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Molecular Interaction profiling of EGFR kinase Inhibitors: Comparative docking study of pyrazole derivatives with Mutant type and Wild type protein.

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ABSTRACT

This study focuses on the design, molecular docking and comparative analysis of twenty-four pyrazole derivatives to investigate their potential as anticancer agents. Molecular docking was performed using AutoDock Vina against both mutation-type (PDB ID:4HJO) and wild-type (PDB ID:1XKK) forms of a cancer-associated protein. Among the screened compounds six derivatives F4, F8, F12, F16, F20 and F24 demonstrated the highest binding affinities with the mutant protein exhibiting binding energies ranging from -10.9 to -10.6 kcal/mol. These ligands share a common structural feature an ortho-nitrophenyl hydrazine moiety at 1st position and hydroxy or methoxy groups at 4th position of the pyrazole ring contributing to enhanced interactions within the mutant proteins active site. Comparatively lower binding affinities were observed with the wild-type protein with -10.3 to -10.1 kcal/mol, indicating selective binding towards the mutation-type. Since mutations in DNA often lead to altered protein function and are a hallmark of cancer, the observed selectivity underscores the therapeutic potential of these derivatives in targeting mutant proteins in cancer treatment.

Keywords: Pyrazole, EGFR kinase, molecular docking, mutant and wild type.

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INTRODUCTION

Cancer is a complex and multifactorial disease marked by the deregulation of cellular signaling pathways, often driven by genetic mutations [1]. Among the key molecular targets implicated in tumorigenesis is the epidermal growth factor receptor (EGFR), a transmembrane tyrosine kinase receptor involved in regulating cell proliferation, survival and differentiation [2]. Aberrant EGFR signaling, resulting from overexpression or mutations is commonly associated with various cancers including breast cancer, non-small cell lung cancer (NSCLC), colorectal cancer and glioblastoma [3].

Mutations within the EGFR kinase domain such as deletions or substitutions can lead to constitutive receptor activation, promoting uncontrolled cellular growth and resistance to standard therapies. These mutant forms differ structurally and functionally from the wild-type EGFR, making them critical targets for selective inhibition in precision cancer therapy [4].

Pyrazole, a five-membered heterocyclic compound with two adjacent nitrogen atoms has attracted significant attention in medicinal chemistry due to its several biological actions including anticancer, anti-inflammatory and antibacterial effects [5]. The structural flexibility of pyrazole allows for strategic modifications at various positions facilitating its optimization as a pharmacophore for kinase inhibition [6]. Several pyrazole-based derivatives have demonstrated promising anticancer activity through EGFR inhibition, attributable to their capacity to occupy the ATP-binding site and form stable interactions with critical residues in the kinase domain [7].

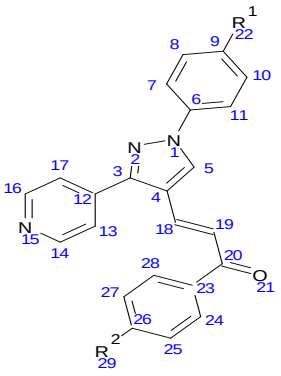
In the current investigation, a variety of new pyrazole derivatives were developed and computationally analysed for their binding potential against both EGFR mutant and wild-type proteins using molecular docking techniques. The aim is to identify structurally favourable ligands with high affinity and selectivity toward both EGFR mutant and EGFR wild-type contributing to the development of targeted anticancer agents.

MATERIALS AND METHODS

Protein and ligand preparation

The crystal structures for EGFR mutant type (PDB ID: 4HJO) and EGFR wildtype (PDB ID: 1XKK) were extracted from RSCB Protein Data Bank http://www.rcsb.org [8, 9]. The docking was carried out on monomeric form of the crystal structure. The AMP-PNP complexed with crystal structure was dismissed to leave a clean docking canvas [10]. The PDB files of the protein and ligand were prepared using AutoDock Tools version 1.5.7 (ADT; Scripps Research Institute, La Jolla, San Diego, USA). The three-dimensional protein target was stripped of all water molecules. Polar hydrogens were incorporated into the proteins to establish hydrogen bonding during docking. Kollman and Gasteiger charges were also added to reconstruct the molecular electrostatic potential. Kollman charges were computed based on quantum mechanics whereas Gasteiger charges were generated based on electronegativity equilibration [11]. Twenty-four pyrazole analogues were designed and sketched using Chemsketch and constructed as in Table no 1. The sdf files for ATP and Gefitinib were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov) and produced using AutoDock Tools [12].

