PROJECT REPORT

On

"SYNTHESIS, CHARACTERIZATION & ANTIBACTERIAL STUDY OF SULPHANILAMIDE SCHIFF BASES"

Submitted by

TREASA DIYA

(AM20CHE015)

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

DEPARTMENT OF CHEMISTRY
AND
CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)
ERNAKULAM

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DEPARTMENT OF CHEMISTRY
AND
CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)

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M.Sc. CHEMISTRY PROJECT REPORT

Name	**: Treasa Diya**
Register Number	**: AM20CHE015**
Year of Work	**: 2021 - 2022**

This is to certify that the project **"SYNTHESIS, CHARACTERIZATION & ANTIBACTERIAL STUDY OF SULPHANILAMIDE SCHIFF BASES"** is the work done by **TREASA DIYA.**

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Submitted to the Examination of Master's degree in Chemistry

Date: 9/6/2022

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CERTIFICATE

This is to certify that the project work entitled "**SYNTHESIS, CHARACTERIZATION & ANTIBACTERIAL STUDY OF SULPHANILAMIDE SCHIFF BASES**" is the work done by **Treasa Diya** under the guidance of **Dr. Shanty A. A, Assistant Professor,** Department of Chemistry and Centre for Research, St. Teresa's College, Ernakulam in partial fulfilment of the award of the Degree of Master of Science in Chemistry at St. Teresa's College, Ernakulam affiliated to Mahatma Gandhi University, Kottayam.

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Project Guide

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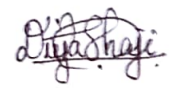
DECLARATION

I hereby declare that the project work entitled "**SYNTHESIS, CHARACTERIZATION & ANTIBACTERIAL STUDY OF SULPHANILAMIDE SCHIFF BASES**" 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.



TREASA DIYA

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Treasa Diya

Contents

Chapter 1

INTRODUCTION

1.1 SCHIFF BASE

Schiff base named after Hugo Schiff, are formed when any primary amine reacts with an aldehyde or a ketone under specific conditions. Structurally, a Schiff base (also known as imine or azomethine) is a nitrogen analogue of an aldehyde or ketone in which the carbonyl group has been replaced by an imine or azomethine group [1].

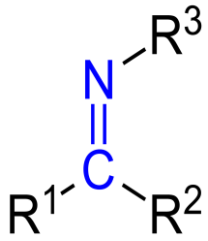

Fig 1: General structure of Schiff Base

Schiff base are some of the most widely used organic compounds. They are used as pigments and dyes, catalysts, intermediates in organic synthesis, and as polymer stabilizers. Schiff bases have also shown to exhibit a broad range of activities, including antifungal, antibacterial, antimalarial, anti-proliferative, anti-inflammatory, antiviral and antipyretic properties [1].

1.1.1 Synthesis of Schiff bases

The first preparation of imines was reported in the 19th century by Schiff (1864). Since a variety of methods for the synthesis of imines have been described. The classical synthesis reported by Schiff involves the condensation of a carbonyl compound with an amine under azeotropic distillation [1].

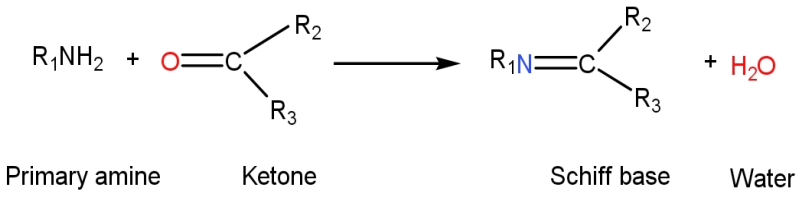

Fig 2: General reaction of synthesis of Schiff Base

1.1.2 Biological applications of Schiff base

Schiff bases have many important applications in the field of medicine and pharmaceuticals. Schiff base has potential biological interest, not only have they played a seminal role in the development of modern chemistry, but also, they can also be found at key points in the development of inorganic biochemistry, catalysis, optical materials and also in other fields [11]. Anti-bacterial activities of substituted Schiff bases like nitro & phenyl derivatives possess more active but activity was lesser than the standard drug. Nitro and halo derivatives of Schiff

bases are reported to have antimicrobial and antitumor activities. Schiff bases have anti-inflammatory, allergic inhibitors reducing activity radical scavenging, analgesic and anti-oxidative action. Schiff bases, derived mostly from variety of heterocyclic rings, may reported to possess a broad spectrum of pharmacological activities with a wide variety of biological properties [12]. Development of new chemotherapeutic Schiff bases is now attracting the attention of medicinal chemist. They are known to exhibit a variety of potent activities. The pharmacologically useful activities include antibacterial, anticonvulsant, anti-inflammatory, anticancer, anti-hypertensive, anti-fungal, antipyretic, antimicrobial, anti-HIV, cytotoxic activity, hypnotic and herbicidal activities [13]. Schiff bases appear to be an important intermediate for a number of enzymatic reactions that involve the interaction of an enzyme with an amino or a carbonyl group of the substrate. One of the most important types of catalytic mechanism is the biochemical process which involves the condensation of a primary amine in an enzyme usually that of a lysine residue, with a carbonyl group of the substrate to form an imine, or Schiff base. Schiff bases have been utilized as synthons in the preparation of a number of industrial and biologically active compounds via ring closure, cycloaddition, and replacement reactions.

1.1.2.1 Antibacterial activity

Schiff bases derived from salicylaldehyde show potent antibacterial activities, like N-(salicylidene)-2-hydroxyaniline reported as antituberculosis, while Schiff bases of 5-chlorosalicylaldehyde show enhanced antibacterial activity against Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), and Micrococcus

luteus (M. luteus) [14]. This shows effective activity against the infectious bacteria. Schiff bases are identified as promising antibacterial agents. Schiff bases containing 2, 4-dichloro-5- fluorophenyl moieties also take part in effective inhibition of bacterial growth [15]. Schiff base of Isatin derivatives (Figure 1.2) shown anti-HIV and antibacterial activity. Wadher et al. [16] reported the Schiff bases of 4, 4'- diaminodiphenylsulphone (a) and substituted 2-azetidinone (b), compounds shown to be more potent anti-microbial agent.

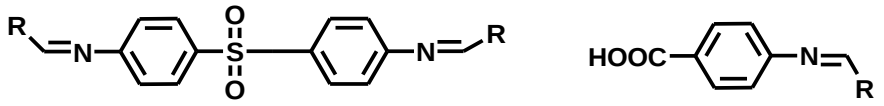

Fig 3: Schiff base of Isatin derivatives

1.1.2.2 Anticancer activity

Schiff bases and their metal complexes were reported as anticancer activities; Cu complexes with vanillin Schiff bases [18] and 5-dimethyl-2-phenyl-4-[(pyridin-2-ylmethylene)-amino]-1,2-dihydro-pyrazol-3-one Schiff bases shown good anticancer activities. Some of the bis-Schiff base analogs of chiral gossypol (Figures 1.4) (a) were reported by Zhang et al. [19] as anti-cancer agents. Chetan et al. [20] synthesized Schiff base compounds with piperazine (b) in linker region and hydroxamate as Zinc Binding Group (ZBG). They were screened against three cancer cell-lines against HL60, human promyelocytic leukemia cell-line.

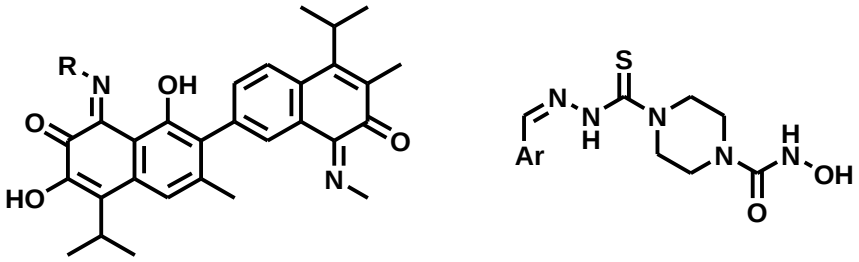

Fig 4: Schiff bases of chiral gossypol (a) & piperazine (b)

Mohsen et al. [21] found the cytotoxic effect of sulfapyridine polyhydroxyalkylidine (or arylidene) Schiff's bases (Figure 1.5 (a)), against breast carcinoma cell lines MCF7 and cervix carcinoma cell line HELA.

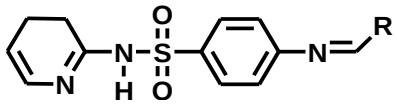

Fig 5: Schiff base of arylidene (a)

1.1.2.3 Antiviral activity

Schiff bases can play a vital role due to their reported antiviral nature. Schiff bases which can be derived from isatin and bisisatin are reported to show activities against different strains of viruses [22]. Schiff bases derived from Ziagen were reported as anti-HIV therapy [23]. Schiff bases of 2-phenylquinazoline4-(3)-H-one was reported to show antiviral activity against some strains of viruses like feline corona virus, influenza viruses, and herpes simplex virus [24]. Schiff base ligand of isatin marked antiviral activity, and

according to this fact is very useful in the treatment of HIV treatment [25]. Gossypol derivatives having high antiviral activity. While increase gossypol, it used in medical therapy is replaced by its derivatives, because of their much lower toxicity. Kumar et al. [26] reported the antiviral activity of 2-hydroxy substitution on a series of 3-(benzylideneamino)-2- phenylquinazoline-4(3H)-one (Figure 1.6) (a).

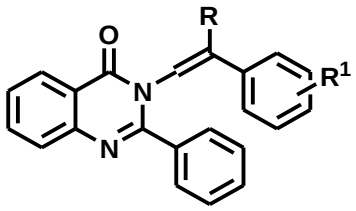

Fig 6: 3-(benzylideneamino)-2- phenylquinazoline-4(3H)-one (a)

1.1.2.4 Anti-inflammatory activity

Schiff bases that derived from 2-(2,6-dichloroanilino) and 4-amino-1,5-dimethyl2-phenylpyrazol-3-one [27] have been reported as anti-inflammatory activities [28]. The transition metal complexes of Schiff bases containing aldose group have also been reported for anti-inflammatory activities [29]. Schiff bases of 2-amino-5-aryl-1, 3, 4-thiadiazole derivatives (Figure 1.7 (a) with different aromatic aldehyde were reported as analgesic, anti-inflammatory, anti-bacterial (Staphylococcus aureus and E. coli) and antitubercular activity (Mycobacterium tuberculosis) by Pandey et al. [30].

