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A COMPUTATIONAL APPROACH FOR THE IDENTIFICATION OF FGFR2 INHIBITORS TARGETING CANCER BY MOLECULAR DOCKING AND *IN SILICO* ADME STUDIES

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ABSTRACT

Cancer remains one of the leading global health burdens, caused by uncontrolled cell proliferation and complex genetic alterations. Among the targets implicated in tumour progression, Fibroblast Growth Factor Receptor 2 (FGFR2) has emerged as a promising candidate due to its critical role in cell proliferation, differentiation, survival, and migration. Dysregulated FGFR2 signalling is associated with multiple cancer types, including breast, gastric, and lung cancers. The present study employed a computational approach to identify potential FGFR2 inhibitors using structure-based virtual screening and *in silico* ADME profiling. The crystal structure of FGFR2 was retrieved from protein data bank and active site prediction was carried out using CASTp. The predicted active site residues are LEU487, GLY488, GLY490, PHE492, GLY493, LYS517, GLU534, ALA567, SER568, LYS569, ASN571, GLU574, ARG579, ASP626, ASN631, ASP644. Docking was carried at the predicted active site of FGFR2 protein and binding analysis showed the residues LEU487, GLY488, LYS517, ASN571, GLU574, ASP626, ASN631of FGFR2 protein to have consistent interactions with the ligands. The ligand M1 showed highest docking score of -10.5 and docking scores of other ligands were in the range of -8.1 to -10.5. The docked ligands were further screened using SwissADME to eliminate potentially toxic molecules. All the top scoring molecules showed ADME properties within permissible ranges. These findings highlight the potential of the obtained compounds as promising candidates for FGFR2 targeted cancer therapy.

Keywords

Fibroblast Growth Factor Receptor 2, FGFR2 inhibitors, molecular docking, virtual screening, ADME studies, cancer therapy, structure-based drug design, computational drug discovery, SwissADME, PyRx

INTRODUCTION

Cancer is characterized as a disease in which certain cells proliferate out of control and spread to other bodily organs, a process known as metastasis. A more recent definition, states that cancer cells are prone to evolution by natural selection due to the genetic and epigenetic alterations that take place inside them. Cancer onset and treatment, results in emotional and psychological burden on individuals, patients may experience various physical and psychological changes. Common issues include fatigue, lymphedema, early menopause, cognitive deficits, neuropathy, and chronic pain [1].

Millions of people are affected by cancer every year, making it one of the leading causes of mortality globally. In 2024, 2 million new cancer cases and 6 million cancer deaths were projected to occur in the United States [2]. In India, the projected number of new cancer cases in India was 1 million with a crude incidence rate of 100.4 per

100,000 individuals. An estimated 12.8% increase in cancer incidence by 2025 is expected as compared to 2020. In terms of new cancer diagnoses, the most prevalent are: skin (non-melanoma) (1.20 million cases); stomach (1.09 million cases); colon and rectum (1.93 million cases); prostate (1.41 million cases); breast (2.26 million cases); and lung (2.21 million cases). Approximately 4 million youngsters are diagnosed with cancer each year. These statistics show the severity of the disease and the urgency to identify better and safe drugs to treat cancer [3-5].

Role of fibroblast growth factor receptor2 in cancer

Fibroblast growth factors (FGFs) and their receptors, FGFRs are responsible for various cellular functions such as cell proliferation, survival, migration and differentiation. Due to their diverse cellular functions, the mechanisms by which they cause cancer are difficult to understand and thus play a critical role in the development of cancer [6,7]. FGFRs belong to the family receptor tyrosine kinases which consists of four transmembrane receptors, FGFR 1- 4. FGF ligands interact with these four receptors, FGFR 1 – 4 and induce dimerization, stimulate their kinase activity and also activate downstream signalling pathways. The downstream pathways include the extracellular signal-regulated kinase (ERK), mitogen activated protein kinase (MAPK) pathway [8-10].

Among the many molecular factors driving cancer, fibroblast growth factor (FGF) proteins play an important role in tumorigenesis, angiogenesis, and metastasis. A transmembrane receptor tyrosine kinase essential for coordinating cellular processes such as survival, differentiation, and proliferation is called fibroblast growth factor receptor 2 (FGFR2). For tissue balance and cellular functions to be maintained, FGFR2 signaling must be properly regulated. Genetic changes or aberrant expression can cause dysregulated FGFR2 signaling, which can lead to a number of illnesses, most notably cancer. FGFR signal transduction triggers the activation of several cascade pathways, such as signal transducer and activator of transcription proteins (STAT), PI3K-PKB, RAS-MAPK, and phospholipase C gamma (PLCγ). FGFRs' aberrant signaling is linked to several cancer forms, such as colorectal, gastric, prostate, bladder, endometrial, breast, and lung cancers [11,12].

