PROJECT REPORT

On

"MOLECULAR DESIGN AND BIOLOGICAL ACTIVITY OF NOVEL QUERCETIN IMINES : A MULTI-ANALYTICAL APPROACH TO ANTI-INFLAMMATORY DRUG DISCOVERY"

Submitted by

DIVYA P.J.
(AM23CHE004)

In partial fulfillment for the award of the Post graduate Degree in Chemistry

DEPARTMENT OF CHEMISTRY
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CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)
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DECLARATION

I hereby declare that the project work entitled "**MOLECULAR DESIGN AND BIOLOGICAL ACTIVITY OF NOVEL QUERCETIN IMINES : A MULTI- ANALYTICAL APPROACH TO ANTI-INFLAMMATORY DRUG DISCOVERY**" submitted to Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous) affiliated to Mahatma Gandhi University, Kottayam, Kerala is a record of an original work done by me under the guidance of **Dr. Shanty A.A. Assistant Professor** Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous), Ernakulam. This project work is submitted in the partial fulfillment of the requirements for the award of the Degree of Master of Science in Chemistry.

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Chapter 1

Introduction

1. SCHIFF BASE

Schiff bases, characterized by their azomethine (-C=N-) group are versatile compounds with significant potential in medicinal chemistry. Their structural diversity and ease of synthesis have made them attractive candidates for therapeutic applications, particularly as an anti-inflammatory agents. This work provides a detailed analysis of the formation mechanisms of Schiff bases, their biological activities, and their application in suppressing inflammation. It also includes experimental methodologies and recent advancements in their evaluation through molecular docking studies. The target protein, 5U73N is specifically discussed to highlight its relevance in the inflammatory succession. Inflammation, a protective response of the immune system can become pathological when deregulated, leading to chronic inflammatory conditions (1).

Schiff bases, first reported by Hugo Schiff in 1864, are formed through the condensation of primary amines with aldehydes or ketones, resulting in compounds containing a functional imine group (-C=N-) (Figure 1.1). These compounds have gained prominence in pharmaceutical research due to their diverse biological activities including antimicrobial, antifungal, anticancer and anti-inflammatory properties(2).

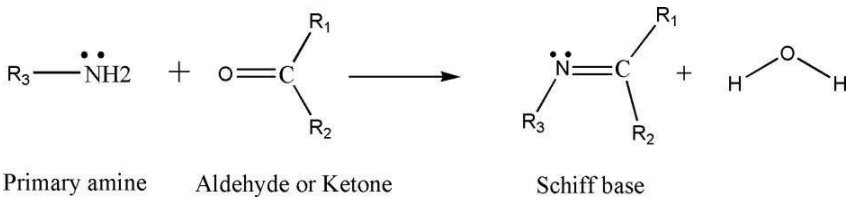


Figure 1.1: General scheme of formation of Schiff base (2)

[Here R1 may be an aryl or alkyl group and R2 may be an aryl, alkyl, or hydrogen; if it is hydrogen then the compound is aldehyde otherwise ketone].

The synthesis of Schiff bases, introduced by Hugo Schiff in 1864, has progressed from basic condensation reactions between primary amines and carbonyl compounds to more advanced methods using modern techniques (3). Early methods involved heating, solvents like ethanol, and sometimes acidic conditions to form the imine linkage. Over time, improvements such as using base catalysts, solvents like methanol and better temperature control led to higher yields.

In the late 20th and early 21st centuries, innovations like microwave-assisted synthesis, solvent-free methods and green solvents made the process more efficient and eco-friendly. Advances in metal catalysis and the development of multifunctional Schiff base ligands have broadened their use in catalysis, drug discovery and material science (4). Today Schiff bases are synthesized efficiently and selectively used in medicine, renewable energy and nanotechnology.

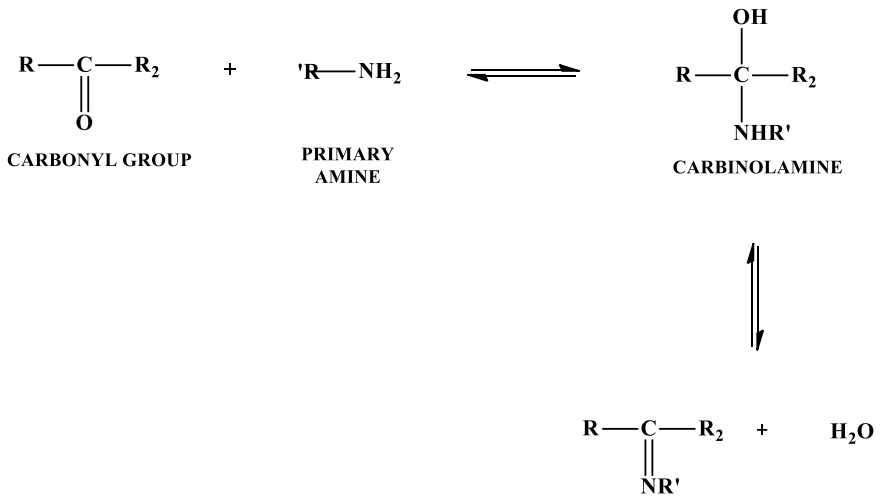


Figure 1.2: Mechanism showing the formation of Schiff Base

Aldehyde or Ketone combines with primary amine to give carbinolamine where the double bond between C and O gets broken and leads to the formation of an OH bond (5). An acid –base catalyzed dehydration occurs in the alcoholic group carbinolamine, an unstable addition molecule. After water is removed , carbinolamine yields an imine with a C=N bond (Figure 1.2). The formation of imine is a reversible reaction. Addition followed by elimination are two types of reaction taking place in the formation of Schiff base.

The azomethine group, in the Schiff Base compounds, has the general formula $RHC=NR_1$, where R and R_1 are heterocyclic, cycloalkyl, or aryl groups that can be substituted in many ways. These substances are also known as “imines” or “azomethines” (6). Aryl substituted Schiff bases are more stable compared with alkyl substituted because of its alternative double bonds. Research has shown that the presence of a single pair of electrons in the nitrogen atom’s sp^2 hybridized orbital of the azomethine group has important chemical and biological ramifications. Schiff bases

are frequently using as an excellent chelating agents, due to their unique C=N group characteristics, synthetic flexibility and relative simplicity of synthesis. The versatility of Schiff base ligands and the uses of their complexes in biology, chemistry, and industry make research in this field extremely desirable.

The most important research in pharmaceutical science today is the development of innovative chemotherapeutics with unique bioactivities and functionality to combat newly emerging diseases (7). Because of their numerous and valuable scientific applications, Schiff base metal complexes have been the focus of coordination chemistry research during the course of several decades of rigorous study on metal-based pharmaceuticals. They may be used as antibacterial, antimicrobial, anticancer, antiviral, anti-inflammatory, analgesic, antifungal, and many other medicinal medicines.

1.1 APPLICATIONS AND IMPORTANCE OF SCHIFF BASE

Schiff base complexes have a wide range of applications with the help of ongoing research, it is expected to uncover even more. Schiff Base has applications in the food industry, dye industry, analytical chemistry, catalysis, agrochemical, and biological activities (8). These Schiff bases can form metal complexes with almost all transition elements. Therefore, they have a vital role in the development of modern coordination chemistry. Now, research shows that these Schiff base complexes have biological interest too. Therefore, these can be found to be key points in the inorganic biochemistry, catalysis, and optical materials.

Schiff base complexes are highly valued for their wide-ranging applications, primarily due to their ability to coordinate with metal ions, exhibit catalytic activity, and display thermal stability and photochromic

properties (9). These complexes are employed in several important catalytic processes, including hydrogen peroxide decomposition, isomerization, allylic alkylation, hydrosilylation, annulation, and carbonylation reactions. For instance, Schiff base complexes with metals like vanadium (V), nickel (Ni), manganese (Mn), and molybdenum (Mo) are commonly used as catalysts in the epoxidation of alkenes, while iron (Fe) and copper (Cu) complexes are effective in the oxidation of catechol (10). The high thermal stability of Schiff base-metal complexes makes them suitable for reactions conducted at high temperatures. In addition to their catalytic roles, Schiff bases are also used in dyeing, where they form coordination compounds with metal ions that help impart fast, durable colors to materials such as leather, wool, and food packaging (11). Schiff bases derived from aniline and phenyl groups are sometimes referred to as "azodyes", known for their vibrant colors and resistance to fading.

Schiff bases are also significant in optoelectronics, particularly in optical computing, where they are used to measure and control radiation intensity in imaging systems and as components in molecular memory storage (12). Their photochromic properties make them ideal for use in reversible optical memory, photo detectors, and solar technologies. Schiff base complexes serve as photo stabilizers, solar collectors, and solar filters, leveraging their ability to change color under light exposure. Additionally, their high thermal stability makes them valuable as stationary phases in gas chromatography, a critical technique in chemical analysis.

With applications such as spanning catalysis, dyeing, optics, and chromatography, Schiff base complexes are crucial in both industrial and research fields (13). Their versatility continues to drive significant

research into new and innovative applications, making Schiff base chemistry an important and rapidly evolving area of study.

1.2 BIOLOGICAL APPLICATIONS OF SCHIFF BASE

Schiff bases, compounds characterized by the imine functional group (-C=N-), are widely recognized for their biological activity and therapeutic potential (14). Since their discovery, Schiff bases have attracted significant interest in medicinal chemistry due to their diverse pharmacological properties, including antimicrobial, anticancer, anti-inflammatory, antioxidant, and neuroprotective effects. The versatility of Schiff bases is primarily attributed to their ability to coordinate with metal ions, allowing them to form stable metal-ligand complexes, which can enhance their biological activity and bioavailability. Schiff bases can interact with biological macromolecules, such as proteins and enzymes, and their metal complexes have shown promise in mimicking metalloenzyme activity, further expanding their therapeutic potential. Schiff bases derived from salicylaldehyde and primary amines exhibited significant antibacterial and antifungal activities against a wide range of pathogens, including multidrug-resistant bacteria (15). The research also indicated that these Schiff bases could disrupt bacterial biofilm formation, which is a major factor in chronic infections.

Recent research has highlighted their diverse biological activities, including anticancer, antimicrobial, neuroprotective, and antioxidant effects, making them promising candidates for drug development and therapeutic applications (16). Ongoing studies into the mechanisms of action and the design of more efficient Schiff base derivatives will likely expand their role in the treatment of a wide range of diseases, solidifying their significance in modern pharmacology and medicinal chemistry.

1.2.1 ANTI INFLAMMATORY ACTIVITY

Schiff bases, have gained considerable attention in recent years for their potential anti-inflammatory properties (17). These compounds, especially when coordinated with metal ions, can significantly modulate the biological pathways involved in inflammation, offering promising alternatives to traditional anti-inflammatory drugs. Inflammation is a complex biological process that involves the activation of various signaling pathways, enzymes, and cytokines. Schiff bases have been shown to influence key elements of this process, including the inhibition of pro-inflammatory mediators such as cytokines (e.g., TNF-α, IL-6, IL-1β), nitric oxide (NO), and enzymes like cyclooxygenase-2 (COX-2) and lipoxygenase (LOX), both of which play vital roles in the production of prostaglandins and leukotriene(18), key molecules involved in inflammation. By targeting these molecules, Schiff bases help to reduce the intensity and duration of the inflammatory response.

Recent studies demonstrated the anti-inflammatory effects of Schiff base derivatives and their metal complexes in treatment of diseases. One such study investigated, Schiff base derivatives synthesized from salicylaldehyde and primary amines (19), which exhibited potent anti-inflammatory activity by reducing the levels of pro-inflammatory cytokines and inhibiting the activation of inflammatory pathways such as NF-κB.NF-κB is a crucial transcription factor that regulates the expression of genes involved in immune responses and inflammation. Schiff base compounds were shown to modulate the activity of NF-κB, effectively controlling the production of inflammatory mediators (20). This ability to regulate NF-κB highlights the potential of Schiff bases in reducing chronic inflammation,which is indicative of several autoimmune and inflammatory

diseases. The anti-inflammatory activity of Schiff bases is further enhanced when they are coordinated with metal ions such as copper, zinc, and nickel. Metal-Schiff base complexes have shown additional benefits by stabilizing the ligand structure and enhancing the compound's bioactivity. For example, copper and zinc Schiff base complexes have been demonstrated to significantly inhibit the production of NO in activated macrophage cells, a critical mediator of inflammation. These complexes also reduce the expression of pro-inflammatory cytokines like TNF-α and IL-1β, which are key players in the inflammatory cascade. Moreover, Schiff base-metal complexes often exhibit antioxidant properties, helping to mitigate oxidative stress, which is commonly associated with inflammation (21) . By reducing the oxidative damage that causes inflammation, these complexes offer a dual mechanism of action: both anti-inflammatory and antioxidative. Diseases caused by microorganisms, such as bacteria or fungi, are typically less severe initially but can gradually increase in fatality. Nonsteroidal anti-inflammatory drugs (NSAIDs) and analgesics, commonly used as anti-inflammatory medicines, have been associated with several side effects, including myocardial infarction, congestive heart failure, nausea, vomiting, dyspepsia, stomach ulceration/bleeding, diarrhea, hypertension, and fluid and salt retention. Research conducted by Mohammad Sayad Alam and his co-workers using Pyrazolon derivatives, particularly 4-aminoantipyrine (4-amino-1,5-dimethyl-2-phenylpyrazole-3-one) and its derivatives, have demonstrated a range of biological effects, such as anti-inflammatory, analgesic, antiviral, antipyretic and antibacterial properties and also serve as potent inhibitors of prostanoid synthesis, platelet thromboxane, and cyclooxygenase isoenzymes .

1.2.2 ANTIDEPPRESENT ACTIVITY

Schiff bases have gained increasing attention for their potential antidepressant activity (22), with recent studies highlighting their efficacy in the treatment of depression. The antidepressant activity of Schiff bases is primarily attributed to their ability to interact with the central nervous system (CNS) and influence key neurotransmitter systems, such as serotonin (5-HT), dopamine (DA), and norepinephrine (NE) (23). These neurotransmitters play a crucial role in regulating mood. Imbalances in their levels are commonly associated with depression. Schiff base derivatives, particularly those involving transition metal complexes, are being investigated for their neurochemical effects and ability to modulate these neurotransmitter pathways, thus offering a potential alternative to conventional antidepressants, which often come with side effects.

Schiff bases of isonicotinoyl hydrazone N-[(1Z)-sustituted aromatic) methylidene] pyridine-4 carbohydrazides were found to have considerable antidepressant and nootropic properties in vitro(24) . Compounds substituted with nitro, halogen, and dimethoxy groups also had strong antidepressant properties (25).

1.2.3 ANTIMALARIAL ACTIVITY

The malaria genus is caused by Plasmodium. In general, Plasmodium is made up of four species. P. Vivax, P. Falciparum, P. Ovale, and P. Malaria. Serious health issues could result from malaria. Nowadays, the hunt for novel medications to treat this illness is essential. Antimalarial medications can be made from Schiff bases (26). N-[(1E)-(5-nitro-1-naphthyl) methylene]-1-(2-(tri-fluoromethyl)phenyl] methanamine was the most effective antimalarial agent among 5 nitroisoquinoline Schiff bases (27).

1.2.4 ANTIOXIDANT ACTIVITY

Antioxidants are naturally occurring chemicals that protect living organisms from damage caused by harmful molecules called free radicals (28). These are produced by the cells in the body in response to free radicals. Free radicals play an important role in the pathogenesis of many diseases, including cancer, diabetes, liver damage, autoimmune diseases, heart disease, atherosclerosis, and aging. Therefore, antioxidants with the potential to scavenge free radicals play an important role in the treatment and prevention of these diseases. Antioxidants are often used as catalysts for antibiotics. Antioxidant compounds have a high ability to scavenge free radicals. Antioxidants play an important role in retarding or preventing the oxidation of oxidizable substances (substrates) (29). In vivo, antioxidant compounds prevent damage to macromolecules and cells by interfering with free radical molecules. Therefore, the importance of searching for antioxidants has increased dramatically in recent years. Currently, synthetic antioxidants are widely used as they are cheap and effective compared to natural anti-oxidant.

1.2.5 ANTI CANCER ACTIVITY

Schiff base complexes, especially those involving transition metals like copper, zinc, nickel, and iron, have shown promising anticancer properties in recent research. These complexes exert their anticancer effects through various mechanisms, including apoptosis induction, cell cycle arrest, and the generation of reactive oxygen species (ROS), which cause oxidative stress and DNA damage in cancer cells. Studies have demonstrated their efficacy in reducing cancer cell proliferation and inducing cell death in various cancer types, such as breast, colon, and lung cancers. For example,

copper (II)-Schiff base complexes have been shown to induce apoptosis in breast cancer cells, while nickel (II)-Schiff base complexes inhibit migration and invasion in colon cancer cells (30).

The anticancer activity of Schiff base complexes is further enhanced by their ability to modulate key signaling pathways involved in tumour growth and survival, such as the $P_{I3}K$/Akt and NF-κB pathways (31). Additionally, these complexes can inhibit metastasis by preventing cancer cells from spreading to other tissues. With their multi-target mechanisms, Schiff base complexes offer a promising alternative to traditional chemotherapy, potentially overcoming issues of resistance and metastasis. Ongoing research continues to optimize these complexes, suggesting that Schiff bases may be a valuable addition to cancer treatment strategies, with fewer side effects compared to conventional chemotherapeutic agents (32).

By condensing salicyaldehyde with 2-amino-4-phenyl-5-methyl thiazole, a Schiff base ligand was created (33) in good yield, the ligand forms compounds with Zn (II), Cu (II), Ni (II), and Co (II). Elements analysis, magnetic susceptibility, molar conductance, infrared spectra, ^{1}H and ^{13}C NMR, mass, electronic absorption, and ESR spectroscopy were used to describe the produced compounds. In order to compare the anticancer activity of the synthesized compounds with that of doxorubicin as a reference medication, the compounds' anticancer activity was investigated against many human tumour cell lines, including breast cancer MCF-7, liver cancer HepG2, lung carcinoma A549, and colorectal cancer HCT116. In comparison to the inhibition in the four cell lines (HepG2, MCF7, A549, and HCT116), the study demonstrated that Zn(II) complex

exhibited potent inhibition against human TRK by the ratios of 80, 70, 61, and 64%, respectively (34).

1.3 QUERCETIN

Flavonoids are a class of compounds commonly found in plants. They all are derivatives of the plant compound, flavone. These compounds together with synthetic analogues, are biologically active in variety of ways (35). Quercetin is a flavanol occurring in fruits and vegetables with proven beneficial impact on health. It is commonly found in red onions, capers, kale and is also ingested from tea, coffee, cocoa, wine, beer, and vinegar. In food, quercetin occur in a bounded form with sugar, phenolic acids, alcohols etc (36). It was discovered by the Nobel prize winner Szent-Gyorgyi. It got its name in 1857 which is derived from quercetum (oak forest)(37). Quercetin is said to be one of the most widely used bioflavonoids for the treatment of metabolic and inflammatory disorders.

Quercetin has a molecular formula $C_{15}H_{10}O_7$ (Figure 1.3) and a molecular mass of 302.236 g/mol. It has a yellow crystalline powdered appearance. It is poorly soluble in hot water, quite soluble in alcohol and lipids and is in soluble in cold water.

