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Design, synthesis, and antiproliferative activity evaluation of novel N-hydroxyurea derivatives as possible dual HDAC-BET inhibitors

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ABSTRACT

Histone Deacetylase (HDAC) and bromodomain (BET) enzymes represent interesting targets for development of new anticancer molecules. In this work, novel hydroxamates derivative were designed through the implication of modeling docking studies utilizing Glide tool in the Maestro platform 13.0.135, 2021-4 of Schrodinger suite, LLC, New York, 2021 to assess the binding affinity of the designed compounds into HDAC and BET enzymes. Compounds with decent docking scores and virtual dual inhibition activities were selected for synthesis. The proposed molecules were synthesized employing conventional organic synthesis methods through amidation reaction followed by nucleophilic substitution reaction to replace the *p*-bromo with amine group. The N-hydroxyurea containing final compounds IVa and IVb were afforded using CDI and NH_3OH. The ADME properties were virtually assessed utilizing QikProp Schrodinger, New York, NY, 2021. The virtual ADME results were indicated the drug-likeness properties for the final compounds with no major violation for the rule of five. In comparison to vorinostst, compound Vb that involves N-hydroxyurea as Zinc-Binding Group (ZBG), aromatic linker, and sulfonamide cap group showed optimum *in silico* selectivity and potency toward HDAC2, HDAC6, and HDAC8 (-10.19, -7.00, -10.87 kcal/mol, respectively), and while interacting into bromodomain (BRD4) with acceptable docking score of -5.48 kcal/mol. The preliminary antiproliferative activity indicated that compounds Va and Vb inhibited the growth of colon cancer cells (LS-174T) in a submicromolar IC50 of 0.47 μM and 0.18 μM, respectively.

Keywords: n-hydroxyurea derivatives, zinc-binding group, docking study, hdac inhibitors, bromodomain

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INTRODUCTION

Histone Deacetylases (HDACs) are a family of enzymes that responsible for the reversible acetylation of lysine side chains on the surfaces of enzymes and other proteins comprises a vital regulatory strategy in myriad cellular processes such as transcription, the cell cycle, and metabolism [1]. The chemistry of lysine acetylation requires "writers"–acetyl transferases that utilize acetyl-CoA as a co-substrate and "erasers"–deacetylases that catalyze the hydrolysis of acetyllysine to yield lysine and acetate. A total of 18 different isoforms of HDACs that are classified into four classes have been discovered. Overexpression of HDACs is well documented in a variety of diseases such as neurodegenerative diseases, Human Immunodeficiency Virus (HIV), cardiovascular diseases, and cancer [2]. Inhibition of HDAC isoforms have received considerable attention, in particularly in cancer curing. Four HDAC inhibitors have been clinically used for the treatment of cancer [3, 4]. Structures of HDAC inhibitors are generally characterized by a Zinc Binding Group (ZBG), a cap, and a linker for connection. The binding of ZBGs to the zinc ion and surrounding residues play the decisive role in the inhibitory activity of HDAC inhibitors. While the cap and linker are maximizing the HDAC inhibitors potency and selectivity.

Bromodomain-containing proteins are "reader" epigenetic proteins that are containing four members, namely Bromodomain 2, Bromodomain 3, Bromodomain 4, and Bromodomain [5]. These proteins interact with acetylated histones and assemble other proteins to create complexes that enhance the process of transcription, including initiation and elongation. BRD4 is the most extensively studied member of this family and is strongly associated with the control of gene expression and the development of tumors. BRD4 is found in both gene promoters and enhancers and has been seen to selectively accumulate on regulatory areas known as "super-enhancers". Strong correlation between HDACs and BET proteins were identified. It found that a dual modulation of both enzymes is resulting in a synergistic antiproliferative effect [6]. The development of new dual HDAC-BET inhibitor with unique pharmacophores is highly demanded. Following our previous work [7-10], we applied structure-based drug design employing Schrodinger software to develop novel molecules

that involving N-hydroxyurea moiety as zinc-binding group inside HDAC enzymes while the amide end is possibly interacting with BET enzyme (Figure 1).