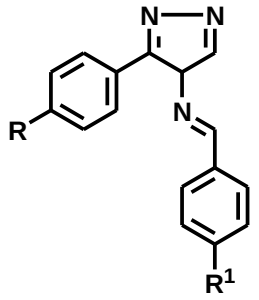

Fig 7: Schiff bases of 2-amino-5-aryl-1, 3,4-thiadiazole derivatives (a)

Analgesic and ulcerogenic activities of novel Schiff base (Figure 1.8)(a) were reported by Ramchandran et al. [31], Zhou et al.[32] discovered anti-inflammatory properties of novel Schiff's bases which can be able to treat chronic pain from inflammation.

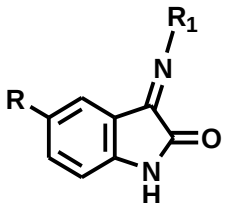

Fig 8: Schiff base analgesic and ulcerogenic

1.1.2.5 Anti-depressant activity

Pharmacological evaluation of N'-[(1Z) -(substituted aromatic) methylidene] pyridine-4-carbohydrazides as anti-depressants agent reported by Thomas et al [33]. Compounds N' - [(1Z) - (2, 5 – dimethoxyphenyl) methylidene] pyridine – 4 – carbohydrazides (Figure 1.9 (a) with 2,5-dimethoxy substitution on the aryl

ring and para-nitro substitution on the aryl ring exhibited the highest anti-depressants activity.

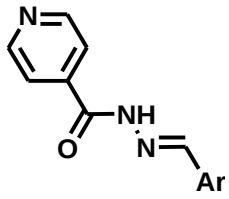

Fig 9: Schiff base of carbohydrazides (a)

1.1.2.6 Antimalarial activity

Human malaria mainly caused by four species of the genus such as Plasmodium (P. falciparum, P. vivax, P. ovale and P. malariae). Schiff bases are interesting compounds, which could be part of antimalarial drugs. The compound Ancistrocladidine (Figure 1.10 (a) which is a secondary metabolite produced by plants of the family Ancistrocladaceae and Dioncophyllaceae, which contain an imine group in a molecular chain. Cryptolepine, valid indolchinoline alkaloid, isolated from African plant Cryptolepis sanguinolenta, which may also use in the treatment of malaria, it is the product of multi-stage reaction, in which Schiff base is involved [34].

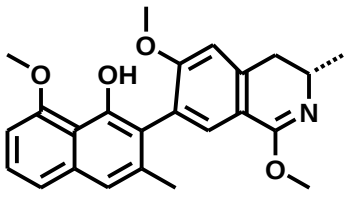

Fig 10: Schiff base of Ancistrocladidine (a)

1.1.2.7 Anti-oxidant activity

The production of reactive oxygen species (ROS) increases with the passage of time, in the human body and leads to many physiological disorders including cardiovascular diseases. Schiff bases and their metal complexes play an important role in the production of reactive oxygen species i.e., antioxidant properties [35]. In vitro antioxidant property of Schiff's bases of benzocoumarin (Figure 1.11 (a)).

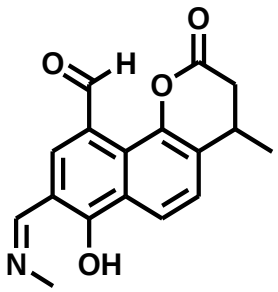

Fig 11: Schiff bases of benzocoumarin(a)

Schiff base structure provides extraordinary reversibility with changing pH values and the stability of the bonds decreases as the pH decreases, because of this feature it is highly preferable for specific pH triggered drug release. By controlled drug release could minimize the unwanted side effects, protect drugs from enzymatic degradation, and allow stimulus responsive release or targeted release.

1.2 SULPHANILAMIDE

Sulphanilamide (also spelled sulfanilamide) is a sulfonamide antibacterial drug. Chemically, it is an organic compound consisting of an aniline derivatized with a sulfonamide group. Powdered sulfanilamide was used by the Allies in World War II to reduce infection rates and contributed to a dramatic reduction in mortality rates compared to previous wars. Sulfanilamide is rarely if ever used systemically due to toxicity and because more effective sulfonamides are available for this purpose. Modern antibiotics have supplanted sulfanilamide on the battlefield; however, sulfanilamide remains in use today in the form of topical preparations, primarily for treatment of vaginal yeast infections mainly vulvovaginitis which is caused by *Candida albicans* [26].

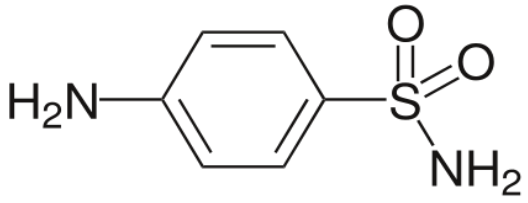

Fig: 12 General structure of sulphanilamide

1.2.1 Uses of Sulphanilamide

It is used to treat vaginal yeast infections. Sulphanilamide reduces vaginal burning, itching and discharge that may occur with this condition. This medication is known as sulphonamide antifungal. It works by stopping the growth of yeast (fungus) that causes the infection [27].

1.2.2 Importance of Sulphanilamide

Sulfanilamide is a sulfonamide antibiotic. The sulfonamides are synthetic bacteriostatic antibiotics with a wide spectrum against most gram-positive and many gram-negative organisms. However, many strains of an individual species may be resistant. Sulfonamides inhibit multiplication of bacteria by acting as competitive inhibitors of p-aminobenzoic acid in the folic acid metabolism cycle. Bacterial sensitivity is the same for the various sulfonamides, and resistance to one sulfonamide indicates resistance to all. Most sulfonamides are readily absorbed orally. However, parenteral administration is difficult, since the soluble sulfonamide salts are highly alkaline and irritating to the tissues. The sulfonamides are widely distributed throughout all tissues. High levels are achieved in pleural, peritoneal, synovial, and ocular fluids. Although these drugs are no longer used to treat meningitis, CSF levels are high in meningeal infections. Their antibacterial action is inhibited by pus [28].

1.3 HETEROCYCLIC ALDEHYDES

Heterocyclic compounds are of very much interest in our daily life. Heterocyclic aldehyde has one or more hetero atoms in their structure. They may be cyclic or non-cyclic in nature. They have a wide range of application. They are predominantly used as pharmaceuticals, as agrochemicals and as veterinary products. They also find applications as sanitizers, developers, antioxidants, as corrosion inhibitors, as copolymers, dye stuff. They are used as vehicles in the synthesis of other organic compounds. Some of the natural products e.g.,

antibiotics such as penicillin's, cephalosporin; alkaloids such as vinblastine, morphine, reserpine etc. have heterocyclic moiety.

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The most common heterocycles are those having five- or six-membered rings and containing heteroatoms of nitrogen (N), oxygen (O), or sulfur (S).

Heterocyclic aldehyde compounds are widely distributed in nature and essential to life; they play a vital role in the metabolism of all living cells. Genetic material DNA is an also composed of heterocyclic bases-pyrimidines and purines. A large number of heterocyclic compounds, both synthetic and natural, are pharmacologically active and are in clinical use. They also find applications as sensitizers, developers, antioxidants, as corrosion inhibitors, as copolymers, dyestuff. They are used as vehicles in the synthesis of other organic compounds [29].

1.4 OBJECTIVES

- To synthesize different types of Sulphanilamide.
- Characterization of synthesized Schiff bases using different spectroscopic techniques.
- To study the antibacterial activity of synthesized Schiff bases.

Chapter 2

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Chemicals required

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

- Sulphanilamide
- Pyrole-2-aldehyde
- Thiophene-2-aldehyde
- Vanillin
- Vetraldehyde
- 4-methyl-5-imidazole carboxaldehyde
- 2-amino-4-methyl phenol
- Fluorenone

2.1.2 Solvents

Solvents used for the synthesis and purification procedure were purchased from Spectrochem Ltd and used without further purification.

- Ethanol
- Methanol
- Petroleum Ether

2.2 METHODS

2.2.1 Elemental analysis

Elemental analysis is a process in which sample of material is analyzed for its elemental composition. Carbon, hydrogen and nitrogen analyses of all synthesized compounds were carried out using a Vario EL CHNS analyzer at Sophisticated Test Instrumentation Centre (SAIF), Cochin University of Science and Technology, Kochi India. Elemental analysis can be quantitative and qualitative.

2.2.2 Fourier Transform Infrared Spectroscopy (FT- IR)

Infrared spectra were recorded on a JASCO FT-IR-5300 Spectrometer in the range 4000- 400 cm^{-1} using KBr pellets at the Department of Applied Chemistry CUSAT. Infrared spectroscopy deals with the IR region of electromagnetic spectrum. From the IR spectra it gives the information about the nature of functional group present in the ligands.

FT-IR stands for “Fourier transform infrared” and it is the most common form of infrared spectroscopy. All infrared spectroscopies act on the principle that when infrared (IR) radiation passes through a sample, some of the radiation is absorbed. The radiation that passes through the sample is recorded. Because different molecules with their different structures produce different spectra, the spectra can be used to identify and distinguish among molecules. In this way, the spectra are like people’s fingerprints or DNA: virtually unique. FTIR is the preferred method of infrared spectroscopy for several reasons. First, it does not

destroy the sample. Second, it is significantly faster than older techniques. Third, it is much more sensitive and precise. The “output” of the interferometer is not the spectroscopy spectrum we use, but a graph known as an “interferogram.” The Fourier transform converts the interferogram into the infrared spectroscopy spectrum graph we recognize and use. FT-IR spectroscopy is used in organic synthesis, polymer science, petrochemical engineering, pharmaceutical industry and food analysis. In other words, it has a wide array of applications, from monitoring processes to identifying compounds to determining components in a mixture.[30]

2.2.3 UV-Visible Spectroscopy

Electronic spectra of the ligands were recorded in DMF on a Thermo electron Nicolet Evolution 300 UV-Vis. Spectrometer. UV-visible spectroscopy deals with UV-visible region of electronic spectrum. UV-visible spectrum is plotted as absorbance against wavelength.

Ultraviolet-visible (UV-Vis) spectroscopy is a widely used technique in many areas of science. UV-Vis spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample. This property is influenced by the sample composition, potentially providing information on what is in the sample and at what concentration. A specific amount of energy is needed to promote electrons in a substance to a higher energy state which we can detect as absorption. Electrons in different bonding environments in a substance require a different specific amount of energy to

promote the electrons to a higher energy state. This is why the absorption of light occurs for different wavelengths in different substances. Humans are able to see a spectrum of visible light, from approximately 380 nm, which we see as violet, to 780 nm, which we see as red. UV light has wavelengths shorter than that of visible light to approximately 100 nm. Therefore, light can be described by its wavelength, which can be useful in UV-Vis spectroscopy to analyze or identify different substances by locating the specific wavelengths corresponding to maximum absorbance.[31]

2.3 SYNTHESIS OF SULPHANILAMIDE SCHIFF BASES

In this present work, mainly six Schiff bases were synthesized. All the compounds were coloured and soluble in organic solvents such as methanol, ethanol, petroleum ether, DMSO, DMF etc.