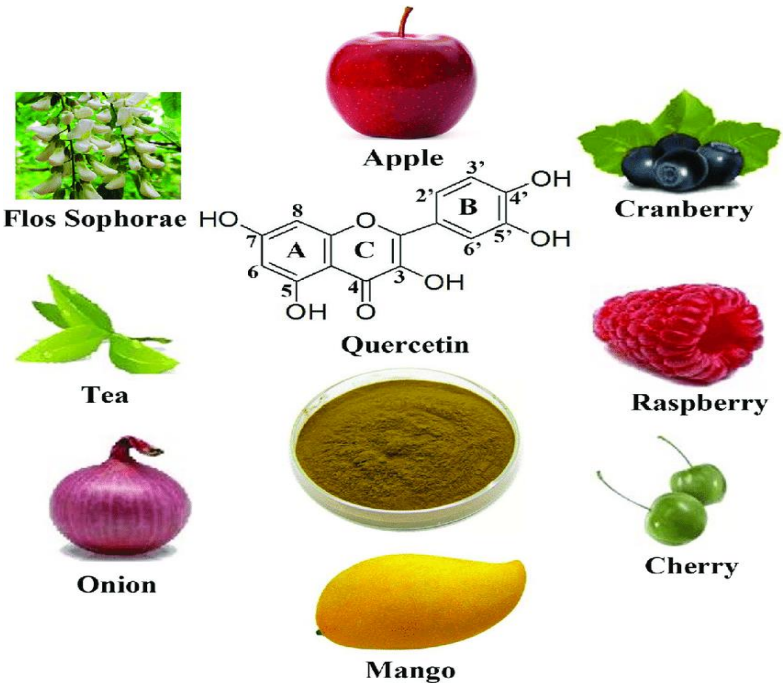


Figure 1.3 : Structure and sources of Quercetin

A molecule of quercetin contains five hydroxyl groups whose presence determines the compounds biological activity and the possible number of derivatives. Quercetin has more polar groups than similar flavonoid compounds. Despite the presence of five hydroxyl groups, the quercetin molecule has a lipophilic character and their derivatives can be both lipo- and hydrophilic depending on the type of substituent in the molecule. A lipophilic quercetin molecule can be easily absorbed by the stomach and the secreted in the bile. Quercetin exists in a planar conformation. Among the five-hydroxyl group, two of them are involved in intramolecular hydrogen bonding with the exocyclic oxygen and the other three participate in intermolecular hydrogen bonding. The exocyclic oxygen plays a pivotal role in the molecular structure of quercetin.

Quercetin is known for its impressive health benefits . Recent research shows that its potent antioxidant properties help combat free radicals, minimizing oxidative stress and safeguarding cells from damage linked to aging and chronic illnesses like cardiovascular and neurodegenerative diseases. With its anti-inflammatory effects, quercetin can aid in managing inflammatory conditions such as arthritis and asthma. It also strengthens the immune system by regulating immune responses and acts as a natural antihistamine, alleviating allergy symptoms. Additionally, quercetin demonstrates antiviral, antibacterial, and antifungal potential, while studies suggest it may help prevent cancer by protecting DNA and inhibiting tumor growth. It supports cardiovascular health by enhancing endothelial function, reducing blood pressure, and improving cholesterol levels. Research also highlights its role in boosting brain health and physical performance by mitigating oxidative damage and enhancing energy production . While foods like onions, apples, berries, and green tea are excellent sources of quercetin, supplements are also available but should be used cautiously, particularly at high doses or by individuals with certain medical conditions. Overall, quercetin is a versatile nutrient that supports overall health and wellness (38).

1.3.1 IMPORTANCE OF QUERCETIN BASED IMINES

Quercetin-based imine compounds represent a fascinating area of research due to their enhanced biological and chemical properties, making them valuable in various fields. These compounds are formed by the condensation reaction of quercetin with amines, leading to the formation of imines that often exhibit improved stability, solubility, and bioavailability compared to quercetin alone. The incorporation of Quercetin with amines enhances the natural antioxidant, anti-

inflammatory, and antimicrobial properties of quercetin, potentially offering broader applications.

One of the key advantages of these compounds lies in their enhanced antioxidant activity. Imine derivatives of quercetin have been shown to more effectively neutralize free radicals and prevent oxidative stress-related damage, making them promising for applications in treating chronic diseases such as cardiovascular disorders, neurodegenerative diseases, and cancer. Their structural modifications also improve their ability to chelate metal ions, which is beneficial in reducing metal-induced oxidative damage and has implications in treating conditions like Alzheimer's disease.

In addition to antioxidant properties, quercetin-based imines exhibit potent antimicrobial and antifungal activities, often surpassing the efficacy of unmodified quercetin (39). These compounds can disrupt microbial membranes and inhibit the growth of pathogens, making them candidates for developing new antimicrobial agents. Their anti-inflammatory effects are also noteworthy, as they help modulate inflammatory pathways and reduce the production of pro-inflammatory mediators, which is crucial for managing diseases like arthritis and asthma.

Quercetin-imine compounds are being explored for their potential as anti-cancer agents, as they can induce apoptosis, inhibit cell proliferation, and suppress tumor growth (40). Moreover, their modified chemical structure enables better interaction with biological targets, enhancing their therapeutic potential. In drug delivery also these compounds are gaining attention for their ability to form stable complexes with metal ions, which can be used for targeted therapy and controlled drug release. Their improved bioavailability and structural versatility make them a valuable

platform for drug design and development, offering exciting possibilities in the treatment of various diseases.

1.3.2 QUERCETIN DERIVATIVES AS AN ANTI INFLAMMATORY AGENTS

Quercetin-based imines have emerged as promising compounds for anti-inflammatory studies due to their enhanced chemical stability, bioavailability, and targeted biological activity (41). These complexes are synthesized through the reaction of quercetin with various amines, forming Schiff bases that often demonstrate superior anti-inflammatory properties compared to quercetin in its unmodified form.

One of the primary mechanisms through which these complexes exert their anti-inflammatory effects is by inhibiting the production of pro-inflammatory mediators such as cytokines, prostaglandins, and reactive oxygen species (ROS). Their antioxidant properties further contribute to reducing oxidative stress, a key factor in chronic inflammation. Additionally, the Schiff base modification enhances quercetin's ability to interact with key enzymes like cyclooxygenase (COX) and lipoxygenase (LOX), effectively suppressing inflammatory pathways (42).

The metal-chelating ability of imines also plays a crucial role in their anti-inflammatory action (43). By binding to transition metal ions, these complexes reduce metal-induced oxidative stress and inflammation, which is particularly beneficial in conditions such as rheumatoid arthritis and other chronic inflammatory diseases. Furthermore, their improved solubility and bioavailability ensure better absorption and targeted delivery to inflamed tissues, enhancing therapeutic efficacy. So Quercetin-based imine complexes represent a significant advancement in anti-inflammatory drug development. Their ability to modulate inflammatory

pathways, reduce oxidative stress, and improve drug delivery makes them highly effective and versatile agents for managing inflammation-associated conditions.

1.4 INFLAMMATION

Inflammation is the response of our body's immune system against the foreign body that enter into our body. Whenever our body encounters an outside invader like a bacteria or virus, toxins or our body suffers an injury our immune system gets activated and releases the substances into the blood called inflammatory markers. The job of these inflammatory markers is to trap the virus or bacteria or heal the injured tissue in case of an injury. These responses may in the form of redness or swelling of the wound (44) (Figure 1.4).

Figure 1.4 Inflammations in human body

There are two different types of Inflammation. ACUTE and CHRONIC inflammation. Acute inflammation are usually short term. Symptoms of acute inflammation include pain, redness or swelling at the site of the infection or injury .Actually acute inflammation is a protective response of the body that helps in healing the body from infection or injury. Even in

the absence of external threats, the body continues to activate the immune system to generate white blood cells and other inflammatory markers. Chronic inflammation is the term used to describe this type of inflammation. Chronic inflammation can last anywhere from a few months to a few years and is typically long-lasting. Chronic inflammation can have a variety of symptoms, such as fever, joint pain, or abdominal pain, which can be dangerous to our health (45).

1.4.1 CAUSES OF INFLAMMATION

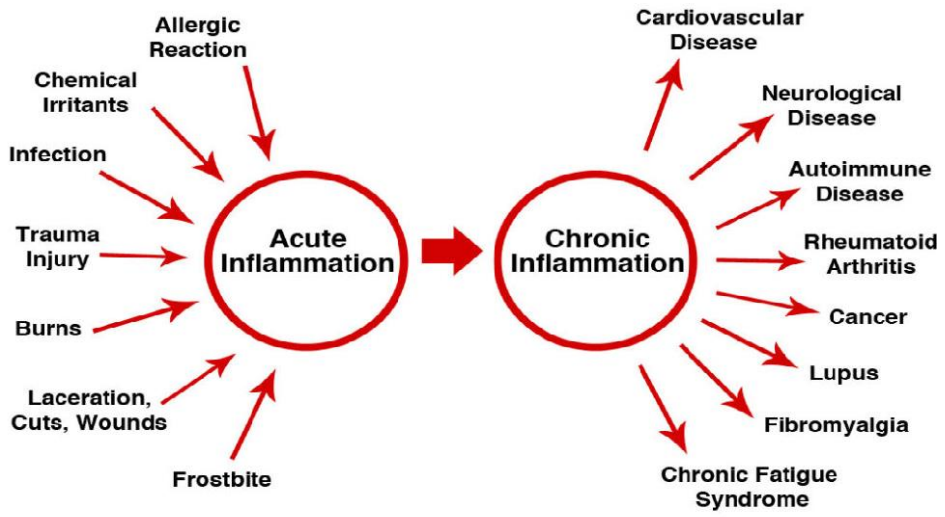


Figure 1.4.1 Acute and chronic inflammation

1.4.2 SYMPTOMS OF INFLAMMATION

Acute inflammation can result in:

- Pain or soreness
- Blushed skin where the damage occurred.
- Swelling.

- Fever

Inflammation that is chronic can last for months or even years. It is associated with or may be associated with a number of disorders, including diabetes, cardiovascular disease (CVD), arthritis and other joint diseases, allergies and chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, psoriasis.

1.4.3 PREVENTION OF INFLAMMATION

A healthy diet, regular exercise, and other practices are the first and most effective anti-inflammatory measures that may be implemented. Turmeric is one of the best foods to reduce inflammation. The turmeric's curcumin ingredient has been extensively researched for its ability to reduce inflammation.

Other substances that have been proven to have strong anti-inflammatory qualities include lutein, which is found in green leafy vegetables, omega-3 fatty acids, which are found in walnuts and fatty fish, and green moong beans. Processed foods, trans fats (such Vanaspati Dalda), alcohol, and other foods should be avoided at all costs in order to prevent or lessen blood inflammation .An ideal test to identify inflammation is does not exist. Typically, when inflammation is linked to other illnesses, doctors and other health care providers advise a test. Doctors use nonsteroidal anti-inflammatory medications, such as aspirin or Ibuprofen, to treat inflammation (46).

1.4.4 ANTI-INFLAMMATORY DRUGS AND ITS EFFECTS

Anti-inflammatory drugs are medications that help reduce inflammation in the body. They are commonly used to treat conditions such as arthritis, autoimmune disorders, and injuries, where inflammation plays a significant role. These drugs work by targeting various pathways involved in the inflammatory process, ultimately helping to reducing pain, swelling, and other symptoms associated with inflammation.

NSAIDs are the most commonly used anti-inflammatory drugs. These drugs work by inhibiting cyclooxygenase (COX), an enzyme responsible for producing prostaglandins chemicals that promote inflammation, pain, and fever. Common NSAIDs are aspirin, Ibuprofen etc. which act via inhibition of the cyclooxygenase (COX) isozymes. They continue to be an essential part of the pharmacological treatment of both acute and persistent pain. The biological activities of the COX-1 and COX-2 isozymes are distinct; analgesic effectiveness is predominantly (but not completely) linked to COX-2 inhibition, whereas the inhibition has distinct adverse effects. All available NSAIDs, including acetaminophen and aspirin, are associated with potential side effects, particularly gastrointestinal and cardiovascular effects, related to their relative selectivity for COX-1 and COX (47).

Ibuprofen was first made available to people as an anti-inflammatory medication in England in 1967 and the US in 1974.It possesses strong but mild anti-inflammatory qualities comparable to aspirin, but with much less detrimental effects on the stomach. It is a nonsteroidal agent since it prevents the manufacture of prostaglandins and does not affect the adreno pituitary axis. Ibuprofen has been shown to be effective in rheumatoid

arthritis and osteoarthritis and is probably effective in spondylitis, gout, and Bartter's syndrome (48).

Short-term use of NSAIDs and can be highly effective in managing inflammation and pain, but long-term use requires monitoring for complications such as gastrointestinal damage, kidney issues, and cardiovascular risks.

1.5 MOLECULAR DOCKING

Molecular docking is a crucial computational method used to model the interaction between ligands, such as imine compounds, and biological targets like enzymes or proteins (49). Imines are formed by the reaction of primary amines with carbonyl compounds and are well-known for their wide range of biological activities, including significant anti-inflammatory potential. These compounds have demonstrated their ability to inhibit key inflammatory enzymes, such as cyclooxygenase (COX) and lipoxygenase (LOX), which are central to the inflammation process. Molecular docking provides detailed insights into how these compounds bind to target sites, their interaction mechanisms, and binding affinities, enabling the design of more effective and selective anti-inflammatory agents.

This technique’s importance lies in its ability to predict ligand-target interactions at a molecular level, significantly reducing the reliance on time-intensive experimental methods. Software tools like AutoDock Vina and Gaussian are integral to this process. AutoDock Vina is a popular tool for docking research because it can accurately and efficiently evaluate the binding affinities of ligands and predict their ideal binding poses within a protein's active site. A computational chemistry program called Gaussian, on the other hand, concentrates on improving the electrical characteristics and molecular geometry of Schiff base compounds. It helps with the

structural improvement of ligands for increased biological activity by offering crucial information on molecular orbitals, charge distributions, and energy states.

Researchers can systematically assess the anti-inflammatory properties of imine derivatives by combining these approaches. AutoDock Vina simulates the ligands' interactions with biological targets to find the most promising choices, while Gaussian aids in optimizing the ligands' structural and electrical properties. This integrated approach accelerates the drug discovery process, minimizes costs, and making molecular docking a keystone of modern anti-inflammatory research (50).

1.6 SCOPE OF STUDY

This research focuses on the synthesis, characterization, and evaluation of quercetin-based Schiff base compounds for their potential anti-inflammatory activity through a combination of molecular docking and experimental studies. The scope encompasses the following key aspects:

- Synthesis of five novel Quercertin based schiff base compounds.
- Characterisation of the synthesized compounds using elemental analysis, uv-visible spectosocpy, FT-IR spectroscopy ,NMR spectroscopy(^{1}H and ^{13}C NMR), and Flourescence spectroscopy
- Theoretical and experimental study of anti-inflammatory activities of synthesized schff base compounds.

Chapter 2

Literature Survey

This literature review aims to explore the development of quercetin-based Schiff base compounds and their anti-inflammatory activities, emphasizing the integration of computational and experimental approaches in the past five years . By synthesizing the current knowledge in this domain, the review seeks to highlight the potential of these compounds as innovative solutions to inflammation-related disorders.

Marija Lesjak et al examined six derivatives of quercetin. The antioxidant and anti-inflammatory qualities of six quercetin derivatives - quercetin-3-O-glucuronide, tamarixetin, isorhamnetin, isorhamnetin-3-O-glucoside, quercetin-3,4′-diOglucoside, and quercetin-3,5,7,3′,4′-pentamethylether were assessed in relation to the activity of benchmarks (butylated hydroxytoluene and aspirin) and standard onion extract, the main source of dietary quercetin. The notable bioactivities of the quercetin compounds were similar to those of standards and onions. Derivatization of quercetin's hydroxyl groups decreased its antioxidant effectiveness. However, there was no discernible correlation between quercetin's amount of free hydroxyl groups and its capacity to inhibit the production of inflammatory mediators. In conclusion, after consuming quercetin, its derivatives may circulate throughout the body and act as potent anti-inflammatory and antioxidant agents (51).

A study by Ali Dehghani et al was published in the Journal of Molecular Liquids in 2020 on page 113035, issue 309.The study looked closely at the protective properties of quercetin, a flavonoid group of polyphenols, in an acidic HCl environment (containing 1 M) using testing and accurate modeling (including molecular/atomic-level simulations, MD/MC augmented with DFT). Both UV-Vis and FT-IR methods were used to characterize the structure of the quercetin compound. The surface protective features were examined using FE-SEM, AFM, and contact angle studies. The findings demonstrated that quercetin molecules' coating and shielding protect steel against corrosion attacks. Based on the EIS and weight loss measurements, protection degrees of approximately 95% and 93%, respectively, were reached after an hour of metal subjection.The potentio dynamic polarization curves show that quercetin molecules can limit corrosion activities by a combination cathodic/anodic mechanism. Additionally, it was shown that the adsorption of quercetin molecules in compliance with the Langmuir isotherm ensured the creation of a mono-protective layer. The interactions between the Quercetin/iron complexations and the target metallic adsorbent may include a donor-acceptor interfacial mechanism, according to the DFT simulations. Additionally, the adsorption of metal-organic complexes on the iron surface was ensured by the MC/MD procedures (52).

Many plants include four common flavonol glycoside chemicals with a range of biological activities: luteolin, kaempferol, apigenin, and quercetin.These four flavonol glycoside compounds were analyzed by Ruxia Wang, Yu Chang et al. They examined the ferric reducing antioxidant power, phagocytosis, DPPH and ABTS radical scavenging activities, and NO level. There in vitro antioxidant and anti-inflammatory

properties were the main focus of the current investigation. This study demonstrated that all four medications might reduce NO levels and phagocytosis. As the concentration rose, so did their antioxidant properties. The relative IC_{50} ABTS values for luteolin, kaempferol, apigenin, quercetin, BHT, and VC were 0.59, 0.8506, 0.8243, 0.5083, 1.4497, and 2.1563 μg/ml. Based on test results, they came to the conclusion that quercetin is a perfect antioxidant and anti-inflammatory medication that may be utilized as an adjuvant treatment for inflammatory diseases and oxidative stress. Additionally, this study offered early proof that there is a direct correlation between antioxidant activity and the quantity of phenolic hydroxyl groups. Additionally, molecules with enol groups showed greater anti-inflammatory and antioxidant action than those without enol groups (53).

In addition to its in vitro antioxidant activity against DPPH free radical, Shankar A. N et al investigated quercetin and its 1-6 derivatives' antibacterial efficacy against Bacillus cereus, Salmonella spp., Escherichia coli, and Staphylococcus aureus. The findings demonstrated that all of the examined derivatives of quercetin exhibited lower antioxidant and antibacterial activity than quercetin itself, with the exception of compounds 3 and 4, which shown stronger antibacterial activity against Escherichia coli when compared to quercetin. They concluded that, at the measured doses, quercetin exhibited superior antioxidant and antibacterial activities compared to its 16 derivatives. But compounds 3 and 4's in vitro antibacterial activity data demonstrate a notable increase in gram-negative antibacterial activity against Escherichia coli when compared to quercetin (54).

Rheumatoid arthritis (RA), a systemic autoimmune disease, and the impact of quercetin, either by alone or in combination with methotrexate, on inflammation were the subjects of a study by Amandeep Sodhi et al. Although the hands, wrists, and feet are the joints most frequently afflicted by RA, the temporomandibular joint (TMJ) may also be affected. With their antiproliferative, immunosuppressive, and anti-inflammatory properties, disease-modifying antirheumatic drugs (DMARDs) slow the course of RA.The first line of treatment for RA is currently methotrexate (MX).Leukopenia, drug resistance, and liver and kidney damage are among the relevant side effects that the continuous MX dosage may cause. Preclinical studies have demonstrated that activating transcription factor signaling pathways improves clinical symptoms and lowers pro-inflammatory cytokines in RA patients' plasma levels. QT also suppresses proinflammatory mediators and increases synoviocyte apoptosis in cell culture, which suggests it may be a useful treatment for RA. When compared to untreated arthritic animals, the nociceptive threshold was significantly higher in the animals treated with QT and/or MX. According to the results, QT has a hepatoprotective impact and lowers inflammation in a TMJ experimental model of RA (55).

Moamen S. Refat et al. conducted a study sought to evaluate the novel [Ru(Q)(Cl)2(H2O)2] complex's anti-inflammatory and antioxidant qualities (Ru(III)/Q). The flavonoid molecule quercetin (Q) and ruthenium (III) ions were combined to form a novel and significant combination. Infrared (FTIR) spectroscopic analysis of the Ru (III)/Q complex showed that Q is coordinated as a bidentate with Ru metal ions.The electronic (UV−Vis) and magnetic susceptibility value (1.85 B.M.) spectra revealed the octahedral geometry of the Ru(III) complex. Thermogravimetric study

(TG/DTG) indicates that the Ru (III)/Q compound is reasonably stable up to 300 °C. To evaluate biological activity, 60 male rats were split up into six groups. Histological and ultrastructural studies were used to assess cytokines in the brain and testicular tissues, while the comet assay was performed in the brain tissue. Ru (III)/Q administration, either alone or in combination with DG, reduced oxidative damage to normal levels and blocked apoptotic activities. Thus, Ru (III)/Q protected brain and testicular damage and reduced oxidative stress in male rats.The (Ru (III)/Q) complex considerably reduces the aging neurotoxicity, reproductive toxicity, and anti-hepatic cancer effect caused by D-galactose (DG) (56).

