"Design, Synthesis, Characterization and Biological evaluation of Novel flavone derivatives: Molecular Docking Studies"

Dissertation Submitted to

The Tamil Nadu Dr. M.G.R Medical University, Chennai

In partial fulfillment for the requirement of the Degree of

MASTER OF PHARMACY

(Pharmaceutical Chemistry)

April - 2012

K	M
C	H

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

KMCH COLLEGE OF PHARMACY

KOVAI ESTATE, KALAPATTI ROAD,

COIMBATORE 641-048.

"Design, Synthesis, Characterization and Biological evaluation of Novel flavone derivatives: Molecular Docking Studies"

Dissertation Submitted to

The Tamil Nadu Dr. M.G.R Medical University, Chennai

In partial fulfillment for the requirement of the Degree of

MASTER OF PHARMACY

(Pharmaceutical Chemistry)

Submitted by

P. PARASURAMAN

Under the guidance of

Mr. K. K.SIVAKUMAR, M. Pharm, (Ph.D).,

Asst. Professor,

Department of Pharmaceutical Chemistry

April-2012

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY,

KMCH COLLEGE OF PHARMACY

KOVAI ESTATE, KALAPATTI ROAD,

COIMBATORE 641-048.

Dr. A Rajasekaran, M. Pharm., Ph.D.,
Principal,
KMCH College of Pharmacy,
Kovai Estate, Kalapatti Road,
Coimbatore-641 048, (T.N)

CERTIFICATE

This is to certify that the dissertation work entitled ***"Design, Synthesis, Characterization and Biological evaluation of Novel flavone derivatives: Molecular Docking Studies"*** submitted by **Mr. P.Parasuraman** is a bonafide work carried out by the candidate under the guidance of **Mr. K.K.Sivakumar, M.Pharm,(PhD).,** Asst. Professor to The Tamilnadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutical Chemistry** at the Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore, during the academic year 2011-2012.

Dr. A. Rajasekaran, M.Pharm., Ph.D.
Principal

Mr. **K.K.Sivakumar, M.Pharm, (PhD)**
Asst. Professor,
KMCH College of Pharmacy,
Kovai Estate, Kalapatti Road,
Coimbatore 641 048(T.N)

CERTIFICATE

This is to certify that the dissertation work entitled ***"Design, Synthesis, Characterization and Biological evaluation of Novel flavone derivatives: Molecular Docking Studies"*** submitted by **Mr. P.Parasuraman** to The Tamilnadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of Master **of Pharmacy** in **Pharmaceutical Chemistry** at the Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore, during the academic year 2011-2012.

Mr. **K.K.Sivakumar, M.Pharm, PhD).,**
Asst. Professor
Department of Pharmaceutical Chemistry

DECLARATION

I do hereby declare that the dissertation work entitled ***"Design, Synthesis, Characterization and Biological evaluation of Novel flavone derivatives: Molecular Docking Studies"*** submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of Master **of Pharmacy** in **Pharmaceutical Chemistry** at the Department of Pharmaceutical Chemistry was done by me under the guidance of **Mr**. **K.K.Sivakumar, M.Pharm, (PhD)** Asst. Professor at the Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore, during the academic year 2011-2012.

P.PARASURAMAN

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled ***"Design, Synthesis, Characterization and Biological evaluation of Novel flavone derivatives: Molecular Docking Studies"*** submitted by **Mr. P.Parasuraman (Reg.No.26107135)** to The Tamilnadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutical Chemistry** is a bonafide work carried out by the candidate at the Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore and was evaluated by us during the academic year 2011-2012.

Internal Examiner **External Examiner**

Convener of Examinations

Examination Center **:** KMCH College of Pharmacy, Coimbatore.

Date **:**

ABBREVIATIONS

e.g	Example
%	Percentage
Mg	Milligram
µg	Microgram
mm	Millimeter
^{0}C	Degree centigrade
Fig	Figure
UV-VIS	Ultraviolet and visible spectroscopy
IR	Infrared spectroscopy
^{1}HNMR	Nuclear Magnetic Resonance
Std	Standard
TLC	Thin layer chromatography
KBr	Potassium bromide
IC	Inhibitory concentration
EC	Effective concentration
DMSO	Dimethyl sulfoxide

ABSTRACT

A series of Schiff bases of flavones have been synthesized by both conventional and microwave irradiation method, purified and characterized on the basis of spectral data (IR, ^{1}HNMR and Mass spectra). The title compounds were screened for their preliminary anti-microbial activity against bacteria (Gram positive and Gram negative strains) and fungi by disc diffusion method. The minimum inhibitory concentration (MIC) was determined by 2-fold dilution technique. Majority of the tested compounds showed mild to significant preliminary anti-microbial activity at 100µg/disc when compared to standard drugs ciprofloxacin and Clotrimazole at 5µg/disc concentration level. Among the synthesized compounds **DPN 07, DPN 08, DPN 11** was found to be moderate activity against most of the screened bacteria and fungi. MIC results indicate that the compound **DPN 07, DPN 08** have anti-bacterial activity at 6.25µg/ml and 12.5µg/ml concentrations level. The entire screened compound exhibited *in vitro* anti-malarial activity against *Plasmodium falciparum,* in which Compounds **DPN 08, DPN 11& DPN 12** exhibited potent activity when compared with standard. The DPPH, FRAP and ABTS *in-vitro* anti-oxidant methods result of the synthesized compound review that high reactivity of hydroxyl substituent's influence the anti -oxidant activity**.** None of the tested compounds show *in-vitro* anti-cancer activity. Compound **DPN 07, DPN 08, DPN 11** and **DPN12** were found to be more potent compound, so this can be used as lead for further studies.

INTRODUCTION

1.1.1 CHEMISTRY:

Flavonoids based on the backbone of 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one) (Fig 1.1). Flavones are phenols that are widely present in the flowers, leaves and fruit tissues of living plants as flavonoid glycosides. The aglycones present in some flavones are poisonous to plants and can be separated only from dead timber. Deriving their name from their yellow color, and have dual roles. While sometimes they draw pollinating insects with their color, in other times their toxic property enable them act as repellents to insects that harm the plants. At the same time, tests have shown that flavanoids are also capable of regulating the growth of the plants.

Flavonoids are either bitter or sweet in taste and recent chemical tests have proved that minor altercations in their cell structure can change their taste from bitter to sweet or the other way around. This is significant, as it shows that the bitter and sweet taste receptors in our tongue are situated close to each other and also analogous in structure.

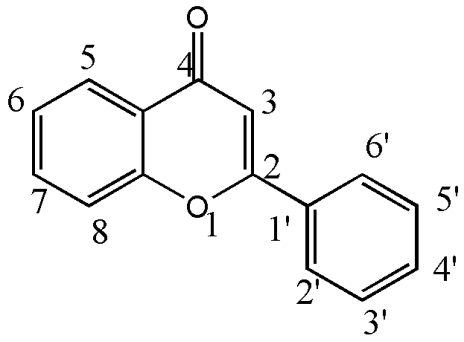


Fig 1.1

Flavonoids are one of the most abundant natural product groups occurring in the plant kingdom. Although the term flavonoids has been interchangeably used by many, including researchers. Flavonoids generally have a common C_5-C_3-C_6 flavone skeleton in which the three carbon bridge between the phenyl group is usually enclosed with oxygen .based on the degree of unsaturation and oxidation of the three carbon segment (c -ring), flavonoids are divided into several sub classes most of the flavones reported in the

literature are glycosides of a relatively small number of flavonoid aglycones, which are generally water soluble and accumulated in the vacuoles of plant cells.

The structural features of the B –ring and the hydroxylation and glycosylation patterns on all A, B, and C rings of the flavones skeleton have made flavonoids one of the largest and diverse phytochemical group.

A significant role that has been under active research in recent years is their possible health beneficial effect to humans. Flavonoids have been found to posses potent anti oxidant activities. Increasing evidence from epidemiological studies suggests that diets high in flavonoids are contributing significantly to lower risks of cardio vascular diseases and cancer in humans, for this reason, both healths' Canada and the US food and drug administration have allowed health claims for fruits and vegetables consumption and lowered risks of heart disease.

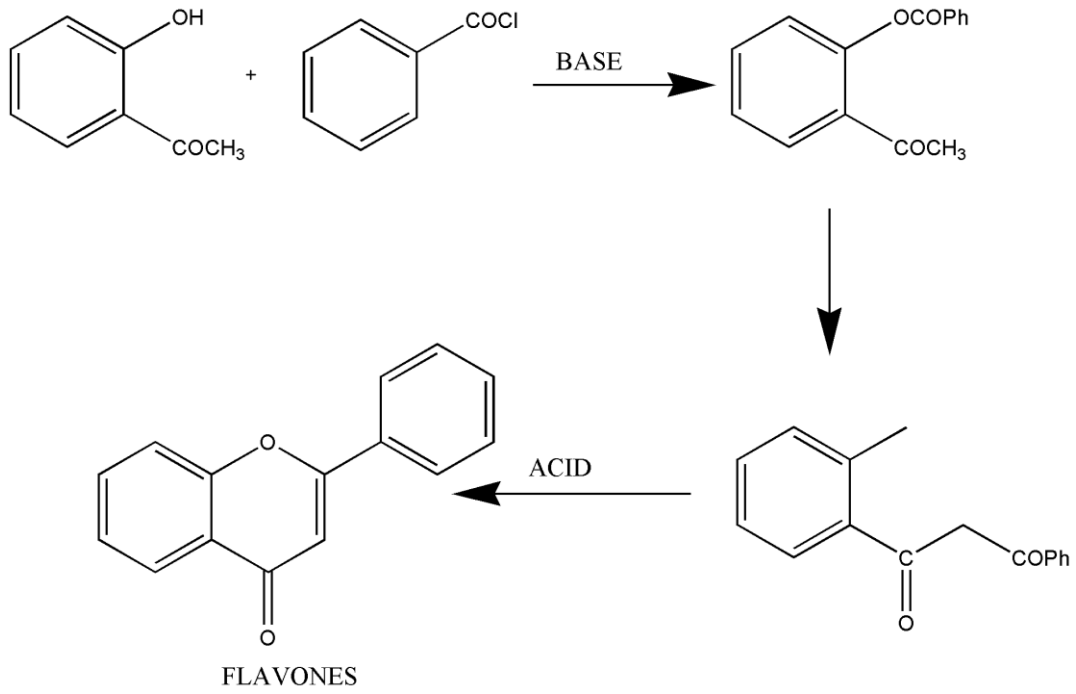
Flavones are mainly found in cereals and herbs. In the West, the estimated daily intake of flavones is in the range 20-50 mg per day [(1)]. In recent years, scientific and public interest in flavones has grown enormously due to their putative beneficial effects against atherosclerosis, osteoporosis, diabetes mellitus and certain cancers [(2)]. Flavones intake in the form of dietary supplements and plant extracts has been steadily increasing.

Flavones have effects on CYP (P450) activity [(3)] which are enzymes that metabolize most drugs in the body. By these observations many research shows synthesized flavonoids by following synthetic route formed,

- Baker-Venkataraman rearrangement [(4)]
- Allan-Robinson reaction[(5)]
- Auwers synthesis[(6)]
- Algar-Flynn-Oyamada reaction[(7)]

\

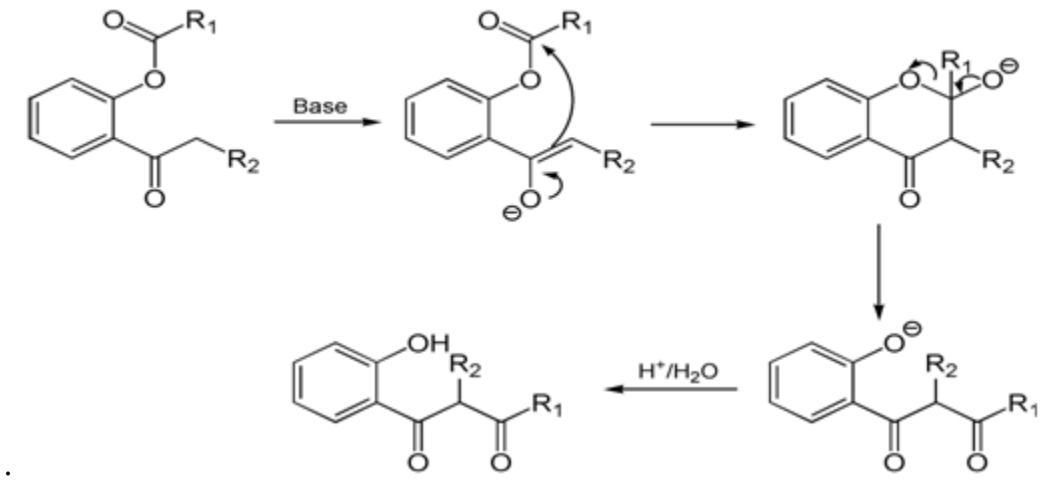
Baker–Venkataraman rearrangement



Scheme 1.1

In the first step, 2-hydroxyacetophenone or its derivative is treated with an aromatic acid chloride to form an aryl ester, which in the second step, is reacted with a base. The ester is transformed into diaryl 1, 3-diketone through an intramolecular rearrangement. Treatment of the latter with an acid leads to the formation of the corresponding flavone. **(Scheme 1.1)** A variety of 2-hydroxyaryl alkyl ketones and a number of aromatic acid chlorides work very well. Thus a large number of substituted flavones are accessible through this simple synthetic procedure.

This rearrangement reaction proceeds via enolate formation followed by acyl transfer with a base being a catalyst, it attacks hydrogen atom in Acetophenone and an enolate is formed. Then, the enolate group attacks the carbon in the phenol ester to form a cyclic alkoxide. Finally, it opens up a phenolate which is reprocessed by acid to undergo protonation **(Scheme 1.2)**



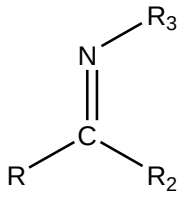
Scheme 1.2

From the above view point, we here on report the synthesis of flavones by **Baker–Venkataraman rearrangement method & Microwave irradiation technique and their anti Microbial, anti malarial, anti oxidant, anti cancer activity.**

1.1.2 SCHIFF BASE

A Schiff base is a compound with a functional group that contains a carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group, not hydrogen [8]. Schiff bases in a broad sense have the general formula $R^1R^2C=NR^3$, where R is an organic side chain. In this definition, Schiff base is synonymous with azomethine. Some restrict the term to the secondary aldimines (azomethines where the carbon is connected to a hydrogen atom), thus with the general formula RCH=NR'[9].

The chain on the nitrogen makes the Schiff base a stable imine. A Schiff base derived from aniline, where R^3 is a phenyl or a substituted phenyl, can be called an anil [10].

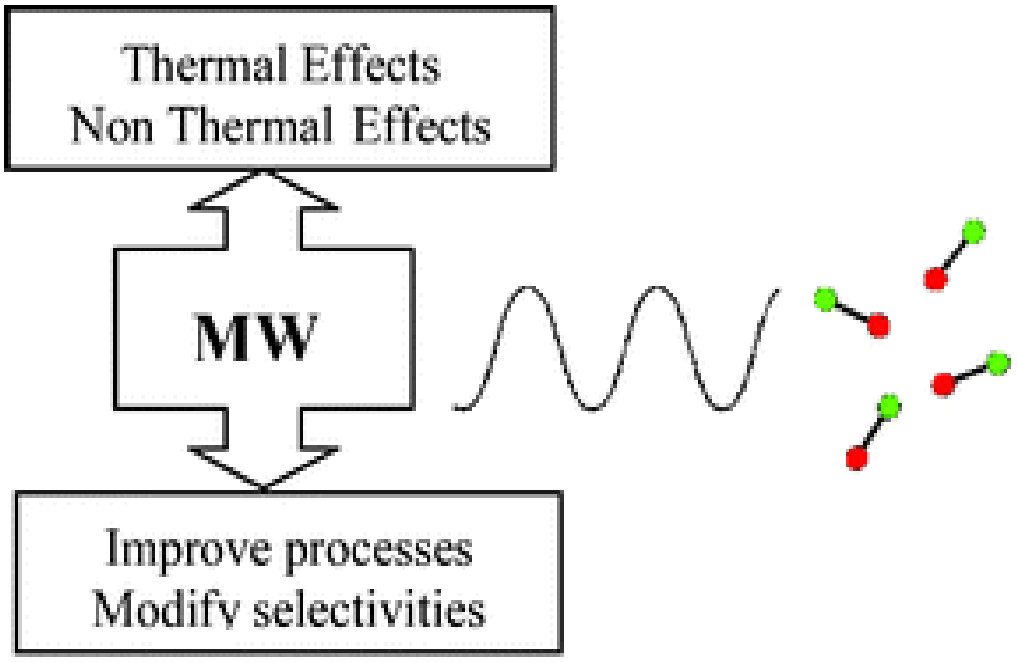


Schiff bases can be synthesized from an aromatic amine and a carbonyl compound by nucleophic addition forming a hemiaminal, followed by a dehydration to generate an imine.

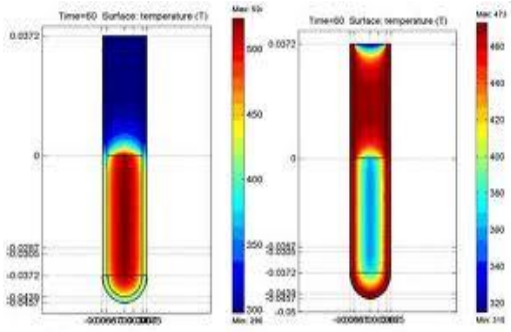
1.2 Micro wave irradiation technique

Environmental pressure to reduce waste and reuse materials has driven studies into 'Green' chemistry. Chemical and pharmaceutical industries are always under pressure to develop more environmentally friendly organic reaction methodologies using nonhazardous catalysis. Microwave irradiation is used for a variety of organic reactions due to short reaction time, cleaner reactions, easier work-up and better yield. Thus the microwave oven procedure is now well established in MORE chemistry [(11)]. More recently, the emphasis has shifted in favour of microwaves-assisted methods under solvent-free conditions, providing an opportunity to work in open vessels, thus avoiding the risk of the development of high pressure. Inorganic solid support organic transformations are gaining much attention due to simplified product isolation, mild reaction conditions, high selectivity etc. Solvent free microwave assisted chemical reactions in combination of solid supported reagents, were used to carry out a wide range of reaction in shorter time as compare to other conventional reaction methods [(12)]. The use of solvent free conditions with heterogeneous catalysts represents one of the more powerful green chemical procedures [(13)].

Microwave irradiation has been successfully applied in organic chemistry. Spectacular accelerations, higher yields under milder reaction conditions and higher product purities have all been reported. Indeed, a number of authors have described success in reactions that do not occur by conventional heating and even modifications of selectivity (chemoregio- and stereo selectivity). The effect of microwave irradiation in organic synthesis is a combination of thermal effects, arising from the heating rate, superheating or "hot spots" and the selective absorption of radiation by polar substances. Such phenomena are not usually accessible by classical heating and the existence of non-thermal effects of highly polarizing radiation—the "specific microwave effect"—is still a controversial topic. The thermal effects and the current state of non-thermal microwave effects are effectively used in organic synthesis.



MW heating has gained popularity in the last decades as it remarkably accelerates a wide variety of reactions and minimizes thermal decomposition of the products. Since the initial work of Gedye and Giguere, a rapidly increasing number of reports and reviews have been published demonstrating the importance of such methodology. However, to the best of our knowledge, the potentially of this method has not been exploited yet for the type of reactions of interest in this case .The use of MW irradiation offers many advantages over conventional heating: it remarkably decreases reaction times, requires less solvent, thus facilitating reaction workups and increases yields.



Microwave Heating Conventional Heating

1.4 ANTI MICROBIAL [14]

1.4.1 Antibacterial

Synthetic antibacterial compounds are divided into two major classes: topical agents and systemic agents. The topical agents are termed disinfectants, antiseptics, and preservatives, depending on how they are used. Antiseptics and disinfectants differ from systemic agents in that they show little selective toxicity between the microbes and the host. Furthermore, most of them do not aid wound healing and may even impair it. Nevertheless, there are indispensable uses for disinfectants in hospital sanitation, including sterilization of surgical instruments, public health methods, and in the home. Antiseptics have important applications in the preoperative preparation of both surgeons and patients. They also are used in treating local infections caused by microorganisms refractive to systemic antimicrobial agents.

1.4.1a. Mechanisms of bacterial resistance

The outer membrane of Gram-negative bacteria is composed of lipopolysaccharide, proteins, and lipids [15]. It presents a barrier to many chemical agents, including quaternary ammonium compounds and triphenylmethane dyes. In contrast, the cytoplasmic membrane of Gram-positive bacteria, excepting spores and mycobacteria, has greater permeability to most agents [16]**.** Spores have a special coat and/or cortex that may not be permeable to hydrogen peroxide and chlorine disinfectants. Bacterial cells may also have efflux mechanisms that extrude agents such as quaternary ammonium compounds, dyes, and mercury compounds from their interiors [17].

1.4.1b. Systemic synthetic antibacterial

Prontosil rubrum [18], a sulfonamido-azo dye, was the first clinically useful systemic antibacterial agent to be discovered. This discovery was done in the early 1930s and the development of sulfonamides and sulfones as a class of antibacterial agents. Their broad antimicrobial spectrum provided, for the first time, drugs for the cure and prevention of a variety of bacterial infections; their widespread clinical use brought about a sharp decline in morbidity and mortality of treatable infectious diseases, and thus proved of great medical and public health importance. Recognition of the inhibition of the action of sulphonamides by extracts which was &own to be attributed to the presence of p-

aminobenzoic acid (PABA), required in folate biosynthesis, was the first clear demonstration of metabolites antagonism as a mechanism of drug action; this provided the long sought after mechanistic basis for drug action.

1.4.2 Antifungal [19]

The first antifungal discovered in 1939 was griseofulvin. The introduction of griseofulvin was followed in 1960 by that of amphotericin B, which is still the "gold standard" for the treatment of severe systemic mycoses.

Fungal infection are caused by Eukaryotic organisms and for that reason they generally present more difficult therapeutic problems than do bacterial infections. The fungal cell wall may be considered to be a prime target for selectively toxic antifungal agent because of its chitin structure, absent from human cells. Day by day number of antifungal agent are preparing and against fungi.

Of the five fundamental Kingdoms of Life, the Kingdom Fungi is arguably the most diverse and prevalent. Unlike the kingdom Monera (containing bacteria), fungi are eukaryotic organisms whose cellular fundions consequently resemble those of plants and animals more closely. Thus the issue of selectivity predominates in the quest for safe and effective chemotherapeutic remedies for diseases caused by fungi. As with all chemotherapy, there is a risk-reward ratio to be taken into account; in the context of fungal infections, this ratio may vary greatly, from minor irritations such as athlete's foot to life-threatening systemic infections such as those caused by *Aspergillus fumigatus.* This addresses medicinal aspects of the treatment of fungal diseases of all types, but because most recent research has been directed toward the treatment of systemic infections, emphasis is placed on this aspect.

Antibiotic resistance

Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic. Many antibiotic resistance genes reside on plasmids, facilitating their transfer. If a bacterium carries several resistance genes, it is called multiresistant or, informally, a superbug or super bacterium. The primary cause of antibiotic resistance is antibiotic use both within medicine and veterinary medicine. The greater the duration of exposure, the greater the risk of the development of resistance

irrespective of the severity of the need for antibiotics. As resistance becomes more common there becomes a greater need for alternative treatments. However despite a push for new antibiotic therapies there has been a continued decline in the number of newly approved drugs. Antibiotic resistance therefore poses a significant problem.

1.5 MALARIA:

MALARIA is a mosquito borne infectious disease caused by a eukaryotic protest of genus *Plasmodium*. It is widespread in tropical and subtropical regions, Asia, Africa and parts of America .It is the most serious and widespread parasitic disease because of its prevalence, virulence and drug resistance, having an overwhelming impact on public health in developing regions of the world, and most dreadful protozoal diseases affecting man each year, more than 500 million people are infected and nearly two million people die of malaria, the majority of who are young children. Malaria is commonly associated with poverty, and can indeed be a cause of poverty and a major hindrance to economic development.

Malarial Parasite: [20, 21]

Casual organism for malaria belongs to genus *Plasmodium*. Five species of the Plasmodium parasite can infect humans: the most serious forms of the disease are caused by *Plasmodium falciparum*. Malaria caused by *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malaria* are milder and is not generally fatal. A fifth species, *Plasmodium knowlesi*, is a zoonosis that causes malaria in macaques can also infect humans. *Plasmodium falciparum* is the main cause of severe clinical malaria and Endemic map indicates that. *P.falciparum* account for 95% of the malaria infections and is also responsible for about 90% of the deaths from malaria.

Malaria parasites contain apicoplasts, that play a crucial role in various aspects of parasite metabolism e.g. fatty acid bio-synthesis, 466 proteins have been found to be produced by apicoplast and these are now being looked at as possible targets for novel anti-malarial drugs.

Mosquito vectors and the *Plasmodium* life cycle: [22, 23, 24]

The life cycle of the malarial parasite (*Plasmodium*) is complicated and involves two hosts, humans and *Anopheles* mosquitoes. The parasite's primary (definitive) hosts and transmission vectors are female mosquitoes of the *Anopheles* genus, while humans and other vertebrates are secondary hosts. Young mosquitoes first ingest the malaria parasite by feeding on an infected human carrier and the infected *Anopheles* mosquitoes carry *Plasmodium* sporozoites in their salivary gland. Once ingested the parasite gametocytes taken up in the blood will further differentiate into male or female gametes and then fuse in the mosquito gut. This produces an ookinete that penetrates the gut lining and produces an oocyst in the gut wall. When the oocyst ruptures, it releases sporozoites that migrate through the mosquito's body to the salivary glands, where they are then ready to infect a new human host. Only female mosquitoes transmit the disease. Malaria parasites can also be transmitted by blood transfusions, although this is rare.

Malaria in humans develops via two phases: an exoerythrocytic and an erythrocytic phase. The exoerythrocytic phase involves infection of the hepatic system, or liver, whereas the erythrocytic phase involves infection of the erythrocytes, or red blood cells. When an infected mosquito pierces a person's skin to take a blood meal, sporozoites in the mosquito's saliva enter the bloodstream and migrate to the liver. Once in the liver, these organisms differentiate to yield thousands of merozoites. Following rupture of their host cells, escape into the blood and infect red blood cells, thus beginning the erythrocytic stage of the life cycle. The parasite escapes from the liver undetected by wrapping itself in the cell membrane of the infected host liver cell. Within the red blood cells, the parasites multiply further, again asexually, periodically breaking out of their hosts to invade fresh red blood cells. Several such amplification cycles occur. Thus, classical descriptions of waves of fever arise from simultaneous waves of merozoites escaping and infecting red blood cells.

Some *P.vivax, P. ovale* sporozoites do not immediately develop into exoerythrocytic-phase merozoites, but instead produce hypnozoites that remain dormant for periods ranging from several months (6–12 months is typical) to as long as three years. After a period of dormancy, they reactivate and produce merozoites. Hypnozoites are responsible for long incubation and late relapses in these two species of malaria.

The parasite is relatively protected from attack by the body's immune system because for most of its human life cycle it resides within the liver and blood cells and is relatively invisible to immune surveillance. However, circulating infected blood cells are destroyed in the spleen. To avoid this fate, the *P. falciparum* parasite displays adhesive proteins on the surface of the infected blood cells, causing the blood cells to stick to the walls of small blood vessels, thereby sequestering the parasite from passage through the general circulation and the spleen. This "stickiness" is the main factor giving rise to hemorrhagic complications of malaria. High endothelial venules (the smallest branches of the circulatory system) can be blocked by the attachment of masses of these infected red blood cells. The blockage of these vessels causes symptoms such as in placental and cerebral malaria. In cerebral malaria the sequestrated red blood cells can breach the blood brain barrier possibly leading to coma.

Although the red blood cell surface adhesive proteins (called PfEMP1, for *Plasmodium falciparum* erythrocyte membrane protein 1) are exposed to the immune system, they do not serve as good immune targets, because of their extreme diversity; there are at least 60 variations of the protein within a single parasite and effectively limitless versions within parasite populations. The parasite switches between broad repertoires of PfEMP1 surface proteins, thus staying one step ahead of the pursuing immune system.

Some merozoites turn into male and female gametocytes. Fertilization and sexual recombination of the parasite occurs in the mosquito's gut, thereby defining the mosquito as the definitive host of the disease. New sporozoites develop and travel to the mosquito's salivary gland, completing the cycle.

SYMPTOMS OF MALARIA: [25]

Symptoms of malaria include fever, shivering, arthralgia (joint pain), anemia, vomiting, hemoglobinuria, retinal damage, and convulsions. The classic symptom of malaria is cyclical fever every six hours, occurring every two days in *P. vivax* and *P. ovale* infections, while every three days for *P. malaria*. *P. falciparum* can have recurrent fever every 36–48 hours or a less pronounced and almost continuous fever. Children with malaria frequently exhibit abnormal posturing and cognitive impairments. It causes

widespread anemia . Cerebral malaria is associated with retinal whitening, which may be a useful clinical sign in distinguishing malaria from other causes of fever. Severe malaria is almost exclusively caused by *P. falciparum* infection, and usually arises 6–14 days after infection. Consequences of severe malaria include coma, splenomegaly, severe headache, cerebral ischemia, hepatomegaly, hypoglycemia, hemoglobinuria with renal failure. Severe malaria can progress extremely rapidly and cause death within hours or days. In the most severe cases of the disease, fatality rates can exceed 20%, even with intensive care and treatment.

Diagnosis: [26]

The diagnosis of malaria is confirmed by the blood tests which can be divided into microscopic and non-microscopic tests.

- A microscopic test involves staining and direct visualization of the parasite under the microscope.
- Peripheral Smear study- MP test
- Quantitative Buffy coat (QBC) test
- A non-Microscopic test involves the identification of the parasitic antigen or the anti plasmodial antibodies or the parasitic metabolic products.
- Rapid Diagnostic tests (RDTS)

1.6.1 ANTIOXIDANT

The adverse effects of oxidative stress on human health have become a serious issue. The World Health Organization (WHO) has estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components. Under stress, our bodies produce more reactive oxygen species (ROS) (e.g., superoxide anion radicals, hydroxyl radicals and hydrogen peroxide) than enzymatic antioxidants (e.g., superoxide dismutase glutathione peroxidase (GPx), and catalase) and non-enzymatic antioxidants (e.g., ascorbic acid (vitamin C), tocopherol (vitamin E), glutathione, carotenoids, and flavonoids). This imbalance leads to cell damage [27] and health problems [28].

A lack of antioxidants, which can quench the reactive free radicals, facilitates the development of degenerative diseases [(29)], including cardiovascular diseases, cancers [(30)], neurodegenerative diseases, Alzheimer's disease [(31)] and inflammatory diseases [(32)]. One solution to this problem is to supplement the diet with antioxidant compounds that are contained in natural plant sources [(33)]. These natural plant antioxidants can therefore serve as a type of preventive medicine. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human disease [(34)]. However, synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have been widely used as antioxidants in the food industry and may be responsible for liver damage and carcinogenesis [(35)].

Antioxidants including phenolic compounds (e.g., flavonoids, phenolic acids and tannins) have diverse biological effects such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic effects as a result of their antioxidant activity [(36)].

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols [(37)].

Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells. As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of disease.

Reactive oxygen species (ROS), capable of causing damage to DNA, has been associated with carcinogenesis, coronary heart disease, and many other health problems related to advancing age [38]. In low concentrations, synthetic antioxidants are also in use for many industrial processes e.g. inhibition of radical formation for preventing premature polymerization during processing, storage and transportation of unsaturated monomers. They exert their effects by scavenging or preventing the generation of ROS [39] which can protect the formation of free radicals and retard the progress of many chronic diseases [40] including cancer, neurodegenerative, inflammation and cardiovascular diseases [41].

1.7 CANCER: [42, 43]

The term carcinoma is the medical term for a malignant tumor derived from epithelial cells. It was Celsius who translated *carcino* into the Latin *cancer*, also meaning crab. Galen used "*oncos*" to describe all tumors, the root for the modern word oncology.

Hippocrates described several kinds of cancers. He called benign tumors “oncos”, Greek for swelling and malignant tumors “carcinos”, Greek for crab or crayfish. This name comes from the appearance of the cut surface of a solid malignant tumor; with a roundish hard centre surrounded by a pointy projection, vaugly resembling the shape of crab. He later added the suffix *-oma*, Greek for swelling, giving the name *Carcinoma*.

Since it was against Greek tradition to open the body, Hippocrates only described and made drawings of outwardly visible tumors on the skin, nose, and breasts. Treatment was based on the humor theory of four bodily fluids (black and yellow bile, blood, and phlegm). According to the patient's humor, treatment consisted of diet, blood-letting, and/or laxatives. Through the centuries it was discovered that cancer could occur anywhere in the body, but humor-theory based treatment remained popular until the 19th century with the discovery of cells.

Cancer is the gross distortion of the cell behavior caused by numerous gene mutations and numerous abnormalities in the production of functioning proteins. The

specific abnormalities vary greatly, depending on the type of cancer as well as the type of tissue from which cancer has originated.

Thus, there is not a single description of cancer or oncogenesis, because cancer is not a single disease. It is really a class of diseases all pertaining to unlimited cell growth that is potentially fatal to the organism.

Cancer initiates from a single cell that has been transformed due to particular changes in its DNA. Some events such as exposure to radiation or exposure to chemical carcinogen, creates a change in genome. This may be a DNA mutation, or epigenetic modification. In comparison with normal cell, a neoplastic cell is hyper responsive to growth factors, under responsive to growth inhibitors, and has an increase in metabolic transport capabilities.

A cancer cell tends to have an irregular shape, an abnormally appearing nucleus, is more mobile, is invasive, and generally shows a genomic instability. Thus the cancer cells look different, grow excessively and behave abnormally.

Solid mass of cells is generally called as tumor or a neoplasm. But tumor is not always cancerous. A tumor may also be ***benign***, benign tumors in humans can often identified and removed surgically. To be diagnosed as a cancer, a tumor must become ***malignant***. It must gain capacity to invade surrounding tissues.

Broadly, carcinomas are the cancers of epithelial cells; sarcomas are cancers of connective tissue or muscle cells, and leukemias are cancers of the blood or lymph systems. In the normal human population, over 90% of all human cancers are **carcinomas.**

1.7.2 Causes of Cancer

Cancer is a diverse class of diseases which differ widely in their causes and biology. The common thread in all known cancers is the acquisition of abnormalities in the genetic material of the cancer cell and its progeny. Research into the pathogenesis of cancer can be divided into three broad areas of focus. The first area of research focuses on the agents and events which cause or facilitate genetic changes in cells destined to become cancer. Second, it is important to uncover the precise nature of the genetic damage, and the genes which are affected by it. The third focus is on the consequences of those genetic changes on the biology of the

cell, both in generating the defining properties of a cancer cell, and in facilitating additional genetic events, leading to further progression of the cancer.

- **Chemical carcinogens:** Cancer pathogenesis is traceable back to DNA mutations that impact cell growth and metastasis. Substances that cause DNA mutations are known as mutagens, and mutagens that cause cancers are known as carcinogens. Particular substances have been linked to specific types of cancer. Tobacco smoking is associated with lung cancer and bladder cancer. Prolonged exposure to asbestos fibers is associated with mesothelioma. Many mutagens are also carcinogens, but some carcinogens are not mutagens. Alcohol is an example of a chemical carcinogen that is not a mutagen. Such chemicals are thought to promote cancers through their stimulating effect on the rate of cell mitosis.
- **Ionizing radiation:** Sources of ionizing radiation, such as Radon gas, can cause cancer. Prolonged exposure to ultraviolet radiation from the sun can lead to melanoma and other skin malignancies.
- **Hormonal imbalances:** Some hormones can act in a similar manner to non-mutagenic carcinogens in that they may stimulate excessive cell growth. A well-established example is the role of hyper estrogenic states in promoting endometrial cancer.
- **Immune system dysfunction:** HIV is associated with a number of malignancies, including Kaposi's sarcoma, Non-Hodgkin's lymphoma, and HPV-associated malignancies such as anal cancer and cervical cancer. AIDS-defining illnesses have long included these diagnoses. The increased incidence of malignancies in HIV patients points to the breakdown of immune surveillance as a possible etiology of cancer. Certain other immune deficiency states (e.g. common variable immunodeficiency and IgA deficiency) are also associated with increased risk of malignancy.
- **Heredity:** Most forms of cancer are "sporadic", and have no basis in heredity. There are, however, a number of recognised syndromes of cancer with a hereditary component, often a defective tumor suppressor allele.

- **Other causes:** A few types of cancer in non-humans have been found to be caused by the tumor cells themselves. This phenomenon is seen in Sticker's sarcoma, also known as canine transmissible venereal tumor.

New Research in Cancer Treatment

- Genetically engineered bacteria and viruses
- Anti-cancer vaccines
- Cancer markers
- Cancer growth retarders / inhibitors

1.8.1 Molecular modeling [44, 45]

Molecular modeling is the investigation of molecular structure and properties using computational chemistry and graphical visualization techniques in order to provide possible 3D representation under a given set of circumstances. Most of the molecular modeling systems strive to provide some basic set of features, visualization and manipulation of 3D molecular models including rotatable bonds, structure building, molecular mechanics, and/or dynamics, conformational analysis, electronic properties, molecular surface displays and the calculation of various physical and/ or structural properties. Molecular modeling is composed of several interlinked activities:

- **Molecular Graphics:** It allows the 3D visualization and manipulation of structure to allow visualization of different parts of molecule, to change the orientation of specific function while holding other constant and to look at other different feasible conformations. Stereochemistry relationship including detailed measurement of molecular geometry and conformations, calculations of electron densities, electrostatic potentials, energies and direct comparison of the key structural features of a range of biologically active structures can be done by molecular graphics.
- **Computational Chemistry:** It is concerned with the simulation of atomic and molecular properties of compounds of medicinal interest through equations and with the numeric methods used to solve these equations on the computer.

- **Statistical Modeling:** It encompasses the search for quantitative relationship between the structure or properties of a series of compound and their resultant biological activities**.**

1.8.2 Functions of Molecular Modeling

1. Structure Generation: Molecular structure may be generated by a variety of procedures:

- The crystal structure (if available) can be loaded from Cambridge crystallographic data file.
- 2D structure can be converted to 3D by software programs such as Chem. Office
- The structure can be built up by stitching together small fragments.
- By modifying a known structure.

2. Structure Visualization: One of the most popular uses of molecular modeling system is to visualize molecular structures in a desired form. Different methods are here to represent molecular structures:

- Ball and Stick representation
- Colored Stick representation
- Space Fill representation
- Stereo Line representation

3. Conformation Generation: The biological activity of a drug molecule is supposed to depend on one single, unique conformation hidden among all the low energy conformations. Only the bioactive conformation can bind to the specific macromolecular environment at the active site of the receptor protein. It is widely accepted that bioactive conformation is not necessarily identical with the lowest energy-conformation. However, on the other hand it cannot be the conformation that is so high in energy that it is excluded from the population of conformations in solution. With the help of molecular modeling various conformations of a molecule can be explored. There are three methods for exploring of the conformations of a molecule:

- Systematic or Grid search.

- Model building methods.
- Random methods

1.8.3 Molecular Interaction (Docking) [46]

The interaction of a drug with its receptor is a complex process. Many factors are involved in the intermolecular association such as hydrophobic; Van der Waal's, hydrogen bonding and electrostatic forces.

The process of "DOCKING" a ligand to binding sites tries to mimic the natural course of interaction of the ligand and its receptor via a lowest energy pathway. Usually the receptor is kept rigid while the conformation of the drug molecule is allowed to change. The molecules are physically moved closer to one another and the preferred docked conformation is minimized. Molecular docking is a study of how two or more molecular structures, for example drug and enzyme or receptor of protein, fit together. The most important application of docking software is virtual screening. In virtual screening the most interesting and promising molecules are selected from an existing database for further research. This places demands on the used computational method; it must be fast and reliable.

1.8.4 AutoDock 4.2

AutoDock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure.

AutoDock 4 actually consists of two main programs: *AutoDock* performs the docking of the ligand to a set of grids describing the target protein; *auto grid* pre-calculates these grids. In addition to using them for docking, the atomic affinity grids can be visualized. This can help, for example, to guide organic synthetic chemists design better binders.

AutoDock has applications in:

- X-ray crystallography;
- Structure-based drug design;

- Lead optimization;
- Virtual screening (HTS);
- Combinatorial library design;
- Protein-protein docking;
- Chemical mechanism studies.

With AutoDock Tools or ADT set up, run and analyze AutoDock dockings and isocontour Auto Grid affinity maps, as well as compute molecular surfaces, display secondary structure ribbons, compute hydrogen-bonds, and do many more useful things.
AutoDock Tools or ADT is used to

- View molecules in 3D, rotate & scale in real time.
- Add all hydrogen's or just non-polar hydrogen's.
- Assign partial atomic charges to the ligand and the macromolecule (Gasteiger or Kollman United Atom charges).
- Merge non-polar hydrogen's and their charges with their parent carbon atom.
- Set up rotatable bonds in the ligand using a graphical version of Auto Tors.
- Set up the Auto Grid Parameter File (GPF) using a visual representation of the grid box, and slider-based widgets.
- Set up the AutoDock Parameter File (DPF) using forms.
- Launch Auto Grid and AutoDock.
- Read in the results of an AutoDock job and graphically display them.
- View isocontoured Auto Grid affinity maps.

1.8.5 Energy Calculation and Energy Minimization [47]:

It was hypothesized that a ligand or drug binds to the enzyme or receptor in its most stable form i.e. 'minimum energy state' form and hence properties of this energy optimized molecule will give the information regarding physicochemical requirements which govern the biological activities. This forms the basis of energy calculation and energy minimization. Energy minimizing procedures can be divided in to two classes:

- ❖ First derivative techniques (e.g. Steepest Descent, Conjugate Gradient, and Powell method).
- ❖ Second derivative techniques (e.g. Newton-Raphson).

LITERATURE REVIEW

2.1 ANTI MICROBIAL ACTIVITY:

Zakir Hussain *et al* (2006) [48] synthesized and reported 4'-position containing electronegative groups in flavones (Fig 2.1) exhibited enhanced anti microbial than other synthesized compounds by agar well diffusion method.

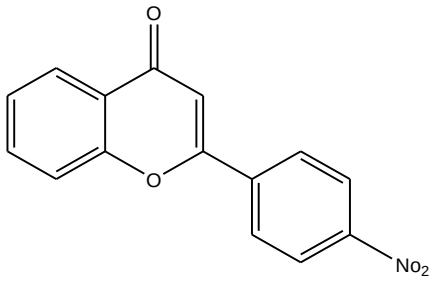


Fig: 2.1

Suresh Babu *et al* (2006) [49] synthesized and reported Substituted 5-hydroxy-2-phenyl-7-ethoxy-4H-chromen-4-one (Fig 2.2) shows more potent anti bacterial activity due to increase chain length by Serial dilution method.

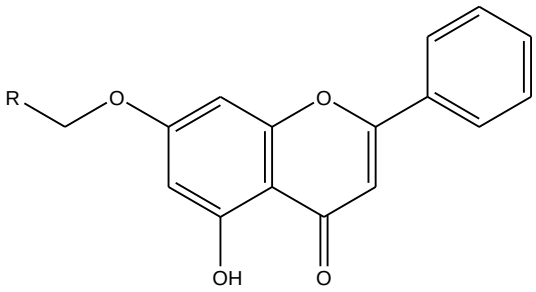


Fig 2.2

Hakan Goker *et al* (2005) [50] Synthesized and reported amidino benzimidazoles substituted flavone (Fig2.3) exhibited enhanced anti microbial activity due to N-bulky alkyl substituent at the position C-6 by macro-broth dilution assay.

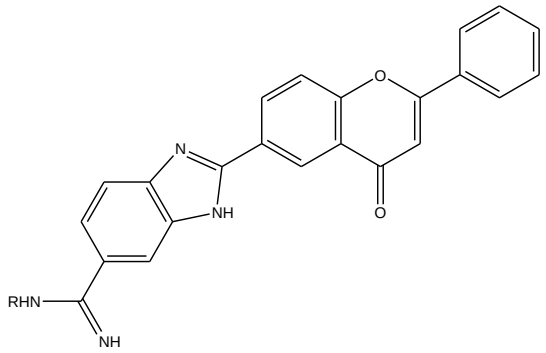


Fig: 2.3

Brown *et al* (2008) [51] Synthesized and reported 2-(4-bromophenyl)-7-methoxy-4H-chromen-4-one (Fig 2.4) as a more potent anti fungal due to the electronegative halogen in the side chain by agar well diffusion method

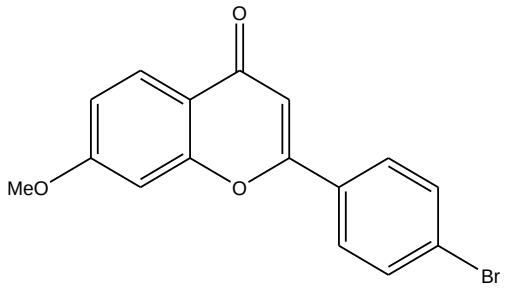


Fig 2.4

Gabriel Sagrera *et al* (2011) [52] Synthesized and reported substituted bioflavonoids (Fig 2.5) show potent activity against fungi in high concentration by Serial dilution method.

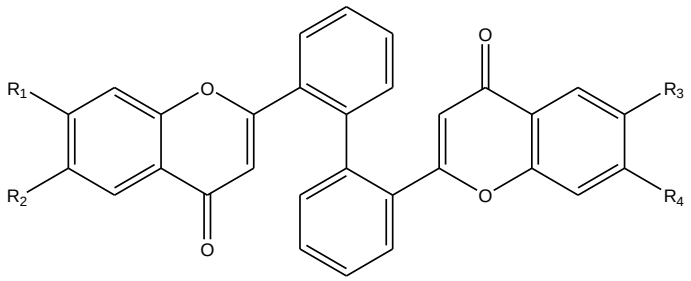


Fig 2.5

Roberta Bernini *et al* (2008) [53] Synthesized and reported mentholated flavone (Fig 2.6) shows enhanced activity against fungi even at low concentration due to racemic modification by agar well diffusion method.

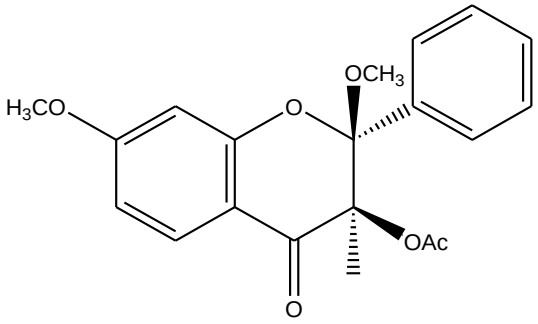


Fig: 2.6

2.2 ANTI MALARIAL ACTIVITY:

G.Auffret *et al* (2007) [54] synthesized and reported piperazinyl flavone derivatives (Fig 2.7) shows potent anti malarial activity against *Plasmodium falciparum* by [^{3}H] hypoxanthine incorporation method.

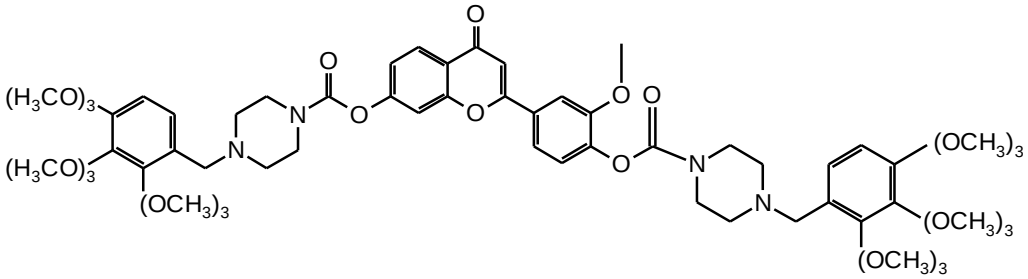


Fig 2.7

Lee *et al* (2007) [55] Synthesized and reported flavone derivative (Fig 2.8) shows enhanced anti malarial activity against *Plasmodium falciparum* even at low concentration by Giemsa stained slide method.

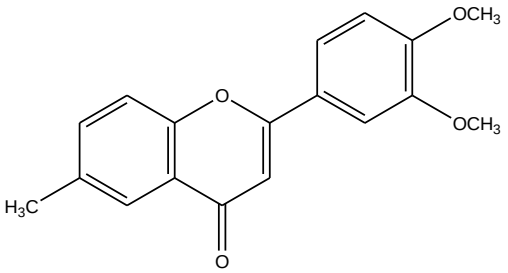


Fig 2.8

R. Ortet *et al* (2010) [56] synthesized and reported methoxy flavone (artementin) (Fig 2.9) shows potent anti malarial activity at very low concentration by Giemsa stained slide method.

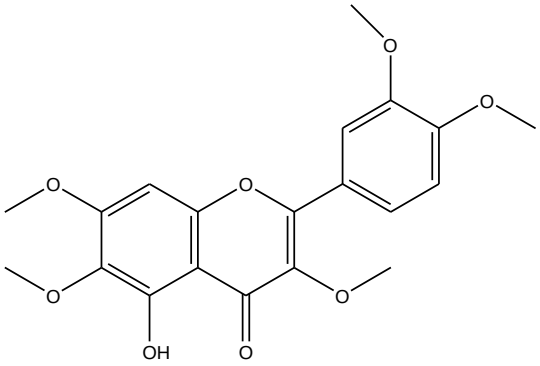


Fig 2.9

Pranorm Khaomek *et al* (2008) [57] Isolated and reported prenylated flavonoids (Fig 2.10) from *Erythrina fusca* shows enhanced anti-malarial activity than standard chloroquine by Micro-test (Mark III) method.

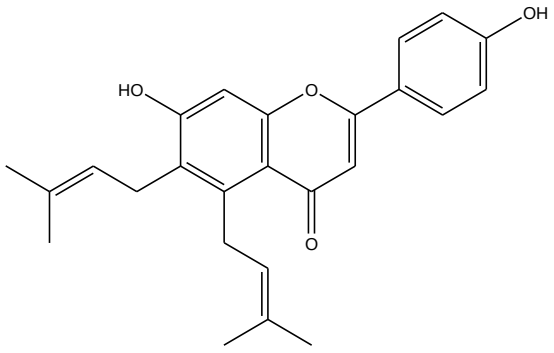


Fig 2.10

2.3 ANTI OXIDANT:

W.Chen *et al* (2006) [58] reported flavones with ortho hydroxy-amino group (Fig 2.11) shows stronger antioxidant activity than the derivatives with mono hydroxy or ortho dihydroxy group

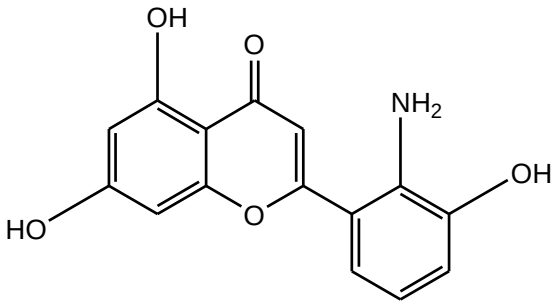


Fig 2.11

S.H. Kim *et al* (2009) [59] Synthesized and reported glucose-containing flavones (Fig 2.12) showed potent DPPH and superoxide anion radical scavenging and lipid per oxidation inhibition activities.

