Virtual screening and protein-ligand based molecular docking studies for the major protein (EGFR) in Non-small cell lung cancer (NSCLC)

Submitted By

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Under the Guidance of

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Special thanks to my parents for their support. They have been by my side to make life better and help me overcome difficulties and become better.

Date: 15th June,2021

Place: Delhi

Rahul

Regd. No. 197801

MSc. Biotechnology

CERTIFICATE

I hereby certify that the title of the thesis is **"Virtual Screening and protein-ligand based molecular docking studies for the major protein (EGFR) in non-small cell lung cancer (NSCLC)".** submitted by **"Rahul" (Regd. No. 197801)** the title of "Master of Biotechnology" awarded to the **"Jaypee University of Information Technology, Waknakhat, Solan, Himachal Pradesh"** is a report of his research under my guidance and supervision.

This thesis meets all the requirements of the JUIT degree type. Depending on the university, not all the content of thesis has been submitted to different courses or diploma of a particular university regulation.

Date: 15/06/2021

Place: JUIT, Waknaghat

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DECLARATION

I “Rahul” Certify that the all information and data in this thesis is original, it has done by myself under the supervision of my guide Dr. Tiratha Raj Singh. I have confirmed that that all data follow the ethical code of conduct of the JUIT. I have not copied any data from anywhere if I used some written material from any sources so, given credit to them by citing them in text of the reference of thesis.

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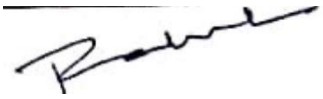

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Abstract:

Introduction: NSCLC is the biggest problem for the world, up to 70% patient are incurable in the world. EGFR (Epidermal Growth factor receptor) is main protein which is response in signaling pathway, Mutation conformation give the alteration in lung cancer patient. Many of data give the approx. figure of cases of male, female and both sexes in the world. According to data we get the median age when person is infected by NSCLC, that median age is 70. EGFR is receptor protein, it's a protein where intercellular and extracellular domain in this protein inhibitor bind on the extracellular domain and that's inhibit the signaling pathways like PI3K and RAS pathways. Ligand, act as an inhibitor which bind with protein and inhibit the signaling pathways which are responsible to cause apoptosis.

Methodology: Bioinformatics tools used for the research work, there many research tools used like Auto dock vina, Pymol, Discovery studio visualizer, and some online tools like NCBI, PDB, PUBCHEM and some more. First, we collect data and screening first protein is on the bases of resolution and ligand screening then start docking by using Autodock vina and docking by two ways: one is single molecule (ligand) docking and second is multi-ligand docking. Multi ligand used Perl programing, after docking analyses the result of docking which give the information where ligand bind with protein and give amino acid residue information which amino acid at which position.

Conclusion: after all this docking process first, we get the binding affinity of ligand with best hit and maximum number of hits, were ligand with protein. Then analyses docking result by discovery studio visualizer and its give 3D protein-ligand interaction structure and 2D protein ligand interaction structure which give the information about amino acid, hydrogen bond and pi-bond. Then after analyses we get four ligand which have maximum binding affinity with protein (EGFR 4uv7), that is TKI258, Afatinib, Dacomitinib and Oluminitib.

Key Words: NSCLC, EGFR, Ligand, Protein, Docking, Tools, Binding affinity and Mutation

Content

Appendix 1

List of Figure:

Figure 5:11 In these figure are showing some different inhibitor which bind with protein (EGFR Extracellular domain) A. TKI258, B. PDL-1, C. Osimertinib, D. Oluminitib, E. Nazartinib, F. Naquotinib, G. Meverletinib, I. Dacomitinib, J. Afatinib, K. Abitinib

Appendix 2

List of Table:

Appendix 3

List of Abbreviation:

NSCLC	Non-small cell lung cancer
AJCC	American joint committee on cancer
USA	Epidermal growth factor Receptor
WHO	World health organization
DNA	Deoxyribonucleic acid
RCSB	Research Collaboratory for Structural Bioinformatics
PDB	Protein Data Base
TKI	Tyrosine Kinase Inhibitors
MAPK	Mitogen-activated Protein kinases
MDS	Molecular dynamic simulation

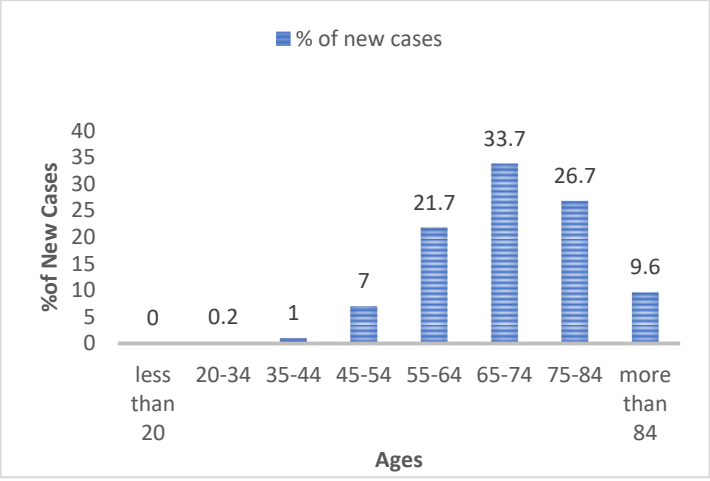
Chapter 1

Introduction:

We are living in the pandemic era and cancer is also a major problem in this era. There are many kinds of cancer, lung cancer is one of the types, NSCLC is classified in different-different subtype which are adenocarcinoma, squamous cell carcinoma or epidermoid carcinoma, and large cell carcinoma. According to American Joint Committee on Cancer (AJCC), Due to NSCLC up to 70% of patients are incurable, since the stage of diagnosed at a metastatic, staged IIIB–IV. The Epidermal Growth Factor (EGFR) is involved in signalling pathways that promote cell proliferation and migration. These mutations, such as G719S, L858R, T790M, G719S/T790M, or T790M/L858R, can modify and give conformation at that location, as well as therapeutic responses from lung cancer patients against the mutations that cause NSCLC. NSCLC (non-small cell lung cancer) is the most common type of lung cancer. According to Surveillance, Epidemiology, and End Result Program, in last years, New estimated case in 2019 is 228,150 that's 12.9% of all new cancer cases in USA. This incidence for men and women, estimated death in 2019 is 142,670 that's 23% of all cancer death.

Table 1:1 shows in which age how much % new cases came.

Ages	% of new cases
less than 20	0
20-34	0.2
35-44	1
45-54	7
55-64	21.7
65-74	33.7
75-84	26.7
more than 84	9.6



In 2019, By this data of lung and bronchus cancer, most frequent new cases detect in median age at diagnosis 70.

According to WHO Estimated number of new cases in 2018, worldwide, all cancers, both sexes, all ages: Lung 2,093,876 (11.6%) (Data source: Globocan 2018 Graph production: Global Cancer).

The Global Cancer Observatory gives an statistical data of India in 2018

Table 1:2 Shows new cases according to sex (male, female, or both)

Sex	**Number of New Cases**	**Percentage**
Both Sex	67,795	5.9%
Male	48,698	8.5%
Female	19,097	3.3%

Number of new cases of lung cancer is 67,795 (5.9%), in both sexes, all ages. number of new cases in males, all ages is 48,698 (8.5%) and number of new cases in females, all ages is 19,097 (3.3%). Number of Deaths is 63,475 (8.1%)

The incidence, mortality and prevalence rate by cancer site: GLOBOCAN 2018 India data

Table 1:3 According to GLOBOCAN 2018 India Data, Mortality and Prevalence rate.

Lung cancer	Number	Rank	Percentage	Cumm. Risk
New cases	67,795	4	5.9	0.65
Death	63,475	3	8.1	0.60

5-year prevalence (all ages) number is 65,805 and prop. 4.86

There are many targeted therapies which reduce or give the first line of treatment of against EGFR mutations.

Chapter 2

EGFR

A transmembrane protein, EGFR, is a protein that spans several membranes. The EGFR gene codes for the Epidermal Growth Factor receptor protein. Receptor protein crosses cell membranes, with one end within the cell and the other outside. The tyrosine kinase receptor EGFR, also known as ErbB1 and HER1, is a type of tyrosine kinase receptor. There are intracellular and extracellular domain positions that allow the receptor to attach to and bind to other proteins known as Ligands.

The following are the Ligands that EGFR binds to: Transforming Growth Factor-alpha (TGFA), Heparin-binding EGF-like Growth Factor (HBEGF), Amphiregulin (AREG), Betacellulin (BTC), Epiregulin (EPR), and Epigenin are all examples of EGF (canonical ligand) (EPGN).

The EGFR gene mutation happens during a person's lifetime (Somatic) and is exclusively found in cancer cells. Somatic mutation is common in a kind of lung cancer known as non-small cell lung cancer (NSCLC), specifically a variant known as adenocarcinoma. Somatic EGFR gene mutations linked to lung cancer erase genetic material in the Exon 19 region of the gene [Del 19; 60% at position 858 (L858R)] or cause a change in DNA synthesis. [Del 19; ~ 60% at position 858 (L858R)] or change in DNA building blocks (nucleotides) in another region called Exon 21 (point mutation L858R ~ 30%).

Mechanism action of EGFR

Cell membrane have transmembrane receptor this receptor is called ligand binding domain. Ligand binding site attached with single transmembrane (made up by peptide chain) and it linked by cytosolic side.

