ISSN: 0974-2115
Journal of Chemical and Pharmaceutical sciences

INSILICO DESIGNING AND DEVELOPMENT OF DRUG INHIBITOR FOR CONSEQUENCES OF KRAS GENE IN TREATMENT OF NON-SMALL CELL LUNG CANCER

*[1]AMMAJI.SHAIK, [1]P.AFROZ, [2]MANAS RANJAN BARIK, [1]NANDA KISHORE AGARWAL
[1]Department of pharmaceutical chemistry, Nimra College of Pharmacy.
[2]DNA Labs India, Ameerpet, Hyderabad.
***corresponding author: E.mail:shaik.ammaji8@gmail.com, Phone No.9705521675.**

ABSTRACT

In this study mainly focused on non-small cell lung cancer KRAS (Kristen rat sarcoma virus) gene is bind to EGFR (epidermal growth factor) that produce chemical signal that will involve for cell differentiations and cell growth in normal cell function of lungs. In this signals controlled by protein tyrosine kinase it is only mediator for controlling the KRAS signals. KRAS is a membrane-bound guanosine triphosphate (GTP)/guanosinediphosphate (GDP)-binding (G) protein that serves as a "molecular switch," converting signals from the cell membrane to the nucleus. These chemical signals lead to protein synthesis and regulation of cell survival, proliferation, and differentiation in NSCLC (non small cell lung cancer). KRAS mutation occur that will causes uncontrolled signals produces these KRAS gene protein tyrosine kinase unable to control these signals that lead to uncontrolled cell growth, protein synthesis and proliferation and differentiation of cells. So drug will bind to KRAS gene prevent the signals and inhibit the action of KRAS in cell differentiation, cell growth. This paper deals to design a drug 6-[(ethyl amino) oxy]-4-[(3-ethynyl-5-methylphenyl) amino]-5, 6-dihydroquinazoline-7-carboxylic acid that inhibit the action of KRAS mutated gene signals that causes inhibition of cell growth, differentiation in NSCLC.

KEY WORDS: EGFR, GTP, GDP, NSCLC, KRAS.

1. INTRODUCTION

Lung cancer is the uncontrolled growth of abnormal cells in the lung. Lung cancer is mainly divided into two types 1.Small cell lung cancer. 2.Non -small cell lung cancer, small cell lung cancer is malignant cell formed from the tissue of the lungs and it is mainly seen in smokers it easily spread throughout the body (Kazutsugu,2003). NSCLC (non-small cell lung cancer) is usual type of cancer it spared slowly when compare to small cell lung cancer. They mainly divided into three types depending on shape and composition of the cells (Jill and Christopher,2007; Roberto,2005; Niranjan,2008; Joseph,2001).

- **Adenocarcinomas** are found in an outer area of the lung.
- **Squamous cell carcinomas** are usually found in the lungs next to the air tube (bronchus).
- **Large cell carcinomas** can occur in any part of the lung. They tend to grow and spread faster than other types (By Joel,2010).

About 85 to 90% of lung cancers are non-small cell lung cancer (NSCLC). About 25 to 30% of all lung cancers are squamous cell carcinomas (By Joel,2010; Makoto,2010). These cancers start in early versions of squamous cells, which are flat cells that line the inside of the airways in the lungs. 40% of lung cancers are adenocarcinomas. These cancers start in early versions of the cells that would normally secrete substances such as mucus. This type of lung cancer occurs mainly in people who smoke (or have smoked), but it is also the most common type of lung cancer seen in non-smokers. It is more common in women than in men, and it is more likely to occur in younger people than other types of lung. 10 to 15% of lung cancers are large cell carcinomas. It may appear in any part of the lung. It tends to grow and spread quickly throughout the body (Makoto,2010).

