"Pharmacophore Modelling, Virtual Screening, Docking and ADMET studies of PI3K inhibitors for the Treatment of Cancer"

A PROJECT SUBMITTED TO

NIRMA UNIVERSITY

In partial fulfilment of the requirements for the degree of

Bachelor of Pharmacy

BY

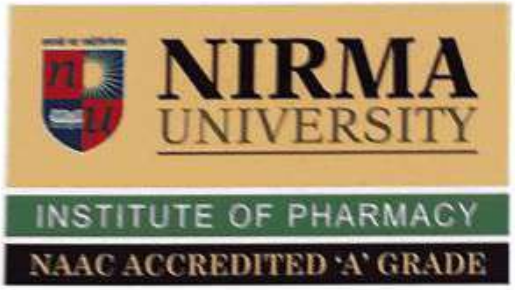
JADVANI ASHRUTI H. (16BPH007)

Semester VIII

UNDER THE GUIDANCE OF

DR. CHARMY KOTHARI - GUIDE

DR. HARDIK BHATT - Co GUIDE



INSTITUTE OF PHARMACY

NIRMA UNIVERSITY

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GUJARAT, INDIA

APRIL 2020

CERTIFICATE

This is to certify that ***"Pharmacophore Modelling, Virtual Screening, Docking and ADMET studies of PI3K inhibitors for the Treatment of Cancer"*** *is the bonafide work carried out by JADVANI ASHRUTI (16BPH007), B.Pharm semester VIII under our guidance and supervision in the Institute of Pharmacy, Nirma University, Ahmedabad during the academic year 2019-2020. This work is up to my satisfaction.*

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CERTIFICATE OF SIMILARITY OF WORK

This is to undertake that the B.Pharm. Project work entitled ***"PHARMACOPHORE MODELLING, VIRTUAL SCREENING, DOCKING AND ADMET STUDIES OF PI3K INHIBITORS FOR THE TREATMENT OF CANCER"*** *Submitted by* ***JADVANI ASHRUTI*** *(16BPH007), B.Pharm. Semester VIII is a bonafide research work carried out by me at the Institute of Pharmacy, Nirma University under the guidance of Dr. Charmy Kothari and Dr. Hardik Bhatt. I am aware about the rules and regulations of Plagiarism policy of Nirma University, Ahmedabad. According to that, the research work carried out by me is not reported anywhere as per best of my Knowledge.*

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DECLARATION

I, JADVANI ASHRUTI (16BPH007), student of VIIIth Semester of B.Pharm at Institute of Pharmacy, Nirma University, hereby declare that my project entitled ***"PHARMACOPHORE MODELLING, VIRTUAL SCREENING, DOCKING AND ADMET STUDIES OF PI3K INHIBITORS FOR THE TREATMENT OF CANCER"*** *is a result of culmination of my sincere efforts. I declare that the submitted project is done solely by me and to the best of my knowledge; no such work is done by any other person for the award of degree or diploma or for any other means. I also declare that all the information was collected from various primary sources (journals, patents, etc.) has been duly acknowledged in this project report.*

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Thank You.

Author

JADVANI ASHRUTI

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ABSTRACT

Cancer is the second leading causes of deaths worldwide. Nearly 24% of total deaths worldwide are due to cancer. Cancer refers to group of diseases which are marked by rapid proliferation of cells which leads to tumour formation. PI3k are group of enzymes that are responsible for cell proliferation, differentiation and cell growth. Signals from the growth factors activate the tyrosine kinase receptors or the G protein coupled receptors which activates the downstream signalling of AKT and m-TOR. PI3K signalling pathway is responsible for cell growth and survival. PI3K enzymes are capable of phosphorylating the –OH group of phosphotidylinositol at various sites. Mutations at various sites in this signaling pathway lead to excessive cell proliferation and inhibition of apoptosis leading to cancer. Therefore PI3K can be targeted to develop anticancer agent. There are various pi3k inhibitors approved by FDA like LY294002 and Wortmannin. The first marketed pi3k inhibitors are LY294002 and Wortannin. Wortmannin was the first naturally derived from a fungus *Penicillium funiculosum* and is a non specific inhibitor of pi3k. LY294002 was the first synthetic agent containing a morpholine ring and is a potent pi3k inhibitor. But both of these agents are nonspecific in their action and have low bioavailability. As they play role in cell proliferation they can be suitable target for cancer therapy. The aim is to generate a new molecule that can be identified as a potent hit and can be designed into a lead molecule having activity more than the reference molecule (FDA approved drug) and can be synthesised and can enter the clinical trials.

1.Introduction

1.1 INTRODUCTION TO CANCER

Cancer is a disease related to excessive proliferation cells. Our body is made up of cells. These cells are responsible for carrying out all the necessary functions. The cells grow, divide and ultimately leads to death. Each cell is continuously dividing and has specific life span. The growth, division and death of the cells is under a tight regulation. When the cells escapes this regulatory the normal activities of cells are disturbed and they start dividing rapidly. This can be caused due to variety of reasons like exposure to harmful chemicals, mutagens, lifestyle or virus. The rapidly dividing cells tends to accumulate to produce tumors which is a characteristic of cancer. These cells invade the nearby tissues and also dislodge from the place of origin and enter the circulation. It is transported by the circulatory systems to different body organs where they lodge themselves. These cells signals the nearby cells to replicate and thus the normal cells are converted into neoplastic cells. This is known as metastasis. The tumor formation begins at the site. When the cells do no metastasized and remains confined at one site it is known as benign tumors. Benign tumors can be cured easily as compared to metastatic tumors.

There has been an increase in the number of cancer cases throughout the world. With every passing decade the prevalence of cancer is increasing by two to three folds. This is due to changes in lifestyle, bad habits, exposure to constant pollution and various other reasons. The large part of ongoing research is concentrated on cancer. But yet no permanent cure has been developed. Each cancer is different and so the present medication cannot cure every cancer. Cancer is also a refractory disease. Most of the patients is seen to have encountered with cancer again after few years even when it was completely cured in the first time.

Many researches are going to find new targets and new derivatives to cure cancer. A wide classes of drugs have already been approve to treat specific types of cancers which include alkylating agents, steroids, plant derivatives like Vinblastine and Vincristine, anti-metabolites like folate antagonists, etc. These drugs have been useful in treating cancers like Lymphomas, Hodgkin’s and breast cancers. But these drugs are associated with many side effects.

According to a press release by WHO is 2018, the number new cancer incidents have reached around 18 million and almost 9 million deaths have occurred in 2018. It also stated that one out of every five men and six women will suffer from cancer at least once in their lifetime.