Signaling pathway used in

- MAPK Pathway
- JAK/STAT Pathway
- PI3K/AKT Pathway ……. activate mTOR
- KRAS/RAF pathway …… activate MEK …… MAPK ……. ErK

- When these pathways activate by SH2 domain activation and doing phosphorylation they cause Metastasis, Angiogenesis and Cell Proliferation.

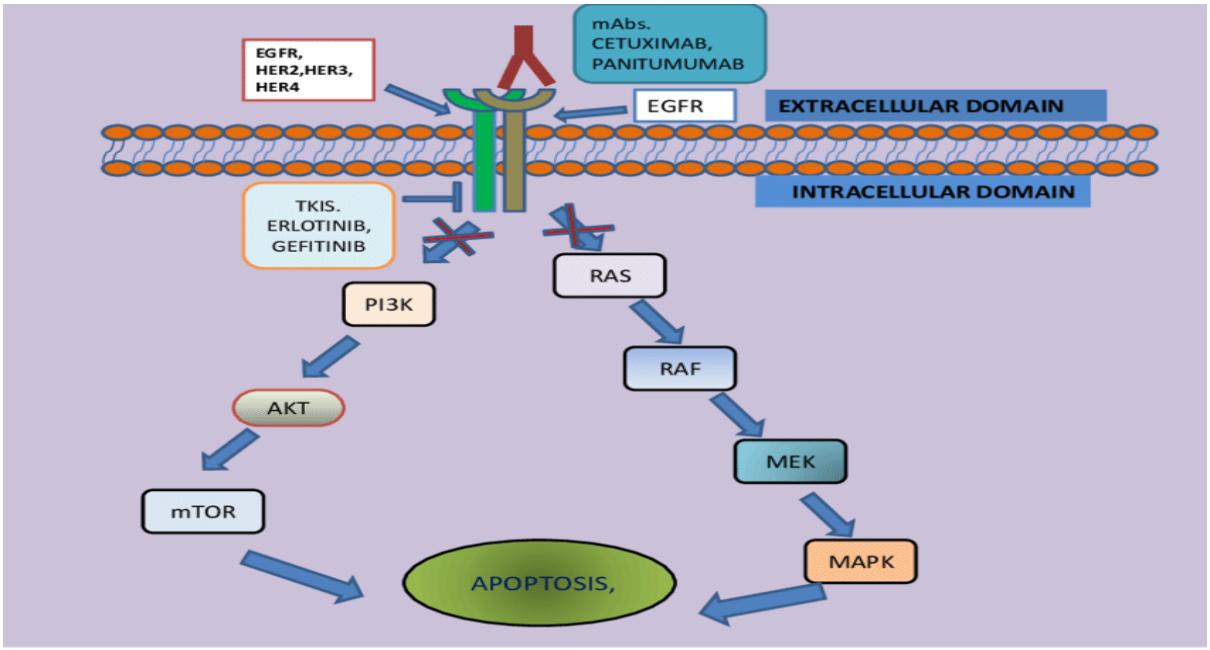


Figure 2:1 It shows the mechanism of EGFR, how inhibitors work in intracellular domain and inhibit the pathways of Apoptosis.

Source of Figure:

EGFR Signaling and its inhibition by EGFR inhibitors in NSCLC - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Mechanism-of-action-of-EGFR-inhibitors-TKIs-and-mAbs-bind-to-the-intracellular-and_fig3_273191177 [accessed 27 May, 2021]

Chapter 3

Material and Method

Some Bioinformatics tool used for docking process, we used to collect some protein data by using RCSB PDB.

Collect ligand data by using PUBCHEM, ZINC database there and many data of ligand we get EGFR inhibitor i.e., Tyrosine kinase inhibitors we find from there, and make a collection of ligands for docking.

PYMOL: Pymol used to visualize the PDB structure here

AutoDock Vina:

AutoDock MGL Tools

Perl Programing

In methodology first we collect data of my selective protein and ligand data collection from RCSB PDB and PUBCHEM. Then first we prepare receptor first for docking, protein is our Receptor and we select a protein EGFR extracellular domain (PDB ID 4uv7)

Preparation of protein

I get my protein from RCSB PDB. My protein PDB ID is 4uv7, it is extracellular domain of EGFR, We select protein on the bases of resolution and Its resolution is 2.1 angstroms. After that we use Autodock MGL tool for preparing tool.

Where first we delete water molecule than add polar hydrogen and kollmann charges.Save this file in PDBQT format.

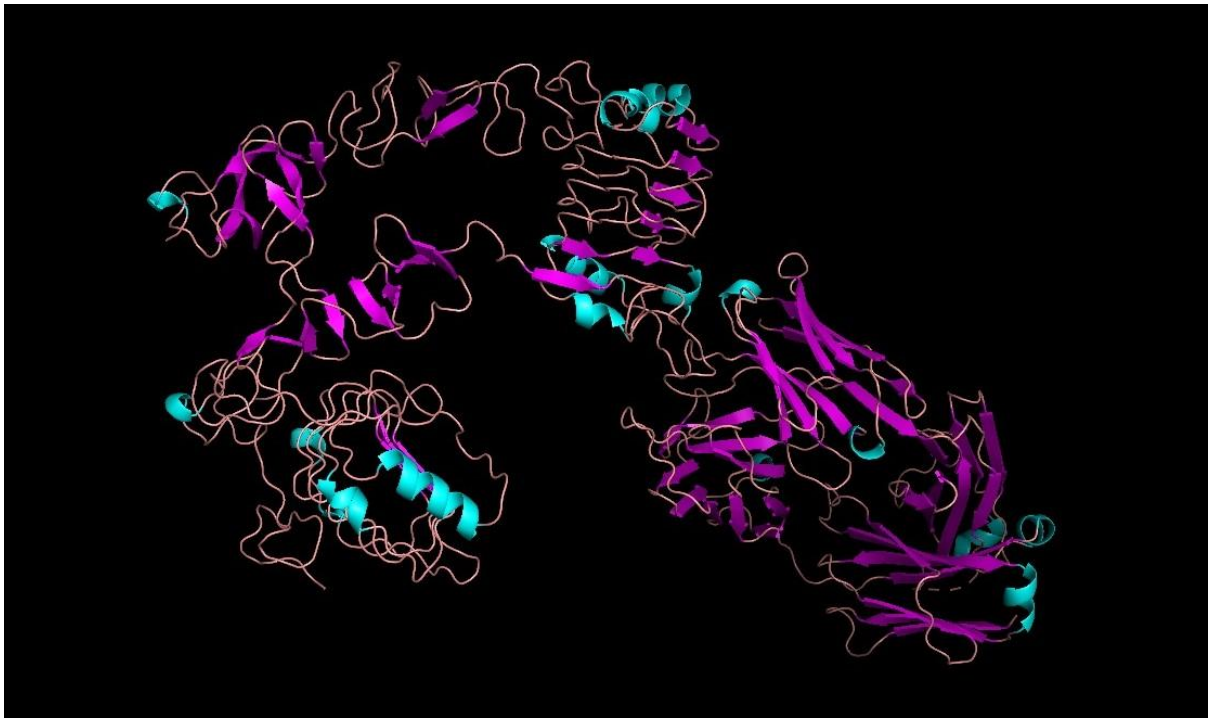


Figure 3:1 Protein Structure of EGFR extracellular domain, protein create by pymol

Preparation of Grid

This selected protein first select by using Autodock MGL tool and form grid box. It's given the center axis and many sites where ligand bind and in each point within the grid map stories the potential energy of "probe" atom or functional group that is done to all the atom in the macro molecules. Help to make docking calculation extremely fast.

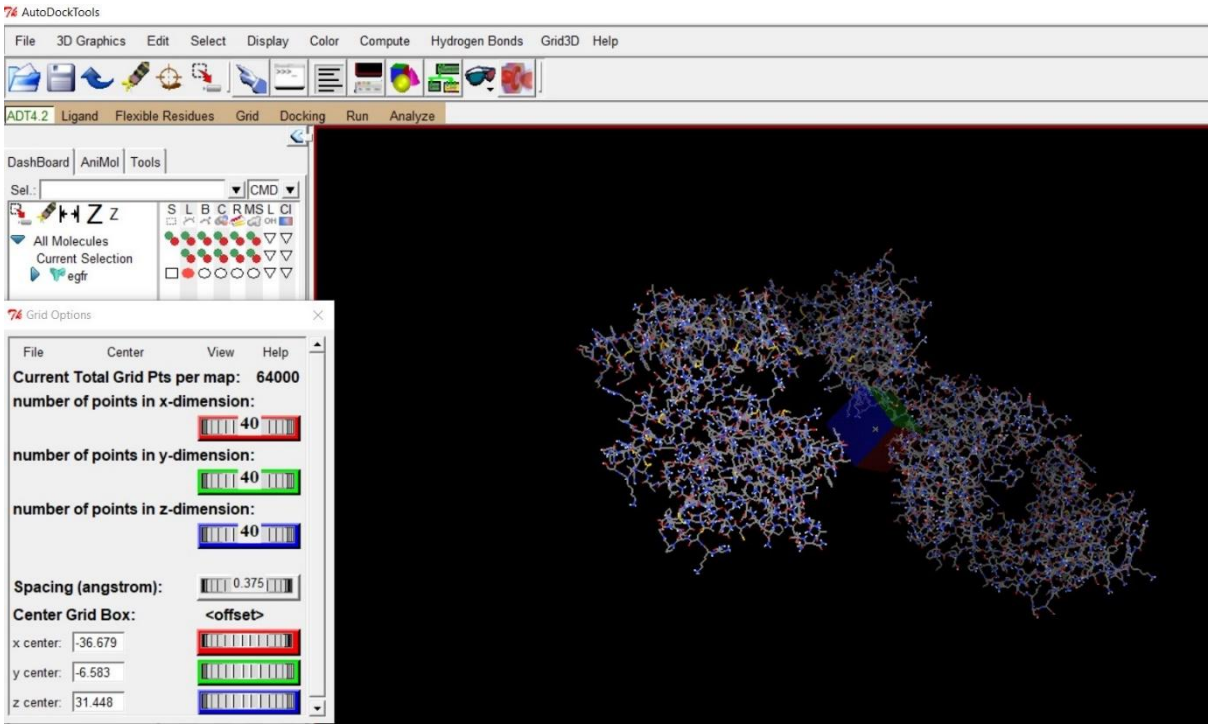


Figure 3:2 Protein Preparation By using Autodock MGL tool, picture showing Grid formation in protein and shows Dimension, Spacing and Center Grid Box

Preparation of Ligand

First by using Pymol SDF file convert in PDB file. After that we use Autodock MGL tool and import my ligand PDB file there and covert in PDBQT format.