1.1. Risk factors for non-small cell lung cancer

a.Tobacco smoking: Smoking is by far the leading risk factor for lung cancer. In the early 20th century, lung Cancer was much less common than some other types of cancer. But this changed once manufactured cigarette became readily available and more people began smoking. About 80% of lung cancer deaths are thought to result from smoking. The risk for lung Cancer among smokers is many times higher than among non-smokers (Joseph,2001; Melissa,2009).

b.Secondhand smoke: If you don't smoke, breathing in the smoke of others (called Secondhand smoke or environmental tobacco smoke) can increase your risk of developing lung cancer (Kazutsugu,2003).

c.Radon: Radon is a naturally occurring radioactive gas that results from the breakdown of uranium in soil and rocks. It cannot be seen, tasted, or smelled (Stephen,1999). According to the US Environmental Protection Agency (EPA), radon is the second leading cause of lung cancer.When it is breathed in, it enters the lungs, exposing them to small amounts of radiation. This may increase a person's risk of lung cancer (By Joel,2010).

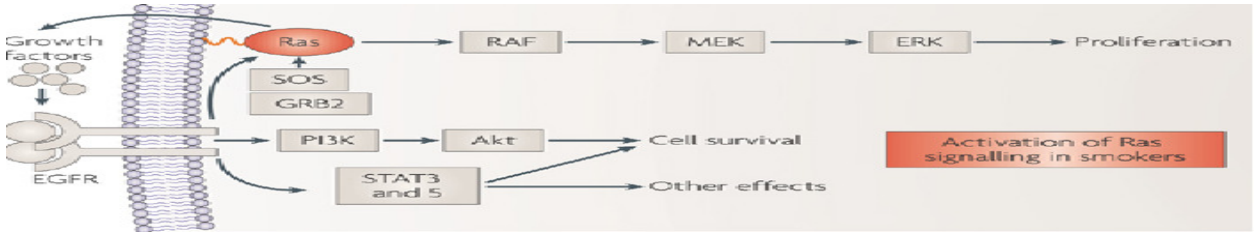

Fig.1. Pathophysiology of non-small cell lung cancer (Williams,2010)

In smokers, mutations of the KRAS gene often occur, resulting in the release of growth factors, including transforming growth factor-α (TGFα), which is a ligand for EGFR. In addition, KRAS directly activates the PI3K–Akt pathway. Thus, the end result of KRAS or EGFR mutations are virtually identical, and mutations of both genes in adenocarcinomas of the lung are rarely seen. Other methods of activation of these pathways include gene amplification and mutations in BRAF, PIK3CA (a subunit of PI3K), and ERBB2 (also known as HER2) (Subhalaxmi,2011; Fabio Vandin,2011).

1.2.KRAS Signaling pathway: GEFS (guanine nucleotide exchange factor), KRAS (Kristen rat sarcoma viral oncogene homolog), GRF (guanine nucleotide releasing factor), RAP (receptor associated protein). KRAS is a gene which is activated by GEFR once it get activated KRAS (protein) bind to GRF they will activate lymphocyte specific protein tyrosine kinase it is mediator for signals of KRAS. They control chemical signals that undergo phosphorylation convert GTP to GDP they important for cell growth. KRAS is a membrane-bound guanosine triphosphate (GTP)/guanosine diphosphate (GDP)-binding (G) protein that serves as a "molecular switch," converting signals from the cell membrane to the nucleus. These chemical signals lead to protein synthesis and regulation of cell survival, proliferation, and differentiation (Jill and Siegfried,2007).Some cases tyrosinekinase directly act on RAP protein they bind on GDP they also control cell differentiation.In Non- small cell lung cancer KRAS get mutated they undergo act on tyrosine kinase they will give more signal for conversion GTP to GDP that will causes uncontrolled cell division and differentiation.

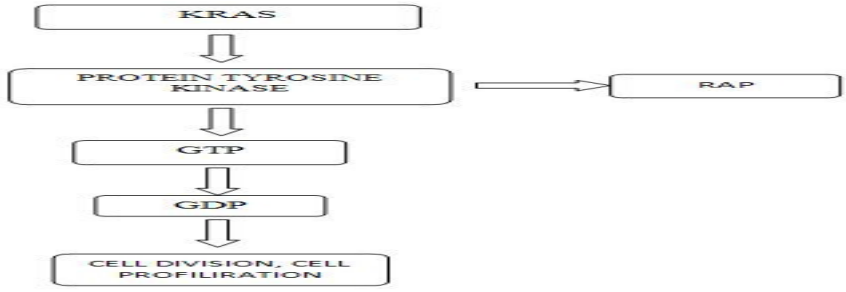

Fig.2.KRAS signaling pathway

1.3.Symptoms (Niranjan,2008)

