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Research Article

Discovery of Promising Inhibitors for VEGFR-2 Using Computational Methods

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Abstract

VEGFR-2 (vascular endothelial growth factor receptor 2) is widely acknowledged as a highly effective target for the advancement of tumor treatment based on angiogenesis. The growth of tumors, their spread to other parts of the body, and their resistance to many drugs are strongly influenced by the formation of new blood vessels (angiogenesis) and the identification of small compounds that can target VEGFR-2. The potential ability of these molecules to inhibit angiogenesis is of great interest to researchers studying anti-cancer treatments. Several small molecule inhibitors targeting VEGFR-2 have received approval for treating various types of malignancies. One of the most recent updates is tivozanib, which has been approved by the FDA specifically for treating relapsed or refractory advanced renal cell carcinoma (RCC). Nevertheless, the inherent and acquired resistance of the protein, toxicity of compounds, and extensive array of adverse effects continue to be significant concerns. These factors contribute to the limited duration of clinical effectiveness and the ineffectiveness of antiangiogenic medications. We utilized a blend of computational techniques and strategies for the purpose of identifying new, promising, and small molecule inhibitors of VEGFR-2. Our aim was to find alternatives to the chemical structures and components of the existing inhibitors. The present research aimed to employ several sophisticated computer-aided procedures and approaches in the field of drug design and discovery. The techniques employed encompassed ligand- and structure-based virtual screening, estimation of binding free energy, and study of RMSD (Root Mean Square Deviation). The aim is to identify new promising small molecules that may effectively bind to VEGFR-2 and possess the capability to hinder the process of angiogenesis. Five indole derivatives were designed. According to docking study outcomes, these derivatives could be considered as notable candidates as VEGFR-2 inhibitors.

Keywords: VEGFR-2 Inhibitors, Molecular Docking, Design, Indole Derivatives

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INTRODUCTION

Cancer is a serious disease characterized by abnormal and uncontrolled growth and spread of cells. It is the second leading cause of death globally, after cardiovascular diseases [1-2]. VEGFR-2 is well acknowledged as a powerful therapeutic molecular target for the development of cancer treatment interrelated to angiogenesis [3]. VEGF interaction to VEGFR causes conformational changes, specifically the exposure of the ATP binding site and the consequent dimerization of VEGFR [4]. The initiation of many downstream signal transduction pathways, including the p38-MAPK, Raf/MEK/ERK, and PI3K/PKB pathways, is triggered by the autophosphorylation or dephosphorylation of particular tyrosine residues of VEGFR [4-5]. The activation of these pathways facilitates cell proliferation, enhances cell vascular permeability, and eventually results in angiogenesis [6-7]. VEGFR-2 is composed of three distinct structural domains: the extracellular domain responsible for binding ligands, the

transmembrane domain, and the tyrosine kinase domain. VEGFR-2 inhibitors bind to the ATP-binding site in the catalytic domain of the receptor, inhibiting its dimerization and autophosphorylation activities [7]. Numerous monoclonal antibodies, such as ramucirumab, bevacizumab, and aflibercept, have been approved in recent decades for the treatment of various types of cancers, involving renal carcinoma, non-small cell lung cancer, metastatic colorectal cancer, and thyroid cancer. Additionally, multiple small molecule inhibitors of the VEGFR-2, such as sorafenib, vandetanib, axitinib, sunitinib, regorafenib, lenvatinib, pazopanib, nintenanib, and apatinib, have also been approved for these cancer treatments [8-13]. The growth, spread, and resistance to multiple drugs of tumors are heavily influenced by angiogenesis, the formation of new blood vessels [4]. Among the receptors that play a crucial role in regulating angiogenesis is VEGFR-2. Therefore, there is significant interest in anti-cancer research to discover small molecules that can target VEGFR-2 and potentially inhibit angiogenesis. Although several VEGFR-2 inhibitors have been identified, the inherent and acquired resistance of the protein and the diverse array of side effects associated with the current agents continue to be significant challenges, resulting in limited effectiveness and the failure of antiangiogenic medications [14-15]. The concerns arise from the significant structural similarity of VEGFR proteins within the family, which leads to a lack of selectivity in VEGFR-2 binding small molecules. As a result, these compounds can interact with all other receptors in the VEGF family. In addition, the catalytic domain of VEGFR-2, which is responsible for the interaction with small molecule inhibitors, exhibits a significant structural resemblance to other tyrosine kinase receptors, including platelet-derived growth factor receptors (PDGFRs), colony-stimulating factor 1 receptor (CSF1R), and fms such as tyrosine kinase 3 (FLT-3) and c-Kit. This similarity can lead to unexpected side effects due to unpredictable reactions [16]. VEGFR-2 small molecule inhibitors can be categorized into two main types: type 1 inhibitors, also known as DFG-in inhibitors, and type 2 inhibitors, also known as DFG-out inhibitors. Sorafenib, lenvatinib, apatinib, and tivozanib are type 2 inhibitors that bind to the hinge area and the hydrophobicity region (HYD-II), also known as an allosteric site. Type 2 inhibitors are considered to be indirect ATP competitive inhibitors that can interact with additional amino acid residues in the binding site. This interaction allows for the potential enhancement of selectivity of small molecule inhibitors towards the VEGFR-2 protein [17-19]. This study focused on utilizing a variety of advanced computer-aided methodologies and approaches in drug design and discovery. These methods included ligand- and structure-based virtual screening, binding free energy estimates, and RMSD (Root Mean Square Deviation) analysis. The objective is to discover new and promising small compounds that bind to VEGFR-2 and have the ability to inhibit angiogenesis. A total of five indole derivatives were designed, based on the results of the docking investigation. These compounds could be regarded as prominent VEGFR-2 Inhibitors