2.3.1 Synthesis of Schiff base from Vanillin and Sulphanilamide (S_1)

Equimolar amounts of vanillin (0.1767g, 0.00116moles) and sulphanilamide (2g, 0.00116moles) was dissolved in methanol separately. Add the vanillin into the round bottom (RB) flask and then it is refluxed under oil bath at higher temperature. Then after 5 minutes, sulphanilamide dissolved in methanol was added into the RB flask. Then the mixture was refluxed for 6 hours at high temperature. A yellow solution is obtained. It is then poured into a beaker and allowed for slow evaporation. A light yellow coloured powder were obtained.

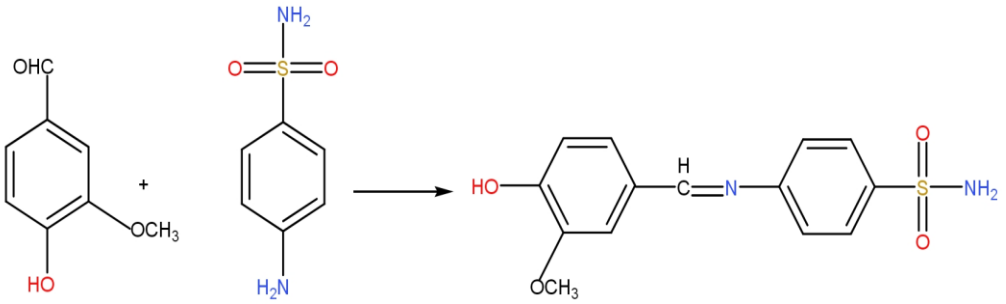


Fig 13: Synthesis of Schiff Base from Vanillin and Sulphanilamide (S_1)

2.3.2 Synthesis of Schiff base from Vetraldehyde and Sulphanilamide (S_2)

Equimolar amounts of vetraldehyde and sulphanilamide was dissolved in methanol separately. Add the vetraldehyde into the RB flask and then it is refluxed under oil bath at high temperature. Then after 5 minutes the sulphanilamide dissolved in methanol was added into it. And then the mixture is refluxed for 6 hours at high temperature. A colourless solution is obtained. It is then poured into a beaker and allowed for slow evaporation.

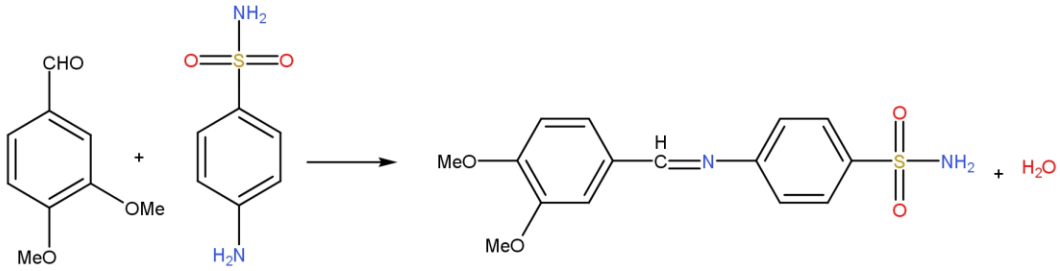


Fig 14: Synthesis of Schiff Bade from Vetraldehyde and Sulphanilamide (S_2)

2.3.3 Synthesis of Schiff base from Pyrrole-2-aldehyde and Sulphadiazine (S_3)

Equimolar amounts of pyrole-2-aldehyde (0.1g, 0.001051moles) and sulphanilamide (0.18099g, 0.001051moles) was dissolved in methanol separately. Add the pyrole-2-aldehyde into the RB flask and then it is refluxed

under oil bath at high temperature. Then after 5 minutes the sulphanilamide dissolved in methanol was added into it. And then the mixture is refluxed for 6 hours at high temperature. A colourless solution is obtained. It is then poured into a beaker and allowed for slow evaporation.

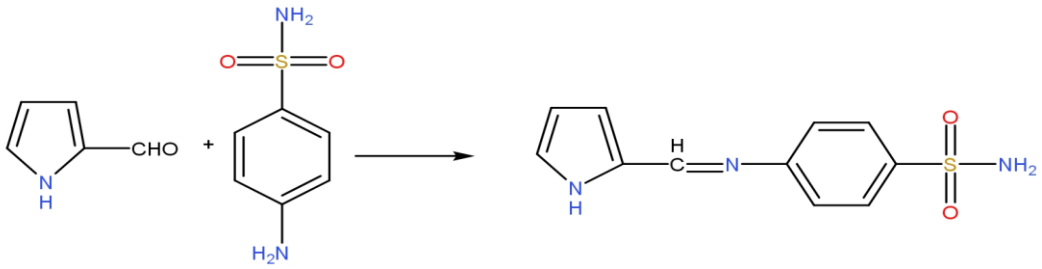


Fig 15: Synthesis of Schiff base from Pyrrole-2-aldehyde and Sulphanilamide (S_3)

2.3.4 Synthesis of Schiff base from Thiophene-2-aldehyde and Sulphanilamide (S_4)

Equimolar amounts of thiophene-2-aldehyde (0.05420mL, 0.00105moles) and sulphanilamide (0.18099g, 0.00105moles) was dissolved in methanol separately. Add thiophene-2-aldehyde into the RB flask and then it is refluxed under oil bath at high temperature. Then after 5 minutes the sulphanilamide dissolved in methanol was added into it. And then the mixture is refluxed for 6 hours at high temperature. A brown colour solution is obtained. It is then poured into a beaker and allowed for slow evaporation.

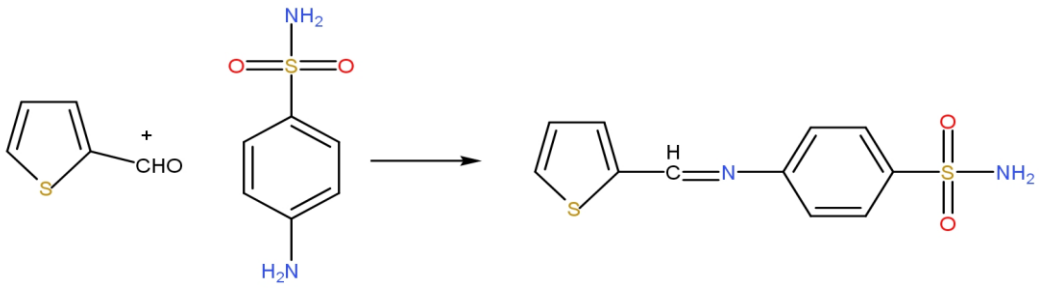


Fig 16: Synthesis of Schiff base from Thiophene-2-aldehyde and Sulphanilamide (S_4)

2.3.5 Synthesis of Schiff base from 4-methyl-5-imidazole carboxaldehyde and Sulphanilamide (S_5)

Equimolar amounts of 4-methyl-5-imidazole carboxaldehyde (0.1g, 0.0009081moles) and sulphanilamide (0.1563g, 0.0009081moles) was dissolved in methanol separately. Add 4-methyl-5-imidazole carboxaldehyde into the RB flask and then it is refluxed under oil bath at high temperature. Then after 5 minutes the sulphanilamide dissolved in methanol was added into it. And then the mixture is refluxed for 6 hours at high temperature. A colour solution is obtained. It is then poured into a beaker and allowed for slow evaporation.

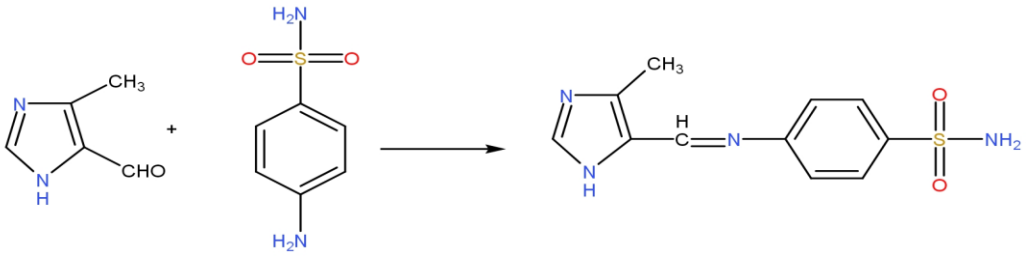


Fig 17: Synthesis of Schiff base from 4-methyl-5-imidazole carboxaldehyde and Sulphanilamide (S_5)

2.3.6 Synthesis of Schiff base from 4-methyl-5-imidazole carboxaldehyde and 2-amino-4-methyl phenol (S_6)

Equimolar amounts of 4-methyl-5-imidazole carboxaldehyde (0.1783g, 0.006124moles) and 2-amino-4-methyl phenol (0.2g, 0.006124moles) was dissolved in methanol separately. Add 4-methyl-5-imidazole carboxaldehyde into the RB flask and then it is refluxed under oil bath at high temperature. Then after 5 minutes the 2-amino-4-methyl phenol dissolved in methanol was added into it. And then the mixture is refluxed for 6 hours at high temperature. A brown colour solution is obtained. It is then poured into a beaker and allowed for slow evaporation. Brown powder is obtained.

