In Silico Profiling of Histone Deacetylase 8 Inhibitory Activity: A Computational Analysis of Novel Dipeptide-Based Compounds Cross-Linked with Hydroxamic Acid

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Abstract:

This study involved the development of innovative compounds consisting of dipeptide cross-links combined with hydroxamic acid. Our objective was to assess their binding affinities with histone deacetylase 8 (HDAC8) by conducting a docking study, comparing the results with the reference ligand, suberoylanilide hydroxamic acid (SAHA).

Docking scores were measured in terms of ΔG (Kcal/mol), and the recorded scores for compounds 2A-D were found to be higher than that of SAHA, with values of 87.36, 80.46, 79.42, and 74.14, respectively. Notably, compound 2A, a dipeptide consisting of L-tryptophyl-L-tyrosine linked to a hydroxamic acid moiety, exhibited the highest docking score of 87.36. This finding suggests that compound 2A may possess the most potent HDAC8 inhibitory activity among the other designed compounds. Furthermore, we utilized the SwissADME server to predict the physicochemical properties and additional ADME parameters for the designed compounds. The analysis revealed that all investigated compounds exhibited a high potential for passive oral absorption and demonstrated no penetration into the blood-brain barrier. Compound 2A, 2B, and 2D exhibited one Lipinski's rule violation each, whereas Compound 2C demonstrated no such violations in all parameters. Additionally, compounds 2A and 2C exhibited potential as P-glycoprotein (P-gp) substrates. SAHA did not exhibit inhibition of any of the cytochrome P450 (CYP) enzymes used in this study, whereas compounds 2B, 2C and 2D displayed possible inhibitory activities. These compelling findings provide encouraging prospects for the future synthesis of the designed compounds and warrant further evaluation through in vitro and in vivo biological studies.

Key words: Dipeptide cross-links, Hydroxamic acid, Histone deacetylase 8 (HDAC8), Binding affinities, Docking study, Physicochemical properties, ADME parameters.

فحص الأنشطة المثبطة لإنزيم إزالة الأسيتيل من الهيستون 8: تحليل حاسوبي لمركبات جديدة معتمدة على الببتيد الثنائي والمرتبطة بحامض الهيدروكساميك

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الخلاصة:
في هذه الدراسة، قمنا بتصميم مركبات جديدة تحتوي على روابط البيتيد الثنائي المرتبطة بحامض الهيدروكساميك. هدفنا كان تقييم قدرتها على الارتباط بإنزيم إزالة الأسيتيل من الهيستون ٨ (HDAC8) من خلال إجراء دراسة للربط، ومقارنة النتائج مع المركب المرجعي، حامض الهيدروكساميك سبيرويل انيليد .(SAHA) تم قياس درجات الربط بوحدة ΔG (كيلو سعرة / مول)، وتبين أن درجات الربط المسجلة للمركبات ٢ A-Dكانت أعلى من تلك لـSAHA ، بقيم تبلغ ٨٧,٣٦، ٨٠,٤٦، ٧٩,٤٢، و٧٤,١٤ على التوالي. ولاحظ بشكل خاص أن المركب A٢، وهو ببتيد يتألف من حمض ال-تريبتوفيل ال-التيروزين وحمض الهيدروكساميك، حقق أعلى درجة ربط بقيمة ٨٧,٣٦. تشير هذه النتيجة إلى أن المركب A2 قد يكون لديه أعلى نشاط مثبط لإنزيم إزالة الأسيتيل من الهيستون ٨ بالمقارنة مع المركبات الأخرى المصممة. علاوة على ذلك، استخدمنا خدمات موقعSwissADME للتوقع بالخواص الفيزيوكيميائية والمعايير الإضافية لامتصاص الدواء وتوزيعه واستقلابه وإخراجه للمركبات المصممة. أظهر التحليل أن جميع المركبات المدروسة تظهر إمكانية امتصاص فموي سهل وعدم اختراق الحاجز الدماغي. تم ملاحظة خرق قاعدة لبينسكي في مركب 2A و 2B و2D عدا مركب 2C والذي لم يخرق اي من القواعد، في حين انطبقت جميع المركبات المصممة على المعايير الأخرى. بالإضافة إلى ذلك، أظهرت المركبات A2 و C2 إمكانية كونها مواد أساسية للجليكوبروتين .P لم تظهر SAHA أي تثبيط لأي من أنزيمات سيتوكروم P450 المستخدمة في هذه الدراسة، بينما أظهرت المركبات ٢ Bو ٢ Cو ٢ Dأنشطة تثبيطية محتملة. توفر هذه النتائج المقنعة آفاقاً مشجعة لتحقيق المركبات المصممة في المستقبل وتستحق التقييم اللاحق من خلال دراسات حيوية في الأنظمة الحيوية وفي الجسم.

كلمات مفتاحية: روابط البيتيد الثنائي، حامض الهيدروكساميك، إنزيم إزالة الأسيتيل من الهيستون ٨(HDAC8) ، قدرة الربط، دراسة الارساء الجزيئي، الخواص الفيزيوكيميائية ، معاملات ADME.