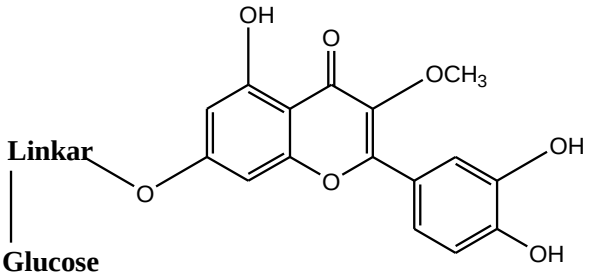


Fig 2.12

A.Gomes *et al* (2009) [60] Synthesized and reported 2-tyrylchromones and flavones (Fig 2.13) showed significant anti oxidant property by ROS and RNS scavenging assays.

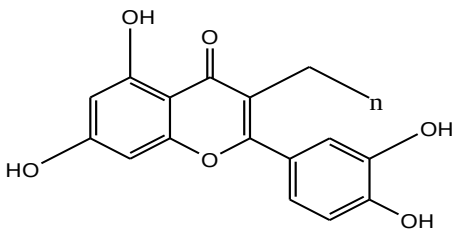


Fig 2.13

J. Gong *et al* (2009) [61] Synthesized and reported 6-OH-5, 7-dimethoxy analogue (Fig 2.14) showed significant anti oxidant mechanism involved was proposed as inhibition of xanthine oxidase, which was superior to allopurinol.

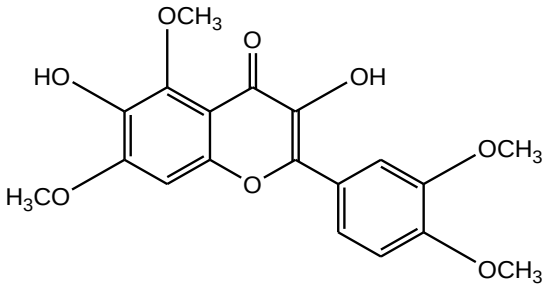


Fig 2.14

C. M. M. Santos *et al* (2010) [62] synthesized and reported the aryl flavones (Fig 2.15) showed potent Superoxide radical scavenging, Hydrogen peroxide scavenging, Singlet oxygen scavenging, Peroxyl radical scavenging, Nitric oxide scavenging and Peroxynitrite scavenging inhibition activities due to increase in number of hydroxyl group.

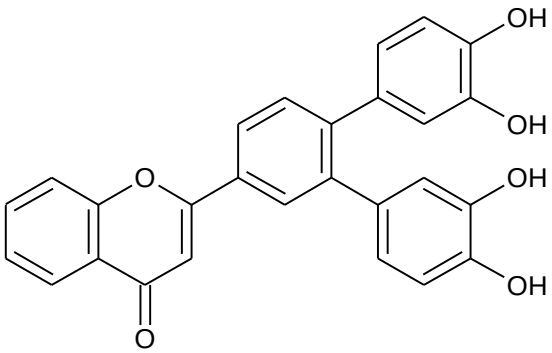


Fig 2.15

2.4 ANTI –CANCER ACTIVITY:

Kataoka *et al* (2004) [63] reported 2-(2'-amino-3'-methoxyphenyl)-4H-1-benzothiopyran-4-one (Fig 2.16) shows a more potent inhibitory effect cell proliferation.

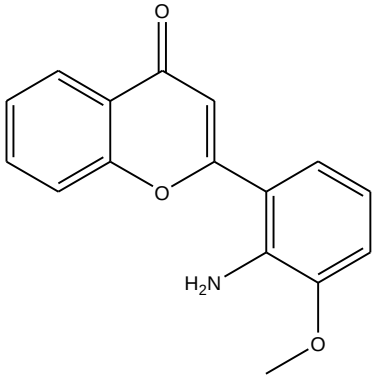


Fig 2.16

Blank *et al* (2004) [64] Synthesized and reported 2'-nitroflavone, (Fig 2.17), 2', 6-dinitroflavone (Fig: 2.18) Shows most potent anti proliferative activity.

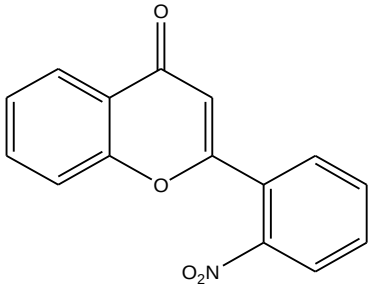


Fig 2.17

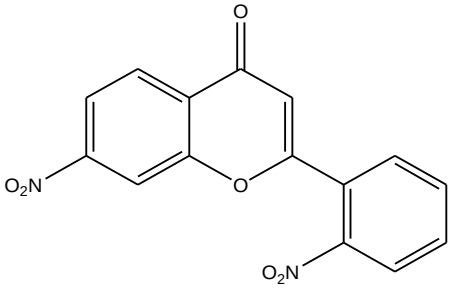


Fig 2.18

T.C. Wang *et al* (2005) [65] Synthesized and reported methyloxime-containing flavone and isoflavone derivatives (Fig 2.19) shows strong anti proliferative effect on HeLa cell line.

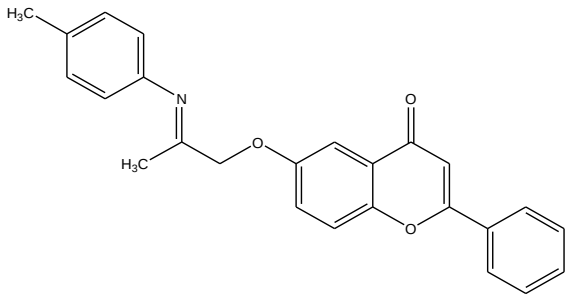


Fig 2.19

Poerwono *et al* (2010) [66] Synthesized and reported increase of the lipophilicity of the derivatives to interact with biological targets provided by the allyl or prenyl (Fig 2.20) groups can be a basis to explain the improvement of antitumor activity.

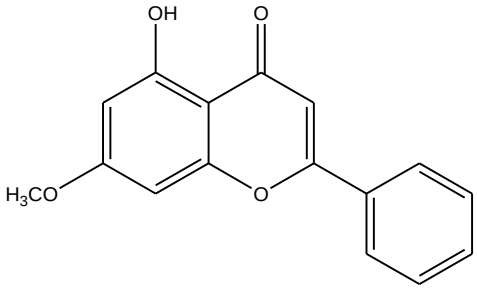


Fig 2.20

C. Pouget et al (2001) [67] reported methoxylated flavanones (Fig 2.21) were found to be potent inhibitors for their antiproliferative activity against MCF-7 human breast cancer cells.

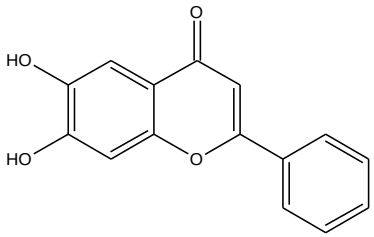


Fig 2.21

H. Liu *et al* (2004) [68] Synthesized and reported 6-chloro-2-(3, 5-dimethoxyphenyl)-4H-chromen-4-one (Fig 2.22) shows a more potent inhibitory effect cell proliferation against HepG-2 cells.

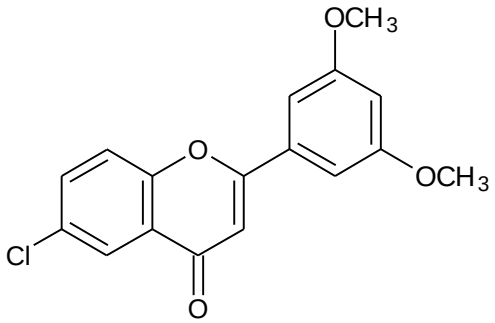


Fig 2.22

2.5 Anti-Hepatotoxicity

Viswanathan Pugalendi *et al* (2010) [69] reported 5, 7 dihydroxy flavone (Fig 2.23) posses hepatoprotective activity which is evidenced by lowered serum hepatic marker enzyme activities.

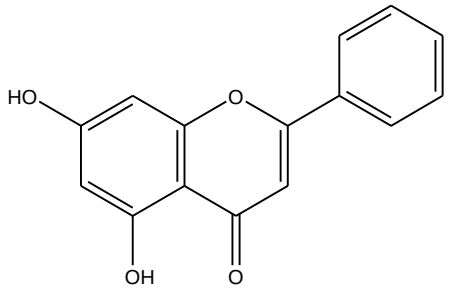


Fig: 2.23

Sharstry *et al* (2010) [70] reported 3, 5, 7-trihydroxy-2-(3, 4-dihydroxyphenyl)-4H-chromen-4-one (Fig 2.24) exhibited enhanced hepatoprotective activity against CCl_4 induced rats.

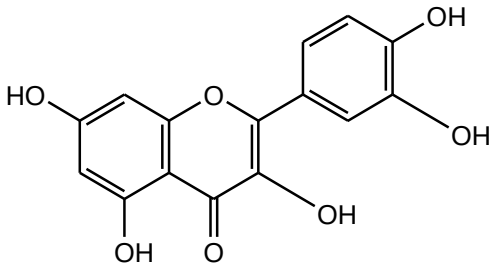


Fig: 2.24

2.6 ANTI-ALZHIMERS ACTIVITY:

R. Sheng et al. (2009) [71] reported the potent inhibitory action on Acetylcholine esterase of flavonoids (Fig 2.25) derivative which was superior to donepezil.

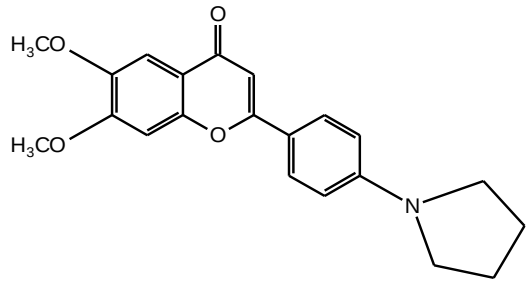
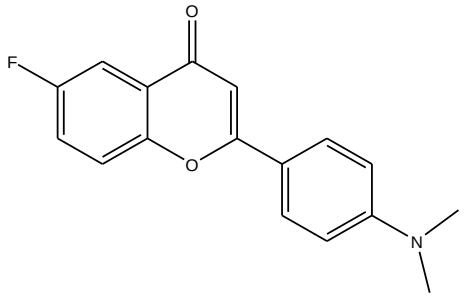


Fig 2.25

M. Ono et al.(2009) [72] reported that Fluoropegylated (Fig 2.17) flavones with the dimethylamino group shown anti - Alzheimer's activity in brain of Alzheimer's disease model mice, mechanism involved was proposed as prevention of intensely stained β-amyloid plaques.



2-(4-(dimethylamino)phenyl)flavones

Fig 2.26

2.7 ANTI HIV ACTIVITY:

Veljkovic *et al* (2007) [73] reported flavones compounds (Fig 2.27) shows antiretroviral for AIDS therapy due to their significant anti-HIV-1 activity and low toxicity.

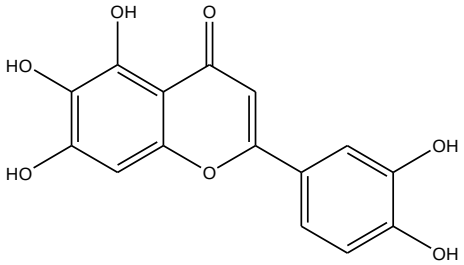


Fig 2.27

2.8 ANTI COAGULANT ACTIVITY:

Vasquez-Martinez *et al* (2007) [74] reported flavones derivative (Fig 2.28) shows potent inhibitors of human platelet mechanism involved by lipoxygenase inhibitors

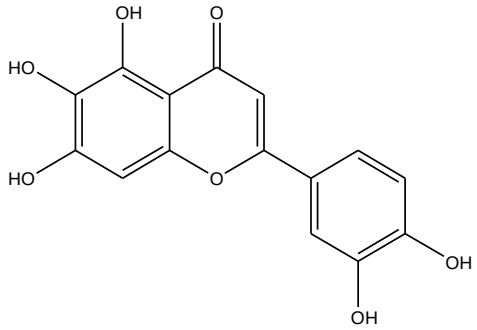


Fig 2.28

2.9 ANTI DIABETIC ACTIVITY:

Hampson *et al* (2006) [75] synthesized and reported flavones (Fig 2.29) show anti diabetic activity mechanism involved was proposed as glycogen phosphorylase inhibitors.

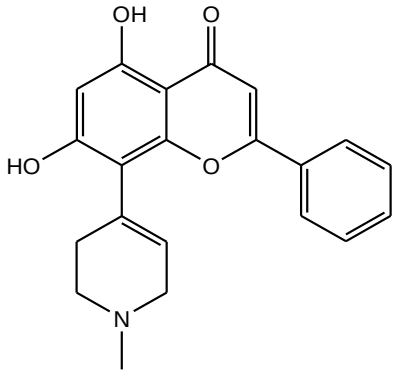


Fig 2.29

2.10 CARDIO VASCULAR ACTIVITY:

Zou *et al* (2010) [76] reported 5-acetyl-7-hydroxy-2-phenyl-4H-chromen-4-one (Fig 2.30) prevent the Vascular endothelial dysfunction disease.

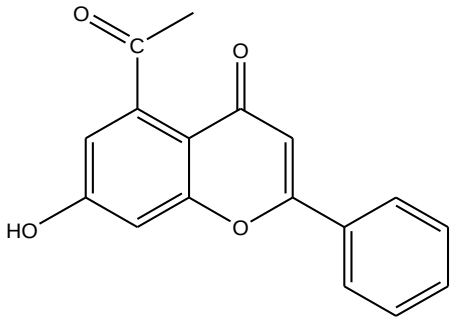


Fig: 2.30

2.11 ANTI-TUBERCULAR ACTVITY

P. Puangsombat et al (2007) [77] reported isolated 5-hydroxy-2-(2,4-dihydroxyphenyl)-7-methoxy-6-((E)-3-methylbut-1-enyl)-3-(3-methylbut-2-enyl)-4H-chromen-4-one (artocarpin) (Fig 2.31) shows potent anti-tubercular activity at low concentration by using microplate Alamar blue assay (MABA) method.

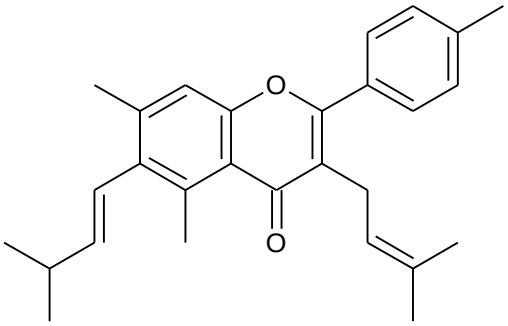


Fig 2.31

AIM AND OBJECTIVE

3.1 OBJECTIVES

In the past 60 years, antibiotics have been critical in the fight against infectious disease caused by bacteria and other microbes. However, disease-causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem. Another part of the problem is due to increasing use, and misuse, of existing antibiotics in human and veterinary medicine and in agriculture.

Antimicrobial resistance is not a new problem but one that is becoming more dangerous; urgent and consolidated efforts are needed to avoid regressing to the pre-antibiotic era. Drug resistance is becoming more severe and many infections are no longer easily cured, leading to prolonged and expensive treatment and greater risk of death.

Microbial development of resistance, as well as economic incentives, has resulted in research and development in the search for new antibiotics in order to maintain a pool of effective drugs at all times. While the development of resistant strains is inevitable, the slack ways that we administer and use antibiotics has greatly exacerbated the process [(78)].

Among the various factors that have contributed to the current resurgence of malaria, growing resistance to anti-malarial drugs is perhaps the most important. The rising all-cause mortality rate among African children is attributable directly to malaria and specifically to the rapidly increasing resistance to anti-malarial drugs. Drug resistance is also emerging as a serious problem in the Indian subcontinent. The number of effective drugs available to treat malaria is small and the rate at which resistance is growing is outpacing the development of new anti-malarials. Nearly all the anti-malarials that are in use today were developed almost 30 years ago and, in general, the pharmaceutical companies, particularly the multinationals, have little interest in developing new cure despite the enormous need. [(79)]

Flavones have attracted continuing interest because of their varied biological activities namely anti-bacterial, anti-fungal, anti-Malarial, anti-viral, anti-oxidant,

hepatoprotective, anti-inflammatory, anti-cancer, cardiovascular, anti-osteoporotic effects, anti-allergic, Anti-Alzheimer and anti-thrombotic activity.

Zakir Hussain *et al* (2006) synthesized and reported 4'-position containing electronegative groups in flavones (Fig 3.1) exhibited enhanced anti microbial activity than other synthesized compounds.

Brown *et al* (2008) reported 2-(4-bromophenyl)-7-methoxy-4H-chromen-4-one (Fig 3.2) as a more potent anti fungal due to the electronegative halogen in the side chain.

Lee *et al* (2007) reported flavone derivative (Fig 3.3) shows enhanced anti malarial activity against *Plasmodium falciparum* even at low concentration (EC_{50} = 1.0 μg/mL).

R. Ortet *et al* (2010) reported methoxy flavone (artementin) (Fig 3.4) shows potent anti malarial activity at low concentration ((IC_{50}= 3.37μg/ml).

J. Gong *et al* (2009) reported 6-OH-5, 7-dimethoxy analogue (Fig 3.5) showed significant anti oxidant mechanism involved was proposed as inhibition of xanthine oxidase.

A.Gomes *et al* (2009) reported flavones (Fig 3.6) showed significant anti oxidant property by ROS and RNS scavenging assays.

Kataoka *et al* (2004) reported 2-(2'-amino-3'-methoxyphenyl)-4H-1-benzothiopyran-4-one (Fig 3.7) shows a more potent inhibitory effect cell proliferation.

Blank *et al* (2004) reported 2'-nitroflavone, 2', 6-dinitroflavone (Fig 3.8) shows most potent anti proliferative activity.

The structural similarities of previously reported anti-microbial, anti-malarial, anti-oxidant, anti-cancer and our newly synthesised compounds (3.9) are shown below. Hence it is rational that to screen these compounds for cytotoxic, anti-malarial, anti microbial and antioxidant activities**.**

REPORTED COMPOUNDS:

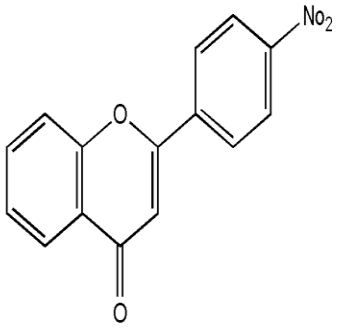


Fig 3.1

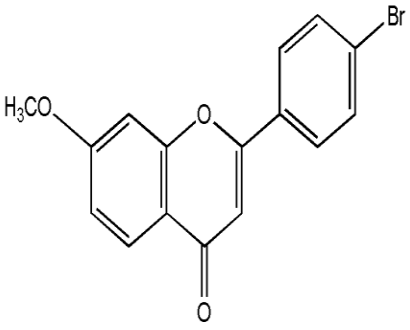


Fig 3.2

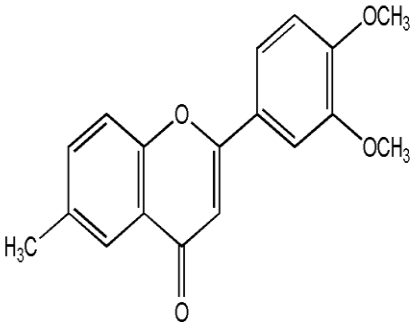


Fig 3.3

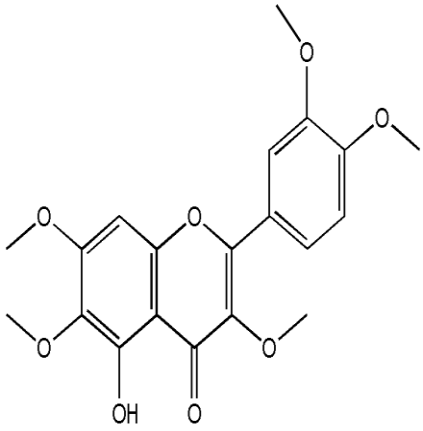


Fig 3.4

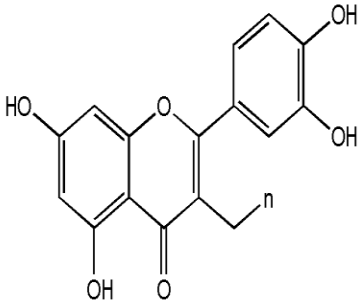


Fig 3.5

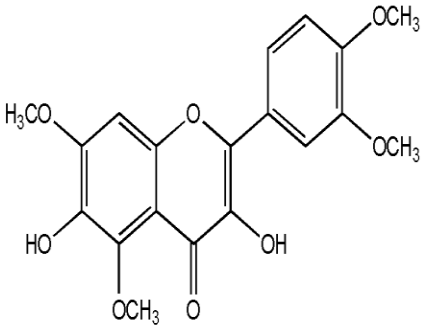


Fig 3.6

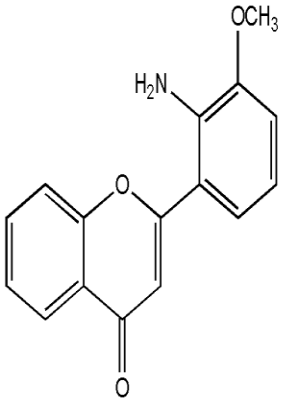


Fig 3.7

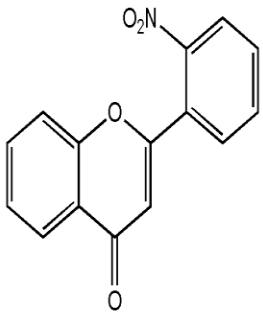


Fig 3.8

PROPOSED COMPOUND:

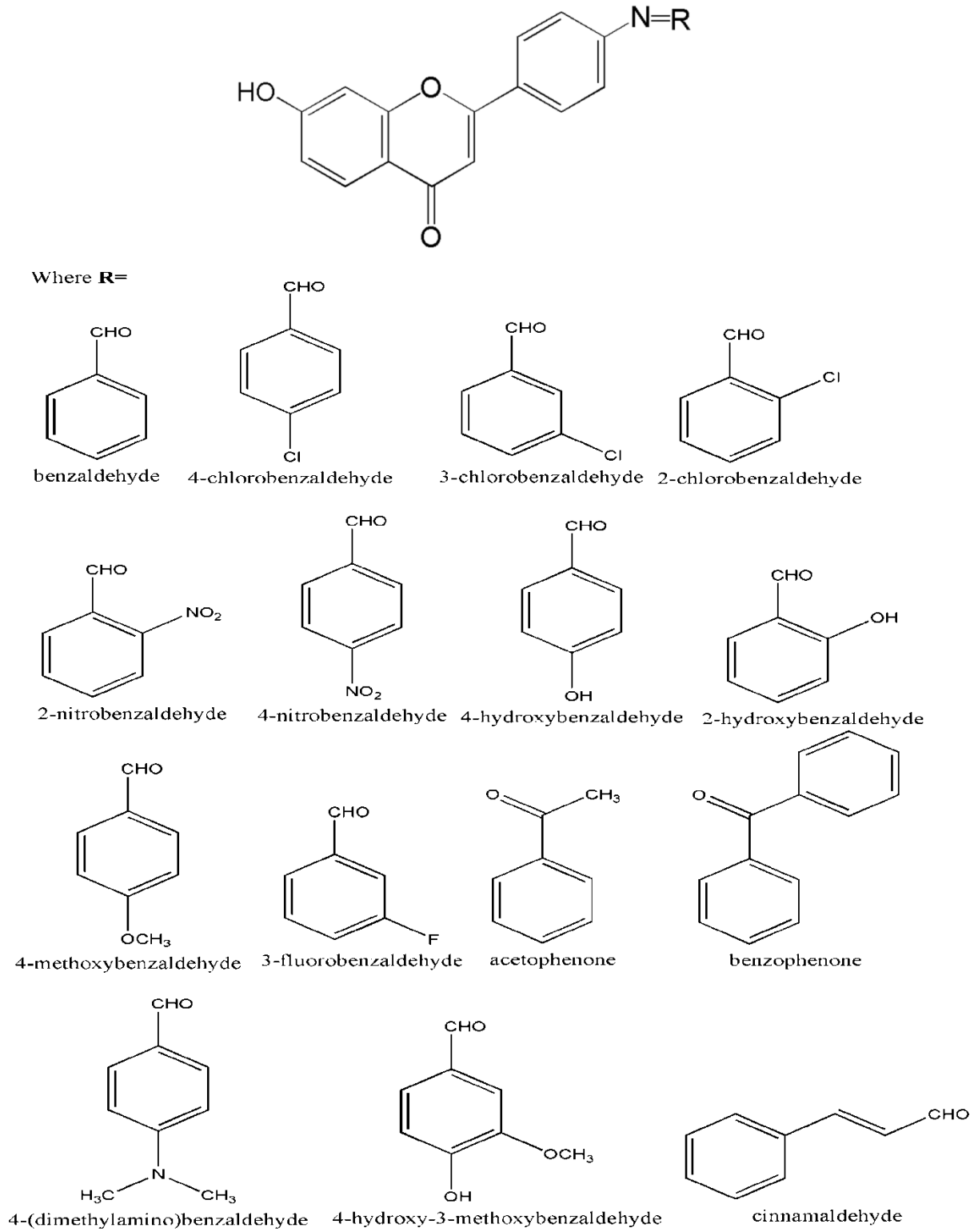


Fig 3.9

With this background the aim of our work is

To synthesize, some newer novel flavones bearing various aryl carbonyl compounds, with the carefully selected auxophores like chloro, nitro, Hydroxy and methoxy aldehydes groups and to facilitate biological activities of lead molecule by the appropriate synthetic route for *In-vitro* Anti-Malarial, *In-vitro* anti-microbial, *In-vitro* Anti-cancer and *In-vitro* antioxidant activities.

Molecular Docking studies

Molecular docking study was on *pf*-DHFR-TS- Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PDB: 1J3I) protein on flavone moiety with substituted carbonyl compounds.

PLAN OF WORK

4. PLAN OF WORK:

- Literature review and scheme development
- Synthesis by conventional method.
- Synthesis by microwave irradiation method
- Physicochemical and Characterization studies
- Evaluation of *In-vitro* antibacterial activity against 8 pathogenic bacteria and 4 Pathogenic fungal activity by Disc Diffusion Method and Minimum Inhibitory Concentration
- Evaluations of *In-Vitro* anti malarial activity by candle jar method.
- Evaluation *In-vitro* antioxidant study by DPPH, ABTS, FRAP assay method.
- Evaluation *In-vitro* anti-cancer activity by MTT assay method.
- Molecular docking studies

EXPERIMENTAL

5.0 GENERAL:

DETERMINATION OF PHYSICOCHEMICAL PROPERTIES OF SYNTHESIZED COMPOUNDS

MELTING POINT ANALYSIS

Melting points of the synthesized compounds were determined in a one end fused capillary tube method by using Thermionic Model–C-LMP- 1 CAMPVEEL Serial. No. 0712022 melting point apparatus, and were uncorrected.

THIN LAYER CHROMATOGRAPHY ANALYSIS

Purity of the compounds was checked by TLC using silica gel G (0.5mm thickness) coated over glass plate (12 x 20 cm). For the determination R_f value the dried silica gel G coated over glass plate were used.

Preparation of TLC plate: By using distilled water silica gel G slurry is prepared and poured on to a glass plate which is maintained on a level surface. The slurry is spread uniformly on the surface of the glass plate. After setting, the plates are dried in an oven at 50˚C or 15 minutes for activating the TLC plate

Chromatogram was developed by ascending technique when solvent front travelled appropriate distance; plates were taken out and dried. The location of spot was detected by using iodine chamber.

R_f = Distance travelled by solute / Distance travelled by solvent

SOLUBILITY

Solubility the solubility of synthesised compound was tested in different solvents such as polar, semi polar, and non polar solvents.

Polar solvent: A solvent in whose molecules there is either a permanent separation of positive and negative charges, or the centres of positive and negative charges do not coincide; these solvents have high dielectric constants, are chemically active, and form coordinate covalent bonds; examples are water and carboxylic acids etc.

Semi polar solvent: Semi-polar solvents may induce a certain degree of polarity in non-polar molecules and may thus act to improve the miscibility of polar and non-polar liquids; examples are alcohols and ketones etc.

nonpolar solvent: a liquid solvent without significant partial charges on any atoms, as in the hydrocarbons, or where the polar bonds are arranged in such a way that the effects of their partial charges cancel out, as in carbon tetrachloride. Liquid hydrocarbons are the most common examples.

The following table 5.1 indicates the meanings of the terms used in statements of approximate solubilities.

Table: 5.1

Descriptive term	Approximate volume of solvent in milliliters per gram of solute
Very soluble	less than 1
Freely soluble	from 1-10
Soluble	from 10-30
Sparingly soluble	from 30-100
Slightly soluble	from 100-1000
Very slightly soluble	from 1000-10,000
Insoluble or practically insoluble	more than 10,000

Clog P Value

Calculated Log P value is computer program which calculates the relative hydrophobicity of a molecule. The value of ClogP tells you how hydrophobic a molecule. The higher the ClogP value, the more hydrophobic the molecule is. Clog P value is calculated using ChemDraw Ultra 8.0.

SPECTRAL STUDIES

CONFIRMATION OF THE STRUCTURE OF SYNTHESIZED COMPOUNDS

ULTRA VIOLET SPECTRAL ANALYSIS

The maximum absorbance or λmax of synthesized compounds were determined at 0.01%w/v concentration in ethanol by using Shimadzu 2000, UV1700 ultraviolet Spectrophotometer at KMCH College of pharmacy. The maximum absorbance was measured in nm.

INFRARED SPECTRAL ANALYSIS

The IR Spectra of the synthesized compounds were recorded at KMCH college of pharmacy by JASCO-FT/IR -1700, Serial no B016861016 spectrophotometer in KBr disc. The IR value was measured in cm^{-1}.

NUCLEAR MAGNETIC RESONANCE SPECTRAL ANALYSIS

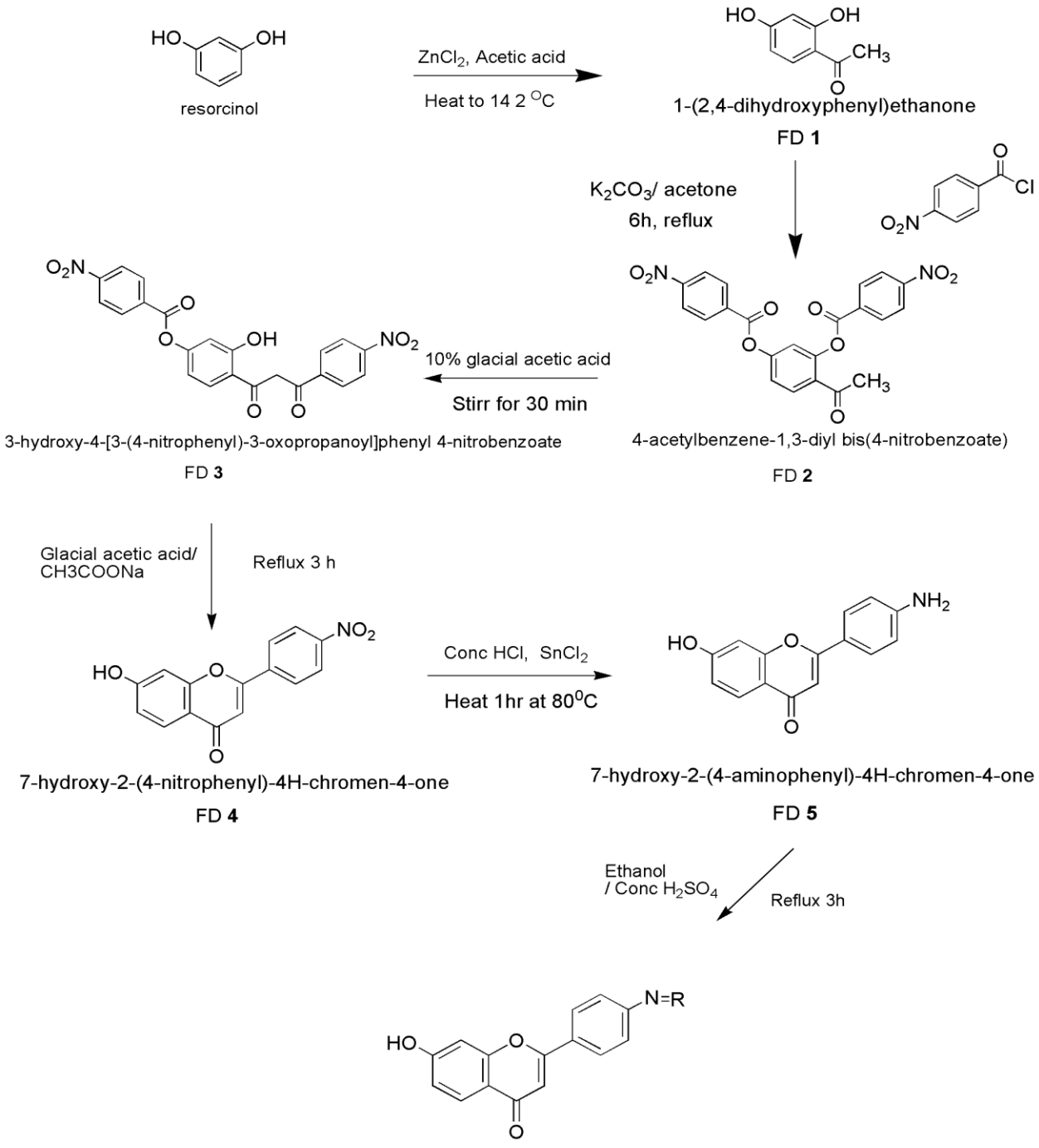
The NMR Spectra of the synthesized compounds were recorded at IIT Madras by Bruker 300 MHz FT- NMR using TMS (Tetra Methyl Silane) as internal standard. The PMR (Proton Magnetic Resonance) spectroscopic values are measured in δ ppm in DMSO-d_6. Compounds were particulars of work done on DSX-300/AV-III 400/DRX-500/AV-III 500(S)/ (L) AV-700 NMR spectrometer.

MASS SPECTRAL ANALYSIS

The Mass Spectra of the synthesized compounds were recorded at IIT Madras in MS (EI) JEOL GC MATE 700 EV.

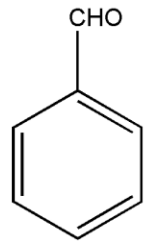
METHODOLOGY

SERIES I: CONVENTIONAL METHOD

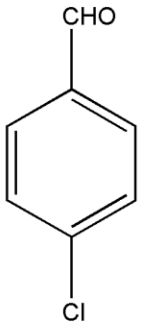


Scheme 5.1 Synthesis of 4'- Aryl carbonyl substituted amino phenyl flavones by conventional method (DPN 1-15)

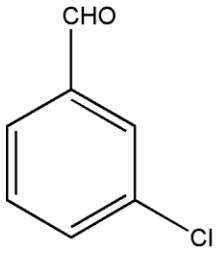
Where **R**=



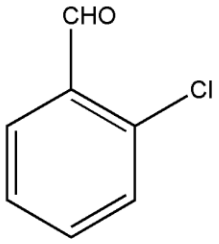
benzaldehyde



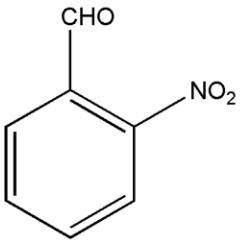
4-chlorobenzaldehyde



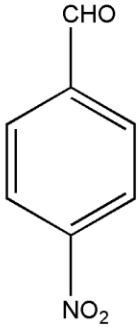
3-chlorobenzaldehyde



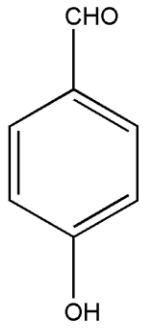
2-chlorobenzaldehyde



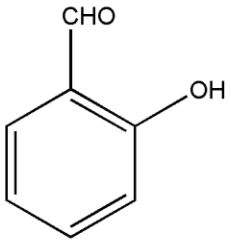
2-nitrobenzaldehyde



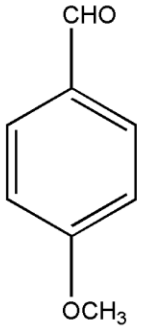
4-nitrobenzaldehyde



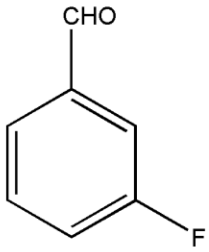
4-hydroxybenzaldehyde



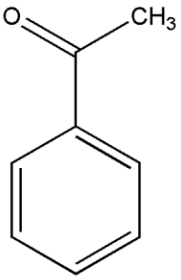
2-hydroxybenzaldehyde



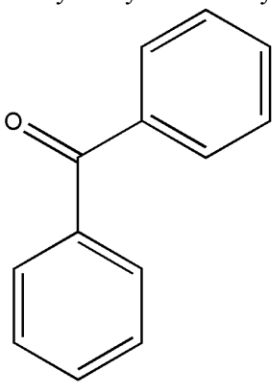
4-methoxybenzaldehyde



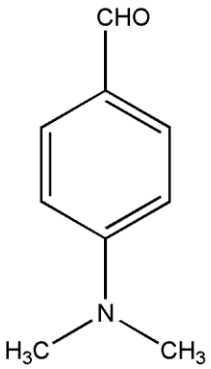
3-fluorobenzaldehyde



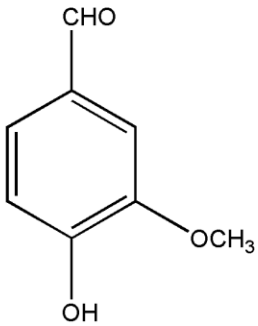
acetophenone



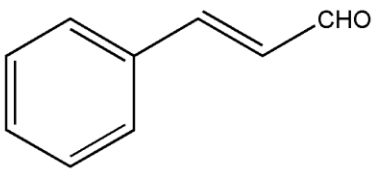
benzophenone



4-(dimethylamino)benzaldehyde



4-hydroxy-3-methoxybenzaldehyde



cinnamaldehyde

PROCEDURE

5.1a Preparation of resacetophenone (FD 1) [80]

Dissolve anhydrous zinc chloride (1.2M, 16.5g) in glacial acetic acid (15.8 ml) by heat at about 120^0C on a sand bath. To this mixture add resorcinol (1M, 11 g) with constant stirring and continue heat upto 159^0C. Remove the heat source and the reaction mixture was kept on sand bath for 20 minutes. The resulting solution was diluted with a mixture of 25 ml of concentrated hydrochloric acid and 25 ml of water. The dark red solution was placed in an ice bath and cooled to 5^0C. The resulting orange yellow precipitate was collected and recrystallized with ethanol. The percentage yield: 65%, mp: 145^0-150^0 C. The compound was confirmed by IR spectra (3118 cm^{-1} –OH, 1689 cm^{-1} for keto group, 1592 cm^{-1} for aryl ring).

5.1b Preparation of 4-acetylbenzene-1, 3-diyl bis (4-nitrobenzoate) (FD 2) [81]

Resacetophenone (0.025 M, 3.8g), dry potassium carbonate (4g), acetone (6M, 12 ml) and p-nitro benzoyl chloride (0.035M, 6.51g) were added slowly in a round bottom flask and reflux it for 6 hours. The Chocolate brown crude color product was collected, immediately used for next step.

5.1c Preparation of 3-hydroxy-4-[3-(4-nitrophenyl)-3-oxopropanoyl] phenyl 4-nitro benzoate (FD 3) [81]

The crude product 4-acetylbenzene-1, 3-diyl bis (4-nitrobenzoate) (**FD 2**) were mixed with 10% glacial acetic acid and stirred for 30 minutes. The chocolate brown color product was obtained, rinsed with water, filtered, dried and recrystallized with ethanol. The percentage Yield: 58%, Mp: 160^0-165^0 C, Rf = 0.61 (Benzene: Pyridine: Ammonia [8:2:1 ratio]). The compound was confirmed by IR spectra (1768 cm^{-1} for esters, 1685 cm^{-1} for keto , 3116 for –OH group,1602 cm^{-1} for aryl ring, 1518 cm^{-1} N=O group)

5.1d Preparation of 7-hydroxy-2-(4-nitrophenyl)-4*H*-chromen-4-one (FD 4) [81]

Dissolve 3-hydroxy-4-[3-(4-nitrophenyl)-3-oxopropanoyl] phenyl 4-nitro benzoate **(FD 3)** (0.01 M, 4 gm) in 30 ml of acetone in a round bottom flask. Dissolve sodium acetate (0.004 M, 0.3gm) in glacial acetic acid (10 ml) in a beaker. Mix both the solution and reflux it for 3 hours. Pour the resulting mixture in crushed ice (150 gm) and

stir well. Filter the dark straw color product, dried and recrystallized with ethanol. The percentage yield: 60%, Mp: 190^{0}-195^{0}C. Rf = 0.58 (Benzene: Pyridine: Ammonia [8:2:1 ratio]). The compound was confirmed by IR spectra (peaks absent for esters, 1689 cm^{-1} for keto, 1248 cm^{-1} for Ar-O-R , 3111 for –OH group 1607 cm^{-1} for aryl ring, 1524 cm^{-1} N=O group).

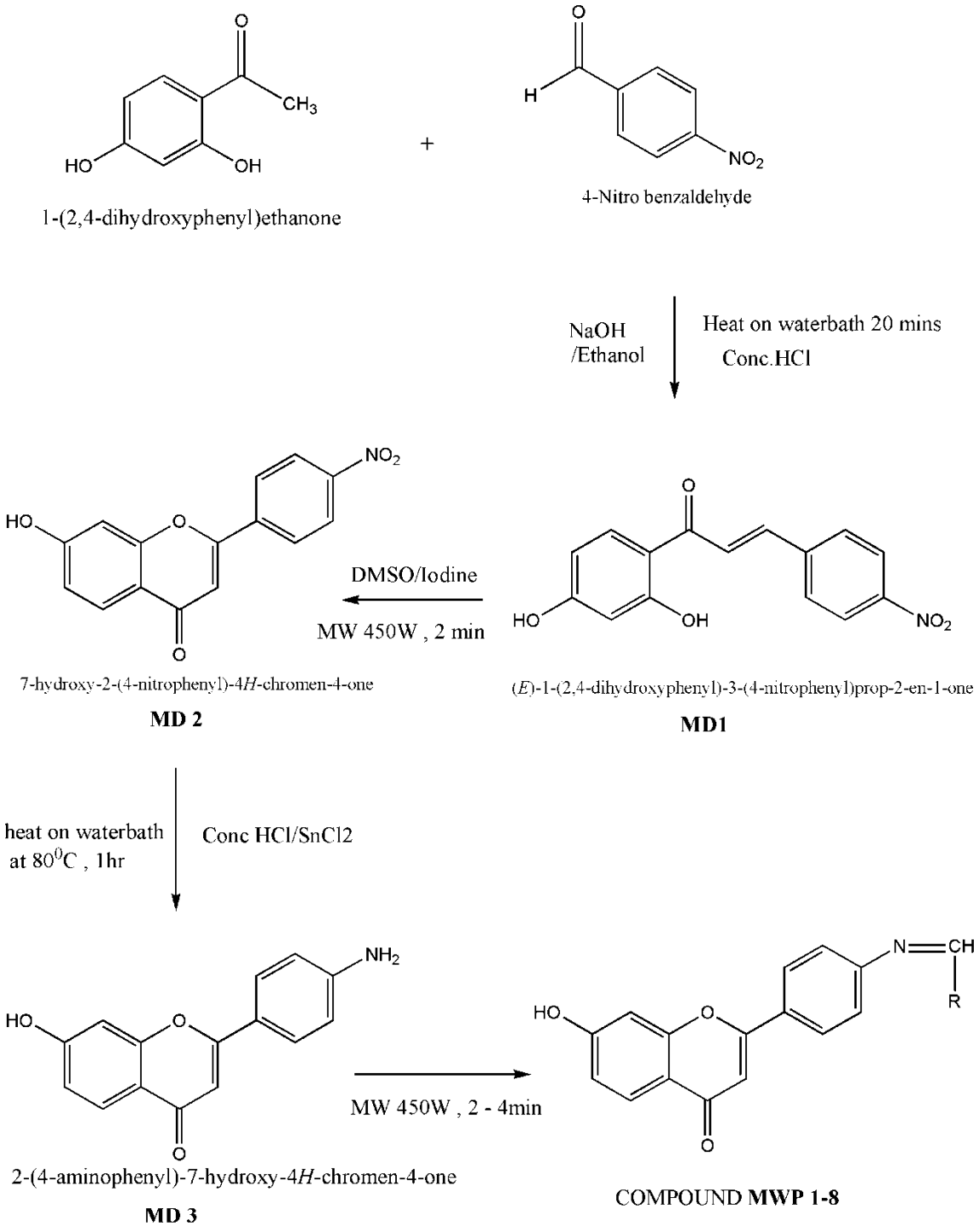
5.1e Preparation of 7-hydroxy-2-(4-aminophenyl)-4H-chromen-4-one (FD 5) [82]

Dissolve 7-hydroxy-2-(4-nitrophenyl)-4*H*-chromen-4-one **(FD 4)** (0.01M, 2.82g) in conc. HCl (13 ml), ethanol (100 ml), and $SnCl_2$ (2.00 g, 10.0 mmol) were added slowly. The suspension was heat at 80 ^{0}C for 1 hour, cool to room temperature. The solid was filtered, washed with concentrated HCl, water and dilute ammonium, and dried in vacuum to give dark brown color product of 7-hydroxy-2-(4-aminophenyl)-4H-chromen-4-one (FD 5). The percentage yield: 60%, Mp: 225^{0}-230^{0}C, Rf = 0.56 (Benzene: Pyridine: Ammonia [8:2:1 ratio]).

5.1f Preparation of 7-hydroxy-2-(4-Substituted amino phenyl)-4*H*-chromen-4-one (DPN 1-15) [83]

To a suspension equal mole of aryl aldehydes / Ketones and **7-hydroxy-2-(4-aminophenyl)-4H-chromen-4-one (FD 5)** (0.005 mol) in ethanol (10 ml). The suspension was heated until a clear solution was obtained. A few drops of conc. sulfuric acid were added as a catalyst and the solution was refluxed for 3 h on a water bath. The precipitated solid was filtered off and recrystallized from ethanol. Physiochemical parameters and analytical data were given in the table 5.1 & 5.5.

SERIES II: MICROWAVE IRRADIATION METHOD



Scheme 5.2: Synthesis of 4'- Aryl carbonyl substituted amino phenyl Flavones by Microwave irradiation method

PROCEDURE

5.2a Preparation of (E)-1-(2, 4-dihydroxyphenyl)-3-(4-nitrophenyl) prop-2-en-1-one (MD 1): [84]

A solution of sodium hydroxide (2.5 mmole in 10ml water) in ethanol (10 ml) was added to mixture of P-Nitro Benzaldehyde and resacetophenone (1mmole each) in a conical flask. Heat on water bath for about 20 minutes, the reaction mixture was quenched into the ice and then conc. HCl was added slowly with stirring till the reaction mixture became acidic. A wine red solid was separated, filtered and washed with ice water followed by recrystallization using absolute alcohol to afford 7-hydroxy-2-(4-nitrophenyl)-4H-chromen-4-one. The percentage Yield: 78%, Mp: 236^0-238^0 C, Rf = 0.67 (Benzene: Pyridine: Ammonia [8:2:1 ratio]).

5.2b Preparation of 7-hydroxy-2-(4-nitrophenyl)-4H-chromen-4-one (MD 2): [84]

7-hydroxy-2-(4-nitrophenyl)-4H-chromen-4-one (1mmole) was suspended in DMSO (2ml) and to this solution iodine (0.02 mmole) was added. The mixture was subjected to microwave irradiation for 2 minutes at 450 W. Then it is diluted with water and extracted with diethyl ether. The organic layer was washed with 20% aqueous sodium thiosulphate, water and dried over anhydrous sodium sulphate. Then it is concentrated to get the orange yellow product 7-hydroxy-2-(4-nitrophenyl)-4H-chromen-4-one. The percentage Yield: 71%, Mp: 160^0-165^0 C, Rf = 0.58 (Benzene: Pyridine: Ammonia [8:2:1 ratio]).

5.2c Preparation of 7-hydroxy-2-(4-aminophenyl)-4H-chromen-4-one (MD 3) [82]

Dissolve 7-hydroxy-2-(4-nitrophenyl)-4*H*-chromen-4-one **(MD 2)** (0.01M, 2.82g) in conc. HCl (13 ml), ethanol (100 ml), and $SnCl_2$ (2.00 g, 10.0 mmol) were added slowly. The suspension was heat at 80 ^{0}C for 1 hour, cool to room temperature. The solid was filtered, washed with concentrated HCl, water and dilute ammonium, and dried in vacuum to give dark brown color product of 7-hydroxy-2-(4-aminophenyl)-4H-chromen-4-one (MD 3). The percentage yield: 98%, Mp: 227^0-231^0C Rf = 0.61 (Benzene: Pyridine: Ammonia [8:2:1 ratio]).

5.2d Preparation of 7-hydroxy-2-(4-Substituted amino phenyl)-4*H*-chromen-4-one (Compound MWP 1-8) [85]

Equal mole of 7-hydroxy-2-(4-aminophenyl)-4H-chromen-4-one **(MD 3)** and aromatic aldehydes and ketones were mixed together at ambient temperature in a conical flask. The mixture was subjected to microwave irradiation for an optimized time. The crude products were recrystallized with ethanol. The Physiochemical parameters and analytical data were given in the table 5.2 and 5.5.

5.3 DOCKING ANALYSIS

Compounds DPN 1-15 were used for docking on Dihydrofolate reductase and SGPT (PDB ID: 1VDR) Docking studies of designed compounds were carried out using AutoDock module version 4.1. The software package running on multi-processor Linux PC. AUTODOCK has previously been validated & applied successfully to predict the binding orientation of many ligands.