Figure 1: The structure of the designed pyrazole derivatives



Molecular docking

AutoDock Vina version 1.2.3 software was used to perform the molecular docking simulations (The Scripps Research Institute, La Jolla, San Diego, USA). Discovery Studio Biovia 2021 (Dassault Systems, San Diego, California, USA) was employed to visualize and modify receptor and ligand structures. All twenty-four pyrazole analogues, ATP, and gefitinib were docked at the expected binding location [13]. We have also superimposed our docking results 4HJO with gefitinib and also 1XKK with gefitinib. The protein grid box, which is the active site for docking was set up using AutoDock Tools to enclose the a fore mentioned residues. The grid box dimensions for EGFR mutant at 2.50 Å are 46 × 44 × 63 and centered at 24.8621 × 19.2636 × 7.3683 while for $EGFR_{wt}$ at 2.40 Å are 54 × 49 × 58 and centered at 19.1689 × 42.6250 × 36.8915. The docking was then performed using AutoDock Vina, where the docking scores (in kcal/mol) were generated and the binding poses ranked from the highest to the lowest according to the order of binding affinity. The protein-ligand interactions were visualized with Biovia Discovery Studio Visualizer and the hydrogen and hydrophobic interactions within the protein-ligand complex were identified [14, 15].

Table 1: The designed pyrazole analogues

Sl No	Ligand	R_1	R_2
1	Compound F1	H	H
2	Compound F2	p-Cl	H
3	Compound F3	2,4-NO_2	H
4	Compound F4	o-NO_2	H
5	Compound F5	H	o-OH
6	Compound F6	p-Cl	o-OH
7	Compound F7	2,4-NO_2	o-OH
8	Compound F8	o-NO_2	o-OH
9	Compound F9	H	p-OH
10	Compound F10	p-Cl	p-OH
11	Compound F11	2,4-NO_2	p-OH
12	Compound F12	o-NO_2	p-OH
13	Compound F13	H	m-OH
14	Compound F14	p-Cl	m-OH
15	Compound F15	2,4-NO_2	m-OH
16	Compound F16	o-NO_2	m-OH
17	Compound F17	H	o-OCH_3
18	Compound F18	p-Cl	o-OCH_3
19	Compound F19	2,4-NO_2	o-OCH_3
20	Compound F20	o-NO_2	o-OCH_3
21	Compound F21	H	p-OCH_3
22	Compound F22	p-Cl	p-OCH_3
23	Compound F23	2,4-NO_2	p-OCH_3
24	Compound F24	o-NO_2	p-OCH_3

Structure-activity relationship between pyrazole and EGFR

The structure-activity relationship (SAR) of pyrazole derivatives as epidermal growth factor receptor (EGFR) inhibitors reveals that specific substitutions on the pyrazole ring are critical for modulating biological activity [16]. Pyrazole, a five-membered heterocycle with two adjacent nitrogen atoms, serves as a privileged scaffold in the design of kinase inhibitors due to its ability to participate in hydrogen bonding and π-π stacking interactions within the ATP-binding pocket of EGFR [17]. Substituents at the 1, 3, and 5 positions of the pyrazole core significantly influence the binding affinity and selectivity. The introduction of electron-donating or electron-withdrawing groups at the 3 or 5 positions improves EGFR inhibition by enhancing favourable interactions with critical amino acid residues such as Met793 and Thr790 in the receptor's hinge region [18]. Incorporation of aryl or heteroaryl moieties at these positions often improves lipophilicity and increases binding strength through hydrophobic and van der Waals interactions. Additionally, linkers such as amide or urea groups connecting pyrazole to pharmacophores may further stabilize the ligand-receptor complex [19]. Optimized pyrazole derivatives have demonstrated

promising anticancer activity by selectively targeting EGFR, inhibiting downstream signaling pathways involved in cell proliferation and survival. Thus, rational modifications on the pyrazole scaffold are essential for enhancing EGFR-targeted anticancer efficacy [20].