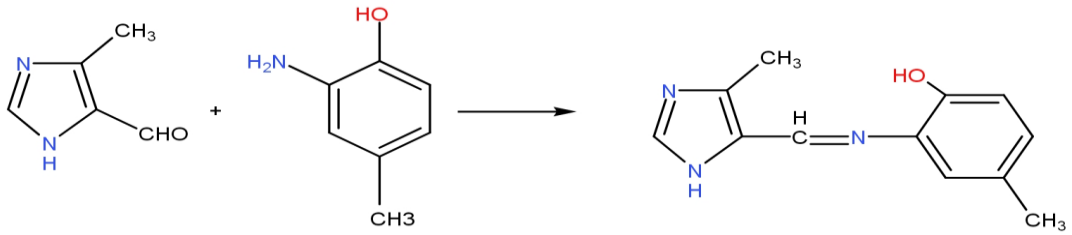


Fig 18: Synthesis of Schiff base from 4-methyl-5-imidazole carboxaldehyde and 2-amino-4-methyl phenol (S_6)

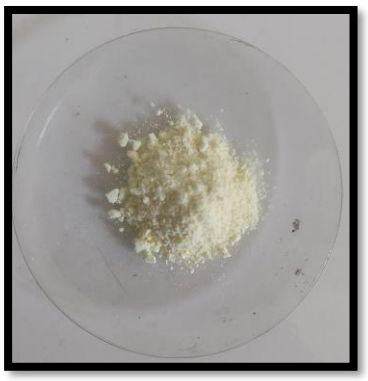
CHAPTER 3

RESULTS AND DISCUSSION

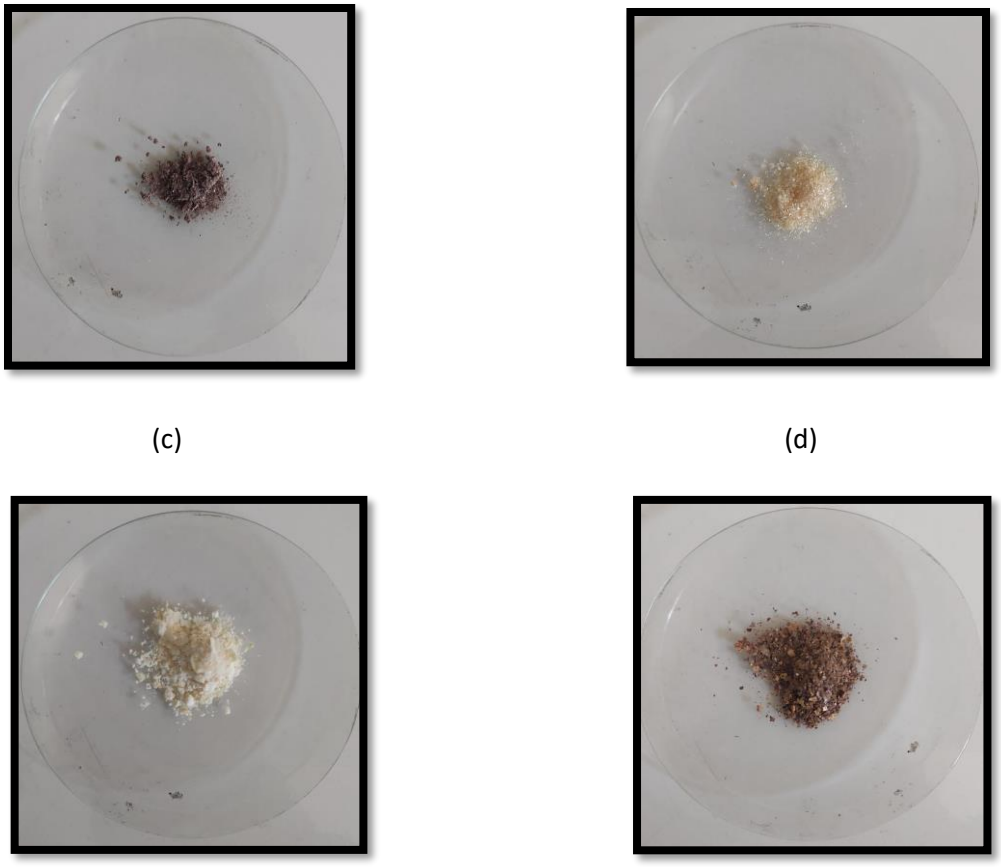
In this present work, mainly six Schiff bases were synthesized. All the compounds were coloured and soluble in organic solvents such as methanol, ethanol, petroleum ether, DMSO, DMF etc. The synthesized Schiff bases were characterized using Elemental analysis, FT-IR, UV-Visible spectroscopy.

3.1 PHYSICAL APPEARANCE

(a)



(b)



(c) (d)

(e) (f)

Fig 19: (a) S_1, (b)S_2, (c) S_3, (d) S_4, (e) S_5, (f) S_6

3.2 ELEMENTAL ANALYSIS

The analytical data for synthesized Schiff bases are given below in the Table 1

Table 1: Analytical data of Schiff base ligands

COMPOUNDS	EMPIRICAL FORMULA	FORMULA WEIGHT	COLOUR	YIELD (%)	CALCULATED (FOUND %)			
					C	H	N	S
S_1	$C_{14}H_{14}N_2O_4S$	306.34	Yellow	80	54.84 (54.89)	4.56 (4.61)	9.09 (9.14)	10.42 (10.47)
S_2	$C_{15}H_{16}N_2O_4S$	320.36	Pale yellow	90	56.19 (56.24)	4.98 (5.03)	8.69 (8.74)	9.97 (10.01)
S_3	$C_{11}H_{11}N_3O_2S$	249.29	Brown	75	53.05 (53.00)	4.4 (4.45)	16.81 (16.86)	12.81 (12.86)
S_4	$C_{11}H_{10}N_2O_2S_2$	266.34	Golden yellow	80	49.66 (49.61)	3.83 (3.78)	10.47 (10.52)	24.03 (24.08)
S_5	$C_{11}H_{12}N_4O_2S$	264.30	Pale yellow	70	49.94 (49.99)	4.63 (4.58)	21.15 (21.20)	12.08 (12.13)
S_6	$C_{12}H_{13}N_3O$	215.25	Brown	90	67.01 (66.96)	6.13 (6.09)	19.47 (19.52)	-

3.3 FT-IR STUDIES

The IR bands of Schiff bases give important information about the various functional groups present in it. The band shows in the range 1730-1580 cm^{-1} which shows the band of azomethine (HC=N) group which confirms the formation of Schiff base.

3.3.1 IR Spectrum of Schiff base from Vanillin and Sulphanilamide (S_1)

The Schiff base (S_1) is synthesized from Vanillin and Sulphanilamide drug in 1:1 molar ratio to yield imine compound. In certain regions and characteristics band in the fingerprint and other regions, IR spectra for the compound showed typical characteristics. In the spectrum a band at 3380 cm^{-1} shows the presence of OH group. A band at 1150 cm^{-1} shows the presence of OCH_3 group. A band at 1586 cm^{-1} confirms the presence of azomethine group (CH=N).[32]

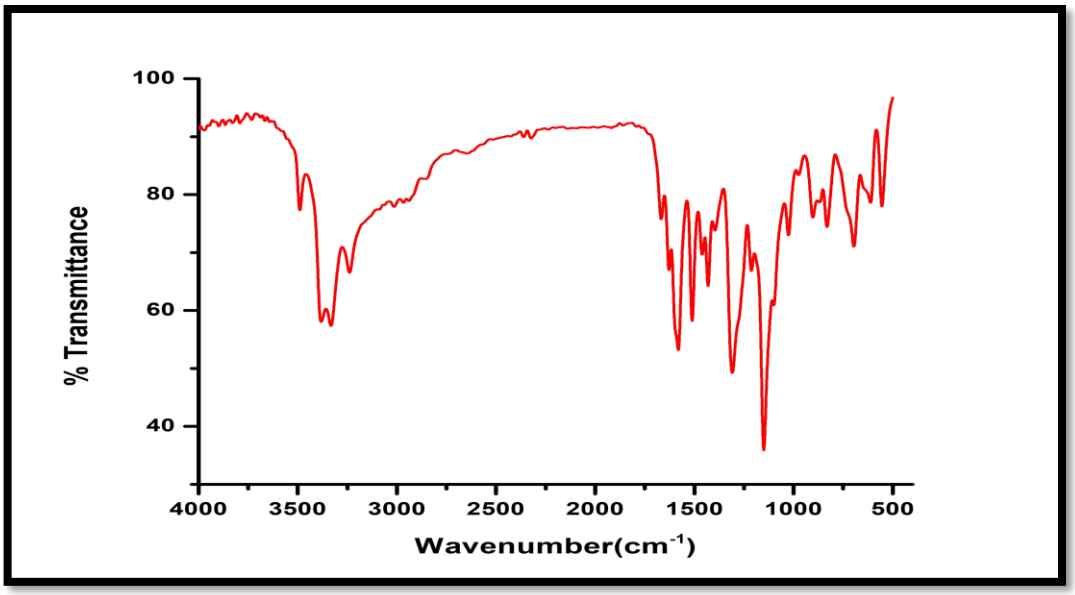


Fig 20: IR Spectrum of S_1

3.3.2 IR Spectrum of Schiff base from Vetraldehyde and Sulphanilamide (S_2)

The Schiff base (S_2) is synthesized from Vetraldehyde and Sulphanilamide drug in 1:1 molar ratio to yield imine compound. In certain regions and characteristics band in the fingerprint and other regions, IR spectra for the compound showed typical characteristics. In the spectrum a band at 3348 cm^{-1} shows the presence of OH group. A band at 1070 cm^{-1} shows the presence of OCH_3 group. A band at 1318 cm^{-1} indicates the presence of S=O group. A band at 1635 cm^{-1} confirms the presence of azomethine group (CH=N).[33]

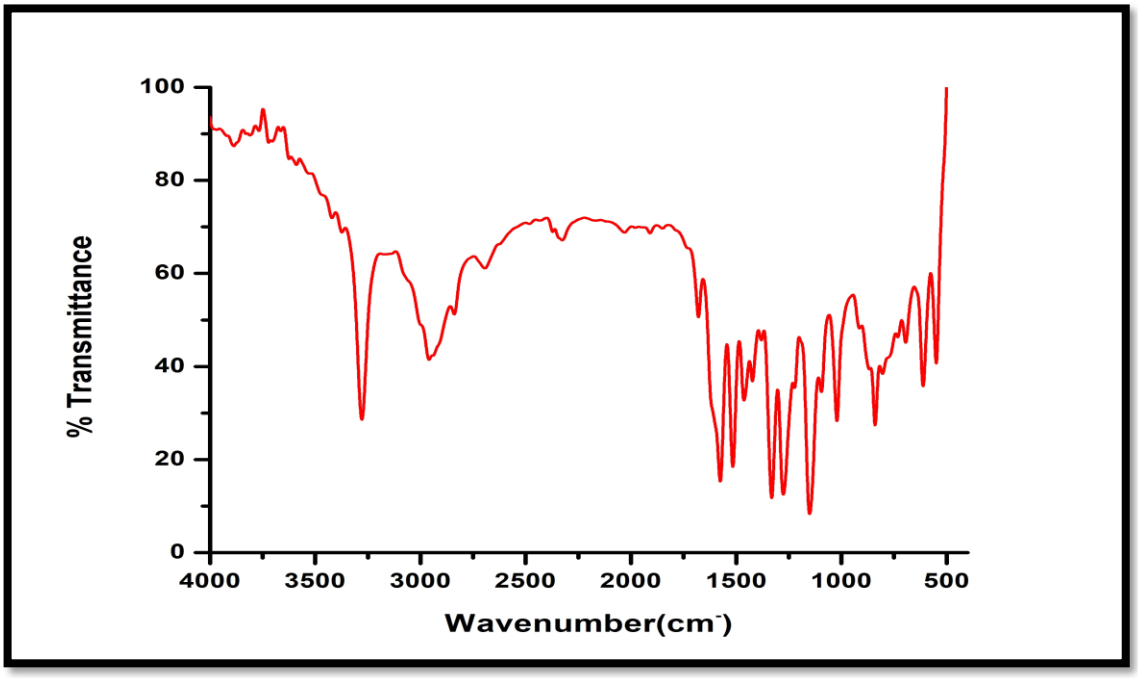


Fig 21: IR spectrum of S_2

3.3.3 IR Spectrum of Schiff base from Pyrrole-2-aldehyde and Sulphanilamide (S_3)

The Schiff base (S_3) is synthesized from Pyrrole-2-aldehyde and Sulphanilamide drug in 1:1 molar ratio to yield imine compound. In certain regions and characteristics band in the fingerprint and other regions, IR spectra for the compound showed typical characteristics. In the spectrum a band at 3479 cm^{-1} shows the presence of N-H group. A band at 1310 cm^{-1} shows the presence of S=O group. A band at 1627 cm^{-1} confirms the presence of azomethine group (CH=N).[34]

