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

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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

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الخلاصة:

تضمن البحث تحضير جزيئات هجينة جديدة لمجموعة البنزوثايازول المرتبطة بحمض الهيدروكسامك 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 الهجيمة أقل طاقة (-٨,١١٧، -٦,٣٢٢، -٨,١٦، -٧,٩٣٩، - ٩,٤٦، على التوالي) من المصدر المرجع

Vorinostat (suberoylanilide hydroxamic acid، SAHA) ، باعتباره الرابط المرجعي ، الذي سجل قيمة أقل بكثير من المركبات (٥,٣٧٥)، باستخدام برنامج Maestro (Schrödinger ، الإصدار ٢٠٢٢-١). واتضح من النتائج تسجل المركب 2E (وهو البنزثايزول-باراميتوبنزوك-هيدروكسامك اسد) أقل درجات الربط (-٩,٤٦٠). قد يشير هذا إلى أن هذا المركب هو أكثر الجزيئات الهجينة فعالية. لم تسجل اي انتهاكات لقاعدة ليبينسكي وجميع الجزيئات الهجينة وتتوافق مع جميع المعطيات الواردة في قواعد ليبنسكي. تم استخدام برنامج SwissADME في الالتحام الجزيئي في التقييم الالكتروني للتنبؤ بالخصائص الفيزيائية والكيميائية وADME للمركبات المحضرة. أظهرت جميع الجزيئات الهجينة انخفاضًا في امتصاص الفم السلبي المحتمل وعدم إمكانية اختراق جدار الجهاز العصبي BBB. يمكن اعتبار الجزيئات الهجينة 2B , 2D ركائز P-gp 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 الانزيمات المذكورة.

الكلمات المفتاحية: بنزوثايزول ,الحوامض الامينية ,الجزيئات الهجينة ,فارينوستات ,مثبطات الهيستون دي استليز

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 (١). 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**).

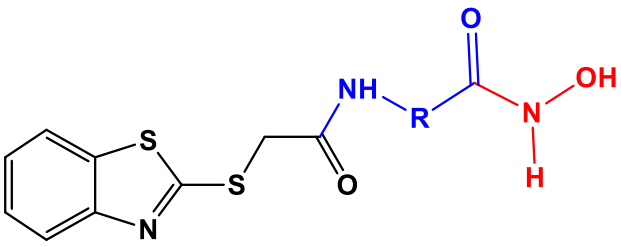
In view of these findings, a series of hybrid molecules of BZT cross-linked with hydroxamic acid via a linker or an amino acid was suggested. This approach may lead to improvement of antibacterial spectrum and/or antitumor activities. Basically, it includes reaction of the carboxyl group of the BZT derivative 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 containing a sulfide and a carboxyl group. The chemical structures of the new hybrids and their SMILES (Simplified Molecular-Input Line-Entry Systems) notations were constructed using Chemdraw ultra 10.0. SAHA was used as the reference compound.



2A-E

R: 2A = Ala ($-CH_2$), 2B =Cys ($-CH_2SH$), 2C = Aminopropionic acid $(-CH2)_2$, 2D = Aminohexanoic acid $(-CH_2)_5$, 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 (**١1**).

Results and Discussion

All the synthesized hybrid molecules of BZT cross-linked with hydroxamic acid via an amino acid or an alkanoic 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. 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**.