- Bone pain or tenderness,
- Eyelid drooping
- Hoarseness or changing voice
- Joint pain
- Nail problems
- Swallowing difficulty
- Swelling of the face
- Weakness

1.4.Treatment:

- **Surgery:** If the cancer has not spread, removing the tumors by surgery is the most common form of treatment for non-small cell lung cancer. If the cancer has spread and surgery is not possible (this is more common in small cell lung cancer) then radiotherapy and chemotherapy are used (alone or in combination) to control symptoms by reducing the size of the tumors (By Joel,2010; Makoto,2010).
- **Radiotherapy:** is a general term for the treatment of cancer using x-rays. It is done by directing painless high-energy beams at the areas of the lung that need treatment. Radiotherapy works by killing cancerous cells and can be used either on its own or in combination with surgery and/or chemotherapy (Kazutsugu,2003).
- **Chemotherapy:** is the general term for the treatment of cancer using drugs. The drugs that are used are designed to kill off cancer cells while causing less damage to normal cells. There are many different types of chemotherapy drugs which can be used on their own or, more commonly, in combinations. Patients with different types of lung cancer are likely to receive different combinations of chemotherapy drugs (Charles,2002).

2.EXPERMENTAL SECTION

2.1.Target Identification: We know the pathway of non-small cell lung cancer and how it occur mainly two genes are responsible for lung cancer. EGFR and KRAS are mainly causes non-small cell lung cancer in earlier EGFR inhibitors used for treatment of NSCLC. In smokers KRAS gene mutation is observed that causes NSCLC. EGFR inhibitors not treat the KRAS mutated genes. So I mainly focused on KRAS gene.To retrive and validate the KRAS associated protein sequence using computational tools such as NCBI (national center for biotechnological information), UniProtKB (The UniProt Knowledgebase), GeneCards (The human gene compendium) and KEGG (Kyoto Encyclopedia of Genes and Genomes)

2.2.Chemical Library: A chemical library or compound library is a collection of chemical compounds used for treating diseases. Selection of chemical compounds through highthroughput screening. It consists in series of compounds. Each compound has associated information and its physicochemical properties such as chemical structure, molecular weight, molecular formula, logP, hydrogen donor, hydrogen bond acceptor, etc. For this library of screening Accelyrs Discovery Studio, ChemSpider, PubChem, ChemBank, Bidd etc. databases were used. There are millions of compounds available in these databases. Through the help of these tools we can find a new compound against a KRAS-associated protein and tested for their ability to modify / inhibit the target protein. In compound screening the major part to test that compound is having drug likeness or must passed ADME properties. We have used Accelyrs Discovery Studio for the present work**.**

2.3.Lead optimization: There are many tools available for designing of lead/drug such as Discovery Studio, HyperChem, ChemDraw, ChemSketch etc. When a drug is a complex chemical mixture, this activity is depending on the active ingredient or pharmacophore but can be modified by the other constituents. Activity is generally dosage-dependentand it is not uncommon to have effects ranging from beneficial to adverse for one substance when going from low to high doses. Activity depends critically on fulfillment of the ADME criteria. To be an effective drug, a compound not only must be active against a target, but also possess the appropriate ADME (Absorption, Distribution, Metabolism,and Excretion) properties necessary to make it suitable for use as a drug. The drug must possess the TOPKATparameter for its novel properties.TOPKAT is nothing but the properties prediction of that drug. The properties such as molecule's bioavailability, it is carcinogenic or not, lethal dose (LD50), value of developmental toxicity Prediction etc. The all values are calculated by protocols of Discovery studio**.**

2.4.Molecular stimulation and docking: High-throughput screening (HTS) of compound libraries is used to discover novel leads for drug development. When a structure is available for the target, computer-based screening using Molecular docking. Molecular docking is a computer simulation procedure to predict the conformation of a receptor-ligand complex, where the receptor is usually a target protein and the ligand is small designed molecule. It is used for stimulation process for ligand position is estimated that will used for binding with the receptor. Molecular docking stimulations may be used for reproducing experimental data

through docking validations. Where protein-ligand conformations are obtained INSILICO and structures obtained from X-ray crystallography or nuclear magnetic resonance. Furthermore, docking is one of main tools for virtual screening Procedures, where a library of several compounds is "docked" against receptor from that select a best possible binding drug. Before going for docking we minimize the energies of ligand (drug molecule) and receptor (targeted protein) all these studies carried out by using discovery studio with help of minimization tool these mainly help for protein-ligand interaction.

