SRI VENKATESWARA INTERNSHIP PROGRAM
FOR RESEARCH IN ACADEMICS
(SRI-VIPRA)

SRI-VIPRA

Project Report of 2025: SVP-2533

"Drug Designing: *In silico* identification of Potential Inhibitors by Protein Ligand Docking and Simulation"

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SRIVIPRA PROJECT 2024

Title : Drug Designing: *In silico* identification of Potential Inhibitors by Protein Ligand Docking and Simulation

Name of Mentor: Dr. Sumit Raj **Name of Department: Zoology** **Designation: Assistant Professor**	**Photo** 

List of students under the SRIVIPRA Project

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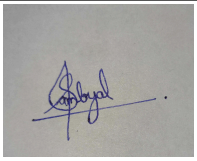
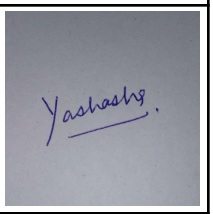
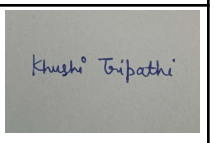
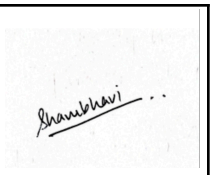
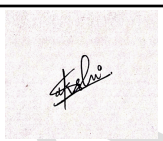
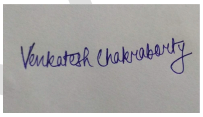
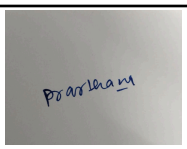
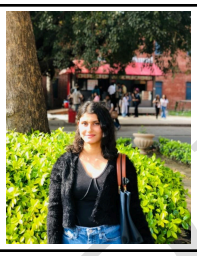
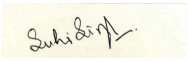
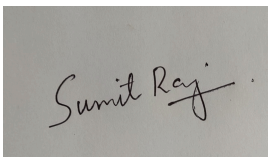
2		Vishakha Sambyal	2023047	B.Sc. (H) Zoology	
3		Yashasha Sharma	1123112	Bsc. Life Sciences (P)	
4		Khushi Tripathi	1123067	BSc.Life Sciences	

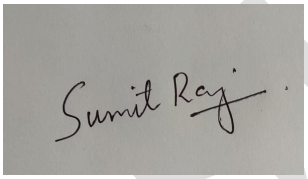
5		Shambhavi	1122148	B.Sc. Life Sciences	
6		Sakshi Sharma	1123085	B.Sc.(P) Life Science	
7		Venkatesh Chakraborty	1122106	B.Sc. Life Sciences	
8		Prarthana Rohatgi	1122122	Bsc Lifescience	
9		Suhani Singh	1123114	BSc. Prog Life Sciences	
10		Srijani Kaur	1323032	Biological sciences	



Signature of Mentor

Certificate of Originality

This is to certify that the aforementioned students from Sri Venkateswara College have participated in the summer project SVP-2533 titled "Drug Designing: *In silico* identification of Potential Inhibitors by Protein Ligand Docking and Simulation**"**.The participants have carried out the research project work under my guidance and supervision from 1st July, 2024 to 30th September 2025. The work carried out is original and carried out in an online/offline/hybrid mode.



Signature of Mentor

Acknowledgements

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ABSTRACT

Cancer remains a leading global health challenge, necessitating the development of novel therapeutic strategies. Inhibiting angiogenesis, a process crucial for tumour growth and metastasis mediated by Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), has emerged as a promising anticancer approach. This study employed in-silico methods to identify VEGFR2 inhibitors from this underexploited resource.

A library of 40 ligands, comprising 35 natural, 5 synthetic and 2 reference standard ligands, was subjected to molecular docking against VEGFR2. Tocotrienol and silicristin exhibited the highest binding affinities and were selected.

In silico ADME analysis indicated that tocotrienol possesses favourable drug-like properties, demonstrating good volume distribution and enhanced metabolic stability relative to sorafenib and silicristin. Collectively, our findings highlight tocotrienol as a promising lead candidate for anticancer drug development and further in vitro and in vivo validation.

INTRODUCTION

Cancer is still a significant global health crisis, posing a tremendous threat to human well-being. The incidence of cancer continues its rapid ascent, largely attributed to evolving lifestyles. Approximately 20 million new cancerous tumour cases and 9.7 million cancer-related deaths occurred in 2022, and are still rising. By the end of 2050, cancer cases will increase by 77% (Bizuayehu et al.,2022). While conventional treatments such as surgery, chemotherapy and radiotherapy have demonstrated efficacy, there is an urgent need for improved therapeutic strategies that minimise adverse effects on healthy tissues. One such effective treatment is targeted drug therapy. However, despite the effectiveness of many synthetic drugs, concerns regarding long-term complications and side effects persist (Pucci et al.,2019). The development of pharmaceuticals from natural sources offers the potential to mitigate these risks and reduce drug development costs, thereby increasing accessibility for patients.

