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Original Article

Peptide-based drug as atherosclerosis multitarget therapy from *Lytechinus variegatus* spine: An in silico study

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Abstract

Atherosclerosis is a leading cardiovascular disease characterized by the buildup of plaques within arterial walls. The aim of this study was to investigate the potential of peptides derived from *Lytechinus variegatus* spines as novel therapeutic agents for atherosclerosis using an in silico approach. Key proteins involved in atherosclerosis were selected as target proteins: vascular endothelial growth factor receptor (VEGFR), protein kinase B (AKT1), epidermal growth factor receptor (EGFR), mitogen-activated protein kinase 8 (MAPK8), and endothelin-1 (ET-1). Comprehensive analysis involving ligand and protein preparation, toxicity, and allergenicity assessments, absorption, distribution, metabolism and excretion (ADME) predictions, and molecular docking were conducted to evaluate the safety, pharmacokinetic properties, binding affinity (kcal/mol), root mean square deviation (RMSD) (Å), as well as a 2D and 3D visualization. Toxicity predictions revealed that peptide 9 was non-toxic and non-allergenic, with a lethal dose 50 (LD_{50}) of 3,000 mg/kg, indicating its safety. Peptide 9 demonstrated the most promising results, effectively inhibiting VEGFR2 (-10,90 kcal/mol), AKT1 (-10,56 kcal/mol), EGFR (-9,82 kcal/mol), MAPK8 (-9,64 kcal/mol), and ET-1 (-11,41 kcal/mol) with strong binding affinities and specificity. These interactions suggested that peptide 9 from *Lytechinus variegatus* spines may serve as a competitive multitarget inhibitor, offering potential multitarget therapeutic activity against atherosclerosis. Peptide 9 also had high water solubility and did not affect the concentration or excretion of other drugs or compounds, minimizing the risk of drug-drug interactions.

Keywords: Atherosclerosis, *Lytechinus variegatus*, molecular docking, peptide-based drug, multitarget therapy

Introduction

Atherosclerosis is a disease characterized by the accumulation of lipids, fibrous elements, and calcification within the large arteries [1]. This process begins with endothelium activation, followed by a cascade of events, which implies the vessel narrowing and activation of

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inflammatory pathways, leading to atheroma plaque formation. Atherosclerosis is considered the major cause of cardiovascular disease (CVD) complications, which remains the leading cause of death worldwide [2]. In 2019, over 17 million people died from CVD, with 85% of these deaths attributed to heart attacks and strokes, which are mainly caused by atherosclerosis in the arteries [3]. Hyperlipidemia, hypertension, smoking and diabetes are the major risk factors for atherosclerosis and are implicated in its plaque initiation, progression, and rupture [1].

For the effective treatment of atherosclerosis, it is crucial to target key signaling pathways involved in the disease pathogenesis. The proteins mitogen-activated protein kinase 8 (MAPK8), epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), endothelin-1 (ET-1), and protein kinase B (AKT1), play significant roles in vascular homeostasis, endothelin function, cell proliferation, and inflammatory responses, all of which contribute to atherosclerosis development [4-7]. The dysregulation of these pathways contributes to the development and progression of atherosclerosis plaques, leading to severe cardiovascular complications [4-7]. Inhibiting these molecular markers offers a therapeutic strategy to halt or reverse plaque formation. However, current anti-atherosclerosis treatments primarily manage risk factors but fail to directly target plaque-causing cells or resolve underlying inflammation. These treatments also have significant side effects and remain costly [3]. Thus, this underscores the need for the exploration of novel anti-atherosclerosis therapies that are safer, more targeted, and affordable.

The investigation of marine bioactivity has emerged as a promising path for discovering anti-atherosclerosis drugs [8]. *Lytechinus variegatus* is known to contain several peptides in both coelomic fluid and spines, but its potential as an anti-atherosclerosis agent has not been extensively explored. Therefore, the aim of this study was to investigate a peptide-based drug strategy for atherosclerosis therapy by evaluating the potential (binding affinity and type of interaction) of the identified peptide of *L. variegatus* spine in targeting MAPK8, EGFR, VEGFR, ET-1, and AKT1 through an in silico approach.

Methods

In this research, several stages of methods were conducted to investigate peptide-based drug strategies as potential atherosclerosis therapies by evaluating the binding affinity and interaction type of identified peptides from *L. variegatus* spines against key target proteins involved in atherosclerosis—MAPK8, EGFR, VEGFR2, ET-1, and AKT1—using in silico studies. This research used several methods: protein and ligand preparation, toxicity, and allergenicity prediction, molecular docking, and absorption, distribution, metabolism, and excretion (ADME) prediction (**Figure 1**).

Protein and ligand preparation

All *L. variegatus* spine peptide sequences were retrieved from peptidomics profiling [9]. *L. variegatus* spine peptides were then visualized using the UCSF Chimera v.1.17.1 application to obtain the crystal structure of each peptide. Ligand controls for each target protein were retrieved from the PubChem database (accessed: March 15, 2024). VEGFR2 (PDB: 4ASE), AKT1 (PDB: 3MVH), EGFR (PDB: 1XKK), MAPK8 (PDB: 4YR8), and ET-1 (PDB: 5X93) crystal structures were downloaded from the PDB database (accessed: March 15, 2024). Next, all ligands and proteins were prepared using the MOE v2022.02 application by removing all water molecules, preserving and neutralizing sequence, and refining them to a root mean square (RMS) gradient of 0.001 kcal/mol/Å2.

Toxicity and allergenicity analysis

To screen the peptide toxicity and allergenicity, the web servers of ProTox-III (https://tox.charite.de/protox3/; accessed September 2, 2024) and AllerTop v2.0 (https://www.ddg-pharmfac.net/AllerTOP/; accessed March 15, 2024) were used. The toxicity test results classified the peptides into several categories based on the LD_{50} value and target organ toxicity [10]. Class I compounds are deadly if swallowed ($LD_{50} \leq 5$), class II compounds are deadly if swallowed ($5 < LD_{50} \leq 50$), class III compounds are toxic if swallowed ($50 < LD_{50} \leq 300$), class IV compounds are harmful if swallowed ($300 < LD_{50} \leq 2{,}000$), class V compounds may be harmful if

swallowed (2,000<LD_{50}≤5,000), and class VI compounds are non-toxic (LD_{50}>5,000). Meanwhile, the allergenicity test results categorized the peptides into two groups: allergen (SVM≥0.5) and non-allergen (SVM<0.5). Peptides that are accepted for molecular docking were those with non-allergic properties and predicted toxicity class of 4–6 (LD_{50}>300 mg/kg) [11].