Introduction

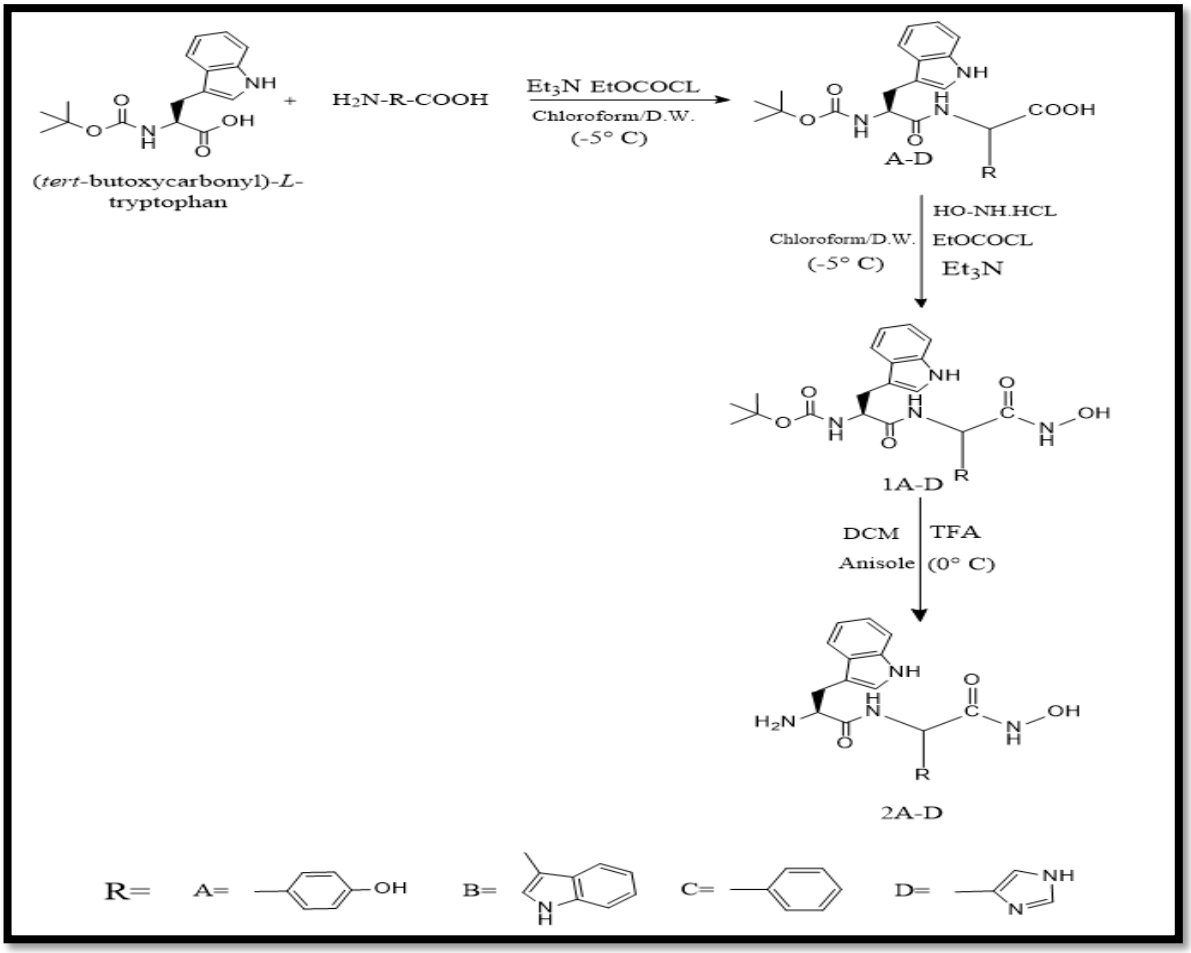
Indole, a heterocyclic compound consisting of a benzene ring fused with a pyrrole ring, has attracted significant attention in medicinal and pharmaceutical research due to its diverse biological activities and structural versatility. Indole derivatives have emerged as valuable scaffolds for the development of pharmaceutical agents, exhibiting promising potential in drug discovery [1]. These compounds have demonstrated various medicinal and pharmacological activities, making them attractive candidates for therapeutic applications [2].The medicinal properties of indole derivatives have been extensively explored, particularly in the field of oncology [3]. Several indole-based compounds have exhibited anticancer activity by targeting specific enzymes like HDACs and signaling pathways involved in tumor growth and metastasis [4]. Additionally, these derivatives have shown promise as anti-inflammatory agents [5], antibacterial [6], antiviral agents[7] and anti-fungal [8], further highlighting their broad range of biological activities [2]. Panobinostat is an indole containing HDAC inhibitor that was approved by the Food and Drug Administration in February 2015 as shown in **figure 1** [9]. Histone deacetylases (HDACs) have emerged as significant targets in the field of epigenetics research, as they play a crucial role in the regulation of gene expression [10]. Epigenetic modifications, such as histone acetylation, are essential for modulating gene transcription and have been associated with various biological processes, including carcinogenesis and tumor development [11]. The acetylation of lysine residues in histone tails by Histone Acetylation Transfer (HATs) neutralizes their positive charge, leading to the relaxation of histone-DNA interactions and enabling transcription factors to access DNA, ultimately promoting gene activation [12]. The dysregulation of HDACs in cancer cell lines and tumor tissues underscores their critical role in tumor development. To counteract the effects of HDACs, histone deacetylase inhibitors (HDACIs) have been developed [13]. These inhibitors have shown promise in inducing acetylation of histone and non-histone proteins, resulting in the activation of specific genes and the repression of others [14]. This modulation of gene expression influences cellular processes such as cell cycle arrest, differentiation, and apoptosis, ultimately