The Tumour Microenvironment (TME) plays a critical role in the cell signalling, proliferation and angiogenesis of the blood vessels, significantly influencing tumour progression and metastasis. The characteristics of the TME vary across different organs, but the aberrant vascular development within this microenvironment is a common feature. Key regulatory proteins within the TME include Tyrosine Kinases (TKs). While the normal functioning of TKs is tightly regulated, mutations or dysregulation in these proteins can drive oncogenesis, particularly in solid tumours. TKs are pivotal for cancer cell survival, proliferation, signalling, growth, and metabolism (Tan et al.,2018). These enzymes are broadly classified into receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (NRTKs). Among the approximately 52 RTKs and 32 NRTKs, several play crucial roles in cancer development. NRTKs are often proto-oncogenic, whereas RTKs are cell surface receptors involved in transducing signals from extracellular biochemical molecules. Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), a subclass of RTKs, is a key driver of carcinogenesis (Zhang & Li.,2023). Primarily involved in vascular development and permeability, VEGFR2 plays a critical role in tumour angiogenesis and has been implicated in the progression of various cancers, including breast, lung, glioblastoma, gastrointestinal, liver (hepatocellular), kidney (renal cell), ovarian, bladder cancer, osteosarcoma (Guo.et al,2010). Molecular docking and simulation are invaluable preclinical methods in drug discovery and development. They offer a cost-effective and safe methodology for computationally identifying potential inhibitors of specific therapeutic targets. Molecular docking allows for the screening of ligand binding conformations that may impede the function of carcinogenic receptors. Various computational algorithms are employed to explore the conformational flexibility of both receptors and ligands (Pinzi.,2019). Ligand conformations are evaluated for a given receptor, and the top-scoring ligands are prioritised based on their predicted binding affinity, indicative of their inhibitory potential.

REVIEW OF ARTICLES

Receptor Tyrosine Kinases

Receptor Tyrosine Kinases (RTKs) are crucial mediators of cell-cell communication and a wide array of physiological functions. Following activation by ligand binding, the intricate signalling pathways initiated by RTKs are typically downregulated by various cellular proteins. The RTK structure consists of an Extracellular ligand binding domain (ELBD), a single transmembrane helix that helps to align residues in the catalytic activity site, an intracellular region that contains a juxtamembrane region for kinase activity inhibition, a tyrosine kinase domain (TKD) for phosphorylation and a carboxyl terminal tail (Zhang & Li.,2023). RTKs are integral to several key signalling pathways, including the Mitogen-Activated Protein Kinase (MAPK) pathway, primarily regulating cell proliferation; the Phosphoinositide-3-Kinase (PI3K) pathway, implicated in cell survival, metastasis, and the recruitment of inflammatory factors; and the Phospholipase C gamma (PLCγ) pathway, which plays a role in signalling hormonal responses (Wei & Hui, 2010).

Normal physiological activation of RTK

The RTKs' activation is mediated by site-specific ligand binding on the receptor. These ligands can be growth factor ligands, ligands for specific signalling for initiation and differentiation. They attach themselves to the ELBD and initiate dimerisation of the receptor molecules. Dimerisation causes autophosphorylation of the TKD, which activates the RTK (Zhenfang, Christine.,2018). There are four types of dimerisation,

1. Dimer initiation by two ligand molecules without contact with ELBD
2. Dimer initiation by two receptor molecules
3. Homodimers of a ligand attach to two receptor molecules for dimer initiation
4. A combination of attachment of a bivalent ligand and direct receptor-receptor contacts.

The dimerisation activates the kinase activity of TKD. Before the activation of TKD, it will be in a cis-inhibition state with the help of the juxtamembrane region. This region interacts with the C terminal tail of the TKD, which maintains inhibition. The activation and the feedback inhibition of the RTK depict the normal functioning of the cell.

Oncogenic activation and functioning of RTK

Mutations in RTK genes alter protein function, leading to overproduction of RTKs or disruption of the inhibitory mechanisms, mainly when mutations occur in the juxtamembrane region. For example, mutations in cysteine residues can promote excessive dimerisation, resulting in abnormal autophosphorylation and increased cell proliferation, signalling and growth. However, mutations alone are not solely responsible for oncogenic activity; other factors include: (1) Overexpression and genomic amplification of RTKs, which amplify signalling and disrupt normal cellular growth. (2) Chromosomal

rearrangements, which produce fusion proteins that sequester inhibitory molecules, preventing their regulation of auto-phosphorylation. (3) Autocrine activation, where tumour cells autonomously produce growth-stimulating signals. (4) Duplication of tyrosine kinase domains, which further contributes to constitutive RTK activation (Saraon, Pathmanathan.,2021).