RESULT AND DISCUSSION

A comparative molecular docking investigation was carried out to determine the binding affinities of chosen pyrazole derivatives with both EGFR mutant (PDB ID: 4HJO) and EGFR wild-type (PDB ID: 1XKK) target proteins. The docking results revealed a notable enhancement in binding affinity for the mutant protein, with binding energies ranging from -7.8 to -10.9 kcal/mol. A Compound F4, F16, F8, F12, F20 and F24 shows highest binding energy with -10.9, -10.8, -10.7, -10.7, -10.6 and -10.6 kcal/mol with two conventional hydrogen bonding with residues Met769 and Thr766 as mentioned in the table no 2. In contrast, the same ligands exhibited significantly lower binding energies when docked with the EGFR wild-type protein with binding energy of -9.9, -10.3, -10.1, -9.8, -9.8 and -9.9 kcal/mol with conventional hydrogen bonding of one and two with residues Asp855, Met793, Lys745, Thr854 and Asp800 indicating that the structural alterations in the mutant protein favour stronger ligand-receptor interactions mentioned in the figure 4-9.

Figure 2: ATP (red) and gefitinib (*blue*) binding sites in EGFR mutant (*left*) and EGFRwt (*right*)

Figure 3: ATP (*red*) and allosteric (*blue*) binding sites for pyrazole analogues in EGFR mutant (*left*) and EGFRwt (*right*)

Table 2: Comparative analysis of EGFR mutant type and EGFR wild type protein.

EGFR (Mutant type)					EGFR (wildtype)				
Ligand	**Binding affinity (kcal/mol)**	**No of H bonds**	**H bond residues**	**Hydrophobic interacting residues**	**Ligand**	**Binding affinity (kcal/mol)**	**No of H bonds**	**H bond residues**	**Hydrophobic interacting residues**
F1	-10.1	1H	Met769	Cys773 Val702 Leu764	**F1**	-9.6	2H	Asp855 Met793	Val726 Lys745 Leu718 Leu788 Cys797
F2	-10.1	1H	Met769	Cys773 Val702 Leu834 Leu753 Leu764	**F2**	-9.9	2H	Thr854 Asp855	Lys745 Ala743 Val726 Leu718
F3	-10.5	2H	Met769 Thr766	Val702 Lys721	**F3**	-10.0	2H	Met793 Thr854	Leu718 Ala743 Arg841 Leu788 Lys475

F4	**-10.9**	2H	Met769 Thr766	Val702 Cys773	**F4**	-9.9	1H	Asp855	Val726 Ala743
F5	-9.9	1H	Met769	Val702 Leu764	**F5**	-9.8	1H	Asp855	Arg841 Val726 Lys745 Ala743 Leu844
F6	-10.0	1H	Met769	Cys773 Val702 Leu764 Leu753 Leu834	**F6**	-9.8	3H	Met793 Thr854 Asp855	Leu718 Ala743 Lys745 Leu788
F7	**-10.6**	2H	Met769 Thr766	Val702	**F7**	**-10.2**	2H	Met793 Lys745	Leu718 Ala743 Arg841 Leu788
F8	**-10.7**	2H	Met769 Thr766	Val702	**F8**	**-10.1**	2H	Met793 Thr854	Ala743 Leu718 Arg841 Lys745 Leu788
F9	-9.9	1H	Met769	Val702 Cys773	**F9**	-9.5	2H	Asp855 Asp800	Val726 Lys745 Leu718 Leu788 Cys797
F10	-10.2	2H	Met769 Asp831	Val702 Leu764 Leu834 Leu753 Met742	**F10**	-9.8	3H	Gln791 Met766 Leu777	Ala743 Arg841
F11	-10.4	2H	Met769 Thr766	Val702 Lys721	**F11**	-10.0	3H	Gln791 Met766 Leu777	Ala743 Arg841
F12	**-10.7**	2H	Met769 Thr766	Val702 Cys773	**F12**	-9.8	2H	Asp855 Asp800	Ala743 Val726 Cys797
F13	-9.9	1H	Met769	Val702 Cys773 Leu764	**F13**	-9.8	1H	Arg841	Lys745 Ala743 Val726 Leu844
F14	-10.3	2H	Met769 Asp831	Val702 Leu764 Leu834 Leu753 Met742	**F14**	-10.1	1H	Phe856	Ala743 Arg841 Met766 Leu777
F15	-10.3	2H	Met769 Thr766	Val702 Lys721	**F15**	**-10.3**	3H	Met793 Thr790 Lys745	Leu718 Ala743 Arg841
F16	**-10.8**	2H	Met769 Thr766	Val702	**F16**	**-10.3**	2H	Met793 Lys745	Ala743 Leu718 Arg841
F17	-9.7	1H	Met769	Val702 Leu768 Leu764	**F17**	-8.7	1H	Val717	Leu844 Ala743 Val726 Leu1001
F18	-10.0	1H	Met769	Lys704 Val702 Leu768 Leu764 Leu753 Met742 Leu834	**F18**	-10.1	2H	Met793 Thr790	Leu844 Ala743 Cys775 Leu777 Arg841 Lys745 Leu788
F19	-10.5	2H	Met769 Thr766	Val702 Leu768	**F19**	**-10.3**	3H	Met793 Thr790 Lys745	Leu844 Ala743 Leu777 Cys775 Leu788
F20	-10.6	2H	Met769 Thr766	Val702 Leu768 Leu764	**F20**	-9.8	1H	Met793	Leu718 Ala743 Leu788 Cys797 Arg841