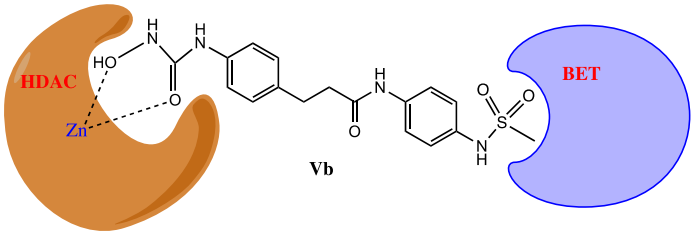


Fig. 1. The proposed dual interaction of Vb with both HDAC and BET enzymes

EXPERIMENTAL

Materials and method

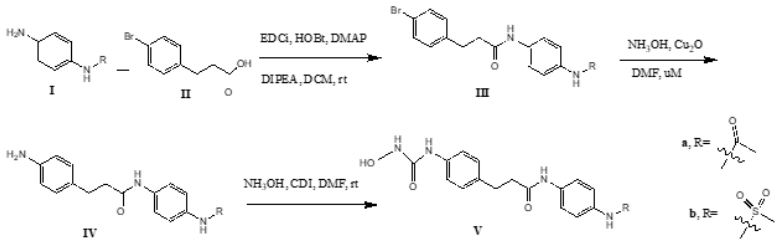
Chemicals and solvents were utilized as obtained from the supplier, and used without additional purification. The 0.2 mm pre-coated TLC-sheets Alugram® Xtra SIL G/UV254 (Macherey-Nagel, Germany) were used, and the visualization was carried out under a 254 nm UV lamp-TLC to monitor the reaction progression. The melting points were determined in open capillary tubes utilizing the Stuart SMP3 melting point apparatus (UK). Fourier Transform Infrared Spectroscopy (FT-IR) was carried out using Shimadzu IRAffinity-1 Spectrometer (Shimadzu, Japan) and Specac® Quest ATR- diamond type (UK). Nuclear magnetic resonance (NMR) spectroscopy was performed by Bruker 500 MHz-Avance III spectrometer (USA).

Molecular docking

Docking study was performed using a licensed Glide within maestro software from Schrodinger's modeling software version 13.0135. The reference compound for modeling is vorinostat [11]. The Ligprep module was utilized to build the chemical structure for the compounds **Va** and **Vb**. The crystal structure for targets, HDAC2 (4LXZ), HDAC8 (1W22), HDAC6 (2VQO), BRD4 (7AMC) were downloaded from protein data bank and prepared using protein preparation wizard from Maestro including preprocessing of the protein to assign bond order, replacing missed hydrogen, adding terminal oxygen to the chain, deleting water beyond 3A°, optimizing H-bond assignments employing default setting, and energy minimization [12-16]. For proteins that involve dimer or trimer, one chain having no amino acid breaks with in the active stie were selected. The active site for docking assigned using grid generation for the co-crystallized ligand obtained from PDB using default setting limiting the size of the grid to 15A° *15A°*15A° [17, 18]. The prepared ligands were docked against the different isoforms of HDACs and BRD4 utilizing High Precision (HP) docking study. The results of the docking were visualized by observing the fitness of the ligand to the active site, docking score, type of bonds involved, number of bonds, energy of Van der Waals and Columbus forces, G-score and other factors [19].

ADMET study

For a drug development effort, it is critical to begin with a moderately hydrophilic chemical as improving a hit molecule to a candidate drug often boosts lipophilicity due to affinity enhancement. The default setup of QIKPROP from licensed Schrodinger suite was used to predict pharmacokinetic properties of the designed compounds. The model compound is vorinostat with hydroxamate which replaced with N-hydroxyurea moiety as ZBG [19].



