Original Article

In-Depth In Silico Investigation Of Gitalin And Glaziovine As Possible VEGFR-2 Kinase Inhibitors For The Treatment Of Cancer

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Abstract

Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) kinase inhibitors have been found to effectively suppress the process of angiogenesis or lymphangiogenesis, thereby exhibiting notable anticancer properties. In the current study, we have chosen two naturally occurring compounds, Gitalin and Glaziovine, as the focus of investigation. The objective is to assess their potential as inhibitors of VEGFR-2 kinase activity. Fro in silico ADMET analysis it was concluded that these compounds possess most favorable drug-likeness properties and can be treated as lead molecules for further analysis by molecular docking. Gitalin exhibited -8.3 kcal/mol of binding affinity and formed seven conventional, one carbon and one Pi-Donor hydrogen bond with Glu885, Asp1046, Arg1027, Glu885, Lys868 and Asp1028. It also displayed hydrophobic (Alkyl) Interactions with Leu1049, Lys868, Val914, Val916 and Ala881. Glaziovine displayed -8.1 kcal/mol and formed one carbon hydrogen bond with Glu885. It also showed Hydrophobic (Alkyl, Pi-Alkyl) Interactions with Met1016, Leu1019, Cys1024 and His1026. From molecular docking it was observed that both the compounds formed more stable complex than native ligand. Gitalin displayed formation of seven conventional hydrogen bonds with target which indicates it has potential to modulate the activity of target enzyme. This compound (Gitalin) can be tested further using in vitro and in vivo models for VEGFR-2 kinase activity to gather more quality data to claim its use clinically. Further lead optimization needed by applying numerous experimental techniques.

Keywords: VEGFR-2; Gitalin, Glaziovine; ADMET; Cancer

1. Introduction

VEGFR-2, or vascular endothelial growth factor receptor 2, is a glycoprotein characterized by a molecular weight that falls between the range of 210 to 230 kilodaltons. The presence of this entity is mostly seen in vascular endothelial cells and hematopoietic stem cells. The Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2) assumes a pivotal function in facilitating the cellular reactions to Vascular Endothelial Growth Factor A (VEGF-A) by its unique interaction to this ligand. The strong association between VEGFR-2 and VEGFR-1 may be attributed to the existence of both common and unique ligands. Nevertheless, it is important to highlight that VEGFR-2 has a much elevated degree of kinase activity, whereas VEGFR-1 operates as a receptor tyrosine kinase with diminished activity[1,2]. The receptor listed above plays a crucial role in regulating responses inside endothelial cells, particularly in relation to vascular endothelial growth factor (VEGF). The restrictions listed above cover the many characteristics of permeability, proliferation, invasion, and migration. The key signaling pathways linked with the human Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2) following interaction with VEGF have been identified as the autophosphorylation sites Y1175 and Y1214. The autophosphorylation of Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2) is a prerequisite for the activation of several downstream pathways. This pivotal occurrence

is noted to be excessively triggered in certain sorts of tumors. The signaling pathways outlined above are of great importance in the mechanism of tumor angiogenesis, a process that facilitates the growth of tumors by supplying oxygen and vital nutrients to the tumor mass. The phenomenon of VEGFR-2 overexpression has been shown in a range of cancer types, such as ovarian, thyroid, melanoma, and medulloblastoma[3–6].

VEGFR-2 inhibitors, also known as kinase insert domain receptor (KDR) inhibitors, belong to a category of substances that selectively target and suppress the function of the tyrosine kinase receptor VEGFR-2. The inhibitors have shown significant anticancer activity by efficiently suppressing the processes of angiogenesis or lymphangiogenesis. Generally, these compounds are small, synthetically generated entities that have a strong ability to bind competitively to the adenosine triphosphate (ATP)-binding site located in the tyrosine kinase domain. The inhibition of many signaling pathways implicated in tumor development, including proliferation, metastasis, and angiogenesis, may be accomplished by using a selective inhibitor targeting VEGFR-2[7,8].

