INSILICO, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NOVEL ISATIN ANALOGUES

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MASTER OF PHARMACY

IN

PHARMACEUTICAL CHEMISTRY

Submitted by

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Under the Guidance of

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OCTOBER- 2017

CERTIFICATE

This is to certify that the thesis entitled **"*INSILICO*, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NOVEL ISATIN ANALOGUES"** submitted by **Reg. No 261515352** was carried out in the Department of Pharmaceutical Chemistry, Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil – 626126, which is affiliated to The Tamil Nadu Dr. M. G. R Medical University, Chennai, under the Direct Supervision and Guidance of **Dr.N.Venkateshan**, Principal, Arulmigu Kalasalingam College of Pharmacy for the Partial fulfillment of Degree of Master of Pharmacy in the department of Pharmaceutical Chemistry.

Place : Krishnankoil

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EVALUATION CERTIFICATE

This to certify that the dissertation work entitled "***INSILICO*, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NOVEL ISATIN ANALOGUES**" submitted by **Reg. No 261515352** to The Tamil Nadu Dr. M. G. R Medical University, Chennai, in Partial fulfillment of the requirement for the award of the Degree of Master of Pharmacy in the department of Pharmaceutical Chemistry is evaluated by,

Date :

Center: Arulmigu Kalasalingam College of Pharmacy,
Anand Nagar,
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Examiners:

1.

2.

Dedicated

To

My Parents and God

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I bow my head to my parents for their blessings and all the pains they have taken for me. This project was undertaken with guidance, co-operation and assistance of distinguished persons cited below who have contributed towards the successful completion of this project.

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KANIGA P

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CHAPTER-I

Introduction

INTRODUCTION

The branch of science concerned with the substance of which matter is composed, the investigation of their properties and reactions, and the use of such reactions to form new substances are called as chemistry. Chemistry is the central science because it bridges other natural sciences, including physics, geology and biology. The atom is the basic unit of chemistry. It consists of a dense core called atomic nucleus surrounded by a space called the electron cloud. The nucleus is made up of positively charged protons and uncharged neutrons, while the electron cloud consists of negatively charged electrons which orbit the nucleus. In a neutral atom, the negatively charged electrons balance out the positive charge of the protons. A chemical element is a pure substance which is composed of a single type of atom, characterized by its particular number of protons in the nuclei of its atoms, known as atomic number and represented by the symbol Z. The mass number is the sum of the number of protons and neutrons in a nucleus. Although all the nuclei of all atoms belonging to one element will have the same atomic number, they may not necessarily have the same mass number, atoms of an element which have different mass number are known as isotops. A pure chemical substance composed of more than one element is called as compound.

Drug design

Drug design, often referred to as rational drug design or simply rational design, is the inventive process of finding new medications based on the knowledge of a biological target.The drug is most commonly an organic small molecule that activates or inhibits the function of a bio molecule such as a protein, which in turn results in a therapeutic benefit to the patient. In the most basic sense, drug design involves the design of molecules that are complementary in shape and charge to the bio molecular target with which they interact and therefore will bind to it. Drug design frequently but not necessarily relies on computer modeling techniques. This type of modeling is sometimes referred to as computer-aided drug design. Finally, drug design that relies on the knowledge of the three-dimensional structure of the bio molecular target is known as structure-based drug

design. In addition to small molecules, biopharmaceuticals and especially therapeutic antibodies are an increasingly important class of drugs and computational methods for improving the affinity, selectivity, and stability of this protein-based therapeutics have also been developed.

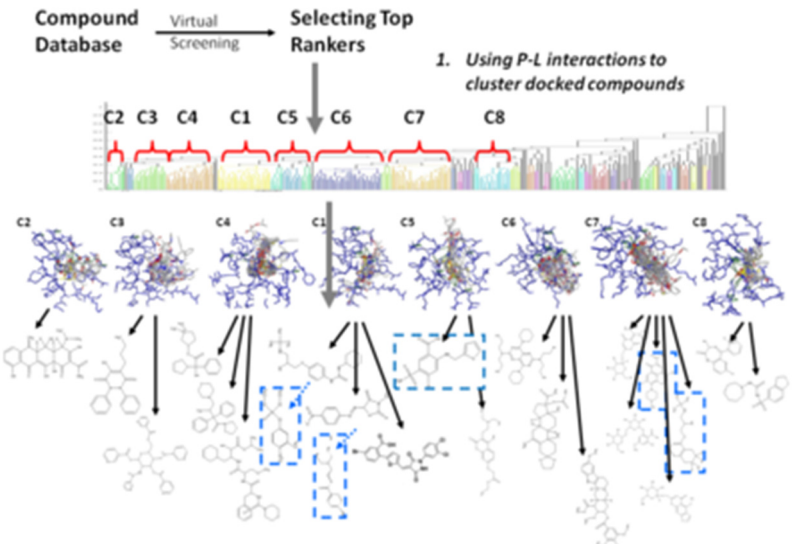


FIG.1: FLOWCHART OF A USUAL CLUSTERING ANALYSIS FOR STRUCTURE-BASED DRUG DESIGN

MOLECULAR DOCKING

In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions.

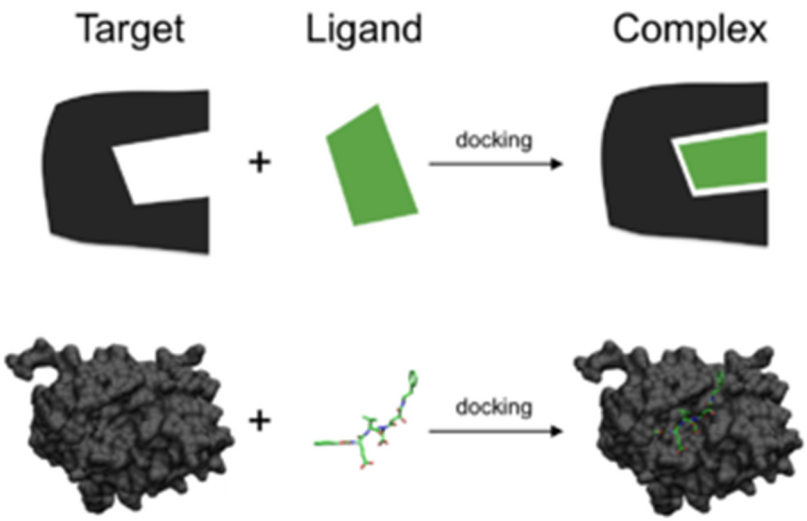
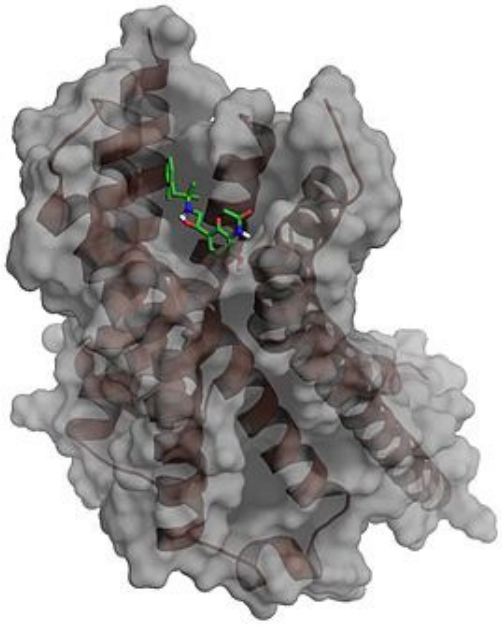


FIG.2: SCHEMATIC ILLUSTRATION OF DOCKING A SMALL MOLECULE LIGAND (GREEN) TO A PROTEIN TARGET (BLACK) PRODUCING A STABLE COMPLEX.



The associations between biologically relevant molecules such as proteins, nucleic acids, carbohydrates, and lipids play a central role in signal transduction. Furthermore, the relative orientation of the two interacting partners may affect the type of signal produced (e.g., agonism vs antagonism). Therefore, docking is useful for predicting both the strength and type of signal produced.

Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. Characterization of the binding behavior plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes.

Docking approaches[1]

Two approaches are particularly popular within the molecular docking community. One approach uses a matching technique that describes the protein and the ligand as complementary surfaces. The second approach simulates the actual docking process in which the ligand-protein pair wise interaction energies are calculated. Both approaches have significant advantages as well as some limitations.

Mechanics of docking

To perform a docking screen, the first requirement is a structure of the protein of interest. Usually the structure has been determined using a biophysical technique such as x-ray crystallography or NMR spectroscopy, but can also derive from homology modeling construction. This protein structure and a database of potential ligands serve as inputs to a docking program. The success of a docking program depends on two components: the search algorithm and the scoring function.

Search algorithm[2]

The search space in theory consists of all possible orientations and conformations of the protein paired with the ligand. However, in practice with current computational resources, it is impossible to exhaustively explore the search space—this would involve enumerating all possible distortions of each molecule (molecules are dynamic and exist in an ensemble of conformational states) and all possible rotational and translational orientations of the ligand relative to the protein at a given level of granularity. Most docking programs in use account for the whole conformational space of the ligand

(flexible ligand), and several attempt to model a flexible protein receptor. Each "snapshot" of the pair is referred to as a pose.

A variety of conformational search strategies have been applied to the ligand and to the receptor. These include:

- Systematic or stochastic torsional searches about rotatable bonds
- Molecular dynamics simulations
- Genetic algorithms to "evolve" new low energy conformations and where the score of each pose acts as the fitness function used to select individuals for the next iteration.

Ligand flexibility

Conformations of the ligand may be generated in the absence of the receptor and subsequently docked or conformations may be generated on-the-fly in the presence of the receptor binding cavity, or with full rotational flexibility of every dihedral angle using fragment based docking. Force field energy evaluations are most often used to select energetically reasonable conformations, but knowledge-based methods have also been used.

Receptor flexibility:

Computational capacity has increased dramatically over the last decade making possible the use of more sophisticated and computationally intensive methods in computer-assisted drug design. However, dealing with receptor flexibility in docking methodologies is still a thorny issue. The main reason behind this difficulty is the large number of degrees of freedom that have to be considered in this kind of calculations. Neglecting it, however, leads to poor docking results in terms of binding pose prediction. Multiple static structures experimentally determined for the same protein in different conformations are often used to emulate receptor flexibility.

Alternatively rotamer libraries of amino acid side chains that surround the binding cavity may be searched to generate alternate but energetically reasonable protein conformations.

Application[3]

A binding interaction between a small molecule ligand and an enzyme protein may result in activation or inhibition of the enzyme. If the protein is a receptor, ligand binding may result in agonism or antagonism. Docking is most commonly used in the field of drug design — most drugs are small organic molecules, and docking may be applied to:

- Hit identification – docking combined with a scoring function can be used to quickly screen large databases of potential drugs in silico to identify molecules that are likely to bind to protein target of interest (see virtual screening).
- Lead optimization – docking can be used to predict in where and in which relative orientation a ligand binds to a protein (also referred to as the binding mode or pose). This information may in turn be used to design more potent and selective analogs.
- Bioremediation – Protein ligand docking can also be used to predict pollutants that can be degraded by enzymes.

CANCER

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Not all tumors are cancerous; benign tumors do not spread to other parts of the body. Possible signs and symptoms include a lump, abnormal bleeding, prolonged cough, unexplained weight loss and a change in bowel movements. While these symptoms may indicate cancer, they may have other causes. Over 100 types of cancers affect humans. Tobacco use is the cause of about 22% of cancer deaths. Another 10% is due to obesity, poor diet, lack of physical activity, and excessive drinking of alcohol. Other factors include certain infections, exposure to ionizing radiation and environmental pollutants. In the developing world nearly 20% of cancers are due to infections such as hepatitis B, hepatitis C and human papilloma virus infection. These

factors act, at least partly, by changing the genes of a cell. Typically many genetic changes are required before cancer develops.

Definition

Cancers are a large family of diseases that involve abnormal cell growth with the potential to invade or spread to other parts of the body. They form a subset of neoplasms. A neoplasm or tumor is a group of cells that have undergone unregulated growth and will often form a mass or lump, but may be distributed diffusely. All tumor cells show the six hallmarks of cancer. These characteristics are required to produce a malignant tumor. They include: Cell growth and division absent the proper signals, Continuous growth and division even given contrary signals, Avoidance of programmed cell death, Limitless number of cell divisions, Promoting blood vessel construction, Invasion of tissue and formation of metastases. The progression from normal cells to cells that can form a detectable mass to outright cancer involves multiple steps known as malignant progression

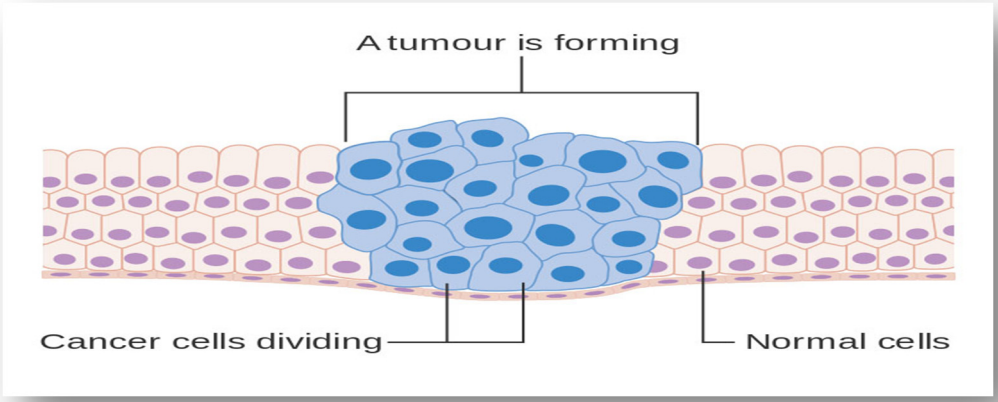


FIG.3: DIVISION OF CANCER CELLS

Cell cycle and Regulation[4]

During cell cycle, each cell divides into two daughter cells having identical genetic material. Each of these cells may immediately re-enter a new cell-cycle or pass into a non-proliferative resting state.

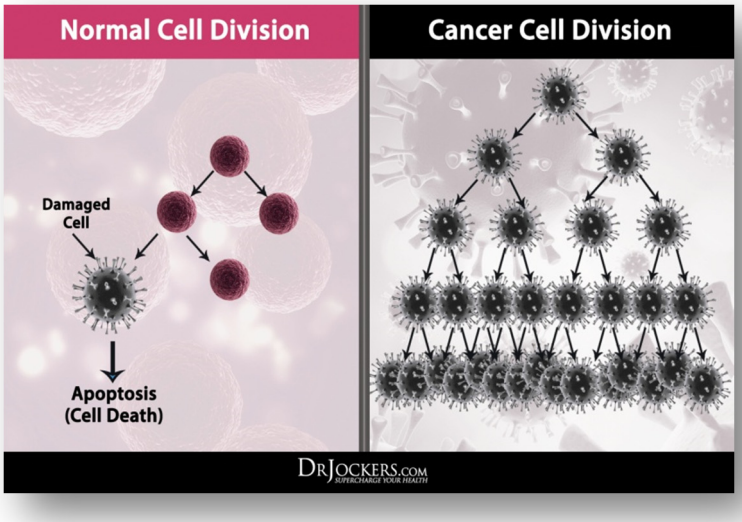


FIG.4: CELL DIVISION

The growth and division of cells can be defined into four prominent phases of cell-cycle. These include:

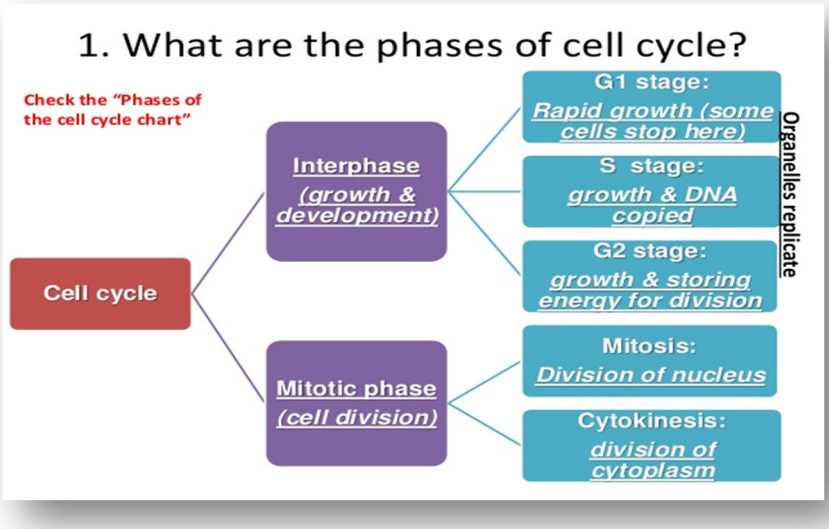


FIG.5: PHASES OF CELL CYCLE

Signs and symptoms[5]

When cancer begins, it produces no symptoms. Signs and symptoms appear as the mass grows. The findings that result depend on the cancer's type and location. Many frequently occur in individuals who have other conditions. Cancer is a great imitator. Thus, it is common for people diagnosed with cancer to have been treated for other diseases, which were hypothesized to be causing their symptoms. People may become anxious or depressed post-diagnosis. The risk of people with cancer is approximately double in suicide.

Local symptoms

Local symptoms may occur due to the mass of the tumor or its ulceration. Masses in breasts or testicles may produce observable lumps. Although localized pain may occur in advanced cancer, the initial swelling is usually painless. Some cancers can cause a buildup of fluid within the chest or abdomen.

Systemic symptoms

General symptoms occur due to effects that are not related to direct or metastatic spread. These may include: fever, excessive fatigue and changes to the skin. Hodgkin disease, leukemias and cancer of the liver or kidney can cause a persistent fever. Some cancers may cause specific groups of systemic symptoms, termed paraneoplastic syndrome. Examples include the appearance of myasthenia gravis in thymoma and clubbing in lung cancer.

Metastasis

Cancer can spread from its original site by local spread, lymphatic spread to regional lymph nodes or by hematogenous spread via the blood to distant sites, known as metastasis. When cancer spreads by a hematogenous route, it usually spreads all over the body. However, cancer 'seeds' grow in certain selected site only ('soil') as hypothesized in the *soil and seed hypothesis* of cancer metastasis. The symptoms of metastatic cancers depend on the tumor location and can include enlarged lymph nodes (which

can be felt or sometimes seen under the skin and are typically hard), enlarged liver or enlarged spleen, which can be felt in the abdomen, pain or fracture of affected bones and neurological symptoms.

Prevention[6]

Cancer prevention is action taken to lower the risk of getting cancer. This can include maintaining a healthy lifestyle, avoiding exposure to known cancer causing substance, and taking medicines or vaccines that can prevent cancer from developing.

Screening

Unlike diagnostic efforts prompted by symptoms and medical signs, cancer screening involves efforts to detect cancer after it has formed, but before any noticeable symptoms appear. This may involve physical examination, blood or urine tests or medical imaging. Cancer screening is not available for many types of cancers. Even when tests are available, they may not be recommended for everyone.

Universal screening or mass screening involves screening everyone. Selective screening identifies people who are at higher risk, such as people with a family history. Several factors are considered to determine whether the benefits of screening outweigh the risks and the costs of screening.

Management:

Many treatment options for cancer exist. The primary ones include surgery, chemotherapy, radiation therapy, hormonal therapy, targeted therapy and palliative care. Which treatments are used depends on the type, location and grade of the cancer as well as the patient's health and preferences. The treatment intent may or may not be curative.

Anti-Cancer drugs[7]

The drugs which are used for the treatment of cancer are called as anti-Cancer drugs. It is also called as Anti-Neoplastic drugs.

Classification of drugs

They are classified as,

alkylating agent, anti-Metabolites, antibiotics, plant products, enzymes, hormone, immuno theraphy, mono clonal antibodies, radio therapeutic agents, cyto protective agents and miscellaneous

Alkylating agent

Cylophosphamide, chlorambucil, mechlorethamine, ifosfamide, melphalan, busulfan, carmustine, lomustine, semustine, ethylenimine, thiotepa, Dacarbazine & procarbazine.

Anti-Metabolites

Cytarabine, fluorouracil, floxuridine, capecitabine, 6-thioguanine, mercaptopurine, fludarbine, pentostatin, cladribine, Methotrexate & azathioprine.

Antibiotics

Doxorubicin, daunorubicin, idarubicine, bleomycin sulfate**,** mitomycin C**,** actinomycin D & mithramycin

Plant products

Vincristine, vinblastine, vinorelbine, etoposide, Teniposide & paclitaxel

Enzymes

L-Asparaginase**,** pegaspargase

Hormones

Mitotane, megestrol, tamoxifen, letrozole, Dromostanolone & pipobroman

Immuno theraphy

Interferon α-2a, interferon α-2b, interferon α-n3, aldesluekin, diftitox, Denileukin & Bacillus Calmette-Guerin(BCG)

Monoclonal antibodies

Rituximab, Gemtuzumab & ozogamicin

Radio therapeutic agents

Chromic phosphate P 32, sodium phosphate P 32, sodium iodide I 131, strontium 89 chloride & smarium SM 153 lexidronam

Cyto protective agents

Mesna, Amifostine & dexrazoxane

Miscellaneous

Cisplastin, carboplatin, hydroxy urea, hexamethylamine, altreamin, mitoxantrane, gallium nitrate, arsenic trioxide, bexavotene, sargramostim, Filgrastim & profimer sodium

Mechanism of action[8]

There are two main types of cell death: apoptosis and necrosis. Necrotic cell death is caused by gross cell injury and results in the death of groups of cells within a tissue. Apoptosis is a regulated form of cell death that may be induced or is preprogrammed into the cell (e.g. during development) and is characterized by specific DNA changes and no accompanying inflammatory response.

It can be triggered if mistakes in DNA replication are identified. Loss of this protective mechanism would allow mutant cells to continue to divide and grow, thereby conserving mutations in subsequent cell divisions. Many cytotoxic anticancer drugs and radiotherapy act by inducing mutations in cancer cells which are not sufficient to cause cell death, but which can be recognized by the cell, triggering apoptosis.

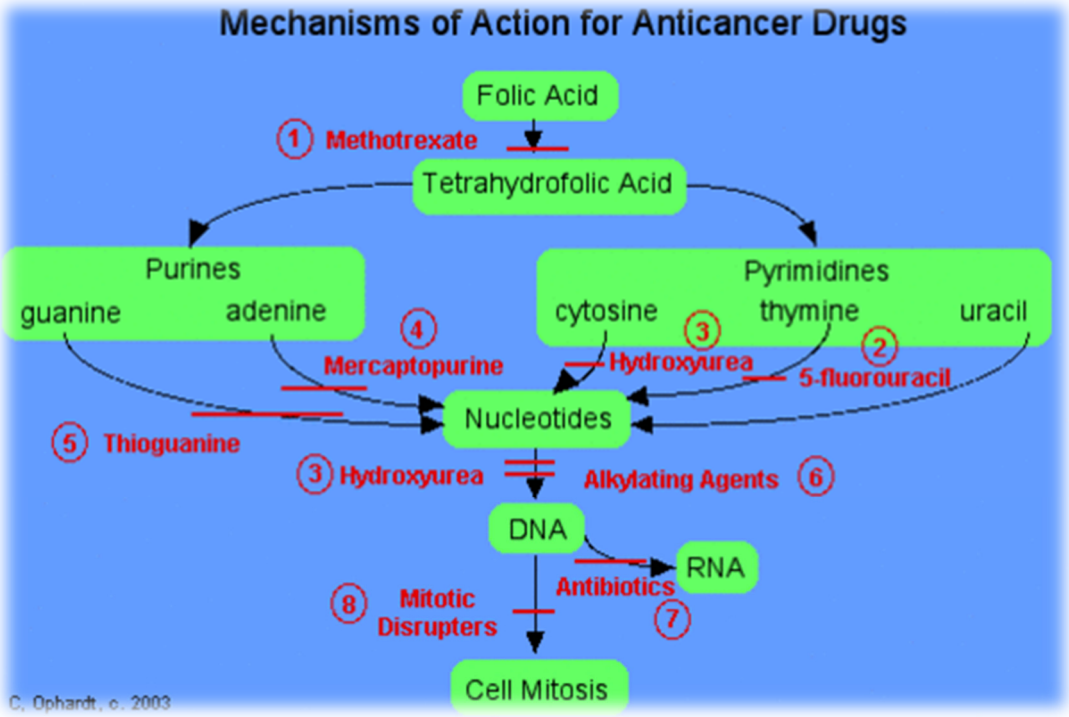


FIG.6: MECHANISM OF ACTION OF ANTI-CANCER DRUGS

MICRO ORGANISMS

Microbes are tiny organism too tiny to see without a microscope yet they are rich on earth. They live everywhere like air, soil, rock, water, poles, deserts & deep-sea.Study of microorganisms is called Microbiology. Study of bacteria and viruses is called Bacteriology and virology respectively.Microorganisms play important role to humans and they contribute recycling other organisms and decomposition the waste products.Some further advantageous activities of microbes are:

Use in food

Microbes are used in baking, other food making processes and also used the fermentation process in the production of dairy products like cheese.