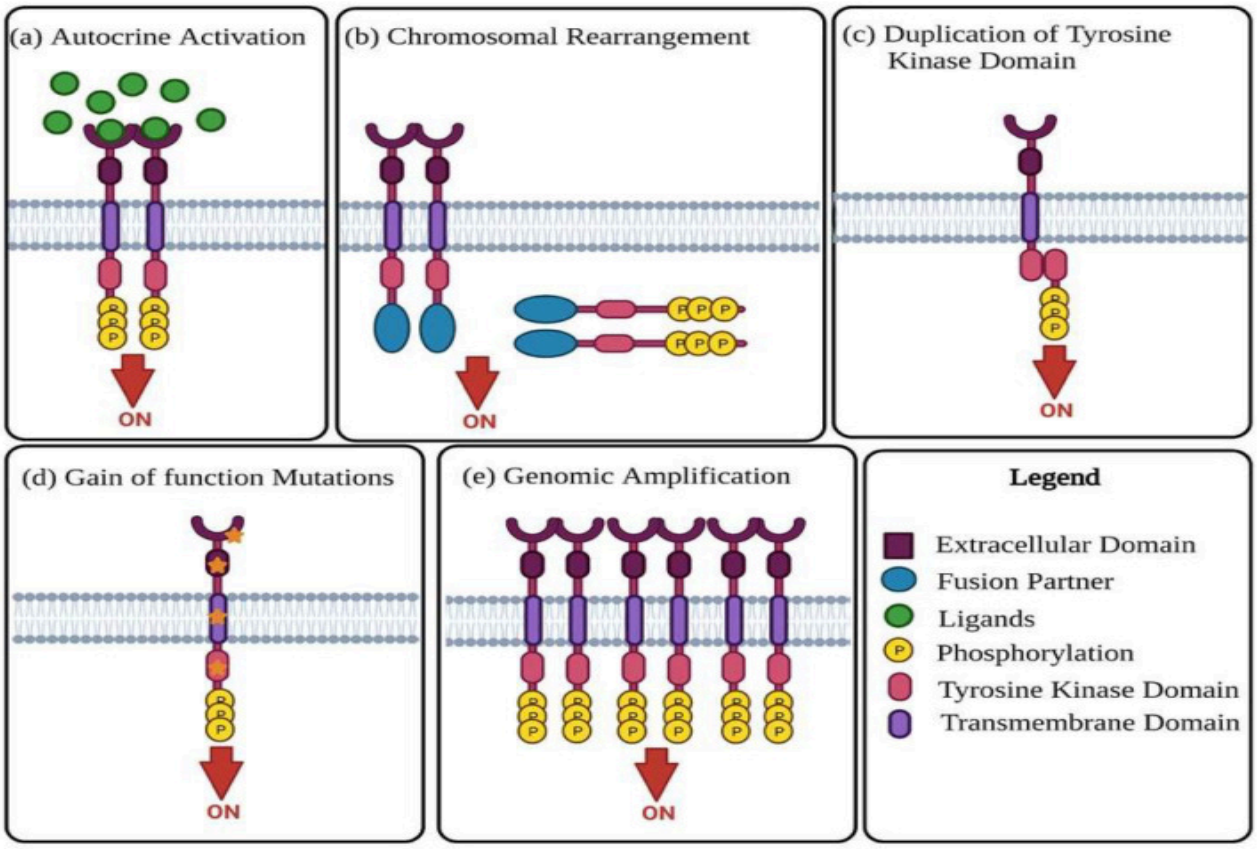

Figure- Mechanisms of oncogenic RTK activation. (a)Representation of autocrine activated RTK signalling. (b) Chromosomal rearrangement results in the creation of a hybrid fusion oncoprotein.(c) Duplication of the tyrosine kinase domain causes dimers in the absence of ligands. (d) Illustration of probable gain-of-function mutations. (e) Representation of RTK genomic amplification.
https://pubmed.ncbi.nlm.nih.gov/36140214/

These aberrant activation mechanisms disrupt normal protein expression, leading to uncontrolled cell proliferation, a hallmark of solid tumours. A critical consequence is the loss of feedback inhibition, which normally regulates RTK dimerisation and phosphorylation. While specific mutations may vary, most RTK subtypes share common pathways of oncogenic activation, resulting in sustained proliferative signalling and tumour progression.

VEGFR2

VEGFR2 is a subclass of RTK mainly involved in vasculogenesis and angiogenesis. This receptor helps in new blood vessel formation from previous blood vessels. VEGFR2 is involved in vascular development and permeability in solid tumour development (Maruyama.,2014).

Structure of VEGFR2

The VEGFR2 contains 5 main domains and 3 subdomains within the TKD domain. This protein consists of ligand-binding extracellular domain (ECD), transmembrane domain (TMD), juxtamembrane domain (JMD), TKD, which consists of ATP-binding domain (TKD1), kinase insert domain (KID), phosphotransferase domain (TKD2) and a carboxy-terminal domain (CTD). All these domains are included in 1356 amino acids (aa), including a single peptide of 19 aa. The molecular weight of a mature VEGFR2 is about 230kD. There are two other forms of VEGFR2, which include a non-glycosylated form with 150kD of MW and an intermediate molecule with 200kD MW (Wang, Bove.,2020).