In the present research, two naturally occurring chemicals, namely Gitalin and Glaziovine, have been selected as the primary subjects of inquiry. The aim of this study is to evaluate the capacity of the compounds to serve as inhibitors of the kinase activity of Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2).

2. Material and Methods

2.1 Pharmacokinetics predictions

The Lipinski rule of five and the pharmacokinetic (ADME) characteristics of molecules were investigated using PubChem[9], molinspiration[10], and SwissADME[11] servers. ADMETlab 2.0 is a totally revamped version of the AMDETlab web server, which is commonly used for predicting the pharmacokinetics and toxic characteristics of various compounds (https://admetmesh.scbdd.com/)[12].

2.2 Molecular docking studies

Molecular docking is a vital component within the realm of computer-assisted drug development and structural molecular biology. The technique of "ligand-protein docking" enables scientists to predict the interaction between a ligand and a protein using a pre-existing knowledge of the protein's three-dimensional structure. The establishment of an accurate scoring system for dockings in high-dimensional regions is of utmost importance. Virtual screening may be conducted on a vast collection of compounds, whereby the outcomes are evaluated and potential structural concepts are suggested about the mechanism by which the ligands obstruct the target. This process has significant value in the optimization of lead compounds[13–17].

In order to achieve further optimization, the molecules underwent binding affinity studies with the target enzyme. All the selected compounds and the native ligand were docked against the Crystal structure of the KDR (VEGFR2) kinase domain in complex with a type-II inhibitor bearing an acrylamide using Autodock vina 1.1.2 in PyRx 0.8[18]. ChemDraw Ultra 8.0 was used to draw the structures of the compounds and native ligand (mole. File format). All the ligands were subjected for energy minimization by applying Universal Force Field (UFF)[19]. The crystal structure of the enzyme with PDB ID: 6XVK was obtained from RCSB Protein Data Bank (PDB) (https://www.rcsb.org/structure/6XVK). Discovery Studio Visualizer (version-19.1.0.18287) was used to refine the enzyme structure, purify it, and get it ready for docking[20]. A three-dimensional grid box with an exhaustiveness value of 8 was created for molecular docking[18]. BIOVIA Discovery Studio Visualizer was used to locate the protein's active amino acid residues. The approach outlined by Khan et al. was used to perform the entire molecular docking procedure, identify cavity and active amino acid residues[21–27]. Fig. 1 shows the revealed cavity of enzyme with the native ligand.

