DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF Pim-1 INHIBITOR FOR ANTICANCER ACTIVITY

A dissertation submitted to

The Tamilnadu Dr M.G.R Medical University

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MADRAS MEDICAL COLLEGE

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CERTIFICATE

This is to certify that the dissertation entitled **"DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF Pim-1 INHIBITOR FOR ANTICANCER ACTIVITY"** submitted by the candidate bearing Register No 26108340 in partial fulfillment of the requirements for the award of the degree of MASTER OF PHARMACY in PHARMACEUTICAL CHEMISTRY by The Tamilnadu Dr M.G.R Medical University is a bonafide work done by him during the academic year 2011-2012 at the Department of Pharmaceutical Chemistry , College of Pharmacy, Madras Medical College , Chennai -03.

Dr. A. JERAD SURESH, M.Pharm., Ph.D., M.B.A.,
Principal,
Professor and Head,
Department of Pharmaceutical Chemistry,
College of Pharmacy,
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Madras Medical College,
Chennai-600 003.

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DEDICATED TO OUR BELOVED PARENTS, TEACHERS, ALMIGHTY & FRIENDS ....

CONTENTS

LIST OF ABBREVIATIONS

CMC	- Carboxy Methyl Cellulose
ECM	- Extracellular matrix
HBA	- Hydrogen Bond Acceptor
HBD	- Hydrogen Bond Donor
HY	- Hydrophobic
IC_{50}	- Inhibitory Concentration
IR	- Infrared Spectroscopy
LD_{50}	- Lethal Dose
MAPK	- Mitogen Activated Protein Kinase
NMR	- Nuclear Magnetic Resonance Spectroscopy
OECD	- Organisation for Economic Cooperation and Development
OPLS	- Optimized Potentials for Liquid Simulations
QSAR	- Quantitative Structure Activity Relationship
RMSD	- Root Mean Square Difference
SEM	- Standard Error of the Mean
HTVS	- high-throughput virtual screening
SP	- standard precision
XP	- extra precision

Introduction

I.INTRODUCTION

I.1.1 CANCER [1],

The division of normal cell is precisely controlled.new cells are only formed for growth or to replace dead ones. Cancerous cells divide repeatedly out of control even though they are not needed, they crowd out other normal cells and function abnormally .they can also destroy the correct functioning of major organs

- **Benign tumours** do not spread from their site of origin, but can crowd out (squash) surrounding cells eg brain tumour, warts.
- **Malignant tumours** can spread from the original site and cause secondary tumours. This is called metastasis. They interfere with neighbouring cells and can block blood vessels, the gut, glands, lungs etc.

Neoplasia literally means the process of "new growth," and a new growth is called a neoplasm. The term tumor was originally applied to the swelling caused by inflammation. Neoplasms also may induce swellings, but by long precedent, the non-neoplastic usage of tumor has passed into limbo; thus, the term is now equated with neoplasm. Oncology (Greek oncos = tumor) is the study of tumors or neoplasms. Cancer is the common term for all malignant tumors. Although the ancient origins of this term are somewhat uncertain, it probably derives from the Latin for crab, cancer—presumably because a cancer "adheres to any part that it seizes upon in an obstinate manner like the crab."

I.1.2 TYPES OF CANCERS

According to conventional allopathic medicine there are over 150 types of cancers that can be categorized as follows:

- **carcinomas** are cancer that formed in the Lung, breast, prostate, skin, stomach, and colon and are characterized by solid tumors.
- **Sarcomas** are cancers that form in the bone and the soft tissues surrounding organs. They are solids and are the most rare and deadly forms of malignant tumors.
- **Leukemia** forms in the blood and the bone marrow. These are non-solid tumors and are characterized by abnormal production of white blood cells.
- **Lymphomas** are cancers of the lymph nodes. They are divided into two categories, Hodgkin's and Non-Hodgkin's.
- **Myelomas** are rare tumors that form in the antibodies producing plasma cells in various tissues.

I.1.3 CAUSES FOR CANCER [2,3]

- Sunlight
- Chronic Exposure to Electromagnetic Fields (EMFs)
- Ionizing Radiation
- Pesticide/Herbicide Residues
- Industrial Toxins
- Polluted, Chlorinated and Fluoridated Water
- Tobacco
- Hormone Therapies
- Wrong Diet and Nutrition

- ❖ Emotional Stress
- ❖ Intestinal Toxicity and Digestive Impairment
- ❖ Viruses(Hepa,titis B virus Epstein-Barr virus
- ❖ Blocked Detoxification Pathway
- ❖ Cellular Oxygen Deficiency
- ❖ Genetic Factors

I.1.4 CANCER STAGING [2,3]

Type of cancer stages

- In situ
- Local
- Regional
- Distant

Staging tells us the extent of the disease.

- Treatment depends on the stage of the specific cancer.
- Staging helps determine the patient's prognosis (prediction of course and outcome of disease, especially chances of recovery).

The Stage of Cancer [2,3]

This describes how far cancer has spread. It is usually from stage I to IV, and often followed by "A" or "B" to further delineate the severity within each stage. In general, stage I cancers are small localized cancers that are usually curable, while stage IV usually represents inoperable or metastatic cancer. Stage II and III cancers are usually locally advanced and/or with involvement of local lymph nodes. It is important to note that the staging system is different for each kind of cancer. For solid

tumors, stages I-IV are actually defined in terms of a more detailed staging system called the "TNM" system. In the TNM system, TNM stands for Tumor, Nodes, and Metastases. Each of these is separately classified with a number to give the total stage. For example, a T2N1M0 cancer means the patient has a T2 tumor, N1 lymph node involvement, and no distant metastases. Again, the definitions of T, N and M are specific to each cancer.

I.1.5 CANCER INCIDENCE

In some measure, an individual's likelihood of developing a cancer is expressed by national incidence and mortality rates. For example, residents of the United States have about a one in five chance of dying of cancer. There were, it is estimated, about 556,000 deaths from cancer in 2003, representing 23% of all mortality, a frequency surpassed only by deaths caused by cardiovascular diseases. These data do not include an additional 1 million, for the most part readily curable, non-melanoma cancers of the skin and 100,000 cases of carcinoma in situ, largely of the uterine cervix but also of the breast. The major organ sites affected and the estimated frequency of cancer deaths are shown. The most common tumors in men are prostate, lung, and colorectal cancers. In women, cancers of the breast, lung, and colon and rectum are the most frequent. Cancers of the lung, female breast, prostate, and colon/rectum constitute more than 50% of cancer diagnoses and cancer deaths in the U.S. population.

I.1.6 CANCER STATISTICS

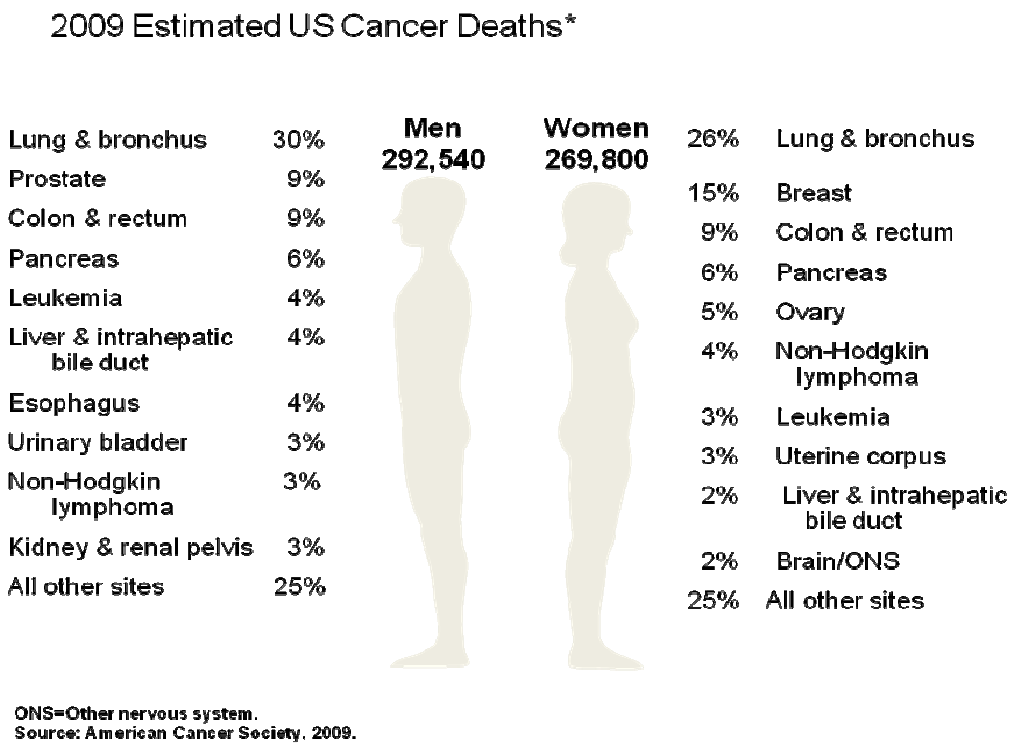


Fig.I.1Cancer statistics

I.1.7 CANCER EVENT [2,3]

First, we must understand that cancer-prone events occur in our bodies in clusters. These include:

- Genetic instability in the nucleus
- Abnormal expression of genes, resulting in too few proteins that Inhibit cancer and too many that facilitate it
- Abnormal cell-to-cell communications
- Induction of angiogenesis
- Invasion and metastasis
- Immune evasion

I.1.8 COLO RECTAL CANCER [4]

Colorectal cancer, commonly known as bowel cancer, is a cancer from uncontrolled cell growth in the colon, rectum, or appendix.

The colon is the part of the digestive system where the waste material is stored. The rectum is the end of the colon adjacent to the anus. Together, they form a long, muscular tube called the large intestine (also known as the large bowel). Tumors of the colon and rectum are growths arising from the inner wall of the large intestine. Benign tumors of the large intestine are called polyps. Malignant tumors of the large intestine are called cancers. Benign polyps do not invade nearby tissue or spread to other parts of the body. Benign polyps can be easily removed during colonoscopy and are not life-threatening. If benign polyps are not removed from the large intestine, they can become malignant (cancerous) over time. Most of the cancers of the large intestine are believed to have developed from polyps. Cancer of the colon and rectum (also referred to as colorectal cancer) can invade and damage adjacent tissues and organs. Cancer cells can also break away and spread to other parts of the body (such as liver and lung) where new tumors form. The spread of colon cancer to distant organs is called metastasis of the colon cancer. Once metastasis has occurred in colorectal cancer, a complete cure of the cancer is unlikely

Most colorectal cancer occurs due to lifestyle and increasing age with only a minority of cases associated with underlying genetic disorders. It typically starts in the lining of the bowel and if left untreated, can grow into the muscle layers underneath, and then through the bowel wall. Screening is effective at decreasing the chance of dying from colorectal cancer and is recommended starting at the age of 50

and continuing until a person is 75 years old. Localized bowel cancer is usually diagnosed through sigmoidoscopy or colonoscopy.

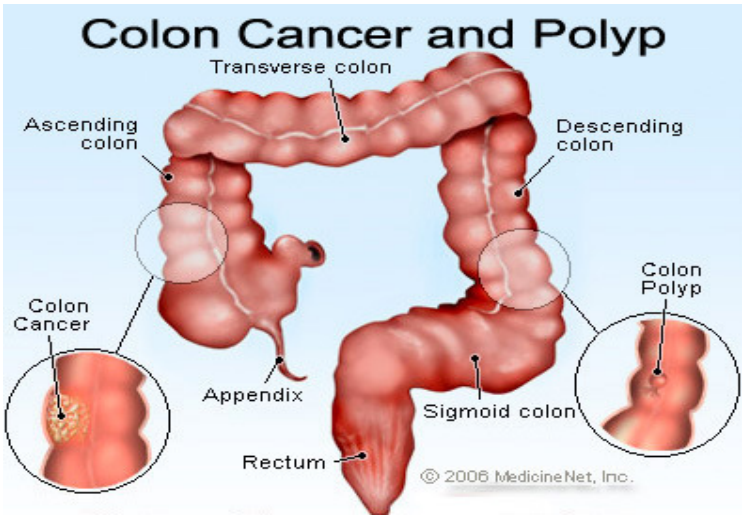


Fig.I.2 Colon cancer

Globally, cancer of the colon and rectum is the third leading cause of cancer in males and the fourth leading cause of cancer in females. The frequency of colorectal cancer varies around the world. It is common in the Western world and is rare in Asia and Africa. In countries where the people have adopted western diets, the incidence of colorectal cancer is increasing.

I.1.9 CAUSES OF COLON CANCER [5]

- Inflammatory bowel disease (ulcerative colitis and Crohn's disease)
- primary sclerosing cholangitis
- High fat content in diet
- Genetics and colon cancer
    - FAP (familial adenomatous polyposis)
    - AFAP (attenuated familial adenomatous polyposis)

- HNPCC (hereditary nonpolyposis colon cancer)
- MYH polyposis syndrome is a recently discovered hereditary colon cancer syndrome

I.1.10 SYMPTOMS

Colo –rectal cancer symptoms come in two general varieties:

1. Local
2. Systemic

Local Colo-rectal Cancer Symptoms

* Changes in your bowel habits,
* Constipation
* Diarrhea
* Intermittent
* Bright red or dark red blood in your stools
* Stools that are thinner than normal ("pencil stools")
* Abdominal (midsection) discomfort, bloating, frequent gas pains

Systemic Colo-rectal Cancer Symptoms:

* Unintentional weight loss
* Loss of appetite
* Unexplained fatigue (extreme tiredness)
* Nausea or vomiting
* Anemia (low red blood cell count or low iron in your red blood cells)
* Jaundice (yellow color to the skin and whites of the eye).

DIAGNOSIS [6]

- Barium enema X-ray
- Colonoscopy
- Ultrasonography
- CT scan
- Chest X-ray
- Biopsy

I.1.11 TREATMENT [7]

- Chemotherapy
- Biological therapy
- Radiation therapy
- Laser treatment
- Photodynamic therapy
- Stem cell transplant

I.2.1 Pim1

The human pim-1 (proviral integration site for moleny murine leukaemia virus or MULV) oncogene is localized on chromosome 6p21.2, a fragile site M) involved in certain leukemias [8]. Its cDNA contains an open reading frame of 313 codons with 94% homology to the mouse counterpart. The RNA transcript is 2.9 kilo bases (kb) long [9]. The pim1 protein is a serine/threonine kinase[10,11]. Two ubiquitously expressed have been isoforms of human pim1 protein (35&34 kda) have been

identified [12]. In vitro human pim1 autophosphorylates and exhibits phosphotransferase activity towards various exogenous substrates [13]. The crystals structure of pim1 reveal that it is a constitutively active kinase: phosphorylation of pim1 is not necessary for it kinase activity regulation but contributes to its stability [14]. Immunoperoxidase staining using monoclonal antibodies have a shown that pim1 protein is predominantly located in the cytoplasm, although nuclear or nucleo-cytoplasmic patterns of localization have been described [15].

I.2.2 Crystal Strucuture of Proto Oncogen Kinase Pim1

Pim 1 is aproto-oncogene originally identified as a preferential proviral integration site in moloney murine leukemia virus induced T-cell lymphomas [16]. Pim1 is the first described member of a uniquefamily of serine/ \threonine kinase with significant sequence homology to pim1 [17,18] several substrates of pim1 phosphorylation have been identified, including c-myb[19], BAD [20,21], SCOS [22], cdc25-A [23], HP1 [24],PAP-1 [25]. Pim1 contains an insertion in the hinge region and a proline residue at a key position (123) critical for ATP binding othe kinases have anon-proline residue at this position where the backbone NH of the residue makes a conserved hydrogen bond to ATP. It was confirmed by x-ray crystallography

Pim1 is often expressed in both normal and transformed cells to different degrees. It is expreesed in many cell lines derived from human lymphoid and myeloid malignancies, as well as in several human solid tumor cells. In humans the pim-1 oncogene is expressed in lymphoid and haematopoitic malignancies, squamous cell carcinomas of the head and neck region , gastric carcinomas and colorectal carcinomas.

Pim1 is astrees reponse kinase which is regulated by cytokines [26-28] growth factors [29] hormones [30] by condition like ischemia [31] and cellular hypoxia [32] as well as by infective agent such as the Epstein-Barr virus [33] and helicobacter pylorus[34].

I.2.3 Molecular Function of Pim1

Pim1 has been implicated in signal tranductional and transcriptional regulation, as well as cell cycle regulation and survival. These biological functions have been well and extensively reviwed elsewhere and are not within the scope of this review [35,36] here we provide a very general if necessary when addressing the expression of pim1 in individual tissue types.

The effect of pim1 in signal transduction is medicated by several players,adapter protein socl and soc3 are involved in negative regulation of cytokine induced JAK-STAT signaling [37] the nuclear adapter protein p^{100}(a pim1 binding partner) is an activation factor of transcription factor c.myb [38]. In addition, the $NFAT_c$ protein is involved in relaying signals from t-cell receptor [39]

Furthermore several pim1 substances have been identified, adding to the evidence that pim1 can regulate nuclear transcription. Induced HP1 (hetro chromatin- associated protein 1) and PAP1 (PIM1 associated protein) function in transcriptional repression by the silencing of chromatin and regulation of mRNA spilicing, respectively [40.41].

I.2.4 Human Pim1 and malignancy:

Human pim1 has multiple role in tumorigenesis. It promotes early transformation, cell proliferation [42], and cell survival [43,44]. In addition it may have a role in angiogenesis

and vasculogenesis as a downstream effector of the VEGFA/F1K1 pathway [45]. Pim1 expression is correlated with tumor aggressiveness [46,47] and is a marker of poor prognosis [48] pim1 expression can be predictive of tumor outcome following chemotherapy [49] and surgery [50] and has been correlated with the enhanced metastatic potential of the role of pim1 in specific tumor types.

- B-cell non-Hodgkin lymphomas
- Leukemia
- Prostate cancer
- Squamous cell carcinoma
- Gastrointestinal tumours
- Pancreas cancer

I.3 COMPUTATIONAL CHEMISTRY

Computational chemistry [51] is a branch of chemistry that uses principles of computer science to assist in solving chemical problems. It uses the results of theoretical chemistry, incorporated into efficient computer programs, to calculate the structures and properties of molecules and solids [52]. Its necessity arises from the well-known fact that apart from relatively recent results concerning the hydrogen molecular ion, the quantum n-body problem cannot be solved analytically, much less in closed form. While its results normally complement the information obtained by

chemical experiments, it can in some cases predict hitherto unobserved chemical phenomena. It is widely used in the design of new drugs and materials.

Calculate the following properties [53]

- structure (i.e. the expected positions of the constituent atoms),
- absolute and relative (interaction) energies,
- electronic charge distributions,
- dipoles and higher multipole moments,
- vibrational frequencies,
- reactivity or other spectroscopic quantities,
- cross sections for collision with other particles.

The methods employed cover both static and dynamic situations. In all cases the computer time and other resources (such as memory and disk space) increase rapidly with the size of the system being studied. That system can be a single molecule, a group of molecules, or a solid. Computational chemistry methods range from highly accurate to very approximate; highly accurate methods are typically feasible only for small systems. *Ab initio* methods [54] are based entirely on theory from first principles. Other (typically less accurate) methods are called empirical or semi-empirical because they employ experimental results, often from acceptable models of atoms or related molecules, to approximate some elements of the underlying theory.

Both *ab initio* and semi-empirical approaches involve approximations. These range from simplified forms of the first-principles equations that are easier or faster to solve,

to approximations limiting the size of the system (for example, periodic boundary conditions), to fundamental approximations to the underlying equations that are required to achieve any solution to them at all[5]. For example, most *ab initio* calculations make the Born–Oppenheimer approximation, which greatly simplifies the underlying Schrödinger equation by freezing the nuclei in place during the calculation. In principle, *ab initio* methods eventually converge to the exact solution of the underlying equations as the number of approximations is reduced. In practice, however, it is impossible to eliminate all approximations, and residual error inevitably remains. The goal of computational chemistry is to minimize this residual error while keeping the calculations tractable.

In some cases, the details of electronic structure are less important than the long-time phase space behavior of molecules. This is the case in conformational studies of proteins and protein-ligand binding thermodynamics. Classical approximations to the potential energy surface are employed, as they are computationally less intensive than electronic calculations, to enable longer simulations of molecular dynamics. Furthermore, cheminformatics uses even more empirical (and computationally cheaper) methods like machine learning based on physicochemical properties. One typical problem in cheminformatics is to predict the binding affinity of drug molecules to a given target [(55)].

I.4.1 DRUG DESIGN

Drug design, sometimes referred to as rational drug design or more simply rational design, is the inventive process of finding new medications based on the knowledge of a biological target [(56)]. In the most basic sense, drug design involves the design of

small molecules that are complementary in shape and charge to the biomolecular target with which they interact and therefore will bind to it. Drug design frequently but not necessarily relies on computer modeling techniques.[57] This type of modeling is often referred to as computer-aided drug design. Finally, drug design that relies on the knowledge of the three-dimensional structure of the biomolecular target is known as structure-based drug design.

The phrase "drug design" is to some extent a misnomer. What is really meant by drug design is ligand design (i.e., design of a small molecule that will bind tightly to its target).[58] Although modeling techniques for prediction of binding affinity are reasonably successful, there are many other properties, such as bioavailability, metabolic half-life, lack of side effects, etc., that first must be optimized before a ligand can become a safe and efficacious drug. These other characteristics are often difficult to optimize using rational drug design techniques.

Typically a drug target is a key molecule involved in a particular metabolic or signaling pathway that is specific to a disease condition or pathology or to the infectivity or survival of a microbial pathogen. Some approaches attempt to inhibit the functioning of the pathway in the diseased state by causing a key molecule to stop functioning. Another approach may be to enhance the normal pathway by promoting specific molecules in the normal pathways that may have been affected in the diseased state.

I.4.2 TYPE OF DRUG DESIGN

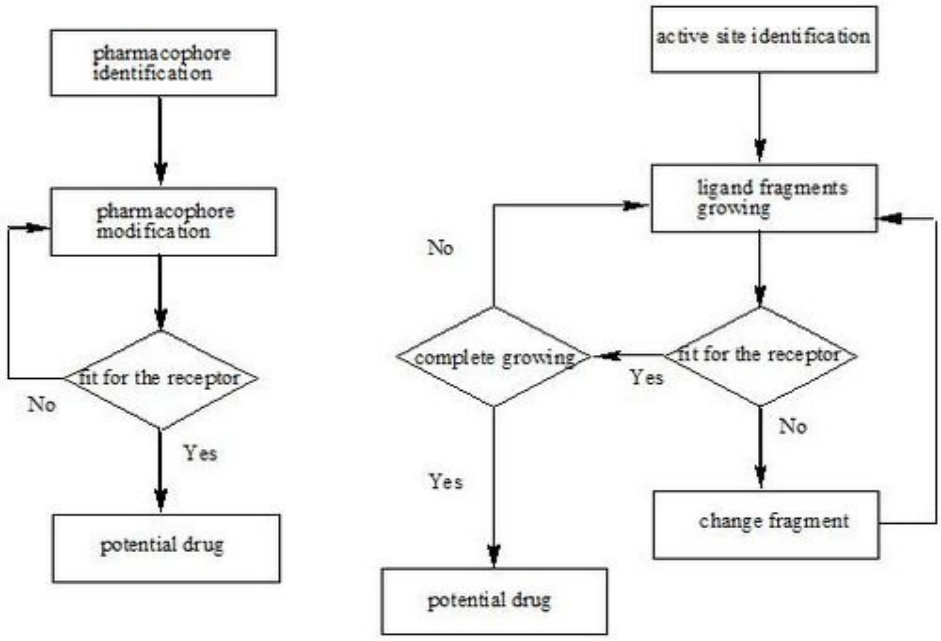


Fig.I.3 Type of drug design

There are Two major types of drug design. The first is referred to as **ligand-based drug design** and the second, **structure-based drug design**.

Ligand-based Drug design

Ligand-based drug design (or **indirect drug design**) relies on knowledge of other molecules that bind to the biological target of interest. These other molecules may be used to derive a pharmacophore model that defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target.[59] In other words, a model of the biological target may be built based on the knowledge of what binds to it, and this model in turn may be used to design new molecular entities that interact with the target.

Alternatively, a quantitative structure-activity relationship (QSAR), in which a correlation between calculated properties of molecules and their experimentally determined biological activity, may be derived. These QSAR relationships in turn may be used to predict the activity of new analogs.

Structure-based Drug Design

Structure-based drug design (or **direct drug design**) relies on knowledge of the three dimensional structure of the biological target obtained through methods such as x-ray crystallography or NMR spectroscopy.[60] If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein. Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics and the intuition of a medicinal chemist. Alternatively various automated computational procedures may be used to suggest new drug candidates.

As **experimental methods** such as X-ray crystallography and NMR develop, the amount of information concerning 3D structures of biomolecular targets has increased dramatically. In parallel, information about the structural dynamics and electronic properties about ligands has also increased. This has encouraged the rapid development of the structure-based drug design.

Active site identification

Active site identification is the first step in this program. It analyzes the protein to find the binding pocket, derives key interaction sites within the binding pocket,

and then prepares the necessary data for Ligand fragment link. The basic inputs for this step are the 3D structure of the protein and a pre-docked ligand in PDB format, as well as their atomic properties. Both ligand and protein atoms need to be classified and their atomic properties should be defined, basically, into four atomic types:**hydrophobic atom**: All carbons in hydrocarbon chains or in aromatic groups.

- **H-bond donor**: Oxygen and nitrogen atoms bonded to hydrogen atom(s).
- **H-bond acceptor**: Oxygen and sp2 or sp hybridized nitrogen atoms with lone electron pair(s).
- **Polar atom**: Oxygen and nitrogen atoms that are neither H-bond donor nor H-bond acceptor, sulfur, phosphorus, halogen, metal, and carbon atoms bonded to hetero-atom(s).

The space inside the ligand binding region would be studied with virtual probe atoms of the four types above so the chemical environment of all spots in the ligand binding region can be known. Hence we are clear what kind of chemical fragments can be put into their corresponding spots in the ligand binding region of the receptor. When we want to plant "seeds" into different regions defined by the previous section, we need a fragments database to choose fragments from. The term "fragment" is used here to describe the building blocks used in the construction process. The rationale of this algorithm lies in the fact that organic structures can be decomposed into basic chemical fragments. Although the diversity of organic structures is infinite, the number of basic fragments is rather limited. Before the first fragment,

i.e. the seed, is put into the binding pocket, and other fragments can be added one by one, it is useful to identify potential problems. First, the possibility for the fragment combinations is huge.