3. RESULTS

From the designed library of molecule only one lead should be screened out from the ADME and TOPKAT parameter. The best molecule has been selected for further analysis by using molecular stimulation and docking technique the best drug molecule were identified which is satisfied the all rules and possess the inhibitor property. The inhibitor shows the highest binding affinity towards the receptor cavity. The drug is 6-[(ethyl amino) oxy]-4-[(3-ethynyl-5-Methyl phenyl) amino]-5, 6-dihydroquinazoline-7- Carboxylic acid passing all ADMET (absorption, distribution, metabolism elimination parameters) and TOPKAT (toxicity result) parameter as shown in Fig.4 and Fig.5.

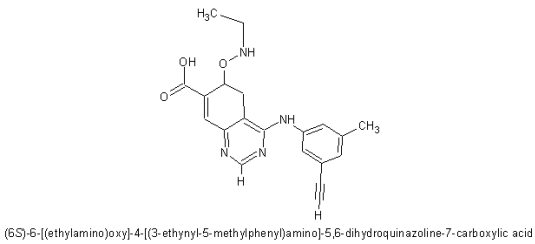

Fig.3. 6-[(ethyl amino) oxy]-4-[(3-ethynyl-5-Methyl phenyl) amino]-5, 6-dihydroquinazoline-7-Carboxylic acid

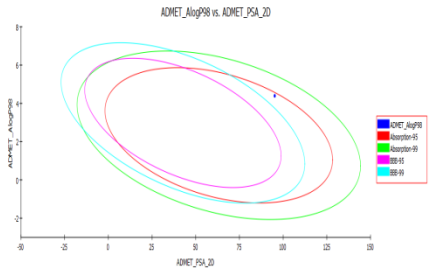

Fig.4. Graphical representation of ADMET absorption

$C_{19}H_{18}N_4O_3$
Molecular Weight: 350.37122
ALogP: 3.655
Rotatable Bonds: 5
Acceptors: 7
Donors: 3

- ABSORPTION: 0
- HEPATOTOXICITY: 1
- HEPATOTOXIC PROBABILITY: 0.602

Prediction
Model: NTP Carcinogenicity Call (Male Rat) (v3.2)
Computed Probability of Carcinogenicity = 0.021
Model: FDA Carcinogenicity Female Mouse Single vs Mult (v3.1)
Probability of Multiple Carcinogenicity = 0.001
Model: Developmental Toxicity Potential (DTP) (v3.1)
Computed Probability of DTP = 0.987
Model: Rat Oral LD50 (v3.1)
Computed Rat Oral LD50 Log (1/Moles) = 1.149
Computed Rat Oral LD50 = 10 g/kg
Lower 95% Confidence Limits = 2.6 g/kg
Upper 95% Confidence Limits = 10 g/kg
Model: Skin Irritation (v6.1)
Probability of SEV = 1.000
Model: Aerobic Biodegradability (v6.1)
Probability of Biodegradability = 0.000

Fig.5.Toxicity prediction like NTP (National toxicity program) carcinogenicity, biodegradability, Rat oral LD50 (lethal dose 50) and LC50 (lethal concentration 50) properties of designed drug molecule

The compound 6-[(ethyl amino) oxy]-4-[(3-ethynyl-5-Methyl phenyl) amino]-5, 6-dihydroquinazoline-7- Carboxylic acid shows best TOPKAT result for inhibition of KRAS gene in non–small cell lung cancer associated protein. These compound used for docking with the receptor.

ISSN: 0974-2115
Journal of Chemical and Pharmaceutical sciences

3.1.DOCKING: The selected compound used for docking with KRAS protein that should be similarity with pdb id 3gft resulted docking score is 15.3399. This result shows that a group of amino acid residues located on the binding cavity such as GLN43 in associated target protein of KRAS. This interaction/affinity plays an important role in ligand binding. The docking result showed that almost all inhibitors were involved in Hydrogen binding with GLN43E chain.

