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Evaluation of *in silico* anticancer activity of some striazine derivatives as VEGFR2 inhibitors

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

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

[2]*Hong Bang International University, Vietnam*

ABSTRACT

The s-triazine derivatives have been shown to have diverse biological activities, especially anticancer activity. Fifty s-triazine derivatives were screened for anticancer activity through inhibition of VEGFR2 (vascular endothelial growth factor receptor-2) by molecular docking method using AutoDock Vina software. Compounds 20 and 40 showed the strongest interactions among all tested compounds with the binding affinity values of -10.8 and -10.5 Kcal/mol, respectively compared to reference drugs Gedatolisib (-9.1 Kcal/mol) and Paclitaxel (-7.8 Kcal/mol) at the active site of VEGFR2. These compounds established one carbon-hydrogen bond at amino acid HIS1026, specifically exhibiting better electrostatic and hydrophobic interactions than the reference drugs Gedatolisib and Paclitaxel. Moreover, compounds 20 and 40 also showed interactions with the VEGFR2 receptor that resemble the reference drug Gedatolisib at amino acids such as ARG1027, ASP1046, and HIS1026. Therefore, these compounds could be a potential lead molecule for anticancer activity.

Keywords: *triazine, anticancer, in silico, molecular docking, VEGFR2*

1. INTRODUCTION

s-Triazines are six-membered nitrogen-containing heterocycles with promising pharmacological activities including antiviral, antibacterial, antifungal, anti-inflammatory, anti-Alzheimer's, and anticancer properties [1]. The *s*-triazine scaffold is extensively used to synthesize a wide spectrum of pharma-cologically relevant compounds. In addition, *s*-triazine scaffold presents in the core structure of a vast list of important drugs such as azacitidine (treat myelodysplastic syndrome), enasidenib (antileukemia), dioxadet (antitumor), bimiralisib (anti-breast cancer), and gedatolisib (anti-breast cancer) (Figure 1).