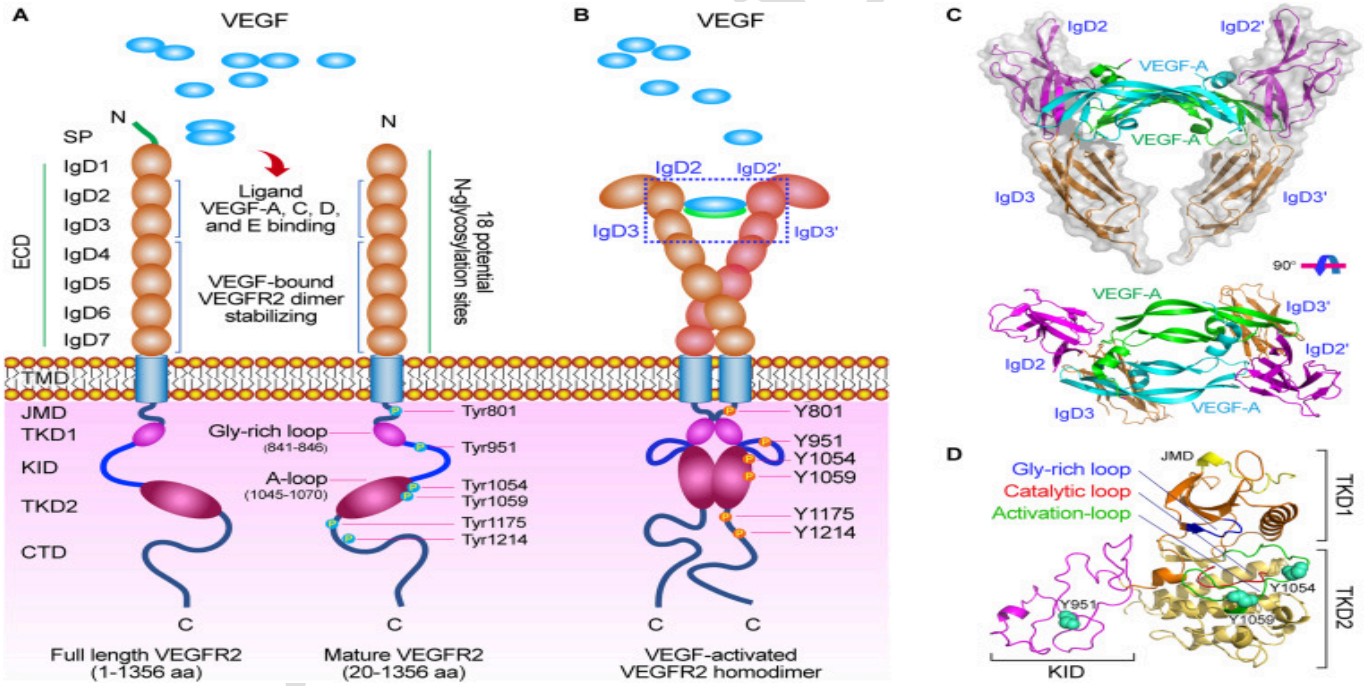

Figure 2.3 : The molecular structure of VEGFR-2.(A) VEGFR-2 is composed of an extracellular domain (ECD) including seven Ig-like subdomains, a TMD, a JMD, a TKD and CTD. (B) VEGF- activated VEGFR-2 homodimer. (C) Molecular structure of VEGF-A binding to IgD2 and IgD3 of VEGFR-2 (PDB ID: 3V2A)(D) Molecular structure of TKD of VEGFR-2 including TKD1, KID, and TKD2 (C-Lobe)..https://pmc.ncbi.nlm.nih.gov/articles/PMC7701214/

The Critical Role of VEGFR2 in Angiogenesis

VEGFR2 serves as the primary mediator of angiogenesis by regulating endothelial cell survival, proliferation, signalling, and migration. This receptor tyrosine kinase promotes the formation of organ-specific capillaries from pre-existing vasculature through multiple coordinated pathways. For endothelial cell survival, VEGFR2 initiates the TSAd-Src-PI3K-PKB/AKT signalling cascade. Activation of PI3K generates secondary messengers PIP3 and PIP2, which stimulate PKB/AKT to phosphorylate and inhibit pro-apoptotic factors Bcl-2-associated death promoter (BAD) and caspase 9, thereby preventing programmed cell death (Alberts et al.,2002).

In terms of proliferation, VEGFR2 activates a multi-step pathway beginning with PLCγ phosphorylation. The activated PLCγ hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). DAG subsequently triggers the PKC- Raf-MEK-ERK cascade, where ERK translocates to the nucleus to initiate DNA synthesis and cellular proliferation (Wang & Bove, 2020). For cellular migration, VEGFR2 coordinates signals through SHB, PI3K and NCK pathways. The SHB pathway is particularly crucial for cytoskeletal reorganisation and directional movement. Additionally, VEGF-activated VEGFR2 stimulates nitric oxide (NO) production, which serves dual functions as a potent vasodilator and regulator of endothelial barrier integrity (Fukumura & Gohongi, 2001).

Computational Approaches in Early-Stage Drug Discovery

The foundation of modern drug development begins with in-silico analysis, where molecular docking and simulation techniques play a pivotal role in identifying potential therapeutic compounds. Molecular docking serves as a powerful computational tool that predicts how small molecules interact with target proteins. This process evaluates the binding conformation of ligands within receptor sites, providing crucial insights into their potential to disrupt pathogenic protein functions (Pinzi & Rastelli.,2019). The initial phase of computer-aided drug design (CADD) involves two fundamental computational steps: searching and scoring. In the searching step, the ligand molecule systematically explores potential binding sites within the receptor's active pocket (Yu et al.,2017), generating multiple possible binding conformations through sophisticated sampling algorithms. Following this exploration, each identified binding pose undergoes evaluation in the scoring step, where specialised scoring functions calculate and assign binding affinity estimates. The process concludes by ranking all generated conformations based on their calculated scores, ultimately selecting the highest-ranking pose that demonstrates the most favourable binding characteristics for further drug development consideration. This sequential approach of conformational sampling followed by energetic evaluation forms the cornerstone of modern virtual screening methodologies in pharmaceutical research (Maia et al.,2020).