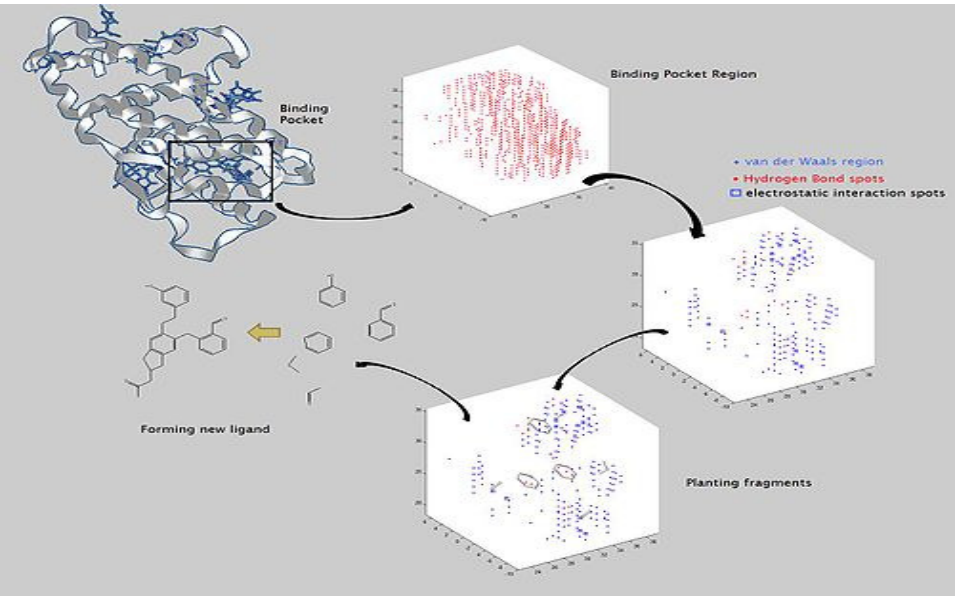


Fig.I.4 Flow chart of drug design

A small perturbation of the previous fragment conformation would cause great difference in the following construction process. At the same time, in order to find the lowest binding energy on the Potential energy surface (PES) between planted fragments and receptor pocket, the scoring function calculation would be done for every step of conformation change of the fragments derived from every type of possible fragments combination. Since this requires a large amount of computation, one may think using other possible strategies to let the program works more efficiently. When a ligand is inserted into the pocket site of a receptor,

conformation favor for these groups on the ligand that can bind tightly with receptor should be taken priority. Therefore it allows us to put several seeds at the same time into the regions that have significant interactions with the seeds and adjust their favorite conformation first, and then connect those seeds into a continuous ligand in a manner that make the rest part of the ligand having the lowest energy. The conformations of the pre-placed seeds ensuring the binding affinity decide the manner that ligand would be grown. This strategy reduces calculation burden for the fragment construction efficiently. On the other hand, it reduces the possibility of the combination of fragments, which reduces the number of possible ligands that can be derived from the program. These two strategies above are well used in most structure-based drug design programs. They are described as "**Grow**" and "**Link**". The two strategies are always combined in order to make the construction result more reliable.[61]

I.4.3 Scoring method

Scoring functions for docking

Structure-based drug design attempts to use the structure of proteins as a basis for designing new ligands by applying accepted principles of molecular recognition. The basic assumption underlying structure-based drug design is that a good ligand molecule should bind tightly to its target. Thus, one of the most important principles for designing or obtaining potential new ligands is to predict the binding affinity of a certain ligand to its target and use it as a criterion for selection.

One early method was developed by Böhm[62] to develop a general-purposed empirical scoring function in order to describe the binding energy. The following "Master Equation" was derived:

$$\Delta G_{\text{bind}} = -RT \ln K_{\text{d}}$$

$$K_{\text{d}} = \frac{[\text{Receptor}][\text{Acceptor}]}{[\text{Complex}]}$$

$$\Delta G_{\text{bind}} = \Delta G_{\text{desolvation}} + \Delta G_{\text{motion}} + \Delta G_{\text{configuration}} + \Delta G_{\text{interaction}}$$

where:

- desolvation – enthalpic penalty for removing the ligand from solvent
- motion – entropic penalty for reducing the degrees of freedom when a ligand binds to its receptor
- configuration – conformational strain energy required to put the ligand in its "active" conformation
- interaction – enthalpic gain for "resolvating" the ligand with its receptor

I.4.4 Rational drug design

In contrast to traditional methods of drug discovery, which rely on trial-and-error testing of chemical substances on cultured cells oranimals, and matching the apparent effects to treatments, rational drug design begins with a hypothesis that modulation of a specific biological target may have therapeutic value. In order for a biomolecule to be selected as a drug target, two essential pieces of information are required. The first is evidence that modulation of the target will have therapeutic value. This knowledge may come from, for example, disease linkage studies that show an association between mutations in the biological target and certain disease

states. The second is that the target is "drugable". This means that it is capable of binding to a small molecule and that its activity can be modulated by the small molecule.

Once a suitable target has been identified, the target is normally cloned and expressed. The expressed target is then used to establish a screening assay. In addition, the three-dimensional structure of the target may be determined.

The search for small molecules that bind to the target is begun by screening libraries of potential drug compounds. This may be done by using the screening assay (a "wet screen"). In addition, if the structure of the target is available, a virtual screen may be performed of candidate drugs. Ideally the candidate drug compounds should be "drug-like", that is they should possess properties that are predicted to lead to oral bioavailability, adequate chemical and metabolic stability, and minimal toxic effects. Several methods are available to estimate druglikeness such Lipinski's Rule of Five and a range of scoring methods such as Lipophilic efficiency. Several methods for predicting drug metabolism have been proposed in the scientific literature, and a recent example is SPORCalc.[63] Due to the complexity of the drug design process, two terms of interest are still serendipity and bounded rationality. Those challenges are caused by the large chemical space describing potential new drugs without side-effects.

I.4.5 COMPUTER AIDED DRUG DESIGN

Computer-aided drug design uses computational chemistry to discover, enhance, or study drugs and related biologically active molecules.

The most fundamental goal is to predict whether a given molecule will bind to a target and if so how strongly. Molecular mechanics or molecular dynamics are most often used to predict the conformation of the small molecule and to model conformational changes in the biological target that may occur when the small molecule binds to it. Semi-empirical, ab initio quantum chemistry methods, or density functional theory are often used to provide optimized parameters for the molecular mechanics calculations and also provide an estimate of the electronic properties (electrostatic potential, polarizability, etc.) of the drug candidate that will influence binding affinity.

Molecular mechanics methods may also be used to provide semi-quantitative prediction of the binding affinity. Also, knowledge-basedscoring function may be used to provide binding affinity estimates. These methods use linear regression, machine learning, neural netsor other statistical techniques to derive predictive binding affinity equations by fitting experimental affinities to computationally derived interaction energies between the small molecule and the target.[64,65]

Ideally the computational method should be able to predict affinity before a compound is synthesized and hence in theory only one compound needs to be synthesized. The reality however is that present computational methods are imperfect and provide at best only qualitatively accurate estimates of affinity. Therefore in practice it still takes several iterations of design, synthesis, and testing before an optimal molecule is discovered. On the other hand, computational methods have accelerated discovery by reducing the number of iterations required and in addition have often provided more novel small molecule structures.

Drug design with the help of computers may be used at any of the following stages of drug discovery:

1. hit identification using virtual screening (structure- or ligand-based design)
2. hit-to-lead optimization of affinity and selectivity (structure-based design, QSAR, etc.)
3. lead optimization optimization of other pharmaceutical properties while maintaining affinity

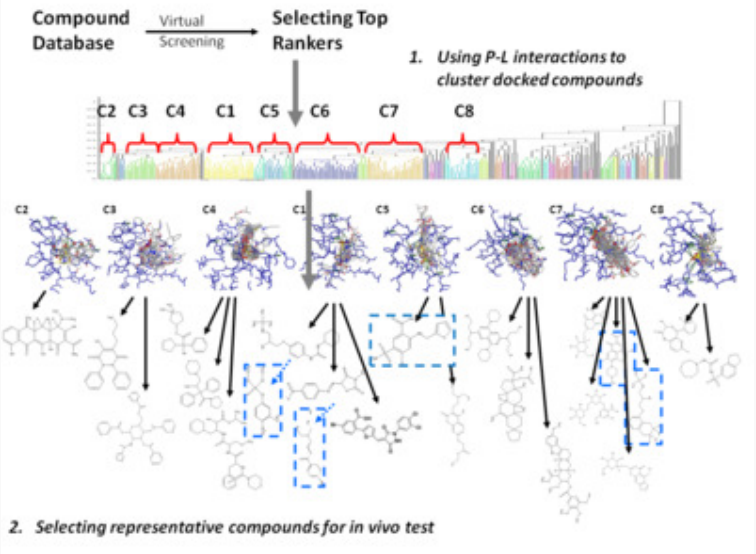


Fig.I.5 Flowchart of a Usual Clustering Analysis for Structure-Based Drug Design

In order to overcome the insufficient prediction of binding affinity calculated by recent scoring functions, the protein-ligand interaction and compound 3D structure information are used to analysis. For structure-based drug design, several post-screening analysis focusing on protein-ligand interaction has been developed for improving enrichment and effectively mining potential candidates:

- Consensus scoring[66,67]
    - Selecting candidates by voting of multiple scoring functions
    - May lose the relationship between protein-ligand structural information and scoring criterion
- Geometric analysis
    - Comparing protein-ligand interactions by visually inspecting individual structures
    - Becoming intractable when the number of complexes to be analyzed increasing
- Cluster analysis[68,69]
    - Represent and cluster candidates according to protein-ligand 3D information
    - Needs meaningful representation of protein-ligand interactions.

A particular example of rational drug design involves the use of three-dimensional information about biomolecules obtained from such techniques as X-ray crystallography and NMR spectroscopy. Computer-aided drug design in particular becomes much more tractable when there's a high-resolution structure of a target protein bound to a potent ligand. This approach to drug discovery is sometimes referred to as structure-based drug design. The first unequivocal example of the application of structure-based drug design leading to an approved drug is the carbonic anhydrase inhibitor dorzolamide, which was approved in 1995.[70,71]

Review of literature

II REVIEW OF LITERATURE

- The review of literature is limited to last 10 years because the establishment of pim kinase had been from the past 10 years.

- Focus of the literature review revolves around receptor site identification, Pharmacophore modeling, Docking study significance etc

II.1TARGET SITE IDENTIFICATION

- **Tarm moroy., et al.,[72] (1993)** reported the Expression of a pim-1 transgene accelerate lymphoproliferation and inhibits apoptosis in lpr/lpr mice. Transgenic mice expressing the Pim-1 kinase are predisposed to develop T-cell lymphomas with a long latency period of about 7-9 months. However, the exact functional basis of the oncogenic activity of Pim-1 remains obscure.
- **D wingett., et al.,[73] (1996)** reported the pim-1 proto-oncogene expression in antiCD_3 mediated T cell activation is associated with protein kinase activation and is independent of Raf-1. We have studied pim-1 proto-oncogene expression in human T cell responses to Ag receptor-generated signals. The pim-1 gene encodes a serine/threonine protein kinase that is expressed primarily in cells of hematopoietic lineage and is implicated in the intracellular signaling processes accompanying lymphocyte activation. We show here that pim-1 mRNA expression is rapidly induced after receptor cross-linking with anti-CD3 Abs.
- **Chrystal K.palaty., et al.,[74] (1997)** reported Identification of the Autophosphorylation sites of the Xenopus laeuis pim-1 proto-oncogene-encoded protein kinase . Pim-1 is an oncogene-encoded serine/threonine kinase

expressed primarily in cells of the hematopoietic and germ line lineages. Previously identified only in mammals, pim-1cDNA was cloned and sequenced from the African clawed frogXenopus laevis.

- **Tony J pircher., et al.,[75] (2000)** repoted pim-1 kinase protect hemopoitic FDC cellfrom genotoxin-induced death.

- **Marcos. M., et al., [76] (2001)** reported cell cycle as a critical decision in the treatment of cancer as to cycle or not.

- **James Thompson., et al.,[77] (2003)** reported the Attenuation of Androgen Recptor-Dependent transcription by the serine/theronine kinase pim-1. oncogenic serine/threonine kinase, Pim-1, was reported to be overexpressed in prostate cancer.To elucidate whether Pim-1 is capable of modulating androgen signaling, we studied the effects of Pim-1 on androgen receptor (AR)-dependent transcription.
- **Hitoshi.O., et al.,[78] (2004)** showed the pathways of apoptotic and non apoptotic death in tumor cells.

- **Kju-Taekim., et al.,[79] (2005)** reported pim-1 is up-regulated by constitutely activated FLT3 and play a role in FLT3-mediated cell survival. *Pim-1* was found to be one of the most significantly down-regulated genes upon FLT3 inhibition. Pim-1 is a proto-oncogene and is known to be up-regulated by signal transducer and activator of transcription 5 (STAT5), which itself is a downstream target of FLT3 signaling.

- **Abhinau kumar., et al.,[80] (2005)** showed the crystal structure of proto-oncogene kinase pim1:A Target of Aberrent somatic hyper mutation in diffuse large cell lymphoma. While pim1 has been shown to be involved in several hematopoietic cancers, it was also recently identified as a target of aberrant somatic hypermutation in diffuse large cell lymphoma (DLCL), the most common form of non-Hodgkin's lymphoma.
- **Jacobs MD., et al.,[81] (2005)** reported the pim-1 ligand –bound structure reveal the mechanism of serine/theronine kinase inhibition by LY294002. Pim-1 kinase
- was originally identified in Maloney murine leukemia virusinduced T-cell lymphomas and is associated with multiple cellular functions such as proliferation, survival, differentiation, apoptosis, and tumorigenesis
- **Charline peng., et al.,[82] (2007)** reported the pim kinase substrate identification and specificity. The Pim family of Ser/Thr kinases has been implicated in the process of lymphomagenesis and cell survival. Known substrates of Pim kinases are few and poorly characterized. In this study we set out to identify novel Pim-2 substrates using the Kinase Substrate Tracking and Elucidation (KESTREL) approach.
- **Nilesh shah., et al.,[83] (2008)** reported the potential role for the pim1 kinase in human cancer-A molecular and therapeutic appraisal. The Pim1 kinase is a true oncogene implicated in early transformation and tumour progression in haematopoietic malignancies and prostate carcinomas
- **Rebbeka grundler., et al.,[84] (2009)** reported the Dissection of Pim serine_threonine kinases in FLT3-ITD–induced leukemogenesis reveals Pim1

as regulator of CXCL12–CXCR4-mediated homing and migration. FLT3-ITD–mediated leukemogenesis is associated with increased expression of oncogenic Pim serine/threonine kinases.

- **Xieu Feng Hu., et al.,[85] (2009)** reported the Pim-1–specific mAb suppresses human and mouse tumor growth by decreasing Pim-1 levels, reducing Akt phosphorylation, and activating apoptosis. Overexpression of Pim-1 plays a critical role in progression of prostatic and hematopoietic malignancies. Here we describe the generation of a mAb specific for GST–Pim-1, which reacted strongly with most human and mouse cancer tissues and cell lines of prostate, breast, and colonorigin but only weakly (if at all) with normal tissues
- **Jongchain kim., et al.,[86] (2010)** reported the pim1 promotes human prostate cancer cell tumorigenicity and C-myc transcriptional activity. We overexpressed Pim1 in three human prostate cell lines representing different disease stages including benign (RWPE1), androgen-dependent cancer (LNCaP) and androgen-independent cancer (DU145).
- **Sengjie Guo., et al.,[87] (2010)** reported the Overexpression of pim-1 in Bladder cancer. Expression and localization of Pim-1 in human normal and malignant bladder specimens were examined by Immunohistochemistry and Pim-1 staining score was compared with several clinicopathologic parameters.
- **Laurent Brault., et al.,[88] (2010)** showed the Pim serine_threonine kinases in the pathogenesis and therapy of hematologic malignancies and solid cancers. Whereas elevated levels of Pim1 and Pim2 were mostly found in hematologic malignancies and prostate cancer, increased PIM3 expression was observed in different solid tumors

- **Methvin Isaac., et al.,[89] (2011)** reported the oncogenic pim kinase family regulation drug resistance through multiple mechanisms. Resistance to chemotherapeutic drugs is a significant clinical problem for the treatment of cancer patients and has been linked to the activation of survival pathways and expression of multidrug efflux transporters.
- **Amir T.Faith., et al.,[90] (2012)** showed a potential therapeutic target for FLT3-ITD AML: pim1 kinase. Pim1, a serine/threonine kinase, is up-regulated in FLT3-ITD AML and may be involved in FLT3-mediated leukemogenesis. We employed a Pim1 inhibitor.
- **Yasid Alvarado., et al.,[91] (2012)** reported the pim kinases in hematological cancer. The PIM genes represent a family of protooncogenes that encode three different serine/threonine protein kinases (Pim1, Pim2 and Pim3) with essential roles in the regulation of signal transduction cascades, which promote cell survival proliferation and drug resistance.

II.2 PHARMACOPHORE MODELING

- **Marc D Jacobs., et al.,[92] (2005)** reported the pim-1 ligand-bound structure reveal the mechanism of serine/theronine kinase inhibition by LY294002. The crystal structures of Pim-1 complexed with staurosporine and adenosine were determined. Although a typical two-domain serine/threonine protein kinase fold is observed, the interdomain hinge region is unusual in both sequence and conformation; a two-residue insertion causes the hinge to bulge away from the ATP-binding pocket, and a proline residue in the hinge removes a conserved main chain hydrogen bond donor

- **Sheldon holder., et al.,[93] (2007)** reported the comparative molecular field analysis of flavanoid inhibitors of the pim-1 kinase. Comparative molecular field analysis (CoMFA) is a 3-D QSAR technique that has been widely used, with notable success, to correlate biological activity with the steric and electrostatic properties of ligands.
- **Sheldon holder., et al.,[94] (2007)** reported the Characterization of a potent and selective small-molecule inhibitor of the Pim1 kinase. We have used experimental approaches to identify a selective, cell-permeable, small-molecule inhibitor of the pim-1 kinase to foster basic and translational studies of the enzyme.
- **Vanda pogacic., et al.,[95] (2007)** reported the structural analysis identifies Imidazo [1,2-b] pyridazine as pim kinase inhibitors with invitro antileukemic activity. Using protein stability shift assays, we identified a family of imidazo[1,2-b]pyridazines to specifically interact with and inhibit Pim kinases with low nanomolar potency.
- **Albert C.pierce., et al.,[96] (2008)** showed the Docking Study Yields Four Novel Inhibitors of the Protooncogene Pim-1 Kinase.
- **Kevin Qian., et al.,[97] (2009)** showed the Hit to Lead Account of the Discovery of a New Class of Inhibitors of Pim Kinases and Crystallographic Studies Revealing an Unusual Kinase Binding Mode.
- **Rufane Akuae-Gedu., et al.,[98] (2009)** reported the Synthesis, Kinase Inhibitory Potencies, and in Vitro Antiproliferative Evaluation of New Pim Kinase Inhibitors .

- **Stefania olla., et al.,[99] (2009)** reported the Indolyl-pyrrolone as a new scaffold for pim-1 inhibitors. In this work, we applied a virtual screening protocol aimed at identifying small molecules able to inhibit Pim1 activity.
- **Silmae Dovdou., et al.,[100] (2010)** reported the Inhibitors of Pim-1 Kinase_ A Computational Analysis of the Binding Free Energies of a Range of Imidazo [1,2-b] Pyridazines.
- **Miviam Lopez-Ramos., et al.,[101] (2010)** showed the New potent dual inhibitors of CK2 and Pim kinases: discovery and structural insights. Protein kinase casein kinase 2 (CK2) is a serine/threonine kinase with evidence of implication in growth dysregulation and apoptosis resistance, making it a relevant target for cancer therapy.
- **Xiangy., et al.,[102] (2011)** showed the the discovery of novel bezofuran 2-b carboxylic acid as potent pim-1 inhibitors.
- **Nishighchi GA., et al.,[103] (2011)** reported the Discovery of novel 3,5 disubstituted indole derivative as potent inhibitors of pim-1,pim-2 and pim-3 protein kinases.
- **JI-Xia Ren., et al.,[104] (2011)** reported the Discovery of Novel Pim-1 Kinase Inhibitors by a Hierarchical Multistage Virtual Screening Approach Based on SVM Model, Pharmacophore, and Molecular Docking.
- **Carmen Blanco-Aparicio., et al.,[105] (2012)** reported the pim-1 kinase inhibitors ETP-45299 suppress cellular proliferation and synersizes with P13K inhibition. Hence pharmacologic inhibitors of Pim 1 are of therapeutic interest for cancer. ETP-45299 is a potent and selective inhibitor of Pim 1 that inhibits

the phosphorylation of Bad and 4EBP1 in cells and suppresses the proliferation of several non-solidand solid human tumor cell line

- **Keiko Tsuganezawa et al[106] 2012** showed the a novel pim-1 kinase inhibitor targeting residue that bond the substrate peptide. Using the method, among approximately 700 candidate compounds selected by virtual screening, we identified a novel Pim-1 kinase inhibitor targeting its peptide binding residues
- **Kilian Haber., et al.,[107] (2012)** reported the 7,8-Dichloro-1-oxo-ß-carbolines as a Versatile Scaffold for the Development of Potent and Selective Kinase Inhibitors with Unusual Binding Modes. The innate promiscuity of kinase inhibitors largely results from ATP-mimetic binding to the kinase hinge region.
- **Pastor J., et al.,[108] (2012)** showed the Hit to lead evaluation of 1,2,3-triazolo[4,5-b] pyridine as pim kinase inhibitors. Pim kinases have become targets of interest due to their association with biochemical mechanisms affecting survival, proliferation and cytokine production. 1,2,3-Triazolo[4,5-b]pyridines were identified as PIM inhibitors applying a scaffold hopping approach

II.3 DOCKING STUDY SIGNIFICANCE

- **Andrew L H.,[109] (2002)** carried and assessment of molecular targets that represent an opportunity for therapeutic intervention.
- **Paul, D.L.,[110](2002)** showed an overview on the significance of receptor based virtual screenings.
- **Jack K., [111] (2003)** showed the basis of the hydrophobic effect as one of the important for docking.

- **Gregory, L.W., et al.,[112] (2005)** reported the evaluation of 10 docking programs and 37 scoring function as an assessment of docking programs and scoring functions.

- **Ajay N. J., [113] (2006)** showed the importance and varies aspect of scoring functions for protein- ligand docking.

- **Gerhard,K., [114](2006)** showed the review and process description involved in virtual ligand screening.

Aim and Objectives

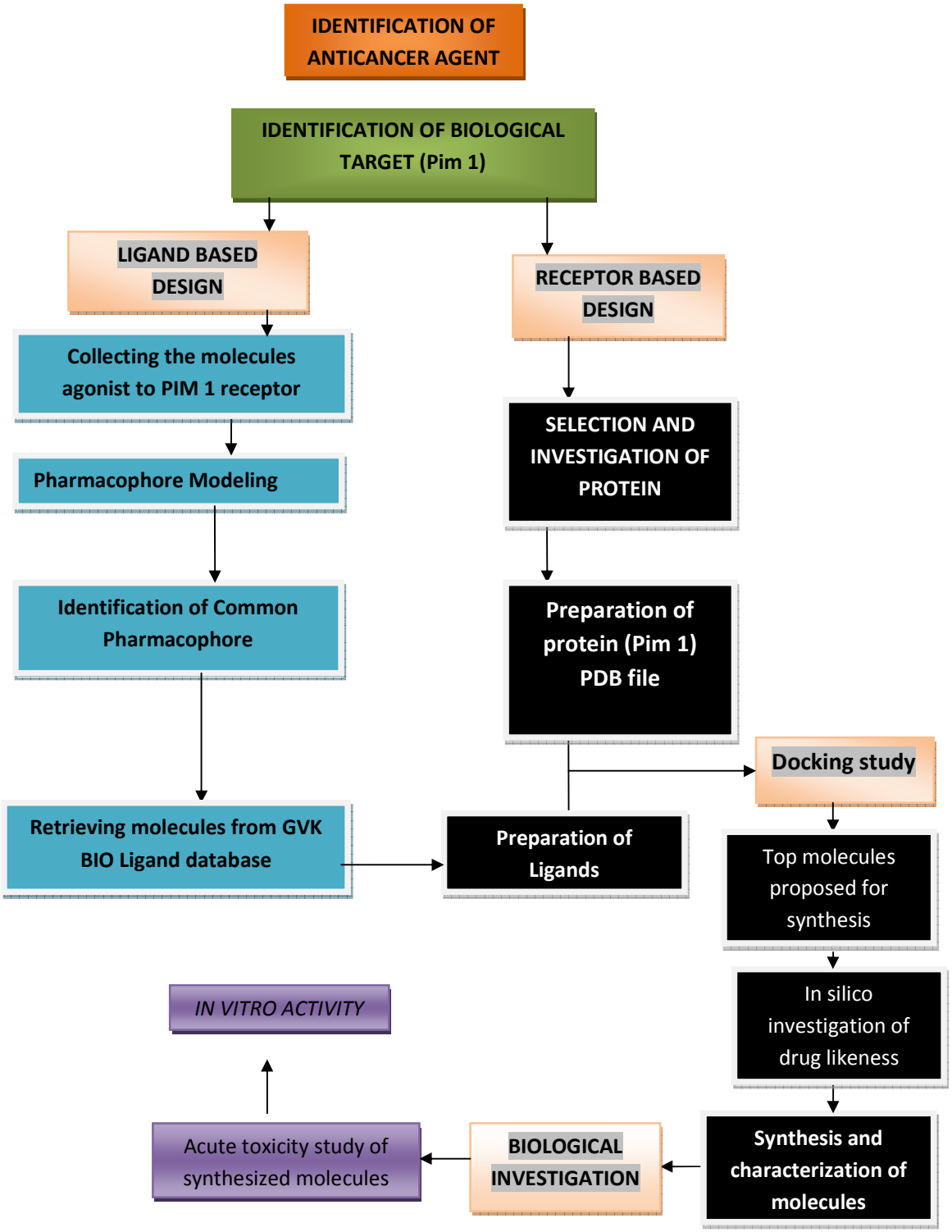
III AIM AND OBJECTIVES

The course of the study and research has been to identify new molecule for anticancer capable to inhibit pim 1 hence the following processes were carried out.