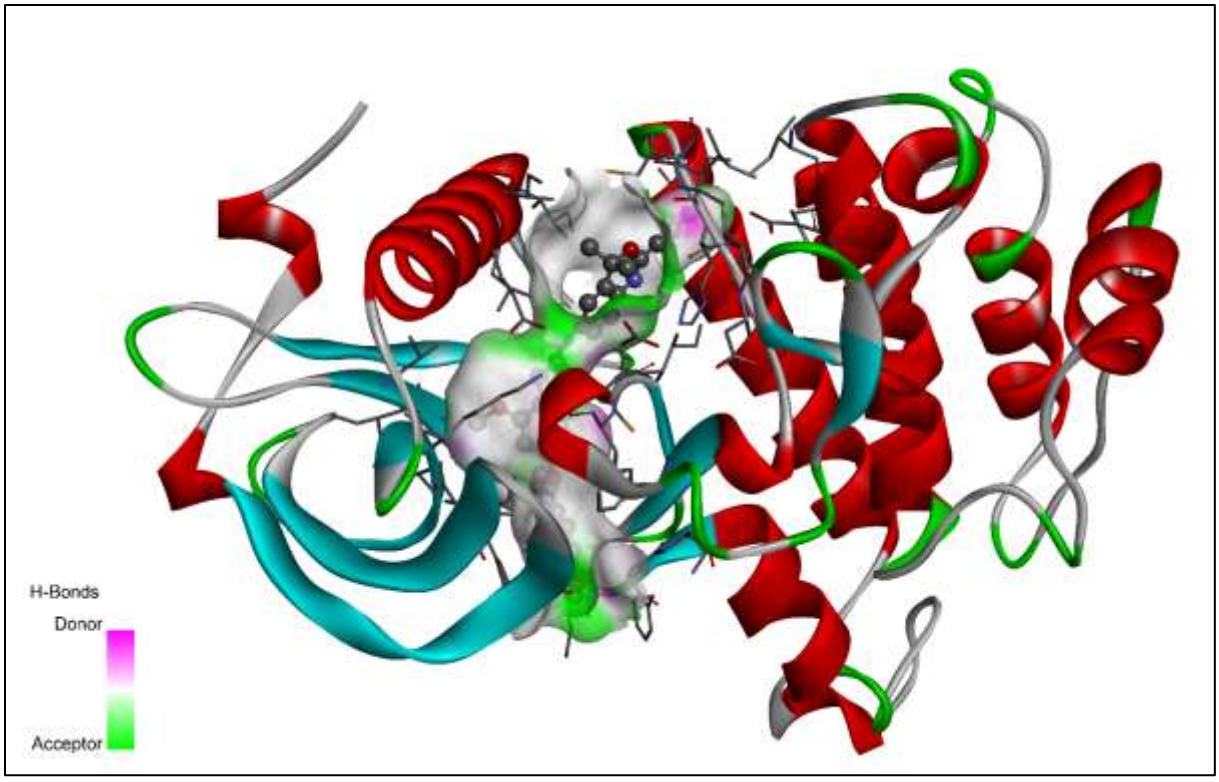


Figure 1. The 3D ribbon view of the enzyme with native ligand in the cavity

3. Results and Discussion

3.1 In silico ADMET Analysis

The main causes of medication failure may be ascribed to inadequate efficacy and safety. The aforementioned criteria have great relevance throughout the whole of the drug discovery and development process, since the characteristics related to absorption, distribution, metabolism, excretion, and toxicity (ADMET) of compounds have a substantial impact on the resulting results. Table 1 provides a thorough overview of the physicochemical features shown by a range of compounds. In the context of physicochemical analysis, it is observed that the values of all the molecules under investigation fall within the acceptable range. The inclusion of logP and logS as components of the Lipinski rule of five was necessitated by the importance of the drug's lipophilicity. In the current study, it was observed that all the examined parameters fell within the acceptable range. Furthermore, these parameters exhibited optimal oral bioavailability, suggesting their potential for development as oral delivery agents[28,29].

The characteristics of the compounds that are indicative of their potential use as drugs are shown in Table 2. Calculations were made using a variety of factors, including QED, NPscore, Lipinski rule, Pfizer rule, GSK rule, Golden Triangle, and Chelator rule. The vast majority of the compounds exhibited a desirable range of quantitative estimate of drug-likeness (QED)[30,31]. The natural product-likeness score, commonly referred to as the NPscore, is typically observed to exhibit values within the -5 to 5 range. Based on the observed correlation, it can be inferred that a higher score is indicative of a heightened probability that the molecule under investigation belongs to the category of natural products (NPs)[32,33]. Both compounds had characteristics that were comparable to those of nanoparticles (NPs). Both of the compounds being studied exhibit adherence to the GSK rule and the Golden Triangle rule, indicating the possibility of a more favorable ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profile.

The absorption parameters of the molecules are presented in Table 3. The optimal Caco-2 permeability is observed when the value exceeds -5.15 Log unit. Regrettably, none of the molecules examined in this study exhibited the desired level of Caco-2 permeability[34]. The compound known as Gitalin has been found to exhibit activity as a substrate for P-glycoprotein (Pgp). Both of the molecules exhibited a level of inhibitory human intestinal absorption (HIA) that can be classified as moderate. The bioavailability of the molecules at F20% and F30% demonstrated values that fell within the acceptable range. Table 4 presents a comprehensive depiction of the distribution and metabolism profile of molecules. The compound known as Gitalin exhibited a plasma protein binding (PPB) value of less than 90%, indicating a relatively low affinity for binding to plasma proteins. The volume distribution (VD) of all the molecules in the study fell within the acceptable range of 0.04-20L/kg, as determined by optimal conditions. Both of the molecules exhibited a moderate potential for penetrating the blood-brain barrier (BBB). The compound known as Gitalin has demonstrated the potential to inhibit cytochrome P450 enzymes (CYP)[12].