Preparation of configuration text file

Write and make a text file on notepad first receptor, ligand, center x , center y center z and size and space of file then we start the docking

receptor = 4uv7.pdbqt, ligand = ligand.pdbqt, center_x = -36.384, center_y = -6.511, center_z = 31.473, size_x = 40, size_y = 40, size_z = 40, energy range = 4, exhaustiveness = 8

Chapter 4

Virtual Screening

Virtual Screening is an important process to detect or find the potent inhibitor/Ligand which have maximum tendency of bind with protein or get the binding affinity by using computational approach. We can screen large and huge data at a time and get a good result for scientific approach. We have used the Perl script and screened a large data library by using Autodock Vina. After that the script sort some potential compounds on the basis of its top binding affinity.

Single ligand docking by using Autodock vina

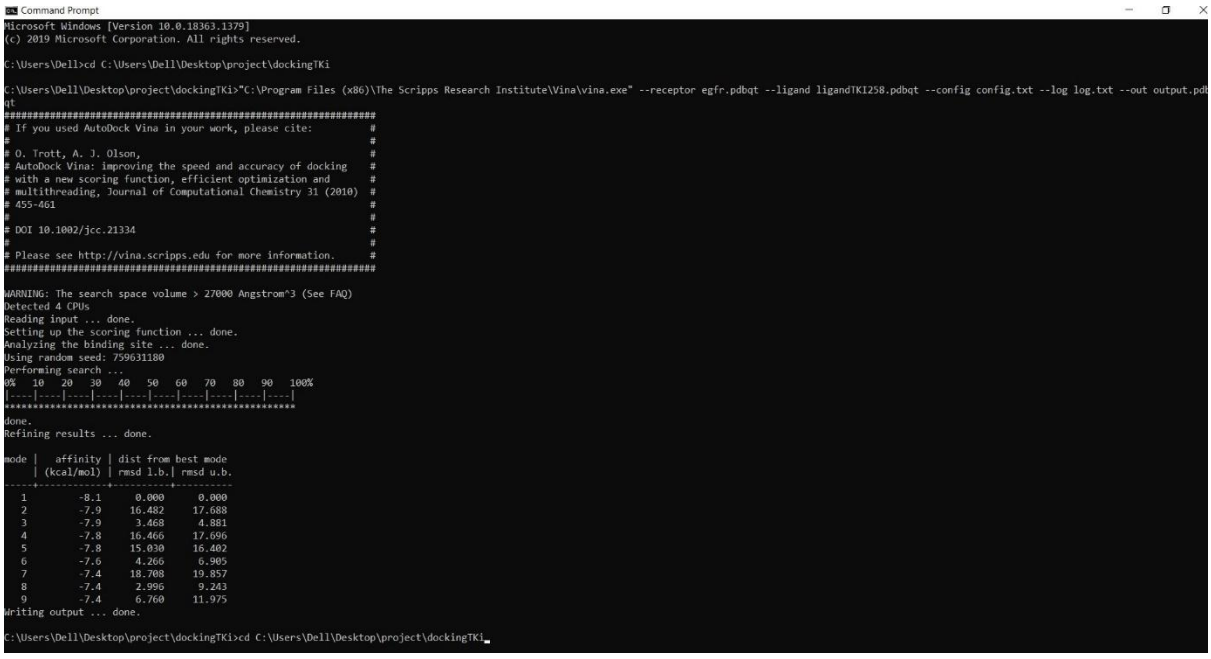


Figure 4:1 showing command on command prompt.

After preparation step, start the molecular docking and its give maximum number of hits by protein- ligand interaction. TKI258 (Dovitinib) shows 9 hits with maximum binding affinity -8.1 kcal/mol.

Table 4:1 This shows maximum number of hits by TKI258 interaction with different-different binding affinity (kcal/mol) and distance from best mode with protein (RMSD LB and RMSD UB).

Mode	Binding Affinity (kcal/mol)	Distance from best mode	
		RMSD L.B	RMSD U.B
1	-8.1	0.000	0.000
2	-7.9	16.482	17.688
3	-7.9	3.468	4.881
4	-7.8	16.466	17.696
5	-7.8	15.030	16.402
6	-7.6	4.266	6.905
7	-7.4	18.708	19.857
8	-7.4	2.996	9.243
9	-7.4	6.760	11.975

Multiligand docking with single protein:

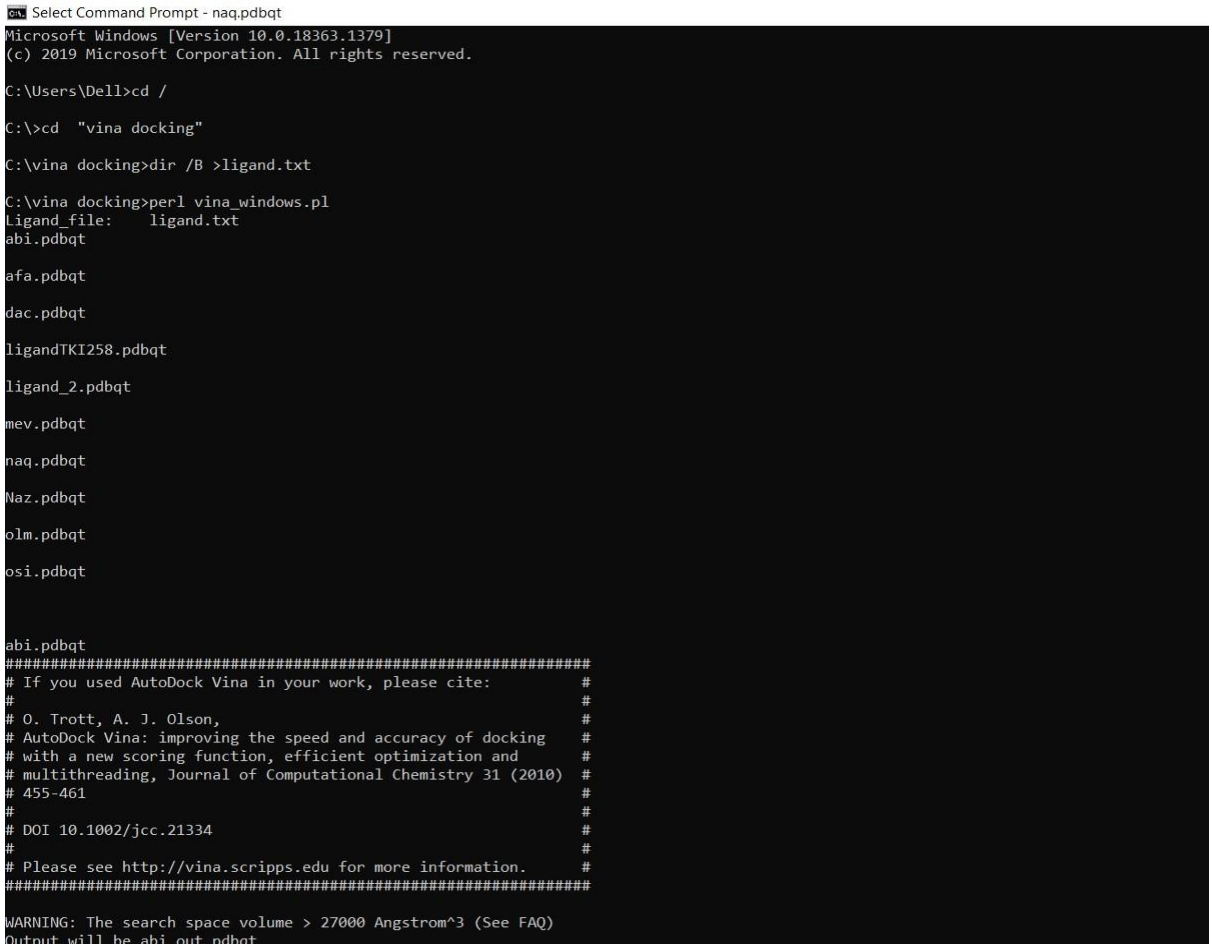


Figure 4:2 This Picture shows multi docking on command prompt by Perl programming command and dock multi ligand together.

For screening, taking many compounds together from PubChem and docking by using Autodock Vina. It is easy and less time-consuming method. Here we have used vina docking with Perl programming and it is multithreaded and improved algorithm for virtual screening and very fast result with binding affinity. Hence, we have used this software for virtual screening and then these compounds which were showing the >= -7.5 Kcal/mol binding affinity were adopted for further future scientific study and MDS (Molecular dynamic simulation).