- Identification of Common Pharmacophore for Pim1.
- Preparation of database of ligands.
- Scaffold identification by docking.
- Investigation of ADME parameters of selected molecules.
- Synthesis of molecules related to scaffhold.
- Characterization of synthesized molecules by TLC, IR spectroscopy, Nuclear Magnetic Resonance spectroscopy and mass spectroscopy.
- Acute toxicity study in albino mice
- In vitro Anticancer Investigation of synthesized molecules against HCT cell lines

Materials And Methodology

IV. FLOW OF WORK



IV.2 DRUG DESIGN

IV. 2a PHARMACOPHORE MODELING

IV.2a.i Pharmacophore

Pharmacophore means "a molecular framework that carries (phoros) the essential features responsible for a drug (pharmacon) biological activity". A pharmacophore is defined as "a set of structural features in a molecule that is recognized at a receptor site and is responsible for that molecule's biological activity".

Pharmacophore features

The typical chemical features are as follows:

- Hydrogen bond acceptor
- Hydrogen bond donor
- Hydrophobic
- Hydrophobic aliphatic
- Hydrophobic aromatic
- Positive ionizable
- Negative ionizable
- Ring aromatic.

All the above pharmacophore features need to match different chemical groups with similar properties.

Pharmacophore Studies: (Catalyst) [46]

CATALYST (Tm), an Accelrys developed software is used for pharmacophore generation. Catalyst treats molecular structures as templates comprising of chemical functions positioned in space that will bind efficiently with complementary functions on the respective binding proteins. Catalyst generates pharmacophore hypotheses in terms of the 3D arrangement of chemical functional group elucidating the activity variations of the compounds. The total energy cost of the each generated hypothesis can be calculated from the difference between the observed activity value and the activity value estimated by the hypothesis based on the pharmacophore features.

Cerius 2 [47]

Cerius2 is a product of accelrys. Cerius2 has a variety of force fields available. The default force field is UFF, which stands for universal force field.Cerius2 offers abilities for modeling materials structure properties, and processes with appliances in catalysis, crystallization and polymer science. Cerius2 is a suite of molecular modeling and simulation package for smaller molecules.

IV.2a.ii Chemical Feature based based models from Catalyst4.11

Catalyst is a program package from accelyrs. The program provided a modeling environment and consists of several modules, which can be bought independently. Below is a description of the module important for database search and hypothesis generation. In Catalyst, 3D-pharmacophore model and queries for searching 3D database are called hypothesis.

HipHop

Generate a set of common feature pharmacophore model from set of compounds known to be active (No activity data) at a target

HypoGen

Develop SAR hypothesis model from a set of molecules for which activity values (IC50 or Ki) on a given biological target are known.

HypoRefine

Permits consideration of exclusion volume in Pharmacophore-based 3D QSAR optimization. The result is better model predictivity activity is determined by considerations of molecular shape.

Exclusion volume

An exclusion volume can be added to a hypothesis (or to a template molecules) to specify one or more spherical spaces that must not contain any atoms or bonds. An exclusion volume can represent a region in space that might impinge sterically on a receptor. An exclusion volume can be interpreted as a geometrical constraint, and this is how it is treated in catalyst.

Compare/Fit

Provide the ability to fit compounds and hypotheses, and determine their degree of similarity, both geometrically and functionally. In a database search, COMPARE fits the original hypothesis onto the hit molecules obtained from the search and a score are calculated according to the geometrical fit.

Cost Parameters

1**. Fixed cost** represents the simplest possible hypothesis (initial) that fits the data perfectly.

2. Null hypothesis

It is the cost when each molecule estimated a mean activity. It acts like a hypothesis with no feature.

3**. Error cost**

The bits needed to describe the error in the leads. It increases as the RMS difference between estimated and measured activities for training set molecules increases.

4. Weight cost

The bits required to describe the feature weights.

5. **Configuration cost**

The bits required to describe the types and relative position of the feature in the hypothesis. A fixed cost that depends on the

- Main assumption made by HypoGen is that active molecules
- Should map more features than an inactive molecules
- Complexity of the hypothesis space being optimized

IV.2a.iii PHARMACOPHORE STUDIES

Pharmacophore elucidation is a molecular alignment problem, the aim being to superimpose a set of active ligands, all of which bind to the same protein of unknown 3D structure, so that the features they have in common become evident.

The model is a collection of chemical features distributed in 3D space that is intended to represent groups in molecules that participate in important binding interaction ions between drugs and their receptor. Estimated activity computed by comparing how well the chemical feature of a subject molecules overlap with the chemical features in the model (hypo). The ability of molecules to adjust their conformation in order to fit a receptor better is accommodated by considering molecules as collections of energetically reasonable conformation (confo model) during analysis.

The step involved in the development of pharmacophore model:

- Visual identification of common structural and chemical feature

- Measurements of 3D aspects of pharmacophore
- Development of pharmacophore model
- Validation of model
- Refinement of model

Data collection and development of database

Pim1 inhibitors data have been collected with their biological activity data from various medicinal chemistry as well as life science journals and developed a unique database using MDL ISIS/Base. Our pim1 inhibitors database contains 91 compounds were selected based on diversity of both chemical structure and biological activity.

Training set selection and conformational generation

The most critical aspect of pharmacophore hypothesis generation is the selection of the training set. The 91 molecules were arranged in decreasing order of their activity. The most diverse 24 molecules were carefully selected as the training set.

Highly active (+++ or < 1µM), moderatively active(++or 1-10µM) and inactive(+ or >10µM) compound were added to training set to obtain critical information on pharmacophore requirements for pim1 inhibition.

Before starting the pharmacophore generation process, conformation models for the molecules was developed by poling algorithm, which seeks to provide a broad coverage of conformational space using the best conformer generation method with a maximum conformational energy of 20kcal/mol above the lowest energy conformation found. The number of conformers generated for each compound was limited to a maximum number of 250.this training set was then used to generate quantitative pharmacophore model.

Generate of HipHop (Qualitative) model

HipHop hypothesis were produced by comparing a set of conformational model and a number of 3D configurations of chemical features shared among the training set molecules with diverse scaffolds.

The automatic hypothesis generation with HipHop was done by the following procedure:

- For each compound a best quality conformational model was generated with default parameters;max number =250 and energy range = 20 kcal/mol.
- An automatic hypothesis generation was run . The hypothesis was allowed to contain the features: 0-5 “hydrophobic”, 0-5 “HB Acceptor feature” (default), 0-5 “HB donor”, 0-5 “Positive ionisable”, and 0-5 “Ring aromatic “based on Lipinski rules.
- All the parameters were set to default. Therefore the program will stop after 10 hypothesis are generated.

Generation of HypoGen(Quantitative)model

The training set of 24 molecules defined earlier was used in HypoGen pharmacophore model, which could be used for quantitative estimation of activities while screening large databases.

- The automatic hypothesis generation with hiphop was done by the following procedure:
- Input structures were selected from the criteria that they should share the same binding mode, be structurally diverse, and the affinity data should cover several orders of magnitude.
- For each compound a best quality conformational model was generated with default parameters;max number =250 and energy range = 20 kcal/mol.

- The compound were associated with an uncertainty of 3
- An automatic hypothesis generation was run. The hypothesis was allowed to contain the features :0-5 “hydrophobic”, 0-5 “HB Acceptor feature” (default), 0-5 “HB donor”, 0-5 and 0-5 “Ring aromatic “.
- All the parameters were set to default. Therefore the program will stop after 10 hypotheses are generated.

Generated quantitative Pharmacophore models are used to predict biological activities of novel compounds. The quality of hypogen model is described in term of cost analysis.

Generate of Hyporefine hypotheses

The automatic hypothesis generation with hyporefine was done by same procedure as hypogen but with excluded volume.

Cost analysis during Pharmacophore Model

During an automated hypothesis generation run, catalyst considers and discards many thousands of models. It distinguishes between alternatives by applying a cost analysis. Here the simplest model is used. Simplicity is defined using the minimum description length principle from information theory. Catalyst uses bits for language, so the program assigns costs to hypothesis in terms of the number of bits required to illustrate them fully. The overall cost of a hypothesis is determined by summing three cost factors, a weight cost, an error cost and a configuration cost. Error cost has the major effect in establishing hypothesis cost. The differences between these two costs are essential, the greater the differences the higher the possibility for finding useful models.

If a returned hypothesis has a cost that differs from the null hypothesis by 40 – 60bits there is a high probability it has a 75-90% chance of demonstrating a true correlation in the data.

As the difference becomes less than 40 bits, the likelihood of the hypothesis demonstrating a true correlation in the data rapidly drops below 50 percent under these conditions it may be difficult to find a model that can be shown to be predictive. The output from the catalyst hypothesis generations job is the ten lowest cost hypotheses establish during the analysis that are different from each other. It is not often not possible to categorize between these by any simple statistical procedure particularly if the cost difference are small (<10bits).

A visual evaluation procedure is used to select the best model comparing the regression graphs, obtained by correlating the train set data, Using the compare fit it was seen how the most active compounds map to the model. This is where chemical relevance established. To predict in a useful way, a model should be capable of explaining the difference in activity between training set members in a way that is chemically reasonable. The process is also dependent on how good the conformation coverage is for each training set. Often, a fit produced during the automatic generation phase can be improved using the best fit compare operation, compound whose activities.

Generation of Pharmacophore Models

Following the basic principle of training set selection, 24 compounds were exquisitely chosen with their shown in figure IV.1. Training and test set compounds taken from our in-house database were imported into CATALYST™, and submitted for conformational analysis.

Training set compound were used for generating pharmacophore model

Pharmacophore model were developed using hypogen module implement in CATALYST and the top 10 scoring hypothesis were exported. Analyses of the best ranking pharmacophore model revealed that four chemical feature that are two H-bond acceptor,

one hydrophobic aliphatic and ring aromatic feature could effectively map all the chemical feature. The activity of each training set compound is estimated using regression parameters. The parameters are computed by the regression analysis using the relationship of geometric fit values versus the negative logarithm of activity. The grater the geometric fit, the greater the activity prediction of the compound. The fit function does not only check if the feature mapped or not; it also contains a distance term which measure the distance term which measure the distance that separates the feature on the molecules from the centroid of the hypothesis feature. Both terms are used to calculate the geometric fit value. The error value shows the ratio of estimated activity to experimental activity. A positive error values indicates that the estimated IC50 is higher than the experimental IC50 while a negative error value indicates that the estimated IC50 is lower than the experimental IC50.

Pharmacophore Model validation and knowledge based screening

The best pharmacophore hypothesis was used initially to screen 30 pim-1 inhibitors test set. The same model has also been used to select potent molecules from 1000 library molecules designed using scaffold hoping (knowledge based screening).

Pharmacophore Model validation

The main purpose of validating a qualitative model is to determine whether our model is able to identify active structures and forecast their actively accurately. The purpose of the pharmacophore hypothesis generation is not just to predict the activity of the training set compounds accurately but also to verify whether the pharmacophore model are capable of predicting the activity for any given compounds and classifying them correctly as active or inactive.

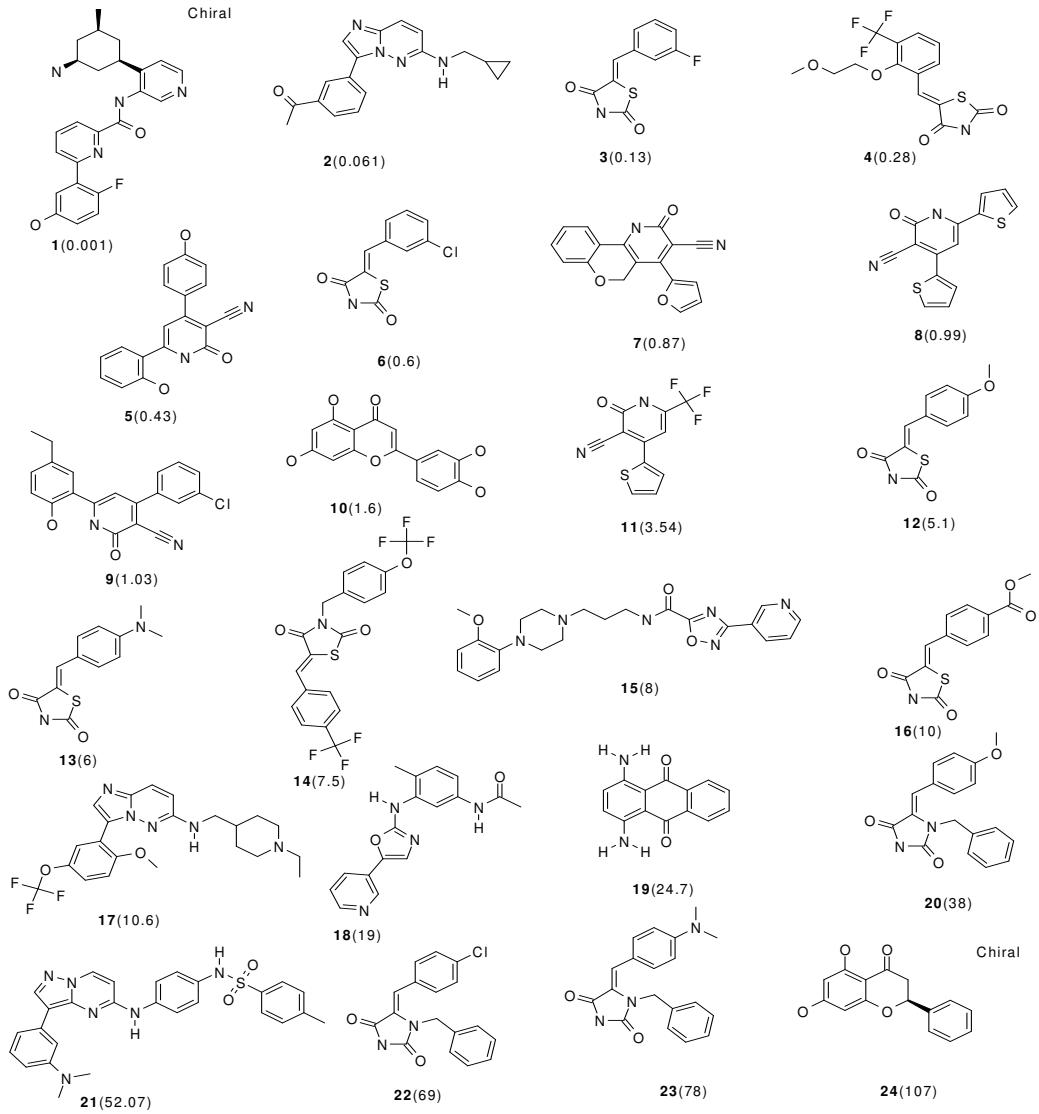


Fig. IV.1 Training set 24 molecules for pim-1 inhibitors. IC50 values of each molecule are given in parentheses

In order to validate our Pharmacophore hypothesis, we have used a training set comprising of molecules which had experimental pim-1 inhibitory activity belonging to different activity ranges and the randomize the activity data associated with the training set compounds, and randomized training sets are used to generate pharmacophore hypothesis using the same features and parameter as developed for the original hypothesis. If

randomized data set results in the generation of a Pharmacophore with similar or better cost values, RMSD, and correlation, then the original hypothesis is considered to have been generated by chance.

The best Pharmacophore hypothesis was used initially to screen the pim-1 inhibitors. All queries were performed using the best flexible search database/SS method. The hyporefine was used to screen the known highly active, moderate active, low active inhibitors of the test set.

The correlation between experimental and predicted value for the test set molecules against hypo-1 model along with training set molecules for pim-1 inhibitors. The features mapped by this pharmacophore are two hydrogen bond acceptor, Hydrogen bond donor and one hydrophobic.

The high active compound is identified from the result of hyporefine process and the compound study on perfect fit for structure as a scaffold is performed. Adding then in appropriate position of scaffold identifies the respective attachment groups.The activity of catalyst compound is then populated by applying Lipinski rule identified the property of new catalyst compound. Underestimated nearly always give improved value when fitted under best fit compare.

During automatic hypothesis generation, catalyst uses the fast fit compare algorithm to measure fits of molecule to a model. The best fit compare always starts with the fast fit and attempts to improve the fit by allowing the induced conformation to flex over a given energy range. In effect, more of the relevant conformation space is examined during this process. Look for model that explains the activity of most of the training set members in a

chemically reasonable way and estimated their activities to within a power of 10 of measured values. This critical step toward establish productiveness of the model, if the model successfully predicts the activities of these outsiders and does so while mapping the compounds in chemically reasonable ways, it is good candidate for use as a model to guide future synthesis.

Knowledge based screening

A total of 1000 molecules were generated based on the knowledge of binding interaction of ligand with the protein and also the common features necessary for the biological activity of molecules. These molecules were drawn using cerius software and were screened for their activities using the developed Pharmacophore models. For the final screening of the compounds, the hits were analyzed in the Pharmacophore model and the most active hits were identified. Best flexible search method in CATALYST was used for database searching to retrieve similar feature compounds.

IV.2.b DOCKING STUDY

Docking procedures aim to identify correct posses of ligands in the binding pocket of the protein and to identify and to predict the affinity between the ligands and the protein, in other words docking describes a process by which molecules fit together in three dimensional space.

Basic requirements for molecular docking approach require the following components. A target protein structure with or without a bound ligand ,the molecules of interest or a database containing existing or virtual compounds for docking posses and a computational framework that allows the implementation of the desired docking and scoring procedures for which the following steps are involved .

- **Selection and investigation of protein**
- **Protein preparation**
- **Receptor grid generation**
- **Ligand database creation**
- **Ligand preparation**
- **Ligand receptor docking and predicting activity**
- **Scaffhold identfication**
- **Docking of molecules going to be synthesized**

IV.2.b.i SELECTION AND INVESTIGATION OF PROTEIN

The protein selection is carried out from the PDB (protein data bank). Protein data bank is resource for studying biological macromolecules. It contains information about experimentally determined structures of proteins, nucleic acids and complex

assemblies. Also providing a variety of tools and resources users can perform simple and advanced searches based on annotations, relating to sequences, structures and function.

The protein PDB file was selected for Pim1 and further evaluated by its Resolution value, R Free, R value and Ramachandran plot.

IV.2.b.ii PROTEIN PREPARATION

At first the protein which needs to be prepared is uploaded in the main menu by the option import PDB file and then the following steps are carried out Pim1 proteins.

In this portion we amend the structures and perform the basic preparation tasks for the preparation of raw protein downloaded from the protein data bank which needs to be prepared for accurate results. The task includes the following.

- Align to options
- Assign bond order option
- Add hydrogen option
- Crete zero order bond to metals option
- Create disulfide bonds option
- Convert selenomethionines to methionines option
- Fill in missing side chains using prime option
- Cap termini option
- Fill in missing loops using prime option
- Delete water beyond NA from het group's option and text box.
- Preprocess button

The above steps are done in the"Maestro 9.1" protein preparation wizard. The workflow contains this tool which carries the steps necessary for preparation of protein. After processing all the above steps we will be able to say whether the protein is ready to be forwarded to the next step.

IV.2.b.ii RECEPTOR GRID GENERATION

Defining the Receptor (We need to define the part of the system in the workspace to be treated as a Receptor (Active site), PDB file of PIM1 (which were prepared earlier by protein preparation Wizard) were loaded on the workspace to generate their respective Grid files)

Receptor Site Information

The choice of receptor site can be done in 2 ways

1) Centroid of workspace ligand
2) Centroid of selected residues

The process of receptor grid generation is done by using Glide® of Maestro 9.1 molecular modeling suite. This software helps the identification of receptor grid according to our needs.

The process is carried out after the particular protein been prepared in the protein preparation wizard and then the application tool helps us to complete the grid generation. This gives an output file which can be save it as Grid output file in zip format.

The information about the receptor site of two receptors is:

Pim1 (3R04)

As per the literature of this particular pdb file, 3R04 gives information that hydrophobic interactions play a vital role in pim1 receptor binding. Hinge region residues P125 and surrounded by A65, R122, L44, L174. The preferable conserved H-bonding interactions between water and E85, D186. The Hydrophobic interaction and H bonding needs to be investigated, so as to validate our affinity with receptor site.

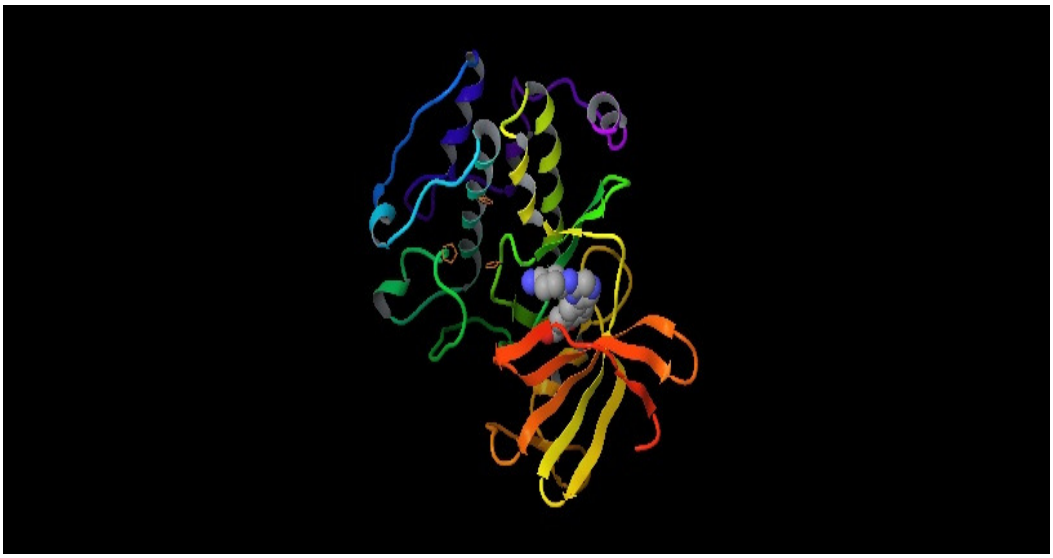


Fig IV.2 Receptor site of Pim1(3R04)

IV.2.b.iii LIGAND DATABASE PREPARATION

Database of the collection of ligand molecules which contain various heterocyclic rings and different fragments as substitutions, can be used to evaluate the affinity of the molecules against different biological targets for their affinity. All molecules were sketched in the database had been sketched using maestro 9.1 using the build tool. The fragments which had been used were shown in the following figures.

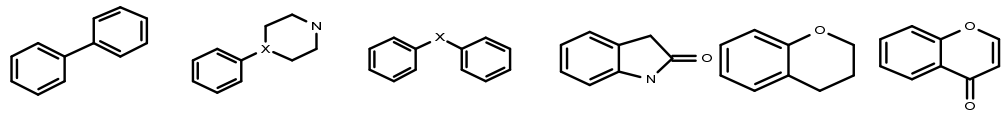


Fig IV.3 Fragments used in the database creation

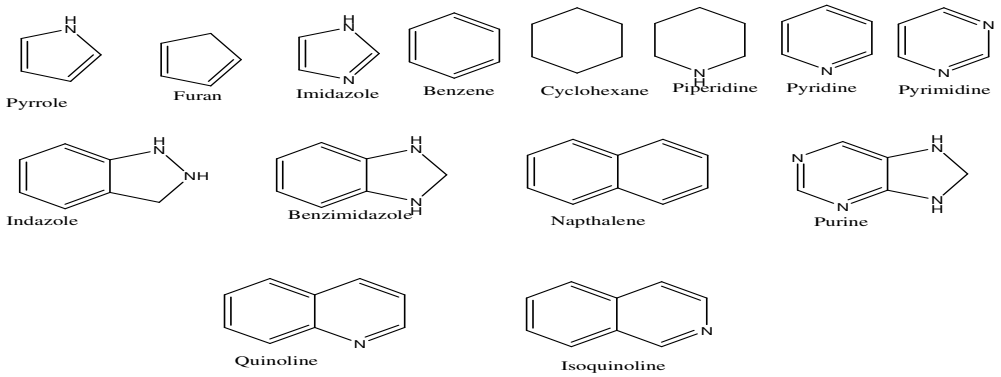


Fig IV.4 Some Heterocyclic nucleus used in the study

Fig IV.5 Fragments used as a side chain

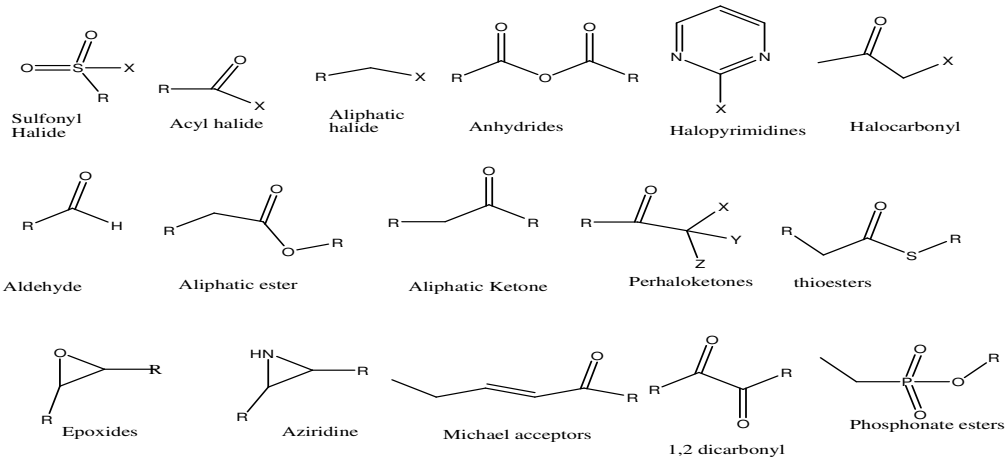


Fig IV.6 Toxicity Filters

IV.2.b. iv LIGAND PREPARATION

Ligands built in the database need to be prepared by which we would get ligands of very high quality so the task includes are

- **Convert structure format.**

- **Select structures.**
- **Add hydrogen atoms.,**
- **Remove unwanted molecules,**
- **Neutralize charged groups.,**
- **Generate ionization states ,**
- **Generate tautomers ,**
- **Filter structures,**
- **Generate alternative chiralities.,**
- **Generate low-energy ring conformations,.**
- **Remove problematic structures,**
- **Optimize the geometries.**
- **Convert output file.**

IV.2.b.v LIGAND RECEPTOR DOCKING AND PREDICTING ACTIVITY

Docking is been done by using Glide® (an application tool of Maestro 9.1)

Glide® is designed to assist in high-throughput screening of potential ligands based on binding mode and affinity for a given receptor molecule.

After the preparation of protein, the prepared ligands were docked with the respective proteins of Pim1.