Materials and Methods

Designing of the new derivates structures

ChemDraw 19 was used to design the new derivate structures (Figure: 1), which includes the designed new derivatives

Docking process method [20-23]

The Auto Dock Vina program was employed to dock the intended derivatives to the ATP binding site of VEGFR-2. The crystal structure of VEGFR-2, the Juxta-membrane and Kinase Domains, in Complex with Sorafenib (PDB code: 4ASD), was obtained from the RCSB Protein Data Bank and used to generate the binding sites. The targeted proteins were synthesized by supplementing deficient amino acids, eliminating water molecules, correcting unoccupied valence atoms, and optimizing the protein peptide energy utilizing Chemistry at Harvard Macromolecular Mechanics (CHARMM) force fields. Essential protein amino acids were meticulously selected and arranged for the purpose of screening. The tested compounds were obtained by creating their 2D structures using Chem-Bio Draw Ultra 17.0. Subsequently, these structures were stored in the SDF (Structure Data file) format. The ligands that were evaluated underwent protonation and their energy was reduced using the Merck Molecular Force Field (MMFF94) with a root mean square deviation (RMSD) of 0.1 kcal/mol. Molecular docking was performed utilizing docking procedures, where the target pocket was held in a fixed position while the ligands were allowed to move freely. The reduced structures that had been stored were utilized for this purpose. During the refining process, each molecule was allowed to establish twenty unique protein interaction sites. The docking process was used to score the best-fit postures with the VEGFR-2 active site based on their affinity interaction energy. The 3D-orientation was created using the visualizer of Discovery Studio Software 2016.

Validation of the molecular docking method

The reference ligand, Sorafenib, was utilized and docked into the VEGFR-2 ATP binding site, and the reliability and reproducibility of the recommended docking algorithm were assessed utilizing the RMSD. Upon docking reference ligand, Sorafenib, onto VEGFR-2 ATP binding site, it displayed an RMSD value of 1.69 Å, which falls below the permitted range of approximately 2.0 Å, suggesting that the algorithm applied for comparison with the crystallographic structure is valid [24]

