In Silico Prediction of Binding Affinities of Hybrid Molecules of Benzothiazole Linked with Hydroxamic Acid by Disulfide Bond and Certain Linkers with HDAC8 Enzyme

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Article Info:

Received Aug 2023
Revised Sept 2023
Accepted Oct 2023
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DOI : https://doi.org/10.32947/ajps.v24i3.1066

Abstract :

A new hybrid molecule of Benzothiazole cross-linked with hydroxamic acid through an amino acid or aminoalkanoic acid were synthesised. All the synthesized hybrid molecules (1-5) were subjected to molecular docking studies to evaluate their binding affinities with histone deacetylase enzyme (HDAC8, PDB ID: 1T69) and recorded lower ΔG (-8.276, -10.093, -8.647, -6.315, -8.676 kcal/mole, respectively) than the reference ligand (Vorinostat, suberoylanilide hydroxamic acid, SAHA -5.375 kcal/mole). Molecular docking studies were performed using the maestro software (Schrödinger, version 2022-1). Moreover, compound 2, which is Benzothiazole-p-amino benzoic acid-hydroxamate has recorded the lowest binding score (-10.093). This may indicate that this compound is the most potent hybrid molecule. There were no violations from Lipinski's rule and all the synthesized hybrid molecules comply with all parameters. Swiss ADME 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 1and 3 may be considered as P-gp substrates.

Key words: Benzothiazole, Amino acids, Molecular hybridization, Vorinostat, HDACs inhibitors.

تقييم درجة ارتباط الجزيئات الهجينة للبنزوثيازول المرتبطة مع حامض الهيدروكسامك بواسطة ثنائي الكبريت مع روابط معينة مع انزيم الهستون دي استيليز بطريقة البرامج الالكترونية

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الخلاصة:

تضمن البحث تحضير جزيئات هجينة جديدة لمجموعة البنزثايزول المرتبطة مع حامض الهايدروكسامك اسد من خلال حمض اميني او حمض اميني الكانوك. وفحصت جميع الجزيئات الهجينة المحضرة (1-5) من خلال برنامج الإرساء الجزيئي لتقييم وقياس درجة ارتباطهما بإنزيم هستون دي استيليز (HDAC8 PDB ID:1T69). سجلت المركبات الهجينة اقل طاقة ارتباط (على التوالي-8.676, -6.315, -8.647, -10.093, -8.276) مقارنة مع المصدر المرجع Vorinostat

suberoylanilide hydroxamic acid, SAHA)، بإعتباره الرابط المرجعي، باستخدام برنامج Maestro (Schrödinger)، الإصدار (2022-1). وأتضح من النتائج تسجيل المركب 2 (وهو البنزثايزول - باراميثوبنزوك - هيدروكسامك أسد) اقل درجات الربط (10.093-). قد يشير هذا إلى أن هذا المركب هو أكثر الجزيئات الهجينة فعالية. لم تسجل أي انحراف لقاعدة ليبينسكي وجميع الجزيئات الهجينة وتتوافق مع جميع المعطيات الواردة في قواعد ليبنسكي. تم استخدام برنامج Swiss ADME في دراسة الالتحام الجزيئي الكترونيا لتوقع بالخصائص الفيزيائية والكيميائية وخواص ال ADME للمركبات المحضرة. أظهرت جميع الجزيئات الهجينة إنخفاضاً محتمل إمتصاص الفموي السلبي المحتمل وعدم إمكانية إختراق جدار الجهاز العصبي BBB. يمكن إعتبار الجزيئات الهجينة المحضرة 1,3 ركائز P-gp

الكلمات المفتاحية: بنزوثايزول، الحوامض الامينية، الجزيئات الهجينة، فارينوستات، مثبطات الهيتستون دي استليز

1.Introduction

Benzothiazole (BTA) is a fused ring of benzene and thiazole and represent a potential scaffold in drug design [**1**]. BTA derivatives have various biological effects such as anti-cancer, anti-tumor, anti-inflammatory, anti-viral, anti-bacterial, anti-proliferative activity, antidiabetic, anticonvulsive, anti-tuberculosis, anti-leishmaniosis, anti-histamine and anti-fungal effects [**2**]. The metalloenzyme carbonic anhydrase was significantly inhibited by the BTA scaffold [**3**]. A potent, highly specific, and distinctive class of anticancer medications is the substituted 2-(4-aminophenyl)-BTs has been studied in vitro and has shown antitumor efficacy against human cell lines with ovarian, breast, lung, kidney, and colon carcinomas [**4**].

