SYNTHESIS, CHARACTERIZATION AND MICROBIAL ACTIVITY OF NOVEL SCHIFF BASES OF *o*-PHENYLENEDIAMINE

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

INTEGRATED MASTER OF SCIENCE (HONS.)
in
CHEMISTRY
(Minor Subject : Biochemistry)

By

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CERTIFICATE - I

This is to certify that the thesis entitled, **"Synthesis, characterization and microbial activity of novel Schiff bases of *o*-phenylenediamine"** submitted for the degree of **Integrated Master of Science (Hons.)** in the subject of **Chemistry** (Minor subject: **Biochemistry**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Gaganpreet Kaur (L-2012-BS-70-IM)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE - II

This is to certify that the thesis entitled, **"Synthesis, characterization and microbial activity of novel Schiff bases of *o*-phenylenediamine"** submitted by **Gaganpreet Kaur** (L-2012-BS-70-IM) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Integrated Master of Science (Hons.)** in the subject of **Chemistry** (Minor subject: **Biochemistry**) has been approved by the Student's Advisory Committee along with the Head of the Department after an oral examination on the same.

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ABSTRACT

Schiff bases are the subject of extensive interest due to their versatile applications and similarity of structure with natural biological compounds. N- substituted benzylidene benzene-1,2-diamines (I-V) and N, N$'$-bis substituted benzylidene benzene-1,2-diamines (VI-X) were synthesized by reacting different aldehydes with *o*-phenylenediamine in the presence of catalytic amount of sodium hydroxide (10%) by taking 1:1 and 2:1 molar ratio respectively. Physical data of all the synthesized compounds was recorded. All the compounds were characterized by their IR and ^{1}H NMR spectra and were evaluated for microbial activity against *Psuedomonas* sp., *Klebsiella* sp., *Burkholderia* sp. and *Fusarium oxysporum.* at different concentrations. The N, N$'$-bis substituted benzylidene benzene-1,2-diamines were found to be more effective than N- substituted benzylidene benzene-1,2-diamines. The compound VIII with halogen substitution at para position was found to be more effective than all other compounds. The other halogenated compounds followed the order Br > Cl > F. All the compounds were found to be less effective than the standard ampicillin and carbendazim (bavistin) at all the concentrations.

Keywords: *o*-phenylenediamine, Schiff bases, microbial activity, *Psuedomonas* sp., *Klebsiella* sp., *Burkholderia* sp., *Fusarium oxysporum.*

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ਸ਼ਿਫ ਬੇਸ ਦੇ ਬਹੁਪੱਖੀ ਉਪਯੋਗਾਂ ਅਤੇ ਕੁਦਰਤੀ ਜੈਵਿਕ ਕੰਪਾਊਂਡਾਂ ਨਾਲ ਬਣਤਰ ਦੇ ਸਮਾਨਤਾ ਦੇ ਕਾਰਨ ਵਿਆਪਕ ਰੁਚੀ ਦਾ ਵਿਸ਼ਾ ਹਨ। ਐਨ-ਸਬਸਟੀਚਿਊਟਡ ਬੈਨਜ਼ਾਈਲੀਡੀਨ ਬੈਨਜ਼ੀਨ 1,2-ਡਾਈਅਮੀਨਸ ਅਤੇ ਐਨ-ਐੱਨ’ ਬਿਸ ਸਬਸਟੀਚਿਊਟਡ ਬੈਨਜ਼ਾਈਲੀਡੀਨ ਬੈਨਜ਼ੀਨ 1,2-ਡਾਈਅਮੀਨਸ ਨੂੰ ਸੋਡੀਅਮ ਹਾਈਡ੍ਰੋਕਸਾਈਡ (10%) ਦੀ ਮੌਜੂਦਗੀ ਵਿੱਚ ਵੱਖ-ਵੱਖ ਐਲਡੀਹਾਈਡਾਂ ਅਤੇ ਆਰਥੋ-ਫਿਨਾਈਲੀਨਡਾਈਅਮੀਨ ਨੂੰ ਕ੍ਰਮਵਾਰ 1:1 ਅਤੇ 2:1 ਮਾਤਰਾ ਵਿੱਚ ਲੈ ਕੇ ਤਿਆਰ ਕੀਤਾ ਗਿਆ। ਸਾਰੇ ਉਤਪਾਦਕ ਕੰਪਾਊਂਡਾਂ ਦੇ ਭੌਤਿਕ ਗੁਣ ਲਏ ਗਏ । ਸਾਰੇ ਕੰਪਾਊਂਡਾਂ ਦੀ ਸਪੈਕਟਰਲ ਡਾਟਾ (ਆਈ ਆਰ ਅਤੇ ਪ੍ਰੋਟੋਨ ਐਮ ਐਨ ਆਰ) ਨਾਲ ਪਹਿਚਾਣ ਕੀਤੀ ਗਈ ਅਤੇ ਇਨ੍ਹਾਂ ਕੰਪਾਊਂਡਾਂ ਦੀ ਵੱਖ-ਵੱਖ ਸੰਚਵਤਾਵਾਂ (ਘਣਤਾਵਾਂ) ਤੇ *ਸੁਡੋਮੋਨਾਸ* ਸਪ., *ਕਲੈਬਸ਼ੈਲਾ* ਸਪ., *ਬੁਰਖੋਲਡਰਿਆ* ਸਪ ਅਤੇ *ਫੁਉਜ਼ੇਰਿਅਮ ਔਗਜ਼ੀਸਪੋਰਮ* ਵਿਰੁੱਧ ਕੀਟਨਾਸ਼ਕ ਸਰਗਰਮੀ ਦੀ ਜਾਂਚ ਕੀਤੀ ਗਈ। ਐਨ-ਐੱਨ’ ਬਿਸ ਸਬਸਟੀਚਿਊਟਡ ਬੈਨਜ਼ਾਈਲੀਡੀਨ ਬੈਨਜ਼ੀਨ 1,2-ਡਾਈਅਮੀਨਸ, ਐਨ-ਸਬਸਟੀਚਿਊਟਡ ਬੈਨਜ਼ਾਈਲੀਡੀਨ ਬੈਨਜ਼ੀਨ 1,2-ਡਾਈਅਮੀਨਸ ਮੁਕਾਬਲੇ ਵਧੇਰੇ ਅਸਰਦਾਰ ਪਾਏ ਗਏ । ਬਾਕੀ ਕੰਪਾਊਂਡਾਂ ਦੇ ਮੁਕਾਬਲੇ ਕੰਪਾਊਂਡ VIII ਜਿਸ ਦੇ ਵਿੱਚ ਹੈਲੋਜ਼ਨ ਪੈਰਾ ਪੋਜ਼ੀਸ਼ਨ ਤੇ ਲੱਗਾ ਹੋਇਆ ਸੀ, ਵੱਧ ਅਸਰਦਾਰ ਪਾਇਆ ਗਿਆ। ਬਾਕੀ ਹੈਲੋਜ਼ਨ ਕੰਪਾਊਂਡਾਂ ਦਾ ਅਸਰ ਕ੍ਰਮਵਾਰ ਬਰੋਮੋ > ਕਲੋਰੋ > ਫਲੋਰੋ ਪਾਇਆ ਗਿਆ। ਸਾਰੇ ਕੰਪਾਊਂਡ ਸਟੈਂਡਰਡ ਐਮਪੀਸੀਲਿਨ ਅਤੇ ਕਾਰਬੈਂਡਾਜਿਮ (ਬਵਿਸਟਨ) ਨਾਲੋਂ ਘੱਟ ਅਸਰਦਾਰ ਪਾਏ ਗਏ ।

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ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ

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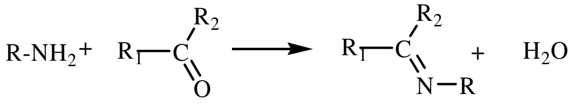
CHAPTER - I

INTRODUCTION

Schiff bases are the subject of extensive interest due to their versatile applications in various fields. Schiff bases are gaining importance in present scenario day by day (Kajal *et al* 2013). These are widely studied due to their selectivity, sensitivity and flexibility towards central atom and similarity of structure with natural biological compounds (Malik and Nema 2016) and also because of versatile coordination behaviour and mixed soft hard donor character (Tyagi *et al* 2011). Schiff bases are the products of condensation reaction between primary amines and compounds having carbonyl moiety as reported by German chemist Hugo Schiff in 1864 (Cimerman *et al* 2000). Schiff bases have characterstic feature of possessing azomethine group with general formula RHC=N-R_1, where R and R_1 can be various alkyl, aryl, cyclo alkyl or heterocyclic groups which may also have substitution at various positions (I). These compounds are often termed as anils, imines or azomethines (Asharf *et al* 2011).



(I)

Several Schiff bases are considerably chemically and biologically important and this can be attributed to the presence of lone pair of electrons in sp^2 hybridised orbital of nitrogen atom. The inhibition efficiency of Schiff bases is much more greater than that of the corresponding amines and aldehyde due to the presence of an -HC=N- group in the molecules (Asharf *et al* 2011).

Schiff bases formed from aromatic aldehydes are more stable as compared to aliphatic aldehydes due to effective conjugation and thus, aliphatic aldehydes are readily polymerizable due to less stability. In various condensation reactions aldehydes are seen to react faster than ketones for the formation of Schiff bases due to presence of less hindered reaction centre in aldehydes (Singh and Agarwal 2015). Schiff base formation generally takes place under base or acid catalysis with heat (Antony *et al* 2016). The experimental conditions for Schiff base formation depend on the nature of amine and carbonyl compounds (Abood 2014).

Schiff bases are generally excellent chelating agents due to their synthetic flexibility, easiness of preparation and the special property of -HC=N group. They form five or six membered ring with metal ions when –OH or –SH is present close to the azomethine group (Elmali *et al* 1999). They serve as the backbone for the synthesis of various heterocyclic compounds (Zemede and Kumar 2014-15). Schiff bases are easily degradable, so purification of these should be done with great care (Anis *et al* 2013). Formation of Schiff bases is normally favoured by dehydrating agents (Yimer 2015). Schiff bases are important intermediates for the synthesis of various bioactive compounds (Yang and Sun 2006, Kapadnis *et al* 2016). They also act as fundamental material and are used as chiral auxillaries in asymmetric synthesis (Surve *et al* 2016).

Schiff bases have been found to plethora of biological activities such as antimicrobial (Nagajothi *et al* 2013), antimalarial (Adedibu and Jashua 2011), anticonvulsant (Abdel-Galil *et al* 2003), anti-flammatory (Deka *et al* 2012), anticancer (Mirian *et al* 2011), anti-tumour (Shabani *et al* 2010), anti-HIV (Pandeya *et al* 1999), neurological disorders (Dave and Bansal 2013), diuretic activities (Nagajothi *et al* 2013), antiproliferative (Cheng *et al* 2010) and herbicidal (Holla *et al* 2000). These are known to be neoplasm inhibitors (Surve *et al* 2016). The Schiff base core compounds are also used for industrial purpose (Mandewale *et al* 2015). Various biological system involving transamination and racemisation reaction are very well elucidated by the import of presence of -N=CH- (imine) group in Schiff bases.

These act as intermediate in biologically important transamination reactions. These also serve as starting material for synthesis of heterocyclic compounds such as benzoxazines, formazans and 2 azetidinones which are bioactive compounds (Murhekar and Khadsan 2011). Also in coordination chemistry due to their stability of oxidative and reductive ease of preparations (Prabhakaran *et al* 2013). Schiff bases were used as flexidendate ligands and these also coordinate through O atom of deprotonated phenolic group and N atom of azomethine group (Kumar *et al* 2014). The conventional method of Schiff bases synthesis involves refluxing the carbonyl compound as well as amines in the presence of an organic solvent.

In general, aldehydes are more reactive than ketones at room temperatures and lesser reaction times are needed (Weingarten *et al* 1967). The extra carbon of ketone donates electron density to the carbon of azomethine and hence makes the ketone less electrophilic compared to aldehyde (Fessenden and Fessenden 1998). Schiff bases of aromatic aldehydes having an effective conjugated system are more stable while aliphatic aldehydes are relatively unstable and readily polymerizable. Further the equilibrium should be shifted which is done by removing water either azeotropically by distillation or using suitable drying agents (Anchal and Shipra 2010). In the past few years, environmental friendly methods of synthesis have gained considerate attention due to which solvent free protocols have been developed. Schiff bases were used in electrochemical and optical sensors in various chromatographic methods which enhances sensitivity and selectivity of organic reagents (Yahyazadeh and Azimi 2013).

Schiff bases can be divided into monodentate, bidentate and multidentatae (Arif *et al* 2001).The preparation of Schiff bases in which water is used as a solvent or reaction medium avails several advantages as (a) it is low cost, non-inflammable, harmless, and safe to use (b) their uniqueness in physical and chemical properties often increase the reactivity or selectivity, which sometimes is unattainable in organic solvents, and (c) hence eliminating the need of extra efforts to dry the substrates and reagents before their use and therefore reduces/eliminates the use of drying agents, energy as well as time (Naqvi *et al* 2009 and Behal *et al* 2016).

Schiff bases are synthesized by different methods. Various Schiff bases are derived by refluxing method as reported by (Gao and Zheng 2003). Naqvi *et al* (2009) reported Schiff bases by environmentally benign synthetic methods. Jarrahpour and Khalili (2006) synthesized Schiff bases by condensing amine and carbonyl compound in water suspension method. The grindstone method is ecofriendly to synthesize Schiff bases. This method resulted in higher yield and shorter time reaction and cleanser reaction conditions as reported by Zarei and Jarrahpour (2011). Use of microwave irradiation as a source of energy have been also used. The reaction under microwave assisted conditions are solvent free or less solvent conditions are attractive offering reduced pollution, low cost and offer high yield together with sampling in processing and sampling. A Schiff bases synthesis was reported by Chaudhary (2012) and Rashmi *et al* (2017) by using microwave method. Also Schiff bases were synthesized by energy efficient greener and eco-friendly methodologies (Khadsan *et al* 2010). Zamani *et al* (2011) reported Schiff base as sensing material. Schiff bases were also formed under solvent free condition catalysed by P_2O_5 (Devidas 2011). Schiff bases were also synthesized using sonicator and UV chamber reported by Bendale *et al* 2011.

A large number of Schiff bases with aromatic and hetero aromatic nucleus are known to possess biologically active compounds. This biological potential may be further enhanced by linking an active nucleus with another nucleus. The heterocyclic compounds with nitrogen and sulfur also showed significant activity against microbes. The difference in the magnitude of the antimicrobial activity of the Schiff bases might be due to the difference in cell wall structure of the microbes, solubility and stability of the Schiff bases (Zemede and Kumar 2014-15).

o-Phenylenediamine is an aromatic diamine which is a precursor of many heterocyclic compounds. Many Schiff bases are formed from *o*-phenylenediamine which exhibited antibacterial activity (Ajaily *et al* 2007) and other biological activities (Fasina *et al* 2012). *o*-Phenylenedimaine Schiff bases registered clinical properties and various applications including biological, analytical (Raman *et al* 2001), pesticides and dyes or corrosion inhibitor. In the presence of metal cation the *o*-phenylenediamine can form chelate ring using *in situ* method of synthesis because of kinetic template effect. *o*-Phenylenediamine can act as key intermediate and is used in the production of corrosion inhibitors, pharmaceuticals compounds, fungicides and various pigments. It is also used to remove sulfur from ores and coloration by aldehydes in polymeric products (Geary 1971).

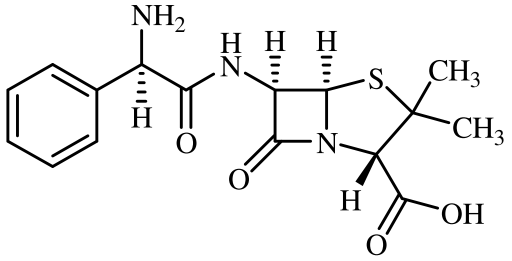
Symmetrical Schiff bases derived from aromatic 1, 2-diamies such as *o*-phenylenediamine had received attention due to their flexibility, rich coordination chemistry and application in catalysis (Jacobsen 2000). Schiff bases synthesized from *o*-phenylenediamine have been shown to be useful models in understanding of biological systems such as irregular binding of peptides. In presence of metal cation, due to kinetic template effect *o*-phenylenediamine can form chelate ring.

A novel Schiff base was made by Gull and Hashmi (2014) derived from 1,2-diphenylethane-1,2-dione with *o*-phenylenediamine and benzophenone. The synthesized compound was tested against antimicrobial bacteria *i.e*, *Klebsiella pneumonia* which showed 9.7 μg ml^{-1} minimum inhibitory concentration and also against *Fusarium solani* which showed 8.5 μg ml^{-1} minimum inhibitory concentration. According to Chittilappilly and Yusuf (2008) ligand salab showed antimicrobial activity against *Psuedomonas aeruginosa.*

The interesting advantage of preparation of Schiff base ligands is the activity of antimicrobial (Yimer 2015). Antimicrobial activity is a property of both organic and inorganic substances and the exploitation of such activity is of considerable importance in the development of antiseptics, sanitizers, germicides, bacterisides and disinfectants (Fasina *et al* 2014). A microbial agent is a substance that enhances, kills or inhibits the growth of microorganisms such as bacteria, fungi or protozoans.Therefore microbial drugs promote, kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). There are handful of fundamental ways in which microbial agent can exert their action.

- Growth or inhibition of bacterial cell wall synthesis.
- Interference with protein and nuclei acid synthesis.
- Accelerate or inhibit the essential metabolic pathway that exists in the bacterium but does not exist in the host.

Ampicillin (II) was used as a standard for microbial activity. Ampicillin is an antibiotic used to prevent and treat a number of bacterial infections, such as respiratory tract infections and urinary tract infections.



(II)

Keeping in view the importance of Schiff bases of *o*-phenylenediamine, the present work will be carried out on the synthesis of Schiff bases of *o*-phenylenediamine and their microbial activity with the following objectives.