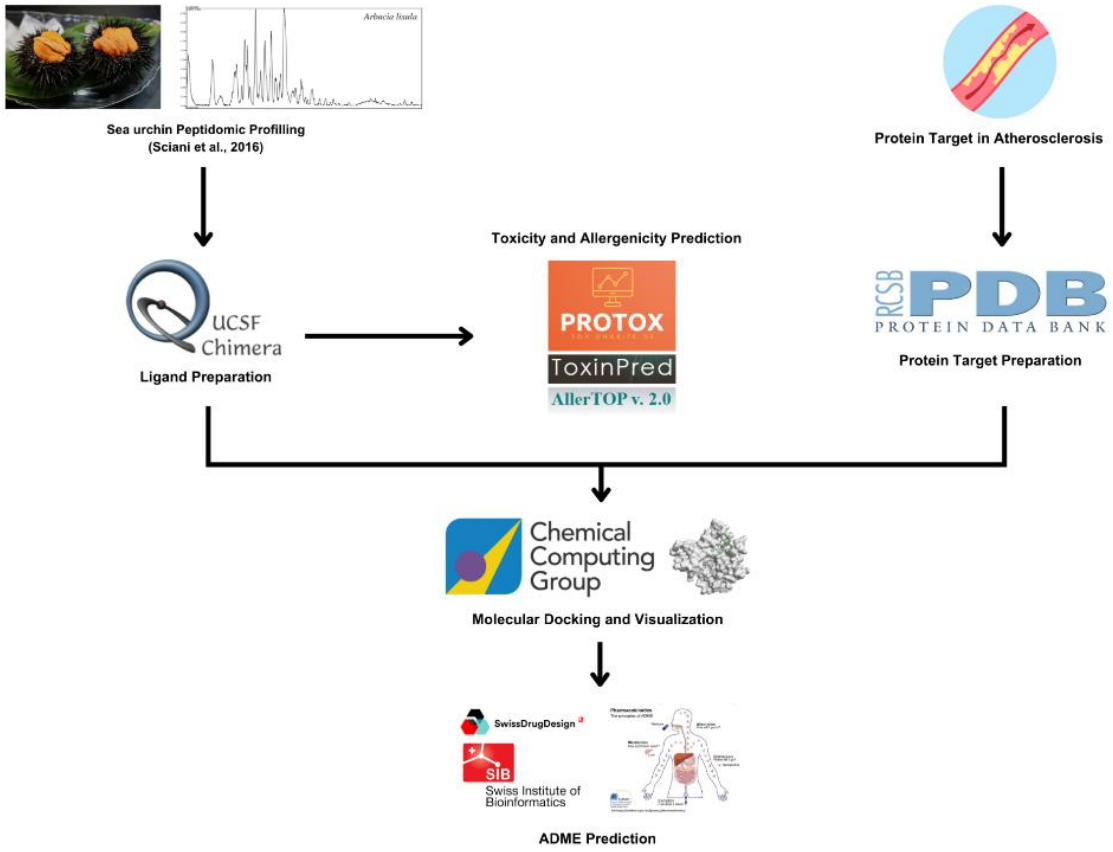


Figure 1. Research workflow illustration.

Molecular docking and visualization

The molecular docking analysis was carried out using the MOE v2022.02 application. To perform the molecular docking analysis, the binding sites were specifically adjusted according to the active site of each target protein. The binding site settings were automatically adjusted based on the amino acid residues of each protein's natural ligand using the Site Finder feature of the MOE application. Molecular docking results include binding affinity (kcal/mol), root mean square deviation (RMSD) (Å), as well as 2D and 3D visualization [12]. Peptides are considered potential inhibitors of target proteins if they exhibit a binding affinity that is much stronger (more negative) than that of the control ligand and have an RMSD<2Å [13]. An RMSD value <2.0 Å helps ensure that the docking results are close to the true binding mode, reflecting high accuracy and reliability in the molecular docking predictions. In addition, molecular docking results were visualized and analyzed based on the type and similarities of the interaction bonds between the selected peptide and the control ligand at the binding site of the target protein. The more remarkable similarity between the amino acid residues formed between the selected peptide and the control ligand indicates that the peptide has potential as a specific inhibitor of the protein target [12,13].

Physiochemical and pharmacokinetic prediction

The PDB format of the selected peptide was used to predict the ADME properties on the SwissADME server (http://www.swissadme.ch/index.php; accessed: January 31, 2025). Upon inserting the PDB format and running the prediction, data on the pharmacokinetics of the selected peptide were collected. The compound's drug-likeness was evaluated on the SCFbio website (http://www.scfbio-iitd.res.in/; accessed: December 10, 2024) using Lipinski's rule of five criteria. Drug-like compounds must meet the following criteria to comply with Lipinski's rule

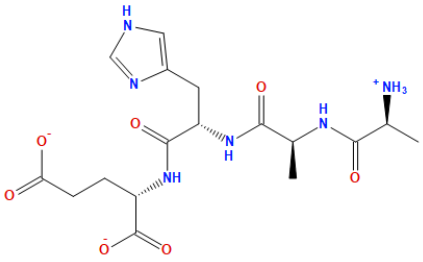
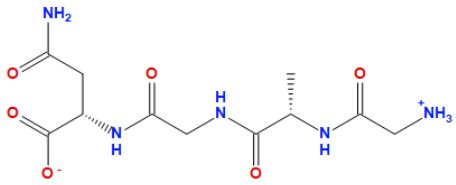
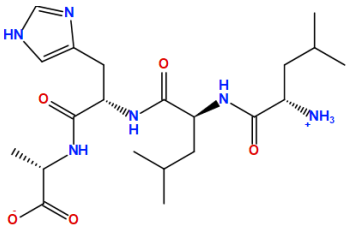
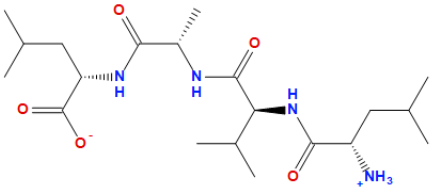
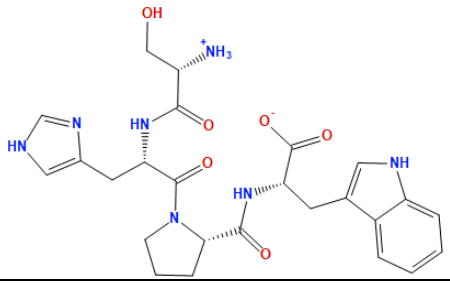
of five: molecular weight (MW) <500 Da, number of hydrogen bond donors (HBD) ≤5, number of hydrogen bond acceptors (HBA) ≤10, and octanol-water partition coefficient (log P) ≤5. A drug-like compound must have no more than one violation of these rules.

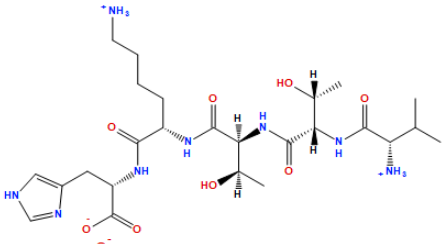
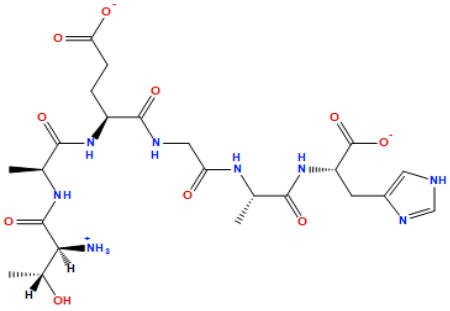
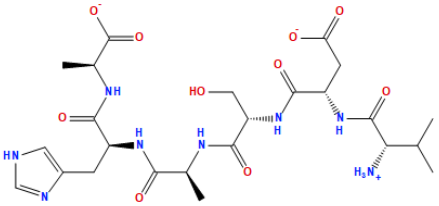
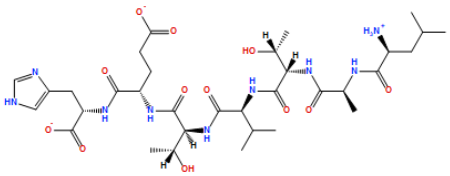
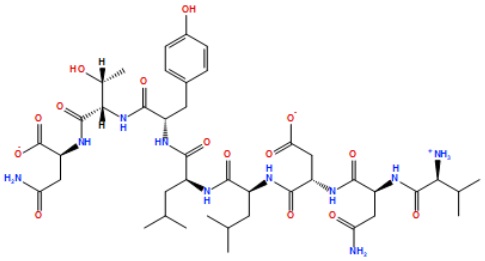
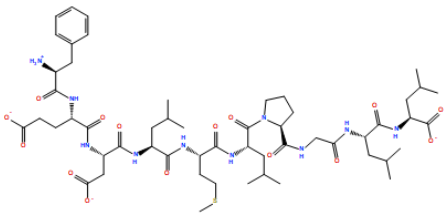
Results

Toxicity and allergenicity analysis

The toxicity and allergenicity predictions for peptides derived from the spines of *L. variegatus* showed promising results. The majority of peptides demonstrated LD_{50} values between 2,400 and 5,000 mg/kg body weight, corresponding to toxicity class 5, which denotes compounds that are possibly hazardous but generally considered to exhibit low acute toxicity. Notably, Peptide 10 (VNDLLYTN) exhibited a lower LD_{50} value of 1,000 mg/kg, classifying it as a class 4 compound, indicative of a comparatively higher toxic potential. Despite variations in toxicity, all peptides were predicted to be non-allergenic. Detailed results of these peptides' toxicity and allergenicity prediction are presented in **Table 1**.