Use in science

Microbes are also vital tools in biotechnology, biochemistry, genetics and molecular biology.

Human digestion

The bacteria that live within the human digestive system supply to gut immunity, synthesizes vitamins and ferment complex indigestible carbohydrates.

In medicines

Microbes are used to make vaccines which can be stimulate the production of antibodies substances to make sure future defense against with unwanted microbes.

Most important types of microbes are bacteria, viruses, fungi, protozoa

Bacteria[9]

Bacteria constitute a large domain of prokaryotic microorganisms. Typically a few micrometer in length, bacteria have a number of shapes including balls, commas, rods, cubes and spirals.These are very useful in many fields like preparation of antibiotics, in human digestion,in fermentation etc.But they are spread out many infectious disease and which are main reason for universal mortality.

Some example for bacterial diseases are, gonorrhea, syhilis, anthrax, tuberculosis, cholera, typhoid, fever, pneumonia, tetanus

Virus[10]

A virus is a small infectious agent that multiply only inside the living cells of other organisms.These are among the smallest microbes than bacteria.It consists genes which are present in one or more molecules of DNA or RNA which surrounded by a protein coat.

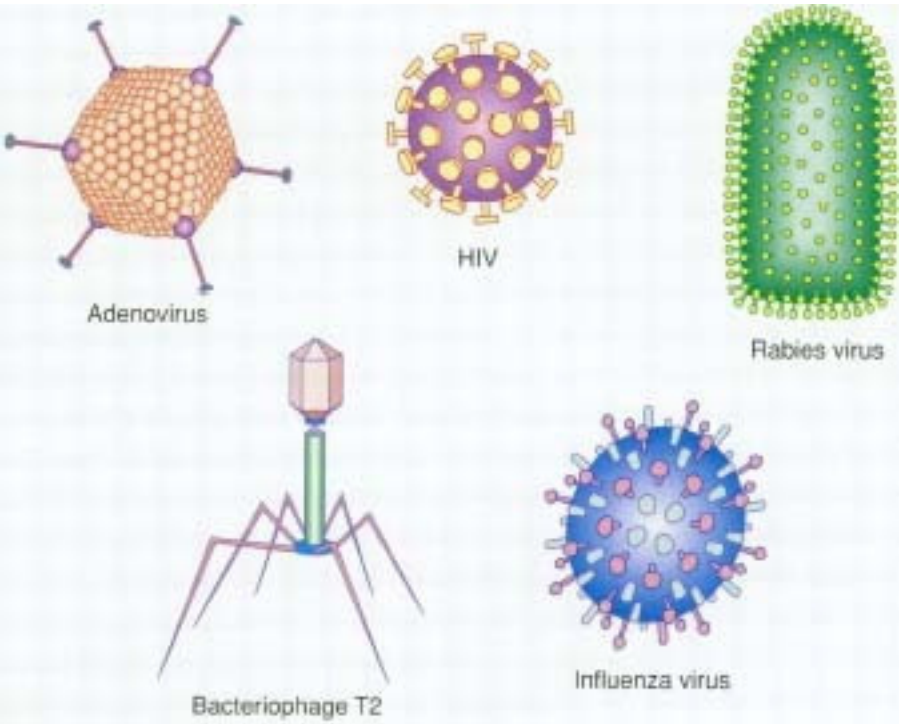


FIG.7: TYPES OF VIRUS

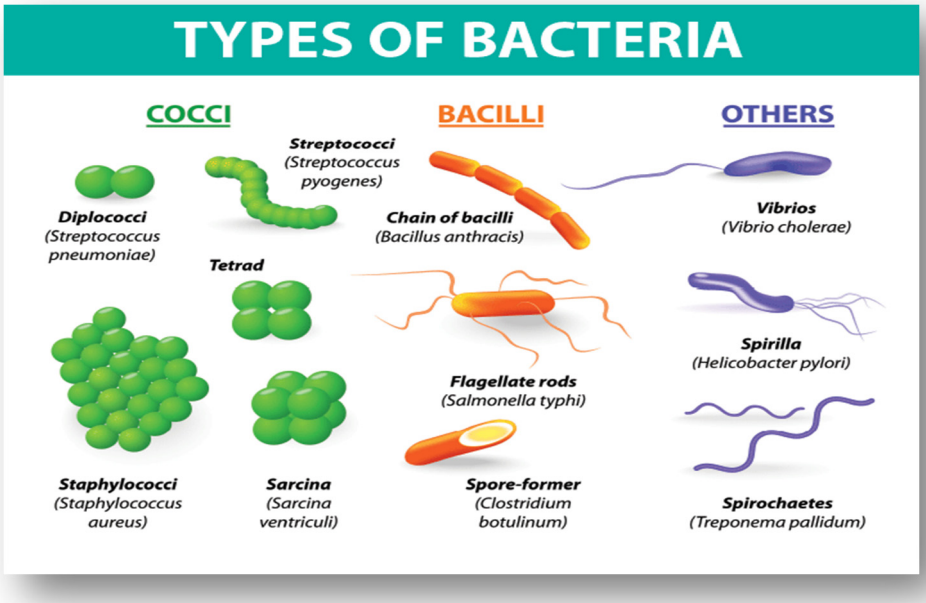


FIG.8: TYPES OF BACTERIA

Antimicrobials

An antimicrobial is an agent which kills micro organisms or inhibits their growth.Anti-microbial can be classified into two types depends upon their function. Microbicidal which means antimicrobial agents that kill microbes while inhibit their growth are called biostatic.

Mechanism of action of antibiotics[11]

Different antibiotics have different modes of action, due to the nature of their structure and degree of affinity to certain target sites within bacterial cells.

1. Cell wall synthesis
2. Protein synthesis
3. Cytoplasmic membrane permeability
4. Nucleic acid synthesis
5. Anti metabolic synthesis

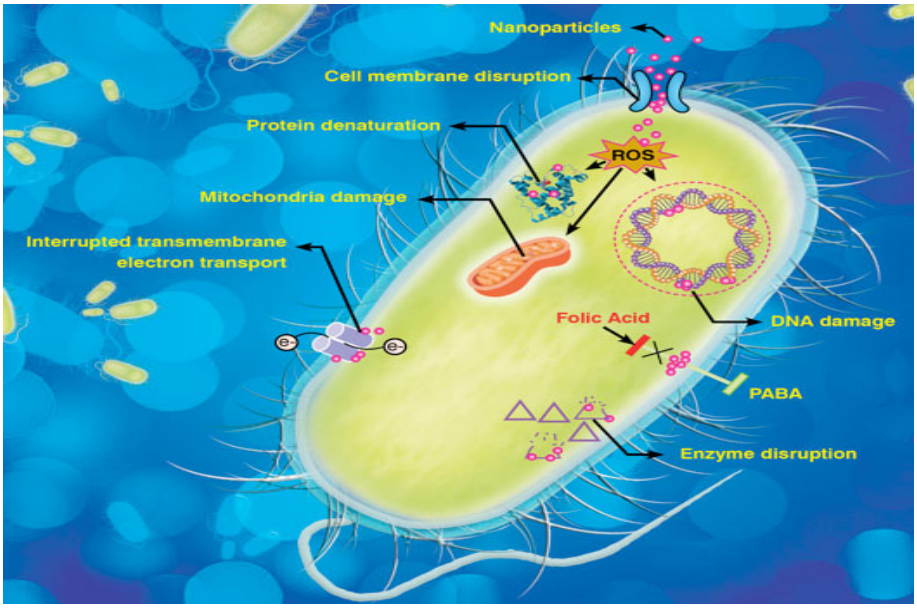


FIG.9: MECHANISM OF ACTION OF ANTIBIOTICS

Antibiotic resistance[12]

Antibiotic resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs, chemicals, or other agents designed to cure or prevent infections. The bacteria survive and continue to multiply causing more harm. Bacteria can do this through several mechanisms.

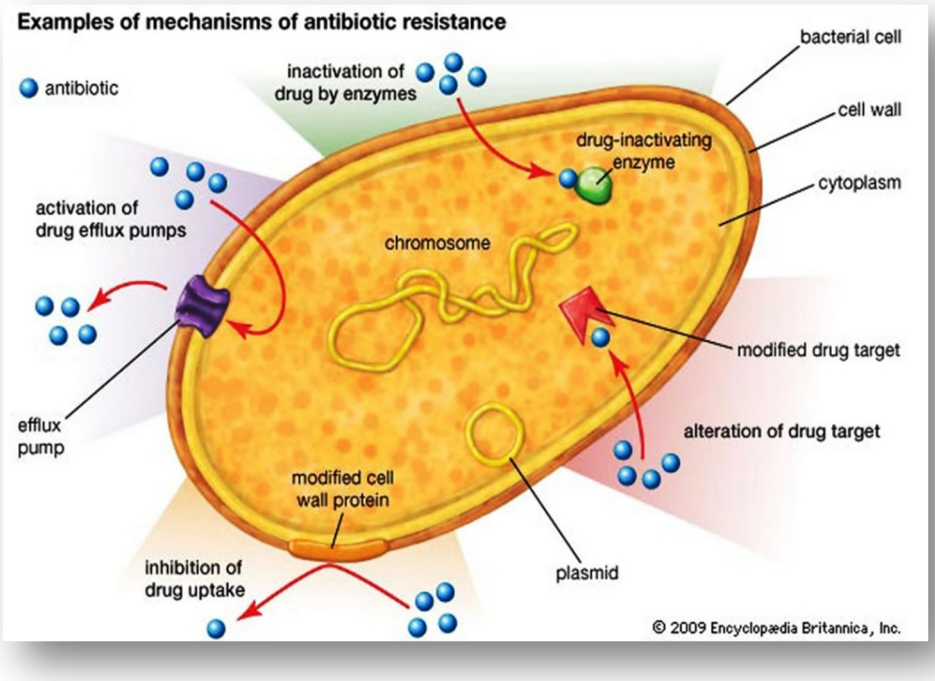
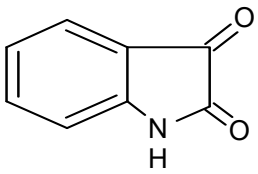


FIG.10: ANTI-BIOTIC RESISTANCE

SUBJECT INTRODUCTION

ISATIN[13, 14]

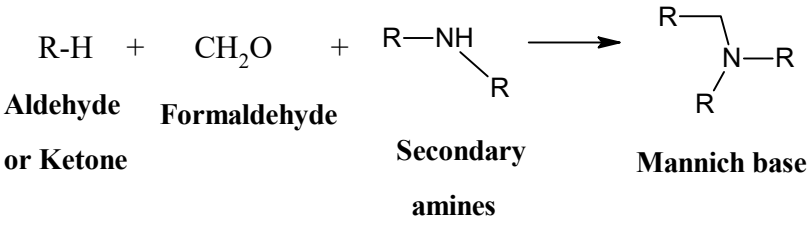
Isatin(indoline-2,3-dione), is an indole derivatives (Ex: Indozoline, Fluvaisatin), possessing an indole nucleus with two chemically distinct cyclic carbonyl groups, keto and lactam.



Isatin

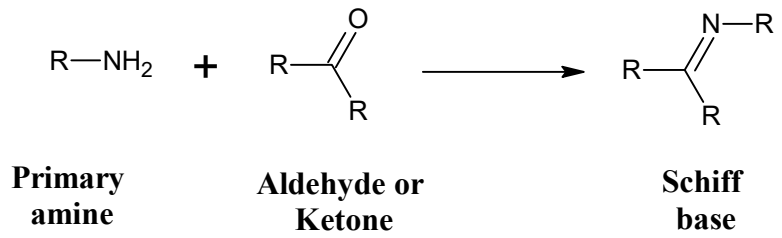
MANNICH BASE[15, 16]

A Mannich base is a beta-amino-ketone, is an end product in the Mannich reaction, is the condensation reaction in which the compound containing active hydrogen atom is allowed to react with formaldehyde and an NH-amine derivative.



SCHIFF BASE[17, 18]

A Schiff base is a nitrogen analog of an aldehyde or ketone in which the C=O group is replaced by C=N-R group. It is usually formed by condensation of an aldehyde or ketone with a primary amine.



CHAPTER-II

LITERATURE REVIEW

LITERATURE REVIEW

Saleh A. Bahashwan *et al*[19], 2013 investigated a new series of poly fused pyrazolothienopyrimidine derivatives (2–14) were synthesized and their anti-parkinsonism, hypoglycemic and anti-microbial activities were evaluated. Some of the newly synthesized compounds exhibited better pharmacological and biological activities than the reference controls with low concentrations. The structures of newly synthesized compounds were confirmed by chemical, elemental and spectroscopic evidences. The detailed synthesis, spectroscopic data, and pharmacological activities were reported.

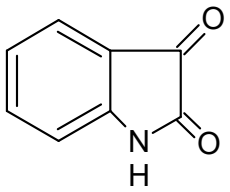
Sanjana Chandran *et al*[20]., 2017 investigated the objective of the present study was to identify the proteome pattern, isolate and study the functions of selective proteins from *Ferula asafoetida* root exudate using chromatographic techniques. The root exudate proteins were fractionated using ion-exchange and gel filtration chromatography. A range of bioactive protein fractions were then separated in sufficient quantity which is the focus of this study. Based on studies, here we report three main proteins with molecular weights 14 kDa, 27 kDa, and 39 kDa. The biological and pharmacological activities of both purified and unpurified proteins obtained were extensively studied to understand their significance. The study revelaed that 27 kDa protein interestingly stabilized trypsin activity in 24 h of time and retained about 64% of the enzyme activity. Analyses confirmed 40°C and pH 8.0 are the optimum temperature and pH respectively. The 39 kDa protein remarkably increased the activity of chymotrypsin and the 14 kDa protein showed anti-bacterial activity against *Pseudomonas aeruginosa*. Invariably all of the three purified proteins showed enhanced anti-oxidant activity. In conclusion, results here obtained suggested that the primary metabolites (proteins) in asafoetida are mainly responsible for its versatile biological and pharmacological activities.

Areej M.Assaf *et al*[21]., 2013 reported *Mercurialis annua* L., *Bongardia chrysogonum* L., and *Viscum cruciatum* Sieb have been traditionally used by local herbalists in Jordan for the treatment of hematopoietic neoplasms.To determine the anti-cancer, anti-inflammatory and anti-microbial potentials of the three extracts against two of the most common hematopoietic malignancies in the Jordanian populations; Burkitt's lymphoma and multiple myeloma.The anti-cancer activity was

tested against the two cell lines (BJAB Burkitt's lymphoma and U266 multiple myeloma) using the MTT and trypan blue assays. The agar dilution assay was used to study the anti-microbial activity against gram-positive bacteria, gram-negative bacteria, anaerobic bacteria and yeast. The pro-inflammatory cytokines interleukin (IL) -1β, IL-8 and tumor necrosis factor-α (TNF-α) were measured in the pretreated cell lines using ELISA assay to determine the anti-inflammatory activity of *Viscum cruciatum* Sieb against the two cell lines.

Dun-Jia Wang *et al*[22]., 2010 proposed several new trifluoromethyl-1*H*-pyrazoles were prepared by reaction of hydrazine monohydrate with 1, 3-diketones. Their structures were confirmed by elemental analysis, IR, ^{1}H NMR and EI-MS spectroscopy. The anti-microbial activities of the newly synthesized compounds were examined by disc diffusion method against *Escherichia coli*, *Staphylococcus aureus*, *Pyricularia oryzae* and *Rhizoctnia solani*. All the trifluoromethyl-1*H*-pyrazoles exhibited a certain degree of anti-bacterial and anti-fungal activities.

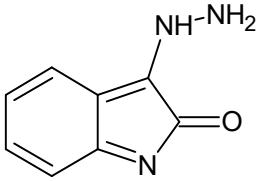
Larry L. Klein *et al*[23]., 2013 investigated isatins (**1**) are valuable intermediates for heterocyclic chemistry. Most of the common methods for their production are less than adequate when the number and lipophilicity of substituents on the targeted isatin are increased. Our group desired such molecules and identified an alternative method for their production.



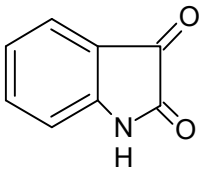
Isatin (**1**)

Nikolai M.Evdokimov *et al*[24]., 2016 proposed that in a search of small molecules active against apoptosis-resistant cancer cells, a series of isatin-based heterocyclic compounds were synthesized and found to inhibit proliferation of cancer cell lines resistant to apoptosis. The synthesis of these compounds involved a condensation of commercially available, active methylene heterocycles with isatin proceeding in moderate to excellent yields. The heterocyclic scaffolds prepared in the current

investigation appear to be a useful starting point for the development of agents to fight cancers with apoptosis resistance, and thus, associated with dismal prognoses.

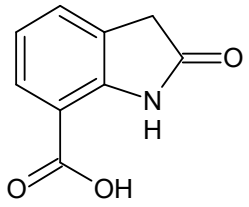


Isatin 3 hydrozone (**2**)



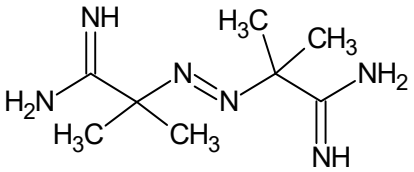
Isatin (**3**)

Muhhammad Arshad *et al*[25]., 2017 reported two new isatin derivatives (E)-*N*′-(1-allyl-2-oxoindolin-3-ylidene)-4-methylbenzenesulfono-hydrazide(**5**) and *(*E*)*-*N*′-(1-allyl-2-oxoindolin-3-ylidene)-4-chlorobenzenesulfono-hydrazide (**6**) were synthesized in good yields by adopting two component synthetic methodology.The structure elucidation was accomplished with the help of UV–vis., FT-IR and NMR (^{1}H and^{13}C) spectroscopic techniques. Suitable crystals were grown by slow evaporation method and structures were confirmed unequivocally with the help of single crystal X-ray diffraction analysis. Both isatin derivatives **5** and **6** exist in triclinic crystal packing having space group P-1. Crystal structures of both compounds showed that the geometries are stabilized by several intermolecular hydrogen bonds. Quantum mechanical calculations performed at density functional theory (DFT) level confirmed the experimental spectroscopic (UV–vis., FT-IR and ^{1}H NMR) as well as X-ray diffraction results. Kinetic stability, reactivity, electrophilicity and nucleophilic behavior of both the derivatives was elaborated using frontier molecular orbitals (FMOs) and molecular electrostatic potential (MEP) analyses. Enzyme inhibition potential of both compounds were tested *in vitro* against *Bacillus pasteurii* urease and both compounds retarded the enzymatic activity with IC_{50} values of 39.46 ± 0.12 μM and 148.35 ± 0.16 μM respectively.



Oxoindolin (**4**)

Liang Ma *et al*[26]., 2013 investigated in a new series of mannich base of 1,3,4-oxadiazole derivatives possessing 1,4-benzodioxan (6a–6ae) were synthesized and characterized by ^{1}H NMR, ESI-MS and elemental analysis. The structure of 6b was further confirmed by single crystal X-ray diffraction. All these novel compounds were screened for their *in vitro* antioxidant activity employing 2, 2′-diphenyl-1-picrylhydrazyl radical (DPPH), 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) cationic radical ($ABTS^{+}\cdot$) and ferric reducing antioxidant power (FRAP) scavenging assays. Due to the combination of 1, 4-benzodioxan, 1, 3, 4-oxadiazoles and substituted phenyl ring, most of them exhibited nice antioxidant activities. In all of these three assays mentioned above, compounds 6f and 6e showed significant radical scavenging ability comparable to the commonly used antioxidants, BHT and Trolox. Seven compounds with representative substituents or activities were selected for further assays in chemical simulation biological systems—inhibition of microsomal lipid peroxidation (LPO) and protection against 2, 2′-azobis (2-amidinopropane hydrochloride) (AAPH) (**5**) induced DNA strand breakage, in which 6f and 6e were demonstrated to be of the most potent antioxidant activities.



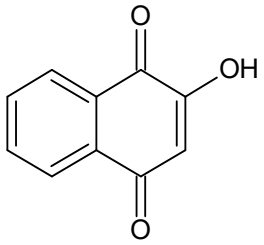
AAPH (**5**)

Bhupendra Mistry *et al*[27]., 2017 reported a new mannich base series of piperazine linked berberine analogues was furnished in this study to screen the antioxidant and anticancer potential of the resultant analogues. Alkoxy group at a C-9 position of berberine was converted to hydroxyl functionality to enhance the ability of final scaffolds binding to the target of drug action mainly through hydrophobic effect,

conjugation effect, whereas mannich base functionality was introduced on the C-12 position of berberine. Scaffolds were investigated for their free radical scavenging antioxidant potential in FRAP and DPPH assay, whereas tested to check their Fe^{+3} reducing power in ABTS assay. The radical scavenging potential of the final derivatives 4a–j was found excellent with IC_{50}, <13 μg/mL and < 8 μg/mL in DPPH and ABTS assay, respectively, whereas some analogues showed significant Fe^{+3} reducing power with absorption at around 2 nm in the FRAP assay. Anticancer effects of titled compounds were inspected against cervical cancer cell line Hela and Caski adapting SRB assay, in which analogues 4a–j presented <6 μg/mL of IC_{50}, and >30 of therapeutic indices, thus exerting low cytotoxic values against Malin–Darby canine kidney (MDCK) cell lines at CC_{50}s >125 μg/mL. Hence, from the bioassay outcomes it can be stated that these analogues are dual active agents as the scavengers of reactive oxygen species and inhibitors of the cancerous cells as compounds with halogen functional group have overall good pharmacological potential in assays studied in this research. Correct structure of the final compounds was adequately confirmed on the basis of FT-IR and ^1H NMR as well as elemental analyses.

Gheorghe Roman *et al*[28]., 2015 proposed the biological activity of mannich bases, a structurally heterogeneous class of chemical compounds that are generated from various substrates through the introduction of an amino methyl function by means of the mannich reaction, is surveyed, with emphasis on the relationship between structure and biological activity. The review covers extensively the literature reports that have disclosed mannich bases as anticancer and cytotoxic agents, or compounds with potential antibacterial and antifungal activity in the last decade. The most relevant studies on the activity of mannich bases as anti-mycobacterial agents, anti-malarials, or antiviral candidates have been included as well. The review contains also a thorough coverage of anticonvulsant, anti-inflammatory, analgesic and antioxidant activities of mannich bases. In addition, several minor biological activities of mannich bases, such as their ability to regulate blood pressure or inhibit platelet aggregation, their anti-parasitic and anti-ulcer effects, as well as their use as agents for the treatment of mental disorders have been presented. The review gives in the end a brief overview of the potential of mannich bases as inhibitors of various enzymes or ligands for several receptors.

Aamir Ahmad *et al*[29]., 2017 investigated lawsone (**6**) is a known naphthoquinone dye from the henna plant *Lawsonia inermis*. Out of a series of four new ferrocene modified mannich bases of 1a, the 2-pyridyl derivative 2a was distinctly more active than its analogs 2b–d in breast, prostate and pancreatic cancer cells. 2a also exhibited greater anti-proliferative effects when compared with the known anticancer active mannich bases 1b and 1c in the androgen-receptor negative PC-3 prostate and Pgp-expressing KB-V1/Vbl cervix carcinoma cell lines. Compound 2a reached sub-micromolar activities in these aggressive cancer cells and, thus, features a promising drug candidate for the efficient treatment of hormone- or multidrug-resistant cancer types.



