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Molecular docking study of anticancer activity of some s-triazine derivatives as HDAC6 inhibitors

Pham Canh Em[1,2], Le Thi Tuong Vi[1] and Truong Ngoc Tuyen[1,*]

[1]University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam

[2]Hong Bang International University, Vietnam

ABSTRACT

A novel series of s-triazine derivatives was designed and screened for in silico anticancer activity in histone deacetylase 6 (HDAC6) target by molecular docking method using AutoDock Vina. Compound 12 showed the strongest interactions among all tested compounds with the affinity value of -11.3 Kcal/mol compared to the reference drugs Gedatolisib (-8.9 Kcal/mol) and Paclitaxel (-9.0 Kcal/mol) at the active site of HDAC6. In particular, compound 12 established strong hydrogen bonds and showed hydrophobic interactions that resemble Gedatolisib and Paclitaxel at amino acids such as SER150, LYS142, TRP261, and ALA145. Therefore, this compound could be a potential lead molecule and support for experimental testing against an HDAC6 enzyme as an anticancer agent.

Keywords: *triazine, anticancer, in silico, molecular docking, HDAC6*

1. INTRODUCTION

s-Triazine derivatives are heterocyclic compounds that exhibit diverse and potential biological activities such as antiangiogenic, antimalarial, antibacterial, anti-tuberculosis, antiviral, and anticancer properties [1]. The *s*-triazine ring system belongs to a class of broadly used molecules. In particular, s-triazine is part of many approved drugs such as altretamine (anti-ovarian cancer), tretamine (antineoplastic), enasidenib (antileukemia), gedatolisib (anti-breast cancer), and bimiralisib (anti-breast cancer) (Figure 1). Several attempts have been made to modify the *s*-triazine nucleus to improve its biological activity, especially developing targets based on cancer cells to discover new compounds with improved efficacy and affinity and limited side effects when compared to the parent drugs.

Histone deacetylase 6 (HDAC6) is a member of the HDAC family whose main substrate is α-tubulin. HDAC6 has become a target for drug development to treat cancer due to its major contribution to oncogenic cell transformation. Besides, HDAC6 can be used as a prognostic marker since HDAC6 overexpression correlates with tumorigenesis and improved survival. HDAC6 inhibition leads to apoptosis in multiple myeloma cells. Moreover, HDAC6 is required for the activation of HSF1 (a heat shock factor 1), HSP (an activator of heat-shock protein-encoding genes), and CYLD (a cylindromatosis tumor suppressor gene). In addition, upregulation of HDAC6 increased cell motility in breast cancer MCF-7 cells, and the interaction of HDAC6 with cortactin regulates motility that contributes to cancer metastasis. HDAC6 also affects transcription and translation by regulating Hsp90 (heat-shock protein 90) and SGs (stress granules), respectively [2-4].

The development of cancer resistance has resulted in research and development in search of new anticancer drugs to maintain an effective drug supply at all times. It is important to find out newer, safer, and more effective anticancer drugs. Besides, in recent years there has been significant progress in improving the receptor flexibility in docking, making it possible to more easily rank the compound potency or precisely predict the target before having experimental *in vitro* results. Therefore, the purpose of this study is to design and *in silico* molecular docking of some new *s*-triazine derivatives for anticancer activity on the HDAC6 target. This is the scientific basis for studies to synthesize and evaluate the *in vitro* and *in vivo* biological activities of potential *s*-triazine derivatives.

Corresponding author: Assoc.Prof.Dr Truong Ngoc Tuyen
Email: truongtuyen@ump.edu.vn