The tabulation of molecules' excretion and toxicity profile can be observed in Table 5. Both of the molecules demonstrated a moderate rate of clearance and displayed a relatively short half-life. The toxicity profile of the suggested molecules exhibited favorable properties, with a significant number of values falling within the acceptable range[12]. Table 6 presents a comprehensive overview of the environmental toxicity profile of various molecules, as indicated by their bioconcentration factors, IGC50 values, LC50FM values, and LC50DM values. The molecules exhibited an environmental toxicity profile that was found to be optimal and fell within the acceptable range.

Table 1. Physicochemical properties calculated for molecules

Code	Physicochemical Properties							
	Molecular Weight	Volume	nHA	nHD	nRot	TPSA	logS	logP
NL	536.210	550.341	9	1	8	104.160	-6.564	5.197
Gitalin	700.410	699.517	18	12	28	301.240	-1.239	-0.185
Glaziovine	297.140	307.207	4	1	1	49.770	-1.500	1.634

Table 2. Drug-likeness properties of molecules

Code	Medicinal Chemistry						
	QED	NPscore	Lipinski Rule	Pfizer Rule	GSK Rule	Golden Triangle	Chelator Rule
NL	0.292	-0.294	Rejected	Accepted	Rejected	Rejected	0
Gitalin	0.056	0.154	Rejected	Accepted	Rejected	Rejected	0
Glaziovine	0.864	1.909	Accepted	Accepted	Accepted	Accepted	1

Table 3. An absorption parameters of molecules

Code	Absorption						
	Caco-2 Permeability	MDCK Permeability	Pgp-inhibitor	Pgp-substrate	HIA	F20%	F30%
NL	-5.395	1.7e-05	+++	---	---	--	+
Gitalin	-7.154	0.00015	--	+++	+++	+++	+
Glaziovine	-4.641	2.7e-05	---	----	----	---	---

Table 4: Distribution and metabolism profile of molecules

Code	Distribution				Metabolism				
		VD	BBB	Fu	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4

	PPB (%)		Penetration		Inhibitor	Substrate	Inhibitor	Substrate	Inhibitor	Substrate	Inhibitor	Substrate	Inhibitor	Substrate
NL	99.372	0.648	---	1.203	--	++	++	++	+++	+++	--	+++	+++	++
Gitalin	9.119	0.405	---	63.077	---	---	---	---	---	---	---	---	---	---
Glaziovine	88.782	1.116	+++	4.972	---	+	--	++	---	+	-	+	---	++

Table 5. Excretion and toxicity profile of molecules

Code	Excretion		Toxicity									
	CL	T1/2	H-HT	DILI	AMES Toxicity	Rat Oral Acute Toxicity	FDA MDD	Skin Sensitization	Carcinogencity	Eye Corrosion	Eye Irritation	Respiratory Toxicity
NL	4.953	0.248	+++	+++	-	-	++	++	+	---	---	+++
Gitalin	2.309	0.776	-	---	---	---	---	---	--	---	---	--
Glaziovine	6.701	0.885	-	---	---	--	+++	++	+++	---	---	+++

Table 6. Environmental toxicity profile of molecules

Code	Environmental toxicity			
	Bioconcentration Factors	IGC50	LC50FM	LC50DM
NL	2.142	5.021	6.296	5.947
Gitalin	0.273	2.699	3.857	4.513
Glaziovine	1.348	4.286	4.972	5.525