FGFR2 is overexpressed in colorectal cancer, and it is associated with poor survival and lymph node metastases. Through the JAK/STAT3 pathway, it increases PD-L1 expression, which aids in immune evasion by triggering T cell death. FGFR2 amplification is frequently seen in gastric cancer, which promotes cell survival and proliferation via the EGFR family signaling pathways. By upregulating thrombospondin-1 through the PI3K/Akt/mTOR pathway, FGFR2 signaling promotes invasion and migration [13-15].

Prostate cancer epithelial cells overexpress FGFR2, which is associated with poor differentiation and may facilitate stromal cell paracrine stimulation, which could accelerate the growth of the cancer. FGFR2 plays a role in the genesis and progression of cutaneous squamous cell carcinoma, and blocking it is being investigated as a potential treatment approach. FGFR2 is linked to a higher risk of breast cancer and interacts with HER2 to promote tumor growth and decrease trastuzumab sensitivity. These studies show the implication of FGFR2 in various cancers and the necessity of targeting FGFR2 protein as a potential target to treat cancer [16,17]. Table 1 shows the different types of cancer associated in FGFR2 dysregulation. In the present study we have carried out structure-based drug design study to identify to potential lead molecules for cancer targeting FGFR2.

Table 1: Cancers associated with FGFR2 dysregulation

Cancer type	Role of FGFR2
Gastric cancer	FGFR2 Amplification
Breast cancer	FGFR2 Over expression
Lung cancer	FGFR2 Mutations
Endometrial cancer	FGFR2 Mutations
Cholangiocarcinoma	FGFR2 Fusion proteins

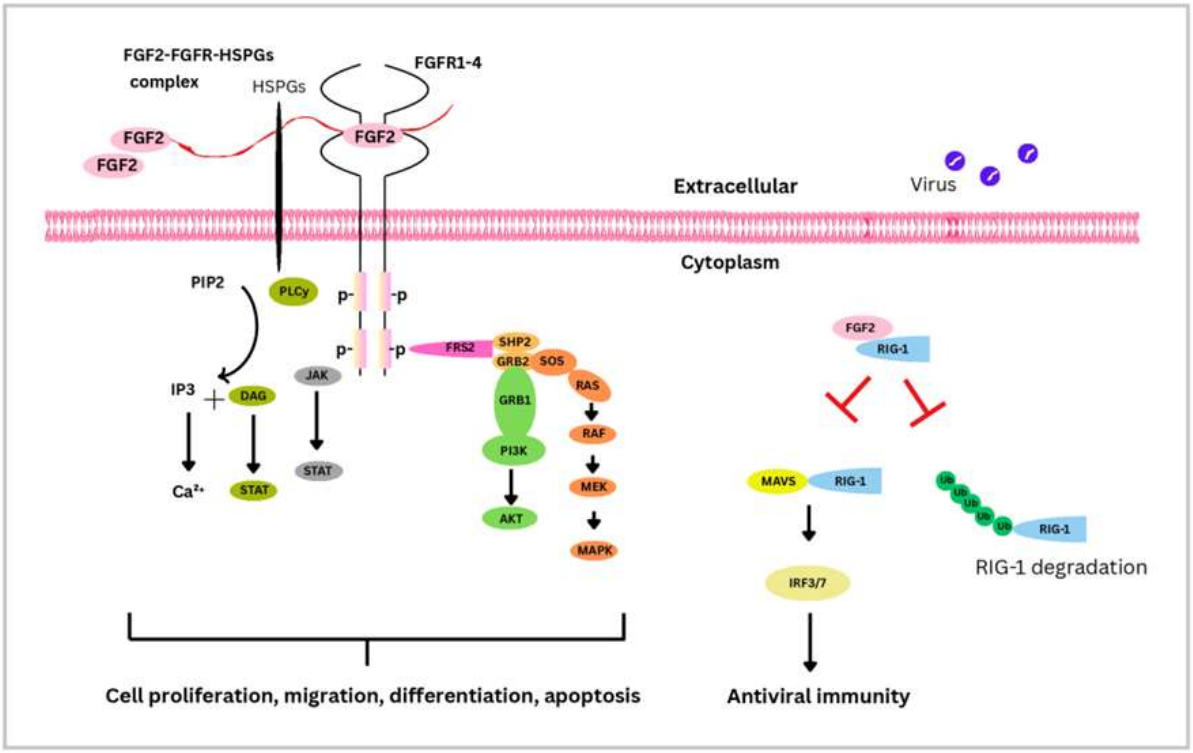


Fig1: FGFR2 Signaling pathway [18]