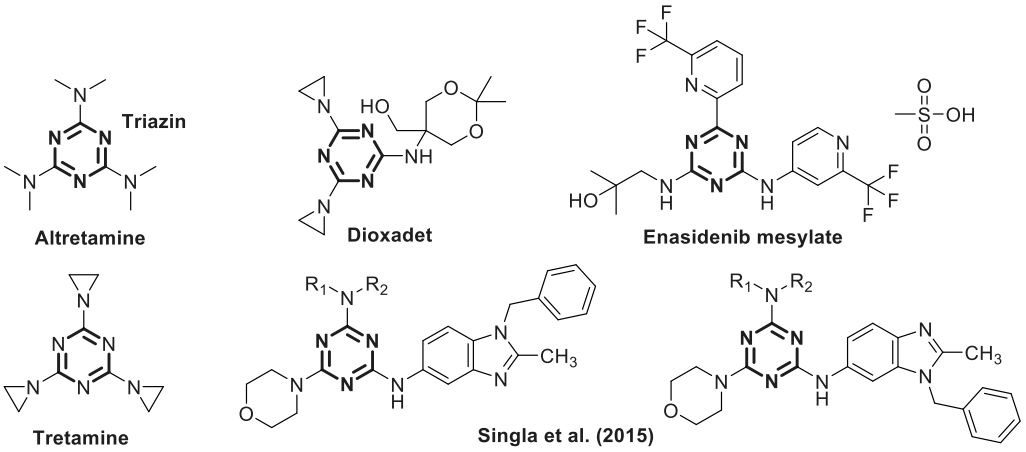


Figure 1. Drugs and compounds containing *s*-triazine nucleus with anticancer activity

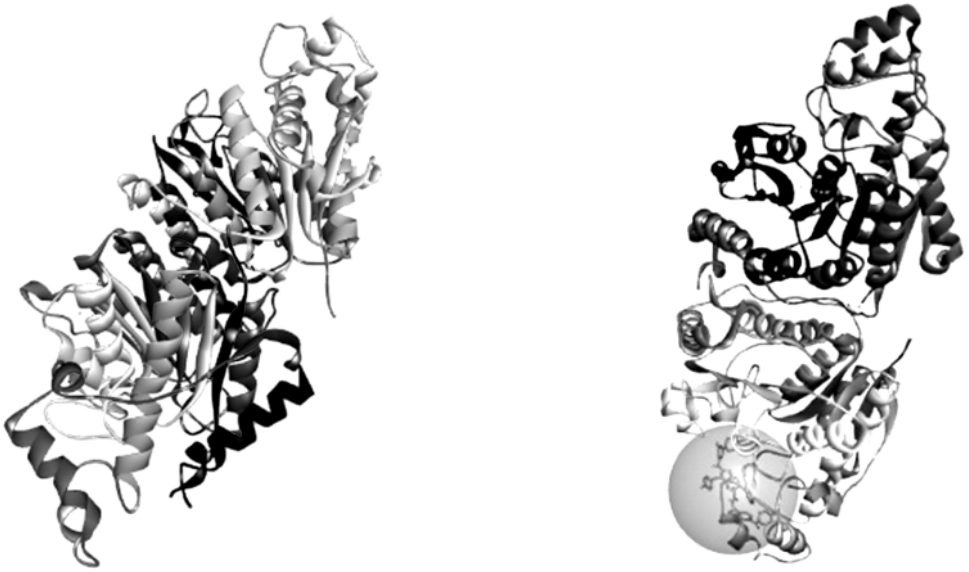
2. METHOD

2.1. Ligand preparation

The structure of ligands was drawn in ChemBioDraw Ultra 19. Then, the energy of these ligands was minimized using the MM2 force field method and saved as Sybyl mol2 file format using ChemBio3D Ultra 19 software.

2.2. Protein preparation

The protein molecule of histone deacetylase 6 (PDB ID: 5EEF) was retrieved from the protein data bank (http://www.rcsb.org/) (Figure 2). After all the water molecules had been removed, the receptors were added to only polar hydrogen and Kollman charges. The grid box for docking simulations was set by AutoDock tools (Table 1) [5].