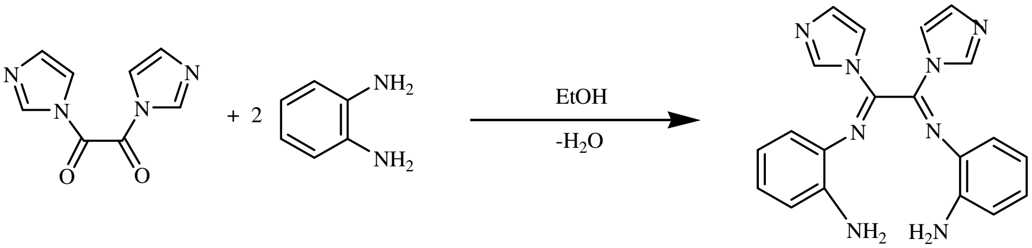
i) To synthesize and characterize different Schiff bases of *o*-phenylenediamine.

ii) To evaluate the synthesized different Schiff bases of *o*-phenylenediamine for their microbial activity.

CHAPTER - II

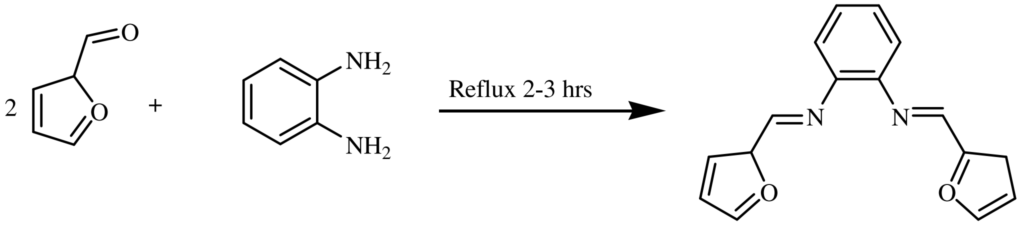
REVIEW OF LITERATURE

Yadava *et al* (2015) synthesized new Schiff base by refluxing *o*-phenylenediamine and 1,1'-oxayldiimidazole in ethanol. This was identified by elemental analysis, conductometric measurements, electronic spectroscopy and characterized by IR. The condensation of *o*-phenylenediamine with keto groups of 1,1'-oxaylimidazoles was confirmed by the presence of >C=N band and the absence of >C=O band at around 1700 cm^{-1}.



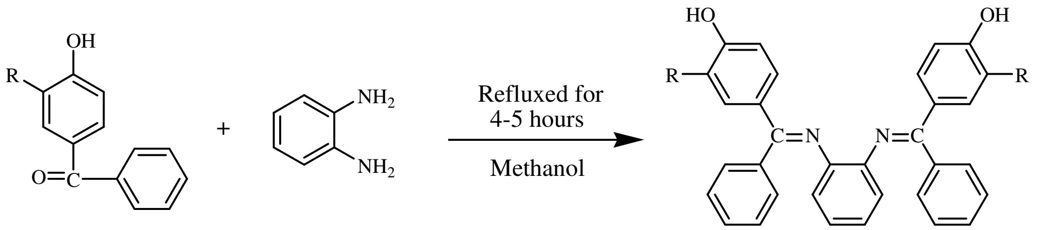
Scheme 1

Synthesis of mononuclear Schiff base was reported by Subramanian *et al* (2015). The Schiff base was derived from *o*-phenylenediamine and furfuraldehyde in ratio 1:2 respectively. These Schiff bases were tested for their antibacterial activity against *Escherichia coli, Staphylococcus aureus*, *Klebsiella, Pseudomonas* and *Bacillus subtilis*. The compounds showed adequate growth inhibitory activity against these micro-oraganisms.



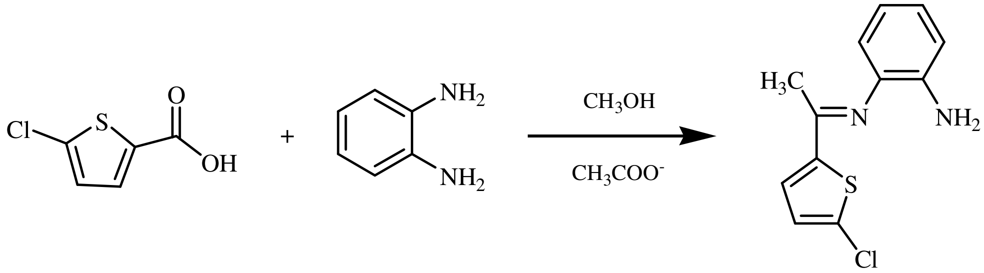
Scheme 2

Asha *et al* (2016) reported the synthesis of Schiff base from *o*-phenylenediamine and substituted hydroxylbenzophenone in 1:2 molar ratio using methanol as a solvent with refluxing and constant stirring. This condensation reaction was further carried out by adding 3 drops of glacial acetic acid for 5 hrs. The water formed during the reaction was removed from reaction mixture with sodium sulphate as dehydrating agent. The newly synthesized Schiff bases were further evaluated for antibacterial activity against *Staphylococcus epidermidis* (gram positive bacteria), *S. aureus* (gram positive bacteria), *Listeria monocytogenes* (gram negative bacteria) and *E. coli* (gram negative bacteria) and antifungal activity against *Candida albicans.*



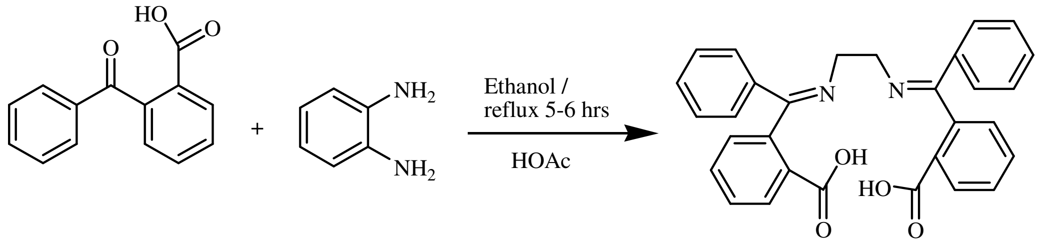
Scheme 3

Ahamed and Yunus in 2014 reported the synthesis of Schiff base which was prepared by the condensation of 2-acetyl 5-chloro thiophene and *o*-phenylenediamine. The solvent was methanol under which this reaction was carried out. Synthesized Schiff base was tested for their biological activity against gram positive bacteria *S. aureus* and *B. subtilis* and gram negative bacteria *Salmonella typi* and *E. coli.*



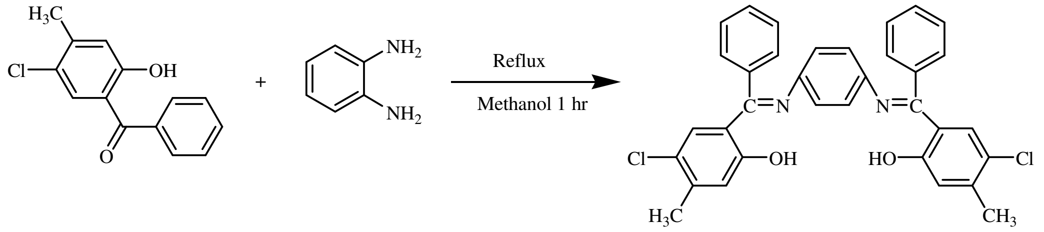
Scheme 4

Shemary (2015) synthesized a Schiff base by addition of ethanolic solution 2-benzoyl benzoic acid ethanolic solution to the same amount of *o*-phenylenediamine along with few drops of glacial acetic acid. These compounds displayed a good activity against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*.



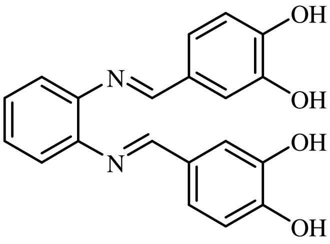
Scheme 5

Schiff base derived from the condensation of 5-chloro-4-methyl-2-hydroxy benzophenone with *o*-phenylenediamine was reported by Prabhakaran *et al* (2013). The mixture was heated and refluxed for 10 minutes. This compound was characterized by elemental and spectral methods. It showed strong absorption around 1600-1615 cm^{-1} due to azomethine group. The phenolic C-O stretching was observed at 1340-1386 cm^{-1}.

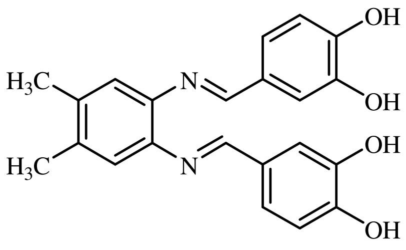


Scheme 6

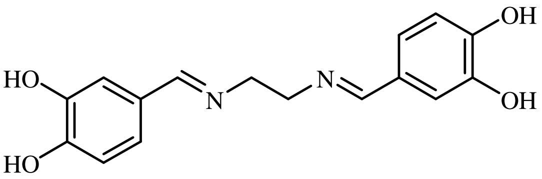
Abdulghani and Khaleel (2013) synthesized three new Schiff bases from 3,4-dihydroxybenzaldehyde and ethylenediamine, *o*-phenylenediamine and 4,5-dimethyl-1,2-phenylenediamine. The reactants were dissolved in ethanol containing 2 drops of piperidine. The reaction was refluxed and continued for stirring. The spectra IR showed strong to moderate absorption band in the range of 3251-3263 cm^{-1}. Asymmetric and symmetric stretching of azomethine group (γ C=N) was observed at 1670-1673cm^{-1} and 1604-1608cm^{-1}.



(III)

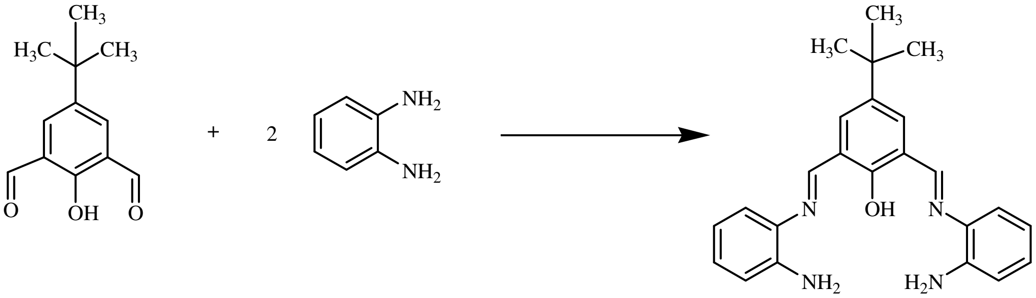


(IV)



(V)

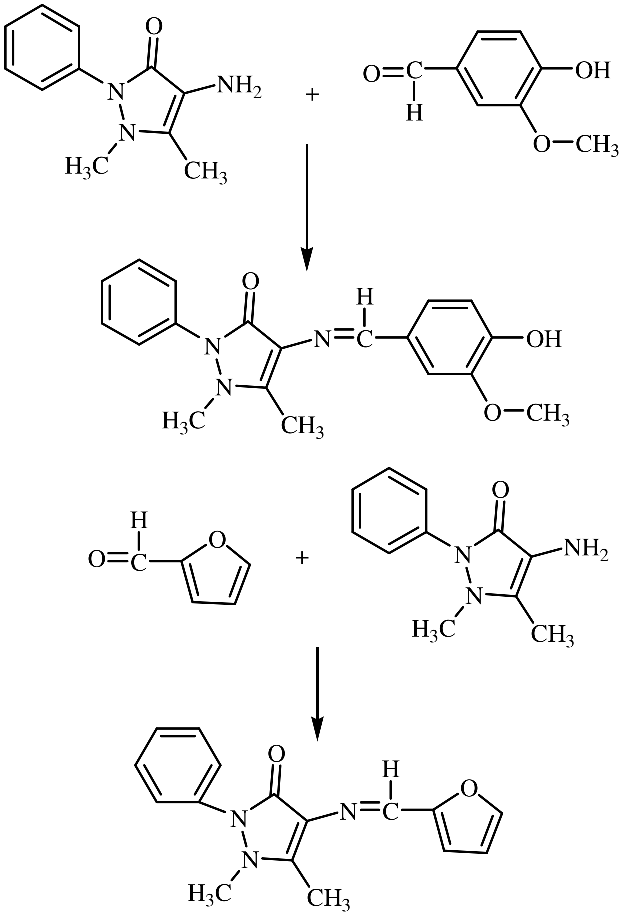
A Schiff base was reported by Prabakarakrishnan *et al* (2015) by condensation of 2,6-diformyl-4-tertiarybutylphenol and *o*-phenylenediamine using grindstone method. These Schiff bases were tested against *K pneumonia, S. aureus, E. coli.* The ligands showed better activity than standard against *S. aureus.* Also the synthesized compounds were tested for their anti-cancer activity using human adherent skin cancer A431 cell line. The ligand exhibited cytotoxicity on the cell line in a concentration dependent manner which ranges from 0 to 70 μg ml^{-1}.

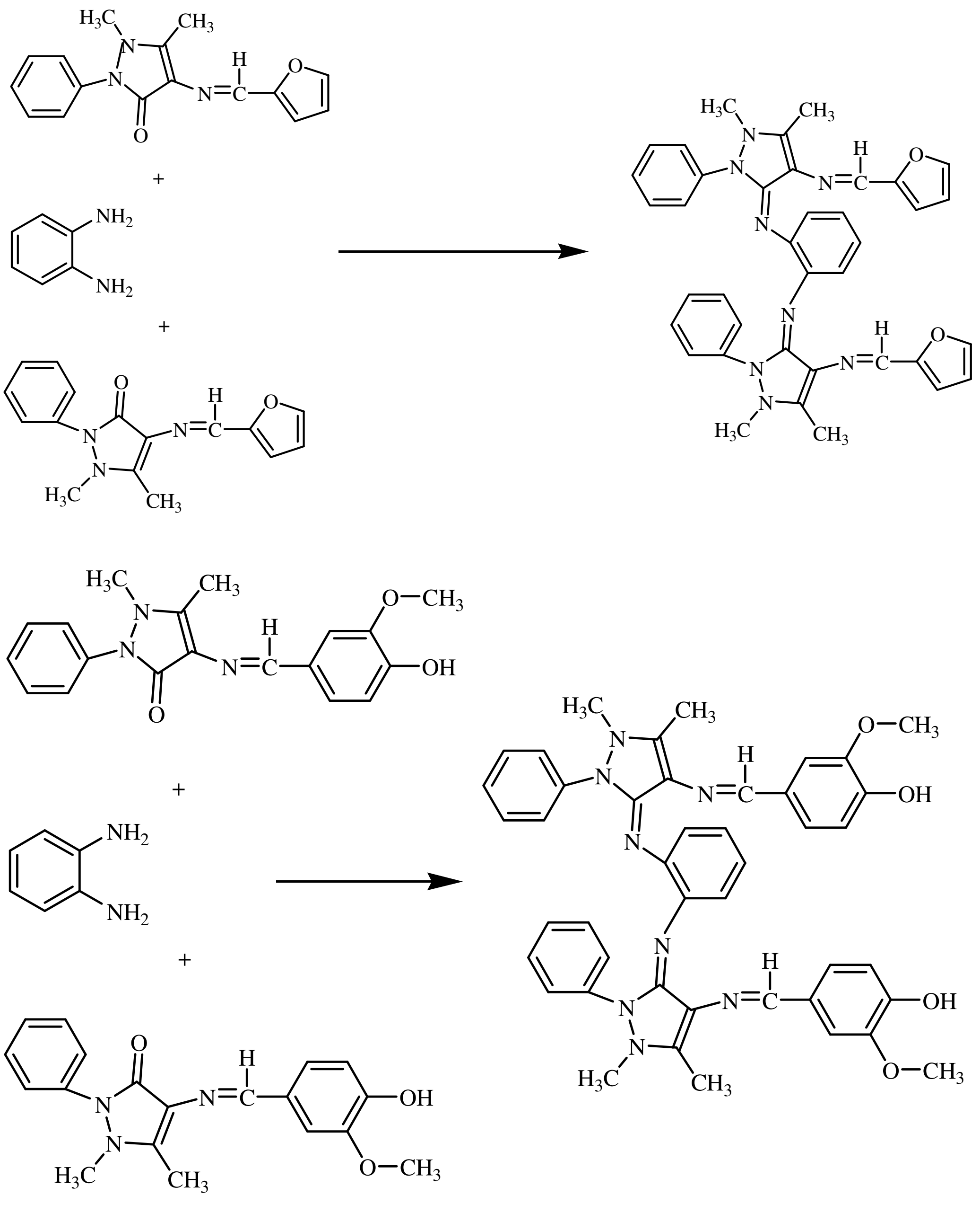


Scheme 7

The Schiff bases derived from salicyaldehyde and *o*-phenylenediamine, o-aminophenol or 2-aminobenzimidazole (salpd, salap, salab respectively) were reported by Chittilappilly and Yusuf (2008). The synthesized Schiff bases except salab were active against all the bacteria *P.aeruginosa*, *K. pneumonia* and *E. coli.*

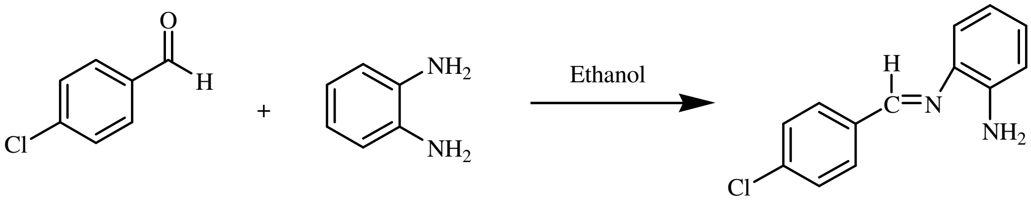
Gayathri and Suresh (2016) prepared two Schiff bases by reacting 4-aminoantipyrine with vanillin and furfural respectively. These Schiff bases were again reacted with *o*-phenylenediamine. Both the reactions were carried out in ethanol. These prepared compounds were tested for antibacterial activity against *Serratia* spp., *Bacillus cereus*, *P. aeruginosa*, *S. aureus* and *E.coli.*





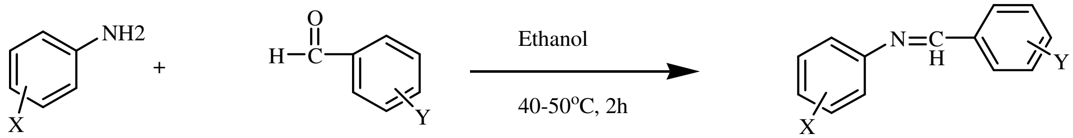
Scheme 8

Synthesis of different substituted Schiff base was reported by Kapadanis *et al* (2016) by reacting *o*-phenylenediamine and *p*-chlorobenzaldehyde by different methods like microwave irradiation, refluxing, stirring and grinding. These were also examined for antimicrobial activity against *Bacillus* and *E. coli.* It was observed that microwave irradiation method was the simplest, cheapest, clean and convenient method and it took shorter time and gave higher yield as compared to other methods.