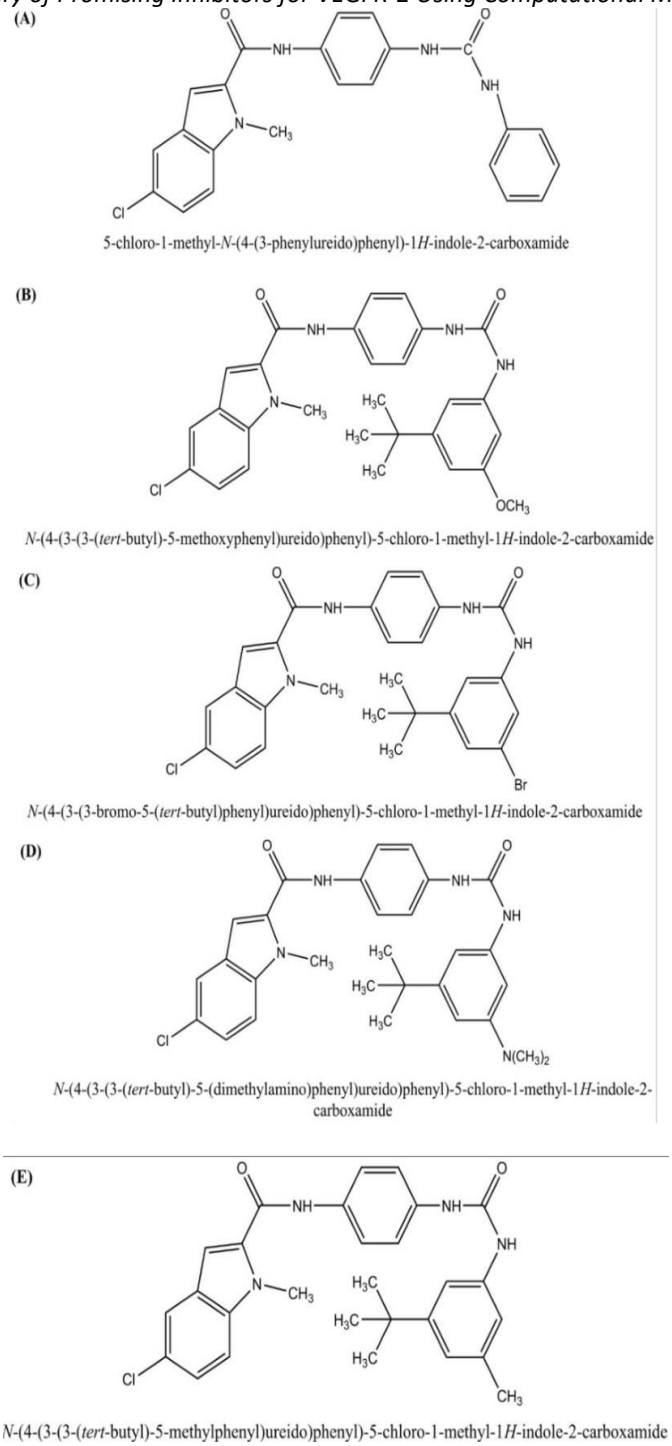


Figure 1: The chemical structures and names of the newly designed derivatives. (A) Derivative I, (B) Derivative II, (C) Derivative III, (D) Derivative IV, (E) Derivative V

ADMET Analyses [25]

Estimation of ADMET (absorption, distribution, metabolism, extraction, and toxicity) features is crucial for realizing the potential pharmacokinetic and safety profiles of the newly obtained candidates. By using established protocols, ADMET parameters of the newly designed (**I-V**) derivatives were studied, and Sorafenib was employed as a reference. These parameters included blood-brain barrier (BBB) permeability, solubility, absorption, hepatotoxicity, CYP2D6 prediction, and Plasma Protein Binding (PPB)**.**

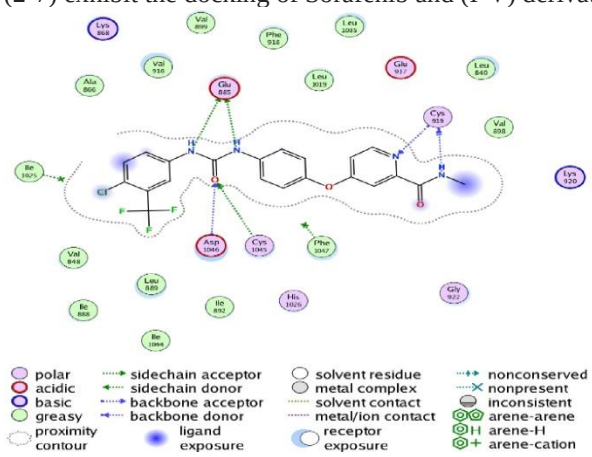
Results and Discussions

The docking scores (ΔG) and binding interactions of the reference ligand, Sorafenib, and the five newly designed indole derivatives against VEGFR-2 ATP binding site are illustrated in Table (1).