Table 1. Toxicity and allergenicity of peptides derived from the spines of *Lytechinus variegatus*

Ligand	Peptide sequence	2D structure	Screening results	
			LD_{50} (mg/kg)	Allergenicity
Peptide 1	AAHE		3,000 (class 5/possibly hazardous)	Non-allergen
Peptide 2	GAGN		2,500 (class 5/possibly hazardous)	Non-allergen
Peptide 3	LLHA		5,000 (class 5/possibly hazardous)	Non-allergen
Peptide 4	LVAL		2,500 (class 5/possibly hazardous)	Non-allergen
Peptide 5	SHPW		2,400 (class 5/possibly hazardous)	Non-allergen

Ligand	Peptide sequence	2D structure	Screening results	
			LD_{50} (mg/kg)	Allergenicity
Peptide 6	VTTKH		3,000 (class 5/possibly hazardous)	Non-allergen
Peptide 7	TAEGAH		3,000 (class 5/possibly hazardous)	Non-allergen
Peptide 8	VDSAHA		3,000 (class 5/possibly hazardous)	Non-allergen
Peptide 9	LATVTEH		3,000 (class 5/possibly hazardous)	Non-allergen
Peptide 10	VNDLLYTN		1,000 (class 4/harmful)	Non-allergen
Peptide 11	FEDLMLPGLL		5,000 (class 5/possibly hazardous)	Non-allergen

Molecular docking results

The molecular docking results of the interaction between peptides derived from the spine of *L. variegatus* and several protein receptors that play crucial roles in atherosclerosis development are presented in **Table 2**. The more negative (lower) the binding affinity value, the stronger the interaction between ligand and protein. This is because the docking score/binding affinity value reflects the change in potential energy when the two interact. The RMSD value indicates deviation in the tested conformation, where a lower RMSD value signifies a smaller deviation from the protein's natural ligand conformation.

Table 2. Predicted binding affinity and RMSD values of peptides derived from the spines of *Lytechinus variegatus* with MAPK8, EGFR, VEGFR2, ET-1, and AKT1 receptors

Target protein	Ligand	Peptide sequence	Molecular docking results	
			Binding affinity (kcal/mol)	RMSD (Å)
MAPK8	MAPK inhibitor (control)	-	-7.82	1.40
	Peptide 1	AAHE	-10.07	2.24
	Peptide 2	GAGN	-7.14	1.93
	Peptide 3	LLHA	-7.69	1.71
	Peptide 4	LVAL	-8.09	1.30
	Peptide 5	SHPW	-9.32	1.78
	Peptide 6	VTTKH	-8.02	1.66
	Peptide 7	TAEGAH	-8.92	1.73
	Peptide 8	VDSAHA	-8.80	1.94
	Peptide 9	LATVTEH	-9.64	1.92
	Peptide 10	VNDLLYTN	-10.50	2.32
	Peptide 11	FEDLMLPGLL	-10.44	2.43
EGFR	Erlotinib (control)	-	-7.70	1.97
	Peptide 1	AAHE	-7.90	1.99
	Peptide 2	GAGN	-6.72	1.88
	Peptide 3	LLHA	-7.66	1.73
	Peptide 4	LVAL	-7.00	1.62
	Peptide 5	SHPW	-8.29	1.84
	Peptide 6	VTTKH	-8.56	1.80
	Peptide 7	TAEGAH	-8.40	1.95
	Peptide 8	VDSAHA	-8.99	1.78
	Peptide 9	LATVTEH	-9.96	1.99
	Peptide 10	VNDLLYTN	-9.82	1.93
	Peptide 11	FEDLMLPGLL	-10.80	3.19
VEGFR2	Benzimidazole urea inhibitor (control)	-	-7.52	1.36
	Peptide 1	AAHE	-7.90	1.99
	Peptide 2	GAGN	-7.07	1.57
	Peptide 3	LLHA	-7.63	1.27
	Peptide 4	LVAL	-7.24	1.47
	Peptide 5	SHPW	-8.21	1.87
	Peptide 6	VTTKH	-7.65	1.91
	Peptide 7	TAEGAH	-9.26	1.39
	Peptide 8	VDSAHA	-8.89	2.28
	Peptide 9	LATVTEH	-11.09	1.90
	Peptide 10	VNDLLYTN	-10.90	2.55
	Peptide 11	FEDLMLPGLL	-11.91	2.60
ET-1	Sitaxentan (control)	-	-7.45	1.77
	Peptide 1	AAHE	-7.89	1.53
	Peptide 2	GAGN	-6.75	1.90
	Peptide 3	LLHA	-8.21	1.73
	Peptide 4	LVAL	-7.66	1.89
	Peptide 5	SHPW	-8.44	1.93
	Peptide 6	VTTKH	-8.80	1.94
	Peptide 7	TAEGAH	-9.12	2.48
	Peptide 8	VDSAHA	-9.03	1.89
	Peptide 9	LATVTEH	-11.41	1.98
	Peptide 10	VNDLLYTN	-12.78	3.52
	Peptide 11	FEDLMLPGLL	-12.33	2.53
AKT1	GDC0068 (control)	-	-9.15	1.68
	Peptide 1	AAHE	-7.76	1.67
	Peptide 2	GAGN	-6.61	1.60
	Peptide 3	LLHA	-8.47	1.53
	Peptide 4	LVAL	-8.17	1.67
	Peptide 5	SHPW	-8.99	1.58
	Peptide 6	VTTKH	-10.64	1.97
	Peptide 7	TAEGAH	-9.69	1.66
	Peptide 8	VDSAHA	-10.31	1.97
	Peptide 9	LATVTEH	-10.56	1.92
	Peptide 10	VNDLLYTN	-10.45	2.13
	Peptide 11	FEDLMLPGLL	-10.85	2.23

AKT1: protein kinase B; EGFR: epidermal growth factor receptor; ET-1: endothelin-1; MAPK8: mitogen-activated protein kinase 8; RMSD: root mean square deviation; VEGFR2: vascular endothelial growth factor receptor

Molecular docking of peptides with MAPK8

The molecular docking results of peptides from *L. variegatus* exhibited lower binding affinities than that of the MAPK inhibitor in the MAPK8 active site. Moreover, the results showed that peptides 2, 3, 4, 5, 6, 7, 8, and 9 have RMSD values <2 Å, indicating that these peptides could closely mimic the natural binding conformation. Among these, peptide 9 was selected due to its lowest binding affinity and favorable RMSD value among the favorable peptides, with a score of -9.64 kcal/mol and 1.92 Å, respectively. Based on 2D visualization analysis, peptide 9 is predicted to create a stronger bond with seven interactions, consisting of two basic hydrophilic interactions (Lys114, Lys151), four polar hydrophilic interactions (Gly32, Tyr36, Asn154, Cys166), and one greasy hydrophobic interaction (Ala35) (**Figure 2**). Meanwhile, the MAPK8 inhibitor creates five interactions with the MAPK8 active site: one acidic interaction (Asp167), two basic hydrophilic interactions (Lys54, Lys114), one polar hydrophilic interaction (Thr110), and one greasy hydrophobic interaction (Met106). This showed that peptide 9 forms the same amino acid residue with the MAPK8 inhibitor in Lys114 at the MAPK8 active site (**Table 3**). These results indicate that peptide 9 is expected to inhibit MAPK8 specifically by blocking the protein's active site.