MATERIALS AND METHODS

Software

Webserver for downloading protein: Protein Data Bank (PDB)

Webserver used for active site prediction: CASTp

Docking: AutoDock Vina module in PyRx

Docking Analysis: BIOVIA Discovery studio

ADME Studies: SwissADME

Structure-based drug design of FGFR2 inhibitors

Structure-based drug design helps in identifying promising inhibitors when the structure of the protein is available [19]. In the present study the three-dimensional structure of the FGFR2 protein (Fibroblast Growth Factor Receptor 2) ie., pdbID 2PVF, was retrieved from the RCSB Protein Data Bank [20] (PDB ID: 2PVF) which corresponds to the crystal structure of the kinase domain of human FGFR2, resolved by X-ray diffraction with resolution of 2.30 Å [21]. The structure of FGFR2 protein, pdbID 2PVF was checked for any missing residues and chain A was used for the study.

Active site identification of FGFR2 protein

Identifying active sites in proteins is essential for understanding their biological roles and plays a key part in drug discovery. CASTp (Computed Atlas of Surface Topography of Proteins) is a computational tool that detects and analyzes the structural and topological features of protein molecules, such as surface cavities and pockets, which frequently correspond to the functional or active regions in the protein. CASTp identifies and quantifies surface pockets, internal cavities, and channels within protein structures. It predicts precise measurements of the volume and surface area of these structural features, aiding in the identification of possible active sites [22].

Retrieval of Ligand Structures

In the present study, the compounds for virtual screening were downloaded from PubChem database [23]. The molecules having anti-cancer activity were downloaded in SDF format and subsequently converted to PDBQT

format using Open Babel for further molecular docking studies [24]. All the ligand structures were subjected to energy minimization to ensure optimal geometry and stability prior to docking.

Virtual Screening

Virtual screening is an efficient and economical technique used to search compound databases for potential lead molecules [25]. It enables extensive screening of small molecule chemical libraries to identify compounds that fit well with targets or proteins in structure-based drug design.

Ligand Optimization

Optimization of ligand structures prior to docking is a critical step to ensure accurate binding predictions and improved docking scores. Ligands were imported into PyRx [26] using OpenBabel in Sybyl mol2 format and subjected to energy minimization using Universal Force Field (UFF). UFF is a versatile force field which can handle wide range of elements across the periodic table [27]. The optimization process involves adjusting torsional angles and molecular geometry to generate the most stable conformations of the ligands. The ligands were subjected to minimization for 200 steps of energy minimization using steepest descent method. After energy minimization, the optimized ligands were exported as pdbqt files, the required format for docking with AutoDock Vina in PyRx [28]. AutoDock Vina module employs RF-Score V2 scoring function, a machine learning-based approach to evaluate ligand binding affinity, allowing for the selection and prioritization of potential lead compounds based on docking scores [29].

In *silico* ADME prediction

Absorption, distribution, metabolism, and elimination (ADME) are vital factors that influence the clinical effectiveness of a drug candidate. Approximately half of all drug failures can be attributed to inadequate ADME characteristics. Significant progress has been made in computational approaches for predicting ADME properties, leading to the development of various tools capable of estimating multiple related parameters and descriptors. The *in silico* methods help minimize animal testing, lower research costs and time, and offer dependable predictions of potential toxicity. In the present study, SwissADME was used to evaluate the *in silico* ADME profiles of the ligands [30].

RESULTS AND DISCUSSION

Protein preparation

In the present study the three-dimensional structure of the FGFR2 (Fibroblast Growth Factor Receptor 2) protein was retrieved from the RCSB Protein Data Bank (PDB ID: 2PVF) which corresponds to the crystal structure of the kinase domain of human FGFR2, resolved by X-ray diffraction with a resolution of 2.30 Å. It has two chains A and B, the number of residues in chain A are 298. The water molecules were removed prior to docking protocol and checked for any stereochemical clashes. Additional chains were removed and chain A was retrieved for docking. The protein was subjected to energy minimisation and converted to pdbqt format to carry out docking in PyRx.