Table 1: The docking scores (ΔG) and binding interactions of the reference ligand, Sorafenib, and the five newly designed indole derivatives against VEGFR-2 ATP binding site, PDB code (4ASD)

Ligand	Energy of Binding (Kcal/mol)	Number of bindings with amino acid residues	RMSD (A^0)
Sorafenib	-9.07	2Glu 885 (3.68 A^0), Cys919 (2.97 A^0), Cys1045 (3.89 A^0), Asp1046 (2.76 A^0)	1.69
I	-9.17	2Glu 885 (2.32 A^0), Asp1046(2.26 A^0), Leu840 (3.12 A^0)	1.76
II	-10.53	2Glu 885 (2.31 A^0), Asp1046(2.22 A^0), Leu840 (3.14 A^0)	1.47
III	-9.85	2Glu 885 (2.29 A^0), Asp1046(2.24 A^0), Cys1045 (2.94 A^0), Leu840 (3.14 A^0)	1.49
IV	-10.12	2Glu 885 (2.31 A^0), Asp1046(2.22 A^0), Leu840 (3.31 A^0)	1.68
V	-9.68	2Glu 885 (2.34 A^0), Asp1046(2.19 A^0), Leu840 (3.26 A^0)	1.59

Figures (2-7) exhibit the docking of Sorafenib and (I-V) derivatives, respectively with VEGFR-2



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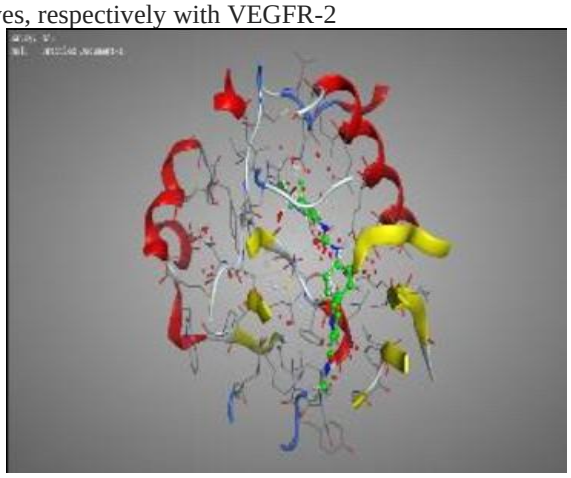

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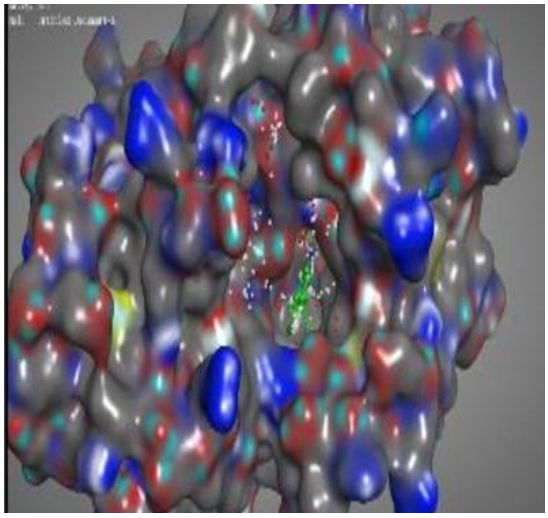

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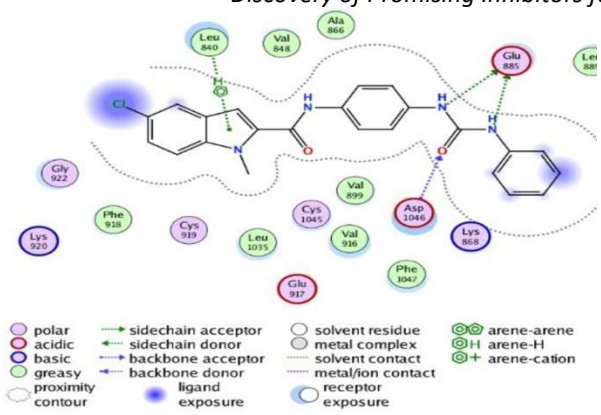
Figure 2: Exhibits docking of Sorafenib with VEGFR-2, Docking of Sorafenib with VEGFR-2, (A) 2D structure of binding Sorafenib with active site of VEGFR-2, (B) 3D structure of binding Sorafenib with active site of VEGFR2, (C) The whole protein picture shows the binding of Sorafenib with VEGFR-2