METHODOLOGY

The steps involved in docking are as follows:

- **Ligand structure**: The chemical structure of each ligand was drawn using build module**.**
- **Ligand preparation**: In order to prepare high quality, all-atom 3D structures for large numbers of drug-like molecules, starting with the 3D structures in SD Maestro format, LigPrep was used. LigPrep produced a single, low-energy, 3D structure with corrected chiralities for each successfully processed input structure.
- **Preparation of protein**: The typical structure file from the PDB is not suitable for immediate use in molecular modeling calculations. A typical PDB structure file consists only of heavy atoms and may include a co-crystallized ligand, water molecules, metal ions, and cofactors. Some structures are multimeric, and may need to be reduced to a single unit. Because of the limited resolution of X-ray experiments, it can be difficult to distinguish between NH and O, and the placement of these groups must be checked. PDB structures may be missing information on connectivity, which must be assigned, along with bond orders and formal charges. This was done using the Protein Preparation Wizard.

- **Receptor Grid Generation**: Receptor grid generation requires a "prepared" structure: an all atom structure with appropriate bond orders and formal charges. AutoDock searches for favourable interactions between one or more ligand molecules and a receptor molecule, usually a protein. The shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. The options in each tab of the Receptor Grid Generation panel allow defining the receptor structure by excluding any co-crystallized ligand that may be present, determine the position and size of the active site as it will be represented by receptor grids, and set up AutoDock constraints. A grid area was generated around the binding site of the receptor.
- **Ligand Docking**: This is carried out using AUTODOCK. AutoDock searches for favourable interactions between one or more ligand molecules and a receptor molecule, usually a protein. Each ligand acts as single molecule, while the receptor may include more than one molecule, e.g., a protein and a cofactor. AutoDock was run in rigid or flexible docking modes; the latter automatically generated conformations for each input ligand. The combination of position and orientation of a ligand relative to the receptor, along with its conformation in flexible docking, is referred to as a ligand pose. The ligand poses that AutoDock generates pass through a series of hierarchical filters that evaluate the ligand's interaction with the receptor. The initial filters test the spatial fit of the ligand to the defined active site, and examine the complementarily of ligand-receptor interactions using a grid-based method patterned after the empirical ChemScore function. Poses that passed these initial screens entered the final stage of the algorithm, which involves evaluation and minimization of a grid approximation to the OPLS-AA non bonded ligand-receptor interaction energy. Final scoring is then carried out on the energy-minimized poses.

Docking Procedure

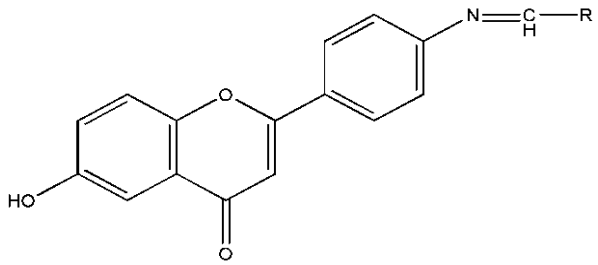
Docking studies of compounds selected compounds were performed using proteins *pf*-DHFR-TS- *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase (PDB ID: 1J3I) obtained from the RCSB Protein Data Bank, *http://www.rcsb.org/pdb*

Experiments were performed using the program AUTODOCK. Coordinates of the full-length substrate-complexed dimmer were prepared for AutoDock 4.0 calculations by running the protein preparation wizard. The p-prep script produces a new receptor file in which all residues are neutralized except those that are relatively close to the ligand (if the protein is complexed with a ligand) or form salt bridges. The impref script runs a series of restrained impact energy minimizations using the Impact utility. Minimizations were run until the average root mean square deviation (rmsd) of the non-hydrogen atoms reached 0.3Å.

AutoDock uses two boxes that share a common centre to organize its calculations: a larger enclosing box and a smaller binding box. The grids themselves are calculated within the space defined by the enclosing box. The binding box defines the space through which the centre of the defined ligand will be allowed to move during docking calculations. It provides a measure of the effective size of the search space. The only requirement on the enclosing box is that it be large enough to contain all ligand atoms, even when the ligand centre is placed at an edge or vertex of the binding box. Grid files were generated using the co crystallized ligand at the centre of the two boxes.

The size of the binding box was set at 20 Å in order to explore a large region of the protein. The three-dimensional structures of the compounds were constructed using the Maestro interface. The initial geometry of the structures was optimized using the OPLS-2005 force field performing 1000 steps of conjugate gradient minimization. The compounds were subjected to flexible docking using the pre-computed grid files. For each compound the 100 top-scored poses were saved and analyzed. The results were tabulated in table 5.6.

Table 5.1: PHYSIOCHEMICAL DATA OF COMPOUNDS DPN 1-15

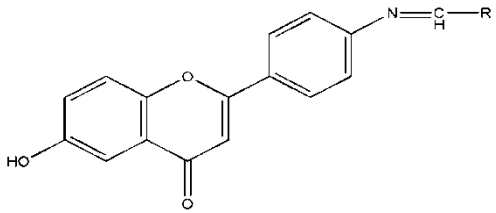


Comp. code	Structure (Ar/R)	Color	Molecular formula	Molecular Wt	MP (^{0}C)	% Yield	*Rf value	Clog P value
DPN-01	Benzaldehyde	Dark yellow	$C_{22}H_{15}NO_3$	341.36	160-165	85%	0.69	3.93
DPN-02	2-Hydroxy Benzaldehyde	Brown	$C_{22}H_{15}NO_4$	357.56	176-180	65%	0.84	4.17
DPN-03	4-Hydroxy Benzaldehyde	Dark Brown	$C_{22}H_{15}NO_4$	357.56	194-198	62%	0.84	4.17
DPN-04	2-Chloro Benzaldehyde	Light Brown	$C_{22}H_{14}ClNO_3$	375.07	145-150	69%	0.85	4.64
DPN-05	3-Chloro Benzaldehyde	Light brown	$C_{22}H_{14}ClNO_3$	375.07	165-170	62%	0.85	4.64
DPN-6	4-Chloro Benzaldehyde	Brown	$C_{22}H_{14}ClNO_3$	375.07	135-140	71%	0.86	4.64
DPN-07	2-Nitro Benzaldehyde	Dark brown	$C_{24}H_{14}N_2O_5$	386.86	109-112	67%	0.82	3.67
DPN-08	4-Nitro Benzaldehyde	Brown	$C_{24}H_{14}N_2O_5$	386.86	118-122	60%	0.83	3.67
DPN-09	3-Fluro Benzaldehyde	Brown	$C_{22}H_{14}FNO_3$	359.35	167-170	78%	0.62	4.07

DPN-10	Anisaldehyde	Pale Red	$C_{23}H_{17}NO_4$	371.39	138-142	74%	0.87	4.28
DPN-11	4-Dimethyl amino Benzaldehyde	Dark Red	$C_{24}H_{20}N_2O_3$	384.43	105-110	81%	0.56	4.62
DPN-12	Vanillin	Grayish	$C_{23}H_{17}NO_5$	387.11	120-125	62%	0.77	4.03
DPN-13	Cinnamaldehyde	Dark Brown	$C_{24}H_{17}NO_3$	367.12	85-90	65%	0.58	4.27
DPN-14	Acetophenone	Dark green	$C_{23}H_{17}NO_3$	355.12	90-95	52%	0.62	5.15
DPN-15	Benzophenone	Dark Brown	$C_{28}H_{19}NO_3$	417.14	80-85	55%	0.74	6.17

*Solvent system- Benzene: Pyridine: Ammonia (8:2: 1 ratio) Detection- UV Chamber
Solubility- DMSO and benzene

Table 5.2: PHYSIOCHEMICAL DATA OF COMPOUNDS MWP 1-8

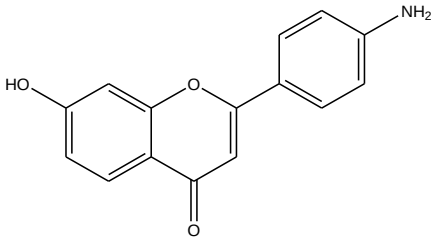
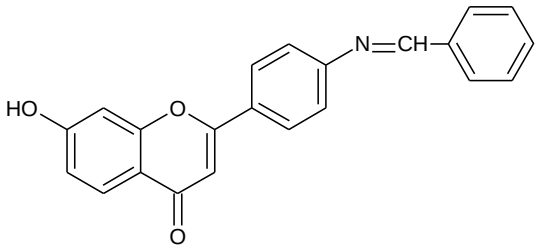


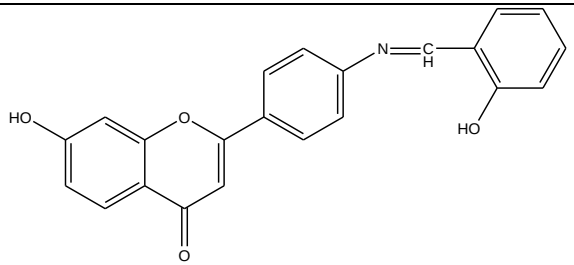
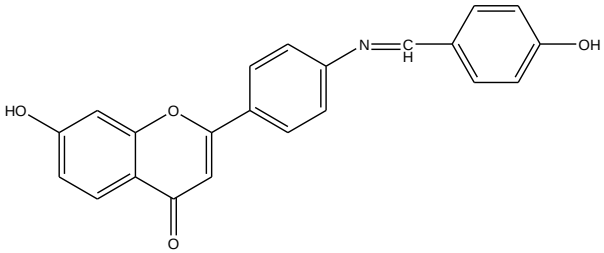
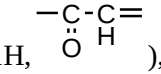
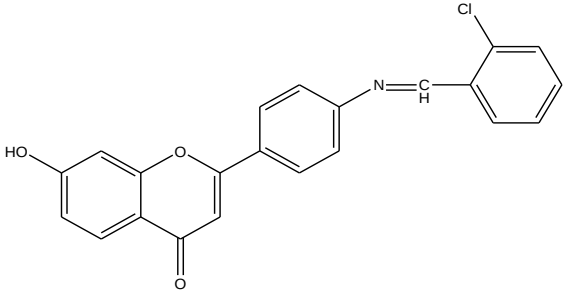
Comp. code	Structure (Ar/R)	Molecular formula	Color	Molecular Wt	MP (^{0}C)	%Yield	*Rf value	Clog P value	Time (sec)
MWP-01	Benzaldehyde	$C_{22}H_{15}NO_3$	Dark yellow	341.36	163-167	94%	0.70	3.93	150
MWP -02	4-Hydroxy Benzaldehyde	$C_{22}H_{15}NO_4$	Dark Brown	357.56	193-196	95%	0.83	4.17	180
MWP -03	4-Chloro Benzaldehyde	$C_{22}H_{14}ClNO_3$	Brown	375.07	136-140	98%	0.87	4.64	180
MWP -04	4-Nitro Benzaldehyde	$C_{24}H_{14}N_2O_5$	Brown	386.86	119-121	96%	0.84	3.67	180
MWP -05	Anisaldehyde	$C_{23}H_{17}NO_4$	Pale Red	371.39	138-143	97%	0.86	4.28	150
MWP -06	4-Dimethyl amino Benzaldehyde	$C_{24}H_{20}N_2O_3$	Dark Red	384.43	108-110	94%	0.55	4.62	210
MWP -07	Vanillin	$C_{23}H_{17}NO_5$	Grayish	387.11	121-125	97%	0.79	4.03	180
MWP -08	Acetophenone	$C_{23}H_{17}NO_3$	Dark green	355.12	91-94	98%	0.64	5.15	210

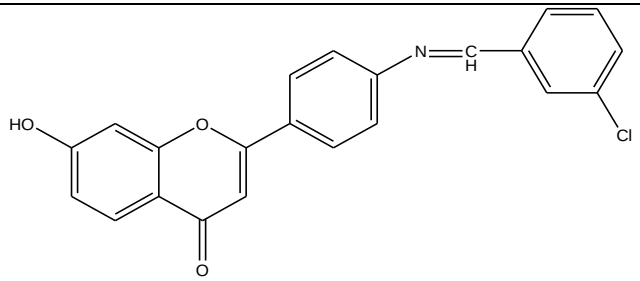
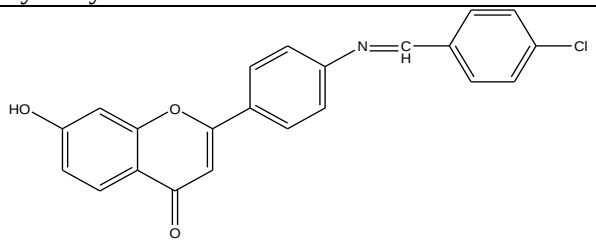
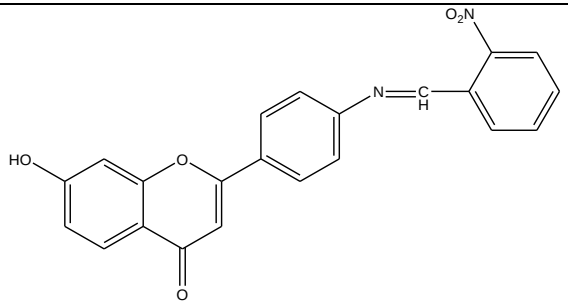
Table 5.3: COMPARISON BETWEEN CONVENTIONAL METHOD AND MICROWAVE IRRADIATION METHOD IN PERCENTAGE YIELD

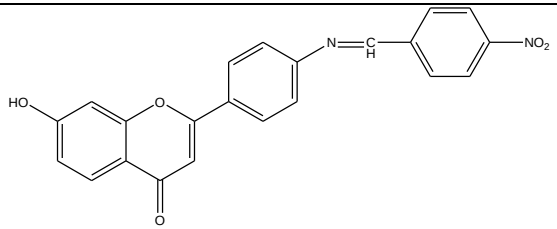
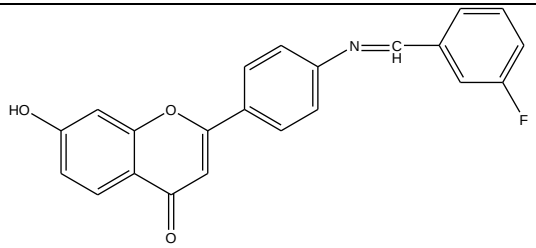
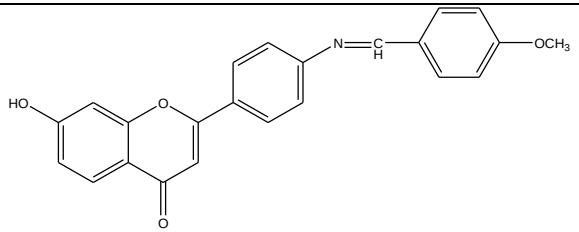
S.NO	Substituent Compound	Percentage yield	
		Conventional method	Microwave irradiation method
1	Benzaldehyde	85%	94%
2	4-Hydroxy Benzaldehyde	62%	95%
3	4-Chloro Benzaldehyde	71%	98%
4	4-Nitro Benzaldehyde	60%	96%
5	Anisaldehyde	74%	97%
6	4-Dimethyl amino Benzaldehyde	81%	94%
7	Vanillin	62%	97%
8	Acetophenone	52%	98%

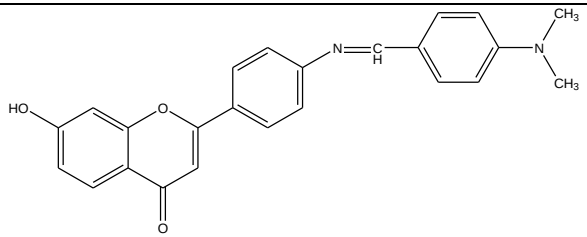
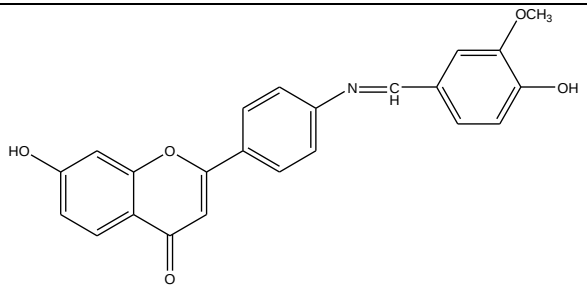
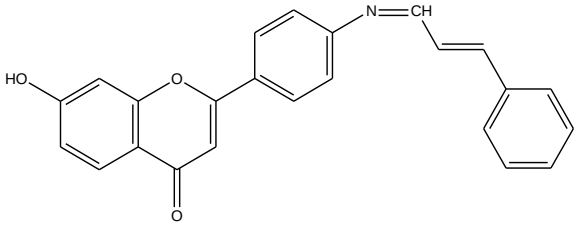
Table 5.4: SPECTRAL DATAS OF SYNTHESISED COMPOUND DPN 1-15

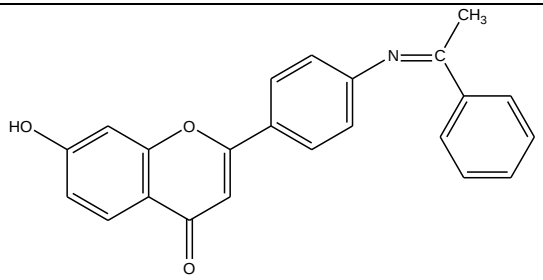
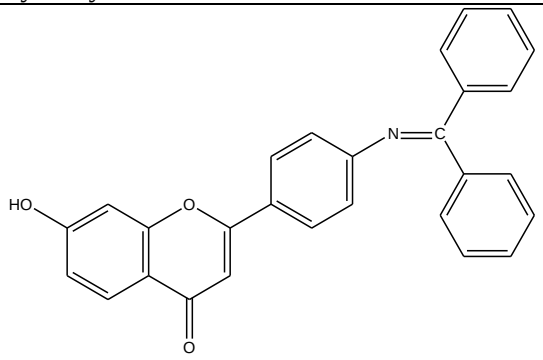
Comp. code	Molecular Structure	λ_{max} (nm)	(IR) υ_{max}(KBr/cm^{-1})	^{1}HNMR (δ- ppm)	MASS SPECTROSCOPIC DATA		
					Mol. wt	Base peak (m/z)	Molecular ion peak (m/z)
FD 5	 2-(4-aminophenyl)-7-hydroxy-4H-chromen-4-one	329	1402 (C=C aryl) 714 (C-C aromatic region) 1693 (C=O) 1108 (C-O-C) 3116 (-OH) 1540 (-NH_2 Primary)	13.63 (s,1H, -OH), 3.3(S,2H, -NH_2) 12.18 (S,1H, −C(=O)-C=CH−) 8.19-8.14 (m, 4H, $C_{2,3,5,6}$ -H of Aryl), 8.30-8.40 (m, 3H, $C_{1',3',4'}$ aryl ring)	253.05	77.44	253.25
DPN-01	 2-[4-(benzylideneamino)phenyl]-7-hydroxy-4*H*-chromen-4-one	446	1605 (C=C aryl) 716 (C-C aromatic region) 1691 (C=O) 1106 (C-O-C) 3119 (-OH)				

DPN-02	 7-hydroxy-2-{4-[(2-hydroxybenzylidene) amino]phenyl}-4*H*-chromen-4-one	420	1604 (C=C aryl) 715(C-C aryl) 838 (p-substituent) 1692 (C=O) 1107 (C-O-C) 3115 (-OH aromatic) 1349(-OH 1^0 alcohol)				
DPN-03	 7-hydroxy-2-{4-[(4-hydroxybenzylidene) amino]phenyl}-4*H*-chromen-4-one	420	1602 (C=C aryl) 717 (C-C aryl) 838 (p-substituent) 1689 (C=O) 1104 (C-O-C) 3119 (-OH aromatic) 1349(-OH 1^0 alcohol)	13.62 (s,1H, -OH), 12.18 (S,1H, ), 8.14-8.18 (m, 7H, $C_{2,3,5,6}$, $C_{1',3',4'}$ -H of Aryl ring), 8.29-8.41 (m, 4H, $C_{A,C,E,F}$ –H Aryl ring) 2.65-3.59(s,1H,-N=CH-)			
DPN-04	 2-(4-(2-chlorobenzylideneamino)phenyl)-7-hydroxy-4H-chromen-4-one	465	1602 (C=C aryl) 714 (C-C aromatic region) 1693 (C=O) 1106 (C-O-C) 3114 (-OH) 798 (o-C-Cl)				

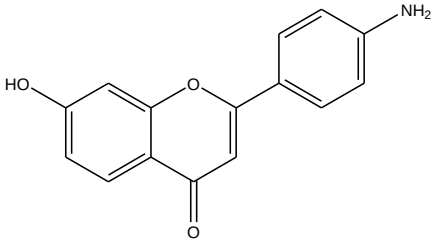
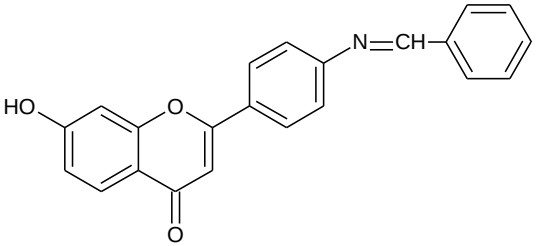
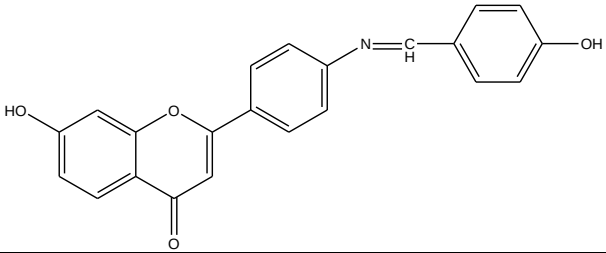
DPN-05	 2-(4-(3-chlorobenzylideneamino)phenyl)-7-hydroxy-4H-chromen-4-one	465	1604 (C=C aryl) 716 (C-C aromatic region) 1698 (C=O) 1223 (C-O-C) 3114 (-OH) 800 (C-Cl)				
DPN-06	 2-(4-(4-chlorobenzylideneamino)phenyl)-7-hydroxy-4H-chromen-4-one	465	1602 (C=C aryl) 717 (C-C aromatic region) 1698 (C=O) 1223 (C-O-C) 3114 (-OH) 820 (C-Cl)	13.1 (s,1H, -OH), 12.58 (S,1H, −C-C= O H), 7.48-7.72 (m, 4H, $C_{2,3,5,6}$ -H of Aryl), 7.79-7.94 (m, 3H, $C_{1',3',4'}$ -H aryl ring) 8.14-8.34 (m, 3H, $C_{A,B,C}$ −H Aryl ring) 3.40-3.59 (s, 1H, -N=CH-)	375.07	250.90	375.77
DPN-07	 2-(4-(2-nitrobenzylideneamino)phenyl)-7-hydroxy-4H-chromen-4-one	444	1603 (C=C aryl) 716 (C-C aromatic region) 1698 (C=O) 1105 (C-O-C) 3113 (-OH) 1530 (N=O)				

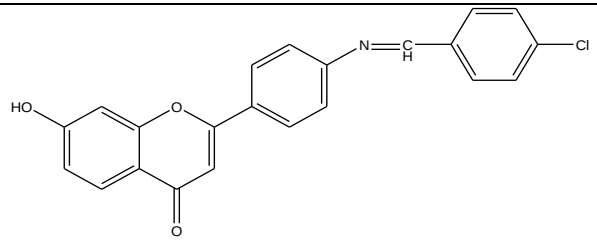
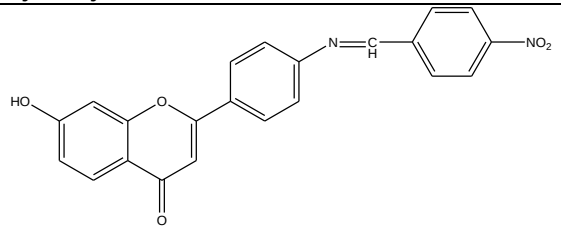
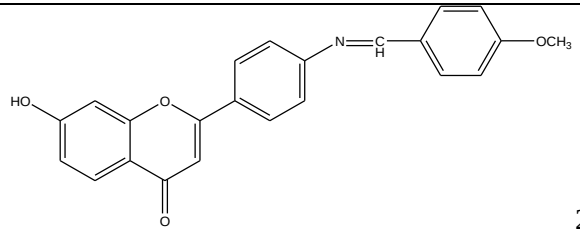
DPN-08	 2-(4-(4-nitrobenzylideneamino)phenyl)-7-hydroxy-4H-chromen-4-one	444	1603 (C=C aryl) 717 (C-C aromatic region) 1698 (C=O) 1225 (C-O-C) 3114 (-OH) 1528 (N=O)	13.62 (s,1H, -OH), 12.18 (S,1H, −C(=O)-C(H)=), 6.95-6.99 (m, 4H, $C_{2,3,5,6}$ -H of Aryl), 8.00-8.19 (m, 3H, $C_{1',3',4'}$ -H aryl ring) 8.29-8.41 (m, 4H, $C_{A,B,D,E}$ −H Aryl ring) 3.40-3.59 (s, 1H, -N=CH-)	386.86	149.46	386.32
DPN-09	 2-(4-(3-fluorobenzylideneamino)phenyl)-7-hydroxy-4H-chromen-4-one	498	1604 (C=C aryl) 717 (C-C aromatic region) 1691 (C=O) 1107 (C-O-C) 3115 (-OH) 876 (C-F)				
DPN-10	 2-(4-(4-methoxybenzylideneamino)phenyl)-7-hydroxy-4H-chromen-4-one	468	1599 (C=C aryl) 717 (C-C aromatic region) 1692 (C=O) 1023 (C-O-C) 3116 (-OH) 605 (C=C alkyl) 834 (p-substituted)				

DPN-11	 2-(4-{[4-(dimethylamino)benzylidene]amino}phenyl)-7-hydroxy-4*H*-chromen-4-one	342	1590 (C=C aryl) 1400(C=C stretching) 719 (C-C aromatic region) 1712 (C=O) 1268 (C-O-C) 3125 (-OH) 834 (p-substituted) 1168(3^0amine)		384.43	152.61	384.43
DPN-12	 7-hydroxy-2-{4-[(4-hydroxy-3-methoxybenzylidene) amino]phenyl}-4*H*-chromen-4-one	380	1596 (C=C aryl) 716 (C-C aromatic region) 1666 (C=O) 1269 (C-O-C) 3118 (-OH Phenol) 1154 (-OH 1^0) 821(di substituted)				
DPN-13	 2-((4Z)-4-((E)-3-phenylallylideneamino)phenyl)-7-hydroxy-4H-chromen-4-one	476	1603 (C=C aryl) 719 (C-C aromatic region) 1694 (C=O) 1106 (C-O-C) 3119 (-OH) 1620 (C=C alkenes)				

DPN-14	 2-((Z)-4-(1-phenylethylideneamino)phenyl)-7-hydroxy-4H-chromen-4-one	628	1605 (C=C aryl) 716 (C-C aromatic region) 1401 (Ar-C) 1693 (C=O) 1107 (C-O-C) 3117 (-OH) 1350 (Methyne C-H bend)				
DPN-15	 2-(4-(biphenyl methylene amino)phenyl)-7-hydroxy-4H-chromen-4-one	539	1600 (C=C aryl) 719 (C-C aromatic region) 1401 (Ar-C) 1691 (C=O) 1109 (C-O-C) 3119 (-OH) 1350 (Methyne C-H bend)				

SPECTRAL DATAS OF SYNTHESISED COMPOUND MWP 1-8

Comp. code	Molecular Structure	λ_{max} (nm)	(IR) υ_{max}(KBr/cm^{-1})
MD 03	 2-(4-aminophenyl)-7-hydroxy-4H-chromen-4-one	329	1402 (C=C aryl) 714 (C-C aromatic region) 1693 (C=O) 1108 (C-O-C) 3116 (-OH) 1540 (-NH_2 Primary)
MWP -01	 2-[4-(benzylideneamino)phenyl]-7-hydroxy-4*H*-chromen-4-one	446	1608 (C=C aryl) 719 (C-C aromatic region) 1695 (C=O) 1104 (C-O-C) 3121 (-OH)
MWP -02		420	1605 (C=C aryl) 716 (C-C aryl) 836 (p-substituent) 1692 (C=O) 1106 (C-O-C) 3116 (-OH aromatic) 1351(-OH 1^0 alcohol)

	7-hydroxy-2-{4-[(4-hydroxybenzylidene) amino]phenyl}-4*H*-chromen-4-one		
MWP -03	 2-(4-(4-chlorobenzylideneamino)phenyl)-7-hydroxy-4H-chromen-4-one	465	1603 (C=C aryl) 718 (C-C aromatic region) 1694 (C=O) 1224 (C-O-C) 3119 (-OH) 822(C-Cl)
MWP -04	 2-(4-(4-nitrobenzylideneamino)phenyl)-7-hydroxy-4H-chromen-4-one	444	1607 (C=C aryl) 719 (C-C aromatic region) 1694 (C=O) 1222 (C-O-C) 3115 (-OH) 1532 (N=O)
MWP -05	 2-(4-(4-methoxybenzylideneamino)phenyl)-7-hydroxy-4H-chromen-4-one	468	1602 (C=C aryl) 714 (C-C aromatic region) 1698 (C=O) 1020 (C-O-C) 3117 (-OH) 606 (C=C alkyl) 832 (p-substituted)

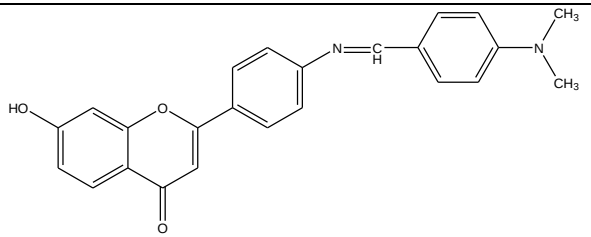
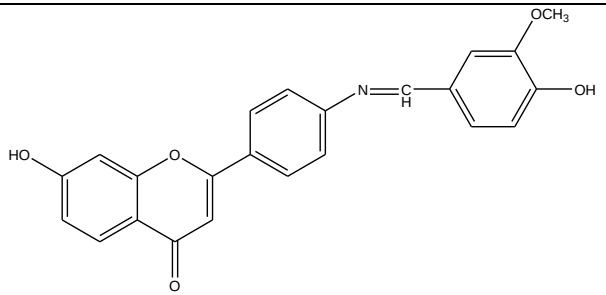
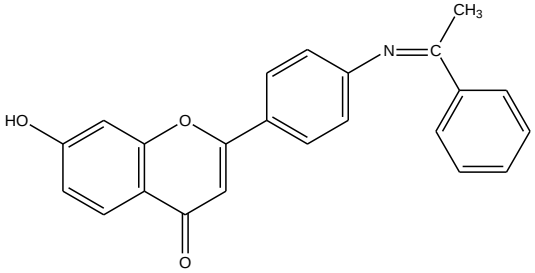
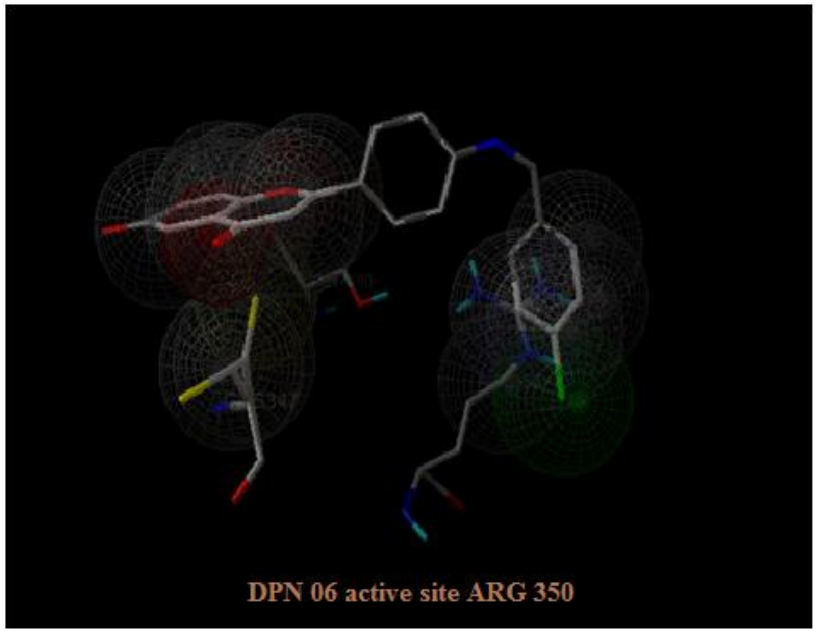
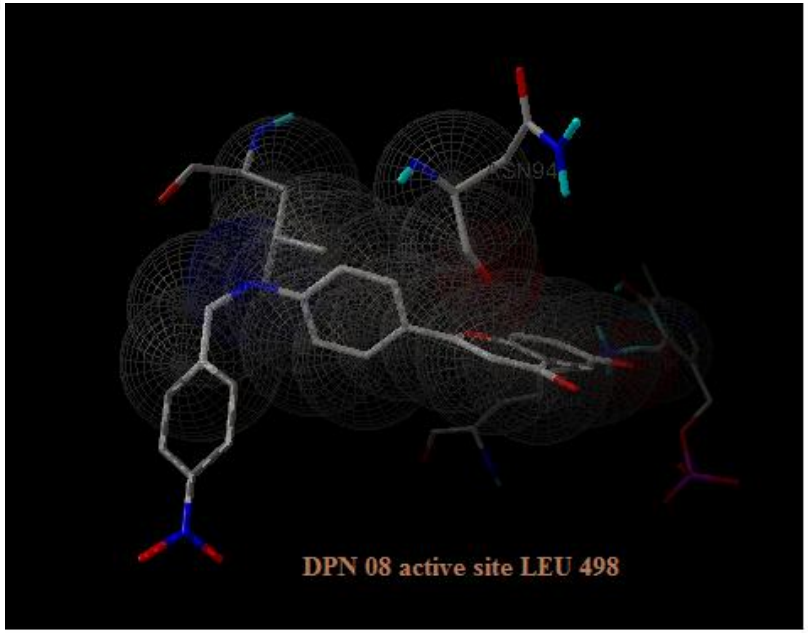
MWP -06	 2-(4-{[4-(dimethylamino)benzylidene]amino}phenyl)-7-hydroxy-4*H*-chromen-4-one	342	1593 (C=C aryl) 1404(C=C stretching) 715 (C-C aromatic region) 1706 (C=O) 1258 (C-O-C) 3120 (-OH) 832 (p-substituted) 1162 (3^0amine)
MWP -07	 7-hydroxy-2-{4-[(4-hydroxy-3-methoxybenzylidene) amino]phenyl}-4*H*-chromen-4-one	380	1598 (C=C aryl) 718(C-C aromatic region) 1669 (C=O) 1272 (C-O-C) 3112 (-OH Phenol) 1148 (-OH 1^0) 822(di substituted)
MWP -08	 2-((Z)-4-(1-phenylethylideneamino)phenyl)-7-hydroxy-4H-chromen-4-one	628	1602 (C=C aryl) 720 (C-C aromatic region) 1399 (Ar-C) 1699 (C=O) 1103 (C-O-C) 3116 (-OH)

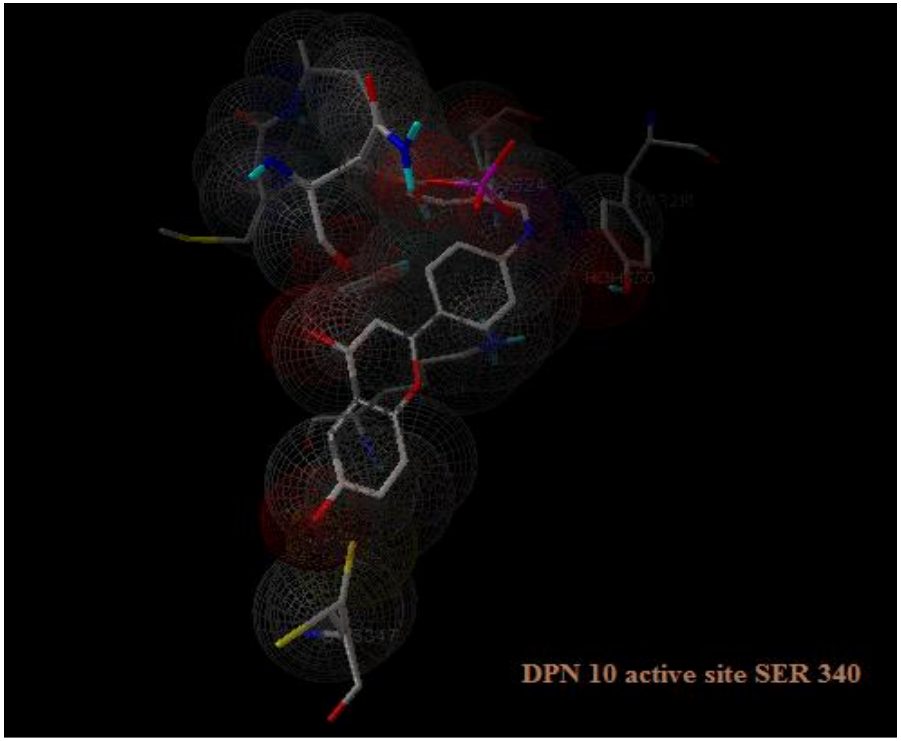
Table 5.6: DOCKING SCORE

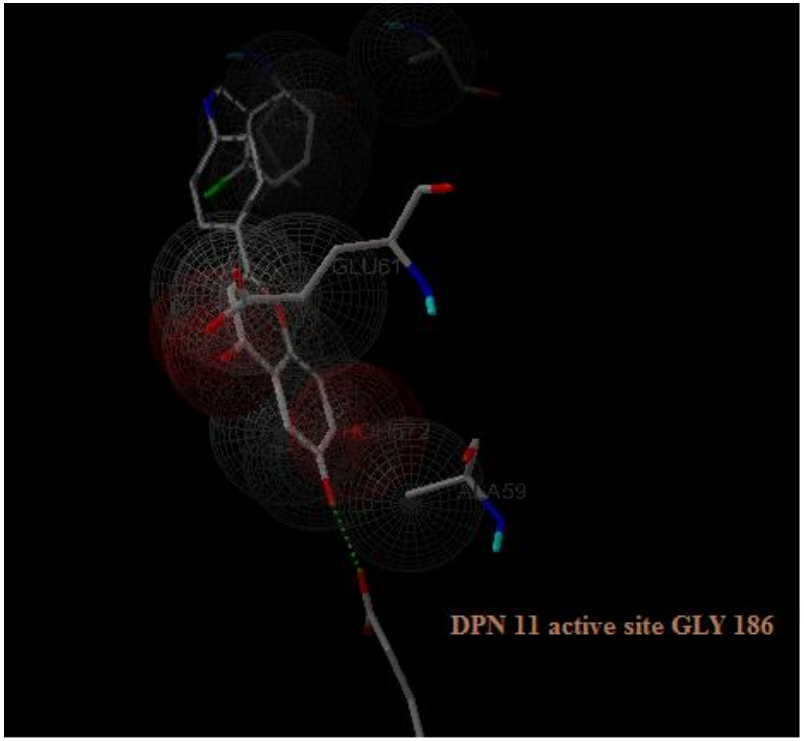
S.No	Compound code	Docking score	Amino acid (Active binding site)
1	DPN-03	-	-
2	DPN-06	-6.07	ARG 350
3	DPN-08	-9.81	LEU 498
4	DPN-10	-7.3	SER 340
5	DPN-11	-6.9	GLY 186
6	DPN-12	-6.37	TY 440
7	DPN-13	-	-
8	DPN-14	-3.5	GLY 144

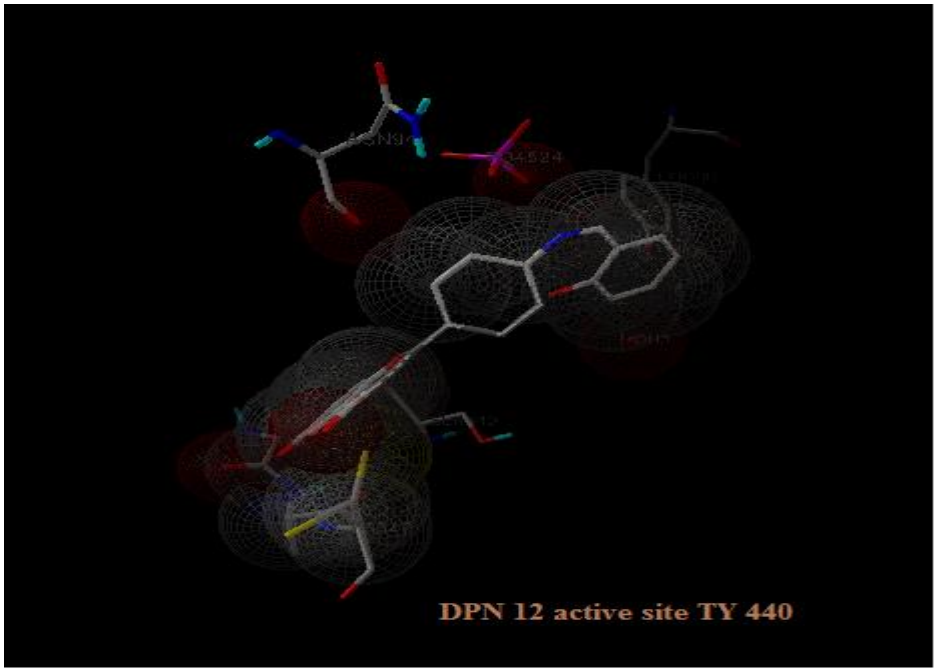
DOCKING INTERACTION:






DPN 10 active site SER 340

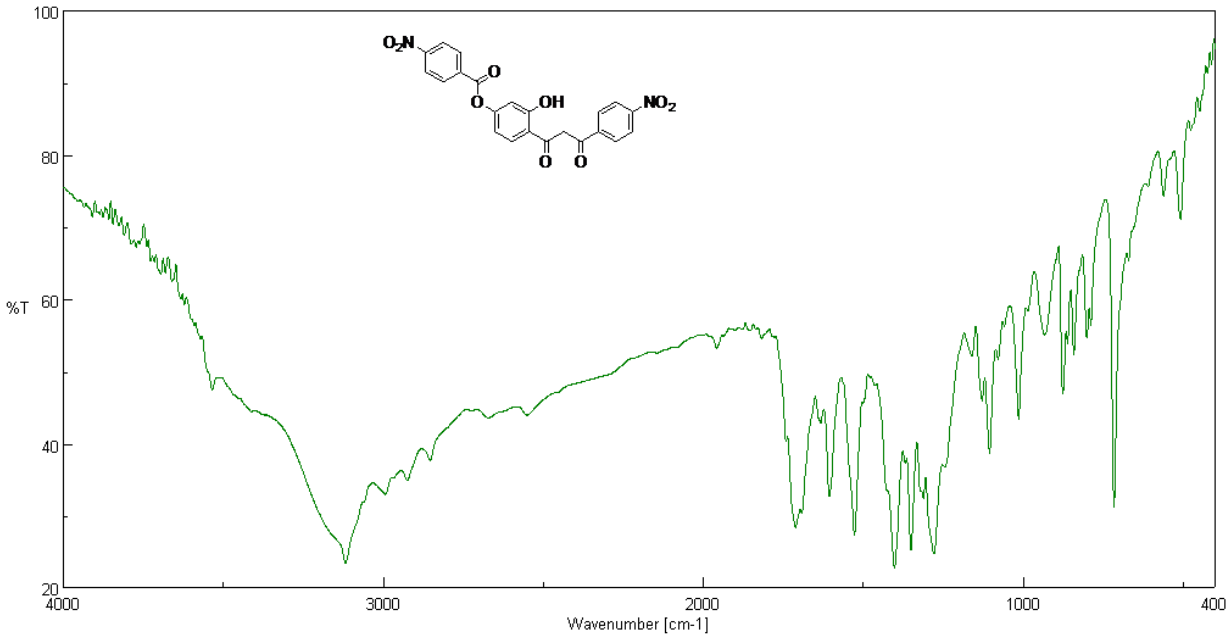

DPN 11 active site GLY 186



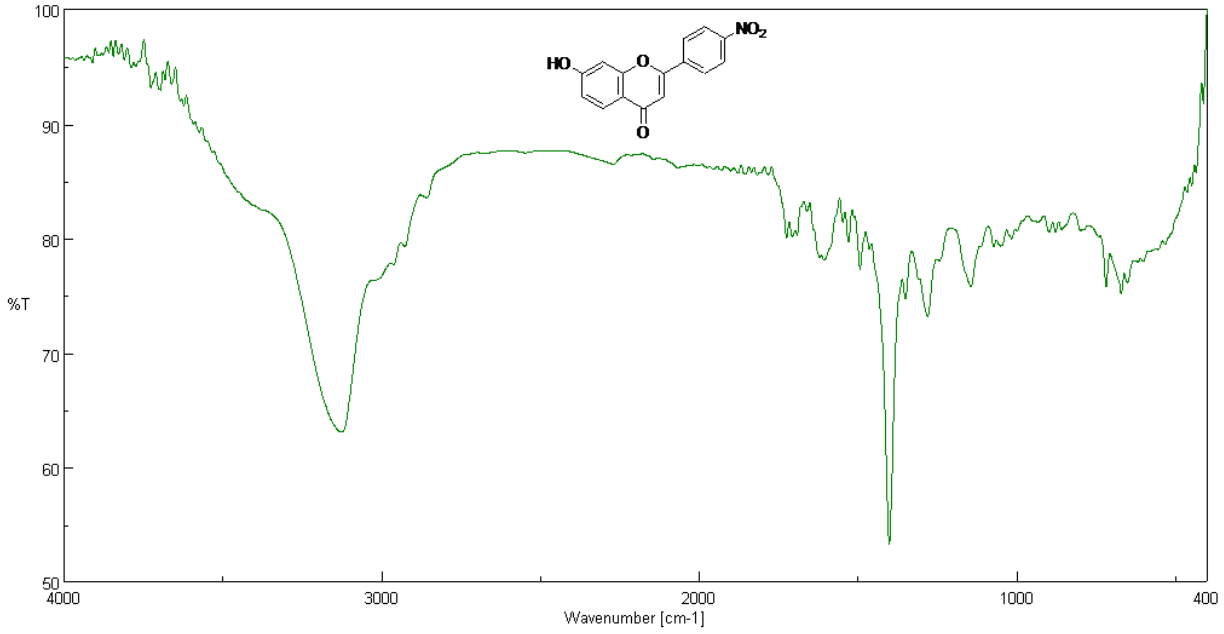
Graphical representation of Docking interaction

SPECTRAL DATAS OF SYNTHESISED COMPOUND BY CONVENTIONAL METHOD

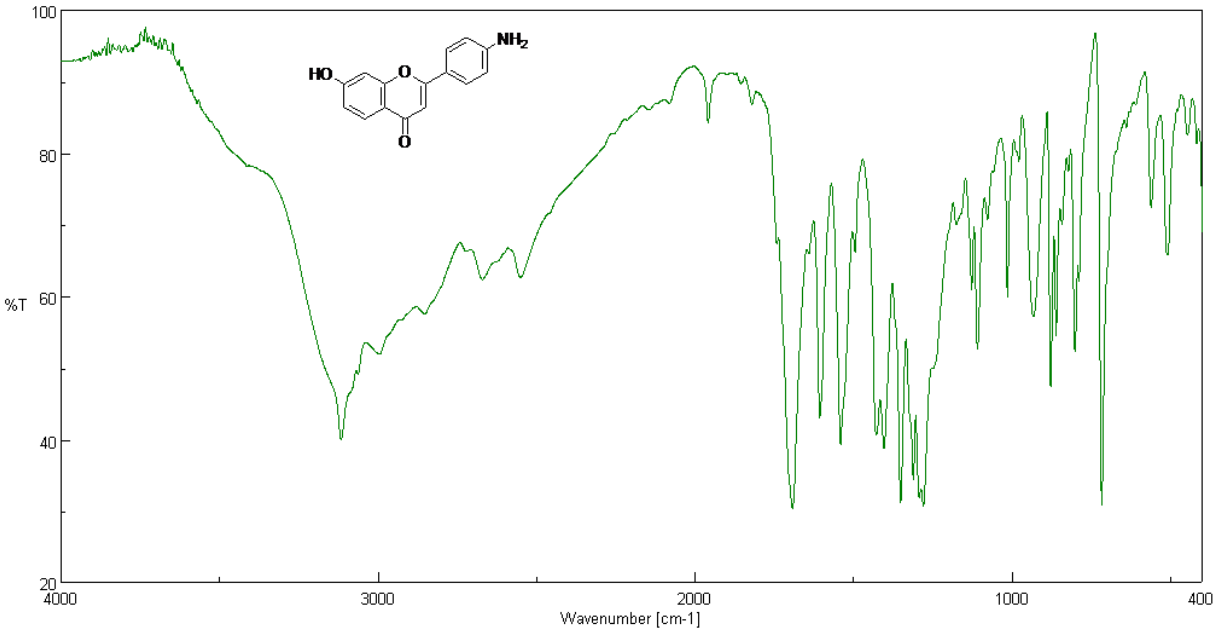
IR SPECTRA:



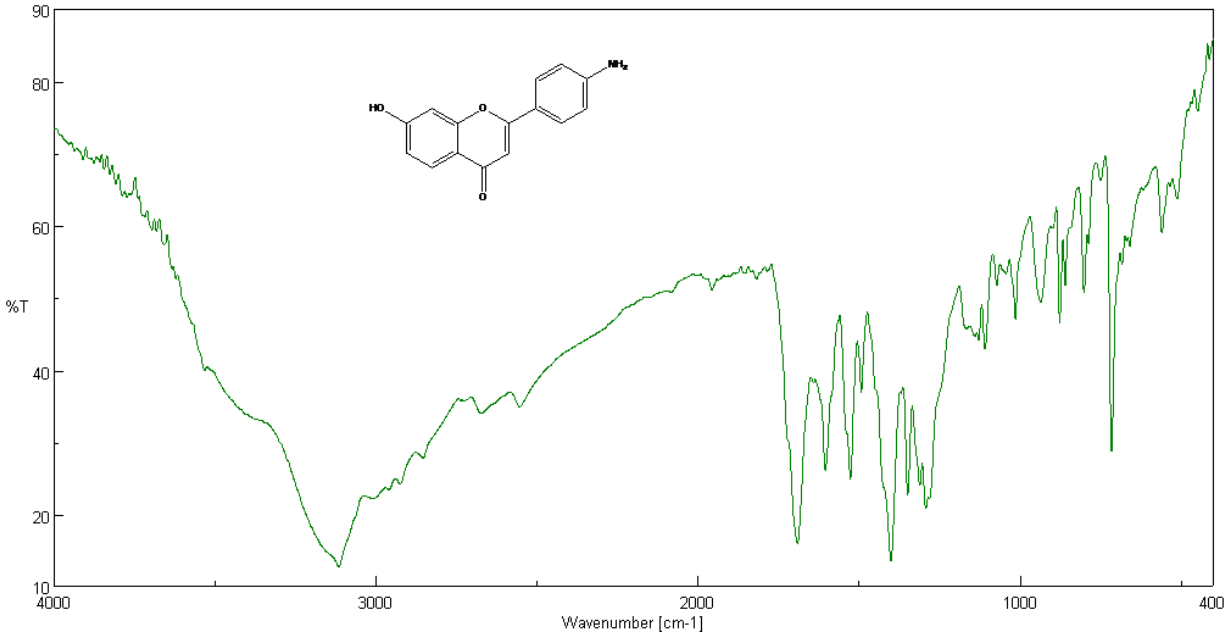
FD 3



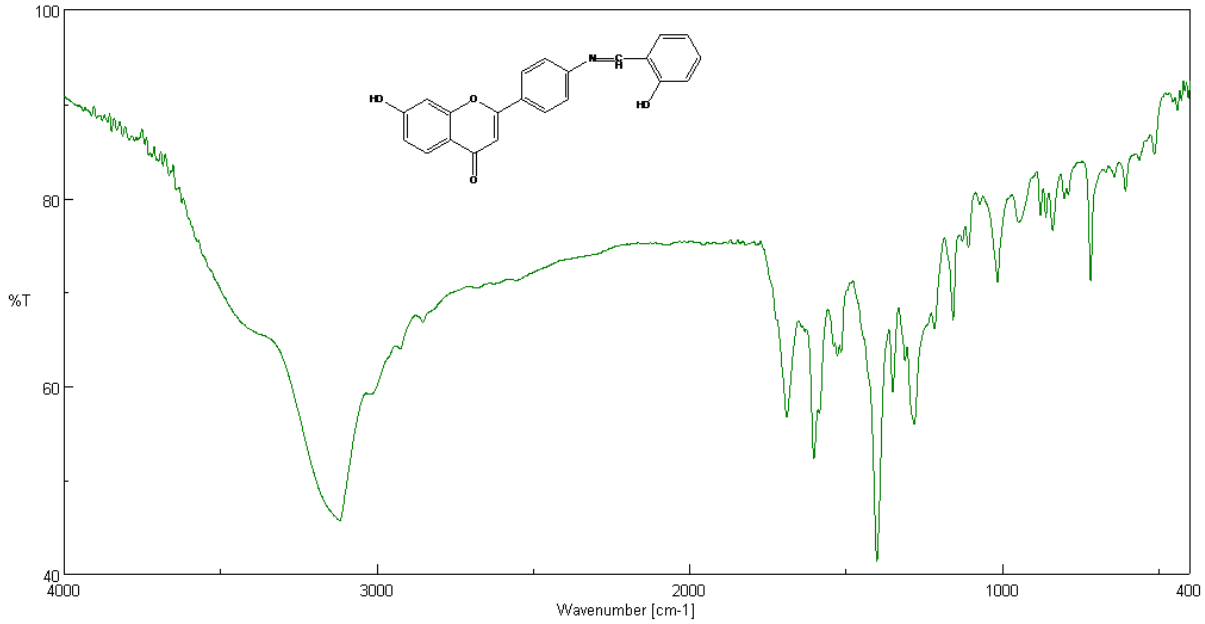
FD 4


100
80
60
40
20
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]
HO
O
O
NH2

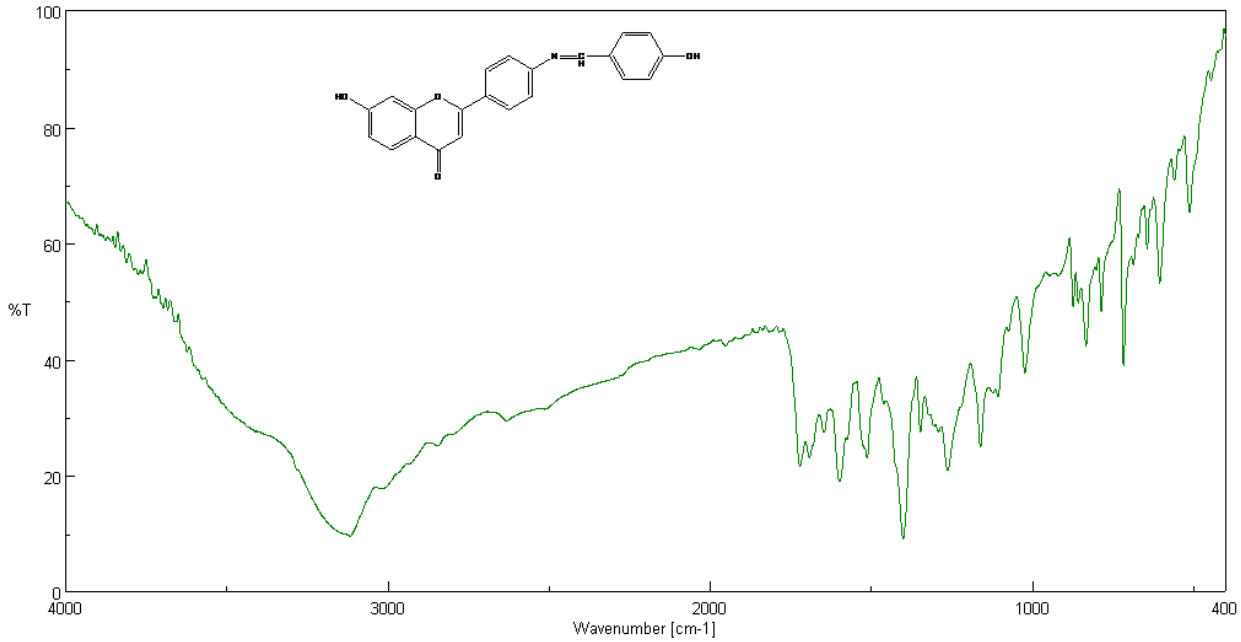
FD 5


90
80
60
40
20
10
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]
HO
O
O
NH2

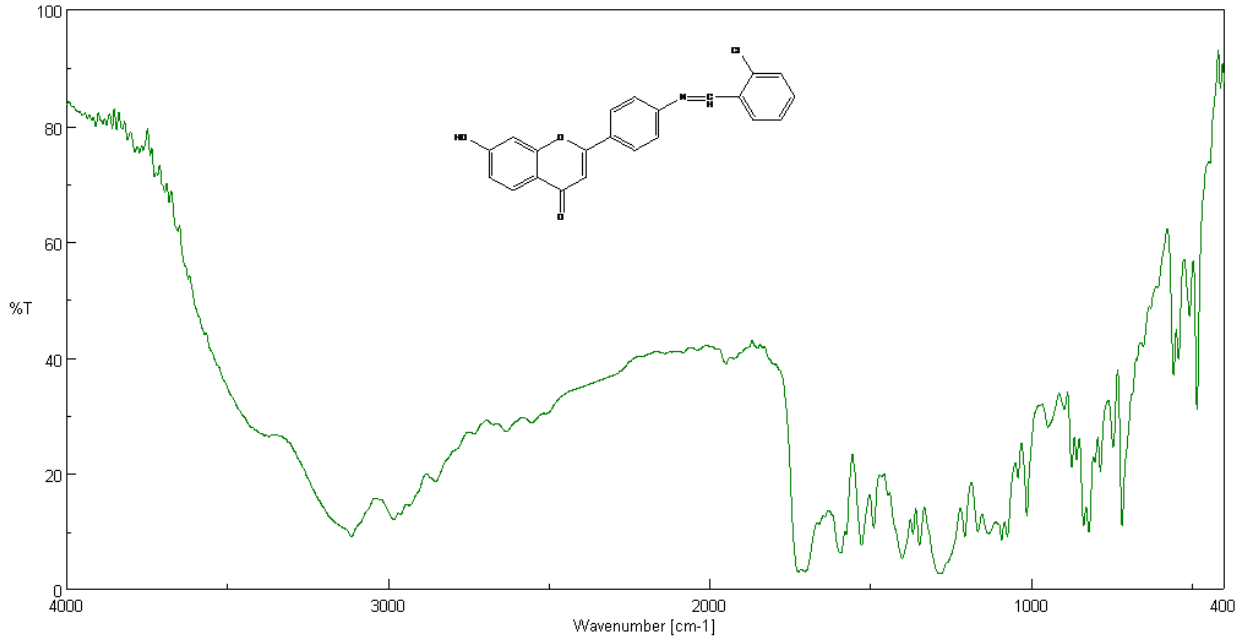
DPN 01


100
80
60
40
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]

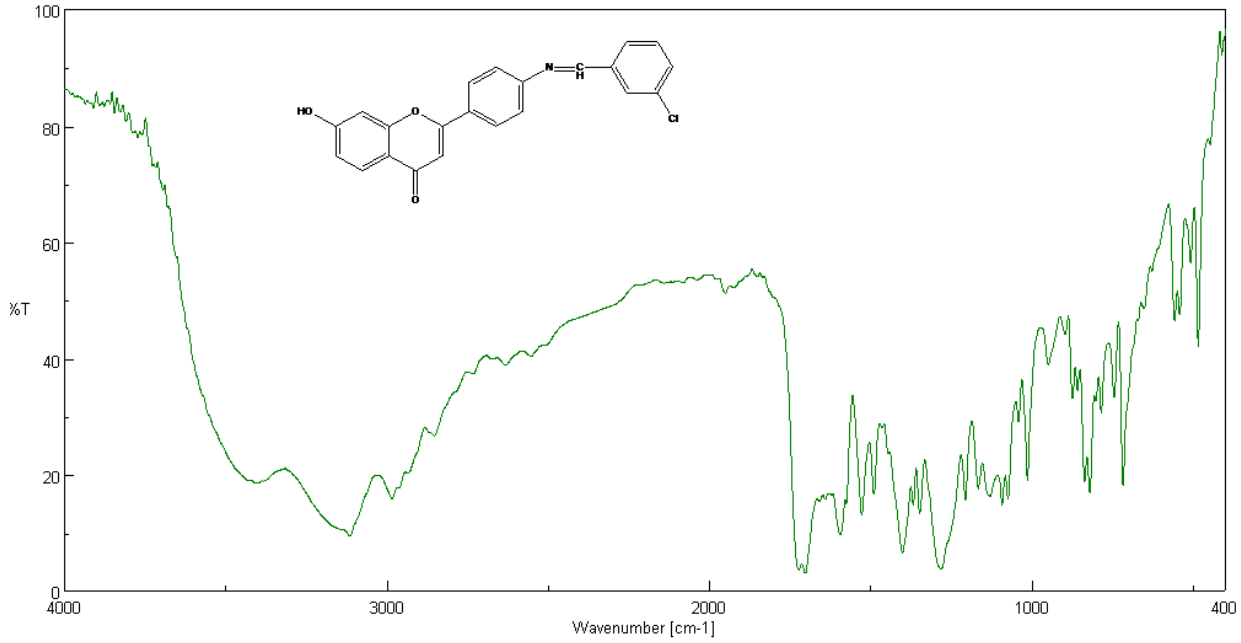
DPN 02


100
80
60
40
20
0
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]

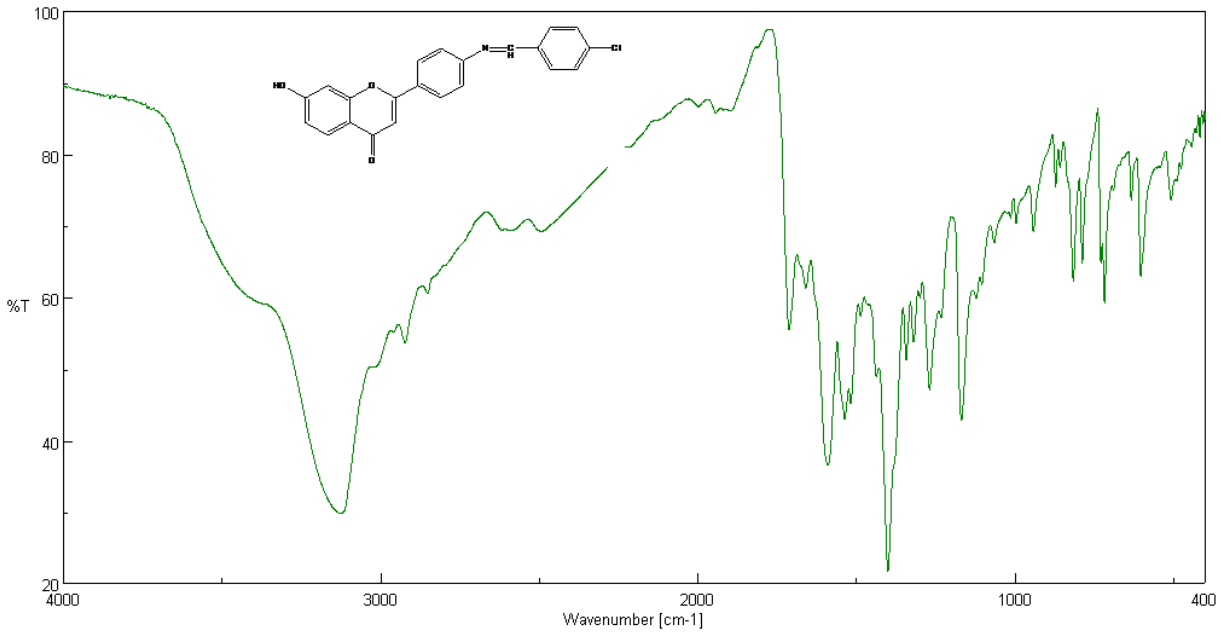
DPN 03


100
80
60
40
20
0
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]

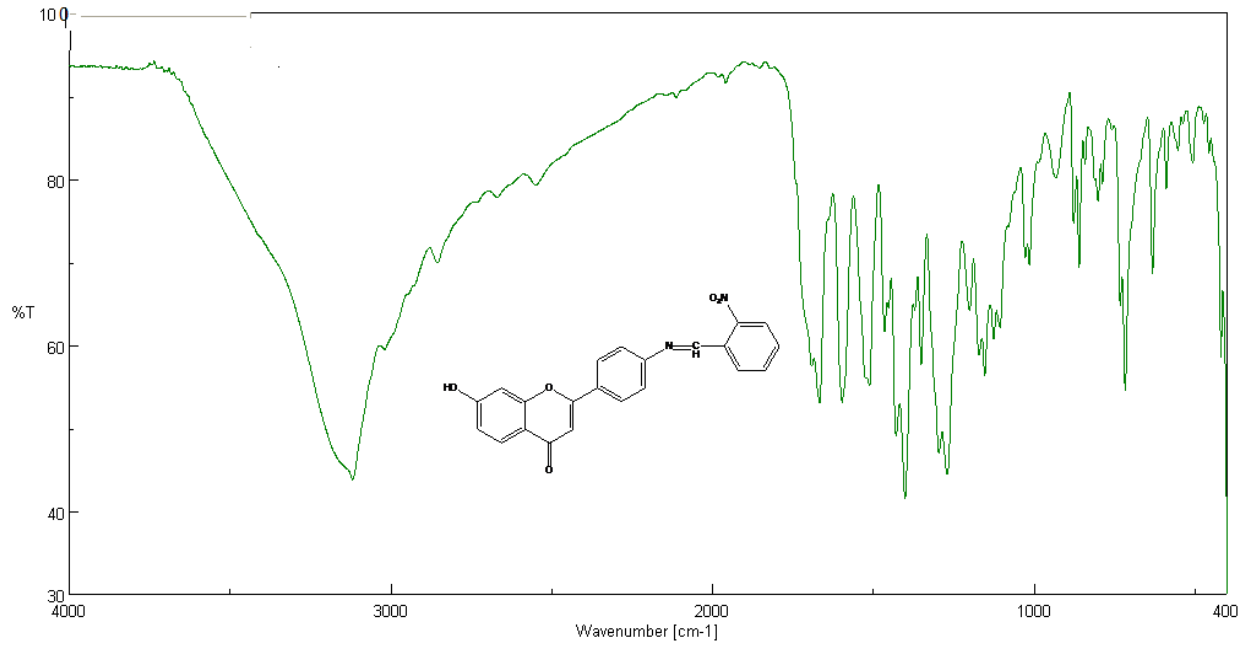
DPN 04


100
80
60
40
20
0
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]

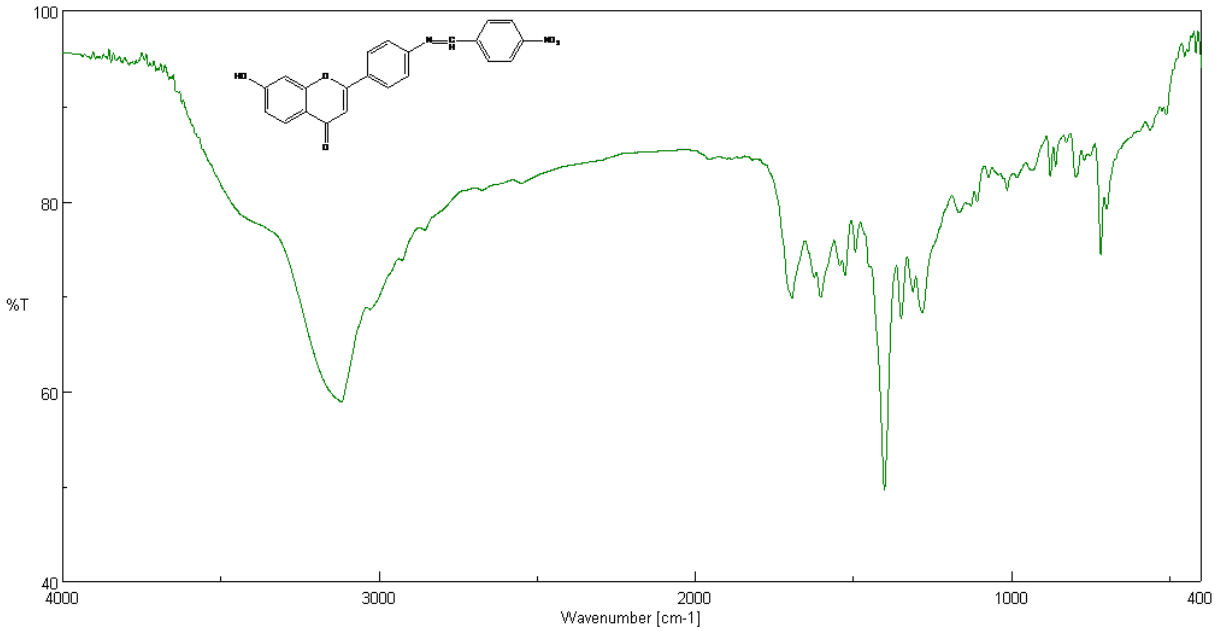
DPN 05


100
80
60
40
20
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]

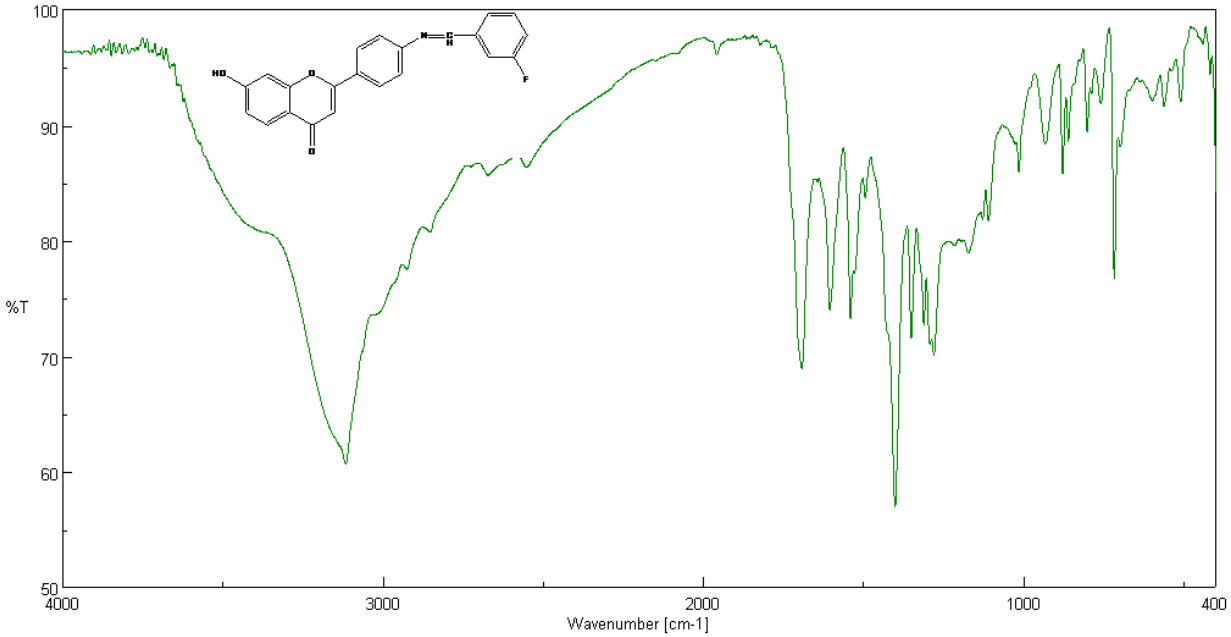
DPN 06


100
80
60
40
30
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]

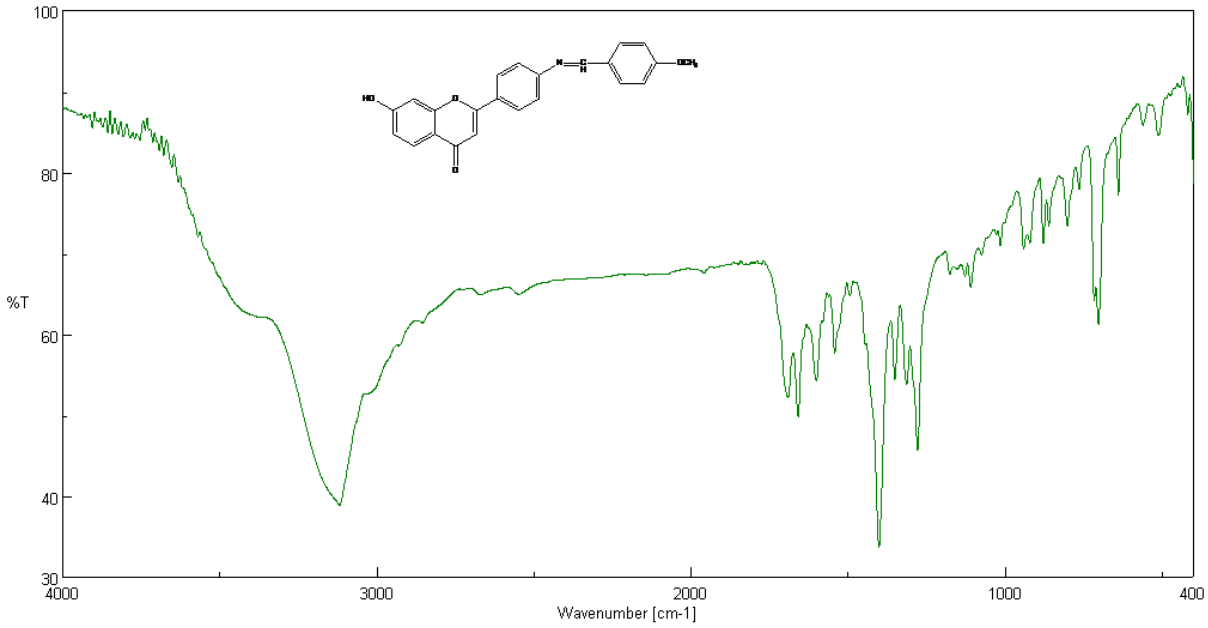
DPN 07


100
80
60
40
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]

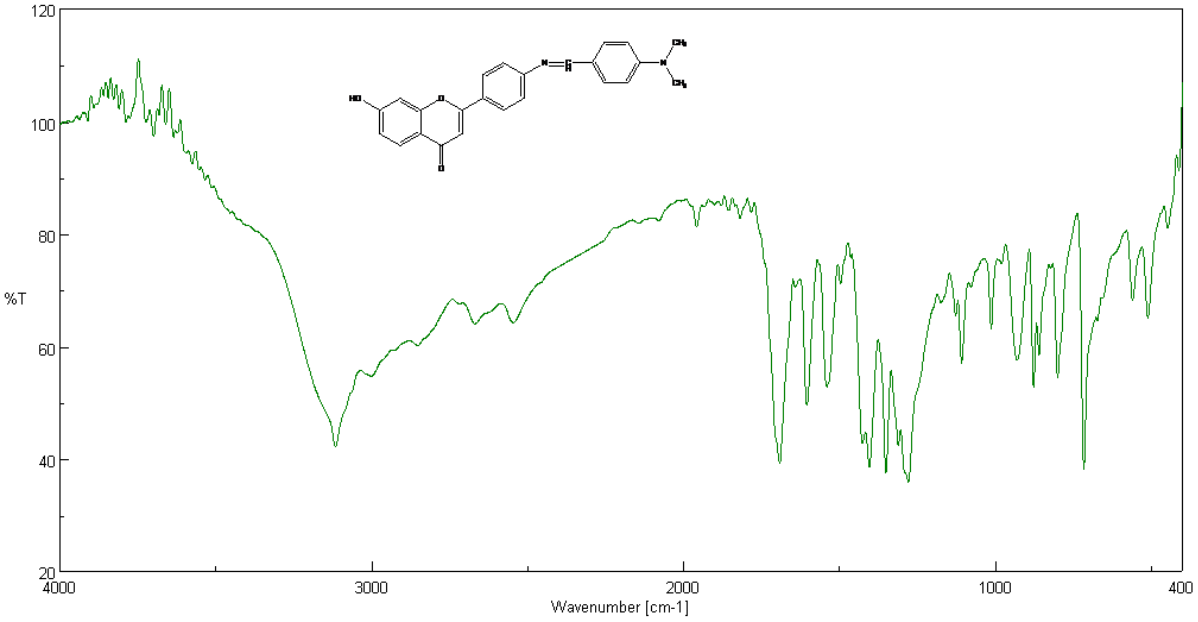
DPN 08


100
90
80
70
60
50
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]

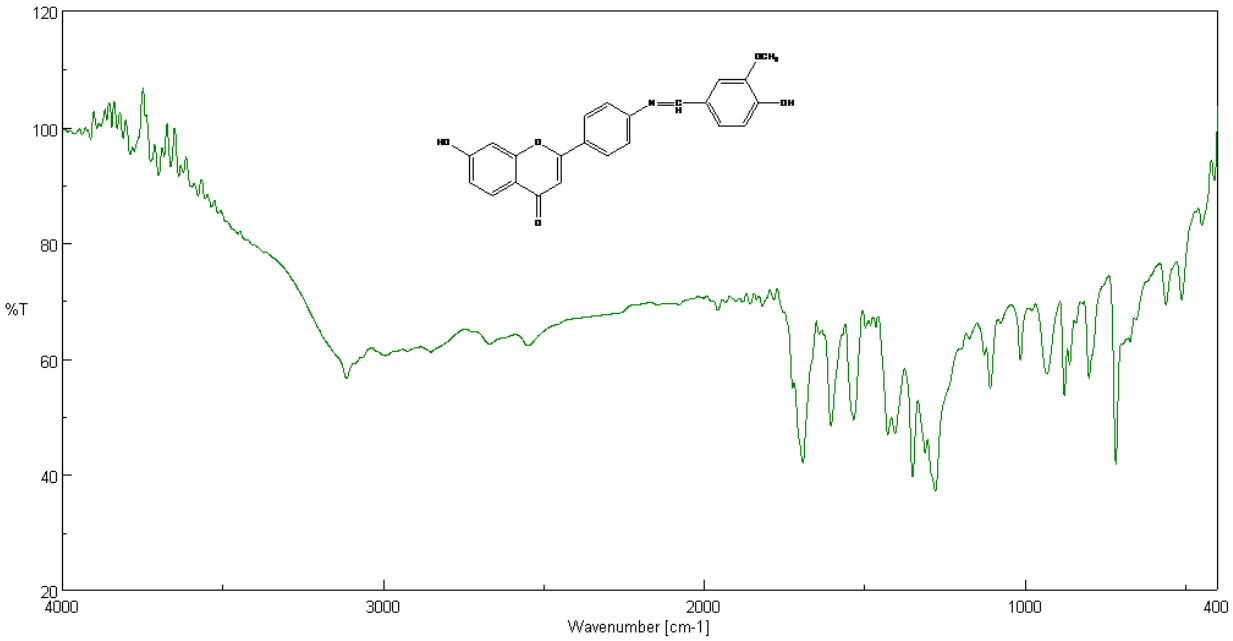
DPN 09


100
80
%T
60
40
30
4000
3000
2000
1000
400
Wavenumber [cm-1]

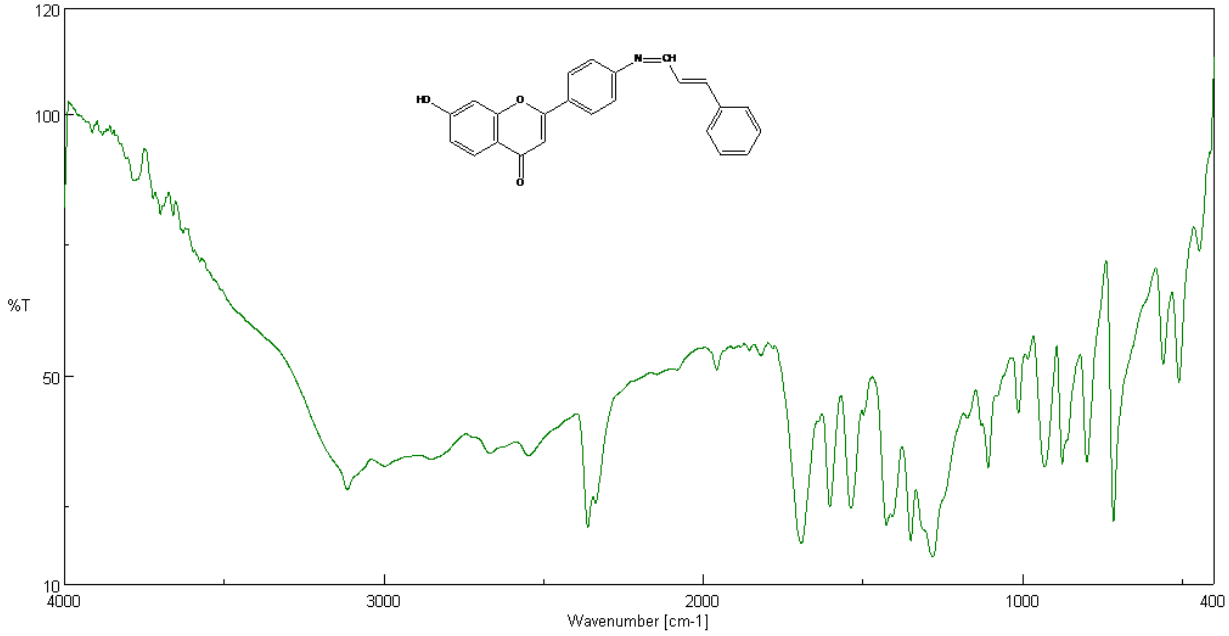
DPN 10


120
100
80
%T
60
40
20
4000
3000
2000
1000
400
Wavenumber [cm-1]

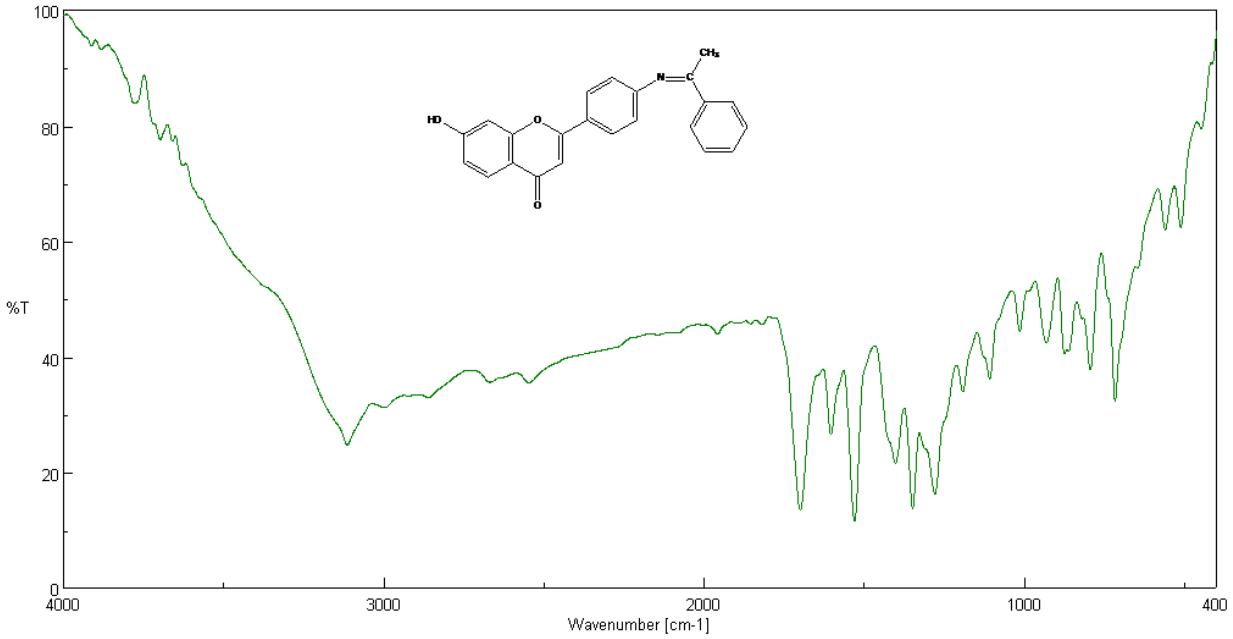
DPN 11


120
100
80
60
40
20
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]

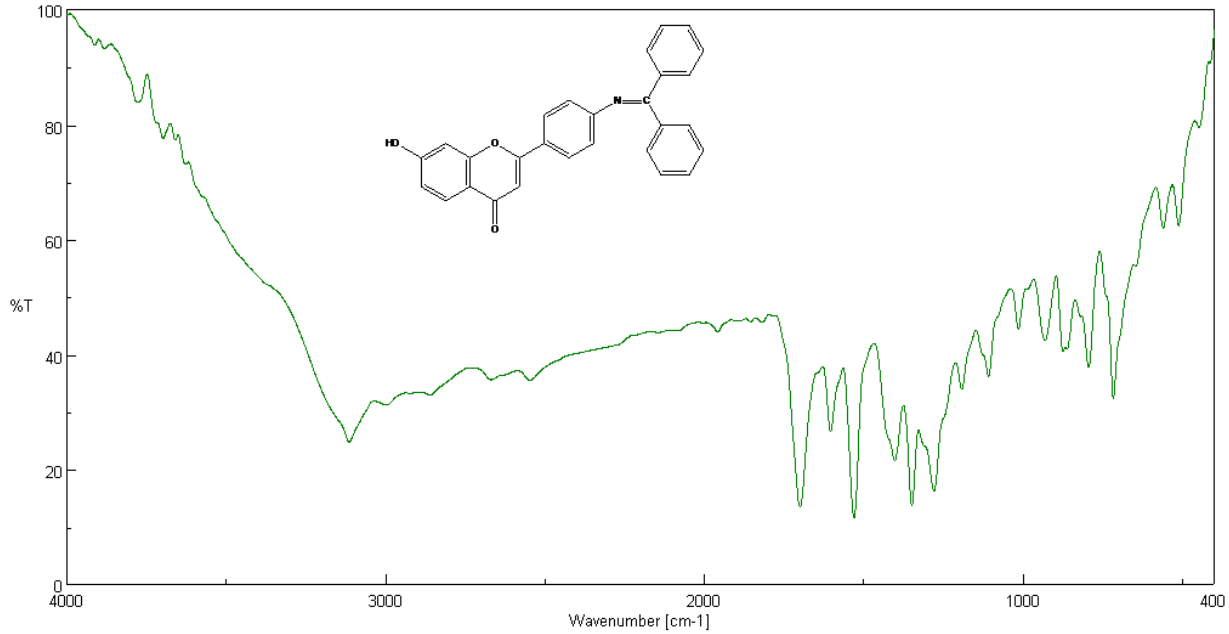
DPN 12


120
100
50
10
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]

DPN 13


100
80
60
40
20
0
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]
HO
O
N
CH3

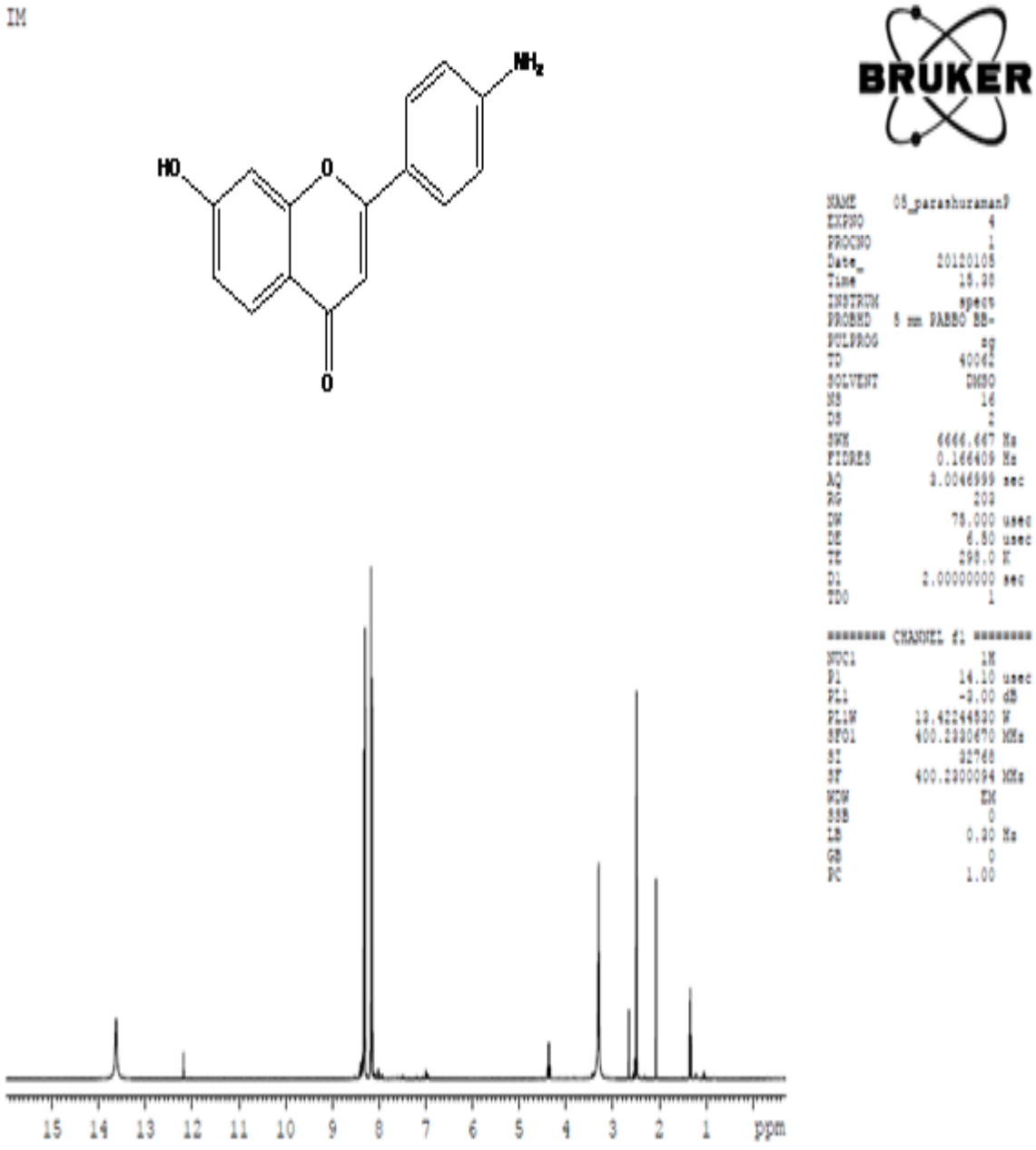
DPN 14

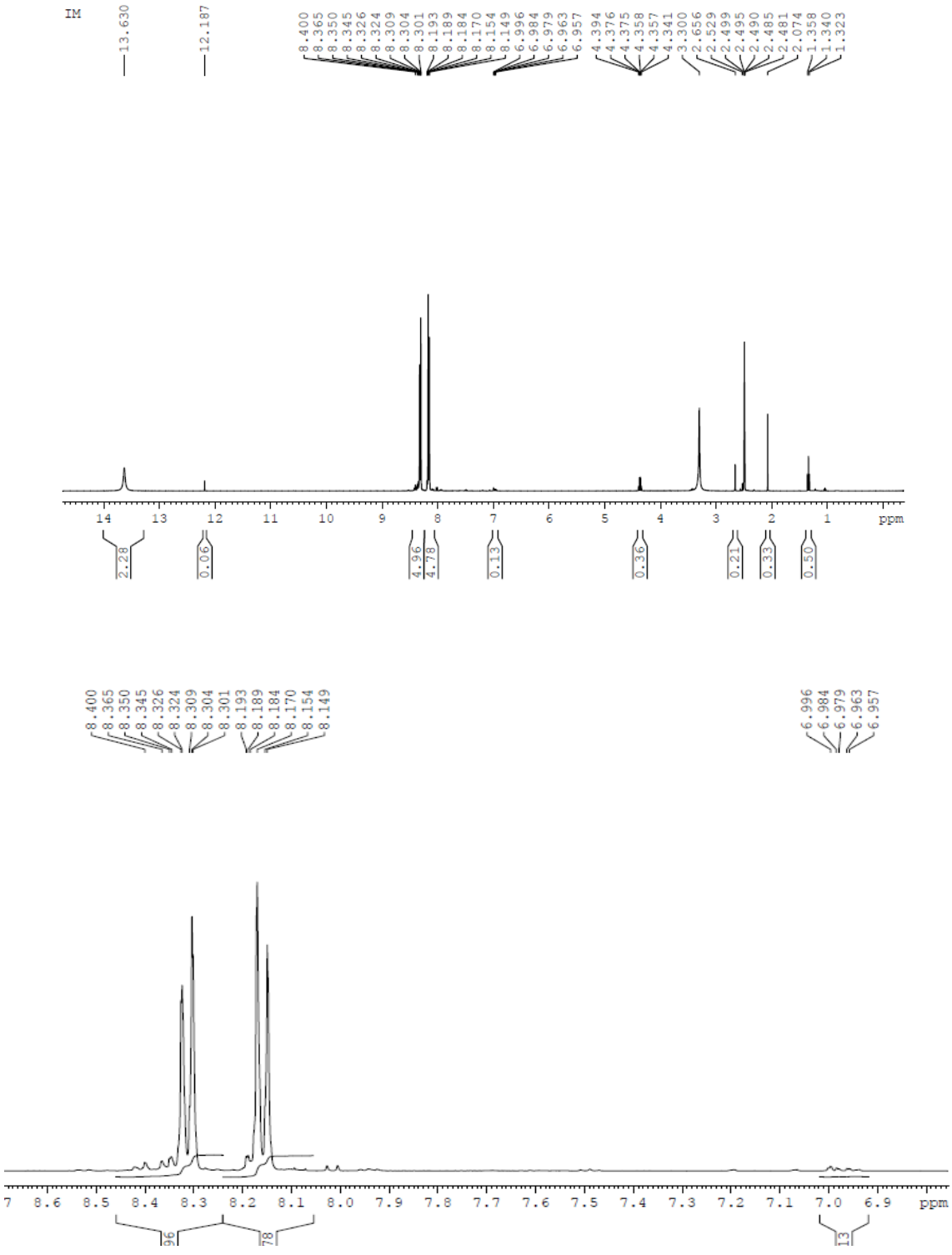

100
80
60
40
20
0
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]
HO
O
N

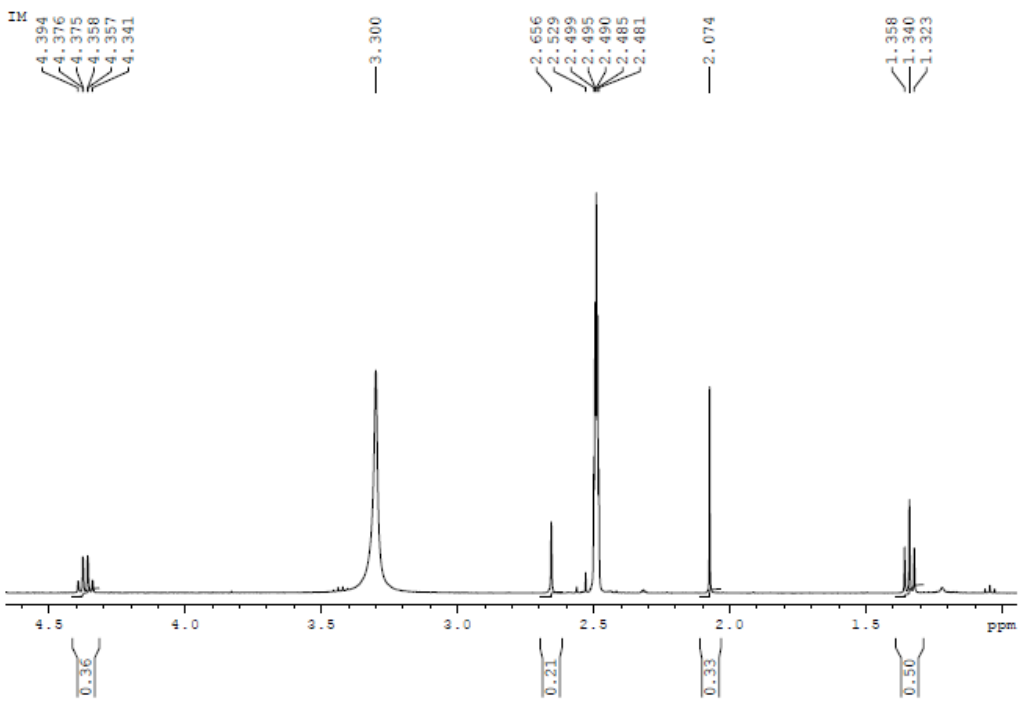
DPN 15

NMR SPECTRA

COMPOUND FD 05

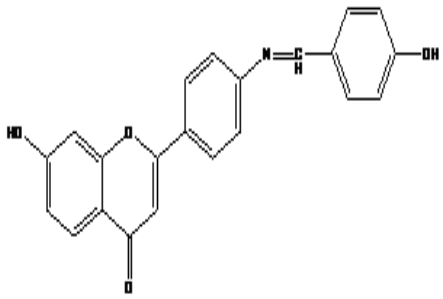



IM
13.630
12.187
8.400
8.365
8.350
8.345
8.326
8.324
8.309
8.304
8.301
8.193
8.189
8.184
8.170
8.154
8.149
6.996
6.984
6.979
6.963
6.957
4.394
4.376
4.375
4.358
4.357
4.341
3.300
2.656
2.529
2.499
2.495
2.490
2.485
2.481
2.074
1.358
1.340
1.323
ppm
2.28
0.06
4.96
4.78
0.13
0.36
0.21
0.33
0.50
1.86
1.78
1.13

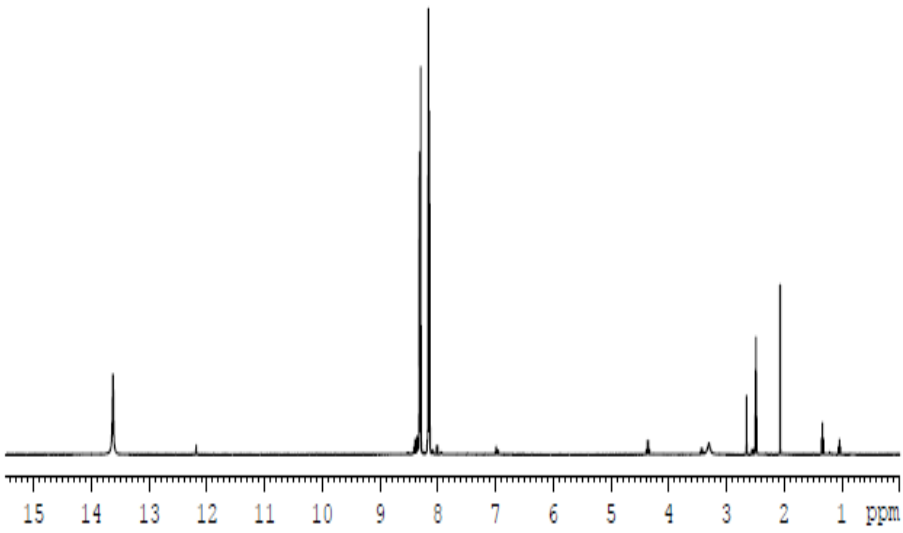

IM
4.394
4.376
4.375
4.358
4.357
4.341
3.300
2.656
2.529
2.499
2.495
2.490
2.485
2.481
2.074
1.358
1.340
1.323
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
0.36
0.21
0.33
0.50