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F21	-9.9	1H	Met769	Val702 Cys773 Leu764	**F21**	-9.6	2H	Met793 Asp855	Leu718 Cys797 Val726 Lys745 Leu788
F22	-10.0	2H	Met769 Asp831	Val702 Leu764 Leu753 Met742	**F22**	-9.9	1H	Met793	Phe856 Leu777 Leu718 Arg841 Ala743
F23	-10.5	2H	Met769 Thr766	Val702 Cys773	**F23**	-9.0	3H	Lys745 Asp800 Tyr998	Ala743 Leu844 Cys797 Leu1001
F24	-10.6	2H	Met769 Thr766	Val702 Cys773	**F24**	-9.9	1H	Met793	Leu718 Ala743 Arg841 Leu788 Lys745 Leu777 Met766
Gefitinib	-9.2	1H	Lys721	Leu764 Leu753 Leu834 Leu820	**Gefitinib**	-8.5	-	-	Lys745 Ala743 Val726 Leu858
ATP	-7.7	5H	Gly700 Phe699 Arg817 Asn818 Asp831	-	**ATP**	-7.8	3H	Thr790 Asp855 Asn842	-

The superior binding affinity in the EGFR mutant protein complexes is primarily attributed to specific structural features of the ligands. All high-affinity derivatives possess an ortho-nitrophenyl hydrazine moiety at the 1-position of the pyrazole ring with hydroxyl and methoxy acetophenone substitutions at the 4-position of the pyrazole ring particularly in ortho, meta and para configurations. These electron-donating and withdrawing groups are presumed to enhance hydrogen bonding and π-π stacking interactions within the active site of the mutant protein, thereby stabilizing the ligand-protein complex.

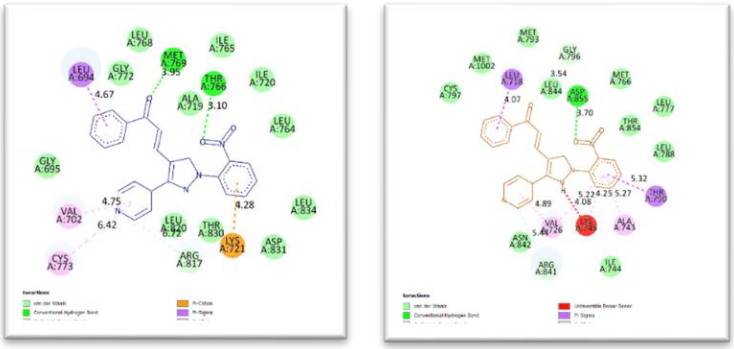

Figure 4: Interactions of (a) $EGFR_{mutant}$ Compound F4 (left) and (b) $EGFR_{wt}$ Compound F4 (right)

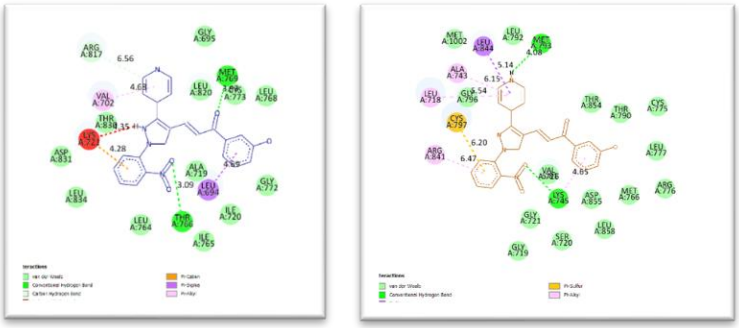

Figure 5: Interactions of (a) $EGFR_{mutant}$ Compound F16 (left) and (b) $EGFR_{wt}$ Compound F16 (right)