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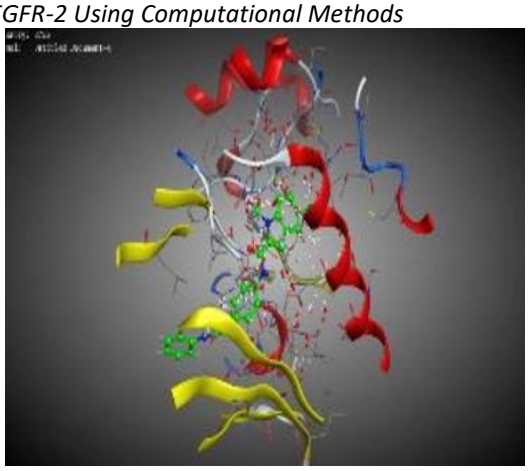

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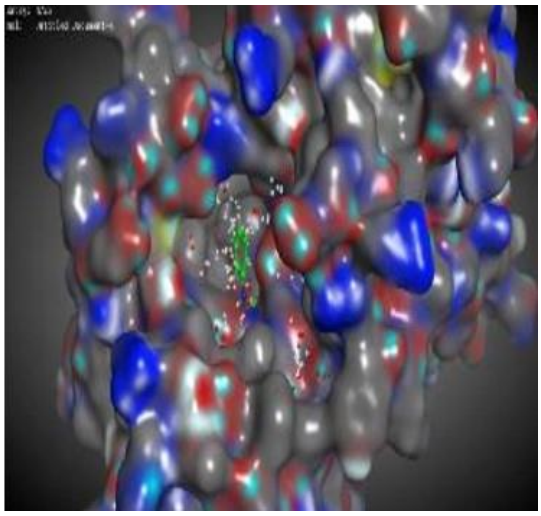

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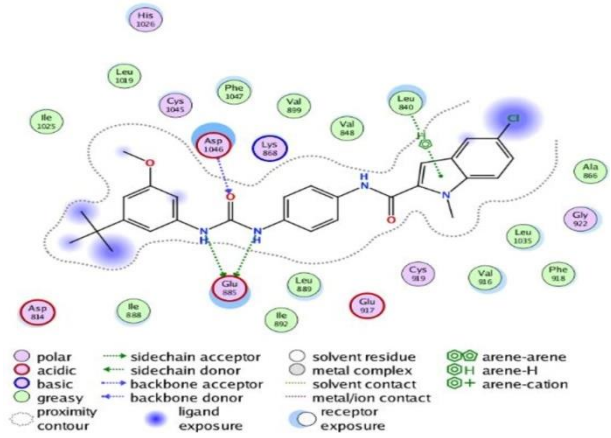
Figure (3) exhibits docking of the derivative **I** with VEGFR-2, Docking of compound **I** with VEGFR-2, (A) 2D structure of binding compound **I** with active site of VEGFR-2, (B) 3D structure of binding compound **I** with active site of VEGFR2, (C) The whole protein picture shows the binding of compound **I** with VEGFR-2



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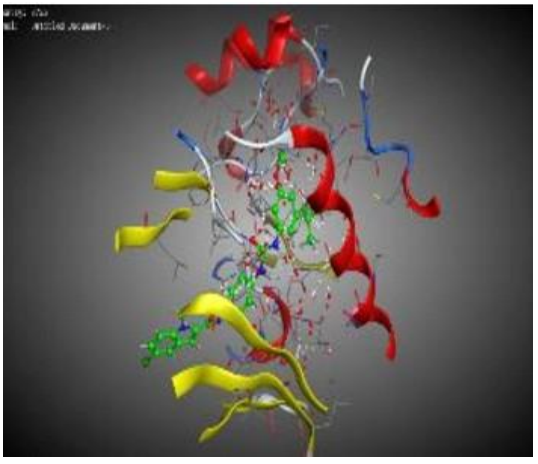

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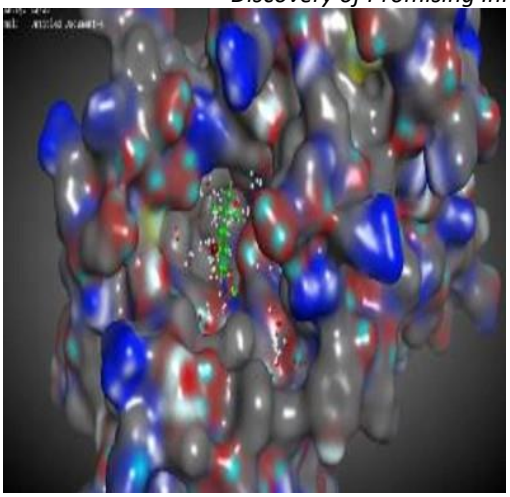