The ligands Database which consist of 1000 molecules which had been already prepared by ligprep were first initially docked by using Glide in HTVS (high throughput virtual screening) mode and Standard mode (sp).

Specifying the Receptor Grid

To specify the receptor grid for the docking job, we click on Browse in the Receptor grid section of the Settings tab to open a file selector and choose a grid file (.grid) or a compressed grid archive (.zip).

Selecting the Docking Precision

There are three choices of docking .

• HTVS (high-throughput virtual screening)—High-throughput virtual screening (HTVS) docking is intended for the rapid screening of very large numbers of ligands. HTVS has much more restricted conformational sampling than SP docking, and cannot be used with score-in-place.

• SP (standard precision)—Standard-precision (SP) docking is appropriate for screening ligands of unknown quality in large numbers.

• XP (extra precision). Extra-precision (XP) docking and scoring is a more powerful and discriminating procedure, which takes longer to run than SP. XP is designed to be used on ligand poses that have a high score using SP docking. We recommend that you run your database through SP docking first, then take the top 10% to 30% of your final poses and dock them using XP,

PREDICTING THE ACTIVITY OF TOP MOLECULES

The top 100 molecules which were found to be top scored in the docking study were further refined by predicting the activity which was previously established.

To calculate activities for the hits based on the QSAR model which was established earlier were used to predict the activity of ligands in the top list. At first the 100

molecules were taken in the project table and then Catalyst was used to predict the activity by taking the common Pharmacophore of Pim1.

IV.2.b. vi SCAFFOLD IDENTIFICATION

Scaffold identification is the process of identifying the template commonly occurring among the top class of molecules which may able to help us to identify a new class of molecules with the best of activity.

In this the top 35 molecules which we identified after the database screening and prediction of activity with the help of 3D QSAR study we were able to identify the scaffold

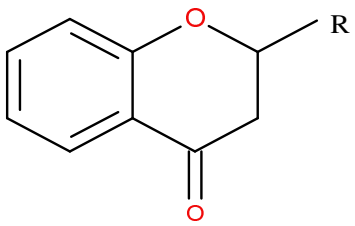


Fig IV.7 scaffold identified from the docking study

The synthesis of the following compounds from the scaffhold had been found to be synthetically possible to synthesis. These molecules were than tagged as PV1, PV2, PV3 and PV4.

The molecules molecular name was again investigated as a literature review to find out the information in NISCAIR.

DOCKING OF MOLECULES GOING TO BE SYNTHESIZED

Docking of selected molecules from the scaffhold is done by XP mode against Pim1 for evaluating the degree of binding by checking or evaluates the following parameters for detailed study with the receptor.

Table IV.1

S.NO	XP Term	Description
1	GScore	Total GlideScore; sum of XP terms
2	LipophilicEvdW	Lipophilic term derived from hydrophobic grid potential and fraction of the total protein ligandvdW energy
3	PhobEn	Hydrophobic enclosure reward
4	PhobEnHB	Reward for hydrophobically packed H-bond
5	PhobEnPairHB	Reward for hydrophobically packed correlated H-bonds
6	HBond	ChemScore H-bond pair term
7	Electro	Electrostatic rewards
8	SiteMap	SiteMap ligand/receptor non-Hbonding polar/hydrophobic and hydrophobic/hydrophilic complementarity terms
9	LowMW	Reward for ligands with low molecular weight
10	Penalties	Polar atom burial and desolvation penalties, and penalty for intra-ligand contacts

11	HBPenal	Penalty for ligands with large hydrophobic contacts and low H-bond scores
12	PhobicPenal	Penalty for exposed hydrophobic ligand groups
13	RotPenal	Rotatable bond penalty

IV.2.c IN SILICO SCREENING OF DRUG LIKENESS

The molecules which are to be synthesized need to be investigated for its drug likeness. So computational process helps in a vital way to calculate or evaluate the ADME parameter beforehand. In silico screening of drug likeness is done by Qikprop. Qikprop has 50 characters for accessing the ADME parameters necessary for good drugs. QikProp is a quick, accurate, easy-to-use absorption, distribution, metabolism, and excretion (ADME) prediction program designed by Professor William L. Jorgensen. QikProp predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules, either individually or in batches.

Table IV.2 (50 parameters calculating the drug likeness)

S.No	Property or descriptors	Description	Range or recommended values
1	**#stars**	Number of property or descriptor values that fall outside the 95% range of similar values for known drugs. Outlying descriptors and predicted properties are denoted with asterisks (*) in the .out file. A large number of stars suggest that a molecule is less drug-like than molecules With few stars. (combination of all descriptors)	0 – 5
2	**#amine**	Number of non-conjugated amine groups.	0 – 1
3	**#amidine**	Number of amidine and guanidine groups.	0
4	**#acid**	Number of carboxylic acid groups.	0 – 1
5	**#amide**	Number of non-conjugated amide groups.	0 – 1
6	**#rotor**	Number of non-trivial (not CX3), non-hindered (not alkene, amide,small ring) rotatable bonds.	0 – 15
7	**#rtvFG**	Number of reactive functional	0 – 2

		groups	
8	**CNS**	Predicted central nervous system activity on a –2 (inactive) to +2 (active) scale.	–2 (inactive), +2 (active)
9	**mol_MW**	Molecular weight of the molecule .	130.0 – 725.0
10	**SASA**	Total solvent accessible surface area (SASA) in square angstroms using a probe with a 1.4 Å radius.	300.0 – 1000.0
11	**FOSA**	Hydrophobic component of the SASA (saturated carbon and attached hydrogen).	0.0 – 750.0
12	**FISA**	Hydrophilic component of the SASA (SASA on N, O, and H on heteroatoms).	7.0 – 330.0
13	**PISA**	π (carbon and attached hydrogen) component of the SASA.	0.0 – 450.0
14	**WPSA**	Weakly polar component of the SASA (halogens, P, and S).	0.0 – 175.0
15	**volume**	Total solvent-accessible volume in cubic angstroms using a probe with a 1.4 Å radius.	0.0 – 175.0
16	**donorHB**	Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution. Values are averages taken over a number of configurations, so they can be non-integer.	0.0 – 6.0
17	**accptHB**	Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution. Values are averages taken over a number of configurations, so they can be non-integer.	2.0 – 20.0
18	**dip^2/V**	Square of the dipole moment divided by the molecular volume. This is the key term in the Kirkwood-Onsager equation for the free energy of solvation of a dipole with volume V.	0.0 – 0.13
19	**ACxDN^.5/SA**	Index of cohesive interaction in solids.	0.0 – 0.05
20	**glob**	Globularity descriptor $(4\pi r2) / (SASA)$where r is the radius of a	0.75 – 0.95

		sphere with a volume equal to the molecular volume. Globularity is 1.0 for a spherical molecule.	
21	**QPpolrz**	Predicted polarizability in cubic angstroms.	13.0 – 70.0
22	**QPlogPC16**	Predicted hexadecane/gas partition coefficient.	4.0 – 18.0
23	**QPlogPoct‡**	Predicted octanol/gas partition coefficient.	8.0 – 35.0
24	**QPlogPw**	Predicted water/gas partition coefficient.	4.0 – 45.0
25	**QPlogPo/w**	Predicted octanol/water partition coefficient	–2.0 – 6.5
26	**QPlogS**	Predicted aqueous solubility, log S. S in mol dm–3 is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid.	–6.5 – 0.5
27	**CIQPlogS**	Conformation-independent predicted aqueous solubility, log S. S in mol dm–3 is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid.	–6.5 – 0.5
28	**QPlogHERG**	Predicted IC50 value for blockage of HERG K+ channels.	concern below –5
29	**QPPCaco**	Predicted apparent Caco-2 cell permeability in nm/sec. Caco-2 cells are a model for the gutblood barrier. QikProp predictions are for non-active transport.	<25 poor, >500 great
30	**QPlogBB**	Predicted brain/blood partition coefficient.	–3.0 – 1.2
31	**QPPMDCK**	Predicted apparent MDCK cell permeability in nm/sec. MDCK cells are considered to be a good mimic for the blood-brain barrier	<25 poor, >500 great
32	**QPlogKp**	Predicted skin permeability, log *K*p.	–8.0 – –1.0
33	**IP(ev)**	PM3 calculated ionization potential.	7.9 – 10.5
34	**EA(eV)**	PM3 calculated electron affinity.	–0.9 – 1.7
35	**#metab**	Number of likely metabolic reactions.	1 – 8

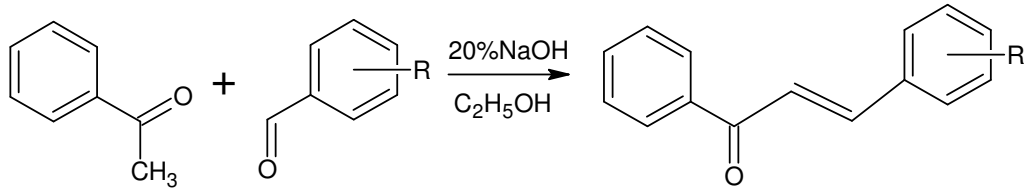
36	**QPlogKhsa**	Prediction of binding to human serum albumin.	–1.5 – 1.5
37	**HumanOralAbsorption**	Predicted qualitative human oral absorption: 1, 2, or 3 for low, medium, or high.	1-3
38	**PercentHuman-OralAbsorption**	Predicted human oral absorption on 0 to 100% scale.The prediction is based on a quantitative multiple linear regression model. This property usually correlates well with HumanOral- Absorption, as both measure the same property.	>80% is high <25% is poor
40	**SAFluorine**	Solvent-accessible surface area of fluorine atoms.	0.0 – 100.0
41	**SAamideO**	Solvent-accessible surface area of amide oxygen atoms.	0.0 – 35.0
42	**PSA**	Van der Waals surface area of polar nitrogen and oxygen atoms.	7.0 – 200.0
43	**#NandO**	Number of nitrogen and oxygen atoms.	2 – 15
44	**RuleOfFive**	Number of violations of Lipinski's rule of five. The rules are: mol_MW < 500, QPlogPo/w <5, donorHB ≤ 5, accptHB ≤ 10. Compunds that satisfy these rules are considered drug-like.(The "five" refers to the limits, which are multiples of 5.)	maximum is 4
45	**RuleOfThree**	Number of violations of Jorgensen's rule of three. The three rules are: , QPlogS > -5.7, QPPCaco > 22 nm/s, # Primary Metabolites < 7.	maximum is 3
46	**#ringatoms**	Number of atoms in rings.	
47	**#in34**	Number of atoms in 3- or 4-membered rings.	
48	**#in56**	Number of atoms in 5- or 6-membered rings.	
49	**#noncon**	number of ring atoms not able to form conjugated aromatic systems (e.g. sp3 C).	
50	**#nonHatm**	Number of heavy atoms (nonhydrogen atoms).	

IV.3 SYNTHESIS

IV.3.i GENERAL SCHME

The synthesis involves the two steps first step involve condensation of reactants under cooling conditions. Second step involve cyclization of reactant under thermal condition.

General scheme
Step: 1



Step: 2

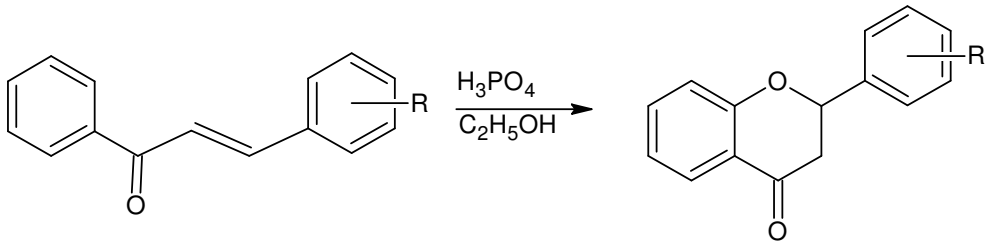
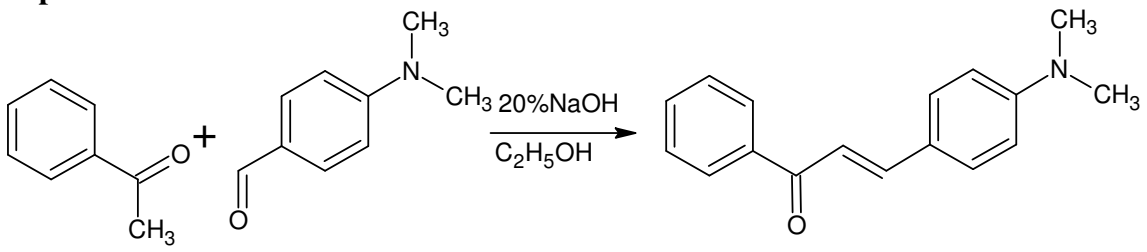


Fig IV.8 General Scheme involved in the synthesis of molecules.

IV.3.ii SYNTHESIS

The syntheses of 4 molecules were carried out in the following way.

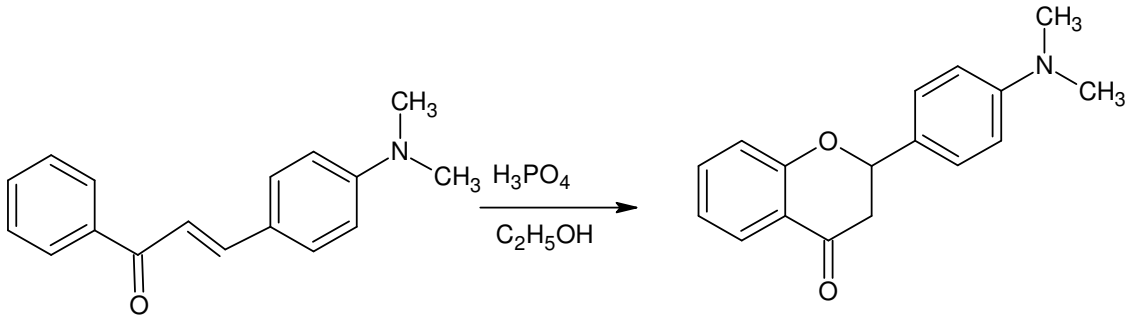
PV 1
Step: 1



IV.9.i Reaction involved in the synthesis of PV1

Chalcones were prepared by base catalyzed condensation with 5.85ml of acetophenone and 7.45gm of P- dimethyl amino benzaldehyde in ethyl alcohol was added to a 20% solution of sodium hydroxide drop wise with stirring. The reaction mixture was kept at 0°C for two days then diluted with water and acidified with 2M hydrochloric acid. The precipitated chalcone was collected and recrystallised form alcohol to yield pure chalcone. Yield 82%.

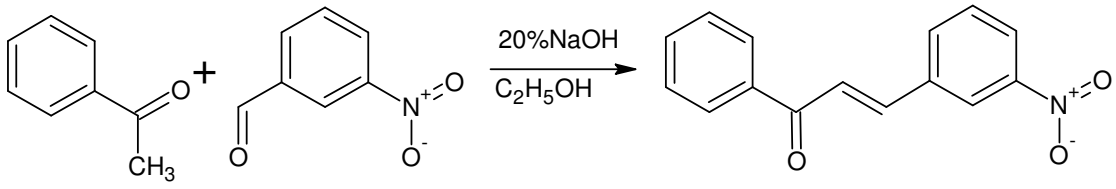
Step:2



IV.9.ii Reaction involved in the synthesis of PV1

Flavanone were prepared by refluxing the corresponding chalcone obtained from step1 with phosphoric acid 10 gm in 150ml ethanol for 2 days.concentration of the solution and dilution with water gave precipitated product.Recrystallised with ethanol. Yield 80%.

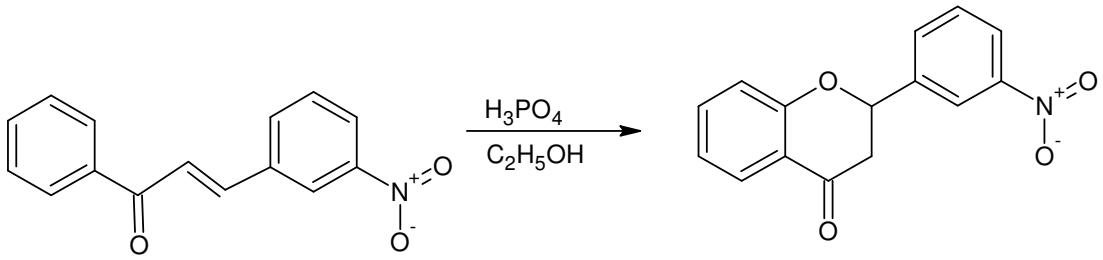
PV2
Step:1



IV.10.i Reaction involved in the synthesis of PV2

Chalcones were prepared by base catalyzed condensation with 5.85ml of acetophenone and 7.55gm of 3-nitrobenzaldehyde in ethyl alcohol was added to a 20% solution of sodium hydroxide drop wise with stirring. The reaction mixture was kept at 0°C for two days then diluted with water and acidified with 2M hydrochloric acid. The precipitated chalcone was collected and recrystallised form alcohol to yield pure chalcone. Yield 83%.

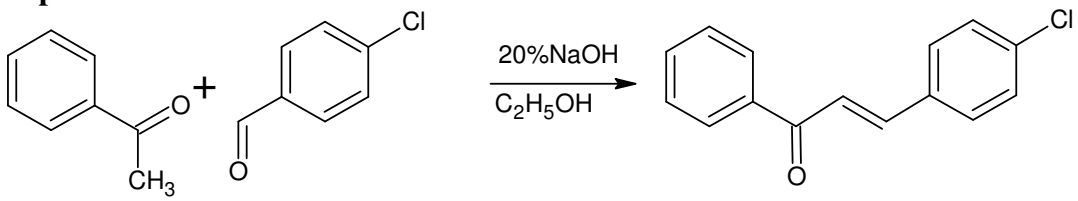
Step:2



IV.10.ii Reaction involved in the synthesis of PV2

Flavanone were prepared by refluxing the corresponding chalcone obtained from step1 with phosphoric acid 10 gm in 150ml ethanol for 2 days.concentration of the solution and dilution with water gave precipitated product.Recrystallised with ethanol. Yield 82%.

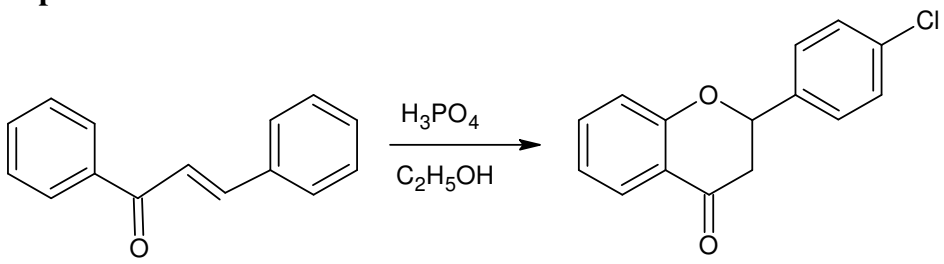
PV3
Step: 1



IV.11.i Reaction involved in the synthesis of PV3

Chalcones were prepared by base catalyzed condensation with5.85ml of acetophenone and 7gm of P-chlorobenzaldehyde in ethyl alcohol was added to a 20% solution of sodium hydroxide drop wise with stirring. The reaction mixture was kept at 0°C for two days then diluted with water and acidified with 2M hydrochloric acid. The precipitated chalcone was collected and recrystallised form alcohol to yield pure chalcone. Yield 85%.

Step:2

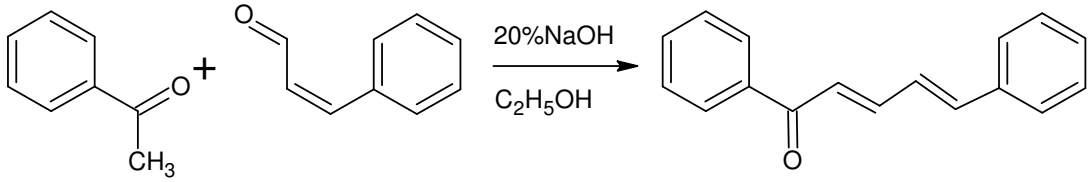


IV.11.ii Reaction involved in the synthesis of PV3

Flavanone were prepared by refluxing the corresponding chalcone obtained from step1 with phosphoric acid 10 gm in 150ml ethanol for 2 days. concentration of the solution and dilution with water gave precipitated product. Recrystallised with ethanol. Yield 83%.

PV4

Step:1

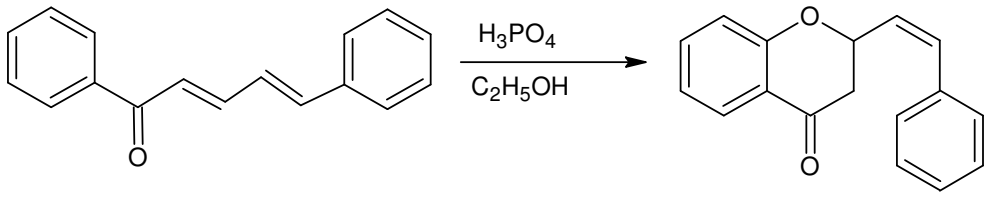


IV.12.i Reaction involved in the synthesis of PV4

Chalcones were prepared by base catalyzed condensation with 5.85ml of acetophenone and 6.25ml of cinnamaldehyde in ethyl alcohol was added to a 20%

solution of sodium hydroxide drop wise with stirring. The reaction mixture was kept at 0°C for two days then diluted with water and acidified with 2M hydrochloric acid. The precipitated chalcone was collected and recrystallised form alcohol to yield pure chalcone. Yield 88%.

Step:2



IV.12.ii Reaction involved in the synthesis of PV4

Flavanone were prepared by refluxing the corresponding chalcone obtained from step1 with phosphoric acid 10 gm in 150ml ethanol for 2 days.concentration of the solution and dilution with water gave precipitated product.Recrystallised with ethanol. Yield 86%.

V.3.iii RECRYSTALLISATION:

Ethanol was added to the synthesized compounds and heated so as to dissolve completely. The clear solution thus obtained was filtered immediately and set aside for cooling. As the temperature decreases crystals were found to appear. The process was repeated to obtain a pure crystalline form.

V.3.iv CHARACTERIZATION AND IDENTIFICATION

The synthesized compounds were recrystalized and identified by TLC. The melting point of the products were found and presented uncorrected. The characterization was carried out using sophisticated instruments like IR, NMR and Mass Spectroscopy and supplemented with 3D images and characteristic properties through the aid of computer software.

METHODS INVOLVED

The synthesized compounds were characterized by using the following methods:

MELTING POINT:

The melting points of the compounds were determined by capillary tube method. The temperatures at which the synthesized compounds started losing its crystallinity were found and are presented uncorrected. It was observed that the compounds did not undergo decomposition during the process.

THIN LAYER CHROMATOGRAPHY:

Precoated aluminum TLC-GF binder was used. Solution of the reactants and products in ethanol were prepared. Various mobile phases were tried out of which methanol and chloroform was found to be suitable.

Stationary Phase: Precoated silica gel GF plates

INFRARED ABSORPTION SPECTROSCOPY[115]

Infra red spectroscopy is a valuable tool for determination of organic structure. Most important advantage of infra red spectroscopy over other usual methods of structural analysis (X-ray diffraction, ESR, etc.,) is that it serves as a introduction tool to ascertain the presence and absence of functional group.

NMR SPECTRA[116]

In NMR spectroscopy radiofrequency waves induces transitions between magnetic energy levels of nuclei of a molecule. NMR enables us to study the number of equivalent protons and their electronic environment. It reveals the different chemical environment in which the proton is present and helps us to ascertain the structure of molecules. The splitting of the signal is due to the different environment of the absorbing proton not with respect to electrons but with respect to the nearby protons.

MASS SPECTROSCOPY[117]

It is an analytical technique used to establish the molecular structure and molecular weight of the analyte under investigation. In mass spectroscopy, the compound under investigation is bombarded with a beam of electrons producing ionic fragments of the original species. The relative abundance of the fragment ion formed depends on the stability of the ion and of the lost radical. The resulting charged particles are then separated according to their masses. Mass spectrum is a record of information regarding various masses produced and their relative abundances

IV.4 BIOLOGICAL EVALUATION

IV.4.a ACUTE TOXICITY STUDY

This acute toxicity study was designed as per the OECD Guideline for Testing of Chemicals, Acute Oral Toxicity (Acute Toxic Class Method), Guideline 423.

Principle and Purpose:

Acute toxicity testing determines the toxicity of a chemical or drug substances after a single administration.The main purpose of acute toxicity studies is to evaluate the degree of toxicity in a quantitative and qualitative manner with the purpose of comparing it with other drug substances (e.g. other drug candidates for the same indication). Further, acute toxicity testing provides information about the acute toxicity effects of a chemical in a quantitative manner i.e. it generates information about mechanism of acute toxicity.

The method of determination has changed in the last three decades mainly for animal welfare reasons. Producing mortality in animals in order to determine LD50 (dosis letalis media) is no longer the main purpose of acute toxicity testing. Today, acute toxicity focuses on levels of acute tolerance, nature of acute toxicity symptoms in the sub-lethal range, and dose levels which cause mortality in few animals i.e. quality has replaced quantity.

The test is based on stepwise procedure with use of minimum number of animals per step; sufficient information is obtained on the acute toxicity of the test substance to enable its classification.

The substance is administered orally to a group of experimental animals at one of the defined dose. The substance is tested sing a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e;

- No further testing is needed.

- Dosing of three additional animals, with the same dose
- Dosing of three additional animals at the next higher or the next lower dose levels.

