

In-Silico drug design of novel indole-Pyrazole Derivatives as Anti Lung Cancer Agents

BINU VARGHESE*, NANCY THOMAS,
ABELJOSE, ANKITHPA, AHALYAMBHASI, ARYASANTHOSH, NANDHA
NAKS

Triveni Institute of pharmacy, Kechery, Thrissur, Kerala

Corresponding Author: BINU VARGHESE, MPHARM, ASSOCIATE PROFESSOR, Department OF Pharmaceutical Analysis

Date of Submission: 01-04-2026

Date of Acceptance: 10-04-2026

ABSTRACT

Cancer is a condition marked by abnormal cell proliferation that has the potential to invade or indicate other health issues. Human beings are affected by more than 100 different types of cancer. Lung cancer is a malignant neoplasm characterized by uncontrolled proliferation of abnormal cells in the lung tissue and remains one of the leading causes of cancer-related mortality worldwide. Non-small cell lung cancer (NSCLC) accounts for nearly 85% of all lung cancer cases and is commonly associated with genetic alterations such as EGFR mutations and ALK and ROS1 rearrangements, which play a crucial role in tumor progression and survival. Therefore the focus of our research is to identify potential ligands targeting EGFR, ALK, and ROS1 proteins involved in NSCLC. Indole-pyrazole derivatives were used as ligands in this study. The target proteins were obtained from the Protein Data Bank (PDB) and were used for interaction studies between designed ligands and target protein. The selected Indole-Pyrazole derivatives undergoes *in-silico* molecular modelling studies. In docking studies, ligands were docked against EGFR, ALK and ROS1 receptors. Docking studies and hydrogen bond interaction were performed using PyRx and Discovery studio visualizer. All the designed ligands show good binding affinity toward the targets. Among the selected targets, compounds PI48, PI38 and PI21 showed the highest docking score against ALK; compound PI38 exhibited the best docking score against ROS1; and compound PI28 demonstrated the highest docking score against EGFR, indicating their potential as promising lead molecules for the treatment of NSCLC.

KEYWORDS:

NSCLC, EGFR inhibitors, ALK inhibitors, ROS1 inhibitors, Molecular docking, Protein Data Bank, PyRx, Indole-Pyrazole derivatives.

I. INTRODUCTION

INDOLE

Indole is a planar bicyclic molecule in which a benzene ring is fused to 2,3-positions of a pyrrole ring. According to Huckles rule, indole is aromatic in nature [1]. Indole was synthesized for the first time by Adolf Von Baeyer from the oxidation of indigo [2]. Various indole derivatives can be synthesized by substituting the indole ring at the N-1, C-2 to C-6 or C-7 positions with the goal of improving its characteristics [3].

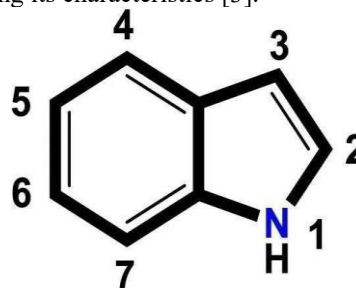


Figure 1: Structure of Indole with positions

SAR of Indole

The indole is planar aromatic structure enables π - π stacking interactions and the pyrrolic N-H group facilitates hydrogen bonding, both essential for biological activity [4], [5]. The free N-H at N-1 is important for receptor binding, while N-alkylation may increase lipophilicity but bulky groups can reduce activity due to steric hindrance [6], [7]. Substitution at C-2 enhances metabolic stability and selectivity [8], and attachment of a pyrazole ring at C-3 improves binding affinity and molecular rigidity [9], [10]. Electron-donating groups at C-4 enhance antioxidant and anti-inflammatory activity [11], whereas electron-withdrawing groups at C-5 improve anticancer and antimicrobial effects [12].

Substitutions at C-6 and C-7 further influence receptor affinity, pharmacokinetics, and lipophilicity, contributing to overall biological activity [13], [14].

PYRAZOLE

Pyrazole is defined as an one of the most important five-membered heterocyclic ring compound that contains three carbon atoms and two adjacent nitrogen atoms substituted at Ortho position. Pyrazole was firstly synthesized from acetylene and diazomethane by the German chemist Hans Von Pechmann in the year 1898 [15].

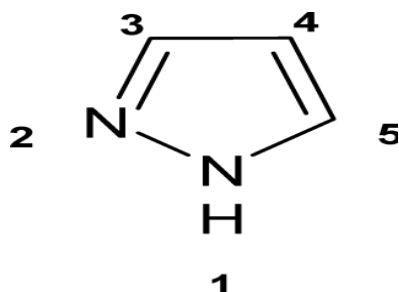


Figure 2: Structure of Pyrazole with positions.

SAR of Pyrazole

Substitution at different positions of pyrazole markedly affects biological activity. Modification at N-1 affects basicity, hydrogen bonding, metabolic stability, and receptor binding, where small substituents improve activity while

bulky groups may reduce potency due to steric hindrance. Substitution at N-2, though less common, can influence binding and selectivity, and rigid groups at this position enhance antimicrobial and anti-tubercular activity. The C-3 position is a major site for substitution; phenyl or heteroaryl groups, particularly mono- or dichloro-substituted phenyl rings, enhance anticancer activity, whereas bulky groups decrease activity. Substituents at C-4 affect potency, selectivity, and pharmacokinetics, with suitable groups improving target binding potency. Overall, careful substitution on the indole ring is essential for optimizing biological activity. Modification at C-5 plays an important role in lipophilicity and metabolic stability, and substituted phenyl rings at this position enhance anticancer activity, while unsubstituted C-5 may lower.

CANCER

Cancer is not just one disease, but a generic term used to encompass a group of more than two hundred diseases sharing common characteristics. Cancers (carcinomas) are characterized by their unregulated growth and spread of cells to other parts of the body [16], [17]. Cancer can result from abnormal proliferation of any of the different kinds of cells in the body, so there are more than a hundred distinct types of cancer, which can vary substantially in their behavior and response to treatment.

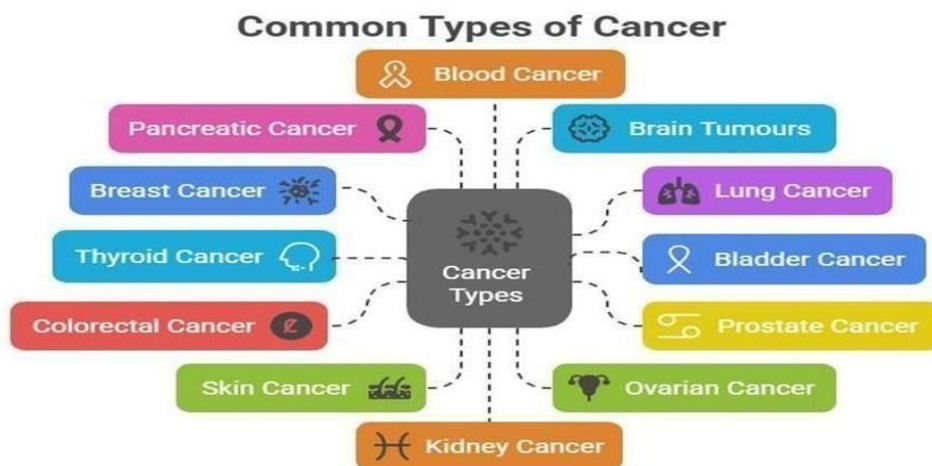


Figure 3: Common types of cancer

LUNG CANCER

Lung cancer remains one of the most frequently diagnosed malignancies and the leading cause of cancer-related mortality worldwide, representing a major global health burden. According to recent international cancer statistics (IARC 2022), approximately 2.5 million new cases were reported globally, accounting for a significant proportion of total cancer incidence. Furthermore, lung cancer is responsible

for nearly 1.8 million deaths annually, reflecting its aggressive nature and the challenges associated with early diagnosis and effective treatment.

Lung cancer is broadly classified into two major histological types:

1. **Small Cell Lung Cancer (SCLC)**
2. **Non-Small Cell Lung Cancer (NSCLC).**

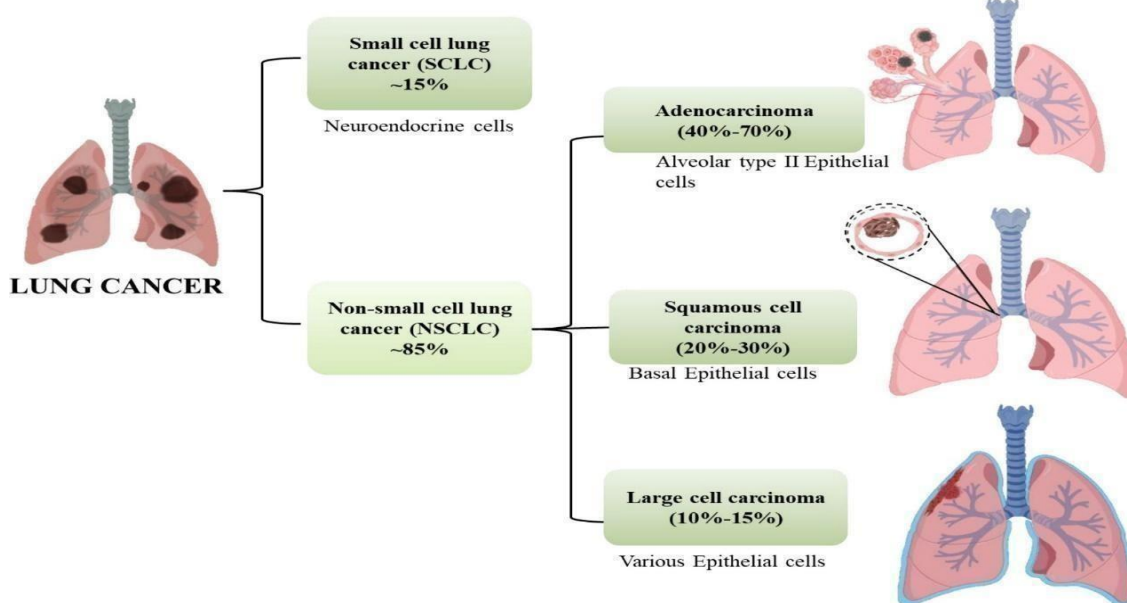


Figure 4: Types of lung cancer

1. Small Cell Lung Cancer (SCLC)

Small cell lung cancer is a highly aggressive and rapidly growing type of lung cancer, accounting for about 20% of all lung cancer cases [18]. It is strongly associated with cigarette smoking and is usually diagnosed at an advanced stage because it spreads quickly to other parts of the body [19]. Microscopically, the cancer cells are smaller than those of non-small cell lung cancer and have a characteristic appearance. SCLC commonly originates in the central part of the lung, particularly along the walls of large bronchi, and forms fast-growing tumors that metastasize widely [20],[21].

2. Non-Small Cell Lung Cancer (NSCLC)

Non-small cell lung cancer is the most common type of lung cancer, representing approximately 80% of all cases. It is broadly classified into three main subtypes: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. Adenocarcinoma is the most frequent subtype, especially in women and non-smokers, and typically arises in the peripheral regions of the lung [22],[23]. Squamous cell carcinoma commonly develops in the central bronchi and accounts for 30–40% of cases [24],[25]. Large cell carcinoma consists of poorly differentiated large tumor cells and is the least common subtype. The TNM classification system is used to determine the stage of NSCLC based on tumor size, lymph node involvement, and distant metastasis, which guides prognosis and treatment decisions [26],[27].

❖ Stages of Lung Cancer

- Stage I: Localized within lung.
- Stage II: Spread to nearby lymph nodes or multiple tumors in same lobe.
- Stage III: Spread to nearby structures/lymph nodes.
- Stage IV: Metastasis to distant organs [28]

❖ Signs and Symptoms 65

Cough, Dyspnoea, Chest pain or Discomfort, Wheezing, Fatigue, Weight loss, Haemoptysis, Loss of appetite, Shoulder pain, Superior vena cava syndrome, Horner's syndrome.