Yumiko Nishimura et al. conducted a experiment suggests that quercetin, an antioxidant, may be used to protect cells against intracellular Ca^{2+} excess. Quercetin is known to protect cells against oxidative damage. Oxidative stress-induced increases in intracellular Ca^{2+} concentration are one of the mechanisms that lead to cell death. They therefore hypothesized that quercetin could protect cells that are suffering from an overabundance of intracellular calcium ions. To test the notion, the effects of quercetin on cells undergoing intracellular Ca^{2+} excess and oxidative stress were examined using rat thymocytes and a flow cytometer equipped with the proper flourescence probes. Comparable quercetin (1–30 μM) doses were employed to shield cells from H_2O_2-induced oxidative stress and intracellular Ca^{2+} overload brought on by a calcium ionophore.H_2O_2 and calcium ionophore-induced cell death were significantly lessened when exogenous Ca^{2+} was eliminated. Quercetin also significantly delayed the Ca^{2+}-dependent cell death process, although it had no effect on the rise in intracellular Ca^{2+} concentration brought on by H_2O_2 or calcium ionophore.In summary, quercetin can protect cells from oxidative damage

even when the intracellular Ca^{2+} concentration rises.These results suggest quercetin is also used to protect cells from excess intracellular Ca^{2+}(57).

The beneficial effects of the genus Bifidobacterium on health are widely acknowledged. When Bifidobacterium adolescentis was exposed to quercetin and similar polyphenols, Yoshikiyo Okada et al discovered that the bacteria secreted more anti-inflammatory chemicals. They investigated the characteristics of the anti-inflammatory substances secreted by B. adolescentis. The activated macrophage levels of inflammatory mediators were reduced by quercetin-infused B. adolescentis culture supernatant. Even after quercetin washout, the increase in anti-inflammatory activity caused by quercetin remained, but spontaneous quercetin degradant was unable to increase anti-inflammatory activity. The bioactive components of the culture supernatant may be acidic, nonphenolic, heat-stable biomolecules with molecular weights less than 3 kDa, as determined by physicochemical investigation. Acetate and lactate have no effect on nitric oxide production. The anti-inflammatory substances found in B. adolescentis may be small molecules rather than short-chain fatty acids when combined. Stearic acid was tentatively identified as a bioactive candidate molecule as a result of these findings (58).

To investigate the anti-inflammatory effects of quercetin and isoquercitrin, A. P. Rogerio et al .used a mouse model of asthma. Two intranasal ovalbumin challenges were followed by an immunization (ovalbumin/aluminum hydroxide, s. c.) for BALB/c mice. Following the first vaccination, the mice received daily gavages of either isoquercitrin (15 mg/kg) or quercetin (10 mg/kg). Positive control: s.c. dexamethasone at a dose of 1 mg/kg. Lung parenchyma, blood, and bronchoalveolar

lavage fluid (BALF) were tested for leucocytes twenty-four hours after the last ovalbumin challenge. Interleukin-5 (IL-5) was investigated in BALF and lung homogenates.Both quercetin and isoquercitrin are potent eosinophilic inflammatory suppressors that may be useful in the treatment of allergies (59).

In the study done by Pouya Ghamari et al synthesized a Schiff base by combining the quercetin extracted from Origanum vulgare L.with ethanolamine. Origanum vulgare L. is a rich source of phenolic compounds, among which quercetin exhibits remarkable antioxidant properties capable of scavenging free radicals. The Schiff base was then used as a precursor for the synthesis of five novel metal complexes comprising Cu(II), Ni(II), Zn(II), Fe(II), and Co(II) ions. The structures of both the ligand and the metal complexes were characterized by analytical techniques, including uv visible spectroscopy, Fourier-transform infrared spectroscopy (FT-IR), elemental analysis, molar conductivity, proton and carbon nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR), and energy-dispersive X-ray analysis (EDX). The antimicrobial activity of the synthesized metal complexes against Escherichia coli and Pseudomonas aeruginosa bacteria and Candida albicans fungi were evaluated. The results demonstrated that the synthesized metal complexes exhibits promising antimicrobial activity, highlighting their potential as therapeutic agents (60).

Hamdi Temel et al modified quercetin with various boronic acids and new types of quercetin-based boronic (QB) compounds were synthesized. These compounds were synthesized to obtain more effective molecules by doping the biological activity properties of quercetin in its structure. 6-methoxy naphthalene boronic acid, 1,4-phenyl diboronic acid, 4-methoxy

phenylboronic acid, 6-methoxy-3-pyridinylboronic acid, 3-formyl-4-methoxyphenyl boronic acid compounds were used. The synthesized compounds were characterized by mass, ^{13}C and ^{1}H NMR, and FTIR spectroscopy.The antioxidant, anticholinesterase, antiurease, antityrosinase and toxic-cytotoxic effects of these molecules were tested. Quercetin based boron compounds were synthesized and examined in vitro in this study and were also analysed by molecular docking. As a result of molecular docking analysis, the binding energy parameter values and active binding site amino acid residues of these compounds were determined. It was also analysed that the Quercetin based boronic compounds showed better efficacy in many biological studies (61).

The natural flavonoid of quercetin revealed a wide range of biological activities by the modulation of various targets and signaling pathways. However, quercetin's low solubility and poor bioavailability have restricted its applicability, as a result of this Seyedeh Alizadeh et al had attempted to design and synthesize numerous novel quercetin derivatives using various methodologies in order to modify quercetin's constraints, the physico-chemical properties of quercetin's molecular scaffold to make it applicable for drug development. Therefore, the biological activities of quercetin derivatives, as well as the relationship between activity and chemical structure and their mechanism of action, were investigated in this study. Thus these Quercetin based molecules can be applied in the discovery of medications for a number of diseases (51).

Chapter 3

Materials and Methods

This chapter focussing on the importance of reagents and solvents used, method of synthesis of novel five imine compounds, their characterisation techniques, and on molecular docking and experimental methods of anti-inflammatory activities**.**

3.1 REAGENTS

All the reagents were used as analytical grade, purchased from Sigma Aldrich and were used as received.

Quercetin

3- amino benzoic acid

Sulphanilamide

4-amino antipyrene

2-amino 5 methyl pyridine

2-amino 4-nitro phenol

3.2 IMPORTANCE OF AMINE GROUPS USED

3.2.1 3-AMINO BENZOIC ACID

3-Amino benzoic acid, sometimes referred to as meta-aminobenzoic acid, is an aromatic molecule that has a carboxyl group (-COOH) and an amino group ($-NH_2$) joined to a benzene ring. It is a crucial precursor in organic synthesis, especially for the synthesis of metal coordination compounds

and Schiff base complexes. Because of their diverse biological actions, such as their antibacterial, anti-inflammatory, and antioxidant properties, these compounds are useful in medicinal chemistry. The compound's ability to form stable complexes with various metal ions and functional groups makes it useful for the synthesis of dyes, polymers, and other industrial chemicals.

3.2.2 SULPHANILAMIDE

Numerous studies have been conducted on sulfanilamide, a significant sulphonamide medication. A key component of its structure is an amine group ($-NH_2$) joined to a benzene ring, which functions by imitating para-aminobenzoic acid (PABA) to prevent the manufacture of folate by bacteria. This functional group is also an important location for changes in drug development and the investigation of structure-activity connections in medicinal chemistry. In pharmacological research, sulphanilamide advances knowledge of drug metabolism, enzyme interactions, and possible toxicities. Environmental studies are also used to assess its impact as a pharmaceutical contaminant, and nanotechnology is used to create sophisticated medication delivery systems. Because of its distinct chemistry and historical significance, it is essential to scientific study and teaching.

3.2.3 4 –AMINO ANTIPYRENE

4-Aminoantipyrine is a derivative of antipyrene that is distinguished by the presence of an amine group ($-NH_2$) at the pyrazolone ring's fourth position. Its amine group increases its chemical reactivity, enabling it to react with aromatic or phenolic chemicals to generate colored complexes.

In analytical chemistry, 4-aminoantipyrine is frequently used in spectrophotometric assays to detect phenols and other compounds in biological and environmental samples because of these properties. Additionally important to its pharmacological characteristics is the amine group, which also acts as a location for other drug development changes.

3.2.4 2-AMINO 5- METHYL PYRIDINE

A pyridine derivative known as 2-Amino-5-methylpyridine has a methyl group ($-CH_3$) at the fifth position of the pyridine ring and an amine group ($-NH_2$) at the second position. The molecule becomes more reactive in a range of chemical processes, such as the creation of coordination complexes with metal ions, amides, and Schiff bases, due to the presence of amine groups that enhances the compounds nucleophilicity. Because of its special arrangement of functional groups, it can be used in agrochemical development, medicinal research, and synthetic organic chemistry

3.2.5 2- AMINO 4-NITRO PHENOL

2-Amino-4-nitrophenol is an aromatic molecule that has a nitro group ($-NO_2$) at the fourth position on a phenol ring and an amine group ($-NH_2$) at the second position. For enhancing the compound's reactivity and nucleophilicity, the amine group has a major impact on its physical and chemical characteristics. Because of this functional group, it can take part in a variety of processes, including hydrogen bonding interactions, amide production, and coupling reactions. The amine group's interaction with the nitro and hydroxyl groups gives the substance its utility in organic synthesis, color production, and the creation of agrochemicals and

medications. Also the amine group plays a crucial role in determining its electronic and biological activity.

3.3 SOLVENTS

Solvent used for the synthesis and purification procedure were purchased from spectrochem Ltd and used without further purification.

i. Methanol

ii. Ethanol

iii. Petroleum Ether

iv. DMSO

3.4 CHARACTERISATION TECHNIQUES

3.4.1 ELEMENTAL ANALYSIS

CHN Elemental Analysis can be used to determine the elemental content of a sample in terms of carbon, hydrogen, and nitrogen. It works with a range of sample kinds, including solids, liquids, and volatile substances. Understanding the composition of the components helps determine the structure of the sample material. It offers a precise and clean measurement of the components in a sample. CHN elemental analysis is based on the combustion of the sample. Consistent composite gases of the elements C, H, and N are produced when the sample burns. After that, gas chromatography is used to measure it (62- 63).

CHN Elemental Analysis was recorded at the SAIF, Cochin University of Science and Technology, Kochi, India.

3.4.2 INFRARED SPECTROSCOPY

Infrared radiation is defined as electromagnetic radiation with a wavelength longer than visible light. IR spectroscopy is used to study the interaction between matter and infrared radiation. Two substances' infrared spectra can never be compared. A change in the dipole moment of the compound or sample during vibration is the primary requirement for IR spectroscopy. Since every kind of link has a distinct intrinsic frequency of vibration, no two molecules with different structures will have precisely the same infrared absorption pattern, or infrared spectrum. The reason for this is because two molecules of the same kind in two distinct compounds are situated in two quite dissimilar surroundings. (64).

Infrared spectra were recorded in the range 4000-400 cm^{-1} and it is recorded on a Thermo Scientific Nicolet 912A0712 iS5 FTIR spectrometer at Department of Chemistry, Bharata Mata College, Thrikkakara, Kochi, India.

3.4.3 ULTRAVIOLET SPECTROSCOPY

In the visible (VIS) and ultraviolet (UV) regions of the electromagnetic spectrum, which cover wavelengths between 190 and 800 nm, the great majority of organic molecules and functional groups are transparent. For this reason, absorption spectroscopy is not very useful in this wavelength range. But occasionally, these spectrum portions can offer insightful information. Combining such data with the information provided by nuclear magnetic resonance (NMR) and infrared spectra can reveal important structural concepts. The principle is Beer Lambert's Law. Both

bonded and unbonded molecules' electrons absorb energy and are stimulated to reach higher energy levels. (65).

Ultraviolet-Visible Spectroscopy were recorded using JASCO U-770 in the range 200 to 700 nm at Department of zoology, St. Teresa's College (Autonomous), Ernakulam, Kochi, India.

3.4.4 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Nuclear magnetic resonance spectroscopy is a method used to study molecules. It involves recording the interactions between radio frequency electromagnetic radiation and the nuclei of molecules in a high magnetic field. NMR spectroscopy is used in both research and quality control. The chemical shift of a certain photon is the difference between its absorption location and the absorption position of a reference proton. It can determine the content, purity, and molecular structures of a material. Additionally, mixtures with known compounds are examined. The reorientation of atomic nuclei bearing non-zero nuclear spin in an external magnetic field is the cornerstone of NMR spectroscopy. NMR spectroscopy can provide both quantitative and qualitative information about the composition of a material(66).

NMR spectroscopy was recorded in the range 0-20 ppm for ^{1}H NMR and 0-220 ppm for ^{13}C NMR at Department of Applied Chemistry, Cochin University of Science and Technology, Kalamassery, Kochi, India

3.4.5 FLOURESCENCE SPECTROSCOPY

The foundation of fluorescence spectroscopy is the idea that some molecules, referred to as fluorophores, absorb light at a particular wavelength (excitation) and then release light at a longer wavelength (emission). This process happens when the fluorophore's electrons are

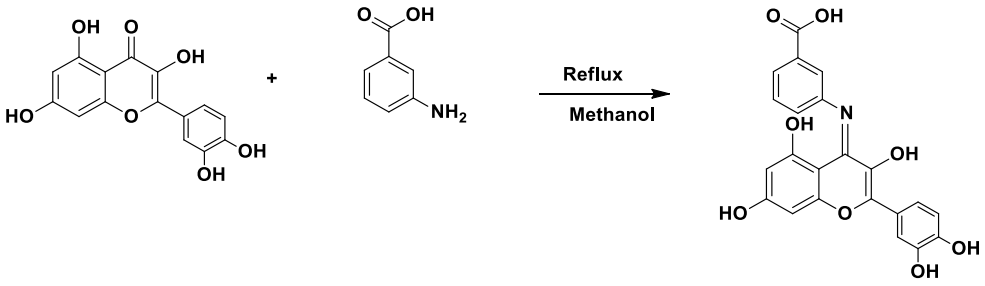
excited to a higher energy state by the absorbed energy, and then they return to the ground state when photons are released. The Stokes shift, which helps differentiate fluorescence from the excitation source, is the difference between the excitation and emission wavelengths. Quantum yield, solvent environment, and molecular interactions are some of the variables that affect the intensity of the light that is released, which is proportional to the fluorophore concentration. Many areas , including biochemistry, environmental monitoring, and others, use fluorescence spectroscopy for sensitive detection and analysis. It offers comprehensive information on a molecule's electronic structure, environment, and interactions, making it an effective tool for structural elucidation of compounds. A compound's molecular structure can be elucidated from the wavelengths of light it absorbs and emits when it fluoresces. The arrangement of atoms, functional groups, and conjugated systems in the compound determines the particular excitation and emission spectra. Shifts in the emission wavelength or intensity, can reveal how a molecule interacts with its surroundings. This information might be useful in determining the compound's structure or how it interacts with other molecules (67).

Flourescence spectroscopy were recorded in the range 200 to 700 nm and it is recorded using the instrument RF 6000 (A40245902040SA) at instrumental lab, St. Teresa’s College (Autonomous), Ernakulam, Kochi, India.

3.5 SYNTHESIS OF SCHIFF BASE

3.5.1 Synthesis of schiff base from Quercetin and 3-amino benzoicacid (QABD)

In an Round bottom (RB) flask (Scheme 1), quercetin in methanol and 3-amino benzoic acid in methanol were mixed in a 1:1 ratio and heated for six hours under reflux. The resulting solution was allowed to slowly evaporate after cooling and concentration. The light yellow precipitate was filtered, collected, and washed with ethanol before being dried and recrystallized. The crystals were collected. (Figure 3.5.1).

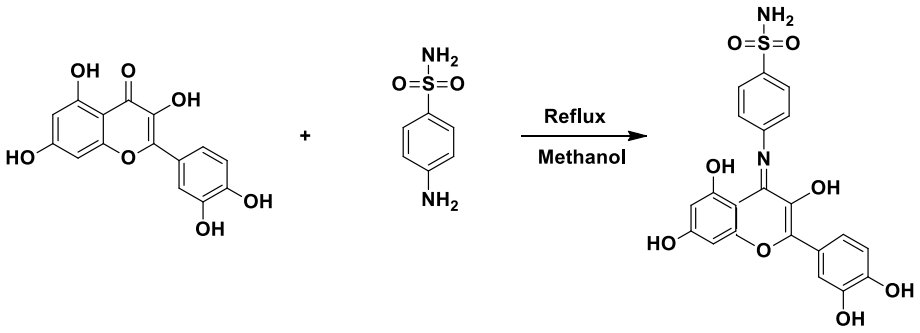


Scheme 1: Preparation of QABD

Figure3.5.1: Crystals of QABD

3.5.2 Synthesis of Schiff Base from Quercetin and Sulphanilamide (QSAM)

In an RB flask, quercetin and sulfanilamide were mixed in a 1:1 ratio and heated for six hours under reflux (Scheme 2). The resulting solution was allowed to cool and concentrate before slowly evaporating. Filtration, collection, and ethanol washing were followed by recrystallization and drying of the greenish yellow precipitate. The crystals were collected. (Figure 3.5.2).

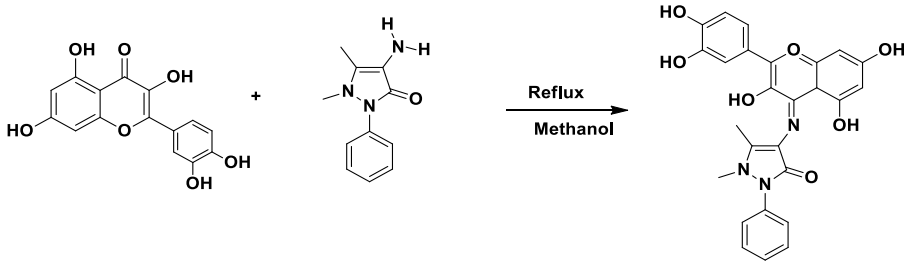


Scheme 2: Preparation of QSAM

Figure 3.5.2: Crystals of QSAM

3.5.3 Synthesis of Schiff Base from Quercetin and 4- amino antipyrene (QAAP)

A 1:1 mixture of quercetin and 4-amino antipyrene in methanol was heated in an RB flask under reflux for six hours (Scheme 3). The resulting solution was allowed to cool and concentrate before slowly evaporating. Filtration, collection, and ethanol washing were followed by recrystallization and drying of the light brown precipitate. The crystals were collected.. (Figure 3.5.3).



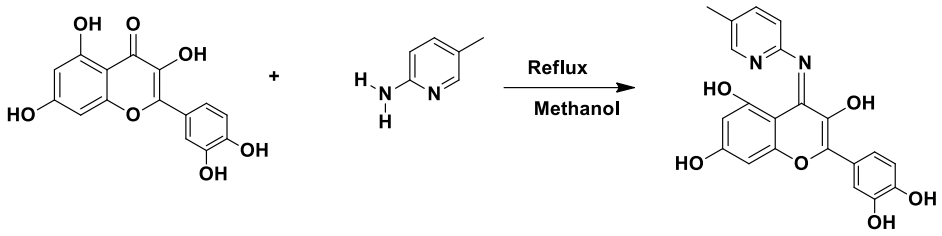
Scheme 3: Preparation of QAAP

Figure 3.5.3 : Crystals of QAAP

3.5.4 Synthesis of Schiff Base from Quercetin and 2-amino 5- methyl pyridine (QAMP)

A 1:1 mixture of quercetin and 2-amino 5 methyl pyridine in methanol was heated in an RB flask under reflux for six hours (Scheme 4). The resulting solution was allowed to cool and concentrate before slowly evaporating. Filtration, collection, and ethanol washing were followed by

recrystallization and drying of the light yellow precipitate. The crystals were collected.(Figure 3.5.4).