Lawsone (6)

Maria susai Boobalan *et al*[30]., 2014 reported the antioxidant active mannich base 1-[anilino (phenyl) methyl] pyrrolidine-2,5-dione (APMPD) have been synthesized and its FT-IR and FT-Raman vibrational spectra were recorded within the region of 4000 cm^{-1}, 50 cm^{-1} respectively. The molecular geometric parameters of APMPD have been computed using HF and DFT model theories. The energies of APMPD are calculated for all the eight possible conformers using B3LYP method at 6-311++G (d, p) basis set. From the computational results, the M1 conformer was identified as the most stable conformer of APMPD. The stable conformer was compared with experimental crystal geometry, which again fortifies the results of conformer analysis. The fundamental vibrations of the molecule are assigned according to the characteristic region and the literature report. The predicted highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energy gap provide vivid idea on charge transfer behavior of APMPD. The molecular electrostatic potential (MEP) and Mulliken charge analysis indicate the feasible electrophilic and nucleophilic reactive sites on APMPD. The thermodynamic

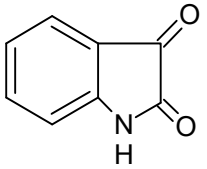
properties (heat capacity, entropy, and enthalpy) of the title compound at various temperatures are calculated in gas phase.

Poul Erik Hansen *et al*[31]., 2016 proposed mannich bases of 2-Hydroxy-3, 4, 5, 6-tetrachlorobenzene are chosen as an exemplary case for tautomeric mannich bases. Molecular structures are calculated. OH stretching frequencies are rationalized based on DFT calculations. Intrinsic deuterium isotope effects on ^{13}C chemical shifts in the M-form are estimated based on OH bond lengths. The observed deuterium isotope effects on ^{13}C chemical shifts are demonstrated to be largely of equilibrium type except at ambient temperatures.

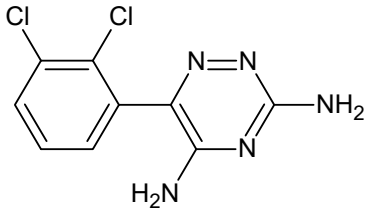
Gilish Jose *et al*[32]., 2017 reported the synthesis and biological evaluation of a new series of pyrrolo [3, 2-*c*] pyridine Mannich bases (7a-v). The mannich bases were obtained in good yields by one-pot three component condensation of pyrrolo [3, 2-*c*] pyridine scaffold (6a-c) with secondary amines and excess of formaldehyde solution in AcOH. The chemical structures of the compounds were characterized by ^{1}H NMR, ^{13}C NMR, LC/MS and elemental analysis. Single crystal X-ray diffraction has been recorded for compound 7k ($[C_{23}H_{29}ClN_4]^{+2}$, H_2O). The *in vitro* antimicrobial activities of the compounds were evaluated against various bacterial and fungal strains using agar diffusion method and broth micro dilution method. Compounds 7e**,** 7f**,** 7r**,** 7t**,** and 7u were showed good gram-positive antibacterial activity against *S. aureus, B. flexus, C. sporogenes and S. mutans*. Furthermore, *in vitro* anti-mycobacterial activity was evaluated against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) using MABA. Compounds 7r**,** 7t**,** and 7u were showed good anti-tubercular activity against *Mtb* (MIC ≥6.25 μg/mL). Among the tested compounds, 1-((4-chloro-2-(cyclohexylmethyl)-1*H*-pyrrolo [3, 2-*c*] pyridin-3-yl) methyl) piperidine-3-carboxamide (**7t**) was showed excellent anti-mycobacterial activity against *Mtb* (MIC <0.78 μg/mL) and low cytotoxicity against the HEK-293T cell line (SI >>25). Molecular docking of the active compounds against glutamate racemase (MurI) and *Mtb* glutamine synthetase were explained the structure-activity observed *in vitro*.

A.A.Kulkarni *et al*[33]., 2017 investigated a series of various schiff's and mannich base derivatives (N1–2 & ND1–6) of Lamotrigine (**7**) with (**8**) and substituted isatin were synthesized to get more potent anticonvulsant agents. The starting material for the

synthesis of various new schiff's and mannich base derivatives was isatin (1H-indole-2, 3-dione) which in turn was prepared from substituted isonitrosoacetanilide using aniline. Lamotrigine reacts with isatin & substituted isatin gave Schiff's bases (N1–2) which on reaction with various secondary amines (dimethylamine, diethylamine, morpholine) produced Mannich bases (ND1–6). The structures of newly synthesized compounds were characterized by using TLC, UV, FT-IR, ^{1}HNMR and studied for their anticonvulsant activity. Anticonvulsant activity of all the derivatives was evaluated by MES method using phenobarbitone sodium & lamotrigine as standard drugs and % reduction of time spent by animals in extension, flexion, clonus, and stupor phase were noted. Compounds ND-4 and ND-6 showed significant anticonvulsant activity when compared with that of standard drugs. The remaining all compounds show moderate activity. Biological activity data of the synthesized derivatives revealed that, the synthesized derivatives are good anticonvulsant agents as compared to lamotrigine.



Isatin (7)



Lamotrigine (8)

Neelima *et al*[34]., 2016 reported schiff base metal complexes are well-known to intercalate DNA. The La (III) complexes have been synthesized such that they hinder with the role of the topoisomerases, which control the topology of DNA during the cell-division cycle. Although several promising chemotherapeutics have been developed, on the basis of Schiff base metal complex DNA intercalating system they did not proceed past clinical trials due to their dose-limiting toxicity. Here in,we discuss an alternative compound, the La (III) complex, [La $(L^{1})_2Cl_3$]. $7H_2O$ based on a schiff base ligand 2, 3-dihydro-1*H*-indolo-[2, 3-*b*]-phenazin-4(5*H*)-ylidene) benzothiazole–2-amine (L^{1}), and report *in vitro* cell studies. Results of antitumor activity using cell viability assay, reactive oxygen species (ROS) generation and

nuclear condensation in PC-3 (Human, prostate carcinoma) cells show that the metal complex is more potent than ligand. La (III) complexes have been synthesized by reaction of lanthanum (III) salt in 1:2 M ratio with ligands L^1 and 3-(ethoxymethylene)-2, 3-dihydro-1*H*-indolo [2, 3-*b*]-phenazin-4(5*H*)-ylidene) benzathiazole-2-amine (L^2) in methanol. The ligands and their La (III) complexes were characterized by molar conductance, magnetic susceptibility, elemental analyses, FT–IR, UV–Vis, $^1H/^{13}C$ NMR, thermogravimetric, XRD, and SEM analysis.

CHAPTER-III

RESEARCH OBJECTIVES

RESEARCH OBJECTIVES

Nitogen atom containing analogues possess significant pharmacological activities. Various hetero cyclic nucleuses containing nitrogen as a hetero atom such as Indole, Imidazole, Benztriazole, Benzoxazole, Triazole, Tetrazole, and Benzimidazole possess varied pharmacological activities. Imidazole moiety is a versatile lead molecule. It is nitrogen containing heterocyclic ring which possess wide range of biological activities such as anti-bacterial, anti-cancer, anti-tubular, anti-fungal, analgesic and anti-HIV activities.

Among, the various synthetic products, the first choice of selection of nucleus in our current research work are isatin. Isatin nucleus have attracted the attention of medicinal chemists due to their wide range of biological activities like as anti-microbial, anti-cancer, anti-convulsant activity and acts as a anxiogenic, sedative and potent antagonist on atrial natriuretic peptide receptors in *In-vitro*. Isatin derivatives reported exhibit interesting pharmacological activities. The work was planned to perform that isatin was substituted by benzylamine at C-3 position to produce Benzylimino-isatin. The Benzylimino-isatin was substituted by various secondary amines at N-1 position to produce Benzylimino-isatin *Mannich* bases, exhibits interesting pharmacological activities.

The present study is aimed to carry out the synthesis of Benzylimino-isatin *Mannich* base derivatives. For newer derivatives, Benzylimino-isatin as lead molecule by combining several secondary amines followed by formaldehyde will be synthesized as per literature method. Then, structures will be assigned by FT-IR and ^{1}H NMR analysis. Further, the compounds are evaluated for biological activities such as anti-cancer and anti-microbial activities.

In this aim, our current research work was initiated.

CHAPTER-IV

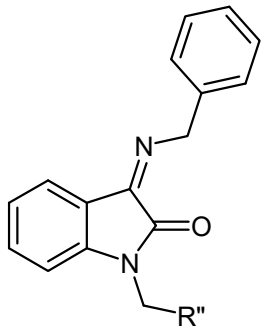
PLAN OF THE WORK

Isatin nucleus is a versatile lead molecule and has attracted the attention of medicinal chemists and has wide range of biological activities such as anti-microbial, anti-cancer, anti-convulsant activity and acts as an anxiogenic, sedative and potent antagonist on atrial natriuretic peptide receptors in *in-vitro*. Isatin derivatives reported exhibit interesting pharmacological activities.

On the basis of these considerations, and in continuation to the previous efforts of our laboratory team in the area of synthesis of anti proliferative agents as well as antibacterial agents

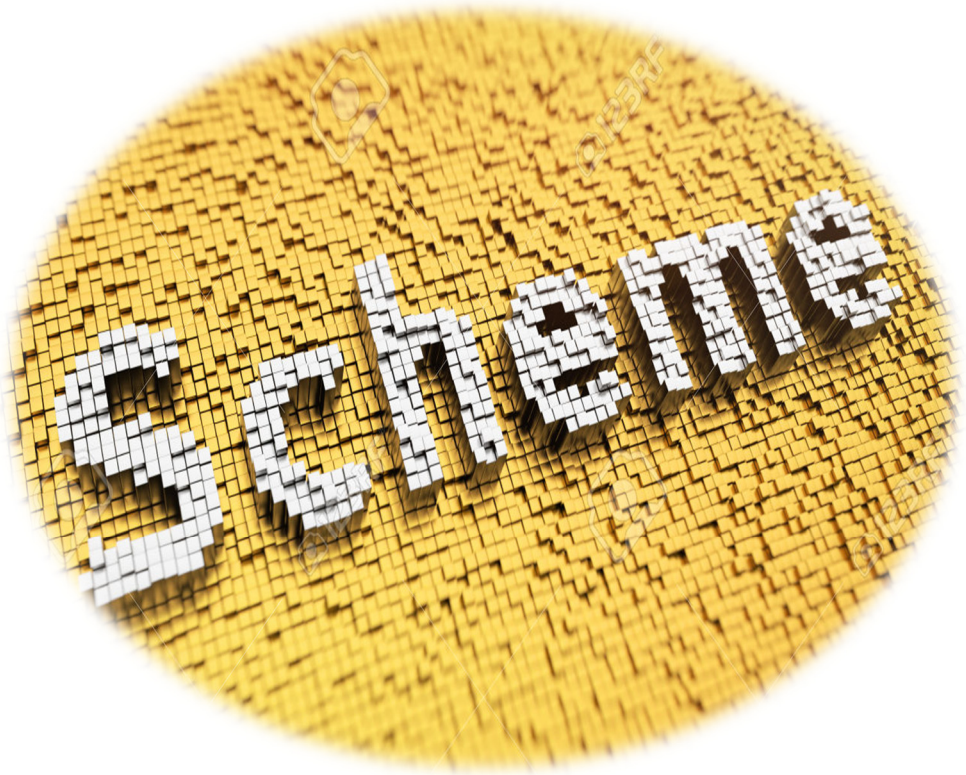
The plan of this thesis were

1. To manually design the synthetic compounds bearing benzylimino-isatin scaffold.
2. The above designed compounds subjected to docking study by the way using Molegro Virtual Docker (MVD)
3. To select the compounds based on the best docking scores for further studies.
4. To synthesize of benzylimino-isatin *Mannich* bases by using specific reagents and conditions.
5. To characterize the newly synthesized compounds by means of their FT-IR and ^{1}H NMR.
6. To evaluate anti-microbial activity by disc diffusion method and well diffusion method and *In vitro* anti cancer activity against HeLa cancer cell line.



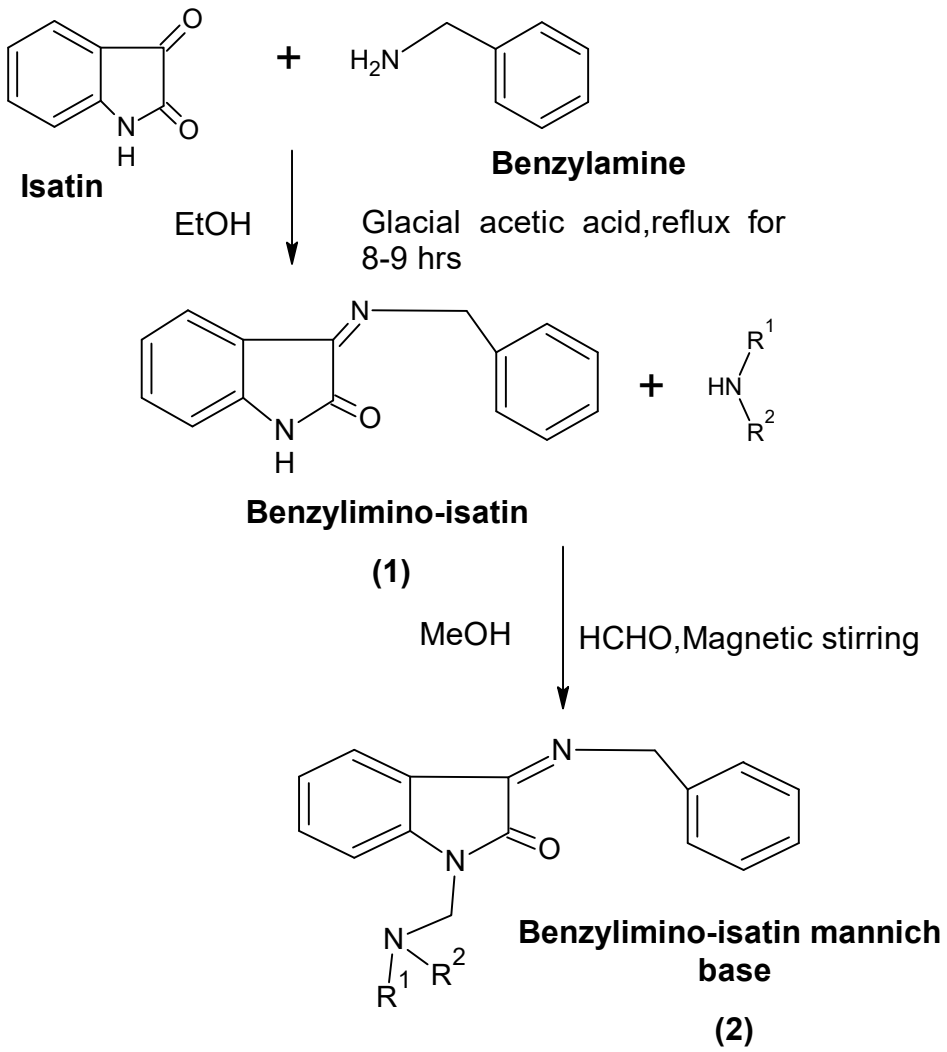
Benzylimino-isatin Lead Compound

CHAPTER-V



SCHEME OF THE WORK

The scheme of the work deals with the synthesis of Benzylimino-isatin Schiff base and Benzylimino-isatin *mannich* base.



R"= Dimethylamine

Diphenylamine

Piperazine

1-Methyl piperazine

Morpholine

Pthalimide

CHAPTER-VI

MATERIALS AND METHODS

Software used in docking study

The docking study was performed using Molegro Virtual Docker Evaluation Version (MVD 2013.6.0), which focused on molecular docking simulations

Chemicals

All the chemicals used were analytical grade and were purchased from UNIVERSAL SCIENTIFIC APPLIANCES in Madurai.

TABLE 1: Company name of the chemicals used in synthesis.

S.NO	CHEMICALS	COMPANY NAME
1.	Isatin	Sisco Research Laboratory
2.	Benzylamine	High Purity Laboratory Chemicals
3.	Glacial acetic acid	Fisher Scientific
4.	Dimethlyamine	Loba Chemie
5.	Piperazine	HiMedia Laboratories
6.	Pthalimide	Santai Labs
7.	Diphenylamine	Sisco Research Laboratory
8.	1-Methly piperazine	Spectrochem
9.	Morpholine	Spectrochem
10	Formaldehyde	Sai Chemicals
11.	Ethanol	Sisco Research Laboratory
12.	Methanol	Molychem
13.	Chloroform	Sisco Research Laboratory
14.	DMSO	Fischer inorganics & aromatics Ltd
15.	Ethyl acetate	Chem India Petrochems
16.	Hexane	Roshan Chemical Industry
17.	Benzene	Alpha Chemika
18.	Pet. ether	Lab-Chem Corporation
19.	Silica gel G	Thomas baker

Instruments

Infra-red spectrophotometer

IR spectra were measured using SHIMADZU IR TRACER-100 IR spectrophotometer at IRC, Kalasalingam University, Srivilliputtur, Virudhunagar (DT).

Nuclear magnetic resonance spectrophotometer

^{1}H NMR spectra was measured at Indian Institute of Science, Bangalore using SHIMADZU-400 instrument by $CDCl_3$ as solvent.

TABLE 2: Instruments and its model

S.NO	INSTRUMENT	MODEL
1.	Digital balance	ELB 300 SHIMADZU
2.	Magnetic stirrer	MCS 66
3.	Rota vaccum evaporater	RVO 400
4.	Melting point apparatus	M-565
5.	FT-IR Spectrophotometer	IRTRACER-100 SHIMADZU
6.	^{1}H-NMR Spectrometer	SHIMADZU-400
7.	MTT Assay reader	Bio-Rad-680

EXPERIMENTAL METHODS

DOCKING STUDY

Reductase enzyme (PDB Code 1kf6) was retrieved from Brookhaven protein data bank. The docking study was performed using Molegro Virtual Docker Evaluation Version (MVD 2013.6.0), which focused on molecular docking simulations. While performing molecular docking, both the protein and ligand molecules were imported into the workspace. All the crystallographic water molecules were removed from the protein during import process. Further, protein and ligands were subjected to molecules preparation. The option to detect cavities in the preparation window was used to identify cavities within the enzyme 1kf6. During this computational procedure, maximum numbers of cavities were fixed to 10, grid resolution 0.80 Å and probe size 1.2 Å; while the other parameters were set as default. The objective of protein preparation was to remove errors like bond order, bond position, explicitly hydrogen, flexible torsions etc.

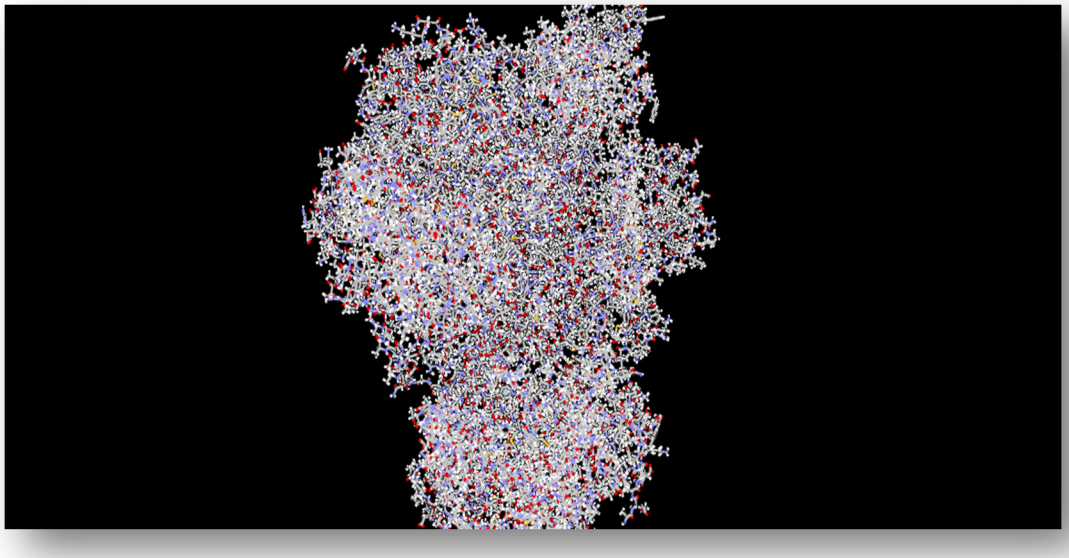


FIG.11: PROTEIN STRUCTURE OF 1KF6

While performing docking, the binding radius, grid resolution and maximum iterations parameters were set to 15 Å, 0.3 Å and 2,000 respectively. The docking algorithm was set to simplex evolution population

size 50, RMSD thresholds 1.00 Å for cluster similar poses, and RMSD threshold 1.00 Å for ignoring similar poses (for multiple runs only), and 5 independent runs were conducted, each of these runs returned to a single final solution (pose). Only negative lowest-energy representative cluster returned from each of them after completion of docking and similar poses were removed keeping the best scoring one. The clusters were ranked through comparison of the conformation of the lowest binding energy in each cluster. The first lowest binding free energy pose was selected for the analysis of the docking results and the other best docking complex also was analyzed for various intermolecular interactions. In the beginning, a total of five different cavities with different surface area and volume were mapped in E. coli Quinol-Fumarate Reductase with Bound Inhibitor HQNO enzyme (1kf6) using the option detect cavity in MVD software. The volume and surface area of these cavities, Cavity 1 had highest volume **(6637.06 Å3)** along with largest surface area **(18324.5 Å2)**.

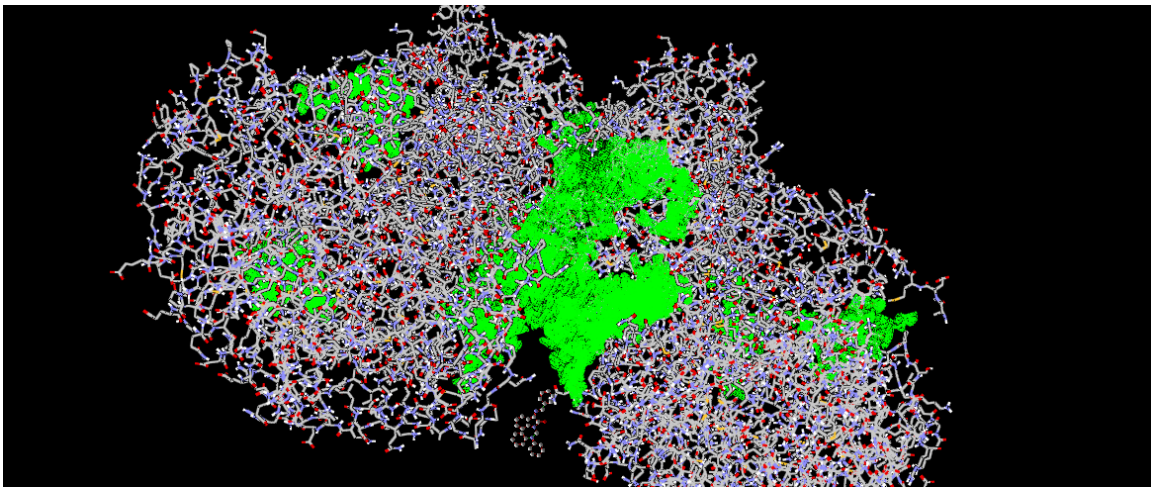


FIG.12: ENZYME OF 1KF6 SHOWING ALL FIVE CAVITIES AS 1,2,3,4 AND 5.