Table 1: A set of designed compound displaying and their ADMET properties

Molecules	1	2	3	4	5
ADMET BBB(blood brain barrier)	4	4	4	4	4
ADMET BBB level	2	4	4	4	4
ADMET absorption level	0	0	0	0	0
ADMET solubility	-6.59	-4.763	-4.763	-5.247	-5.583
ADMET hepatotoxicity	1	1	1	1	1
ADMET hepato probability	0.993	0.668	0.668	0.682	0.609
ADMET CYP2D6 (cytpchrome P 2D6)	0.603	1	1	0	0

Table-2 molecular properties result

Molecules	1	2	3	4	5
logP	4.309	3.449	3.655	2.963	5.55
Molecular weight	393.436	336.345	350.371	322.318	363.41
Number of Hydrogen acceptors	7	7	7	7	6
Number of Hydrogendonars	1	3	3	3	2
Number of rotatable bonds	10	4	5	4	7
Number of rings	3	3	3	3	3
Number of aromatic rings	3	2	2	2	3
Molecular fraction	0.19	0.339	0.283	0.361	0.213

Molecule names:

1.6(S)-4-[(3-etynyl-5-methylphenyl) amino]-6-(methylamine) oxy-5,6 dihydroquinazoline-7-carboxylic acid.
2.6(S)-4-[4-ethynyl-5-methylphenyl) amino]-6-9methylamine) oxy-5, 6-hydroquinazoline-7-carboxylic acid
3.6(s)-5-[4-ethynyl-5-methylphenyl) amino]-6-9[methyl amine] oxy 5,6-hydroquinazoline-7-carboxylic acid.
4.6(s)-5-[ethynyl-5-methylphenyl) amino]-6,9[methylamine] oxy 5,6-hydroxyquinazoline-7-carboxylic acid.
5.6-[(ethyl amino) oxy]-4-[(3-ethynyl-5-Methyl phenyl) amino]-5, 6-dihydroquinazoline-7- Carboxylic acid

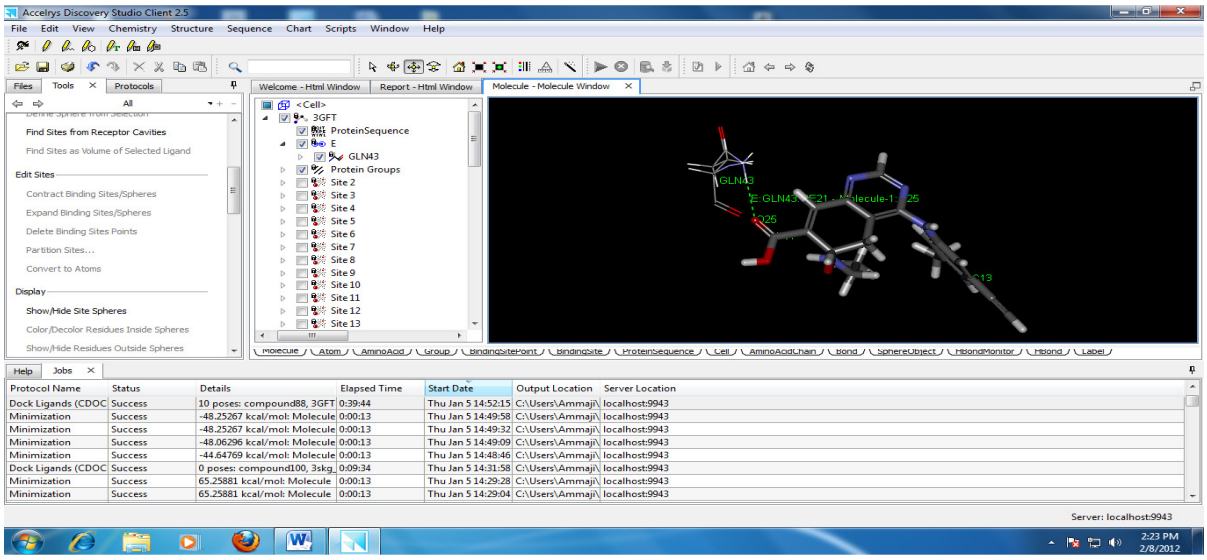

Fig.6. Binding orientation of designed compound. 6-[(ethyl amino) oxy]-4-[(3-ethynyl-5-Methyl phenyl) amino]-5, 6-dihydroquinazoline-7- Carboxylic acid with the target associated protein 3GFT