Structure **Active site**

Figure 2. Structure and active site of HDAC6 target

Table 1. Grid box parameters for HDAC6 target

Target	Size			Center		
	x	y	z	x	y	z
HDAC6	30	42	30	-18.649	-42.547	-12.834

HDAC6 - Histone deacetylase 6

2.3. Molecular docking

All the minimizations were performed by AutoDock Vina docking simulation protocol with AMBER force field and the partial charges were automatically calculated. AutoDock Vina actually uses a united-atom scoring function (one that involves only the heavy atoms) with combines knowledge-based and empiric scoring function features as well as

supports the AutoDock4.2 scoring function. Besides, AutoDock Vina was compiled and run under Windows 10.0 Professional operating system. Discovery Studio 2021 was also used to deduce the pictorial representation of the interaction between the ligands and the target protein [6-7].

3. RESULTS AND DISCUSSION

HDACs have an important role in transcription regulation, protein modification, and post-translational modification. The role of HDACs in tumorigenesis is demonstrated by charac-teristics including proliferative phenotype, undif-ferentiated features, tumor vessel formation, distant metastasis, and aneuploidy. HDACs are increased in malignant cells such as in solid tumors and hematological cancers. Additionally, HDACs are strongly associated with the acquisition of malignant phenotypes during carcinogenesis as well as aberrant overexpression of HDACs is somewhat diverse among specific subtypes. Many anticancer drugs (vorinostat, romidepsin, belinostat, panobinostat, etc) have also been approved by the US Food and Drug Administration (US FDA). On the other hand, HDAC6 was highly overexpressed in colon and breast cancer cells as well as provoked cell motility, which results in metastasis. HDAC6 inhibitors targeting pro-liferation, differentiation, angiogenesis, and migration are a potential cancer treatment strategy. Moreover, heterocyclic hybrid de-rivatives, especially benzimidazole/imidazole - *s*-triazine, have shown potential anticancer activity in inhibiting HDAC6 [3-4]. Therefore, HDAC6 is the target of choice in the present study.

Rationale and structure-based design as anticancer agents: Structure-activity relation-ship studies of the *s*-triazine ring suggested the disubstituted and trisubstituted *s*-triazine derivatives with the presence of saturated cyclic amines exhibit potential anticancer activity against many cancer cell lines. The designed derivatives, anticancer drug Gedatolisib, and anticancer derivatives of Singla et al., 2015 share three common essential structural features i) A planar s-triazine moiety. ii) Aromatic ring with different substituted groups. iii) The groups of saturated cyclic amines (Figure 3) [1]. In particular, changes in many different cyclic amine substituents on the *s*-triazine nucleus were investigated to evaluate anticancer activity through the HDAC6 target (Table 2). The designed derivatives are new compounds and can be synthesized according to the researched process.