The docking score of the interaction between the active site of enzyme 1kf6 and ligand molecules (**IM1 - 20**) has been depicted in **Table 3.**

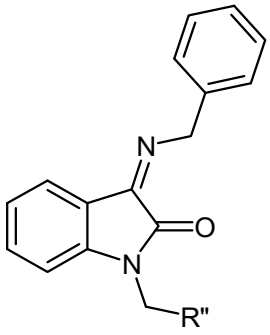
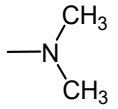
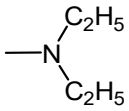
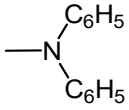
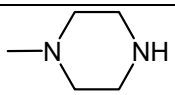
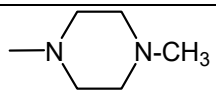
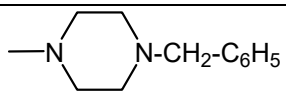
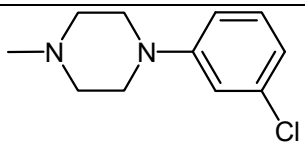
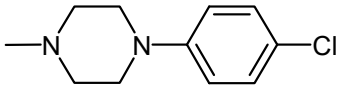
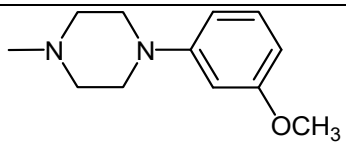
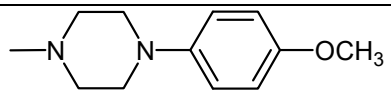
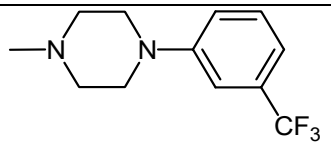
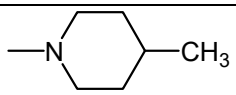
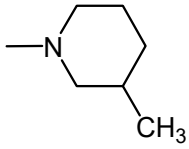
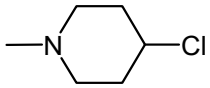
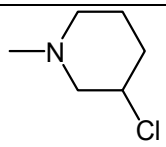
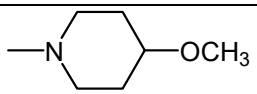
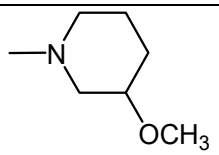
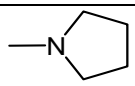
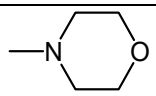
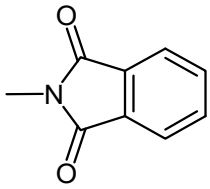


Table 3: Docking Scores of benzylimino-isatin Mannich bases (***Compound IM1 - 20***) in the cavity of 1kf6 enzyme:

Com. Code	R"	Mol-dock score	Re-rank score	H-bond score
IM1		**-146.225**	**-105.988**	**-3.693**
IM2		-121.342	-109.877	-2.142
IM3		**-183.245**	**-140.876**	**-1.402**
IM4		**-167.199**	**-123.731**	**-3.887**
IM5		**-177.219**	**-129.985**	**-2.241**
IM6		-138.142	-117.346	-1.187
IM7		-126.763	-107.934	-1.754
IM8		-129.543	-112.347	-1.564
IM9		-110.654	-103.741	-0.942

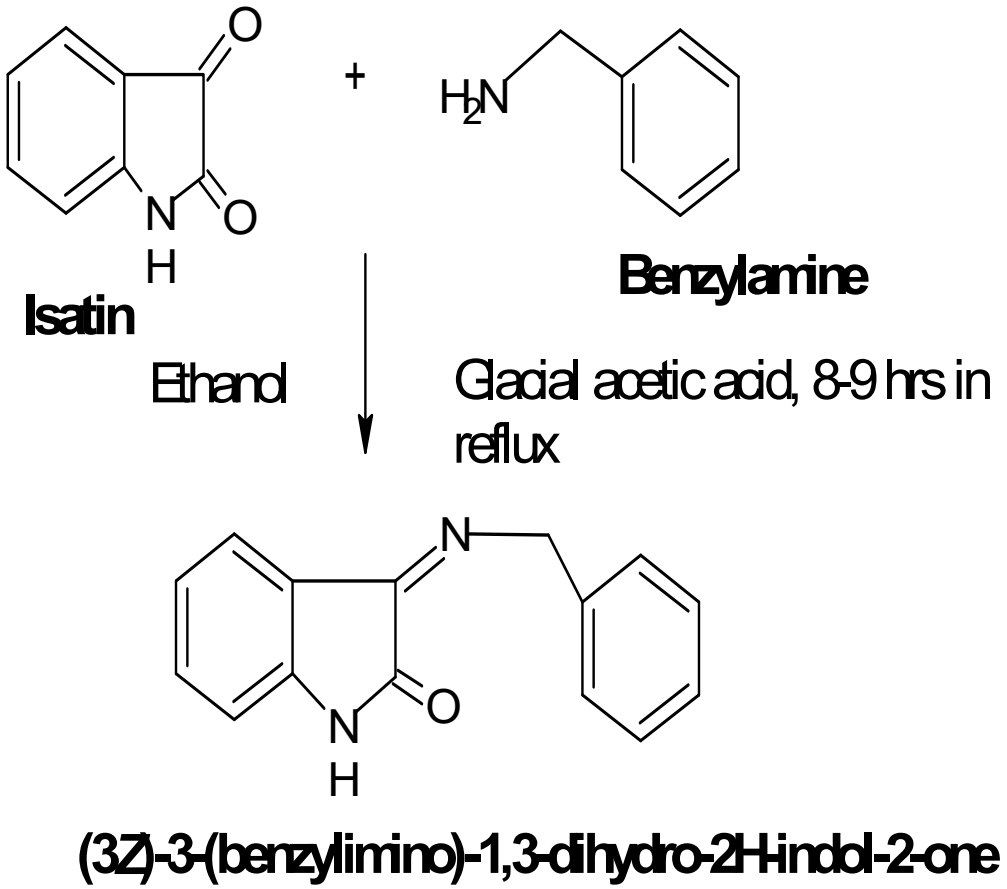
IM10		-112.384	-106.273	-1.046
IM11		-98.483	-78.285	-0.282
IM12		-132.482	-112.392	-1.842
IM13		-137.158	-107.384	-1.743
IM14		-121.354	-102.387	-1.372
IM15		-129.475	-99.452	-1.492
IM16		-84.453	-71.564	-0.995
IM17		-104.264	-93.657	-1.548
IM18		-85.375	-76.457	-0.578
IM19		**-186.228**	**-135.796**	**-1.511**
IM20		**-143.335**	**-114.225**	**-2.298**

SYNTHESIS

Step 1

Synthesis of benzylimino-isatin [(3Z)-3-(benzylimino)-1, 3-dihydro-2H-indol-2-one] (IS1):

Indole-2, 3-dione (isatin 0.001 mole) was dissolved in ethanol (30 mL) in a 250 ml round bottomed flask fitted with a condenser. Benzylamine (0.001 mole) was dissolved in ethanol (10 mL) added to the mixture, followed by 3-4 drops of glacial acetic acid. The reaction mixture was heated under reflux for 8-9 hrs. The precipitate formed was filtered, recrystallized from ethanol and dried at hot air oven.The purity of the product was ascertained by TLC and the R_f value is 0.6271.Finally,the obtained titled compound is [(3Z)-3-(benzylimino)-1, 3-dihydro-2H-indol-2-one].

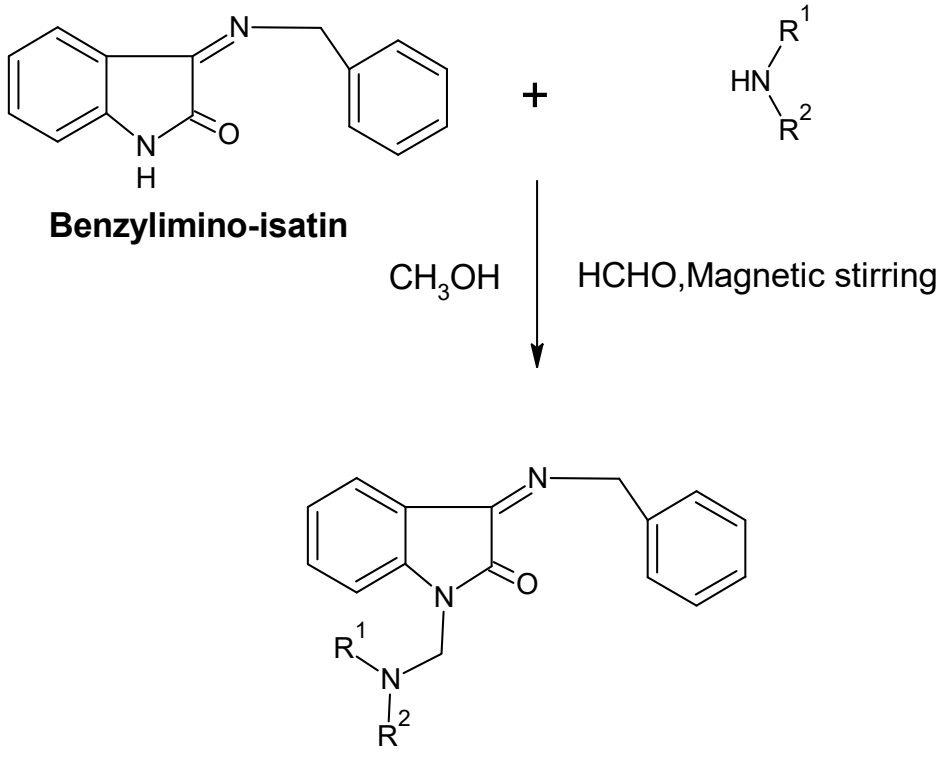


Step 2

Synthesis of benzylimino- isatin *mannich* bases

General procedure

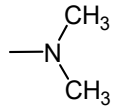
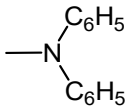
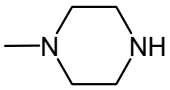
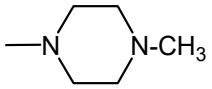
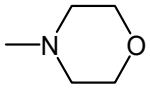
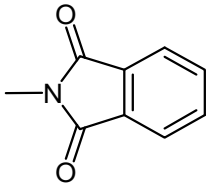
The above synthesized [(3Z)-3-(benzylimino)-1, 3-dihydro-2H-indol-2-one] (0.01 mole) was dissolved in methanol (10 mL) and add appropriated secondary amines separately (0.01 mole), then add formaldehyde (0.01 mole) (37%) was added to the mixture with stirring. The stirring was held by magnetic stirrer continued for 3h and then it was left at room temperature for 24h. The precipitate was collected and recrystallized from methanol and dried at hot air oven. The purity of the product was ascertained by TLC. Finally, the titled compounds was obtained and characterized by spectral datas.



Benzylimino-isatin

Benzylimino-isatin mannich base

TABLE: 4 Synthesized compounds

S.No	Compound code	R''	IUPAC name of the synthesized compounds
1.	**IM1**		[(3Z)-3-(benzylimino)-1-[(dimethylamino) methyl]-1, 3-dihydro-2H-indol-2-one].
2.	**IM3**		[(3Z)-3-(benzylimino)-1-[(diphenylamino) methyl]-1, 3-dihydro-2H-indol-2-one].
3.	**IM4**		[(3Z)-3-(benzylimino)-1-[(piperazin-1-yl methyl)-1, 3-dihydro-2H-indol-2-one].
4.	**IM5**		[(3Z)-3-(benzylimino)-1-[(4-methylpiperazin-1-yl) methyl]-1,3-dihydro-2H-indol-2-one].
5.	**IM19**		[(3Z)-3-(benzylimino)-1-[(morpholin-4-yl methyl)-1,3-dihydro-2H-indol-2-one].
6.	**IM20**		2-{[(3Z)-3-(benzylimino)2-oxo-2,3-dihydro-1H-indol-1yl)methyl]}-1H-isoindole-1,3(2H)-dione.

BIOLOGICAL EVALUATION

IN VITRO STUDIES

- Anti-microbial activity
- Anti-cancer activity

Invitro anti-microbial studies:

a) Well diffusion method

b) Disc diffusion method

Media preparation:

Bacterial medium (Muller Hinton Agar)

36 g of Muller Hinton media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 15 LB pressure for 15 minutes. The sterilized media were poured into petri dishes. The solidified plates were pored with 6 mm dia.cork borer. The plates with wells were used for the antibacterial studies.

Test against standard controls

Commercially available antibiotic disc ciprofloxacin (10 µg) and was used as standard control for the entire test microorganism.

Bacterial inoculums:

Bacterial inoculums were prepared by inoculating a loopful of test organisms such as *E.coli, B.subtilis, P.aeruginosa* and *S.aureus* in 5 mL nutrient broth and incubated at 37ºC for 5 to 8hrs till a moderate turbidity was developed. The turbidity was matched with 0.5 Mc Farland standard (WHO drug information, 1993) and the culture was diluted with sterile distilled water if necessary which corresponds to the cell density of 1.5×10^8 (CFU/mL).

FIG.13: LAMINAR AIR FLOW CHAMBER

Well diffusion method[36]

Antibacterial of the synthetic compound was tested using well diffusion method. The prepared culture plates were inoculated with different selected strains of bacteria using streak plate method. Wells were made on the agar surface with 6mm cork borer. The synthesized compounds were loaded into the well using sterile syringe. The plates were incubated 24 hours at 37 ± 2ºC bacterial plates. The plates were observed for inhibition zone formation around the well. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The various concentration of benzylimino-isatin (**IS1**) and benzylimino-isatin *Mannich* base derivatives (IM1, IM3, IM4, IM5, IM19 & IM20) were used throughout the study. The derivatives of 5µg/0.1mL,10µg/0.1mL,25µg/0.1mL,50µg/0.1mL & 100µg/0.1 mL were tested against 4 different bacterial pathogens such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* for their antimicrobial activity. It was demonstrated by well diffusion assay and reported.

Disc diffusion method[37]

Principle

Paper discs impregnated with specific antibiotics or the test substances were placed on the surface of the Muller Hinton agar medium inoculated with the target organisms, which was recommended for the diffusion of antimicrobial agents as described in NCCLS approved standard. The plates incubated and the zones of inhibition around each disc were measured.

Procedure

Petri dishes were prepared with a base layer of Mueller Hinton agar. At twenty-four hours culture of selected bacteria like *Bacillus subtilis,Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli* were mixed with physiological saline solution and the turbidity was corrected by adding sterile physiological saline until a Mc Farland turbidity standard of 0.5 (10^8 CFU/mL). Afterwards, a top layer of Mueller Hinton agar inoculated with 0.2% microbial suspension was poured over the petri dishes. Sterile filter discs (6 mm in diameter) were impregnated with benzylimino-isatin (**IS1**) and various derivatives of benzylimino-isatin mannich bases such as IM1, IM3, IM4, IM5, IM19 & IM20 (5, 10, 25, 50, 100 µg/0.1mL) dissolved in DMSO and placed on the inoculated plates. Ciprofloxacin was used as control. The plates were incubated at 35ºC for 18 hours microbial growth inhibition was determined as the diameter of the inhibition zones around the discs and reported.

***Invitro* anti-cancer studies**

Anti-cancer activity was studied for the various concentrations (10µg/1mL, 50µg/1mL, 100µg/1mL, 250µg/1mL & 500µg/1mL) of benzylimino-isatin (IS1) and benzylimino-isatin *Mannich* base derivatives (IM1, IM3, IM4, IM5, IM19 & IM20) against cervical (HeLa) cancer cells.

Procedure[35]

The HeLa cells were trypsinized and seeded into 96-wellplates (1000 cells per well) containing Iscove's Modified Dulbecco's medium with 10% serum, with the medium being changed according to the experimental design after 24 h.

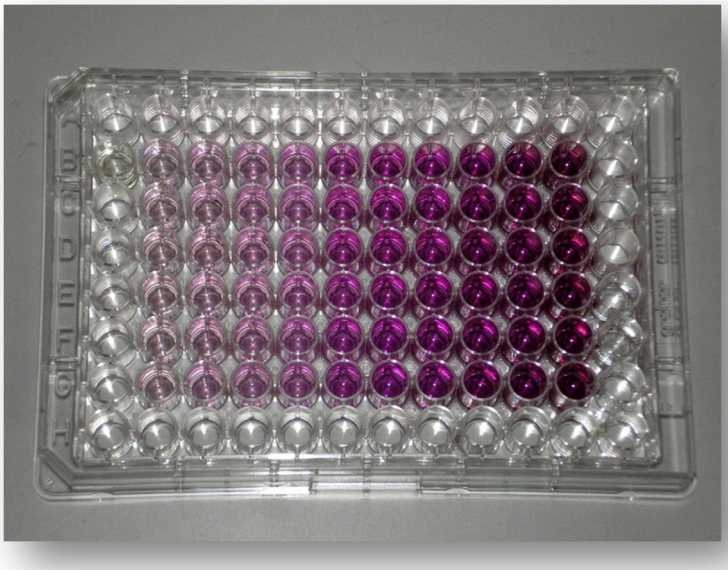


FIG.14: MTT assay 96 well plates

The cells were treated with various concentrations (10µg/1mL, 50µg/1mL,100µg/1mL,250µg/1mL & 500µg/1mL) of synthetic chemical such as benzylimino-isatin (IS1) and benzylimino-isatin *Mannich* base derivatives (IM1, IM3, IM4, IM5, IM19 & IM20) and incubated at 37ºC in a humidified incubator with 5% CO_2 . After 24 h of incubation, 10µL of MTT was added to each well, and the plates were incubated for a further 4 hrs. Following incubation the formazan crystals were dissolved in 100 mL of dissolving buffer and the absorbance read at 595 nm using a plate reader (Bio-Rad, Model 680). 5FU is used as standard.IC_{50} values were determined further based on a 50% reduction in cell viability. The cells were stained with trypan blue and live cells were enumerated in order to determine % growth inhibition. The experiments were done in triplicates. The morphology changes on cancer cells observed under phase contrast microscope.

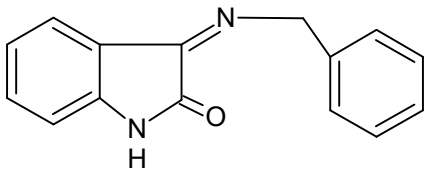
CHAPTER-VII

CHARACTERIZATION

CHARACTERIZATION

IS1

Structure**:**



Compound code : IS1

IUPAC name : (3Z)-3-(benzylimino)-1, 3-dihydro-2H-indol-2-one

Colour : Brown

Melting point : 75-78ºC

Molecular formula : $C_{15}H_{12}N_2O$

Molecular weight : 236.26

Percentage yield : 70.75%

R_f value : 0.6271

IR (KBr, cm^{-1}) : 1024, 1618, 1716, 2819, 2881, 3199

NMR **(**400 $CDCl_3$) δ [ppm] : 4.73 (s, 2H, CH_2 - benzyl)

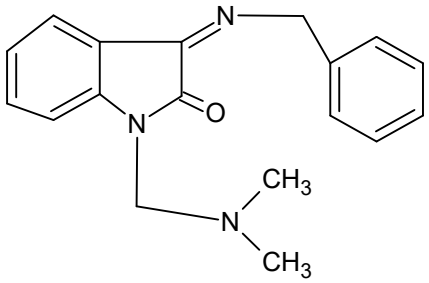
7.00-7.11 (m, 5H, ArH of benzyl)

7.25-7.41 (m, 4H, ArH of Isatin)

7..89 (s, 1H,NH of isatin,D_2O Exchangable)

IM1

Structure:



Compound code : IM1

IUPAC name : (3Z)-3-(benzylimino)-1-[(dimethylamino) methyl]-1,3- dihydro-2H-indol-2-one

Colour : Dark brown

Melting point : 80-90ºC

Molecular formula : $C_{18}H_{19}N_3O$

Molecular weight : 293.36

Percentage yield : 60.76%

R_f value : 0.7673

IR (KBr, cm^{-1}) : 1053, 1610, 1716, 2870, 2941, 3059

NMR (400 $CDCl_3$) δ [ppm] : 2.72 (s, 6H $(CH_3)_2$)

3.63 (s, 2H, N- CH_2- N)

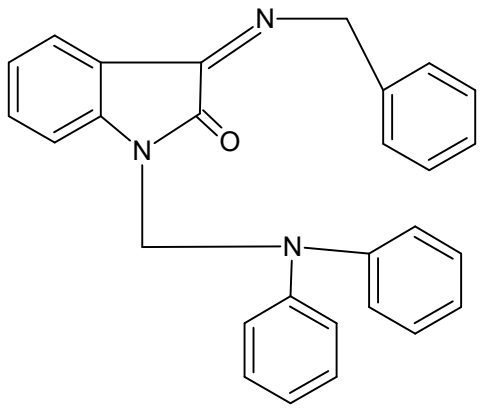
4.71(s,2H,CH_2 of benzyl)

7.05-7.19 (m, 5H, ArH of benzyl)

7.39-7.85 (m,4H, ArH of Isatin)

IM3

Structure**:**



Compound code : IM3

IUPAC name : (3Z)-3-(benzylimino)-1-[(diphenylamino)methyl]-1,3-dihydro-2H-indol-2-one

Colour : Brown

Molecular formula : $C_{28}H_{23}N_3O$

Molecular weight : 417.50

Percentage yield : 76.78%

R_f value : 0.5627

IR (KBr, cm^{-1}) : 1018, 1651, 2831, 2945

NMR (400 $CDCl_3$) δ [ppm] : 3.81 (s, 2H, N- CH_2-N)

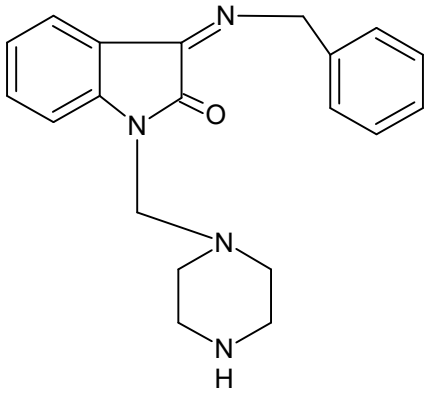
4.70 (s, 2H, CH_2 of benzyl)

7.05-7.19(m, 5H, ArH of benzyl)

7.39-7.85 (m,4H, ArH of Isatin)

IM4

Structure:



Compound code : IM4

IUPAC name : (3Z)-3-(benzylimino)-1-[(piperazin-1-yl methyl)-1, 3- dihydro-2H-indol-2-one

Colour : Pale yellow

Melting point : 70-76ºC

Molecular formula : $C_{20}H_{22}N_4O$

Molecular weight : 334.41

Percentage yield : 73.12%

R_f value : 0.7797

IR (KBr, cm^{-1}) : 1051, 1610, 1718, 2804, 2879, 3057

NMR (400 $CDCl_3$) δ [ppm] : 2.50-2.92 (m, 10H, piperazinyl H)

4.15 (s, 2H, N-CH_2-N)

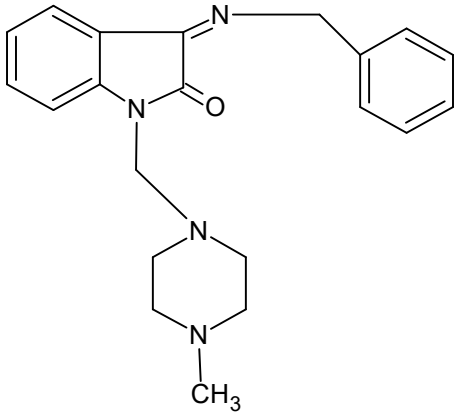
5.32 (s, 2H, CH_2 of benzyl)

7.00-7.20(m,5H,ArH of benzyl)

7.74-7.89 (m, 4H, ArH of Isatin)

IM5

Structure:



Compound code : IM5

IUPAC name : (3Z)-3-(benzylimino)-1-[(4-methylpiperazin-1yl) methyl]-1,3-dihydro-2H-indol-2-one

Colour : Dark red

Molecular formula : $C_{21}H_{24}N_4O$

Molecular weight : 348.44

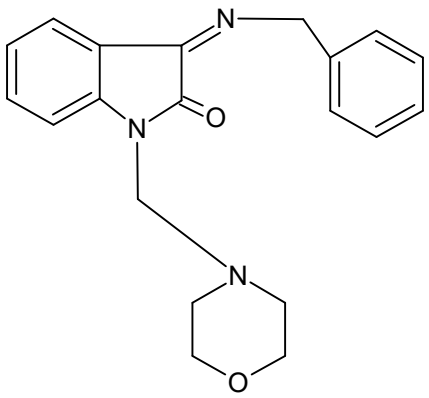
Percentage yield : 90.76%

R_f value : 0.9426

IR (KBr, cm^{-1}) : 1020, 2831, 2945

IM19

Structure**:**



Compound code : IM19

IUPAC name : (3Z)-3-(benzylimino)-1-[(morpholin-4-yl methyl)-1,3-dihydro-2H-indol-2-one

Colour : Pale red

Molecular formula : $C_{20}H_{21}N_3O_2$

Molecular weight : 335.39

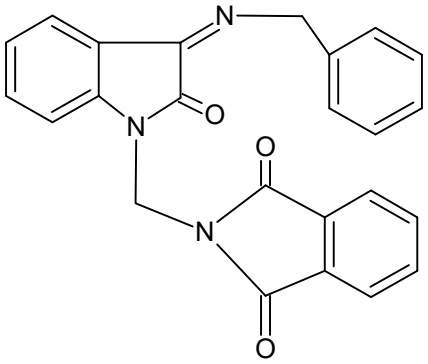
Percentage yield : 83.88%

R_f value : 0.9787

IR (KBr, cm^{-1}) : 1020, 1651, 2831, 2943

IM20

Structure:



Compound code : IM20

IUPAC name : (3Z)-3-(benzylimino)2-oxo-2, 3-dihydro-1H-indol1yl)methyl]-1H-isoindole-1,3(2H)-dione

Colour : Pale yellow

Melting point : 70-79ºC

Molecular formula : $C_{24}H_{17}N_3O_3$

Molecular weight : 395.41

Percentage yield : 87.12%

R_f value : 0.6235

IR (KBr, cm^{-1}) : 1060, 1610, 1707, 2895, 2954, 3489

CHAPTER-VIII

RESULTS & DISCUSSION

Docking studies of different benzylimino-isatin derivatives (**IM1, IM2, IM3, IM4, IM5, IM6, IM7, IM8, IM9, IM10, IM11, IM12, IM13, IM14, IM15, IM16, IM17, IM18, IM19 & IM20**) were performed successfully inside the highest volume cavity measure the affinity were Mol Dock, re-rank and H-bond score of the above designed compounds with *E. coli* Quinol-Fumarate Reductase with Bound Inhibitor HQNO enzymes (1kf6). It facilitated us to identify relevant H-bond interaction (via H-bond score) that occurs between each ligand and the amino acid residues of the active site of enzyme in order to obtain conformations achieved with these molecules. Although the key moieties of all the compounds were similar but each individual compound showed interaction up to a variable extent.