To quantify binding strength, researchers employ various scoring functions that assess protein-ligand interactions. Force-field-based functions calculate physical interactions like electrostatic forces and hydrogen bonding, while empirical functions derive binding affinities from regression models of known compounds. Knowledge-based functions utilise statistical analysis of structural databases, and consensus scoring combines multiple approaches for enhanced reliability (Kitchen et al., 2004). These computational tools collectively form a robust framework for virtual screening, significantly accelerating the drug discovery process by identifying the most promising candidates before costly laboratory testing begins.

MATERIALS AND METHODS

Receptor selection

VEGFR is responsible for blood vessel development, and targeting this protein reduces cell proliferation and cell signalling, leading to the retardment of vascular development in TME. From the information retrieved from the literature, the best VEGFR2 structure was selected. The selected target VEGFR2 is activated by the VEGF A at the Extracellular ligand-binding domain. The crystal structure of VEGFR2 Juxtamembrane and Kinase Domains (PDB ID:4ASD) was downloaded from RCSB-PDB (https://www.rcsb.org/) in PDB format. This structure was experimentally determined using X-ray diffraction, and it had a good resolution of 2.03 Å (McTigue et al.,2012).

Receptor preparation

Receptor preparation is done to remove the atoms which are not in the core structure of the protein. The receptor 4ASD was prepared for screening by removing the heteroatoms and all the connector molecules from the PDB file format. This edited molecule is converted to pdbqt format using Open Babel 3.0.0 through a Linux terminal. The hydrogen atoms and the Gasteiger partial charges were added during the conversion to the pdbqt file format. The pdb format possesses information about atoms and their coordinates, but pdbqt includes all the information of pdb along with charges and torsion angles required for assigning binding affinity.

```
obabel 4ASD.pdb -O 4ASD.pdbqt -xr -p 7.4 --partialcharge gasteiger
```

The -xr removes the HOH molecules and treats the receptor as a rigid molecule, -p 7.4 adjusts the protonation state at 7.4 pH and addition of Gasteiger partial charges. The pdbqt format is used for the screening process.

Preparation of ligands

The preparation of ligands is necessary as the ligands downloaded from PubChem do not possess charges and torsion angles. The electrostatic interactions and torsion determine the binding affinity during docking. The ligand molecules in the SDF format were converted to pdbqt format. Hydrogen atoms and Gasteiger partial charges were added to ligands during the conversion.

```
obabel *.sdf -O *.pdbqt --partialcharge gasteiger --add-hydrogen
```

Screening of ligand molecules

Screening of the ligands is the initial step for docking. Screening was performed to quickly evaluate a large set of compounds and identify those with favourable predicted binding

affinities. Out of 42 ligand molecules, the ligands with the best binding affinity were screened out. The screening was done through AutoDock Vina 1.1.2, and a shell script was written, which included the details of the config file. A config file is used to specify the parameters required for screening. The 4ASD grid box centre and box size parameters were retrieved from blind docking through DockThor (https://dockthor.lncc.br/v2/).The grid box centre specifies the X, Y, Z coordinates to find the binding sites, and the grid box size defines the 3D region around the centre that the ligand is allowed to explore.

Docking of ligand molecules

In the docking of ligands, the top five ligands with the best binding affinity, along with the reference molecules, were selected and allowed for docking. Screening is a rapid process to filter large compound libraries based on predicted binding affinities, while docking involves detailed prediction of binding poses and interaction energies with the target protein. Docking was performed after screening to focus computational efforts on top candidates with potential activity (Meng et al., 2011). The docking was done through DockThor (https://dockthor.lncc.br/v2/). The prepared receptor molecule was uploaded in PDB format, and the best ligand molecules were converted to mol2 format using Open Babel and uploaded one by one in DT. The grid box parameters were retrieved by blind docking, and the prepared molecules were submitted to DockThor, and the results were retrieved. Blind docking was performed in DockThor to search the entire surface of the target protein without restricting the docking to a specific active site. This approach helps identify potential novel binding sites and ensures no important interaction regions are overlooked, especially when the exact binding pocket is uncertain

```
# Grid box parameters
CENTER_X= -22.4555
CENTER_Y= -1.176
CENTER_Z= -5.3145
SIZE_X=40
SIZE_Y=40
SIZE_Z=40
```

ADME Analysis

The absorption, distribution, metabolism and excretion (ADME) analysis for two compounds with the best binding affinity and a reference molecule was done. The Simplified Molecular Input Line Entry System (SMILES) of the compounds and reference molecule were retrieved from PubChem and uploaded to ADMETlab 3.0 (https://admetlab3.scbdd.com/), an integrated online platform for pharmacokinetic profiling. The absorption parameters, such as Human intestinal absorption (HIA), Caco-2 permeability and P-glycoprotein (P-gp) substrate/inhibitor status for absorption, were

analysed. The volume of distribution (VD), blood-brain barrier (BBB) permeability, and plasma protein binding (PPB) for distribution analysis, interactions with major cytochrome P450 (CYP) isoforms for metabolism and total clearance and renal OCT2 substrate prediction for excretion analysis were done (Vugmeyster.,2012). This evaluation was done to find out the drug likeliness of the potential inhibitors.