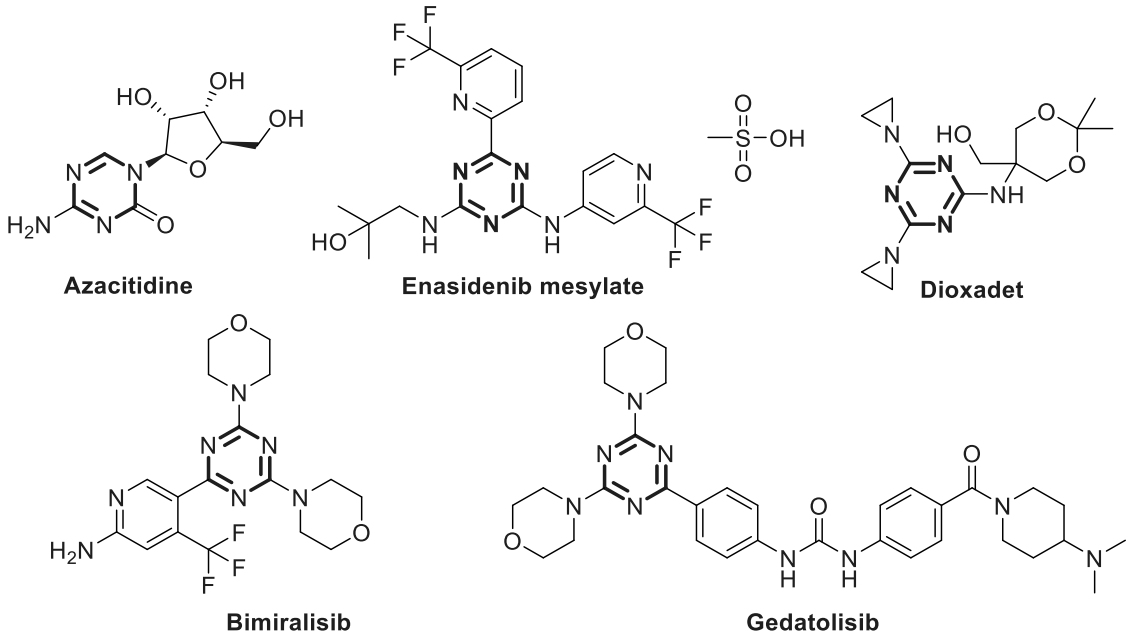


Figure 1. Several drug compounds containing *s*-triazine moiety

Corresponding author: Assoc.Prof.Dr Truong Ngoc Tuyen

Email: truongtuyen@ump.edu.vn

Vascular endothelial growth factor (VEGF) plays an important role in many pathologies, including vascular disease and cancer. VEGF is the key mediator of angiogenesis in cancer, in which it is up-regulated by oncogene expression, a variety of growth factors, and hypoxia. Besides, VEGF receptor-1 (VEGFR1) has emerged as a predictive biomarker for anti-VEGF treatment in cancer. VEGFR1 is upregulated during periods of vascular reperfusion in ischemic tissue in both hypoxic tumor cells and tumor endothelial cells. Meanwhile, VEGFR-2 are crucial player in vasculogenenis and angiogenesis. VEGFR-2 regulates endothelial cell survival mainly by the activation of the TSAd-Src-PI3K-PKB/AKT signaling pathway. General blocking of this signaling system with small molecule inhibitors is an established strategy for cancer treatment. Therefore, inhibition of VEGFR2 has shown promise in suppressing tumor growth and metastasis [2 - 5].

The development of new anti-cancer drugs with safer and more effective characteristics is urgently needed because of increasing cancer drug resistance [4 - 5]. Therefore, the study's purpose is to *in silico* molecular docking of some new *s*-triazine derivatives for anticancer activity on the VEGFR2 receptor.

2. METHOD

2.1. Ligand preparation

The structure of ligands was drawn in Chem-BioDraw Ultra 19. The energy of these ligands was minimized using ChemBio3D Ultra 19 software.

2.2. Protein preparation

The protein molecule of vascular endothelial growth factor receptor 2 (PDB ID: 5EW3) was retrieved from the protein data bank (rcsb.org) (Figure 2). All the water molecules were removed, the receptors were then added to only polar hydrogen and Kollman charges. The grid box for docking simulations was set by AutoDock tools (Table 1) [5].

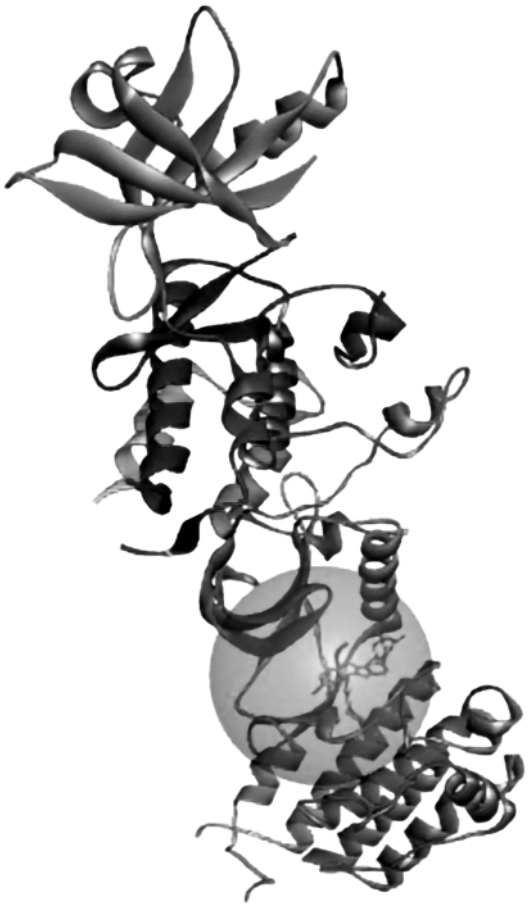


Figure 2. Active site of VEGFR2 receptor

Table 1. Grid box parameters for VEGFR2 receptor

Target	Size			Center		
	x	y	z	x	y	z
VEGFR1	36	34	34	18.728	9.165	12.278

VEGFR2 - Vascular endothelial growth factor receptor-2

2.3. Molecular docking

The ligands were docked with the target to determine the docking parameters using AutoDock Vina with the help of Grid-based ligand docking. The pictorial representation of the interaction between the ligands and the target protein was performed by BIOVIA Discovery Studio 2021 software [6 - 7].