Prior to docking process first preparation of protein and ligand were performed to using the Autodock tools. The protein and ligand were prepared by same process which we have given

above in chapter 3. Protein is retrieved on the bases of protein resolution; the resolution of protein EGFR (4uv7) is 2.10 Angstrom.

Chapter 5

Result:

Table 5:1 there are many inhibitors which have different binding affinity and highlighted shows the maximum binding affinity, which bind with Protein (EGFR 4uv7)

Sr. No	Ligand Name	Pubchem ID	Binding affinity (Kcal/mol)	Protein
1	TKI 258	135398510	-8.0	EGFR
2	PDL-1	91663303	-7.1	EGFR
3	Afatinib	10184653	-8.2	EGFR
4	Abitinib	72734520	-7.6	EGFR
5	Dacomitinib	11511120	-8.2	EGFR
6	Mavelertinib	91668194	-7.7	EGFR
7	Naquotinib	71667668	-7.8	EGFR
8	Nazartinib	72703790	-7.8	EGFR
9	Olmutinib	54758501	-8.0	EGFR
10	Osimertinib	71496458	-7.6	EGFR

Table 5:2 Result Analysis of docking of protein-ligand (inhibitor) binding interaction by using 3D BIOVIA Discovery Studio Visualizer and give amino acid residue, where ligand interact with protein at particular amino acid at given position.

Result Analysis	**Visualization software**	**protein**	**Ligand**	**Docking score**	**Amino acid residue**
Autodock Vina	3D BIOVIA Discovery studio visualizer	EGFR 4UV7	Abitinib	-8.2	PRO 362, ARG 353, TRP 386, ALA 385, SER 30, PRO 365, PRO 387, GLU 388, ASP 31, PRO 349
			Afatinib	-7.6	PRO 102, PHE 352, ASP 31, ARG 353, TYR 32
			Dacomitinib	-8.2	THR L:97, HIS L:31, TRP L:99, PHE A:357, VAL A:350, LEU A:325
			Mavelertinib	-7.7	ASN L:33, HIS A:359, THR A:360, THR A:330
			Naquotinib	-7.7	ASN A:389, GLU A:388, SER H:30, PRO A:365, PHE A:352, PRO A:349, ARG A:353, PRO A:362
			Nazartinib	-7.7	THR A:330, ASN A:328, THR A:360, VAL A:350, ARG H:57, ASP A:355, LEU A:325, THR A:358
			Oluminitib	-8.0	PRO A:387, PRO A:365, PHE A:352, ASP H:31, ALA A:385, ARG A:353, LEU A:363, PRO A:349
			Osimertinib	-7.6	TRP A:386, PRO A:349,

					ALA A:385, ARG A:353, ASN A:420, PRO A:362, GLN A:384
			TKI 258	-8.2	ALA A:385, PHE A:352, GLU A:388, TRP A:386, ARG A:353, PRO A:362
			PDL-1	-7.1	ARG 353, PRO 349, PHE 352, PRO 365, PRO 102

2-D Ligand-Protein Interaction Structure

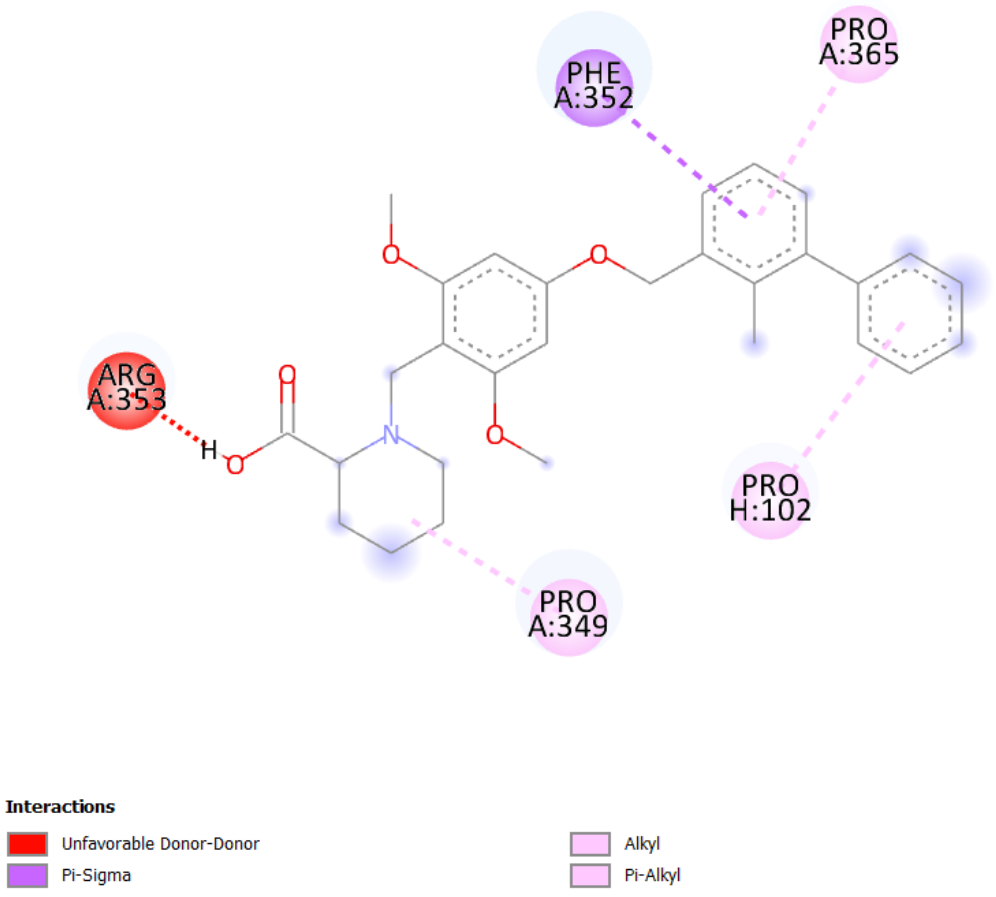


Figure 5:1 2D Structure of Ligand-Protein Interaction {Ligand is PDL-1 and Protein is EGFR(4uv7)}

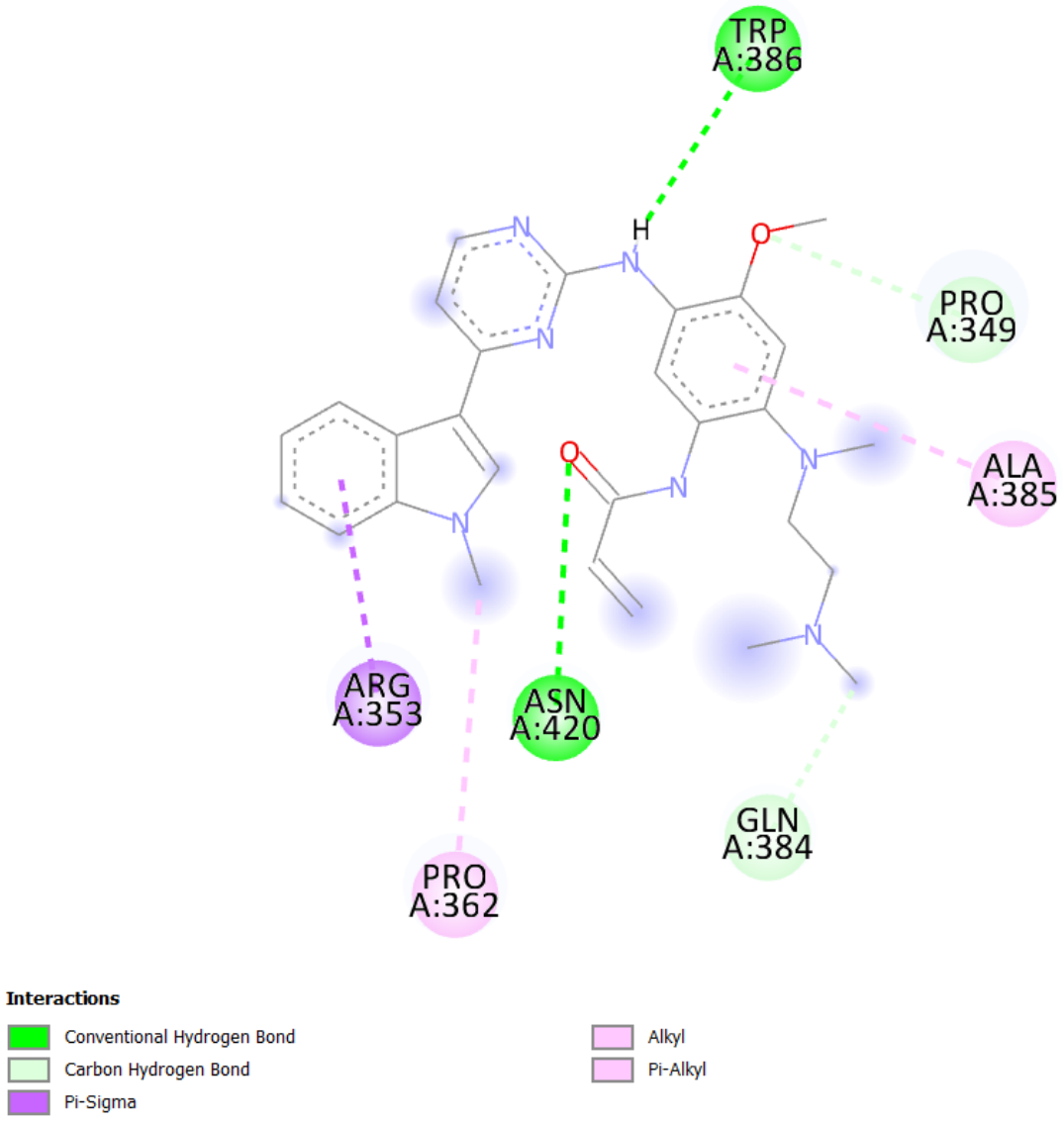


Figure 5:2 2D Structure of Ligand-Protein Interaction {Ligand is Osimertinib and Protein is EGFR(4uv7)}