❖ Pathogenesis of Lung Cancer

Lung cancer develops through a multistep process involving genetic mutations, epigenetic alterations, and abnormal cell signaling that lead to uncontrolled

cell growth [29]. Long-term exposure to carcinogens such as tobacco smoke and pollutants causes DNA damage in bronchial epithelial cells [30]. Mutations in oncogenes like KRAS, EGFR, and ALK activate pathways such as MAPK and PI3K/AKT, promoting cell proliferation and survival [31]. Inactivation of tumor suppressor genes including TP53 and RB1 removes normal growth control mechanisms [32]. In NSCLC, EGFR mutations and ALK/ROS1 rearrangements are common [33], while SCLC is strongly associated with TP53 and RB1 loss [34]. Chronic inflammation, oxidative stress, and epigenetic changes further enhance tumor progression, angiogenesis, and metastasis, which are key hallmarks of lung cancer [35],[36],[37].

❖ Treatment of lung cancer

The treatment of lung cancer depends on the type of cancer and the stage of the disease. The main treatment options include surgery, chemotherapy, and radiotherapy, either alone or in combination. Surgery involves removal of the tumor and may include wedge resection, segmental resection, lobectomy, or pneumonectomy. Chemotherapy uses anticancer drugs such as alkylating agents, antimetabolites, plant alkaloids, and antitumor antibiotics to destroy cancer cells; it is the primary treatment for small cell lung cancer and is also used in non-small cell lung cancer before or after surgery or along with radiotherapy [38][39]. Radiotherapy uses high-energy X-rays to kill cancer cells and may be given externally (external beam radiation) or internally (brachytherapy) [40]. Combination therapy (chemoradiation) is often used to improve treatment outcomes.

Materials and methods

In-Silico Molecular Modelling

Molecular modelling is a computational approach used to design and discover new drugs for different diseases.

Software used

The software used includes Chemsketch, Molinspiration, SwissADME, ProTox-3.0, PyRx Autodock, Vina and Discovery studio visualizer.

Methodology of molecular docking

Ligand designing and optimization

Based on literature review, Indole and pyrazole were selected. A total 30 ligands were designed by introducing different substituents at 2nd, 3rd or 4th position of the phenyl ring attached to

the 3rd position of the pyrazole ring. 2D and 3D structures were drawn using chemsketch. Ligands were optimized based on Molecular weight, Lipophilicity, size and shape.

Lipinski Rule of five

Drug-likeness of ligands was evaluated using Molinspiration. Open Molinspiration website and select calculation of molecular properties. Draw the molecule in the JME window and calculate save properties.

ADME parameter prediction

Absorption, Distribution, Metabolism and Excretion properties were predicted using SwissADME. Molecules were submitted in SMILES format or drawn using molecular sketch. Calculations were initiated by clicking the Run button. Results were displayed on the same webpage and ADME properties were grouped for each molecule.

Target Identification and Retrieval

Protein crystal structures were obtained from the Protein Data Bank (PDB)

SL NO	TARGETS	PDB ID
1	Anaplastic Lymphoma Kinase inhibitor	2XP2
2	ROS proto-oncogene tyrosine kinase inhibitor	9QEK
3	Epidermal Growth Factor Receptor inhibitor	4WQK

Table 1: selected targets and its PDB ID

Target therapy is effective in treating Non-Small Lung Cancer (NSCLC). These selected targets were ALK, ROS1, EGFR. ALK inhibitors block abnormal ALK proteins involved in tumour growth by binding to the ATP-binding site, preventing cancer cell proliferation. EGFR is a tyrosine kinase receptor involved in cell growth. EGFR inhibitors block ATP binding and inhibit tumour growth in EGFR-mutated lung cancer. ROS1 gene rearrangements cause cancer progression. ROS1 inhibitors suppress tumour growth and induce apoptosis.

Active site Identification and Preparation

Proteins were loaded into Discovery studio Visualizer. Single protein chain was selected and cleaned. Active site residues were identified using co-crystallized ligands. Residues were confirmed using literature data. Missing hydrogen atoms were added. ATOM list of active site residues were prepared using Discovery studio visualizer.

Energy minimization

Proteins were minimized using PyRx AutoDock vina. Hydrogen-added proteins were optimized. Energy minimization corrected unfavourable conformations. Ensured stable protein structures for docking.

Molecular Docking

Docking studies were performed using PyRx AutoDock Vina. Docking steps are involved by Download PyRx software. Load protein and ligand files. Convert files to PDBQT format. Define docking grid box around active site. Run docking calculations and binding affinity values obtained (kcal/mol). More negative values can be better binding affinity. Docked complexes were analyzed using Discovery Studio Visualizer. Binding orientation and interactions were visually inspected.

Ligand series can be Designed

The designed compounds were Indole-Pyrazole hybrids with different electron-donating or electron-withdrawing substituents at the 2nd, 3rd or 4th position of the phenyl ring.

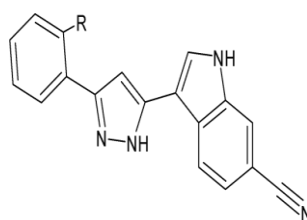


Figure 5: General structure at 2nd position of phenyl ring

SL NO	COMPOUNDS	R
1	PI21	OH
2	PI22	Cl
3	PI23	Br
4	PI24	F
5	PI25	I
6	PI26	CH ₃
7	PI27	OCH ₃
8	PI28	NO ₂
9	PI29	NH ₂
10	PI210	N(CH ₃) ₂

Table2: Targeted analogues at 2nd position

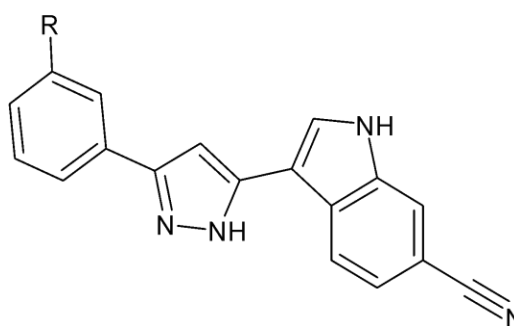


Figure6: General structure at 3rd position

SL NO	COMPOUNDS	R
1	PI31	OH
2	PI32	Cl
3	PI33	Br
4	PI34	F
5	PI35	I
6	PI36	CH ₃
7	PI37	OCH ₃
8	PI38	NO ₂
9	PI39	NH ₂

Table3: Targeted analogues at 3rd position

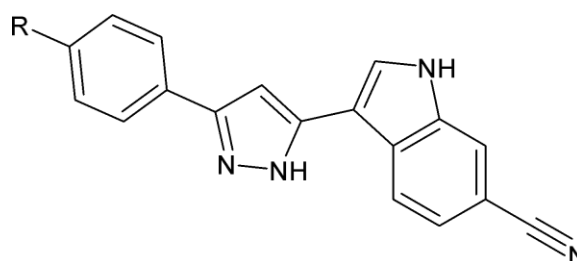


Figure7:Generalstructureat4thposition

SL NO	COMPOUNDS	R
1	PI41	OH
2	PI42	Cl
3	PI43	Br
4	PI44	F
5	PI45	I
6	PI46	CH3
7	PI47	OCH3
8	PI48	NO2
9	PI49	NH2
10	PI410	N(CH3)2

Table4:targetedanaloguesat4thposition

II. RESULTS AND DISCUSSION

INSILICO MOLECULAR MODELING

Computational analysis was done to compute ligand protein binding affinity of the compound. The interactions showed efficient docking score which was considered as a good score ligand protein interactions. This study explored action of indole – pyrazole derivatives against their target proteins. In-silico molecular modelling helped to get the preliminary findings about the lead compound. This information led to develop these useful compounds of simple structure exhibiting anti- lung cancer activity.

Drug-likeness assessment

The derived analogues were evaluated for their drug-likeness. It was done by calculating the parameters like Lipinski-rule of 5 and some of their extension parameters like number of rotatable bonds and TPSA. The drug-likeness assessments of the compounds were shown in the following table 5:

Compound Code	CLogP	Molecular Weight	nON	nOHNH	nrotb	TPSA	nviolations
PI41	3.07	300.32	5	3	2	88.49	0
PI42	4.22	318.77	4	2	2	68.27	0
PI43	4.36	363.22	4	2	2	68.27	0
PI44	3.71	302.31	4	2	2	68.27	0
PI45	4.63	410.22	4	2	2	68.27	0
PI46	3.99	298.35	4	2	2	68.27	0
PI47	3.60	314.35	5	2	3	77.50	0
PI48	3.50	329.32	7	2	3	114.09	0
PI49	2.62	299.34	5	4	2	94.29	0
PI410	3.65	327.39	5	2	3	71.50	0
PI31	3.04	300.32	5	3	2	88.49	0

PI32	4.20	318.77	4	2	2	68.27	0
PI33	4.33	363.22	4	2	2	68.27	0
PI34	3.69	302.31	4	2	2	68.27	0
PI35	4.61	410.22	4	2	2	68.27	0
PI36	3.97	298.35	4	2	2	68.27	0
PI37	3.58	314.35	5	2	3	77.50	0
PI38	3.48	329.32	7	2	3	114.09	0
PI39	2.60	299.34	5	4	2	94.29	0
PI310	3.62	327.39	5	2	3	71.50	0
PI21	3.28	300.32	5	3	2	88.49	0
PI22	4.18	318.77	4	2	2	68.27	0
PI23	4.31	363.22	4	2	2	68.27	0
PI24	3.66	302.31	4	2	2	68.27	0
PI25	4.58	410.22	4	2	2	68.27	0
PI26	3.95	298.35	4	2	2	68.27	0
PI27	3.56	314.35	5	2	3	77.50	0
PI28	3.46	329.32	7	2	3	114.09	0
PI29	2.98	299.34	5	4	2	94.29	0
PI210	3.60	327.39	5	2	3	71.50	0

Table 5: Drug-likeness assessment of the Indole-Pyrazole derivatives

These results showed that value of all derivatives relied within the optimal range.all the compoundshavemolecularweightlessthan500daltons andpossessednumberof hydrogenbonddonorsandhydrogenbondacceptorsfall theanalogues ≤ 5 and ≤ 10 respectively.Allthevaluesofpartitioncoefficient andnumber of rotatable bonds were coming under the limit of 5 and 10.

ADMETparametersbymeansofSwissADME&ProTox3.0

SwissADME was a computer software system for predictive modeling of absorption, distribution, metabolism and elimination of chemical substances in the human body. ProTox 3.0 is an advanced, web-based virtual toxicity lab used by toxicologists and medicinal chemists to predict how small molecules and chemical compounds will affect the human body. The results for the ADMET parameters by means of SwissADME were shown in the table 6:

SL NO	Compound Code	Absorption level	Solubility Level	Metabolism	Toxicity
1	PI41	High	Soluble	Yes	No
2	PI42	High	Soluble	Yes	No
3	PI43	High	Soluble	Yes	No
4	PI44	High	Soluble	Yes	No
5	PI45	High	Soluble	Yes	No
6	PI46	High	Soluble	Yes	No
7	PI47	High	Soluble	Yes	No
8	PI48	High	Soluble	Yes	No
9	PI49	High	Soluble	Yes	No
10	PI410	High	Soluble	Yes	No
11	PI31	High	Soluble	Yes	No
12	PI32	High	Soluble	Yes	No
13	PI33	High	Soluble	Yes	No

14	PI34	High	Soluble	Yes	No
15	PI35	High	Soluble	Yes	No
16	PI36	High	Soluble	Yes	No
17	PI37	High	Soluble	Yes	No
18	PI38	High	Soluble	Yes	No
19	PI39	High	Soluble	Yes	No
20	PI310	High	Soluble	Yes	No
21	PI21	High	Soluble	Yes	No
22	PI22	High	Soluble	Yes	No
23	PI23	High	Soluble	Yes	No
24	PI24	High	Soluble	Yes	No
25	PI25	High	Soluble	Yes	No
26	PI26	High	Soluble	Yes	No
27	PI27	High	Soluble	Yes	No
28	PI28	High	Soluble	Yes	No
29	PI29	High	Soluble	Yes	No
30	PI210	High	Soluble	Yes	No

Table6:ResultsofADMETparameter

Molecular docking

Indole-pyrazole derivatives were subjected to molecular docking against ALK, ROS 1 and EGFR receptors. *In-silico* studies were done by using different softwares like PyRx, ChemsKetch, Molinspiration, SwissADME and Discovery studiovisualizer. PyRx serves as primary docking tool. The prepared ligands were validated by docking operation using PyRx and Discovery studio visualizer. The docking score of the ligands were compared with reference standard.