3. RESULTS AND DISCUSSION

Rationale and structure-based design as anticancer agents: The designed molecules consisted of a heterocyclic ring (*s*-triazine) as the central core that can act as a scaffold to carry three functionalized branches at 2-, 4- and 6-positions. The disubstituted s-triazine derivatives with the presence of saturated cyclic amines exhibit potential anticancer activity against many cancer cell lines [1]. In addition, 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].

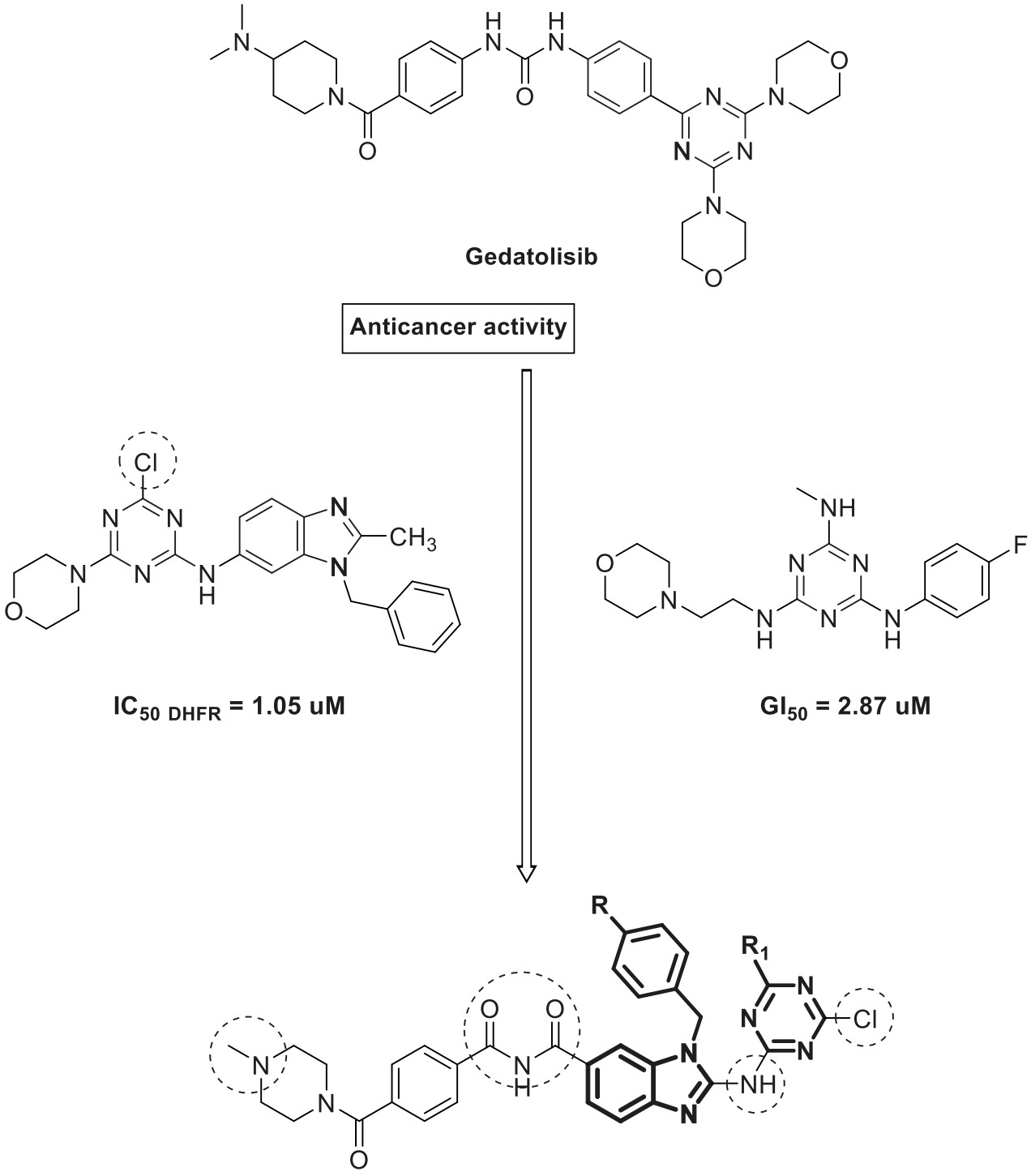


Figure 3. Design of some novel *s*-triazine derivatives

The designed derivatives contained s-triazine nuclei with three substituents including benzimidazole-2-amino groups with different benzyl moieties at a 2 position, different cyclic amine groups, and chloro group. The cyclic amine substituents were modified on the *s*-triazine nucleus to form many different derivatives to evaluate *in silico* anticancer activity in the VEGFR2 receptor (Table 2).