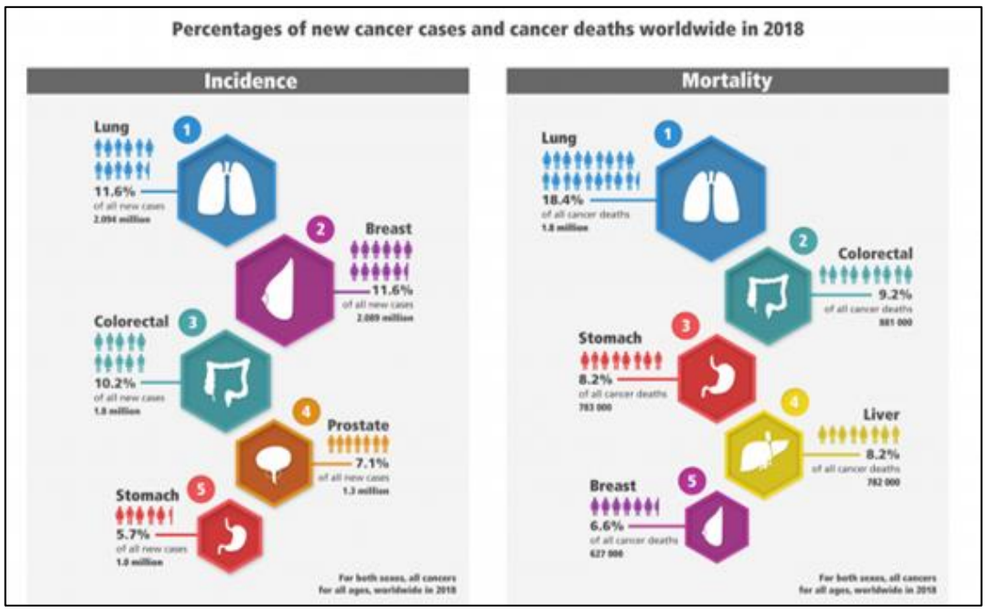


Figure 1 Global Cancer data by WHO, 2018

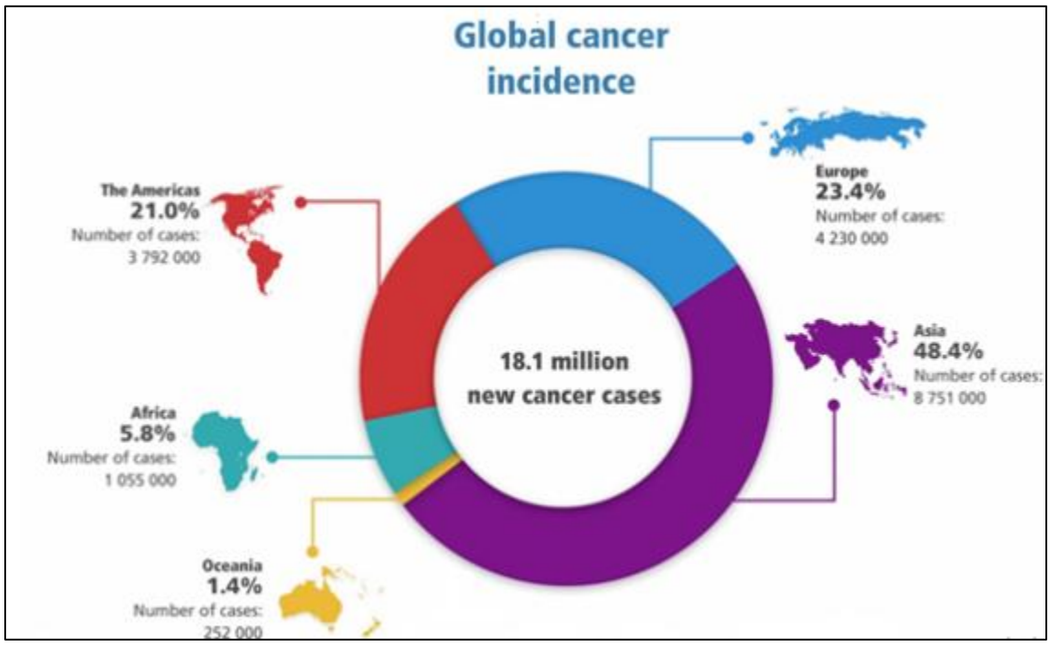


Figure 2 Percentage of new cancer cases and deaths worldwide in 2018

1.2 TYPES OF CANCER

Cancer is basically classified into two categories: Benign and Malignant

Benign Tumor

They are generally non-cancerous. The cells proliferate and accumulate to produce tumors. But the cells from this tumors remain confined at one place and so the cancer doesn't spread to other parts. They can be treated easily but they may convert into malignant tumors.

Malignant Tumor

The cells that divide uncontrollably and displace from the site of origin to the other are known as malignant tumors. The cells move from the site through blood or lymphatic system and lodges at a different site and initiates the tumor formation at that place. The cells are known to be metastasized. Such cancers are difficult to control and cure.

The benign and malignant tumors can be classified on the basis of site of origin

a. **Carcinoma**

They are the most common type of cancer which originates from skin, lungs, breast, pancreas and glands.That is cancer that arises from epithelial tissues. They are solid tumors which can metastasize and spread throughout the body they usually do not.

The most common types of carcinomas include:

- Basal cell carcinoma
- Squamous cell carcinoma
- Renal cell carcinoma
- Invasive ductal carcinoma
- Adenomacarcinoma

b. Sarcoma

These are the cancers of bones and soft tissues that connect the bones or tissues or support different organs which include blood vessels, tendons, and nerves. Bone sarcoma also known as osteosarcoma arises from bones and spreads to other bones in later stages. Soft tissue sarcomas are those that appear in muscles, fats and blood vessels

c. Lymphoma

This cancer arises in the lymphocytes. Lymphocytes are responsible for the fighting off the disease and are a part of the immune system. These include the cells of spleen, thymus, bone marrow etc.

The lymphomas are classified into

- Hodgkin's lymphoma
- Non-Hodgkin's lymphoma

They differ in the type of lymphocytes that are affected

d. Leukemia

It is the cancer of white blood cells. It originates in the cells of bone marrow and spreads throughout the body. The classification depends on the extent of duration and the type of cells affected.

e. Melanoma

This is the cancer of skin and the cells that make pigments of skin.

1.3 CAUSES OF CANCER

1. Mutations

The main cause of cancer is mutation. Mutation is change or alteration in genetic composition of an individual. The genes control the functioning of the cell any alteration changes the protein structure which deviates from its original activity. These mutations are mainly due to mutagens. The mutagens can be radiations, UV rays or any chemical substances.

2. Smoking

It is one of the leading cause of cancer. The smoke is responsible for damaging the linings of the lungs. The smoke contains many carcinogens which enters into the blood stream and may cause cancer of different body parts. The cancers caused by smoking can be prevented.

3. Obesity

Due to sedentary lifestyle and lack of physical activities there has been an increased in obesity and overweight. This enhances the chances of cancer especially pancreas cancer, colon cancer, breast cancer (in postmenopausal women). Increase in adiposity makes an individual fall prey to cancer. [1]

4. Infections

The infectious agents like viruses doesn't cause cancer but they increase the chances of cancer. The most common viruses that are known to cause cancer

are Human Papilloma Virus, H. Pylori, Hepatitis B and C viruses, etc. Every one out of five cancers is due to these viruses. They are mostly responsible for the cancers of stomach, liver and cervix.

5. Pharmaceutical drugs

The overuse of many drugs can increase the chances of cancer. Many drugs have severe side effects and may lead to cancer. The abuse of anabolic steroids have cause severe damage to many organs leading to liver cancer, adenocarcinomas, etc. These steroids causes hormonal imbalance and causes prostate cancer.

6. Alcohol

The over consumption of alcohol for a longer period is the major cause of liver, larynx, breast and esophageal cancer. Combination of alcohol and tobacco or smoking heightens the risk of cancer. Alcohol is metabolized by the body into acetaldehyde and also due to oxidation it may form free radicals of DNA and proteins. These are the main risk factors for causing cancer.

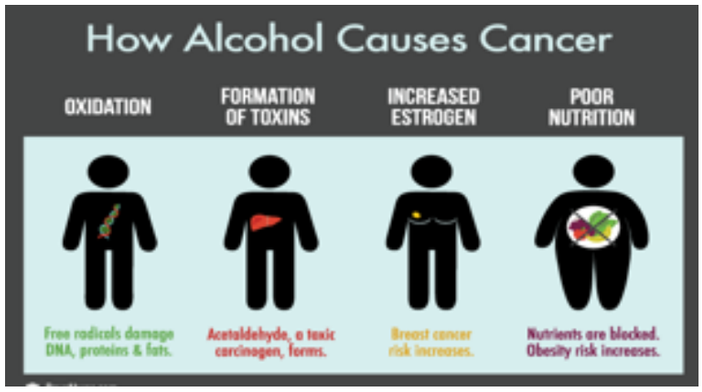


Figure 3 how alcohol causes cancer

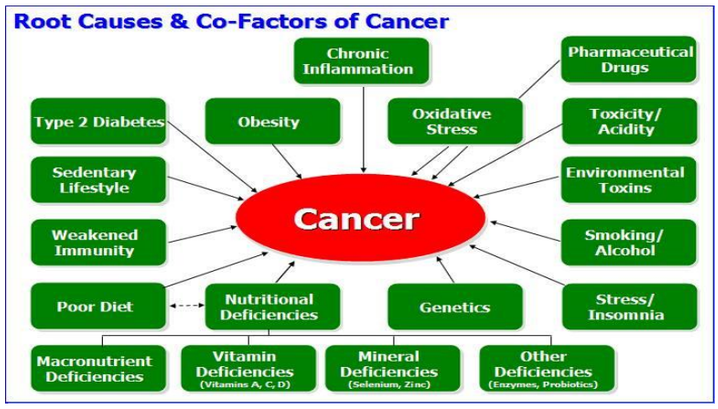


Figure 4 Causes and Co- Factor of cancer

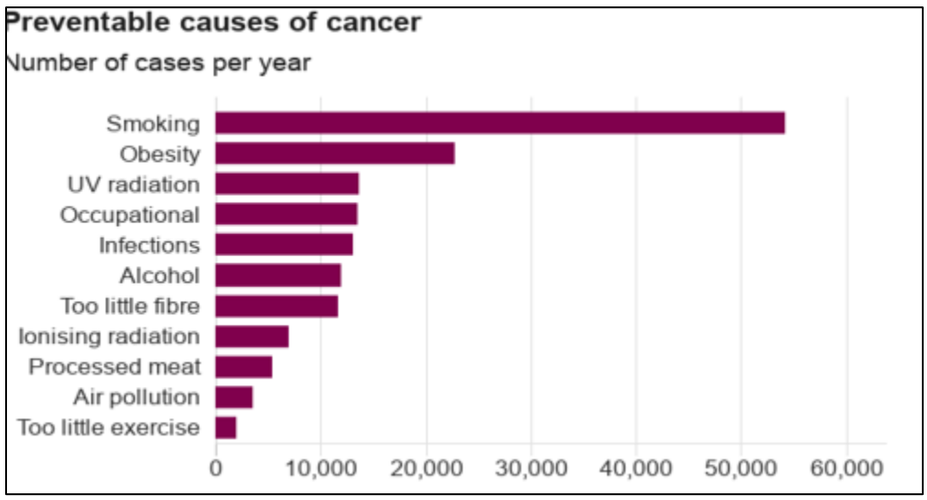


Figure 5 Number of cases of preventable cancer per year

1.4 PATHOPHYSIOLOGY

Cancer on the cellular level cancer is due to changes or mutations in deoxyribonucleic acid. This changes are due to exposure to any mutagens. Cancer arises due to uncontrolled division of cells. The cells are responsible for carrying out normal functioning of the body. The cell division takes place under strict regulation. Sometimes due to mutagens or any other external factors the cells may escape this regulation and start dividing controllably. Mutagens like UV rays, radiations, or any chemical substances causes ionization of proteins and nucleic acids. This leads to change in the structure of protein material and hence alteration in their activity.