Active site identification

The FGFR2 protein, pdbID 2PVF was submitted to CASTp for the identification of active site residues. The active site residues predicted for FGFR2 protein from CASTp are given in Table 2 and fig 2 shows the graphical representation of the active site, where the active site residues are represented as red spheres and the protein is represented as grey cartoon. Fig 3 represents the sequence of 2PVF protein showing different active sites predicted by CASTp, different active site pockets are represented by different colour code for identification. In the present study, we have taken the pocket having the highest volume given in Table 2. The active site residues predicted by CASTp are LEU487, GLY488, GLY490, PHE492, GLY493, LYS517, GLU534, ALA567, SER568, LYS569, ASN571, GLU574, ARG579, ASP626, ASN631, ASP644. The predicted active site residues were used for grid generation during docking.

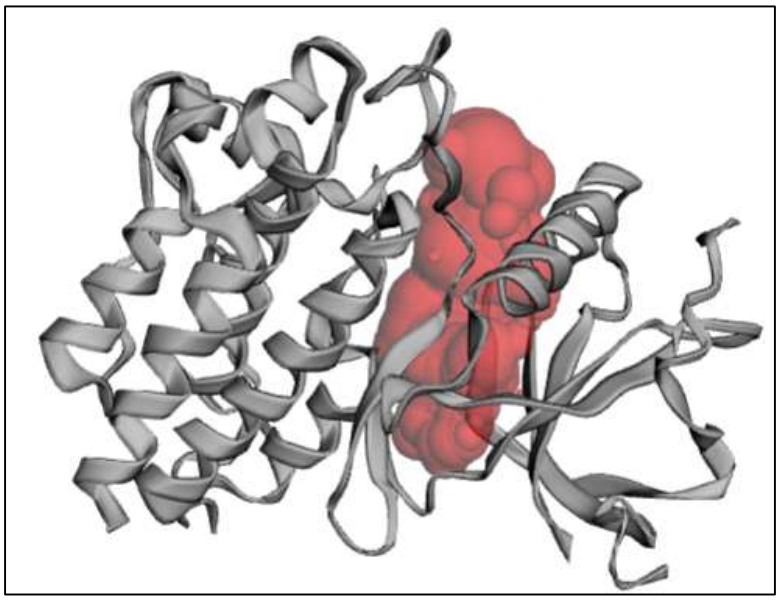


Fig2: Active site of FGFR2 protein. The protein is represented as grey colour cartoon model, and active sites residues are represented as red colour spheres

Table 2: Predicted active site pocket, area and volume for FGFR2 protein

PocID	Area (SA) Å2	Volume (SA) Å3
1	789.095	754.124

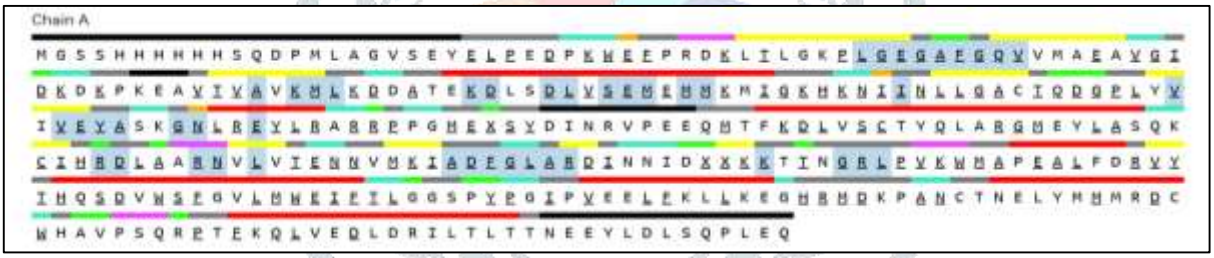


Fig 3: The active site residue pockets predicted by CASTp are represented by different colours