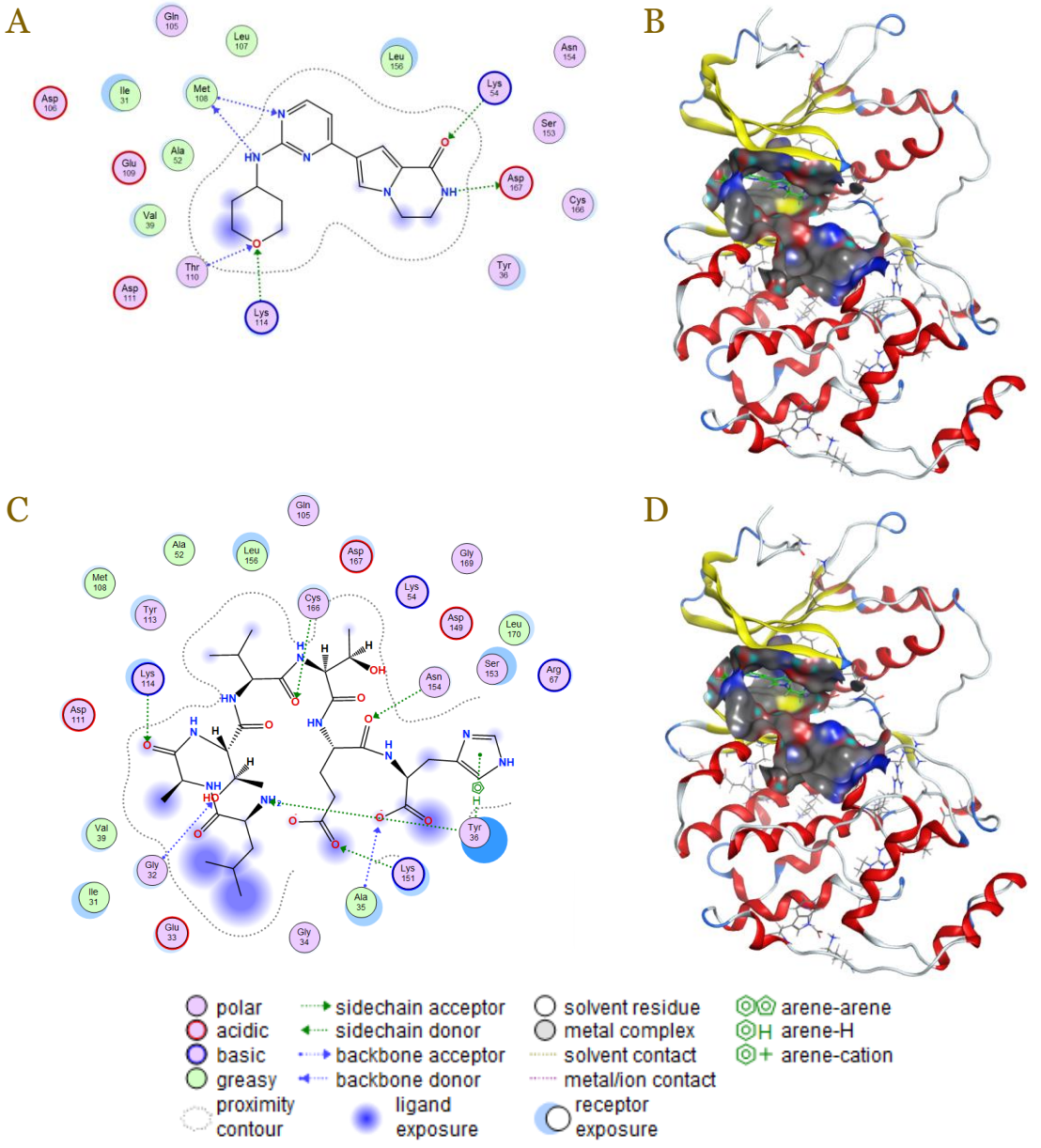


Figure 2. Molecular docking results of MAPK8 protein. (A) 2D visualisation of MAPK inhibitor with MAPK8 protein; (B) 3D visualisation of MAPK inhibitor with MAPK8 protein; (C) 2D visualisation of peptide 9 with MAPK8 protein; (D) 3D visualisation of peptide 9 with MAPK8 protein.

Molecular docking of peptides with EGFR

Peptides 6, 8, and 9 from *L. variegatus* demonstrated a strong binding affinity as inhibitors of the EGFR protein. Generally, the binding affinities of all *L. variegatus* peptides were significantly stronger than erlotinib (control ligand). Among these favorable peptides, peptide 9 was selected based on the lowest binding affinity (-9.96 kcal/mol). Peptide 9 forms three interactions with the active site of EGFR. The 2D interaction visualization shows two acidic hydrophilic (Asp776 and Asp831) and one polar hydrophilic (Cys773) interaction. Similarly, control erlotinib forms three interactions (Lys721, Met769, and Asp831) with the active site binding pocket of EGFR, involving a basic hydrophilic (Lys721), acidic hydrophilic (Asp831), and a hydrophobic greasy interaction (Met769) (**Figure 3** and **Table 3**). Peptide 9 and erlotinib create the same interaction at Asp831, indicating that peptide 9 can potentially replace erlotinib as a specific EGFR inhibitor.