The best Docking Score of the compounds (**table 5**) were selected for the synthesis as well as biological evaluation, among various compounds, **IM19 ([(3Z)-3-(benzylimino)-1-[(morpholin-4-ylmethyl)-1,3-dihydro-2H-indol-2-one])** showed highest MolDock score (-186.228) as well as re-rank score (-135.796) as compared to the other benzylimino-isatin derivatives. While compound **IM16** showed poor MolDock score (-84.453) as well as re-rank score (-71.564) as compared with other *Mannich* bases of benzylimino-isatin derivatives. Docking view, hydrophobic and steric interactions and secondary view of **IM19 ([(3Z)-3-(benzylimino)-1-[(morpholin-4-ylmethyl)-1,3-dihydro-2H-indol-2-one])** with 1kf6 enzyme have been shown in Figs. (15, 16 & 17) respectively.

Table. 5: Selected Docking Scores of compounds in the cavity 1 of 1kf6 Enzyme

Com. Code	Mol-dock score	Re-rank score	H-bond score
IS1	-136.065	-104.478	-3.179
IM1	-146.225	-105.988	-3.693
IM3	-183.245	-140.876	-1.402
IM4	-167.199	-123.731	-3.887
IM5	-177.219	-129.985	-2.241
IM19	-186.228	-135.796	-1.511
IM20	-143.335	-114.225	-2.298

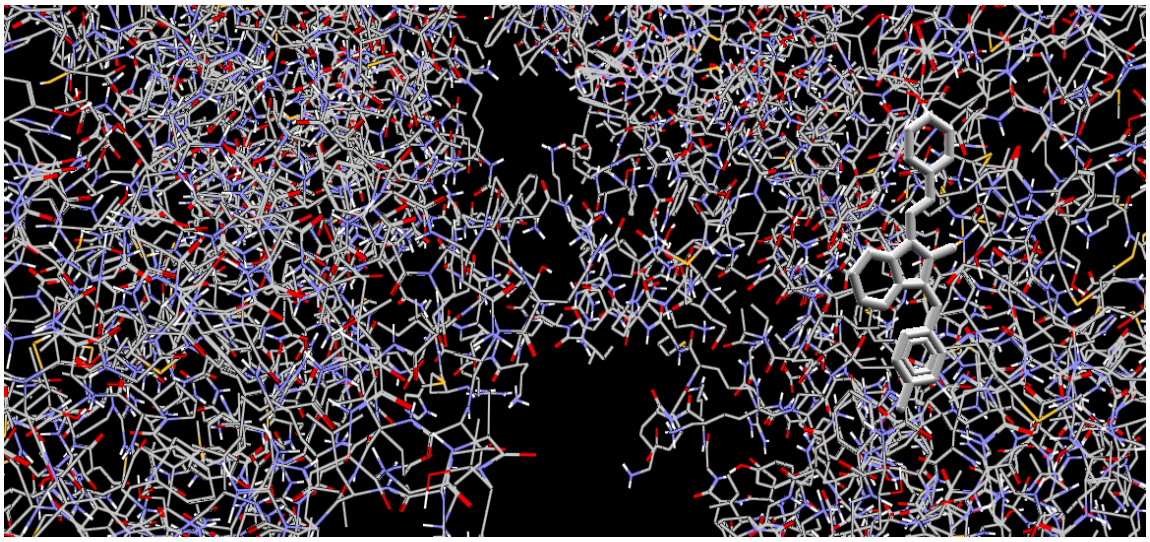


FIG.15: DOCKING VIEW OF LIGAND (IM19) AND PROTEIN (1KF6)

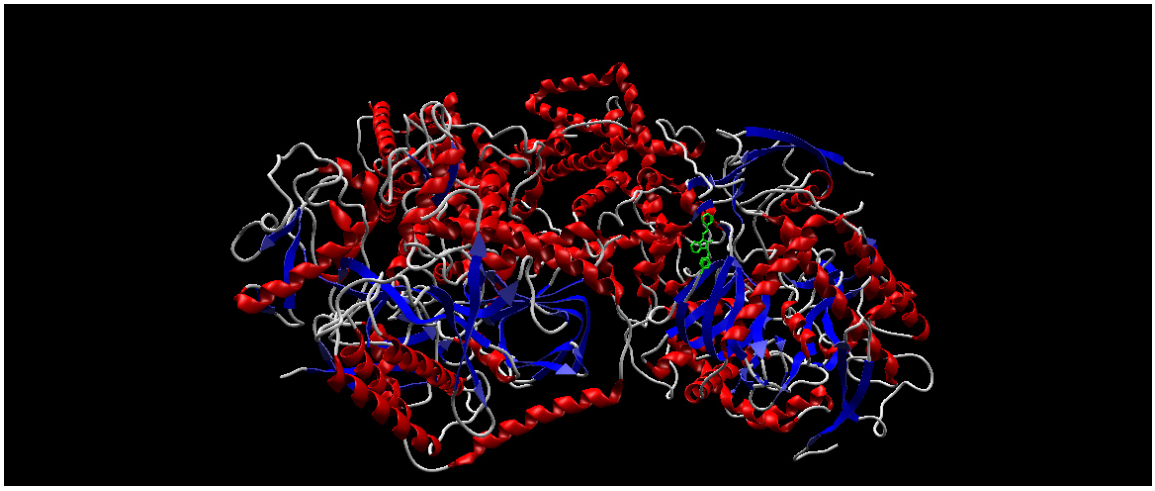


FIG.16: SECONDARY VIEW OF COMPOUND IM19 (GREEN COLOUR) WITH 1KF6 ENZYME HAVING PDB ID: 1KF6 (RED AS A-HELICES AND BLUE AS B-SHEETS)

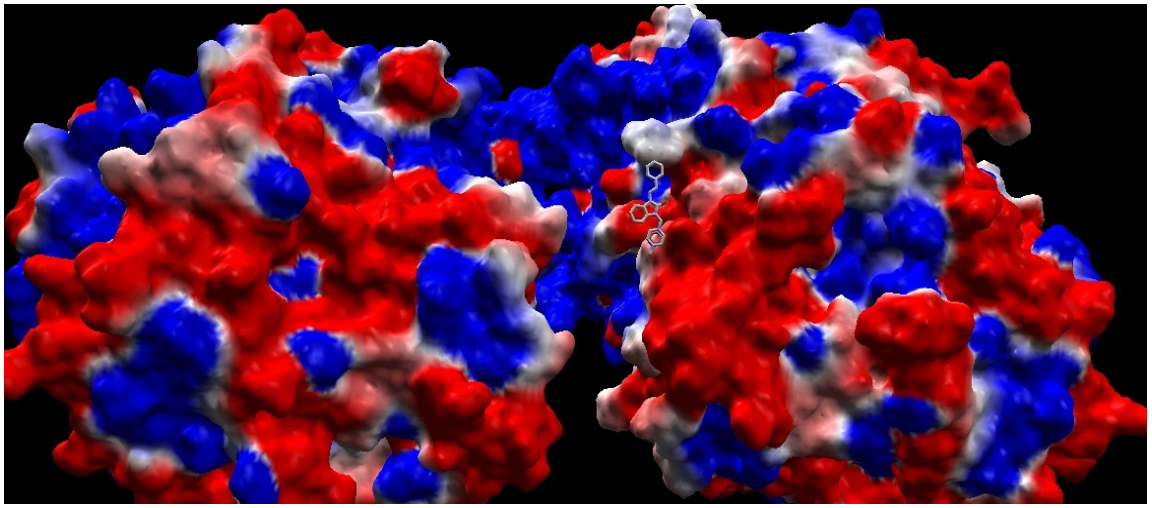


FIG.17: HYDROPHOBIC INTERACTIONS OF COMPOUND IM19 WITH 1KF6 ENZYME SHOWING HYDROPHOBIC AND HYDROPHILIC SURFACES

SOLUBILITY DATA

The title compounds of **IS1 [(3Z)-3-(benzylimino)-1, 3-dihydro-2H-indol-2-one)]** were synthesized from isatin and benzylamine in the presence of glacial acetic acid/EtOH, reflux with 8-9 h, as reported literature. The *Mannich* bases of benzylimino-isatin **IM1 ([(3Z)-3-(benzylimino)-1-[(dimethylamino)methyl] -1,3-dihydro-2H-indol-2-one])**, **IM3 ([(3Z)-3-(benzylimino)-1-[(diphenylamino) methyl]-1, 3-dihydro-2H-indol-2-one])**, **IM4 ([(3Z)-3- (benzylimino)-1- [(piperazin-1-yl methyl)-1, 3-dihydro-2H-indol-2-one])**, **IM5([(3Z)-3- (benzylimino)-1- [(4-methylpiperazin-1-yl) methyl] -1,3-dihydro- 2H-indol-2-one]), IM19([(3Z)-3- (benzylimino)-1-[(morpholin-4-ylmethyl) -1,3-dihydro-2H-indol-2-one])** and **IM20 (2-{[(3Z)-3-(benzylimino)2-oxo-2,3-dihydro-1H-indol-1yl)methyl]}-1H-isoindole 1,3(2H)-dione)** were prepared by the reaction of benzylimino isatin Schiff bases **IS1[(3Z)-3- (benzylimino)- 1,3-dihydro- 2H-indol-2-one)]** with appropriate secondary amines in the presence of HCHO at room temperature. The solubility data of the synthesized compounds were posted on **table 6**. From the table all the compounds were freely soluble in ethanol, methanol and DMSO moreover all the synthesized compounds were insoluble in water, hexane and pet.ether.

TABLE.6: SOLUBILITY DATA OF THE SYNTHESIZED COMPOUNDS

S. No	Com. Code	Solvents							
		MeOH	EtOH	$CHCl_3$	Et. acetate	Water	Hexane	Pet ether	DMSO
1.	**IS1**	+++	+++	++	+	-	-	-	++
2.	**IM1**	+++	+++	++	+	-	-	-	++
3.	**IM3**	+++	+++	++	+	-	-	-	++
4.	**IM4**	+++	+++	++	+	-	-	-	++
5.	**IM5**	+++	+++	++	+	-	-	-	++
6.	**IM19**	+++	+++	++	+	-	-	-	++
7.	**IM20**	+++	+++	++	+	-	-	-	++

+++ Freely soluble; ++ readily soluble; + Soluble; - Insoluble.

CHARACTERIZATION

The structures of the targeted compounds were confirmed by the means of FT-IR, ^{1}H NMR spectral data. The synthesized compounds **IS1 [(3Z)-3-(benzylimino)-1, 3-dihydro-2H-indol-2-one)]** and **IM1 [(3Z)-3-(benzylimino)-1-[(dimethylamino) methyl]-1,3-dihydro-2H-indol-2-one**], **IM3 [(3Z)-3-(benzylimino)-1-[(diphenylamino) methyl]-1, 3-dihydro-2H-indol-2-one], IM4 [(3Z)-3-(benzylimino)-1-[(piperazin-1-yl methyl)-1,3-dihydro-2H-indol-2-one],IM5[(3Z)-3-(benzylimino)-1-[(4-methylpiperazin-1-yl)methyl]-1,3-dihydro-2H-indol-2-one],IM19[(3Z)-3-(benzylimino)-1-[(morpholin-4-yl methyl)- 1,3-dihydro-2H-indol-2-one]** and **IM20 [(2-{[(3Z)-3-(benzylimino)2-oxo-2,3-dihydro-1H-indol-1yl)methyl]}-1H-isoindole-1,3(2H)-dione)]** of benzylimino-isatin *Mannich* bases, IR spectra were showed peak at 1640 for the conformation of C=N (azomethine) and one intensive peak were showed at 1715 for the conformation of C=O (Ketone), all the compounds shows peaks at 2860 and 2840 for the conformation of *Mannich* bases N-CH_2-N.

The synthesized compound benzylimino-isatin **IS1 [(3Z)-3-(benzylimino)-1, 3-dihydro-2H-indol-2-one)]** showed a singlet at 4.81 ppm which assigned for CH_2 of C_6H_5-CH_2. Multiplet shows at 7.0-7.14 which assigns 5H for aromatic ring for benzyl and multiplet shows 7.27-7.72 ppm which assign for aromatic ring for isatin and one singlet shows at 8.0 ppm which assign for NH proton for isatin.

The synthesized compounds of benzylimino-isatin *Mannich* bases, all the compounds shows singlet at 4.81 ppm which assign for 2H proton for the CH_2 of benzyl, one multiplet shows at 7.0-7.14 ppm which assign 5H for aromatic ring for benzyl and multiplet shows at 7.27-7.72 ppm which assign for 4H for isatin aromatic proton and one singlet at 4.03 shows 2H for N-CH_2-N of *Mannich* bases.

ANTIBACTERIAL ACTIVITY

The antibacterial activities of synthesized compounds **IS1 [(3Z)-3-(benzylimino)-1, 3-dihydro-2H-indol-2-one)] , IM1 [(3Z)-3-(benzylimino)-1-[(dimethylamino) methyl]-1, 3-dihydro-2H-indol-2-one], IM3 [(3Z)-3-(benzylimino)-1-[(diphenylamino) methyl]-1, 3-dihydro-2H-indol-2-one], IM4 [(3Z)-3-(benzylimino)-1-[(piperazin-1-yl methyl)-1, 3-dihydro-2H-indol-2-one], IM5 [(3Z)-3-(benzylimino)-1-[(4-methylpiperazin-1-yl) methyl]-1,3-dihydro-2H-indol-2-one], IM19 [(3Z)-3-(benzylimino)-1-[(morpholin-4-yl methyl)-1,3-dihydro-2H-indol-2-one]** and **IM20 [2-{[(3Z)-3-(benzylimino)2-oxo-2,3-dihydro-1H-indol-1yl)methyl]}-1H-isoindole-1,3(2H)-dione]** were evaluated by the determination of their zone of inhibition by using disc diffusion method as well as well diffusion method. A panel of gram-positive (*S. aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633) and gram negative (*E. coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027) bacteria was used. The quinolone antibacterial agent ciprofloxacin was used as reference drug.

Disc diffusion method

The entire compound shows moderate antibacterial activity. Compounds **IM3 [(3Z)-3-(benzylimino)-1-[(diphenylamino) methyl]-1, 3-dihydro-2H-indol-2-one], IM4 [(3Z)-3-(benzylimino)-1-[(piperazin-1-yl methyl)-1, 3-dihydro-2H-indol-2-one]** and **IM20 [2-{[(3Z)-3-(benzylimino)2-oxo-2,3-dihydro-1H-indol-1yl)methyl]}-1H-isoindole-1,3(2H)-dione**] were shows good activity about 19 mm zone of inhibition at 100µg/0.1ml concentration in gram –ve bacteria (*P. aeruginosa*) while compare with standard fluoroquinolone antibacterial ciprofloxacin have 30 mm zone of inhibition at 100µg/0.1ML concentration in gram –ve bacteria (*P. aeruginosa*) as well as gram +ve bacteria (*B. subtilis*). The antibacterial results by disc diffusion method were posted at **table 7**.

Well diffusion method:

The Compounds **IM3 [(3Z)-3-(benzylimino)-1-[(diphenylamino) methyl]-1, 3-dihydro-2H-indol-2-one], IM4 [(3Z)-3- (benzylimino)-1-[(piperazin-1-yl methyl)-1, 3-dihydro-2H-indol-2-one]** and **IM20 [2-{[(3Z)-3-**

(benzylimino)2-oxo-2,3-dihydro-1H-indol-1yl)methyl]}-1H-isoindole-1,3 (2H)- dione] were shows good activity about 20 mm zone of inhibition at 100µg/0.1ML concentration in gram –ve bacteria (*P. aeruginosa*) while compound 6 shows significant activity about 20mm zone of inhibition at 50 µg/ml concentration in gram –ve bacteria (*P. aeruginosa*). The standard fluoroquinolone antibacterial ciprofloxacin have 30mm zone of inhibition at 50 µg/ml concentration in gram –ve bacteria (*P. aeruginosa*) as well as gram +ve bacteria (*B. Subtilis*). The antibacterial results by well diffusion method were posted at **table 8**.

TABLE.7: ANTIMICROBIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS BY DISC DIFFUSION METHOD

S. No	Com. Code	Zone of inhibition (mm)											
		S. aureus			*B. subtilis*			*P.aeruginosa*			*E. Coli*		
		25	50	100	25	50	100	25	50	100	25	50	100
1.	IS1	9	9	9	9	10	9	10	12	14	9	10	10
2.	IM1	9	11	13	10	10	14	10	12	13	9	9	11
3.	IM3	10	10	12	10	11	11	10	14	19	9	10	11
4.	IM4	9	9	9	11	14	14	11	12	19	9	9	11
5.	IM5	9	9	10	9	10	14	10	12	14	9	10	10
6.	IM19	11	11	12	10	14	12	9	10	15	9	9	11
7.	IM20	9	9	9	9	9	9	10	15	19	9	10	11
8.	Ciprofloxacin	20 (10 µg/ml)			30 (10 µg/ml)			30 (10 µg/ml)			20 (10 µg/ml)		

Concentration 25, 50, 100 µg/ml.

TABLE.8: ANTI-MICROBIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS BY WELL DIFFUSION METHOD

S.No	Com. Code	Zone of inhibition (mm)											
		S. aureus			*B. subtilis*			*P.aeruginosa*			*E. Coli*		
		25	50	100	25	50	100	25	50	100	25	50	100
1.	IS1	10	10	10	10	11	15	11	13	15	10	11	11
2.	IM1	12	12	13	11	15	10	10	20	20	10	10	12
3.	IM3	11	11	13	11	11	12	10	10	20	10	11	12
4.	IM4	10	10	10	12	15	15	12	13	20	10	10	12
5.	IM5	10	10	10	10	11	15	11	13	15	10	11	11
6.	IM19	12	12	13	11	15	13	10	20	20	10	10	12
7.	IM20	10	10	10	10	10	10	10	10	20	10	11	11
8.	Ciprofloxacin	20 (10 µg/ml)			30 (10 µg/ml)			30 (10 µg/ml)			20 (10 µg/ml)		

Concentration 25, 50, 100 µg/ml.