The proto-oncogenes are expressed in cells such that they control the cell division. They inhibit the aberrant growth and division of cells. Due to mutation sometimes an incorrect proto-oncogene is expressed it leads to malignancies. The aberrant expression or suppression of tumour supressing genes can cause conversion of proto-oncogenes into oncogenes.

The neoplastic cells differ from the normal cells in many ways. The neoplastic cells shows local invasiveness, disorganization, metastasis and persistent growth.[2] The cancer cells attack the neighbouring cells and escapes the contact inhibition. This is achieved by the cancer cells by either destroying the neighbouring cells or by producing some changes in the neighbouring healthy cells. This is known as local invasiveness. Metastasis refers to passive movement of cancer cells from sit of origin to another through lymphatic system. The cancer cells are rapidly dividing cells. They undergo mitosis continuously and hence leads to persistent growth. This persistent division of cells tend to accumulate. The adhesion of one type of cells leads to disorganisation of the normal structure of the cells and tissues.

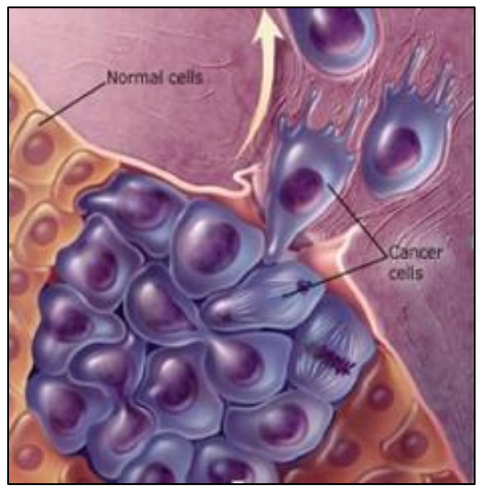


Figure 6 Pathophysiology of Cancer

1.5 TREATMENTS

Cancers if diagnosed at an early stage can be completely cured. As the cancer progresses the aggressive therapies are to be employed. During the last stages of cancer palliative treatments are offered to the patients and the relatives to give them the symptomatic relief. Various combination therapy treatments are offered. The treatments offered to the patients depends on the type of cancer and the stage of cancer. The various treatment options available are:

1. Chemotherapy
2. Radiotherapy
3. Surgery
4. Palliative Therapy

1. Chemotherapy:

Chemotherapy is an aggressive form of therapy used to treat cancer. It involves administration of various drugs through intravenous route. It is most widely used to cure cancer and is often used in combination with other therapies like radiotherapy, hormone therapy, or surgery. It is given to reduce the amount of cancer cells, to shrink the tumor, to get them ready for the other therapies and to reduce the symptoms and pain in the later stage. The chemotherapy drugs acts by various mechanisms like alkylating the DNA molecule, inhibiting mitotic spindle formation, or by acting as false substrate. These

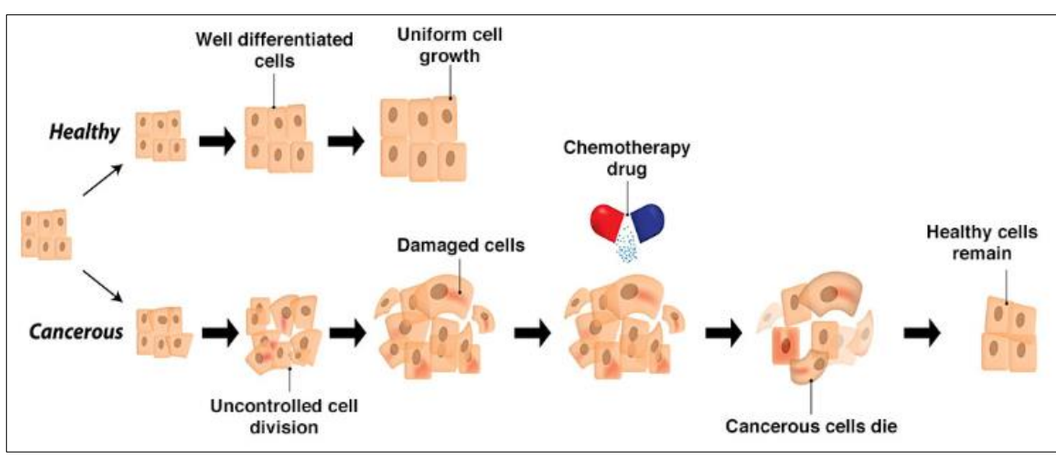


Figure 7 Chemotherapy Treatment

drugs are associated with various side effects like nausea, vomiting, hair loss, weight loss, kidney and liver failure, infections, anemia, etc

2. Radiotherapy

Radiotherapy uses radiations to destroy the cancerous cells. At low dose x rays are used to observe the internal body parts. When these radiations are administered at high doses they damage the DNA and causes death of the cells. These radiations causes fragmentation of the DNA or causes mutations in DNA strands. It requires few weeks for DNA to be mutated and to be destroyed which is then removed by the body's immune system. The radiations can be given as an external beam which provides a localized effect whereas it can also be given in the solid or liquid forms which is ingested and attacks the cancerous cells.

3. Surgery

Surgery involves removal of the cancerous cells or the tumor. The tumor along with the neighboring cells are surgically removed to ensure that all the cancerous cells are removed. It is the oldest therapy and is very effective in some cancers. The surgery can be for diagnosis, prevention, curing or removal of cancer. A part of the tissue is surgically removed to check for the cancerous growth, known as biopsy.

4. Palliative Therapy

Palliative therapy is employed during the last stage of cancer or for the untreatable cancers. The sole purpose of this therapy is to reduce the symptoms and to alleviate the painful conditions. This therapy can be employed at any stage to improve the quality of living and to prolong the living period by few years even when cancer cannot be cured.

2.Phosphoinositide 3-kinase

2.1 INTRODUCTION TO PI3K

Phosphatidylinisitol-3-kinase are the lipid kinases which are widely expressed and phosphorylates the phosphoinositides. They phosphorylate at the D-3 position of Phosphoinositides ring and converts phosphatidylinositol into phosphatidylinositol-3,4-bisphosphate and phosphotidylinisitol-3,4,5- triphosphate. PIP2 and PIP3 are the byproducts of this phosphorylation. They regulate the downstream process and signal transduction and are the secondary messengers responsible for the cell growth and its vital functions like cell division, cell differentiation, chemo taxis and for the survival of the cell.

PI3K consists eight members which are categorized into three classes based on their domain structure, substrate that it acts on to and the activation molecules. There are major three class of PI3K that are class 1, class 2and class 3. Class 1 comprises of 1A and 1B. Class 1A has three catalytic subunits which are p110α, p110β and p110δ which interacts with one of the regulatory subunit and forms heterodimers. The regulatory subunits are p85α, p85β, p85γ, p50α and p55α. Class 1B consists of heterodimer of p110γ and regulatory p101 subunits. [3] Class 2 consists of C2α, C2β and C2γ but it has no regulatory subunit. Class 2 is responsible for converting PI to PIP2 and PIP3 to PIP4. Class 3 differs from Class 2 as it produces only PIP2 from PI and consist of a catalytic and regulatory subunits which are present in the form of heterodimer. The catalytic and regulatory subunits are Vps34 and Vps15/p150 respectively. Class 4 is a serine/threonine protein kinase.

When an activation moiety binds to the PI3K receptor it activates the downstream signaling process. Over activation of this pathway or mutations are responsible for many diseased conditions.

2.2 CLASSES OF PI3K

There are 15 proteins in the PI3K family which are similar in their kinase domain structure but differ in the substrates that they act on. It is majorly categorized into four classes:

Class I

Class I is divided into IA and IIB and IA is predominant in cancer. It consists of a catalytic and regulatory subunits. The catalytic subunit is p110 which is categorized into p110α, p110β, p110δ and the regulatory subunit is p85 which is categorized into p85α, p85β, p55α, p55γ and p50α. The catalytic subunit is responsible for formation of PIP3 and the regulatory subunit is responsible for the binding to the activator molecules. The class IA enzymes are activated by tyrosine kinase receptors whereas p110β and p110γ are

activated by GPCR. Class IB enzymes consist of p110γ abundantly present in leukocytes and its regulatory subunits are p101 and p87. [4]

Class II

The class II family consists of only catalytic unit and has no regulatory subunit. It comprises of C2α, C2β and C2γ. Class II has a center moiety which contains C2 domain, helical domain and a catalytic domain. It also has a RAS biding site. The class II proteins are conserved proteins. The N terminal differs significantly from other classes as it has no regulatory subunit binding site. The Class II protein are activated by various factors like insulin, cytokines, macrophages etc. The also play role in secretion of insulin. [5]

Class III

The class III proteins are similar to the Class I proteins in their structure. They also contain regulatory as well catalytic subunits. They are present in the form of heterodimers. They are important in several processes that are involved in immune system.