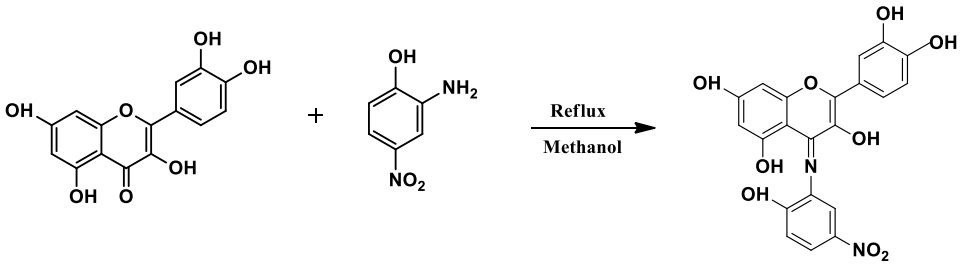


Scheme 4: Preparation of QAMP

Figure3.5.4: Crystals of QAMP

3.5.5 Synthesis of Schiff Base from Quercetin and 2-amino-4-nitrophenol (QANP)

2-Amino-4-nitrophenol in methanol and quercetin in methanol were mixed in a 1:1 ratio and heated in an RB flask under reflux for six hours. (scheme 5).The resulting solution was allowed to cool and concentrate before slowly evaporating. Filtration, collection, and ethanol washing were followed by recrystallization and drying of the greenish yellow precipitate. The crystals were collected. (Figure 3.5.5).



Scheme 5: Preparation of QANP

Figure 3.5.5 : Crystals of QANP

3.6 THEORETICAL STUDY OF ANTIINFLAMMATORY ACTIVITIES

3.6.1 Computational chemistry

The computational chemistry of Schiff base compound's anti-inflammatory properties include investigating and forecasting how these compounds will interact with biological targets linked to inflammation using computational tools and molecular modeling methodologies. These methods aid in forecasting the ways in which these substances may interact with inflammatory targets such enzymes, receptors, and signaling cascades. We can learn more about imines potential as anti-inflammatory drugs by using molecular docking (68). The development of novel, more

effective imine derivatives for the treatment of inflammatory illnesses is made easier by these computational techniques.

3.6.2 Guassian software

A crucial tool for the computational analysis of Schiff base ligands in anti-inflammatory research is Gaussian software. Schiff base compounds with improved anti-inflammatory activity can be designed with the use of Gaussian by predicting interactions with biological targets, calculating electrical characteristics, and optimizing ligand structures. Despite not carrying out molecule docking directly, Gaussian is a crucial component of the computational toolset for researching and creating potent anti-inflammatory drugs due to its strength in quantum mechanical computations, electrostatic analysis, and thermodynamic property predictions (69).

3.6.3 Density Functional Theory (DFT) calculations

The electronic structure of molecules can be studied using DFT, a quantum mechanical modeling technique. This theory states that functionals are functions of other functions. In this situation, the characteristics of a many-electron system can be ascertained using the spatially dependent electron density. Consequently, the density functional theory was developed. This theory is based on two theorems proposed by Hohenberg and Kohn: (a) the electron density of every molecule is functionally connected to its ground state property and (b) any trial electron density function will produce an energy greater than the genuine ground state energy. DFT is one of the most popular and versatile methods in condensed-matter physics, computational chemistry, and computational

physics. DFT approaches with hybrid functionals are just as precise as high-level ab intio procedures, but they take less computer time. When researching the anti-inflammatory properties of Schiff base compounds, DFT is an effective approach. It offers comprehensive information on electronic characteristics, molecular stability, and interactions with biological targets (70). Researchers can create more potent imine compounds for anti-inflammatory studies by combining DFT results with docking.

3.6.4 Determination of anti-inflammatory activities using molecular docking

The schiff base's three-dimensional structure for the anti-inflammatory investigation was determined using DFT calculations at the B3LYP/6-31-G (d,p) level. For DFT calculations, the Gaussian 09 software package was utilized. Geometry optimization are provided using DFT computations. The RCSB protein data bank was used to download the protein molecule 5U73N. Using Auto dock Vina software, the optimized schiff bases were docked with protein 5U73N. Information regarding the kind of ligand-protein interactions, binding energy, stability, and structural activity linkages is therefore obtained, which helps to build a new, more powerful schiff base medication for the treatment of inflammatory illnesses.

3.7 EXPERIMENTAL STUDY OF ANTIINFLAMMATORY ACTIVITIES

The anti-inflammatory activities of Quercetin based imines was studied using in vitro HRBC membrane stabilization method.

3.7.1 MATERIALS USED

Shiff bases

Human blood

Alsever solution

Isoalanine

Diclofenac sodium.

3.7.2 METHODS

I. Preparation of Sample

A stock solution was prepared by dissolving 20 mg of sample in 1 mL of methanol, resulting in a concentration of 20 mg/ml. These stock solution were then utilized for the anti-inflammatory assay (HRBC).

II. Preparation of Human Red Blood Cells (HRBC) Suspension

An identical volume of sterile Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride in water) was combined with freshly drawn whole human blood. After centrifuging the blood for 10 minutes at 3000 rpm, the packed cells were rinsed three times with isosaline (0.85%, pH 7.2). One milliliter of the produced HRBC suspension was combined with an equivalent volume of plant extracts at three distinct concentrations 0.5, 2.5, 5, 10 and 12.5 mg/mL

(T1-T5) in order to evaluate the anti-inflammatory action. The standard was 50 mg/ml of diclofenac sodium. To stabilize the HRBC membranes, the test mixtures including the HRBC solution with plant extracts were incubated for 30 minutes at 56°C. The intact RBCs were separated from the supernatant solution by centrifuging the assay mixtures one more at 3000 rpm after incubation. To determine how much hemoglobin had been released, the absorbance of the supernatant solution was measured with a spectrophotometer at 560 nm. HRBC that had been heated without plant extract was used as a control (71- 72).

The percentage of membrane protection was calculated using the formula;

$$Percentage\ of\ membrane\ protection = \frac{(OD\ of\ Control - OD\ of\ test)}{OD\ of\ control} X100$$

Chapter 4

Results and discussion

A novel five Quercetin based schiff bases was synthesized by the condensation of Quercetin with 3-amino benzoic acid, Sulphanilamide, 4-amino antipyrene, 2-amino 5-methyl pyridine and 2-amino 4-nitro phenol. The synthesized compounds are abbreviated as QABD,QSAM,QAAP,QAMP,QANP. This chapter foccussing on the analysis and discussion of results from different characterisation techniques like CHN elemental analysis, IR spectrosopy,UV-Visible spectroscopy, NMR (^{1}H and ^{13}C NMR) spectroscopy and fluorescence spectroscopy separately for each compounds for confirming that the compounds has been successfully synthesized . Additionaly results of molecular docking as well as experimental study of anti-inflammatory activites are also conducted in this chapter.

4.1 QUERCETIN + 3-AMINO BENZOIC ACID (QABD)

4.1.1 CHN ELEMENTAL ANALYSIS

Compound	Empirical Formula	Formula weight	Color	Observed and Calculated (%)		
				C	H	N
QABD	$C_{22}H_{15}O_8N$	421	Light Yellow	63.45 (63.25)	3.94 (3.67)	3.22 (3.01)

Table 4.1.1 : Elemental analysis

The elemental analysis obtained is in good agrrement with assigned chemical formula.

4.1.2 INFRARED SPECTROSCOPY

The peaks shown in the IR Absorption spectrum give important information about the different functional groups present in the Schiff base. For QABD the peak obtained in the range 1663cm^{-1} indicates the presence of imine groups(73) and the peak obtained in the range 3409 cm^{-1} the presence of OH groups (74).From this data we can confirm that schiff bases are successfully formed. The figure 4.1.2 indicates the IR spectra of schiff base QABD.

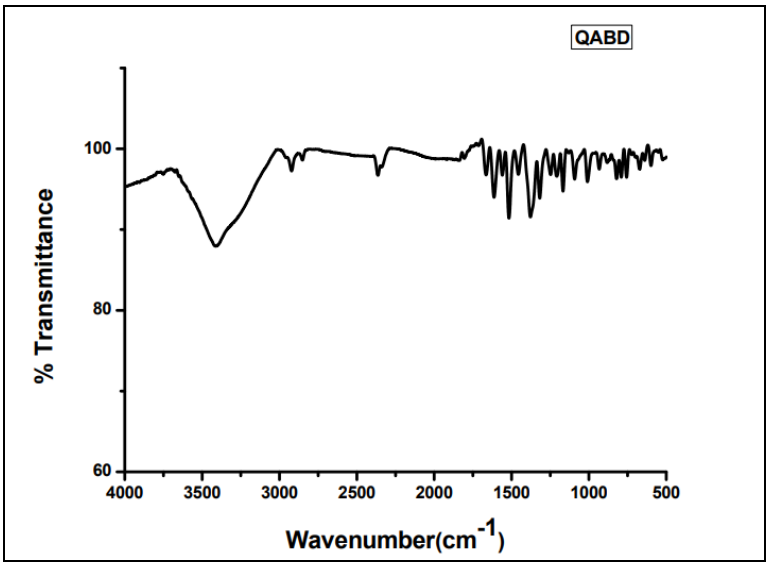


Figure 4.1.2 : IR spectra of QABD

4.1.3 ULTRAVIOLET SPECTROSCOPY

UV-Visible Spectroscopy is used to study the electronic structure and its dynamics in atom and molecules. The UV –Visible spectra of compound QABD was taken in methanol. The figure 4.1.3 indicates the UV-Visible spectra of QABD. The peak at 250 nm is due to $\pi - \pi^*$ transitions and peak at 370nm is due to $n - \pi^*$ transitions (75) which clearly indicates the presence of aromatic group as well as azomethine group leading to the confirmation that compound has been successfully synthesized.

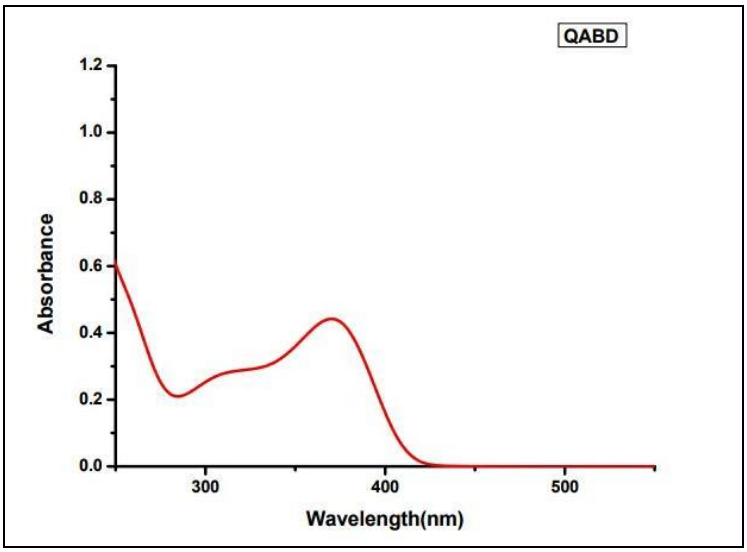


Figure 4.1.3 : UV-Visible spectra of QABD

4.1.4 NMR SPECTROSCOPY

For unknown compounds, NMR can either match against spectral libraries or infer the basic structure directly. Once the basic structure is known, NMR can be used to determine molecular conformation in solution as well as study physical properties at the molecular level such as conformational exchange, phase changes, solubility, and diffusion. To achieve the desired results, a variety of NMR techniques are available.

Both ^{1}H and ^{13}C NMR of QABD compound was taken in methanol using TMS as an internal standard (Figure 4.1.4 a and 4.1.4.b).

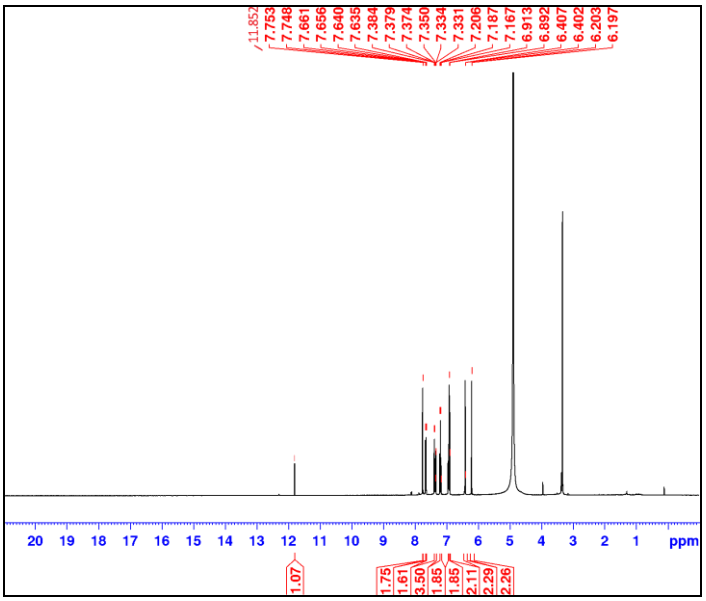


Figure 4.1.4 a : ^{1}H NMR of QABD

^{1}H NMR(CH_3OH δppm) : 6.2(2H, Ar-H), 6.9(2H, Ar-H), 6.8(2H, Ar-H), 6.9(1H, Ar-H), 7.1(1H, Ar-H), 7.2(2H, OH), 7.3(1H, OH), 7.6(2H, OH),7.7(1H, OH), 11.8(1H, CH3COOH)

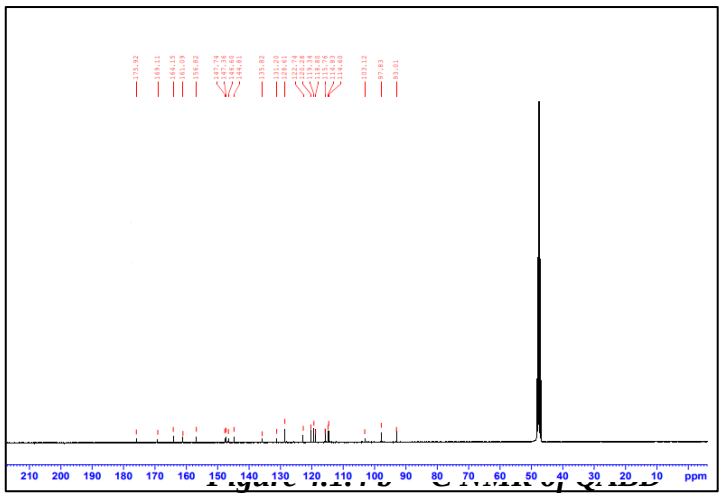


Figure 4.1.4 b : ^{13}C NMR of QABD

^{13}C NMR (CH_3OH δ ppm); 93.70(Ar-C), 98.63 (Ar-C), 104.23(Ar-C), 114.29,116.79(Ar-C), 120.031(Ar-C), 122.49 (Ar-C),130.28(Ar-C),132.46(Ar-C), 136.24(Ar-OH)147.22 (Ar-C-OH),148.23(Ar-C-OH),157.65(Ar-CO).161.27(Ar-C-OH),168.15(Ar-C-OH),169.11(Ar-C-OH),175.92(ArC=N).

4.1.5 FLOURESCENCE SPECTROSCOPY

In QABD, of 10^{-3}M concentration in methanol for an excitation wavelength of 350 nm obtained an emission wavelength at 430 nm (figure 4.1.5 a) and it corresponds to strong blue emission in the visible spectrum, indicative of efficient fluorescence due to its aromatic or conjugated structure. Under a UV chamber, QABD exhibits a solid-state fluorescence of yellow-green color (figure 4.1.5 b), which changes to blue and green fluorescence specifically in methanol and hexane compared to the other

solvents tested (figure 4.1.5 c). The distinct fluorescence behavior highlights the sensitivity of QABD to its environment and to solvent interactions.

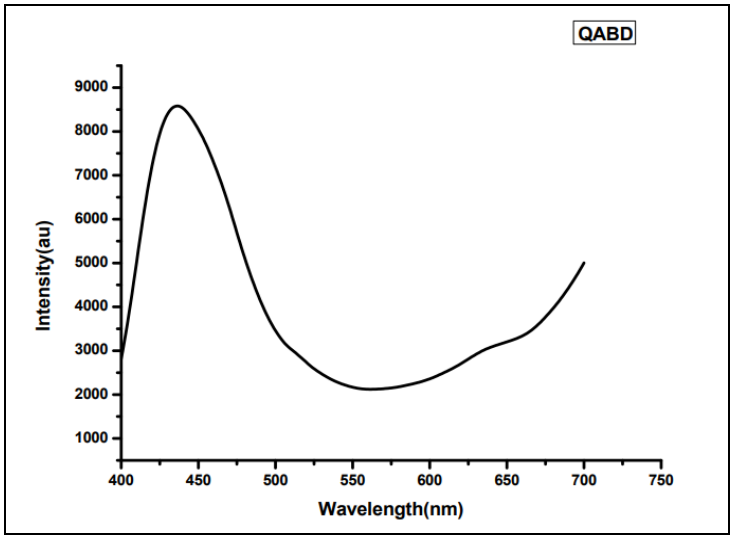


Figure 4.1.5 a : Flourescence spectra of QABD

Normal light

Figure 4.1.5 b Solid state flourescence under UV chamber

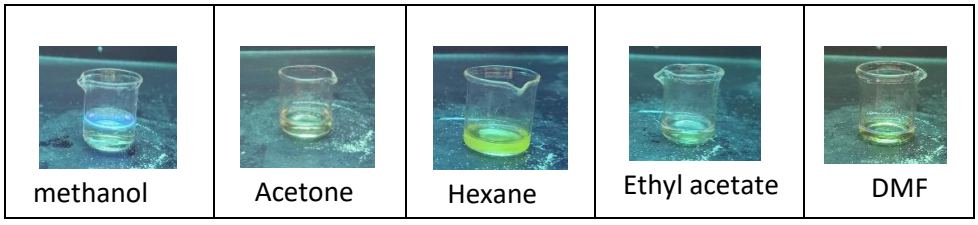


Figure 4.1.5 c liquid state fluorescence of QABD under UV chamber

4.2 QUERCETIN + SULPHANILAMIDE (QSAM)

4.2.1 CHN ELEMENTAL ANALYSIS

Compound	**Empirical Formula**	**Formula weight**	**Color**	**Observed and Calculated (%)**		
				C	**H**	**N**
QSAM	$C_{21}H_{16}O_8N_2$	424	Greenish Yellow	56.78 (56.06)	4.36 (4.36)	5.76 (5.31)

Table 4.2.1 : Elemental analysis

The elemental analysis obtained is in good agrrement with assigned chemical formula.

4.2.2 INFRARED SPECTROSCOPY

The peaks shown in the IR Absorption spectrum give important information about the different functional groups present in the Schiff base. For QSAM the peak obtained in the range 1665cm^{-1} indicates the presence of imine groups(73) and the peak obtained in the range 3430 cm^{-1} the presence of OH groups (74).From this data we can confirm that schiff bases are successfully formed. The figure 4.2.2 indicates the IR spectra of schiff base QSAM.

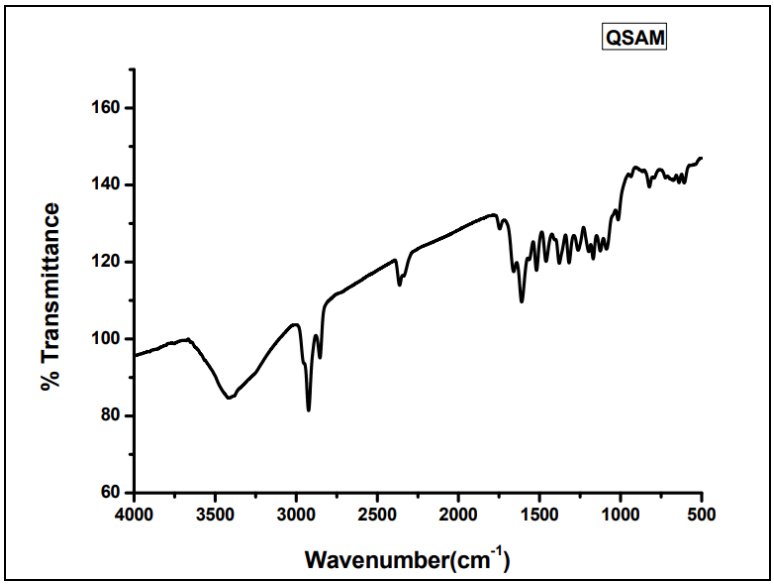


Figure 4.2.2 : IR spectra of QSAM

4.2.3 ULTRAVIOLET SPECTROSCOPY

UV-Visible Spectroscopy is used to study the electronic structure and its dynamics in atom and molecules. The UV –Visible spectra of compound QSAM was taken in methanol. The figure 4.2.3 indicates the UV-Visible spectra of QSAM. The peak at 260 nm is due to π – π* transitions and peak at 369 nm is due to n –π* transitions (75) which clearly indicates the presence of aromatic group as well as azomethine group leading to the confirmation that compound has been successfully synthesized.