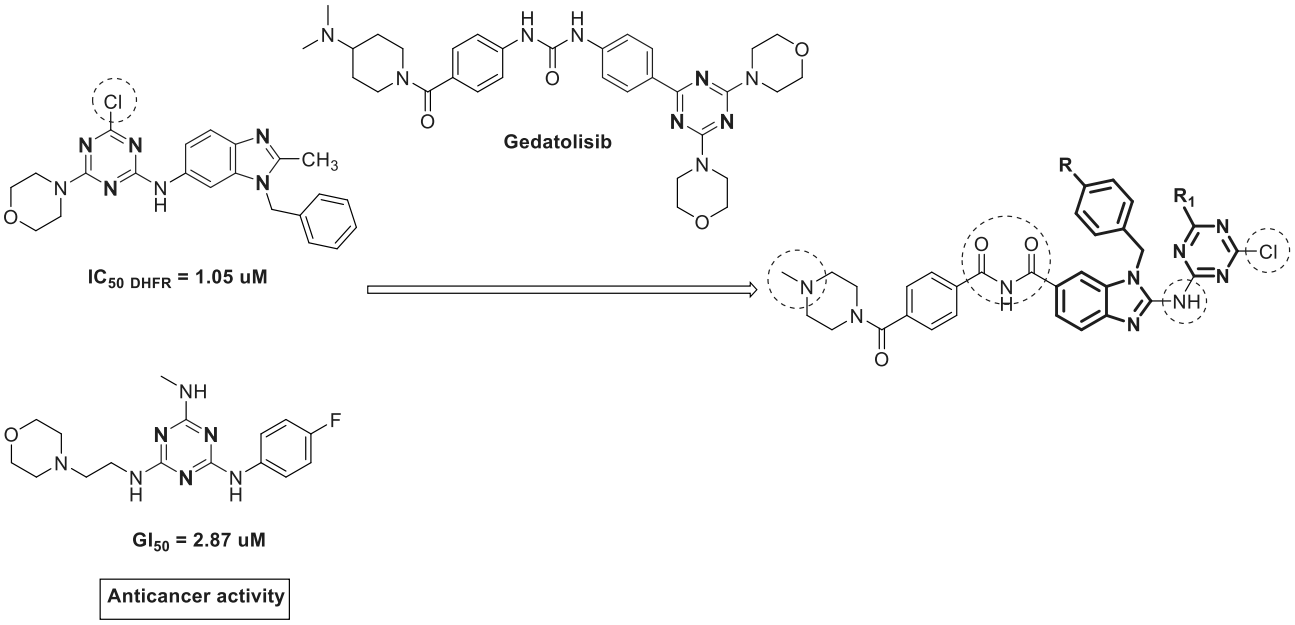


Figure 3. Design of some novel *s*-triazine derivatives

To determine the optimal structure for anticancer activity, 50 ligands were docked with HDAC6 target using Autodock Vina software and compared with reference drugs with strong anticancer activity such as Gedatolisib and Paclitaxel. The docking results of the target-ligand interaction are shown in Table 3-4 and Figure 5.

Table 2. Structure of designed *s*-triazine derivatives

Comp.	R	R_1	Comp.	R	R_1
1	Br	Piperidinyl	26	F	Pyrrolidinyl
2	Br	4-Methylpiperidinyl	27	F	4-(Dimethylamino)piperidinyl
3	Br	Morpholino	28	F	3-Chloropyrrolidinyl
4	Br	Piperazinyl	29	F	4-Chloropiperidinyl
5	Br	4-Methylpiperazinyl	30	F	4-Bromopiperidinyl
6	Br	Pyrrolidinyl	31	OMe	Piperidinyl
7	Br	4-(Dimethylamino)piperidinyl	32	OMe	4-Methylpiperidinyl
8	Br	3-Chloropyrrolidinyl	33	OMe	Morpholino
9	Br	4-Chloropiperidinyl	34	OMe	Piperazinyl
10	Br	4-Bromopiperidinyl	35	OMe	4-Methylpiperazinyl
11	Cl	Piperidinyl	36	OMe	Pyrrolidinyl
12	**Cl**	**4-Methylpiperidinyl**	37	OMe	4-(Dimethylamino)piperidinyl
13	Cl	Morpholino	38	OMe	3-Chloropyrrolidinyl
14	Cl	Piperazinyl	39	OMe	4-Chloropiperidinyl
15	Cl	4-Methylpiperazinyl	40	OMe	4-Bromopiperidinyl
16	Cl	Pyrrolidinyl	41	$N(Me)_2$	Piperidinyl
17	Cl	4-(Dimethylamino)piperidinyl	42	$N(Me)_2$	4-Methylpiperidinyl
18	Cl	3-Chloropyrrolidinyl	43	$N(Me)_2$	Morpholino
19	Cl	4-Chloropiperidinyl	44	$N(Me)_2$	Piperazinyl
20	Cl	4-Bromopiperidinyl	45	$N(Me)_2$	4-Methylpiperazinyl
21	F	Piperidinyl	46	$N(Me)_2$	Pyrrolidinyl
22	F	4-Methylpiperidinyl	47	$N(Me)_2$	4-(Dimethylamino)piperidinyl
23	F	Morpholino	48	$N(Me)_2$	3-Chloropyrrolidinyl
24	F	Piperazinyl	49	$N(Me)_2$	4-Chloropiperidinyl
25	F	4-Methylpiperazinyl	50	$N(Me)_2$	4-Bromopiperidinyl