On the other hand, to determine the optimal structure for anticancer activity, 50 ligands were docked with VEGFR2 receptor compared to anticancer drugs (Gedatolisib and Paclitaxel). The docking results of the receptor-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

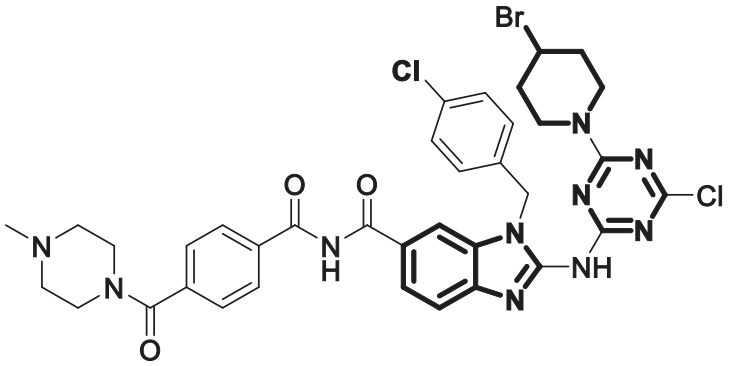
All tested *s*-triazine compounds showed good affinity between -9.2 to -10.8 Kcal/mol with the VEGFR2 receptor. Compounds 20 (-10.8 Kcal/mol) and 40 (-10.8 Kcal/mol) exhibited the strongest interaction with the VEGFR2 receptor, compared to the reference drugs Gedatolisib (-9.1 Kcal/mol) and Paclitaxel (-7.8 Kcal/mol). The structures of these compounds are shown in Figure 4. The structure of compounds 20 and 40 contain a 4-bromopiperidinyl group on the 1,3,5-triazine nucleus. However, compound 20 has a structure containing an *N*-(4-chlorobenzyl) group at position 1 on the benzimidazole ring, whereas compound 40 has a structure containing an *N*-(4-methoxybenzyl) group at position 1 on the benzimidazole ring.