Scheme 1. The organic synthesis for final compounds

Organic synthesis

General method for amide synthesis (IIIa and IIIb:

To a mixture of p-amino acetanilide Ia (0.75 g, 5 mmol, 1 equiv) or N-(4-aminophenyl) methane sulfonamide Ib (1.15 g, 5 mmol, 1 equiv), EDCI (1.91 g, 10 mmol, 2 equiv) in dry DCM (5 ml/g), were added 3-(4-bromo phenyl) propanoic acid (II) (1 g, 5 mmol, 1 equiv), HOBt (1.35 g, 10 mmol, 2 equiv), DMAP (0.06 g, 0.5 mmol, 0.1 equiv). e reaction was stirred overnight at room temperature. A^{2}er completion, the residue was washed with 5% HCl, 10% sodium bicarbonate, and dried by filtering through Mg_2SO_4. Solvent was removed under vacuum to obtain a crude residue which then purified utilizing column chromatography using (EtOAc: n-hexane) to afford a powder of (III a and IIIb) in yield 80-95%. IIIa, m.p. 117-120. IR (u, cm^{-1}): 3290 (N-H amide), 3101 (C-H aromatic ring), 1654 (C=O amide), 1543 (C=C). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (d, *J*=46.4 Hz, 2H), 7.87–7.40 (m, 4H), 7.33 (s, 1H), 7.21–6.97 (m, 2H), 6.64 (s, 1H), 2.97 (d, *J*=42.6 Hz, 2H), 2.64 (d, *J*=42.6 Hz, 2H), 1.60 (s, *J*=37.7 Hz, 3H). IIIb, m.p. 130-134. IR (u, cm^{-1}): 3294 (N-H), 3124-3043 (C-H str), 1658 (C=O amide), 1600 (C=C aromatic ring), 1512, 1315 (SO_2 str.). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (dd, *J*=9.1, 5.1 Hz, 3H), 7.47–7.33 (m, 6H), 7.10 (t, *J*=6.8 Hz, 1H), 3.28–2.81 (m, 4H), 2.06 (s, 3H) (Scheme 1).

General method for synthesis of compounds IVa and IVb:

To a mixture of copper oxide (Cu_2O) (15 mg, 5.0 mol%) IIIa or IIIb (2.0 mmol), ammonium hydroxide (29%) solution (20.0 mmol) were added 1.3 mL of DMF and in a microwave vial that equipped with magnetic stir bar. The sealed vessel is stirred at microwave at 90°C for 3 h. As the reaction was completed the crude was washed with water (8 mL) and dried by filtering through Mg_2SO_4. Solvent was removed under vacuum to attain a crude gray powder IVa and IVb in yield 55%-65%. The products were used in the next step without further purification. IVa, IR (u, cm^{-1}): 3286,3254 (NH_2 str.), 3051-2927 (C-H str.), 1654 (C=O amide), 1612 (C=C aromatic ring). IVb, IR (u, cm^{-1}): 3344,3232 (NH_2 str.), 3016-2931 (C-H str.), 1654 (C=O amide), 1597-1512 (C=C aromatic ring), 1469, 1312 (SO_2 str.).

General method for synthesis of N-hydroxyurea derivatives (Va and Vb):