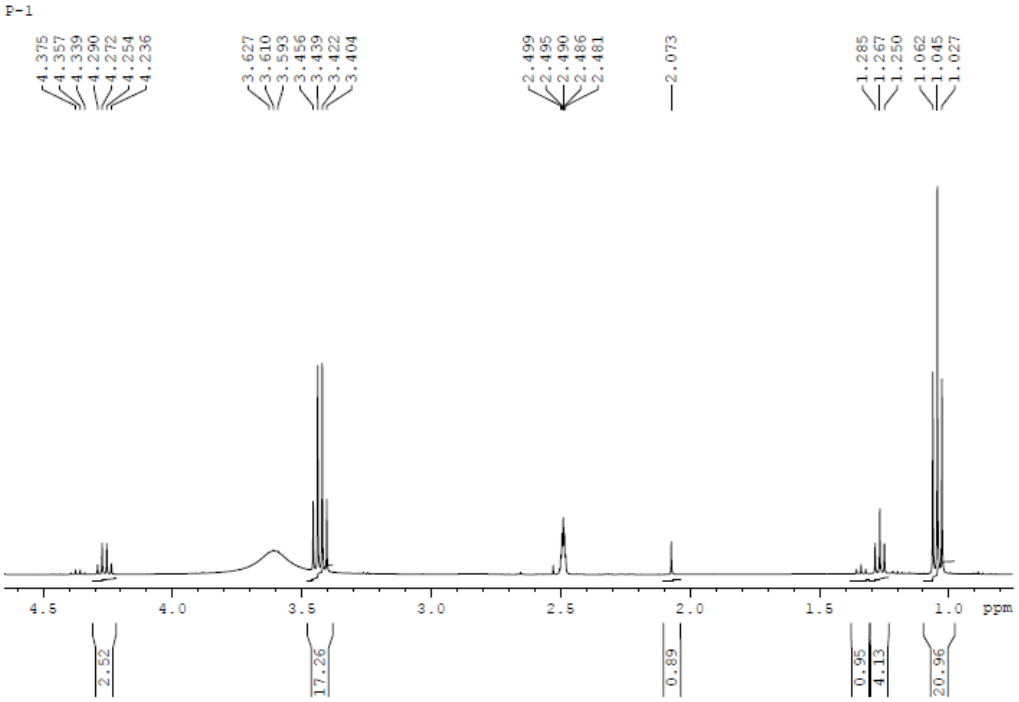
COMPOUND DPN 03

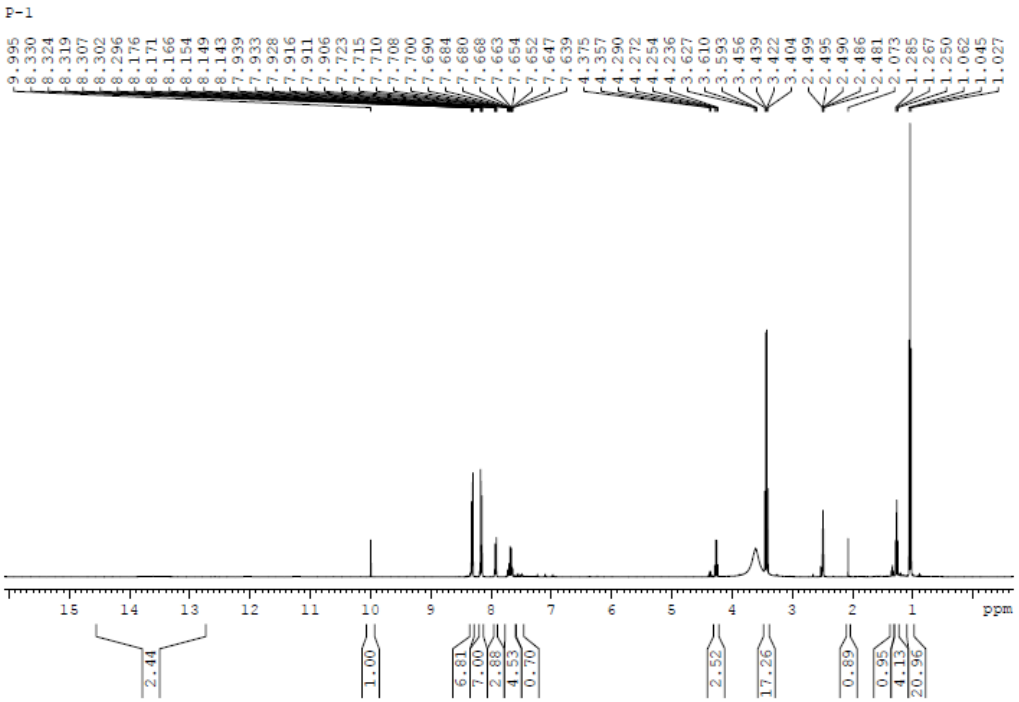
P-2

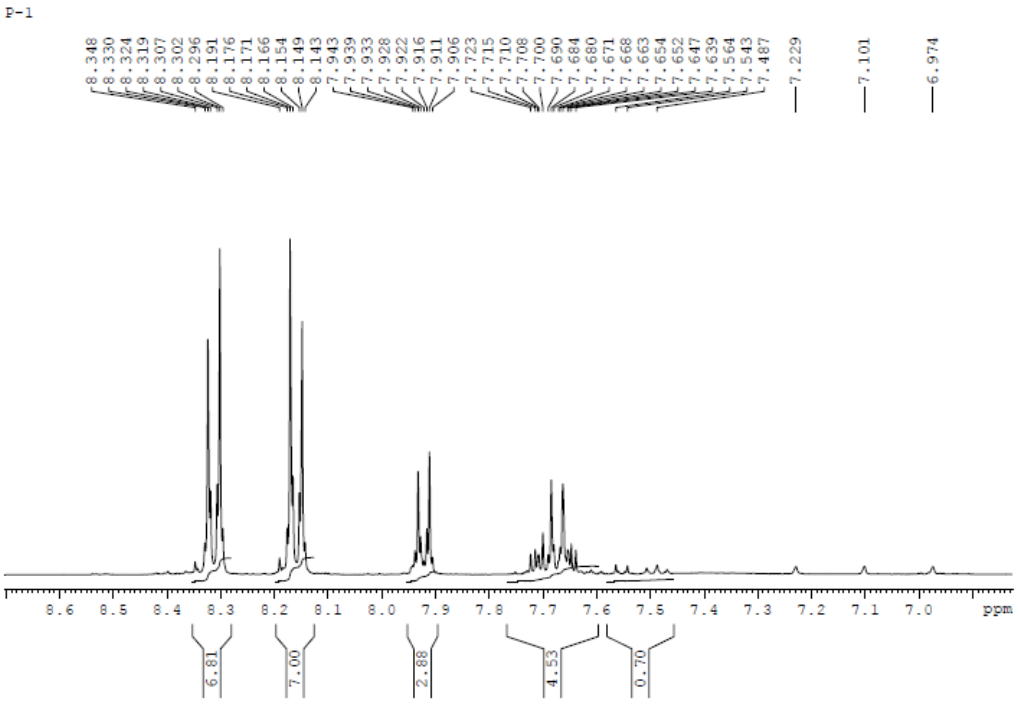



```
NAME          05_parashuramanP
EXPNO                        2
PROCNO                       1
Date_               20120105
Time                    15.27
INSTRUM                  spect
PROBHD   5 mm PABBO BB-
PULPROG                     zg
TD                       40760
SOLVENT                   DMSO
NS                          16
DS                           2
SWH                 6793.478 Hz
FIDRES                0.166670 Hz
AQ                    2.9999859 sec
RG                         203
DW                      73.600 usec
DE                       6.50 usec
TE                      298.0 K
D1                   2.00000000 sec
TD0                          1

======== CHANNEL f1 ========
NUC1                        1H
P1                      14.10 usec
PL1                     -3.00 dB
PL1W               13.42244530 W
SFO1               400.2330132 MHz
SI                       32768
SF                 400.2300095 MHz
WDW                         EM
SSB                          0
LB                       0.30 Hz
GB                           0
PC                        1.00
```

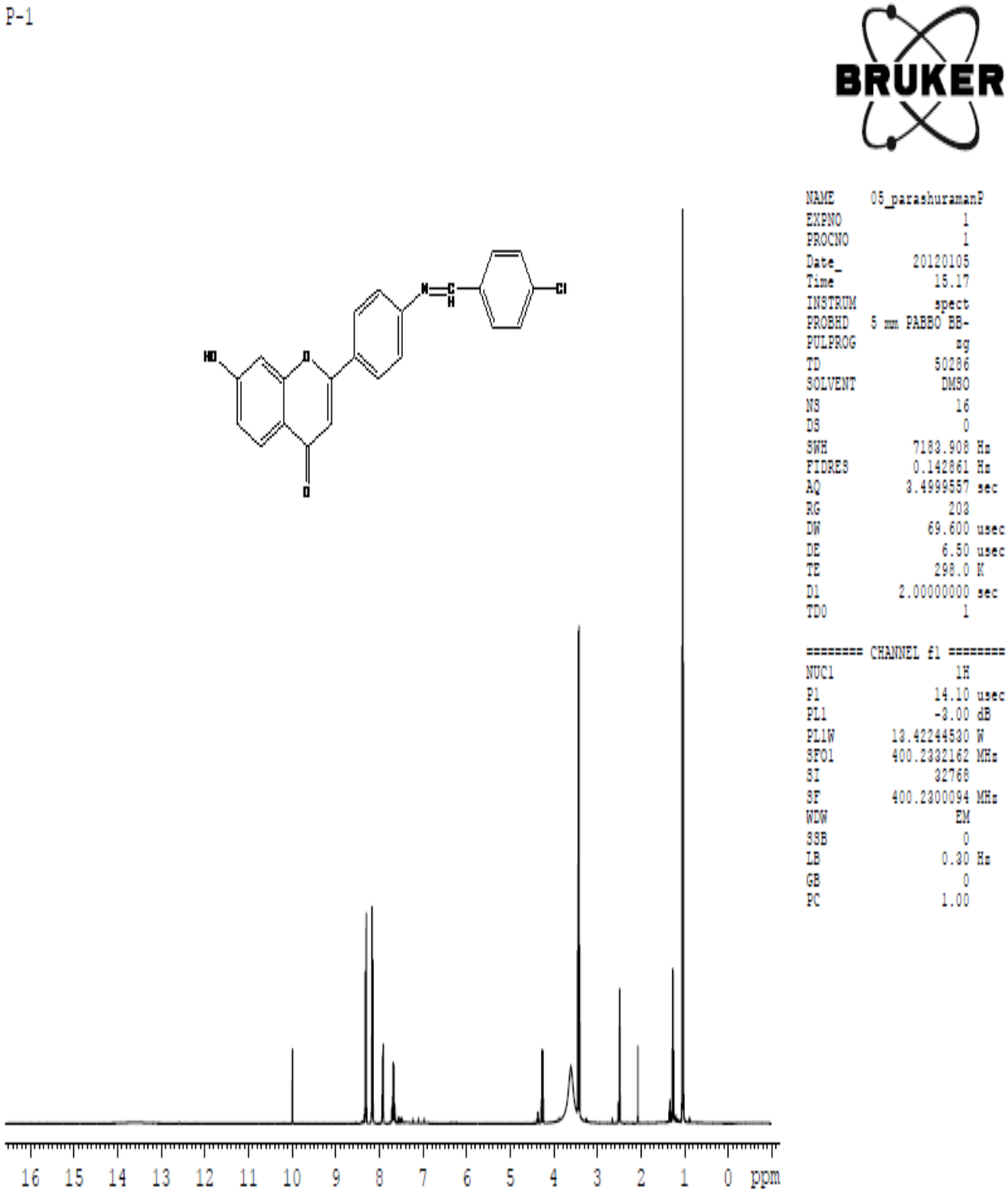



P-1
4.375
4.357
4.339
4.290
4.272
4.254
4.236
3.627
3.610
3.593
3.456
3.439
3.422
3.404
2.499
2.495
2.490
2.486
2.481
2.073
1.285
1.267
1.250
1.062
1.045
1.027
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
2.52
17.26
0.89
0.95
4.13
20.96

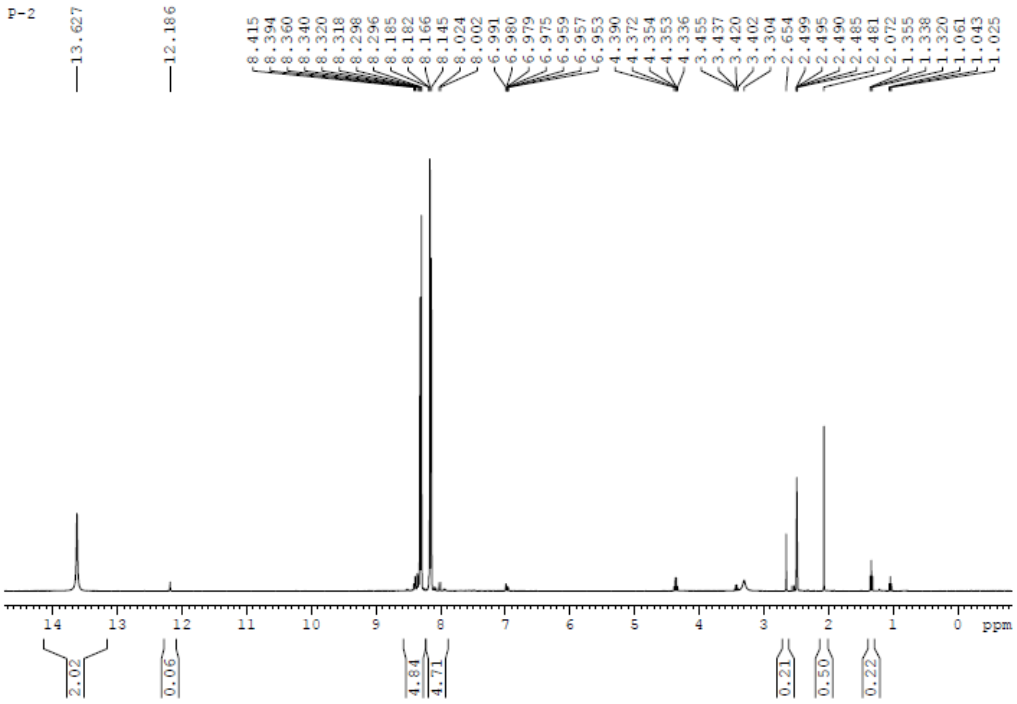

P-1
9.995
8.330
8.324
8.319
8.307
8.302
8.296
8.176
8.171
8.166
8.154
8.149
8.143
7.939
7.933
7.928
7.916
7.911
7.906
7.723
7.715
7.710
7.708
7.700
7.690
7.684
7.680
7.668
7.663
7.654
7.652
7.647
7.639
4.375
4.357
4.290
4.272
4.254
4.236
3.627
3.610
3.593
3.456
3.439
3.422
3.404
2.499
2.495
2.490
2.486
2.481
2.073
1.285
1.267
1.250
1.062
1.045
1.027
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
2.44
1.00
6.81
7.00
2.88
4.53
0.70
2.52
17.26
0.89
0.95
4.13
20.96

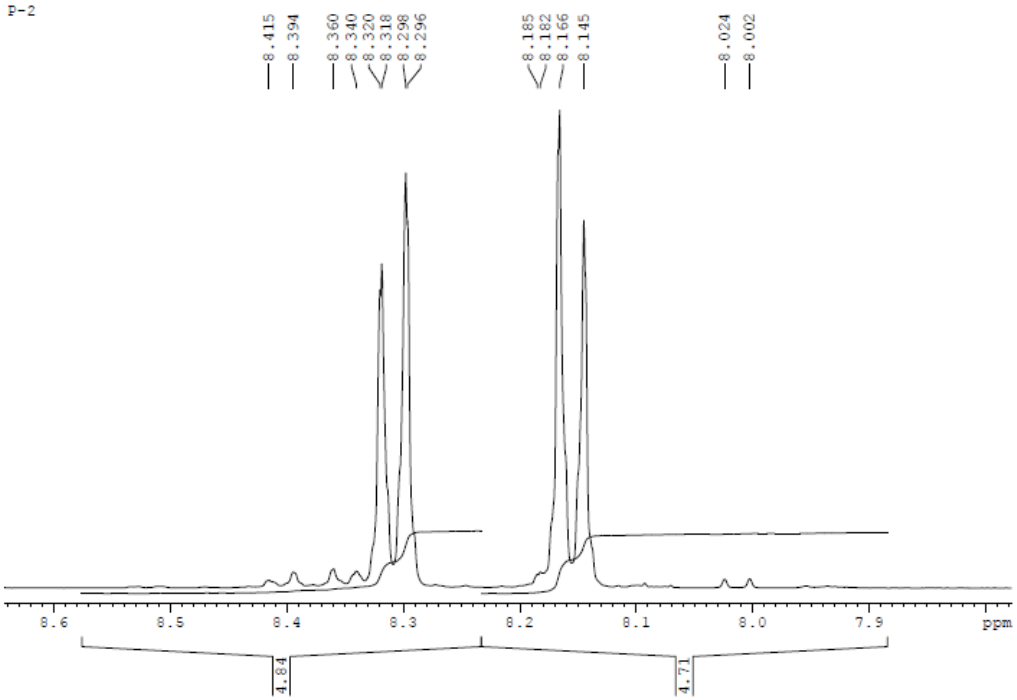

P-1
8.348
8.330
8.324
8.319
8.307
8.302
8.296
8.191
8.176
8.171
8.166
8.154
8.149
8.143
7.943
7.939
7.933
7.928
7.922
7.916
7.911
7.906
7.723
7.715
7.710
7.708
7.700
7.690
7.684
7.680
7.671
7.668
7.663
7.654
7.652
7.647
7.639
7.564
7.543
7.487
7.229
7.101
6.974
8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 ppm
6.81
7.00
2.88
4.53
0.70

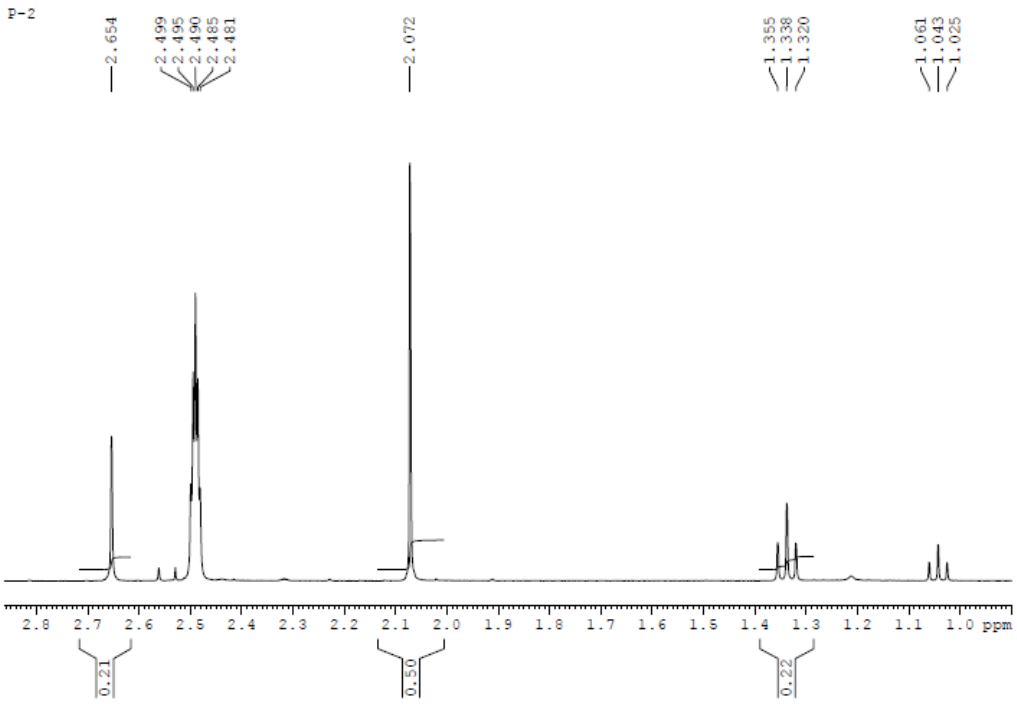
COMPOUND DPN 06

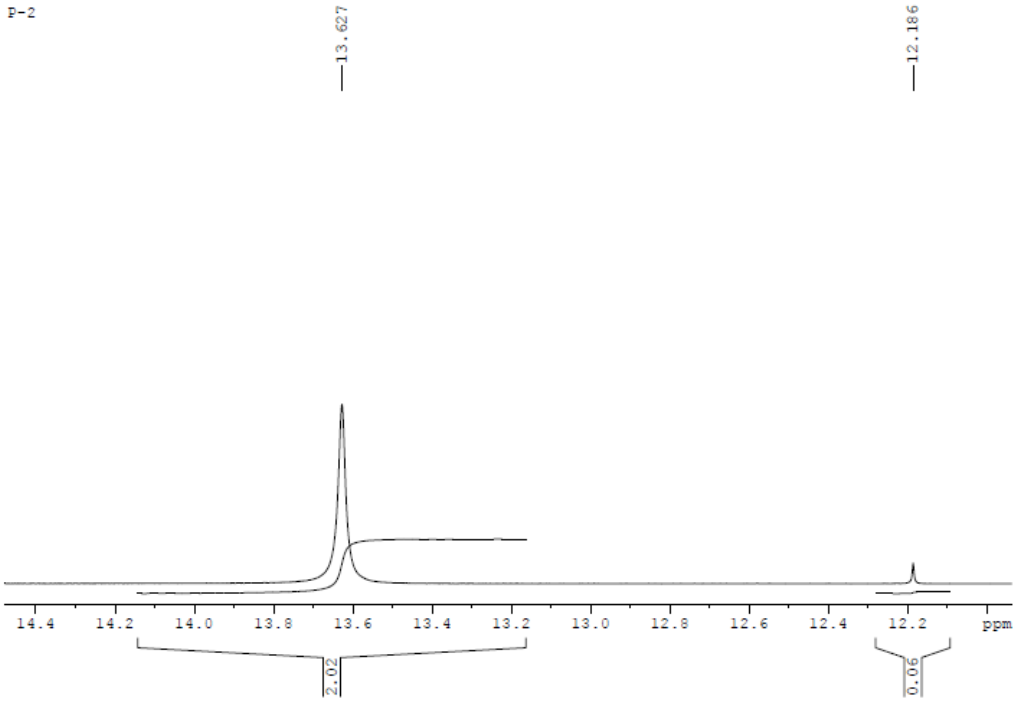



```
NAME      05_parashuramanP
EXPNO                    1
PROCNO                   1
Date_             20120105
Time                 15.17
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG                 zg
TD                   50286
SOLVENT               DMSO
NS                      16
DS                       0
SWH               7183.908 Hz
FIDRES            0.142861 Hz
AQ                3.4999557 sec
RG                     203
DW                  69.600 usec
DE                   6.50 usec
TE                  298.0 K
D1               2.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                    1H
P1                  14.10 usec
PL1                 -3.00 dB
PL1W            13.42244530 W
SFO1            400.2332162 MHz
SI                   32768
SF              400.2300094 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```


P-2
13.627
12.186
8.415
8.394
8.360
8.340
8.320
8.318
8.298
8.296
8.185
8.182
8.166
8.145
8.024
8.002
6.991
6.980
6.979
6.975
6.959
6.957
6.953
4.390
4.372
4.354
4.353
4.336
3.455
3.437
3.420
3.402
3.304
2.654
2.499
2.495
2.490
2.485
2.481
2.072
1.355
1.338
1.320
1.061
1.043
1.025
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
2.02
0.06
4.84
4.71
0.21
0.50
0.22

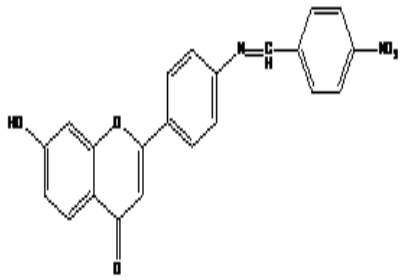

P-2
8.415
8.394
8.360
8.340
8.320
8.318
8.298
8.296
8.185
8.182
8.166
8.145
8.024
8.002
8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 ppm
4.84
4.71

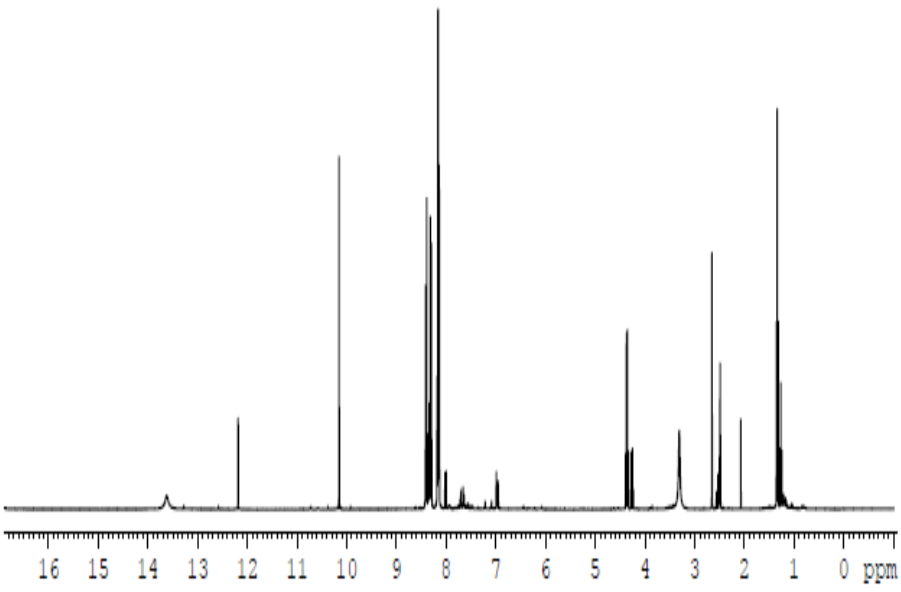

P-2
2.654
2.499
2.495
2.490
2.485
2.481
2.072
1.355
1.338
1.320
1.061
1.043
1.025
2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 ppm
0.21
0.50
0.22


P-2
13.627
12.186
14.4 14.2 14.0 13.8 13.6 13.4 13.2 13.0 12.8 12.6 12.4 12.2 ppm
2.02
0.06

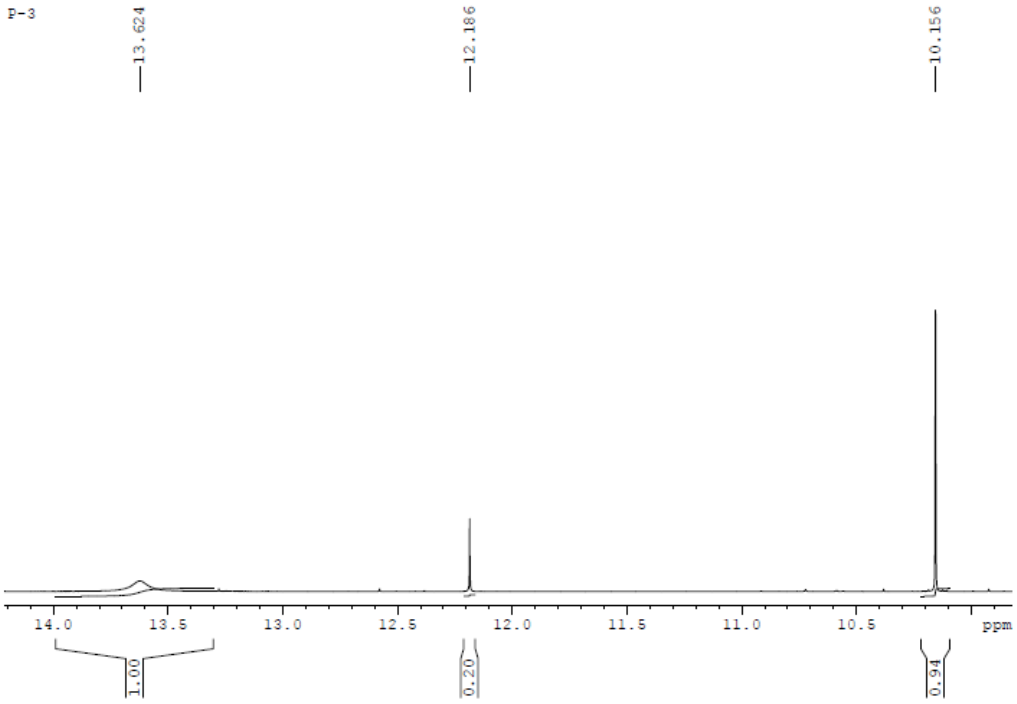
COMPOUND DPN 08

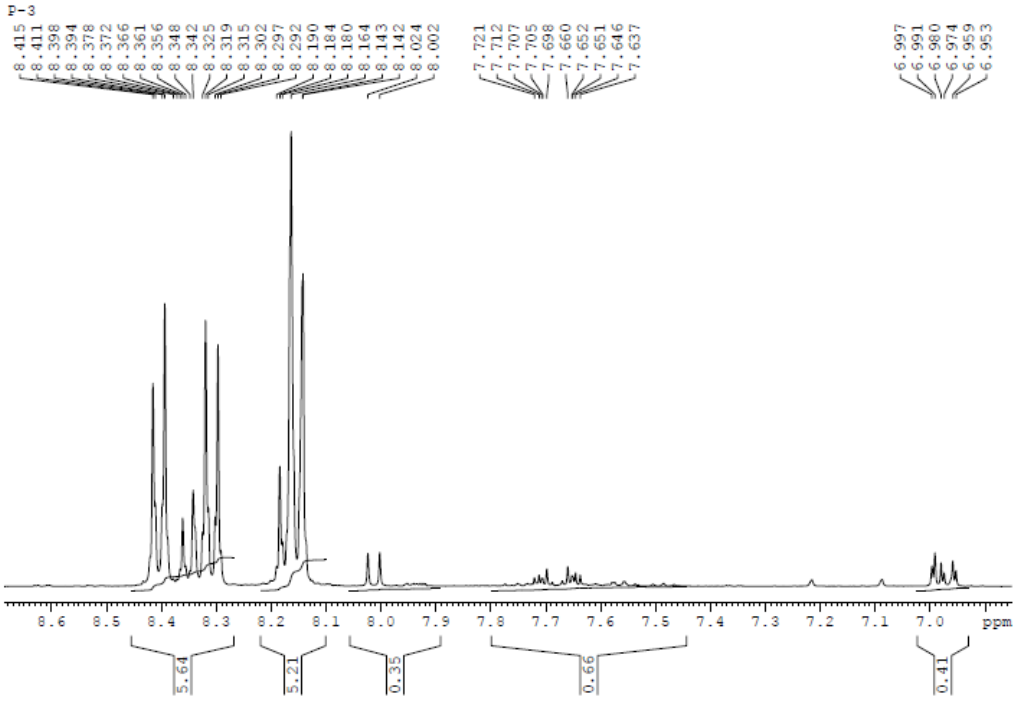
P-3

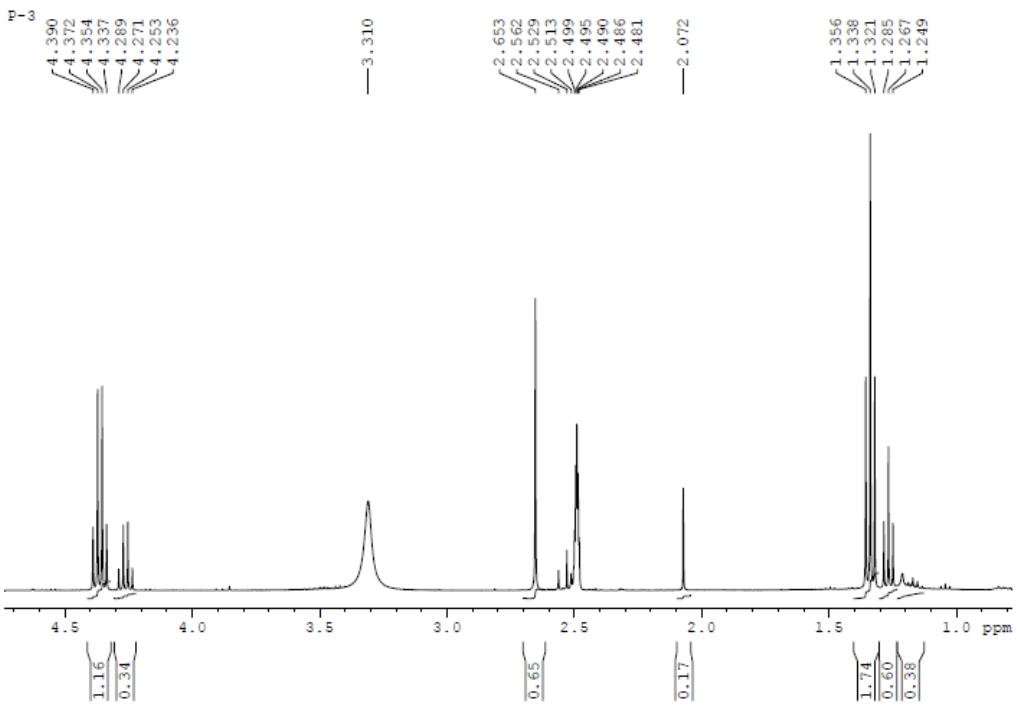




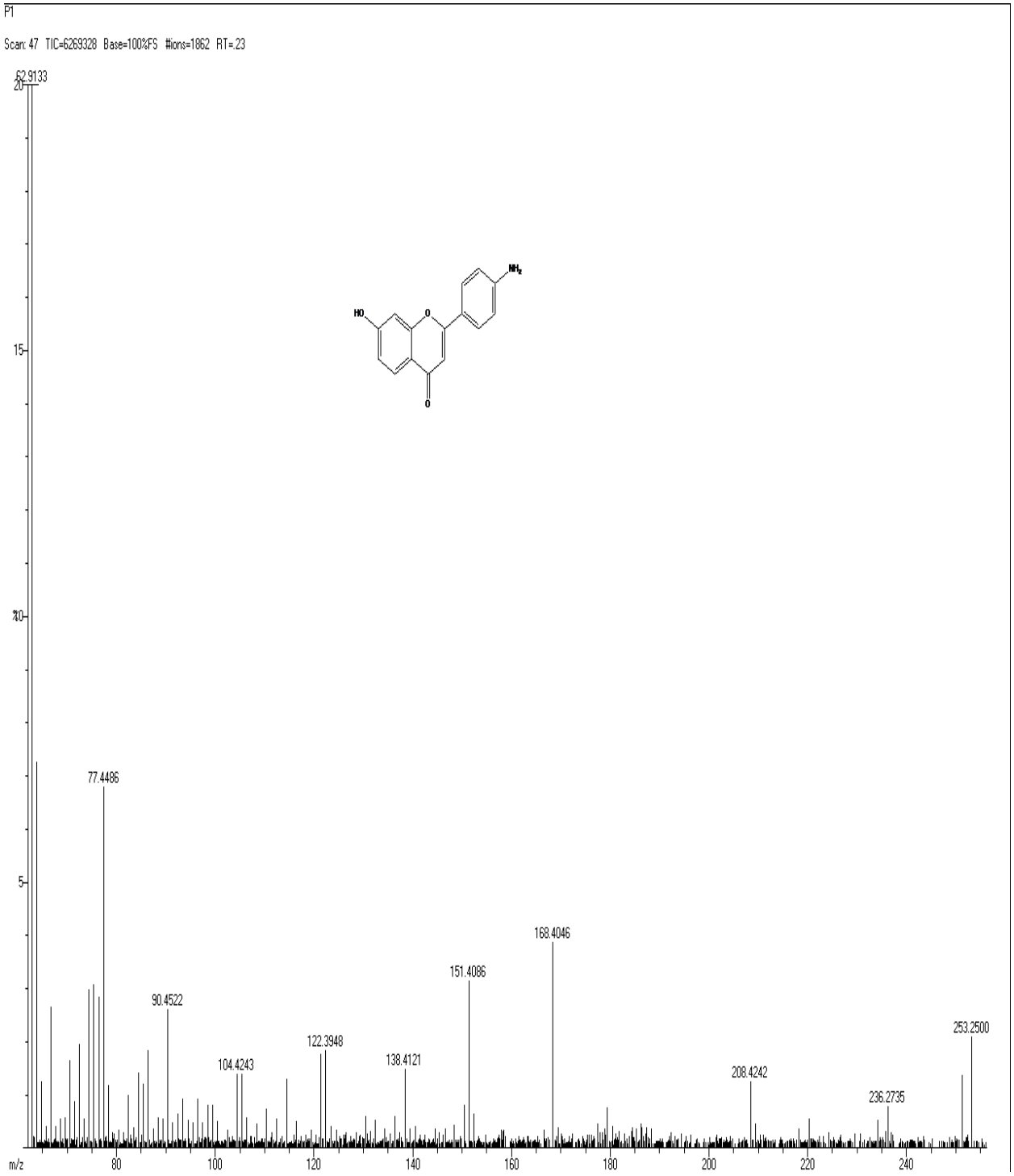

```
NAME        05_parashuramanP
EXPNO                  3
PROCNO                 1
Date_           20120105
Time                15.33
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG                zg
TD                 40062
SOLVENT             DMSO
NS                    16
DS                     2
SWH             7211.539 Hz
FIDRES          0.180009 Hz
AQ              2.7776821 sec
RG                   203
DW                69.333 usec
DE                 6.50 usec
TE                298.0 K
D1             2.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 14.10 usec
PL1                -3.00 dB
PL1W         13.42244530 W
SFO1         400.2332079 MHz
SI                 32768
SF           400.2300094 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```


P-3
13.624
12.186
10.156
14.0
13.5
13.0
12.5
12.0
11.5
11.0
10.5
ppm
1.00
0.20
0.94

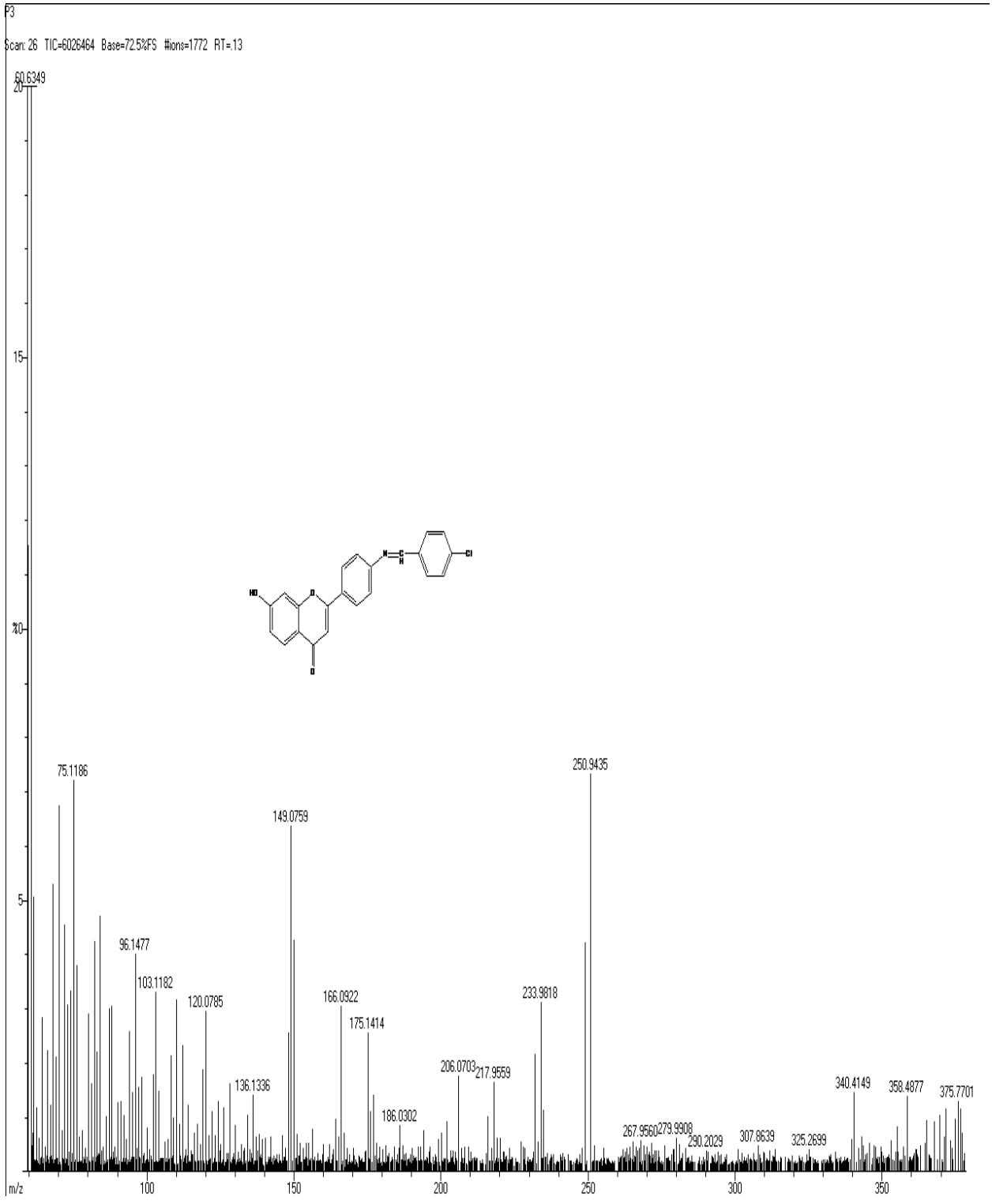

P-3
8.415
8.411
8.398
8.394
8.378
8.372
8.366
8.361
8.356
8.348
8.342
8.325
8.319
8.315
8.302
8.297
8.292
8.190
8.184
8.180
8.164
8.143
8.142
8.024
8.002
7.721
7.712
7.707
7.705
7.698
7.660
7.652
7.651
7.646
7.637
6.997
6.991
6.980
6.974
6.959
6.953
8.6
8.5
8.4
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
ppm
5.64
5.21
0.35
0.66
0.41


P-3
4.390
4.372
4.354
4.337
4.289
4.271
4.253
4.236
3.310
2.653
2.562
2.529
2.513
2.499
2.495
2.490
2.486
2.481
2.072
1.356
1.338
1.321
1.285
1.267
1.249
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0 ppm
1.16
0.34
0.65
0.17
1.74
0.60
0.38

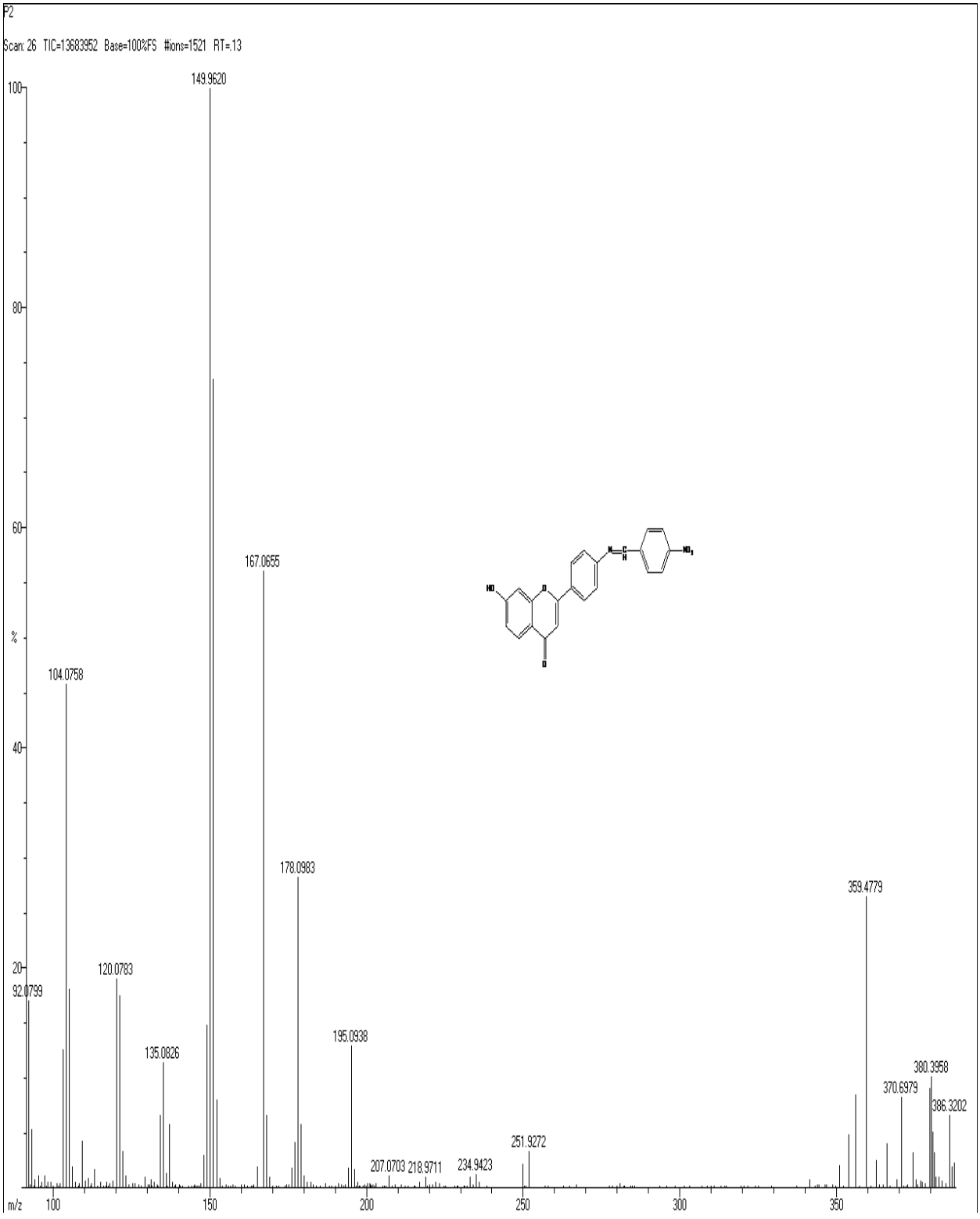
MASS SPECTRA:



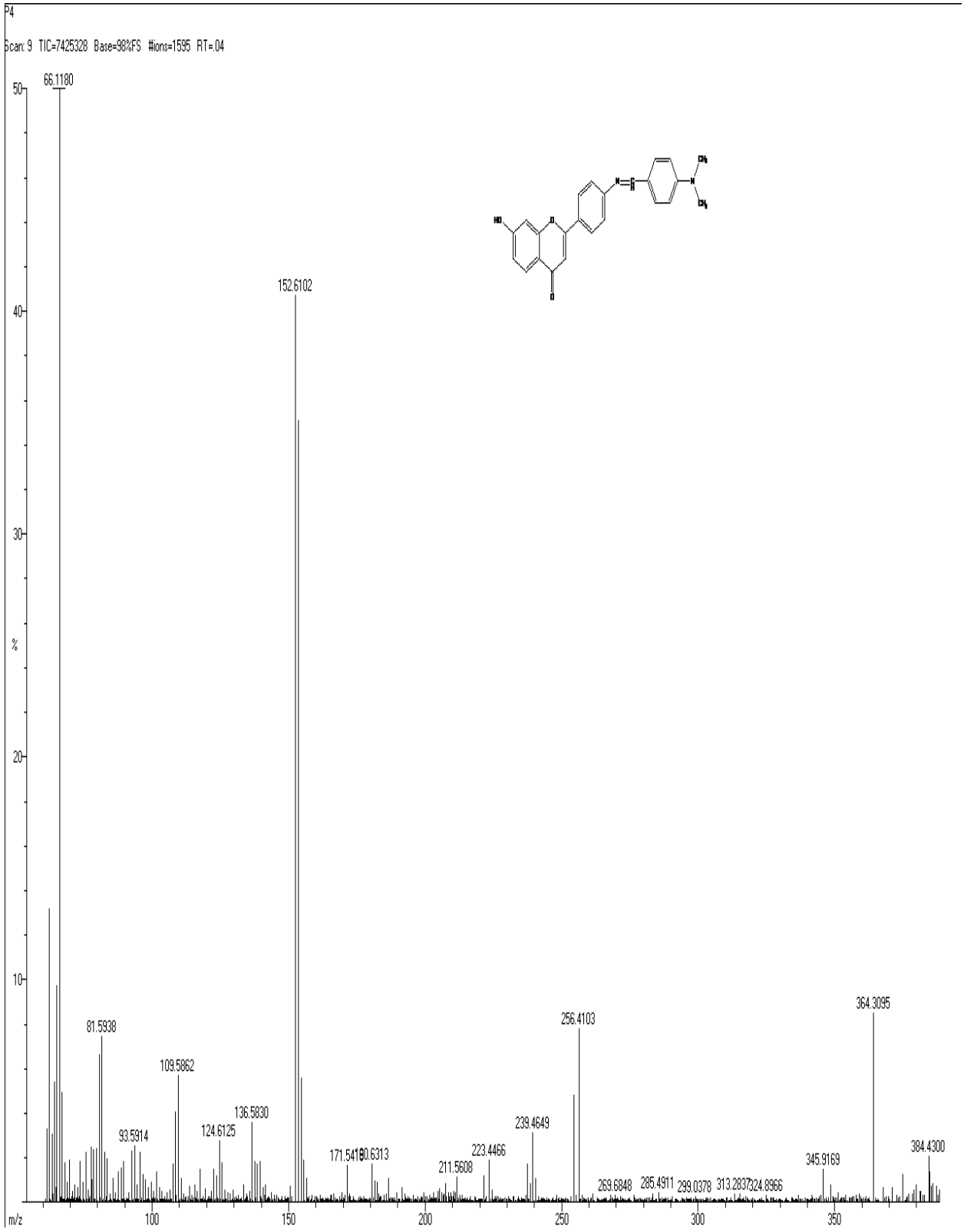
FD 05


P3
Scan: 26 TIC=6026464 Base=72.5%FS #ions=1772 RT=.13
60.6349
20
15
%0
5
75.1186
250.9435
149.0759
96.1477
103.1182
120.0785
166.0922
175.1414
233.9818
206.0703
217.9559
136.1336
186.0302
267.9560
279.9908
290.2029
307.8639
325.2699
340.4149
358.4877
375.7701
m/z
100
150
200
250
300
350

DPN 06


P2
Scan: 26 TIC=13683952 Base=100%FS #ions=1521 RT=.13
149.9620
167.0655
104.0758
178.0983
359.4779
120.0783
92.0799
195.0938
135.0826
380.3958
370.6979
386.3202
251.9272
207.0703
218.9711
234.9423
100
80
60
%
40
20
m/z
100
150
200
250
300
350

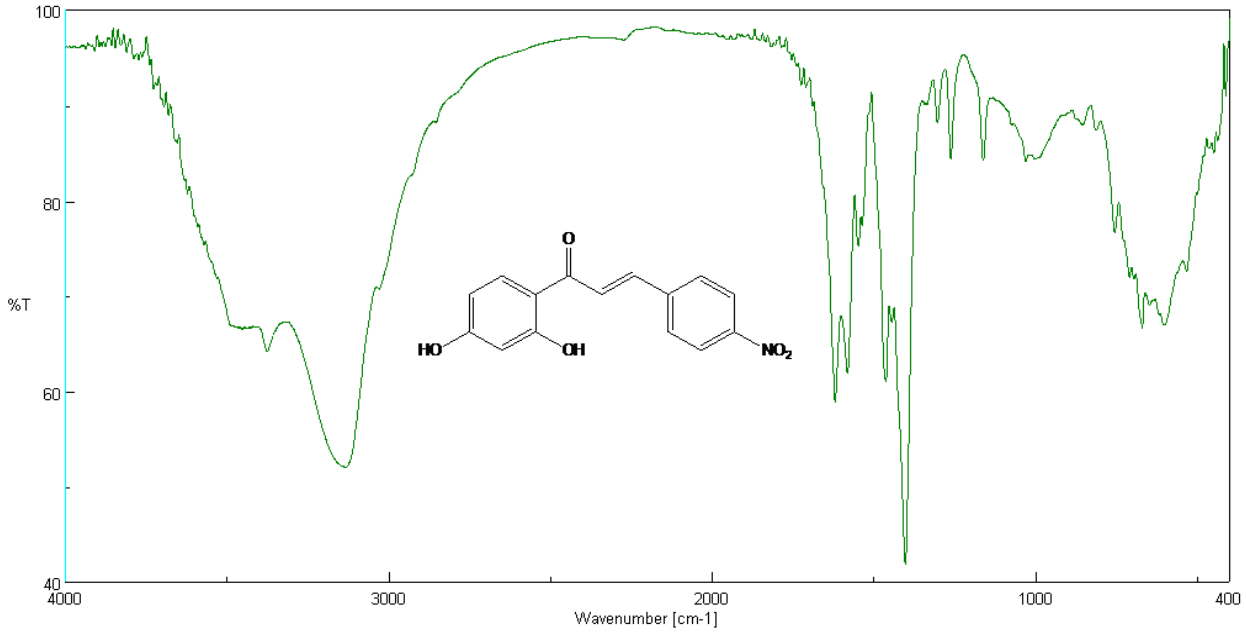
DPN 08


P4
Scan: 9 TIC=7425328 Base=98%FS #ions=1595 RT=.04
66.1180
152.6102
81.5938
109.5862
93.5914
124.6125
136.5830
171.5418
180.6313
211.5608
223.4466
239.4649
256.4103
269.6848
285.4911
299.0378
313.2837
324.8966
345.9169
364.3095
384.4300
%
m/z
50
40
30
20
10
100
150
200
250
300
350