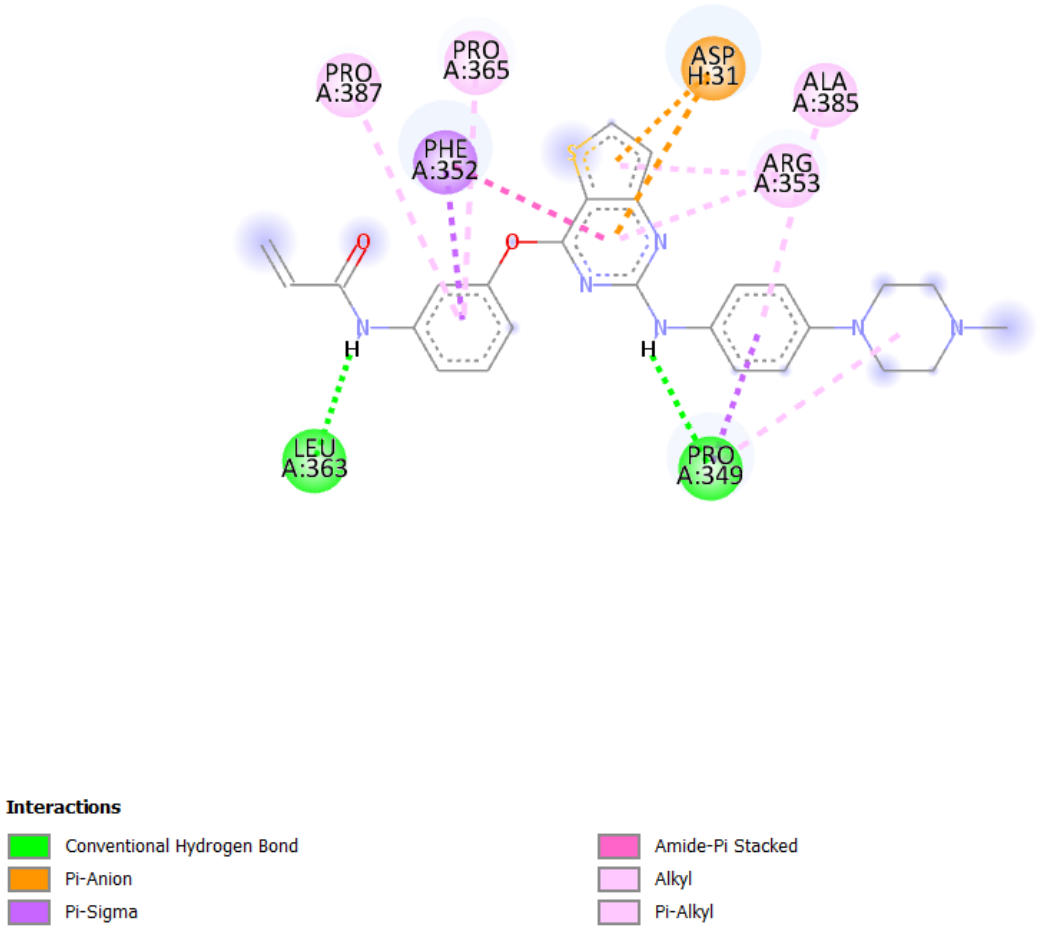


Figure 5:3 2D Structure of Ligand-Protein Interaction {Ligand is Olmutinib and Protein is EGFR(4uv7)}

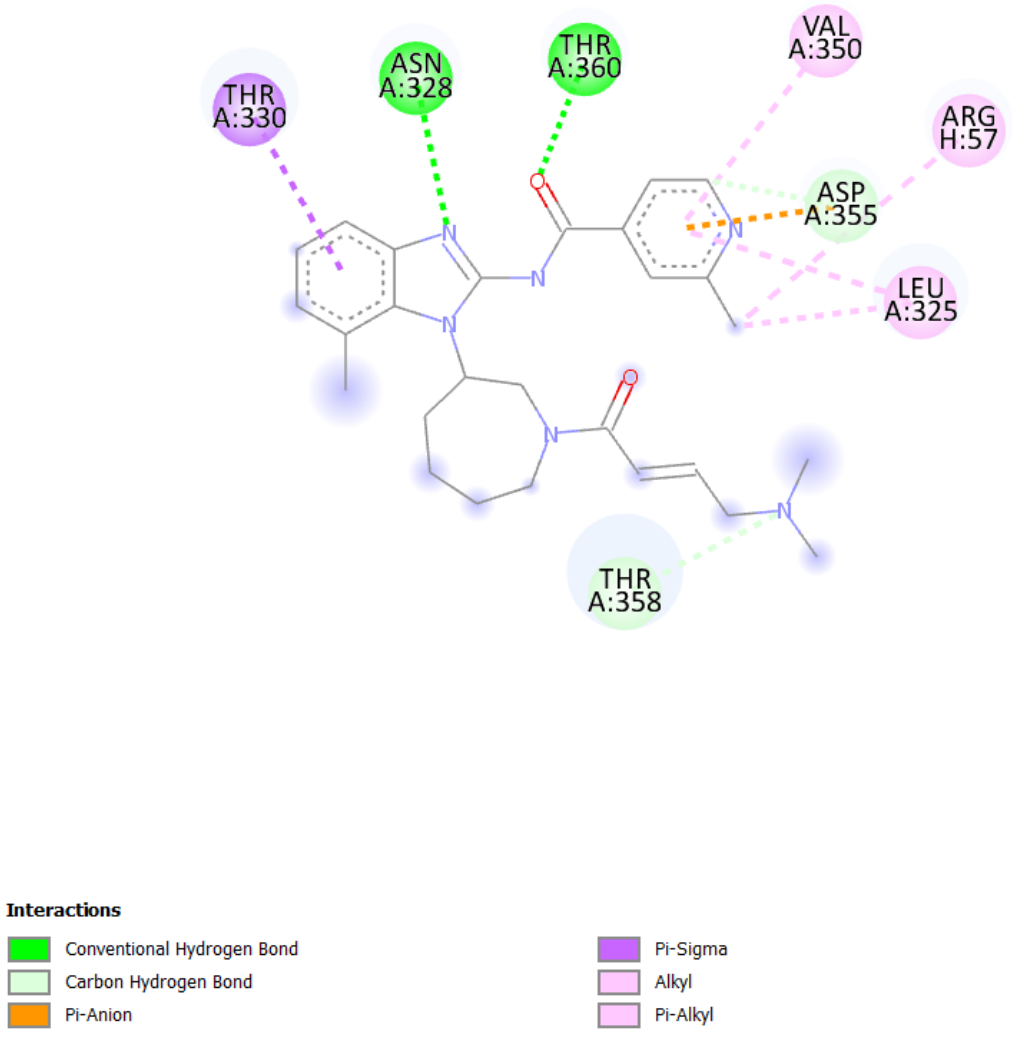


Figure 5:4 2D Structure of Ligand-Protein Interaction {Ligand is Nazartinib and Protein is EGFR(4uv7)}

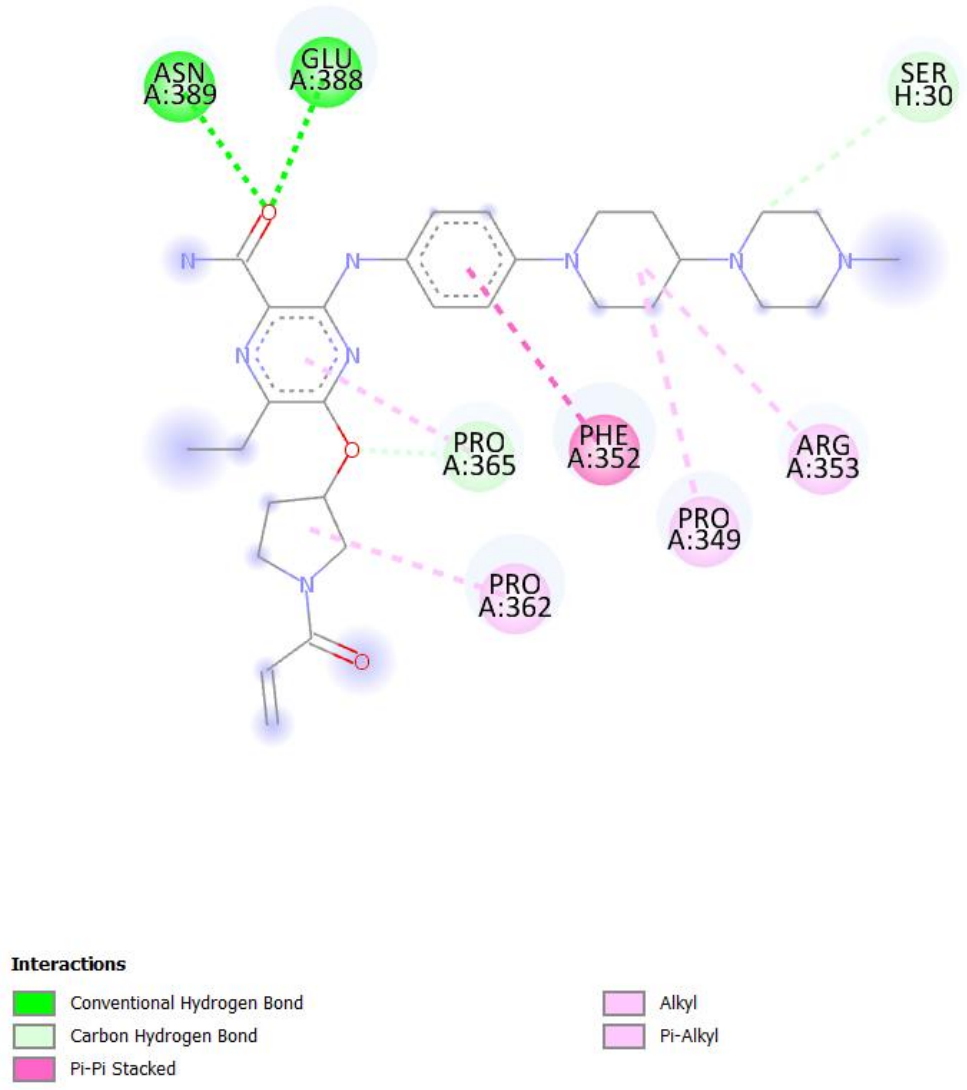


Figure 5:5 2D Structure of Ligand-Protein Interaction {Ligand is Naquotinib and Protein is EGFR(4uv7)}