RESULTS

Ligand selection

The ligands were selected based on their medicinal properties. The ligands were selected from IMPPATT and literature, and SDF formats were downloaded from PubChem. The information about the list of ligands is given in the Table

S.No	Ligand	Organism	Source
1	Muscone	*Moschus moschiferus* (Male musk deer)	Jifu and Yi, 2004
2	Oridonin	*Isodon trichocarpus* (Blackflower Teacost)	Fujita et al., 1970
3	Orientin	*Agrostemma githago* (Corn cockle)	IMPPAT
4	Osthole	*Cnidium monnieri* (Monnier's snowparsley)	Lateif et al., 2018
5	Paeoniflorin	*Paeonia lactiflora* (Chinese peony)	Ma et al., 2020
6	Peonidin	*Paeonia emodi* (Himalayan peony)	Cooper, 2019
7	Phloretin	*Lawsonia inermis* (Henna)	IMPPAT
8	Phytosterols	Various plants	Li et al., 2020
9	Pinocembrin	*Pinus roxburghii* (Chir pine)	IMPPAT
10	Piperlongumine	*Piper longum* (Long pepper)	IMPPAT
11	Plumericin	*Plumeria rubra* (Frangipani)	IMPPAT
12	Panax notoginseng saponins	*Panax notoginseng* (Chinese ginseng)	Qu et al., 2020
13	Puerarin	*Pueraria montana* (Kudzu vine)	IMPPAT
14	Pulegone	*Acinos alpinus* (Alpine rock thyme)	IMPPAT
15	Quercetin	*Abies pindrow* (West Himalayan fir)	IMPPAT

16	Sappanone A	*Caesalpinia sappan* (Sappanwood)	Zhi Wang et al., 2023
17	Sappanone B	*Caesalpinia sappan* (Sappanwood)	IMPPAT
18	Saringosterol	*Cydonia oblonga* (Quince)	IMPPAT
19	Saxitoxin	Mussels, clams, oysters	Hurley et al., 2014
20	Scandoside	*Oldenlandia corymbosa*	IMPPAT
21	Schaftoside	*Desmodium styracifolium*	Lie et al., 2017
22	Scropolioside B	*Scrophularia dentata* (Figwort)	Tiantian et al., 2014
23	Scutellarin	*Clerodendrum indicum* (Blue glory)	IMPPAT
24	Sesamin	*Sesamum indicum* (Sesame)	Jose, 2005
25	Shikimic acid	*Illicium griffithii* (Himalayan star anise)	Borah et al., 2021
26	Shikonin	*Alkanna tinctoria* (Dyer's alkanet)	IMPPAT
27	Silibinin	*Silybum marianum* (Milk thistle)	IMPPAT
28	Silicristin	*Silybum marianum* (Milk thistle)	IMPPAT
29	Silymarin	*Silybum marianum* (Milk thistle)	IMPPAT
30	Sinensetin	*Ageratum conyzoides* (Billygoat weed)	IMPPAT
31	Sinomenine	*Sinomenium acutum* (Orientvine)	IMPPAT
32	Stachyose	*Butomus umbellatus* (Flowering rush)	IMPPAT
33	Taxifolin	*Cedrus deodara* (Himalayan cedar)	IMPPAT
34	Tocotrienol	*Cratoxylum cochinchinense* Cow (Yellow Wood)	IMPPAT

35	Usnic acid	*Chukrasia tabularis* (Chittagong wood)	IMPPAT
36	Top_1	Synthetic ligand	–
37	Top_2	Synthetic ligand	–
38	Top_3	Synthetic ligand	–
39	Top_4	Synthetic ligand	–
40	Top_5	Synthetic ligand	–
41	Anlotinib	Reference molecule	–
42	Sorafenib	Reference molecule	–

Screening of ligand molecules

AutoDock Vina was used for the screening of ligands. These phytochemicals were screened out, and a total of nine molecules were selected, including seven molecules with the best binding affinity and two reference molecules. Among the seven, a synthetic ligand designed based on anlotinib (Top_3), tocotrienol and silymarin topped the binding affinity score with -10.4, -9.9 and -9.9 . The reference sorafenib had a very high binding affinity of -11.6, greater than anlotinib. The results of AutoDock Vina are given in the Table below.

S.No	**Ligand**	**Binding Affinity (kcal/mol)**	**RMSD LB**	**RMSD UB**
1	Sorafenib (Reference)	-11.6	0	0
2	Top_3	-10.4	0	0
3	Silymarin	-9.9	0	0
4	Tocotrienol	-9.9	0	0
5	Sesamin	-9.4	0	0
6	Scutellarin	-9.3	0	0
7	Paeoniflorin	-9	0	0

8	Silicristin	-9	0	0
9	Silibinin	-8.9	0	0
10	Phloretin	-8.8	0	0
11	Pinocembrin	-8.7	0	0
12	Sappanone_A	-8.7	0	0

13	Top_5	-8.7	0	0
14	Anlotinib (Reference)	-8.6	0	0
15	Quercetin	-8.5	0	0
16	Saringosterol	-8.5	0	0
17	Usnic acid	-8.5	0	0
18	Top_4	-8.4	0	0
19	Orientin	-8.2	0	0
20	Piperlongumine	-8.2	0	0
21	Puerarin	-8.2	0	0
22	Sappanone_B	-8.2	0	0
23	Schaftoside	-8.2	0	0
24	Taxifolin	-8.2	0	0
25	Top_1	-8.2	0	0
26	Scropolioside_B	-8.1	0	0
27	Sinensetin	-8	0	0