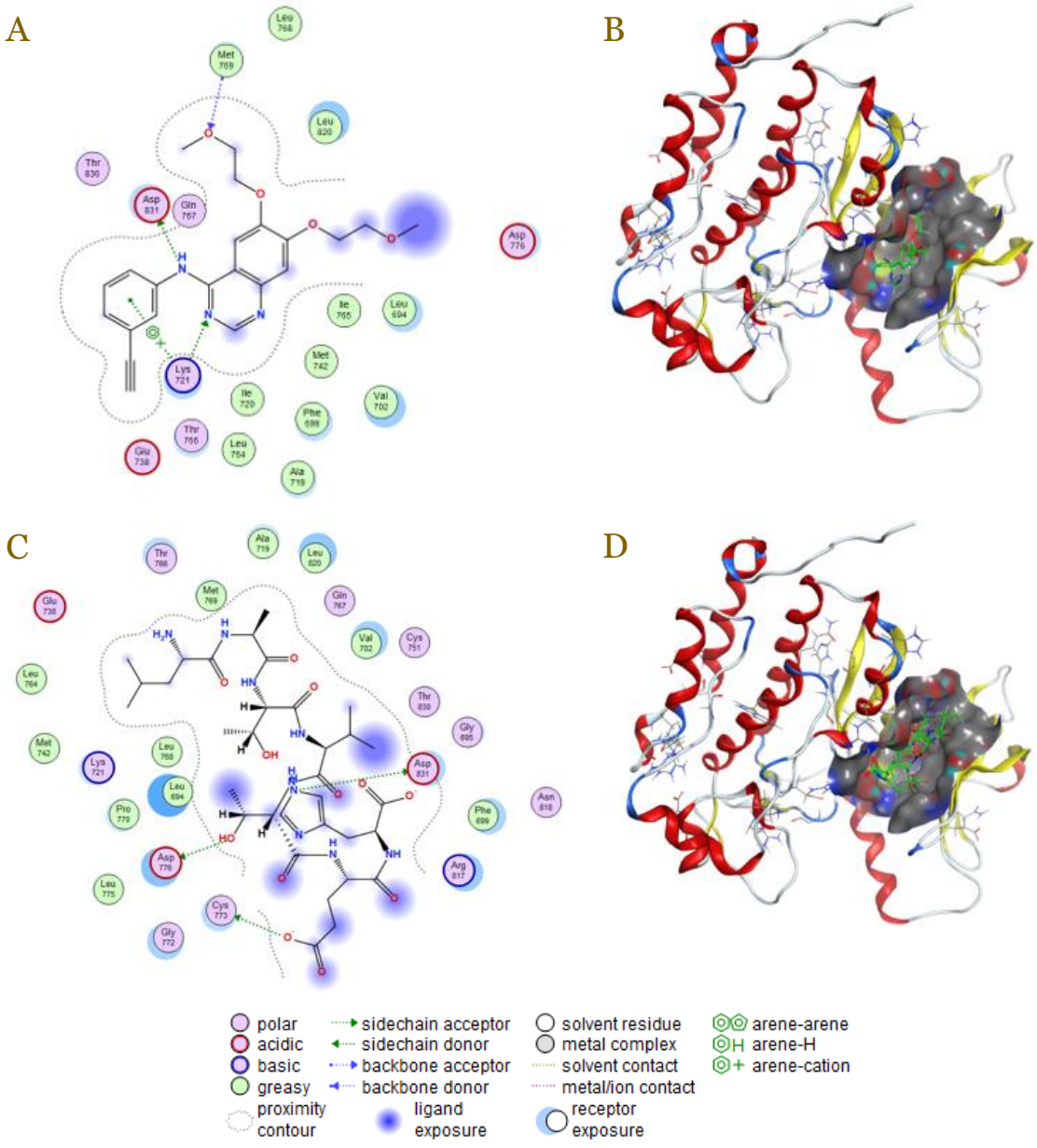


Figure 3. Molecular docking results of EGFR protein. (A) 2D visualisation of erlotinib with EGFR protein; (B) 3D visualisation of erlotinib with EGFR protein; (C) 2D visualisation of peptide 9 with EGFR protein; (D) 3D visualisation of peptide 9 with EGFR protein.

Molecular docking of peptides with VEGFR2

Peptides 1, 3, 5, 6, 7, and 9 demonstrated potential activities to replace benzimidazole urea inhibitors due to their lower binding affinity scores and favorable RMSD values. However, peptide 9 was selected among these favorable peptides based on the lowest binding affinity (-11.91

kcal/mol). The 2D analysis results show that peptide 9 and benzimidazole urea inhibitors interact with the same amino acid residues (Asp1044 and Arg1049). In addition, the interaction of VEGFR2 protein with peptide 9 is predicted to be more stable than with benzimidazole urea inhibitor, as peptide 9 forms more interactions: one acidic hydrophilic interaction (Asp1044), three basic hydrophilic interactions (Arg 1029, Arg1049, Arg1030), one polar hydrophilic interaction (Gly1046) and two greasy hydrophobic interactions (Ala1048, Leu1047). In contrast, the VEGFR2-benzimidazole urea inhibitor complex interaction consists of only three interactions: one acidic hydrophilic interaction (Asp1044), one basic hydrophilic interaction (Arg1049), and one polar hydrophilic interaction (Asn1031) (**Figure 4** and **Table 3**). These results suggest that peptide 9 has the potential to replace the benzimidazole urea inhibitor as a specific VEGFR2 inhibitor because it forms the same interactions with residues as in the benzimidazole urea inhibitor and has a stronger affinity with VEGFR2.

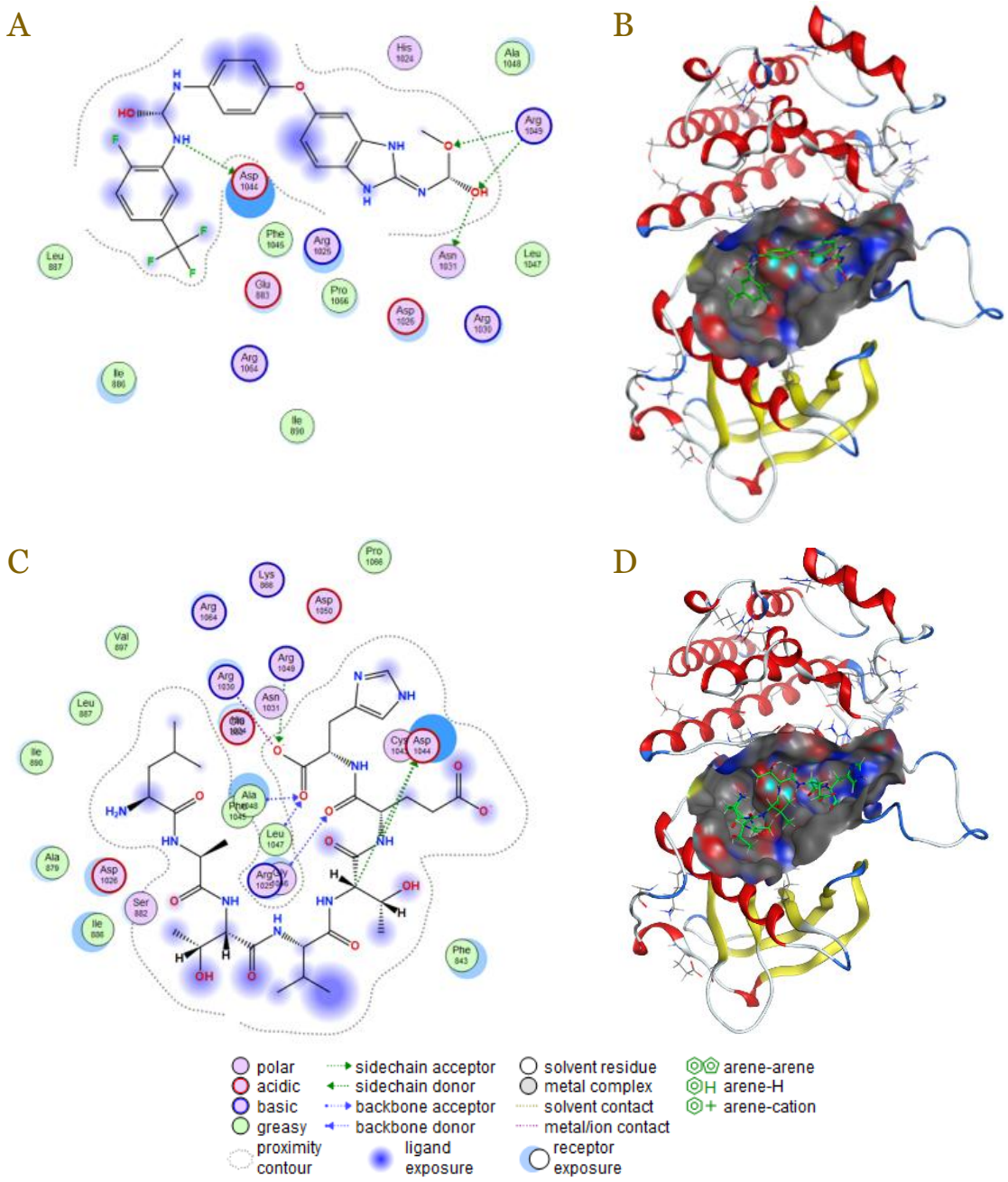


Figure 4. Molecular docking results of VEGFR2 protein. (A) 2D visualisation of benzimidazole urea inhibitor with VEGFR2 protein; (B) 3D visualisation of benzimidazole urea inhibitor with VEGFFR2 protein; (C) 2D visualisation of peptide 9 with VEGFR2 protein; (D) 3D visualisation of peptide 9 with VEGFR2 protein.