leading to cancer cell death [15]. The synthesis and evaluation of HDAC inhibitors have led to the discovery of hydroxamic acid-based compounds, particularly those containing an indole amide residue at the terminus, as potent inhibitors [16]. Further modifications of the indole ring have significantly enhanced the potency of these inhibitors, resulting in a series of highly active compounds with notable antiproliferative activity [17]. Several HDAC inhibitors, including Vorinostat (SAHA), Romidepsin, Belinostat, and Panobinostat, have been approved by the U.S [18–20]. Food and Drug Administration (FDA) for the treatment of cancer [21]. These inhibitors exhibit their anticancer activity by regulating gene expression and modifying the acetylation status of histone and non-histone proteins. Their therapeutic potential has been investigated in clinical trials, either as standalone treatments or in combination with other anticancer agents [22]. In this study, we propose a rational design of new molecules incorporating tryptophan derivatives as a cap, cross-linked with hydroxamic acid (the Zn+2 binding group), and utilizing various amino acids as linkers. This approach aims to optimize the pharmacological activities of the proposed compounds, particularly their antitumor activity. Previous studies have demonstrated the intriguing biological activity of Indole derivatives, rendering them potential candidates for the development of novel HDAC inhibitors [16]. In conclusion, indole derivatives have garnered considerable interest in pharmaceutical research due to their diverse biological activities and structural versatility [23]. HDACs have emerged as significant targets in epigenetics research, and the development of HDAC inhibitors, including indole-based compounds, holds promise for cancer treatment. The rational design of new molecules incorporating dipeptide hydroxamic acid derivatives presents an opportunity to optimize the pharmacological activities of these compounds, particularly their antitumor effects. Further investigations are warranted to explore the therapeutic potential of these novel HDAC inhibitors in preclinical and clinical settings [16].

Experimental work

The structure of the proposed compounds 2A-D

The design of the proposed compounds 2A-D New compounds containing Indole (as a cap) cross-linked with a hydroxamic acid group (as a ZBG zinc binging group) through amino acid linkers, and a scheme was proposed to synthesize the designed compounds 2A-D, Scheme 1, which was constructed by using ChemOffice software (ChemDraw Professional -19.1.0.8). Basically, it includes the reaction of Boc-l-tryptophan carboxylic acid with the primary amine group of the other amino acid to form an amide bond as a dipeptide, which reacts with the primary amino group of different linkers to form an amide linkage, Boc-dipeptide compounds [24]. Then, Boc-dipeptide compounds will react with hydroxylamine hydrochloride, leading to the formation of the Boc final proposed compounds, 1A-D [24,25], and the deprotection of these compounds to give the deprotected final compounds, 2A-D [26,27].



Scheme 1. A proposed strategy for the synthesis of the designed compounds, 2A-D.

Computer System and Software

Throughout the course of the present study, an ASUS computer system, specifically the LAPTOP-ASUS ROG STRIX model, was utilized. The system was characterized by its distinctive hardware specifications, including an Intel(R) Core(TM) i7-9750H CPU operating at a base frequency of 2.60GHz (with a boost frequency of 2.59 GHz). Furthermore, the system was equipped with 16.0 GB of installed RAM, with 15.9 GB being available for use. In order to facilitate the research endeavors, the study involved the downloading and installation of fully licensed software applications. Notably, the CCDC GOLD suite V. 2021.2.0 and Chemdraw Professional software V.19.1.0.8 were acquired and integrated into the research procedures. Additionally, the Swiss ADME online software was employed as a supplementary tool within the study.

Computational methods for characterizing the compounds evaluated by the ADME program.

The SwissADME online server (www.swissadme.ch) was employed to conduct pharmacokinetic, absorption, distribution, metabolism, and excretion (ADME) studies, as well as to analyze various physicochemical properties of the compounds designed in this research [28,29]. The chemical structures of the newly designed compounds were created using chemAxon's Marvin JS software and subsequently converted into SMILE names. To assess the lipophilicity and polarity of the small molecules, BOILED Egg software was utilized, **Figure 2** [30].