Scheme 9

Ibrahim *et al* (2006) synthesized ten different Schiff bases from aromatic aldehydes and anilines and its derivatives. The reaction was carried out in absolute ethanol. This was done with stirring for two hrs. The temperature was maintained at 40-50^{o}C. The identification and characterization of synthesized compounds was done by elemental analysis and ^{1}H-NMR respectively. The aromatic protons displayed as multiplet signals in the range of 6.8-7.8 ppm whereas a sharp singlet peak appeared at 8.5-8.6 ppm for -CH=N- proton.

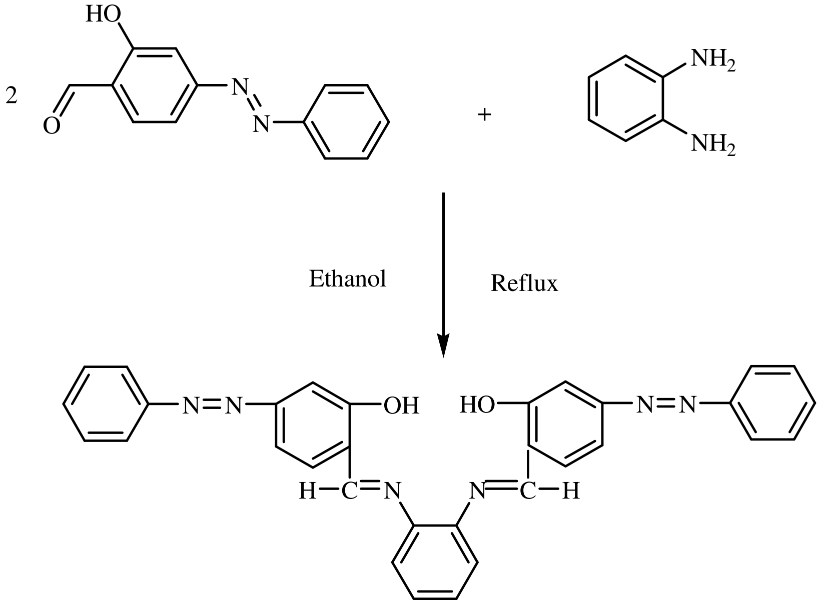


X = H, *o*-NO_2, *p*-NO_2, *o*-COOH, *p*-OCH_3, *o*-Br

Y = H, *m*-NO_2, *m*- OH, *p*-OH, *o*-OH, *o*-OCH_3, *m*-OCH_3, *p*-OCH_3, *p*-$N(CH_3)_3$

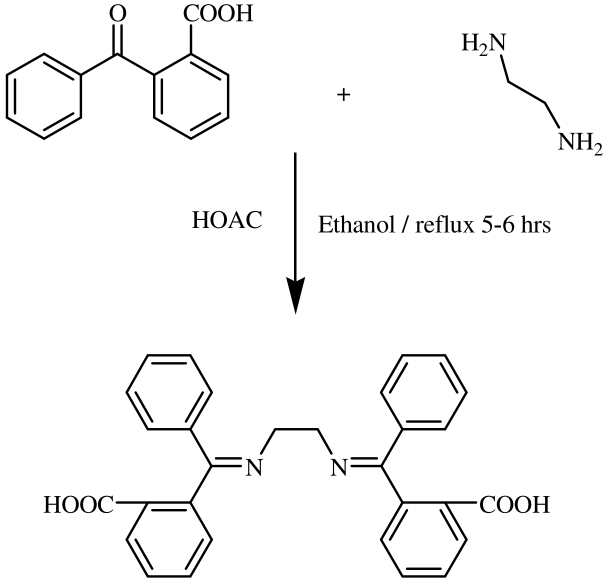
Scheme 10

Aliyu *et al* (2013) synthesized a Schiff base formed from *o*-phenylenediamine and 4-(benzeneazo) salicylaldehyde. Ethanol was used as a solvent and the reaction mixture was refluxed for two hrs. The Schiff base was determined with dissociation constant (pKa). These synthesized compounds were screened for antifungal activity against *Asperigillus nigar* and *C. albicans* and antibacterial activity against *E. Coli* and *S. aureus* showed good activity.



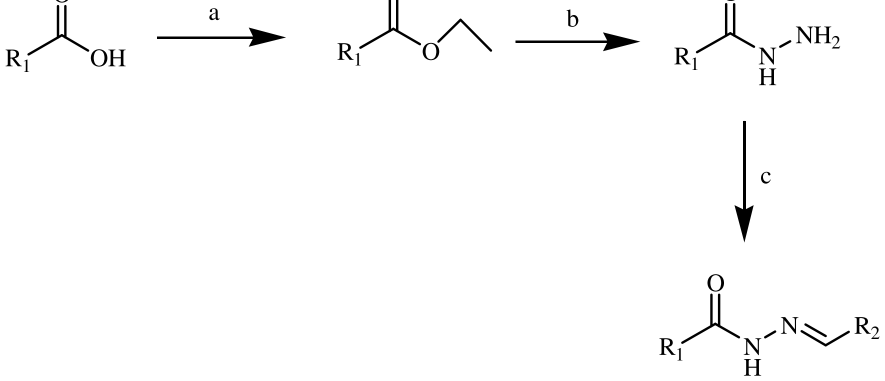
Scheme 11

Shemary and Zaidan (2016) reported tetradentate Schiff base prepared from 2-benzoyl benzoic acid and ethylene diamine in the presence of glacial acetic acid. The reaction mixture was refluxed and then stirred for 5-6 hrs. ^{13}C-NMR of Schiff base showed the peak carbon of carboxyl group at 161.07 ppm and of carbon HC=N at 143.50 ppm. The synthesized Schiff bases were then tested for antifungal and antibacterial potential against *P. aeruginosa, E. coli, B. subtilis* and *S. aureus.*



Scheme 12

Bhat *et al* (2014) synthesized a series of different hydrazides Schiff bases which were prepared from various types of aldehydes and hydrazides. The prepared compounds were tested for antioxidant and antimicrobial activity. The microorganisms on which the antimicrobial activity was screened were *S. aureus, E. coli, B. subtilis* and *Salmonella typhi.* For antioxidant activity free radical scavenging ability by DPPH (1,1-diphenyl-2-picryl hydrazyl) assay and ABTS (2,2’-azino-bis[3-ethylbenzthiazoline-6-sulphonic acid) assay was done.

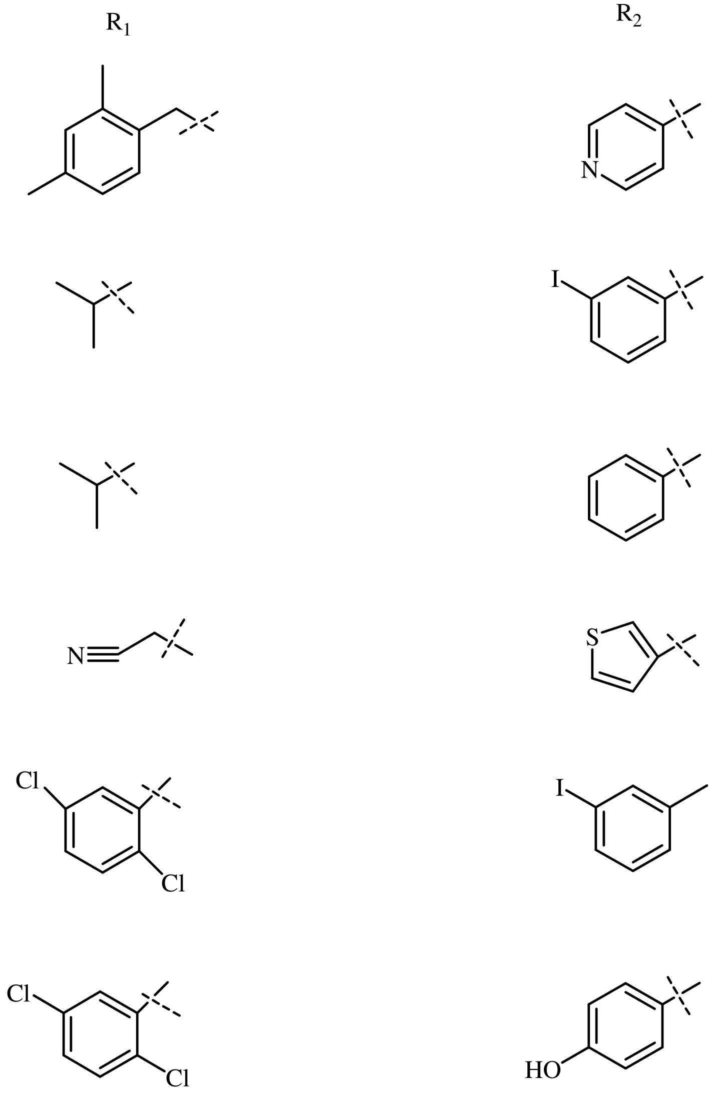


Scheme 13

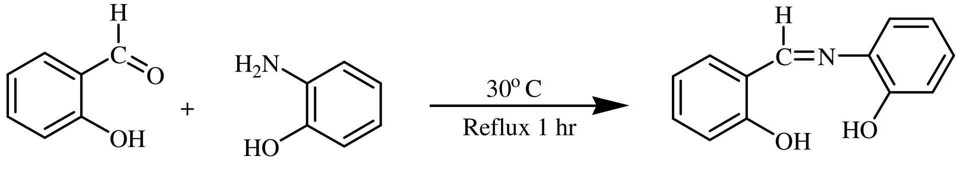
a) Ethanol, reflux for 5 hrs

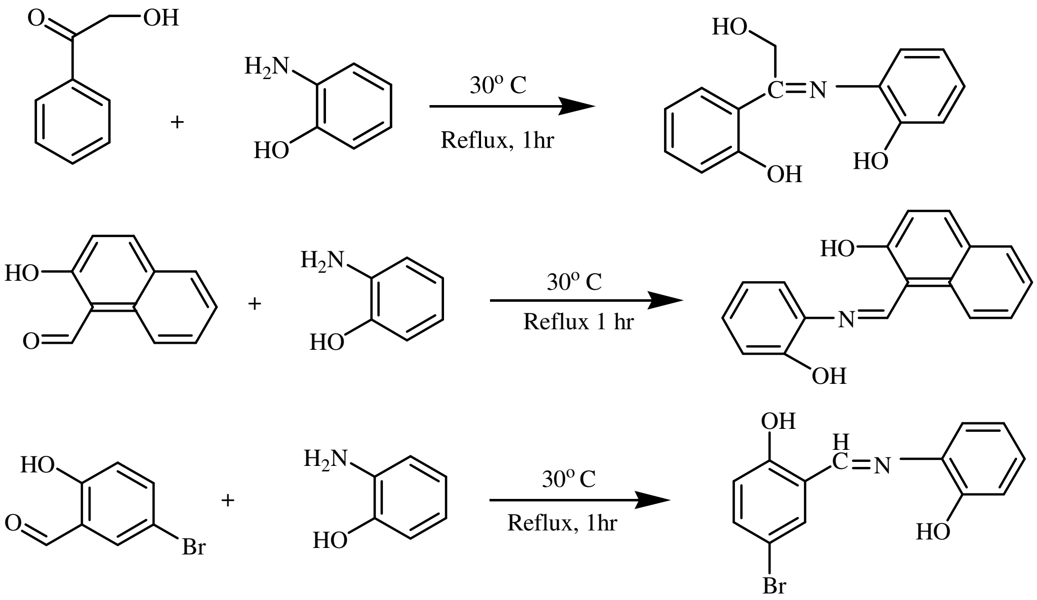
b) Hyrazine hydrate, ethanol, reflux for 6 hrs

c) R_2-CHO, ethanol, reflux for 4 hrs



Uddin *et al* (2014) reported synthesis of dibasic tridendate Schiff bases of *o*-aminophenol and different aldehydes. The condensation of salicylaldehyde, 5-bromosalicyaldehyde, 2-hydroxy-1-naphthaldehyde and 2-hydroxyacetophenone with *o*-aminophenol was done with stirring. These Schiff bases were then evaluated for antibacterial activity against *S. typhi*, *B. cereus*, *E. coli, Bacillus megaterium* and *S. aureus.*

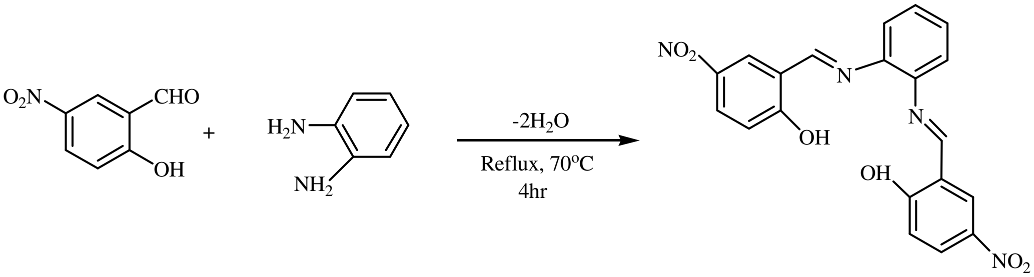




Scheme 14

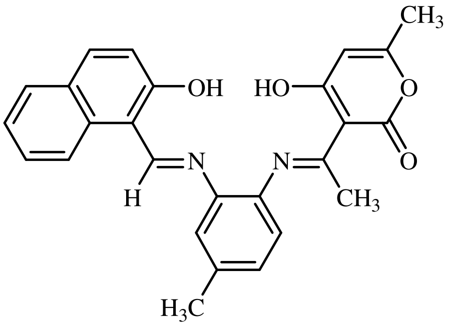
A series of symmetric bis-Schiff bases of salicylaldehyde derivatives like 3-methoxysalicyaldehyde, 4-methoxysalicylaldehyde and 5-methoxysalicyaldehyde and *p*-phenylenediamine were reported by Uzarevic *et al* (2010). The reaction was done with stirring and refluxing for one hour. The synthesized compounds consisted of 2 subunits which assume tautomeric forms: enol-imino form and keto-amino form.

Schiff bases derived from *o*-phenylenediamine and 5-nitrosalicylaldehyde were synthesized by Fasina *et al* (2014). The antimicrobial activity of Schiff base against some clinically important bacteria, namely *S. aureus, E. coli, P. aeruginousa* and *S. typhi* showed that the activity was dependent on the geometry of compound and the substituent present on benzene ring.



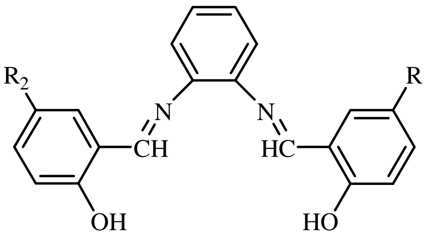
Scheme 15

Unsymmetrical tetradentate Schiff bases were reported by Shelke *et al* (2013). These Schiff bases were formed from 4-methyl-*o*-phenylenediamine and 3-acetyl-6-methyl-pyran-2,4-dione and 2-hydroxy-1-naphthaldehyde. The synthesized compounds were analysed for antifungal activity against *Trichoderma* and *Aspergillus niger* also for antibacterial activity against *E. coli* and *S. aureus.*



(VI)

Three symmetrical and two unsymmetrical N_2O_2 Schiff bases obtained from the condensation of *o*-phenylenediamine with various aldehydes like salicylaldehydes, 5-nitrosalicylaldehyde and 5-bromosalicylaldehyde as reported by Fasina *et al* (2014). The antimicrobial activity of the compounds tested against the microorganisms *E. coli, Escherchia feacalis* and *S. aureus*. The unsymmetrical Schiff bases were active against these micro-organisms.