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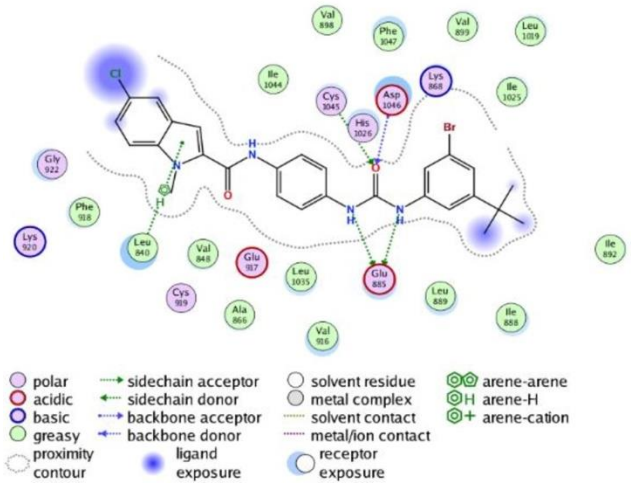
Figure (4) exhibits docking of the derivative **II** with VEGFR-2, Docking of compound **II** with VEGFR2-, (A) 2D structure of binding compound **II** with active site of VEGFR-2, (B) 3D structure of binding compound **II** with active site of VEGFR2, (C) The whole protein picture shows the binding of compound **II** with VEGFR-2



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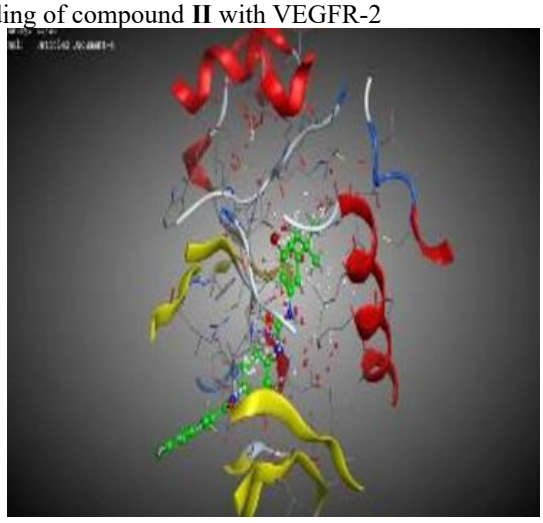

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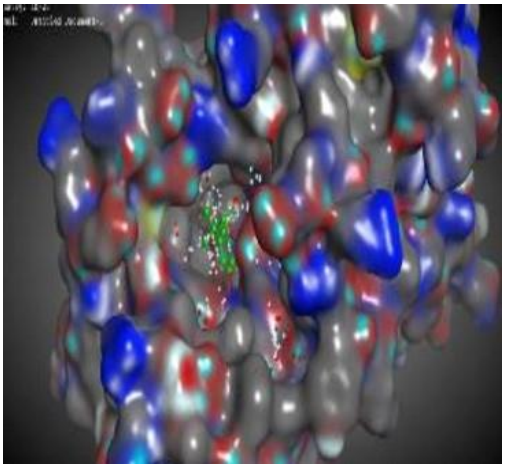

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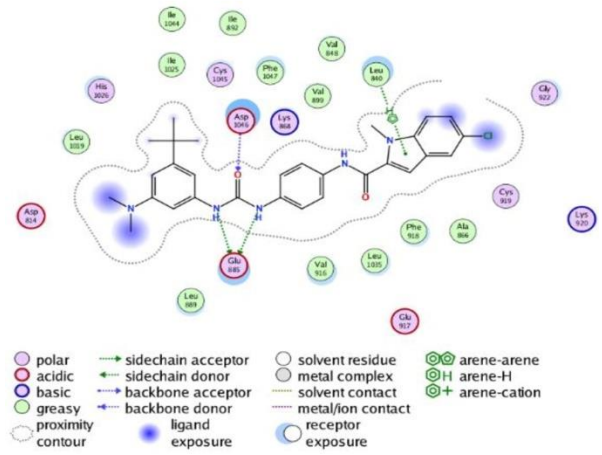
Figure (5) exhibits docking of the derivative **III** with VEGFR-2, Docking of compound **III** with VEGFR2, (A) 2D structure of binding compound **III** with active site of VEGFR-2, (B) 3D structure of binding compound **III** with active site of VEGFR2, (C) The whole protein picture shows the binding of compound **III** with VEGFR-2