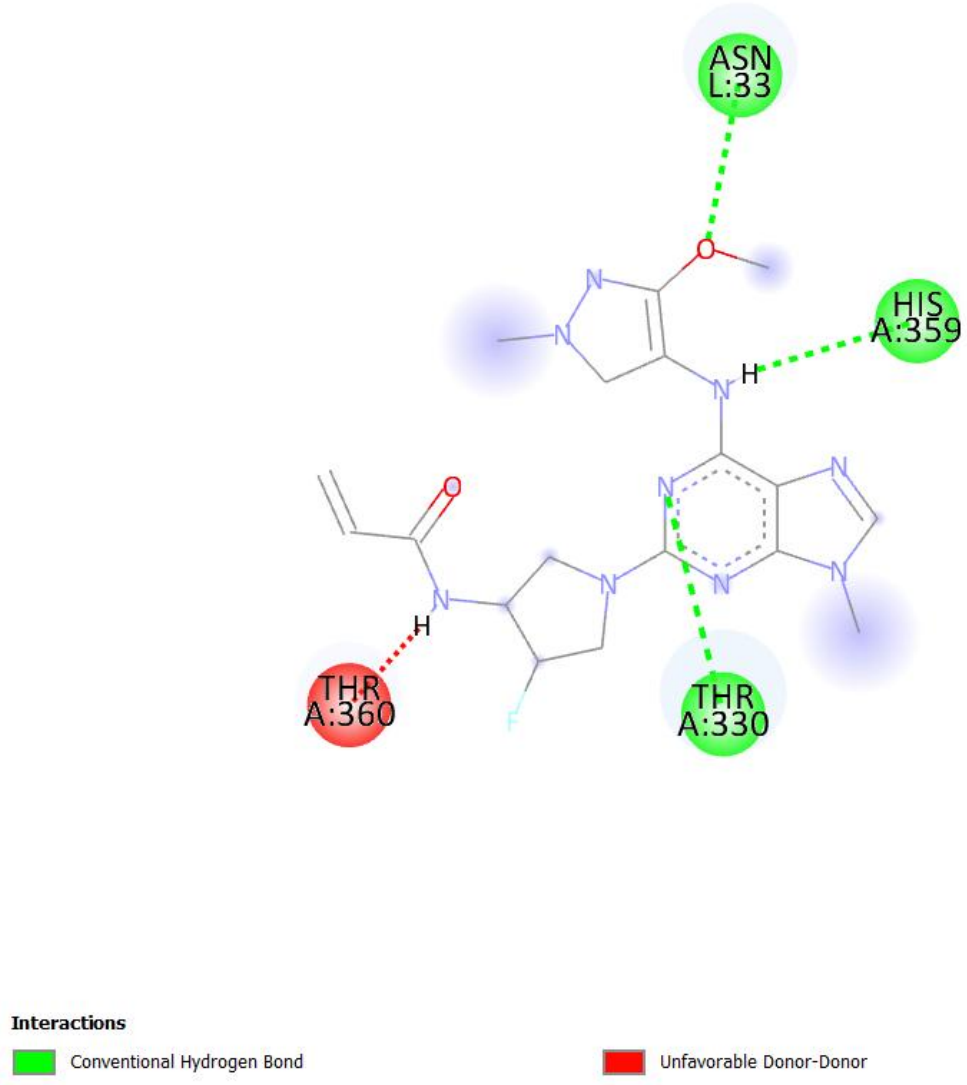


Figure 5:6 2D Structure of Ligand-Protein Interaction {Ligand is Mavelertinib and Protein is EGFR(4uv7)}

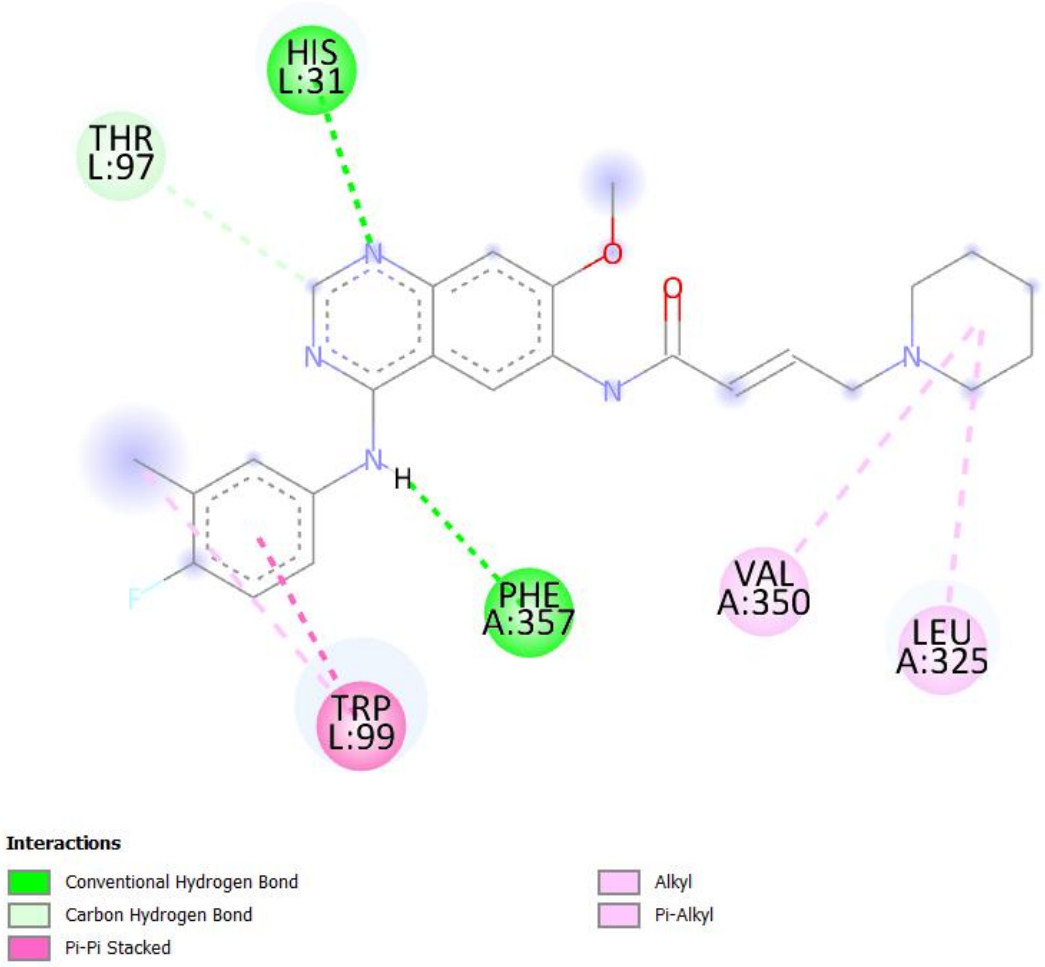


Figure 5:7 2D Structure of Ligand-Protein Interaction {Ligand is Dacomitinib and Protein is EGFR(4uv7)}

