was quenched and neutralized by adding saturated sodium bicarbonate, solvent was removed. Silica column chromatography was performed using gradient eluent (hexane: ethyl acetate). Yield is 70-80% as white powder (19).

**General procedure for synthesis of 4-(hydroxymethyl)phenyl-4-methyl benzamide compound (IIIA)**

To a round bottom flask of 50 ml equipped magnetic stirrer, 1 mmol of compound I (190 mg), 3 mmol of DMAP (366 mg) and 2 equivalent of DIPEA was added. At 0°C, 0.25 mmol of acetyl chloride (20 mg) was added, the reaction mixture was stirred at room temperature for 2 hours, then at room temperature for 6 hours. The reaction mixture monitored by TLC (ethyl acetate: hexane) for the consumption of the starting material. The organic layer was washed with 5% HCl twice, dried over MgSO₄, solvent was removed to get acyl Meldrum as a dark red needle shape powder that undergo further reactions without purification. Yield is 643 mg (87%) of crude.

The resultant acyl Meldrum 2 mmol (374 mg) was added into 15 ml of toluene in 100 ml round bottom flask, N,O-diboc was added to the mixture and heated to 65°C for 8 hours. Monitored by TLC (5 ethyl acetate: 10 hexane), solvent was removed to get an oily mass which further purified using column chromatography on neutral silica gel using ethyl acetate / hexane to yield is 378 mg (65%) of tert-butyloxy carbamoyl (3-oxobutanoyl)carbamate. In the next step 450 mg (1.42 mmol) of tert-butyloxycarbonyl (3-oxobutanoyl)carbamate in 5 ml methanol, 11 mmol concentrated HCl was added and heated to reflux for 3 hours, sodium bicarbonate added to adjust the pH between 2-3, solvent was removed to produce a light brown powder, purified with column chromatography (5 ethyl acetate: 95 hexane: 1 acetic acid), yield is 40 % of compound IV. IR: 3159, 3012, 2939, 1631, 1527. 1H NMR (400 MHz, DMSO) δ 11.03 (s, 1H), 5.74 (s, 1H), 2.26 (s, 3H). 13C NMR (101 MHz, DMSO) δ 170.93, 170.17, 93.82, 12.87.

**General procedure for the synthesis of final compounds (compound V)**

A solution of Di-p-chlorobenzyl Azodicarboxylate (DCAD) (1.1 mmol) in 3 ml dichloromethane in ice bath under argon atmosphere using Schlenk flask of 25 ml was added dropwise to a solution of triphenylphosphine (1.1 mmol), 3-
hydroyisoxazole (1.1 mmol) and benzyl alcohol derivative 1 mmol (compound IIIa and compound IIIb), the mixture is stirred at 0°C for 3 hours, then at room temperature for 12 hours. The solvent was removed to leave a gummy mass that was purified by column chromatography (1.8 ethyl acetate: 8.2 hexane) to get a white powder. Yield is 30%. (21-23).

N-(4-(5-(methylisoxazol-3-yl)oxy) methyl) phenyl)benzamide (compound Va), I.R: 3336, 2924, 2854, 1708, 1561, 1523, 1261. 1H NMR (400 MHz, DMSO) δ 10.17 (s, 1H), 7.80 (d, J = 8.0 Hz, 2H), 7.76 – 7.69 (m, 2H), 7.39 – 7.32 (m, 2H), 7.26 (d, J = 7.9 Hz, 2H), 5.93 (d, J = 1.0 Hz, 1H), 5.08 (s, 2H), 2.31 (s, 3H), 2.23 (s, 3H), 2.18 (s, 1H). 13C NMR (101 MHz, DMSO) δ 171.86, 171.16, 170.86, 170.21, 165.86, 142.13, 139.83, 132.40, 131.39, 129.42, 129.40, 128.99, 126.63, 125.18, 124.97, 119.54, 118.98, 118.83, 118.57, 117.54, 117.16, 116.73, 114.01, 113.95, 113.89, 111.67, 110.91, 109.57, 108.78, 107.39, 106.84, 106.46, 106.16, 105.61, 105.26, 104.81, 104.26, 103.81, 103.36, 102.91, 102.46, 102.11, 101.66, 101.21, 100.76, 99.31, 98.86, 98.41, 97.96, 97.51. HRMS (EI): m/z 420.2041 [M+H]+, calculated for C30H23N2O4 420.2027.

Results and Discussion

Docking Study, the proposed compounds of Va and Vb revealed docking score of -3.75, -4.35 kcal/mol respectively, while vorinostat showed a docking score of -6.16 kcal/mol. After a careful visual inspection of 2D ligand-receptor interaction, compounds Va and Vb impart an accepted receptor fitness through the formation of bidentate zinc chelation, and interaction with several residues inside the active site. Similar to vorinostat, the cap amide carbonyl group for Va and Vb revealed a virtual hydrogen bond formation with PHE208. Additionally, the availability of linker phenyl residue forming a descent π-π stacking with the side chain of HIE180 This interaction is not available for vorinostat due the absence of aromatic moieties in its linker group. Most interestingly, the isoxazole moiety in Va and Vb is chelating the zinc ion in a bidentate manner and forming π-π stacking with HIS 143; while vorinostat hydroxamate group chelating zinc ion and forming two hydrogen bonds with TYR306 and HIS143 residues (Figure 4).

The designed compounds showed an accepted active site fitness supported by filling the active site and bidentate interaction with zinc binding group. The replacing of promiscuous and metabolically unstable hydroxamate moiety with a heterocyclic isoxazole moiety might slightly reduce the virtual binding affinity into the HDAC8 enzyme. However, the descent enzyme fitting, in addition to a promising antiproliferative activity and metabolic profile for novel ZBG of isoxazole might open the avenue for developing biologically active HDAC inhibitors.

