

In Silico Prediction of Binding Affinities of Hybrid Molecules of Benzothiazole cross-linked with Hydroxamic acid by certain linkers

Yazen Abdul-Hameed*, Shakir M. Alwan**, Ashour H. Dawood***

*Pharmaceutical Chemistry Department, College of Pharmacy, Al-Mustanseryia University

**Department of Pharmacy, Al-Farabi University College, Baghdad, Iraq, Author of

***Vise Chancellor Al-Esraa University, Baghdad, Iraq.

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Corresponding Author email:

shakir.alwan@alfarabiuc.edu.iq

orcid: <https://orcid.org/0000-0002-9384-8611>

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Abstract:

New hybrid molecules of Benzothiazole cross-linked with hydroxamic acid through an amino acid or aminoalkanoic acid are suggested. All the synthesized hybrid molecules (**2A-E**) were subjected to molecular docking to evaluate their binding affinities with histone deacetylase enzyme (HDAC8, PDB ID: 1T69) and recorded lower ΔG (**-8.117**, **-6.322**, **-8.16**, **-7.939**, **-9.46**, respectively)

than Vorinostat (suberoylanilide hydroxamic acid, SAHA), as the reference ligand, which recorded a much less value of **-5.375**, using the Maestro software (Schrödinger, version 2022-1). Moreover, compound **2E**, which is Benzothiazole-p-amino benzoic acid-hydroxamate has recorded the lowest binding score (-9.460). This may indicate that this compound is the most active hybrid molecule. There were no violations from Lipinski's rule and all the synthesized hybrid molecules comply with all parameters. SwissADME server was employed for the *in silico* molecular docking for prediction of the physicochemical and ADME properties of the investigated compounds. All hybrid molecules showed low possible passive oral absorption and no penetration into BBB. The hybrid molecules **2B** and **2D** may be considered as P-gp substrates. SAHA does not inhibit any of the CYP enzymes used in this study, while, the hybrid molecules **2B**, **2D** and **2E** have shown possible inhibitory activities.

Key Words: Benzothiazole, Amino acids, Molecular hybridization, Vorinostat, HDACs inhibitors

حاسوبيا معرفة الارتباطات الملزمة للجزيئات الهجينة للبنزوثيازول المرتبط بحمض الهيدروكساميك بواسطة روابط معينة

يزن عبد الحميد شكر*, عاشور داود الساعدي**, شاكر محمد علوان***

*قسم الكيمياء الصيدلانية كلية الصيدلة جامعة مستنصرية

**مساعد رئيس الجامعة جامعة الاسراء -العراق -بغداد

***قسم الصيدلة جامعة الفارابي-العراق -بغداد

الخلاصة:

تضمن البحث تحضير جزيئات هجينة جديدة لمجموعة البنزوثيازول المرتبطة بحمض الهيدروكساميك من خلال حمض أميني أو حمض أميني ألكانويك. وفحصت جميع الجزيئات الهجينة المركبة (2A-E) من خلال برنامج الإرساء الجزيئي



لتقييم وقياس درجة ارتباطها بإنزيم هيستون ديستيلاز (PDB ID: 1T69 HDAC8). سجلت المركبات الهجينة أقل طاقة (-8.117، -6.322، -8.16، -7.939، -9.46، على التوالي) من المصدر المرجع (SAHA)، Vorinostat (suberoylanilide hydroxamic acid)، الذي سجل قيمة أقل بكثير من المركبات (5.375)، باستخدام برنامج Maestro (Schrödinger، الإصدار 2022-1). واتضح من النتائج تسجل المركب 2E (وهو البنزوثيازول-بارامينوزول-هيدروكساميك أسيد) أقل درجات الربط (-9.460). قد يشير هذا إلى أن هذا المركب هو أكثر الجزيئات الهجينة فعالية. لم تسجل أي انتهاكات لقاعدة ليبينسكي وجميع الجزيئات الهجينة وتتوافق مع جميع المعطيات الواردة في قواعد ليبينسكي. تم استخدام برنامج SwissADME في الالتحام الجزيئي في التقييم الإلكتروني للتنبؤ بالخصائص الفيزيائية والكيميائية وADME للمركبات المحضرة. أظهرت جميع الجزيئات الهجينة انخفاضاً في امتصاص الفم السلبي المحتمل وعدم إمكانية اختراق جدار الجهاز العصبي BBB. يمكن اعتبار الجزيئات الهجينة 2B، 2D، P-gp ركائز لم يتمكن SAHA تثبيط فعالية أيًا من إنزيمات CYP المستخدمة في هذه الدراسة، بينما أظهرت الجزيئات الهجينة 2B و 2D و 2E إمكانية محتملة في تثبيط فعالية الإنزيمات المذكورة.