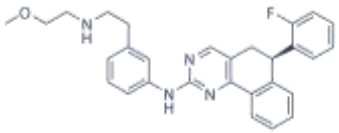
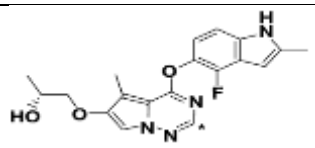
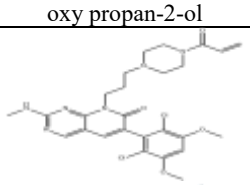
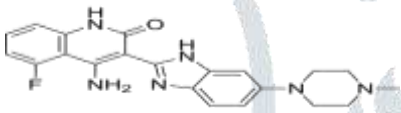
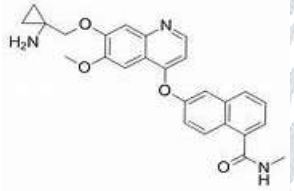
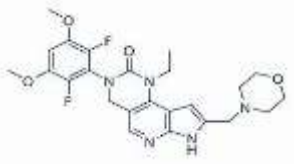
VIRTUAL SCREENING

Structure-based virtual screening was performed targeting the FGFR2 protein using the ligands from PubChem database, which comprises approximately 119 million unique chemical compounds. The compounds were then subjected to ligand preparation, including the generation of stereoisomers with defined chiralities. For each ligand, 7–8 conformers were generated to account for structural flexibility during docking. A total of 352 docked complexes were generated as output after virtual screening. The docking process involved evaluating the binding affinity of each ligand to the active site of the FGFR2 protein using a validated scoring function, *ie*., RF-V2 scoring function. The output included docking scores and predicted binding poses for all complexes.

To prioritize compounds with the highest potential for inhibitory activity, the docked complexes were ranked based on their docking scores, which reflects the predicted binding free energy. The selected complexes were subjected to visual inspection of binding poses using Biovia Discovery studio [31], focusing on the orientation, interactions within the binding pocket (e.g., hydrogen bonds, hydrophobic interactions), and potential key residues involved in ligand binding**.** Based on this ranking, the top 6 ligand–protein complexes are given in Table 3.

Table 3: Ligands showing Docking score with intermolecular interactions and bond distance in Angstroms

S.No.	Structure	Docking score	Intermolecular Interactions	Bond Distance(Å)

1.	 (6R)-6-(2-fluorophenyl)-N-[3-[2-(2-methoxyethyl amino)ethyl]phenyl]-5,6-dihydrobenzo[h]quina zolin-2-amine	-10.5	Hydrogen bonds M1:ARG579 M1:LEU487 M1:LYS569 M1:GLU574	 2.938 2.393 3.411 3.507
2.	 (2R)-1-[4-[(4-fluoro-2-methyl-1H-indol-5-yl)oxy]-5-methylpyrrolo[2,1-f] [1,2,4] triazin-6-yl] oxy propan-2-ol	-9.6	M2:SER568 M2:GLY490 M2:GLY493 M2:LYS517 M2:ALA567	3.265 3.630 3.332 3.308 3.284
3.	 6-(2,6-dichloro-3,5-dimethoxyphenyl)-2-(methyl amino)-8-[3-(4-prop-2-enoylpiperazin-1-yl) propyl] pyrido[2,3-d]pyrimidin-7-one	-9.5	M3:LYS517 M3:ASP644 M3:ASP644 M3:PHE492	 3.291 3.238 3.602 3.544
4	 4-amino-5-fluoro-3-[6-(4-methylpiperazin-1-yl)-1H-benzimidazol-2-yl]-1H-quinolin-2-one	-9.4	M4:GLY488 M4:ASP626 M4:ASP644	3.602 3.699 3.357
5	 6-[7-[(1-aminocyclopropyl)methoxy]-6-methoxy quinolin-4-yl]oxy-N-methylnaphthalene-1-carbo xamide	-8.9	M6:ASP644 M6:ASP626 M6:ASP644 M6:LYS517 M6:ASN631	2.148 1.911 2.949 3.266 3.879
6	 11-(2,6-difluoro-3,5-dimethoxyphenyl)-13-ethyl-4-(morpholin-4-ylmethyl)-5,7,11,13-tetrazatricyclo [7.4.0.02,6] trideca-1,3,6,8-tetraen-12-one	-8.5	M7:ASN571 M7:ASN571 M7:ASN571 M7:GLU574 M7:ALA567 M7:ASP626 M7:ASP644 M7:GLU534 M7:ASP644	2.954 3.025 3.375 3.432 3.641 3.646 3.456 3.576 3.758