28	Top_2	-8	0	0
29	Shikonin	-7.9	0	0
30	Stachyose	-7.9	0	0
31	Scandoside	-7.8	0	0
32	Phytosterols	-7.7	0	0
33	Oridonin	-7.6	0	0
34	Osthole	-7.6	0	0
35	Peonidin	-7.6	0	0
36	Plumericin	-7.5	0	0
37	Saxitoxin	-7.1	0	0
38	Muscone	-6.9	0	0
39	Pulegone	-6.9	0	0
40	Sinomenine	-6.9	0	0
41	Panax notoginseng saponins	-6.3	0	0
42	Shikimic_acid	-5.5	0	0

Molecular Docking

DockThor was used for molecular docking. The best seven and reference molecules were docked and the results were retrieved. Tocotrienol and silicristin had the highest binding affinity.The results from DockThor are given in the Table below.

S.No.	Compound	Affinity	Total Energy
1	Tocotrienol	-8.577	164.662

2	Anlotinib (Reference)	-8.421	24.238
3	Silicristin	-7.946	210.433
4	Scutellarin	-7.863	46.77
5	Sesamin	-7.671	121.45
6	Top_3	-7.644	63.365
7	Silymarin	-7.501	182.188
8	Sorafenib (Reference)	-7.18	28.368
9	Paeoniflorin	-7.111	3642.994

Visualisation

Visualisation is done through PyMol, VEGFR2 protein and the ligands sorafenib, tocotrienol and silicristin were visualised.

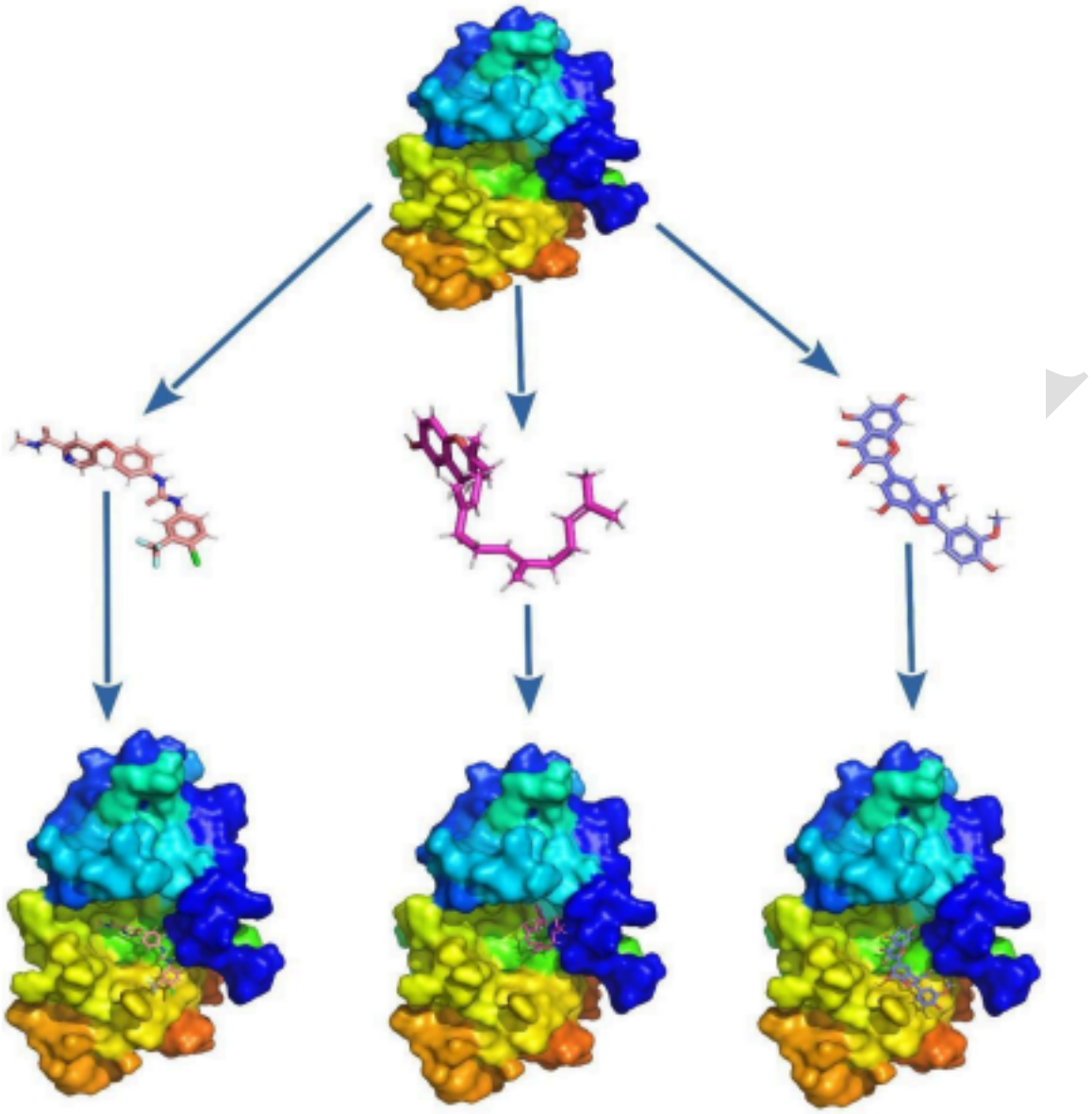

Figure 4.7: Holistic view of complex formation. VEGFR2 is represented in surface form with a pretty colour. The ligands such as sorafenib (pink), tocotrienol (magenta) and silicristin (purple) were also visualised

ADME Analysis

The ADME analysis and toxicity studies were done for the sorafenib, tocotrienol and silicristin with ADMETlab 3.0. These compounds were compared with the reference sorafenib. A total of 32 parameters within adsorption, distribution, metabolism, excretion and toxicity categories were evaluated, and the results were listed in the Table below.