Experimental Protocol:

In the present study the oral toxicity of the synthesized compounds were performed by acute toxic class method. In this methods the toxicity of the synthesized compounds were tested using a procedure, using three mice of a single sex. The various concentration of a test drug as per OECD guidelines are as follows:

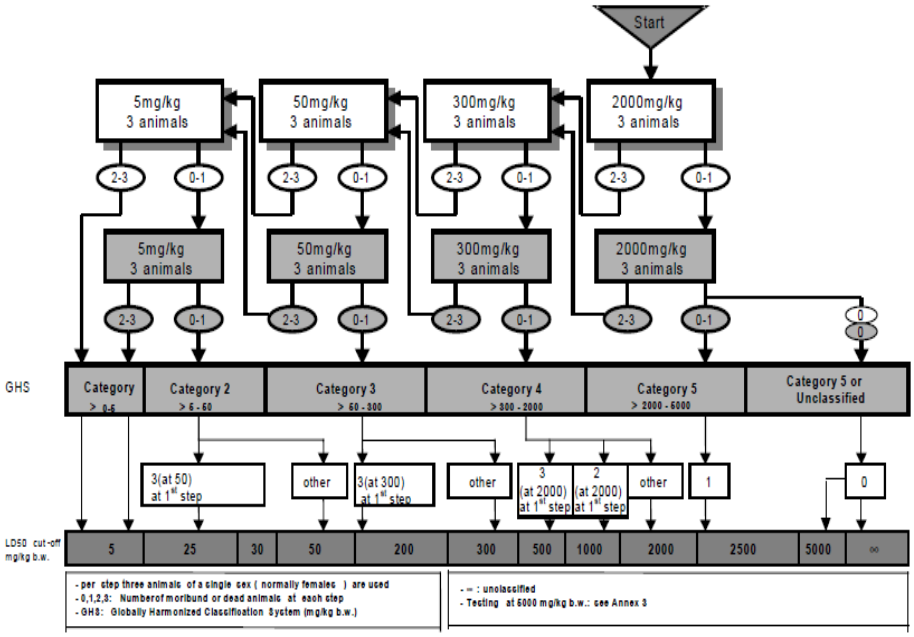
Group I : Synthesized compound – 5mg

Group II: Synthesized compound – 10mg

Group III: Synthesized compound – 300mg

Group IV: Synthesized compound – 2000mg

The mice is fasted prior to drug administration (food alone withheld not water) overnight. After the fasting the animal is weighed and the synthesized compounds were administered orally at a dose of 2000mg/kg body weight. Animals were observed individually after dosing during the first 30 mins; periodically during the first 24 hours with special attention given during the first 24 hours with special attention given during the first 4 hours and daily thereafter for a total of 14 days.As no mortality was observed with the above dose, a series of doses 250 and 500mg were selected for further pharmacological evaluation. The procedure with a starting dose of 2000mg/kg body weight as per OECD Guidelines is as follows:



FigIV.13: Dosing procedure of OECD Guidelines

IV.4.b IN VITRO ACTIVITY

As per literature [119] the expression of Pim1 was found to be in human colorectal cancer cell line (HCT116) cell lines so the synthesized compound in vitro activity was performed on the colorectal cancer cell lines.

IV.4.b.i PROCEDURE INVOLVED

The human colorectal carcinoma cell line (HCT116) was obtained from National Centre for Cell Science (NCCS), Pune, and grown in Dulbeccos Modified Eagles Medium (DMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure[118]

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium with 5% FBS to give final density of $1x10^5$ cells/ml. one hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the extracts and fractions. They were initially dissolved in neat dimethylsulfoxide (DMSO) and further diluted in serum free medium to produce five concentrations. One hundred microlitres per well of each concentration was added to plates to obtain final concentrations of 100, 10, 1.0 and 0.1 µM. The final volume in each well was 200 µl and the plates were incubated at

37^0C, 5% CO_2, 95% air and 100% relative humidity for 48h. The medium containing without samples were served as control. Triplicate was maintained for all concentrations.

MTT assay

MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula and graph was plotted between % Cell inhibition and concentration and from this IC50 was calculated.

% cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Result and Discussion

V.1. DRUG DESIGN

V.1.a.PHARMACOPHORE MODELING

The best hiphop Pharmacophore model indicated the importance of acceptors, ring aromatic, hydrophobic aliphatic, hydrophobic aromatic and hydrophobic features which were further, confirmed in the hypogen generated models.

In the hypogen studies 10 hypotheses were generated using the most diverse 24 molecules from the training set and validated using 91 test molecules. The best 10 hypothesis consists of two hydrogen bond acceptor, one hydrogen bond donor and one hydrophobic feature, their relative distances, angle and other geometric parameters. The cost values, correlation and root mean square deviation values are given in table. The best hypothesis is characterized by the highest cost difference (56.342) lowest RMS error (1.1160) and with correlation (0.88). The fixed cost and null cost are 117.449, 173.791. It is evident that as error, weight and configuration component are very low, the total Pharmacophore cost is also low, the total Pharmacophore cost is also low and close to the fixed cost. Also, as total cost is less than the null cost, this model accounts for all the Pharmacophore features and has a good predictability power. Figure V.1 shows the best Pharmacophore model aligned with the most active and inactive fig V.2 and V.3 represent the best Pharmacophore model aligned with the most active and inactive molecules 53 and 91 with IC50 of 0.001 and 107 respectively. In the fig.V.2 the Pharmacophore features are mapped well to the active molecules. On the other hand in fig.V.3 the features of the Hydrogen bond donor could not fit well since it is a low active molecule.

Table V.110 Pharmacophore models generated by the HypoGen for pim-1 inhibitors

Hypothesis	Total cost	Correlation	RMSD	Δcost	error	features
1	117.449	0.88652	1.1160	56.342	95.673	2HBA,HBD,HY
2	120.871	0.85746	1.2397	52.920	99.169	2HBA,HBD,HY
3	121.194	0.84753	1.2745	52.597	100.207	2HBA,HBD,HY
4	123.076	0.72759	1.6459	50.715	113.236	2HBA,HBD,HY
5	125.237	0.71593	1.6751	48.554	114.398	2HBA,HBD,HY
6	128.936	0.70900	1.6949	44.855	115.080	2HBA,HBD,HY
7	129.403	0.69560	1.7241	44.388	116.399	2HBA,HBD,HY
8	130.066	0.67955	1.7609	43.725	117.938	2HBA,HBD,HY
9	130.406	0.67388	1.7735	43.385	118.449	2HBA,HBD,HY
10	132.659	0.66926	1.7825	41.132	118.856	3HBA,HBD,HY

* (Null cost -total cost), null cost -173.791, fixed cost- 117.449, for theHypo1

weight -1.84, configuration – 19.6775. All cost units are in bits.

* A – hydrogen bond acceptor, D – hydrogen bond donor and HY – hydrophobic

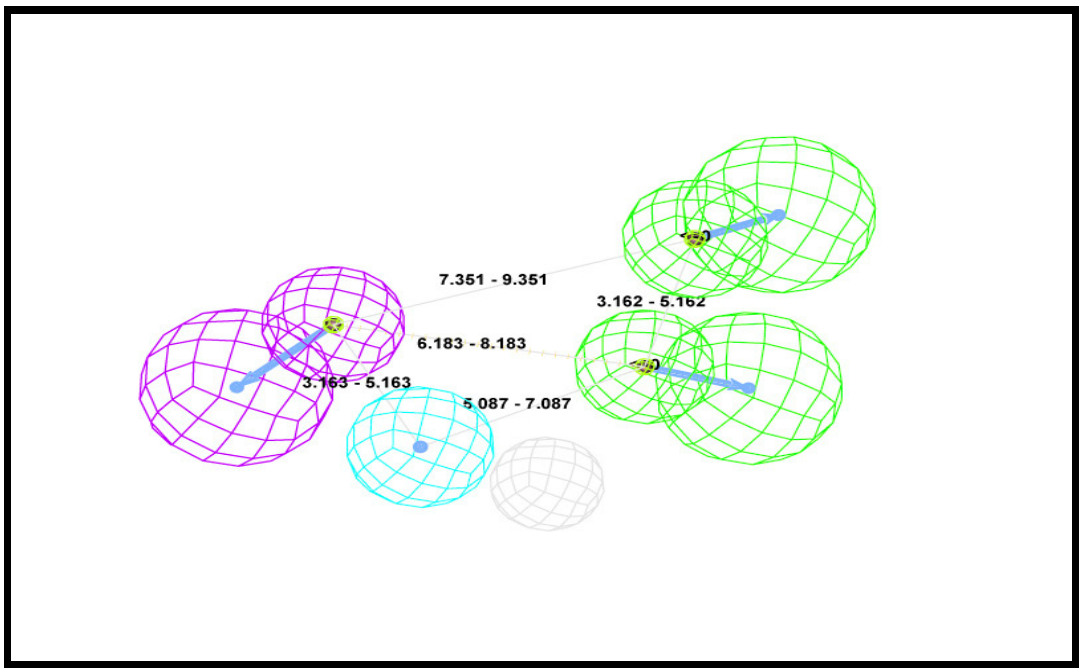


Fig.V.1 HypoGen features with its distance constraints

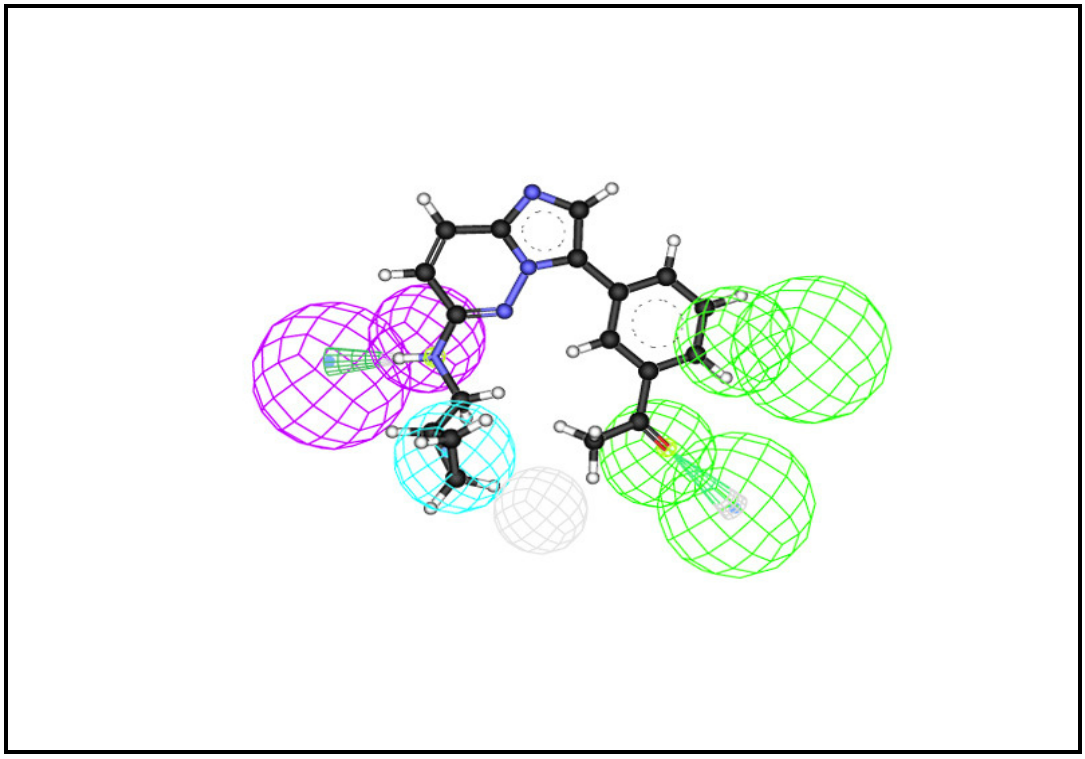


Fig.V.2 Pharmacophore mapped to the most active molecule

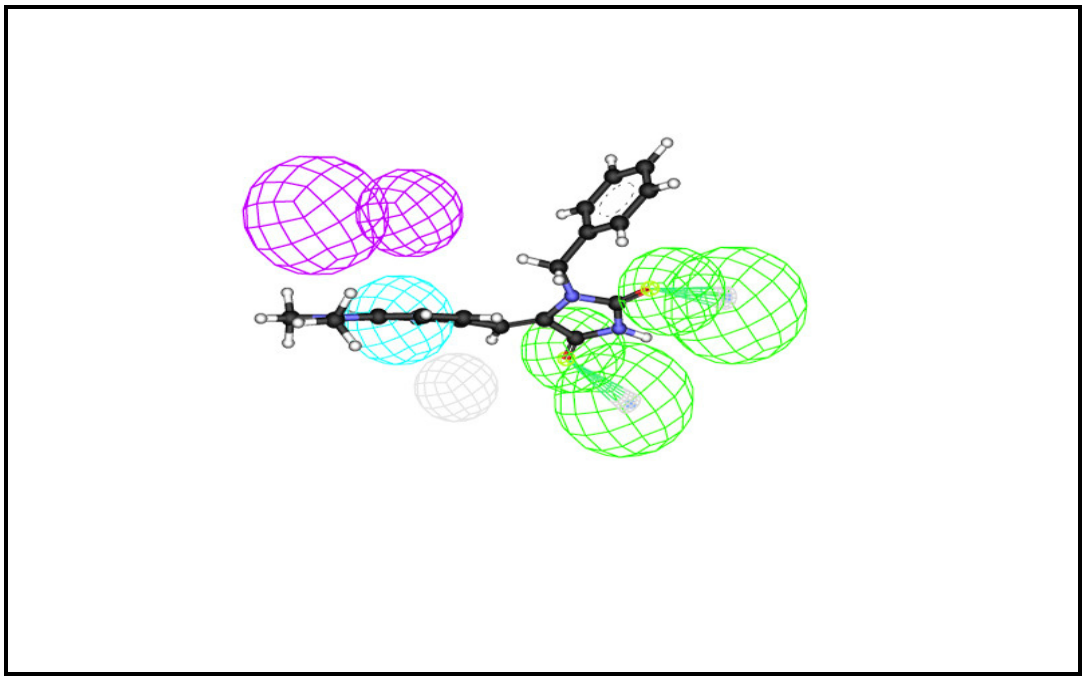


Fig.V.3 Pharmacophore mapped to the low-active molecule

Table.V.2 Experimental and predicted IC50 data of 25 training set molecules against HypoGen model.

Compound name	Exp.ic50	Predicted.ic50	Error	Fit value	Experimental cale	Predicted scale
1	0.001	0.0021	2.1	9.25	+++	+++
2	0.061	0.54	8.8	6.84	+++	+++
3	0.130	1	7.9	6.56	+++	++
4	0.280	0.45	1.6	6.92	+++	+++
5	0.430	2	4.5	6.28	+++	++
6	0.6	1	1.7	6.56	+++	++
7	0.87	1.6	1.8	6.37	+++	++
8	0.99	1.2	1.2	6.50	+++	++
9	1	1.3	1.2	6.47	++	++
10	1.6	3.1	1.9	6.08	++	++
11	3.5	1.3	3.7	5.46	++	+
12	5.1	2.2	-2.3	6.22	++	++
13	6	2.4	-2.5	6.19	++	++
14	7.5	2	-3.8	6.28	++	++
15	8	1.6	-4.9	6.36	++	++
16	10	2.5	-4.1	6.18	++	++
17	11	2.4	-4.4	6.19	+	++
18	19	3.6	-5.2	6.01	+	++
19	25	130	5.3	4.45	+	+
20	38	54	1.4	4.84	+	+
21	52	54	-9.7	5.84	+	++
22	69	62	-1.1	4.77	+	+
23	78	61	-1.3	4.78	+	+
24	110	59	-1.8	4.80	+	+

a .+ indicates that the predicted IC50 is higher than the experimental IC50;

-indicates that the predicted IC50 is lower than the experimental IC50; a value of 1 indicates that the predicted IC50 is equal to the experimental IC5

b. Activity scale – IC50 < 0.1 µM-+++ (highly active) – IC50 1–10 µM-++ (moderately active) – IC50 > 10 µM-+ (low active).

In the training set, out of eight highly active molecules three are correctly predicted as highly active and rest are predicted as moderately active, out of eight medium active molecules seven were predicted as moderately active, and in the eight inactive molecules, for predicted to be three moderatively active rest were predicted as low

active as shown in table.V.2 was inferred that there is less difference between the experimental and the predicted IC50 values and thus the error values are less and the fit value are less and the fit values give a good measure of how precisely the defined features in the best Pharmacophore fit well with each molecules.

Model validation and knowledge based screening

The validity of any Pharmacophore model needs to be ascertained by screening some known inhibitors (test set) in order to check how many active molecules are picked in the screening process, how their predicted activities are correlated with the experimental activities. The best hypothesis was used to screen the 91 pim1 inhibitors of the test set which contain high, medium and low active inhibitors, the result gave a correlation value of 0.887. Some of the tests set molecules with the experimental values are shown in figure V.4

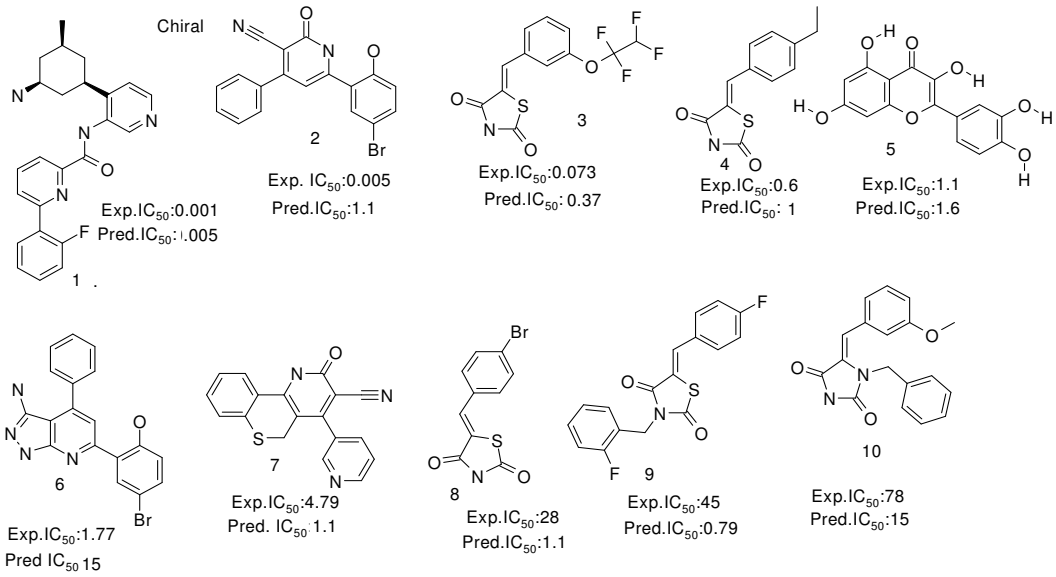


Fig V.4 Some test set molecules for pim-1 inhibitors along with its experimental and Pharmacophore predicted IC50 values.

Knowledge based screening

A total of 1000 molecules were generated based on the knowledge of binding interaction of ligands with the protein and also the common features necessary for the biological activity of molecules. These molecules were drawn using cerius software and were screened for their activities using the developed Pharmacophore models. For the final screening of the compounds, the hits were analyzed in the Pharmacophore model and the most active hits were identified and their structure are shown in fig. glide dock studies were conducted against pim1 inhibitors and designed active molecules against the x-ray structure of pim1

V.1.b DOCKING STUDY

As the docking study gives the conformational sample of molecule which would bind to the particular protein giving rise to useful information's aiding our possibilities of finding a candidate molecule which could be used for the treatment of diseases. So before docking the following steps should be performed as to ensure that the testing of each molecule mimics the real binding happening in the body.

SELECTION AND INVESTIGATION OF PROTEINS

The Pdb downloaded from the protein databank have the following ID PIM1=3R04.The Investigation of protein PDB is very important so as to attain good computational results so the tools for analyzing are resolution, R Value, R Free, Ramachandran plot. These values were found to be range and the results had been shown below

RESOLUTION

Resolution is a measure of the quality of the data that has been collected on the crystal containing the protein or nucleic acid. (Level of details present in the different ion pattern, level of electron density map is calculated)

Resolution values of the receptors are as following PIM1-1.70

Resolution should be less than 2.0.

R VALUE AND R FREE

R value is the quality of the atomic model obtained from the crystallographic data of particular.

Receptor/pdb id	**R Value**	**R Free**
PIM1 3R04	0.179	0.206

And the values of PIM1 R value seems to be close 0 and the value of R free is also close to R value of their receptors

RAMACHANDRAN PLOT

Ramachandran plot is defined as the phi and psi rotation of the adjacent atoms to locate its allowed region, disallowed region etc to know the stability of the protein. Since most of the residues available were used in the Pdb of Pim1 is having more residues in the allowed region and very less residues in the partially allowed region and very little residues in the disallowed region. So the proteins are very good to be used shown in figure V.5.

PROTEIN PREPARATION

A typical PDB structure file consists of heavy atoms, can contain waters, cofactors, and metal ions, and can be multimeric. The structure generally has no information on bond orders, topologies, or formal atomic charges. Terminal amide groups can also be misaligned, because the X-ray structure analysis cannot usually distinguish between O and NH2. Ionization and tautomeric states are also generally unassigned.

Pim1

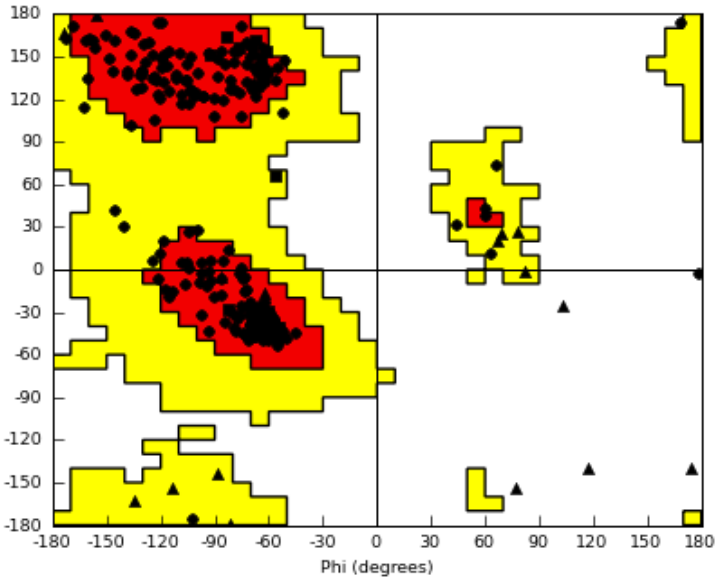


Fig V.5 Ramachandran plot of Pim1 receptor pdb file (3R04)

Partially allowed region Allowed region Disallowed region

. Glide calculations use an all-atom force field for accurate energy evaluation. Thus, Glide requires bond orders and ionization states to be properly assigned and performs better when side chains are reoriented when necessary and steric clashes are relieved. Protein preparation wizard panel is to locate and fix structural defects and prepare them for use.

RECEPTOR GRID GENERATION

Glide searches for favorable interactions between one or more ligand molecules and a receptor molecule, usually a protein. The shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. For receptors that adopt more than one

conformation on binding, you might want to prepare grids for each conformation, to ensure that possible actives are not missed. Glide can, however, handle different hydroxyl conformations with a single grid generation.

Pim1 receptor grid was identified by using this method.

X= 23.2831Å Y= -36.1077Å Z=2.1563Å

As the identification of the receptor site of Pim1 grid region has been defined which comes as a grid output Zip file in their respective names were generated which were used for the docking against the respective set of ligands.

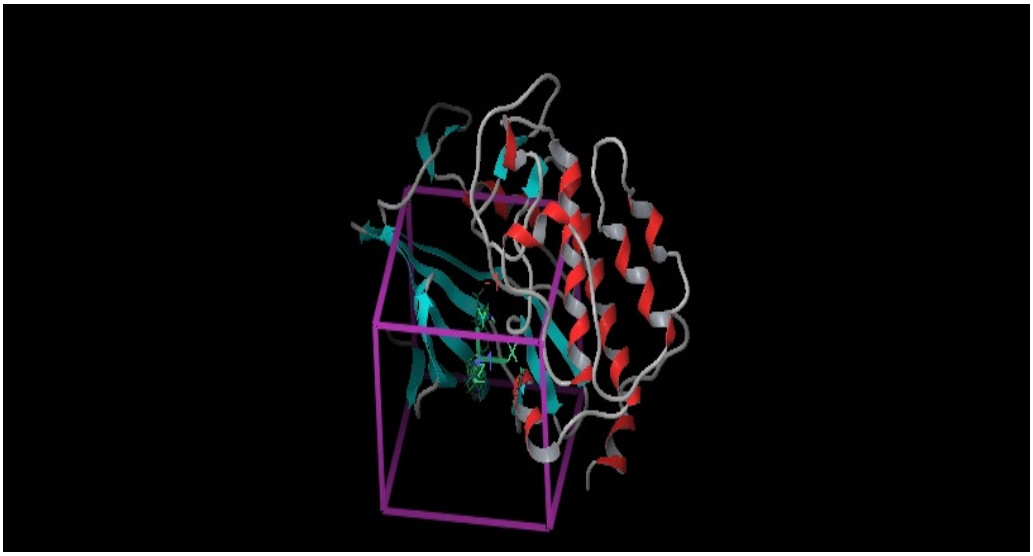


Fig V.6 Receptor grid image of Pim1 receptor (3R04)

LIGAND DATABASE PREPARATION

Ligand database is the large collection of large molecules which could possibly contain the basic nucleus and large variety of functional groups. These molecules were sketched in the Maestro 9.1® by the build tool.

Aromatic or Heterocyclic Nucleus and fragments significance

- Play vital role in the *steric occupancy* in the receptor site.
- *Hydrophilic or Hydrophobic* region mapping necessary for binding against the particular receptors.
- It also play important role in forming Hydrogen bonding by acting as an *H bond donor* or *H bond acceptor.*
- *Positively or negatively ionizable* groups increase the affinity of the ligand with the receptor for evoking the desired pharmacological action.

Toxicity filter significance

- Usually drugs have chances of producing toxicity so to reduce the chances of toxicity we had avoided the functional groups listed in the toxicity filters in the fig Molecules in the database need to be prepared so it was preceded to the next step.

LIGAND PREPARATION

The Schrödinger ligand preparation product LigPrep is designed to prepare high quality, all atom 3D structures for large numbers of drug-like molecules, starting with 2D or 3D structures in SD, Maestro, or SMILES format.

To give the best results, the structures that were docked must have good representations of the actual ligand structures as they would appear in a protein-ligand complex. This means that the structures supplied to glide must have three-dimensional (3D) structures, realistic bond lengths and bond angles. modified Torsional internal coordinates of the ligand during docking, so the rest of the geometric parameters must be optimized beforehand, must consist of a single molecule that has no covalent bonds

to the receptor, with no accompanying fragments, such as counter ions and solvent molecules, must have all their hydrogen's (filled valences).should have appropriate protonation state for physiological pH values etc so the ligands in the database were processed by ligprep.

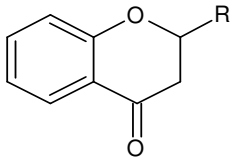
LIGAND RECEPTOR DOCKING AND PREDICTING ACTIVITY

The molecules in the database consist of 1000 molecules and which was prepared in the earlier steps. Than the molecules were investigated for drug likeness by using Qikprop than 960 molecules was filtering and these molecules were docked against Pim1. Top 100 selected were preceded for predicting the activity by using the 3D QSAR established in the Pharmacophore modeling. Top 35 Molecules which showed very good docking score and predicted activity against Pim1 were selected to identify the scaffold.