Figure 1. The 3D poses for the interaction of compound Vb with HDAC8
ADME-TOX Studies

Several structural features and properties should be considered for the designed molecules to considered as drug-like molecule, such as the rule of five and rule of three for orally administered drug also is a vital approach for avoiding expensive late preclinical trial and clinical trials frustration. Compounds Va and Vb showed acceptable estimated pharmacokinetic properties. As the results indicated several hydrogen bonds tendency and metabolic stability. In addition, molecules having decent calculated oral absorptivity and no violation for drug-like molecules rules (Table 1). (27,28)

Table 1. The predicted ADMET data for the synthesized compounds

<table>
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<th>Compound</th>
<th>CNS</th>
<th>#metab</th>
<th>Human Oral Absorption</th>
<th>Percent Human Oral Absorption</th>
<th>RuleOfFive</th>
<th>RuleOfThree</th>
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<td>3</td>
<td>67</td>
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</tr>
<tr>
<td>Vb</td>
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<td>4</td>
<td>3</td>
<td>90</td>
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</table>

Chemical Synthesis

Compound I was prepared by the reaction of 4-aminobenzylalcohol with equimolar tertiary butyl dimethyl silyl chloride (TBDMSCl) in presence of imidazole to selectively protect the hydroxyl group. Compound I underwent an amidation reaction with various acids in the presence of EDC.HCl, DMAP, HOBt, and DIPEA at room temperature followed by simple work-up and purification method to produce compound II in an excellent yield. Compound III was obtained by compound II silyl deprotection using 0.25 equivalent of acetyl chloride in dry methanol to produce benzylalcohol derivatives. The isoxazole moiety (compound IV) was prepared by the reaction of acyl Meldrum with N,O-diboc. The final compounds (compound V) were synthesized through the reaction of compound IV with isoxazole derivative of compound IV in presence of 1.2 equivalent of DCAD, 1.1 equivalent triphenylphosphine (pPh₃) (Scheme 1).
Scheme 1. Synthetic Pathway for Final Compounds

**Antiproliferative Activity**
The preliminary cancer cell growth inhibition assay (MTT assay) indicated that the synthesized compound Va and Vb showed a submicromolar inhibition activity with IC\(_{50}\) of 0.8 μM and 0.88 μM, respectively, that is comparable to vorinostat.
inhibition activity in IC50 of 0.6 μM in colon cancer cells (LS-174T) (Figure 5).

\[ \text{IC50} = 0.6 \mu M \]

**Figure 5.** The IC50 for (A) Compound Va, (B) Compound Vb, (C) Vorinostat, in colon cancer cells (LS-174T).

**Conclusion**

Potential HDAC inhibitors were designed by the instillation of new zinc binding group of isoxazole. The zinc chelation tendency was virtually studied through the molecular docking studies using the licensed Glide software. The final compounds were virtually bound to HDAC8 at lower docking score; however, these molecules are chelating zinc ion in a bidentate manner through the isoxazole amine and oxygen moieties with accepted fitness. Final compounds showed accepted pharmacokinetic properties through the virtual ADMET studies. The designed compounds were successfully synthesized by applying the excellent mild organic synthesis methods with accepted yields. All the intermediates and final products were characterized by FTIR and NMR spectroscopy. The antiproliferative activity study indicated that the synthesized compounds exhibited a promising preliminary cancer cell inhibition which is comparable to the clinically used HDAC inhibitor of vorinostat.

**Funding**

Self-funding.

**Ethics statement**

We confirm as authors that our signing of this form is to guarantee that the submitted manuscript is in accordance with the ethical considerations and we have received the ethical approval from the related institution(s) and no animal or humans involved in this work.

**Author contribution**

The authors confirm contribution to the paper as follows: study conception and design: Ayad Abed Ali, Ali mohammed saeed; data collection: Ali mohammed saeed; analysis and interpretation of results: Ayad abed ali, Ali mohammed saeed.; draft manuscript preparation: Ali mohammed saeed, draft editing and arrangements: Ayad abed ali. All authors reviewed the results and approved the final version of the manuscript.

**Conflict of Interest**

The author declared no conflict of interest.

**References**

7. Al-Hamashi AA, Abdulhadi SL, Ali RMH. Evaluation of Zinc Chelation Ability for Non-
11. RCSB PDB - 1T69: Crystal Structure of human HDAC8 complexed with SAHA [Internet]. [cited 2022 May 13].
Supplementary information

Figure 2. I.R spectrum of 4-aminobenzyl alcohol.

Figure 3. I.R spectra of compound I.
Figure 4. IR spectrum of compound IIa.

Figure 5. IR spectra of compound IIb.

Figure 6. IR spectrum of compound IIIa.
Figure 7. I.R Spectra of 5-methyl, 3-hydroxyisoxazole.
Figure 8. IR spectrum of compound IIIb.

Figure 9. IR spectra of compound Va.

Figure 10. IR spectrum of compound Vb.
Figure 11. $^1$HNMR spectra of compound IIa.

Figure 12. $^1$HNMR spectrum of compound IIb
Figure 13. 1HNMR spectrum of compound IIIa.

Figure 14. 1HNMR spectrum of compound IIIb

Figure 15. 1HNMR spectrum of compound IV

Figure 16 C13 NMR of compound IV
Figure 17. 1HNMR spectrum of compound Va

Figure 18. 1HNMR spectrum of compound Vb

Figure 19. C13NMR spectra of compound Va

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