Comp. – compound

Table 3. The binding affinity of ligands with HDAC6 target at the active site

Compound	Affinity (Kcal/mol)	Compound	Affinity (Kcal/mol)
	HDAC6		HDAC6
1	-10.6	**26**	-10.3
2	-11.1	**27**	-10.5
3	-10.2	**28**	-9.8
4	-11.0	**29**	-11.0

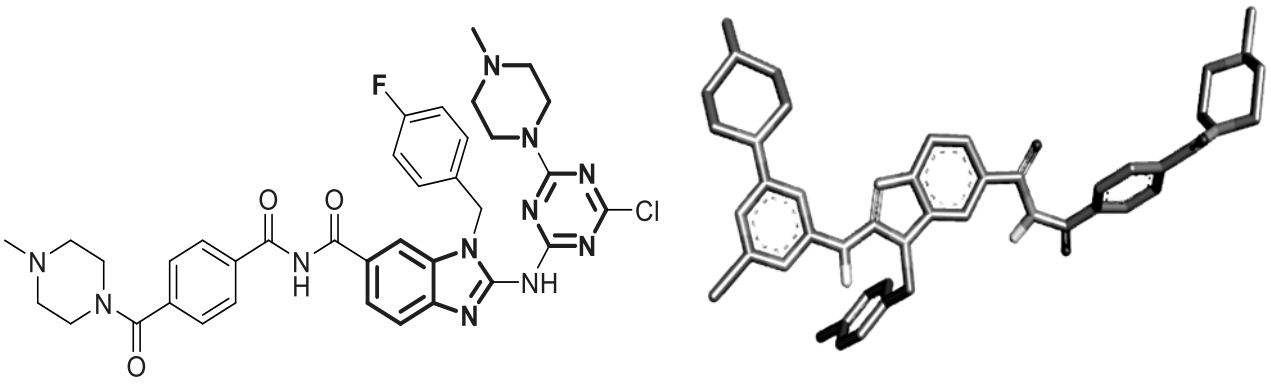
Compound	Affinity (Kcal/mol)	Compound	Affinity (Kcal/mol)
	HDAC6		HDAC6
5	-10.7	**30**	-10.9
6	-10.4	**31**	-10.5
7	-10.4	**32**	-11.0
8	-10.0	**33**	-10.0
9	-11.0	**34**	-11.0
10	-10.9	**35**	-10.4
11	-10.7	**36**	-10.1
12	**-11.3**	**37**	-10.2
13	-10.2	**38**	-10.0
14	-10.2	**39**	-10.8
15	-10.6	**40**	-10.7
16	-10.4	**41**	-10.4
17	-10.4	**42**	-11.0
18	-10.0	**43**	-10.0
19	-11.0	**44**	-10.0
20	-11.0	**45**	-10.4
21	-10.7	**46**	-10.2
22	-11.2	**47**	-10.2
23	-10.2	**48**	-9.9
24	-10.9	**49**	-10.8
25	-10.6	**50**	-10.8
Ged	-8.9	**PTX**	-9.0

Ged: Gedatolisib, PTX: Paclitaxel, HDAC6 - Histone deacetylase 6

All designed *s*-triazine derivatives showed good affinity (-9.8 to -11.3 Kcal/mol) and formed hydrogen bonds with the HDAC6 target. Compounds 22 and 2 showed strong interactions with HDAC6 with binding affinities of -11.2 and -11.1 Kcal/mol, respectively, compared to the reference drugs Gedatolisib (-8.9 Kcal/mol) and Paclitaxel (-9.0 Kcal/mol) at the active site. In particular, compound 12 (-11.3 Kcal/mol) showed the strongest affinity of all tested derivatives in HDAC6 compared to the reference drugs. The 2D and 3D structures of compound 12 are shown in Figure 4. Compound 12 has a structure containing an *N*-(4-fluorobenzyl) group at position 1 on the benzimidazole ring and an *N*-methylpiperazinyl group on the 1,3,5-triazine ring. These may be groups that increase the ability of this compound to interact with the HDAC6 target.