WELL DIFFUSION METHOD

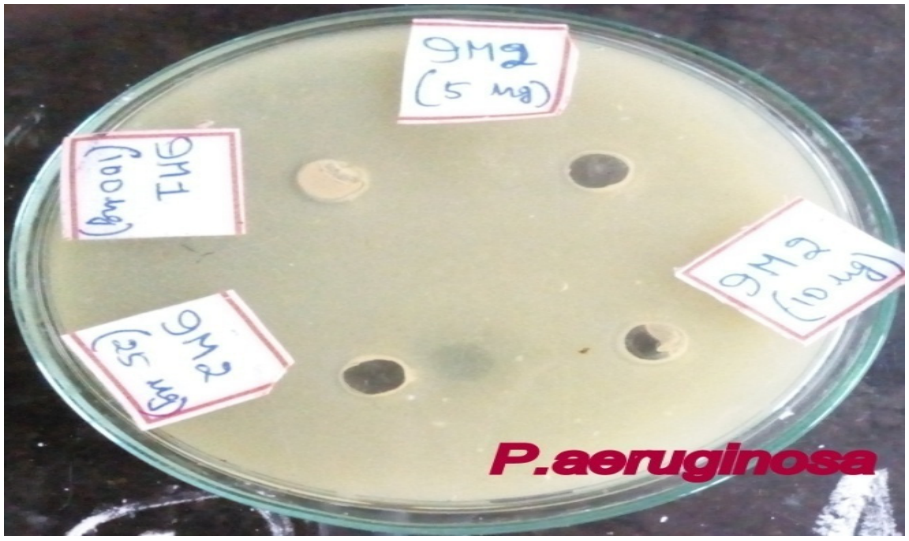


FIG.18: ZONE OF ANTI-MICROBIAL INHIBITION OF IM1 (100 µG/0.1 ML) AND IM2 (5, 10 & 25 µG/0.1 ML) AGAINST *P.AERUGINOSA*

FIG.19: ZONE OF ANTI-MICROBIAL INHIBITION OF IM2 (50 & 100 µG/0.1 ML) AND IM3 (5 & 10µG/0.1 ML) AGAINST *P.AERUGINOSA*

FIG.20: ZONE OF ANTI-MICROBIAL INHIBITION OF IM3 (25, 50 & 100 µG/0.1 ML) AND IM4 (5µG/0.1 ML) AGAINST *P.AERUGINOSA*

FIG.21: ZONE OF ANTI-MICROBIAL INHIBITION OF IM4 (10, 25, 50 & 100 µG/0.1 ML) AGAINST *P.AERUGINOSA*

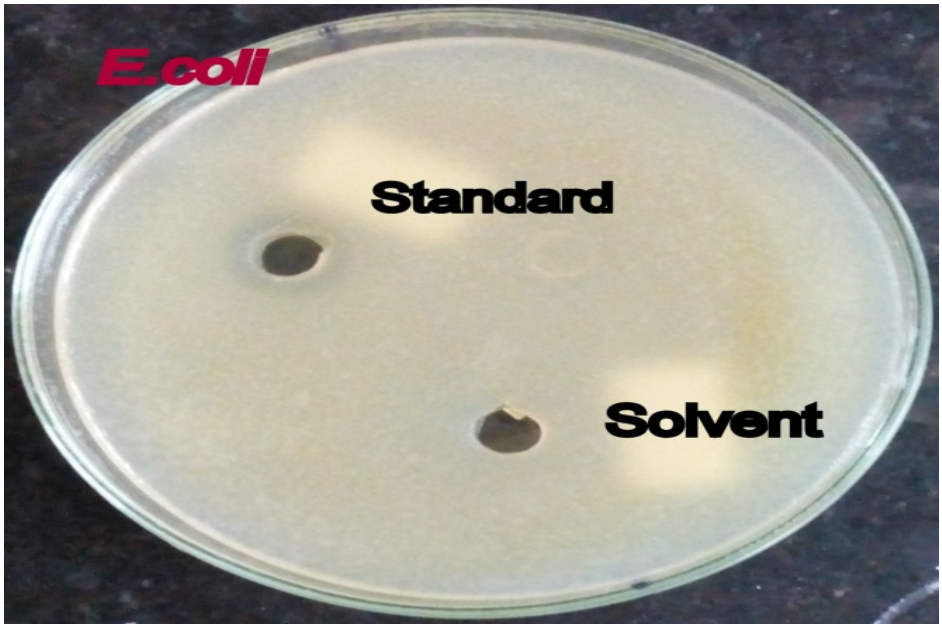


FIG.22: ZONE OF ANTI-MICROBIAL INHIBITION OF STANDARD (CIPROFLOXACIN-10 µG/ML) AND SOLVENT (DMSO) AGAINST *E.COLI*

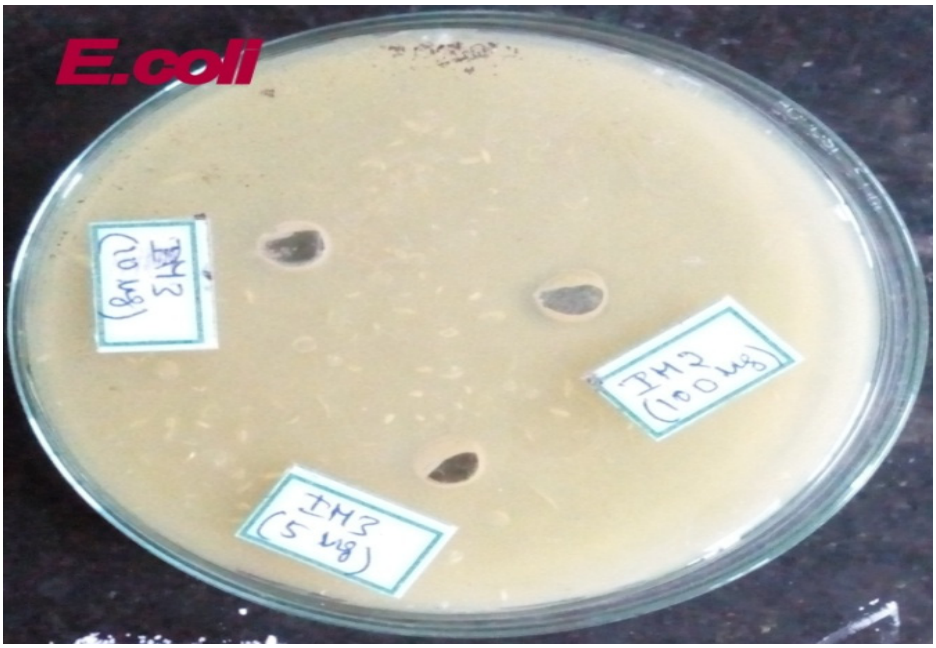


FIG.23: ZONE OF ANTI-MICROBIAL INHIBITION OF IM2 (100 µG/0.1 ML) AND IM3 (5 & 10 µG/0.1 ML) AGAINST *E.COLI*

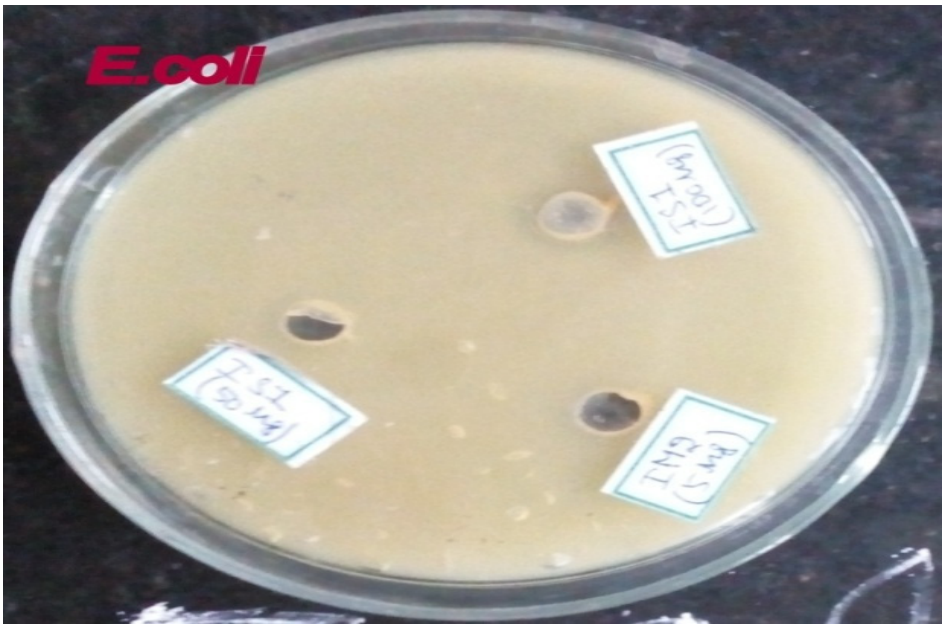


FIG.24: ZONE OF ANTI-MICROBIAL INHIBITION OF IM2 (5 µG/0.1 ML) AND IS1 (50 & 100 µG/0.1 ML) AGAINST *E.COLI*

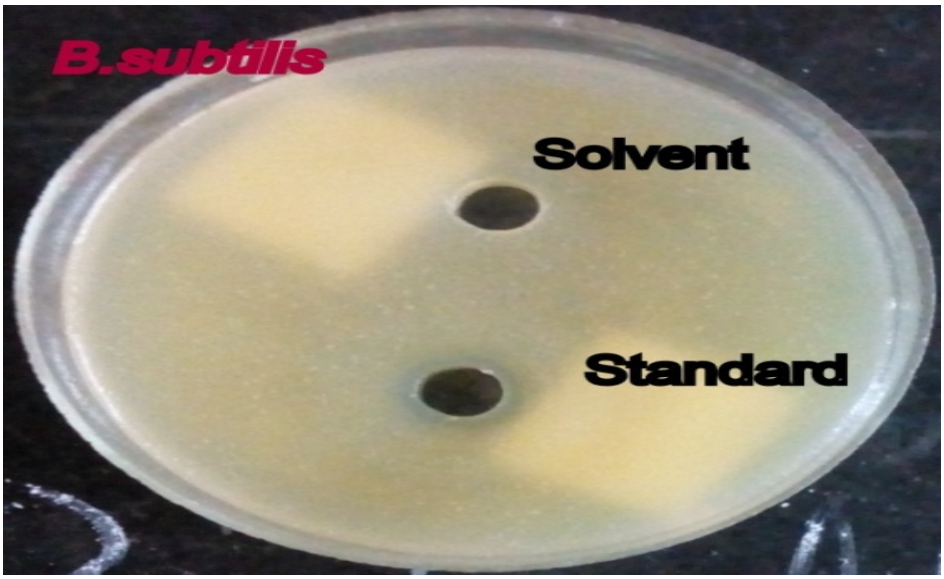


FIG.25: ZONE OF ANTI-MICROBIAL INHIBITION OF SOLVENT (DMSO) AND STANDARD (CIPROFLOXACIN-10µG/0.1 ML) AGAINST *B.SUBTILIS*

FIG.26: ZONE OF ANTI-MICROBIAL INHIBITION OF IM2 (25, 50 &100µG/0.1 ML) AGAINST *B.SUBTILIS*

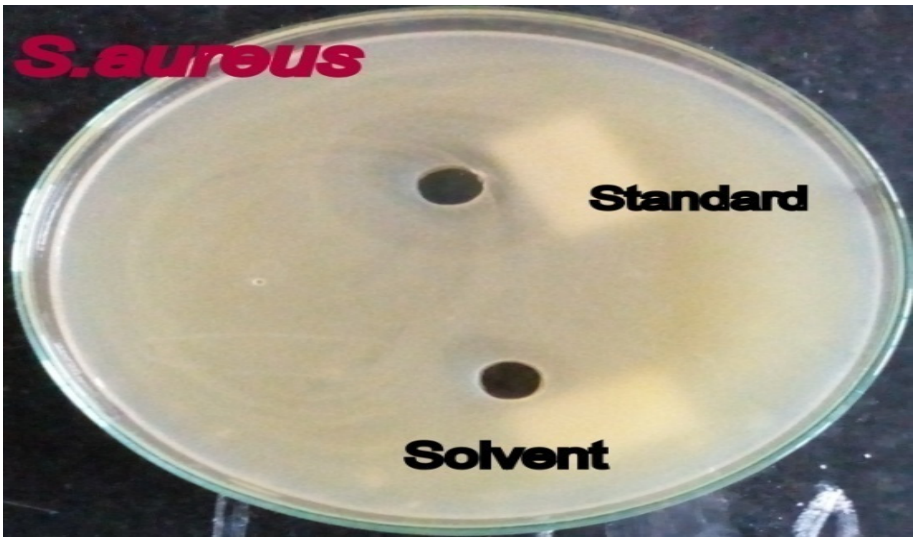


FIG.27: ZONE OF ANTI-MICROBIAL INHIBITION OF SOLVENT (DMSO) AND STANDARD (CIPROFLOXACIN-10µG/0.1 ML) AGAINST *S.AUREUS*

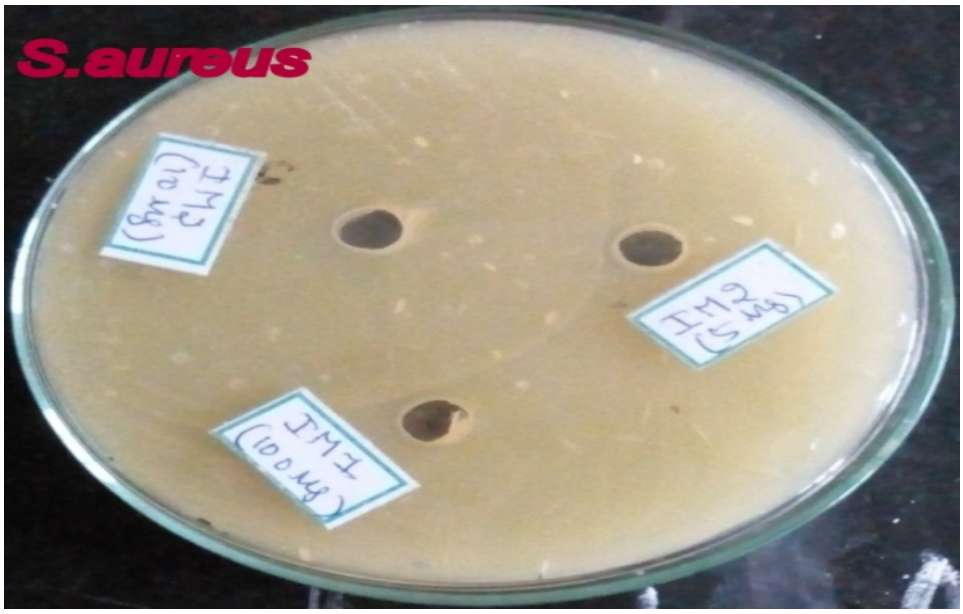


FIG.28: ZONE OF ANTI-MICROBIAL INHIBITION OF IM1 (100 µG/0.1 ML) AND IM2 (5 & 10 µG/0.1 ML) AGAINST *S.AUREUS*

ANTICANCER ACTIVITY

The anticancer activity for synthesized compounds was performed in HeLa cell line by MTT assay method. The results of the synthesized compounds in the form of IC_{50} was posted at table 8.From the **table 9**, the compound **IM3 [(3Z)-3-(benzylimino)-1-[(diphenylamino) methyl]-1, 3-dihydro-2H-indol-2-one]** and **IM20 2-{[(3Z)-3-(benzylimino)2-oxo-2,3-dihydro-1H-indol-1yl)methyl]}-1H-isoindole-1,3(2H)-dione** has good activity about 0.327, 0.392 µg/ml respectively while other compounds shows moderate activity. 5FU used as standard and it have IC_{50} about 0.21 µg/ml.

TABLE.9: *IN VITRO* CELL GROWTH INHIBITORY (IC_{50}) VALUES OF THE SYNTHESIZED COMPOUNDS

S.No	Compound code	IC_{50} (µg / mL)
1.	IS1	0.423
2.	IM1	0.426
3.	IM3	0.392
4.	IM4	0.417
5.	IM5	0.432
6.	IM19	NT
7.	IM20	0.327
8.	5FU	0.21

IC_{50} **:** Compound concentration required to inhibit tumor cell proliferation by 50%.

NT: Not tested

CHAPTER-IX

SUMMARY AND CONCLUSION

Our current research work deals with manually designed, library of compounds (IM1-20) bearing benzylimino-isatin scaffold that performed docking study with *E.coli Quinol-Fumarate Reductase* with Bound Inhibitor HQNO enzyme (**1kf6)** [PDB code 1kf6] using Molegro Virtual Docker Evaluation version (MVD 2013.6.0) the best compounds were selected based on their Moldock score in order to synthesis of benzylimino-isatin (IS1)([(3Z)-3-(benzylimino)-1,3-dihydro-2H-indol-2-one)]) and benzylimino-isatin mannich base derivatives with isatin as a parent moiety. Benzylimino-isatin (IS1) ([(3Z)-3-(benzylimino)-1,3-dihydro-2H-indol-2-one)]) and benzylimino-isatin mannich bases such as IM1 [(3Z)-3-(benzylimino)-1-[(dimethylamino) methyl]-1, 3-dihydro-2H-indol-2-one], IM3 [(3Z)-3-(benzylimino)-1-[(diphenylamino) methyl]-1, 3-dihydro-2H-indol-2-one], IM4 [(3Z)-3-(benzylimino)-1-[(piperazin-1-yl methyl)-1, 3-dihydro-2H-indol-2-one], IM5 [(3Z)-3-(benzylimino)-1-[(4-methylpiperazin-1-yl) methyl]-1,3-dihydro-2H-indol-2-one], IM19 [(3Z)-3-(benzylimino)-1-[(morpholin-4-yl methyl)-1,3-dihydro-2H-indol-2-one] & IM20 [2-{[(3Z)-3-(benzylimino)2-oxo-2,3-dihydro-1H-indol-1yl)methyl]}-1H-isoindole-1,3(2H)-dione] were synthesized by the suitable experimental procedure.

The synthesized compounds were characterized by melting point, solubility and subjected to various common analytical techniques like TLC, FT-IR and ^{1}H NMR and it is confirmed by means of their FT-IR and ^{1}H NMR spectrum reports were in complete agreement with the chemical structure.

The synthesized compounds were screened for *in-vitro* anti-microbial activity by disc diffusion method as well as well diffusion method and *in-vitro* anti-cancer activity by MTT assay method. Among the evaluated compound, three compounds such as IM3 [(3Z)-3-(benzylimino)-1-[(diphenylamino) methyl]-1, 3-dihydro-2H-indol-2-one], IM4 [(3Z)-3-(benzylimino)-1-[(piperazin-1-yl methyl)-1, 3-dihydro-2H-indol-2-one] & IM20 [2-{[(3Z)-3-(benzylimino)2-oxo-2,3-dihydro-1H-indol-1yl)methyl]}-1H-isoindole-1,3(2H)-dione] have good *in-vitro* anti-microbial activity at a dose of 50 and 100 µg/0.1 mL, when compared to standard drug Ciprofloxacin at a dose of 10 µg/0.1 mL.Among the evaluated compounds, two compounds such as IM3 [(3Z)-3-(benzylimino)-

1-[(diphenylamino) methyl]-1, 3-dihydro-2H-indol-2-one] & IM20 2-{[(3Z)-3-(benzylimino)2-oxo-2,3-dihydro-1H-indol-1yl)methyl]}-1H-isoindole-1,3(2H)-dione have good *in-vitro* anti-cancer activity with IC_{50} 0.392 µg/mL and 0.327 µg/mL aagainst human cervical cancer cell line (HeLa cell line) when compared to standard about 5FU with IC_{50} 0.21 µg/mL.

From the above facts it can be suggested that the benzylimino-isatin mannich base derivatives finds an interesting field of research because of their varied pharmacological activities.

CHAPTER-X

LIST OF ABBREVIATIONS

C_2H_5OH	Ethanol
CH_3COOH	Acetic acid
HCHO	Formaldehyde
CO_2	Carbon dioxide
$CHCl_3$	Chloroform
Pet.ether	Petroleum ether
$CDCl_3$	Deuterated chloroform
DMSO	Dimethyl sulfoxide
Std	Standard
Mm	Millimetre
µg	Microgram
ML	Milliliter
CFU	Colony forming units
WHO	World health organization
IR	Infra-red
NMR	Nuclear magnetic resonance
TLC	Thin layer chromatography
MTT	3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide
IS1	Benzylimino-isatin
IM1	Benzylimino-isatin mannich base 1
IM3	Benzylimino-isatin mannich base 3
IM4	Benzylimino-isatin mannich base 4
IM5	Benzylimino-isatin mannich base 5
IM19	Benzylimino-isatin mannich base 19
IM20	Benzylimino-isatin mannich base 20
E.coli	*Escherichia coli.*
P.aeruginosa	*Pseudomonas aeruginosa*
S.aureus	*Staphylococcus aureus*
B.substilus	*Bacillus subtilis*

CHAPTER-XI

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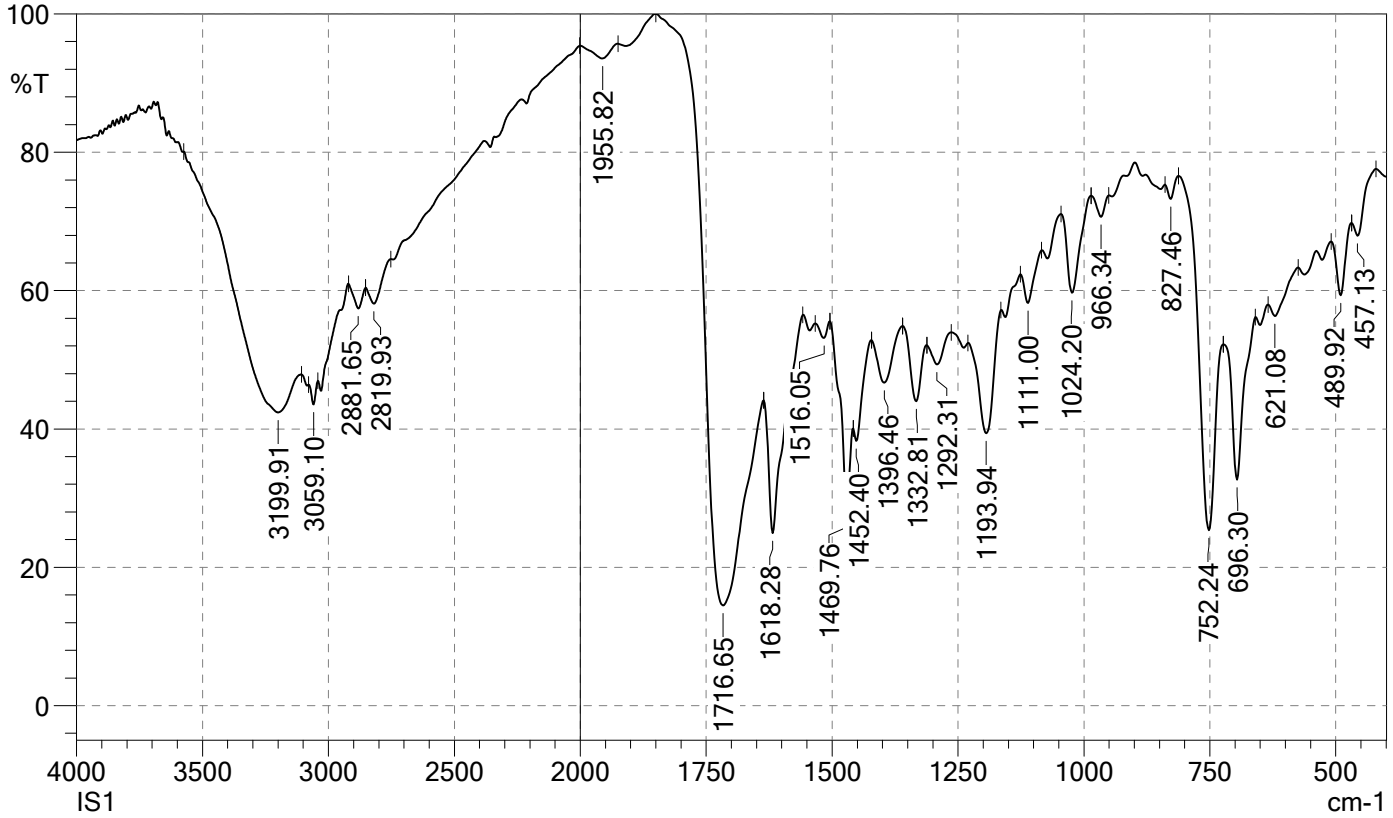
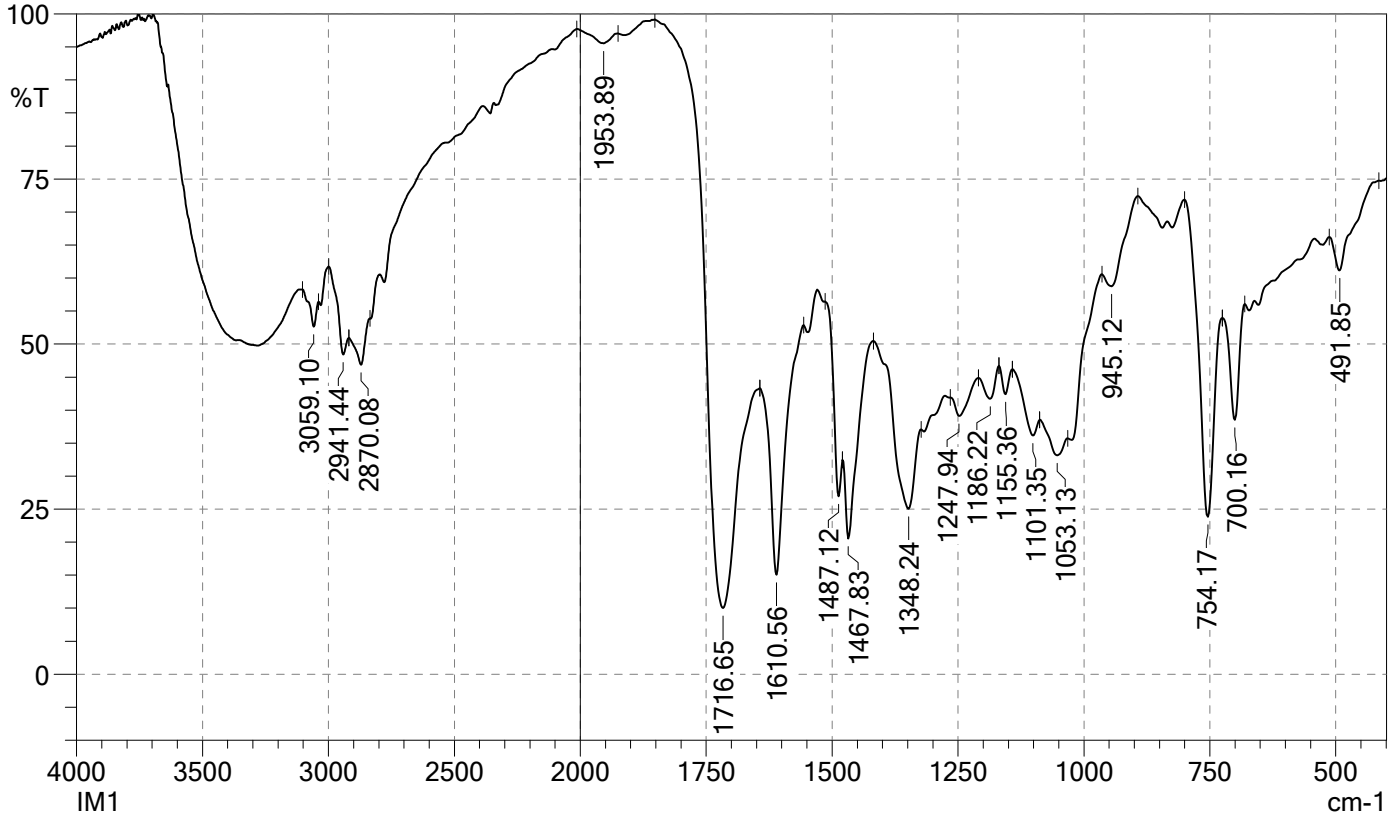
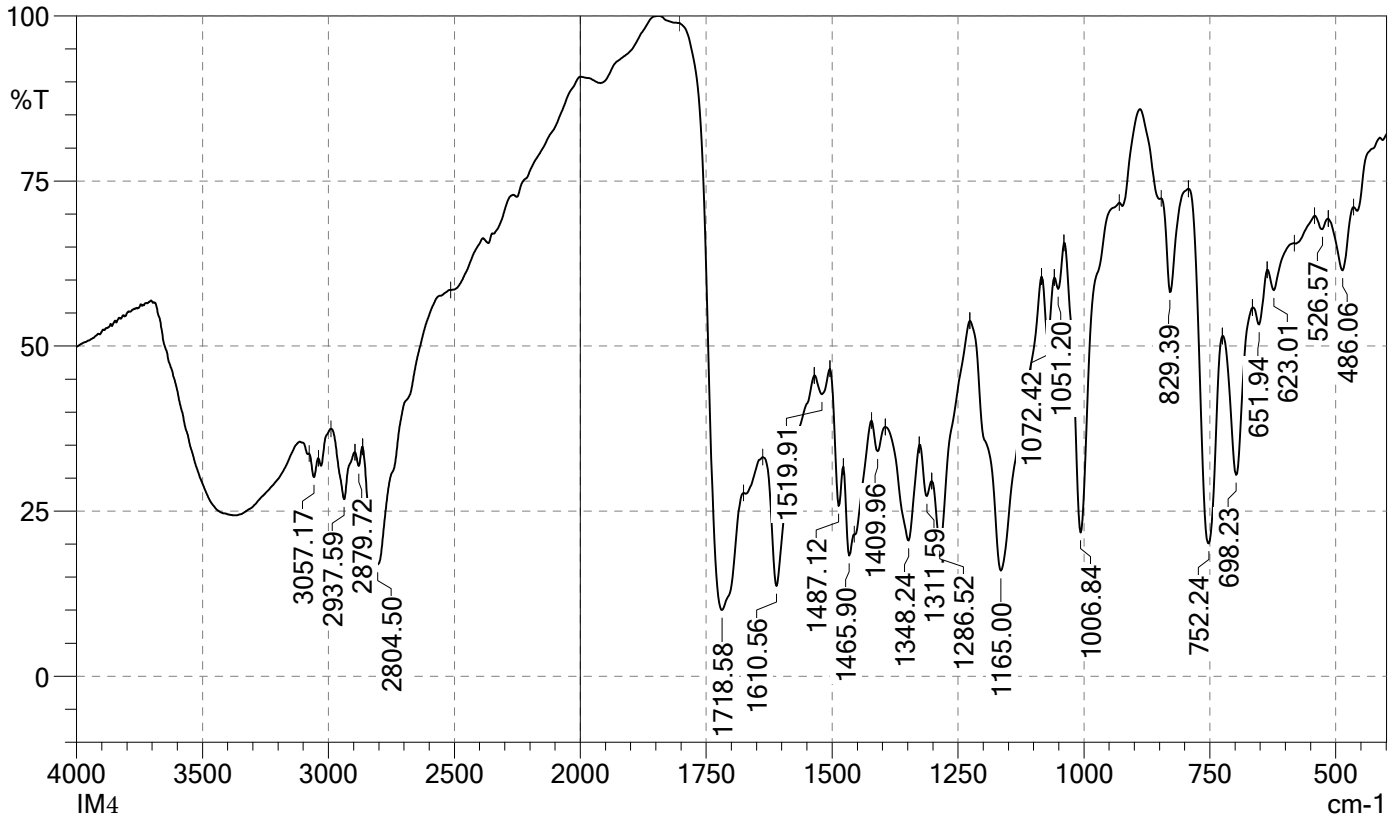
CHAPTER-XII
SPECTRAL EVIDENCE