Compound IVa (80 mg, 0.24 mmol, 1 equiv) or compound IVb (71 mg, 0.24 mmol, 1 equiv), triethylamine (3 ml, 2 mmol, 2 equiv) were dissolved in dry DMF (2 mL) and stirred for 10 min at room temperature. Then CDI (40 mg, 0.24 mmol, 1 equiv) was added and the mixture was stirred at room temperature after 1 h. Later NH_3OH (48 mg, 0.48 mmol, 2 equiv) was added, and the mixture stirred for 12 h at room temperature. The reaction worked-up by extraction with ethyl acetate and water, and the resulting residues were purified utilizing column chromatography using (EtOAc: n-hexane) to afford yellow solid product in yield 65%-75% of final compounds Va and Vb. For Va, m.p. 130-134. IR (u, cm^{-1}): 3290 (O-H str.), 3143 (N-H str.), 3043-2850 (C-H str.), 1654 (C=O amide), 1597-1546 (C=C aromatic ring). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.05 (s, *J*=7.4 Hz, 1H), 8.92 (s, 1H), 7.36 (d, *J*=8.1 Hz, 2H), 7.26 (dd, *J*=11.2, 6.8 Hz, 2H), 7.16 (s, *J*=7.4 Hz, 1H), 7.10-7.02 (m, 7.4 Hz, 4H), 2.85 (dq, *J*=15.1, 6.8 Hz, 2H), 2.73 (d, *J*=6.8 Hz, 2H), 1.09 (s, 3H). Vb, m.p. 140-145. IR (u, cm^{-1}): 3294 (O-H str.), 3232 (N-H str.), 3128- 2850 (C-H str.), 1739 (C=O amide), 1658-1600 (C=C aromatic ring), 1512, 1315 (SO_2 str.). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.1 (s, *J*=7.4 Hz, 1H), 7.50-7.35 (s, 4H), 7.32-6.7 (m, 8H), 3.0 (d, *J*=7.4 Hz, 2H), 2.6 (d, *J*=7.4 Hz, 2H), 2.3 (s, 3H).

Cancer cell cytotoxicity study

In this work, MTT study was used to evaluate the cell growth inhibition vorinostat, Va, and Vb were incubated with colon cancer cells (174-TB16). The stock for all compounds was 0.1 mg/1 ml, with serial dilution at 50% for each (100, 50, 25, 12.5, 6.25, 03.13 mg/ml). 2 ml of each dilution was Added into 198 ml of cancer cell line and incubate for 24 hours at 37°C. After the exposure period was complete, prepare a solution of MTT reagent and add it to each well, incubate the plate for 4 hours to allow MTT to be converted to formazan. After that, each well was filled with 200 µl of the resulting solution. Plate was incubated at 37°C for 4 hours until purple intracellular formazan crystals were visible under an inverted microscope. After the supernatant was removed, 100 µl of DMSO was added to each well to dissolve the resultant formazan crystals. The plate was incubated for 30 minutes at room temperature until the cells lysed and the purple crystals dissolved. The percentage of cell viability or proliferation was calculated by dividing the absorbance; readings of test samples by those of the control samples and multiplying by 100 to determine the IC_{50} value.

RESULTS AND DISCUSSION

Molecular docking

To evaluate the *in silico* potency and selectivity for Va and Vb, a molecular docking study was carried out on variant HDAC isoforms of HDAC2, HDAC6, HDAC8, in addition to BRD4. The docking score for the final compounds on HDAC isoforms was significantly higher than the FDA approved drug of vorinostat (Table 1). The virtual interaction of Vb with HDAC2 indicating an interesting chelation of carbonyl residue of N-hydroxyurea moiety with zinc ion inside the active site. As well as the phenyl containing linker is nicely inserted through the active site channel and forming p-p stacking with Phe210, while the sulfonamide cap group is fitting into the outside rim as the amide group generating a hydrogen bond with Asp104 (Figure 2).

Tab. 1. The docking score (kcal/mol) for compounds Va, Vb, and vorinostat with several HDAC isoforms and BRD4 enzyme

Code	HDAC2	HDAC6	HDAC8	BRD4
Va	-10.27	-7.13	-11.23	-5.95
Vb	-10.18	-7	-10,87	-5.82
Vorinostat	-7.57	-3.61	-7.95	-3.3

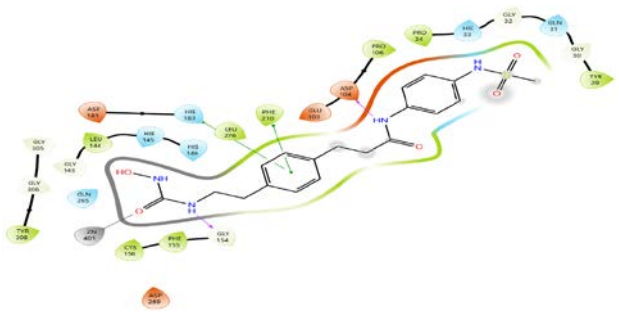


Fig. 2. The 2D interaction diagram of Vb with HDAC2

ADME study

Prior to synthesis or preliminary studies, it is crucial to engage in virtual prediction of medicinal characteristics. Compounds Va and Vb showed acceptable *in silico* pharmacokinetic properties. In addition, molecules having decent calculated oral absorptivity and no violation for drug-likeness rules (Table 2) [20-22].