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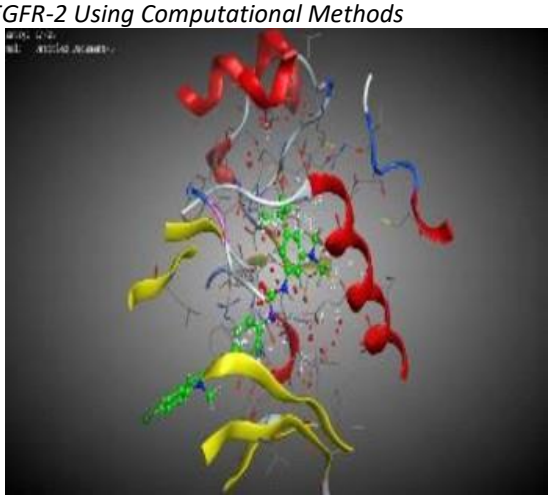

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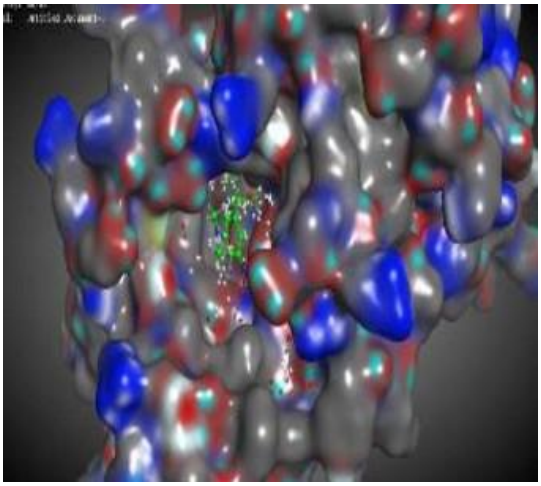
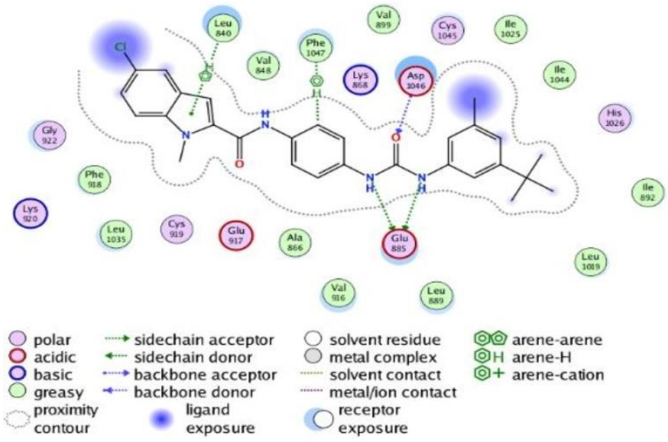


C

Figure (6) exhibits docking of the derivative **IV** with VEGFR-2, Docking of compound **IV** with VEGFR-2, (A) 2D structure of binding compound **IV** with active site of VEGFR-2, (B) 3D structure of binding compound **IV** with active site of VEGFR2, (C) The whole protein picture shows the binding of compound **IV** with VEGFR-2



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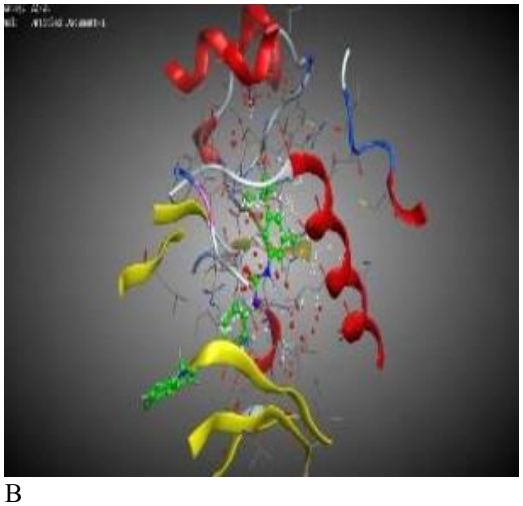