3.2 Molecular Docking Studies

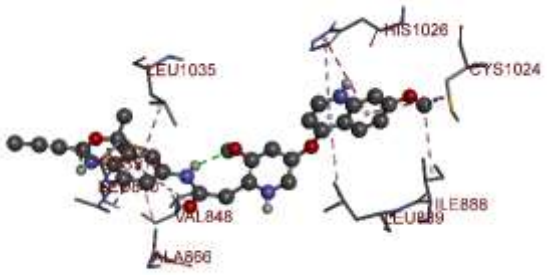
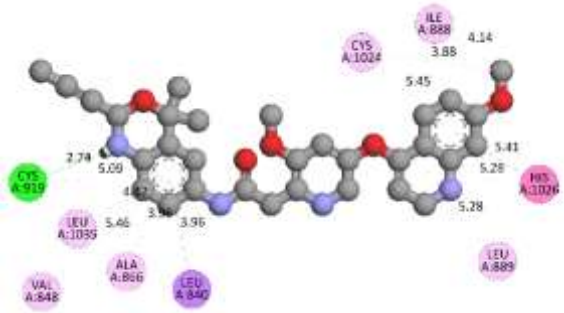
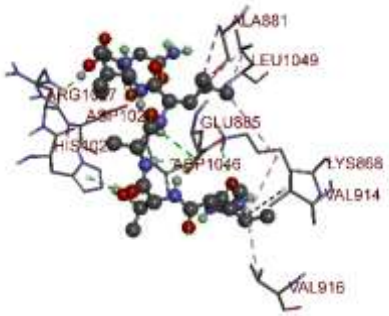
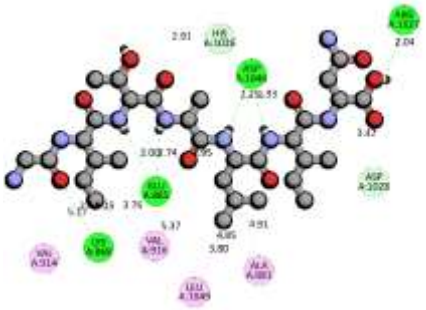
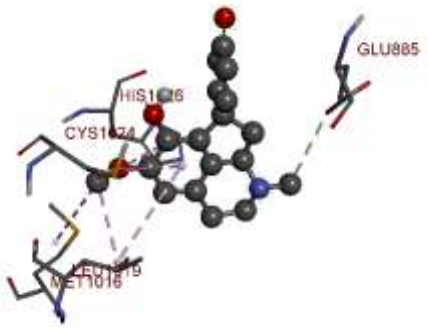
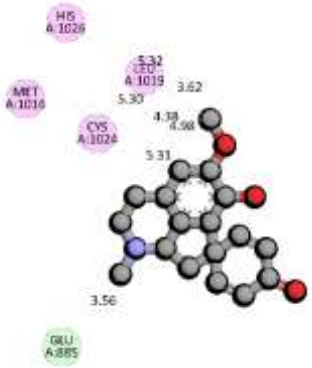
The computer technique known as molecular docking is used to conduct virtual screening of molecules, enabling the assessment of the preliminary activity potential of a ligand against certain biological targets. The attainment of this aim may be aided by assessing the ligand's affinity for binding to the particular target. The molecular docking interactions have been methodically documented and classified in Table 7. The table shown above offers a detailed summary of the many interactions that have been observed during the process of docking. Furthermore, Table 8 presents distinct docking configurations, providing tangible illustrations of the molecular interactions. The binding affinities of the molecules have been compared with the binding mode of native ligand present in the crystal structure of VEGFR-2 kinase (PDB ID: 6XKV). Native ligand exhibited -8.7 kcal/mol of binding affinity and formed two conventional hydrogen bonds with Cys919. It also formed hydrophobic Interactions (Pi-sigma, Pi-Pi T-shaped, Alkyl, Pi-alkyl) with Leu840, His1026, Ile888, Cys1024, Leu889, Val848, Ala866, Cys919, Leu1035, and Cys1024. **Gitalin** exhibited -8.3 kcal/mol of binding affinity and formed seven conventional, one carbon and one Pi-Donor hydrogen bond with Glu885, Asp1046, Arg1027, Glu885, Lys868 and Asp1028. It also displayed hydrophobic (Alkyl) Interactions with Leu1049, Lys868, Val914, Val916 and Ala881. **Glaziovine** displayed -8.1 kcal/mol and formed one carbon hydrogen bond with Glu885. It also showed Hydrophobic (Alkyl, Pi-Alkyl) Interactions with Met1016, Leu1019, Cys1024 and His1026. From molecular docking it was observed that both the compounds formed more stable complex than native ligand. Gitalin displayed formation of seven conventional hydrogen bonds with target which indicates it has potential to modulate the activity of target enzyme. This compound (Gitalin) can be tested further using in vitro and in vivo models for VEGFR-2 kinase activity to gather more quality data to claim its use clinically. Further lead optimization needed by applying numerous experimental techniques.