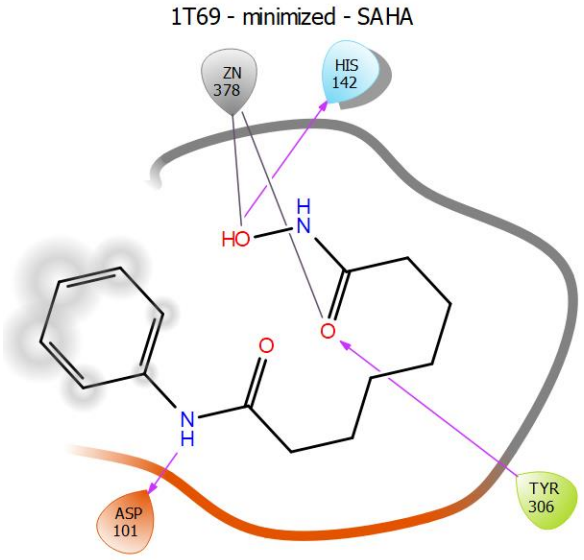


Figure (2): Docking of SAHA on HDAC8 type 1T69 protein

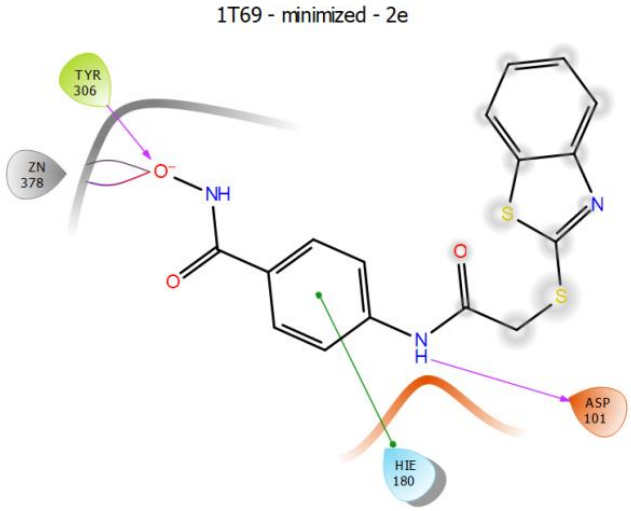


Figure (3): Docking of the hybrid 2E on HDAC8, type 1T69 protein

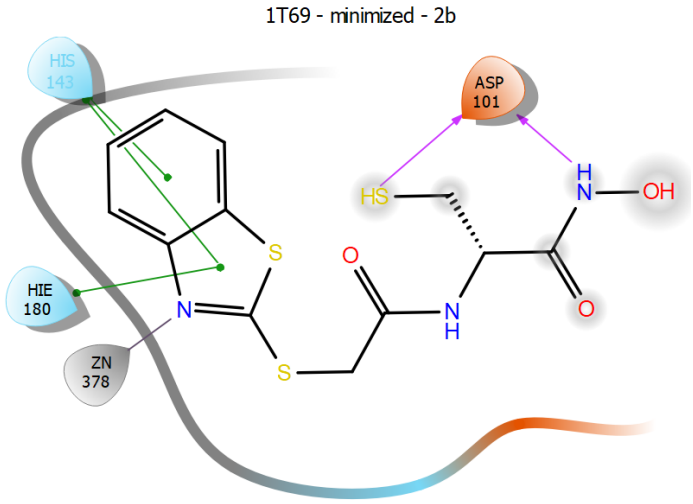


Figure (4): Docking of the hybrid 2B on HDAC8 type 1T69 protein.

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 cephalexin (**15**).

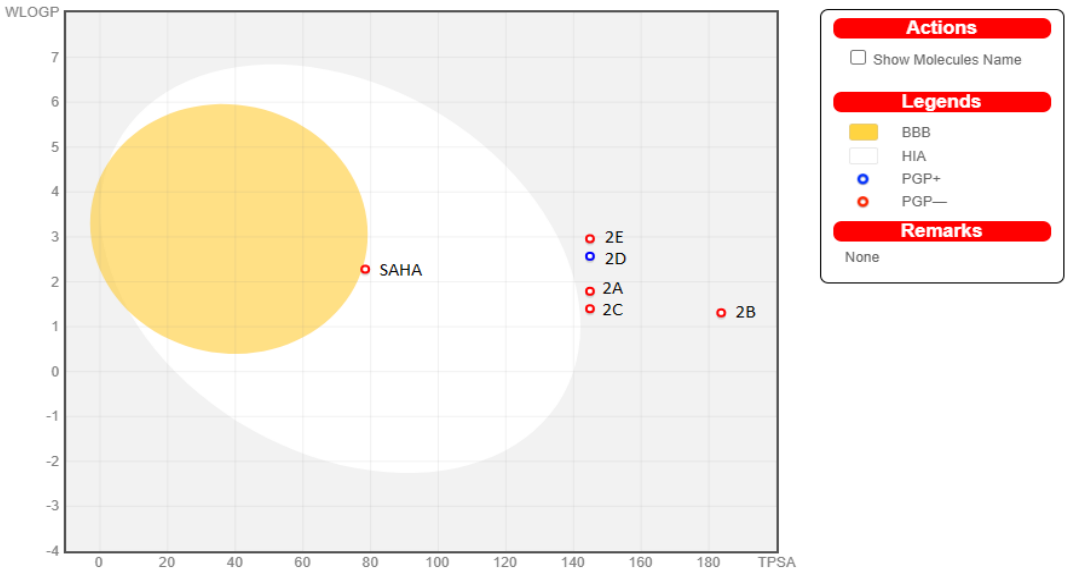


Figure 5. Overview of the BOILED-Egg construction for SAHA and compounds 2A-E

References

1- Ruijter, A. J. M., Gennip, A. H., Caron, H. N., Kemp S., & Kuilenburg, A. B. P. (2003). Histone deacetylases (HDACS): Characterization of the classical HDAC family. Biochemical Journal, 370(3), 737–749. https://doi.org/10.1042/bj20021321

2- Ali, S. A. (2013). Synthesis and preliminary antibacterial study of new 2-mercapto-1, 3- Benzothiazole derivatives with expected biological activity. Al Mustansiriyah Journal of Pharmaceutical Sciences, 13(1), 119–124. https://doi.org/10.32947/ajps.v13i1.187.