الكلمات المفتاحية: بنزوثيازول، الحوامض الامينية، الجزيئات الهجينة، فارينوستات، مثبطات الهيستون دي استيلاز

Introduction

Benzothiazole (BTZ) is a heterocyclic compound, including a fused rings of benzene and a thiazole. Benzothiazole is responsible for the medicinal and pharmacological activities of the naturally occurring products (1). BTZ derivatives have broad spectrum biological activities, such as, antioxidant, anticancer, anti-proliferative, antiviral, anti-diabetic, analgesic, anti-malarial, anti-fungal, anti-histamine and anti-convulsant (2). These compounds perform their action against a variety of cancer cell lines by different mechanisms. Because of the ability of inhibition of tumor-associated carbonic anhydrase by BTZ derivatives they serve as anticancer agents (3). A potent and highly selective class of anticancer drugs known as substituted 2-(4-aminophenyl)-BZT derivatives has shown antitumor efficacy against human cell lines with ovarian, breast, lung, kidney and colon carcinomas (4). Recent research on cancer cell lines has revealed that inhibiting HDACs causes inhibition of cell growth, differentiation, death, and changes in gene expression (5, 6). HDACs inhibitors have relatively low toxicity toward normal cells and inhibit tumor cells from proliferating and surviving. The first FDA-Approved HDACs inhibitor was the hydroxamate, Vorinostat (suberoyl-anilide hydroxamic

acid, SAHA), for the treatment of cutaneous T-cell lymphoma. The development of hybrid multifunctional inhibitors has generated a lot of interest in the search for new medicines. Hybrid molecules have the potential to simultaneously act on two or more cancer-relevant targets, such as, metallo-proteinase. ATP binding cassette subfamily G member 2, human mitochondrial peptide deformylase, nuclear factor kappa light-chain-enhancer of activated B cells, P-glycoprotein, tubulin, and vascular endothelial growth factor (7, 8). Many hydroxamic acid-containing hybrids have showed antiproliferative and anticancer activity and certain hybrids possessed great potency against both drug-sensitive and drug-resistant cancers (9).

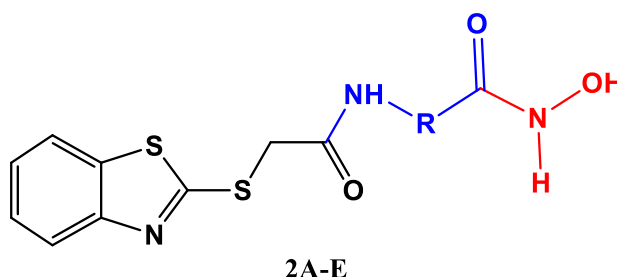
In view of these findings, a series of hybrid molecules of BZT cross-linked with hydroxamic acid via a linker or an amino acid was suggested. This approach may lead to improvement of antibacterial spectrum and/or antitumor activities. Basically, it includes reaction of the carboxyl group of the BZT derivative with the primary amino group of the linker or amino acid forming an amide linkage, which will react with hydroxylamine leading to the suggested hybrid molecules.



Experimental work

Preparation of the investigated hybrid molecules

New hybrid molecules of Benzothiazole cross-linked with hydroxamic acid through an amino acid or aminoalkanoic acid are suggested. These hybrid molecules were prepared starting from 2-mercaptobenzothiazole, which is S-alkylated with bromoacetic acid leading to the formation of Benzothiazole containing a side chain



R: 2A = Ala (-CH₂), 2B =Cys (-CH₂SH), 2C = Aminopropionic acid (-CH₂)₂,
2D = Aminohexanoic acid (-CH₂)₅, 2E = p-Aminobenzoic acid =

Figure 1. The chemical structures of the newly prepared BZT-Hydroxamic acid hybrids.