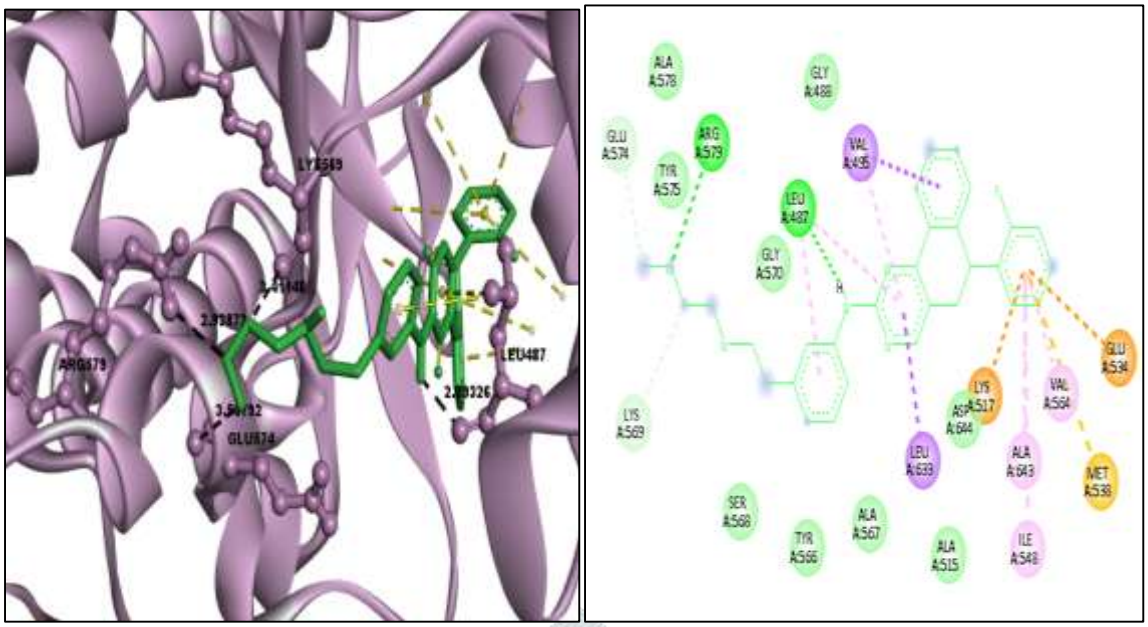


Fig 4:a) Binding interaction of (M1) with FGFR2 protein b) 2-D view of Binding interaction of with FGFR2 protein

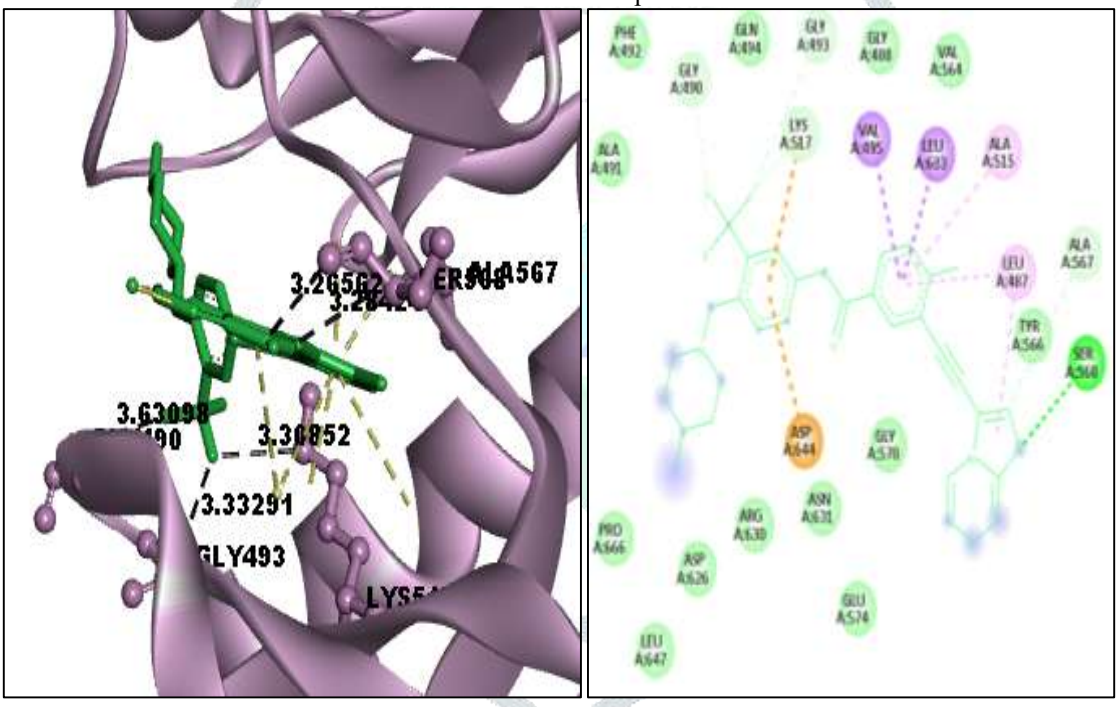


Fig 5: a)Binding interaction of (M2) with FGFR 2protein b) 2-D view of Binding interaction of with FGFR2 protein