Docking scores of Indole-pyrazole derivatives targeting ALK as anti-lung cancer agents

The docking scores obtained from the preliminary docking program by using PyRx were listed in table 7:

SL NO	COMPOUND CODE	DOCKING SCORE OF ALK(kcal/mol)
1	PI41	-8.2
2	PI42	-8.2
3	PI43	-8.3
4	PI44	-8.0
5	PI45	-8.1
6	PI46	-8.3
7	PI47	-7.9
8	PI48	-8.5
9	PI49	-8.1
10	PI410	-8.4
11	PI31	-8.4
12	PI32	-8.1
13	PI33	-8.1
14	PI34	-7.9
15	PI35	-8.2

16	PI36	-8.3
17	PI37	-7.9
18	PI38	-8.5
19	PI39	-8.4
20	PI310	-8.1
21	PI21	-8.5
22	PI22	-8.4
23	PI23	-8.2
24	PI24	-8.3
25	PI25	-7.9
26	PI26	-8.2
27	PI27	-7.8
28	PI28	-8.4
29	PI29	-8.0
30	PI210	-8.0
31	CRIZOTINIB	-8.0

Table 7: PyRx docking for the designed Indole-Pyrazole derivative targeting ALK as anti-lung cancer agents

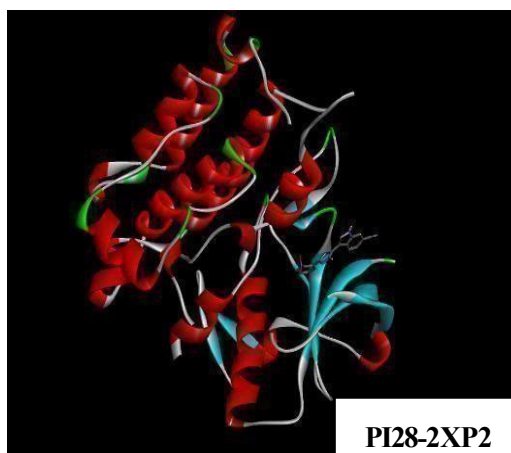
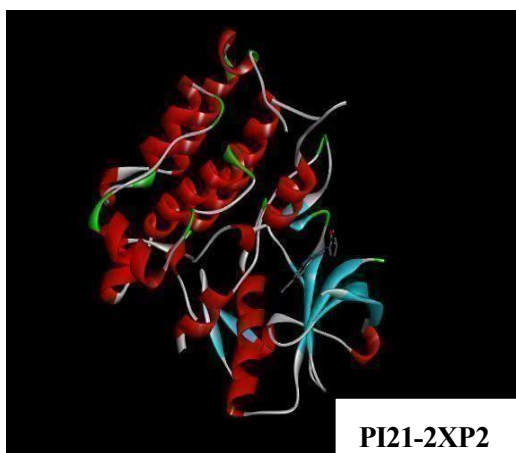
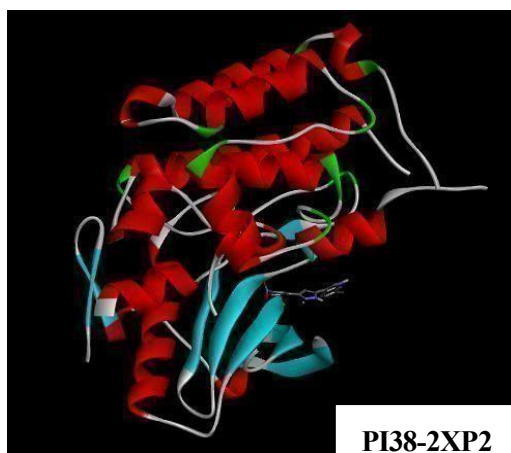
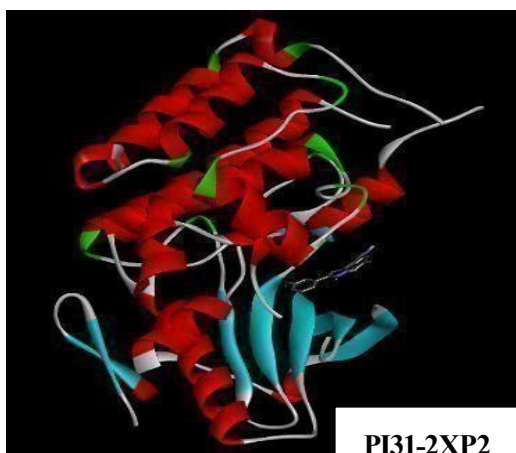
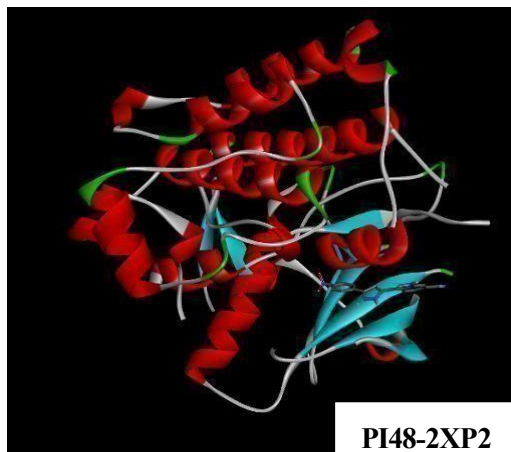
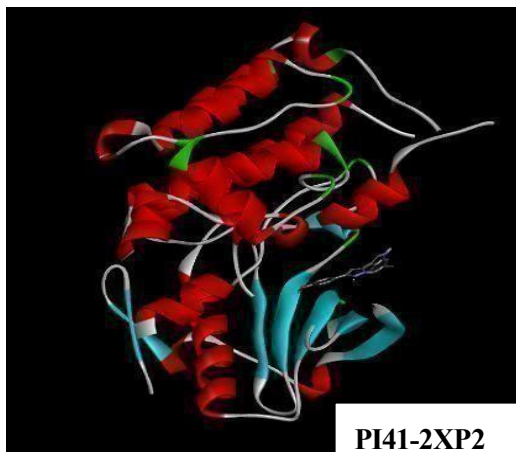
All the compounds were docked against known target. ALK receptor (2XP2) was the NSCLC protein target. In case of binding interactions of protein targets, all the generated ligands showed higher docking score. This can be assured by the pre-screening program of PyRx.

The inhibition of ALK receptors help to stop the constitutive signaling of the EML4-ALK fusion protein, which drives cancer cell proliferation. So the docking score of the compounds targeted ALK were evaluated against the docking score of standard (Crizotinib).

As we go through the docking score of each compound which are the combination of Indole-Pyrazole derivative, almost all the derivatives showed higher docking score than the corresponding drug. PI48, PI38 and PI21 have high docking score (-8.5 kcal/mol). Among them PI27 is having the least score (-7.8 kcal/mol).

If we compare the score between the ligands obtained by the combination of Indole-Pyrazole derivatives, the highest score (-8.5 kcal/mol) was found to be on PI48, PI38, PI21. Whereas, the standard drug which has been available in market is Crizotinib used for NSCLC, whose docking score is -8.0. Therefore, while we compare the score between the compounds PI48, PI38, PI21 and Crizotinib, it seems PI48, PI38 and PI21 has more score than Crizotinib. Therefore, PI48, PI38 and PI21 are best drug for NSCLC.

Some of the ligand-target complex interactions by Discovery studio visualizer were shown in the figure 8:



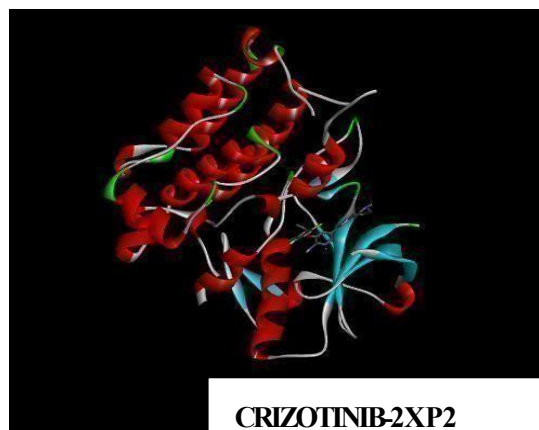
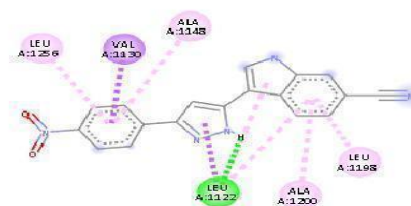
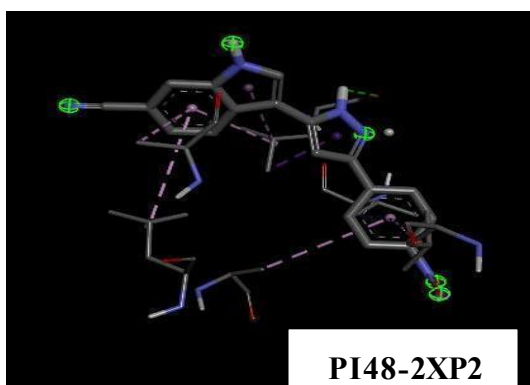
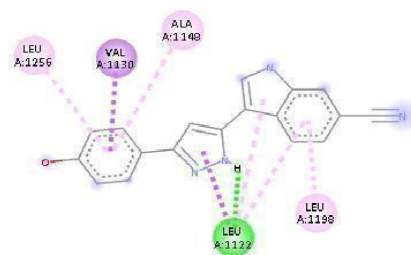
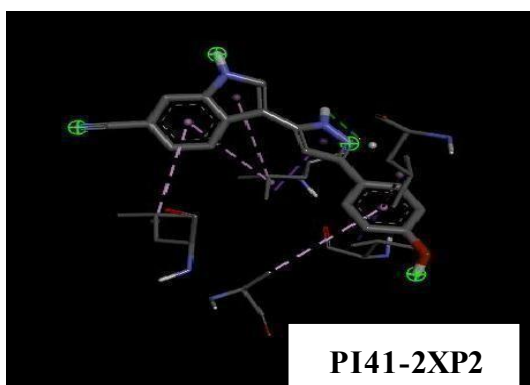
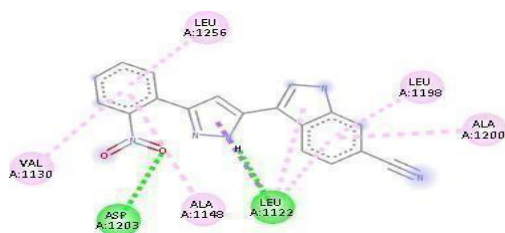
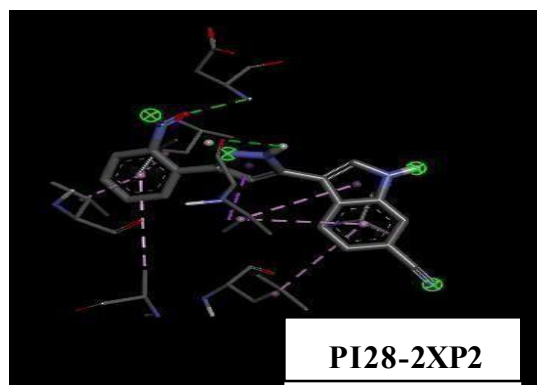
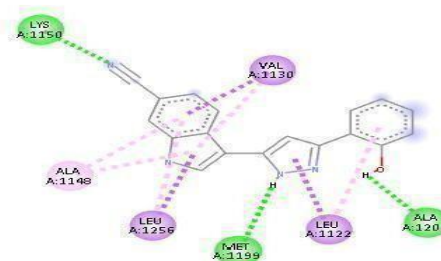
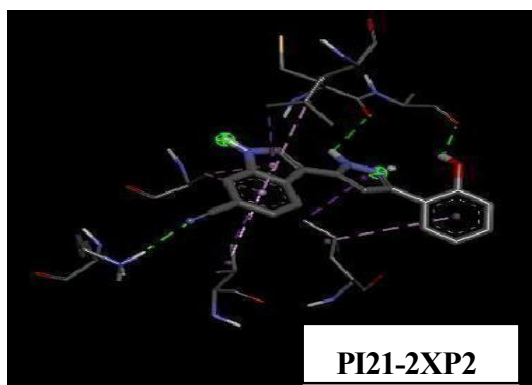
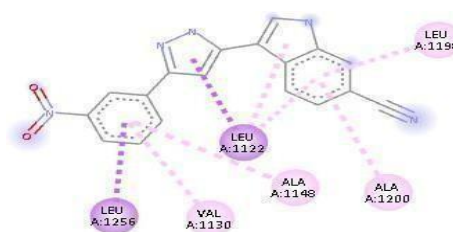
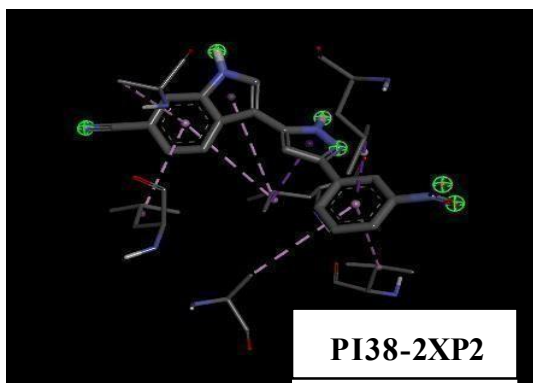
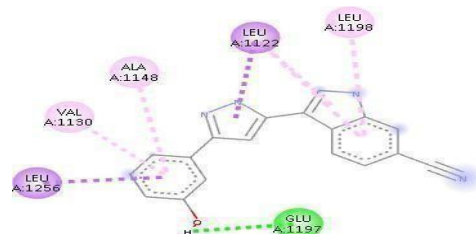
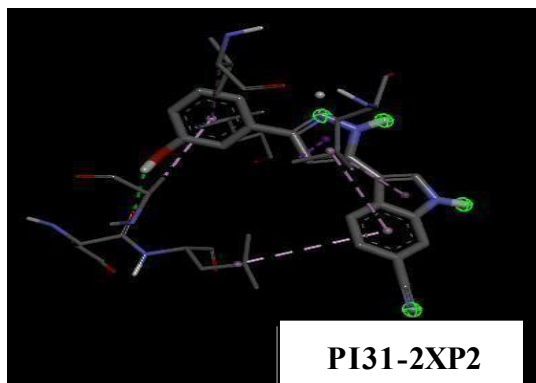


Figure8:Ligand-TargetcomplexinteractionbyDiscoverystudio visualizer

3Dand2Dstructuresofligand-targetcomplexinteractionbydiscoverystudiovisualizer are shown in figure 9:





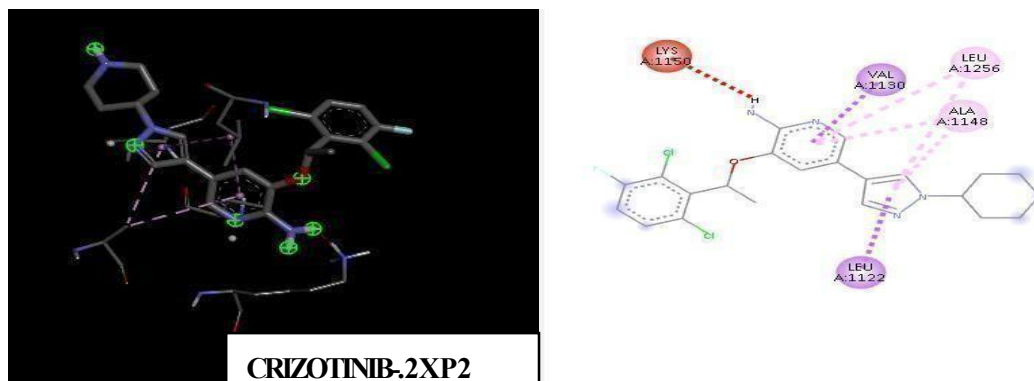
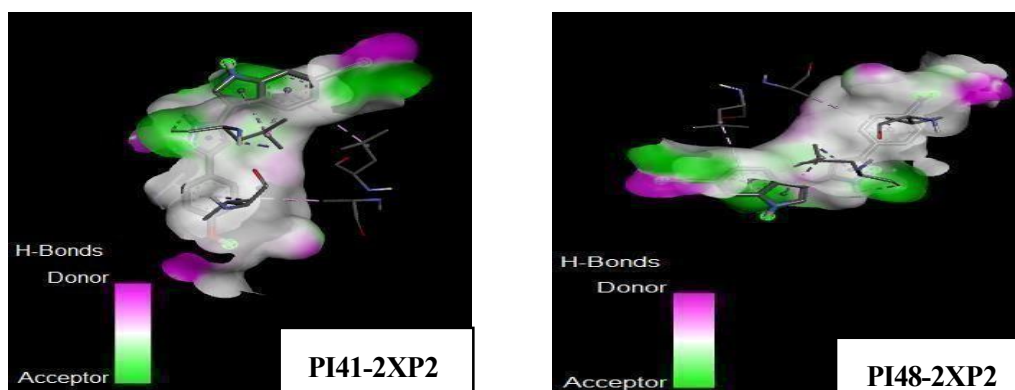


Figure 9 : 3D and 2D structure of ligand-target complex interactions by Discovery studio visualizer.

Number of hydrogen bonding will considerably increase the affinity of ligand- target interaction. PyRx results showed that most of the Indole-Pyrazole derivatives have hydrogen bonding between the ligand-target interactions. Some of the derivatives have more than 1 hydrogen bond for ALK targeted derivatives, commonly LEU:1122. PI21 complex have the highest docking score (-8.5 kcal/mol) as well as highest ligand-target interactions. These hydrogen bond interactions helped to increase the binding energy of ligand-protein interactions .

Some of the ligand-target complexes with hydrogen bond interactions by Discovery studio visualizer were shown in the figure 10:



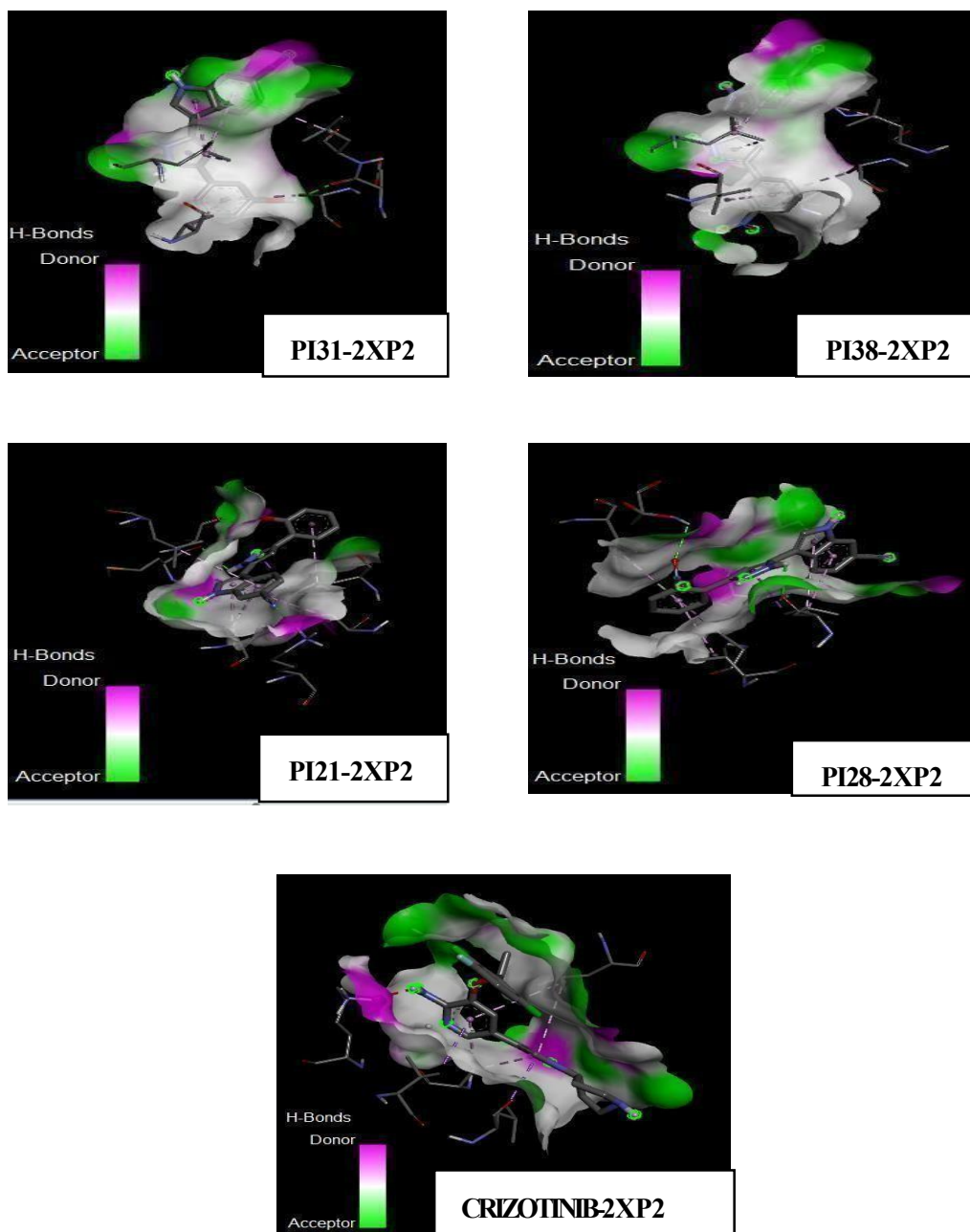


Figure 10: Ligand-target hydrogen bond interactions by Discovery studio visualizer. Docking scores of Indole-pyrazole derivatives targeting ROS1 as anti-lung cancer agents
 The docking scores obtained from the preliminary docking program by using PyRx were listed in the table 8:

SLNO	COMPOUND CODE	DOCKING SCORE OF ROS1(kcal/mol)
1	PI41	-9.1
2	PI42	-9.0
3	PI43	-8.9
4	PI44	-8.5

5	PI45	-9.0
6	PI46	-9.0
7	PI47	-8.3
8	PI48	-9.2
9	PI49	-8.7
10	PI410	-8.8
11	PI31	-9.0
12	PI32	-8.6
13	PI33	-8.8
14	PI34	-8.7
15	PI35	-9.2
16	PI36	-9.0
17	PI37	-8.4
18	PI38	-9.4
19	PI39	-8.9
20	PI310	-8.7
21	PI21	-9.2
22	PI22	-8.1
23	PI23	-8.7
24	PI24	-8.4
25	PI25	-9.1
26	PI26	-7.3
27	PI27	-8.6
28	PI28	-9.1
29	PI29	-8.8
30	PI210	-8.3
31	TALETRECTINIB	-8.1

Table 8: PyRx docking for the designed Indole-Pyrazole derivatives targeting ROS1 as anti-lung cancer agent.