Molecular docking of peptides with ET-1

The molecular docking results between the peptide derived from the spine of *L. variegatus* and ET-1 protein indicate that peptides 1, 3, 4, 5, 6, 8, and 9 have the potential to replace sitaxentan as a competitive inhibitor of ET-1. Among these favorable peptides, peptide 9 was selected based on the lowest binding affinity (-11.41 kcal/mol). The 2D analysis revealed that peptide 9 forms four interactions with the ET-1 active site, consisting of three polar hydrophilic bonds (two interactions with Asn158 and Ser184) and one basic hydrophilic bond (Arg343). Similarly, 2D analysis of control sitaxentan formed four interactions with ET-1 active site, characterized by three basic hydrophilic bonds (Arg 343 and two interactions with Lys 182) and one greasy hydrophobic bond (Leu277) (**Table 3**). Both peptide 9 and sitaxentan exhibit interaction at the amino acid residue Lys182 (**Figure 5**). These findings suggest that peptide 9 can occupy the ET-1 active site similar to sitaxentan.

Table 3. 2D visualization interpretation from molecular docking results

Ligand-protein	Types of interaction	Amino acid residue
Peptide 9-MAPK8	Hydrophilic: acidic	-
	Hydrophilic: basic	Lys114, Lys151
	Hydrophilic: polar	Gly32, Tyr36, Asn154, Cys166
	Hydrophobic: greasy	Ala35
MAPK8 inhibitor-MAPK8	Hydrophilic: acidic	Asp167
	Hydrophilic: basic	Lys54, Lys114
	Hydrophilic: polar	Thr110
	Hydrophobic: greasy	Met106
Peptide 9-EGFR	Hydrophilic: acidic	Asp776 and Asp831
	Hydrophilic: basic	-
	Hydrophilic: polar	Cys773
	Hydrophobic: greasy	-
Erlotinib-EGFR	Hydrophilic: acidic	Asp831
	Hydrophilic: basic	Lys721
	Hydrophilic: polar	-
	Hydrophobic: greasy	Met769
Peptide 9 -VEGFR2	Hydrophilic: acidic	Asp1044
	Hydrophilic: basic	Arg1049, Arg1030
	Hydrophilic: polar	Gly1046
	Hydrophobic: greasy	Ala1048, Leu1047
Benzimidazole urea inhibitor-VEGFR2	Hydrophilic: acidic	Asp1044
	Hydrophilic: basic	Arg1049
	Hydrophilic: polar	Asn1031
	Hydrophobic: greasy	-
Peptide 9-ET-1	Hydrophilic: acidic	-
	Hydrophilic: basic	Arg343
	Hydrophilic: polar	Asn158, Ser184
	Hydrophobic: greasy	-
Sitaxentan-ET-1	Hydrophilic: acidic	-
	Hydrophilic: basic	Lys182, Arg343
	Hydrophilic: polar	-
	Hydrophobic: greasy	Leu277
Peptide 9-AKT1	Hydrophilic: acidic	Glu278, Asp292
	Hydrophilic: basic	Lys276, Lys179
	Hydrophilic: polar	Gly157
	Hydrophobic: greasy	Phe161
GDC0068-AKT1	Hydrophilic: acidic	Glu278, Glu228
	Hydrophilic: basic	-
	Hydrophilic: polar	-
	Hydrophobic: greasy	Ala230, Met281, Val164

Molecular docking of peptides with AKT1

The molecular docking results between peptides derived from *L. variegatus* and AKT1 reveal that peptides 6 (-10,64 kcal/mol), 8 (-10,31 kcal/mol), and 9 (-10,56 kcal/mol) demonstrate potential as AKT1 inhibitors, as evidenced by an RMSD value below 2Å and a binding affinity value lower than GDC0068, a potent AKT1 inhibitor. Peptide 9 has been identified as the most promising candidate for AKT1, as it exhibits a lower binding affinity than the AKT1 inhibitor and is also capable of inhibiting four previous protein targets. The 2D molecular analysis demonstrates that peptide 9 and GDC0068 interact with the same amino acid residue (Glu278).

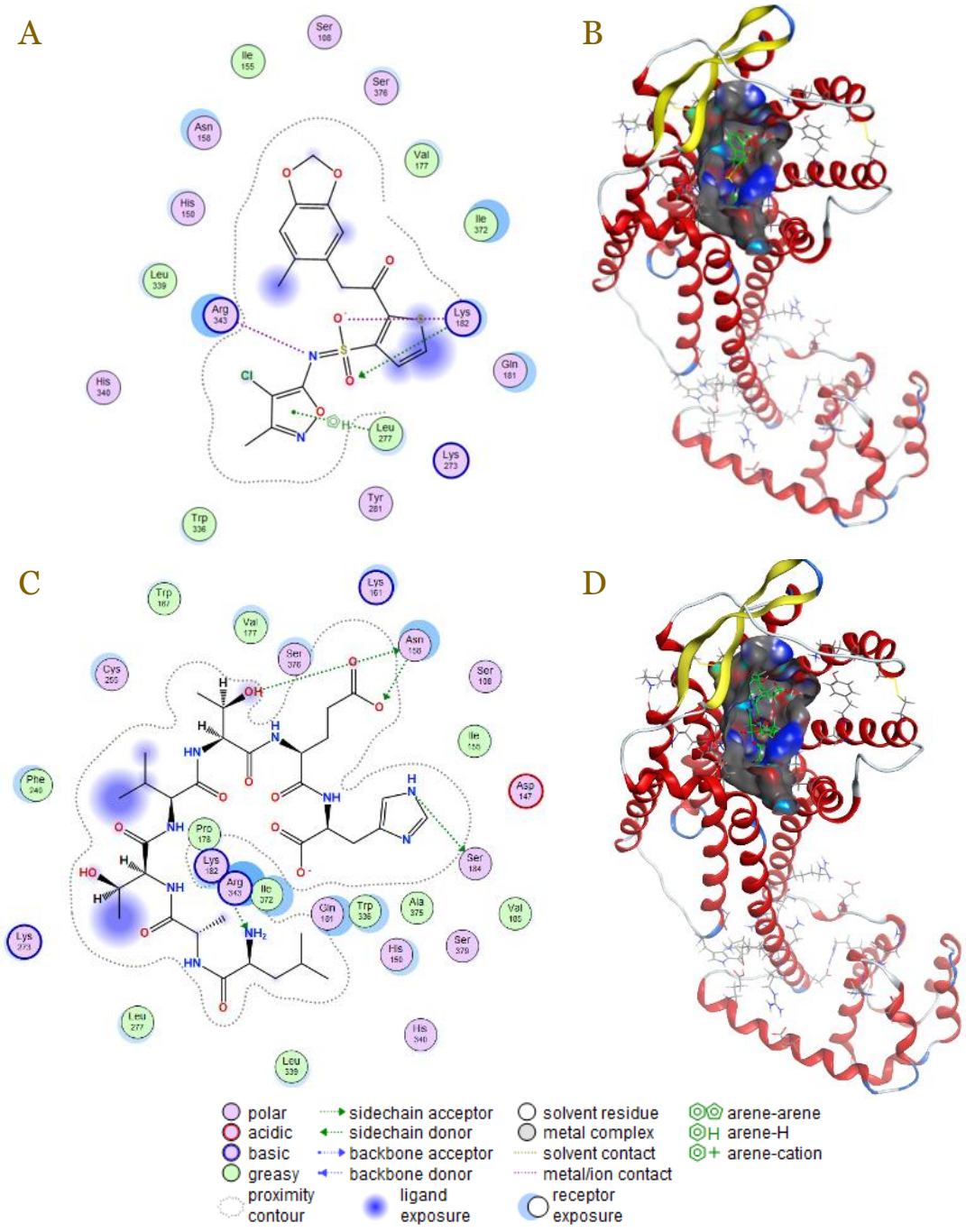


Figure 5. Molecular docking results of ET-1 protein. (A) 2D visualization of sitaxentan with ET-1 protein; (B) 3D visualization of sitaxentan with ET-1 protein; (C) 2D visualization of peptide 9 with ET-1 protein; (D) 3D visualization of peptide 9 with ET-1 protein.