Parameters	Sorafenib	Tocotrienol	Silicristin
Medicinal Chemistry			
Lipinski rule	0 violation	0 violation	0 violation
Pfizer rule	0 violation	1 violation	0 violation
GSK rule	1 violation	1 violation	1 violation
Golden triangle	0 violation	0 violation	0 violation
Absorption			
HIA	0.001	0.002	0.025
Caco2 permeability	-5.166	-4.712	-6.427
Pgp-inhibitor	0.968	0.999	0.509
Pgp-substrate	0.005	0	0.463
Distribution			
Volume of distribution	0.007	0.24	0.051
BBB	3	0.001	0
PPB (%)	99.326	97.328	93.157
Excretion			
Clplasma	4.595	8.412	6.45
T1/2	0.89	0.803	1.959
Toxicity			

Skin sensitivity	0.197	0.97	0.996
Carcinogenicity	0.114	0.167	0.405
Respiratory toxicity	0.611	0.913	0.745
Human hepatotoxicity	0.784	0.716	0.962
Drug-induced nephrotoxicity	0.973	0.262	0.883
Drug-induced neurotoxicity	0.677	0.408	0.36
Ototoxicity	0.549	0.464	0.645
Hematotoxicity	0.757	0.201	0.464
Genotoxicity	1	0.088	0.996
Metabolism			
CYP1A2 inhibitor	0.999	0.632	0
CYP1A2 substrate	0.524	0.043	0.441
CYP2C19 inhibitor	0.963	0.932	0.001
CYP2C19 substrate	0.001	0.996	1.001
CYP2C9 inhibitor	0.877	0.419	0.109
CYP2C9 substrate	0.015	0.996	0.062
CYP2D6 inhibitor	0	0.097	0
CYP2D6 substrate	0.002	0.999	0
CYP3A4 inhibitor	0.06	0.693	0.235

CYP3A4 substrate	0.999	0.006	0.943
CYP2B6 inhibitor	1	0.787	1
CYP2B6 substrate	0	0.001	0
CYP2C8 inhibitor	1	0.97	1
HLM Stability	0.022	0.998	0

DISCUSSION

Molecular docking, ADME, along simulations are essential computational tools in drug discovery, helping predict how potential drug molecules interact with target proteins involved in diseases like cancer. In this study, the VEGFR-2 protein, a key regulator of tumour angiogenesis, was selected as the target. Proper preparation of the receptor (adding hydrogens, assigning charges, and removing interfering heteroatoms) ensured accurate docking results. The ligands, including phytochemicals from Himalayan medicinal plants (like tocotrienol, a vitamin E variant with known anti-cancer effects) and synthetic analogues of anlotinib

(a VEGFR-2 inhibitor), were screened for binding affinity. Tocotrienol and silicristin (a flavonoid from Silybum marianum with antioxidant properties) showed the strongest binding energies (−8.577 and −7.946 kcal/mol, respectively), suggesting they could effectively block VEGFR-2's active site and inhibit angiogenesis.

From a pharmacokinetic perspective, ADME profiling highlighted challenges: all compounds had low intestinal absorption due to poor permeability, likely because of their polar structures, and high plasma protein binding, which may reduce free drug availability. However, tocotrienol's high volume of distribution suggests it can penetrate tissues effectively, a crucial trait for targeting tumour microenvironments. Its stability in human liver microsomes (HLM) and rapid plasma clearance further support its potential as a drug candidate, though formulation adjustments (e.g., nano-encapsulation) may be needed to improve oral bioavailability. Toxicity profiling showed tocotrienol was safer than sorafenib (which inhibits CYP enzymes, risking drug-drug interactions) but had mild skin/respiratory sensitivities, while silicristin's higher toxicity may stem from reactive functional groups that could be modified.

Biologically, tocotrienols' anticancer effects align with their role as a ROS (Reactive Oxygen Species) scavenger and their ability to downregulate pro-angiogenic signals (e.g., VEGF). By binding VEGFR-2, it could starve tumours of blood supply while minimising side effects—unlike synthetic kinase inhibitors like sorafenib, which often cause hypertension and fatigue. Silicristin's moderate efficacy and toxicity suggest it could serve as a lead compound for structural optimisation.

CONCLUSION

This study computationally validates tocotrienol as a potent, natural VEGFR-2 inhibitor with favourable binding, stability, and safety profiles. Its multi-target potential (anti-angiogenic + antioxidant) and synergy with conventional therapies warrant further in vitro and in vivo studies to advance it as a candidate for anti-cancer drug development.

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