Compound 12 established one carbon-hydrogen bond and one π-donor hydrogen bond at amino acids HIS75 and SER150 with bond lengths of 3.46 and 2.81 Å, respectively. In addition, this compound established two strong hydrogen

bonds at amino acids SER150 and VAL151 through the triazine nucleus and -CONHCO- substituent with bond lengths of 2.65 and 2.86 Å, respectively, with the highest affinity and hence is considered as the best dock conformation (Fig 5). Besides, compound 12 also e showed hydrophobic interactions (π-Sigma, π-π Stacked, Amide-π Stacked, π-Alkyl) with the crucial residue of the HDAC6 protein with bond lengths in the range of 3.62-5.93 Å (Table 4). In particular, compound 12 interacted with the HDAC6 target similar to Gedatolisib and Paclitaxel at amino acids in the active site such as SER150, LYS142, TRP261, and ALA145. The results of the docking study predicted that the new compound 12 may show potential anticancer activity similar to or better than strong anticancer drugs Gedatolisib and Paclitaxel.



2D **3D**

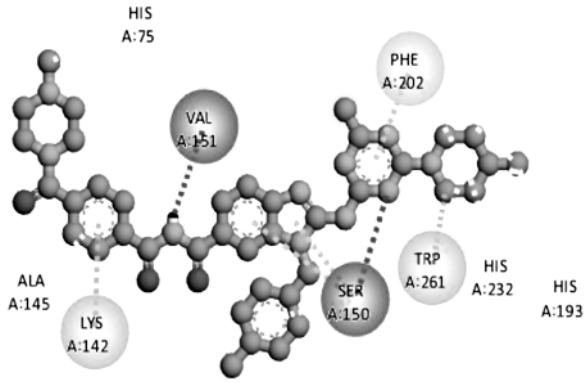
Figure 4. Structure of compound 12 with potential anticancer activity in the HDAC6 target

Table 4. *In silico* molecular docking results of potential compound 12 and standard drugs

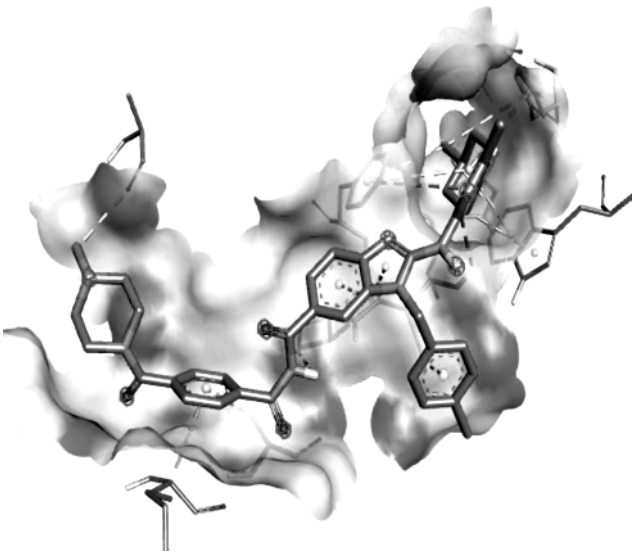
Ligand	Affinity (Kcal/mol)	Distance (Å)	Bond Types	Amino acid
12	**-11.3**	2.65034	Conventional hydrogen bond	SER150
		2.86004	Conventional hydrogen bond	VAL151
		3.46094	Carbon hydrogen bond	HIS75
		2.80885	π-Donor hydrogen bond	SER150
		3.75728	π-Sigma	LYS142
		3.62190	π-Sigma	TRP261
		5.92894	π-π Stacked	PHE202
		4.58641	Amide-π Stacked	SER150, VAL151
		4.72081	Amide-π Stacked	SER150, VAL151
		4.65018	π-Alkyl	HIS193
		4.61907	π-Alkyl	PHE202
		4.57245	π-Alkyl	PHE202
		4.67879	π-Alkyl	HIS232
		4.41806	π-Alkyl	HIS232
		3.78892	π-Alkyl	TRP261
		4.68891	π-Alkyl	TRP261