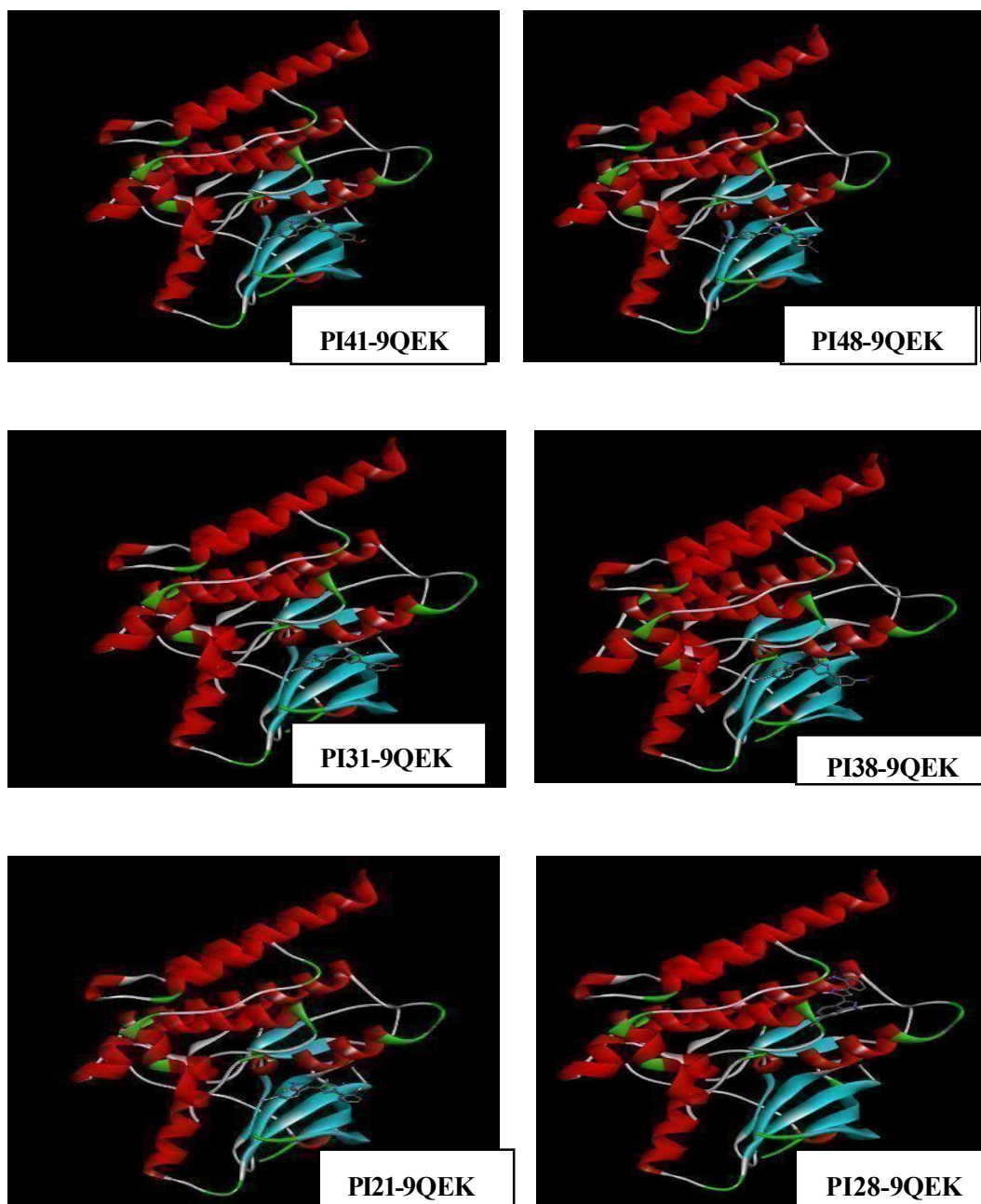
All the compounds were docked against known target ROS1 receptor 9WQK was the NSCLC protein target. In case of binding interaction of protein targets, all the ligands showed high docking score. This can be assured by the pre-screening programme of PyRx.

ROS1 inhibition helps manage and prevent the progression of ROS1-positive NSCLC by targeting abnormal proteins that drive tumor growth. So the docking score of the compounds targeted ROS1 were evaluated against docking score of the standard (Talectrectinib).

As we go through the docking score of each compound which are the combination of Indole-Pyrazole derivative, almost all the derivatives showed higher docking score than the corresponding drug. PI38 have high docking score (-9.4 kcal/mol). Among them PI22 is having the least score (-8.1 kcal/mol).

If we compare the score between the ligands obtained by the combination of Indole-pyrazole derivatives, the highest score (-9.4 kcal/mol) was found to be on PI38 were as the standard drug which has been available in market is Taletrectinib used for NSCLC, whose docking score is -8.1. Therefore, while we compare the score between the compound PI38 and Taletrectinib, it seems PI38 has more score than Taletrectinib. Therefore PI38 are best drug for NSCLC.

Some of the ligand-target complex interactions by Discovery studio visualizer were shown in the figure 11:



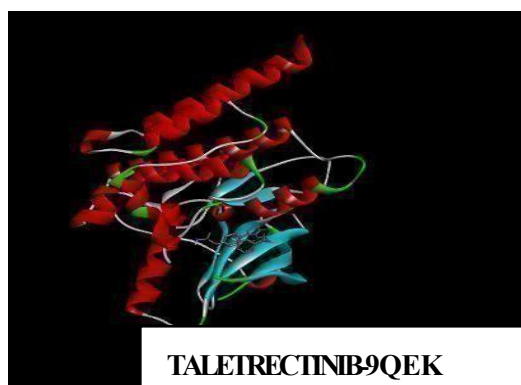
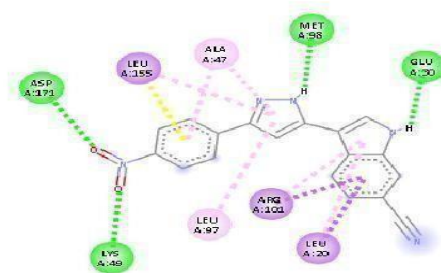
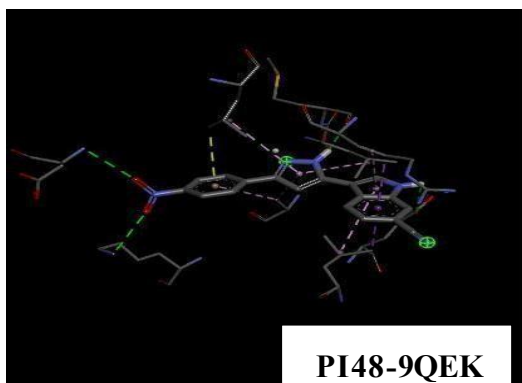
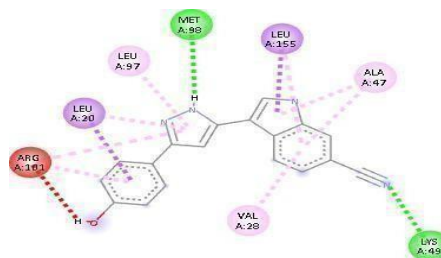
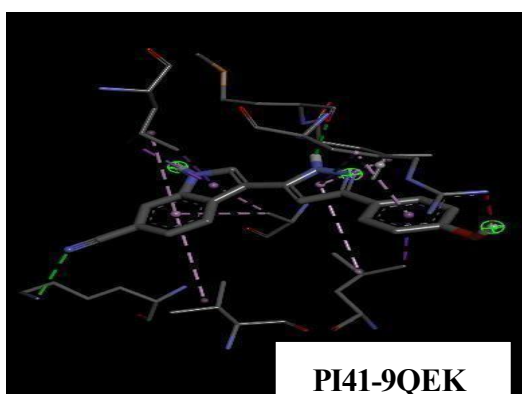
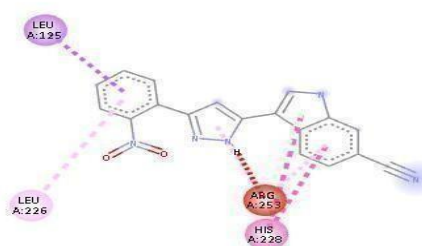
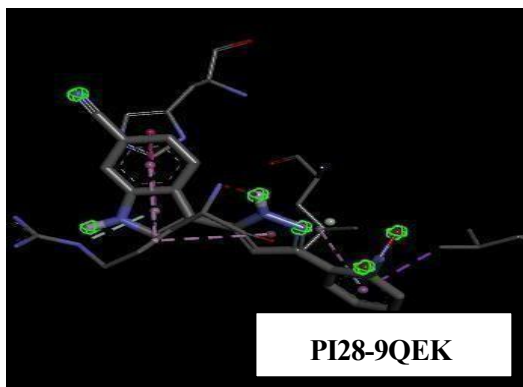
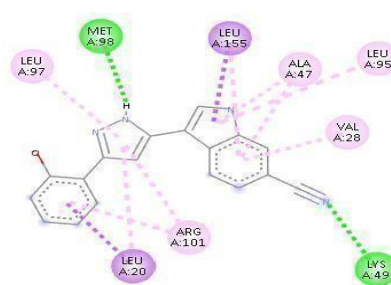
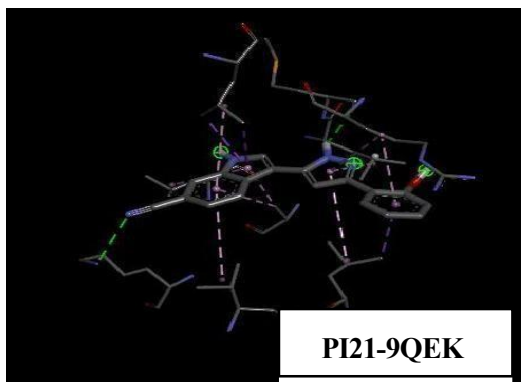
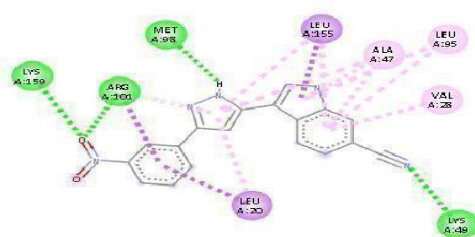
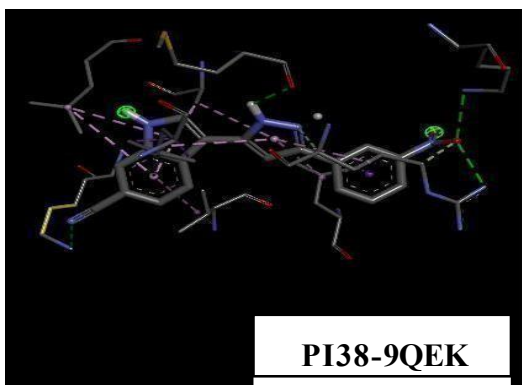
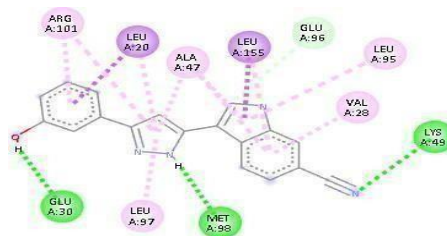
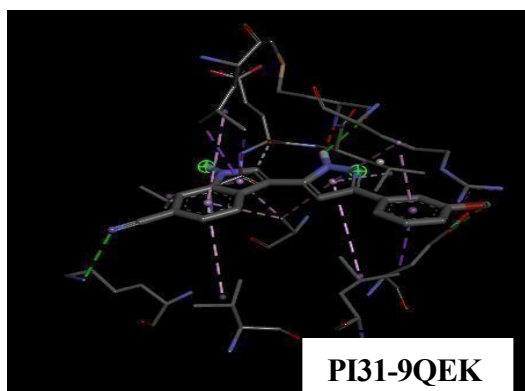


Figure11:Ligand-TargetcomplexinteractionbyDiscoverystudio visualizer.