Ligand/Receptor preparation and Molecular docking protocol

In this study, the chemical structures of the molecules under investigation were

accurately drawn using the ChemDraw Professional software (version 19.1.0.8). Energy minimization of the compounds was then carried out using Chem3D Ultra software (version 19.1.0.8) by applying the MM2 force field. For the docking process, the receptors were obtained from the Protein Data Bank (PDB) website (https://www.rcsb.org/) and loaded into the Hermes module of GOLD. Specifically, the crystal structures of the Human Histone deacetylase Receptor (ER) Protein (PDB code: 1T69) in complex with SAHA (vorinostat) were used as the receptor models. Before initiating the docking process, the receptors were prepared by adding polar hydrogen atoms to ensure accurate ionization and tautomeric positions of the amino acid residues. Crystallographic water molecules not involved in the active site were removed, and the original ligands from the receptor's active sites were extracted, and the three-dimensional position of the metal ion was adjusted. The newly designed ligands were then docked using the 3D structure of the prepared active targets (1T69). The setup of the receptors for the docking process was performed using the Hermes visualizer software within the CCDC GOLD suite. The active sites were determined based on the interaction sites of the original ligands. Protein binding sites within a 10 Å radius of the standard ligands were characterized for the docking process. Default settings were used for the docking procedure, including generating 10 positions and keeping the top-ranked solution. The early termination option was disabled. A configuration template of Chemscore kinase was used, and the piecewise linear potential (ChemPLP) was employed as the scoring function. The results were saved as mol.2 files, providing information about the best binding mode, free energy of binding, and docked poses. These results were carefully examined to determine the optimal binding and interaction of the designed ligands and SAHA with the amino acid residues of the HDAC8 receptors. In **Table 1** and **Figures (3-7)**, the docking scores and visualization images of ligand-receptor complexes are presented, respectively.

Results and Discussion

All the compounds under investigation, namely 2A-D, exhibited a zinc binding pattern with HDAC 8 (PDB ID: 1T69) comparable to that of the reference ligand, SAHA. This observation is illustrated in **Figures (3-7) and** summarized in **Table 1**. However, these compounds displayed higher docking scores in terms of ΔG (Kcal/mol) compared to SAHA, as indicated in **Table 1**. Notably, Compound 2A, which consists of L-tryptophan coupled to a hydroxamic acid group via L-tyrosine amino acid as a linker, demonstrated the highest docking score of 87.36. This suggests that Compound 2A may possess superior inhibitory activity against HDAC-8 when compared to the other designed molecules. The enhanced predicted activity of Compound 2A may be attributed to the rigidity of its aromatic ring, which forces the molecule to adopt a specific conformation oriented towards the zinc metal. This conformation facilitates stronger interactions, as depicted in **Figure 4**. Moreover, Compound 2A may engage in π-π interactions (π-π stacking) with aromatic amino acid residues, such as tyrosine, tryptophan, phenylalanine, and histidine, which line the hydrophobic tunnel of the active site in close proximity to the zinc metal. This interaction further contributes to the compound's binding affinity. The importance of the aromatic linker has been evidenced in the development of several newly investigated HDAC inhibitors, including tubastatin A, belinostat, and quisinostat, as depicted in **Figure 8**.

Table 1: Docking scores for SAHA and compounds 2A–D, as well as their interactions with amino acid residues in the active site of HDACs 8 (PDB ID: 1T69)

Compound	Docking scores of hybrid molecules to HDAC 8 type 1T69 ΔG (kcal/mol)	Amino acid residues involved in the interaction with HDAC 8 type 1T69	
Vorinostat (SAHA)	70	Asp101, HIS180, TYR306 and ZN378	
2A	87.36	Asp101, ASP101, ASP101, TYR306 and ZN378	
2B	80.46	Asp101, ASP101, LYS33 and ZN378	
2C	79.42	Asp101, HIS180, and ZN378	
2D	74.14	Asp101, LYS202, SER276, TYR306 and ZN378	

The red color represents a comparable interaction between the investigated compounds and the reference ligand, SAHA.

The Swiss ADME server was utilized to forecast the physicochemical and ADME (absorption, distribution, metabolism, and excretion) properties of the compounds under investigation. The pharmacokinetic parameters of these compounds were documented, revealing variations in properties based on their chemical structures, as demonstrated in Tables 2 and 3. The predicted outcomes indicate that with the exception of compound 2C, which had no violations, Lipinski's rule was only violated in one instance due to the presence of NH or OH > 5. Nevertheless, all the investigated molecules adhered to the remaining parameters. Nonetheless, they exhibited high passive oral absorption, and there was no penetration into the blood-brain barrier (BBB), as depicted in table 3. This outcome may be attributed to violations of other guidelines, such as Veber, Egan, and Muegge, as all the investigated compounds violated these rules, except for 3C, owing to their total polar surface area (TPSA) being >131.6 for Egan, >140 for Veber, and H-don >5 for Muegge. Table 2 highlights the considerable potential of the investigated compounds for oral bioavailability, which could be further evaluated for targeting the lymphatic system in future studies. Compounds 2A and 2C may be considered substrates of P-glycoprotein (P-gp), thereby suggesting that compounds 2B and 2D may exhibit a lower incidence of cellular resistance in in vitro investigations. SAHA did not demonstrate predictive inhibitory activity against any of the cytochrome P450 (CYP) enzymes employed in this study. However, compounds 2B, 2C, and 2D displayed potential predictive inhibitory activities, as outlined in Table 3. Notably, the cap group and linker of the compounds investigated in this study are regarded as more versatile due to the presence of a greater number of hydrogen bond acceptors and donors, as well as an optimal length conducive to interaction with the target enzyme.