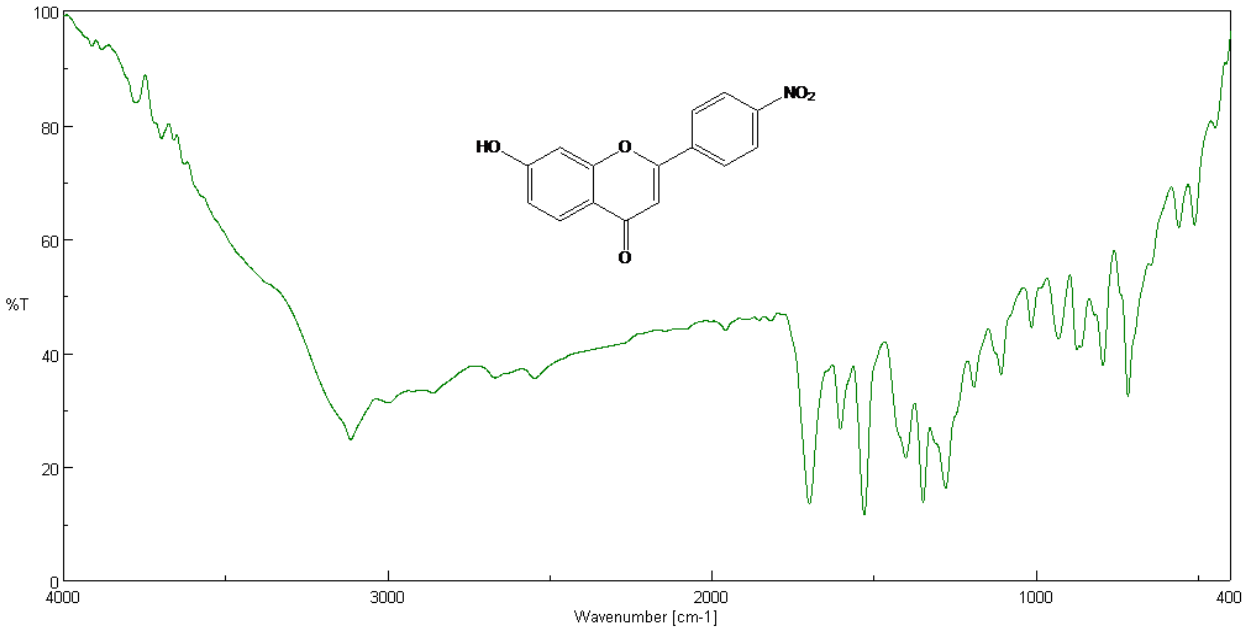
DPN 11

SPECTRAL DATAS OF SYNTHESISED COMPOUNDS BY MICROWAVE IRRADIATION METHOD:

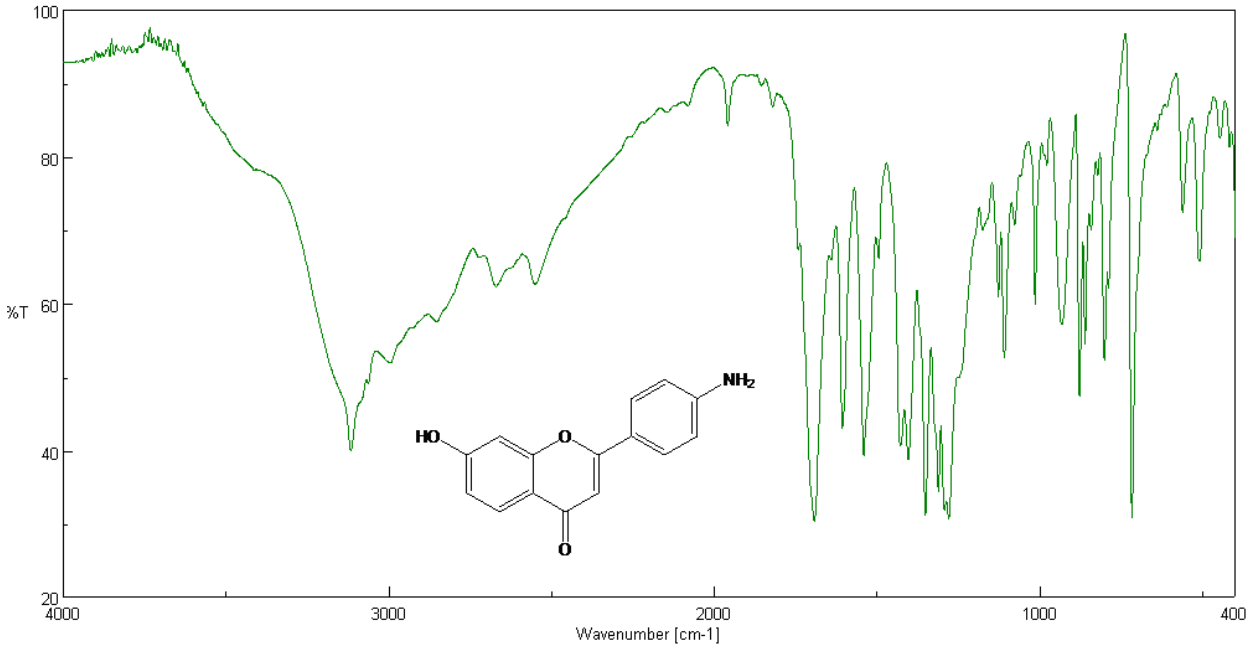
IR SPECTRA:



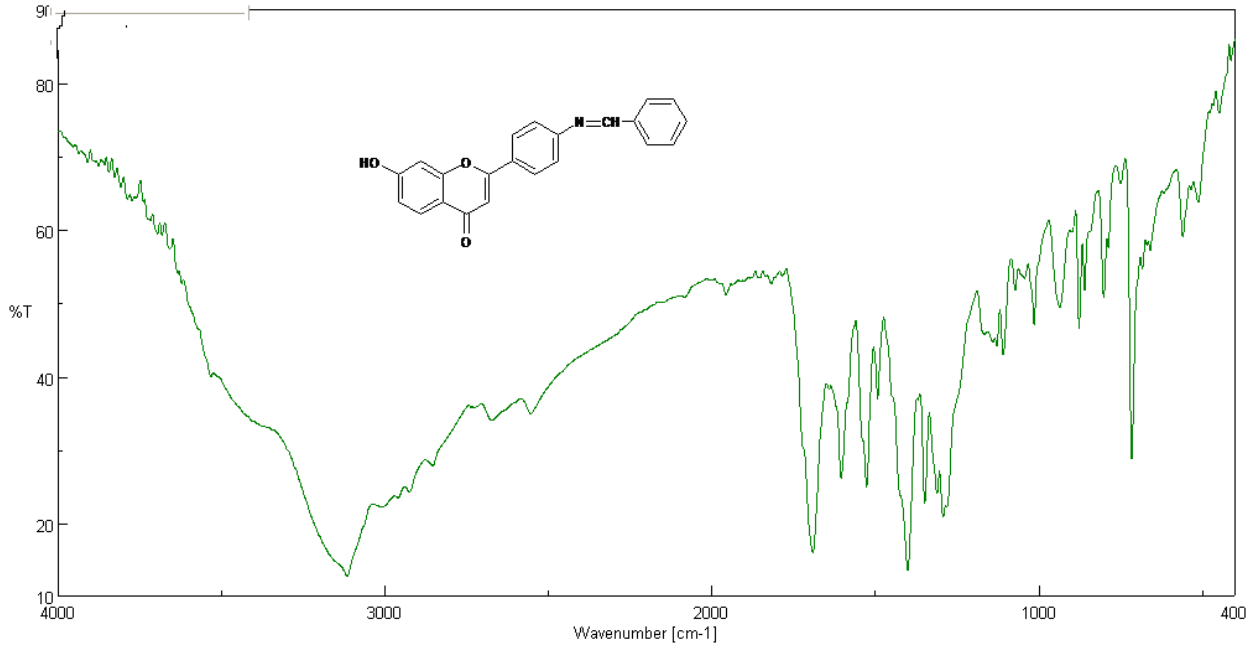
FM 01



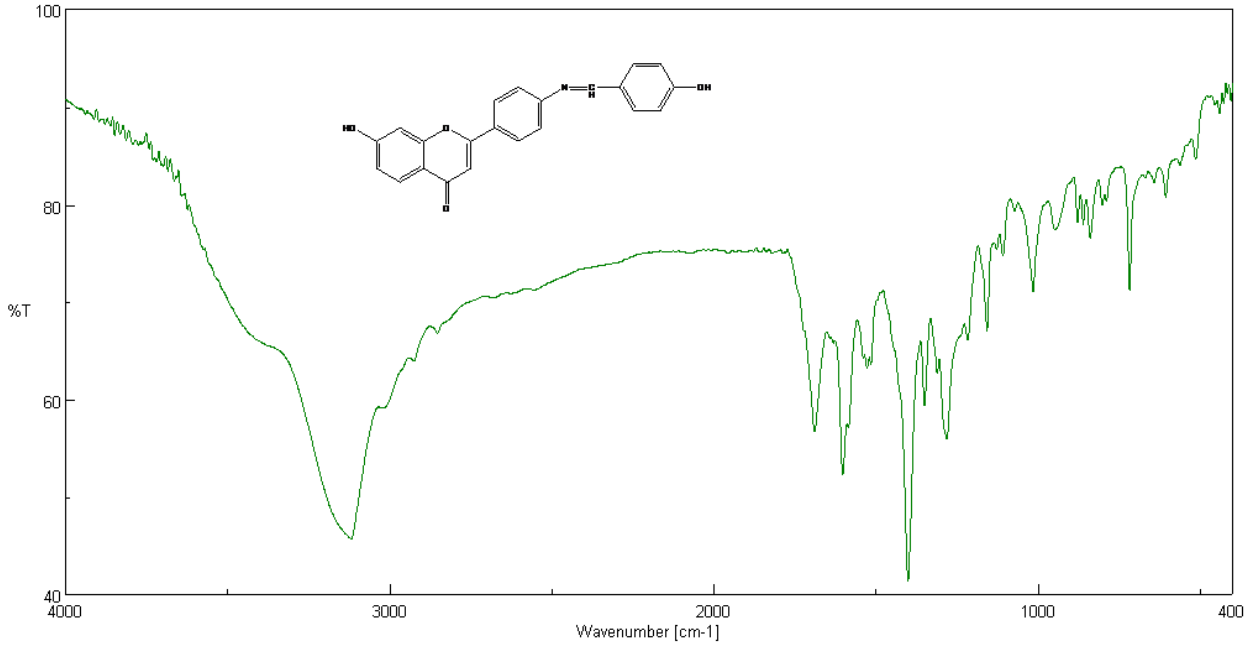
FM 02


100
80
60
40
20
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]
HO
O
O
NH2

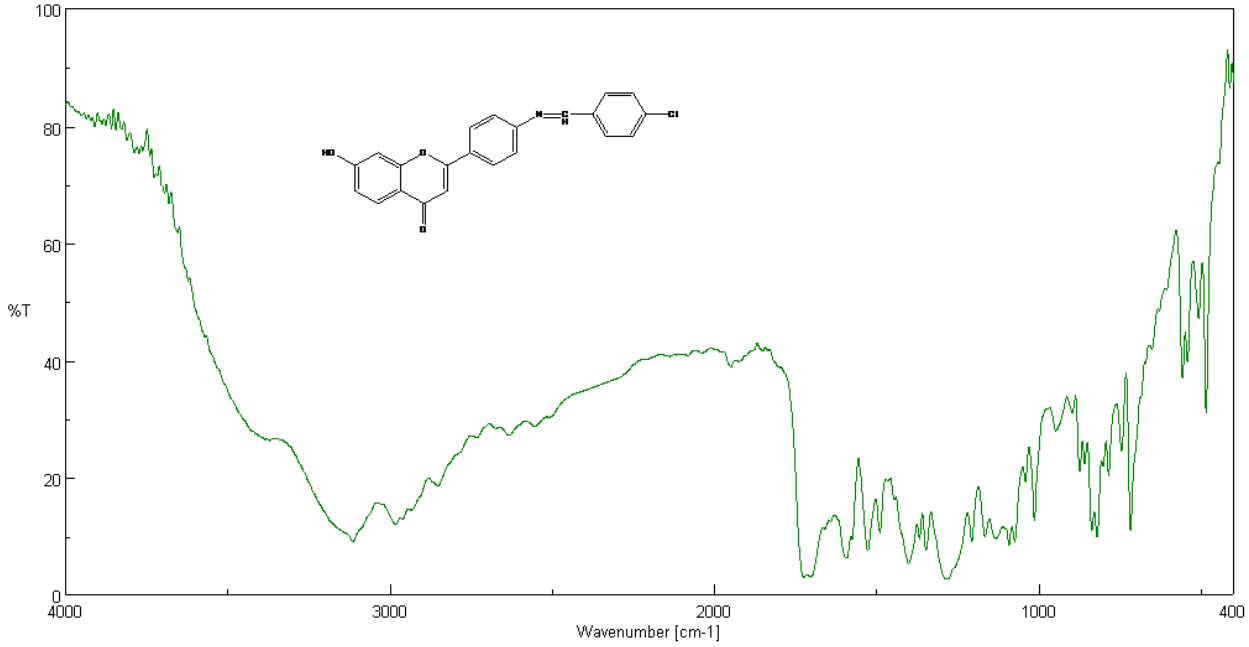
FM 03


90
80
60
40
20
10
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]
HO
O
O
N=CH

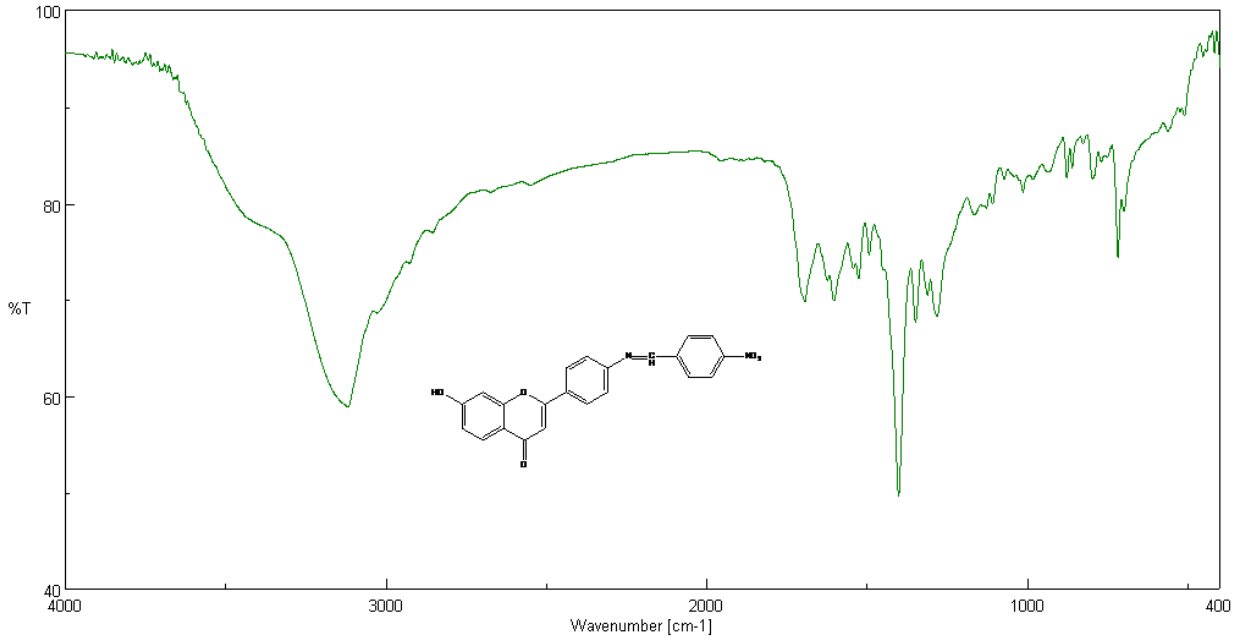
MWD 01


100
80
60
40
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]

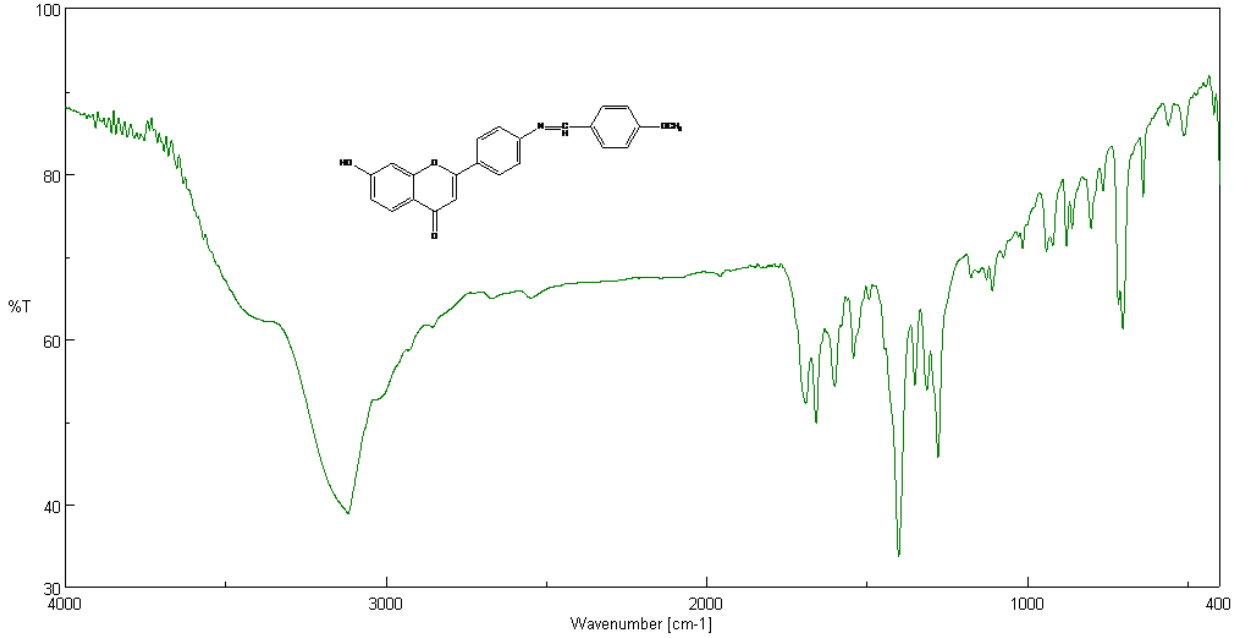
MWD 02


100
80
60
40
20
0
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]

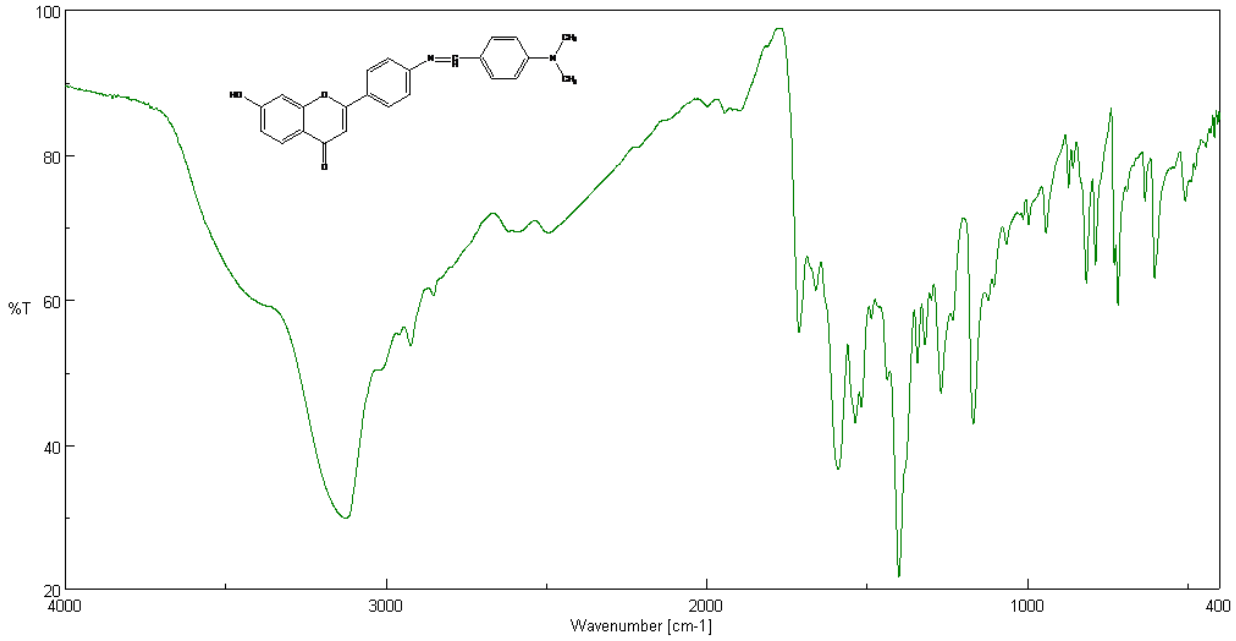
MWD 03


100
80
60
40
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]

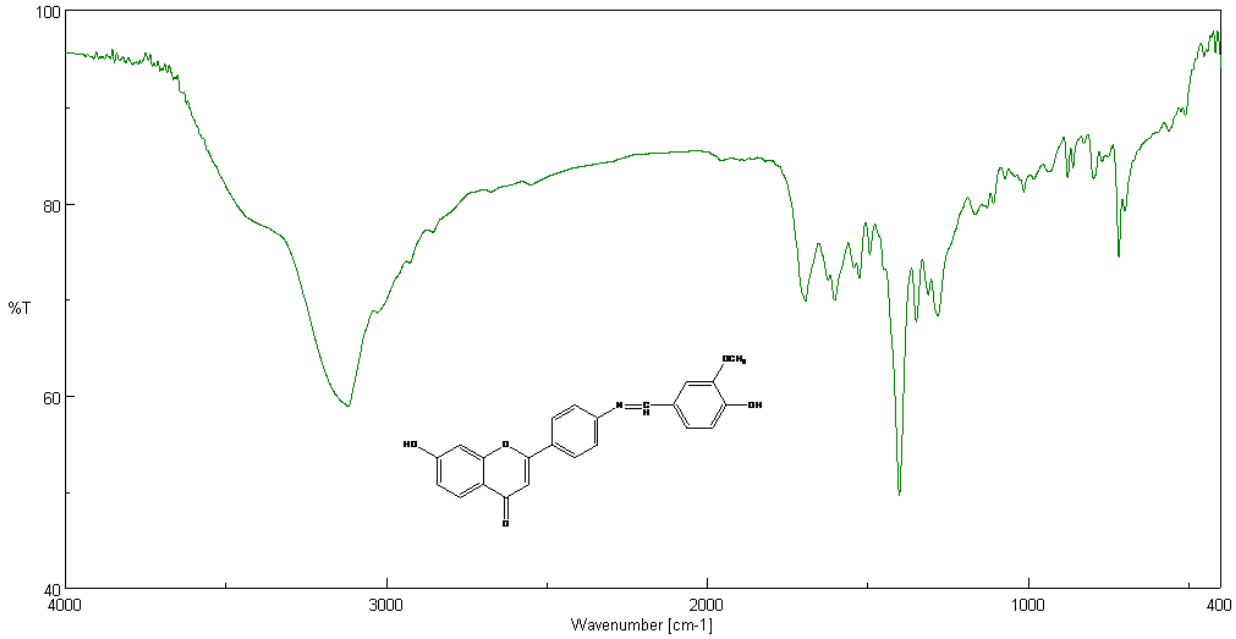
MWD 04


100
80
60
40
30
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]

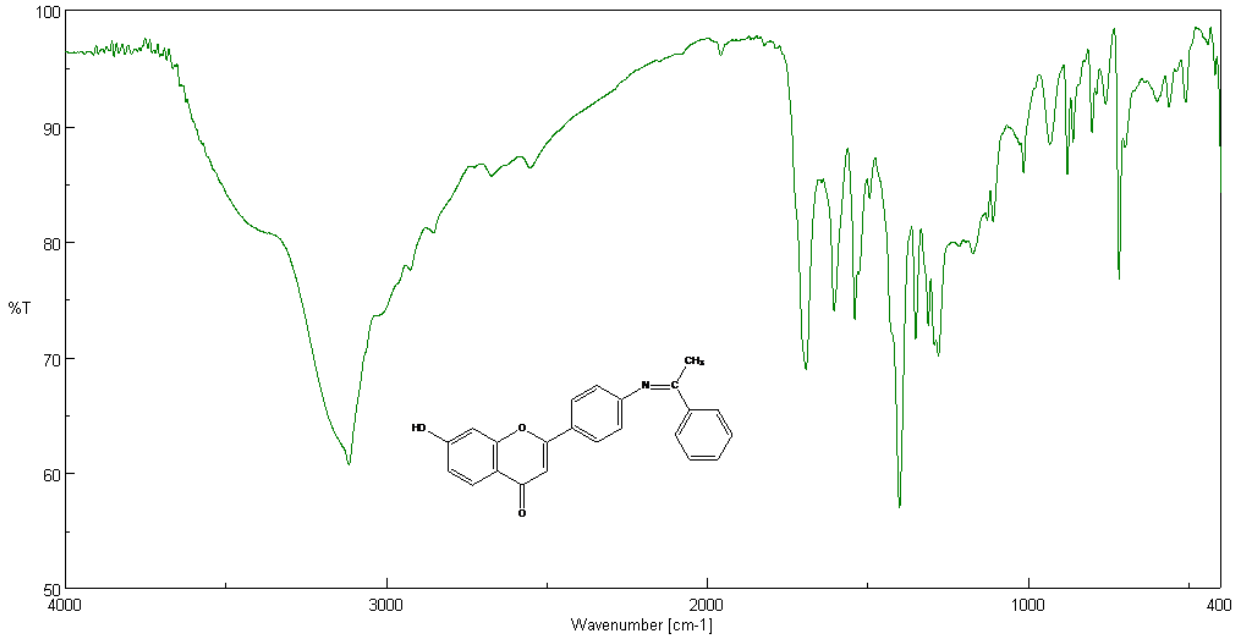
MWD 05


100
80
60
40
20
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]

MWD 06


100
80
60
40
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]

MWD 07


100
90
80
%T
70
60
50
4000
3000
2000
1000
400
Wavenumber [cm-1]
HO
O
O
N
CH3

MWD 08

BIOLOGICAL SCREENING

6.1 *In-vitro* Anti Microbial screening by Zone of inhibition and MIC method

6.2 *In-Vitro* Anti Malarial screening by Candle jar method

6.3 *In-vitro* Antioxidant screening by DPPH, FRAP, ABTS assays method.

6.4 *In-vitro* Anti-cancer screening by MTT assay.

6.1 *IN-VITRO* ANTIMICROBIAL STUDIES [86]

The *in vitro* antibacterial activity of the series of synthesized compounds was evaluated against 8 pathogenic bacteria and 4 pathogenic fungi. The bacteria and fungus strains were procured from the bacterial repository of Department of Biotechnology, KMCH College of Pharmacy Coimbatore.

Dimethyl sulphoxide was used to prepare stock solution for synthesized drugs. Subsequent dilutions of the stock were also made with above solvent. Muller Hinton agar media (Hi-media) for bacteria and Sabouraud's Dextrose Broth for fungus were used to subculture various strain of bacteria and fungus as well for determining the MIC's of synthesized compounds by serial dilution method.

Initially the sensitivity of various microorganisms to the synthesized compounds was determined by disc diffusion method.

Compounds used for activity: DPN 1-15 (100µg/disc)

Standard drugs used for activity:

Ciprofloxacin (antibacterial) (5µg/disc)

Clotrimazole (antifungal) (5µg/disc)

4.1.1 DISC DIFFUSION METHOD

The disc diffusion method, the drug potency is based on measurement of the diameter of zone of inhibition surrounding cylinder disc which are placed on the surface of a nutrient medium previously inoculated with a culture of suitable

microorganisms. Inhibition produced by the test compound is compared with that produced by known concentration of reference standard.

List of bacterial and fungal strains used

Bacteria

1. *Micrococcus luteus* NCIM 2169 (Gram positive)
2. *Bacillus subtilis* NCIM 2063 (Gram positive)
3. *Staphylococcus aureus* NCIM 2079 (Gram positive)
4. *Corynebacterium* NCIM 2268 (Gram positive)
5. *Vibrio cholera* MTCC 1738 (Gram negative)
6. *Escherichia coli* NCIM 2068 (Gram negative)
7. *Pseudomonas aeruginosa* NCIM 2206 (Gram negative)
8. *Rhodosporium rubrum* NCIM 1689 (Gram negative)

Fungus

1. *Monascus purpureus* MTCC 1090
2. *Aspergillus niger* NCIM 1207
3. *Candida albicans* MTCC 3100
4. *Trichophyton rubrum* MTCC 3272

6.1.1a. Antibacterial screening

The antibacterial activities of synthesized compounds were screened in the concentration of 1mg/ml in dimethyl sulphoxide against the above mentioned bacteria in the Muller Hinton agar medium by Disc diffusion method using ciprofloxacin 5µg as standard. The antibacterial activity was evaluated by measuring zone of inhibition in mm. The procedure is given below.

PROCEDURE

Preparation of Nutrient Broth for Bacteria

Ingredients used

Sr. No	Ingredients	Quantity
1	Beef extract	10g

2	Peptone	10g
3	Sodium chloride	5g
4	Water	1000ml

The accurately weighed quantity of above ingredients were transferred to a conical flask, and dissolved in distilled water with the aid of heat with stirring and the pH was adjusted to 7.2 – 7.4 and plugged with non-absorbent cotton, covered by Aluminium foil and sterilized by autoclaving (121˚C at 15 lbs pressure for 15 min).

Preparation and standardization of inoculation

Each bacterial pure culture from the slant culture is picked up aseptically and was transferred into 100 ml of nutrient broth. The inoculated broths were incubated at 37˚C for 24 hrs and growth was arrested by stored in the refrigerator (below 4˚C).

Sample Preparation

1 mg of each sample was dissolved in 1ml DMSO (Dimethyl Sulfoxide) to a final concentration of 1µg/ml. The solvent control shows no antibacterial activity with all the test organisms used. The sterile disc (6 mm in diameter) were impregnated with 10 µg/disc of the sample and tested against microbial cultures.

Media Used

Muller – Hinton Agar Medium, Hi-media India (Pvt) Ltd

Muller Hinton agar medium was prepared and transferred into sterile petri plates aseptically (thickness of 5-6mm). The plates were allowed to dry at room temp. The plates were inverted to prevent condensate falling on the agar surface. The layers of the medium are uniform in thickness, is done by placing the plates on a leveled surface. Standardized bacterial inoculums were applied to the plates and spreaded uniformly over the surface of medium by using a sterile Non-absorbent cotton swab and finally the swab was passed around the edge of the medium. The inoculated plates were closed with the lid and allowed to dry at room temperature. The sample impregnated discs were placed on the inoculated agar medium. All Petri plates were incubated at 37˚C for

24 hrs. After the incubation diameter of zone of inhibition produced by the sample were measured.

The results were tabulated in the table 6.1& 6.2.

6.1.1b. Antifungal screening

The antifungal activities of synthesized compounds were screened in the concentration of 1 mg/ml in dimethyl sulphoxide against the above mentioned fungus in the Sabourand's Dextrose Broth by Disc diffusion method using Clotrimazole 5 µg. The antifungal activity was evaluated by measuring zone of inhibition in mm. The procedure is given below.

PROCEDURE

Preparation of Nutrient Broth for Bacteria

Ingredients used

S.No	Ingredients	Quantity
1	Dextrose	40g
2	Peptone	10g
3	Water	1000ml

Specified amount of dextrose and peptone was taken along with 1000ml of distilled water in a conical flask and heated in a steam bath to dissolve. The pH was maintained at 7.6 ± 0.2 and sterilized in an autoclave at 15 lb pressure, 120°C for 15 minutes. The sterile medium was poured into the sterile Petri dish and allowed to solidify.

Preparation and standardization of inoculums

Each fungal pure culture from the slant culture is picked up aseptically and was transferred into 100ml of nutrient broth. The inoculated broths were incubated at 27˚C-28˚C for 24-48 hrs and growth was arrested by stored in the refrigerator (below 4˚C).

Sample Preparation

1mg of each sample was dissolved in 1ml DMSO (Dimethyl Sulphoxide) to a final concentration of 1µg/ml. The solvent control shows no antibacterial activity with all the test organisms used. The sterile disc (6 mm in diameter) were impregnated with 10µg/disc of the sample and tested against microbial cultures.

Media Used

Media used **Sabouraud's Dextrose Broth**, Hi-media India (Pvt) Ltd

Sabouraud's dextrose broth medium was prepared and transferred into sterile Petri plates aseptically (thickness of 5-6mm). The plates were allowed to dry at room temp. The plates were inverted to prevent condensate falling on the agar surface. The layers of the medium are uniform in thickness, is done by placing the plates on a leveled surface. Standardized bacterial inoculums were applied to the plates and spreaded uniformly over the surface of medium by using a sterile Non-absorbent cotton swab and finally the swab was passed around the edge of the medium. The inoculated plates were closed with the lid and allowed to dry at room temperature. The sample impregnated discs were placed on the inoculated agar medium. All Petri plates were incubated at 27˚C -28˚C for 24 hrs. After the incubation diameter of zone of inhibition produced by the sample were measured.

The results were tabulated in the table 6.5.

6.1.2 MINIMUM INHIBITORY CONCENTRATION (MIC)

The lowest concentration of the test compound that completely inhibited growth on agar plates, disregarding a single colony or a faint haze caused by the inoculums was considered as minimum inhibitory concentration (MIC) of that compound.

The potency of drug is based on inhibition of microbial growth as indicated by measurement of turbidity of a suspension of a suitable microorganism in a fluid medium to which graded amount of test compound have been added. The changes in turbidity produced by known concentration of reference materials are compared with results.

Determination of Minimum Inhibitory Concentration for Synthesized Compounds (MIC) by serial dilution Method:

1. The serial dilutions of known concentration of compound solution were made from the stock (0.1mg/ml) by using Muller Hinton broth using the method described below.
2. The tubes were labelled 1 to 8 and 1 ml of Muller Hinton broth were added to the first 5 tubes and 8^{th} tube, then added 0.5 ml Muller Hinton broth to 6^{th} and 7^{th} tubes.
3. One ml of different synthesized compounds was added to the 1^{st} tube, mixed and transfers 1 ml serially up to tube 5. From the 5^{th} tube transfer 1ml to 6^{th} tube. Mixed and transfer 0.5 ml to the 7^{th} tube. Each tube, 1 to 7 contains 1ml diluted extract.
4. 8^{th} tube was the control.
5. With a standardized micro pipette, added a drop of the diluted broth culture approximately 0.01ml of the test organism to all tubes, including the control, gently mixed and incubated at $37\ ^0C$ for 16 to 18hrs.
6. The highest dilution of particular compounds showing no turbidity was observed and recorded. This was taken as the end point, and this dilution was considered to contain the concentration of drug equivalent to MIC.

The results were tabulated in the table 6.3, 6.4, 6.6.

6.2 *IN- VITRO* ANTI MALARIAL SCREENING:

CANDLE JAR METHOD [87]

PROCEDURE

Preparation of Serum:

- Fresh human whole blood (preferably O+ or A+) was collected in a blood collection bags without anticoagulant. The blood was transferred (before clotting occurs) to the sterile 50ml centrifuge tubes. (In a laminar flow cabinet)
- The blood was stored overnight at $4^{o}C$ to allow clotting.
- The serum and erythrocytes is fractionated by centrifugation. (2500 rpm for 30mins at room temperature)
- The serum was then transferred under sterile conditions to new sterile 50ml tubes.
- The serum was inactivated by incubation in a water bath for 20mins at $65^{o}C$.
- The serum was then stored at $-20^{o}C$ in the 50ml sterile tubes.

P.falciparum culture: Media and Buffers (Serum):

The medium used for culturing of *Plasmodium falciparum* was prepared as follows:

Ingredients used

S.NO	INGREDIENTS	QUANTITY
1	RPMI-1640(sigma)	10.4g
2	HEPES (sigma, cell culture tested)	5.94g
3	D-glucose (sigma, cell culture tested)	4.0g
4	Hypoxanthine(sigma, cell culture tested)	44mg
5	Gentamycin	0.4ml
6	Distilled water	1000ml

The accurately weighed quantities of the above ingredients were transferred into the conical flask, mixed well and leave for 30 minute at room temperature. Add 36ml of 5% $NaHCO_3$ solution was added to the medium to buffer the solution at pH

of 7.4. The solution was filtered using a sterile Millipore filters (0.22mm). Filtered solution was divided into two half's. Transferred half to the 500ml medium bottle and stored at 4^oC (wash medium). 50ml human serum was added to the other half and transferred to a 500ml medium bottle and stored at 4^0C(culture medium).

Preparation of Erythrocytes:

- Erythrocytes were collected in a 10ml vacationer (vacutest) tubes with anticoagulant (EDTA purple).
- The blood was transferred under sterile conditions to the sterile 10ml centrifuge tube.
- It was centrifuged to separate the cells and serum (2500 rpm for 5mins at room temperature).
- The serum and "Buffy-coat" was aspirated using a Pasteur pipette fixed to the vacuum line.
- The volume was then supplemented to 10ml with "wash medium" and mixed well.
- The cells were centrifuged (2500 rpm for 5mins at room temperature).
- Steps 4 to 6 were repeated another two times.
- The washed erythrocytes was resuspended in wash medium and stored at 4^oC.

Cultivation of Parasites:

Preheat the culture medium by placing it in the 37^oC water bath or in the incubator. 0.5ml of culture medium, 20µl of washed erythrocytes and 20µl of *P.falciparum* infected human blood were placed in 96 well plates. The well plates were placed in a candle jar and incubated at 37^oC for 24hrs. Parasite count was made on giemsa stained thin smears.

Preparation of thin smears:

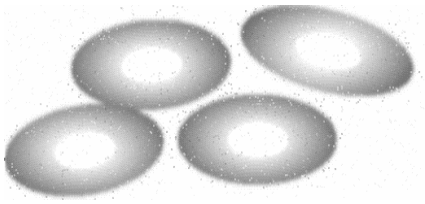
- A small amount of erythrocytes were removed using a Pasteur pipette and placed on a microscopic slide. The second slide was placed on the first and moves it back into the drop, spreading it. The second slide was then moved forward, smearing a film of blood across the first slide.

- The smear was allowed to dry, and then it was fixed with methanol.
- The methanol was discarded and the slide was covered with giemsa stained solution for 3 to 5 minutes to stain. (The giemsa solution consists of approximately 0.5ml phosphate buffer and 10-20 drops of concentrated giemsa stock solution). Then rinsed with water and allowed it to dry, where after it was studied microscopically (100x).
- Parasitemia were counted by counting both uninfected and parasite infected erythrocytes.
- Expressing the parasite count as a percentage of infected erythrocytes as per total erythrocyte count.

Interpretation of microscopic slides:

Different stages of malaria parasite in the red blood cells interpreted.

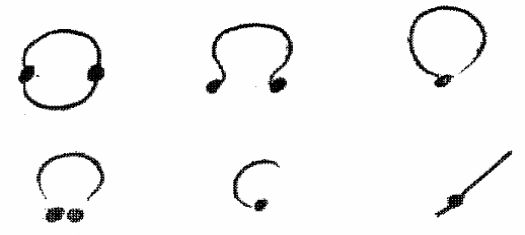
Uninfected erythrocytes



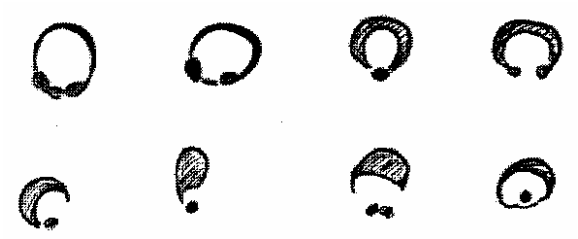
Infected erythrocytes

Different stages of the malarial parasite in the red blood cells.

Ring:

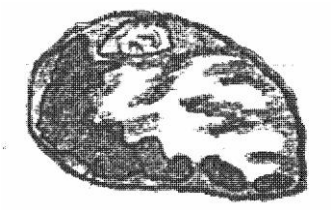


Early Trophozoite:



Trophozoite:

Transition Stage:



Anti-Plasmodial activity of the synthesized compounds:

Various concentrations of the synthesized compounds and standard drug pyremethamine (20,40,60,80 µg/ml) in DMSO, was added to the well plates containing 0.5ml of culture medium, 20 µl of washed erythrocytes and 20 µl of *P.falciparum* infected human blood. Well plates were placed in the candle jar and incubated at 37^{o}C for 24hrs. Parasite count were made on the giemsa stained thin smears prepared at different time intervals (24, 48, 72 hrs). And *in-vitro* anti-plasmodial activity of the synthesized compounds was determined by calculating inhibitory concentration percentage.

Percentage can be calculated using the formula,

$$\mathbf{IC_{50}} = \frac{\text{No of parasitemia in control} - \text{No of parasitemia in treated}}{\text{No of parasitemia in control}} \times 100$$

The results were tabulated in the **table 6.7**.

6.3 *IN-VITRO* ANTI-OXIDANT SCREENING OF SYNTHESIZED COMPOUNDS

- In-vitro anti-oxidant screening of synthesized compounds were done by using three methods
  - ✓ DPPH method
  - ✓ FRAP method
  - ✓ ABTS method

6.3.1 DPPH RADICAL SCAVENING ACTIVITY: [88]

PRINCIPLE:

DPPH molecule a stable free radical by its nature of delocalization of unpaired electron which gives rise to the deep violet color which was characterized by an absorption at about 519 nm. When solution of DPPH (diphenylpicrylhydrazyl) mixed with that of a substance that can donate an electron (i.e. an antioxidant) it is converted to its reduced form with the loss of this violet color to pale yellow color. The primary reaction involved in this assay is given below.

$$\mathbf{Z^{\cdot} + AH = ZH + A^{\cdot}}$$ Where,

Z. -DPPH radical

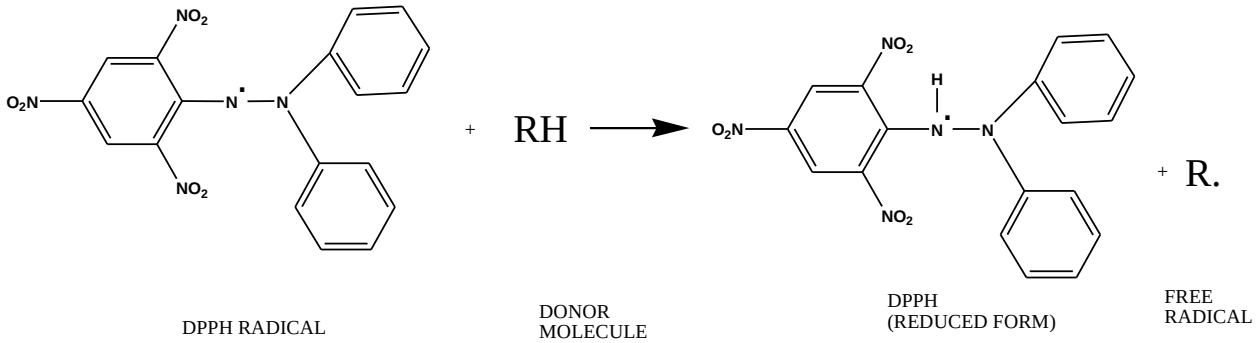
AH -Donor molecule

ZH -Reduced form

A. -Free radical

The result of the antioxidant efficiency is expressed as $EC_{50,}$ determined as the concentration of substrate that causes 50% loss in absorbance.

Reaction:



REAGENTS:

Radical : DPPH

Solvent : Ethanol

Standard : Ascorbic acid

Preparation of 0.3 mm DPPH solution:

It was prepared by dissolving DPPH (5.91 mg) in 50ml of methanol. This stock solution was prepared freshly and kept in the dark at ambient temperature when not used.

Preparation of Sample Stock Solution:

The sample stock solution was prepared by dissolving the compound in suitable solvent (Ethanol) with a final concentration of 1mg/ml.

Preparation of Standard Stock Solution:

The standard stock solution was prepared by dissolving the Ascorbic acid in suitable solvent (Ethanol) with a final concentration of 1mg/ml.

PROCEDURE:

All the compound anti oxidant were screening by DPPH assay method, Sample stock solutions (1.0 mg/ml) were diluted to final concentrations of 10, 20, 40, 60, 80, 100 µg/ml, in ethanol. An ethanolic solution of 1ml of DPPH (0.3mM) was added to 0.5ml of the compound and allowed to react at room temperature in a dark place for 30 minutes. After 30 minutes the absorbance values were measured at 519 nm. All the measurements were taken as a triplicate values from the average of the absorbance values; Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The antioxidant scavenging activity of the DPPH radical was calculated according to the following equation.

% inhibition = (ABS control – ABS test) /ABS control × 100

Where,

Control-Absorbance of ethanol+DPPH

Test -Absorbance of DPPH + Compound /Standard

The percentage antioxidant activity (Percentage inhibition) was extrapolated against concentration of the Compound

The results were tabulated in the **table 6.8**.

6.3.2 FRAP (Free Radical scavenging activity) ASSAY: [89]

Total antioxidant activity is measured by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1999). FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess.

Principle

At low pH, reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has an intense blue color) can be monitored by measuring the change in absorption at 593nm. The reaction is non-specific, in that any half reaction that has lower redox potential, under reaction conditions, than that of ferric ferrous half reaction, will drive the ferrous (Fe III to Fe II) ion formation. The change in absorbance is therefore, directly related to the combined or "total" reducing power of the electron donating antioxidants present in the reaction mixture.

Reagents

FRAP Reagent

a) Acetate buffer 300 mM pH 3.6: Weigh 3.1g sodium acetate trihydrate and add 16 ml of glacial acetic acid and make the volume to 1 L with distilled water.

b) TPTZ (2, 4, 6-tripyridyl-s- triazine) (M.W. 312.34) 10 mM in 40mM HCl (M.W. 36.46)

c) FeCl3. 6H2O (M.W. 270.30) 20 mM

The working FRAP reagent was prepared by mixing a b & c in the ratio of 10:1:1 at the time of use.

Standard: Ascorbic Acid (M.W. 176.13) 1000 µ M

Procedure

The reaction mixture contained 900 µl of freshly prepared FRAP reagent and it was added to different concentrations of test solution. Finally volume was made to 1ml with ethanol. The blank solution was prepared by mixing 900 µl FRAP reagent and 100 µl of methanol. Measured the absorbance at 593 nm, just after 4 minutes from the time of addition of FRAP reagent. An increase in absorbance indicated enhanced reducing potential of plasma. Quantitative calculation for each sample was done using an equation obtained from the standard curve of Fe++-TPTZ.

The equation used: Absorbance = 0.274 x µM of Fe++ + 0.114 [R2 = 0.974]

The results were tabulated in the **table 6.9**.

6.3.3 ABTS Radical scavenging activity: [90]

Reagents

- 2.45 mM ammonium persulphate
- 7 mM 2,2,-azinobis (3-ethylbenzoline-6-sulfonic acid) (ABTS)

Procedure

The ABTS radical scavenging activity of the extract was measured by Rice-Evans et al., (1997). ABTS radical cation ($ABTS^{+\cdot}$) was produced by reacting 5ml of 14mM ABTS solution with 5ml of 4.9mM ammonium persulphate solution and the mixture was allowed to stand in dark place at room temperature for 12-16 hr before use. The mixture was diluted with water to yield an absorbance of 0.7±0.02 at 734nm. Different concentrations (1-10 µg/ml) of Methanolic extract of test solution or standard (Quercetin) were added to 950µl of ABTS radical solution and the final volume was made up to 1ml with methanol. The blank solution was prepared by mixing 950µl ABTS solution and 50µl methanol. After 6 minutes the absorbance was read at 734nm and the percentage inhibition was calculated. The experiment was performed in triplicate.**[41].** The scavenging activity of ABTS radical was calculated by using the following equation.

$$\% \text{ inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where $A_{control}$ was the control absorbance and A_{test} was the sample absorbance in the presence of synthesized compound.

The results were tabulated in the **table 6.10**.

6.4 *IN-VITRO* ANTI-CANCER SCREENING: [91, 92]

The human hepatic carcinoma cell line ($HepG_2$) was obtained from National Centre for Cell Science (NCCS), Pune, and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). All cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediaminetetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium with 5% FBS to give final density of $1x10^5$ cells/ml. one hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the extracts and fractions. They were initially dissolved in neat dimethyl sulfoxide (DMSO) and further diluted in serum free medium to produce five concentrations. One hundred microlitres per well of each concentration was added to plates to obtain final concentrations of 100, 10, 1.0 and 0.1µM. The final volume in each well was 200 µl and the plates were incubated at 37^0C, 5% CO_2, 95% air and 100% relative humidity for 48h. The medium containing without samples were served as control. Triplicate was maintained for all concentrations.