Molecular docking

Molecular docking has been carried out using the Maestro software (Schrödinger, version 2022-1) and the ΔG (kcal/mol) as the docking scores function representing the energy required for binding to receptor. The chemical structure of HDAC8 type 1T69 was retrieved from protein data bank (PDB). The ΔG (kcal/mol) and the amino acids that are involved in the interaction of the hybrid molecules and the reference compound, SAHA, to the target enzyme HDAC8 type 1T69, were listed on **Table 1**. The successful candidates were selected as those that have high binding affinities based on the lowest docking scores (ΔG , Kcal/mole) on a specific target enzyme HDAC8 type 1T69.

Computational methods for the characterization of the investigated hybrid molecules

ADME program

The SwissADME server (**10**) was used to predict the ADME parameters (Absorption, Distribution, Metabolism, and Excretion) and the other physicochemical properties of the newly synthesized hybrids. ChemAxon's Marvin JS was used to draw the chemical structures of all compounds (**1A-E** and **2A-E**) and their SMILES notations. The BOILED EGG approach was used to assess the possibility of passive gastrointestinal absorption and brain penetration, or the polarity and lipophilicity of the investigated small molecules (**11**).

Results and Discussion



All the synthesized hybrid molecules of BZT cross-linked with hydroxamic acid via an amino acid or an alkanolic acid showed lower ΔG than the reference compound SAHA (Table 3). Moreover, Compound 2E, which is the BZT-p-amino benzoic acid-hydroxamic acid has recorded the lowest docking score (-9.460). This may indicate that this compound is the most active among the hybrid molecules. This is the only compound that contain an aromatic moiety inserted within the linker side chain. Figures 2 and 3 show the docking of SAHA and compound 2E on HDAC8 type (1T69). The aromatic ring in compound 2E is expected to provide an

increase in the binding affinity to the surface of the enzyme by hydrophobic interaction. The molecular docking of SAHA and compound 2E is shown on Figures 2 and 3.

Lipinski's rule of five (12), which relates to the features of drugs that must possess certain properties to be passively orally absorbed includes the calculated parameters. Based on Lipinski's rule, the polar fragments OH-NH (proton acceptor/proton donor) values should be <5 and ≤ 10 respectively. There were no violations from Lipinski's rule and all the hybrid molecules comply with all parameters (Table 1).

Table (1): Lipinski parameters for SAHA and the BZT-Hydroxamic acid hybrids.

Compound	Lipinski violations	H-bond donor	H-bond acceptor
SAHA	Yes; 0 violation	3	3
BZT-Ala-Hydroxamic acid 2A	YES; 0 violations: MW<500, N or O>10	4	3
BZT-Cys-Hydroxamic acid 2B	YES; 0 violations: MW<500, N or O>10	3	4
BZT-Amino propionic-Hydroxamic acid 2C	YES; 0 violations: MW<500, N or O>10, NH or OH>5	3	4
BZT-Aminohexanoic-Hydroxamic acid 2D	YES; 0 violations: MW<500, N or O>10	3	4
BZT-p-Amino benzoic-Hydroxamic acid 2E	YES; 0 violations: MW<500, N or O>10	3	4

SwissADME server was employed for the *in silico* prediction of the physicochemical and ADME properties of the investigated hybrid molecules. The pharmacokinetic parameters of these hybrid molecules have been recorded and there was a variation in properties according to chemical structures, as illustrated on Table 2. As shown from the predicted results, all hybrid molecules have low possible passive oral absorption and no penetration into BBB. The hybrid molecules 2B and 2D may be considered as P-gp substrates.

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SAHA does not inhibit any of the CYP enzymes used in this study, while, the hybrid molecules 2B, 2D and 2E have shown possible inhibitory activities (Table 2). The cap group and the linker of the present investigated hybrid molecules are considered more versatile due to the presence of more hydrogen bond acceptors and also the optimal length favorable for the interaction with the target enzyme. A similar case was noticed with acyl urea moiety when incorporated within the linker side chain of certain HDACs inhibitor



(13). The BOILED EGG approach has referred to the physicochemical properties of the investigated compounds (Table 2). These compounds have low predictive

passive oral absorption and no penetration into the blood-brain barrier (BBB), as shown in Figure 5.