Table 7. The binding interactions of molecules with VEGFR-2 kinase

Active amino acid residues	Bond Length	Bond Type	Bond Category	Ligand energy	Docking score
NL					
CYS919	2.73884	Hydrogen Bond	Conventional Hydrogen Bond	933.34	-8.7
	3.06352				
LEU840	3.95706	Hydrophobic	Pi-Sigma		
HIS1026	5.40986		Pi-Pi T-shaped		
ILE888	4.13805		Alkyl		
CYS1024	3.8825				
LEU889	5.27859				
VAL848	5.45953		Pi-Alkyl		
ALA866	3.95797				
CYS919	5.0877				
LEU1035	4.42133				
CYS1024	5.44712				
HIS1026	5.28315				
Gitalin					
GLU885	2.95482	Hydrogen Bond	Conventional Hydrogen Bond	341	-8.3
ASP1046	2.24943				
ASP1046	1.92536				
ARG1027	2.03904				
GLU885	2.73781				
GLU885	2.00324				
LYS868	2.44199				
ASP1028	3.41953		Carbon Hydrogen Bond		
HIS1026	2.90779		Pi-Donor Hydrogen Bond		
LEU1049	4.85438	Hydrophobic	Alkyl		
LYS868	5.37385				
LEU1049	3.80216				
LYS868	4.25216				
VAL914	5.16816				
VAL916	3.75642				
ALA881	4.90853				
Glaziovine					
GLU885	3.56	Hydrogen Bond	Carbon Hydrogen Bond	302.01	-8.1
MET1016	5.30402	Hydrophobic	Alkyl		
LEU1019	3.62238				
CYS1024	4.38475				
LEU1019	4.98085		Pi-Alkyl		
:CYS1024	5.31198				

HIS1026	5.32006				

Table 8. The docking poses of molecules

3D-docking poses	2D-docking poses
	
Native ligand	
	
Gitalin	
	
Glaziovine	

Conclusion

Inhibitors that specifically target the kinase activity of Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) have shown considerable effectiveness in suppressing the biological processes of angiogenesis and lymphangiogenesis. Consequently, these inhibitors have notable anti-cancer properties. The current study focuses on the investigation of two naturally occurring compounds, namely Gitalin and Glaziovine, which have been chosen as the main topics of interest. The objective of this research is to assess the potential of these substances as inhibitors of VEGFR-2 kinase activity. An extensive analysis was done to assess the absorption, distribution, metabolism,

excretion, and toxicity (ADMET) properties of these compounds in order to determine their viability as prospective therapeutic options. The in silico ADMET research demonstrated that these compounds had very favorable drug-likeness characteristics, suggesting their viability as lead molecules for further molecular docking analysis. Based on the results obtained from molecular docking analysis, it was revealed that both compounds exhibited a higher degree of stability while forming a complex compared to the native ligand. Gitalin demonstrated the creation of seven typical hydrogen bonds with the target, suggesting its ability to control the activity of the target enzyme. Further testing of this chemical, Gitalin, may be conducted utilizing in vitro and in vivo models to assess its VEGFR-2 kinase activity. This would provide more high-quality data that might support its potential therapeutic use. Additional lead optimization is required via the use of various experimental methodologies.

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