Table.3.The list of five inhibitor with their C-Docker interaction energy to active site of target receptor

Molecules	1	2	3	4	5
Tagged	No	No	No	No	No
Visible	No	No	No	No	No
Visibility locked	No	No	No	No	No
Calculate charges	No	No	No	No	No
Top hits	10	10	10	10	10
C docking energy	9.06436	9.06436	9.06436	9.06436	9.06436
C docking interactions	18.6849	18.6849	18.6849	18.6849	18.6849

3.2.Pharmacophore: The docked compound with binding pocket of receptor can be easily visualized on three feature of pharmacophore model. Aromatic ring features (yellow), hydrophobic region feature (blue), hydrogen bond acceptor feature (red).

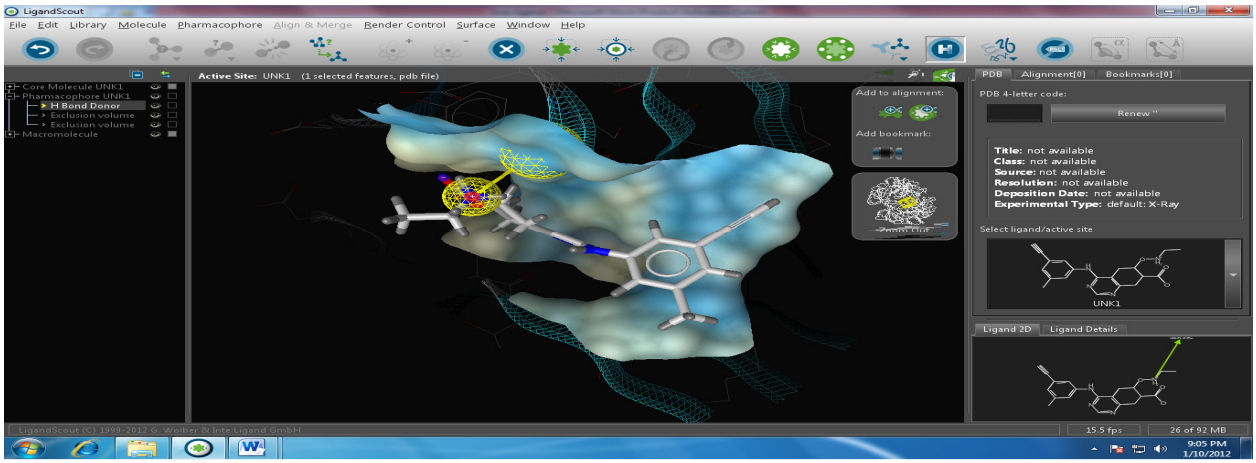

Fig.7. The designed compound with binding pocket of target receptor

3.3.DISCUSSION

The interactions between potent and receptor were studied by using various computational methods. Based on binding energy, and hydrogen bond formed, docking results were analyzed to find out the best ligand which can inhibit the target receptor of KRAS protein. Based on these observations, Ligand 6-[(ethyl amino) oxy]-4-[(3-ethynyl-5-Methyl phenyl) amino]-5, 6-dihydroquinazoline-7- Carboxylic acid has high values to inhibit the target among the all ligands. The INSILICO method adopted in the present study helped in identifying the ligands using the commercial software and online tools for the treatment of acute as well as chronic non-small cell lung cancer. This method reduces the time and cost in designing a drug as well as in analyzing the drug likeliness before it enters the clinical trials. The further studies were carried out by pre-clinical trials.