A powerful and specific pharmacophore that targets different cancer cell types via a distinct mechanism of action. 2-(4-aminophenyl)-Benzothiazole is synthesized and affects breast cancer cell lines in vivo and in vitro. both in vitro and in vivo testing show that 2-(4-aminophenyl)-Benzothiazole influences the development of human ovarian cancer [**5**]. Hydroxamic acid derivatives have recently received more attention as they may be highly effective in treating various cancer-related etiologies. A hydroxamic acid derivative known as SAHA

is considered to be one of the most effective anticancer agents. [**6**], SAHA suppress the growth of the breast cancer cell lines MCF-7, MDA-MB 231, MDA-MB-435, and SKBr-3 by activation of G1 and G2-M capture and death; however, a clinical study in phase II advised that SAHA and their analogue be further investigated in the management of breast cancer as a component of a triple therapy [**7**]. Additionally, it showed a negligible impact on relapsed primary peritoneal cancer or ovarian cancer or diffuse large B-cell lymphoma [**8**]. Hybrid molecules may act simultaneously on two or more cancer-associated targets, such as metalloproteinase. ATP-binding cassette subfamily G, human mitochondrial peptide deformylase, activated B-cell nuclear factor kappa light chain enhancer, P-glycoprotein, tubulin, and vascular endothelial growth factor. [**9, 10**] Many hydroxamic acid-containing hybrids have shown antiproliferative and anticancer activity, and certain hybrids have shown potent activity against both drug-sensitive and drug-resistant cancers [**11**]. Aim of The Work is to synthesize new hybrid molecules containing Benzothiazole and Hydroxamic acid moiety cross-linked through an amino acid or aminoalkanoic acid. This approach may provide improvement of antitumor activities. Initially, this work starts with the prediction of both the binding affinities of the target compounds using molecular docking program on HDACs, and the ADME profile using the Swiss ADME tool

2.Experimental Work

2.1 Materials and Methods

2-Mercapto-Benzothiazole, Hydroxamic acid and Ethylchloroformate (ECF) were purchased from Sigma Aldrich (Germany). Aminoalkanoic acid linkers were supplied

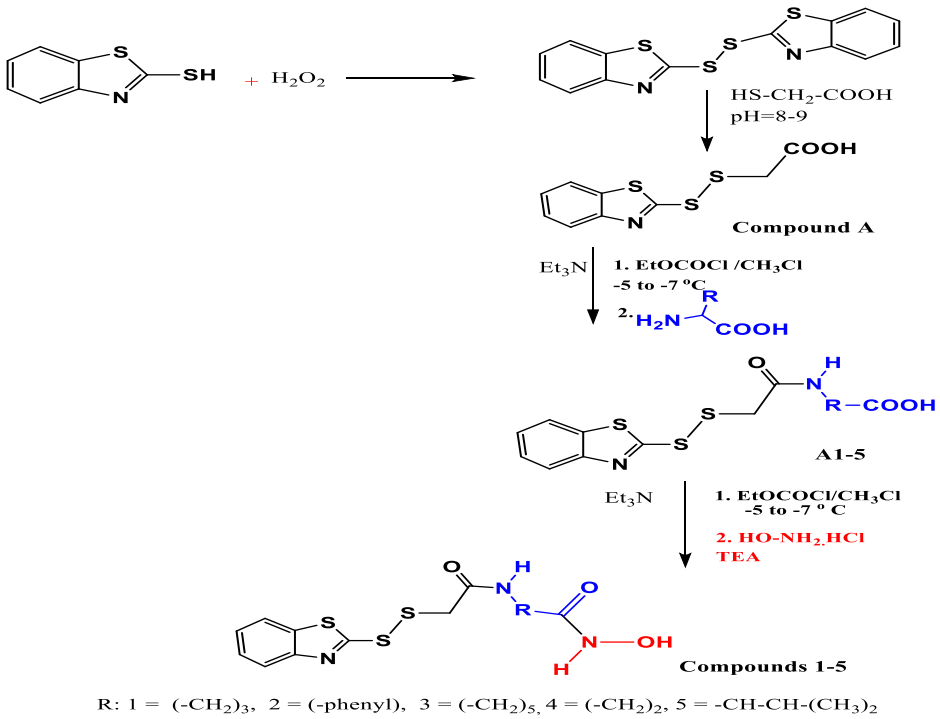
by Alpha-Chemika (India). The melting points were measured using Stuart Electrical melting point apparatus (Germany). FT-IR spectra (4000-400 cm^{-1}) were recorded on a Bruker FT-IR spectrophotometer. The ^{1}H NMR and ^{13}C-NMR were recorded in DMSO-d6 using a Bruker AC-400 (400 MHz and 100 MHZ, respectively) instrument with Tetramethylsilane (TMS), serving as the internal standard. Chemical shifts (d) and coupling constants (J) are stated in ppm and Hz, respectively. The abbreviations engaged for the multiplicity are s (singlet), d (doublet), t (triplet), and m (multiplet) signals.