SCAFFOLD IDENTIFICATION

After investigating the results of docking study and predicted activity

Top 35 molecules were selected and then the common scaffold was identified.



R -$C_6H_5N(CH_3)_2$
R -$C_6H_5NO_2$
R -C_6H_5Cl
R - $C_6H_5CH=CH$

Fig V.8 Scaffold identified from the screening The literature review in NISCAIR indicted that no work had been done using this molecules.

Top molecules identified with the predicted activity and docking score of Pim1

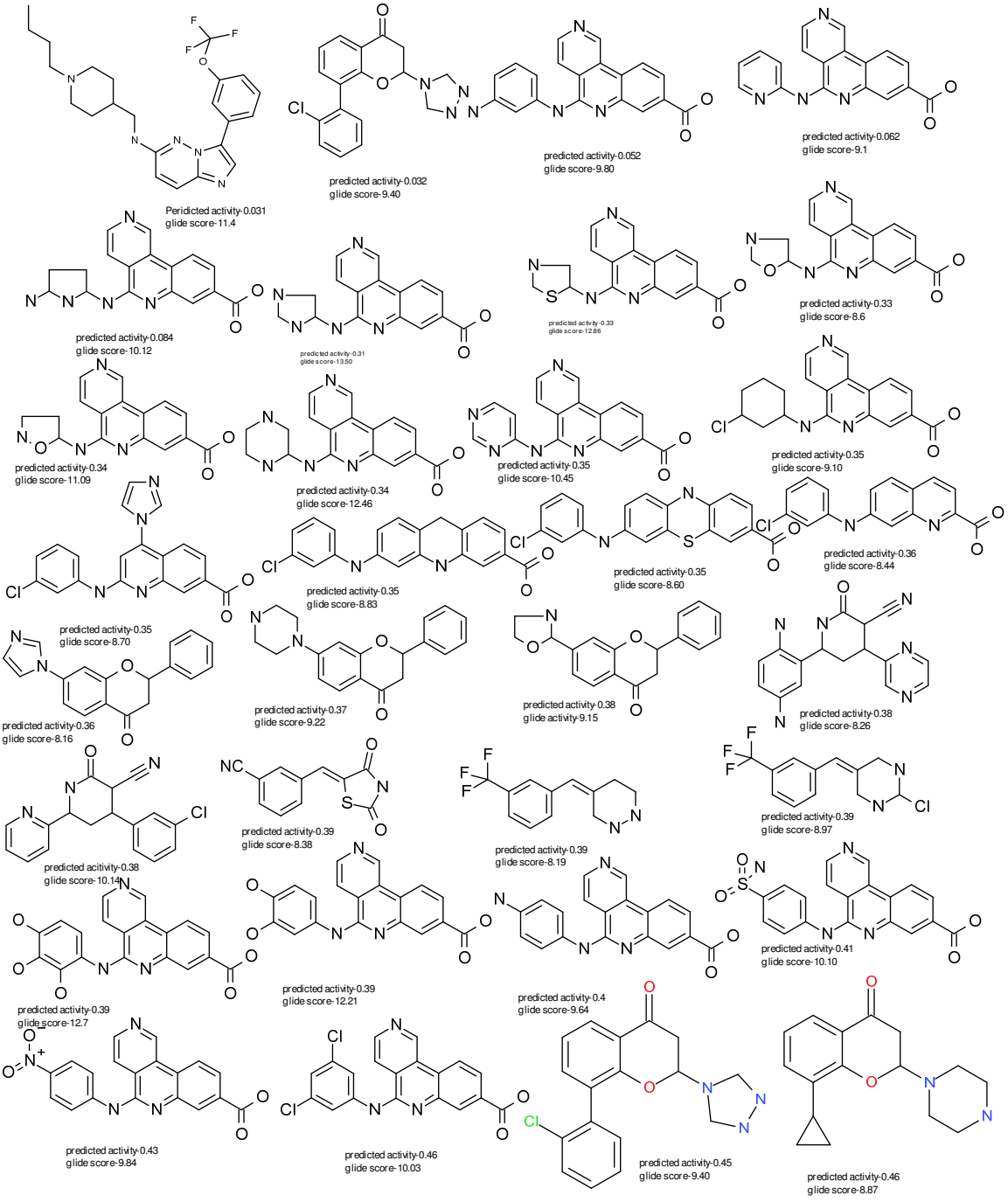


Fig V.7 Molecules list which had been obtained from screening with their Pim1 docking and predicted activity score.

DOCKING OF MOLECULES TO BE SYNTHESIZED

Above molecules identified from the scaffolds were docked against Pim 1 and its interaction at the particular site were identified

PV1

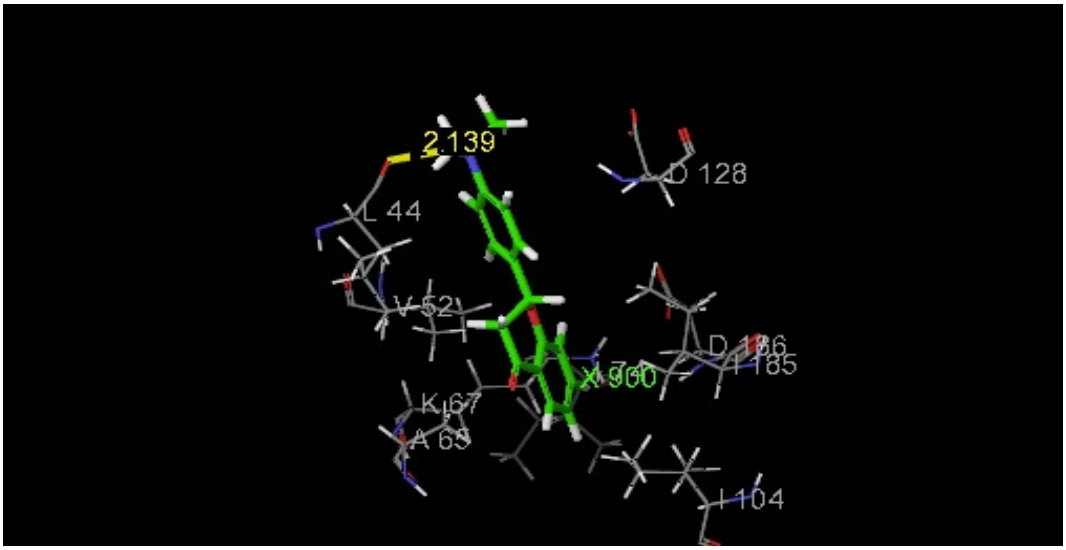


Fig V.9 PV1 ligand been docked in the Pim1 receptor

PV2

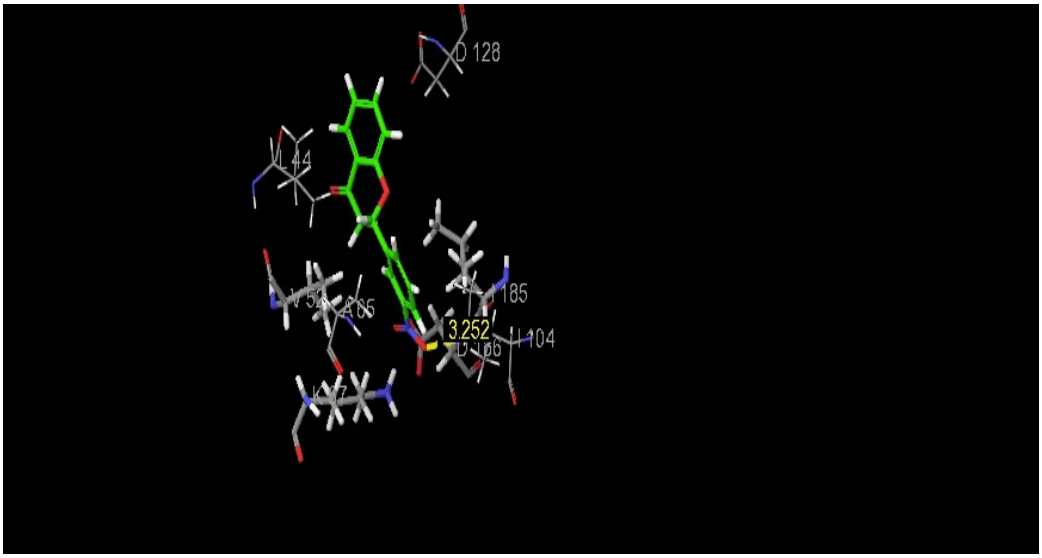


Fig V.10 PV2 ligand been docked in the Pim1 receptor

PV3

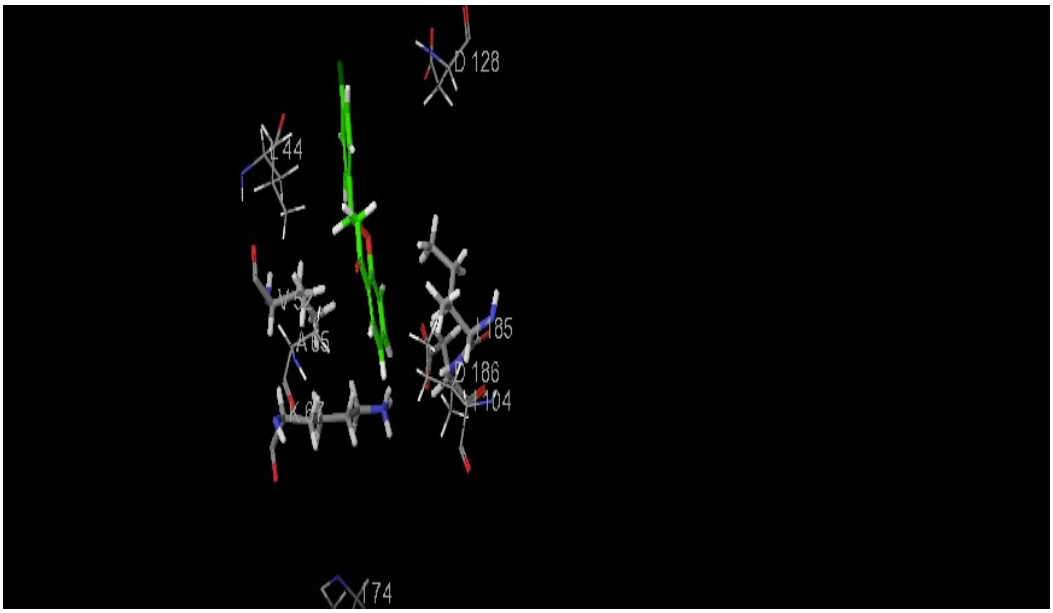


Fig V.11 PV3 ligand been docked in the Pim1 receptor

PV4

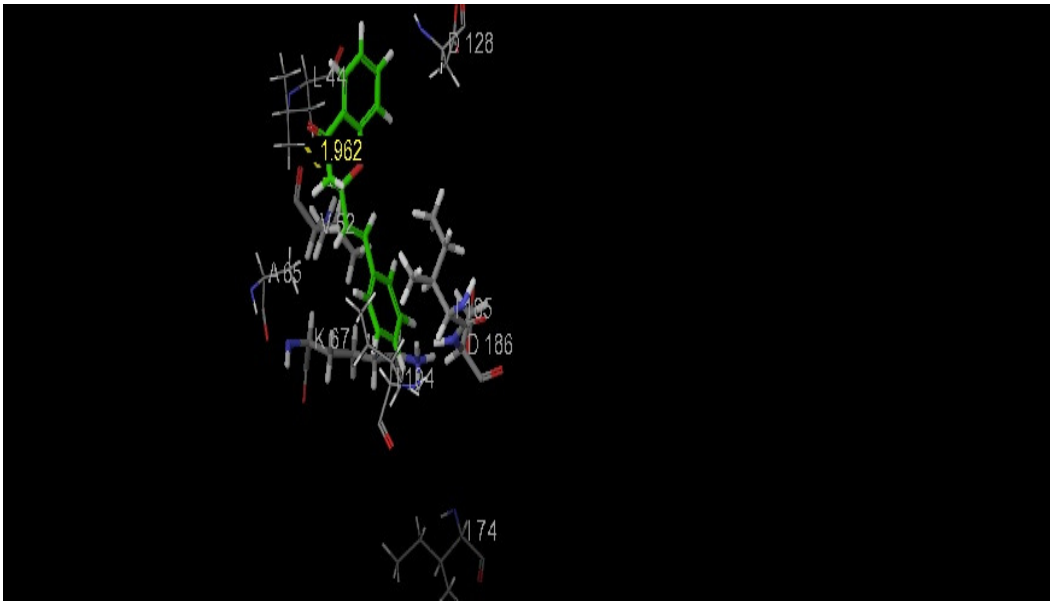


Fig V.12 ligand been docked in the Pim1 receptor

XP DOCKING RESULTS OF MOLECULES DOCKED AGAINST Pim1

Table.V.3 Pim 1 XP docking results

ligand	GScore	LipophilicEvdW	PhobEn	PhobEnHB	PhobEnPairHB	HBond	Electro	Sitemap	LowMW	Penalties	HBPenal	PhobicPenel	RotPenal
Pv1	-9.424	-3.64	-1.2	0	0	-0.7	-1.84	-0.35	-0.5	0	0	0	0.10
Pv3	-8.37	-4.23	-1.35	0	0	-0.7	-0.09	-0.30	-0.5	0	0	0	0.11
Pv4	-8.84	-3.70	-1.3	0	0	-0.45	-0.06	-0.18	-0.5	0	0	0	0.23
Pv2	-7.63	-3.55	-1.2	0	0	-0.7	-0.431	0	-0.5	0	0	0	0.10

V.1.c.IN SILICO IVESTICATION OF DRUG LIKINESS

All the 50 characters calculating varies aspect of ADME was within the range assuring that 4 molecules will most probably be very comfortable passing the ADME.

Table.V.4

S.No	Property or descriptors	Pv1	Pv2	Pv3	Pv4	Range or recommended values
1	**#stars**	0	0	0	0	0 – 5
2	**#amine**	0	0	0	0	0 – 1
3	**#amidine**	0	0	0	0	0
4	**#acid**	0	0	0	0	0 – 1
5	**#amide**	0	0	0	0	0 – 1
6	**#rotor**	1	1	0	2	0 – 15
7	**#rtvFG**	0	0	0	0	0 – 2
8	**CNS**	0	1	1	0	–2 (inactive), +2 (active)
9	**mol_MW**	267.32	269.25	258.7	250.29	130.0 – 725.0
10	**SASA**	550.59	504.49	490.813	523.29	300.0 – 1000.0
11	**FOSA**	203.49	48.06	48.226	78.176	0.0 – 750.0
12	**FISA**	54.089	147.34	50.141	50.161	7.0 – 330.0
13	**PISA**	293	307.09	320.8	394.96	0.0 – 450.0
14	**WPSA**	0	0	71.64	0	0.0 – 175.0
15	**volume**	935.09	846.175	818.68	874.402	0.0 – 175.0
16	**donorHB**	0	0	0	0	0.0 – 6.0
17	**accptHB**	3.75	3.75	2.75	2.75	2.0 – 20.0
18	**dip^2/V**	0	0	0	0	0.0 – 0.13
19	**ACxDN^.5/SA**	0	0	0	0	0.0 – 0.05
20	**glob**	0.8398	0.875	0.861	0.845	0.75 – 0.95
21	**QPpolrz**	33.424	30.02	29.653	31.327	13.0 – 70.0
22	**QPlogPC16**	9.282	9.237	8.879	9.384	4.0 – 18.0
23	**QPlogPoct**	12.515	13.377	11.631	11.767	8.0 – 35.0
24	**QPlogPw**	6.878	7.511	6.157	6.274	4.0 – 45.0
25	**QPlogPo/w**	3.538	2.331	3.596	3.776	–2.0 – 6.5
26	**QPlogS**	-4.525	-3.651	-4.397	-4.368	–6.5 – 0.5
27	**CIQPlogS**	-3.84	-3.881	-4.121	-3.942	–6.5 – 0.5
28	**QPlogHERG**	-5.52	-5.31	-5.296	-5,923	concern below –5

S.No	Property or descriptors	Pv1	Pv2	Pv3	Pv4	Range or recommended values
29	**QPPCaco**	3040.69	396.817	3314.43	3313.014	<25 poor, >500 great
30	**QPlogBB**	-0.008	-0.832	0.289	-0.025	–3.0 – 1.2
31	**QPPMDCK**	1645.83	182.69	4459.9	1805.718	<25 poor,>500 great
32	**QPlogKp**	-1.38	-3.05	-1.314	-0.861	–8.0 – –1.0
33	**IP(ev)**	0	0	0	0	7.9 – 10.5
34	**EA(eV)**	0	0	0	0	–0.9 – 1.7
35	**#metab**	3	4	2	2	1 – 8
36	**QPlogKhsa**	0.287	0.029	0.258	-0.336	–1.5 – +1.5
37	**HumanOral Absorption**	3	3	3	3	1-3
38	**Percent Human-Oral Absorption**	100	87.106	100	100	>80% is high <25% is poor
40	**SAFluorine**	0	0	0	0	0.0 – 100.0
41	**SAamideO**	0	0	0	0	0.0 – 35.0
42	**PSA**	40.987	80.611	35.77	36.634	7.0 – 200.0
43	**#NandO**	3	5	2	2	2 – 15
44	**RuleOfFive**	0	0	0	0	maximum is 4
45	**RuleOfThree**	0	0	0	0	maximum is 3
46	**#ringatoms**	16	16	16	16	
47	**#in34**	0	0	0	0	
48	**#in56**	16	16	16	16	
49	**#noncon**	2	2	2	2	
50	**#nonHatm**	20	20	18	19	

V.2. SYNTHESIS

The compound has synthesized condensation followed by cyclization and characterized using following method.

Table.V.5.**PROPERTIES**

Properties	**PV1**	**PV2**	**PV3**	**PV4**
Molecular Formula	$C_{17}H_{17}NO_2$	$C_{15}H_{11}NO_4$	$C_{15}H_{11}ClO_2$	$C_{17}H_{14}O_2$
Molecular Weight	267.32	269.25	258.69	250.29
Molar Refractivity	78.92	71.15	69.50	77.41
Molar Volume	225.9	199.8	199.9	201.9
Parachor	594.5	546.9	526.9	557.5
Index of Refraction	1.615	1.630	1.611	1.692
Surface Tension	47.9	56.1	48.2	58.1
Density	1.182	1.347	1.293	1.239
Polarizability	31.28	28.20	27.55	30.68

IDENTIFICATION AND CHARACTERIZATION

Thin layer Chromatography

Stationary phase: recoated silica gel plate

Mobile Phase :

Chloroform: Ethanol (3:1) as developing Solvent system for compound-PV1

Ethanol: Water (3:1) as developing Solvent system for compounds- PV2.

Ethanol: Water (4:2) as developing Solvent system for compounds-PV3

DMSO: Ethanol (3:1) as developing solvent for compound-PV4.

Location of spots: UV chamber/and Iodine vapors in a tightly closed chamber.

CHARACTERIZATION

IR SPECTROSCOPY

IR spectra of synthesized compounds was showing presence of aromatic CH stretching vibration between 3100-3000 cm^{-1} and aromatic C=C stretching at 1450 cm^{-1}, The carbonyl keto group shows stretching vibration at the region of 1700-1600 for C=O. The aliphatic CH stretching at 2900-2950 cm^{-1},C-O-C at 1100-1200cm^{-1}.

MASS SPECTROSCOPY

All the synthesized compounds exhibited molecular ion peak (M+) of varying intensities ascertaining the molecular weights of the compounds

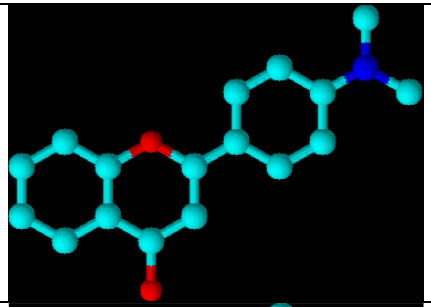
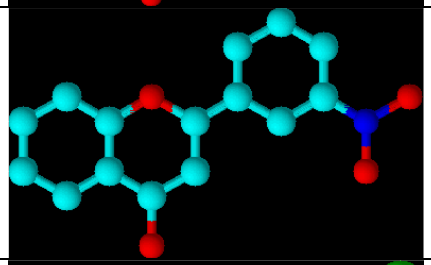
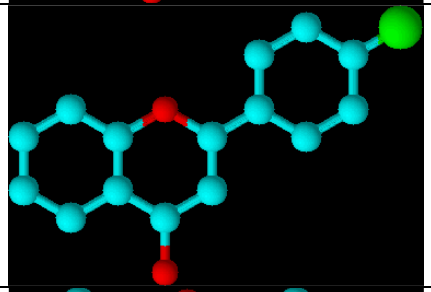
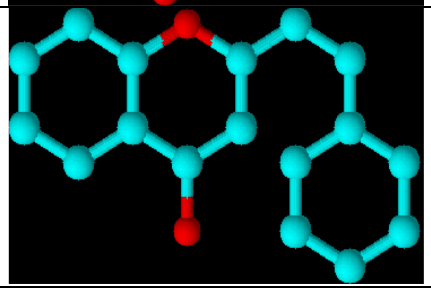
All the above spectral data confirmed the assigned structure of the synthesized compounds.

NMR SPECTROSCOPY

The ^{1}HNMR spectral data of all the synthesized compounds were in conformity with the structure assigned. Compounds showed peak between 3-3.4.2(δppm) which indicates the presence of CH_2 protons.

All the compounds showed multiplet signals for the presence of aromatic protons between 7.2-7.8 (δppm).PV1 shows peak at 2.8(δppm) which indicates the presence of CH_3 protons.

Table.V.6 CHARACTERIZATION AND IDENTIFICATION

S.NO	COMPOUNDS ID	3D STRUCTURE	MELTING POINT	MASS SPECTRA	NMR SPECTRA ppm	IR SPECTRA cm^{-1}
1	PV1		128 °C	267.028 M^+ ion peak	2.8(CH_3) 3.1(CH_2) 4.2(CH) 6.7–7.8 (Aromatic H)	1744(Keto) 3001(Ar- CH) 2901(Ali-CH) 1080(C-O-C) 1620(Ar-C=C)
2	PV2		98 °C	269.66 M^+ ion peak	3.1(CH_2) 4.2(CH) 6.7–7.8 (Aromatic H)	1744(Keto) 3001(Ar- CH) 2901(Ali-CH) 1080(C-O-C) 1620(Ar-C=C)
3	PV3		108 °C	258.27 M^+ ion peak	3.1(CH_2) 4.2(CH) 6.7–7.8 (Aromatic H)	1736(Keto) 3001(Ar- CH) 2908(Ali-CH) 1148(C-O-C) 1636(Ar-C=C)
4	PV4		95 °C	250.08 M^+ ion peak	3.1(CH_2),4.2(CH) 5.3(CH),6.0(CH) 6.7–7.8 (Aromatic H)	1744(Keto) 3001(Ar- CH) 2901(Ali-CH) 1080(C-O-C) 1620(Ar-C=C) 1450(Ali-C=C

PHYSICAL PROPERTIES

Table .V.7

S.NO	COMPOUND ID	MOLECULAR WEIGHT	MOLECULAR FORMULA	SOLUBILITY	APPEARANCE
1	**PV1**	267.32	$C_{17}H_{17}NO_2$	Soluble in Ethanol, chloroform	yellow color crystals
2	**PV2**	269.25	$C_{15}H_{11}NO_4$	Soluble in Ethanol, Dimethylsulphoxide	Light brown color crystals
3	**PV3**	258.69	$C_{15}H_{11}ClO_2$	Soluble in Ethanol, Dimethylsulphoxide	cream color oily crystals
4	**PV4**	250.29	$C_{17}H_{14}O_2$	Soluble in Ethanol, Dimethylsulphoxide	Light yellow color oily crystals

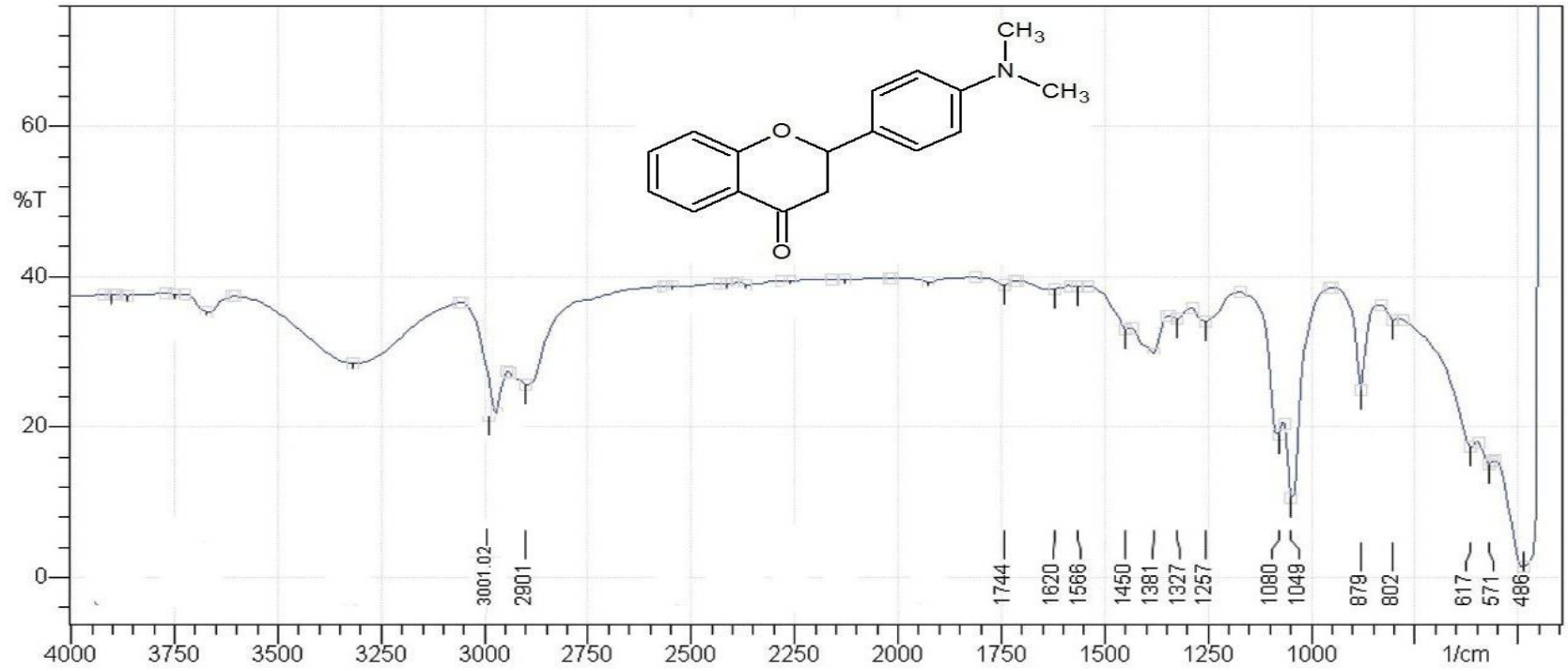


Fig.V.13 IR Spectrum of 2-[4-(dimethylamino)phenyl]-2,3-dihydro-4*H*-chromen-4-one (PV1)