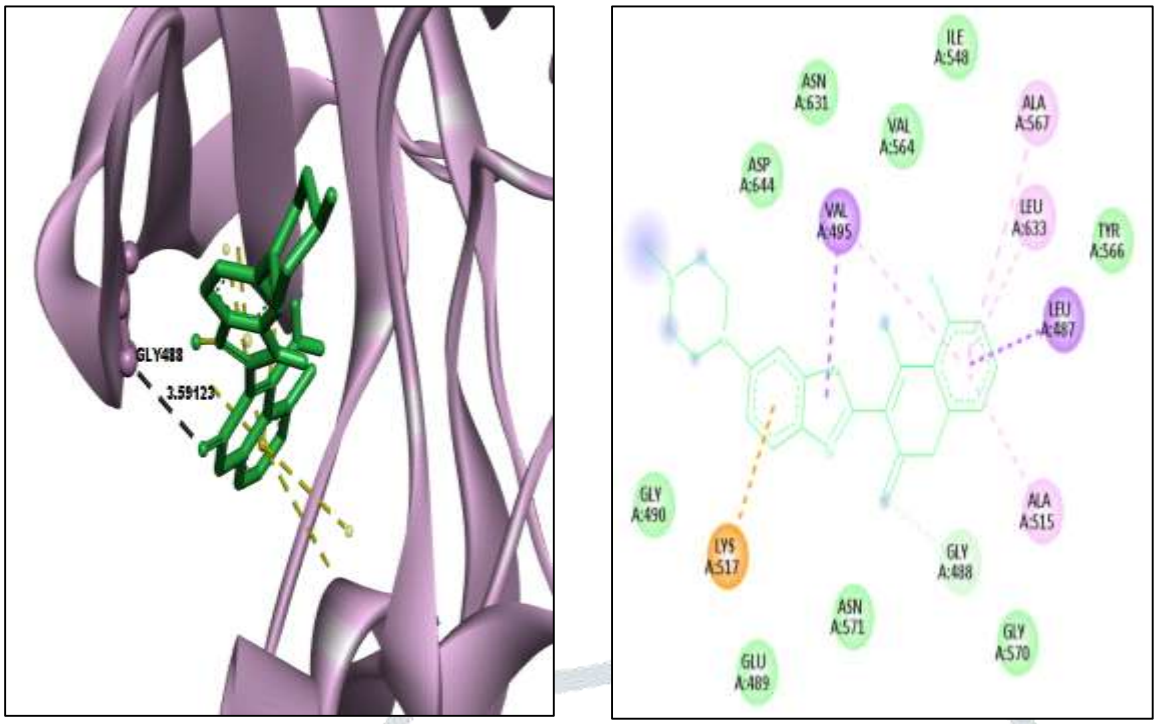


Fig 6: a) Binding interaction of (M3) with FGFR2protein b) 2-D view of Binding interaction of with FGFR2protein