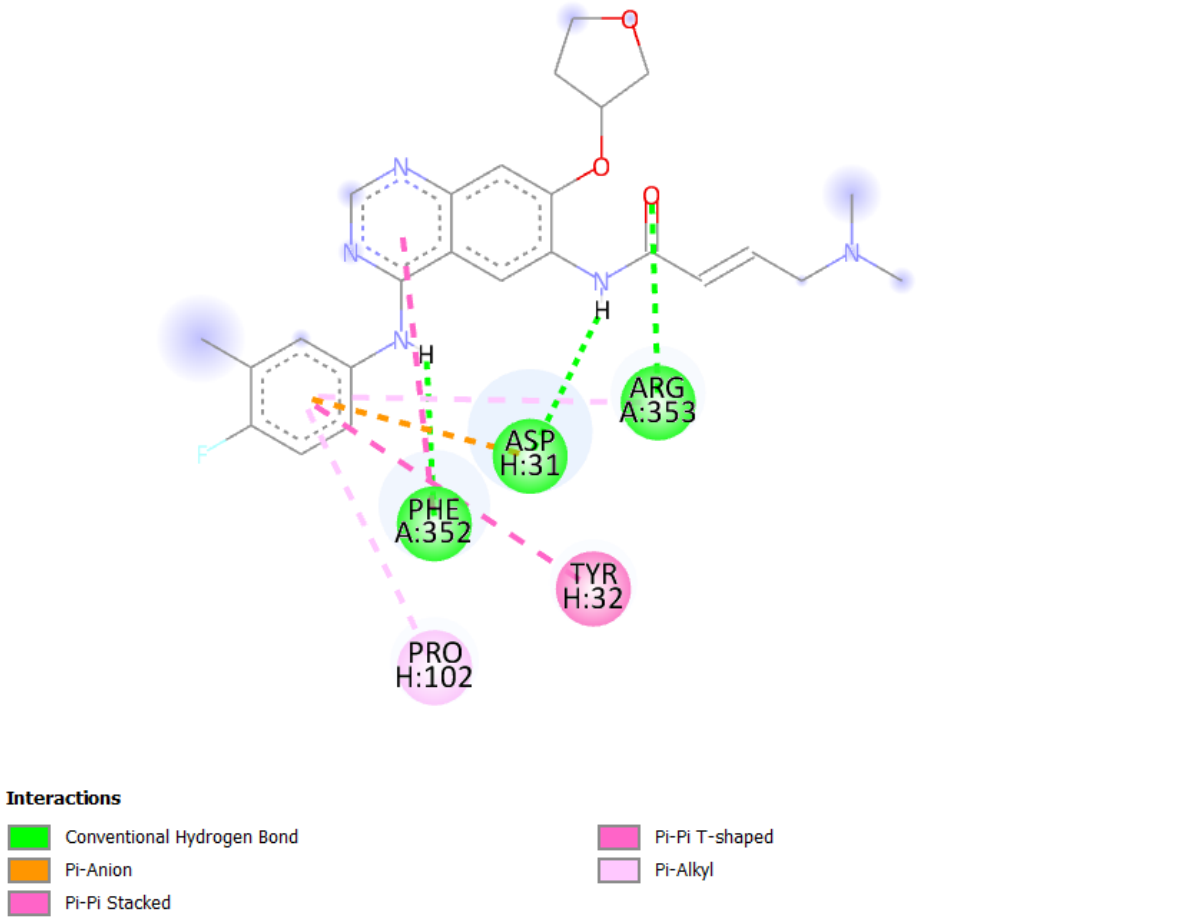


Figure 5:8 2D Structure of Ligand-Protein Interaction {Ligand is Afatinib and Protein is EGFR(4uv7)}

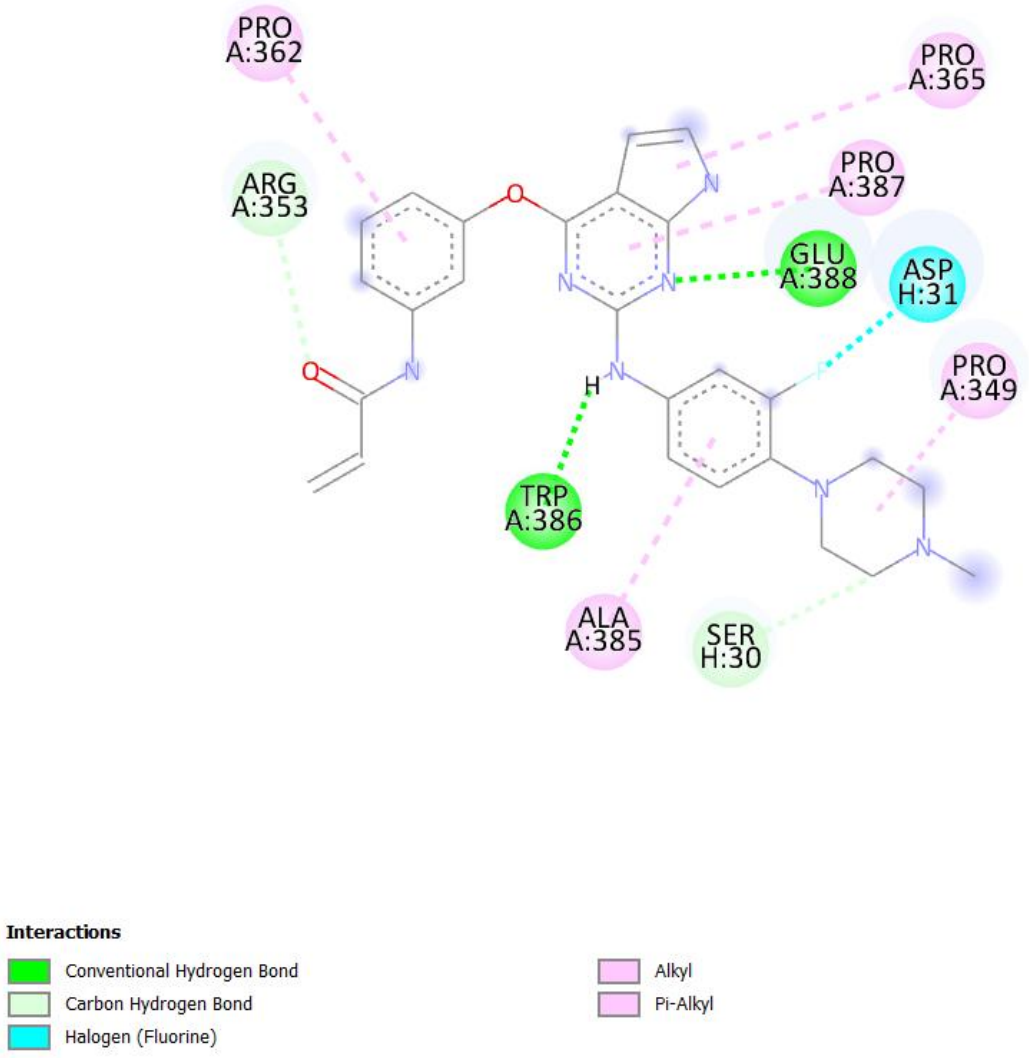


Figure 6:9 2D Structure of Ligand-Protein Interaction {Ligand is Abitinib and Protein is EGFR(4uv7)}

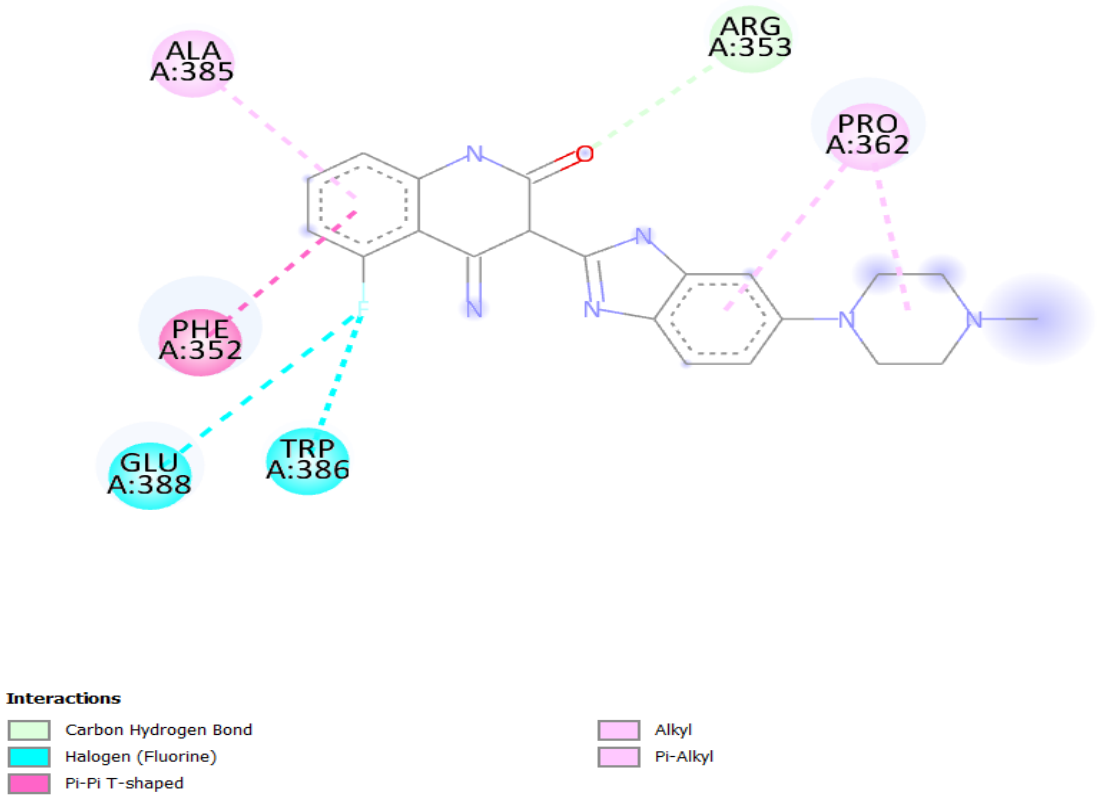
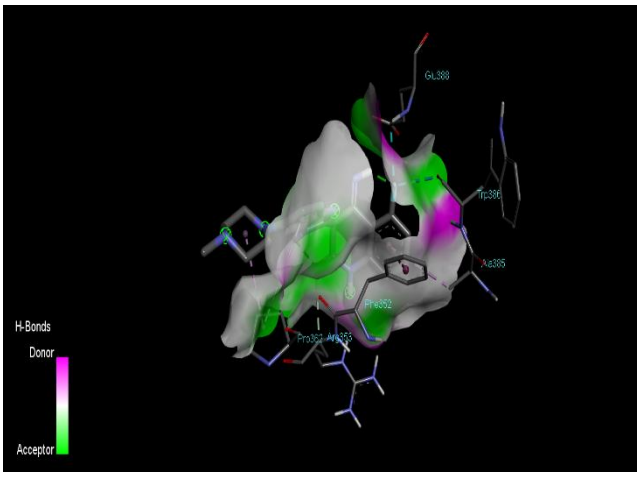