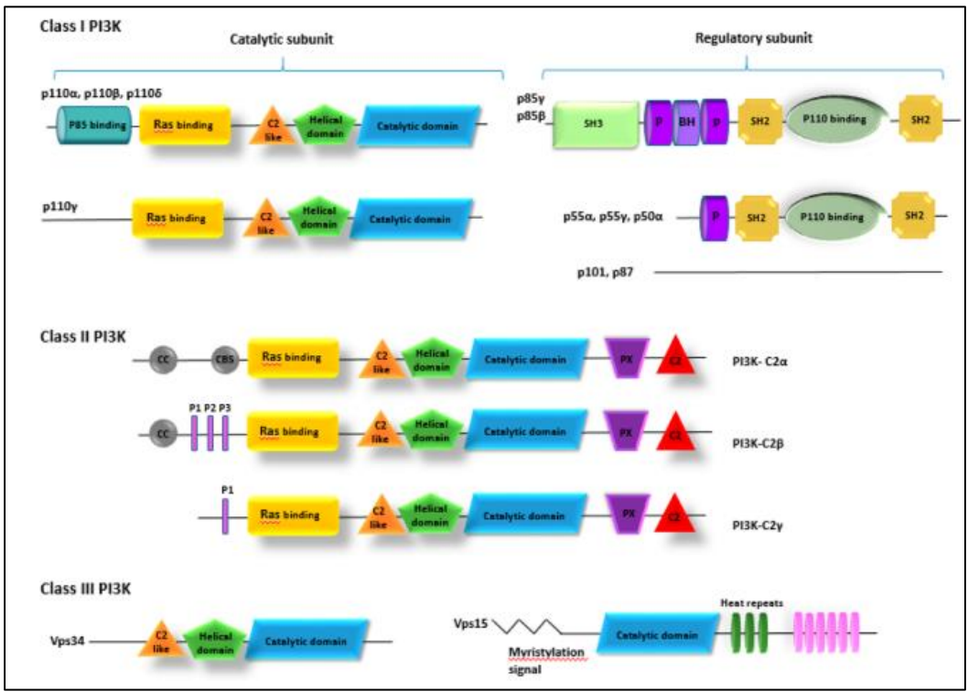


Figure 8 . Structures of the isoforms of the three classes of PI3Ks

2.3 PI3K SIGNALING PATHWAY

PI3K are characterized lipid kinases which are responsible for phosphorylating 3-OH group of the inositol and converting them into PIP2 and PIP3. The class 1 proteins are activated by receptor tyrosine kinase and class II by G- protein coupled receptors. This leads to interaction of the catalytic and regulatory subunits forming the heterodimers. The activation by binding of growth factors lead to auto phosphorylation of the tyrosine

residues. This leads to initiation of the downstream process. The activatedPI3K phosphorylates the Phosphatidylinisitol-3- phosphate and converts it into Phosphatidylinisitol-3,4- bisphosphate further phosphorylation forms Phosphatidylinisitol-3,4,5- triphosphate. These are the second messengers. This activation process is regulated by PTEN protein found in the cytosol. This inhibits conversion of PIP2 into PIP3. The PIP3 further activates the Akt protein via PDK-1. The Akt main functions for cell proliferation and cell division and inhibits apoptosis. It further activates m-TOR (mammalian target of rapamycin) which also plays role in cell growth and survival.

The activation of PI3K by the growth factors lead to production of PIP3 and PIP2 in the inner side of plasma membrane. The interaction of these phospholipids with the Akt leads to translocation where the PDK-1 protein is located. The Akt undergoes conformational changes due to interaction of Akt PH domain and phosphoinositides. The activation ofAkt activates 30 different targets which includes m-TOR.

Phosphatase and tensin homologue is identified as the tumor suppressor gene and negatively regulates this activation process. The deletion or mutation in this gene can lead to uncontrolled cell division and leads to cancer formation. PTEN dephosphorylates the PIP3 and converts into PIP2. It is one of the most frequently mutated gene contributing to cancer. [6]

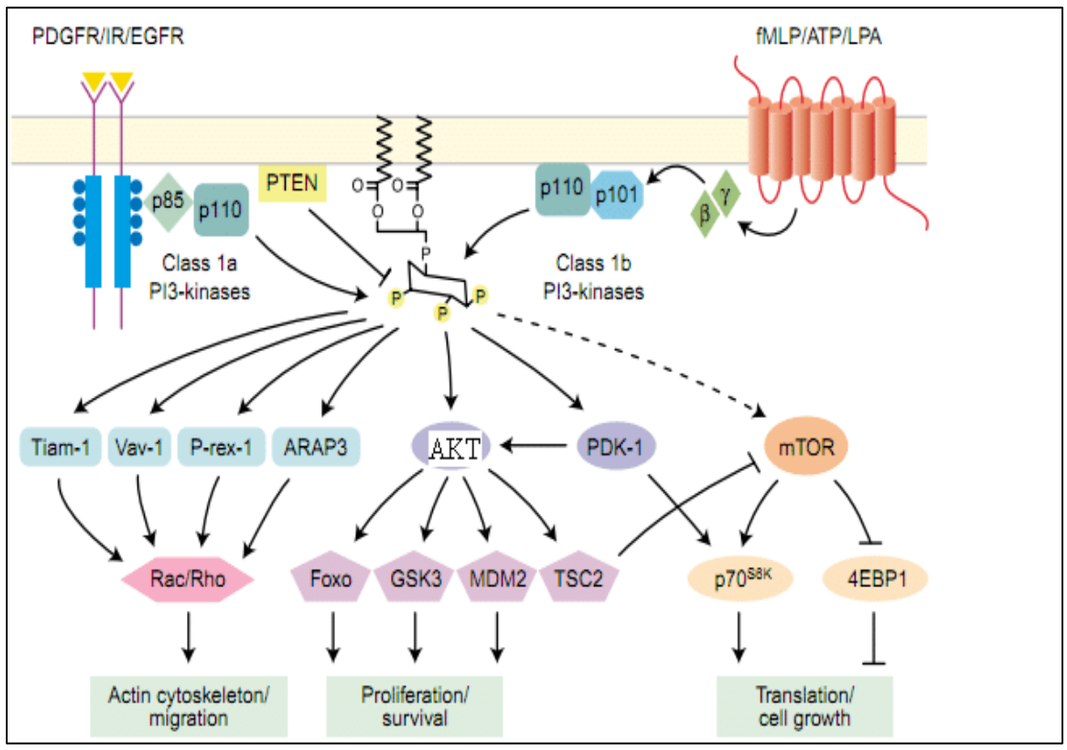


Figure 9 PI3K Signalling Pathway

3.PI3K INHIBITORS

3.1 ROLE OF PI3K IN CANCER

PI3K plays an important role in cell division and proliferation. The activation of PI3K causes activation of AKT which has major role in cell proliferation. It inactivates the pro apoptotic factors such as Fas ligand. This causes inhibition of cell death and prolongation of survival period. The cell death regulation is important for treatment of cancer.

The cell growth and the cell progression involves various proteins which includes GSK-3, m-TOR, IRS-1 and the various Akt targets. They also play role in the protein synthesis, glycogen metabolism and cell cycle regulation. The activation of these proteins is like a chain reaction. Activation of one protein leads to activation of other cellular proteins. These activated cell growth leads to rapid cell division leads to tumor growth and development.

The main factor that leads to cancer is the mutation in the PI3k. The gene which is frequently mutated is present on the chromosomes 3q26 which is altered in most of the cancer. The alteration in PIK3C is leading cause of breast, ovarian and cervix cancer.

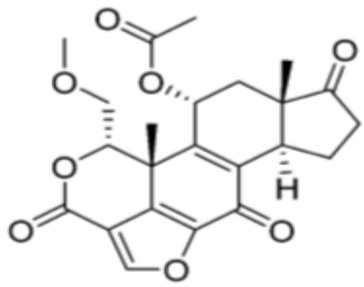
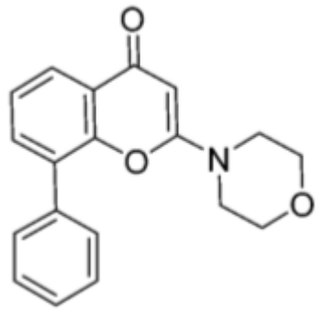
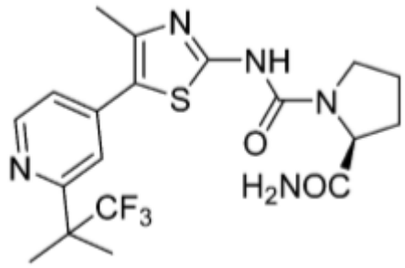
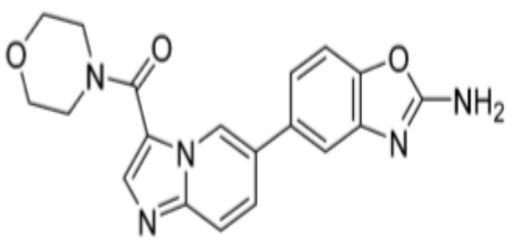
The PI3K is activated by the protein tyrosine kinase or other growth factors. These targets can be self-activated in the absence of these growth factors as well. The overexpression of these targets or the abundance of these receptors on the cell surface amplifies the activation process leading to cancerous development.