Table 2: Drug likeness criteria for SAHA and compounds 2A–D

Compound	Drug likeness according to				
	Lipinski	**Ghose**	**Veber**	**Egan**	**Muegge**
SAHA	Yes	Yes	YES	YES	Yes
2A	Yes, 1 violation: NHorOH>5	Yes	1 violation: TPSA>140	1 violation: TPSA>131.6	1 violation: H-don>5
2B	Yes, 1 violation: NHorOH>5	Yes	Yes	1 violation: TPSA>131.6	1 violation: H-don>5
2C	Yes	Yes	Yes	Yes	Yes
2D	Yes, 1 violation: NHorOH>5	Yes	1 violation: TPSA>140	1 violation: TPSA>131.6	1 violation: H-don>5

Yes: means 0 violations, TPSA= total polar surface area, H-don=Hydrogen donor.

Table 3: Pharmacokinetic characteristics of SAHA and compounds 2A–E

Parameters	**Compounds**				
	SAHA	**2A**	**2B**	**2C**	**2D**
Passive GI absorption	**High**	**High**	**High**	**High**	Low
BBB permeant	**Yes**	No	No	No	No
P-gp substrate	No	**yes**	No	**yes**	No
CYP1A2 inhibitor	No	No	No	No	No
CYP2C19 inhibitor	No	No	**Yes**	No	**Yes**
CYP2C9 inhibitor	No	No	No	No	No
CYP2D6 inhibitor	No	No	**Yes**	**yes**	No
CYP3A4 inhibitor	No	No	No	No	No
Log K_p (skin permeation) cm/s	-6.23	-8.34	-7.87	-8.00	-8.95

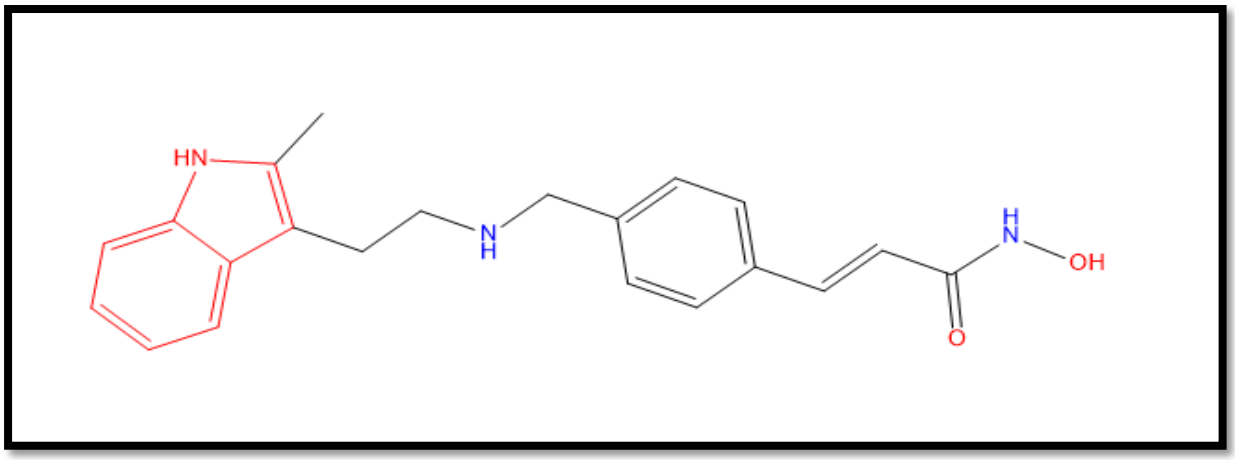


Figure 1. Chemical structure of indole containing HDAC inhibitor Panobinostat.