$R_1=R_2=H$

$R_1=R_2=Br$

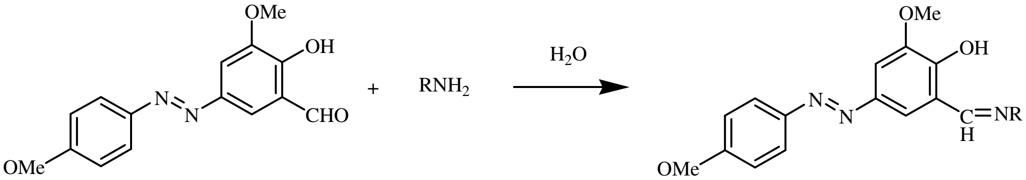
$R_1=R_2=NO_2$

$R_1=H$ $R_2=Br$

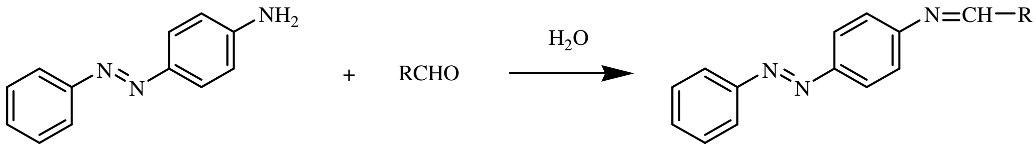
$R_1=H$ $R_2=NO_2$

(VII)

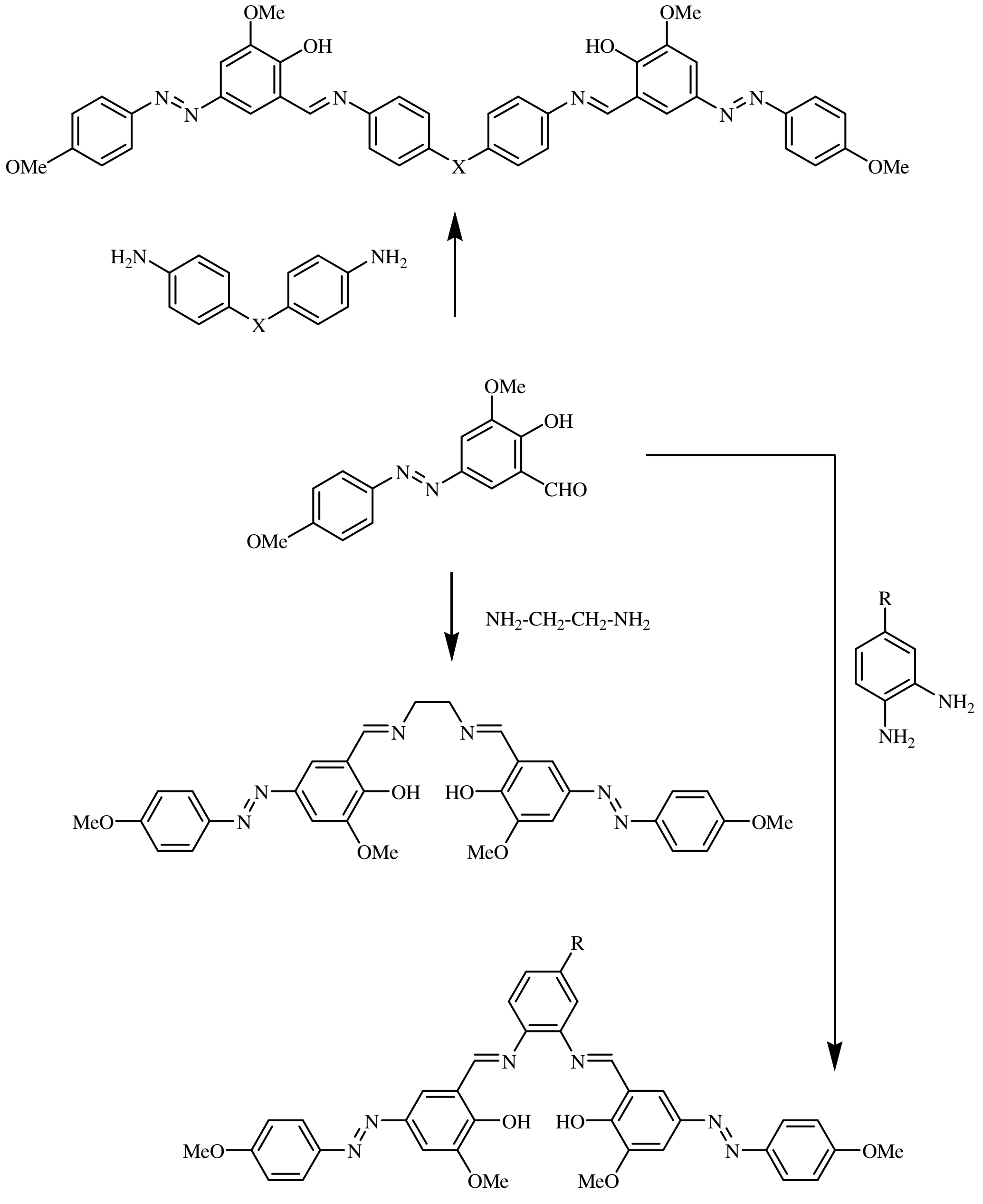
Zarei and Jarrahpour (2011) reported green and efficient synthesis of azo Schiff bases by water based method and grind stone method. These Schiff bases were made under solvent free conditions. These methods neither required refluxing condition nor organic solvent. The yields were excellent and reaction rate was also fast.



R= 4-$MeOC_6H_4$, 3- $MeOC_6H_4$, 4- MeC_6H_4, 3- MeC_6H_4, C_6H_5, $C_6H_5CH_2$, 3-OH C_6H_4, 2,4-diMeO C_6H_3, 4-Cl C_6H_4, 4-$NO_2C_6H_4$



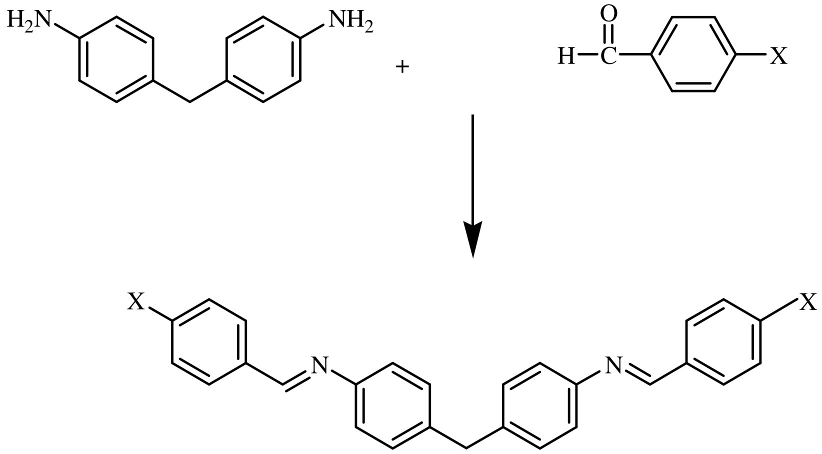
R = 4-NO$_2$C$_6$H$_4$, 4-Cl C$_6$H$_4$, 2-OH C$_6$H$_4$, 4-MeOC$_6$H$_4$, 4- MeC$_6$H$_4$, C$_6$H$_5$, C$_6$H$_5$CH$_2$



X = O, SO$_2$, CH$_2$
R = H, C$_6$H$_5$CO

Scheme 16

Sabah (2014) reported Schiff bases formed from 4,4'-methylen di aniline (MDA) with different aldehydes. The aldehydes were benzaldehyde, *p*-methoxy benzaldehyde, *p*-chloro benzaldehyde, *p*-methoxy benzaldehyde and *p*-chloro benzaldehyde. The reaction mixture was stirred for 30 minutes. The unreacted starting materials were removed by washing with ethanol. Then the products were recrystallized from minimum amount of boiling ethanol. The ring protons of all the synthesized compounds were found in the range of 7.25-7.35 ppm.

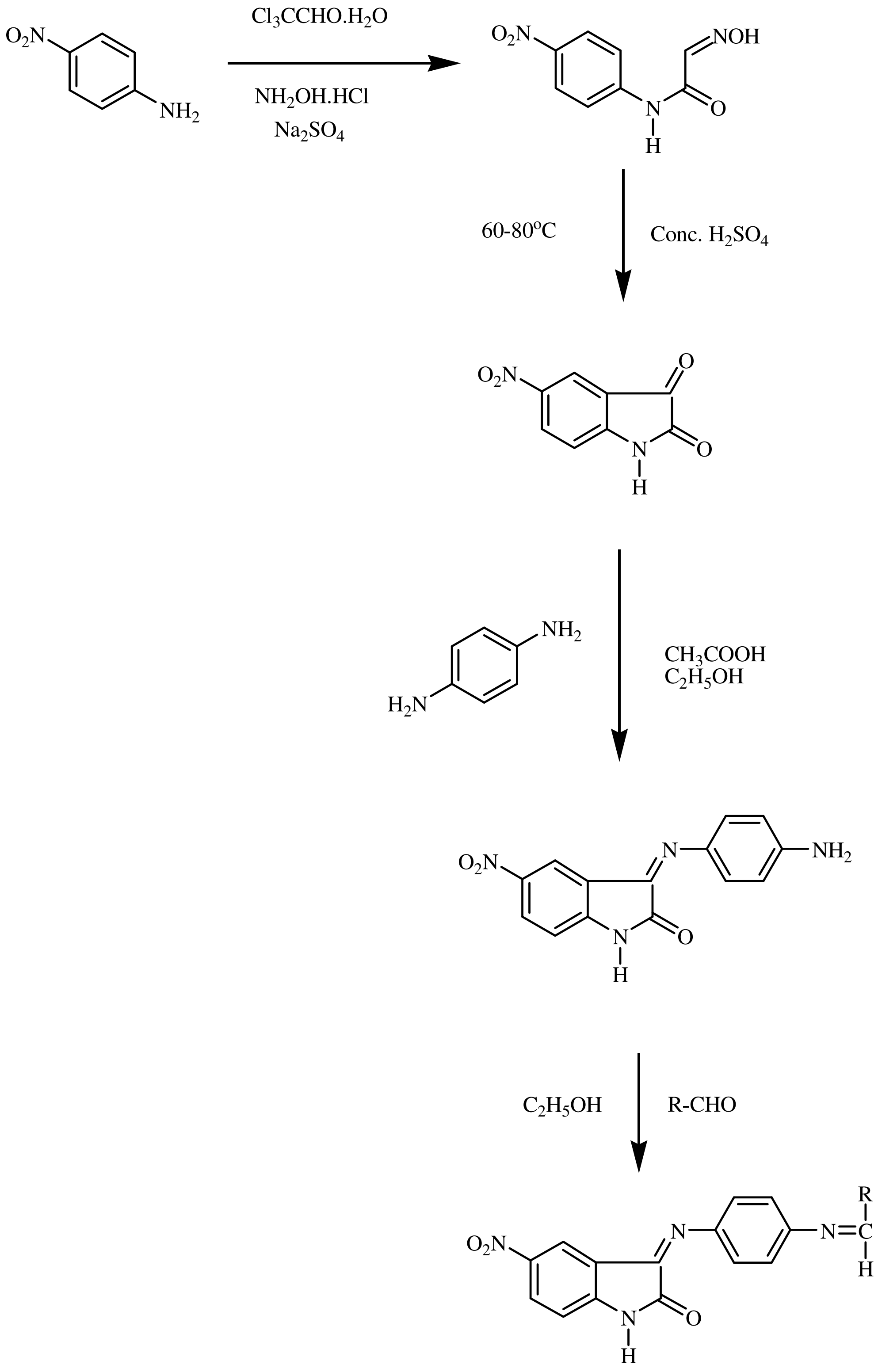


X = H, OCH_3, Cl

Scheme 17

Suresh and Prakash (2011) reported a series of Schiff bases which were derived from 4-aminoantipyrine, furfural and o-phenylenediamine. The synthesized Schiff bases were then characterized by elemental analyses, infrared, UV-visible spectroscopy, NMR spectroscopy, thermal gravimetric analyses, ESR spectral analyses and conductivity measurements. These Schiff bases were further evaluated for antibacterial activity and also the MIC values these were determined.

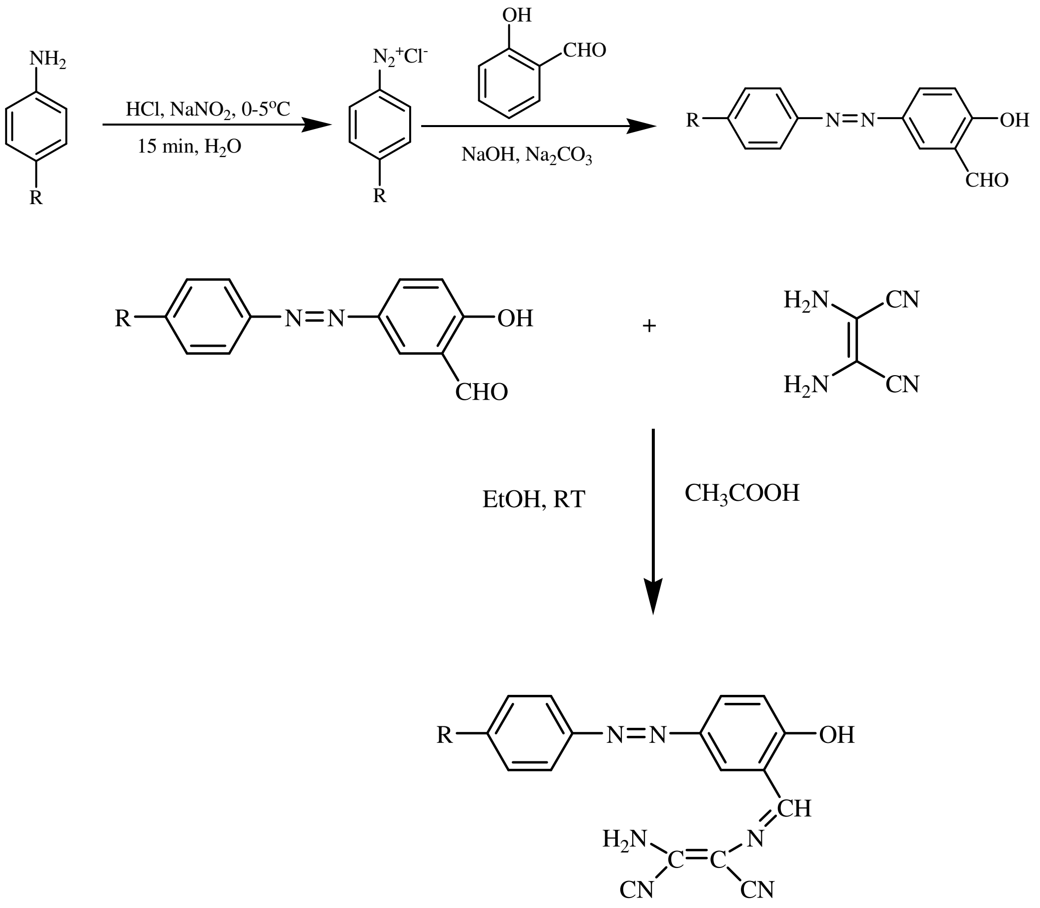
Prakash *et al* (2009) synthesized five novel Schiff bases of 5-substituted isatin. These Schiff bases were tested for antimicrobial activity against different micro-organisms which were *S. aureus, S. epidermidis, Bacillus cereus, E. coli, Micrococcus leteus, P. aeruginosa, K. pneumoniae* and antifungal activity against *A. niger* and *Aspergillus fumigates.* The obtained results showed that compounds tested were more effective towards bacteria than fungi. This may be due to because of electron withdrawing substituents in phenyl ring at 3rd position and nitro group at 5th position which increases the lyophilic character of molecule. This character facilitated the crossing of biological membrane of bacteria hence inhibited the growth.



R = H, *p* -Cl, *p* -OH, *p* -OH, *p* -OCH_3, *p* - NO_2, *o* -OH, *p* -CH_3, *m* -NO_2, *p* -$N(CH_3)_2$

Scheme 18

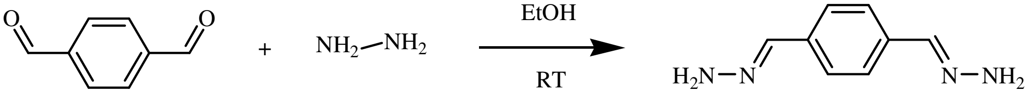
Synthesis of azo Schiff bases was reported by Yahyazadeh and Azimi in 2013. The Schiff bases were made in the presence of catalytic amount of acetic acid. The reaction was done with stirring for 2 hrs. The electronic absorption spectra of compound showed in the UV-Vis region 240-260 nm and 340-370 nm. NO_2, C=O electron attracting substituents increases polarizability. These Schiff bases were unsymmetrical and showed antimicrobial activity.

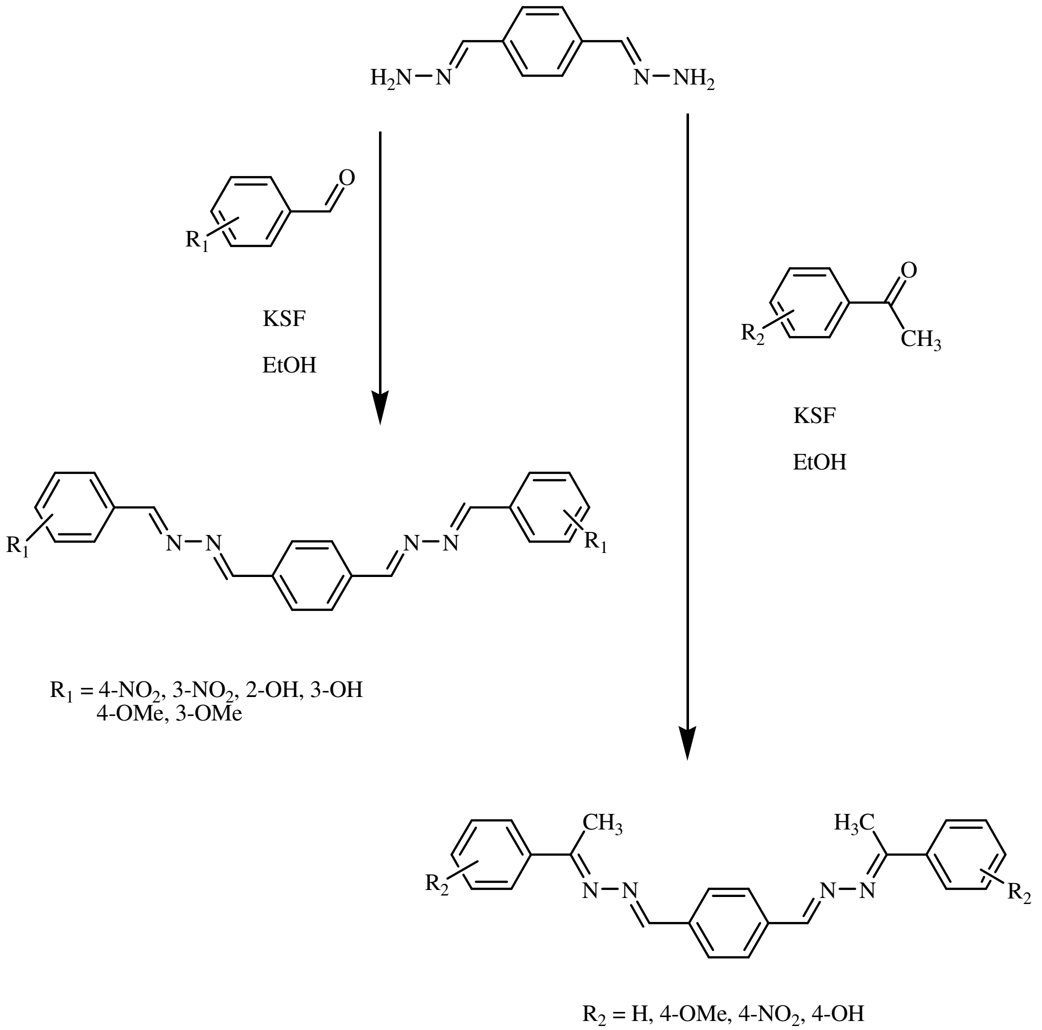


R = H, NO_2, CH_3, $-C(CH_3)=O$

Scheme 19

Synthesis of Schiff bases using KSF was reported by Mahmoodi *et al* (2011) with various aldehydes and ketones. In these reactions aldehydes react faster than ketones. Compounds containing nitro group *i.e* electron withdrawing groups gave high yield.