3Dand2Dstructureofligand-targetcomplexinteractionbyDiscoverystudio visualizer are shown in figure 12:





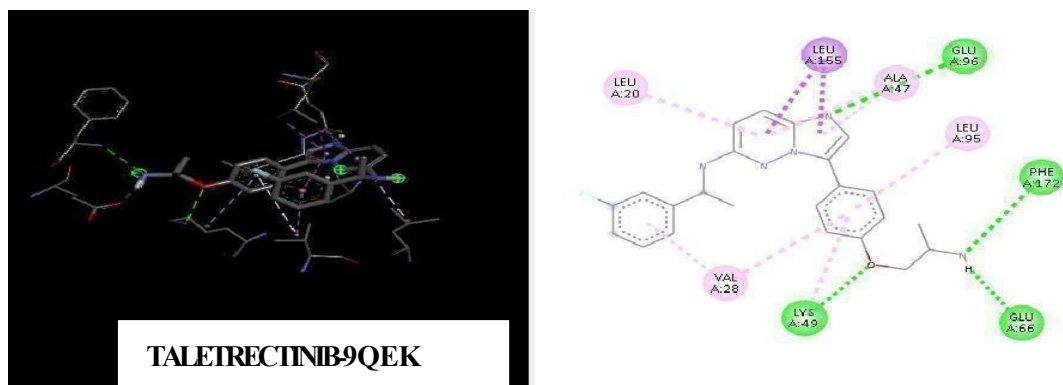
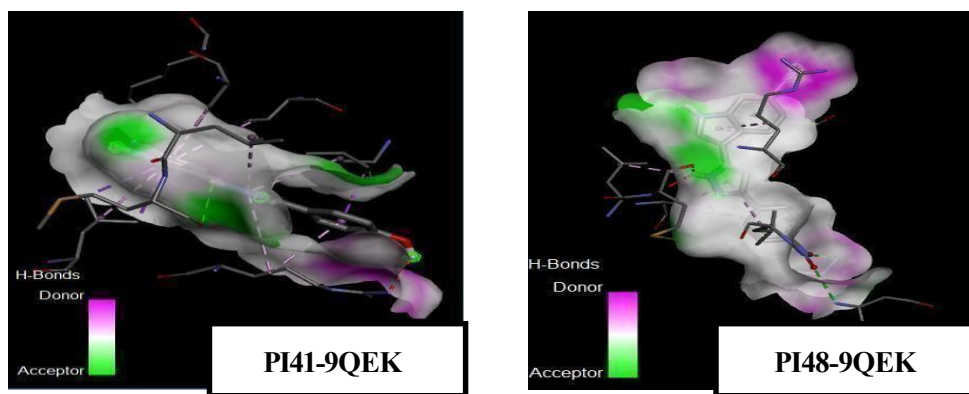


Figure 12: 3D and 2D structure of ligand-target complex interaction by Discovery studio visualizer. Number of hydrogen bonds will considerably increase the affinity of ligand – target interaction. PyRx results showed that most of the Indole-Pyrazole derivatives have hydrogen bond between ligand-target interactions. Some of the derivatives have more than two or three hydrogen bonds for ROS1 targeted derivatives, commonly MET:98, LYS:49, GLU:30. PI48 complex showed 4 hydrogen bond interactions while PI38 complex had the highest docking score (- 9.4 kcal/mol) with 4 hydrogen bond interactions .These hydrogen bond interactions helped to increase the binding energy of ligand protein interactions.

Some of the ligand-target complex with hydrogen bonding interactions by Discovery studio visualizer were shown in figure 13:



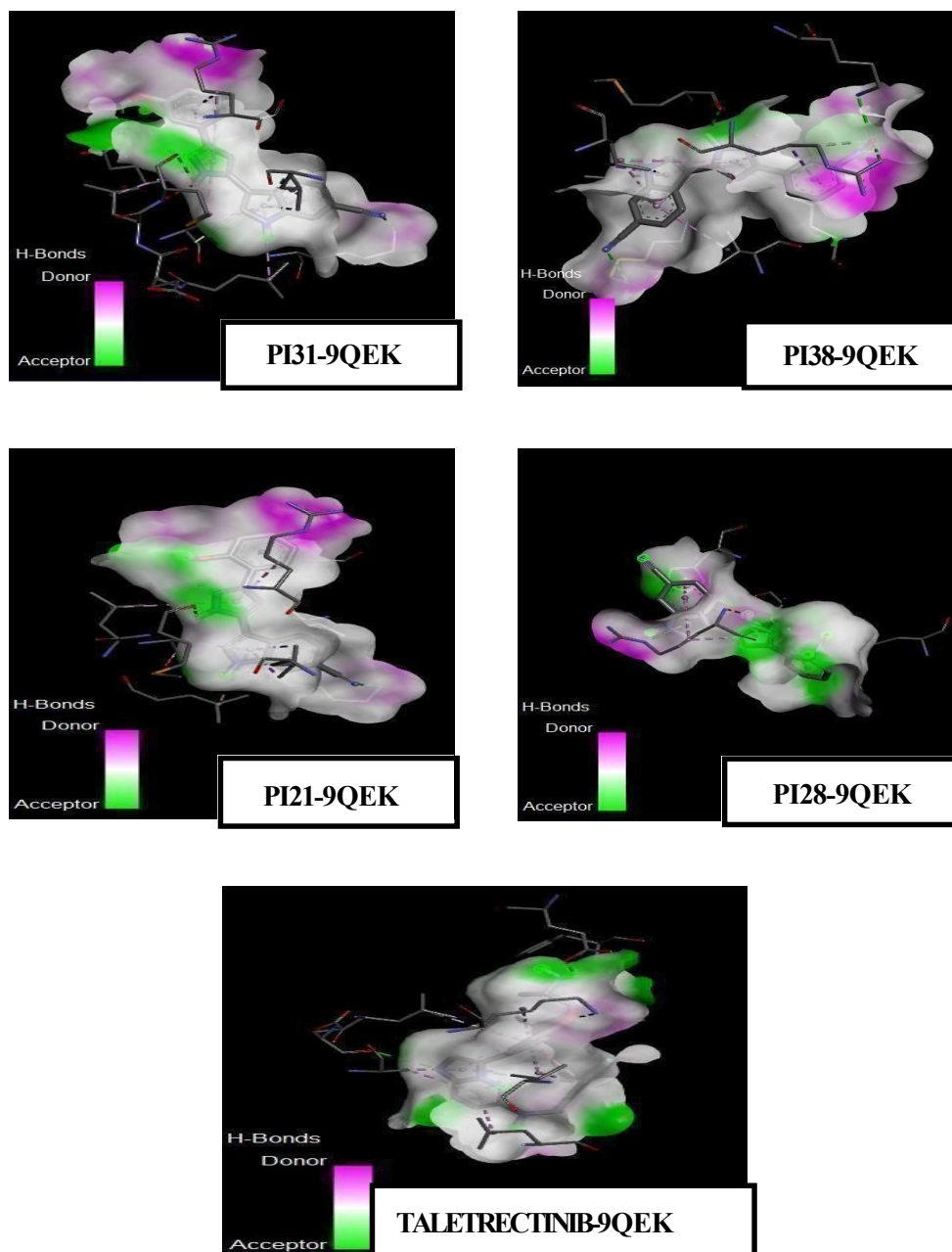


Figure13:Ligand-targethydrogenbondinteractionbyDiscoverystudio visualizer.

DockingscoresofIndole-pyrazolederivativetargetingEGFRasanti-lung cancer agent

The dockingscoresobtainedfromthepreliminarydockingprogrammebyusing PyRx were listed in the table 9:

SL.NO	COMPOUND CODE	DOCKING SCORE OFEGFR(kcal/mol)
1	PI41	-8.5
2	PI42	-7.8

3	PI43	-8.2
4	PI44	-9.2
5	PI45	-7.3
6	PI46	-8.3
7	PI47	-8.0
8	PI48	-8.7
9	PI49	-8.2
10	PI410	-8.1
11	PI31	-9.3
12	PI32	-8.1
13	PI33	-8.3
14	PI34	-9.2
15	PI35	-8.0
16	PI36	-8.5
17	PI37	-8.4
18	PI38	-8.8
19	PI39	-9.2
20	PI310	-7.6
21	PI21	-9.3
22	PI22	-8.0
23	PI23	-8.0
24	PI24	-8.1
25	PI25	-8.1
26	PI26	-8.1
27	PI27	-7.0
28	PI28	-9.8
29	PI29	-9.3
30	PI210	-7.9
31	GEFITINIB	-7.9

Table 9:PyRx docking for the designed Indole-Pyrazole derivative targeting EGFR as anti-lung cancer agents.

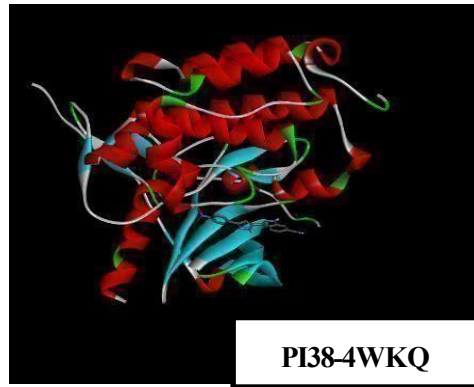
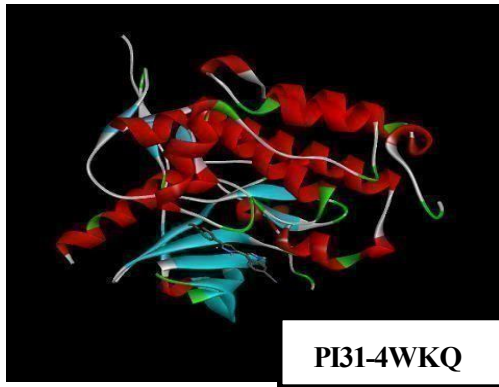
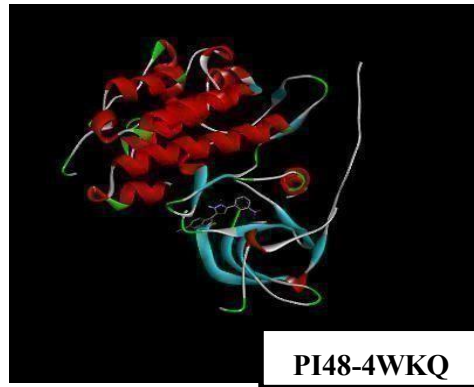
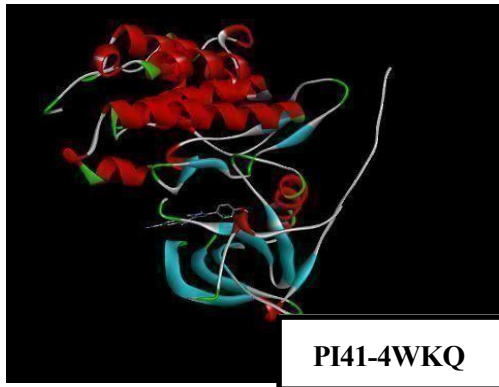
All the compounds were docked against known target. EGFR receptor 4WKQ was the NSCLC protein target. In case of binding interaction of protein targets, all the ligands showed high docking score. This can be assured by the prescreening programme of PyRx.