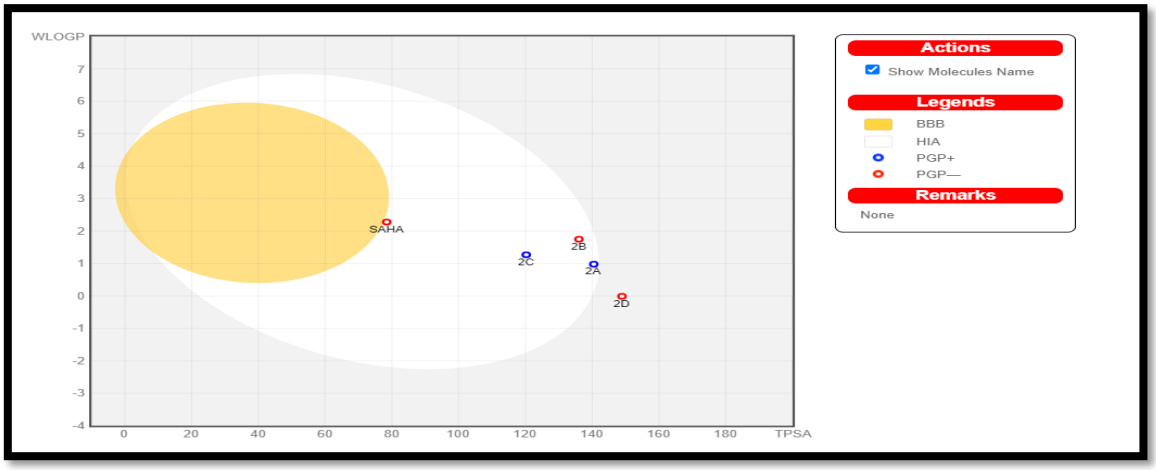


Figure 2. BOILED-Egg structure overview for SAHA and compounds 2A–D.

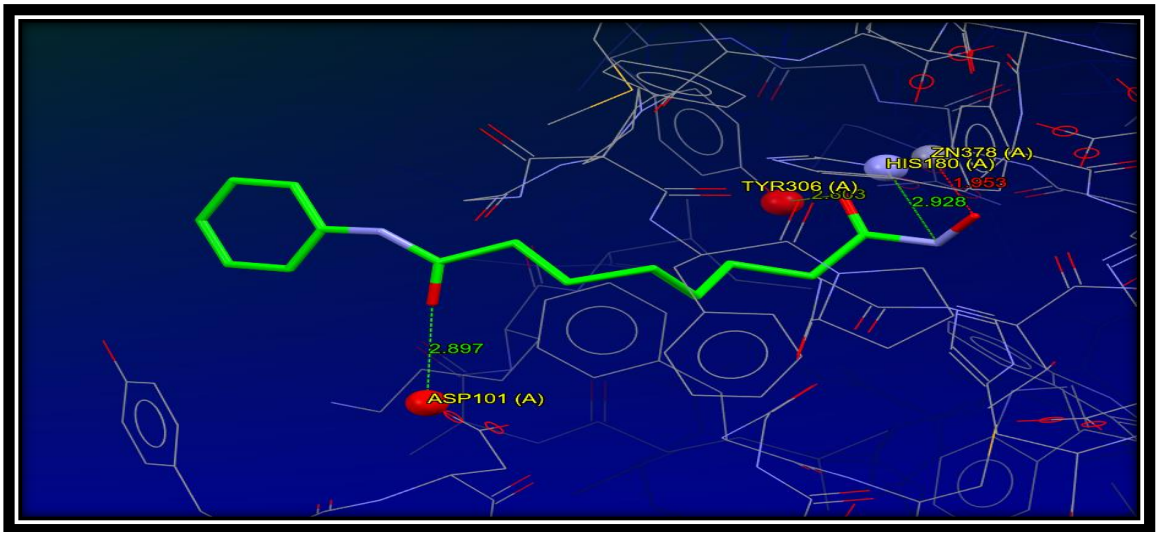


Figure 3. Interaction of Compound SAHA with the amino acid's residues of HDAC 8 catalytic pocket (PDB ID: 1T69)

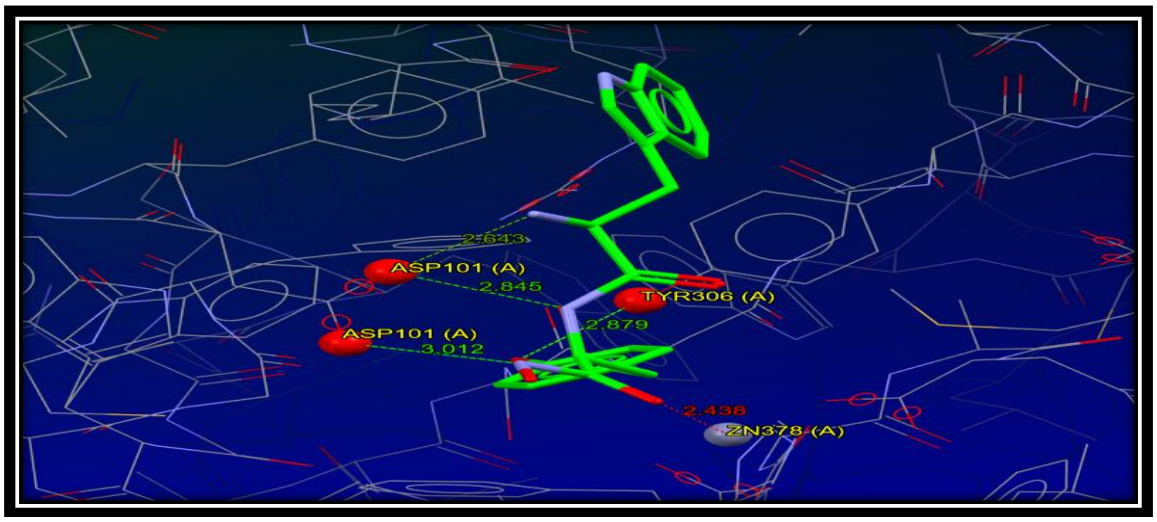


Figure 4. Interaction of Compound 2A with the amino acid's residues of HDAC 8 catalytic pocket (PDB ID: 1T69)