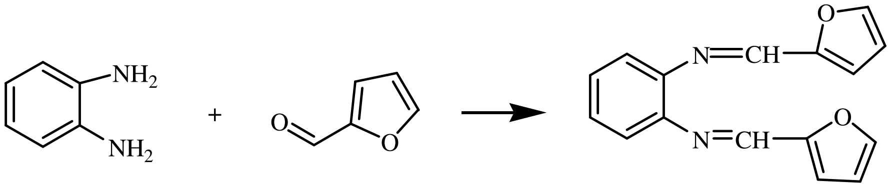


R_1 = 4-NO_2, 3-NO_2, 2-OH, 3-OH
4-OMe, 3-OMe

R_2 = H, 4-OMe, 4-NO_2, 4-OH

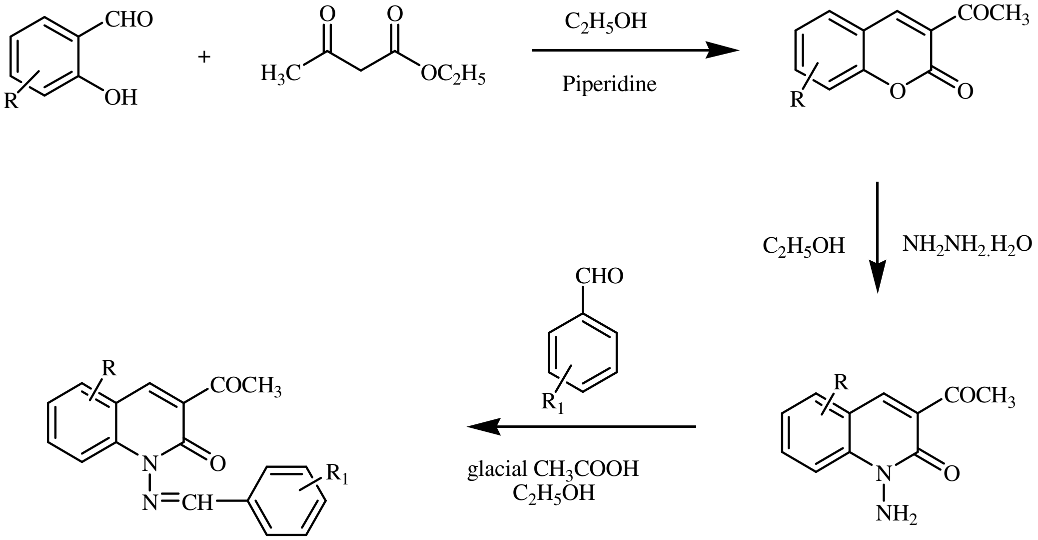
Scheme 20

Mohamed *et al* (2006) reported a Schiff base derived from *o*-phenylenediamine and 2-furancarboxaldehyde mixed in hot solution of ethanol. The mixture was left under reflux for 2 hrs. The solid product was separated by filtration, purified by crystallization from ethanol, washed with diethyl ether, and then dried in a vacuum over anhydrous calcium chloride. The synthesized ligands were tested against bacterial species for antibacterial activity against *E. coli, P. aeruginosa,* and *Staphylococcus Pyogones* as well as fungus *Candida*.



Scheme 21

Kumar *et al* (2014) reported a Schiff bases derived from different aromatic aldehydes and different substituted 3-acetyl-amino-quinolin-2-one in alcohol medium. These Schiff bases were screened for antimicrobial activity against *P. aeruginosa, E. coli, B. subtilis*, *C. albicans* and *S. aureus.*

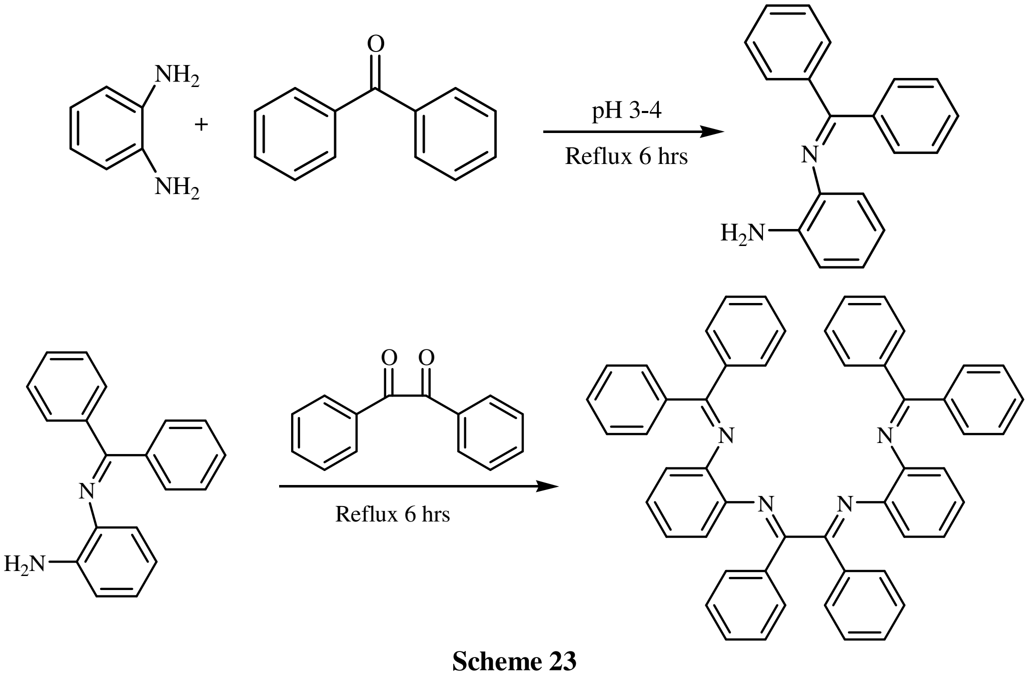


R_1= H, 6-NO_2, 6-Cl

R_2= 3-NO_2, 3,4,5-OCH_3, 4-CH_3.4-OH, 2-Cl, 2-NO_2

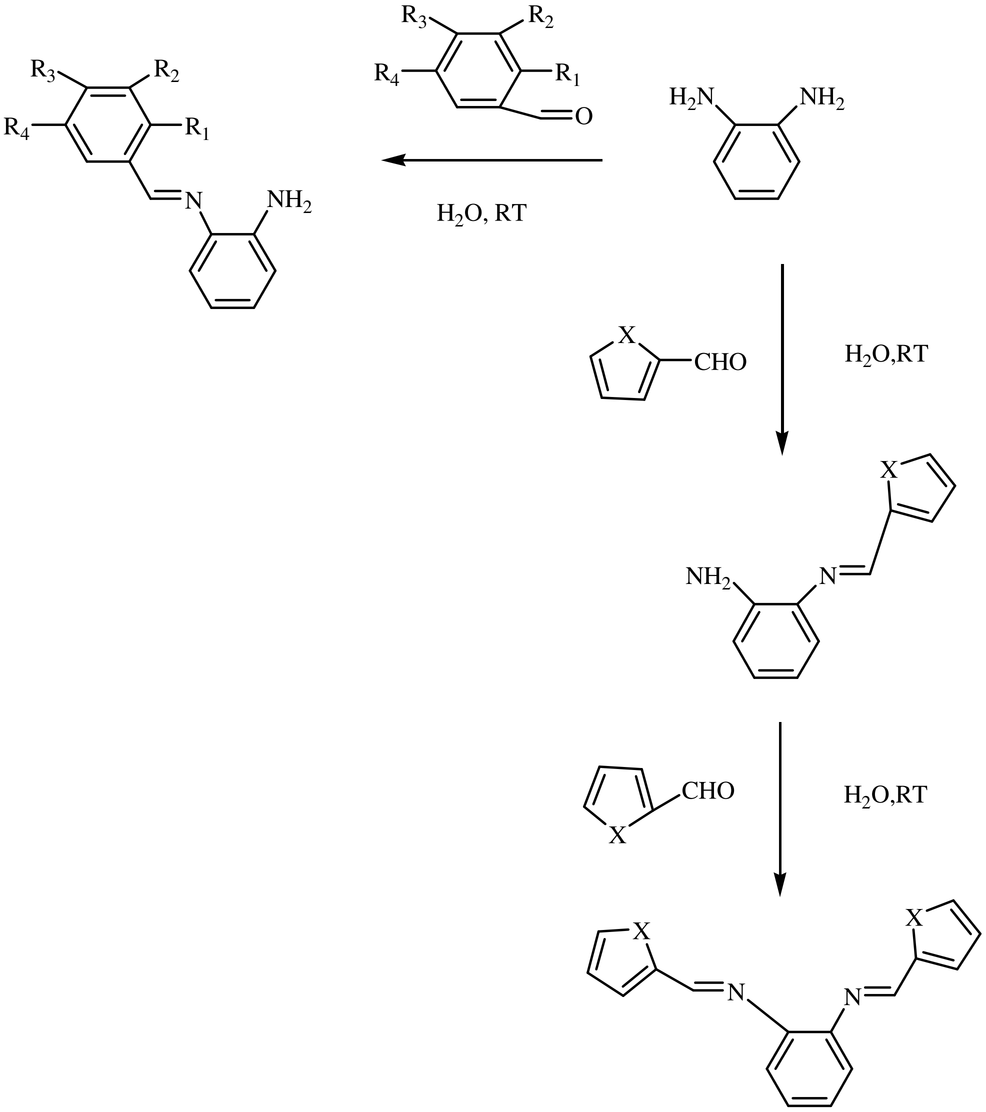
Scheme 22

The Schiff base derived from *o*-phenylenediamine and benzophenone was synthesized by Gull and Hashmi (2014). This Schiff base then treated with 1,2-diphenylethane-1,2-dione. The synthesized compound was studied for its *in vitro* antibacterial activity against *S. aureus, B. subtilis, E. coli, K. pneumonia* and *S. typhi* and antifungal activity against five *A. niger, Fusarium solani, Curvularia lunata, Rizoctonia bataicole* and *C. albicans*



Scheme 23

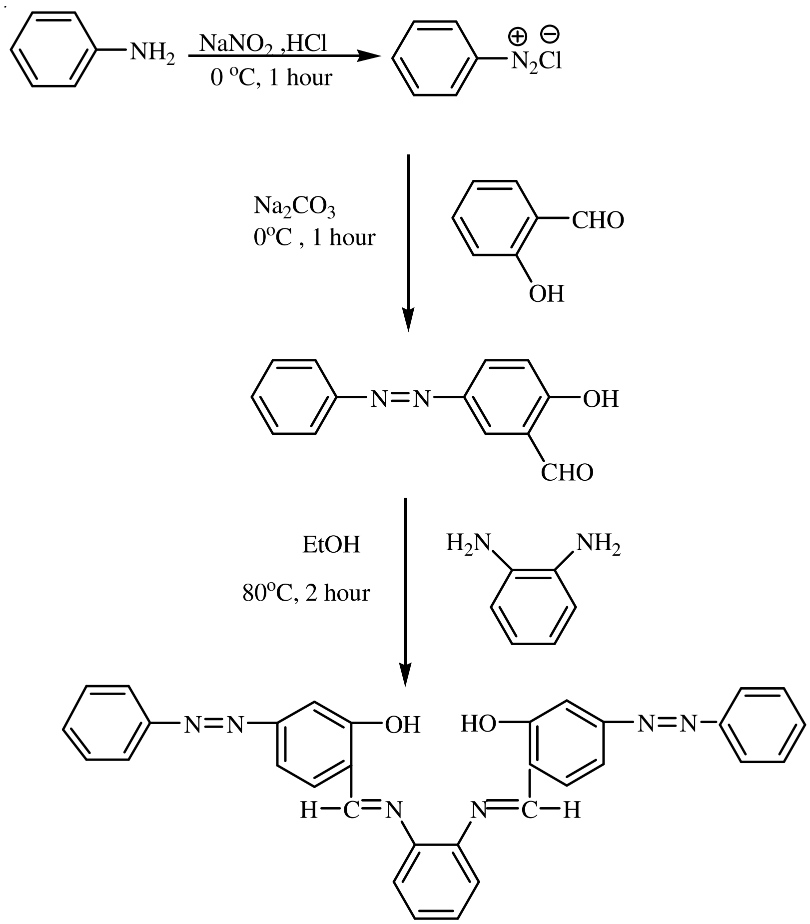
Rao *et al* (2010) reported synthesis of Schiff base in aqueous medium. It is a green alternative approach with effective mass yield and high reaction rates. Conventional methods took more time for the synthesis of the Schiff bases than with the use of water as solvent. So, novel and eco-friendly condensation reaction method permitting the green synthesis of the Schiff bases by stirring 1,2-diaminobenzene with various aldehydes in water as solvent.



R_1, R_2=H, OH; R_3=H, OH, Cl; R_4=H, Cl, Br; X=N-H, S

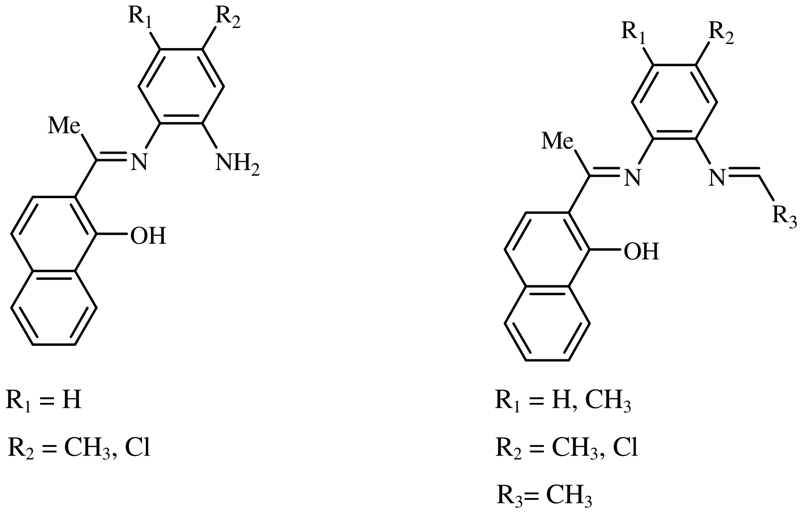
Scheme 24

Schiff bases with N_2O_2 were synthesized by Liu *et al* (2006). 4-(benzeneazo) salicylaldehyde was synthesized with aniline, sodium nitrate and salicylaldehyde. Two tetradendate ligands, N,N'-bis[4 - (benzeneazo) salicylaldehyde] - *o* - phenylenediamine and N, N' - bis [4 - (benzeneazo) salicylaldehyde] ethylenediamine were formed by the 2:1 molar condensation of 4-(benzeneazo) salicylaldehyde with *o*-phenylenediamine or ethylenediamine.



Scheme 25

Esmadi *et al* (2015) reported Schiff bases which were unsymmetrical. These Schiff bases were derived from phenylenediamine derivatives in conjunction with 2-hydroxynaphthalade and 1-hydroxy-2-acetonaphthone. Twelve compounds were formed by making their complexes with copper(II) and nickel(II) which were further screened for biological activity. Out of these only three compounds were found to be mutagenic.



$R_1 = H$

$R_2 = CH_3$, Cl

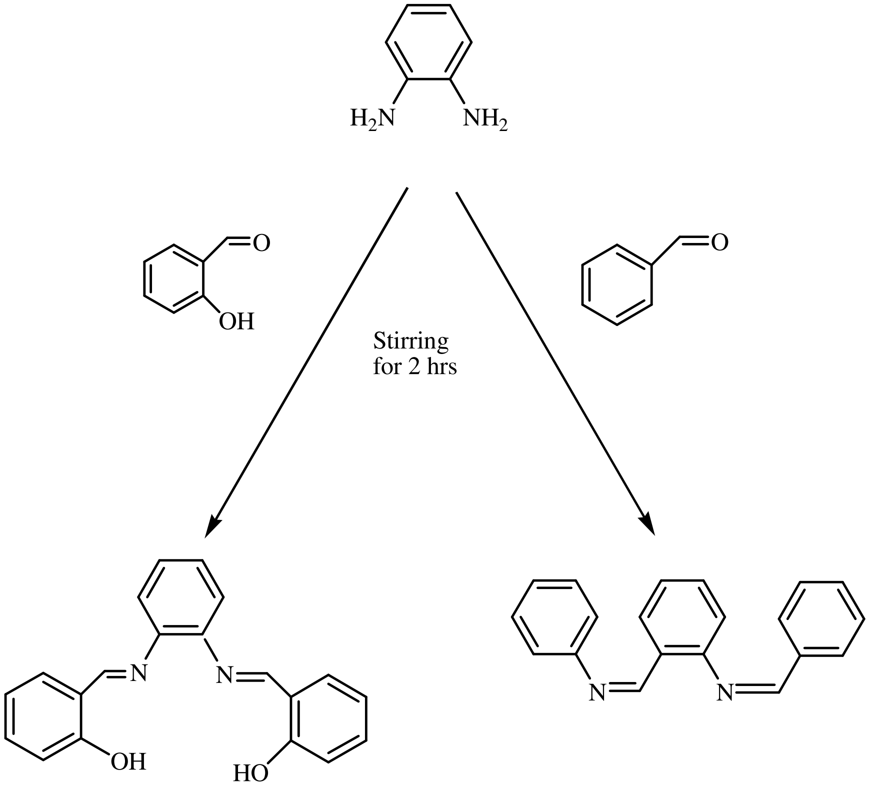
$R_1 = H$, CH_3

$R_2 = CH_3$, Cl

$R_3 = CH_3$

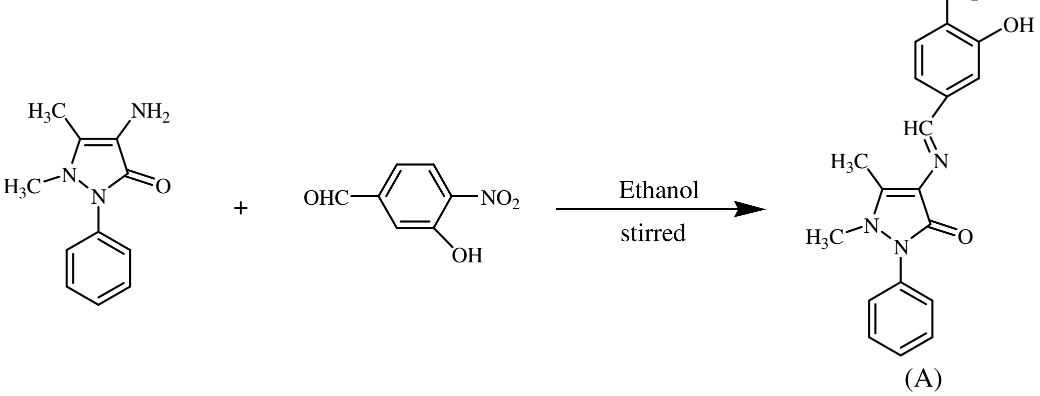
Scheme 26

Two symmetrical ligand Schiff bases were prepared by condensing *o*-phenylenediamine with salicylaldehyde and benzaldehyde. Their antimicrobial activity against *S. aureus, B.cereus, E. coli, P. aeruginosa* and antioxidant activity were determined by Akila *et al* (2012).