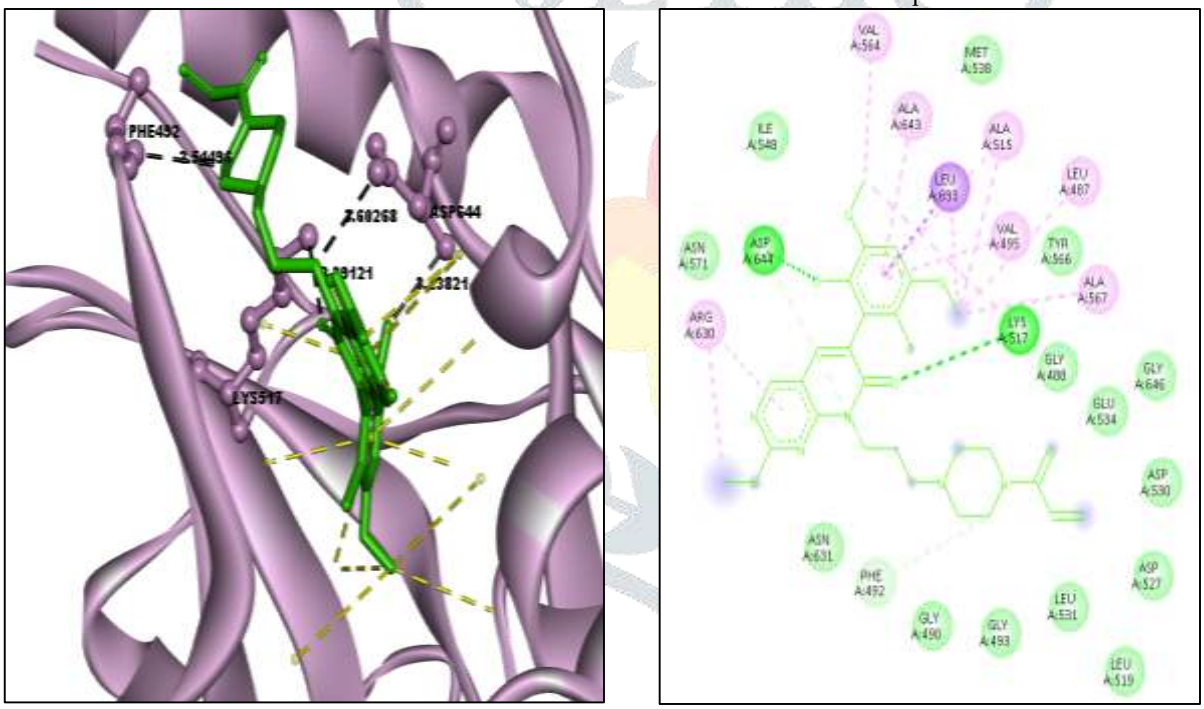


Fig 7:a) Binding interaction of (M4) with FGFR2protein b) 2-D view of Binding interaction of with FGFR2protein

***in silico* ADME Analysis**

Assessing ADME properties of small molecules is a crucial step in drug discovery, aimed at reducing the likelihood of drug candidate failures during clinical trials. In the present study, the ADME properties were predicted using SwissADME, given in Table 4. The predicted physicochemical properties such as molecular weight, number of hydrogen bonds donors, acceptor hydrogen bonds, etc., are in acceptable range. The *in silico* ADME analysis shows that all the compounds obey Lipinski rule of five and have 0 pains alert.

Table 4: Predicted ADME values of the top ten docked molecules for FGFR2 protein

S. No	Mol. Wt	No of HB acceptors	No of HB donors	TPSA	Consensus log $P_{o/w}$	Class	Lipinski rule	Pains
M1	443.49g/mol	6	2	95.70 A^2	3.53	Poorly soluble	Yes 0 violation	0 alert
M2	356.38g/mol	3	3	105.38 A^2	2.79	Poorly soluble	Yes 0 violation	0 alert
M3	446.54g/mol	6	1	77.33 A^2	3.29	Poorly soluble	Yes 0 violation	0 alert
M4	479.96g/mol	5	2	97.32 A^2	1.62	Poorly soluble	Yes 0 violation	0 alert
M5	418.45g/mol	6	1	108.39 A^2	2.00	Moderately soluble	Yes 0 violation	0 alert
M6	446.54g/mol	6	1	77.33 A^2	3.29	Poorly soluble	Yes 0 violation	0 alert

CONCLUSION

Fibroblast growth factor receptors, particularly FGFR2, are implicated in a wide array of cancers including breast, gastric, bladder, and endometrial cancers. Given their critical role in tumor proliferation, angiogenesis and metastasis, FGFRs have emerged as promising targets for anticancer therapeutics. In the present study, identification of potential fibroblast growth factor receptor (FGFR2) inhibitors, using computational techniques such as active site prediction, virtual screening, molecular docking, and *in silico* ADME profiling was carried out. The top scoring molecules obey Lipinski rule of Five and are within acceptable limits for all the other ADME descriptors and the results of top 6 molecules have been reported. Our analysis showed that the top docked compounds exhibited favorable ADME properties, including good gastrointestinal absorption, acceptable lipophilicity, and lack of major cytochrome P450 inhibitory effects. The present computational study further emphasises that the molecules studied can be promising leads for FGFR2 inhibition in cancer treatment.

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