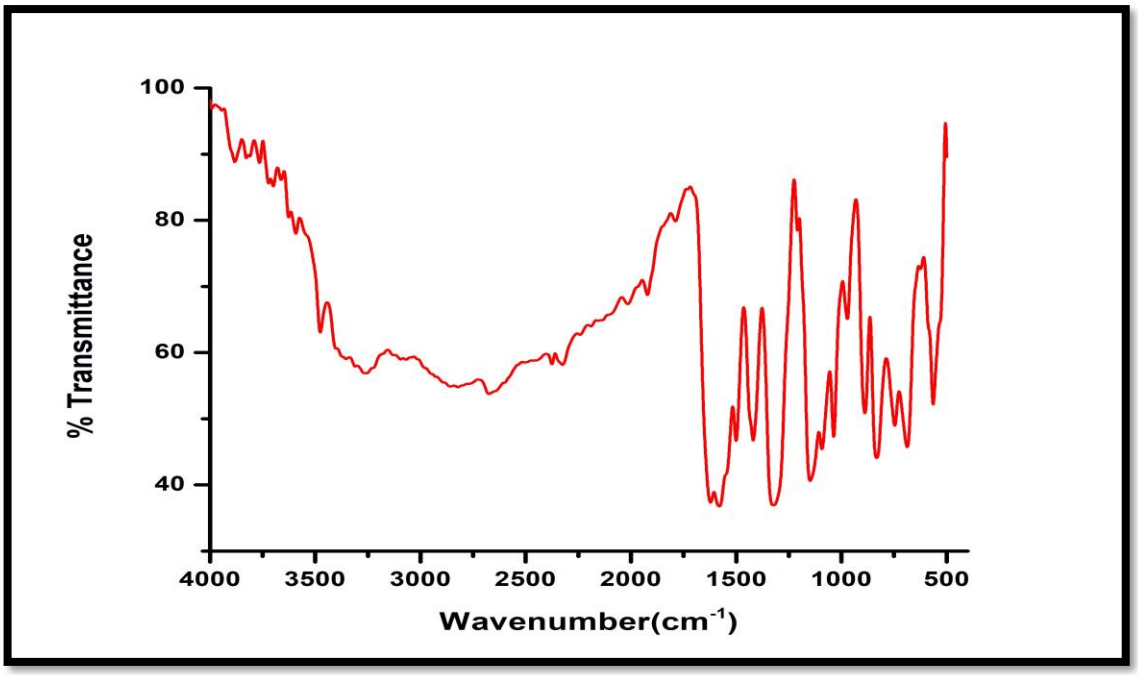


Fig 22: IR spectrum of S_3

3.3.4 IR Spectrum of Schiff base from Thiophene-2-aldehyde and Sulphanilamide (S_4)

The Schiff base (S_4) is synthesized from Thiophene-2-aldehyde and Sulphanilamide drug in 1:1 molar ratio to yield imine compound. In certain regions and characteristics band in the fingerprint and other regions, IR spectra for the compound showed typical characteristics. In the spectrum a band at 3288 cm^{-1} shows the presence of N-H group. A band at 2994 cm^{-1} shows the presence of C-H stretching. A band at 1350 cm^{-1} shows the presence of S=O group. A band at 1615 cm^{-1} confirms the presence of azomethine group (CH=N).[35]

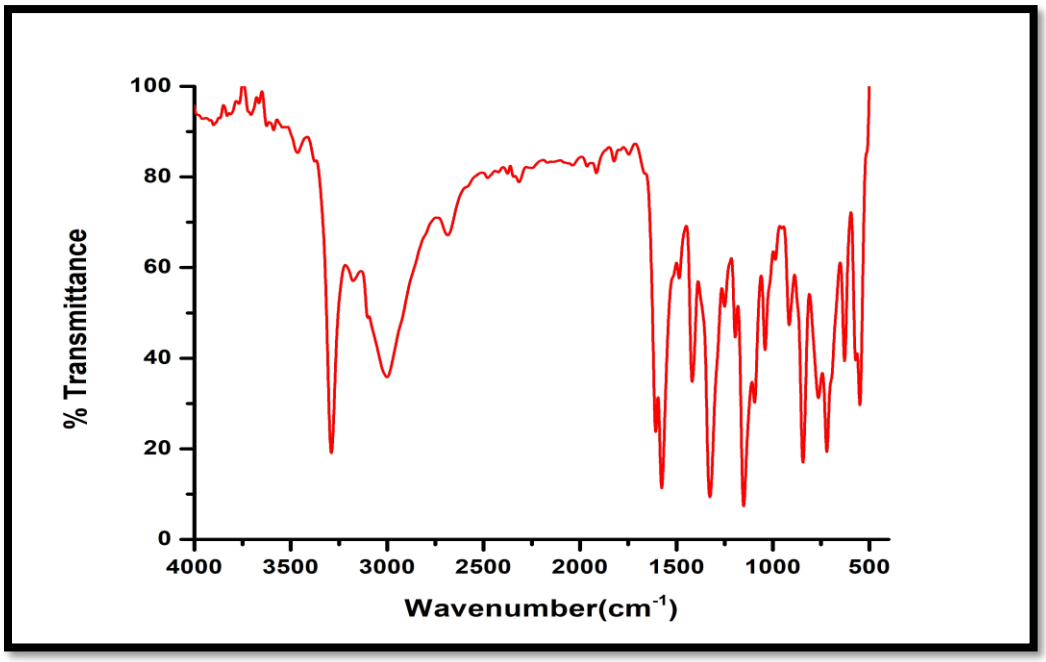


Fig 23: IR spectrum of S_4

3.3.5 IR Spectrum of Schiff base from 4-methyl-5-imidazole carboxaldehyde and Sulphanilamide (S_5)

The Schiff base (S_5) is synthesized from 4-methyl-5-imidazole carboxaldehyde and Sulphanilamide drug in 1:1 molar ratio to yield imine compound. In certain regions and characteristics band in the fingerprint and other regions, IR spectra for the compound showed typical characteristics. In the spectrum a band at 3490 cm^{-1} shows the presence of OH group. A band at 3100 cm^{-1} shows the presence of N-H group. A band at 1363 cm^{-1} shows the presence of S=O group. A band at 1621 cm^{-1} confirms the presence of azomethine group (CH=N).[36]

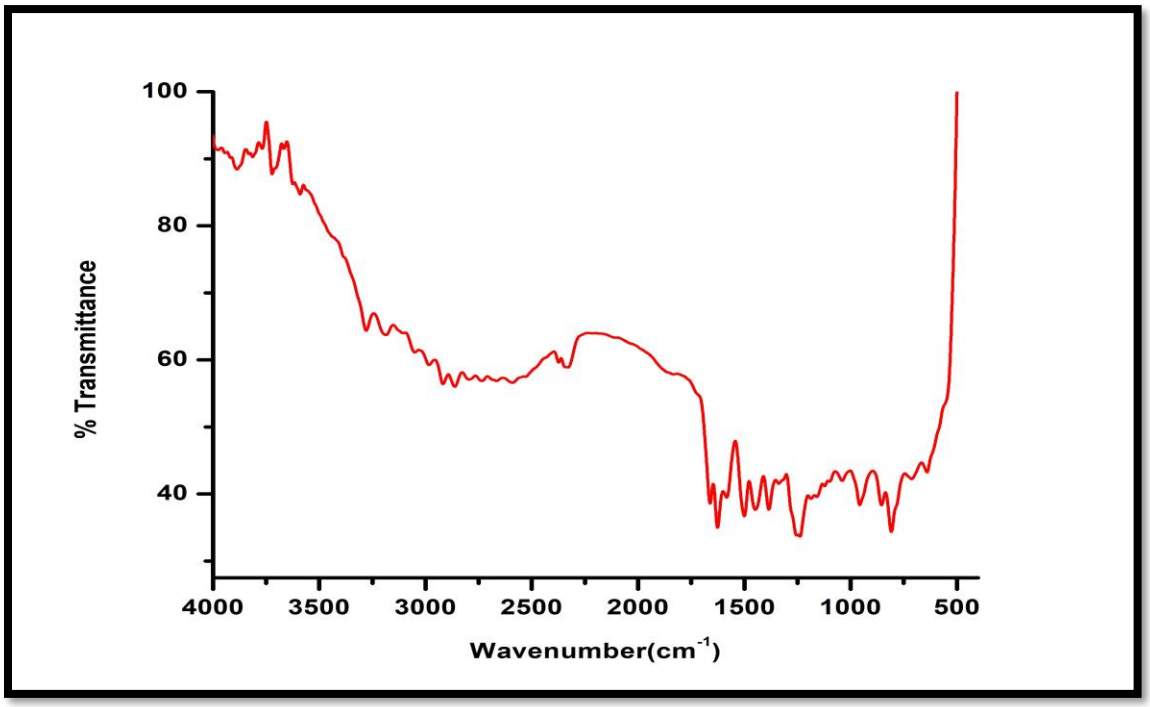


Fig 24: IR Spectrum S_5

3.3.6 IR Spectra of Schiff base from 4-methyl-5-imidazole carboxaldehyde and 2-amino-4-methyl phenol (S_6)

The Schiff base (S_6) is synthesized from 4-methyl-5-imidazole carboxaldehyde and 2-amino-4-methyl phenol in 1:1 molar ratio to yield imine compound. In certain regions and characteristics band in the fingerprint and other regions, IR spectra for the compound showed typical characteristics. In the spectrum a band at 3601 cm^{-1} shows the presence of OH group. A band at 3472 cm^{-1} shows the presence of N-H group. A band at 1609 cm^{-1} confirms the presence of azomethine group (CH=N).[37]