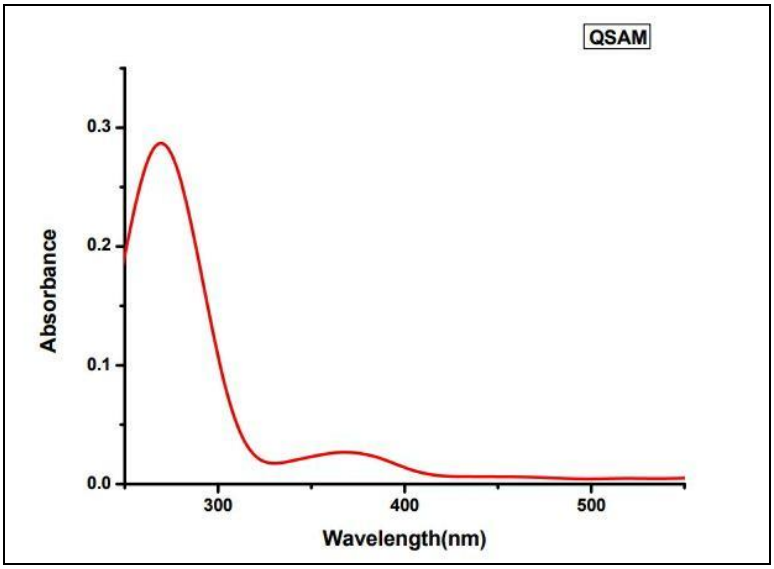


Figure 4.2.3 : UV-Visible spectra of QSAM

4.2.4 NMR SPECTROSCOPY

For unknown compounds, NMR can either match against spectral libraries or infer the basic structure directly. Once the basic structure is known, NMR can be used to determine molecular conformation in solution as well as study physical properties at the molecular level such as conformational exchange, phase changes, solubility, and diffusion. To achieve the desired results, a variety of NMR techniques are available.

Both ^{1}H and ^{13}C NMR of QSAM compound was taken in methanol using TMS as an internal standard (Figure 4.2.4 a and 4.2.4.b).

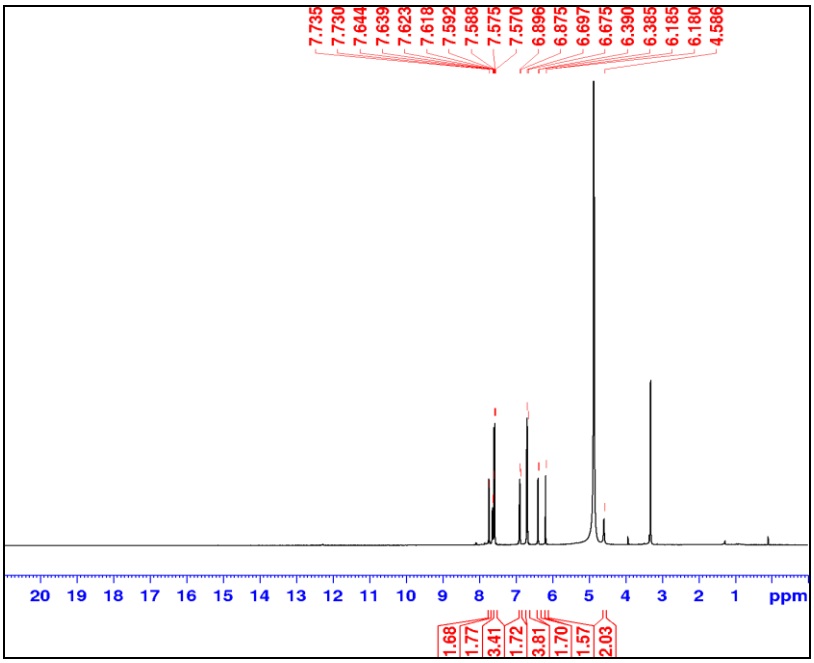


Figure 4.2.4 .a : ^{1}H NMR of QSAM

^{1}H NMR(CH_3OH δppm) : 4.58(2H,N-H),6.1(2H, Ar-H), 6.3(1H, Ar-H), 6.6(2H, Ar-H), 6.8(2H, Ar-H), 7.5(2H, Ar-H), 7.6(2H, Ar-OH), 7.7(2H, OH), 7.8(1H, OH).

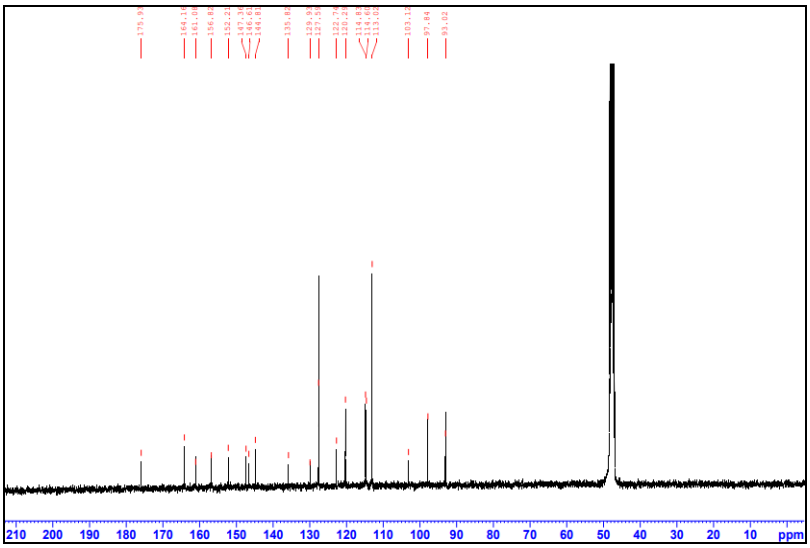


Figure 4.2.4 .b : ^{13}C NMR of QSAM

^{13}C NMR (CH_3OH δ ppm); 93.02 (Ar-C), 97.84 (Ar-C), 103.12(Ar-C), 113.02,114.60 (Ar-C), 120.29(Ar-C), 122.74(Ar-C), 129.39(Ar-OH), 135.82(Ar-OH) ,144.81(Ar-OH), 146.61 (Ar-C-OH), 152.21(Ar-CO),156.82(Ar-CO), 161.08 (Ar-C-OH), 175.53(ArC=N).

4.2.5 FLOURESCENCE SPECTROSCOPY

In QSAM, of 10^{-3}M concentration in methanol at an excitation wavelength of 350 nm gives an emission spectrum at 426 nm (Figure 4.2.5 a) indicating strong emission in the violet-blue region of the visible spectrum. This high fluorescence intensity suggests efficient electronic transitions, likely influenced by the compound's structural features, such as conjugated systems or aromatic groups. Under a UV chamber, QSAM exhibits a green-yellow fluorescence in its solid state (Figure 4.2.5 b), which shifts to a yellow-orange colour specifically in dimethylformamide (DMF), differing from its behavior in other solvents (figure 4.2.5.c). This solvent-dependent fluorescence shift highlights the role of QSAM to its chemical environment and its potential applications in solvent-specific sensing.

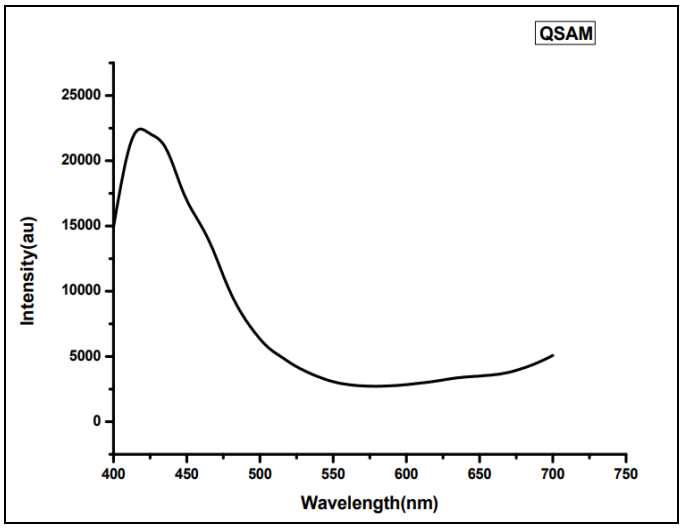


Figure 4.2.5 a : Flourescence spectra of QSAM

Normal light

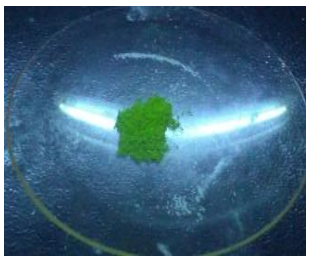


Figure 4.2.5 b Solid state flourescence under UV chamber

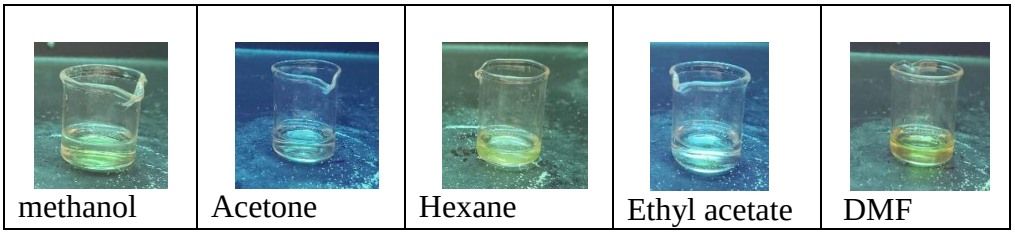


Figure 4.2.5.c liquid state fluorescence of QSAM under UV chamber.

4.3 QUERCETIN + 4- AMINO ANTIPYRENE (QAAP)

4.3.1 CHN ELEMENTAL ANALYSIS

Compound	Empirical Formula	Formula weight	Color	Observed and Calculated (%)		
				C	H	N
QAAP	$C_{25}H_{21}O_7N_3$	475	Light Brown	63.93 (63.16)	4.54 (4.01)	8.60 (8.08)

Table 4.3.1 : Elemental analysis

The elemental analysis obtained is in good agrrement with assigned chemical formula.

4.3.2 INFRARED SPECTROSCOPY

The peaks shown in the IR Absorption spectrum give important information about the different functional groups present in the Schiff base. For QAAP the peak obtained in the range 1663cm^{-1} indicates the presence of imine groups (73) and the peak obtained in the range 3425 cm^{-1} the presence of OH groups (74).From this data we can confirm that schiff bases are successfully formed. The figure 4.3.2 indicates the IR spectra of schiff base QAAP.

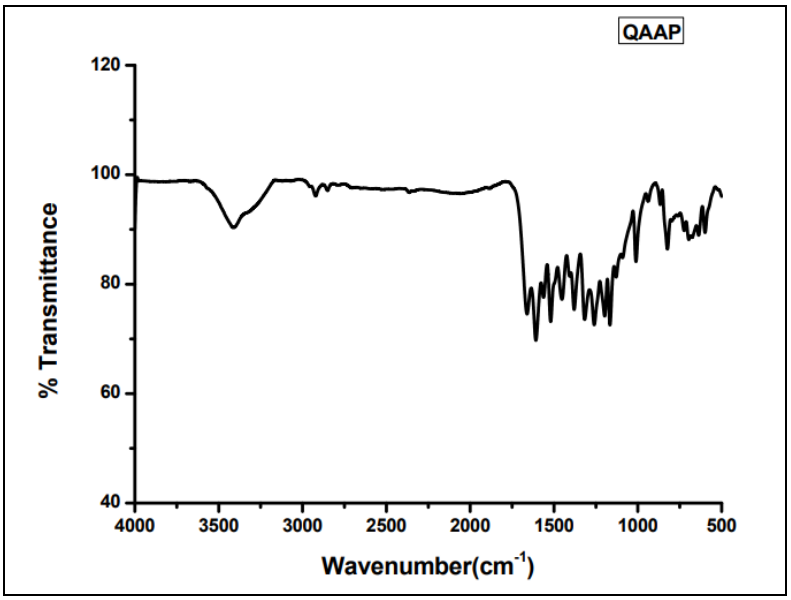


Figure 4.3.2 : IR spectra of QAAP

4.3.3 ULTRAVIOLET SPECTROSCOPY

UV-Visible Spectroscopy is used to study the electronic structure and its dynamics in atom and molecules. The UV –Visible spectra of compound QAAP was taken in methanol. The (figure 4.3.3) indicates the UV-Visible spectra of QAAP. The peak at 258 nm is due to $\pi - \pi^*$ transitions and peak at 373 nm is due to $n - \pi^*$ transitions (75) which clearly indicates the presence of aromatic group as well as azomethine group leading to the confirmation that compound has been successfully synthesized.

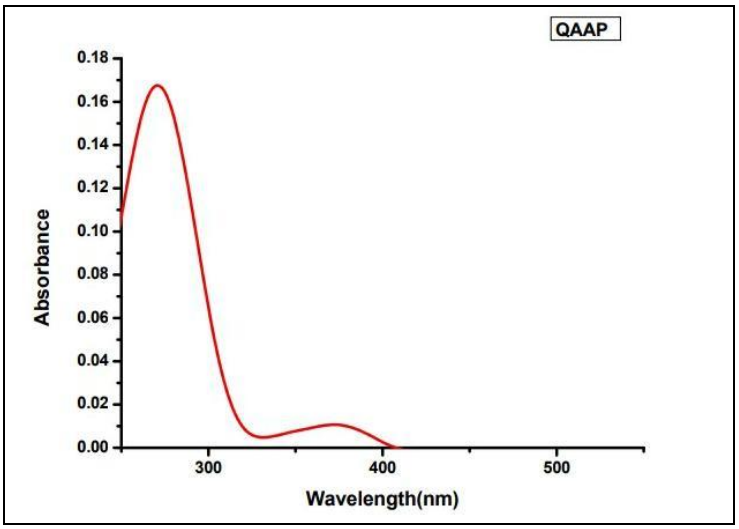


Figure 4.3.3 : UV-Visible spectra of QAAP

4.3.4 NMR SPECTROSCOPY

For unknown compounds, NMR can either match against spectral libraries or infer the basic structure directly. Once the basic structure is known, NMR can be used to determine molecular conformation in solution as well as study physical properties at the molecular level such as conformational exchange, phase changes, solubility, and diffusion. To achieve the desired results, a variety of NMR techniques are available.

Both ^{1}H and ^{13}C NMR of QAAP compound was taken in methanol using TMS as an internal standard (Figure 4.3.4.a and 4.3.4.b).

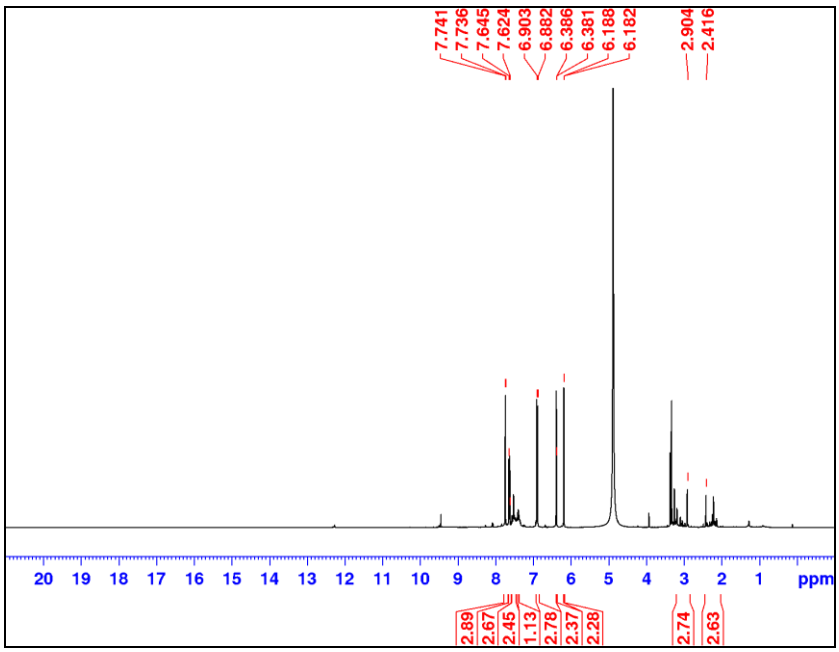


Figure 4.3.4. a : ^{1}H NMR of QAAP

^{1}H NMR(CH_3OH δppm) : 2.4(3H, C-H), 2.9(3H, C-H),6.1(2H, Ar-H), 6.2(1H,Ar-H), 6.3(3H,Ar-H), 6.8(2H,Ar-H), 6.9(1H,Ar-H), 7.4(1H,OH),7.6(2H, OH), 7.7(1H, OH), 7.8(1H, OH).

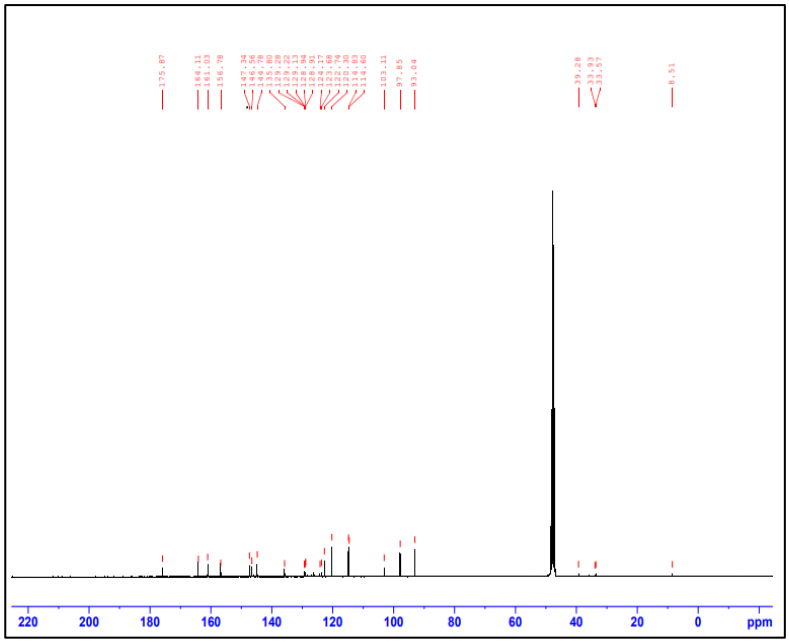


Figure 4.3.4. b ; ^{13}C NMR of QAAP

^{13}C NMR (CH_3OH δ ppm); 33.57(C-H) , 39.28(C-H), 93.04 (Ar-C), 97.85(Ar-C), 103.11(Ar-C), 114.60(Ar-C), 120.30 (Ar-C), 122.74(Ar-C), 123.68,124.27(ArC), 128.91,129.13(Ar-C), 135.80(Ar-OH), 144.78 (Ar-OH), 146.56 (Ar-C-OH), 156.78 (Ar-CO) , 161.03 (Ar-C-OH), 164.11(Ar-C-OH), 175.87 (Ar C=N).

4.3.5 FLOURESCENCE SPECTROSCOPY

In QAAP of 10^{-3}M concentration in methanol, for an excitation wavelength of 350nm shows an emission spectrum at 429 nm (Figure 4.3.5 a) indicating fluorescence with an emission in the blue region of the visible spectrum. This emission suggests the presence of aromatic or

conjugated systems in the compound, enabling electronic transitions that lead to fluorescence. Under a UV chamber, QAAP exhibits a minimal solid-state fluorescence (Figure 4.3.5 b), reflecting limited molecular interactions or restricted energy transfer in its aggregated form. However, when dissolved in solvents, its fluorescence properties are highly solvent-dependent. In DMF, QAAP shows a yellow-orange fluorescence, while in ethyl acetate, it displays a bright orange fluorescence (Figure 4.3.5 c). This behavior highlights QAAP's sensitivity to its surroundings and its potential use as a solvatochromic fluorescent probe.