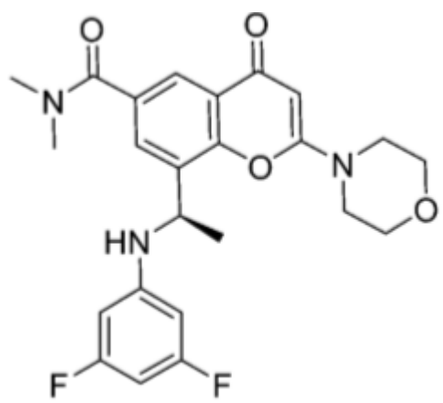
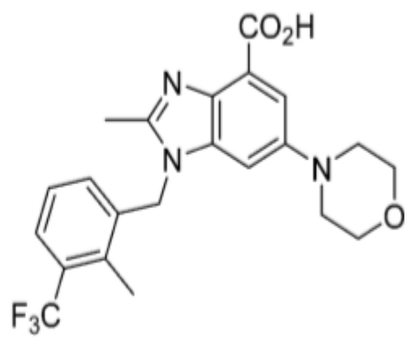
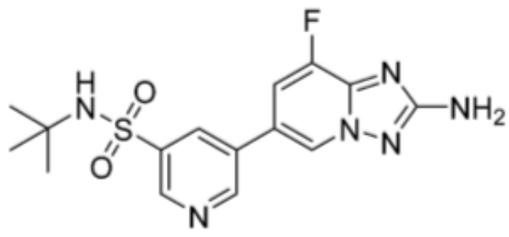
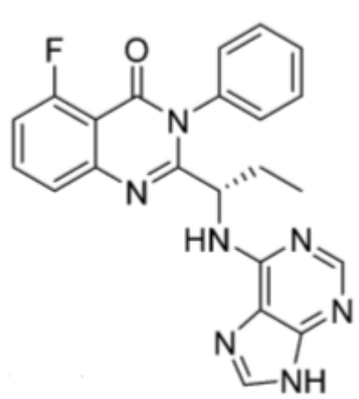
The downstream process after activation of PI3K is the generation of secondary messengers which are the PI3 k products. These products are the substrate for the PTEN which and the dual activity on the lipids and proteins. PTEN is responsible for the negative feedback mechanisms and inhibits super excitation of PI3K. But the mutations in the PTEN causes alterations in the functions and hence the control over the PI3K pathway is lost. When this protein is overexpressed it is said to be tumor suppressor as it inhibits the cell growth and enhance the programmable cell death.[7]

3.2 PI3K INHIBITORS

The first PI3K inhibitors which were first developed are Wortmannin and LY294002. The Wortmannin is a fungal derivative and is developed from Penicilliumfuniculosum. The first synthetic derivative is the LY294002. But after the discovery of other targets and the role of PI3K came into picture these drugs were proved to be not very efficient. This is because they did not have specific action and had varying affinity for different isoforms. They had very less solubility in water and also caused cytotoxicity and hence this lead to development of new PI3K inhibitors which has good solubility and good

specificity. The first isoform specific PI3k inhibitor was a quinazolin derivative which was p110δ specific. [8][6]

1.		**Wortmannin** Pan-PI3k inhibitor Fungal derivative Low solubility and lacks specificity [9]
2.		**LY294002** First synthetic derivative Good solubility Lacks Specificity [9]
3.		**NVP- BYL719** (Alpelisib) P110alpha inhibitor Novartis Phase IB/II [10]
4.		**MLN1117** (Serabilisib) PI3Kalpha inhibitor Intellikine/Millenial Phase IB/II [11]

5.		**AZD8186** AstraZenac PI3Kβ Phase I [11]
6.		**GSK2636771** GlaxoSmithKline PI3Kβ Phase I/IIA [10]
7.		**CZC24832** Cayman Chemical Company PI3Kγ [11]
8.		**CAL101** Idelalisib Calistoga Pharmaceuticals p110δ Phase III [12]

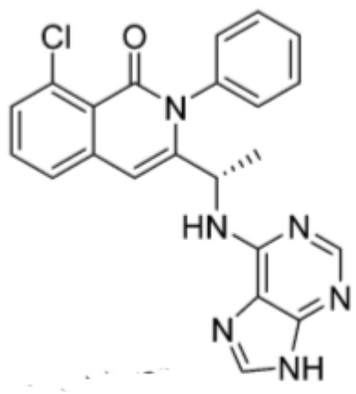
9.		**Duvelisib** Copiktra PI3kγ/δ FDA approved [13]

Table 1: PI3K inhibitors approved by FDA or under clinical trials

4.Aim and Objective

AIM OF THE RESEARCH

To perform Pharmacophore modelling, virtual screening, docking and in-silico ADMET studies of PI3K inhibitors for the treatment of cancer.

Objective

1. To perform an intensive literature search and to identify the different scaffolds that can be used for developing PI3K inhibitor. From the articles various structures with the least, moderate and the highest were used to generate a ligand based model.

2. To generate a pharmacophore model using various softwares like DiscoTech and GASP.

3. To perform virtual screening of the generated model

4. To perform docking of the top hits obtained

5. To perform ADMET studies to determine its physicochemical properties and its toxicity profile.

5.Computer Aided Drug Design

5.1 INTRODUCTION TO CADD

With advances in the fields of molecular biology, biochemistry many novel targets are being exploited. With introduction to new targets, new chemical moieties are also being developed. The use of traditional methods to synthesise new molecules is very tedious and involves screening of lot many drug moieties and also its in-vitro screening that seems to have either same structure or same activity. The ever increasing demand has led to development of computational methods of drug designing. The drug discovery and development process can be speed up, with decrease in the cost by using the computer aided drug design. This will accelerate the process.

The development of new drug moiety takes near about 14-15 years with the total investment of US $ 500-600 million. This can be reduced using the computational hit to lead optimization process which reduces the number of drugs that are synthesis and screened in-vitro.

This technique is based on the interaction of biologically active compounds with the molecular targets which mostly are proteins or nucleic acids. These interactions are mainly influenced by the various physical parameters like molecular surface, electrostatic force, hydrophobic interactions and hydrogen bonding.

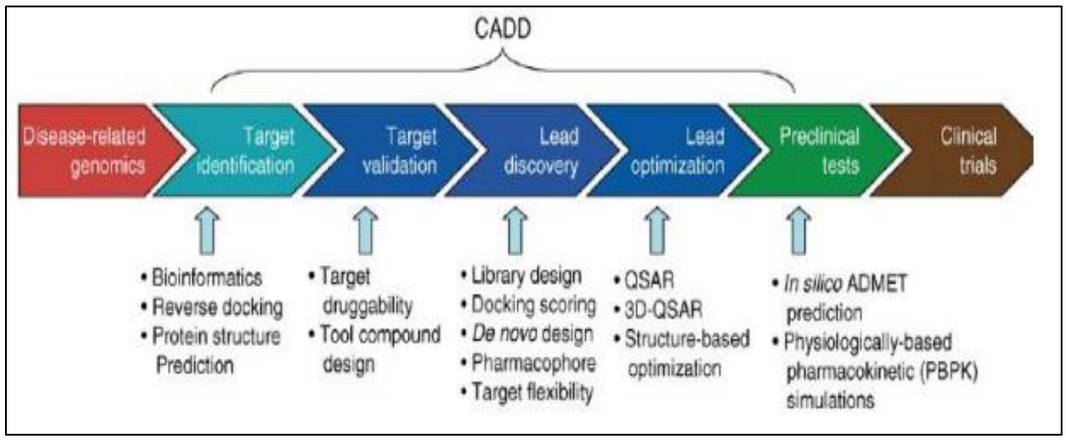


Figure 10 In-silico Computer Aided Drug Design

The drug discovery process through computational method includes major seven steps which is initiated by identifying the disease and its underlying genetic cause. The target is identified which can be exploited to identify its therapeutic interventions. The target is validated using bioinformatics and this leads to identification of the lead molecule. The lead is then optimised using QSAR or structure based optimization. The molecule having biological activity is optimized and then enters into the pre-clinical phase. This phase include the ADMET studies to predict the pharmacokinetics and toxicity profile of the drug.

The steps included in CADD are:

1. Identification of hit molecule through virtual Screening
2. Hit to lead optimization
3. Optimization of lead molecule

The types of drug design are:

1. Ligand based drug design
2. Structure based drug design

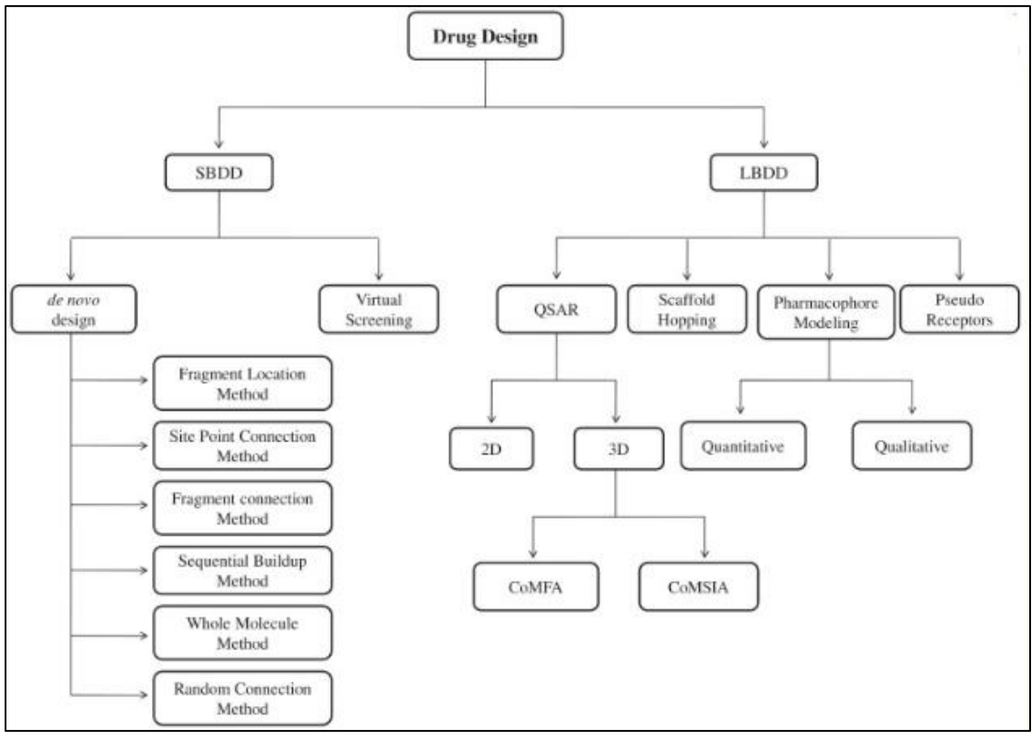


Figure 11 Computer Aided Drug Design

1. Structure based drug design

   SBDD approach is used when the structure of the target is known. When the target is not known it can be identified using the various experimental methods like X-ray diffraction, NMR, etc. The important feature for SBDD is the structure of receptor.

   This involves de-novo and virtual screening. De-novo means starting from the beginning. The target is identified, the chemical entity is developed from the beginning that complimentarily binds to the receptor. Virtual screening involves searching the online databases to identify the lead molecule.[14]

2. Ligand based drug design

When the 3D structure of the receptor target is not known LBDD approach is used. It relies on the knowledge of the molecule that is going to bind to the receptor of interest. 3D QSAR and Pharmacophore modelling are the main approaches in LBDD. [15]

5.2 PHARMACOPHORE MODELLING

According to Paul Ehrlich, "a pharmacophore is the molecular framework that contains all the essential features which are responsible for the activity". IUPAC defines pharmacophore as "an ensemble of electronic and steric features that is necessary to ensure the supra-molecular interactions with a specific biological target and to trigger the biological response."

Pharmacophore is the group of common features that are necessary for the chemical moiety to bring about its action by interacting with the receptor. The pharmacophore generation is necessary step in the drug discovery process to know the receptor – ligand interaction, its ADMET profile, and drug - drug interaction and also for de- novo synthesis. [16]

The pharmacophore has few basic features which are

1. Hydrogen bond donor
2. Hydrogen bond acceptor
3. Aromatic ring
4. Cation
5. Anion
6. Hydrophobic centroid

Ligand based pharmacophore can be generated by stacking the active molecules upon one another and determine the common features in all the molecules that are responsible for the activity. This involves two main steps that are: firstly making the conformational space for the ligands and then stacking the multiple ligands.[17]

5.2.1. DiscoTech

The pharmacophore alignment is done using the various softwares like DiscoTech, GALHAD and GASP. DiscoTech aligns all the molecules and displays all the features available in the structures. The DiscoTech software displays the size, hits, score, tolerance and DMean value.

The size displays the number of features in the molecule.

The HITS displays number of models that are generated using the selected molecules.

The molecules are scored according to its binding ability with the receptor.

The model with the highest DMean value is used for refining.

Applications:

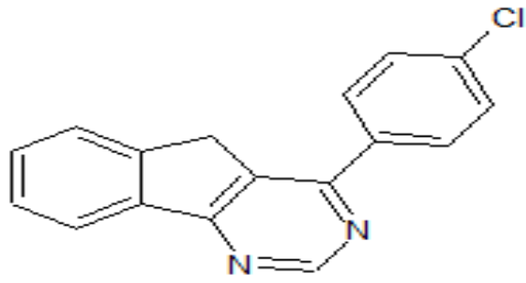
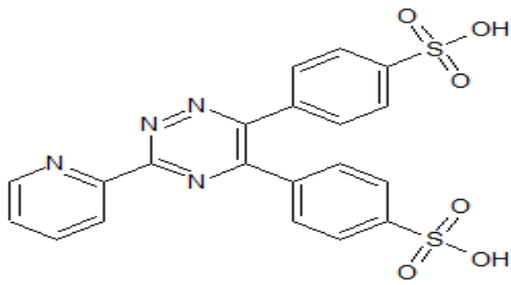
It is used for ligand binding.

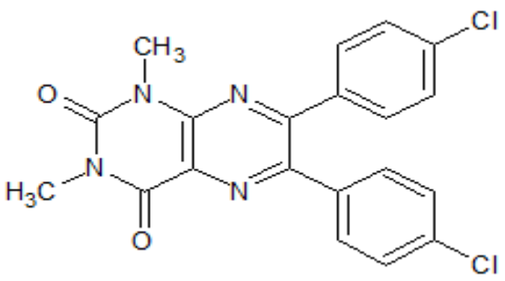
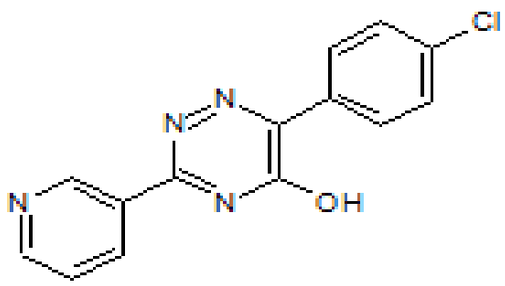
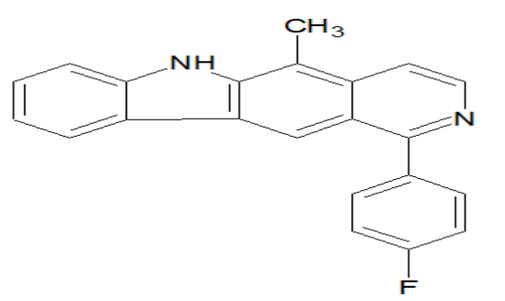
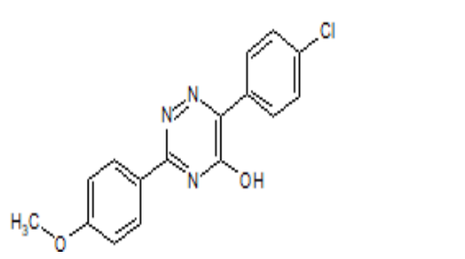
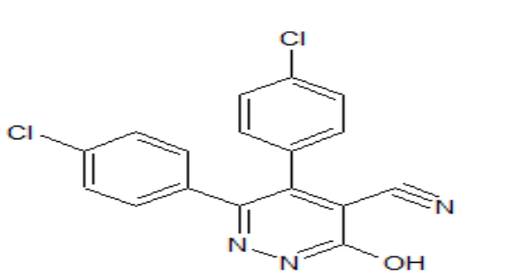
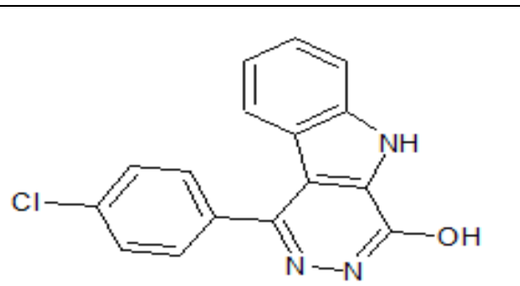
It is used for 3D-QSAR.

It is used for refining the pharmacophore models.

It is used to identify the activity of the functional group.

This experiment was performed on the basis of ligand based drug design. This was started by generating the pharmacophore model using the sybyl software. For this model generation, 13 molecules were selected from various articles whose activity is already known and is within the range of nm to µm. The pharmacophore I generated by combining different molecules and obtaining a final molecule having common features present in the molecules. The pharmacophore was generated using the 13 molecules and the 4 models were generated. Amongst this 4 models, model_003 had the highest Dmean value of 3.5311. This model was obtained by using the GASP software. The features obtained in the pharmacophore model were donor sites, acceptor atoms and hydrophobic atoms. The molecules used for the pharmacophore generation are mentioned in the below table.