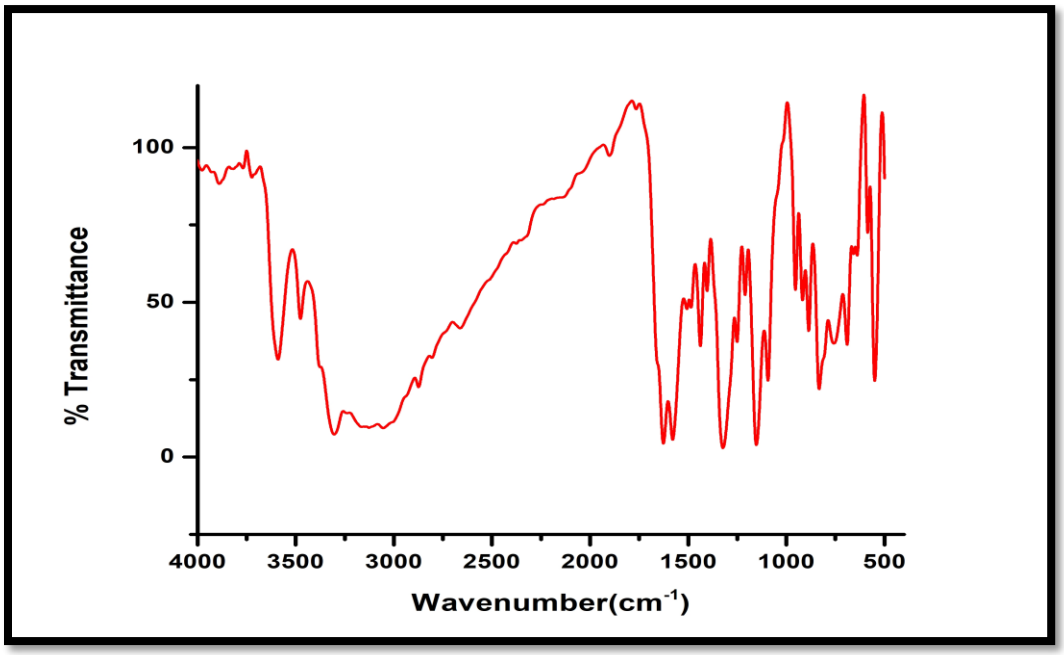


Fig 25: IR Spectrum of S_6

Table 2 : Infrared spectral data of Schiff base ligands (cm^{-1})

COMPOUND	υ ($\boldsymbol{HC=N}$)	υ ($\boldsymbol{NH}$)	υ ($\boldsymbol{S=O}$)
S_1	**1586**	**3325**	**1328**
S_2	**1635**	**3472**	**1318**
S_3	**1627**	**3479**	**1310**
S_4	**1615**	**3288**	**1350**
S_5	**1621**	**3100**	**1308**
S_6	**1609**	**3472**	**-**

3.4 UV–VISIBLE STUDIES

The UV-Visile spectra of synthezised schiff bases were taken in methanol. The UV-visible spectra of the compounds shows bands at 260-320 nm and 330-380 nm. Transition within the aromatic ring assigned to $\pi \rightarrow \pi^*$. Transition within the C=N group assigned to be $n \rightarrow \pi^*$.

3.4.1 UV-Visible Spectrum of Schiff base from Vanillin and Sulphanilamide (S_1)

UV-Visible spectrum for 10^{-3} molar solution of Schiff base from Vanillin and Sulphanilamide is shown in the fig 26. Here we can see that a band at 285 nm is due to π to π^* transition and a band at 325 nm is due to n to π^* transition.[38]

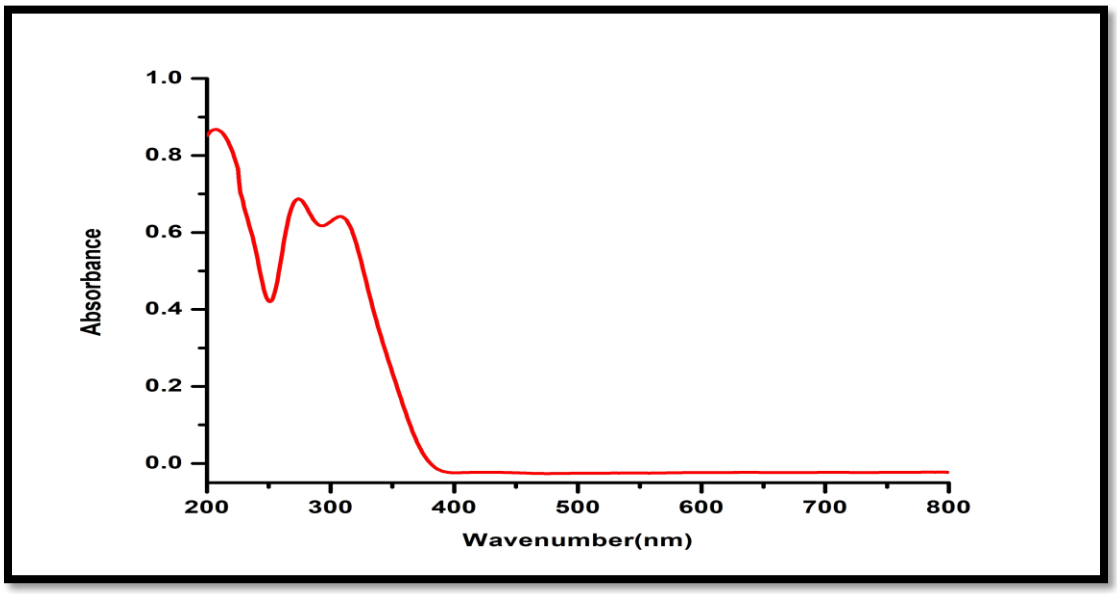


Fig 26: UV-Visible spectrum of S_1

3.4.2 UV-Visible Spectrum of Schiff base from Vetraldehyde and Sulphanilamide (S_2)

UV-Visible spectrum for 10^{-3} molar solution of Schiff base from Vetraldehyde and Sulphanilamide is shown in the fig 27. Here we can see that a band at 275 nm is due to π to π* transition and a band at 330 nm is due to n to π* transition.[39]

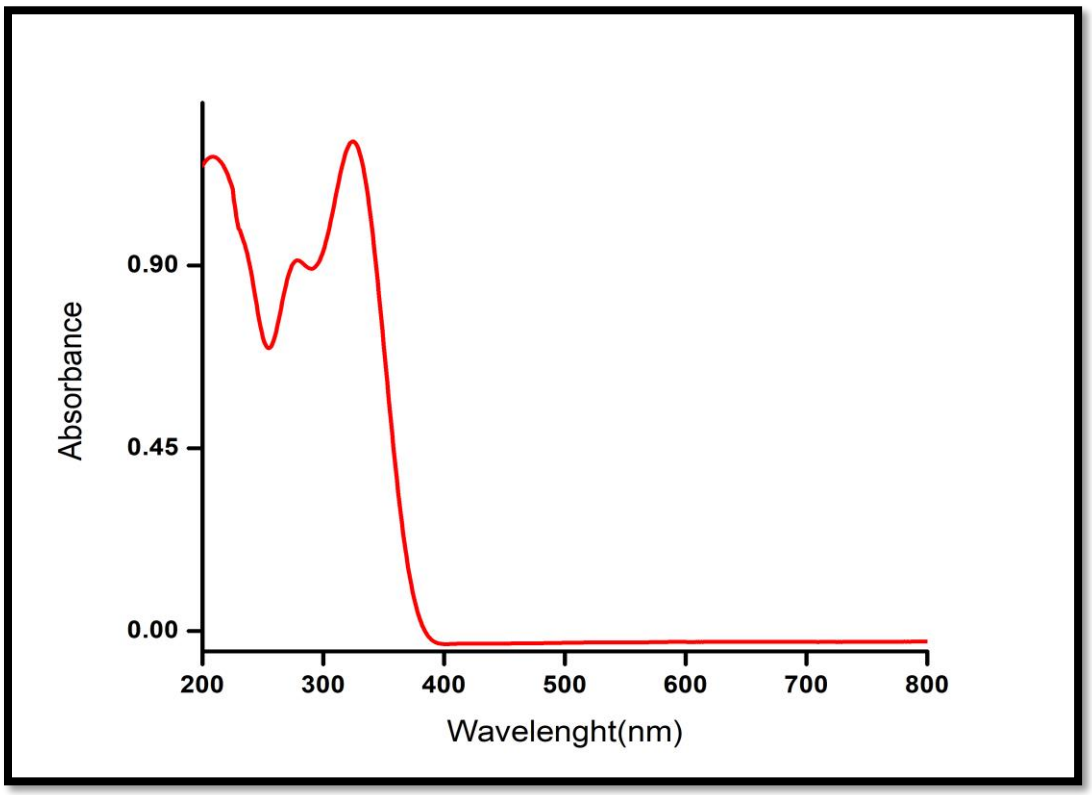


Fig 27: UV-Visible spectrum of S_2

3.4.3 UV-Visible Spectrum of Schiff base from Pyrrole-2-aldehyde and Sulphanilamide (S_3)

UV-Visible spectrum for 10^{-3} molar solution of Schiff base from Pyrrole-2-aldehyde and Sulphanilamide is shown in the fig 28. Here we can see that a band at 290 nm is due to π to π* transition and a band at 330 nm is due to n to π* transition.[40]