Ligand	Affinity (Kcal/mol)	Distance (Å)	Bond Types	Amino acid
		4.09936	π-Alkyl	ALA145
Ged	**-8.9**	3.06047	Conventional hydrogen bond	HIS263
		3.60400	Carbon hydrogen bond	TYR148
		3.67313	Carbon hydrogen bond	LYS147
		3.63679	π-Anion	ASP149
		3.17969	π-Donor hydrogen bond	SER150
		3.87420	π -Sigma	ASP149
		4.89629	Amide- π Stacked	LEU141, LYS142
		5.3595	π-Alkyl	TRP261
		4.33821	π-Alkyl	LYS142
		4.84181	π-Alkyl	ALA145
PTX	**-9.0**	2.82543	Conventional hydrogen bond	SER150
		1.89619	Conventional hydrogen bond	SER150
		2.13424	Conventional hydrogen bond	VAL151
		2.48268	Conventional hydrogen bond	LYS330
		4.33723	π-π Stacked	PHE202
		5.18701	π-π Stacked	TRP261
		4.31021	π-π Stacked	TRP261
		5.02508	π-Alkyl	LYS142
		5.26732	π-Alkyl	ALA145

Ged: Gedatolisib, PTX: Paclitaxel, Hydrophobic interaction (π-Sigma, π-π Stacked, Amide-π Stacked, π-Alkyl)



12 – HDAC6 (2D)



12 – HDAC6 (3D)