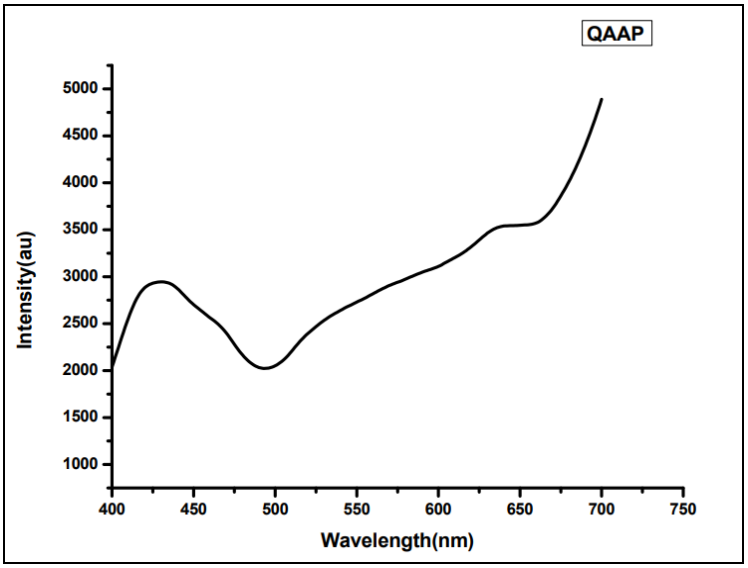


Figure 4.3.5 a ; Flourescence spectra of QAAP

Normal light

Figure 4.3.5 b Solid state flourescence Under uv chamber

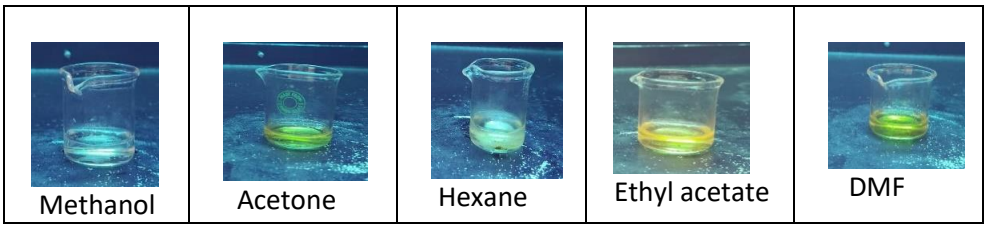


Figure 4.3.5.c liquid state fluorescence of QAAP under UV chamber

4.4 QUERCETIN + 2-AMINO 5 METHYL PYRIDINE (QAMP)

4.4.1 CHN ELEMENTAL ANALYSIS

Compound	Empirical Formula	Formula weight	Color	Observed and Calculated (%)		
				C	H	N
QAMP	$C_{21}H_{16}O_6N_2$	392	Light Yellow	64.45 (64.21)	3.86 (3.62)	7.16 (7.03)

Table 4.4.1 : Elemental analysis

The elemental analysis obtained is in good agrrement with assigned chemical formula.

4.4.2 INFRARED SPECTROSCOPY

The peaks shown in the IR Absorption spectrum give important information about the different functional groups present in the Schiff base. For QAMP the peak obtained in the range 1670cm^{-1} indicates the presence of imine groups (73) and the peak obtained in the range 3410 cm^{-1} the presence of OH groups (74).From this data we can confirm that schiff bases are successfully formed. The figure 4.4.2 indicates the IR spectra of schiff base QAMP.

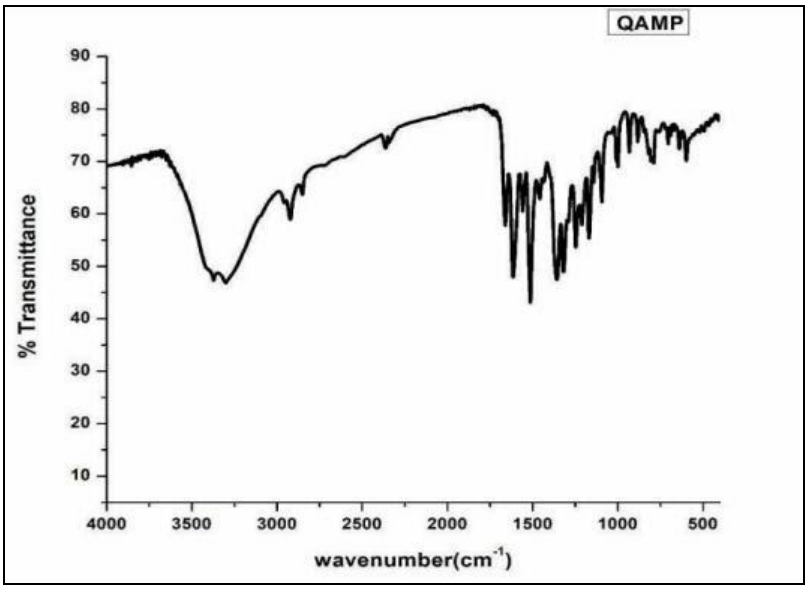


Figure 4.4.2 : IR spectra of QAMP

4.4.3 ULTRAVIOLET SPECTROSCOPY

UV-Visible Spectroscopy is used to study the electronic structure and its dynamics in atom and molecules. The UV –Visible spectra of compound QAMP was taken in methanol. The figure 4.4.3 indicates the UV-Visible spectra of QAMP. The peak at 256 nm is due to π – π* transitions and peak at 369 nm is due to n –π* transitions (75) which clearly indicates the presence of aromatic group as well as azomethine group leading to the confirmation that compound has been successfully synthesized.

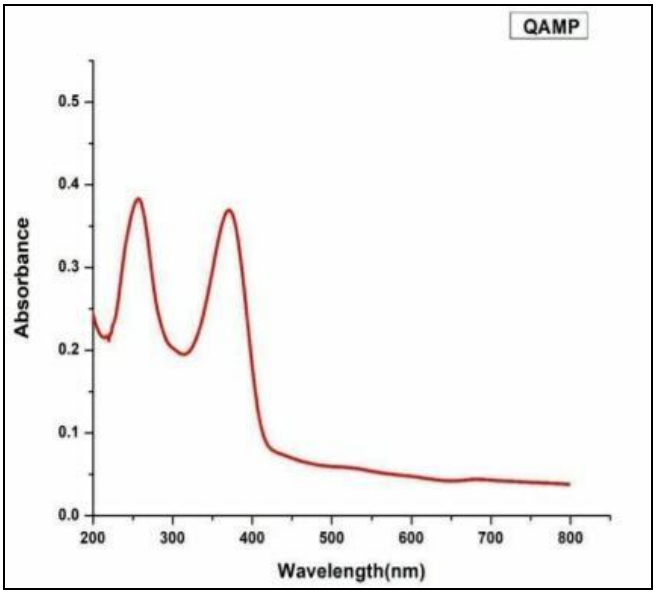


Figure 4.4.3 : UV-Visible spectra of QAMP

4.4.4 NMR SPECTROSCOPY

For unknown compounds, NMR can either match against spectral libraries or infer the basic structure directly. Once the basic structure is known, NMR can be used to determine molecular conformation in solution as well as study physical properties at the molecular level such as conformational exchange, phase changes, solubility, and diffusion. To achieve the desired results, a variety of NMR techniques are available

Both ^{1}H and ^{13}C NMR of QAMP compound was taken in methanol using TMS as an internal standard (Figure 4.4.4 a and 4.4.4.b).

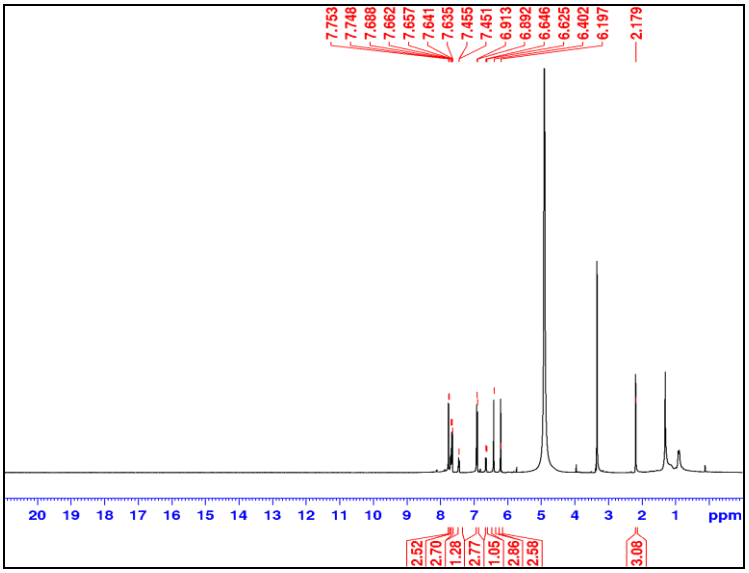


Figure 4.4.4 a : ^{1}H NMR of QAMP

^{1}H NMR(CH_3OH δppm): 2.1(3H, C-H), 6.2(1H, Ar-H), 6.4(2H, Ar-H), 6.6(1H, Ar-H), 6.8(2H, Ar-H), 6.9(1H, Ar-H), 7.2(1H, Ar-H),7.4(1H,OH), 7.6(2H, OH), 7.7(1H, OH),7.8(2H, OH).

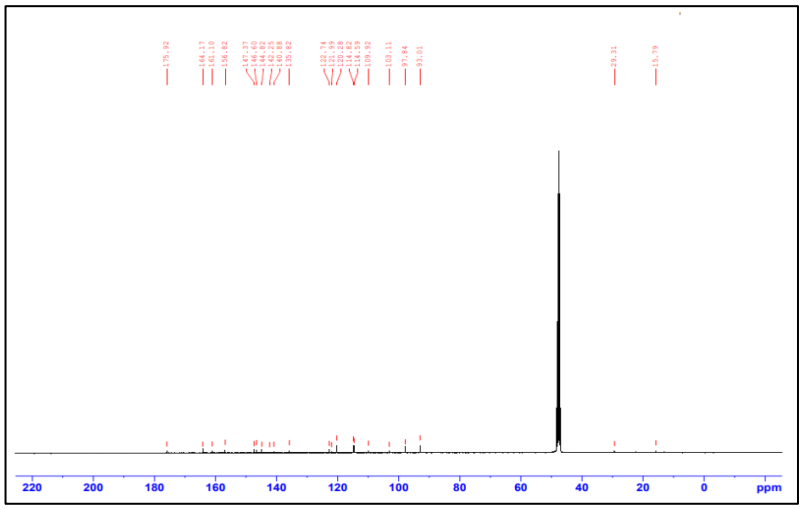


Figure 4.4.4. b ; ^{13}C NMR of QAMP

^{13}C NMR (CH_3OH δ ppm): 29.31 (C-H) ,93.01 (Ar-C), 97.84 (Ar-C), 103.12(Ar-C),109.92(Ar-C),114.59,114.82(Ar-C),120.28(Ar-C), 121.99(Ar-C),122.74(Ar-C), 135.82(Ar-OH) ,140.88(Ar-OH),144.81(Ar-OH), 146.60 (Ar-C-OH), 147.37(Ar-CO),156.82(Ar-CO), 161.10(Ar-C-OH), 175.92(ArC=N).

4.4.5 FLOURESCENCE SPECTROSCOPY

In QAMP of 10^{-3}M concentration at excitation wavelength of 350nm, gives a fluorescence spectrum having an emission wavelength of 429 nm (Figure 4.4.5 a) indicating emission in the blue region of the visible spectrum. Under a UV chamber, QAMP exhibits solid-state fluorescence with a green color (Figure 4.4.5 b). QAMP does not exhibit any noticeable color changes in different solvents under UV light (figure 4.4.5.c), indicating that its fluorescence properties are less influenced by solvent

polarity or interactions. This behavior highlights the rigidity or stability of QAMP's electronic environment, regardless of its phase or medium.

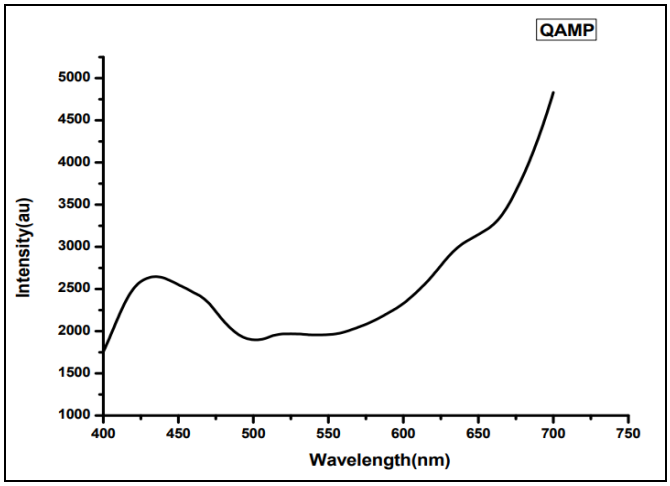


Figure 4.4.5.a ; Flourescence spectra of QAMP

Normal light

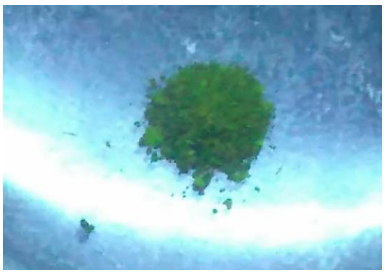


Figure 4.4.5 b ; Solid state flourescence under uv chamber

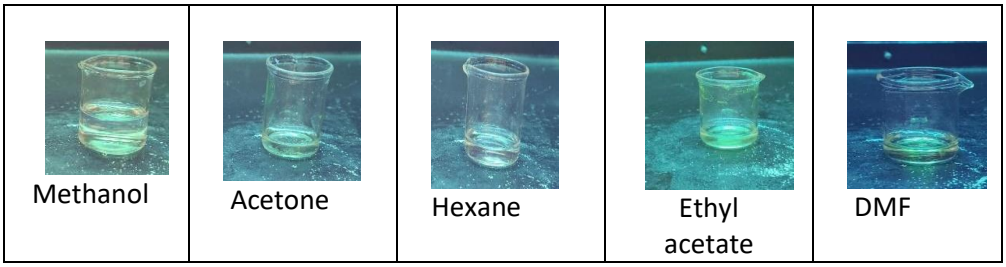


Figure 4.4.5.c ; liquid state fluorescence of QAMP under UV chamber

4.5 QUERCETIN + 2- AMINO 4- NITRO PHENOL (QANP)

4.5.1 CHN ELEMENTAL ANALYSIS

Compound	Empirical Formula	Formula weight	Color	Observed and Calculated (%)		
				C	H	N
QANP	$C_{21}H_{14}N_2O_9$	438.34	Greenish Yellow	57.54 (57.48)	3.22 (3.19)	6.39 (6.38)

Table 4.5.1 : Elemental analysis

The elemental analysis obtained is in good agrrement with assigned chemical formula.

4.5.2 INFRARED SPECTROSCOPY

The peaks shown in the IR Absorption spectrum give important information about the different functional groups present in the Schiff base. For QANP the peak obtained in the range 1661 cm^{-1} indicates the presence of imine groups (73) and the peak obtained in the range 3558 cm^{-1} the presence of OH groups (74).From this data we can confirm that schiff bases are successfully formed. The figure 4.5.2 indicates the IR spectra of schiff base QANP.

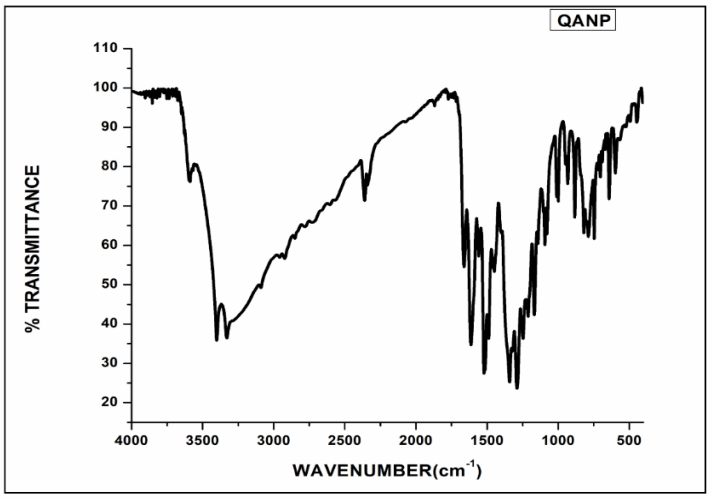


Figure 4.5.2 : IR spectra of QANP

4.5.3 ULTRAVIOLET SPECTROSCOPY

UV-Visible Spectroscopy is used to study the electronic structure and its dynamics in atom and molecules. The UV –Visible spectra of compound QANP was taken in methanol. The figure 4.5.3 indicates the UV-Visible spectra of QANP. The peak at 256 nm is due to $\pi - \pi^*$ transitions and peak at 372 nm is due to $n - \pi^*$ transitions (75) which clearly indicates the presence of aromatic group as well as azomethine group leading to the confirmation that compound has been successfully synthesized .

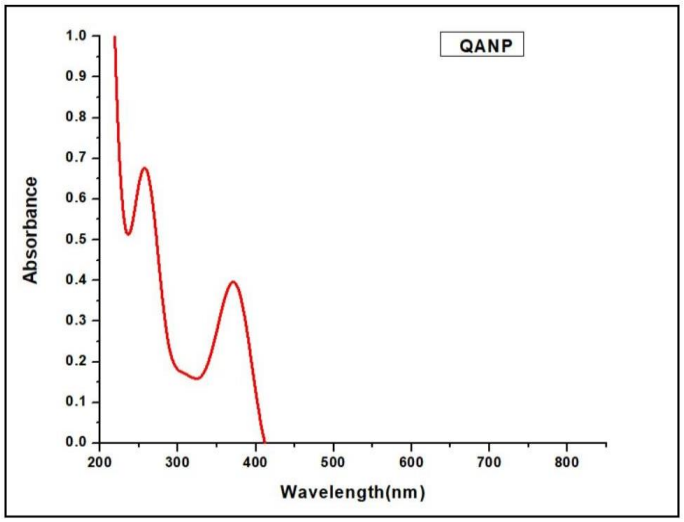


Figure 4.5.3 : UV-Visible spectra of QANP

4.5.4 NMR SPECTROSCOPY

For unknown compounds, NMR can either match against spectral libraries or infer the basic structure directly. Once the basic structure is known, NMR can be used to determine molecular conformation in solution as well as study physical properties at the molecular level such as conformational exchange, phase changes, solubility, and diffusion. To achieve the desired results, a variety of NMR techniques are available

Both ^{1}H AND ^{13}C NMR of QANP compound was taken in methanol using TMS as an internal standard (Figure 4.5.4 a and 4.5.4.b).

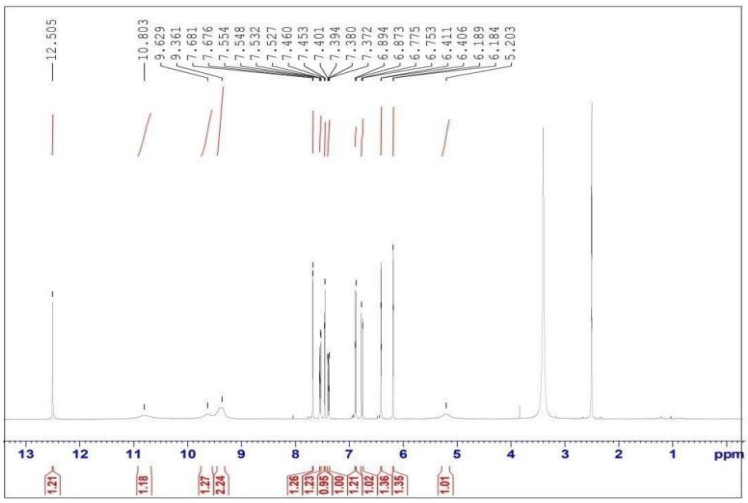


Figure 4.5.4 a : ^{1}H NMR of QANP

^{1}H NMR(DMSOδppm); 5.2(1H,Ar-H),6.2 (1H,Ar-H),6.4(1H,Ar-H), 6.8(IH,Ar-H), 6.9(1H,Ar--H), 7.45(1H,Ar-H) ,7.7(1H,Ar-H), 9.4(2H,Ar-OH),9.6 (1H, ArOH),10.8(1H,Ar-0H),12.5 (IH,Ar-OH).