4.CONCLUSION

The interaction between the KRAS protein and ligand molecule were studied by using various tools and databases, using various techniques. Based upon binding energy and hydrogen bond formation in docking results. So I analysed that it was the best molecule which inhibited the action of targeted receptor. Based on observation ligand 6-[(ethyl amino) oxy]-4-[(3-ethynyl-5-Methyl phenyl) amino]-5, 6-dihydroquinazoline-7- Carboxylic acid having high value to inhibit the action of receptor. So finally I conclude that INSILICO is commercial software tool for treatment of non-small cell lung cancer.

5.ACKNOWLEDGEMENT

I, Ammaji Shaik, would like to thank the Management of Nimra College of Pharmacy for providing all the necessary facilities to do the research work. I also acknowledge Dr. Duraiwal, Principal, Nimra College of pharmacy, S.Padmavathi, Assistant Professor, Nimra College of Pharmacy and Dr. Nanda Kishore Agarwal, Head, Department of pharmaceutical chemistry, Nimra College of Pharmacy, for giving

the guidance and timely help in completing the project. I express my deep sense of gratitude to DNA Labs India, Hyderabad, for guidance and support of research work.

REFERENCES

Anais Baudot, Mutated genes, pathways and processes in tumours, International Journal of Medical Sciences, 10, 2010, 74-81.

Anais Baudot, Mutated genes, pathways and processes in tumours, Scientific report, 67, 2010, 34-40.

By Joel A, Lefferts and Gregory J.Tsongalis, KRAS mutation detection Clinical Chemistry, 56(5), 2010, 698–701.

Cesare Gridelli, Erlotinib in non-small cell lung cancer treatment: current status and future development, European journal of cancer, 21, 2012, 56-62.

Charles J. Sherr, the RB and P53 pathways in cancer, European journal of cancer, 2, 2002, 798-804.

Chikako Kiyohara and Kouichi Yoshimasu, Genetic polymorphisms in the nucleotide excision repair pathway and lung cancer risk: a meta-analysis, European journal of cancers, 4, 2007, 56-62.

Fabio Vandin, Denovo discovery of mutated driver pathway in cancer, Scientific Report, 7, 2011, 12-18.

Gregory J. Riely, Jenifer Marks, KRAS mutations in non-small cell lung cancer, Indian journal of cancer research, 6, 2008, 67-71.

Jill M. Siegfried, Christopher T. Gubish, signaling pathway involved in cyclooxygenase-2 induction by hepatocyte growth factor in non-small cell lung cancer, The American Society for Pharmacology and Experimental Therapeutics, 72, 2007, 65-70.

Joseph R. Nevins, the RB/E2F pathway and cancer, International Journal of Collaborative Research on Internal Medicine & Public Health, 10, 2001, 958-965.

Kazutsugu Uematsu, activation of the WNT pathway in non-small cell evidence lung cancer: evidence of disheveled over expression, genetic research, 6, 2003, 123.

Lishan Wang, Yuanyuan Xiong, Yihua Sun, Hlung DB: an integrated database of human lung cancer research, Nucleic Acids Research, 38, 2010, 234-240.

Makoto Maemonda, Gefitinib or chemotherapy for non-small cell lung cancer with mutated EGFR, the New England journal of medicine, 83, 2010, 576-580.

Melissa Rotunno, phase 1 metabolic genes and risk of lung cancer: multiple polymorphisms and mRNA expression, scientific report, 4, 2009, 560-567.

Niranjan, Study of cancer genes in X- chromosome, Journal of Theoretical and Applied Information Technology, 90, 2008, 890-901.

Roberto Bianco, Key cancer cell signal transduction pathways as Therapeutic targets, European journal of cancers, 34, 2005, 165-169.

Stephen S.Hecht, Tobacco smoke carcinogens and lung cancer, European journal of cancers, 19, 1999, 78-81.

Subhalaxmi nayak, insilico retrival of novel gene using genetic and comparative modeling of immunoglobuline lambda locus a potent target of lung cancer, International Journal of Bioinformatics Research , 3(1), 2011, 103-113.

Williams, New drive mutations in non-small cell lung cancer, International journal of cancer, 6, 2010, 342-348.

Xiaozhus Zhang, Alex Chang, molecular prediction of EGFR-TKI sensitivity in advanced non-small cell lung cancer, International Journal of Medical Sciences, 4, 2008, 4-6.