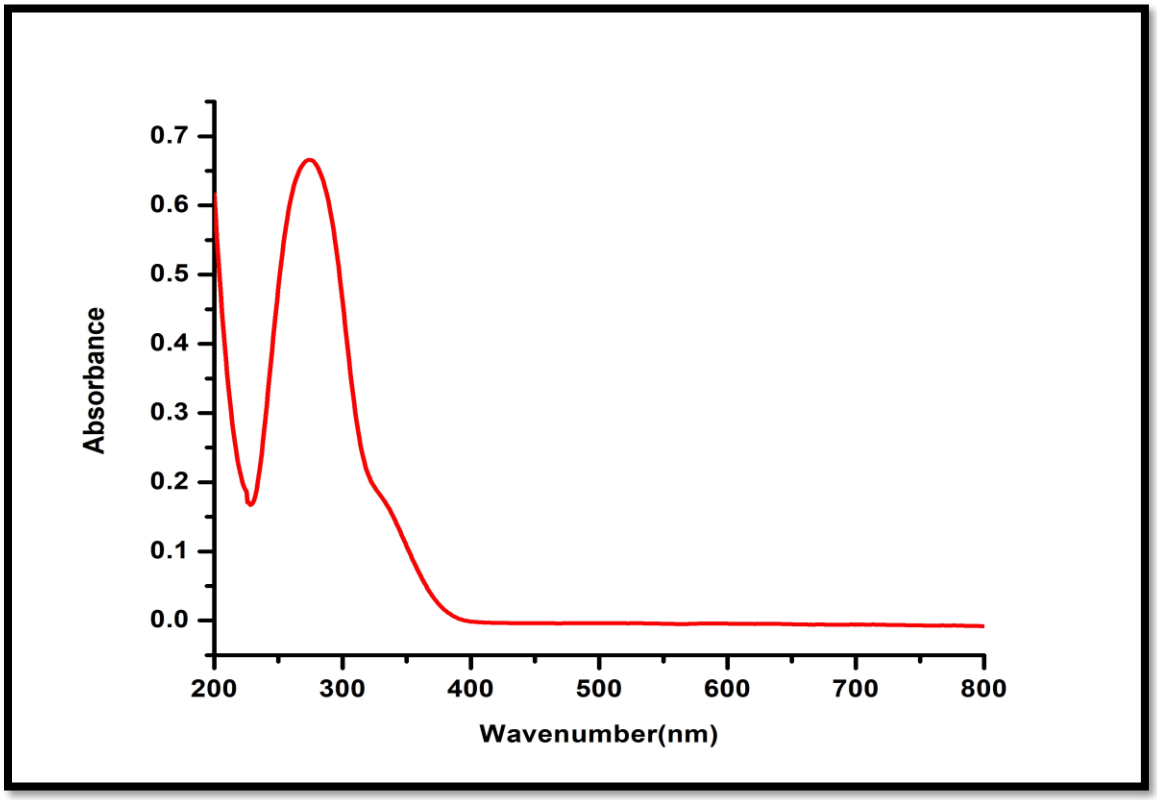


Fig 28: UV-Visible spectrum of S_3

3.4.4 UV-Visible Spectrum of Schiff base from Thiophene-2-aldehyde and Sulphanilamide (S_4)

UV-Visible spectrum for 10^{-3} molar solution of Schiff base from Thiophene-2-aldehyde and Sulphanilamide is shown in the fig 29. Here we can see that a band at 275 nm is due to π to π* transition and a band at 325 nm is due to n to π* transition.[41]

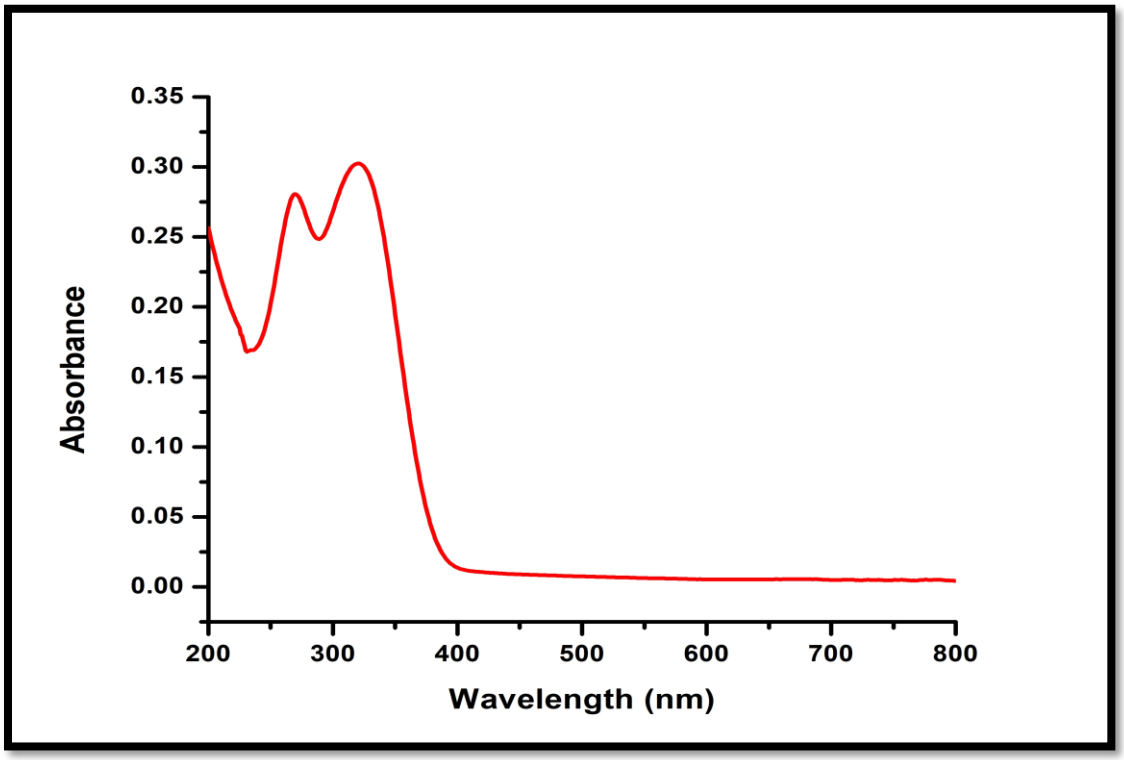


Fig 29: UV-Visible spectrum of S_4

3.4.5 UV-Visible Spectrum of Schiff base from 4-methyl-5-imidazole carboxaldehyde and Sulphanilamide (S_5)

UV-Visible spectrum for 10^{-3} molar solution of Schiff base from 4-methyl-5-imidazole carboxaldehyde and Sulphanilamide is shown in the fig 30. Here we can see that a band at 275 nm is due to π to π* transition and a band at 315 nm is due to n to π* transition.[42]

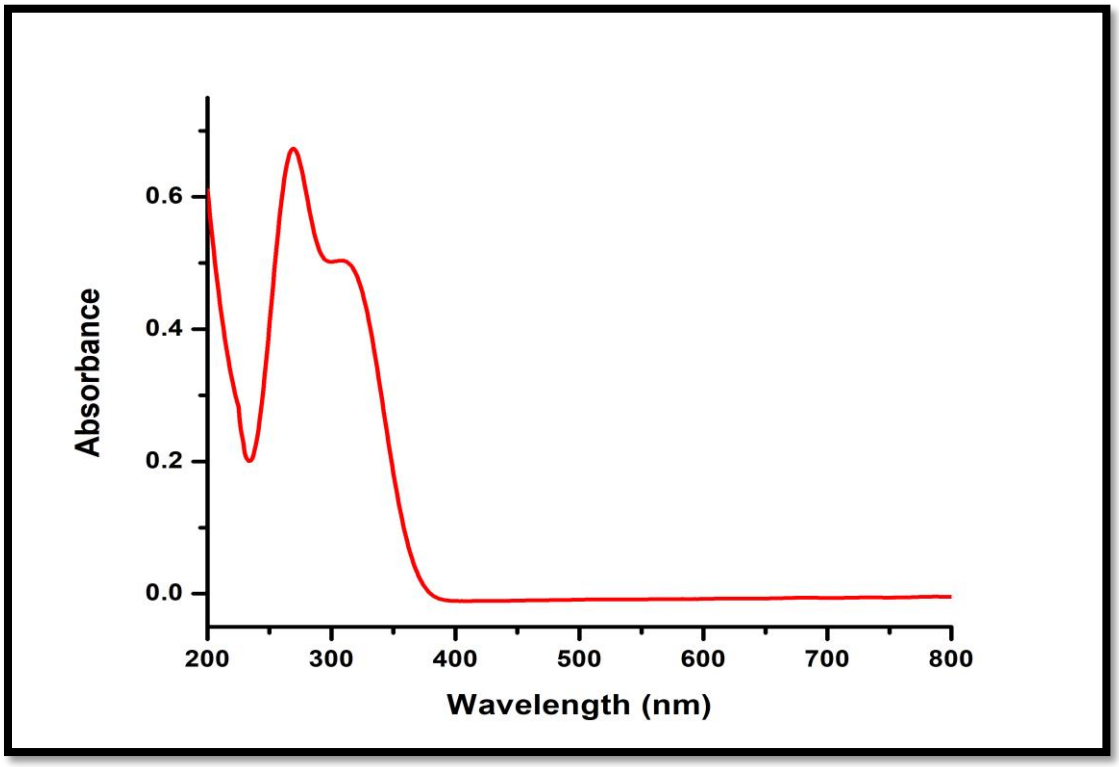


Fig 30: UV-Visible spectrum of S_5

3.4.6 UV-Visible Spectrum of Schiff base from 4-methyl-5-imidazole carboxaldehyde and 2-methyl-4-amino phenol (S_6)

UV-Visible spectrum for 10^{-3} molar solution of Schiff base from 4-methyl-5-imidazole carboxaldehyde and 2-methyl-4-amino phenol is shown in the fig 31. Here we can see that a band at 270 nm is due to π to π* transition and a band at 350 nm is due to n to π* transition.[43]