Additionally, the interaction between AKT1 and peptide 9 is potentially more stable than GDC0068, as peptide 9 forms more interactions. Peptide 9 forms six interactions: two acidic hydrophilic interactions (Glu278, Asp292), two basic hydrophilic interactions (Lys276, Lys179), one polar hydrophilic interaction (Gly157), and one greasy hydrophobic interaction (Phe161). In contrast, GDC0068 forms five interactions: two acidic hydrophilic (Glu278, Glu228) and three greasy hydrophobic (Ala230, Met281, Val164) (**Table 3**). Based on these results, peptide 9 is concluded to interact with AKT1 and elicit an inhibitory response more effectively than GDC0068 (**Figure 6**). This conclusion is further supported by quantitative 3D analysis, which shows that peptide 9 fits perfectly within the AKT1 active site, reinforcing its potential as a reliable AKT1 inhibitor.

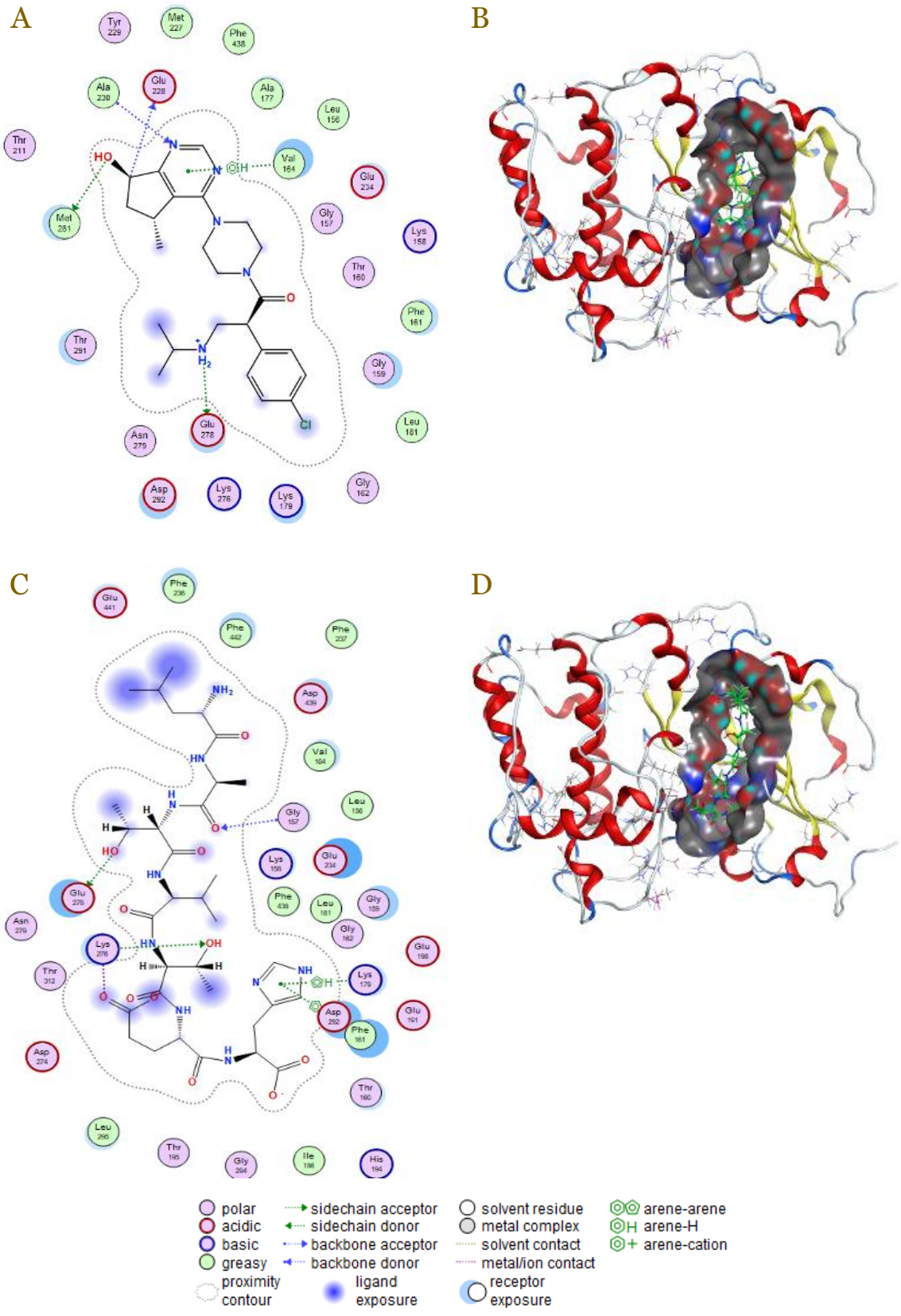


Figure 6. Molecular docking results of AKT1 protein. (A) 2D visualization of GDC0068 with AKT1 protein; (B) 3D visualization of GDC0068 with AKT1 protein; (C) 2D visualization of peptide 9 with AKT1 protein; (D) 3D visualization of peptide 9 with AKT1 protein.

Physicochemical and pharmacokinetics prediction

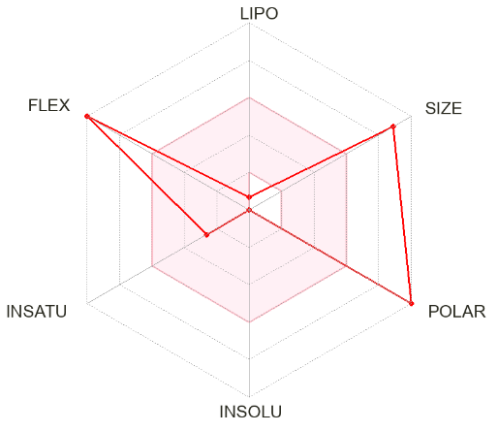
Drug development requires extensive investigation of the potential drug's pharmacokinetic properties. The results showed that peptide 9 violates three of the rules: it has 13 HBA (>5), 29

rotatable bonds (>10), and a molecular weight of 753.84 dalton (>500 dalton) (**Table 4**). This indicates that peptide 9 does not qualify as a drug-like molecule according to Lipinski's rules of five.