3- Witt, O., Deubzer, H. E., Milde, T., & Oehme, I. (2009). HDAC family: What are the cancer relevant targets? Cancer Letters, 277(1), 8–21. https://doi.org/10.1016/j.canlet.2008.08.016.

4- Menta, E., & Palumbo, M. (1997). Novel antineoplastic agents. Expert Opinion on Therapeutic Patents, 7(12), 1401–1426. https://doi.org/10.1517/13543776.7.12.1401

5- Marks, P. A., Richon, V. M., Breslow, R., & Rifkind, R. A. (2001). Histone deacetylase inhibitors as new cancer drugs. Current Opinion in Oncology, 13(6), 477–483. https://doi.org/10.1097/00001622-200111000-00010

6- Bolden, J. E., Peart, M. J., & Johnstone, R. W. (2006). Anticancer activities of histone deacetylase inhibitors. Nature Reviews Drug Discovery, 5(9), 769–784. https://doi.org/10.1038/nrd2133

7- Shaveta, Mishra, S., & Singh, P. (2016). Hybrid molecules: The privileged scaffolds for various pharmaceuticals. European Journal of Medicinal Chemistry, 124, 500–536. https://doi.org/10.1016/j.ejmech.2016.08.039

8- Musso, L., Dallavalle, S., & Zunino, F. (2015). Perspectives in the development of hybrid bifunctional antitumor agents. Biochemical Pharmacology, 96(4), 297–305. https://doi.org/10.1016/j.bcp.2015.06.006

9- Papavassiliou, K. A., & Papavassiliou, A. G. (2013). Histone deacetylases inhibitors: Conjugation to other antitumor pharmacophores provides novel tools for cancer treatment. Expert Opinion on Investigational Drugs, 23(3), 291–294. https://doi.org/10.1517/13543784.2014.857401

10- Sarah Abdul-Razzaq makki, Shakir M Alwan, & Mayada H. Al-Qaissy. (2023). In silico molecular docking, synthesis and preliminary evaluation of antibacterial activity of levofloxacin carboxamides with certain amino acids. Al Mustansiriyah Journal of Pharmaceutical Sciences, 23(1), 22–32. https://doi.org/10.32947/ajps.v23i1.984

11- Daina, A., & Zoete, V. (2016). A boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules. Chem Med. Chem, 11(11), 1117–1121 https://doi.org/10.1002/cmdc.201600182.

12- Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (2012). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews, 64, 4–17. https://doi.org/10.1016/j.addr.2012.09.019.

13- H. Wang, Z.Y. Lim, Y. Zhou, M. Ng, T. Lu, K. Lee, K. Sangthongpitag, K.C. Goh, X. Wang, X. Wub, H.H. Khng, S.K. Goh, W.C. Ong, Z. Bonday, E.T. Sun, Acylurea connected straight chain hydroxamates as novel histone deacetylase inhibitors, Synthesis, SAR, and in vivo antitumor activity, Bioorg. Med. Chem. Lett. 20 (2010) 3314-3321.https://doi.org/10.1016/j.bmcl.2010.04.041

14- J. Jiao, H. Fang, X. Wang, P. Guan, Y. Yuan, W. Xu, Design, synthesis and preliminary biological evaluation of N-hydroxy-4-(3-phenylpropanamido) benzamide (HPPB) derivatives as novel histone deacetylase inhibitors, Eur. J. Med. Chem. 44 (2009) 4470-4476.https://doi.org/10.1016/j.ejmech.2009.06.010

15- Shakir M. Alwan and Jaafar S. Shia, 2023, In Silico Molecular Docking Study for Prediction of Binding Affinities to Penicillin Binding Proteins and β-Lactamases of Amino Acids-Cephalexin conjugates; Journal of Al-Farabi for Medical Sciences, Vol. 1, No. 1.https://doi.org/10.59746/jfms.v1i1.21