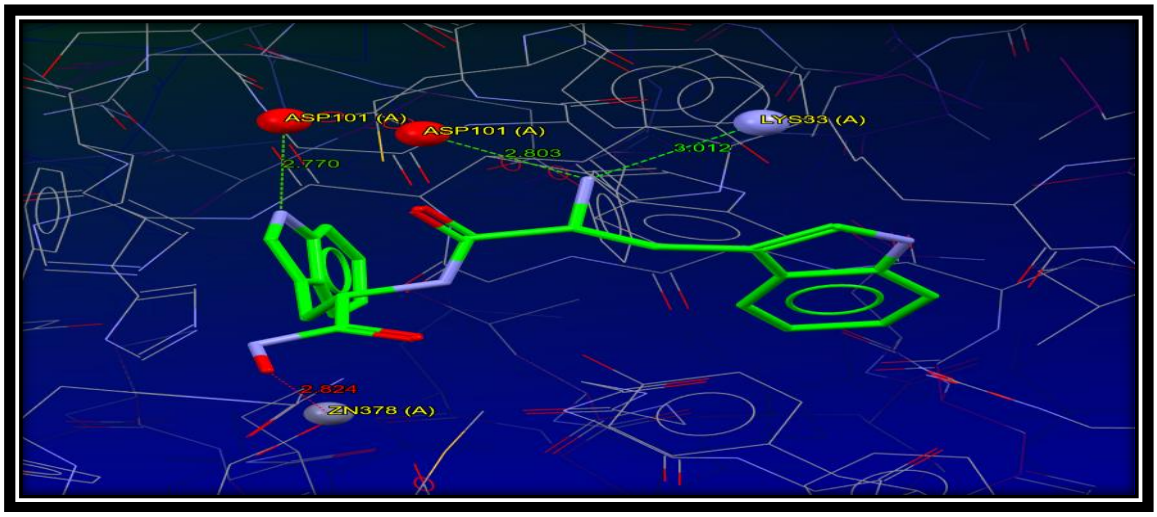


Figure 5. Interaction of Compound 2B with the amino acid's residues of HDAC 8 catalytic pocket (PDB ID: 1T69)

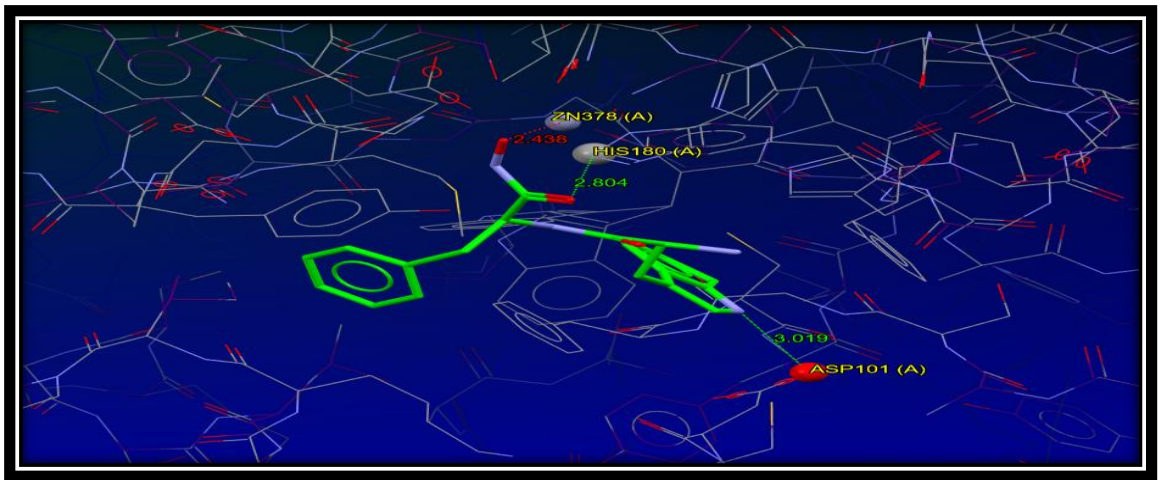


Figure 6. Interaction of Compound 2C with the amino acid's residues of HDAC 8 catalytic pocket (PDB ID: 1T69)