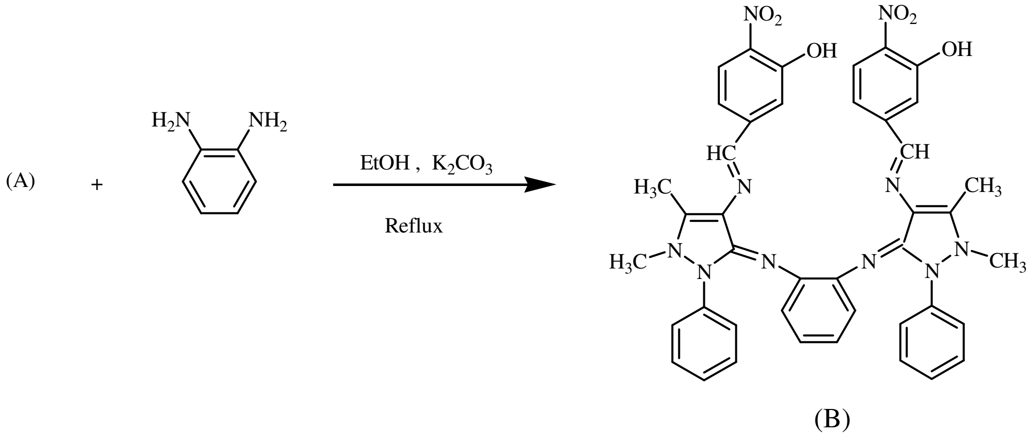


Scheme 27

The Schiff bases reported by Raman *et al* (2007) were synthesized from 4-aminoantipyrine and 3-hydroxy-4-nitrobenzaldehyde (A). The synthesized Schiff base (A) on further rection with *o*-phenylenediamine yielded another Schiff base (B). The *in vitro* biological screening effects of the compounds were tested against the bacteria *S. aureus, E. coli* and *B. subtilis.*



(A)



Scheme 28

CHAPTER - III

MATERIALS AND METHODS

3.1 Instrumentation

This chapter provides information regarding the experimental procedures employed during the course of investigation. The melting points of the synthesized compounds were determined in open capillaries. UV spectra was recorded on UV2600 spectrophotometer of Techcomp company and is expressed in λ max. IR and ^{1}H NMR spectra were got scanned from Sophisticated Analytical Instrumentation Facility (SAIF), Central Instrument Laboratory (CIL), Panjab University, Chandigarh. IR Spectra were recorded on Perkin-Elmer FTIR Spectrophotometer with wavenumber in cm^{-1}. ^{1}H NMR spectra were recorded on Bruker Avance II 400 spectrophotometer using TMS as internal reference. The chemical shifts were expressed in δ (ppm) values and the abbreviations 's'& 'm' stand for singlet and multiplet respectively.

3.2 Chemicals and apparatus used

Chemicals and apparatus were procured from the sources given below.

3.2.1 Chemicals

1. Iodine :Thermo Fisher Scientific India Pvt. Ltd, Mumbai
2. Silica Gel G :Thermo Fisher Scientific India Pvt. Ltd, Mumbai
3. Acetone :Sisco Research Laboratories Pvt. Ltd Mumbai
4. Chloroform :Sisco Research Laboratories Pvt. Ltd Mumbai
5. Dichloromethane :Sisco Research Laboratories Pvt. Ltd Mumbai
6. Ethanol :S.D. Fine Chemicals Ltd, Mumbai
7. Ethylacetate :Sisco Research Laboratories Pvt. Ltd Mumbai
8. Petroleum ether :Sisco Research Laboratories Pvt. Ltd Mumbai
9. 4-Bromobenzaldehyde :Central Drug House (P) Ltd, New Delhi
10. 4-Chlorobenzaldehyde :Sisco Research Laboratories Pvt. Ltd Mumbai
11. 3-Chlorobenzaldehyde :Sisco Research Laboratories Pvt. Ltd Mumbai
12. 4-Fluorobenzaldeyde :Centarl Drug House (P) Ltd, New Delhi
13. 4-Nitrobenzaldehyde :Sisco Research Laboratories Pvt. Ltd Mumbai
14. 3-Nitrobenzaldehyde :Sisco Research Laboratories Pvt. Ltd Mumbai
15. *o*-Phenylenediamine :Loba Chemie Pvt. Ltd, Mumbai
16. Sodium Hydroxide :Central Drug House (P) Ltd New Delhi
17. Agar agar :Central Drug House (P) Ltd New Delhi
18. Bavistin 50 WP :Crystal, India
19. Potato dextrose broth :HiMedia Laboratories Pvt. Ltd, Mumbai
20. Ampicillin :Ranbaxy Pvt.Ltd, Hyderabad
21. Nutrient broth :HiMedia Laboratories Pvt. Ltd, Mumbai

3.2.2 Apparatus

1. Hot- air-oven :MAC, India
2. Magnetic stirrer with hot plate :Tarsons, New Dehli
3. Melting point apparatus :Relitech, Ambala
4. Weighing balance :Mettler Toledo, New Dehli
5. Thin layer chromatographic equipment
    - i. TLC plates 5 x 10 cm : 20 x 20 cm glass plates
    - ii. Development chamber
6. Autoclave :MAC, India
7. Microwave :LG, India
8. Sterile discs :HiMedia Laboratories Pvt. Ltd, Mumbai

3.3 Chromatographic techniques

Since Chromatography is the most useful method for identification of compounds from mixture, therefore, the brief description of chromatographic technique used during the study is as follow:

3.3.1 Thin Layer Chromatography (TLC)

In Thin Layer Chromatography mixture of substances to be separated are adsorbed on silics gel G and eluted from bottom to top. The underlying mechanism is the partitioning of the moving compounds between the liquid phase and also their being reversibly bound to the surface of the adsorbent. The amount transferred to the solvent will be a function of the distribution of the compound, least strongly adsorbed will be in the highest concentration.

3.3.2 Preparation of Thin Layer Chromatographic plates

The plates were coated with adsorbent silica gel G containing calcium sulfate as binder by dipping a pair of plates held back to back in slurry (10 g of silica gel per 100 ml of water), then these plates were air dried at room temperature and finally activated at 110^{o}C for 45 minutes. The chromatoplates were used after cooling for 10-15 minutes. The spotting of chromatoplates was done with the help of capillary tubes. The spot was applied 1 cm upward from the lower end of chromatoplate. After the initial spotting on the stationary phase, the chromatoplate was placed inside the developing chamber and mobile phase was allowed to run up the plate and finally visualization of spots was done in iodine chamber.

3.4 Experimental

N-substituted benzylidene benzene-1,2-diamines

The N-substituted benzylidene benzene-1,2-diamines i.e the unsymmetrical Schiff bases were synthesized by taking the equimolar amount of both the reactants. The compounds (I-V) were prepared by adding 0.01 mole of o-phenylenediamine to 0.01 mole of different substituted aldehydes.

N-(4-Nitrobenzylidene)benzene-1,2-diamine (I)

In a 150 ml flask, a mixture of 4-nitrobenzaldehyde (0.01 mole, 1.5 g) and *o*-phenylenediamine (0.01 mole, 1.08 g) in ethanol (10 ml) was taken. Then the mixture was stirred for 5 minutes so as to mix properly. After that there was addition few drops of NaOH solution (10%) to the reaction mixture (drop-wise). This reaction mixture was kept on stirring for 19 hrs. The brown colored product was then washed with cold water. The separated product was filtered and recrystallized from 95 % ethanol. Purity of compound was checked by thin layer chromatography (TLC). The yield of compound (I) was in 79% and melting point 128-130 °C.

N-(3-Nitrobenzylidene)benzene-1,2-diamine (II)

An equimolar ratio of 3-nitrobenzaldehyde (0.01 mole, 1.5 g) and *o*-phenylenediamine (0.01 mole, 1.08 g) was dissolved in ethanol (10 ml) was taken in 150 ml flask. For the reaction to be carried out in basic conditions, few drops of NaOH solution (10%) were added. Then the reaction mixture was stirred for 21 hrs at room temperature. The progress of reaction was monitored by thin layer chromatography (TLC) from time to time The yellow coloured compound was formed which was further washed with ice cold water. The crude product was then recrytallized with ethanol (95%) to get pure product. Single spot on thin layer chromatographic plate assured the purity of the recrystallized product. The yield of the product was 76%. The melting point was 156-158 °C.

N-(4-Bromobenzylidene)benzene-1,2-diamine (III)

A mixture of 4-bromobenzaldehyde (0.01 mole, 1.85 g) and *o*-phenylenediamine (0.01mole, 1.08 g) was taken in ethanol (10 ml) in a 150 ml flask. Catalytic amount of NaOH solution (10%) was added to it and stirred for 29 hrs at ambient temperature. The completion of reaction was confirmed by TLC. After this, the content was poured into ice cold water. The solid obtained was filtered and washed with cold water. The crude product was recrystallized from ethanol to give corresponding product in 84% yield. The melting point of the compound was 144-146 °C.

N-(3-Chlorobenzylidene)benzene-1,2-diamine (IV)

o-Phenylenediamine (0.01mole, 1.08 g) was dissolved in ethanol (10 ml) and 3-chlorobenzaldehyde (0.01 mole, 1.405 g) was dissolved in ethanol. Both the reactants were mixed and stirred for few minutes. Few drops of NaOH solution (10%) were added to the reaction mixture and kept for stirring. The reaction mixture was stirred for 20 hrs. The crude product formed was washed with iced cold water. The solid product obtained after washing was recrystallized with ethanol. The purity of compound was checked with thin layer chromatography. The melting of the compound was 132-134°C. The yield of the compound was found to be 80%.

N-(4-Fluorobenzylidene)benzene-1,2-diamine (V)

A mixture of 4-fluorobenzaldehyde (0.01 mole, 1.51 g) and *o*-phenylenediamine (0.01mole, 1.08 g) was taken in ethanol (10 ml) in a 150 ml flask. Catalytic amount of NaOH solution (10%) was added to it and stirred for 19 hrs at ambient temperature. The completion of reaction was confirmed by TLC. After completion of reaction, the content was poured into ice cold water. The solid obtained was filtered and washed with cold water. The crude product was recrystallized from ethanol to give corresponding product in 80% yield. The melting point of the compound was 140-142 °C.

N, N$^{'}$-Bis substituted benzylidene benzene-1,2-diamines

N, N$^{'}$-Bis substituted benzylidene benzene-1,2-diamines were prepared by taking 0.01 mole of *o*-phenylenediamine and 0.02 moles of different substituted aldehydes. The 1:2 molar ratio resulted the formation of symmetrical Schiff bases.

N, N$^{'}$-Bis(4-Nitrobenzylidene)benzene-1,2-diamine (VI)

A mixture of 4-nitrobenzaldehyde (0.02 mole, 3.0 g) and *o*-Phenylenediamine (0.01 mole, 1.08g) in ethanol (10 ml) was taken in a 150 ml flask. Then the mixture was stirred for 5 minutes so as to mix properly. After that few drops of NaOH solution (10%) were added reaction (drop-wise). This reaction mixture was kept on stirring for 14 hrs. The brown colored product was then washed with cold water. The separated product was filtered and recrystallized from 95% ethanol. Purity of compound was checked by thin layer chromatography (TLC). The yield of compound (VI) was in 71% and melting point 130-132°C.

N, N$^{'}$-Bis(3-Nitrobenzylidene)benzene-1,2-diamine (VII)

A mixture of *o*-Phenylenediamine (0.01mole, 1.08 g) and 3-nitrobenzaldehyde (0.02 mole, 3.0 g) was prepared in ethanol (10 ml). For mixing of the reactants, the reaction mixture was stirred for few minutes. Few drops of NaOH was added dropwise to the reaction mixture as a catalyst. Then the reaction was stirred at room temperature for 17 hrs. The crude product obtained was washed with iced cold water. Then the final pure product was obtained with recrystallisation from 95% of ethanol. The purity of the product was checked with thin layer chromatography. The melting point of the formed compound was 172-174°C and yield of the compound was 67%.

N, N$^{'}$-Bis(4-Bromobenzylidene)benzene-1,2-diamine (VIII)

Treatment of *o*-Phenylenediamine (0.01mole, 1.08 g) with 4-bromobenzaldehyde (0.02mole, 3.70 g) yielded N, N$^{'}$-bis(4-bromobenzylidene)benzene-1,2-diamine (VIII). The reaction was done in the presence of ethanol. Also the reaction was carried out in the basic conditions. Few drops of 10% NaOH was added to reaction mixture and stirred for 18 hrs. The progress of reaction was checked with thin layer chromatography. Single spot on the TLC plate assured the formation of desired product. The melting point of the resulted compound was 159-161°C. The yield of the synthesized compound was 73%.

N, N'-Bis(3-Chlorobenzylidene)benzene-1,2-diamine (IX)

o-Pheylenediamine (0.01mole, 1.08 g) and 3-chlorobenzaldehyde (0.02mole, 2.810 g) was dissolved in ethanol (10 ml). Both the reactants were stirred for few minutes so as to mix them properly. The reaction was carried out in basic conditions. For this few drops of NaOH solution (10%) was added dropwise to the reaction mixture. The progress of the reaction was checked with spotting on thin layer chromatography. The formed product was washed with ice cold water. The product was then recrystallized with ethanol. The pure product was obtained. The yield of the compound was 59%. The melting point observed was 110-112°C.

N, N'-Bis(4-Fluorobenzylidene)benzene-1,2-diamine (X)

o-Phenylenediamine was weighed (0.01mole, 1.08 g) was dissolved in ethanol (10 ml) and 4-fluorobenzaldehyde (0.02 mole, 1.405 g) was dissolved in ethanol. Both the reactants were mixed and stirred for few minutes. After the mixing of reactants properly few drops of NaOH solution (10%) were added to the reaction mixture and kept for stirring. The reaction mixture was stirred for 18 hrs. The crude product formed was washed with ice cold water. The solid product obtained after washing was recrystallized with ethanol. The progress of the reaction was checked by thin layer chromatography. Single spot on the thin layer chromatography assured the formation of the product. The yield of the compound was 63%. The melting of the compound observed was 124-126°C.

3.5 Physical properties

Table 1 represents the physical parameters of the synthesized compounds (I-X). All the melting points were determined in open capillaries in an electric melting point apparatus and were uncorrected.

3.6 Microbial activity

3.6.1 Procurement of micro-organisms

Pseudomonas sp., *Klebsiella* sp. and *Burkholderia* sp. were procured from Pulses Microbiology Laboratory, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana.

3.6.2 Nutrient agar

Pure cultures of *Pseudomonas* sp. (KF 853103.3), *Klebsiella* sp. (KF 424316.1) and *Burkholderia* sp were maintained on nutrient agar media slants, subcultured once in a month throughout the period of investigation and slants were stored at 3°C in refrigerator.

Composition of nutrient agar medium

Medium was sterilized in autoclave at 15 psi, at a temperature of 121.6 ± 2° C for 20 min, pH of the medium was 7.2.

Ingredients	Quantity
Protease peptone	5.0 g
Beef extract	3.0 g
NaCl	5.0 g
Agar	20.0 g
Distilled water	1000 ml

3.6.3 Evaluation of antibacterial activity

The antibacterial activity of different Schiff bases against *Pseudomonas* sp. (KF 853103.3), *Klebsiella* sp. (KF 424316.1) and *Burkholderia* sp. was tested by disc plate method of Davis and Stout (1971). Stock solutions of test compounds of different concentrations were prepared by dissolving them in DMSO and serial dilutions were made in DMSO. The medium plates were prepared by pouring 15-20 ml of prepared and autoclaved nutrient agar media for *Pseudomonas* sp., *Klebsiella* sp, and *Burkholderia* sp. in sterilized plates. Plates were kept for solidification and stored for 24 hrs to ensure sterility. Prepared suspensions of 48 hrs old broth of test bacteria (0.1 ml on each sterilized plate) were spread under aseptic conditions. Sterile filter paper discs (HiMedia sterile susceptibility discs) moistened with the test compound solutions of different concentrations were placed on the medium inoculated with respective bacterial suspension under aseptic condition. Control was served by sterile disc dipped in DMSO. Petriplates were kept for incubation at 28 ± 2°C for 24 hrs and diameters of growth inhibition zone (mm) were measured. The inhibition bacteria by medium containing the test compound was compared with antibiotic ampicillin as standard along with uninoculated plate as control. All the experiments of antibacterial activity were performed in triplicates.

3.6.4 Determination of minimum inhibitory concentration

Different Schiff bases showing different inhibition at different concentration were further screened for their minimum inhibitory concentration (MIC) for *Pseudomonas* sp., *Klebsiella* sp, and *Burkholderia* sp. in Nutrient agar media. Serial dilutions were done from a stock solutuion of 5.0 mg ml^{-1} concentration. Sterile discs were dipped into different dilutions and placed on the nutrient agar media inoculated with *Pseudomonas* sp., *Klebsiella* sp, and *Burkholderia* sp. Sterile disc dipped in DMSO was used as control. The plates were allowed to incubate for 24 hrs at 28 ± 2°C. The inhibition bacteria by the test compounds was also compared with control (the plates having only bacteria *i.e* no test compound) and minimum inhibitory concentration was recorded for all the three test bacteria.

3.6.5 Procurement of fungus

Cultures of *Fusarium* were procured from Pulses Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana.

3.6.6 Potato Dextrose Agar media (PDA)

A bit of pure culture of *Fusarium oxysporum* was placed on PDA media plates and allowed to grow to form a colony. The full colonized fungus was then stored in a refrigerator for further use.