2.2 General procedure for the synthesis of compound A, A1-5 and target compounds 1-5:

To a solution of 2-mercaptobenzthiazole (10mmol, 1.6725g) in absolute ethanol (30 ml), hydrogen peroxide (H_2O_2, 6 ml, 30%) was added drop wise with continuous stirring. A yellow precipitate was formed, filtered, collected, and washed excessively with distilled water and dried in an oven at 50 ^{o}C. This product was reacted with mercaptoacetic acid (1.5 ml 30%) in KCl (5 ml, 10%) and the pH was adjusted using KOH (10%) drop wise the color changed to mushroom color. The mixture was filtered and the filtrate was separated and acidified with diluted HCl to pH 4-5. A precipitate was obtained washed with distilled water to afford slightly yellow product [12,13].

Compound **A** (10mmol) was suspended in dry chloroform (40ml) containing triethylamine (TEA, 10 mmol) and the mixture was cooled in an ice bath. Ethyl chloroformate (10 mmol) was added dropwise during 15 min and the mixture was stirred for further 15 min. Aminoalkanoic acid (10mmol) was dissolved in a cold distilled water containing TEA (10mmol) and was added immediately all at once with vigorous stirring for 1hr in an ice bath and for further 3hrs at room temperature. A two-layered was obtained, which was separated using a separator funnel and the aqueous layer was removed. The chloroform layer was washed with distilled water and was dried with anhydrous magnesium sulfate, filtered and evaporated under reduced pressure in a rotary evaporator to obtain yellowish-orange residues. The residues were washed with diethyl ether or petroleum ether for few times to afford crystalline powder representing the resultant products **A1-4.**

The target hybrid molecules (**1-4**) were prepared by reacting compounds **A1-4** with hydroxylamine, as depicted in **scheme 1**. This reaction was performed using the mixed anhydride method [**14**], as previously described. Compounds **A1-4** (10mmol) in dry chloroform containing TEA (10mmol) were reacted with ECF (10mmol) and were then reacted with hydroxylamine. HCl (10mmol) in distilled water (10ml) containing TEA (10 mmol).



Scheme 1: Chemical synthesis of the target hybrid molecules

2.3Molecular docking studies

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 2**. 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.

2.4Computational methods for the characterization of the investigated hybrid molecules

ADME program: SwissADME program was used to predict the physicochemical descriptors and pharmacokinetic properties. The permeability through Git and blood brain barrier of the Prepared small molecules have been measured using BOILED-EGG method [15]. The results are summarized on table 1.

3.Results and Discussion

The Swiss ADME server was employed to predict and evaluate the physicochemical and ADME properties of the target hybrid molecules. This method is used to predict which of the synthesized ligands are susceptible for oral absorption, and to rule out the compounds that might fail in the future phases of drug development due to ADME parameters [16].