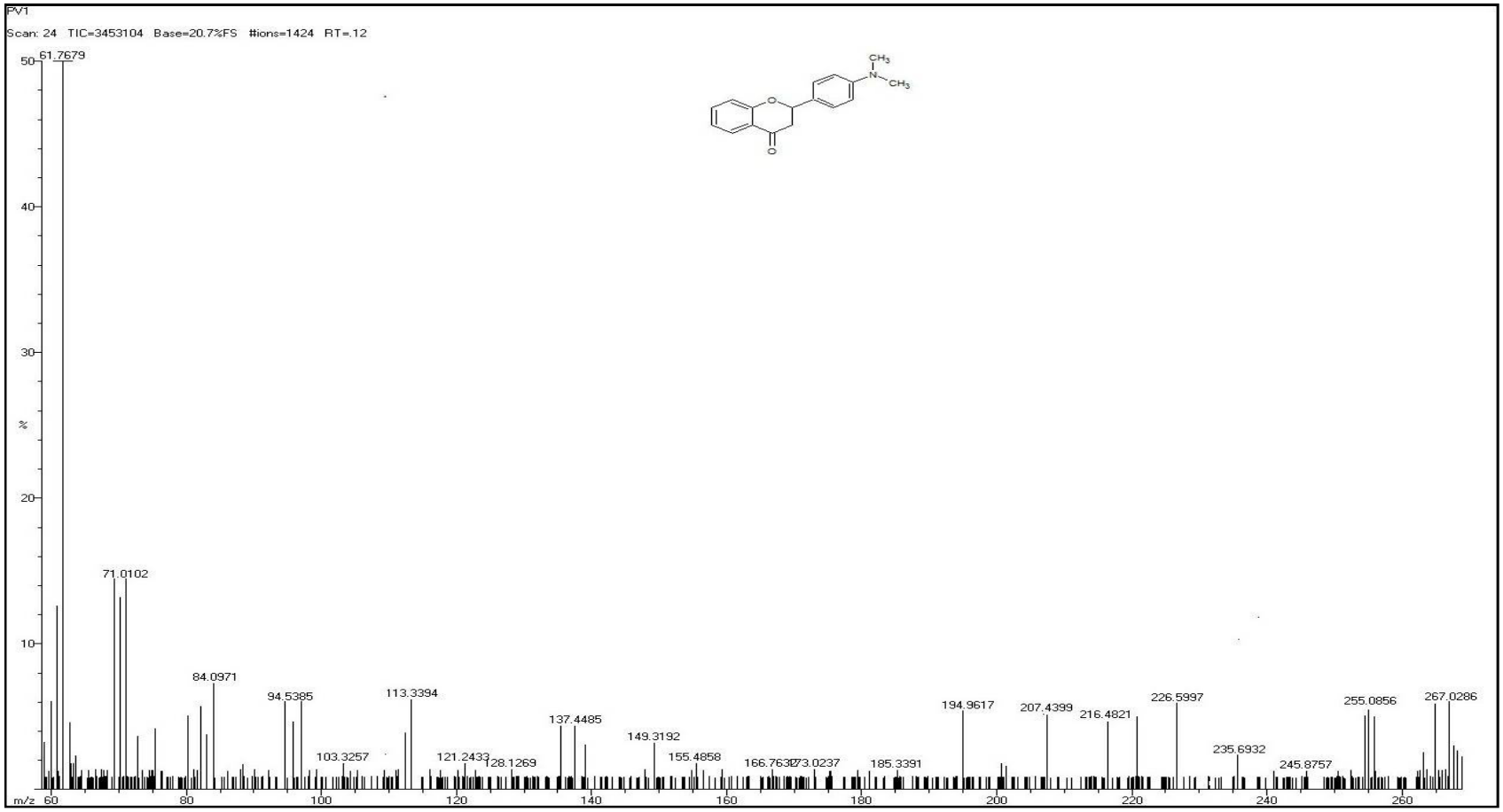


Fig.V.14 MASS Spectrum of 2-[4-(dimethylamino)phenyl]-2,3-dihydro-4*H*-chromen-4-one (PV1)

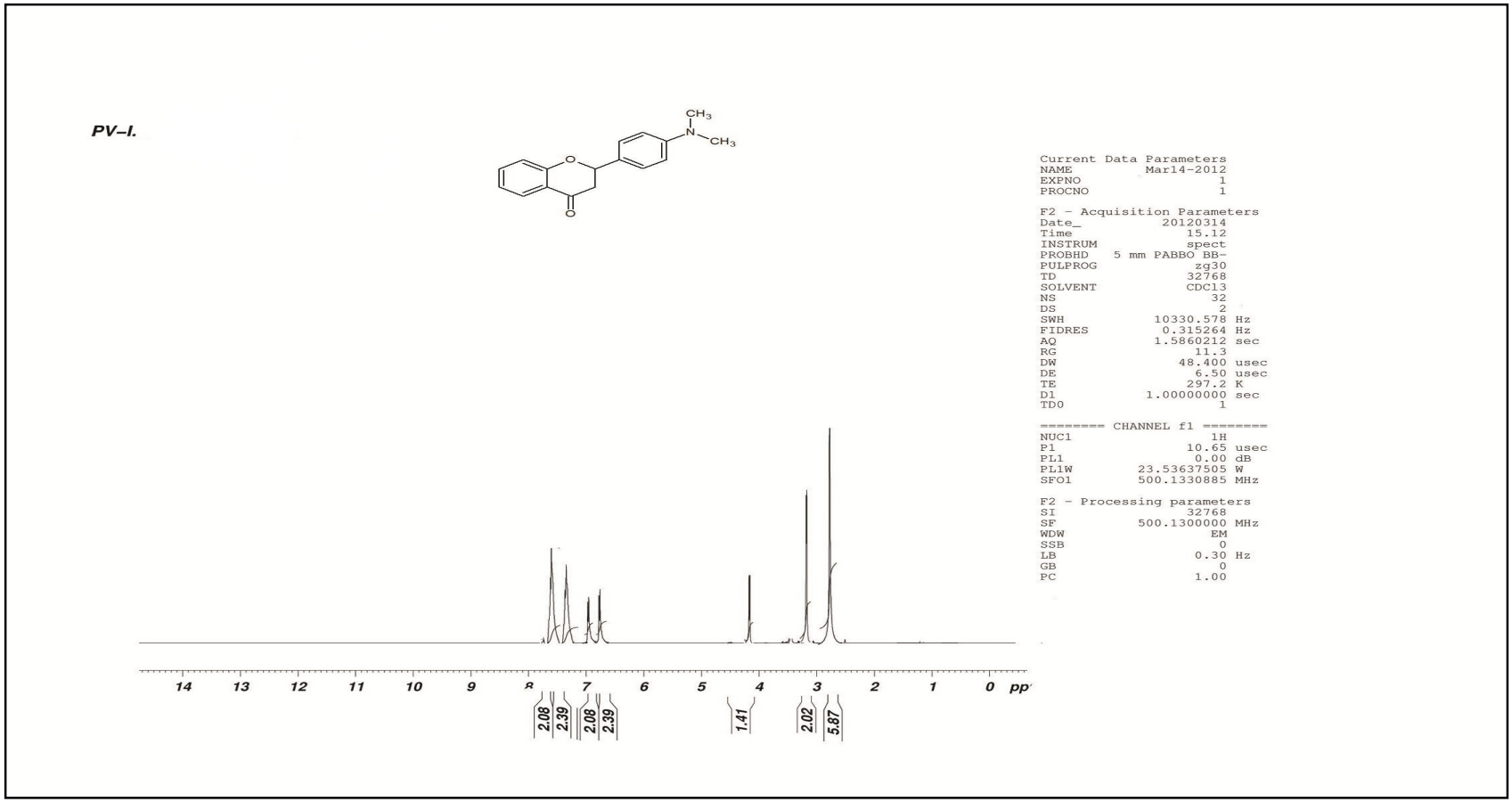


Fig.V.15 NMR Spectrum of 2-[4-(dimethylamino)phenyl]-2,3-dihydro-4*H*-chromen-4-one (PV1)

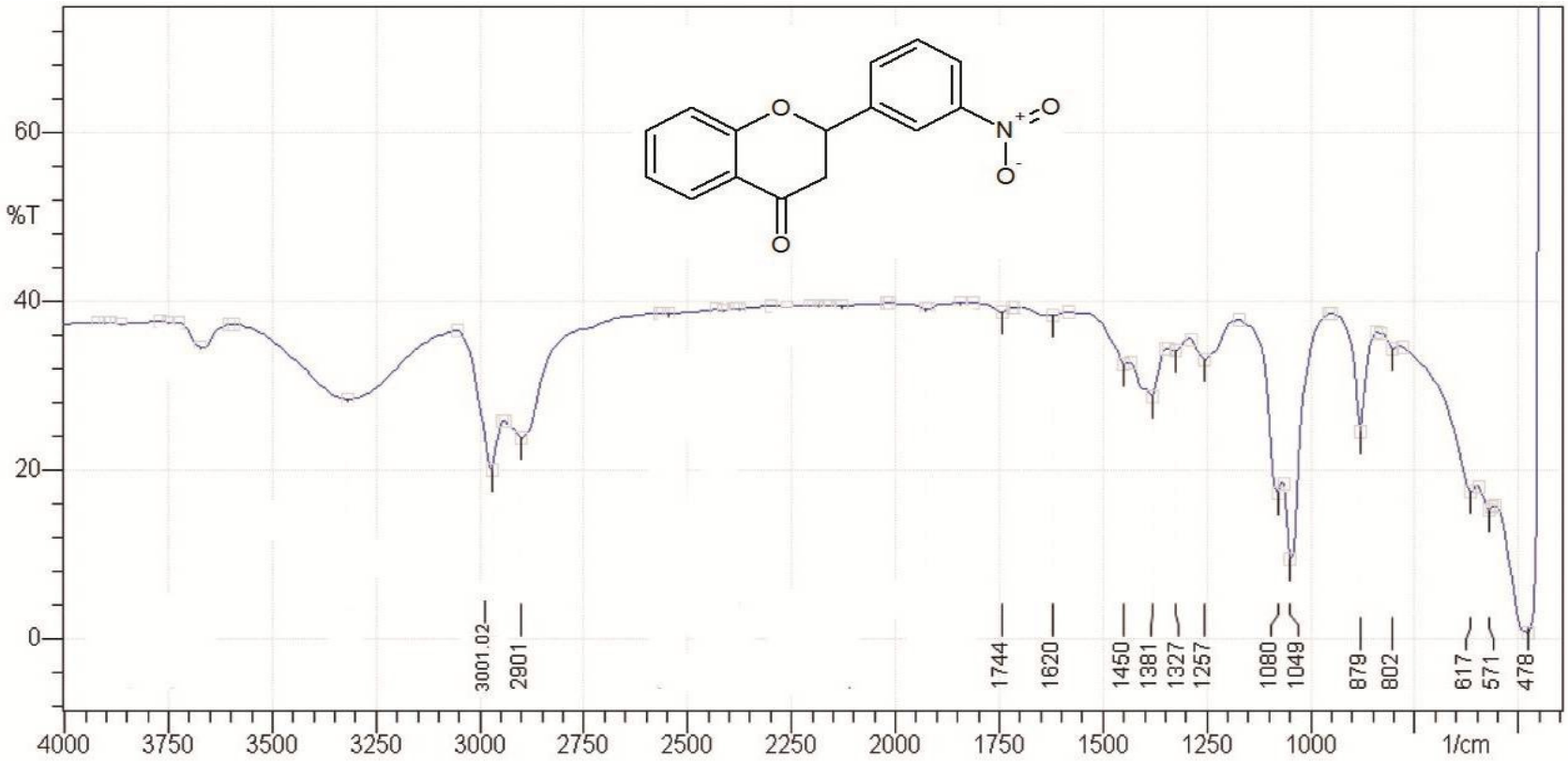


Fig.V.16 IR Spectrum of 2-(3-nitrophenyl)-2,3-dihydro-4*H*-chromen-4-one (PV2)

Fig.V.17 MASS Spectrum of 2-(3-nitrophenyl)-2,3-dihydro-4*H*-chromen-4-one (PV2)

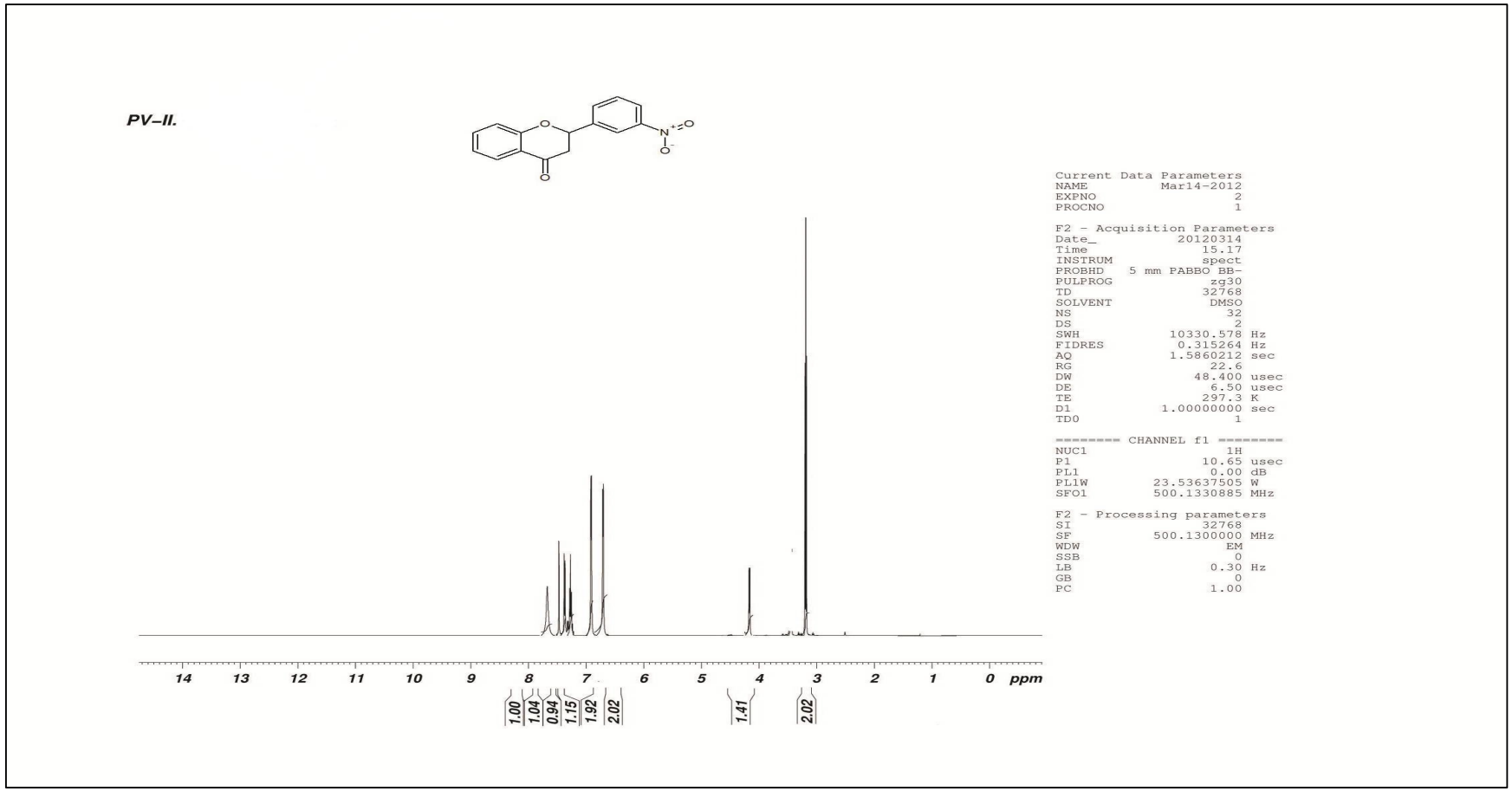


Fig.V.18 NMR Spectrum of 2-(3-nitrophenyl)-2,3-dihydro-4*H*-chromen-4-one (PV2)

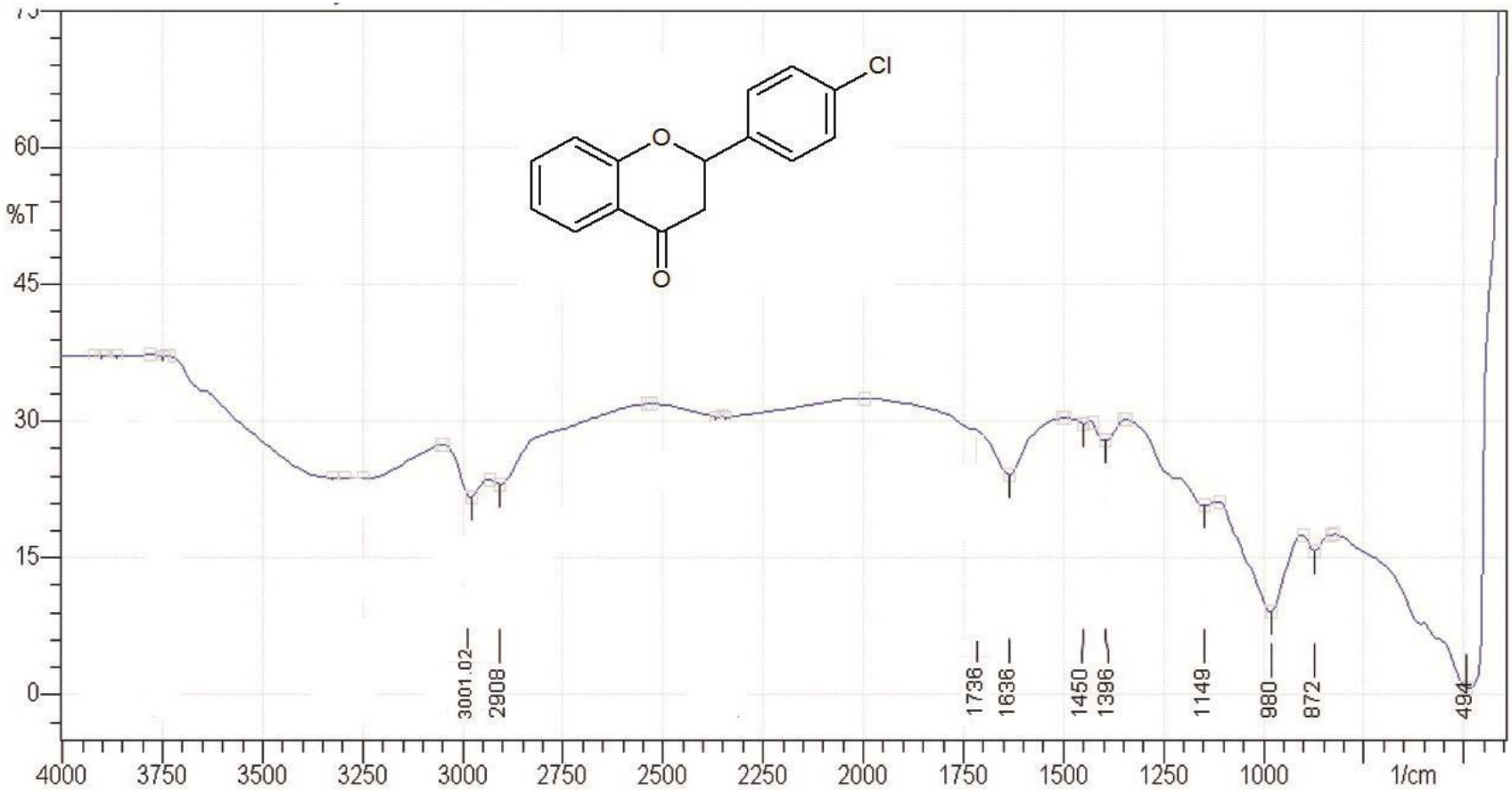


Fig.V.19 IR Spectrum of 2-(4-chlorophenyl)-2,3-dihydro-4*H*-chromen-4-one (PV3)

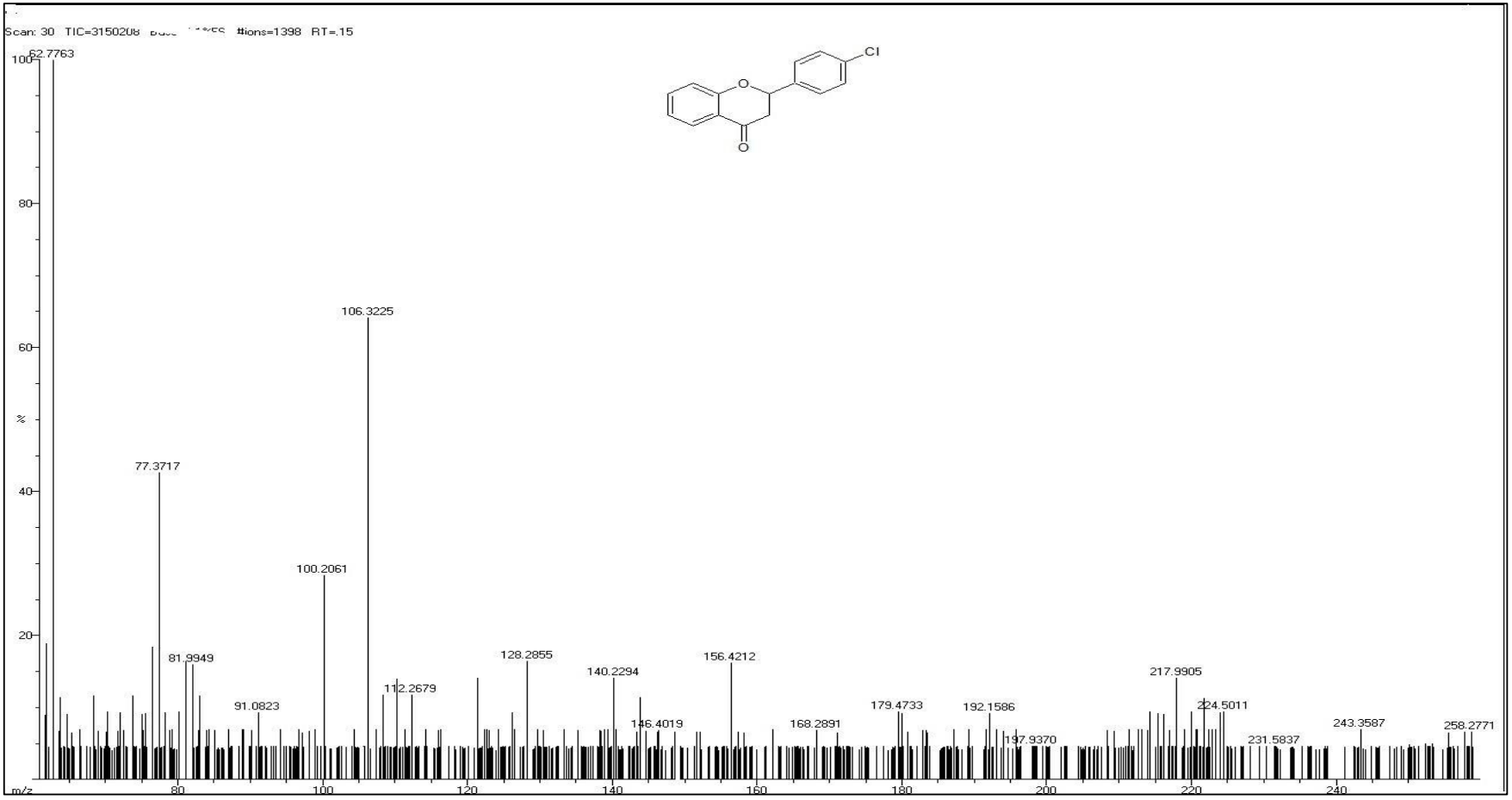


Fig.V.20 MASS Spectrum of 2-(4-chlorophenyl)-2,3-dihydro-4*H*-chromen-4-one (PV3)