MTT assay

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

After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO

and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

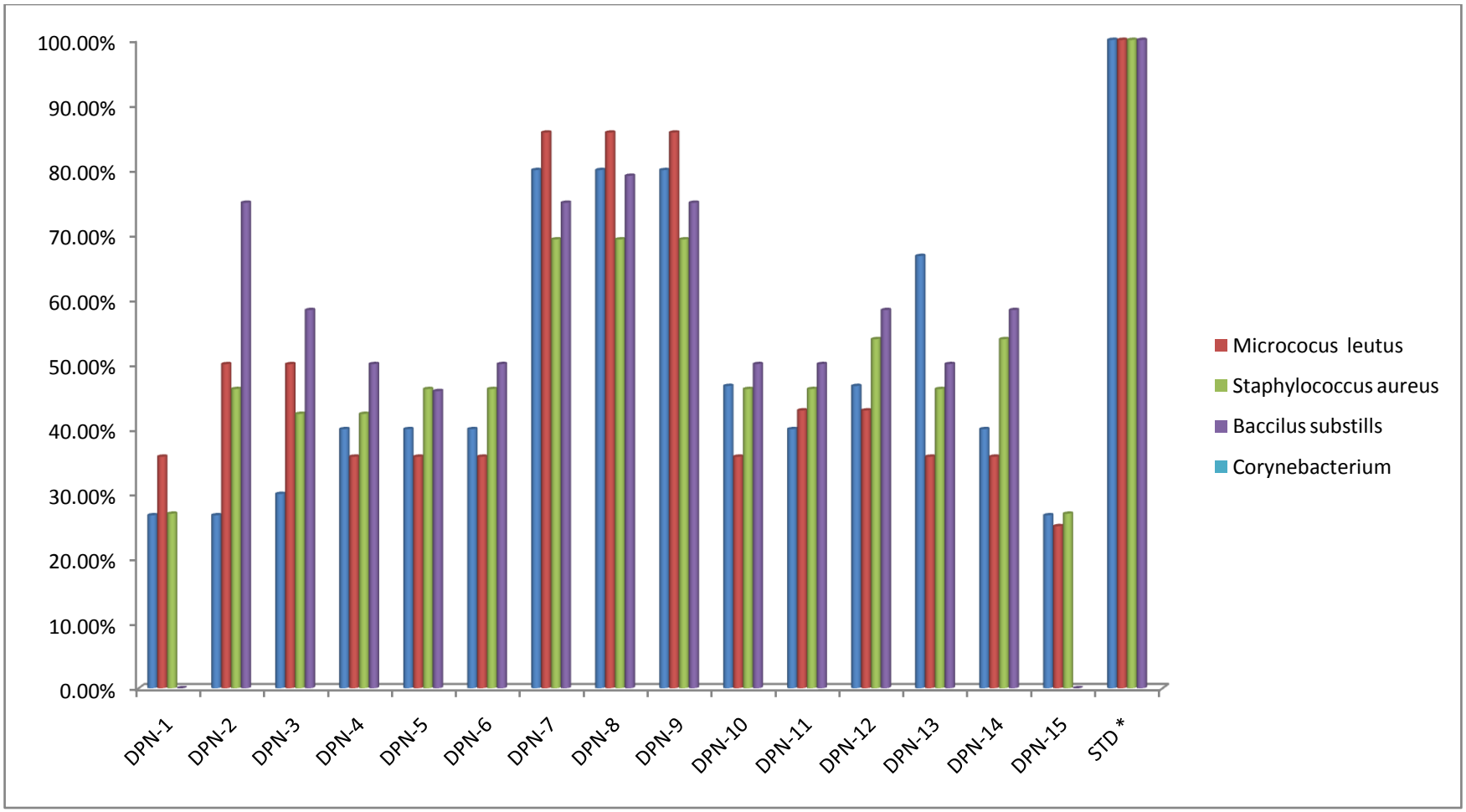
% cell Inhibition = 100- Abs (sample)/Abs (control) x100.

The results were tabulated in the table **6.11, 6.12, 6.13, 6.14**.

Table 6.1: *In -Vitro* Antibacterial Activity (Gram Positive Strains) Data in Zone Of Inhibition (Mm) and Percentage Inhibition by Disc Diffusion Method

Organism	Diameter of zone of inhibition (mm) / percentage of inhibition															
	DPN-1	DPN-2	DPN-3	DPN-4	DPN-5	DPN-6	DPN-7	DPN-8	DPN-9	DPN-10	DPN-11	DPN-12	DPN-13	DPN-14	DPN-15	STD *
Micrococus leutus	08 (26.67%)	08 (26.67%)	09 (29.97%)	12 (39.96%)	12 (39.96%)	12 (39.96%)	24 (79.92%)	24 (79.92%)	12 (39.96%)	14 (46.62%)	24 (79.92%)	18 (46.62%)	20 (66.67%)	12 (39.96%)	08 (26.67%)	30
Staphylococcus aureus	10 (35.71%)	14 (49.98%)	14 (49.98%)	10 (35.71%)	10 (35.71%)	10 (35.71%)	24 (85.71%)	24 (85.71%)	12 (42.85%)	10 (35.71%	24 (85.71%)	12 (42.85%)	10 (35.71%	10 (35.71%	07 (24.99%)	28
Baccilus substills	07 (26.92%)	12 (46.15%)	11 (42.31%)	11 (42.31%)	12 (46.15%)	12 (46.15%)	18 (69.23%)	18 (69.23%)	12 (46.15%)	12 (46.15%)	18 (69.23%)	14 (53.84%)	12 (46.15%)	14 (53.84%)	07 (26.92%)	26
Corynebacterium	-	13 (74.88%)	14 (58.33%)	12 (50.00%)	11 (45.83%)	12 (50.00%)	18 (74.88%)	19 (79.04%)	12 (50.00%)	12 (50.00%)	18 (74.88%)	14 (58.33%)	12 (50.00%)	14 (58.33%)	-	24

***Ciprofloxacin**



***Ciprofloxacin**

Fig 6.1: Graphical Representation of *In-Vitro* Anti-Bacterial (Gram Positive) activity

Table 6.2: *In-Vitro* Antibacterial Activity (Gram Negative Strains) Data in Zone Of Inhibition (Mm) and Percentage Inhibition by Disc Diffusion Method

Organism	Diameter of zone of inhibition (mm) / percentage of inhibition															
	DPN-1	DPN-2	DPN-3	DPN-4	DPN-5	DPN-6	DPN-7	DPN-8	DPN-9	DPN-10	DPN-11	DPN-12	DPN-13	DPN-14	DPN-15	STD *
Escherichia coli	08 (33.28%)	10 (41.63%)	10 (41.63%)	12 (50.00%)	11 (45.83%)	12 (50.00%)	16 (66.56%)	17 (70.72%)	08 (33.28%)	10 (41.63%)	16 (66.56%)	10 (41.63%)	10 (41.63%)	10 (41.63%)	-	24
Pseudomonas aeruginosa	-	14 (49.98%)	14 (49.98%)	10 (35.71%)	10 (35.71%)	09 (32.14%)	20 (71.42%)	20 (71.42%)	08 (28.57%)	12 (42.85%)	20 (71.42%)	12 (42.85%)	10 (35.71%)	10 (35.71%)	08 (28.57%)	28
Rhodospirillum rubrum	10 (55.56%)	14 (77.78%)	10 (55.56%)	08 (44.44%)	10 (55.56%)	12 (66.67%)	14 (77.78%)	14 (77.78%)	12 (66.67%)	11 (61.11%)	14 (77.78%)	10 (55.56%)	12 (66.67%)	14 (77.78%)	09 (50.00%)	18
Vibrio cholerae	10 (50.00%)	12 (60.00%)	13 (65.00%)	14 (70.00%)	14 (70.00%)	14 (70.00%)	14 (70.00%)	15 (75.00%)	10 (50.00%)	10 (50.00%)	14 (70.00%)	12 (60.00%)	08 (40.00%)	12 (60.00%)	-	20

***Ciprofloxacin**

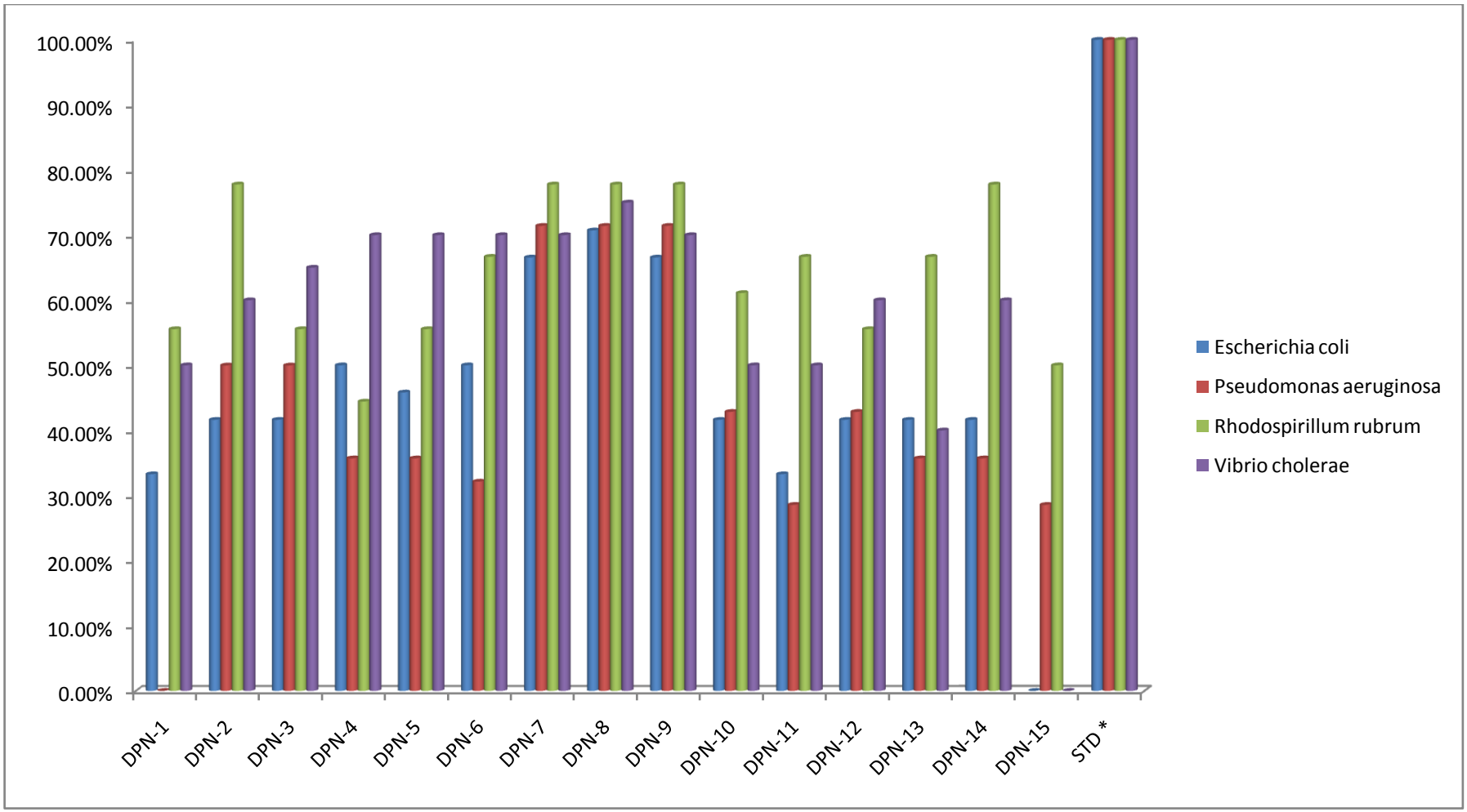


FIG 6.2: Graphical Representation of *In-Vitro* Anti-Bacterial (Gram negative) activity

Table 6.3: *In -Vitro* Antibacterial Activity (Gram Positive Strains) Data in Minimum Inhibitory Concentration

Organism	**MIC µg\ml**														
	DPN-1	DPN-2	DPN-3	DPN-4	DPN-5	DPN-6	DPN-7	DPN-8	DPN-9	DPN-10	DPN-11	DPN-12	DPN-13	DPN-14	DPN-15
Micrococus leutus	100	100	50	50	50	50	12.5	12.5	50	50	12.5	25	25	50	100
Staphylococcus aureus	50	50	50	50	50	50	12.5	12.5	50	50	12.5	50	50	50	100
Baccilus substills	100	50	50	50	50	50	25	25	50	50	25	50	50	50	100
Corynebacterium	-	50	50	50	50	50	25	25	50	50	25	50	50	50	-

***Ciprofloxacin**

Table 6.4: *In-Vitro* Antibacterial Activity (Gram Negative Strains) Data in Minimum Inhibitory Concentration

Organism	**MIC µg/ml**														
	DPN-1	DPN-2	DPN-3	DPN-4	DPN-5	DPN-6	DPN-7	DPN-8	DPN-9	DPN-10	DPN-11	DPN-12	DPN-13	DPN-14	DPN-15
Escherichia coli	50	50	50	50	50	50	25	25	100	50	25	50	50	50	-
Pseudomonas aeruginosa	-	50	50	50	50	50	25	25	100	50	25	50	50	50	100
Rhodospirillum rubrum	50	50	50	100	50	50	50	50	50	50	50	50	50	50	100
Vibrio cholerae	50	50	50	50	50	50	50	25	50	50	50	50	100	50	-

***Ciprofloxacin**

Table 6.5: *In-Vitro* Antifungal Activity Data in Zone Of Inhibition (Mm) and Percentage Inhibition by Disc Diffusion Method

Organism	Diameter of zone of inhibition (mm) / percentage of inhibition															
	DPN-1	**DPN-2**	**DPN-3**	**DPN-4**	**DPN-5**	**DPN-6**	**DPN-7**	**DPN-8**	**DPN-9**	**DPN-10**	**DPN-11**	**DPN-12**	**DPN-13**	**DPN-14**	**DPN-15**	**STD ***
Candida albicans	12 (39.96%)	12 (39.96%)	12 (39.96%)	12 (39.96%)	12 (39.96%)	12 (39.96%)	24 (79.92%)	24 (79.92%)	18 (59.94%)	14 (46.62%)	14 (46.62%)	16 (53.32%)	16 (53.32%)	10 (33.33%)	18 (59.94%)	30
Monascus purpureus	10 (33.33%)	10 (33.33%)	10 (33.33%)	12 (39.96%)	12 (39.96%)	12 (39.96%)	20 (66.67%)	20 (66.67%)	14 (46.62%)	12 (39.96%)	12 (39.96%)	12 (39.96%)	12 (39.96%)	10 (33.33%)	16 (53.32%)	30
Aspergillus niger	18 (59.94%)	16 (53.32%)	16 (53.32%)	18 (59.94%)	18 (59.94%)	18 (59.94%)	28 (93.24%)	28 (93.24%)	16 (53.32%)	24 (79.92%)	24 (79.92%)	22 (73.26%)	22 (73.26%)	20 (66.67%)	20 (66.67%)	30
Trichophyton rubrum	16 (53.32%)	14 (46.62%)	14 (46.62%)	18 (59.94%)	18 (59.94%)	18 (59.94%)	24 (79.92%)	24 (79.92%)	16 (53.32%)	22 (73.26%)	22 (73.26%)	22 (73.26%)	22 (73.26%)	22 (73.26%)	19 (63.27%)	30

***Clotrimazole**

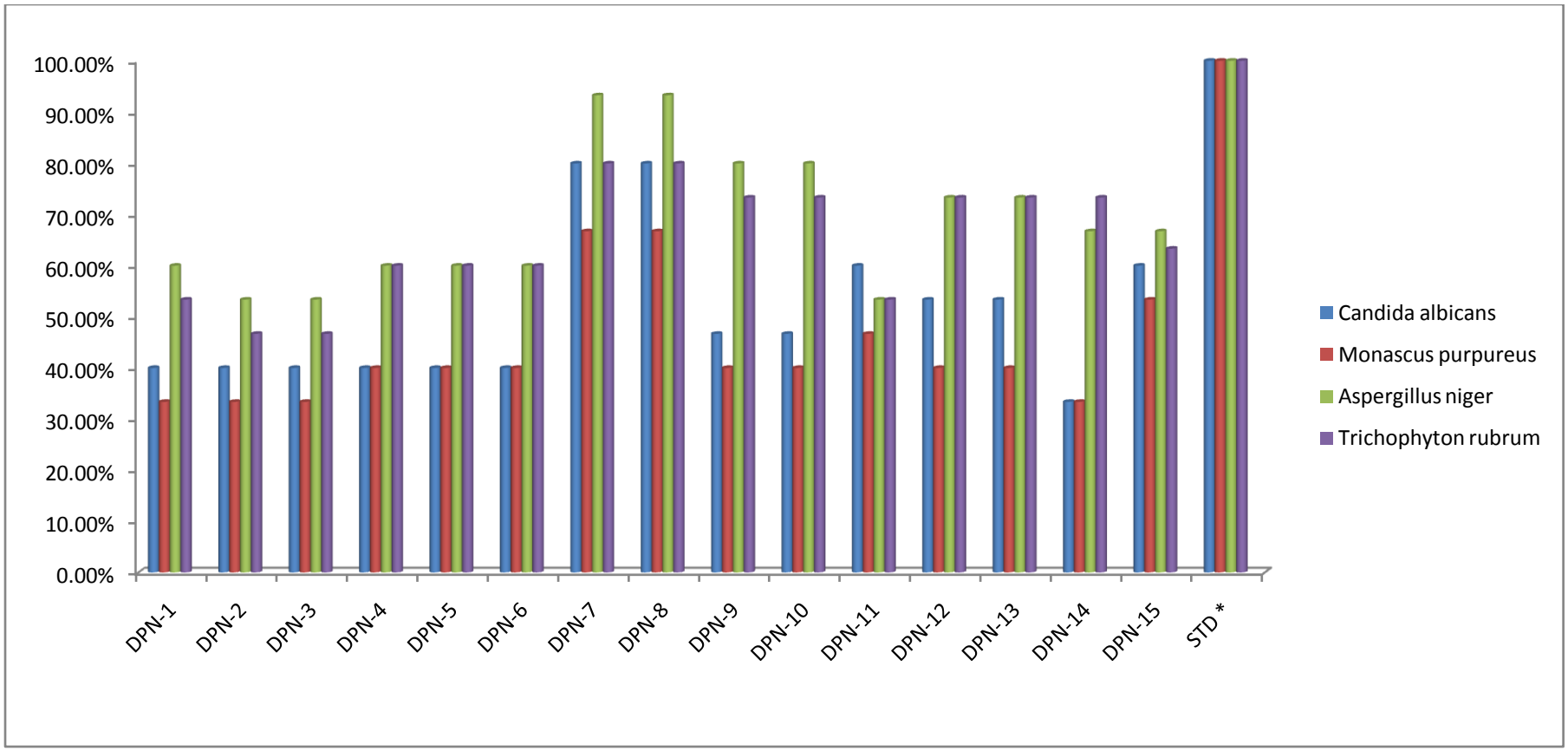


FIG 6.3: Graphical Representation of *In-Vitro* Anti-Fungal activity

Table 6.6: *In-Vitro* Anti-Fungal Activity Data in Minimum Inhibitory Concentration

Organism	**MIC µg\ml**														
	DPN-1	DPN-2	DPN-3	DPN-4	DPN-5	DPN-6	DPN-7	DPN-8	DPN-9	DPN-10	DPN-11	DPN-12	DPN-13	DPN-14	DPN-15
Candida albicans	50	50	50	50	50	50	12.5	12.5	25	50	50	25	25	50	25
Monascus purpureus	50	50	50	50	50	50	12.5	12.5	50	50	50	50	50	50	25
Aspergillus niger	25	50	50	25	25	25	6.25	6.25	50	12.5	12.5	12.5	12.5	25	25
Trichophyton rubrum	50	50	50	25	25	25	12.5	12.5	50	25	25	25	25	25	25

***Clotrimazole**

Table 6.7: *In vitro* anti Plasmodial activity of synthesized compounds against *plasmodium falciparum*

S.No	Compound Code	IC_{50} Values (%) Percentage Inhibition			
		20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)
1	DPN-03	28.46	36.15	41.53	43.84
2	DPN-06	66.92	72.30	80.0	84.61
3	DPN-08	74.61	80.0	82.30	87.69
4	DPN-10	59.23	66.92	74.61	84.61
5	DPN-11	72.30	74.61	80.0	87.69
6	DPN-12	59.23	66.92	74.61	80.0
7	DPN-13	36.15	41.53	43.84	49.23
8	DPN-14	41.53	46.15	57.69	61.53
9	STD*	72.30	80.15	87.69	95.32

*** Pyremethamine**

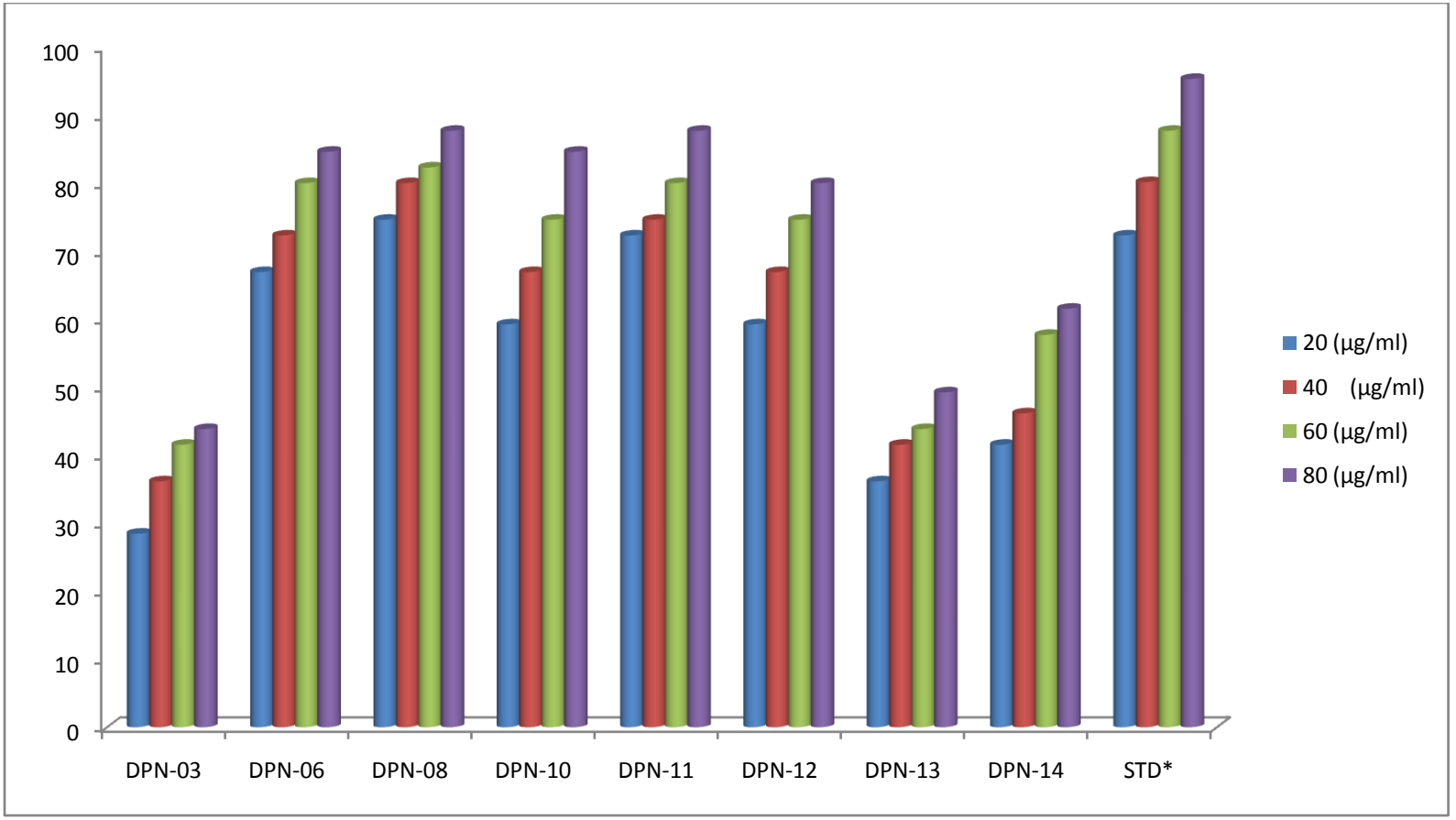


Fig 6.4: Graphical Representation of *In-Vitro* anti Plasmodial activity

TABLE 6.8: *IN-VITRO* ANTI-OXIDENT ACTIVITY BY DPPH ASSAY METHOD

Compound code	Concentration						EC_{50}
	10µg	20µg	40µg	60µg	80µg	100µg	
DPN-01	15.95	22.28	19.56	23.51	36.73	32.23	-
DPN-02	58.34	61.94	66.81	70.19	76.98	81.82	8.57
DPN-03	59.4	62.46	68.42	72.23	77.71	82.36	8.14
DPN-04	56.34	62.46	72.23	74.31	82.94	85.14	8.88
DPN-05	58.49	62.97	73.32	78.65	83.23	87.03	8.57
DPN-06	60.35	63.5	74.31	82.94	85.14	89.37	8.12
DPN-07	40.28	54.89	68.19	90.20	95.64	96.94	18.21
DPN-08	42.81	53.49	78.61	95.8	96.32	97.58	18.69
DPN-09	52.84	65.67	80.18	90.79	95.34	97.58	9.46
DPN-10	23.29	25.34	34.98	19.34	24.65	31.65	-
DPN-11	42.81	53.49	63.35	79.73	83.92	87.61	18.69
DPN-12	50.68	66.89	70.75	73.22	77.66	78.06	9.92
DPN-13	60.66	66.01	83.92	87.61	89.72	93.28	8.24
DPN-14	30.33	39.84	47.15	49.51	55.23	55.77	-
DPN-15	23.12	32.56	34.49	33.87	39.51	40.99	-
STD*	97.64	98.06	98.64	99.03	99.25	99.65	5.12

*Ascorbic acid

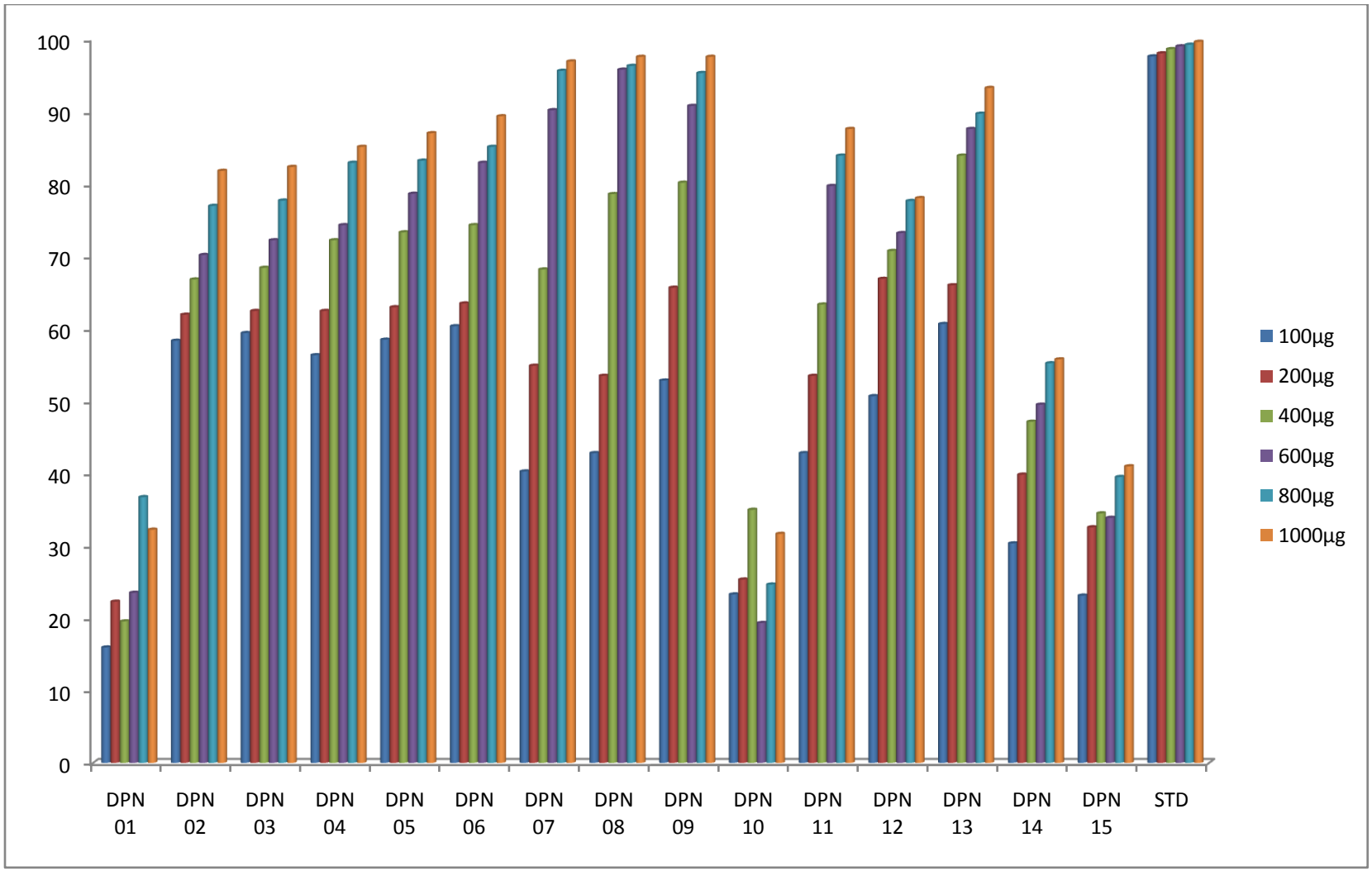


Fig 6.5: Graphical Representation of *In-Vitro* Anti-Oxidant Activity by DPPH Assay Method

Table 6.9: *IN-VITRO* ANTI-OXIDENT ACTIVITY BY ABTS ASSAY METHOD

Compound code	Concentration						EC_{50}
	10µg	20µg	40µg	60µg	80µg	100µg	
DPN-01	59.4	62.46	68.42	72.23	77.71	82.36	8.41
DPN-02	15.95	22.28	19.56	23.51	36.73	32.23	-
DPN-03	16.99	18.28	19.56	23.51	30.73	33.23	-
DPN-04	23.12	32.56	34.49	33.87	39.51	40.99	-
DPN-05	30.33	39.84	47.15	49.51	50.12	52.77	-
DPN-06	31.33	33.84	36.15	40.51	43.23	46.77	-
DPN-07	58.49	62.97	73.32	78.65	83.23	87.03	8.56
DPN-08	40.28	54.89	68.19	90.2	95.64	96.94	18.21
DPN-09	24.12	31.56	33.49	34.87	38.51	42.99	-
DPN-10	59.35	62.5	75.31	82.94	84.14	87.39	8.47
DPN-11	23.12	32.56	34.49	33.87	35.51	44.99	-
DPN-12	60.35	63.5	74.31	82.94	85.14	89.37	8.27
DPN-13	30.33	39.84	47.15	49.51	55.23	55.77	-
DPN-14	23.12	32.56	34.49	33.87	39.51	40.99	-
DPN-15	15.95	22.28	19.56	23.51	36.73	32.23	-
STD*	97.64	98.06	98.64	99.03	99.25	99.65	5.12

***Ascorbic acid**

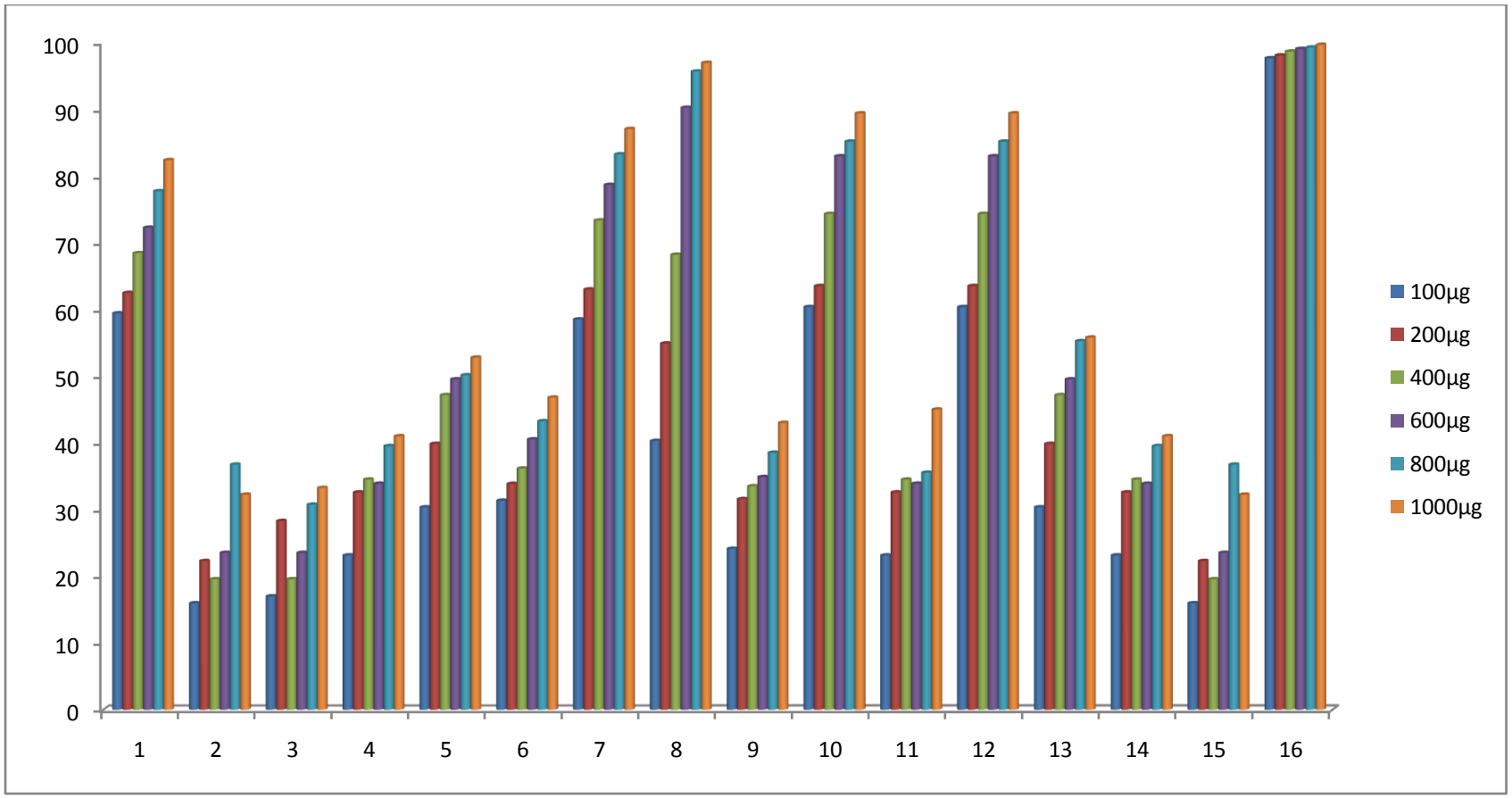
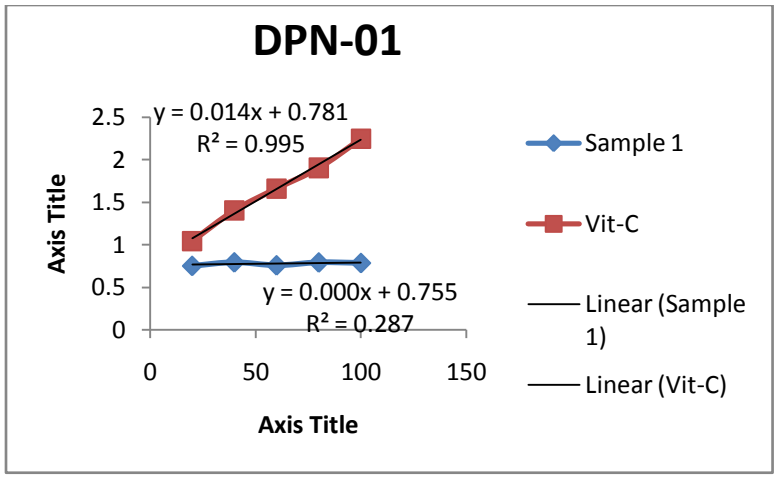


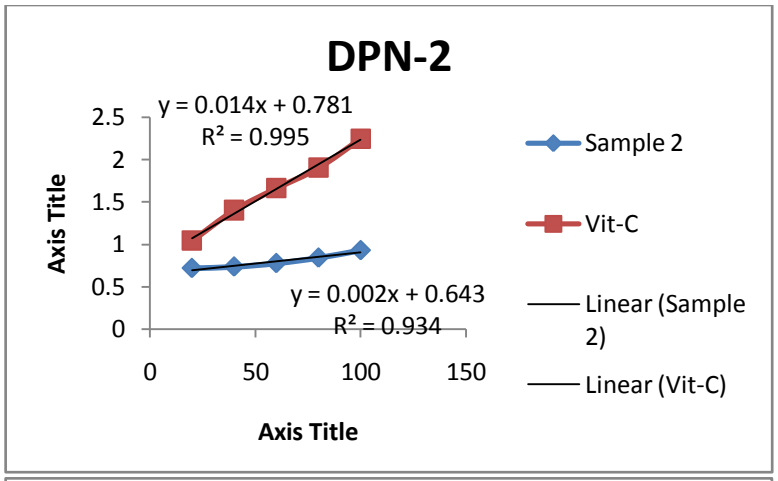
Fig6.5: Graphical Representation of *In-Vitro* Anti-Oxidant Activity by ABTS Assay Method

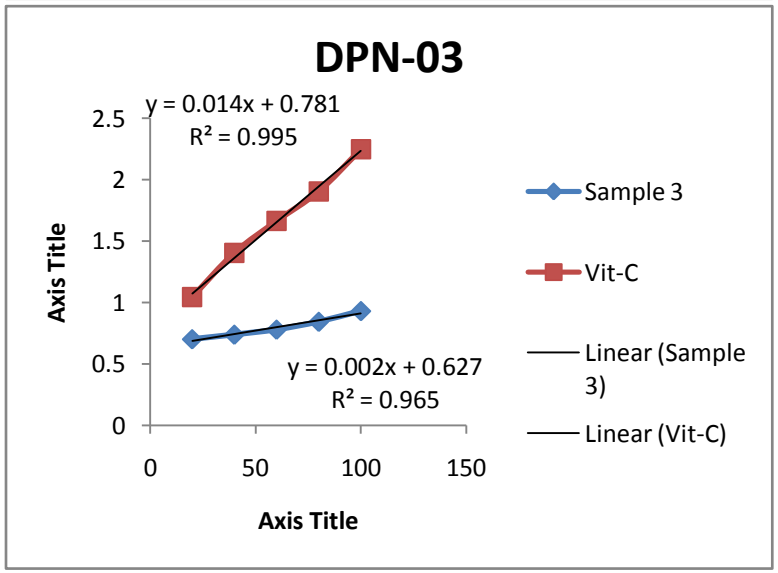
Table 6.9: *In-Vitro* Anti-Oxidant Activity By FRAP Assay Method

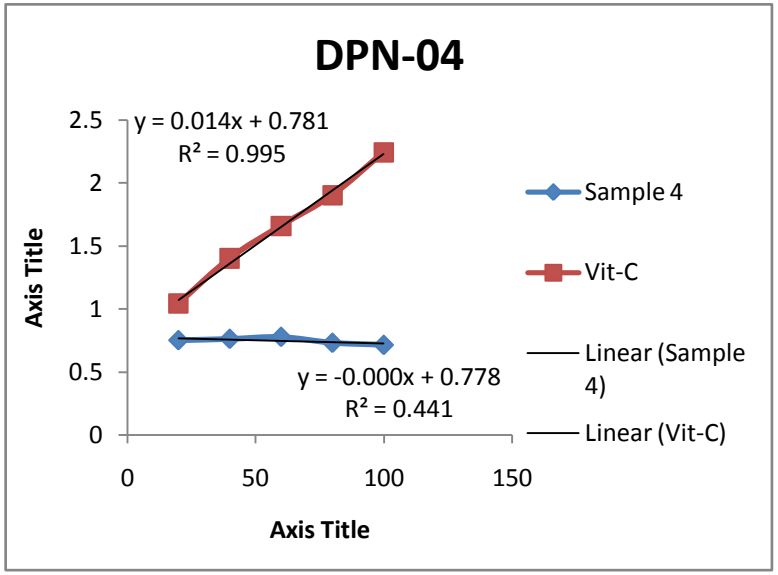
Compound code	Concentration					R^2
	20µg	40µg	60µg	80µg	100µg	
DPN-01	0.7513	0.7921	0.7576	0.7909	0.7848	-
DPN-02	0.7192	0.7364	0.7776	0.8419	0.9298	0.934
DPN-03	0.6992	0.7364	0.7776	0.8419	0.9298	0.965
DPN-04	0.7513	0.7621	0.7776	0.7309	0.7141	-
DPN-05	0.8513	0.8621	0.9776	0.9309	0.9141	-
DPN-06	0.6513	0.6621	0.7776	0.6309	0.8141	-
DPN-07	0.8222	0.6655	0.6974	0.8042	0.7431	-
DPN-08	0.7222	0.7655	0.7974	0.8042	0.7431	-
DPN-09	0.7992	0.8364	0.8776	0.9419	1.0298	-
DPN-10	0.8736	0.9605	0.9909	1.066	1.1081	0.98
DPN-11	1.3417	1.4412	1.6059	1.7892	1.8472	0.978
DPN-12	0.8736	0.9605	0.9909	1.066	1.1081	0.98
DPN-13	0.8857	0.7327	0.8584	0.7331	0.9572	-
DPN-14	0.9525	1.0179	1.1928	1.244	1.3869	0.975
DPN-15	0.6052	0.7547	0.8658	0.7386	0.7452	-
STD*	1.0447	1.403	1.6617	1.9034	2.2443	0.995

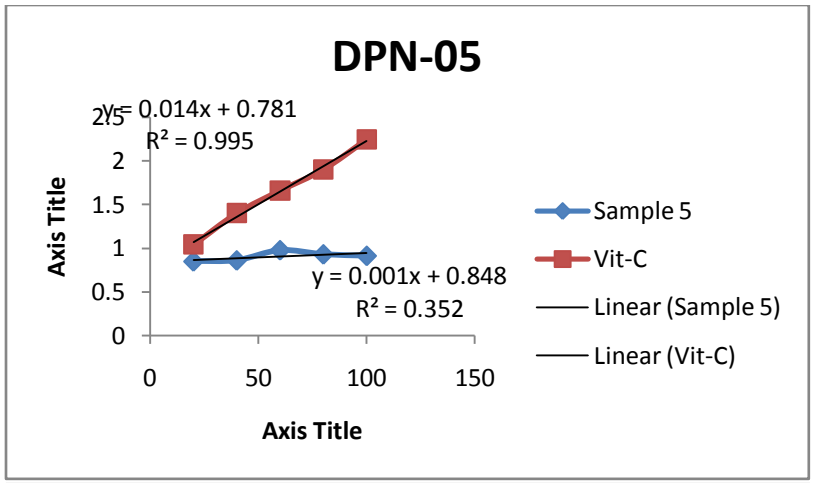
*FERROUS SULPHATE

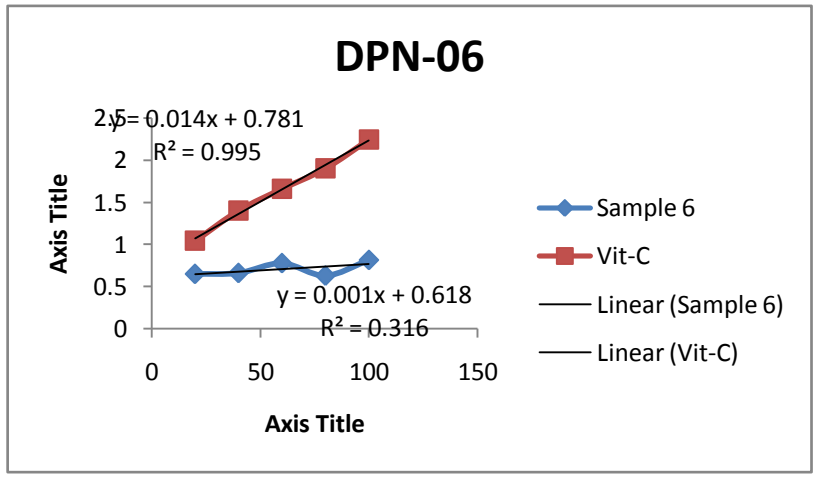

DPN-01
y = 0.014x + 0.781
R² = 0.995
y = 0.000x + 0.755
R² = 0.287
Axis Title
Axis Title
Sample 1
Vit-C
Linear (Sample 1)
Linear (Vit-C)

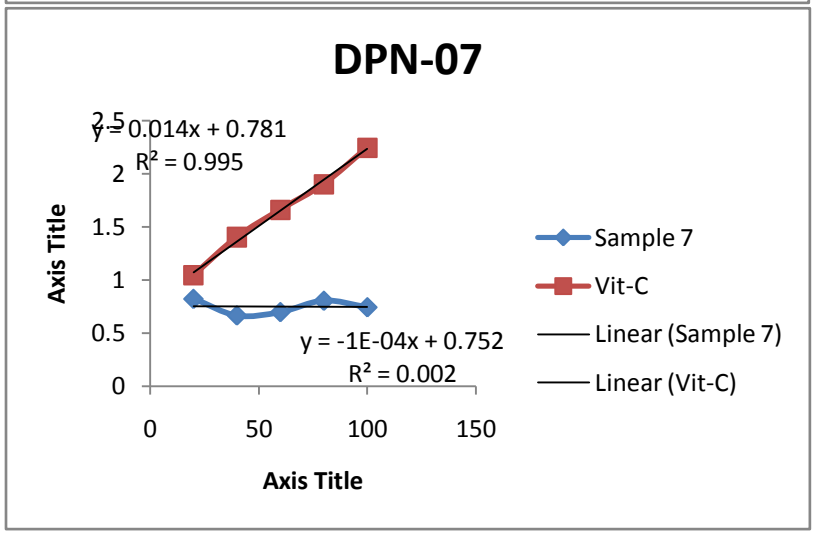

DPN-2
y = 0.014x + 0.781
R² = 0.995
y = 0.002x + 0.643
R² = 0.934
Axis Title
Axis Title
Sample 2
Vit-C
Linear (Sample 2)
Linear (Vit-C)

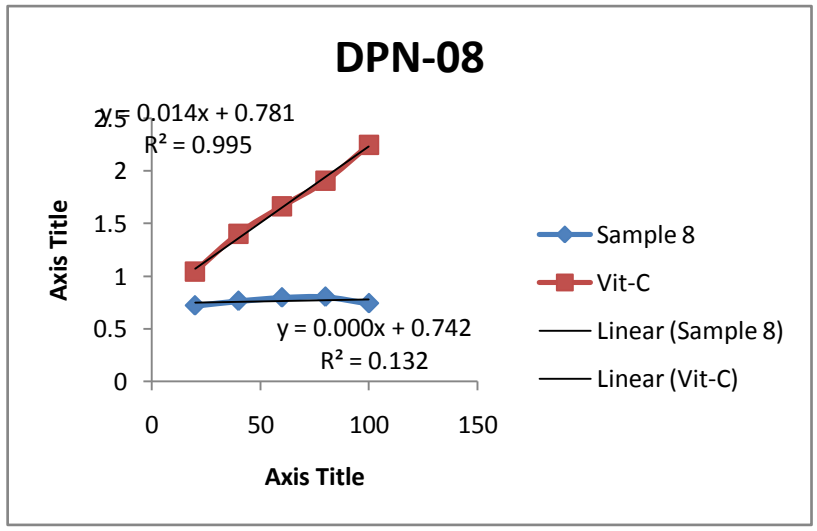

DPN-03
y = 0.014x + 0.781
R² = 0.995
y = 0.002x + 0.627
R² = 0.965
Axis Title
Axis Title
Sample 3
Vit-C
Linear (Sample 3)
Linear (Vit-C)

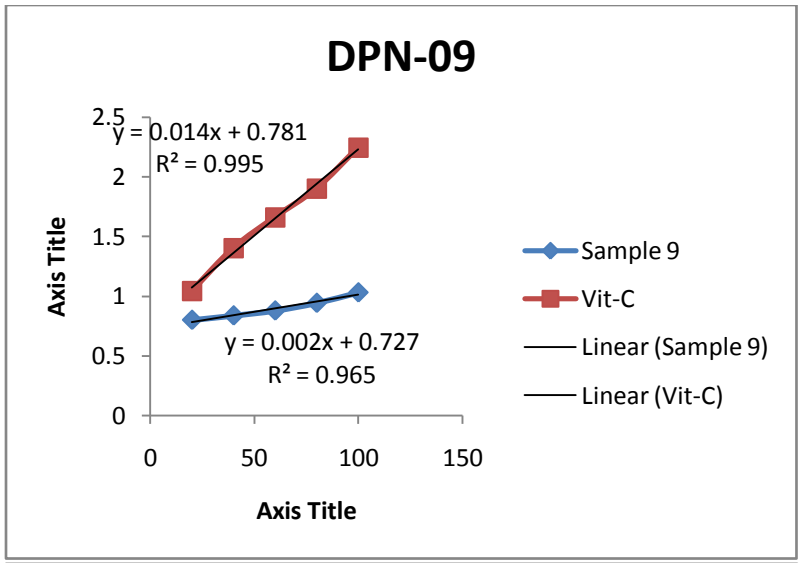

DPN-04
y = 0.014x + 0.781
R² = 0.995
y = -0.000x + 0.778
R² = 0.441
Axis Title
Axis Title
Sample 4
Vit-C
Linear (Sample 4)
Linear (Vit-C)

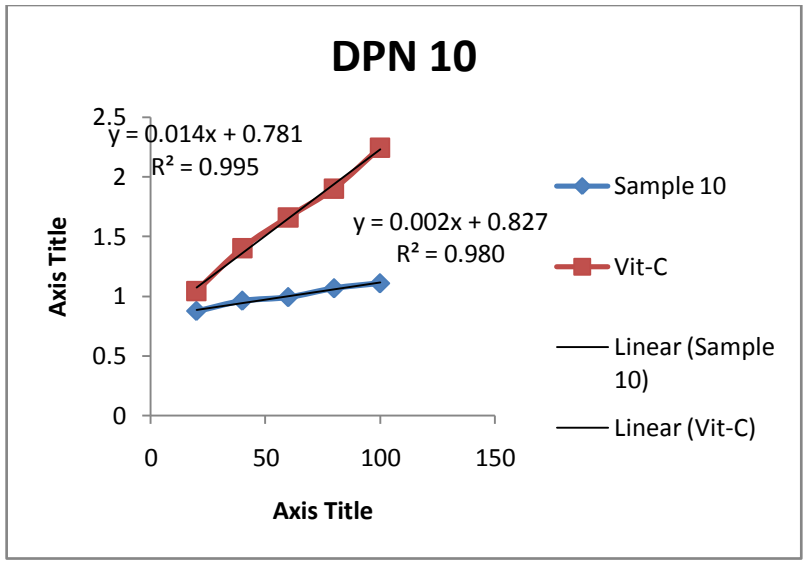

DPN-05
y = 0.014x + 0.781
R² = 0.995
y = 0.001x + 0.848
R² = 0.352
Axis Title
Axis Title
Sample 5
Vit-C
Linear (Sample 5)
Linear (Vit-C)