Table 1: Lipinski parameters and pharmacokinetic properties of BZT-Hydroxamic acid hybrids and SAHA

Compound	H-bond acceptor	H-bond Donor	P-gp	TPSA (A°)	GI Abs	BBB permeant	Bioavailability	Lipinski violation
1	4	3	YES	170.16	Low	NO	0.55	0 violation
2	4	3	NO	170.16	Low	NO	0.55	0 violation
3	4	3	YES	170.16	Low	NO	0.55	0 violation
4	4	3	NO	170.16	Low	NO	0.55	0 violation
5	4	3	NO	157.27	Low	NO	0.55	0 violation
SAHA	3	3	NO	78.43	High	YES	0.55	0 violation

The topological polar surface area (TPSA) was calculated, which is a valuable descriptor for predicting various ADME features, including drug bioavailability and brain access [17]. As a result, molecules having a TPSA >140 A° are likely to be poorly absorbed in the gastrointestinal tract. All of our produced compounds had TPSA values more than 140, and the bioavailability for all ligands was 0.55 suggesting that all compounds comply with the Lipinski rule of five upon bioavailability (M. Wt. less than 500) table 1. The bioavailability score refers to the degree after oral delivery, the extent to which a chemical is absorbed from the gastrointestinal system. The absorption would be excellent if the result was high. All synthesized ligands demonstrated low GI absorption. Furthermore, compounds **1** and **3** showed link with P-gp substrate, which is a well characterized ABC-transporter that transport these compounds across extra-and intracellular membranes. While compounds **2,3**and **5** not considered as substrate of these transporter that leads to prevent resistant of HDAC related carcinogen. The graphical output of all calculations was displayed by BOILED-EGG figure 9 by which two key ADME parameters can be predicted; the passive absorption from GIT and blood brain barrier (BBB) penetration.

Table 2: 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
1	-8.276	His142, Tyr306, Phe152, Zinc378
2	-10.093	His142, Phe152, Tyr306, Zinc378
3	-8.647	Hie180, His142, Tyr306, Zinc378
4	-6.315	His142, Phe152, Tyr306, Zinc378
5	-8.676	Gly151, Gly303, Zinc 378

All the synthesized hybrid molecules recorded lower docking scores, when compared with SAHA, table 2. Interestingly, compound **2** has the highest binding affinity with HDAC8 type 1T69 with docking score of (ΔG kcal/mol of -10.093 table 2, when compared with SAHA (-5.357). figures 3 and 5showed the interaction of SAHA and compound **2** with the target site of HDAC8 type 1T69, respectively. When compared with SAHA, the compound **2** 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 **2** is more potent than the reference standard, SAHA.

Compound **2** 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. [18].