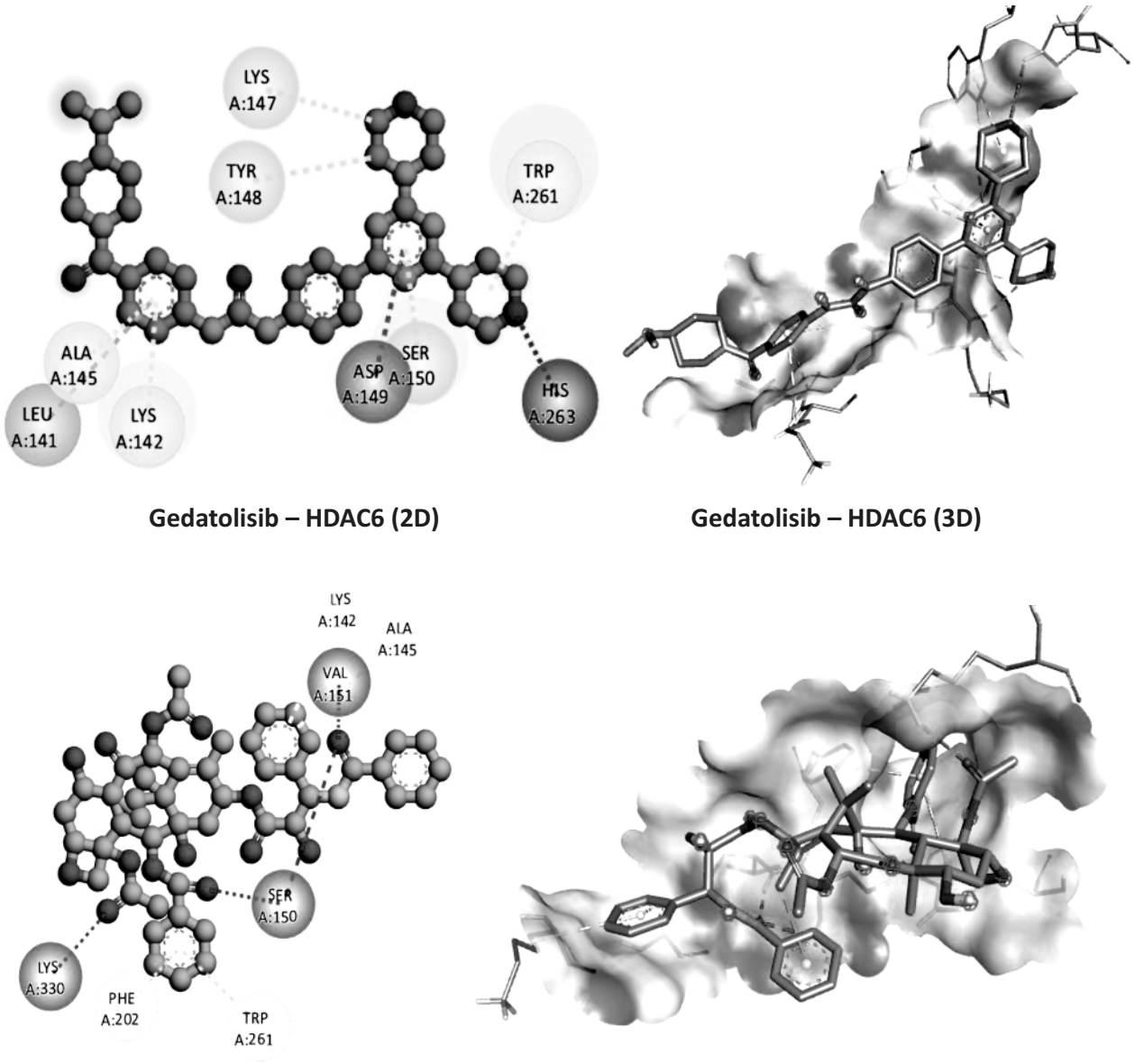


Figure 5. 2D and 3D representation of the interaction of compound 12 and reference drugs (Gedatolisib and Paclitaxel) with histone deacetylase 6 (HDAC6)

Based on the study results, compound 12, Gedatolisib, and Paclitaxel demonstrated hydrophobic interactions through aromatic ring systems such as benzene or *s*-triazine. However, these compounds demonstrated hydrogen bonding through different groups with the HDAC6 target. Gedatolisib showed one strong hydrogen bond through the morpholine ring and Paclitaxel exhibited strong hydrogen bonds through carbonyl groups (> C = O) (Fig. 5). Meanwhile, potential compound 12 demonstrated strong hydrogen bonds with HDAC6 through the -NH- of the imide (-CONHCO-) group and *s*-triazine ring. These may be important structures for potential anticancer activity through HDAC6 inhibition.

4. CONCLUSION

In summary, fifty new *s*-triazine derivatives were designed and evaluated for *in silico* anticancer activity targeting HDAC6. Compound 12 being the most potent anticancer displayed the best strong affinity of -11.3 Kcal/mol with HDAC6 enzyme as well as showed hydrogen bond and hydrophobic interactions that resemble the reference drugs. The imide group (-CONHCO-) and the *s*-triazine ring are

predicted to play an important role in HDAC6 target inhibition. This work paved the way for the synthesis of more potent anticancer *s*-triazine derivatives.

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Nghiên cứu docking phân tử hoạt tính kháng ung thư của một số dẫn chất *s*-triazin như chất ức chế HDAC6

Phạm Cảnh Em, Lê Thị Tường Vi và Trương Ngọc Tuyền

TÓM TẮT

Một loạt dẫn chất s-triazin mới được thiết kế và sàng lọc hoạt tính kháng ung thư in silico trên đích tác dụng histon deacetylase 6 (HDAC6) bằng phương pháp docking phân tử sử dụng AutoDock Vina. Hợp chất 12 cho thấy tương tác mạnh nhất trong tất cả hợp chất thử nghiệm với ái lực là -11.3 Kcal/mol khi so sánh với thuốc đối chứng Gedatolisib (-8.9 Kcal/mol) và Paclitaxel (-9.0 Kcal/mol) tại vị trí tác động của HDAC6. Đặc biệt, hợp chất 12 đã thành lập các liên kết hydrogen mạnh và thể hiện các tương tác kỵ nước giống như Gedatolisib và Paclitaxel tại các acid amin như SER150, LYS142, TRP261 và

ALA145. Do đó, hợp chất này có thể là một phân tử dẫn đầu tiềm năng và hỗ trợ thử nghiệm thực nghiệm chống lại enzym HDAC6 như một tác nhân kháng ung thư.

Từ khóa: *triazin, kháng ung thư, in silico, docking phân tử, HDAC6.*

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