Figure 5:10 2D Structure of Ligand-Protein Interaction {Ligand is TKI 258 and Protein is EGFR(4uv7)}

3 D Ligand-Protein interaction Structure



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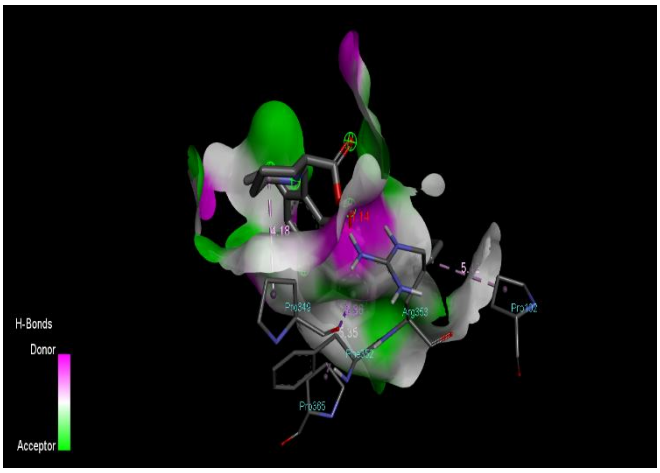

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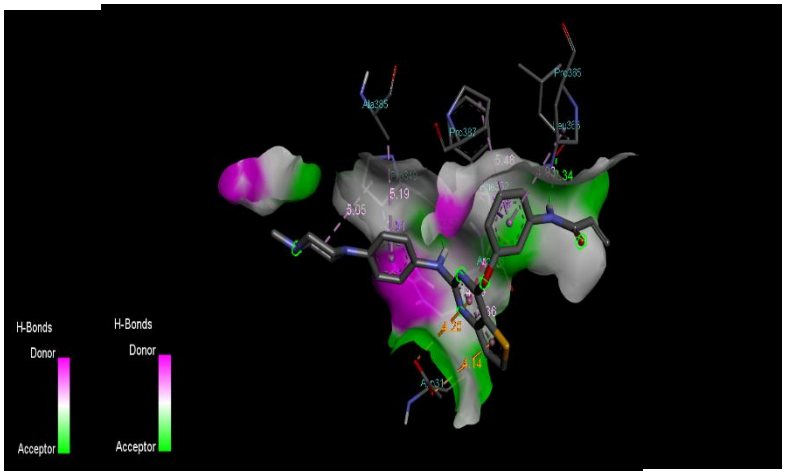

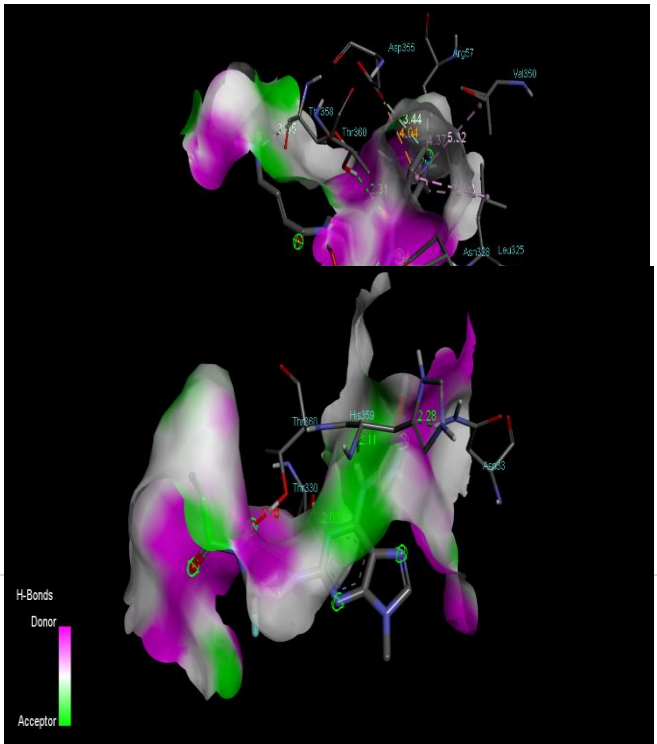

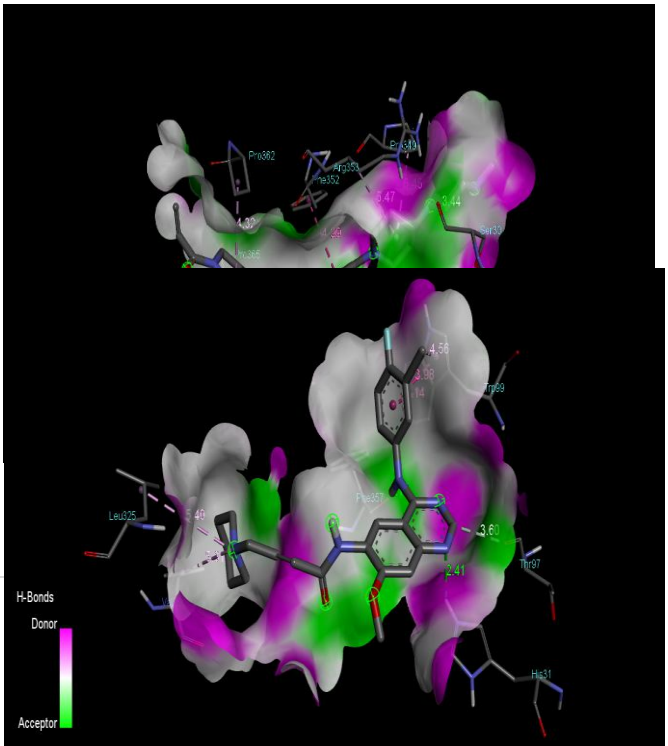

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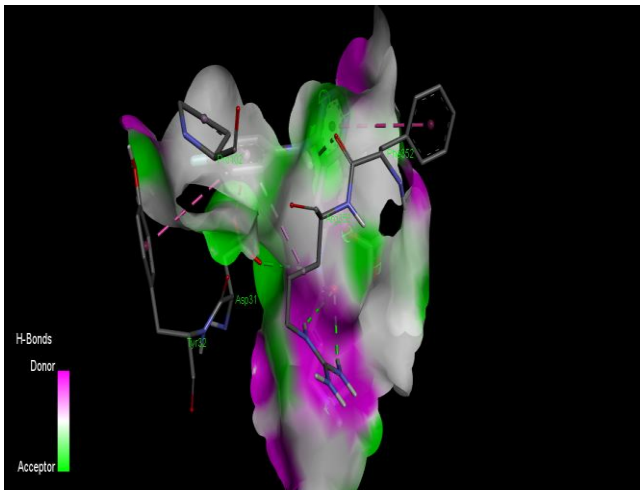

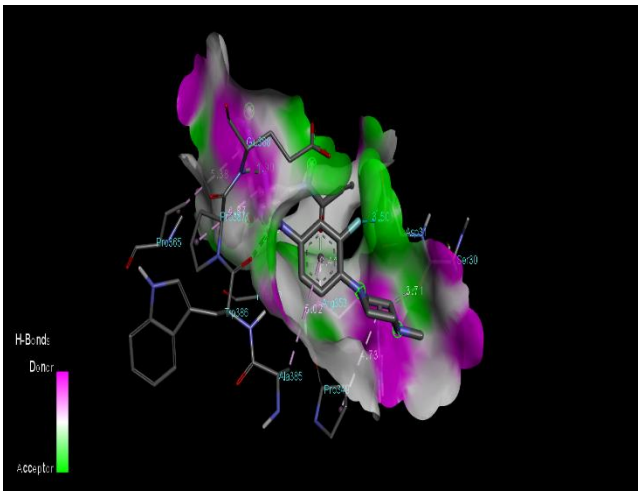

I

J

Figure 5:11 these figures are showing some different inhibitor which bind with protein (EGFR Extracellular domain) A. TKI258, B. PDL-1, C. Osimertinib, D. Olmutinib, E. Nazartinib, F. Naquotinib, G. Mavelertinib, H. Dacotinib, I. Afatinib, J. Abitinib

Conclusion

NSCLC is major disease in this time many of the people suffered by NSCLC. It is due to Mutation in EGFR. There are many medications available with different median PFS (Progressive free survival). EGFR is the key target in NSCLC we have taken as a target and screened with many ligands, which I have retrieved from PUBCHEM. We have started with collecting data of ligand then start molecular docking and to get different binding affinity

with different hits of each inhibitor. We have selected the inhibitor on the bases of binding affinity and after visualize the interaction with the help of DSV and get the information of amino acid residue when ligand attached with protein or region of binding at which position. They all structure represent Amino acid at different position on protein where its bind with ligand. We get four inhibitor which have maximum binding affinity (kcal/mol) these are; afatinib (PubChem ID 10184653), dacomitinib (PubChem ID 11511120), olmutinib (PubChem ID 54758501) and TKI258 (PubChem ID 135398510). And according to ZINC database the ZINC3976838, ZINC72266312, ZINC198970879 AND ZINC3816310.

Appendix's

Virtual screening and protein-ligand based molecular docking studies for the major protein (EGFR) in non-small cell lung cancer (NSCLC)

Appendix 1: List of Figures

Appendix 2: List of Tables

Appendix 3: List of Abbreviation

References

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