Chemical Class	Structure	Activity (Ic50 value)
4-(4-chlorophenyl)-5*H*-indeno[1,2-*d*]pyrimidine Qfit: 97.9100 [18]		9nM
4,4'-[3-(pyridin-2-yl)-1,2,4-triazine-5,6-diyl]di(benzene-1-sulfonic acid) Qfit: 97.8600 [19]		0.05µm

6,7-bis(4-chlorophenyl)-1,3-dimethylpteridine-2,4(1*H*,3*H*)-dione Qfit: 97.8600 [20]		0.8nM
Benzaoxazol-2-one Qfit: 97.8600 [21]		19µm
2-pyrimidine derivatives Qfit: 97.8600		7.89 µm
6-(4-chlorophenyl)-3-(4-methoxyphenyl)-1,2,4-triazin-5-ol Qfit: 97.8600 [22]		0.008 µm
5,6-bis(4-chlorophenyl)-3-hydroxypyridazine-4-carbonitrile Qfit: 97.8300 [23]		0.24 nM
1-(4-chlorophenyl)-5*H*-pyridazino[4,5-*b*]indol-4-ol Qfit: 97.8200 [17]		0.008 µm

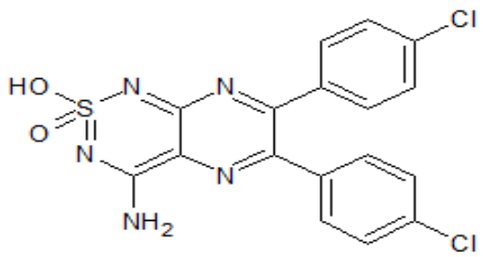
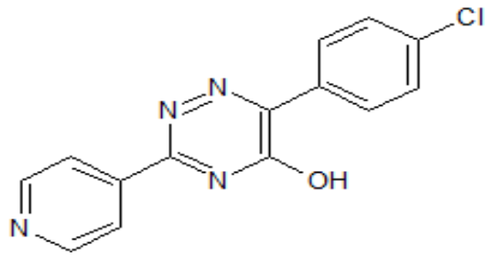
4-amino-6,7-bis(4-chlorophenyl)-2-hydroxy-2λ^6-pyrazino[2,3-*c*][1,2,6]thiadiazin-2-one Qfit: 97.7200 [23]		2.54 nM
6-(4-chlorophenyl)-3-(pyridin-4-yl)-1,2,4-triazin-5-ol Qfit: 97.6300 [16]		0.02 µm

Table 2: Molecules used for pharmacophore generation

Pharmacophore Models obtained through GASP:

1 of 4 rows

	1: FITNESS	2: SIZE	3: HITS	4: DMEAN
1: MODEL_001	3331.3200	4	14	3.5124
2: MODEL_002	3339.2400	4	14	3.5081
3: MODEL_003	3333.5500	4	14	3.5311
4: MODEL_004	3332.5700	4	14	3.5082

Table 3: Models generated through GASP

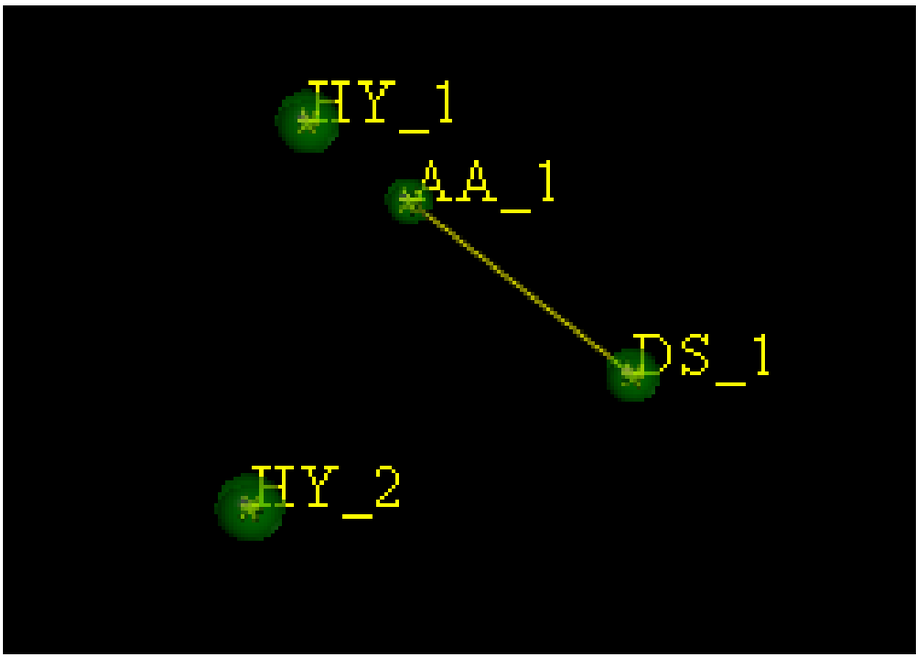


Figure 12: Pharmacophore model. The features include acceptor atom, donor site and two hydrophobic sites.

5.3 VIRTUAL SCREENING

Virtual screening is technique to identify the potential compounds that can interact with the receptors whose structure is already known. Virtual screening is most widely used to identify the potential compounds amongst the hundreds of molecules that will bind efficiently with the receptor.

Virtual screening is a computational technique widely used for discovery of lead molecule. Hundreds of molecules are virtually screened for it binging ability to the protein receptors. The protein structures are obtained from the various online databases which are available like ZINC, NCBI Pubchem, etc.

The main aim of virtual screening is identifying the protein for a particular receptor using different softwares and programs. It is like a computational filter which selects only a few and filters the rest of molecules. This leads to decrease in the number of compounds to be synthesized by the chemists. This helps to select a few molecules which can then be synthesised and can be optimized. [24][23]

Once the pharmacophore is generated, the online databases can be searched for the atoms that are likely to be selected as the hit molecule. These databases screen the available structures which may be mixtures of the molecules having the known activity and some novel molecules. This is known as pharmacophore based virtual screening. The pharmacophore is taken as the basis to select the hit molecule.

5.4 DOCKING

Molecular docking is used in knowing the structure of the molecule and is integral part of computer aided drug designing. It depicts the interaction between the small molecules at the ligand site. The small molecules reacts with the ligand protein. Docking displays the interaction and then scores the molecules depending on the affinity of the interactions. This can be used for virtual screening of the libraries based on the interactions between the molecule and ligand protein. The docking mainly includes two main processes. Firstly adaptation of the small molecule at the receptor site and introduction of various substituents at different position so as to fit the receptor site.[25]

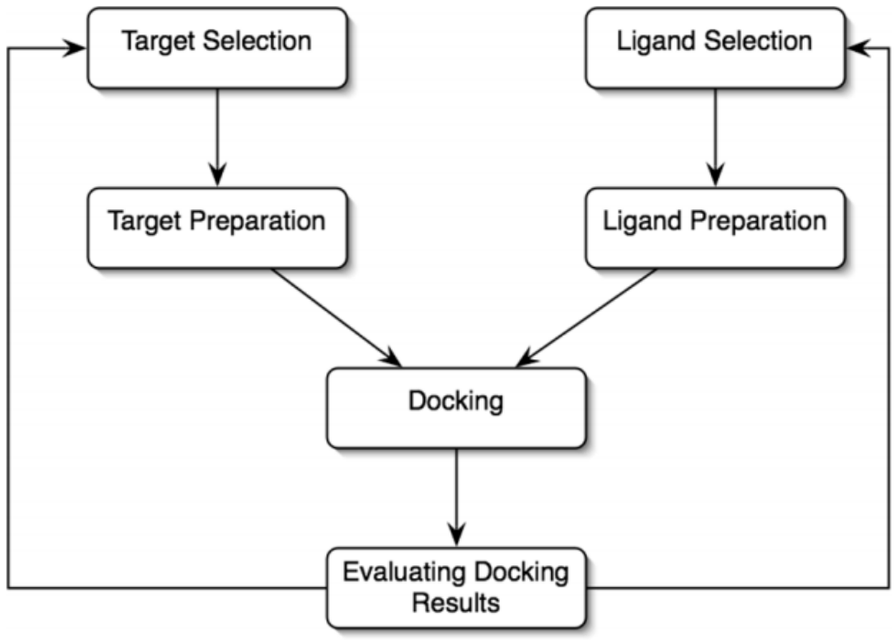


Figure 13 Key steps in docking

The first step in molecular docking is the target selection. The target at which the docking is to be done is identified using various techniques like X- ray crystallography, NMR etc. The target having the known structure can be obtained from the online databases. The crystal structure of the target must be known.

The next step includes preparing the ligand involved in docking. The preparation of ligand depends on the purpose of docking that is either lead discovery or to filter the crude molecules. The softwares used includes Auto – Dock, Surflex Dock, etc. These softwares uses the molecules having the Gasteigerpartial charge. The ease of docking depends on the flexibility of the molecule. The molecules having a rigid structure with few conformational orientation can be docked easily. As the flexibility increase the rotation of the bonds is available which increases the work space making the docking process more tedious and time consuming.

The final step in docking is evaluating the results. The docking involves examining the molecular structure and to score them depending on the best mode of interaction. This can be done by systematic method or stochastic method. The systematic method depends on the singularity of the molecule whereas the stochastic method depends on the randomness of the molecule. This leads to variations in the outcome. The scoring is done on the basis of evaluation parameters. These parameters are based on change in free energy of the molecule and loss of randomness of the molecule.