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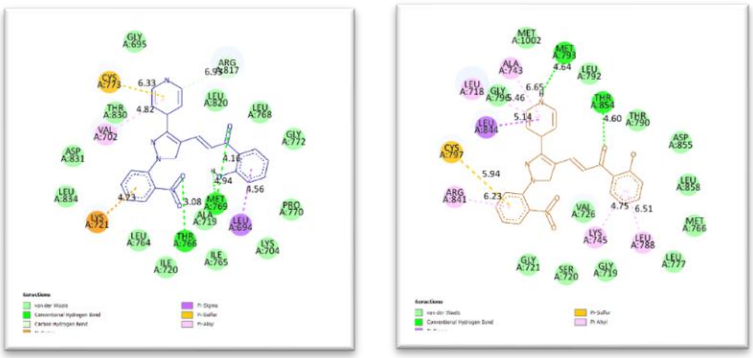

Figure 6: Interactions of (a) $EGFR_{mutant}$ Compound F8 (left) and (b) $EGFR_{wt}$ Compound F8 (right)

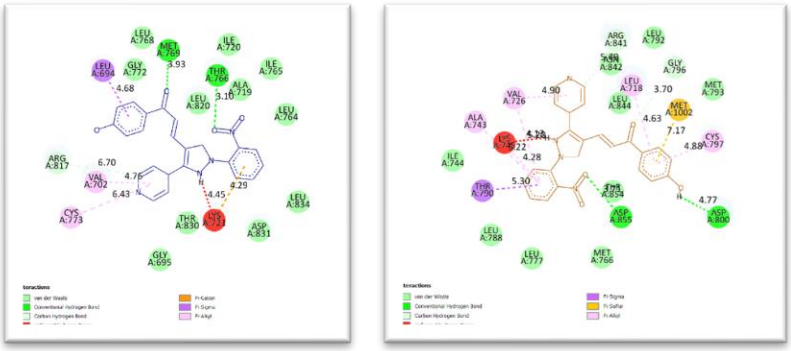

Figure 7: Interactions of (a) $EGFR_{mutant}$ Compound F12 (left) and (b) $EGFR_{wt}$ Compound F12 (right)

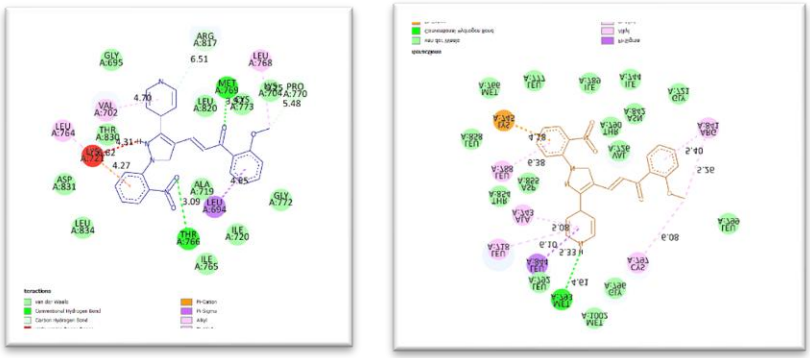

Figure 8: Interactions of (a) $EGFR_{mutant}$ Compound F20 (left) and (b) $EGFR_{wt}$ Compound F20 (right)

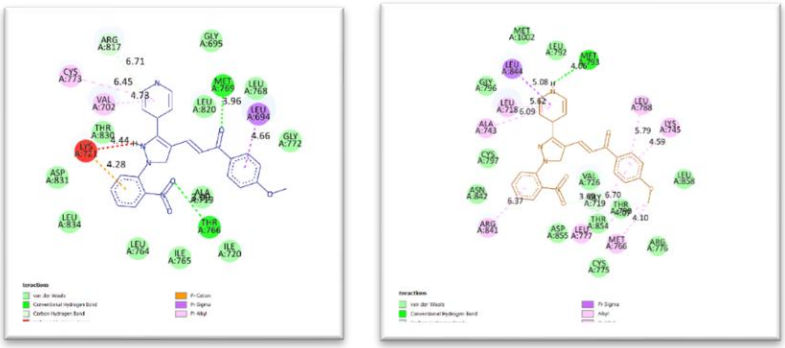

Figure 9: Interactions of (a) $EGFR_{mutant}$ Compound F24 (left) and (b) $EGFR_{wt}$ Compound F24 (right)
Comparison of docking result from pyrazole, ATP and gefitinib

To evaluate the potential of the designed pyrazole derivatives as EGFR kinase inhibitors molecular docking studies were performed using AutoDock Vina. The binding free energies of the derivatives were compared with that of the natural ligand ATP and the standard EGFR inhibitor gefitinib. The docking protocol was validated by redocking the co-crystallized ligand (gefitinib) into the active site of EGFR kinase of both EGFR mutant (PDB ID: 4HJO) and EGFR wild-type (PDB ID: 1XKK) target proteins, which reproduced the binding pose with a low RMSD value, confirming the reliability of the docking method as shown in the figure 2 and 3.