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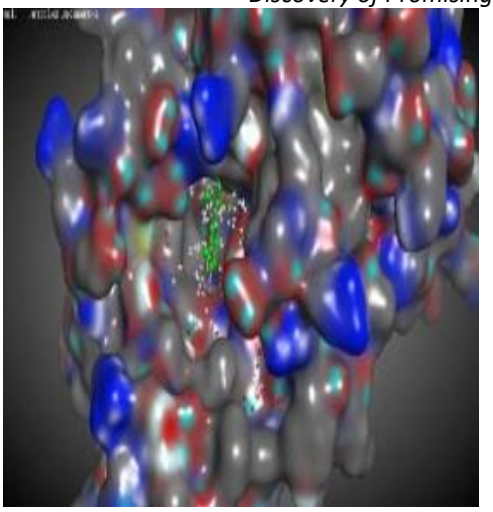

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Figure (7) exhibits docking of the derivative **V** with VEGFR-2, Docking of compound **V** with VEGFR-2, (A) 2D structure of binding compound **V** with active site of VEGFR-2, (B) 3D structure of binding compound **V** with active site of VEGFR2, (C) The whole protein picture shows the binding of compound **V** with VEGFR-2

The reference ligand, Sorafenib, and the new derivatives were bounded to Asp1046 and Glu885 via H-bonds. Cys1045 is of a higher importance for hydrophobic interactions with the small molecules. Results of docking studies indicated that the new derivatives bound higher tightly to the active pocket than the reference ligand, Sorafenib, producing highest docking scores (ΔG). The reference ligand, Sorafenib, and the new derivatives were bounded to approximately same amino acids resides. The new designed derivatives provide lower distance (Aº) with amine acids residues than the reference ligand, Sorafenib, that leading to the higher tightly binding with target. The new designed derivatives are also bounded to the Leu840, leading to the better inhibition of VEGFR-2, may be due to inhibition of ATP binding and allosteric site. Compound II showed highest fitting and affinity, (ΔG = -10.53 Kcal/mol) due to smallest RMSD (1.47 Aº). The results of ADMET analyses of the newly designed derivatives and Sorafenib are illustrated in Table (2).

Table 2: The results of ADMET analyses of the newly designed derivatives and Sorafenib

Compound	BBB level	Solubility level	Absorption level	Hepatotoxicity	Clearance	CYP2D6 Inhibition	PPB prediction
I	moderate	low	high	low	moderate	low	high
II	high	low	low	low	moderate	moderate	high
III	moderate	low	low	low	low	moderate	high
IV	high	low	low	moderate	moderate	low	high
V	high	low	low	low	low	moderate	high
Sorafenib	high	low	low	low	moderate	moderate	high

The BBB permeability level, which reveals the capacity of the derivative to pass through the BBB, exhibited variability between the derivatives. This analysis indicates the ability of agent to treat brain cancer, solubility a critical feature affecting the bioavailability and drug formulation. Absorption level indicates the ability of the derivatives to be absorbed from the gastrointestinal tract. The hepatotoxicity evaluation reveals the possibility of hepatic injury. Clearance analysis exhibits the elimination rate. CYP2D6 prediction exhibits the interaction with CYP2D6 enzyme. The study of PPB, which determines the capacity of a derivative for binding to plasma proteins, revealed that all derivatives and the reference ligand have the ability to bind the plasma proteins, affecting their distribution, metabolism, and elimination features.

Conclusion

VEGFR-2 becomes activated upon interaction with VEGF, which triggers a phosphorylation process that leads to an increase in both endothelial cell proliferation and motility. VEGFR2 facilitated the proliferation and invasion of cancer cells, and increased tumor growth. A new set of Indole-derived compounds have been identified as inhibitors of VEGFR-2, a protein involved in angiogenesis. Sorafenib was used as the reference ligand. All derivatives exhibited higher docking scores (ΔG) than Sorafenib. The reference ligand, Sorafenib, and the novel derivatives were bound to with the same amino acid residues. The new compounds are additionally attached to Leu840, resulting in improved inhibition of VEGFR-2. This may be attributed to the dual inhibition of the ATP binding and allosteric site. Compound II exhibited the highest fitting and affinity, with a ΔG value of -10.53 Kcal/mol, which can be attributed to its shortest RMSD of 1.47 Aº, the ADMET parameters of the newly designed (I-V) derivatives were investigated utilizing standard methods, with Sorafenib serving as a reference ligand.

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