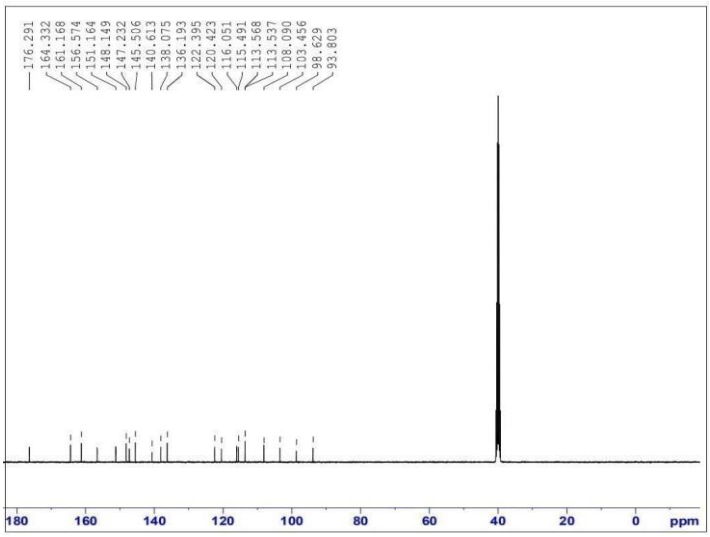


Figure 4.5.4 b : ^{13}C NMR of QANP

^{13}C NMR(DMSOδppm); 93.80 (Ar-C), 98.62 (Ar- C), 103.45(Ar-C),108.09(Ar-C),113.53(Ar-C),113-56 (Ar-C), 115.49 (Ar-C), 116.05 (Ar-C), 120.42(Ar-C),122.39 (Ar-C),136:19 (Ar-C-OH),138.07(Ar-C-OH),140.61(Ar-C-0H),145.50(Ar-C-0H), 147.03 (Ar-C-OH), 148.14 (Ar-C-OH), 151.16(Ar-C-OH),156.57 (Ar-C0), 176.29 (ArC=N).

4.5.5 FLOURESCENCE SPECTROSCOPY

For QANP of 10^{-3}M concentration in methanol, at an excitation wavelength of 350 nm gives a fluorescence spectrum at an emission wavelength in the range 400-700nm, indicating strong emission in the blue-violet region of the visible spectrum. Under a UV chamber, QANP displays green solid-state fluorescence. Also, the fluorescence appears green across all tested solvents under uv chamber (figure 4.5.5.c), highlighting the stability of its emissive behavior irrespective of solvent polarity or hydrogen-bonding capacity. This uniform fluorescence colour in various solvents suggests that the compound's excited-state properties are relatively insensitive to environmental changes, making it as a suitable probe for applications in stable fluorescence imaging or sensing.

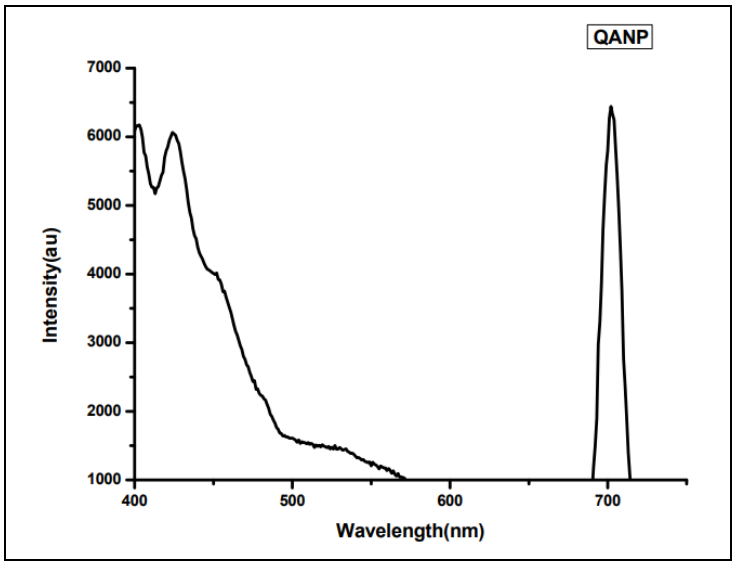


Figure 4.5.5. a : Flourescence spectra of QANP

Normal light

Figure 4.5.5 b Solid state flourescence under uv chamber

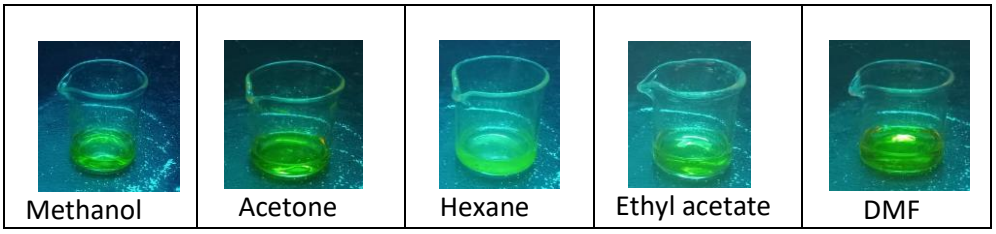
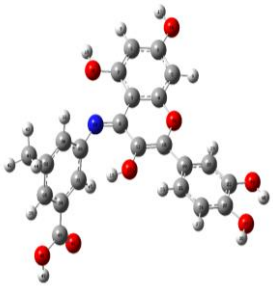
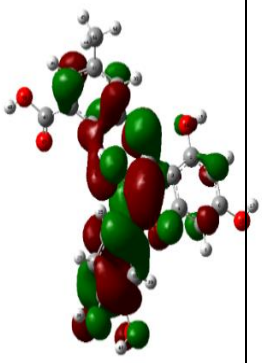
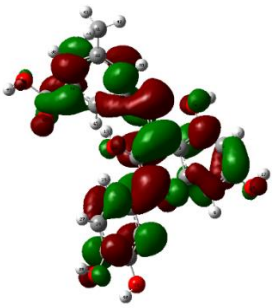


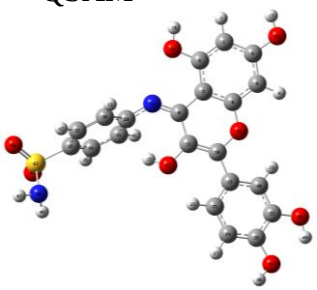
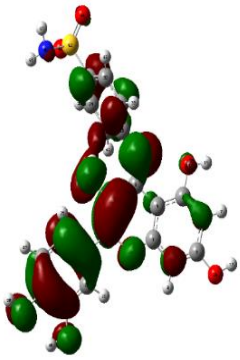
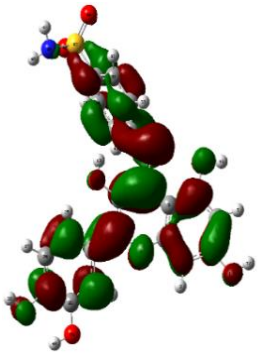
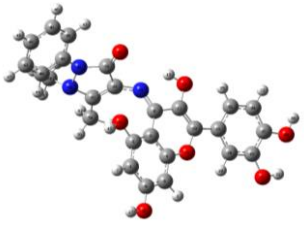
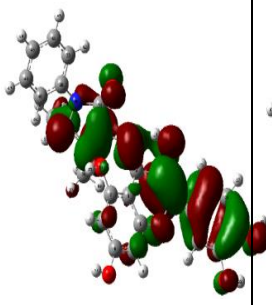
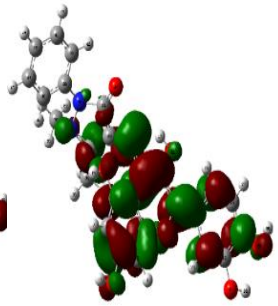
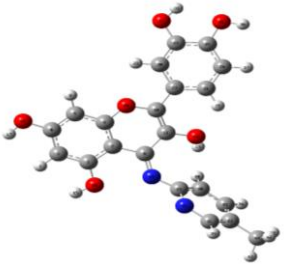
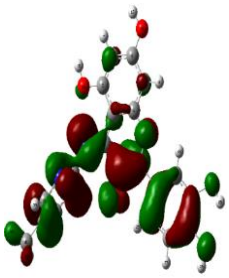
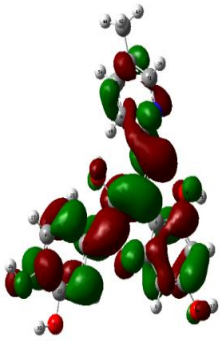
Figure 4.5.5.c liquid state fluorescence of QANP under UV chamber

4.6 ANTI- INFLAMMATORY ACTIVITIES- THEORETICAL STUDIES

4.6.1 DFT Calculations

The geometry optimization of the ligands QABD, QSAM ,QAAP, QAMP, QANP for anti-inflammatory study were obtained by DFT calculations using Gaussian 09 software at B3LYP/6-31- G (d,p) level. The ground state optimized structures and HOMO and LUMO orbitals (76) of the selected ligands are given in table 4.6.1.a

QUERCETIN IMINES (OPTIMIZED STRUCTURES)	HOMO	LUMO
QABD  Energy= -1543.24657739 au		

QSAM  Energy= -1919.27857565 au		
QAAP  Energy= -1694.21455500 au		
QAMP  Energy= -1370.68164086 au		

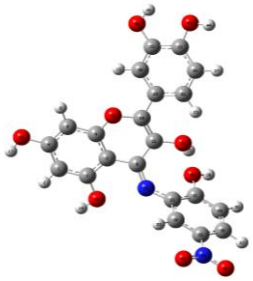
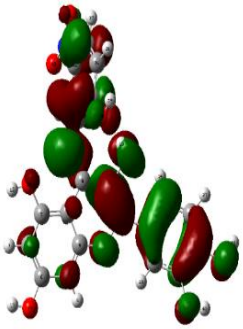
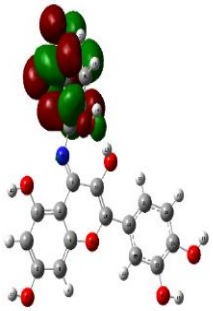
QANP  Energy = -1595.06985460 au		

Table 4.6.1.a The ground state optimized structures and HOMO and LUMO orbitals of the selected ligand.

The HOMO-LUMO energies of the selected ligands are given in table 4.6.1.b

QUERCETIN IMINES	HOMO(au)	LUMO(au)	HOMO-LUMO Energy gap (au)	HOMO-LUMO Energy gap in eV
QABD	-0.18924	-0.05276	0.13648	3.712256
QSAM	-0.19756	-0.06222	0.13534	3.681248
QAAP	-0.18100	-0.05366	0.12734	3.463648
QAMP	-0.19037	-0.05244	0.13793	3.751696
QANP	-0.19382	-0.06966	0.12416	3.377152

Table 4.6.1.b HOMO-LUMO energies in eV

By DFT calculations we get informations about the optimized structures, HOMO-LUMO orbitals and their corresponding energies .The above data helps in predicting how well a ligand

binds into the active site of inflammatory target. HOMO and LUMO energies provide idea about stability and reactivity of ligands. A good balance of HOMO-LUMO energy gap indicates optimal binding interactions without excessive toxicity. A small HOMO-LUMO energy gap indicates higher reactivity, making the ligand more likely to interact with target molecules. A large band gap suggests a stable ligand with lesser reactivity. So from the above table we can conclude that all of the selected ligands shows a good balance of HOMO-LUMO energy gap indicating optimal binding interactions without excessive toxicity.

4.6.2 Molecular docking

The inflammation causing target protein molecule 5U73N were downloaded from RCSB protein data bank. Then the optimized schiff bases were docked with protein 5U73N using Auto dock Vina software. As a result informations about types of ligand – protein interactions,their binding affinity and there by stability of synthesized imine compounds can be compared.

The figure 4.6.2.a – 4.6.2.e represents the docking interaction of optimized imine compounds with target protein 5U73N.

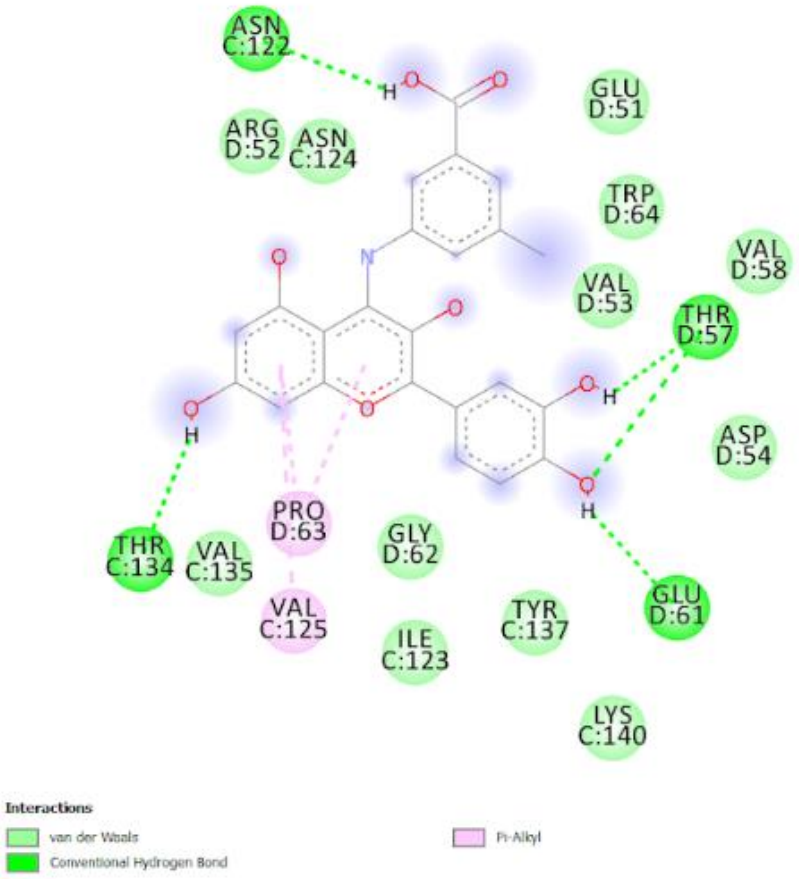


Figure 4.6.2.a ; Docking interaction of imine QABD with protein 5U73N

In QABD the optimized imine compounds and target protein 5U73N interacts through Vanderwal's forces and conventional hydrogen bonding.

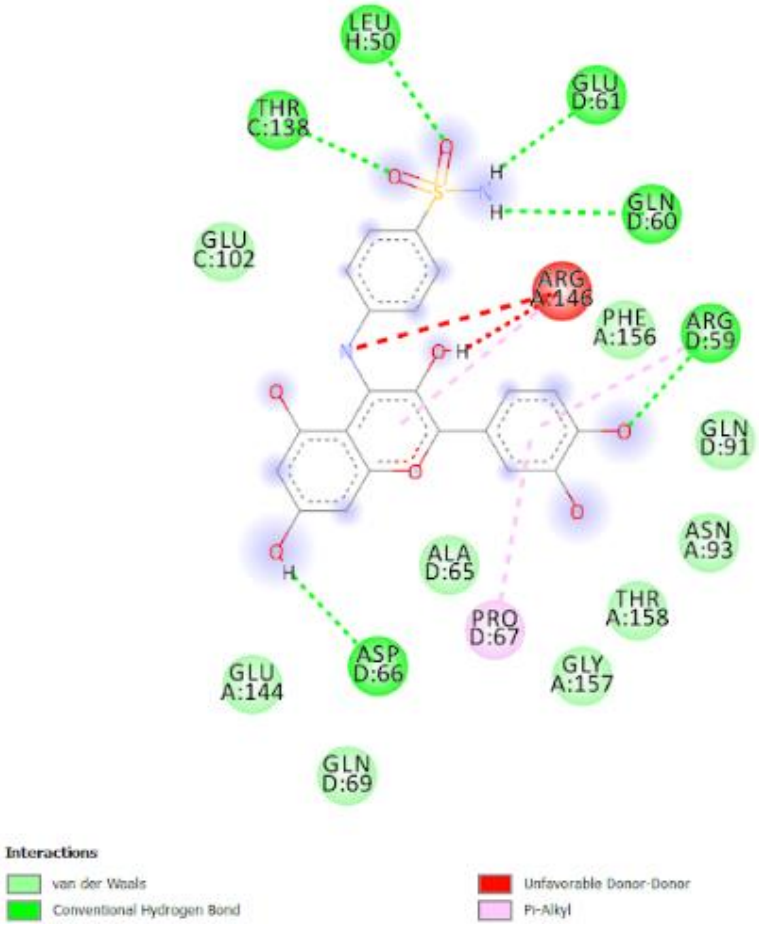


Figure 4.6.2.b ; Docking interaction of imine QSAM with protein 5U73N.

In QSAM the optimized imine compounds and target protein 5U73N interacts through Vanderwal's forces and conventional hydrogen bonding.

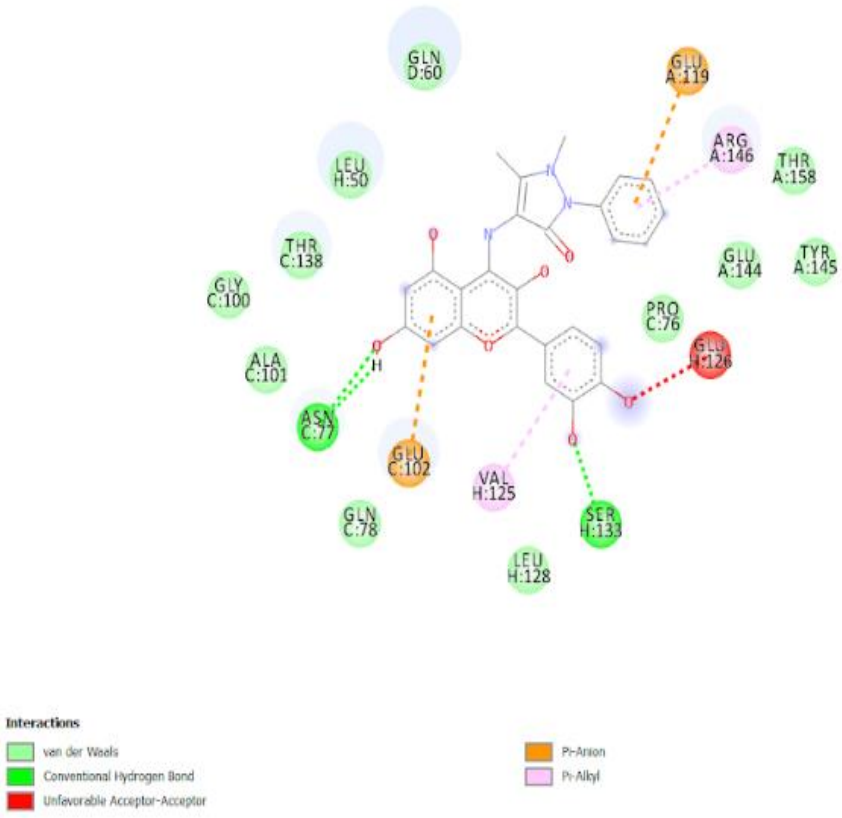


Figure 4.6.2.c ; Docking interaction of imine QAAP with protein 5U73N

In QAAP the optimized imine compounds and target protein 5U73N interacts through Vanderwal's forces and conventional hydrogen bonding.

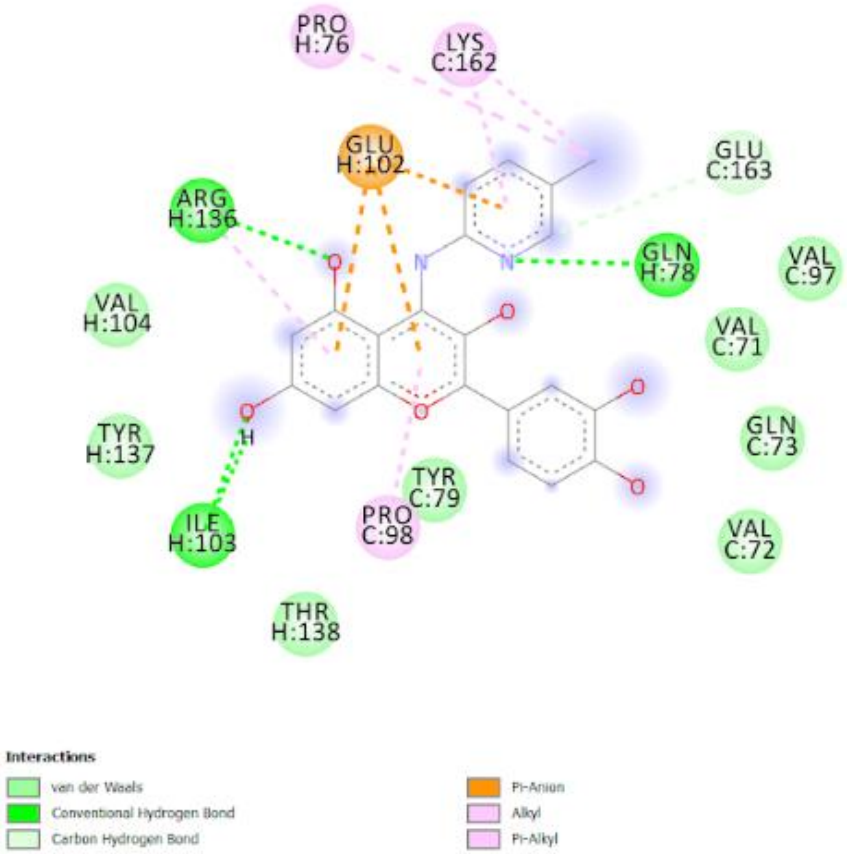


Figure 4.6.2.d; Docking interaction of imine QAMP with protein 5U73N

In QAMP the optimized imine compounds and target protein 5U73N interacts through Vanderwal's forces, conventional hydrogen bonding and carbon hydrogen bond.