Table 3. The binding affinity of ligands with VEGFR1 receptor

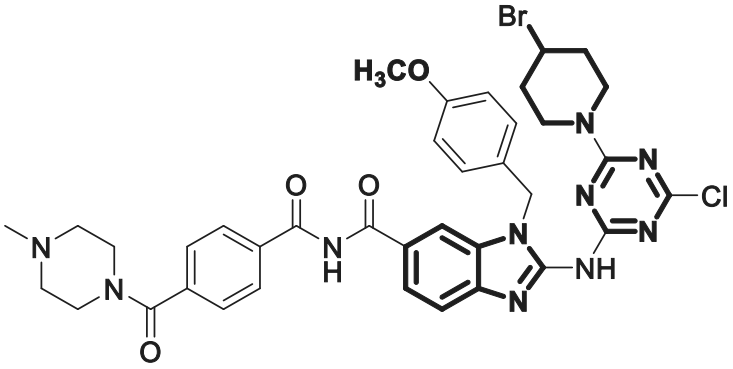
Compound	Affinity (Kcal/mol)	Compound	Affinity (Kcal/mol)
	VEGFR2		VEGFR2
1	-9.8	**26**	-10.1
2	-10.0	**27**	-9.8
3	-9.6	**28**	-10.0
4	-9.6	**29**	-9.9
5	-9.6	**30**	-10.2
6	-9.6	**31**	-9.4
7	-10.0	**32**	-9.7
8	-9.9	**33**	-9.3
9	-9.8	**34**	-9.6
10	-9.9	**35**	-9.4
11	-9.7	**36**	-9.9
12	-9.7	**37**	-9.8
13	-9.6	**38**	-9.8
14	-9.5	**39**	-10.2
15	-9.5	**40**	**-10.5**
16	-9.7	**41**	-9.5
17	-9.9	**42**	-9.6
18	-9.9	**43**	-9.2
19	-9.6	**44**	-9.3
20	**-10.8**	**45**	-9.2
21	-9.8	**46**	-9.6
22	-9.7	**47**	-9.7
23	-10.3	**48**	-9.7
24	-10.2	**49**	-9.4
25	-10.2	**50**	-9.3
Ged	-9.1	**PTX**	-7.8

Ged - Gedatolisib, PTX- Paclitaxel, VEGFR2 - vascular endothelial growth factor receptor-2

Compounds 20 and 40 did not form strong hydrogen bonds and only formed 1 carbon-hydrogen bond at amino acid HIS1026 with bond lengths of 3.43 and 3.45 Å, respectively. Ged and PTX formed more strong hydrogen bonds and carbon-hydrogen bonds than compounds 20 and 40. However, these two compounds exhibited better electrostatic and hydrophobic interactions than the reference drugs Ged and PTX. This may be the reason the binding affinities of these two compounds (-10.5 to -10.8 Kcal/mol) are stronger than the reference drugs (Ged: -9.1 Kcal/mol, PTX: -7.8 Kcal/mol). Compounds 20 and 40 showed three π-Anion interactions with amino acids ASP1028 and ASP1046 with bond lengths in the range of 3.42-3.69 Å. In addition, these two compounds also showed hydrophobic interactions with the VEGFR2 receptor including π-σ, π-π Stacked, Alkyl, π-Alkyl at amino acids LEU1067, PHE1047, ARG1032, PRO1068, ARG1027, ARG1032, and ASP1046 with bond lengths in the range of 3.59-5.53 Å. In particular, compounds 20 and 40 showed interaction with the VEGFR2 receptor at an active site similar to Gedatolisib at amino acids ARG1027, ASP1046, and HIS1026. The docking study results predicted that new compounds 20 and 40 may exhibit potent anticancer activity through interaction with the VEGFR2 receptor. Therefore, these two compounds need to continue studying *in vitro* biological activity to confirm the *in silico* research results.



Compound 20



Compound 40

Figure 4. Structure of compounds 20 and 40 with potential *in silico* anticancer activity

Table 4. MoLecular docking results of potential compounds and standard drugs

Ligand	Affinity (Kcal/mol)	Distance (Å)	Bond types	Amino acid
20	**-10.8**	3.43021	Carbon hydrogen bond	HIS1026
		3.42890	π-Anion	ASP1028
		3.69293	π-Anion	ASP1028
		3.52221	π-Anion	ASP1046
		3.92495	π-σ	LEU1067