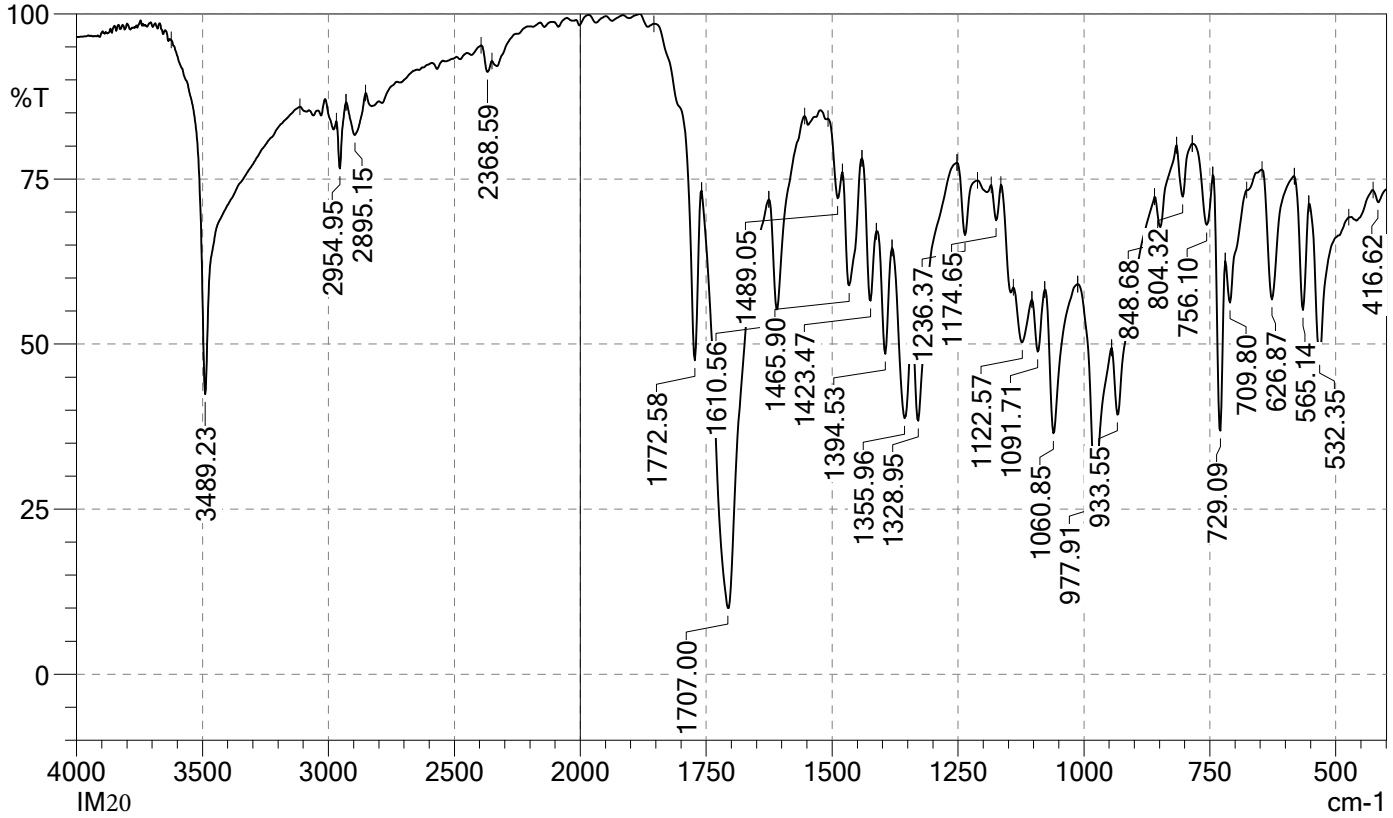
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	457.13	67.95	3.71	468.70	420.48	1317.481	48.711	
2	489.92	59.31	9.07	509.21	468.70	1438.315	160.193	
3	621.08	56.34	2.87	634.58	574.79	2430.518	78.907	
4	696.30	32.65	21.26	723.31	659.66	3423.393	508.428	
5	752.24	25.35	34.84	812.03	723.31	4205.491	1049.000	
6	827.46	73.23	2.64	839.03	812.03	681.284	32.152	
7	966.34	70.67	3.08	985.62	950.91	961.131	49.661	
8	1024.20	59.68	12.33	1045.42	985.62	1978.923	328.229	
9	1111.00	58.23	5.39	1126.43	1083.99	1630.183	106.549	
10	1193.94	39.38	15.71	1230.58	1165.00	3459.371	495.452	
11	1292.31	49.33	3.51	1311.59	1263.37	2344.873	79.817	
12	1332.81	44.02	9.31	1359.82	1311.59	2449.809	207.544	
13	1396.46	46.70	6.97	1421.54	1359.82	3059.894	212.449	
14	1452.40	38.25	3.84	1458.18	1421.54	2001.083	38.931	
15	1469.76	28.13	15.84	1504.48	1458.18	2687.841	273.313	
16	1516.05	53.17	2.29	1533.41	1504.48	1322.180	32.534	
17	1618.28	24.94	22.00	1635.64	1558.48	4545.770	714.691	
18	1716.65	14.50	50.80	1849.73	1635.64	9288.119	3311.258	
19	1955.82	93.57	2.00	2002.11	1924.96	417.698	74.417	
20	2819.93	58.12	3.66	2852.72	2752.42	3935.799	173.801	
21	2881.65	57.42	3.25	2920.23	2852.72	2766.803	113.967	
22	3059.10	43.55	3.14	3078.39	3041.74	2008.357	54.027	
23	3199.91	42.40	11.87	3574.10	3107.32	19725.670	2913.809	



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	491.85	61.16	6.89	513.07	414.70	3118.099	213.026	
2	700.16	38.53	16.61	725.23	680.87	2306.288	310.113	
3	754.17	23.83	37.01	800.46	725.23	3925.378	1135.474	
4	945.12	58.74	5.02	964.41	893.04	2559.620	167.563	
5	1053.13	33.17	3.63	1087.85	1031.92	3620.944	104.817	
6	1101.35	36.14	4.32	1141.86	1087.85	3218.435	106.233	
7	1155.36	42.43	4.03	1168.86	1141.86	1499.134	53.236	
8	1186.22	41.70	4.22	1209.37	1168.86	2285.718	90.187	
9	1247.94	39.12	3.71	1265.30	1209.37	3267.032	100.512	
10	1348.24	25.07	15.55	1417.68	1323.17	5947.207	632.934	
11	1467.83	20.55	15.28	1479.40	1417.68	3909.017	295.920	
12	1487.12	26.93	10.85	1514.12	1479.40	2019.024	90.420	
13	1610.56	15.06	31.89	1643.35	1556.55	5504.541	1000.025	
14	1716.65	10.00	52.99	1851.66	1643.35	8690.824	2705.006	
15	1953.89	95.55	1.72	2013.68	1924.96	296.786	65.295	
16	2870.08	46.86	5.79	2918.30	2835.36	4161.179	213.577	
17	2941.44	48.41	5.59	2999.31	2918.30	3692.518	154.093	
18	3059.10	52.64	4.34	3103.46	3039.81	2827.461	111.987	

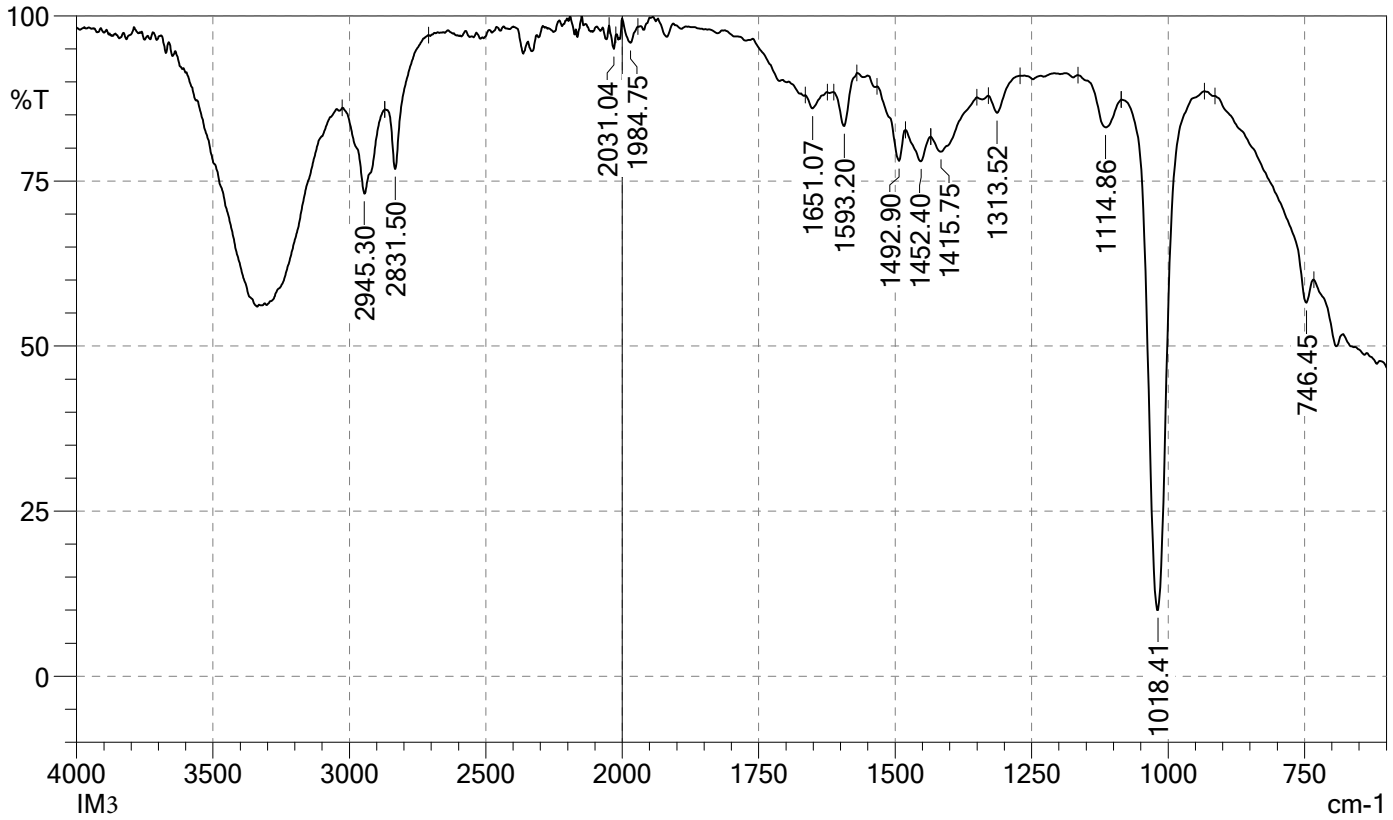


	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	486.06	61.48	8.83	514.99	464.84	1698.913	203.166	
2	526.57	67.71	1.78	542.00	514.99	846.059	23.223	
3	623.01	58.48	4.10	636.51	582.50	2029.765	62.516	
4	651.94	53.27	5.25	665.44	636.51	1268.644	74.169	
5	698.23	30.47	23.02	725.23	665.44	3334.751	566.208	
6	752.24	20.10	40.36	792.74	725.23	3792.330	1273.229	
7	829.39	58.16	14.68	846.75	792.74	1733.540	280.937	
8	1006.84	21.73	45.73	1039.63	929.69	4995.347	1553.262	
9	1051.20	58.66	3.83	1058.92	1039.63	755.436	42.085	
10	1072.42	51.09	9.38	1083.99	1058.92	1106.566	115.196	
11	1165.00	15.98	40.75	1226.73	1083.99	8938.594	2826.762	
12	1286.52	20.19	14.36	1301.95	1226.73	4795.139	409.788	
13	1311.59	27.27	4.42	1327.03	1301.95	1750.685	53.558	
14	1348.24	20.52	15.37	1394.53	1327.03	4760.180	466.300	
15	1409.96	34.05	4.25	1421.54	1394.53	1722.635	54.499	
16	1465.90	18.26	7.91	1477.47	1456.26	1643.578	86.981	
17	1487.12	25.78	11.25	1504.48	1477.47	1778.863	135.781	
18	1519.91	42.72	3.34	1535.34	1504.48	1721.008	56.214	
19	1610.56	13.66	22.78	1637.56	1535.34	7040.021	841.497	
20	1718.58	10.00	41.51	1803.44	1676.14	6319.133	1655.835	
21	2804.50	16.91	21.92	2864.29	2515.18	20825.116	2195.408	
22	2879.72	31.83	2.52	2895.15	2864.29	2062.896	36.850	
23	2937.59	26.77	8.76	2989.66	2895.15	6419.585	343.198	
24	3057.17	30.14	3.21	3076.46	3039.81	2499.253	57.250	



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	416.62	71.49	1.88	426.27	399.26	741.776	23.017	
2	532.35	48.02	22.67	553.57	474.49	2925.407	570.887	
3	565.14	55.16	17.75	582.50	553.57	1009.125	237.531	
4	626.87	56.75	19.38	646.15	582.50	1980.419	448.525	
5	709.80	56.27	8.76	719.45	677.01	1498.238	138.733	
6	729.09	36.89	30.72	744.52	719.45	1140.075	365.627	
7	756.10	68.04	8.94	785.03	744.52	1030.819	139.759	
8	804.32	72.31	7.89	815.89	785.03	712.346	102.513	
9	848.68	67.71	6.64	860.25	815.89	1174.252	118.898	
10	933.55	39.29	13.24	945.12	860.25	3661.073	339.562	
11	977.91	27.57	26.54	1012.63	945.12	3735.909	647.606	
12	1060.85	36.50	21.99	1078.21	1012.63	3228.876	519.239	
13	1091.71	48.86	8.59	1103.28	1078.21	1173.184	107.752	
14	1122.57	50.27	7.45	1139.93	1103.28	1696.609	145.373	
15	1174.65	68.75	5.41	1184.29	1165.00	555.261	56.794	
16	1236.37	66.49	9.99	1251.80	1211.30	1114.137	148.161	
17	1328.95	38.39	18.86	1342.46	1251.80	3415.938	297.882	
18	1355.96	38.79	18.71	1381.03	1342.46	1944.709	368.128	
19	1394.53	48.53	17.14	1411.89	1381.03	1284.921	230.385	
20	1423.47	56.54	14.98	1440.83	1411.89	994.673	202.568	
21	1465.90	58.87	17.90	1479.40	1440.83	1263.233	379.295	
22	1489.05	72.07	6.66	1508.33	1479.40	654.648	78.385	
23	1610.56	55.24	19.41	1625.99	1554.63	2028.235	474.584	
24	1707.00	10.00	62.74	1759.08	1625.99	7170.879	3523.437	
25	1772.58	47.56	29.31	1853.59	1759.08	1681.176	346.835	

26	2368.59	91.21	2.62	2393.66	2351.23	304.273	51.590	
27	2895.15	81.67	5.57	2929.87	2852.72	1228.731	249.568	
28	2954.95	76.59	8.15	2968.45	2929.87	699.994	127.973	
29	3489.23	42.39	51.01	3624.25	3113.11	11252.437	6656.300	



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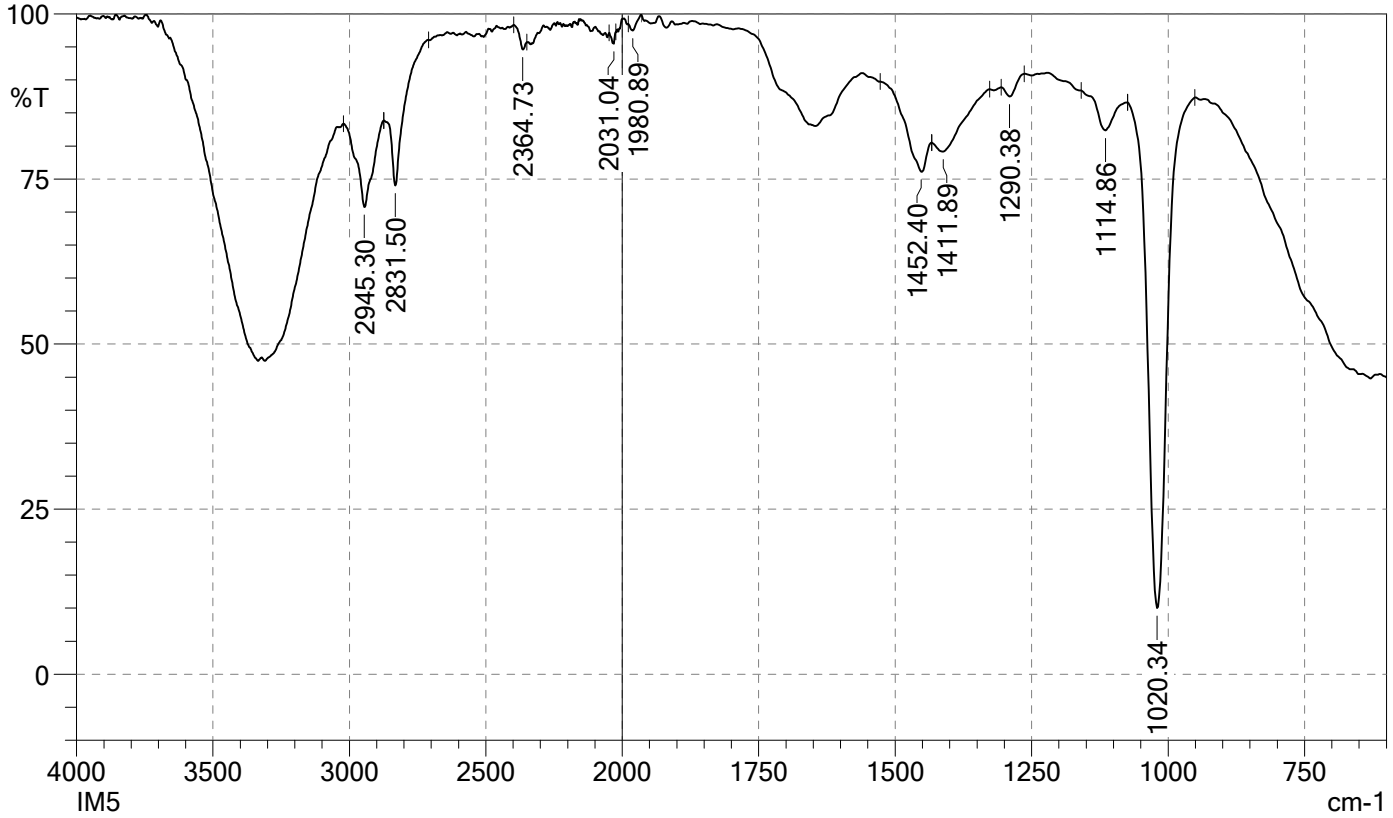
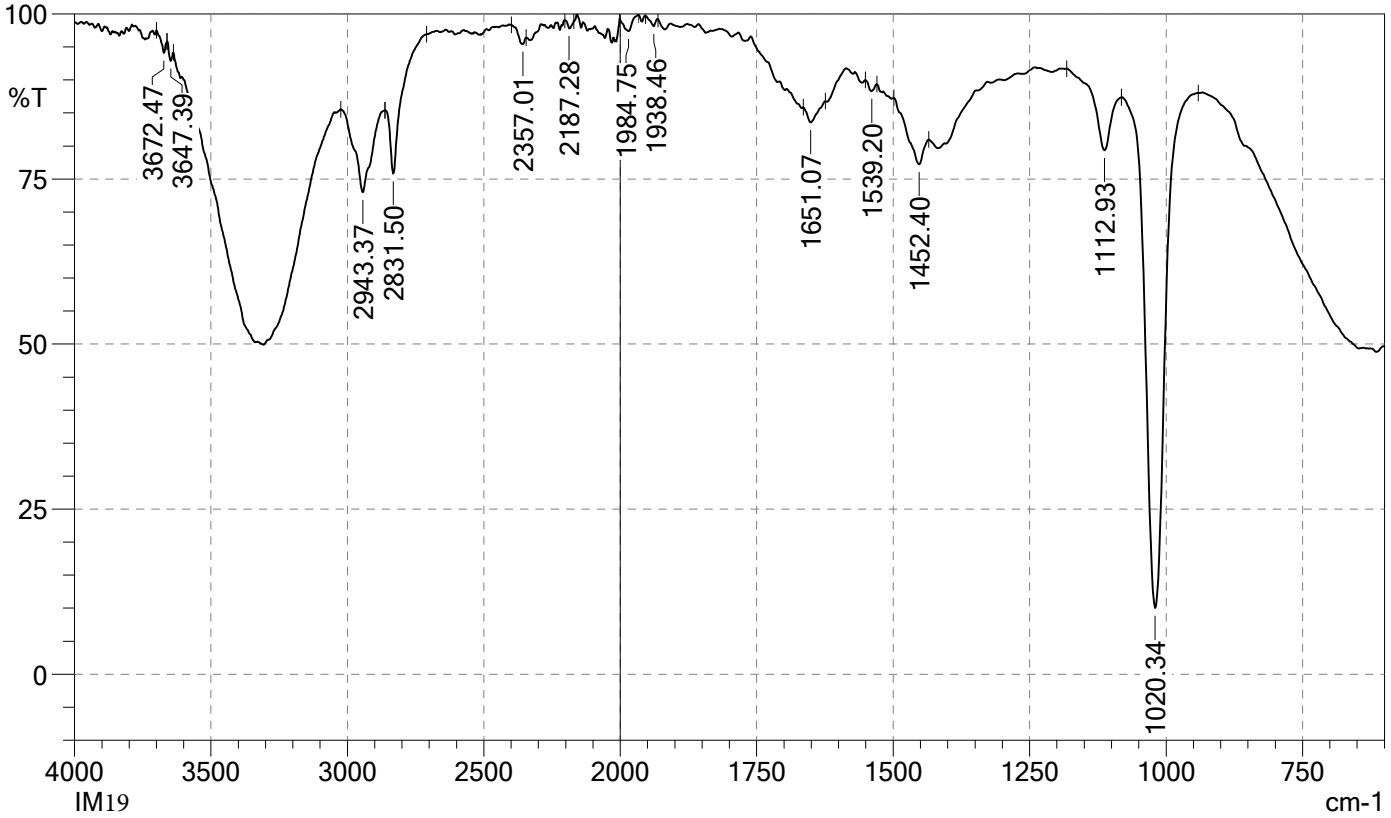
1	746.45	56.56	5.57	914.26	732.95	4526.686	-192.067	
2	1018.41	10.00	77.89	1085.92	933.55	4926.741	3092.515	
3	1114.86	83.11	5.56	1165.00	1085.92	1026.650	170.478	
4	1313.52	85.33	3.40	1328.95	1271.09	675.229	64.006	
5	1415.75	79.43	3.64	1435.04	1350.17	1452.560	153.513	
6	1452.40	77.98	4.12	1481.33	1435.04	916.904	94.215	
7	1492.90	78.04	6.17	1533.41	1481.33	851.142	124.281	
8	1593.20	83.33	6.45	1612.49	1570.06	541.203	113.169	
9	1651.07	85.99	2.18	1664.57	1624.06	515.215	39.247	
10	1984.75	95.92	3.04	2000.18	1971.25	77.741	48.774	
11	2031.04	95.02	2.53	2048.40	2023.33	88.776	34.172	
12	2831.50	76.81	11.74	2870.08	2709.99	1707.950	342.582	
13	2945.30	73.09	12.88	3026.31	2870.08	3039.946	848.618	