EGFR inhibition is a primary targeted therapy for NSCLC patients with EGFR mutations, significantly improving survival by inhibiting tumor proliferation, inducing apoptosis, and blocking cell signaling. So the docking score of the compounds targeted EGFR were evaluated against the docking score of standard (Gefitinib).

As we go through the docking score of each compound which are the combination of Indole-Pyrazole derivative, almost all the derivatives showed higher docking score than the corresponding drug. PI28 have high docking score (-9.8 kcal/mol). Among them PI27 is having the least score (-7.0 kcal/mol).

If we compare the score between the ligands obtained by the combination of Indole-Pyrazole derivative, the highest score (-9.8 kcal/mol) was found to be on PI28 were as the standard drug which has been available in market is Gefitinib used for NSCLC, whose docking score is -7.9. Therefore, while we compare the score between the compound PI28 and Gefitinib, it seems PI28 has more score than Gefitinib. Therefore PI28 are the best drug for NSCLC.

Some of the ligand-target complex interactions by Discovery studio visualizer were shown in the figure 14:



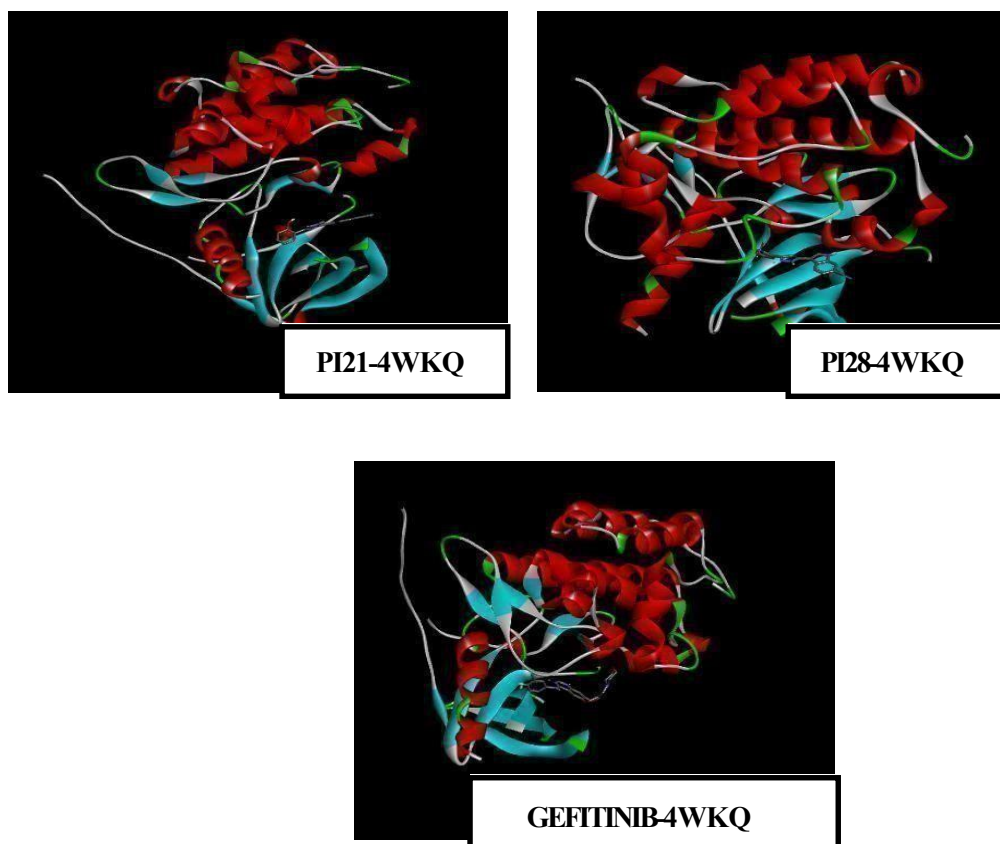
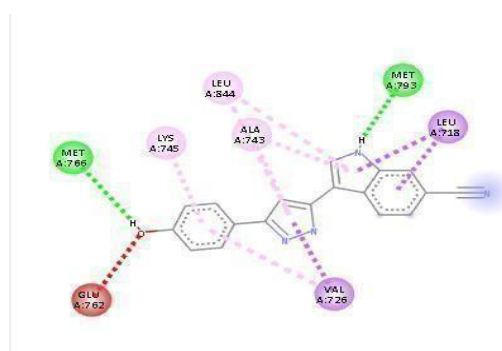
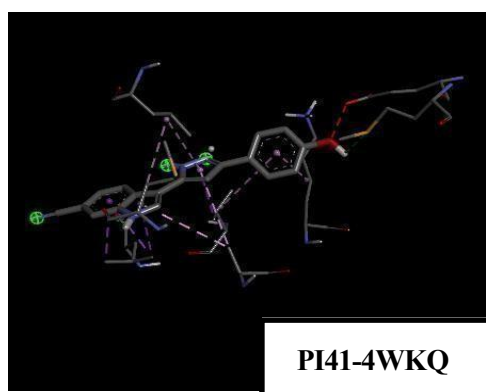
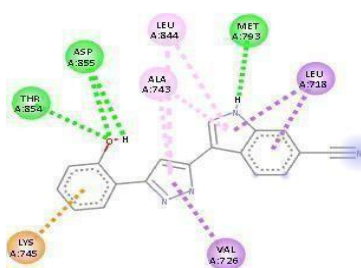
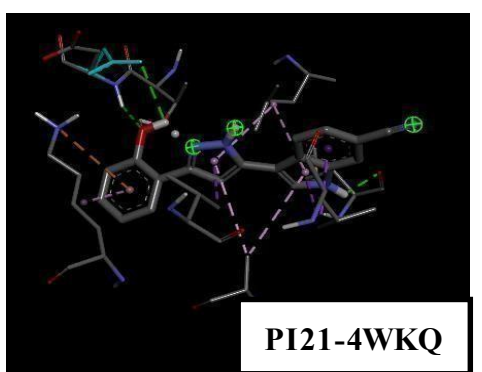
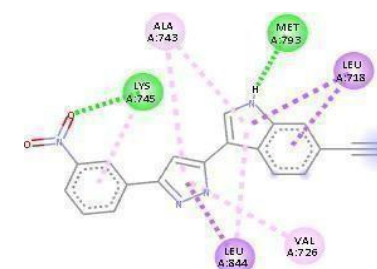
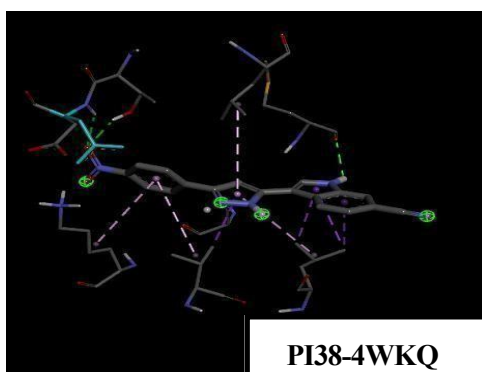
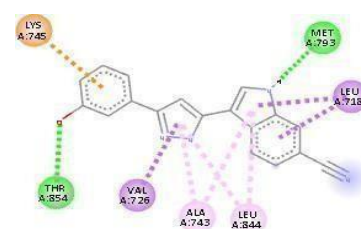
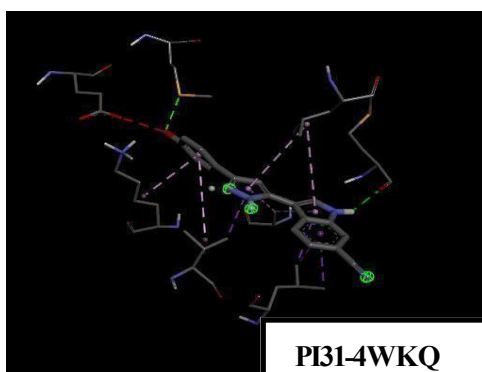
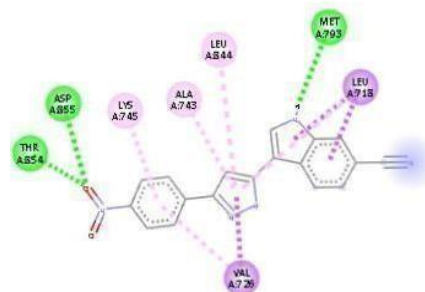
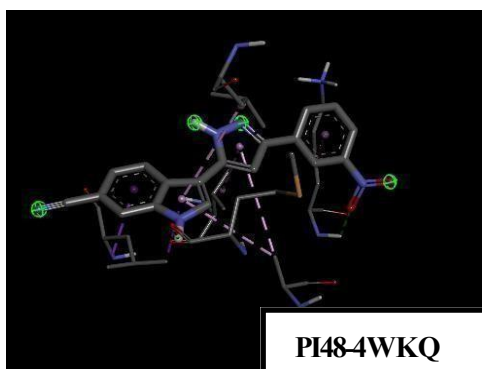


Figure14:Ligand-TargetcomplexinteractionbyDiscoverystudio visualizer.

3Dand2DstructureofLigand-TargetcomplexinteractionbyDiscoverystudio





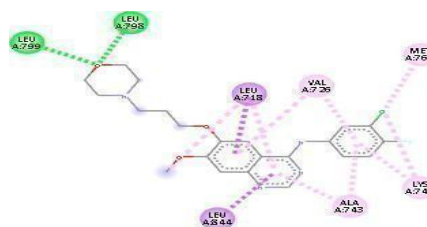
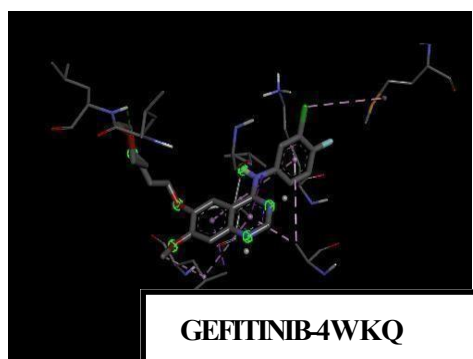
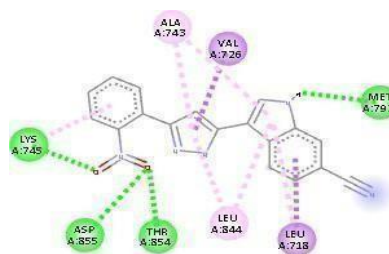
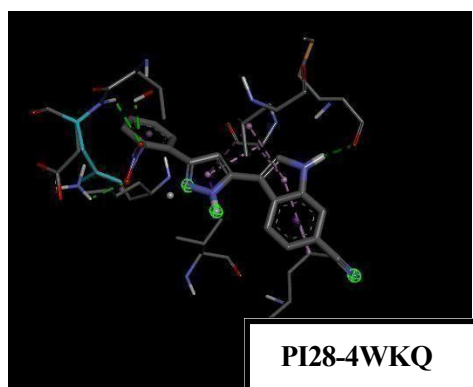


Figure 15: 3D and 2D structure of Ligand-Target complex interaction by Discovery studio visualizer
 Number of hydrogen bond will considerably increase the affinity of ligand-target interaction. PyRx result showed that most of the Indole-Pyrazole derivatives have hydrogen bond between the ligand target interactions. Some of the derivatives have more than 2 or 3 hydrogen bonds for EGFR targeted derivatives, commonly MET:793, ASP:855, THR:854, LYS:745. PI 28 complex have the highest docking score (-9.8 kcal/mol) as well as highest ligand-target hydrogen bond interactions. These hydrogen bond interactions helped to increase the binding energy of ligand-protein interactions. Some of the Ligand-Target complexes with hydrogen bonding interactions by Discovery studio visualizer were shown in figure 16:

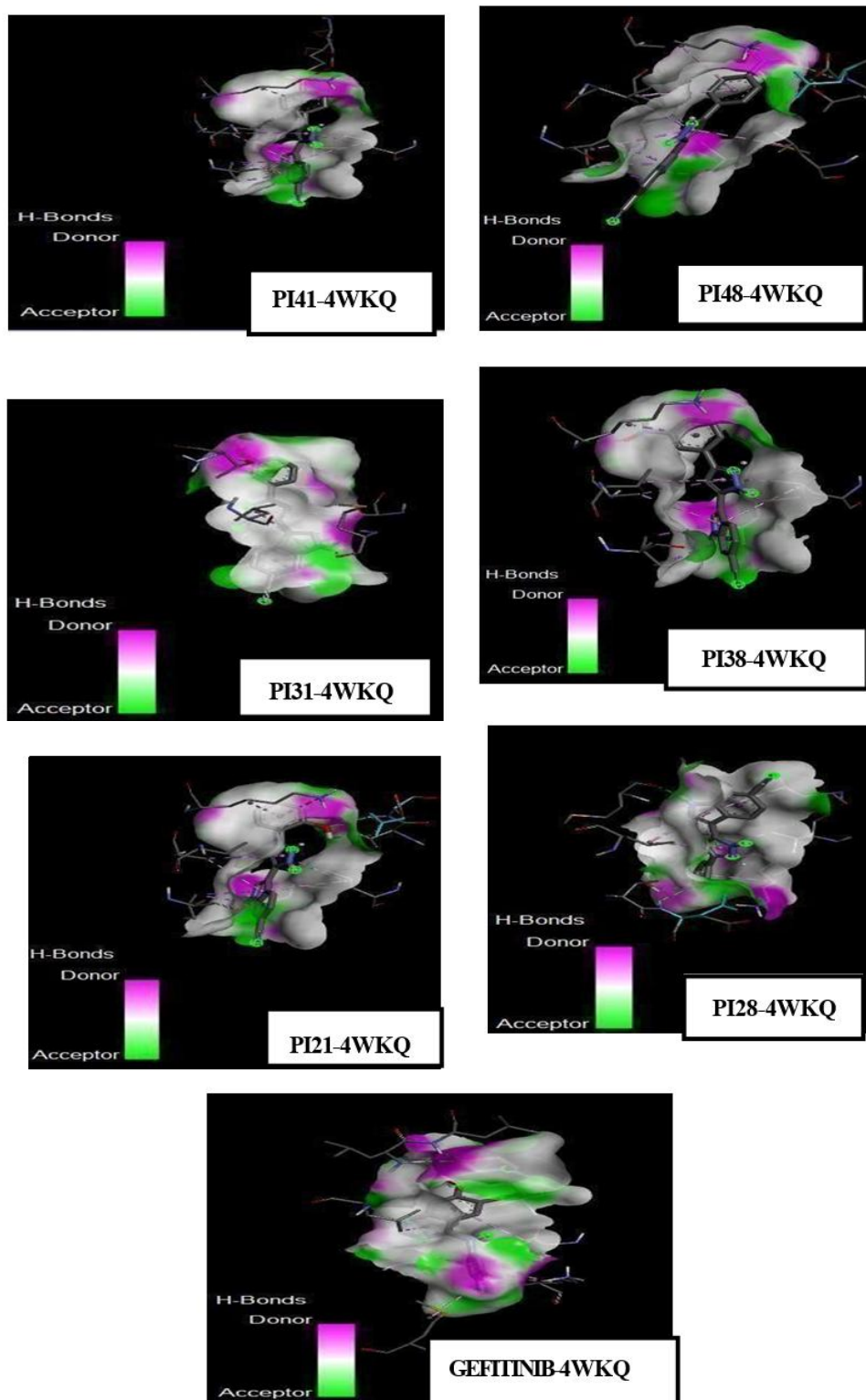


Figure16 :Ligand-TargethydrogenbondinteractionsbyDiscoverystudio visualizer.

III. SUMMARY

The study explores the *In-silico* molecular modelling and docking studies of Indole-Pyrazole derivatives, aiming to assess their potential as anti-lung cancer agents. The study involves the use of several computational tools including Chemsketch, Molinspiration, SwissADME, PyRx and Discovery Studio visualizer, ProTox3.0 to evaluate ligand-protein binding affinities, drug-likeness, toxicity and ADME (Absorption, Distribution, Metabolism and Excretion) properties. The compounds are differentiated by the presence of electron-donating and electron-withdrawing groups at the 2nd, 3rd, 4th position of the phenyl ring substituted at the 3rd position of the Pyrazole ring.

The docking is done by PyRx. PI48, PI38 and PI21 demonstrated the highest docking scores against ALK with -8.5 kcal/mol, outperforming the standard drug Crizotinib (-8.0 kcal/mol). Against ROS1, PI38 had the highest docking score of -9.4 kcal/mol, outperforming the standard drug Taletrectinib (-8.1 kcal/mol). PI28 demonstrated the highest docking score against EGFR with -9.8 kcal/mol, outperforming the standard drug Gefitinib (-7.9 kcal/mol).

Hydrogen bonding significantly enhanced ligand-target interactions. PI48 exhibited one hydrogen bond interaction with ALK, while PI21 showed 3 interactions, indicating strong binding affinity. For ROS1, PI38 showed 4 hydrogen bonds. PI28 exhibited 4 hydrogen bond interactions with EGFR, contributing to its high docking score.

All derivatives adhered to Lipinski's rule of five, suggesting good drug-likeness. The compounds displayed favorable ADME properties, with high solubility and no toxicity.

IV. CONCLUSION

The *In-silico* analysis of Indole-Pyrazole derivatives indicates that these compounds have strong potential as therapeutic agents for NSCLC disease. The docking studies reveal that compounds PI48 and PI21 as ALK and PI38 as ROS1 and PI28 as EGFR exhibit excellent binding affinities, suggesting their efficacy as inhibitors. The combination of the Indole-Pyrazole derivatives demonstrated promising drug-like properties and favorable ADME profiles. These findings suggest their potential as effective drug candidates with the ability to be highly absorbed and distributed within the human body. Further studies and clinical trials are recommended to validate these findings and explore their therapeutic applications.

REFERENCES

- [1]. Lakhdar, S.; Westermaier, M.; Terrier, F.; Goumont, R.; Boubaker, T.; Ofial, A.R.; Mayr, H. Nucleophilic reactivities of indoles. *J. Org. Chem.* 2006, 71, 9088–9095. [Google Scholar] [CrossRef] [PubMed].
- [2]. Ma, Q.; Zhang, X.; Qu, Y. Biodegradation and bio transformation of indole: Advances and perspectives. *Front. Microbiol.* 2018, 9, 2625. [Google Scholar] [CrossRef] [PubMed].
- [3]. Dorababu, A. Indole—A promising pharmacophore in recent antiviral drug discovery. *RSC Med. Chem.* 2020, 11, 1335–1353. [Google Scholar] [CrossRef] [PubMed].
- [4]. Knorr, L. Action of ethylacetoacetate on phenylhydrazine. *I. Chemische Berichte.* 1883. 16. 2597–2599.
- [5]. Kelloff, G.J., Crowell, J.A., Steele, V.E., Lubet, R.A., Malone, W.A., Boone, C.W., Kopelovich, L., Hawk, E.T., Lieberman, R., Lawrence, J.A., Ali, I., Viner, J.L., Sigman, C.C. Progress in cancer chemoprevention: development of diet-derived chemopreventive agents. *Journal of Nutrition* 2000; 130 (2): 467S–471S.
- [7]. Greenwald, P. Cancer chemoprevention. *British Medical Journal* 2002; 324(7339): 714–718.
- [8]. Benign lung tumors: Presentation, diagnosis, and outcome *European Respiratory Journal* Sep 2013, 42 (Suppl 57) P4522.
- [9]. C Zappa and S AMousa “Non-small cell lung cancer: current treatment and future advances” *Translational Lung Cancer Research*, 2016. 5, (3): 288-300. <http://www.cancer.org/acs/groups/cid/documents/webcontent/003117.pdf>.
- [10]. Malik, Prabhat Singh, and Vinod Raina. “Lung Cancer: Prevalent Trends & Emerging Concepts.” *The Indian Journal of Medical Research* 2015 141.1: 5–7. <http://globocan.iarc.fr>
- [11]. Fumihiko Tanaka, Kaze Y. “Circulating Tumor Cells in Lung Cancer: status and future perspectives” *dove press I*: 2010. 77-84.
- [12]. Karine Jacob, Caroline Sollier & Nada Jabado. Circulating tumor cells: detection, molecular profiling and future prospects, *Expert Review of Proteomics*, 2007 4:6, 741-756.
- [13]. Huang CY, Ju DT, Chang CF, Muralidhar Reddy P, Velmurugan BK. A review on the effects of current chemotherapy drugs and natural agents in treating non-small cell lung cancer. *Biomedicine (Taipei)*. 2017;7(4):23.

- [14]. El-Telbany A, Ma PC. Cancer genes in lung cancer: racial disparities: are there any? *Genes Cancer*. 2012;3(7-8):467-480.
- [15]. Siddiqui F, Siddiqui AH. *Cancer, Lung*. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020
- [16]. Cleveland Clinic. Lung cancer [Internet]. Cleveland (OH): Cleveland Clinic; 2022 Oct 31 [cited 2026 Feb 12]. Available from: <https://my.clevelandclinic.org/health/diseases/4375-lung-cancer>
- [17]. Herbst, R.S., et al. (2018). The biology and management of non-small cell lung cancer. *Nature*, 553(7689), 446–454
- [18]. Hecht, S.S. (2012). Tobacco smoke carcinogens and lung cancer. *Journal of the National Cancer Institute*, 104(14), 1145–1157.
- [19]. Pao, W., & Girard, N. (2011). New driver mutations in non-small-cell lung cancer. *The Lancet Oncology*, 12(2), 175–180
- [20]. George, J., et al. (2015). Comprehensive genomic profiles of small cell lung cancer. *Nature*, 524(7563), 47–53.
- [21]. Lindeman, N.I., et al. (2018). Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors. *Journal of Thoracic Oncology*, 13(3), 323–358
- [22]. Rudin, C.M., et al. (2021). Small-cell lung cancer: Molecular subtypes and therapeutic opportunities. *Nature Reviews Cancer*, 21(9), 537–554.
- [23]. Hussain, S.P., et al. (2003). Oxidative stress and chronic inflammation in carcinogenesis: The role of persistent infection and inflammation. *Nature Reviews Cancer*, 3(4), 276–285.
- [24]. Sandoval, J., & Esteller, M. (2012). Cancer epigenomics: Beyond genomics. *Current Opinion in Genetics & Development*, 22(1), 50–55.
- [25]. Hanahan, D., & Weinberg, R.A. (2011). Hallmarks of cancer: The next generation. *Cell*, 144(5), 646–674.
- [26]. Lemjabbar-Alaoui H, Hassan OU, Yang YW, Buchanan P. Lung cancer: Biology and treatment options. *Biochim Biophys Acta*. 2015;1856(2):189-210
- [27]. Gadgeel SM, Ramalingam SS, Kalemkerian GP. Treatment of lung cancer. *Radiol Clin North Am*. 2012;50(5):961-974
- [28]. Sankar V, Kothai R, Vanisri N, Akilandeswari S, Anandharaj G. Lung cancer – A review. *International Journal of Health Sciences and Research*. 2023;13(10):307. doi:10.52403/ijhsr.20231042.