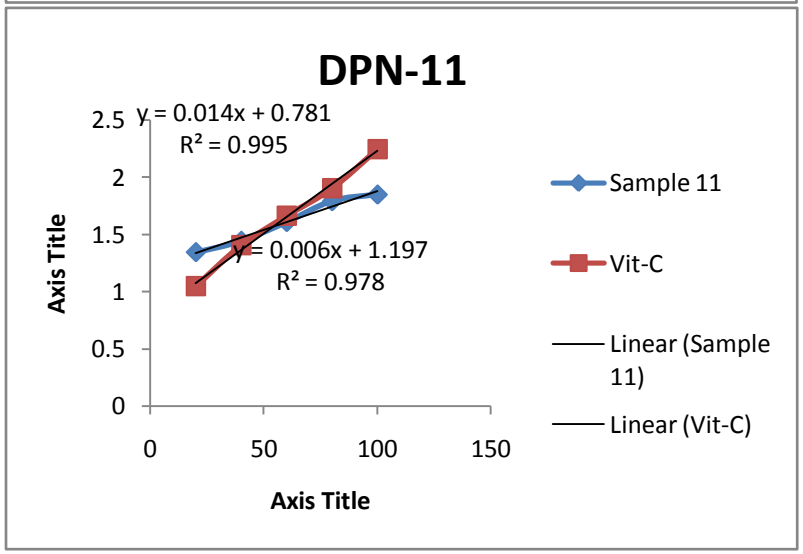

DPN-06
y = 0.014x + 0.781
R² = 0.995
y = 0.001x + 0.618
R² = 0.316
Axis Title
Axis Title
Sample 6
Vit-C
Linear (Sample 6)
Linear (Vit-C)

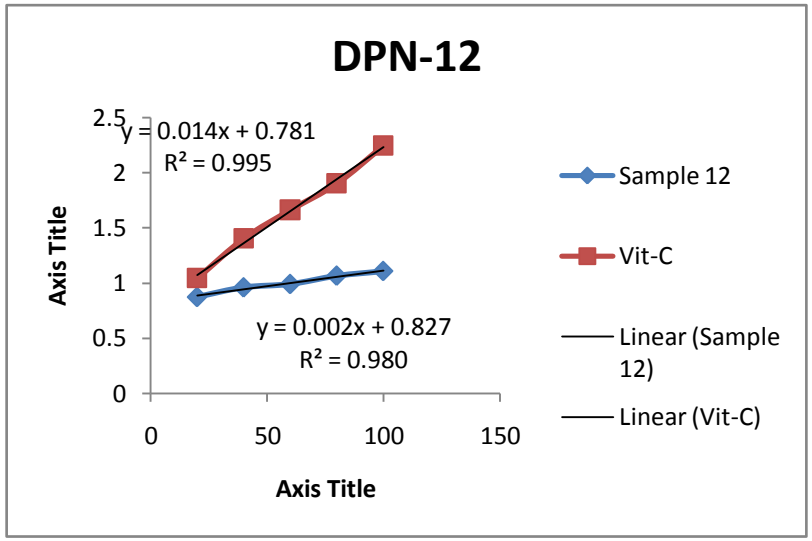

DPN-07
y = 0.014x + 0.781
R² = 0.995
y = -1E-04x + 0.752
R² = 0.002
Axis Title
Axis Title
Sample 7
Vit-C
Linear (Sample 7)
Linear (Vit-C)

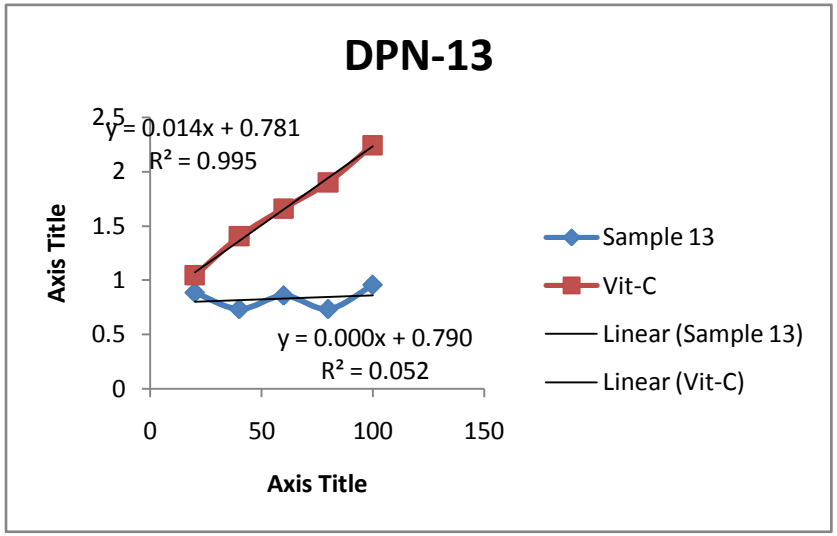

DPN-08
y = 0.014x + 0.781
R² = 0.995
y = 0.000x + 0.742
R² = 0.132
Axis Title
Axis Title
Sample 8
Vit-C
Linear (Sample 8)
Linear (Vit-C)

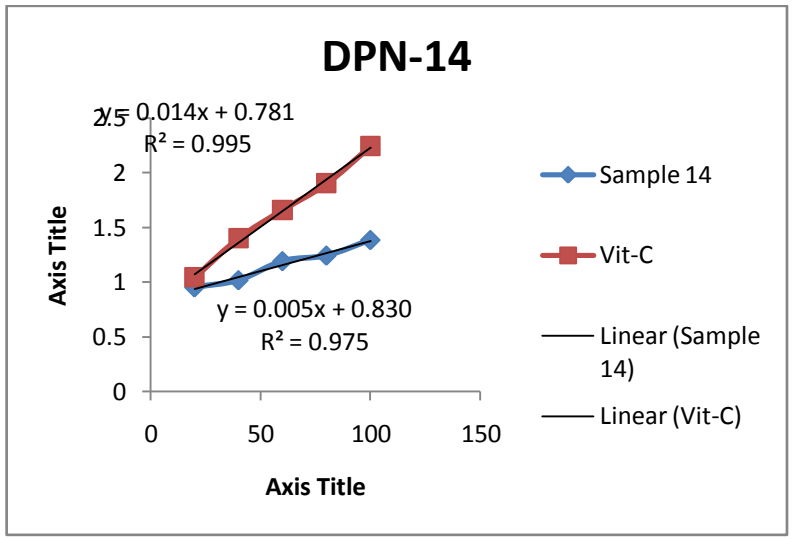

DPN-09
y = 0.014x + 0.781
R² = 0.995
y = 0.002x + 0.727
R² = 0.965
Axis Title
Axis Title
Sample 9
Vit-C
Linear (Sample 9)
Linear (Vit-C)

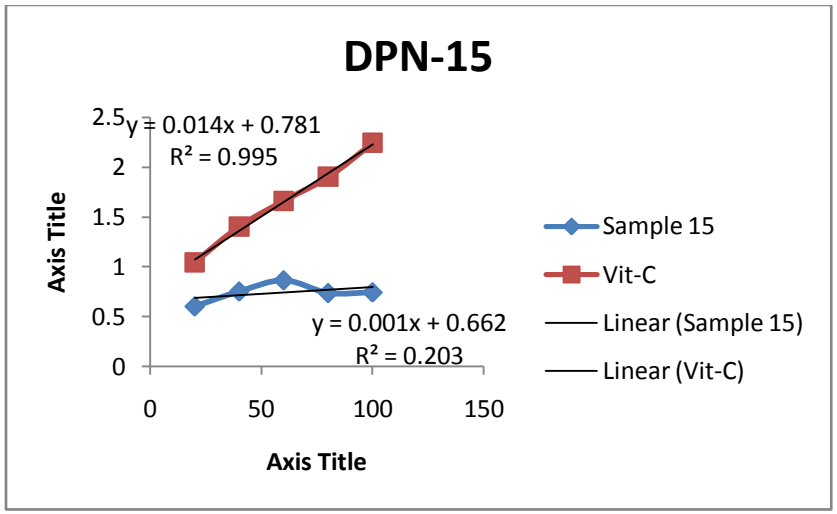

DPN 10
y = 0.014x + 0.781
R² = 0.995
y = 0.002x + 0.827
R² = 0.980
Axis Title
Axis Title
Sample 10
Vit-C
Linear (Sample 10)
Linear (Vit-C)


DPN-11
y = 0.014x + 0.781
R² = 0.995
y = 0.006x + 1.197
R² = 0.978
Axis Title
Axis Title
Sample 11
Vit-C
Linear (Sample 11)
Linear (Vit-C)


DPN-12
y = 0.014x + 0.781
R² = 0.995
y = 0.002x + 0.827
R² = 0.980
Axis Title
Axis Title
Sample 12
Vit-C
Linear (Sample 12)
Linear (Vit-C)


DPN-13
y = 0.014x + 0.781
R² = 0.995
y = 0.000x + 0.790
R² = 0.052
Axis Title
Axis Title
Sample 13
Vit-C
Linear (Sample 13)
Linear (Vit-C)


DPN-14
y = 0.014x + 0.781
R² = 0.995
y = 0.005x + 0.830
R² = 0.975
Axis Title
Axis Title
Sample 14
Vit-C
Linear (Sample 14)
Linear (Vit-C)


DPN-15
y = 0.014x + 0.781
R² = 0.995
y = 0.001x + 0.662
R² = 0.203
Axis Title
Axis Title
Sample 15
Vit-C
Linear (Sample 15)
Linear (Vit-C)

IN-*VITRO* ANTI-CANCER SCREENING

TABLE 6.11: $HepG_2$ cell line inhibition by the **compound DPN 03**

Conc (µM)	**% Cell Inhibition**	**Conc**	**0.1 µM**	**1 µM**	**10 µM**	**100 µM**
0.1	3.548896	ABS	0.402	0.388	0.365	0.355
1	8.359621		0.402	0.379	0.369	0.356
10	13.24921		0.419	0.395	0.366	0.361
100	15.45741	**Avg**	**0.407667**	**0.387333**	**0.366667**	**0.357333**

TABLE 6.12: $HepG_2$ cell line inhibition by the **compound DPN 06**

Conc (µM)	**% Cell Inhibition**	**Conc**	**0.1 µM**	**1 µM**	**10 µM**	**100 µM**
0.1	1.419558	ABS	0.4	0.409	0.395	0.383
1	3.864353		0.416	0.428	0.414	0.4
10	4.810726		0.434	0.382	0.398	0.383
100	8.044164	**Avg**	**0.416667**	**0.406333**	**0.402333**	**0.388667**

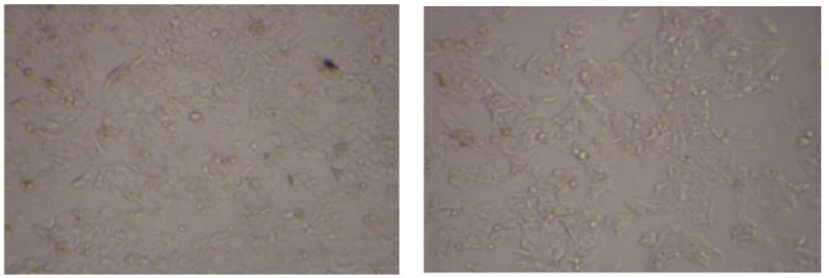
TABLE 6.13: $HepG_2$ cell line inhibition by the **compound DPN 08**

Conc (µM)	**% Cell Inhibition**	**Conc**	**0.1 µM**	**1 µM**	**10 µM**	**100 µM**
0.1	4.022082	ABS	0.41	0.384	0.364	0.342
1	10.41009		0.406	0.374	0.352	0.349
10	15.77287		0.401	0.378	0.352	0.349
100	17.98107	**Avg**	**0.405667**	**0.378667**	**0.356**	**0.346667**

TABLE 6.14: $HepG_2$ cell line inhibition by the **compound DPN 11**

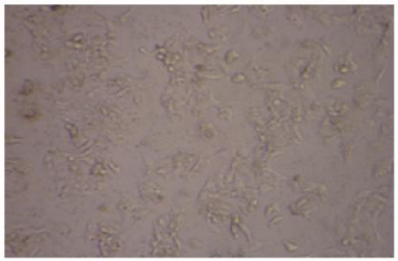
Conc (µM)	**% Cell Inhibition**	**Conc**	**0.1 µM**	**1 µM**	**10 µM**	**100 µM**
0.1	8.753943	ABS	0.38	0.365	0.339	0.329
1	14.27445		0.388	0.368	0.359	0.321
10	16.64038		0.389	0.354	0.359	0.321
100	23.42271	**Avg**	**0.385667**	**0.362333**	**0.352333**	**0.323667**

At concentration 100µm

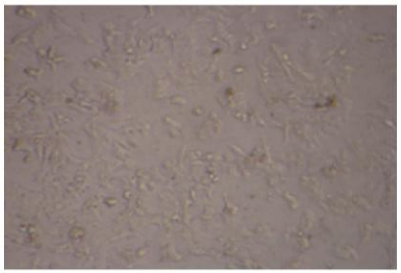


DPN 03

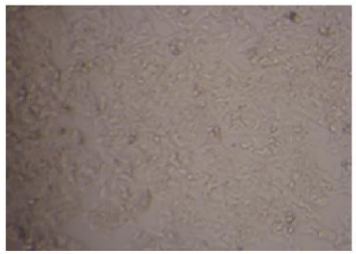
DPN 06



DPN 08



DPN 11



CONTROL

RESULTS AND DISCUSSION:

CHEMISTRY

In the present work 15 different Schiff bases were synthesized in 6 steps by conventional method.

Series I - Conventional method

In the present work 15 different Schiff bases were synthesized in 6 steps.

- Resacetophenone (**FD1)** was synthesized by heating the mixture of zinc chloride and glacial acetic acid with resorcinol at 120^0C, then temperature was raised 142^oC for 20 minutes.
- 4-acetylbenzene-1, 3-diyl bi (4-nitrobenzoate) (**FD 2**) was synthesized by refluxing resacetophenone with p-nitrobenzoyl chloride in presence of potassium carbonate and acetone for 6 hours.
- 3-hydroxy-4-[3-(4-nitrophenyl)-3-oxopropanoyl] phenyl 4-nitro benzoate (**FD 3**) was synthesized by stirring 4-acetylbenzene-1, 3-diyl bis (4-nitrobenzoate) with 10 % glacial acetic acid for 30 minutes.
- 7-hydroxy-2-(4-nitrophenyl)-4*H*-chromen-4-one (**FD 4**) was synthesized from 3-hydroxy-4-[3-(4-nitrophenyl)-3-oxopropanoyl] phenyl 4-nitro benzoate by refluxing them with glacial acetic acid and sodium acetate for 3 hours.
- 7-hydroxy-2-(4-aminophenyl)-4H-chromen-4-one (**FD 5**) was obtained from heating 7-hydroxy-2-(4-nitrophenyl)-4*H*-chromen-4-one with Tin Chloride and conc. HCl for 1 hour.
- Finally 7-hydroxy-2-(4-Substituted amino phenyl)-4*H*-chromen-4-one (**DP 1-15**) is synthesized from 7-hydroxy-2-(4-aminophenyl)-4H-chromen-4-one by refluxing with aryl carbonyl compounds in presence of ethanol for 3 hrs.

Yield of the derivatives were in the range of 52- 85%. Purity of all the newly compounds were checked by melting point and TLC Analysis and the structures were confirmed by UV, IR, NMR and MASS spectral data.

Series II – Microwave Irradiation method

In the present work 8 different Schiff bases were synthesized in 4 steps by microwave irradiation method.

- (E)-1-(2, 4-dihydroxyphenyl)-3-(4-nitrophenyl) prop-2-en-1-one(**FM 1**) was synthesized from resacetophenone with p-nitro benzaldehyde in the presence of sodium hydroxide with ethanol and heated on water bath resulting product was acetified with HCl.
- 7-hydroxy-2-(4-nitrophenyl)-4H-chromen-4-one (**FM 2**) was obtained by irradiating the compound **FM 1** with DMSO/I_2 in Micro oven.
- 7-hydroxy-2-(4-aminophenyl)-4H-chromen-4-one (**FM 3**) is obtained from heating 7-hydroxy-2-(4-nitrophenyl)-4*H*-chromen-4-one (**FM 2**) with Tin Chloride and conc. HCl for 1 hour.
- 7-hydroxy-2-(4-Substituted amino phenyl)-4*H*-chromen-4-one (**MWD 1-8**) was obtained by irradiating the compound **(FM 3)** with equal mole aromatic aldehydes & ketones for an optimized time.

Yield of the derivatives were in the range of 94- 98%. Purity of all the newly compounds were checked by melting point and TLC Analysis and the structures were confirmed by UV, IR spectral data.

DETERMINATION OF PHYSICOCHEMICAL PROPERTIES OF SYNTHESIZED COMPOUNDS

MELTING POINT ANALYSIS

Melting points of all the newly synthesized compounds were checked and uncorrected and the values were given in Table: 1&2.

THIN LAYER CHROMATOGRAPHY ANALYSIS

The reaction time for all the compounds were determined by running TLC and a single spot was obtained. R_f Values of all the newly synthesized compounds were checked and were tabulated in **Table: 1&2**.

SOLUBILITY

Solubility was checked for all the newly synthesized compounds and found that all the compounds were soluble in benzene and DMSO.

CHARACTERIZATION

INFRARED SPECTRAL ANALYSIS

- The lead compound **FD 5** showed the absorption band at 3116 cm^{-1} due to the presence of –OH at 7^{th} position of flavanoid ring and 1108 cm^{-1} due to ether linkage (C-O-C). A peak at 1540 cm^{-1} indicates the presence of primary amine and 1693 cm^{-1} due to keto group (C=O).

- All the Schiff base derivative compounds showed the characteristic peaks in the region 3200-3100 cm^{-1} for associated hydroxyl group, 840- 790 cm^{-1} for Ar, CH=CH stretching, 1640-1620 cm^{-1} , 1300-1050 cm^{-1} ether linkage (C-O-C stretch) and 1600-1400$cm^{-1}$for C=O stretching. Compound **DPN 7, DPN 8** containing NO_2 group showed absorption bands at 1550-1500, for the N=O stretching. The peak at 716-808 cm^{-1} could be assigned to C-Cl stretching in the compound **DPN 4, 5, 6**.

NUCLEAR MAGNETIC RESONANCE SPECTRAL ANALYSIS

- The structures of the new compounds were confirmed by ^{1}H-NMR spectra. All the synthesized compounds showed multiplets in the range δ 8.0-8.4 for the protons of aromatic ring. The spectrum of FD 05 revealed a singlet at δ 3.3 which may be assigned to NH_2 proton.
- The amino group of synthesized shown the broad singlet signals at 3.308 ppm, which were assigned to the N–H protons. Phenolic OH proton of compounds exhibited singlets at13.4 ppm. Ethenyl proton adjacent to C=O (=CH (C=O) exhibited singlets at 12.18 ppm

- Compounds exhibited Multiplet, signals at 7.42-7.79 ppm, and 8.29-8.42 ppm which was assigned to the benzopyran ring is present in compound. The phenyl

group of shown the doublet signals at 6.95-6.99 ppm, which were assigned to the phenyl group at second position of benzopyran ring.

MASS SPECTRAL ANALYSIS

- Electron impact mass spectral analysis was carried out on two randomly selected compounds **FD 05**, **DPN 06**, **DPN 08** and **DPN 11**. Mass spectrums of the compounds were in full agreement with their molecular weights.

BIOLOGICAL EVALUATION

***In-Vitro* Anti-Bacterial Studies**

All the newly synthesized compounds were screened for preliminary antibacterial activity against both gram positive and gram negative bacteria by Disc diffusion method and determination of Minimum inhibitory concentration (MIC) by serial dilution method.

All the newly synthesized compounds were screened for preliminary antibacterial activity at 100µg/disc concentration level by Disc diffusion. The results were compared with the standard drug (Ciprofloxacin) at 5µg/disc concentration level.

All the synthesized compounds were shown mild to moderate activity against all the screened bacterial strains. Among the tested gram positive bacterial strains, all the synthesized compounds were shown more activity against *corny bacterium* with the percentage of inhibition range from 50-79%.

Among the synthesized compounds, compounds **DPN 07**, **DPN 08** and **DPN 11** shown better antibacterial activity than the other synthesized compounds with the percentage of inhibition range from 50-79%. MIC results were shown that most of the synthesized compounds have MIC range from 50 &100 µg/ml concentration levels against tested gram positive bacterial strains.

The newly synthesized compounds **DPN 07, DPN 08** and **DPN 11** were shown MIC value was found to be 12.5 and 25 µg/ml concentration levels against *Micrococcus luteus, Staphylococcus aureus* and *Bacillus subtilis, Corny bacterium* respectively.

Among the tested gram negative bacterial strains, all the synthesized compounds at 100µg/disc concentration were shown more activity against *Rhodospirillum rubrum* and *Vibrio cholerae* the percentage of inhibition range from 50-78%.

Among the synthesized compounds, compounds **DPN 07**, **DPN 08** and **DPN 11** shown better antibacterial activity than the other synthesized compounds with the percentage of inhibition range from 67-78%. Most of the synthesized compounds show MIC value at 50 µg/ml concentration levels.

The newly synthesized compounds **DPN 07, DPN 08** and **DPN 11** were shown MIC value at 25 and 50 µg/ml concentration levels against *Escherichia coli ,Pseudomonas aeruginosa* and *Rhodospirillum rubrum, Vibrio cholerae respectively.*

All the compounds screened shown better activity against gram negative bacteria than gram positive bacteria.

***In-Vitro* Anti-Fungal Studies**

All the newly synthesized compounds were screened for preliminary antifungal activity by disc diffusion method and determination of Minimum inhibitory concentration (MIC) was done by serial dilution method.

All the newly synthesized compounds were screened for preliminary antifungal activity at 100µg/disc concentration level by Disc diffusion. The results were compared with the standard drug (Clotrimazole) at 5µg/disc concentration level.

All the synthesized compounds at 100 µg/disc concentrations were shown mild to moderate activity against all the screened fungal strains.

Among the tested fungal strains, all the synthesized compounds at 100µg/disc concentration were shown more activity against *Aspergillus niger* and *Trichophyton rubrum* with the percentage of inhibition range from 53-93%.

Among the synthesized compounds, compounds **DPN 07**, DPN **08** and **DPN 15** shown better antifungal activity than the other synthesized compounds with the percentage of inhibition value from 53-93%. Most of the synthesized compounds show MIC value at 25 and 50 µg/ml concentration levels.

The newly synthesized compounds **DPN 07, DPN 08** and **DPN 15** were shown MIC value at 6.25 and 12.5µg/ml concentration levels, *Aspergillus niger*, *Trichophyton rubrum* and *Candida albicans***,** *Monascus purpureus respectively*.

***In-Vitro* Anti-Malarial Activity**

The anti-malarial studies were carried out with the randomly selected synthesized Flavone derivatives in the concentration 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml in DMSO against *Plasmodium falciparum* using Candle jar method. Pyremethamine of same concentrations was used as standard. The anti-malarial activities of the compounds were evaluated by estimation of percentage of inhibition of parasethima at different concentration. It could be seen that these newly synthesized derivatives of flavone exhibit weak to good anti-malarial activity. Out of the compounds synthesized DPN 08 & DPN 11 was most potent which exhibit 87.69% inhibition at 80µg/ml concentration. Other derivatives (DPN 06, DPN 10 and DPN 12) also showed inhibition more than 80 % i.e. (84.61%, 84.61% and 80 %,) respectively.

***In-Vitro* Antioxidant Activities**

All newly synthesized compounds (**DPN 1-15**) were screened for *in-vitro* anti-oxidant activity by DPPH, FRAP and ABTS assay method at the concentration of 10µg/ml, 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml. DMSO was used as a solvent, ascorbic acid was used as a standard for DPPH and ABTS, ferrous sulphate was used as a standard for FRAP method.

DPPH ASSAY:

- Free radical scavenging capacity of flavone derivatives has been primarily attributed to the high reactivity of hydroxyl substituent's that participate in the following reaction.

$$\text{Flavone-OH} + R^{\bullet} \longrightarrow \text{Flavone-O}^{\bullet} + RH$$

- The result of *in-vitro* anti oxidant activity by DPPH method indicates that compounds **DPN-02, DPN-03, DPN-04, DPN-05, DPN-06, DPN-07, DPN-08,**

DPN-09, DPN-11, DPN-12 and **DPN-13** have significant anti oxidant activity with EC_{50} value 8.57,8.14, 8.88, 8.57, 8.12, 18.21, 18.69, 9.46, 18.69, 9.92 and 8.24 respectively.

- As in the case of flavones, the electron-withdrawing or -donating character of the substituent's does not play an important role for anti oxidant activity

ABTS ASSAY:

- The result of *in-vitro* anti oxidant activity by ABTS method shows that among the screened compounds, compound **DPN-01, DPN-07, DPN-08, DPN-10** and **DPN-12** have significant anti oxidant activity with EC_{50} value 8.41, 8.56, 18.21, 8.47and 8.27 respectively. The other synthesized compounds showed mild anti oxidant activity. The ABTS *in-vitro* anti oxidant assay method result review that the Schiff base synthesized from aldehydes derivatives shows potent anti oxidant activity when compared with ketones derivatives. The percentage inhibition values were given in the **table 6.9**

FRAP ASSAY:

- The result of *in-vitro* anti oxidant activity by FRAP method indicates that among the screened compounds, compound **DPN-02, DPN-03, DPN-10, DPN-11, DPN-12** and **DPN-1** have significant anti oxidant activity with R^2 value of 0.934, 0.965, 0.98, 0.978, 0.98, 0.975 near to the standard ferrous sulphate value of 0.995 indicating that compounds containing substitution of electron donating group attach at the ring imparted significant anti oxidant property to the resulting Schiff base containing flavone derivatives. The percentage inhibition values were given in the table showed good anti oxidant activity. The R^2 values were given in the **table 6.10.**

In all the above screened method among the synthesized compounds, Compound **DPN 12** bearing substituted Hydroxy and methoxy group showed potent anti oxidant activity when compared with the standard.

IN-VITRO ANTI-CANCER ACTIVITY

- Four of the newly synthesized compounds **(DPN 3, DPN 06, DPN 08** and **DPN 11)** were randomly selected and screened for *in vitro* anti-cancer activity against human hepatic carcinoma cell line ($HepG_2$) by MTT assay in DMSO.
- *In-vitro* anti-cancer results indicate that they are very weekly cytotoxic effect against human hepatic carcinoma cell line ($HepG_2$).

DOCKING

- Docking studies of compounds (DPN 02, DPN 06, DPN08, DPN 10, DPN 11, DPN 12, DPN13, DPN 14) using *pf*-DHFR-TS- Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PDB: 1J3I) protein. In which the compounds DPN 06, DPN08, DPN 10, DPN 11, DPN 12, DPN 14 having docking score -6.07,-9.81,-7.3,-6.9,-6.37, -3.5.

SUMMARY AND CONCLUSION:

- 23 compounds were synthesized by two different methods.
- 15 compounds of Schiff base derivatives by conventional method and 8 compounds of Schiff base derivatives by microwave irradiation method was synthesized, purified and characterized by spectral data's.
- The homogeneity of the synthesized compounds was monitored by ascending thin layer chromatography.
- Both in the conventional and microwave irradiation methods the same molecule is obtained in which the former method took 8 folds of time than the later method. The percentage yield obtained was comparatively higher (above 92%) in microwave method than the conventional method.

ANTI-MICROBIAL ACTIVITY

- Compounds **DPN 1-15** (15 compounds) were screened on various bacterial and fungal strains.

Compounds **DPN 07, DPN 08** and **DPN 11** were found to be more potent anti-bacterial and **DPN 07, DPN 08 and DPN 15** antifungal activity than standard ciprofloxacin and Clotrimazole.

ANTI- MALARIAL ACTIVITY:

- Compounds **DPN 03, DPN 06, DPN 08, DPN 10, DPN 11, DPN 12, DPN 13** and **DPN 14** (8 compounds) were screened against *P.falciparum.*
- Compounds **DPN 06, DPN 08, DPN 10, DPN 11 and DPN 12** showed potent activity when compared with standard pyremethamine.
- It was evident from the docking analysis as well as antimalarial evaluation, title molecules were disclosed admirable scoring and significant bio-activity. It was concluded from docking studies that our molecules were deeply engulfed into the inner groove of *pf-DHFR-TS active site* by making stable ligand receptor posses.

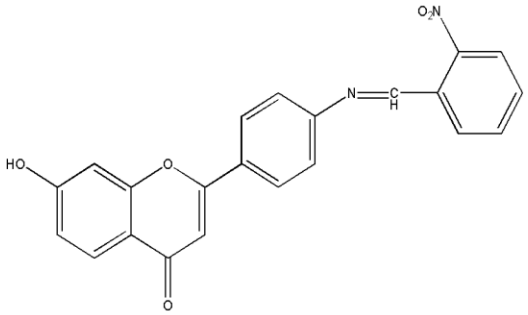
ANTI-OXIDANT ACTIVITY

✓ The DPPH, FRAP and ABTS *in-vitro* anti oxidant methods result of the synthesized compound review that the electron withdrawing group attached at ring Compounds (**DPN 12)** shows potent activity.

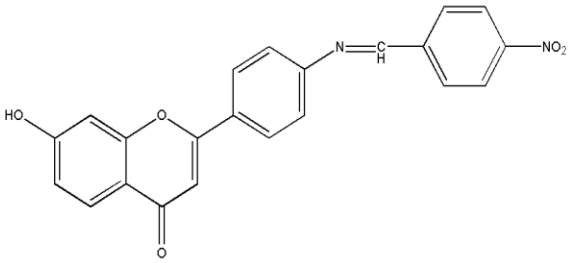
ANTI-CANCER ACTIVITY:

None of the compound showed potent activity against $HepG_2$ Cell.

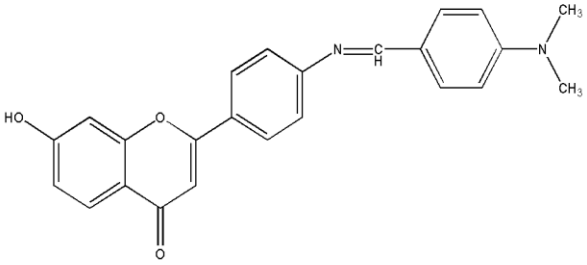
Compound **DPN 07, DPN 08, DPN 11** and **DPN12** were found to more potent compound, so this can be used as lead for further studies.



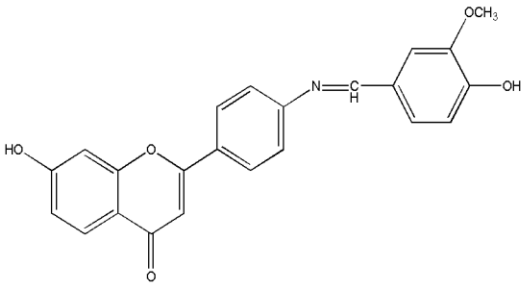
COMPOUND **DPN 07**



COMPOUND **DPN 8**



COMPOUND **DPN 11**



COMPOUND **DPN 12**

REFERENCE

1. Cermak R, Wolffram S, The potential of mongonoids to influence drug metabolism and pharmacokinetics by local gastrointestinal mechanisms, *Curr. Drug Metab* **2006**; 7 (7); 729–44.
2. Cermak R, Effect of dietary flavonoids on pathways involved in drug metabolism, *Drug Metab Toxicol* **2008;** 4 (1): 17–35.
3. Si D, Wang Y, Zhou YH, et al, Mechanism of CYP2C9 inhibition by flavones and flavonols. *Drug Metab.Dispos* **2009**; 37 (3): 629–34.
4. Mahal H.S, Venkataraman.K, Synthetical experiments in the chromone group XIV. Action of sodamide on 1-acyloxy-2-acetonaphthones, *J. Chem. Soc* **1934** ,1767–1769
5. Allan, J Robinson, R. Organic Synthesis *J. Chem. Soc* **1924**, 125, 2192.
6. Auwers et al. Synthesis of flavone, *Ber* **1916**, 49, 809.
7. Algar, J.; Flynn, J. P, Proc. Roy. *Irish Acad* **1934**, 42B, 1.
8. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book") (**1997**). Online corrected version: (**2006**) "schiffbase".
9. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book") (**1997**). Online corrected version: (**2006**) "AZOMETHINS".
10. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book") (**1997**). Online corrected version: (**2006**) "anils".
11. Sayed El., Ashry H. El. and Kassem, A. A., Account of microwave irradiation for accelerating organic reactions. *Arkivoc*, **2006**, ix,1
12. Ferri D., Diezi S., Maciejewski M. and Baiker A., Alumina-catalysed degradation of ethyl pyruvate during enantioselective hydrogenation over Pt/alumina and its inhibition by acetic acid. *Applied Catalysis A*, **2006,** 297, 6165.
13. Gareth W. V., Colin L., Raston, J. and Scotta, L., Recent advances in solvent less organic reactions: towards benign synthesis with remarkable versatility. *Chem. Commun*, **2001**, 2159–2169**.**

14. Hessen TM, Kaye D. Principles of selection and use of antibacterial agents: In vitro activity and pharmacology. *Infect Dis Clin North Am* **2000**; 14: 265–279.
15. J. W. Costerton, J. M. Ingraham, and K.-J. Cheng, Ultrastructure of Butyrivibrio fibrisolvens: a gram-positive bacterium, *Bacteriol. Rev*., **1974**; 38, 87.
16. S. M. Hammond, P. A. Lambert, and A. N.Rycroft, The Bacterial Cell Surface, *Croom Helm*, London, **1984**.
17. G. Jones and M. Midgley, *FEMS Microbiol. Let*. **1985**; 28, 355.
18. M. Midgley, Nagwa S. Iscandar and E.A. Dawes, The interaction of phosphonium ions with Acinetobacter calcoaceticus: evidence for the operation of an efflux system, *Biochimica* **1941**; 28, 209.
19. Paola Vicini, Athina Geronikaki, Matteo Incerti, Franca Zani, John Deardenc and Mark Hewittc, 2-Heteroarylimino-5-benzylidene-4-thiazolidinones analogues of 2-thiazolylimino-5-benzylidene-4-thiazolidinones with antimicrobial activity: Synthesis and structure–activity relationship, *Bioorganic & Medicinal Chemistry*, **2008**; 16, 3714–3724.
20. Mendis K, Sina BJ, Marchesini P, Carter R: The neglected burden of Plasmodium vivax malaria. *Am J Trop Med Hyg* **2001**, 64(1-2 Suppl**):**97–106.
21. Escalante A, Ayala F. The origin of malignant malaria, *Proc Natl Acad Sci USA* **1994**, 91 (24), 11373–7.
22. Garnham, PCC. Malaria parasites and other haemosporidia. *Oxford: Blackwell Scientific Publications*, **1966**,201.
23. Talman A, Domarle O, McKenzie F, Ariey F, Robert V. Gametocytogenesis : the puberty of *Plasmodium falciparum., Malar J* **,2004,** 3-24.
24. Marcucci C, Madjdpour C, Spahn D , Allogeneic blood transfusions: benefit, risks and clinical indications in countries with a low or high human development index *Br. Med Bull* **2004**, 70, 15–28
25. WebMD, Malaria symptoms Last Updated: May 2007,16-20
26. Arhurst DC, Williams JE, Laboratory diagnosis of malaria, *J Clin Pathol* 49 (7) **1996**, 533–38.
27. Aruoma, O.L., Free radicals, oxidative stress and antioxidants in human health and disease *J Am Oil Chem*. **1998**; 75; 199–212.

28. Uchida, K, Role of reactive aldehyde in cardiovascular diseases. *Free Radical Biol Med* 2000; 28; 1685–1696
29. Shahidi, F., Janitha, P.K. and Wanasundara, P.D., Phenolic antioxidants. *Crit Rev Food Sci Nutr* **1992**; 32; 67–103.
30. Gerber, M., Boutron-Ruault, M.C., Hercberg, S, et al, Food and Cancer: state of the art about the protective effect of fruits and vegetables. *Bull Cancer* **2002**; 89; 293–312.
31. Di Matteo, V. and Esposito, E, Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. *Curr Drug Targets CNS Neurol Disord* **2003**; 2; 95–10
32. Sreejayan, N. and Rao, M., Free radical scavenging activity of Curcuminoids. *Drug Res* **1996**; 46, 169–171.
33. Knekt, P., Jarvinen, R., Reunanen, A. and Maatela, J., Flavonoid intake and coronary mortality in Finland: A cohort study. *Brit Med J*, **1996**; 312, 478–481.
34. Sies, H., Strategies of antioxidant defense. *Eur J Biochem*, **1993**; 215, 213–219.
35. Grice, H.P., Enhanced tumour development by butylated hydroxyanisole (BHA) from the prospective of effect on forestomach and oesophageal squamous epithelium. *Food Chem Toxicol* **1988**; 26, 717–723.
36. Chung, K.T., Wong, T.Y., Huang, Y.W. and Lin, Y., Tannins and human health: A review. *Crit Rev Food Sci Nutr* **1998**; 38, 421–464
37. Helmut sies, "Oxidative stress: Oxidants and antioxidants", *Experimental Physiology* **1997**; 82, 291-295
38. Uchida, Role of Reactive Aldehyde in Cardiovascular Diseases *Free Rad. Biol. Med*., **2000**; 28, 1685-1696.
39. Kinsella, E. Frankel, B. German, J. Kanner J, Possible mechanisms for the protective role of antioxidants in wine and plant foods. *J. Food Technol* **1993**; 47, 85-89.
40. Singh N, Rajini P, Free radical scavenging activity of an aqueous extract of potato peel *J.Food Chem* **2004**; 85, 611-616.
41. Prior X, Schaichs K, *J. Agric. Food Chem* **2005**; 5; 4290-4302.

42. Burger's medicinal chemistry and drug discovery, Sixth Edition, Chemotherapeutic Agents, *Wiley inter science A John Wiley and Sons, Inc., Publication*., **2003**; 5, 281-293
43. Anand P, Kunnumakkara AB, Kunnumakara AB, et al. Cancer is a preventable disease that requires major lifestyle changes. *Pharm. Res.***2008**; 25 (9): 2097–2116
44. Gerig, J.T. In NMR in Drug Design; *CRC Press*: **199**4; 15.
45. Perun, T.J.; and Propst, C.L. In Computer Aided Drug Design, Method & Applications; *Marcel Dekker Inc. New York*: **1989**; 12.
46. Holtje, H.D. and Folkers, G. Molecular Modeling, Basic Principles and Applications; *VCH publication*: **1997**; 5, 17-37.
47. Andres Alonso, Joanna Sasin, Nunzio Bottini, Ilan Friedberg, Iddo Friedberg, Andrei Osterman, Adam Godzik, Tony Hunter, Jack Dixon, and Tomas Mustelin; Protein Tyrosine Phosphatases in the Human Genome: Rewiev article published in Cell, 2004; 117, 699–711.
48. Ehsan Ullah Mughal, Muhammad Ayaz, Zakir Hussain, Aurangzeb Hasan et al, Synthesis and antibacterial activity of substituted flavones,4-thioflavones and 4-iminoflavones *J. Bioorg. Med. Chem*. **2006,** 14, 4704–4711.
49. K. Suresh Babu, T. Hari Babu, P. V. Srinivas, K. Hara Kishore, U. S. N. Murthy and J. Madhusudana Rao et al, Synthesis and biological evaluation of novel C (7) modified chrysin analogues as antibacterial agent *J.Bioorg. Med. Chem. Lett.* **2006**, 16, 221–224
50. David W. Boykina, Sulhiye Yıldız and Hakan Goker et al, Synthesis and potent antimicrobial activity of some novel 2-phenyl or methyl-4H-1-benzopyran-4-ones carrying amidinobenzimidazoles *J.Bioorg. Med. Chem.***2005**, 13 1707–1714.
51. Sherif B. Abdel Ghani, Louise Weaver, Zidan H. Zidan, Hussein M. Ali, C. William Keevilb and Richard C. D. Brown et al, Microwave-assisted synthesis and antimicrobial activities of flavonoid derivatives *J. Bioorg. Med. Chem. Lett.* **2008,** 18, 518–522.

52. Ana Bertucci, Alvaro Vazquez, Gustavo Seoane and Gabriel Sagrera et al, Synthesis and antifungal activities of natural and synthetic biflavonoids *J. Bioorg. Med. Chem.* **2011**, 19, 3060–3073.
53. Enrico Mincione , Gianfranco Provenzano , Giancarlo Fabrizi Sabrina Tempest , Marcella Pasqualetti and Roberta Bernini et al, Obtaining new flavanones exhibiting antifungal activities by methyltrioxorhenium-catalyzed epoxidation–methanolysis of flavones *J.Tetrahedron* **2008,** 64, 7561–7566.
54. Mehdi Labaied, Francois Frappier, Philippe Rasoanaivo, Philippe Grellier and Guy Lewin and Gwenola Auffret et al, Synthesis and antimalarial evaluation of a series of piperazinyl flavones *J.Bioorg. Med. Chem. Lett.* **2007,** 17, 959–963.
55. Soon Sung Lim, Hye-Sook Kim, and Dong-Ung Lee et al, *In-vitro* Antimalarial Activity of Flavonoids and Chalcones *J. Bull. Korean Chem. Soc.* **2007**, 28, 12-16.
56. Soizic Prado, Erik L. Regalado, Frederick A. Valeriote, Joseph Mediad, Judith Mendiola, Olivier P. Thomas and Risoleta Orte et al, Furfuran lignans and a flavone from Artemisia gorgonum Webb and their *in vitro* activity against Plasmodium falciparum *Journal of Ethnopharmacology* **2011,** 138, 637– 640.
57. Chikara Ichino, Aki Ishiyama, Hitomi Sekiguchi, Miyuki Namatam, Nijsiri Ruangrungsi, Ekarin Saifah, Hiroaki Kiyohara, Kazuhiko Otoguro, Satoshi Omura, Haruki Yamada and Pranorm Khaomek et al, *In vitro* antimalarial activity of prenylated flavonoids from Erythrina fusca *J Nat Med*, **2008,** 62,217–220.
58. Ping Guam Jargon Song, Wei Cao and Jiang Biand, W. Chen et al. The ortho hydroxy-amino group: Another choice for synthesizing novel antioxidants *J.Bioorg. Med. Chem. Let.* **2006**,16,3582–3585
59. Seung Hwan Kim, Ch. Naveen Kumar, Hyoung JA Kim et al, Glucose-containing flavones—their synthesis and antioxidant and neuroprotective activities *J. Bioorg. Med. Chem. Lett* **2009,** 19, 6009–6013.
60. Ana Gomes, Ondrej Neuwirth, Marisa Freitas et.al, 2, 3-Diarylxanthones as strong scavengers of reactive oxygen and nitrogen species: A structure–activity relationship study., *J.Bioorg. Med. Chem.* **2009,** 17, 7218–7226.

61. Jingxu Gong, Kexin Huang, Feng Wang et al, Preparation of two sets of 5,6,7-trioxygenated dihydroflavonol derivatives as free radical scavengers and neuronal cell protectors to oxidative damage *J. Bioorg. Med. Chem.* **2009,** 17, 3414–3425
62. Daniela Ribeiro, Ana Gomes, Artur M. S. Silva, Jose A. S. Cavaleiro , Eduarda Fernandes and Clementina M. M. Santos Marisa Freitas et al, Synthesis and antioxidant properties of new chromone derivatives *J. Bioorg. Med. Chem.* **2010,** 18, 6776–6784.
63. Tadashi Kataoka, Shin-ichi Watanabe, Eiji Mori et.al, Synthesis and structure–activity relationships of thioflavone derivatives as specific inhibitors of the ERK-MAP kinase signaling pathway *J. Bioorg. Med. Chem.* **2004**, 12, 2397–2407.
64. Viviana C. Blank, Cecilia Poli, Mariel Marder et.al, antitumor activity of some natural flavonoids and synthetic derivatives on various human and murine cancer cell lines *J.Bioorg. Med. Chem. Lett* **2004,** 14, 133–136.
65. Tai-Chi Wang, I.-Li Chen, Pei-Jung et.al, Synthesis, antiproliferative, and antiplatelet activities of oximeand methyloxime-containing flavone and isoflavone derivatives *J.Bioorg. Med. Chem.*, 2005**,** 13, 6045–6053.
66. Hadi Poerwono, Shigeru Sasaki, Yoshiyuki Hattori et al, Synthesis, antiproliferative, and antiplatelet activities of oximeand methyloxime-containing flavone and isoflavone derivatives*J.Bioorg. Med. Chem. Lett*. **2010**, 20, 2086–2089.
67. Christelle Pouget, Fabienne Lauthier, Alain Simon et al, Flavonoids: Structural Requirements for Antiproliferative Activity on Breast Cancer Cells *J.Bioorg. Med. Chem* **2001,** 11, 3095-3097
68. Aijun Dong, Chunmei Gao, Chunyan Tan, Zhenhua Xie, Xuyu Zu Long Qu, Yuyang Jiang and Huachen Liu et al, New synthetic flavone derivatives induce apoptosis of hepatocarcinoma cells, *J.Bioorg. Med. Chem.,***2010** , 18, 6322–6328
69. Ganesan Pushpavalli, Panneerselvam Kalaiarasi, Chinnadurai Veeramani, Kodukkur Viswanathan Pugalendi et al, Effect of chrysin on hepatoprotective

and antioxidant status in D-galactosamine-induced hepatitis in rats *European Journal of Pharmacology* **2010,** 631, 36–41.

70. Biradar SM, Mahadevan KM, Habbu PV and Sharstry RA et al, Isolation and Characterization of Secondary Metabolite from *Amorphophallus paeoniifolius* for Hepatoprotective activity *Research Journal of Pharmaceutical, Biological and Chemical Sciences* **2007**, 1(4), 429-434.
71. Rong Sheng, Xiao Lin, Jing Zhang et al, Design, synthesis and evaluation of flavonoid derivatives as potent AChE inhibitors *J.Bioorg. Med. Chem*. **2009,** 17, 6692–6698
72. Masahiro Ono, Rumi Watanabe, Hidekazu Kawashima et al, ^{99m}Tc/Re complexes based on flavone and aurone as SPECT probes for imaging cerebral b-amyloid plaques, *J.Bioorg. Med. Chem* **2009**, 17, 2069–2076.
73. Veljko Veljkovic, a, Jean-Franc¸ois Mouscadet et al, Simple criterion for selection of flavonoid compounds with anti-HIV activity*J.Bioorg. Med.Chem. Let* **2007,** 17, 1226–1232.
74. Yesseny Vasquez-Martinez, Rachana et al, *J.Bioorg. Med. Chem. Lett* **2007,** 17, 1226–1232
75. Laura J. Hampson, Catherine Arden, Loranne Agius et al, Bioactivity of glycogen phosphorylase inhibitors that bind to the purine nucleoside site *J. Bioorg. Med. Chem.* **2006,** 14, 7835–784.
76. Xiao-Qing Zou, Sheng-Ming Peng, Chang-Ping et al Synthesis, characterization and vasculoprotective effects of nitric oxide-donating derivatives of chrysin *J.Bioorg. Med. Chem.* **2010,** 18, 3020–3025.
77. Surat Boonphong, Apiwat Baramee, Prasat Kittakoop and Pakawan Puangsomba et al, Antitubercular and Antiplasmodial Prenylated Flavones from the Roots of *Artocarpus altilis Chiang Mai J. Sci.* **2007**, 34(3), 339-344.
78. http://www.who.int/topics/drug_resistance
79. R. Ramachandran , Resistance to anti-malaria drugs, *Frontline-India's National Magazine*, **2002,** 19(13), Jun. 22 - Jul. 5.
80. S. R. Cooper *Organic Syntheses*, Coll. Vol. 3, p.761 (1955); Vol. 21, p.103 (**1941**).

81. Jing Jin, Xiao-Bing Wang, Ling-Yi Kong et al, Design, synthesis and biological evaluation of new aryl piperazine derivatives bearing a flavone moiety as a1-adrenoceptor antagonists, *J. Bioorg. Med. Chem. Lett*. 21 (**2011**) 909–911.
82. Chunying Wu, Jingjun Wei, Kuanqiang Gao and Yanming Wang et al, Dibenzothiazoles as novel amyloid-imaging agents, *J. Bioorg. Med. Chem.* 15 (**2007**) 2789–2796.
83. Mrunalini M. Deshpande, Subhash B. Junne, Devdatta V. Saraf and Prafullakumar A. Kulkarni, Synthesis and spectral analysis of some new lanthanide complexes derived from 2,4 and 2,5-dihydroxy acetophenones and screened their antimicrobial activity, *J. Chem. Pharm. Res.*, **2010**, 2(3):453-458
84. M J Menezes, S Manjrekar, V Pai, R E Patre & S G Tilve A facile microwave assisted synthesis of flavones., *Indian Journal of Chemistry* Vol. 48B, September **2009**, pp. 1311-1314
85. Constantinos G. Neochoritis , Tryfon Zarganes-Tzitzikas, Constantinos A. Tsoleridis Julia Stephanidou-Stephanatou, Christos A. Kontogiorgis, Dimitra J. Hadjipavlou-Litina, Theodora Choli-Papadopoulou, One-pot microwave assisted synthesis under green chemistry conditions, antioxidant screening, and cytotoxicity assessments of benzimidazole Schiff bases and pyrimido[1,2-a]benzimidazol-3(4H)-ones., *European Journal of Medicinal Chemistry* 46 (**2011**) 297-306.
86. M R Shivalingam, D Jeslin, Y V Kishore Reddy, B Appa Rao, N Sunitha, Velpuri Amulya and T Jyothibasu, anti bacterial activity of 3- (4- aryl substituted- 3 - yl) - thiazolidin- 4 - one and their derivatives, *international journal of pharmaceutical science and research, IJPSR* **2010**; Vol. 1, Issue 7
87. Trager W & Jensen .J B., Human malaria parasites in Continuous culture., *Science* 193:673-5, **1976**.
88. Ana Gomes, Ondrej Neuwirth, Marisa Freitas et.al, Synthesis and antioxidant properties of new chromone derivatives, Bioorg. Med. Chem2009; 17;218–7226
89. S. Chanda* and R. Dave., *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: *An overview African Journal of Microbiology Research* **2009** Vol. 3(13) pp. 981-996 December.

90. K. Srinivasa Rao , Pradeep Kumar Chaudhury, Anshuman Pradhan., Evaluation of anti-oxidant activities and total phenolic content of Chromolaena odorata., *J. Food and Chemical Toxicology* 48 (**2010**) 729–732.
91. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, **1983;** 65, 55-63.
92. Monks, A., et al, Feasibility of high flux anticancer drug screen using a diverse panel of cultured human tumour cell lines. *Journal of the National Cancer Institute*, **1991**; 83, 757-766.