Ligand	Affinity (Kcal/mol)	Distance (Å)	Bond types	Amino acid
		5.45309	π-π Stacked	PHE1047
		3.58588	Alkyl	ARG1032
		4.33429	π-Alkyl	PHE1047
		5.25515	π-Alkyl	PRO1068
		5.42093	π-Alkyl	PRO1068
		5.49337	π-Alkyl	ARG1027
		5.42259	π-Alkyl	ARG1032
40	**-10.5**	3.44781	Carbon hydrogen bond	HIS1026
		3.47405	π-Anion	ASP1028
		3.66347	π-Anion	ASP1028
		3.49107	π-Anion	ASP1046
		3.79366	π-σ	LEU1067
		3.84734	π-σ	PHE1047
		5.52815	π-π Stacked	PHE1047
		4.71605	Alkyl	ARG1032
		5.29552	Alkyl	LEU1067
		5.22935	π-Alkyl	PRO1068
		5.33792	π-Alkyl	PRO1068
		5.48587	π-Alkyl	ARG1027
		5.38084	π-Alkyl	ARG1032
PTX	**-7.8**	2.52658	Conventional hydrogen bond	ALA844
		3.47228	Carbon hydrogen bond	GLU878
		3.07643	Carbon hydrogen bond	ARG1027
		4.24195	π-Cation	ARG1027
		3.91938	π-Anion	GLU885
		3.96435	π-Anion	ASP1046
		5.13922	π-Alkyl	ARG1027
		5.13856	π-Alkyl	ARG1027
		4.91859	π-Alkyl	ILE888
		5.30818	π-Alkyl	LEU889
Ged	**-9.1**	2.94700	Conventional hydrogen bond	GLY1048
		2.8178	Conventional hydrogen bond	GLY1048
		3.46496	Carbon hydrogen bond	HIS1026

Ged - Gedatolisib, PTX - Paclitaxel, Electrostatic interaction (π-cation, π-anion), Hydrophobic interaction (π-σ, π-π stacked, amide-π stacked, alkyl, π-alkyl)