Table (2): The pharmacokinetic properties of SAHA and BZT-Hydroxamic acid hybrids

Parameters	Compounds					
	SAHA	2A	2B	2C	2D	2E
Passive GI absorption	High	Low	Low	Low	Low	Low
BBB permeant	Yes	No	No	No	No	No
P-gp substrate	No	No	Yes	No	Yes	No
CYP1A2 inhibitor	No	No	No	No	No	No
CYP2C19 inhibitor	No	No	Yes	No	No	Yes
CYP2C9 inhibitor	No	No	No	No	Yes	Yes
CYP2D6 inhibitor	No	No	No	No	No	Yes
CYP3A4 inhibitor	No	No	No	No	Yes	Yes
Log K_p (skin permeation) cm/s	-6.23	-6.79	-7.15	-7.31	-6.75	-6.43

Table (3): The binding energies of hybrid molecules and SAHA to HDAC8 type 1T69.

Compound	Docking scores of hybrid molecules to HDAC 8 type 1T69 ΔG (kcal/mol)	Amino acids residues involved in the interaction with HDAC 8 type 1T69
SAHA	-5.357	His142, Asp101, Tyr306, Zinc378
BZT-Ala-Hydroxamic acid 2A	-8.117	His142, Tyr306, Phe207, Zinc378
BZT-Cys-Hydroxamic acid 2B	-6.322	His142, Aso101, Tyr306, Zinc378
BZT-Amino propionic-Hydroxamic acid 2C	-8.160	His142, Phe207, Tyr306, Zinc378
BZT-Amino hexanoic Hydroxamic acid 2D	-7.939	His142, Lys202, Phe207, His180, Tyr306, Zinc378
BZT-p-Amino benzoic-Hydroxamic acid 2E	-9.460	HIS142, Tyr306, Zinc 378



All the synthesized hybrid molecules recorded lower docking scores, when compared with SAHA, except compound **2B**, which recorded comparable result (**Table 3**). Interestingly, compound **2E** has the highest binding affinity on HDAC8 type 1T69 with docking score of (ΔG kcal/mol of -9.46 (**Table 3**), when compared with SAHA (-5.357). **Figures 2** and **3** showed the interaction of SAHA and **2E** with the target site of HDAC8 type 1T69, respectively. When compared with SAHA, the compound **2E** has interacted on approximately the same amino acids (His142, Tyr306) with the same position of Zinc ions at 378. This result may indicate that this hybrid molecule (**2E**) is more potent than the reference standard, SAHA. Compound **2E** contains an aromatic moiety in the linker chain, while the other

hybrid molecules contain aliphatic side chain. The presence of an aromatic moiety adjacent to the hydroxamic acid group has previously shown to increase the activity of the compound (**14**).

All the hybrid molecules and SAHA showed interaction with the target enzyme, HDAC8, Type 1T69, through the hydroxamic acid moiety and the amide bond, except compound **2B**, which has interacted with hydroxamic acid moiety and the thiol group of Cys (**Figure 4**). This may be due to the presence of the thiol group of Cysteine in this compound, which is more potent than the hydroxyl group of hydroxamic acid and conjugated with Asp101 more tightly, while, Zinc ions have interacted with the N of the thiazole ring, as shown on **Figure 4**.