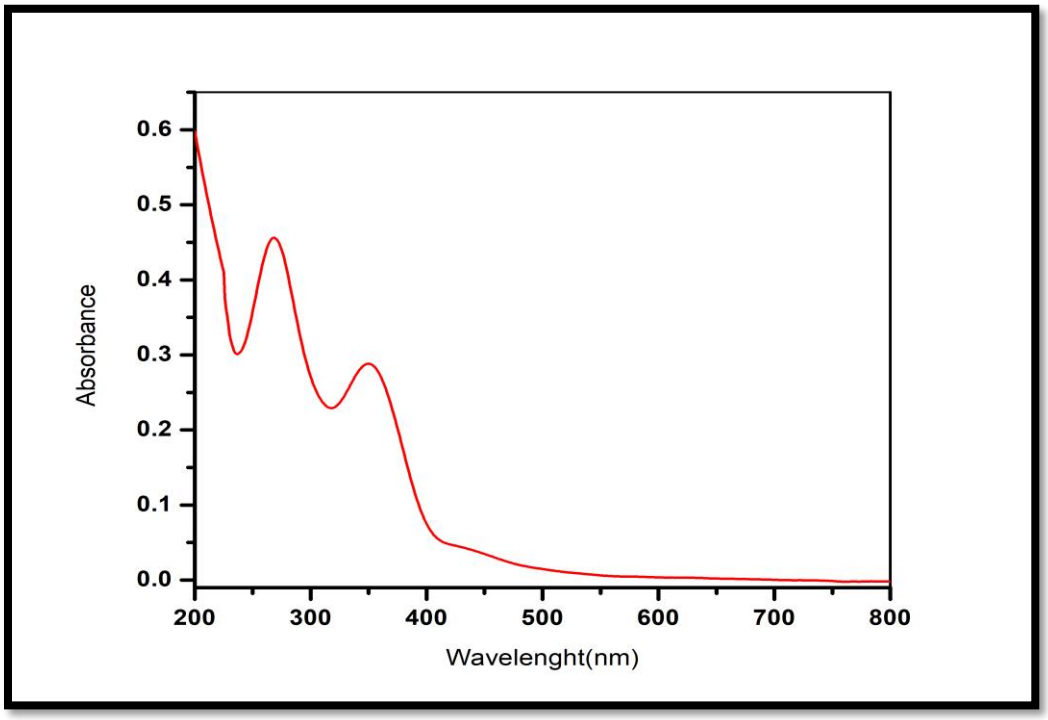


Fig 31: UV-Visible spectrum of S_6

Table 3: UV-VIS SPECTRAL DATA OF SCHIFF BASES IN METHANOL

Compounds	π→π*	n→π*
S_1	285 nm	325 nm
S_2	275 nm	330 nm
S_3	290 nm	330 nm
S_4	275 nm	325 nm
S_5	275 nm	315 nm
S_6	270 nm	350 nm

The synthesized Schiff base (S_6) from 4-methyl-5-imidazole carboxaldehyde and 2-methyl-4-amino phenol when tested fluorescence shows green fluorescence. It is shown in figure 32.

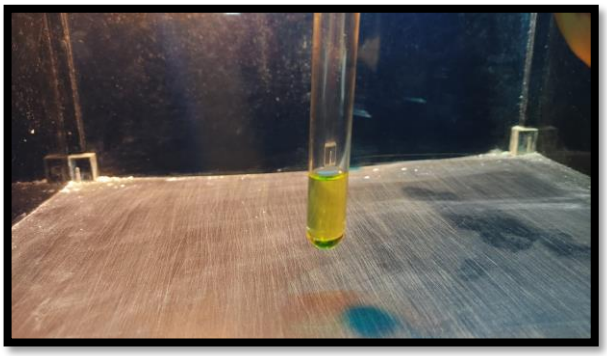


Fig 32: Green Fluorescence of S_6

Chapter 4

ANTIBACTERIAL STUDIES

4.1 INTRODUCTION

Antibacterial activity is the most important characteristic of medical textiles, to provide adequate protection against microorganisms, biological fluids, and aerosols as well as disease transmission. Antibacterial as well as antiviral activity of a molecule is completely associated with the compounds that provincially kill bacteria and virus or slow down their rate of growth, without being extensively toxic to nearby tissues. Antibacterial agents are the most important in fighting infectious diseases. But, with their wide use as well as abuse, the appearance of bacterial resistance toward antibacterial agents has become a major problem for today's pharmaceutical industry.[44]

4.2 Bacterial species used for study

Pseudomonas and *Staphylococcus aureus* are the microorganisms which are used in this present work.

Pseudomonas is a genus of gammoproteobacteria. They belong to the family of *Pseudomonas*. *Pseudomonas* bacteria can be found in many different environments, such as soil, water, and plant and animal tissue. There are many *Pseudomonas* species. Some are parasitic and invade animal tissues. In humans, pathogenic *Pseudomonas* thrive, especially after antibiotic treatment: once they are able to multiply rapidly they can be harmful.[45]

Staphylococcus aureus is a Gram-positive round-shaped bacterium, a member of the Bacillota, and is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin.[46] *Staphylococcus aureus* is a type of bacteria. It stains Gram positive and is non-moving small round shaped or non-motile cocci. It is found in grape-like (staphylo-) clusters. *Staphylococcus aureus* belongs to the family *Staphylococcaceae.* It affects all known mammalian species, including humans.[47]

Fig 33: Psuedomonas

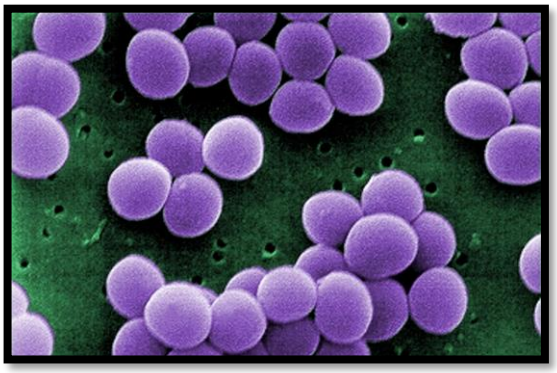


Fig 34: Staphylococcus

4.3 Experimental method

4.3.1 Disc Diffusion Method

Using aseptic techniques, a single pure colony was transferred into a 10 ml of nutrient Broth and was placed in incubator at 37°C for overnight incubation. Sterile MHA plates were prepared and the bacterial inoculum of *Pseudomonas* aeruginosa and *Staphylococcus aureus* were uniformly swabbed in each plate. Test samples of volume 50µl added to the disc and were placed over the agar plates. The plates were incubated at 37°C for 18 hours a period sufficient for the growth. After incubation, the diameter of inhibitory zones formed around each well were measured in mm and recorded.[48]

4.4 Results and Discussion

The antibacterial activity of synthesized Schiff bases was tested against gram positive and gram-negative microorganisms using disc diffusion method. The microorganisms used in the present work includes *Pseudomonas* and *Staphylococcus.* The diameter of zone of inhibition was presented in Table 4. The representation of antibacterial activity of synthesized sulphanilamide Schiff bases are shown in Fig 23 it shows the antibacterial activity against *Psuedomonas* bacteria and the *Staphylococcus* bacteria. The Schiff base S_1 and S_3 shows highest antibacterial activity on the *Psuedomonas* bacteria and Schiff base S_1 and S_6 shows milder activity on *Staphylococcus* bacteria. The Schiff base S_2, S_3, S_4, S_5, S_6 shows no activity against *Staphylococcus* bacteria.

Table 4: Antibacterial study of synthesized Schiff Bases

COMPOUNDS						
BACTERIA	**ZONE DIAMETER (mm)**					
	S_1	**S_2**	**S_3**	**S_4**	**S_5**	**S_6**
***Pseudomonas*[b]**	**11**	**3**	**11**	**8**	**9**	**10**
***Staphylococcus*[a]**	**5**	**-**	**-**	**-**	**-**	**6**

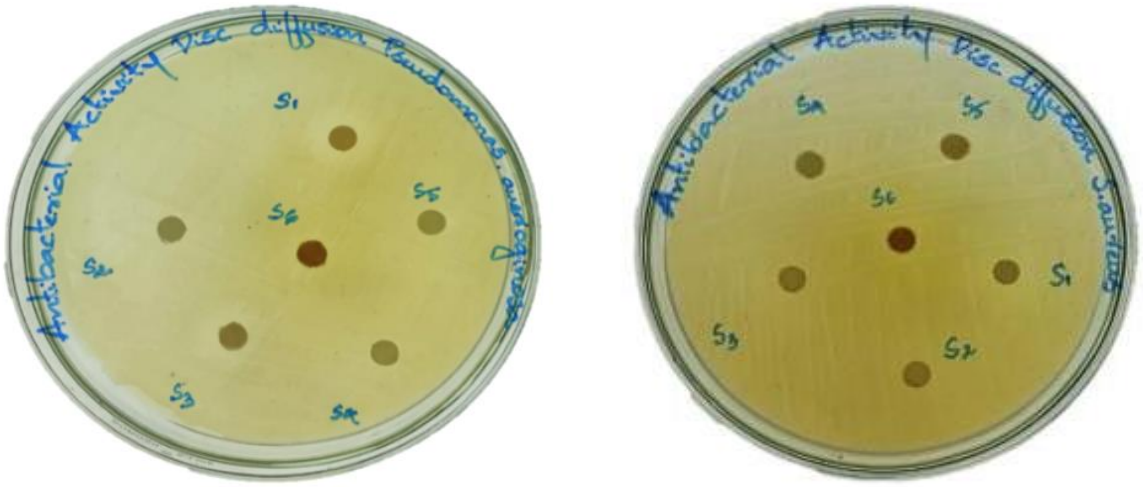


Fig 35:Zone of inhibition against Psuedomonas and Staphylococcus

4.5 CONCLUSION

The antibacterial activity of synthesized Schiff bases was tested against gram positive and gram-negative bacteria using disc diffusion method. The Schiff base S_1 and S_3 shows highest antibacterial activity on the *Psuedomonas* bacteria and Schiff base S_1 and S_6 shows milder activity on *Staphylococcus* bacteria. The Schiff base S_2, S_3, S_4, S_5, S_6 shows no activity against *Staphylococcus* bacteria.

CHAPTER 5

CONCLUSION

Schiff's bases are an important class of organic compounds. Schiff base is a functional group that contains a carbon-nitrogen double bond connected to an aryl or alkyl group. Schiff bases are condensation product of primary amines with carbonyl compounds. Schiff bases are considered as a very important class of organic compounds because of their various applications in biological process and potential application in designing of new therapeutic agents. Schiff bases are reported to show a variety of biological activities including antibacterial, antifungal, antiviral, anti-inflammatory, antitumor etc.

In this work, we describe the synthesis, characterization and antibacterial studies of six sulphanilamide Schiff base ligands. These ligands were successfully synthesised utililizing by a standard condensation reaction by heating to reflux sulphanilamide and corresponding aldehyde.

The synthesised sulphanilamide Schiff bases were characterized using Elemental analysis, FT-IR and UV-Visible spectroscopy. The antibacterial activity of these synthesised compounds were carried out by disc diffusion method.

From the IR spectra of synthesized Sulphanilamide Schiff bases, the band in the range of $1580cm^{-1}$-$1640cm^{-1}$ shows the presence of azomethine group (C=N) which confirms the formation of Schiff base.

Among the six compounds the Schiff base S_1 and S_3 shows higher activity against *Psuedomonas* bacteria and the Schiff base S_1 and S_6 shows milder activity against *Staphylococcus* bacteria.

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