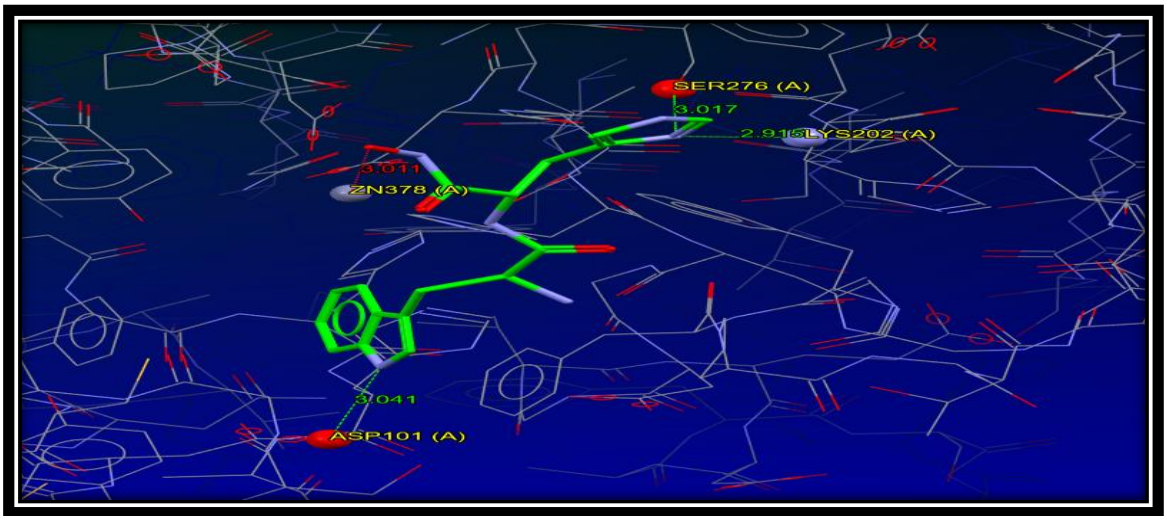


Figure 7. Interaction of Compound 2D with the amino acid's residues of HDAC 8 catalytic pocket (PDB ID: 1T69)

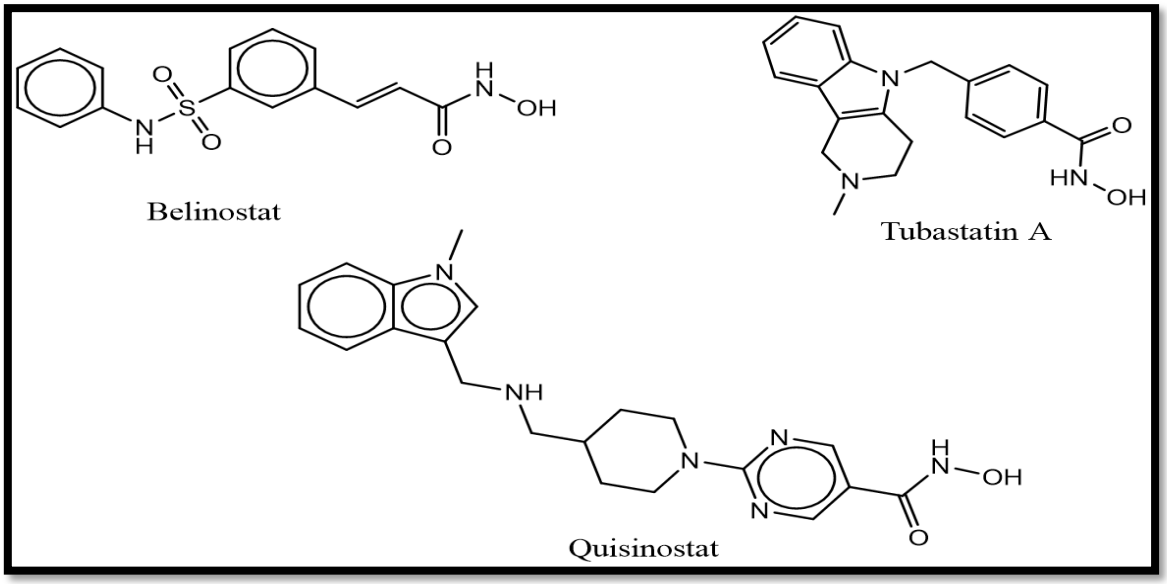


Figure 8. Chemical structures of belinostat, tubastatin A, and quisinostat.

Conclusion

The designed compounds featuring tryptophan cross-linked with hydroxamic acid via amino acid linkers were examined with respect to their binding affinity to HDAC 8 (PDB ID: 1T69) and the prediction of their physicochemical properties and other ADME parameters. The evaluation revealed that all the tested compounds exhibited a higher binding affinity to HDAC 8 compared to the reference ligand, SAHA. Furthermore, their anticipated high oral bioavailability suggests the potential for future investigation regarding their targeting of the lymphatic system. Notably, compounds 2B and 2D are not anticipated to function as P-gp substrates, potentially leading to a reduced occurrence of cellular resistance in in vitro studies. These promising findings provide encouragement for future synthesis of the designed compounds and warrant further assessment through in vitro and in vivo biological studies.

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Divergences of interest

There are no conflicts of interest declared by the authors.

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