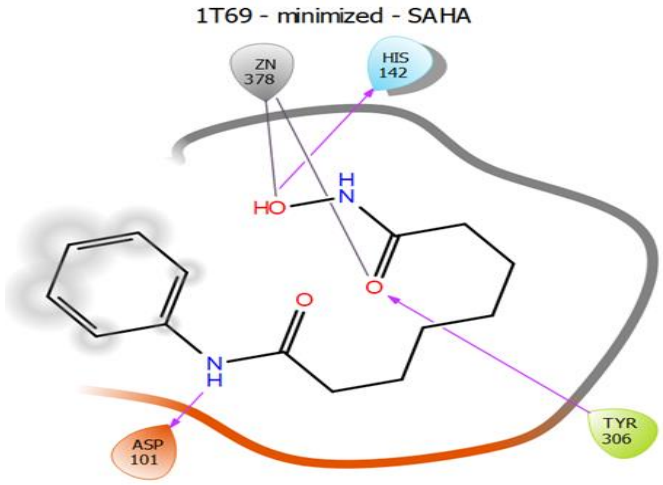


Figure 3: Docking of SAHA on HDAC8 type 1T69 protein

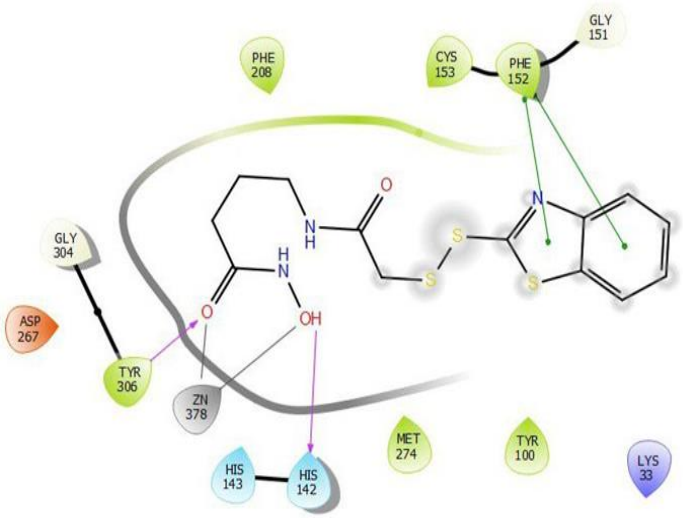


Figure 4: Docking of the hybrid 1on HDAC8, type 1T69 protein

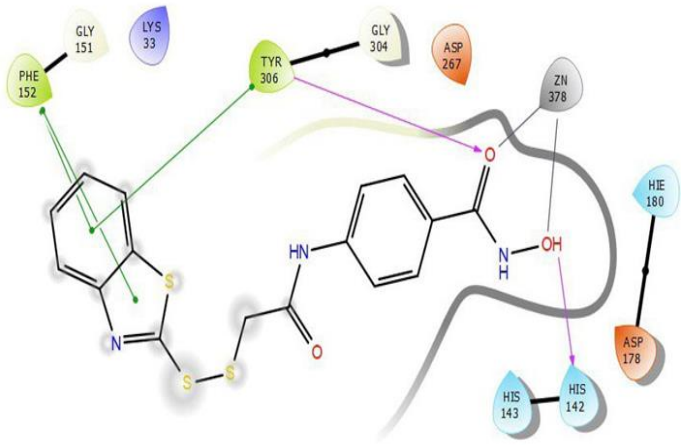


Figure 5: Docking of the hybrid 2 on HDAC8 type 1T69 protein

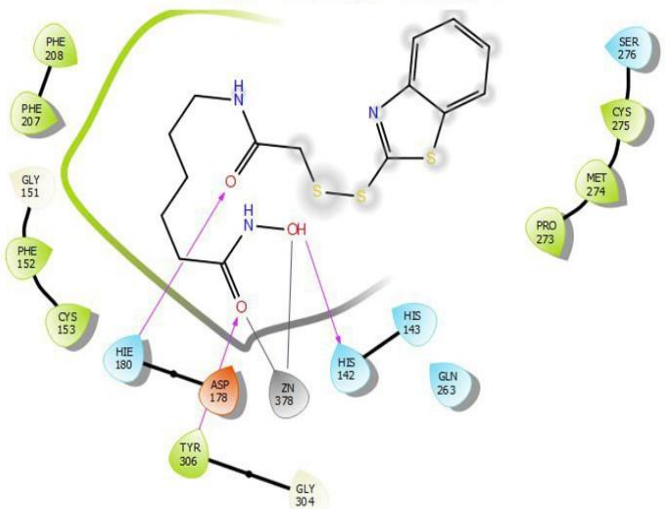


Figure 6: Docking of the hybrid 3 on HDAC8 type 1T69 protein

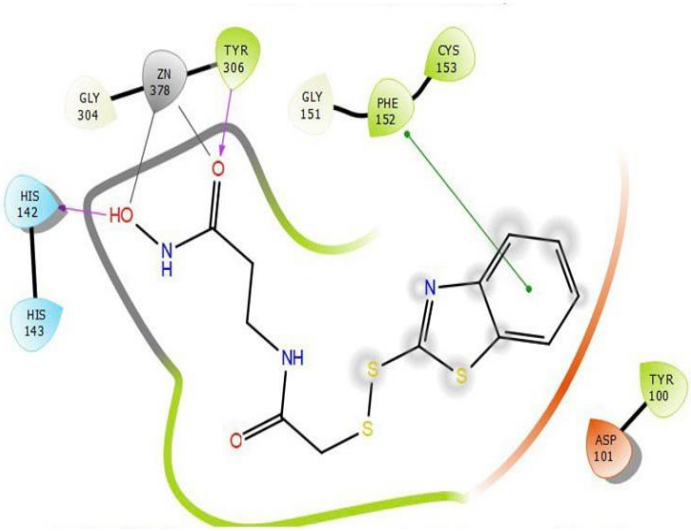


Figure 7: Docking of the hybrid 4 on HDAC8 type 1T69 protein

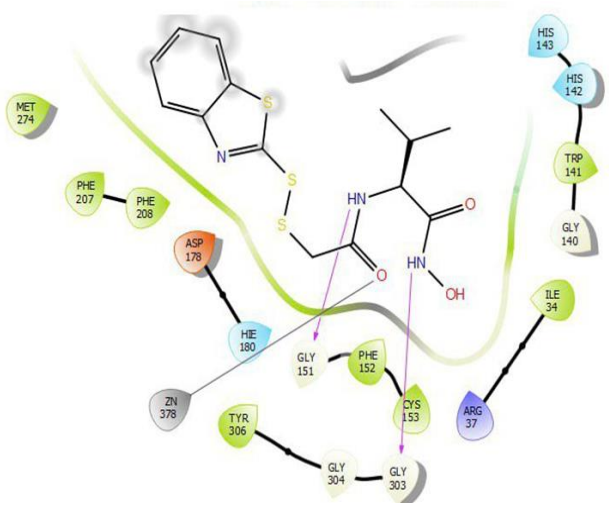


Figure 8: Docking of the hybrid 5 on HDAC8 type 1T69 protein

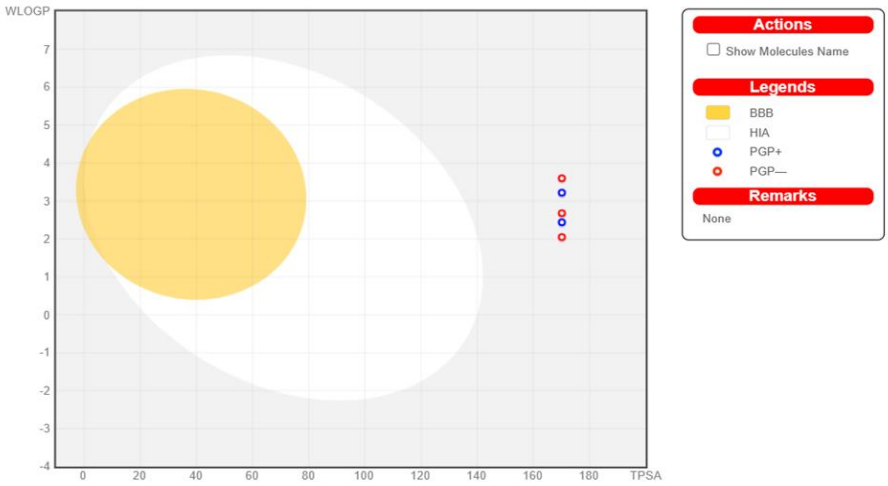


Figure 9: Overview of the BOILED-Egg construction for SAHA and compounds 1-5

Yellow ovule (yolk): molecule that is projected to pass across blood-brain barriers passively. White ovule (white): chemical that the GIT is expected to absorb passively.
PGP+: Blue dots represent chemicals that the PGP is likely to excrete from the CNS.
PGP-: Red dots indicate compounds that the PGP is not expected to remove from the CNS.

Conclusion

All the prepared hybrid molecules of BTA linked with hydroxamic acid moiety through a disulfide bond and an amino acid or aminoalkanoic acid were recorded to have a lower binding scores than SAHA, which may indicate that these hybrids have better activities. Compound **2**, which is the hybrid molecule containing ban aromatic ring in the side chain recorded the lowest docking score, which may refer to its high activity than SAHA.

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