The docking results revealed that most of the pyrazole derivatives exhibited binding affinities in the range of -10.9 to -9.6 kcal/mol. These compounds showed key interactions with crucial amino acid residues within the ATP-binding pocket, including hydrogen bonding with Met769 and hydrophobic contacts with residues such as Cys773, Val702 and Leu764 as shown in the figure 10 and 11. Some derivatives demonstrated π-π stacking interactions that further stabilized the ligand-protein complex.

In contrast, ATP displayed a comparatively lower binding affinity of -7.7 kcal/mol in mutant type and -7.8 kcal/mol in wild type protein. Although it forms several polar interactions, its binding is less stable due to the lack of extensive hydrophobic or aromatic interactions. Gefitinib used as a reference inhibitor, showed a docking score of -9.2 kcal/mol in mutant type and -8.5 kcal/mol in wild type protein, forming strong hydrogen bonds and hydrophobic interactions with key residues, effectively occupying the ATP-binding cleft of EGFR.

The comparative docking analysis indicates that selected pyrazole derivatives possess binding affinities and interaction profiles comparable to or better than ATP and closely mimic those of gefitinib. These findings suggest that the synthesized pyrazole derivatives may serve as promising lead compounds for further development as EGFR-targeted anticancer agents.

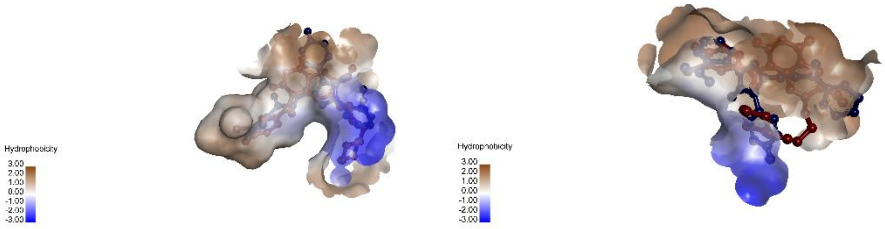

Figure 10: Comparison of adopted conformation by pyrazole analogue (*blue*)and Gefitinib (*red*) after being docked against
(a) $EGFR_{mutant}$ and (b) $EGFR_{wt}$

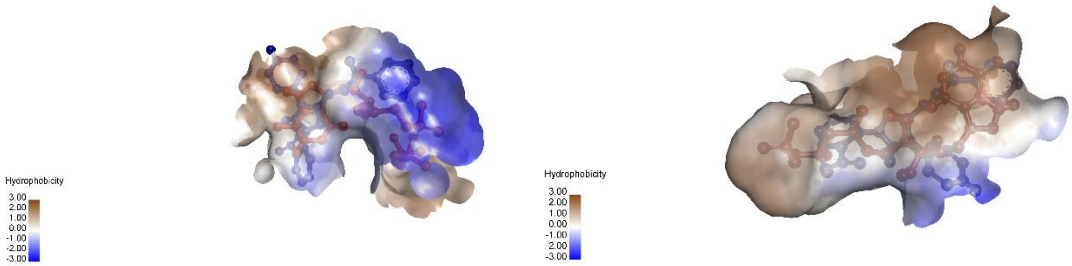

Figure no 11: Comparison of adopted conformation by pyrazole analogue (*blue*) and ATP (*red*) after being docked against
(a) $EGFR_{mutant}$ and (b) $EGFR_{wt}$

This comparative analysis underscores the influence of both electronic and spatial effects introduced by these substituents, suggesting their crucial role in optimizing ligand affinity towards mutant cancer-related proteins, which may guide future structure-based drug design.

CONCLUSION

This study highlights the significance of targeting mutant proteins in cancer research as mutations often arising from genetic alterations in DNA play a crucial role in cancer development and progression.

Among the twenty-four designed pyrazole analogues F4, F8, F12, F16, F20, and F24 exhibited superior binding affinities specifically with the mutant form of the target protein than the wild type protein. These ligands contain ortho-nitrophenyl hydrazine at 1st position and hydroxy/methoxy substitutions at 4th position, showed enhanced interaction due to favourable electronic and spatial features. The findings suggest that mutant proteins are promising targets for the design of selective anticancer agents.

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