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	1020.34	10.00	76.93	1074.35	950.91	4590.217	2982.616	
2	1114.86	82.38	5.09	1159.22	1074.35	1224.119	164.037	
3	1290.38	87.50	2.13	1305.81	1263.37	468.975	40.777	
4	1411.89	79.13	3.00	1433.11	1327.03	1768.118	129.340	
5	1452.40	76.10	6.29	1527.62	1433.11	1599.143	194.168	
6	1980.89	97.47	1.52	1988.61	1965.46	36.264	18.769	
7	2031.04	95.47	1.78	2048.40	2023.33	91.548	22.099	
8	2364.73	94.62	1.96	2397.52	2349.30	174.677	32.471	
9	2831.50	74.00	13.01	2873.94	2709.99	2107.126	462.225	
10	2945.30	70.75	12.87	3022.45	2873.94	3245.503	811.560	



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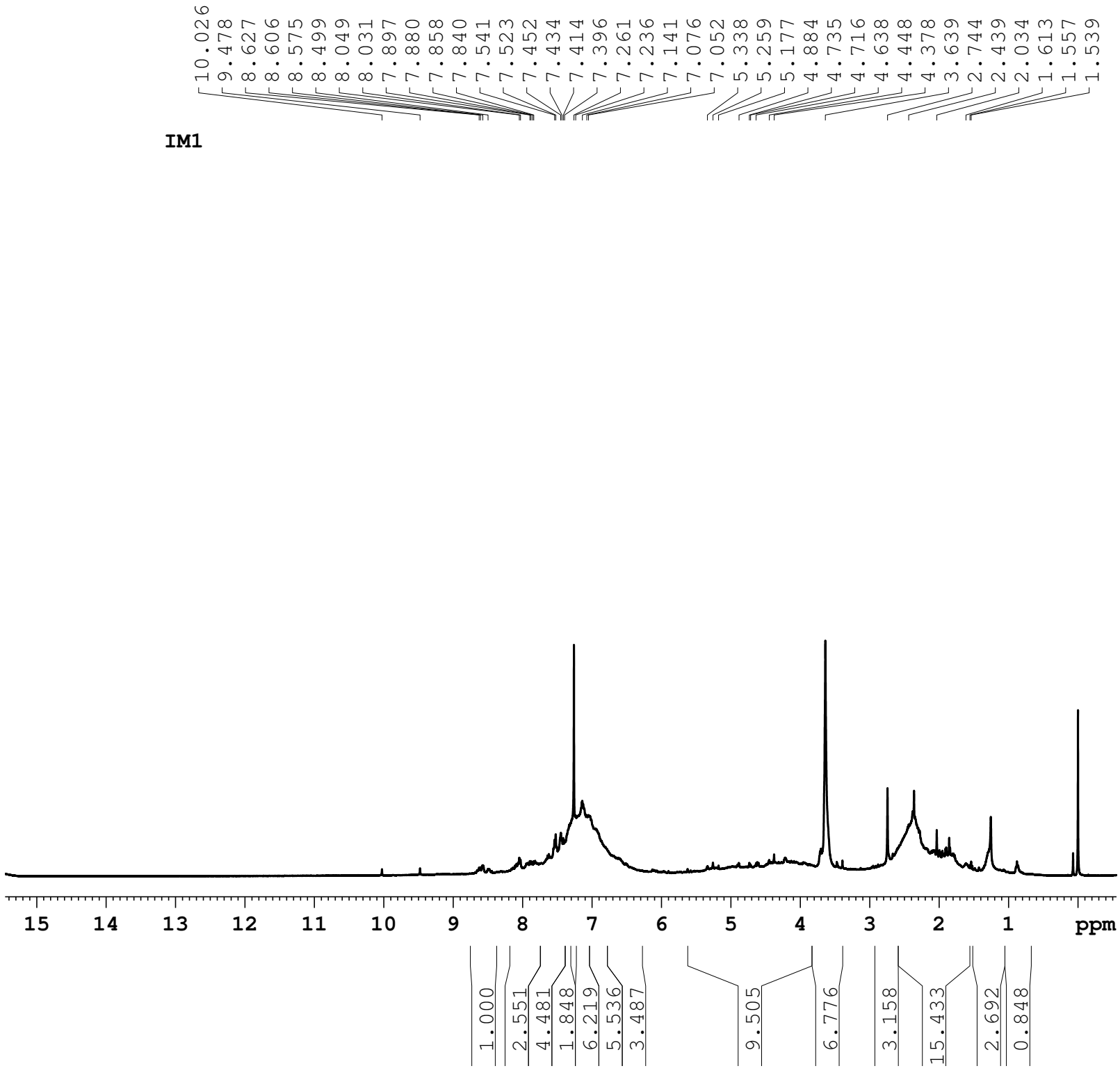
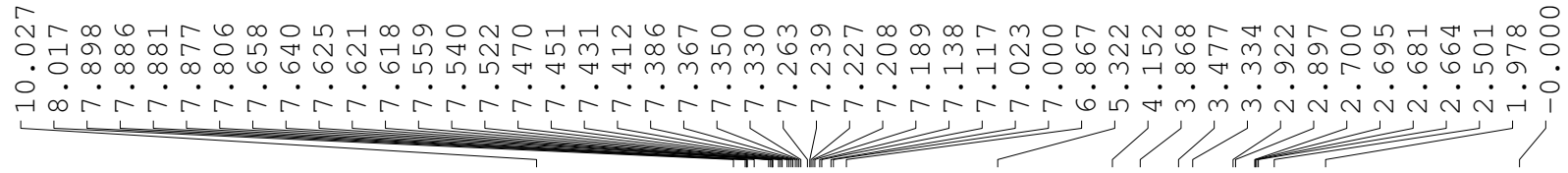
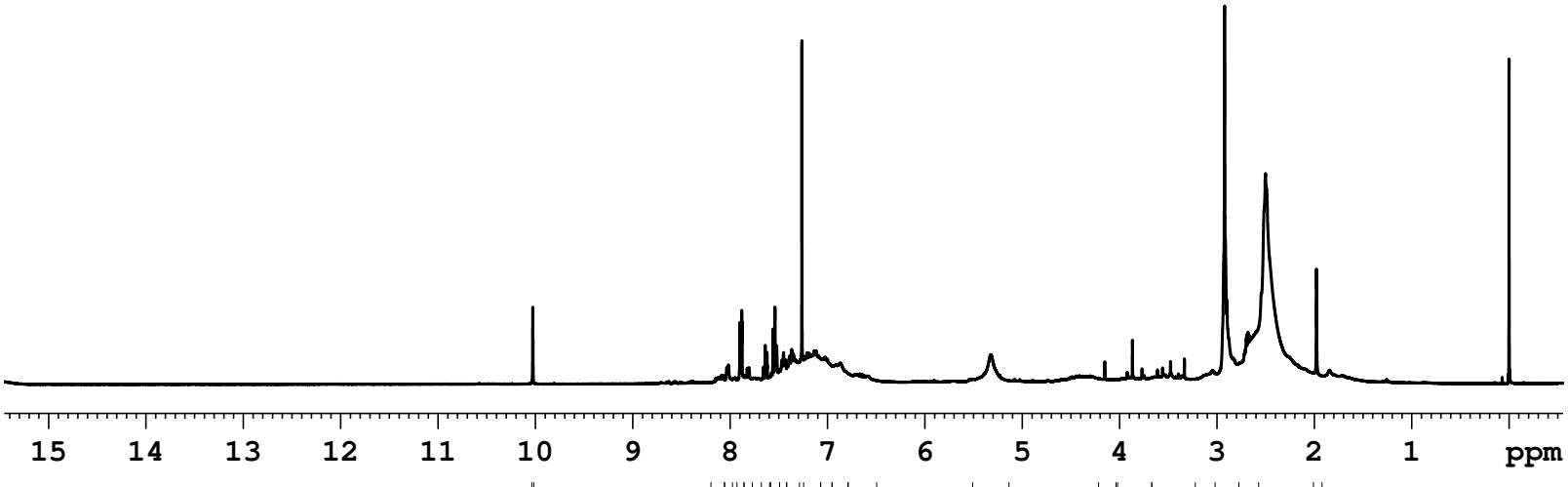
2	1112.93	79.33	9.38	1182.36	1082.07	1320.304	272.259	
3	1452.40	77.20	5.50	1498.69	1435.04	1180.854	171.299	
4	1539.20	88.30	1.37	1550.77	1529.55	233.669	14.904	
5	1651.07	83.56	2.65	1664.57	1624.06	605.820	53.305	
6	1938.46	98.11	1.30	1953.89	1930.74	24.678	13.149	
7	1984.75	97.38	2.20	2002.11	1967.39	50.302	35.815	
8	2187.28	97.82	1.21	2202.71	2169.92	52.040	20.278	
9	2357.01	95.42	1.43	2399.45	2345.44	171.305	31.016	
10	2831.50	75.82	11.93	2862.36	2709.99	1663.351	319.595	
11	2943.37	73.00	12.49	3024.38	2862.36	3175.602	825.682	
12	3647.39	92.87	2.00	3660.89	3637.75	140.465	24.951	
13	3672.47	94.08	2.27	3699.47	3660.89	164.105	36.209	

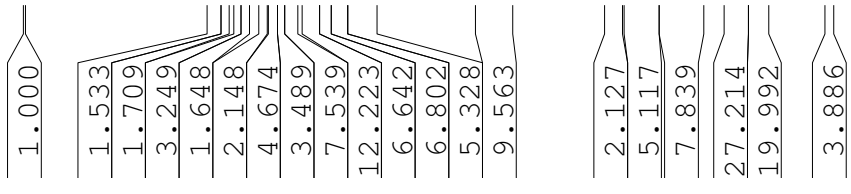



```
Current Data Parameters
NAME                  03_Kaniga
EXPNO                         3
PROCNO                        1

F2 - Acquisition Parameters
Date_                  20170703
Time                      10.04
INSTRUM                   spect
PROBHD    5 mm PABBO BB-
PULPROG                      zg
TD                        32768
SOLVENT                   CDCl3
NS                           32
DS                            0
SWH                    6410.256 Hz
FIDRES                 0.195625 Hz
AQ                     2.5559039 sec
RG                          144
DW                       78.000 usec
DE                        6.50 usec
TE                       298.0 K
D1                    2.00000000 sec
TD0                           1

======== CHANNEL f1 ========
NUC1                         1H
P1                       13.50 usec
PL1                      -3.00 dB
PL1W               13.42244530 W
SFO1               400.2330017 MHz

F2 - Processing parameters
SI                       131072
SF                 400.2300191 MHz
WDW                          EM
SSB          0
LB                        0.30 Hz
GB           0
PC                        1.00
```



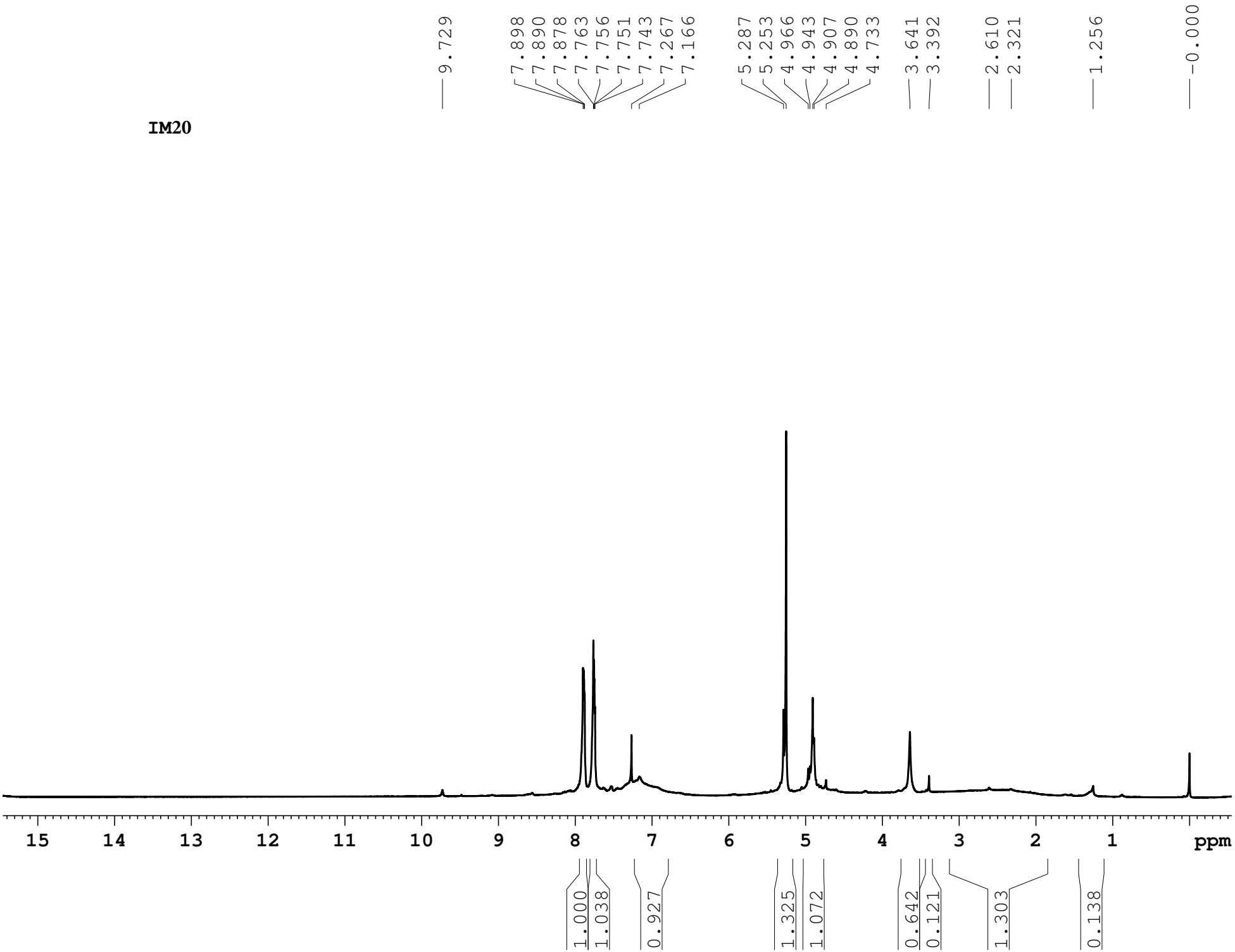
IM4

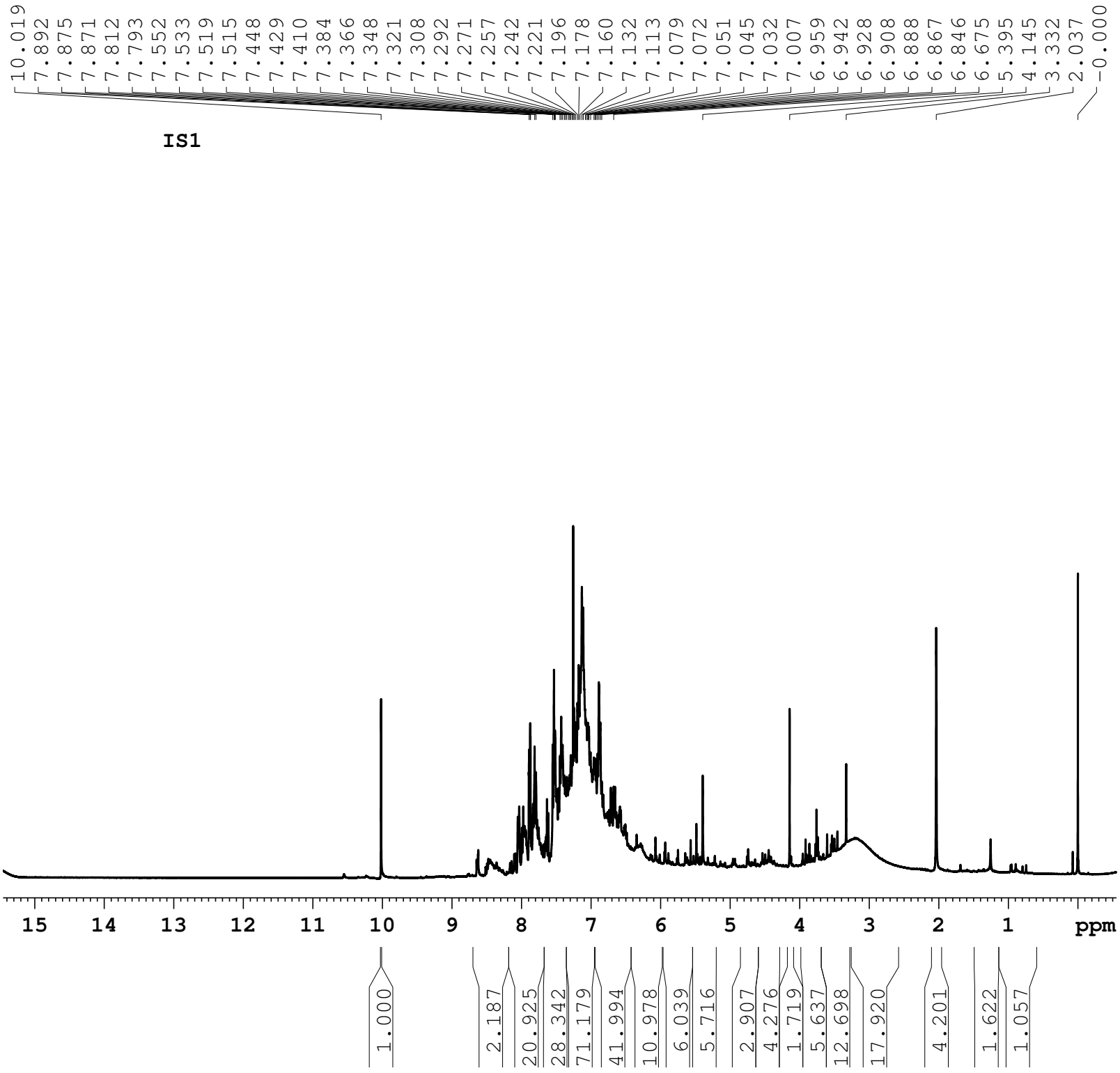





```
Current Data Parameters
NAME                  03_Kaniga
EXPNO                         2
PROCNO                        1

F2 - Acquisition Parameters
Date_                  20170703
Time                       9.58
INSTRUM                   spect
PROBHD    5 mm PABBO BB-
PULPROG                      zg
TD                        32768
SOLVENT                   CDCl3
NS                           32
DS                            0
SWH                    6410.256 Hz
FIDRES                 0.195625 Hz
AQ                     2.5559039 sec
RG                          144
DW                       78.000 usec
DE                        6.50 usec
TE                       298.0 K
D1                    2.00000000 sec
TD0                           1

======== CHANNEL f1 ========
NUC1                         1H
P1                       13.50 usec
PL1                      -3.00 dB
PL1W               13.42244530 W
SFO1               400.2330017 MHz

F2 - Processing parameters
SI                       131072
SF                 400.2300185 MHz
WDW                          EM
SSB             0
LB                        0.30 Hz
GB              0
PC                        1.00
```

IM20


9.729
7.898
7.890
7.878
7.763
7.756
7.751
7.743
7.267
7.166
5.287
5.253
4.966
4.943
4.907
4.890
4.733
3.641
3.392
2.610
2.321
1.256
-0.000
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.000
1.038
0.927
1.325
1.072
0.642
0.121
1.303
0.138




```
Current Data Parameters
NAME                  03_Kaniga
EXPNO                         4
PROCNO                        1

F2 - Acquisition Parameters
Date_                  20170703
Time                      10.13
INSTRUM                   spect
PROBHD    5 mm PABBO BB-
PULPROG                      zg
TD                        32768
SOLVENT                   CDCl3
NS                           32
DS                            0
SWH                    6410.256 Hz
FIDRES                 0.195625 Hz
AQ                     2.5559039 sec
RG                          114
DW                       78.000 usec
DE                        6.50 usec
TE                       298.0 K
D1                    2.00000000 sec
TD0                           1

======== CHANNEL f1 ========
NUC1                         1H
P1                       13.50 usec
PL1                      -3.00 dB
PL1W               13.42244530 W
SFO1               400.2330017 MHz

F2 - Processing parameters
SI                       131072
SF                 400.2300208 MHz
WDW                          EM
SSB          0
LB                        0.30 Hz
GB           0
PC                        1.00
```


THANK YOU