Composition of Potato Dextrose Agar media (PDA media)

Ingredients	Quantity
Potato dextrose broth	24 g
Agar agar	24 g
Distilled water	1000 ml

Medium was sterilized in autoclave at 15 psi, at a temperature of 121.6º C for 20 min and the pH of the media was 7.2.

3.6.7 Evaluation of antifungal activity

Antifungal activity of different Schiff bases was carried out by poisoned food technique (Grover and Moore 1962). A culture of the test fungi was grown in Petri plates on Potato Dextrose Agar (PDA) media and kept in incubator for seven days. Stock solution of test compounds was prepared in dimethyl sulfoxide at five concentrations (5, 10, 25, 50 and 100 mg ml^{-1}) and stored at 4 °C for further use. PDA supplemented with different prepared solutions at five concentrations (5, 10, 25, 50 and 100 mg ml^{-1}) was poured in the Petri plates under aseptic conditions. After solidification, small disc (0.5 cm diameter) of the test fungi was cut with a sterile cork borer and transferred aseptically upside down at the centre of Petri dish. Suitable checks were maintained, where the culture discs were grown under same conditions on PDA. Petri plates were incubated at 25 ± 1 °C for seven days. The average diameter of fungal colonies was measured on 7th day after inoculation. The percentage inhibition to mycelial growth of test fungus on medium containing the test compound was compared with fungicide carbendazim (bavistin) as standard and a plate with control. The percentage mycelial growth inhibition was calculated using the following formula.

$$I = \frac{C - T}{C} \times 100$$

Where, I is inhibition percent,

C is colony diameter in control (cm) and

T is colony diameter in treatment (cm)

3.7 Statistical analysis- The significant difference in the microbial activity of compounds was calculated using Complete Randomized Design (CRD).

Table 1: Physical parameters of different synthesized compounds

Compound	Molecular formula	Molecular weight	Colour	Yield (%)	Melting point (°C)	R_f value
I	$C_{13}H_{11}N_3O_2$	241 g	Mustard	79	128-130	0.65
II	$C_{13}H_{11}N_3O_2$	241 g	Brown	76	156-158	0.70
III	$C_{13}H_{11}BrN_2$	275 g	Green	84	144-146	0.66
IV	$C_{13}H_{11}ClN_2$	230.5 g	Yellow	80	132-134	0.71
V	$C_{13}H_{11}FN_2$	214 g	Yellow	80	140-142	0.69
VI	$C_{20}H_{14}N_4O_4$	346 g	Brown	71	130-132	0.80
VII	$C_{20}H_{14}N_4O_4$	374 g	Dark brown	67	172-174	0.82
VIII	$C_{20}H_{14}NBr_2$	361 g	Bright Yellow	73	159-161	0.65
IX	$C_{20}H_{14}N_2Cl_2$	353 g	Yellow	59	110-112	0.59
X	$C_{20}H_{14}N_2F_2$	321 g	Yellow	63	124-126	0.79

*All Schiff bases were obtained in solid form.

** The solvent system for TLC was Dichloromethane.

CHAPTER - IV

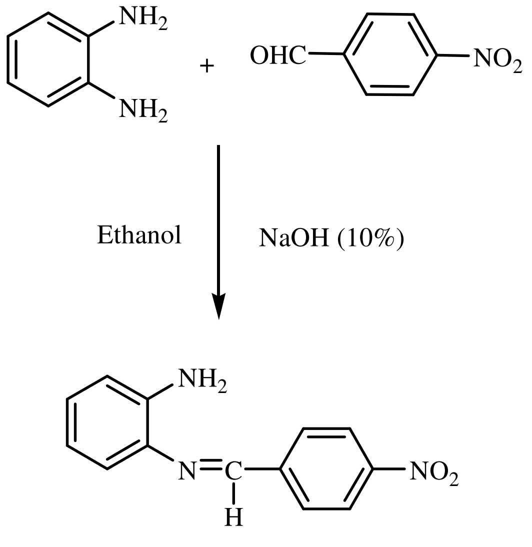
RESULTS AND DISCUSSION

4.1 Synthesis and characterization of N- substituted benzylidene benzene-1,2-diamines (I-V)

N-substituted benzylidene benzene-1,2-diamine were synthesized by reacting *o*-phenylenediamine (0.01mole) and different aldehydes (0.01mole) *i.e* the reactants were taken in equimolar ratio (1:1).

N-(4-Nitrobenzylidene)benzene-1,2-diamine (I)

N-(4-Nitrobenzylidene)benzene-1,2-diamine (I) was synthesized by dissolving *o*-phenylenediamine and 4-nirobenzaldehyde in ethanol in the presence of catalytic amount of 10 % NaOH solution. The progress of reaction was monitored by thin layer chromatography. A single spot on the thin layer chromatographic plate revealed the purity of synthesized Schiff base.



(I)

(Scheme 1)

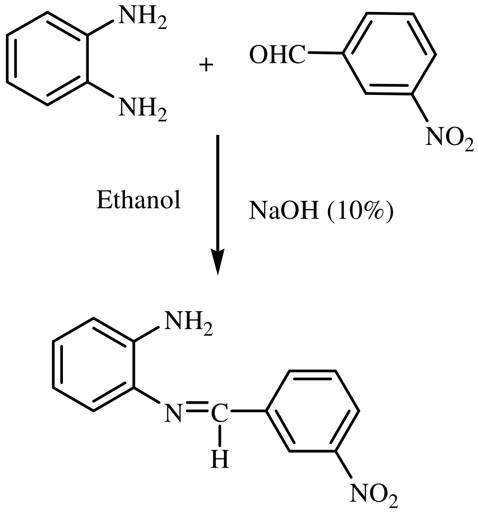
The product obtained was characterized by UV-visible, IR and ^{1}H NMR spectroscopic techniques. The synthesized compound showed absorption maxima at 375 nm in UV-visible region. The prominent bands of the N-(4-Nitrobenzylidene)benzene-1,2-diamine (I) in IR spectrum are as follows

S.No.	Wave number (cm^{-1})	Vibrations	Assignment of peaks
1	3467	Asymmetric $-NH_2$ stretching	$-NH_2$ group
2	3369	Symmetric $-NH_2$ stretching	$-NH_2$ group
3	3062	C-H stretching	Aromatic ring
4	2934	C-H stretching	Aliphatic
5	1607	C=N stretching	Azomethine group
6	1596, 1559, 1491, 1458	C=C stretching	Benzene ring
7	1343	N=O stretching	Nitro group
8	938	=C-H Bending	Aliphatic
9	689	C=C Bending	Aromatic ring

In IR spectrum, a sharp band of C=N stretching at 1607 cm^{-1} and in ^1H NMR, absence of singlet due to aldehydic proton of 4-nitrobenzaldeyde and appearance of singlet at 8.63 due to azomethine proton (-N=CH-) confirmed the formation of desired product. Similar spectral data was also reported by Rashmi *et al* (2017)

N-(3-Nitrobenzylidene)benzene-1,2-diamine (II)

Treatment of *o*-phenylenediamine with 3-nitrobenzaldehyde in the presence of 10% NaOH using ethanol as a solvent afforded N-(3-nitrobenzylidene)benzene-1,2-diamine (II). Thin layer chromatography technique was used to determine the progress of the reaction. Purity of synthesized compound was revealed by single spot on TLC.



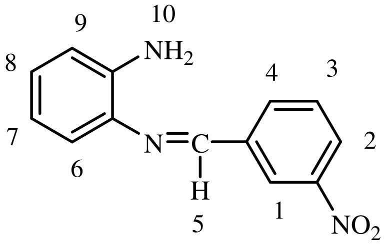
(II)

(Scheme II)

UV-visible, IR and ^{1}H NMR spectroscopic techniques were used to characterize the compound. The absorption maxima of the N-(3-nitrobenzylidene)benzene-1,2-diamine (II) was at 305 nm in UV-visible region. The prominent bands of the synthesized compound in IR spectrum are as follows

S.No.	Wave number (cm^{-1})	Vibrations	Assignment of peaks
1	3486	Asymmetric -NH$_2$ stretching	-NH$_2$ group
2	3467	Symmetric -NH$_2$ stretching	-NH$_2$ group
3	3062	C-H stretching	Aromatic ring
4	2900	C-H stretching	Aliphatic
5	1617	C=N stretching	Azomethine group
6	1590, 1541, 1461, 1434	C=C stretching	Benzene ring
7	1303	N=O stretching	Nitro group
8	931	=C-H bending	Aliphatic
9	647	C=C bending	Aromatic ring

^{1}H NMR spectrum (CDCl$_3$, 'δ', ppm) showed a singlet at 4.07 due to hydrogens present on -NH$_2$ group which confirmed the formation of mono-substituted product instead of di-substituted. product. Another singlet at 8.73 was observed due to –CH=N- group. A multiplet due to eight aromatic hydrogens was observed in range of 6.70-8.59.

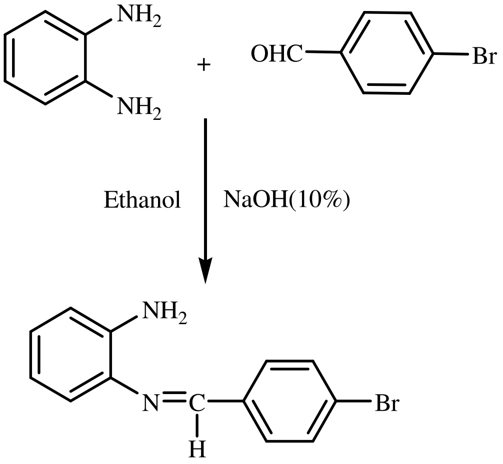


(II)

In IR spectrum, a sharp band of C=N stretching at 1617 cm^{-1} and in ^{1}H NMR, absence of singlet due to aldehydic proton of 3-nitrobenzaldeyde and appearance of singlet at 8.73 due to azomethine proton (-N=CH-) confirmed the formation of desired product.

N-(4-Bromobenzylidene)benzene-1,2-diamine (III)

The reaction of *o*-phenylenediamine and 4-bromobenzaldehyde in ethanol using NaOH (10%) as a base afforded N-(4-bromobenzylidene)benzene-1,2-diamine (III). Thin layer chromatography technique was used to determine the progress of the reaction. The crude product formed was recrystallized with ethanol. Purity of synthesized compound was revealed by single spot on TLC.



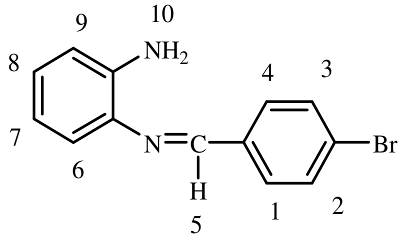
(III)

(Scheme III)

The synthesized product obtained was characterized by UV-visible, IR and ^1H NMR spectroscopic techniques. The absorption maxima of the synthesized compound was at 305 nm in UV-visible region. The prominent bands of the N-(4-bromobenzylidene)benzene-1,2-diamine (III) in IR spectrum are as follows

S.No.	**Wave number (cm^{-1})**	**Vibrations**	**Assignment of peaks**
1	3473	Asymmetric $-NH_2$ stretching	$-NH_2$ group
2	3372	Symmetric $-NH_2$ stretching	$-NH_2$ group
3	3068	C-H stretching	Aromatic ring
4	2981	C-H stretching	Aliphatic
5	1599	C=N stretching	Azomethine group
6	1490, 1460, 1398, 1368	C=C stretching	Benzene ring
7	932	C=C Bending	Aromatic ring
8	630	=C-H Bending	Aliphatic
9	585	C-Br stretching	C-Br group

^1H NMR spectrum ($CDCl_3$, 'δ', ppm) showed a singlet due to two hydrogens attached to nitrogen of $-NH_2$ group at 4.24 which confirmed the formation of mono-substituted product. Another singlet at 8.45 was observed due to azomethine group. A multiplet due to eight aromatic hydrogens was recorded in range of 6.71-7.76.

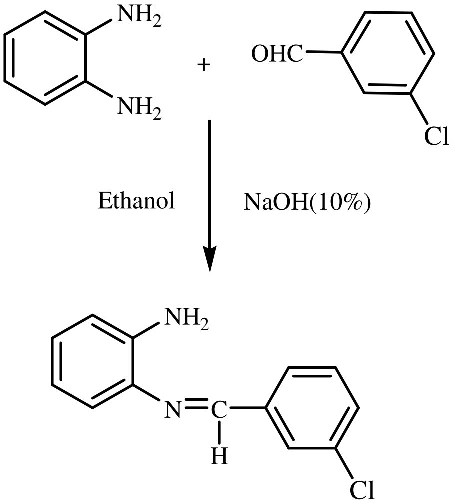


(III)

In IR spectrum, sharp band of C=N stretching at 1599 cm^{-1} and in ^{1}H NMR, absence of singlet due to aldehydic proton of 4-bromobenzaldeyde and appearance of singlet at 8.45 due to azomethine proton (-N=CH-) confirmed the formation of desired mono-substituted product.

N-(3-Chlorobenzylidene)benzene-1,2-diamine (IV)

N-(3-Chlorobenzylidene)benzene-1,2-diamine (IV) was synthesized from *o*-phenylenediamine and 3-chlorobenzaldehyde using ethanol as a solvent. Few drops of 10% NaOH (10%) solution were added as a catalyst. The crude product obtained was purified by recrystallization from ethanol. A single spot on thin layer chromatographic plate depicted the purity of synthesized Schiff base.



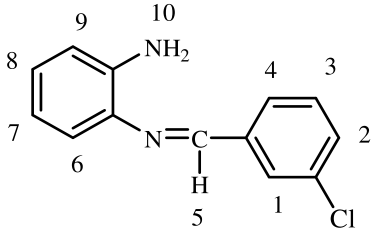
(IV)

Scheme (IV)

Spectroscopic techniques like UV-visible, IR and ^{1}H NMR were used to characterize the product. In UV-Visible region, the absorption maxima of the synthesized compound was at 300 nm. The prominent bands of the N-(3-chlorobenzylidene)benzene-1,2-diamine (IV) in IR spectrum are as follows

S.No.	Wave number (cm^{-1})	Vibrations	Assignment of peaks
1	3397	Asymmetric -NH_2 stretching	-NH_2 group
2	3110	Symmetric -NH_2 stretching	-NH_2 group
3	3075	C-H stretching	Aromatic ring
4	2911	C-H stretching	Aliphatic
5	1590	C=N stretching	Azomethine group
6	1576, 1498, 1472, 1389	C=C stretching	Benzene ring
7	995	=C-H bending	Aliphatic
8	686	C=C bending	Aromatic ring
9	783	C-Cl stretching	C-Cl group

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) showed a singlet due to two hydrogens attached to nitrogen of -NH_2 group at 4.26 which showed the formation of mono-substituted product rather than di-substituted product. A singlet at 9.00 was observed due to -CH=N- group. A multiplet was recorded due to eight aromatic hydrogens was observed in range of 6.74-8.26.

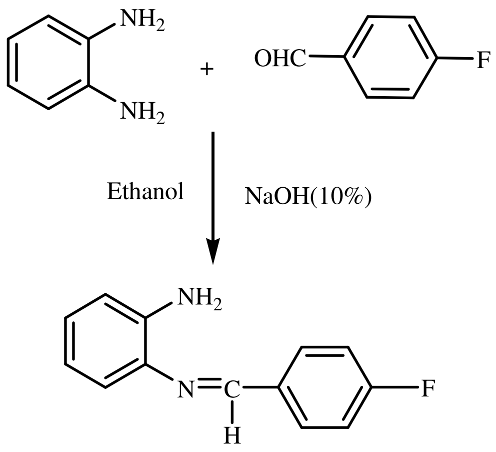


(IV)

In IR spectrum, a sharp band of C=N stretching was found at 1590 cm^{-1} and in ^{1}H NMR, absence of singlet due to aldehydic proton of 3-chlorobenzaldeyde and appearance of singlet at 8.63 due to azomethine proton (-N=CH-) confirmed the formation of desired product which was a mono-substituted product. Kapadnis *et al* (2016) reported the similar spectral data.

N-(4-Fluorobenzylidene)benzene-1,2-diamine (V)

The study of scheme (V) showed the synthesis of N-(4-fluorobenzylidene)benzene-1,2-diamine (V) on reaction of *o*-phenylenediamine with 4-fluorobenzdehyde in the presence of catalytic amount of 10% NaOH solution using ethanol as a solvent. The progress of reaction was checked by thin layer chromatography. A single spot on thin layer chromatographic plate revealed the purity of formed compound.



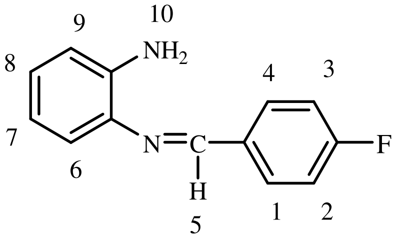
(V)

Scheme (V)

UV-visible, IR and ^{1}H NMR spectroscopic techniques were used to characterize the synthesized compound. The synthesized compound showed absorption maxima at 290 nm in UV-visible region. The prominent bands of the synthesized compound in IR spectrum are as follows

S.No.	**Wave number (cm^{-1})**	**Vibrations**	**Assignment of peaks**
1	3389	Asymmetric -NH$_2$ stretching	-NH$_2$ group
2	3252	Symmetric -NH$_2$ stretching	-NH$_2$ group
3	3043	C-H stretching	Aromatic ring
4	2830	C-H stretching	Aliphatic
5	1627	C=N stretching	Azomethine group
6	1586,1504,1452,1421	C=C stretching	Benzene ring
7	1183	C-F stretching	C-F group
8	964	=C-H bending	Aliphatic
9	690	C=C bending	Aromatic ring

^{1}H NMR spectrum (CDCl$_3$, ‘δ’, ppm) showed a singlet due to hydrogens attached to nitrogen of -NH$_2$ group at 4.33 which showed the formation of mono-substituted product rather than di-substituted product. A singlet at 8.62 was observed due to azomethine group. A multiplet recorded due to eight aromatic hydrogens was observed in range of 6.70-8.31.