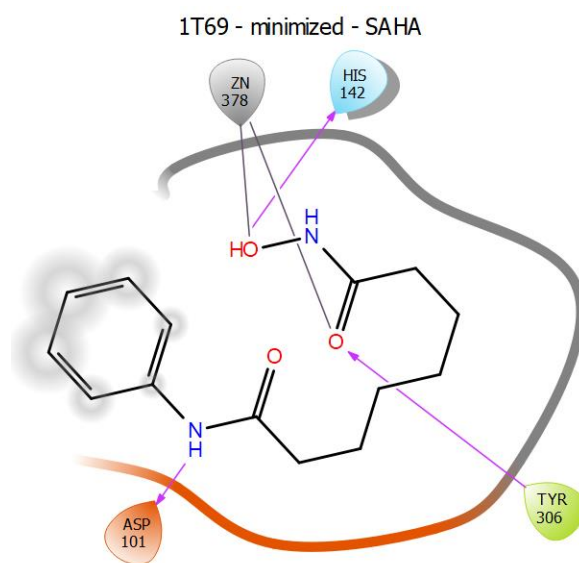


Figure (2): Docking of SAHA on HDAC8 type 1T69 protein

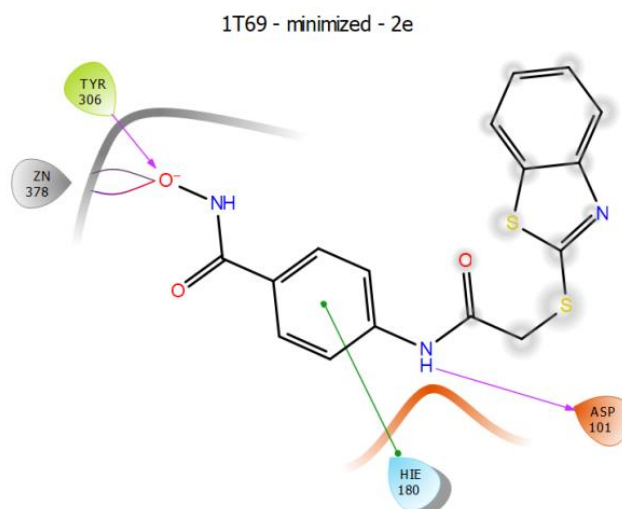


Figure (3): Docking of the hybrid 2E on HDAC8, type 1T69 protein

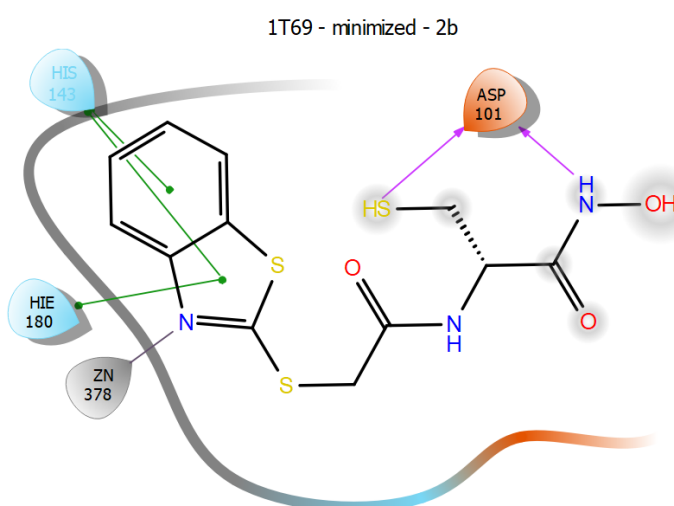


Figure (4): Docking of the hybrid 2B on HDAC8 type 1T69 protein.

A similar method was used for the *in silico* prediction on certain targets to predict the

binding affinities of a number of new derivatives of cephalixin (**15**).

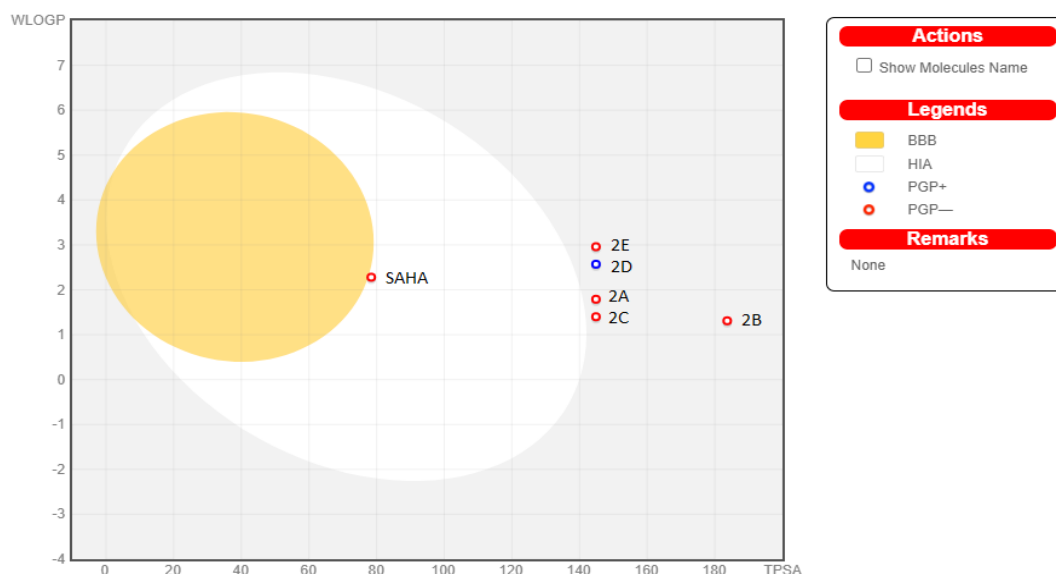


Figure 5. Overview of the BOILED-Egg construction for SAHA and compounds 2A-E

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