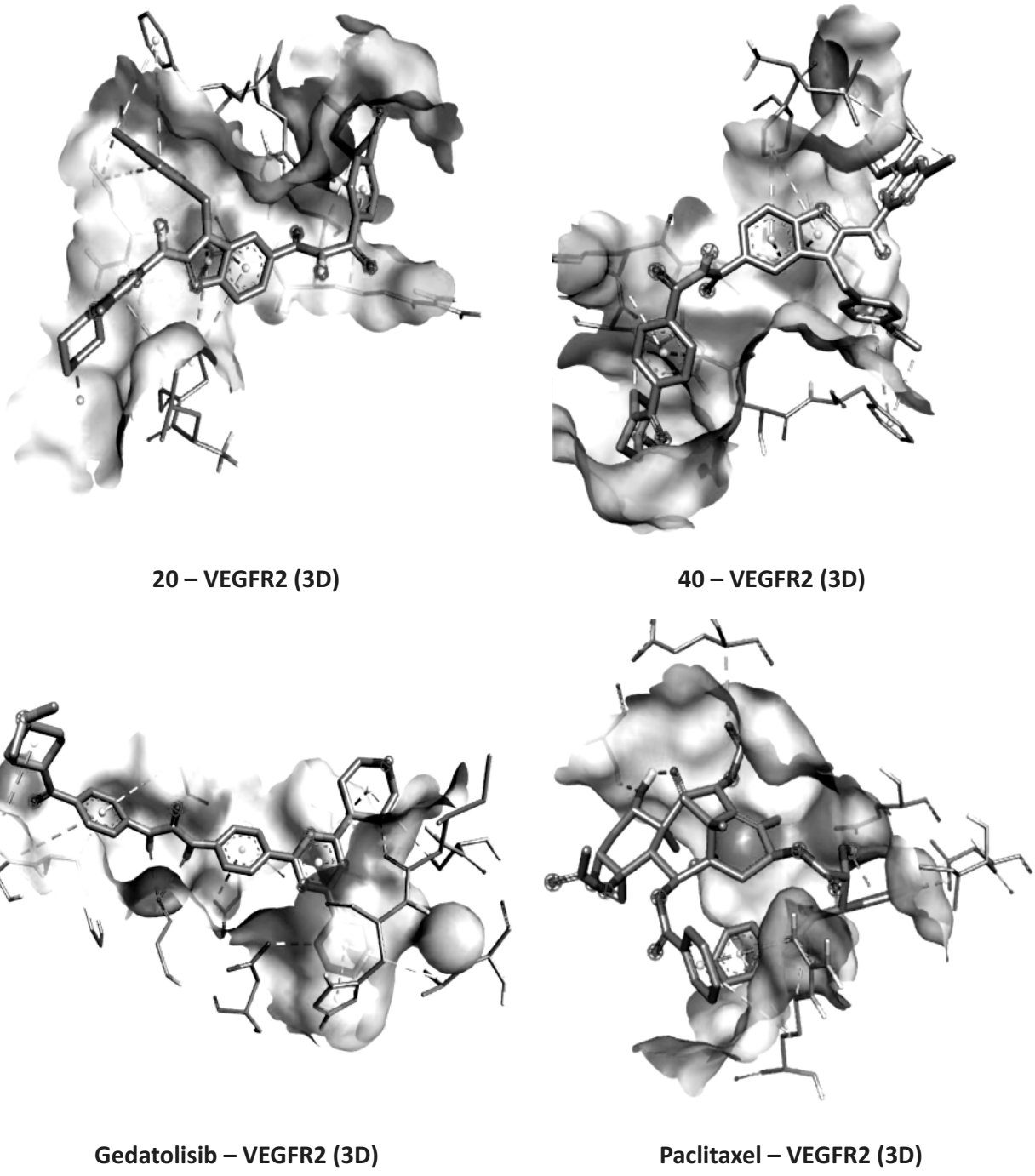


Figure 5. 3D representation of the interaction of potential compounds and reference drugs with VEGFR2

4. CONCLUSION

Compounds 20 (-10.8 Kcal/mol) and 40 (-10.5 Kcal/mol) demonstrated potential *in silico* anticancer activity with strong binding affinity to the VEGFR2 receptor. In particular, the binding affinities of these two compounds are greater than the reference drugs (Gedatolisib: -9.1 Kcal/mol and Paclitaxel: -7.8 Kcal/mol). These two compounds also showed interactions with the VEGFR2 receptor similar to the reference drug Gedatolisib at amino acids ARG1027, ASP1046, and HIS1026.

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Đánh giá hoạt tính kháng ung thư *in silico* của một số dẫn chất *s*-triazin như chất ức chế VEGFR2

Lê Thị Tường Vi[2], Phạm Cảnh Em[1,2] và Trương Ngọc Tuyền[1,*]

TÓM TẮT

Các dẫn chất s-triazin đã được chứng minh có hoạt tính sinh học đa dạng, đặc biệt là hoạt tính kháng ung thư. Năm mươi dẫn chất s-triazin được sàng lọc hoạt tính kháng ung thư thông qua ức chế VEGFR2 (thụ thể yếu tố tăng trưởng nội mô mạch máu 2) bằng phương pháp docking phân tử sử dụng phần mềm AutoDock Vina. Các hợp chất 20 và 40 đã thể hiện tương tác mạnh nhất trong số tất cả các hợp chất thử nghiệm với giá trị ái lực liên kết lần lượt là -10.8 và -10.5 Kcal/mol so với thuốc đối chiếu Gedatolisib (-9.1 Kcal/mol) và Paclitaxel (-7.8 Kcal/mol) tại vị trí tác động của VEGFR2. Các hợp chất này đã hình thành một liên kết carbon-hydrogen tại acid amin HIS1026, đặc biệt thể hiện tương tác tĩnh điện và kỵ nước tốt hơn so với các thuốc đối chiếu Gedatolisib và Paclitaxel. Hơn nữa, các hợp chất 20 và 40 cũng cho thấy sự tương tác với thụ thể VEGFR2 giống với thuốc đối chiếu Gedatolisib tại các acid amin như ARG1027,

ASP1046 và HIS1026. Do đó, các hợp chất này có thể là phân tử dẫn đầu tiềm năng cho hoạt tính kháng ung thư.

Từ khóa: *triazin, kháng ung thư, in silico, docking phân tử, VEGFR2*

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