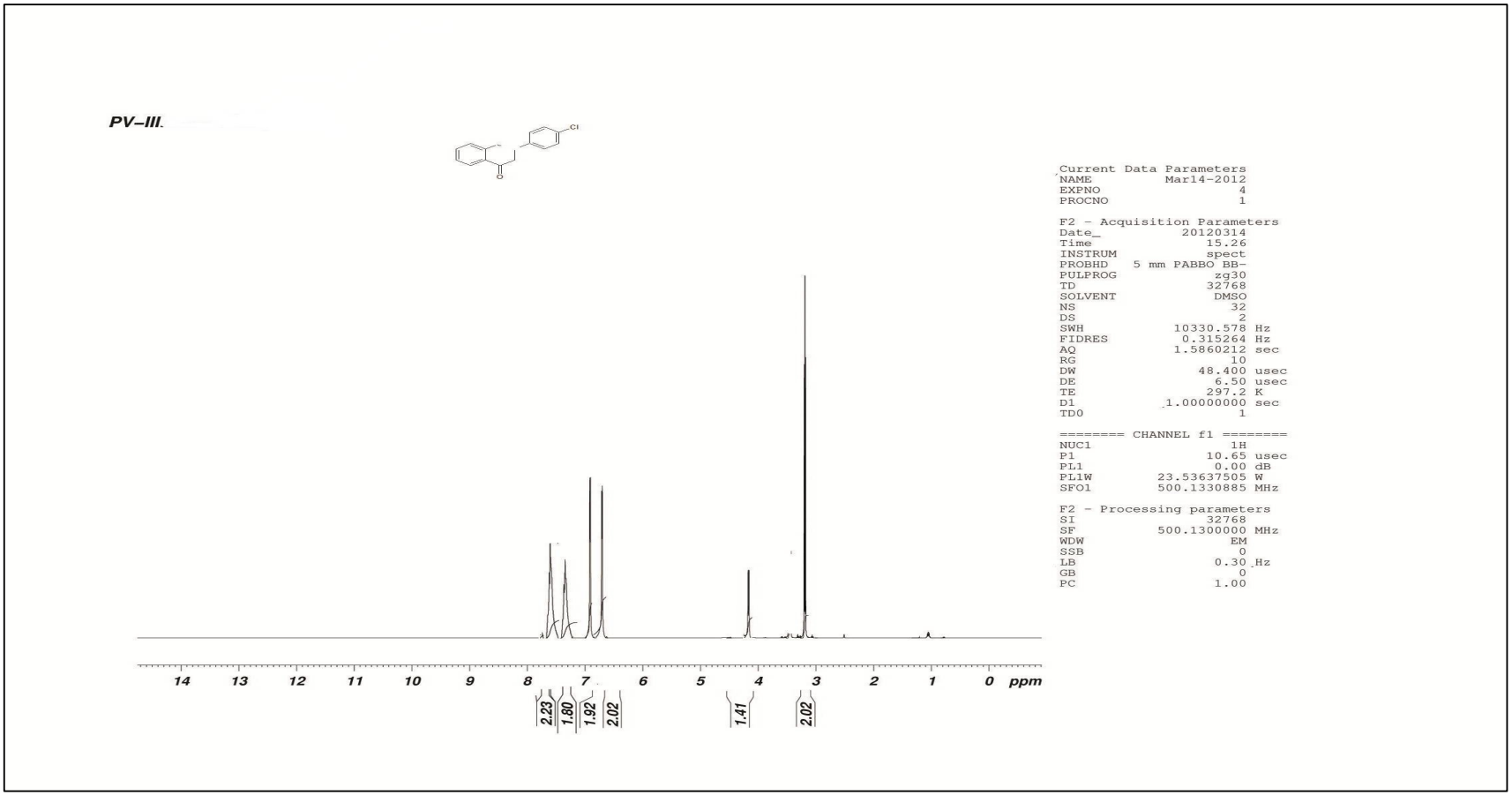



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Fig.V.21 NMR Spectrum of 2-(4-chlorophenyl)-2,3-dihydro-4*H*-chromen-4-one (PV3)

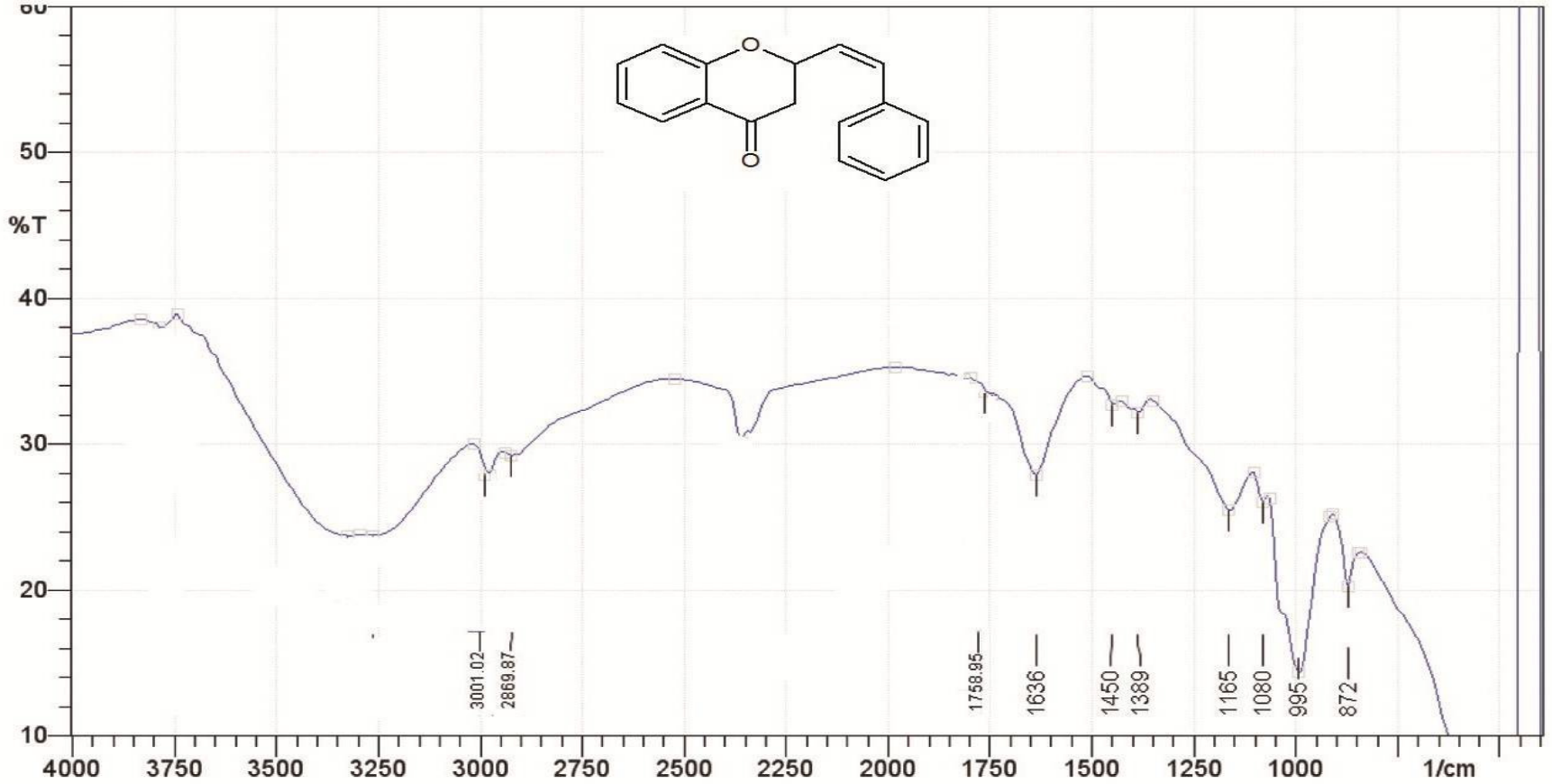


Fig.V.22 IR Spectrum of 2-[(*Z*)-2-phenylethenyl]-2,3-dihydro-4*H*-chromen-4-one (PV4)

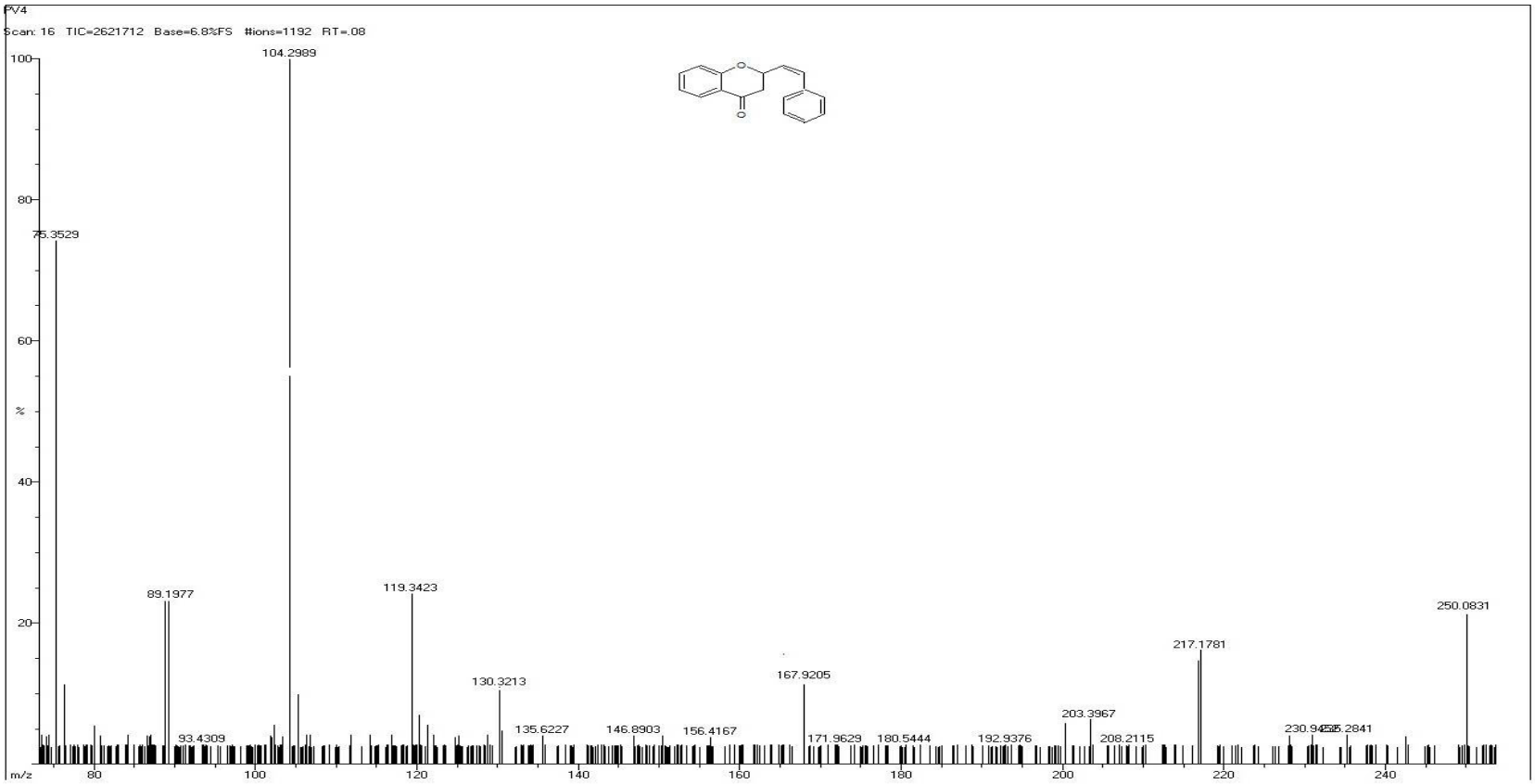


Fig.V.23 MASS Spectrum of 2-[(*Z*)-2-phenylethenyl]-2,3-dihydro-4*H*-chromen-4-one (PV4)

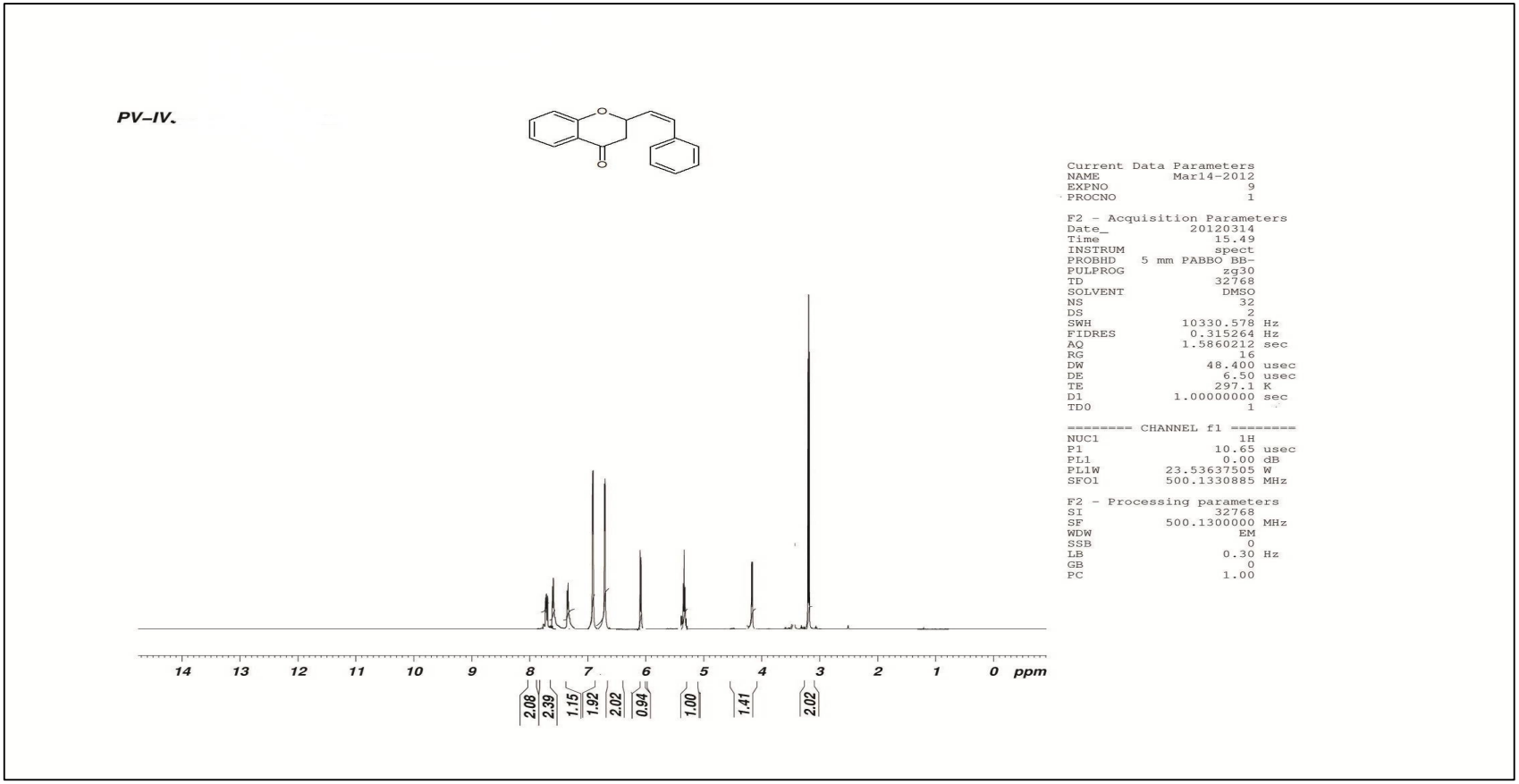


Fig.V.24 NMR Spectrum of 2-[(*Z*)-2-phenylethenyl]-2,3-dihydro-4*H*-chromen-4-one (PV4)

V.3 BIOLOGICAL ACTIVITY

V.3.a ACUTE TOXICITY STUDY

- Acute oral toxicity studies were performed according to the OECD guidelines 423 method.
- The synthesized compounds were administered initially at a starting dose of 2000mg/kg body weight in 1% CMC orally and observed for 14 days mortality due to acute toxicity.
- Continuous observation was made atleast twice a day for the effect on CNS,ANS, motor activity,salivation, skin coloration and other general signs of toxicity were also observed and recorded.
- Since no sign of toxicity was observed at 2000mg/kg body weight to the group animals, the LD50 value of the synthesized compounds(pv1-pv4) were expected to exceed 2000mg/kg body weight and represented as class 5 (2000mg/kg <LD50 > 2500mg/kg)
- The above studies reveal that all the synthesized compounds proved to be non toxic to the animals at test dose level and well tolerated.

V.3.b INVITRO ACTIVITY

In vitro study of the synthesized compounds PV1,PV2 and PV3 showed activity. The % cell inhibition and IC_{50} value after 48 hours has also been tabulated in the table. The compound PV4 did not show the activity.

Table.V.8 %Cell inhibition of PV1

S.NO	Concentration µmol/ml	PV1	
		% cell ihibition	IC50 µmol/ml
1	0.1	0.865470	10.59
2	1.0	18.94514	
3	10	48.64584	
4	100	90.64547	

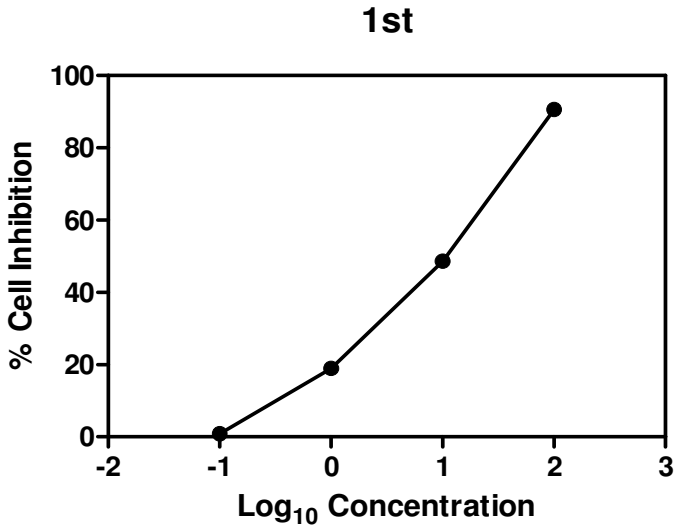


Fig.V.25 IC50 value of PV1

Table.V.9 %Cell inhibition of PV2

S.NO	Concentration µmol/ml	PV1	
		% cell ihibition	IC50 µmol/ml
1	0.1	1.054854	12.80
2	1.0	17.94875	
3	10	45.92576	
4	100	91.36487	

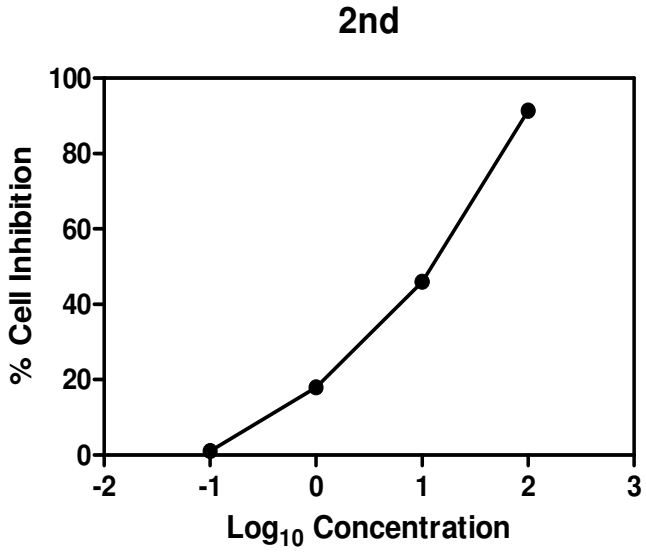


Fig.V.26 IC50 value of PV2

Table.V.10 % Cell inhibition of PV3

S.NO	Concentration µmol/ml	PV1	
		% cell ihibition	IC50 µmol/ml
1	0.1	2.656545	11.27
2	1.0	21.65420	
3	10	49.54285	
4	100	92.65466	

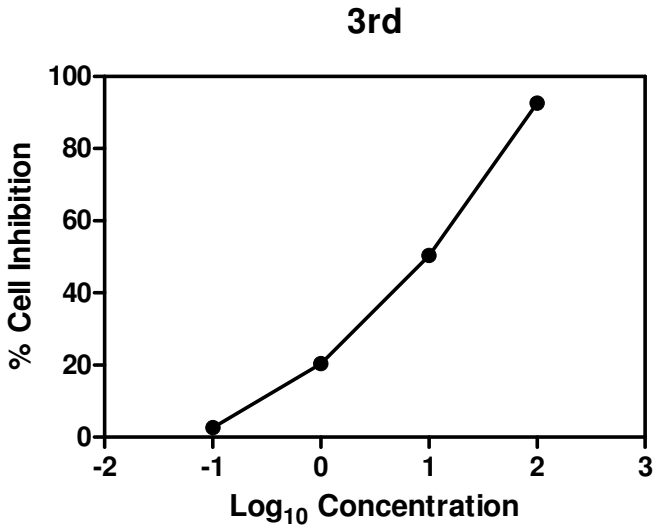


Fig.V.27 IC50 value of PV3

MEDIA

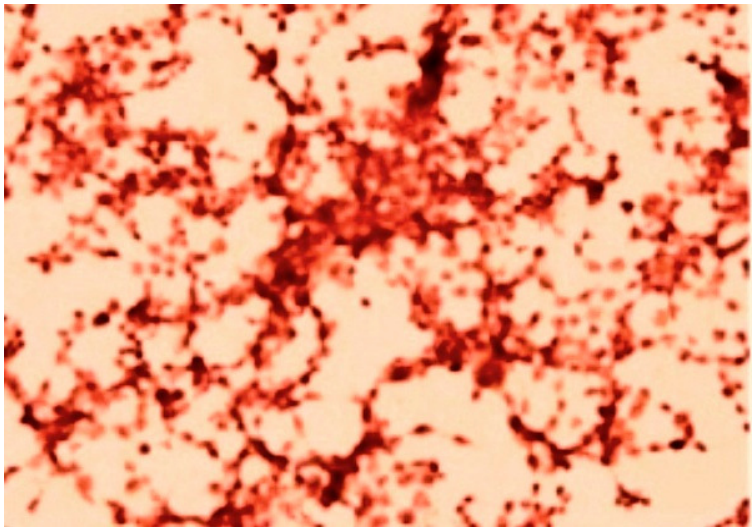


Fig.V.28 media of HCT 116 Cell line

PV1

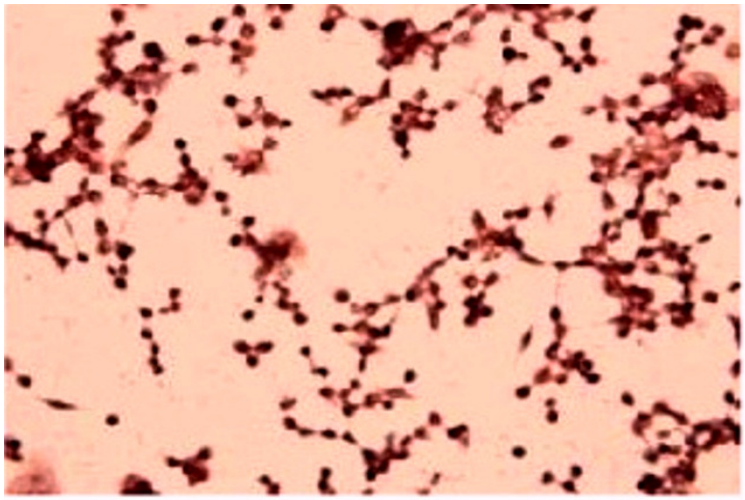


Fig.V.29 Cell Inhibition of PV1

PV2

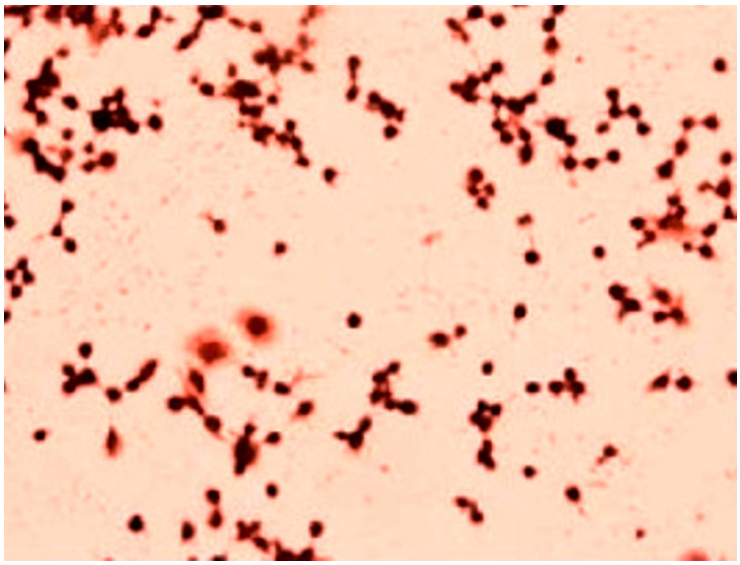
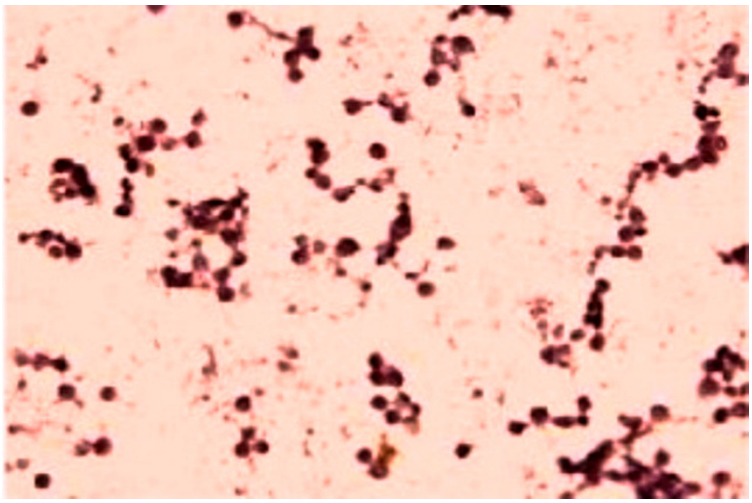


Fig.V.30 Cell Inhibition of PV2

PV3



Fig,V.31 Cell Inhibition of PV2

Summary And Conclusion

VI SUMMARY AND CONCLUSION

- The present work is generating the Pharmacophore hypothesis that can be successfully used to predict biological activity. The models were capable of predicting the activities. The knowledge of Pharmacophore model was used to design and selective of new scaffolds.
- The selection, investigation and preparation of receptor protein from pdb for pim1 and docking of the molecule from the database against prepared Pim 1 receptor protein was carried out. The best scaffold selected from ligand database based, on the Pharmacophore predicted value and docking score against pim1 receptor, were chosen for software based ADME studies.
- The selected scaffold was investigated for the ADME properties. The selected molecules having good ADME properties, were selected for synthesis.
- The synthesis of molecules PV1, PV2, PV3 and PV4 was carried out using the identified scaffold. The Synthetic procedure involved condensation followed by cyclisation.
- The synthesized molecules, where characterized by using IR, NMR and MASS spectrometry.
- Acute toxicity studied of synthesized molecules was carried using albino mice. The LD_{50} value of the synthesized compound was above 2000mg/kg.
- The invitro anticancer activity was done using MTT assay method. The synthesized molecules PV1, PV2 and PV3 showed the activity.PV4 showed the less activity because hydrophobic feature is not available compare to the other compounds.

Future scope of study

VII.FUTURE SCOPE OF STUDY

- The established 3D QSAR and common Pharmacophore which has been identified can be used in the screening of large set of molecules which we want to evaluate for Pim 1 inhibitors gaining new molecule for anticancer treatment.
- As the whole sequence of docking study proposed certain molecules which when evaluated showed activity for molecules (PV1, PV2 and PV3) in μM.
- Since the optimization process also performed, The 4 molecules proposed as per the optimization can be synthesized and can further evaluate for biological evaluation
- These works laid a way to do further studies on these derivatives.
- The further work carried as organ based toxicity and invivo anticancer activity.

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