Tab. 2. ADME study of selected compounds

Title	#rtvFG	CNS	#metab	% Human Oral Absorption	Rule of 5 violation	Rule of 3 violation	M Wt	Donor HB	Accept HB
Vorinostat	1	-2	3	70.953	0	0	264.324	3	6.7
Va	1	-2	3	57.027	0	1	384.434	5	8.7
Vb	1	-2	3	48.162	0	1	420.482	5	10.7

Rtvfg Range of reactive functional group counts, from zero to two a lower value is preferable since it indicates that the functional group is less stable and hence more likely to breakdown to other groups or have a hazardous impact.

Rule of Five Number of times Lipinski's rule of five was broken. Donor HB 5, Receiver HB 10, and Molecular Weight (MW) 500 are the guidelines to follow. Substances meeting these criteria are classified as potential pharmaceuticals.

Rule of Three Jorgensen's rule of three breakdowns in terms of frequency. QPlogS >-5.7, QP PCaco >22 nm/s, and #Primary Metabolites 7 are the three criteria to follow. Orally bioavailable compounds are those that break these criteria less often, if at all.

Chemistry

The synthesis of final compounds was commenced with the amidation reaction between acid (II) with various amines (Ia and Ib) to afford amides (IIIa and IIIb). Using copper oxide and NH_3OH, the *p*-bromo substituted compounds (IIIa and IIIb) were replaced with amines (IVa and IVb), which then reacted with CDI and NH_3OH to afford final compounds (Va and Vb).

Antiproliferative activity

The preliminary MTT assay against colon cancer cell line (LS-174T) indicated that the N-hydroxyurea molecules (Va and Vb) showed a decent inhibition activity with half maximal inhibitory concentration (IC_{50}) of 0.47 mM and 0.185 mM, respectively, which is comparable to the FDA approved drug vorinostat that has an IC_{50} of 0.7 mM (Figure 3) [23, 24].

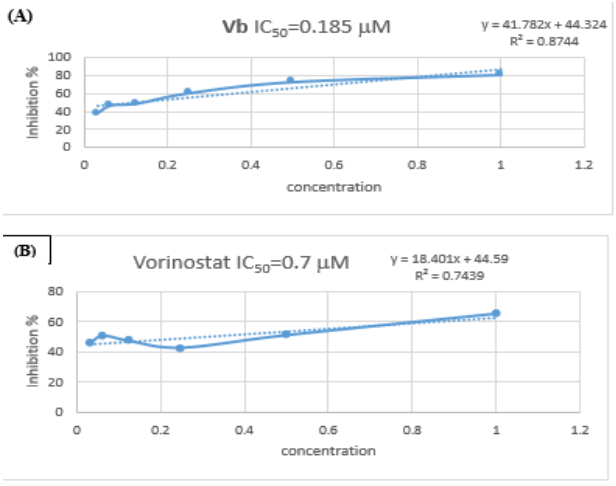


Fig. 3. The dose-response inhibition assay of (A) Vb, (B) Vorinostat, against colon

CONCLUSION

New HDAC/BET inhibitors were designed by the instillation of new zinc binding group of N-hydroxyurea. The molecular docking studies showed an acceptable virtual interaction with both HDAC and BET enzymes. Final compounds were synthesized utilizing conventional organic synthesis methods. The *in silico* ADME studies revealed an acceptable drug-likeness. The preliminary antiproliferative study on cancer cells of LS174T indicated that compound Vb has a promising inhibition activity with a submicromolar IC_{50}.

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AUTHORS CONTRIBUTION

Authors are equally contributed

CONFLICT OF INTERESTS

Author declared that there is no conflict of interest.

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