The molecules are scored depending on the interaction between the ligand and the target molecules which depends on the hydrogen bond donors and acceptors and the hydrophobic areas in the molecules.[26]

5.5 IN – SILICO ADMET PREDICTION

The molecules obtained after high throughput screening showing a good activity are exposed to ADMET studies. Traditionally the ADMET properties of the drug was determined by its action in –vitro and in-vivo using a series of battery of cell lines. This was very time consuming and was very tedious. Determining the pharmacokinetic and toxicity profile is an important step in drug discovery process. Absorption, distribution, metabolism, elimination and toxicity of the promising compounds are determined computationally. It is important to check the body's interaction with the drug molecule. Now this step is being done way before making any final decision regarding the drug compound. If the drug shows toxicity or low absorption characteristics it can be refrained from further optimisation process. Today the use of in-silico approach ensures the clinical safety and reduces the trials on animals. [27]

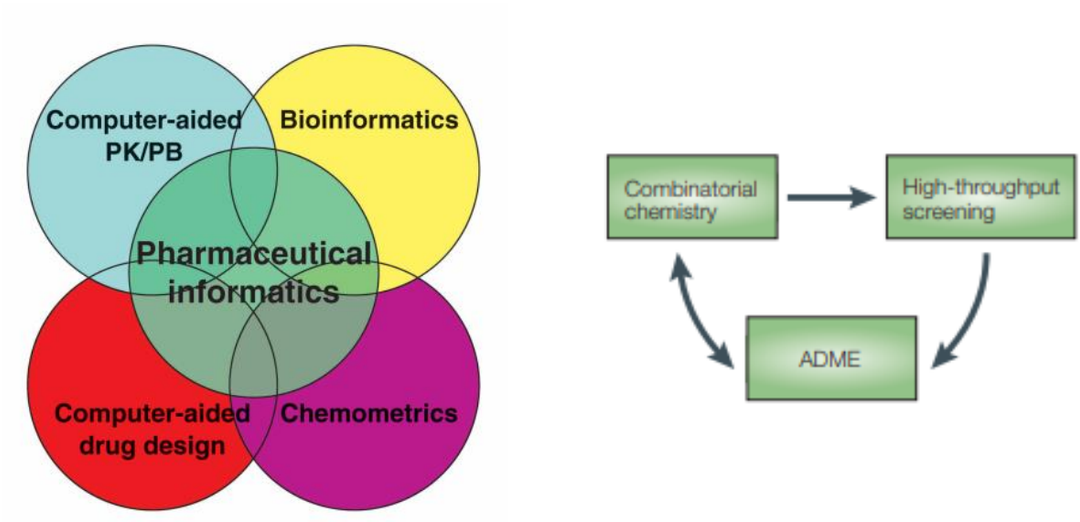


Figure 14 Prediction of ADMET Properties

The in-silico approach aims to determine the ADMET properties of the molecule using various softwares examining the pharmacokinetic parameters of drugs in a simulated environment. It determines the mutagenic or toxicity of the molecule so to ensure the safety of the patients.The drug to be selected is based upon various parameters, descriptors and complex models. [28]

Absorption: This means movement of the drug molecules from the site of administration to the circulatory system. The solubility of drug determines its absorption property. Drugs having good solubility in the aqueous phase is absorbed to great extent from the site of administration. The solubility is taken into consideration while lead optimisation as the changes in molecular weight leads to changes in the absorption properties. The ionization of the molecules also contributes to its absorption. The unionized form of drug is absorbed with greater extent as the ionized form of molecules cannot penetrate the GIT. The permeability of the drug molecule depends on its lipophilicity and its ionization.

Lipophilicity is necessary to penetrate the lipid bi-layer of the cells. Acidic drugs are well absorbed from acidic pH and basic from basic ph.

Distribution: This means the extent at which the drug is being distributed in the body. Volume of distribution is the theoretical volume of blood in which the specific amount of drug is distributed so as to elicit a pharmacologic action. The distribution of drug from the systemic circulation to site of action is necessary for the pharmacological action. Volume of distribution along with clearance determines the half-life of drug. Even when a drug is distributed it may not produce any action. As for any drug to elicit an action it must be distributed in a sufficient amount. A specific amount is required for the pharmacological action. The distribution is widely affected by the protein binding. The free drug is widely distributed but when they are bound to a protein they do not diffuse into the cell because of its high molecular size. The distribution in CNS primarily depends on the ability to cross the blood brain barrier. The BBB is highly lipophilic and a lipophilic drug is required for it to pass the BBB.

Metabolism: It is irreversible conversion of large molecules into smaller organic molecules. It is biochemical process that coverts the large molecules into small organic molecules via various chemical reactions like oxidation, reduction, conjugation, hydrolysis, etc. The drugs clearance level also depends on the solubility and permeability of the drug. The drug having good solubility may still not be able to bring out action because of its high clearance level. The drug clearance can take place through kidneys, liver, biliary routes etc. The clearance of the drug depends on the structural properties and conditions rather than any other parameter. This may pose great challenge in determining the ADMET properties. [29]

Toxicity: Many drugs produces toxic effects on the body. These toxic effects leads to failure of drugs and the withdrawal from the market. The toxicity must be analysed before it can be optimised and marketed. Presently the ADMET softwares uses the approaches like based on the knowledge of the scientific literature and the human experts and the other approach is based on the descriptors related to chemical structure and analytical statics. These descriptors are generated and the interaction between these structures and toxicology. These interactions help to determine the toxicity of the drug molecule. The main properties of these softwares is to determine the mutagenicity, teratogenicity, cardio-toxicity, etc. It is important to evaluate these parameters before the drug can be further developed and can be introduced into the market. [30]

6 CONCLUSION

The cohort study on the computational approach to developing the isoform specific PI3k inhibitor for the treatment of cancer has be done using rational drug discovery method which involved generation of pharmacophore model, virtual screening, molecular docking and the ADMET studies using various softwares.

This study comprised of vaious steps staring from generating a pharmacophre model using 11 molecules whose activity has been already determined. The virtual screening of this model was carried out using the NCI database. The hit molecules obtained aftr virtual screening were based on its Qfit value. These molecules were then docked using the various softwares. The molecules were scored based on the affinity of the interaction between the ligand and the target molecule. Depending on the molecula docking and the various amino acid interaction 2 molecules were selected for the ADMET studies. After the ADMET prediction one hit molecule was obtained which can be optimised into a potent lead molecule and can be marketed as pi3k inhibitor in future.

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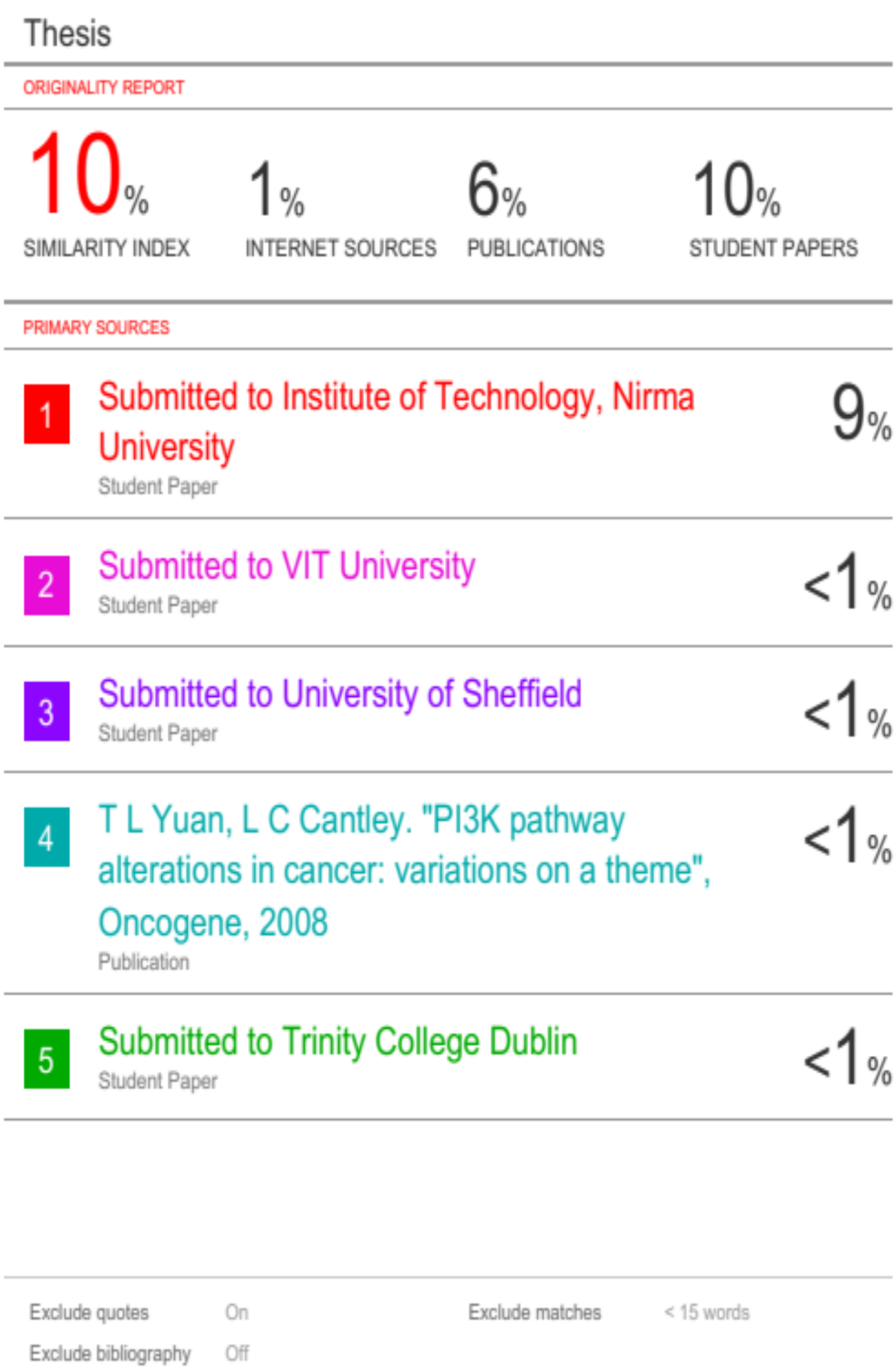

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