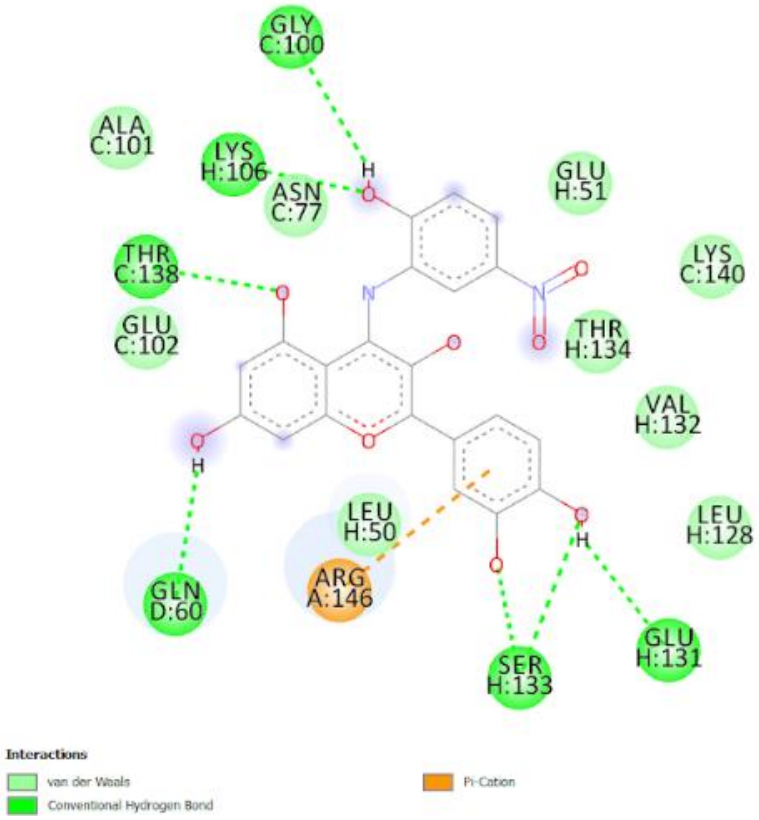


Figure 4.6.2.e ; Docking interaction of imine QANP with protein 5U73N

In QANP the optimized imine compounds and target protein 5U73N interacts through Vanderwal's forces and conventional hydrogen bonding .

Table 4.8 below represents the binding affinity of optimized imine compounds on docking interaction with protein 5U73N

IMINES	**BINDING AFFINITY (kcal/mol)**
QABD	**-9.4**
QSAM	**-9.0**
QAAP	**-9.3**
QAMP	**-8.5**
QANP	**-8.8**

From the above table we can conclude that all of the synthesized imines exhibits a high binding affinity with the protein 5U73N.Among them QABD shows a high binding affinity. High binding affinity shows the presence of strong molecular interactions between imine compounds and targeted protein, indicating that it can be used for inhibiting the functions of enzymatic mediators and this inhibition reduces the synthesis of prostaglandins, a key molecule involved in inflammation. Also upon binding imines may induces conformational changes in the protein structure which can disrupt the above signaling pathway involved in inflammation.

4.7 ANTIINFLAMMATORY ACTIVITIES – EXPERIMENTAL STUDY

The anti-inflammatory activity of the quercertin based imines QABD, QSAM, QAAP, QAMP, QANP were studied using the HRBC membrane stabilization method (77) and are given in figure 4.7.1 – 4.7.5.

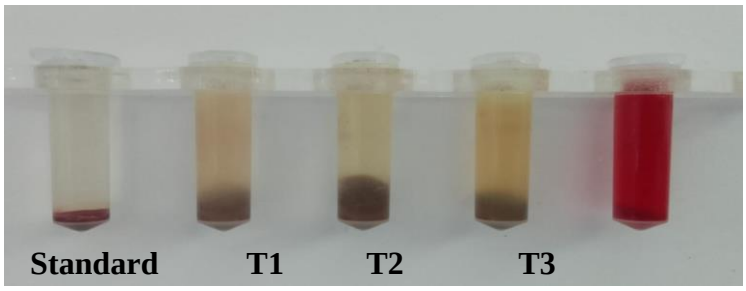


Figure 4.7.1 HRBC membrane stabilization assay of samples- QABD

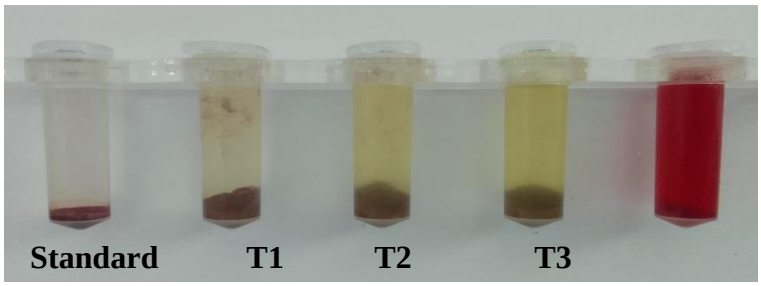


Figure 4.7.2 HRBC membrane stabilization assay of samples- QSAM

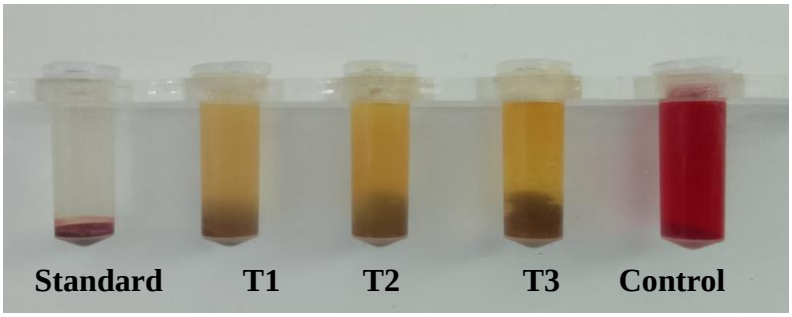


Figure 4.7.3 HRBC membrane stabilization assay of samples- QAAP

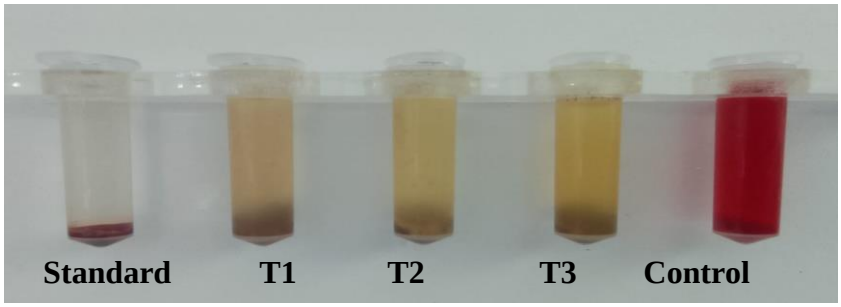


Figure 4.7.4 HRBC membrane stabilization assay of samples- QAMP

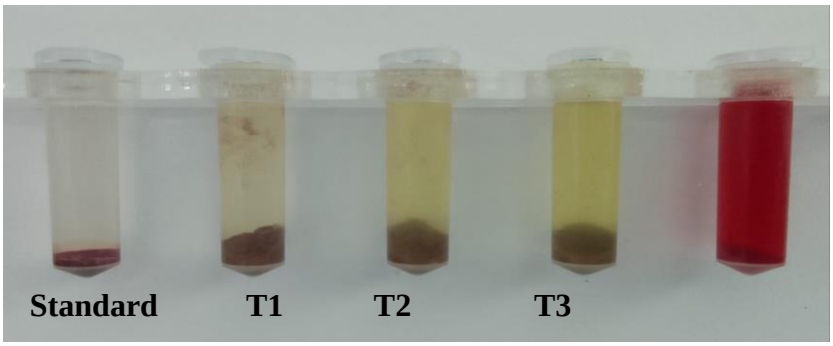


Figure 4.7.5 HRBC membrane stabilization assay of samples- QANP

The % inhibition of the synthesized compounds at five different concentrations were calculated. The % of inhibition values of the same were tabulated in tables 4.7.1 – 4.7.5. To find the IC_{50} value of the Quercetin based imines a graph was plotted with the values % of inhibition against the concentration of the sample which is shown in the figures 4.7.6 -4.7.10

Compound	Concentration of sample (mg/ml)	% of Inhibition
QABD	0.5	49.56
	2.5	70.48
	5	71.73
	10	73.39
	12.5	74.45

Table 4.7.1 Antinflammatory activity of QABD

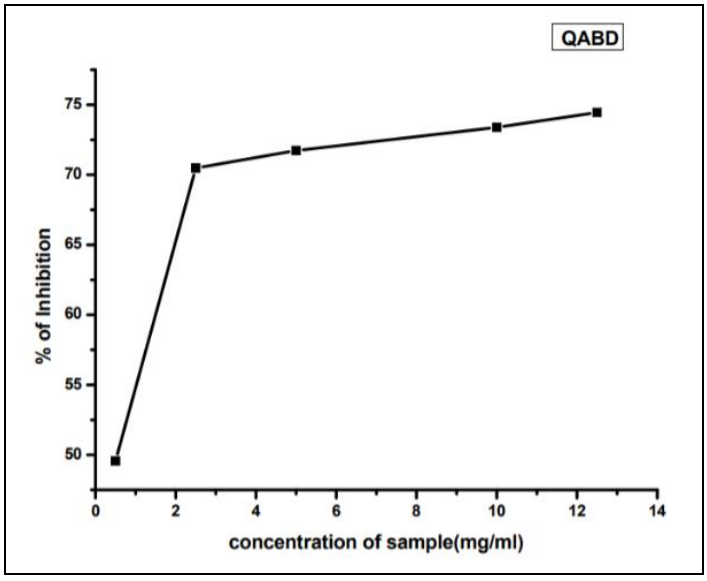


Figure 4.7.6 Antinflammatory activity of QABD

Compound	Concentration of sample (mg/ml)	% of Inhibition
QSAM	0.5	52.75
	2.5	68.81
	5	72.97
	10	74.22
	12.5	76.34

Table 4.7.2 Antinflammatory activity of QSAM

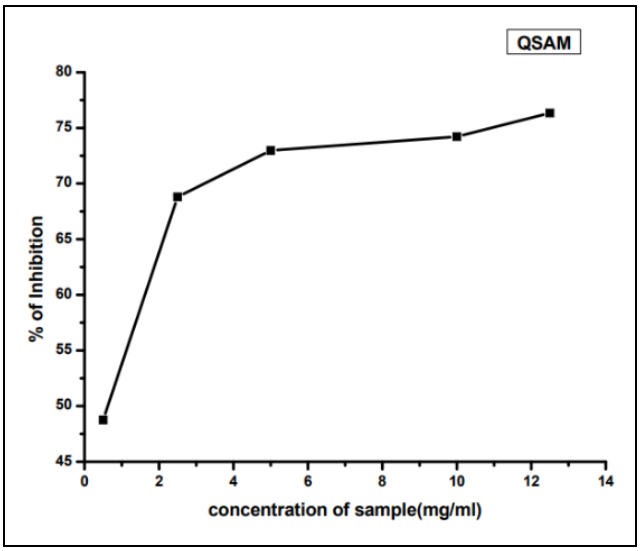


Figure 4.7.7 Antinflammatory activity of QSAM

Compound	Concentration of sample (mg/ml)	% of Inhibition
QAAP	0.5	48.07
	2.5	55.09
	5	59.25
	10	60.91
	12.5	62.08

Table 4.7.3 Antinflammatory activity of QAAP

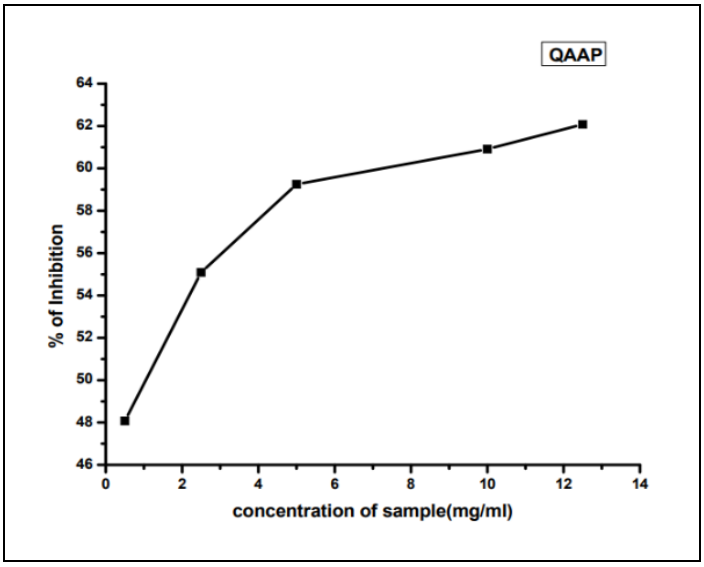


Figure 4.7.8 Antinflammatory activity of QAAP

Compound	Concentration of sample (mg/ml)	% of Inhibition
QAMP	0.5	49.68
	2.5	69.23
	5	71.31
	10	73.8
	12.5	74.52

Table 4.7.4 Antinflammatory activity of QAMP

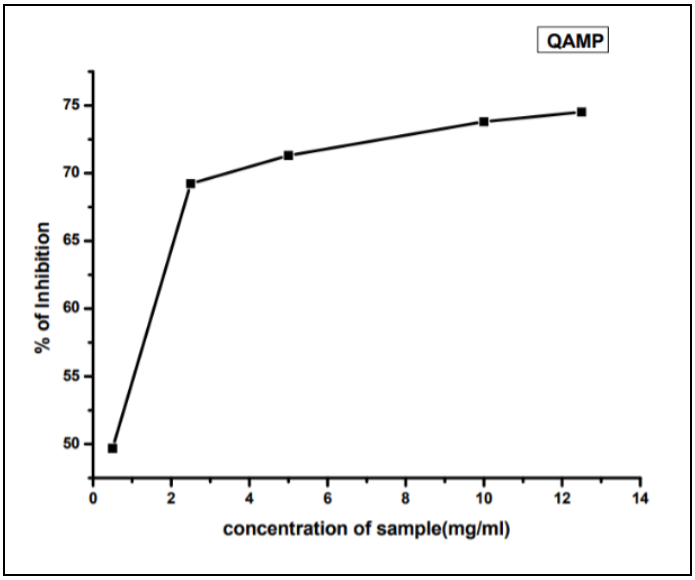


Figure 4.7.9 Antinflammatory activity of QAMP

Compound	Concentration of sample (mg/ml)	% of Inhibition
QANP	0.2	26.3
	2.5	34.7
	5	40.4
	10	54.9
	12.5	65.4

Table 4.7.5 Antinflammatory activity of QANP

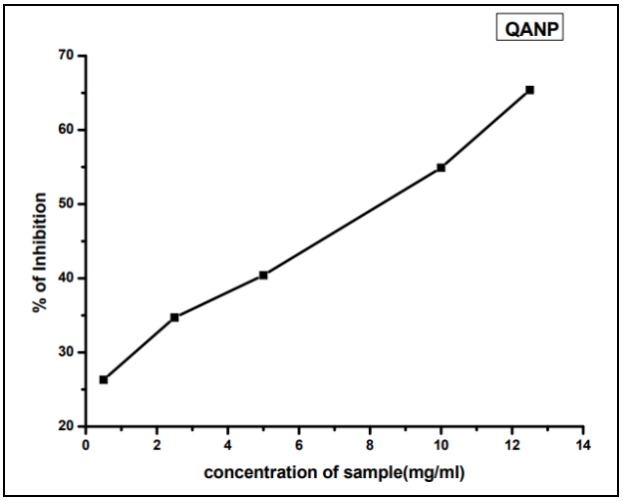


Figure 4.7.10 Antinflammatory activity of QANP

IC_{50} Values of imines

IC_{50} value represents the concentration at which a substance exerts half of its maximal inhibitory effect. This value is typically used to characterize the effectiveness of an antagonist in inhibiting a specific biological or biochemical process (78) . Low IC_{50} value means that the drug is potent at low concentrations and thus will show lower systemic toxicity when administered to the patient.

The IC_{50} values of the compounds QABD, QSAM, QAAP,QAMP and QANP were obtained from the graph and tabulated. The IC_{50} values of QABD, QSAM, QAAP, QAMP and QANP are given in table 4.7.6

COMPOUND	**IC_{50} VALUES (mg/ml)**
QABD	0.5617
QSAM	0.5851
QAAP	1.0854
QAMP	0.5354
QANP	8.5931
Ibuprofen	76.3273

Table 4.7.6 IC_{50} values of imines

The IC_{50} values of synthesized Quercetin based imines were compared with Ibuprofen, a widely used non steroidal anti-inflammatory drug. The IC_{50} values of Ibuprofen was 76.3273 mg/ml , whereas IC_{50} values of all

others are much lower than that of Ibuprofen which indicates that synthesized imine compounds are much effective than that of Ibuprofen,a non steroidal anti-inflammatory drug. And among the five imines QAMP shows better anti-inflammatory activity due to its lower IC_{50} value.

Chapter 5

Conclusions

In the present work five novel Quercetin based imines were synthesized by the condensation of Quercetin with 3-amino benzoic acid, sulphanilamide, 4-amino antipyrene, 2-amino 5-methyl pyridine and 2-amino 4-nitro phenol by reflux method and are abbreviated as QABD, QSAM, QAAP, QAMP, QANP. The synthesized imines are then characterized by following techniques which include Elemental analysis, UV-Visible, IR, ^{1}H NMR, ^{13}C NMR and Flourescence spectroscopic method. All these studies give good results for the formation of Quercetin imine compounds. By fluorescence spectroscopy it is also observed that most of our synthesized imines shows a strong solid state fluorescence under uv chamber.

The anti-inflammatory activity of synthesized imines are also evaluated by both theoretical study as well as by experimental study. To determine anti-inflammatory activities by computational method we used Auto dock vina software. DFT calculations using Gaussian 09 software at B3LYP/6-31- G (d,p) level helped in optimizing the selected imine compounds , analyzing their HOMO-LUMO energies and thereby providing insights into stability and reactivity of compounds. Therefore by DFT calculation we can conclude that all of the selected imines shows a good balance of HOMO-LUMO energy gap indicating optimal binding interactions without

excessive toxicity. Molecular docking were performed using Autodock vina software helped in understanding the interaction patterns and binding energy between optimized imines and protein molecule 5U73N which is downloaded from protein data bank. By molecular docking we concluded that all of our imines shows a high binding affinity. Among them QABD shows a high binding affinity of -9.4 kcal/mol which showing a strong interaction between imine QABD and the target protein, indicating that it can be used for inhibiting the functions of enzymatic mediators, and this inhibition reduces the synthesis of prostaglandins, a key molecule involved in inflammation. . Also a strong binding of imines may induces conformational changes in the protein structure which can disrupt the above signaling pathway involved in inflammation.

In experimental approach we studied anti-inflammatory activities by HRBC membrane stabilization method. From this study IC_{50} values of synthesized Quercetin imines were obtained. The IC_{50} values of QABD, QSAM, QAAP, QAMP and QANP are 0.5617 mg/ml, 0.5851 mg/ml , 1.0854 mg/ml , 0.5354mg/ml and 8.5931 mg/ml respectively. From this study it is found that synthesized imines were effective than Ibuprofen,a non steroidal anti-inflammatory drug. And among the five imines QAMP shows better anti-inflammatory activities due to its lower IC_{50} value.

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