According to the absorption predictions, peptide 9 has a low amount of absorption in the human intestine. Furthermore, the examination of Caco-2 permeability as an indicator of the absorption rate for orally administered medications revealed that peptide 9 was not easily absorbed. Additionally, peptide 9 was unable to pass across the blood-brain barrier (BBB). The Log S value of 0.03 indicates that peptide 9 is also regarded as being highly water soluble. To predict the metabolism, its potential as an inhibitor of liver metabolic enzymes, including Cytochrome P450 family 2 subfamily D member 6 (CYP2D6), Cytochrome P450 family 1 subfamily A member 2 (CYP1A2), Cytochrome P450 family 2 subfamily C member 19 (CYP2C19), and Cytochrome P450 family 3 subfamily A member 4 (CYP3A4), was assessed. Peptide 9 does not act as a CYP2D6, CYP1A2, CYP2C19, or CYP3A4 inhibitor, suggesting that the peptide will not alter the concentration of other drugs that are dependent on the CYP2D6, CYP1A2, CYP2C19, and CYP3A4 enzymes for its activation or elimination (**Table 4**).

To assess the excretion of peptide 9, its potential to act as an inhibitor of organic cation transporter 2 (OCT2) protein in the kidney was examined. The results indicate that peptide 9 does not function as an OCT2 substrate (**Table 4**). This finding suggests that peptide 9 would not affect the excretion of other drugs or compounds in the kidney and would not affect the concentration of other drugs or compounds in the circulatory system when combined.

Table 4. Physicochemical and pharmacokinetics properties of SwissADME analysis

Peptide 9	Formula	$C_{33}H_{55}N_9O_{11}$
	Physicochemical	
	Molecular weight (<500 dalton)	753.84 dalton
	LogP (≤5)	-3.2644
	Rotatable bonds (≤10)	29
	H bond acceptors (<5)	13
	H bond donors (≤10)	10
	Lipinski's rule of five	No (3 violations)
	Pharmacokinetics	
	Water solubility (log S)	0.03 (highly soluble)
	CaCO-2 permeability	0.522 (poor)
	GI absorption	Low
	BBB permeant	No
	CYP1A2 inhibitor	No
	CYP2C19 inhibitor	No
	CYP2C9 inhibitor	No
	CYP2D6 inhibitor	No
	CYP3A4 inhibitor	No
	Renal OCT2 substrate	No

Discussion

This present study demonstrated the therapeutic potential of *L. variegatus* spine-derived peptides as therapeutic agents for atherosclerosis through a combination of toxicity and allergenicity analysis, molecular docking and physicochemical and pharmacokinetics prediction. Based on the results, peptide 9 exhibited non-toxic and non-allergenic properties, with favorable binding affinity and RMSD value. However, peptide 9 did not show optimal pharmacokinetic properties, as its violates three of Lipinski's rules of five, has low intestinal absorption, and is unable to pass across the BBB. Despite these limitations in its pharmacokinetics properties, peptide 9 is highly water soluble, which may contribute to its potential application in drug development.

To infer inhibition potential, we analyzed the binding of peptide 9 at critical active site residues known to be essential for the catalytic activity of these proteins. Catalytic activity plays a crucial role in facilitating biochemical reactions necessary for protein function [14]. Many enzymes and signaling proteins rely on specific active site residues to bind substrates or ATP molecules, enabling phosphorylation or other activation processes. Disrupting these catalytic

interactions can prevent the target proteins from carrying out their normal functions, ultimately affecting the pathways involved in atherosclerosis progression. For example, the interaction with Lys114 in MAPK8, Asp831 in EGFR, and Arg343 in ET-1 occurs at sites necessary for ATP binding or receptor activation, suggesting that peptide 9 may block these proteins' functions. Furthermore, the higher binding affinity compared to known inhibitors supports the hypothesis that peptide 9 acts as a competitive inhibitor rather than a stabilizer. However, further experimental validation, such as enzymatic assays or molecular dynamics simulations, would be necessary to confirm its functional inhibition.

Although peptide 9 exhibited promising binding affinity and specificity, pharmacokinetic (PK) and pharmacodynamic (PD) evaluations are crucial for assessing its clinical potential. The ADME analysis of pharmaceutical substances plays an essential role in identifying their therapeutic efficacy and potential adverse effects [15]. Although peptide 9 has low intestinal absorption, making it unsuitable for oral administration, intravenous delivery emerges as a more suitable route. Importantly, from a metabolic perspective, peptide 9 does not inhibit CYP enzymes, indicating minimal drug-drug interactions for patients with atherosclerosis who often require multiple medications [16]. Moreover, peptide 9 is not an OCT2 substrate, suggesting no interference with renal excretion of other medications, thus supporting its compatibility in combination therapies [17]. Peptide 9's high water solubility has the potential to enhance its distribution [18]. Moreover, toxicity and allergenicity tests confirm that peptide 9 is non-toxic and non-allergenic, enhancing its safety profile for therapeutic use. Despite its poor bioavailability, which needs strategies such as peptide modifications or alternative delivery systems to optimize its clinical utility, peptide 9 holds great promise.

The selected target proteins are critically involved in atherosclerosis progression. MAPK8 plays a significant role in macrophage survival and foam cell formation, contributing to inflammatory plaque development. EGFR is linked to oxidative stress and foam cell transformation, while VEGFR2 mediates pathological angiogenesis, promoting plaque instability [19]. ET-1 is a potent vasoconstrictor associated with vascular remodeling, and AKT1 regulates cell proliferation and survival, influencing plaque stability [15,20]. By inhibiting these targets, a multitarget therapeutic approach could offer significant potential for treating atherosclerosis by modulating key molecular pathways involved in disease progression. Targeting MAPK8 can reduce macrophage survival and foam cell accumulation, potentially decreasing plaque size and inflammation [21]. (17). An in vivo study in a type 1 diabetes mellitus (DM) mouse model exhibited that inhibiting EGFR can suppress oxidative stress, contributing to cardiovascular disease, including atherosclerosis [19]. Studies reported that inhibition of the ET-1 pathway also represents a promising therapeutic approach to reducing vascular inflammation and remodeling in atherosclerosis (24). Furthermore, targeting AKT1 with inhibitors like GDC0068 can reduce plaque formation and inflammation [22].

This study offers valuable insight into the potential of *L. variegatus* spine-derived peptides as therapeutic agents for atherosclerosis, but there are several limitations to consider. Primarily, the research relied on in silico methods, which are useful for initial assessments but may not fully capture the complexities of biological interaction in living organisms. To address this limitation, further research should focus on experimental validation through in vitro and in vivo studies. In vitro assays will help determine the peptides' efficacy in inhibiting key pathways implicated in atherosclerosis, while in vivo studies will provide insight on bioavailability, toxicity, and therapeutic effectiveness in animal models. Additionally, long-term safety assessments and toxicological studies are necessary to confirm the non-toxic and non-allergenic of peptides. Further exploration of peptide optimization, including structural modifications, could enhance their therapeutic potential and reduce side effects, leading to clinical trials and applications.

Conclusion

This study revealed that peptide 9 consistently demonstrated the most effective inhibition across multiple key proteins involved in atherosclerosis, such as MAPK8, EGFR, VEGFR2, ET-1, and AKT1. This peptide was found to be non-toxic, non-allergenic, and exhibited favorable pharmacokinetic properties, such as high-water solubility and minimal risk of drug-drug interactions. This research underscores the potential of *L. variegatus* spine, particularly peptide

9, as a candidate for new peptide-based therapy for atherosclerosis. However, peptide 9 violated several of Lipinski's rules, indicating the need for an advanced drug delivery system to enhance its bioavailability. While these in silico results are promising, further in vitro and in vivo studies are necessary to validate its potential side effects and efficacy.

Ethics approval

Not required

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Not applicable

Declaration of artificial intelligence use

AI tools were used under the supervision of the authors solely to enhance grammar and language clarity. No AI tools were used to generate scientific content, analyze data, or draw conclusions. All intellectual content and critical interpretations are the sole responsibility of the authors.

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