(V)

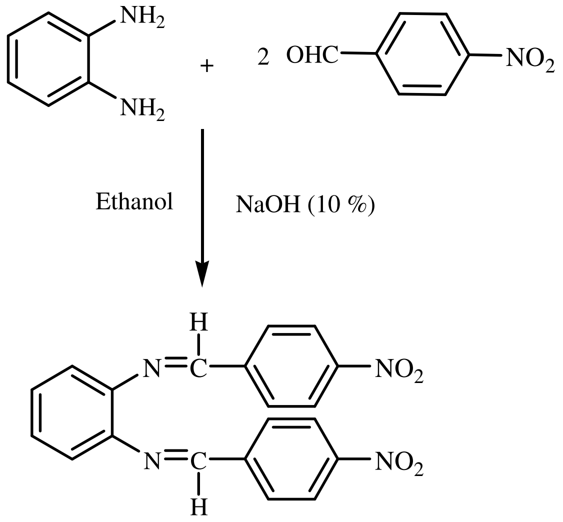
In IR spectrum, sharp band of C=N stretching at 1627 cm^{-1} and in ^1H NMR, absence of singlet due to aldehydic proton of 4-fluorobenzaldeyde and appearance of singlet at 8.62 due to azomethine proton (-N=CH-) confirmed the formation of required product. Similar spectral data was also reported by Behal *et al* 2016.

Synthesis and Characterization of N, N$^{'}$-bis substituted benzylidene benzene-1,2-diamines (VI-X)

N, N$^{'}$-bis substituted benzylidene benzene-1,2-diamines were synthesized by reacting *o*-phenylenediamine (0.01mole) with different aldehydes (0.02 moles). The molar ratio of *o*-phenylenediamine and different substituted aldehydes taken was 1:2.

N, N$^{'}$-Bis(4-Nitrobenzylidene)benzene-1,2-diamine (VI)

o-Phenylenediamine was added to 4-nitrobenzaldehyde in which ethanol. Catalytic amount of NaOH (10%) solution was added and N, N$^{'}$-bis(3-nitrobenzylidene)benzene-1,2-diamine (VII) was yielded. This 1:2 ratio of reactants yielded a di-substituted product instead of mono-substituted product. The crude product obtained was purified by recrystallization from ethanol. The progress of the reaction was checked on thin layer chromatography. A single spot on thin layer chromatographic plate depicted the purity of synthesized Schiff base.



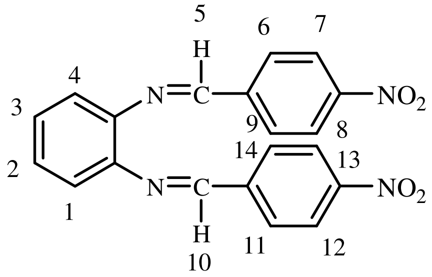
(VI)

Scheme (VI)

The product obtained was characterized by UV-visible, IR and ^{1}H NMR spectroscopic techniques. In the UV-visible region the obtained product showed absorption maxima at 300 nm. The prominent bands of the synthesized compound in IR spectrum are as follows

S.No.	Wave number(cm^{-1})	Vibrations	Assignment of peaks
1	3233	C-H stretching	Aromatic ring
2	2911	C-H stretching	Aliphatic
3	1590	C=N stretching	Azomethine group
4	1493,1457,1439,1409	C=C stretching	Benzene ring
5	1351	N=O stretching	Nitro group
6	925	=C-H bending	Aliphatic
7	696	C=C bending	Aromatic ring

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) as the protons of azomethine group shared same environment so a singlet of two protons of this group appeared at 9.90. In the range of 6.98-8.25 a multiplet was recorded because of due to twelve aromatic hydrogens.

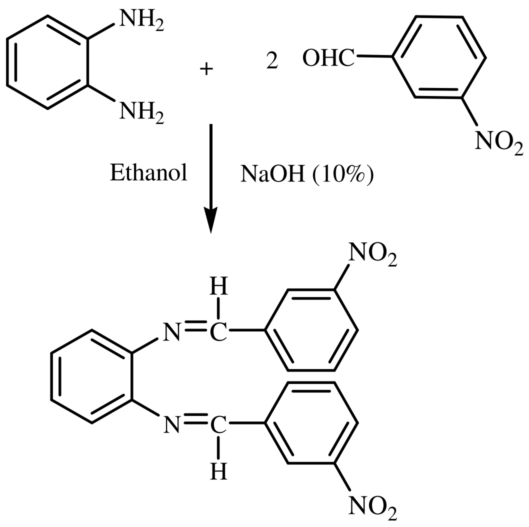


(VI)

In IR spectrum, absence of H-N stretching of *o*-phenylenediamine and in ^{1}H NMR, absence of singlets due to aldehydic proton of 4-nitrobenzaldeyde and protons of both NH_2 groups of revealed the formation of di-substituted product which was further confirmed from the appearance of singlet of two protons at 9.90 due to azomethine proton (-N=CH-). Similar spectral data was showed by Prabhakaran *et al* 2013.

N ,N$'$-Bis(3-Nitrobenzylidene)benzene-1,2-diamine (VII)

Treatment of of o-phenylenediamine with 3-nitrobenzaldehyde yielded N ,N$'$-bis(3-nitrobenzylidene)benzene-1,2-diamine (VII). Ethanol was used as a solvent in the reaction. Few drops of 10 % NaOH solution were added as catalyst. This 1:2 ratio of reactants yielded a di-substituted product. The crude product obtained was purified by recrystallization from ethanol. A single spot on thin layer chromatographic plate depicted the purity of synthesized Schiff base.



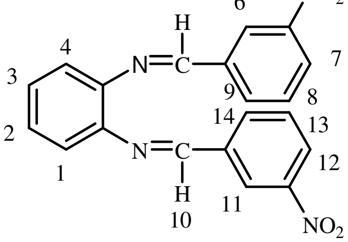
(VII)

Scheme (VII)

UV-visible, IR and ^{1}H NMR spectroscopic techniques were used to characterize the compound. The N ,N$'$-bis(3-nitrobenzylidene)benzene-1,2-diamine (VII) showed λ max at 295 nm in UV-visible region. The prominent bands of the desired compound in IR spectrum are as follows

S.No.	**Wave number (cm^{-1})**	**Vibrations**	**Assignment of peaks**
1	3052	C-H stretching	Aromatic ring
2	2918	C-H stretching	Aliphatic
3	1591	C=N stretching	Azomethine group
4	1544,1492,1422,1390	C=C stretching	Benzene ring
5	1320	N=O stretching	Nitro group
6	963	=C-H bending	Aliphatic
7	685	C=C bending	Aromatic ring

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) a singlet of two protons was appeared at 8.62 of azomethine group. A multiplet was recorded due to twelve aromatic hydrogens in the range of 6.71-8.50.

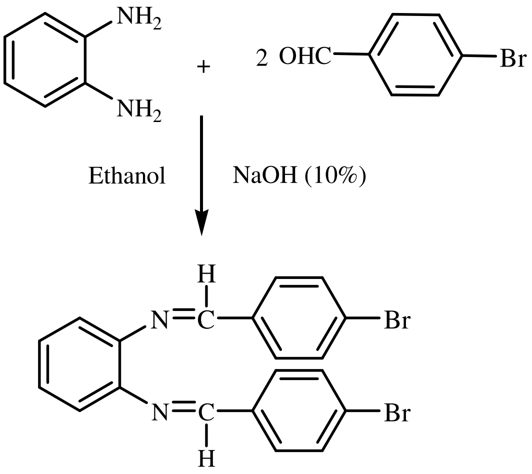


(VII)

In IR spectrum, N-H stretching was absent and in ^{1}H NMR, absence of singlet due to aldehydic proton of 3-nitrobenzaldeyde and protons of both NH_2 group of *o*-phenylenediamine confirmed the formation of di-substituted product which is confirmed from the appearance of singlet at 8.62 due to azomethine proton (-N=CH-).

N, N$^{'}$-Bis(4-Bromobenzylidene)benzene-1,2-diamine (VIII)

N, N$^{'}$-Bis(4-bromobenzylidene)benzene-1,2-diamine (VIII) was synthesized by dissolving *o*-phenylenediamine and 4-bromobenzaldehyde in ethanol in the presence of catalytic amount of 10 % NaOH solution. The progress of reaction was checked by thin layer chromatography. A single spot on thin layer chromatographic plate revealed the purity of synthesized Schiff base.



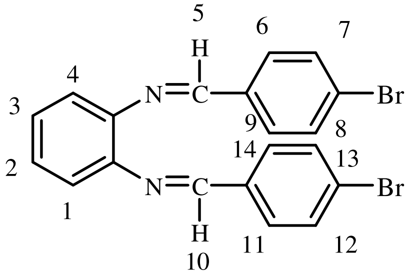
(VIII)

Scheme (VIII)

The product obtained was characterized by UV-visible, IR and ^{1}H NMR spectroscopic techniques. In UV-visible region, the absorption maxima of the obtained compound was at 295 nm. The prominent bands of the obtained compound in IR spectrum are as follows

S.No.	**Wave number (cm^{-1})**	**Vibrations**	**Assignment of peaks**
1	3102	C-H stretching	Aromatic ring
2	2955	C-H stretching	Aliphatic
3	1615	C=N stretching	Azomethine group
4	1577,1463,1451,1404	C=C stretching	Benzene ring
5	977	=C-H bending	Aliphatic
6	689	C=C bending	Aromatic ring
7	590	C-Br stretching	Bromo group

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) exhibited a singlet due to two protons of azomethine group at 8.74. A multiplet was recorded due to twelve aromatic hydrogens was in the range of 6.98-8.12.

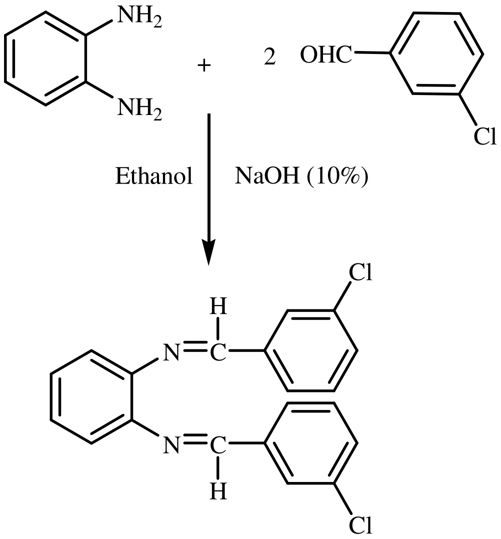


(VIII)

In IR spectrum, there was no N-H stretching and in ^{1}H NMR, absence of singlets due to aldehydic proton of 4-bromobenzaldeyde and protons of both NH_2 group of *o*-phenylenediamine assured the formation of di-substituted product which was confirmed from the appearance of singlet of two protons due to azomethine proton (-N=CH-) at 8.74.

N, N'-Bis(3-Chlorobenzylidene)benzene-1,2-diamine (IX)

o-Phenylenediamine was treated with 3-chlorobenzaldehyde which yielded the product N, N'-bis(3-chlorobenzylidene)benzene-1,2-diamine in which ethanol was used as a solvent. Sodium hydroxide (10%) solution was added as catalyst. The 1:2 ratio of reactants yielded a di-substituted product. The progress of the reaction was checked with thin layer chromatography. The crude product was filtered after adding the reaction mixture in ice cold water and was further recrystallized from ethanol. Single spot on the TLC plate assured the formation of required Schiff base.



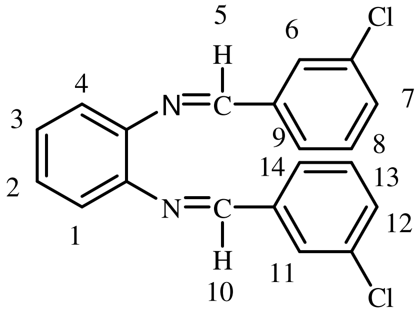
(IX)

Scheme (IX)

UV-visible, IR and ^{1}H NMR spectroscopic techniques were used to characterize the synthesized compound. The synthesized compound showed absorption maxima at 290 nm in UV-visible region. The prominent bands of the N, N$^{'}$-bis(3-chlorobenzylidene)benzene-1,2-diamine in IR spectrum are as follows

S.No.	Wave number (cm^{-1})	Vibrations	Assignment of peaks
1	3212	C-H stretching	Aromatic ring
2	2936	C-H stretching	Aliphatic
3	1679	C=N stretching	Azomethine group
4	1565,1512,1479,1433	C=C stretching	Benzene ring
5	768	C-Cl stretching	Chloro group
6	947	=C-H bending	Aliphatic
7	660	C=C bending	Aromatic ring

^{1}H NMR spectrum (CDCl$_3$, 'δ', ppm) registered a singlet of two protons of azomethine group at 9.91. A multiplet was recorded due to twelve aromatic hydrogens was observed in the range of 6.90-8.26.



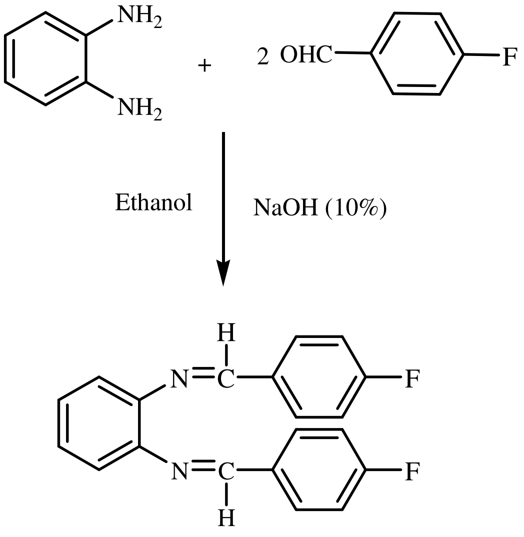
(IX)

In IR spectrum, absence of N-H stretching of *o*-phenylenediamine and in ^{1}H NMR, absence of singlets due to aldehydic proton of 3-chlorobenzaldeyde and protons of both NH$_2$ group of *o*-phenylenediamine assured the formation of di-substituted product which was confirmed from the appearance of singlet of two protons due to azomethine proton (-N=CH-) at 8.28.

N, N$^{'}$-Bis(4-Fluorobenzylidene)benzene-1,2-diamine (X)

Treatment *o*-phenylenediamine with 4-fluorobenzaldehyde yielded N, N$^{'}$-bis(4-fluorobenzylidene)benzene-1,2-diamine (X). Ethanol was used as a solvent in the reaction.

Few drops of 10 % NaOH solution were added as catalyst. This 1:2 ratio of reactants yielded a di-substituted product. The crude product obtained was purified by recrystallization from ethanol. A single spot on thin layer chromatographic plate depicted the purity of synthesized Schiff base.



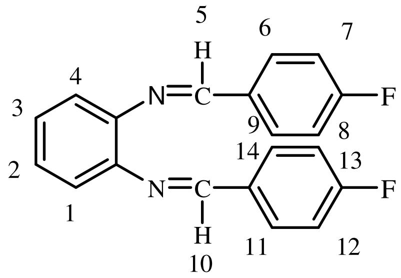
(X)

Scheme (X)

The product obtained was characterized by UV-visible, IR and ^{1}H NMR spectroscopic techniques. N, N$^'$-bis(4-fluorobenzylidene)benzene-1,2-diamine (X) showed maximum absorption at 295 nm. The prominent bands of the obtained in IR spectrum are as follows

S.No.	**Wave number (cm^{-1})**	**Vibrations**	**Assignment of peaks**
1	3160	C-H stretching	Aromatic ring
2	2916	C-H stretching	Aliphatic
3	1609	C=N stretching	Azomethine group
4	1578,1496,1417,1336	C=C stretching	Benzene ring
5	1015	C-F stretching	Fluoro group
6	951	=C-H bending	Aliphatic
7	607	C=C bending	Aromatic ring

^1H NMR spectrum ($CDCl_3$, 'δ', ppm) a singlet at 9.78 appeared due to two protons of azomethine group. A multiplet was recorded due to twelve aromatic hydrogens was in the range of 6.98-8.12.



(X)

In IR spectrum, there was no N-H stretching and in ^1H NMR, there was no singlet of aldehydic proton of 4-fluorobenzaldeyde and protons of both NH_2 group of *o*-phenylenediamine was absent which assured the formation of di-substituted product. Further this was confirmed from the appearance of singlet of two protons due to azomethine proton (-N=CH-) at 9.78.

N-substituted Schiff bases were characterized by the presence of NH_2 band in the region of 3389-3486 cm^{-1} whereas absence of this NH_2 band confirmed the synthesis of N, N'-bis substituted Schiff bases. The characteristic band of -HC=N- (azomethine group) in the range of 1590-1679 cm^{-1} assured formation of Schiff bases. Similar IR spectral data was showed by Rashmi *et al* 2017. ^1H NMR spectrum ($CDCl_3$, 'δ', ppm) showed a multiplet in the range of 6.70-7.76 due to the eight and twelve aromatic hydrogens in mono and di-substituted products respectively. A singlet of two hydrogens of NH_2 in the range of δ 4.04-4.33 was observed only the case of mono-substituted products. Presence of singlets due to one and two hydrogens in mono and di-substituted products was in the range of 8.45-9.91. the results regarding ^1H NMR spectral data are in consonance with the findings of Asha *et al* 2016 ; Kapadnis *et al* 2015.

4.2 Effect of different Schiff bases on microbial activity

Schiff bases synthesized during the study have been subjected for their microbial effect on the growth of *Pseudomonas* sp., *Klebsiella* sp. and *Burkholderia* sp.

Pseudomonas sp.

The data presented in Table 2 exhibited that all compounds were moderately active against *Pseudomonas* sp at 5.0 mg ml^{-1}. Compound VI, VIII and X which are di-substituted containing two nitro, bromo and fluoro groups respectively attached to benzene ring at para position exhibited higher activity than the mono-substituted compounds (I, III and V). Among mono and di-substituted Schiff bases, activity shown by compound III (having one bromo group at para position attached to benzene ring) and VIII (two bromo groups at para position) were most effective at all concentrations. The di-substituted compounds containing chloro group (IX) and fluoro group (X) at benzene ring exhibited inhibition zone of 11mm and 9 mm respectively at 1.0 mg ml^{-1} whereas the mono-substituted compounds of chloro and flouro groups showed inhibition zone of 8 and 7 mm respectively (IV and V). Compounds having one halogen and nitro groups were less effective than compounds containing two halogens and nitro groups. Bromo substitiuted Schiff bases were more effective than chloro and fluoro. The order of activity having was Br > Cl > F. All the compounds registered activity even at low concentration. All Schiff bases were less active than standard ampicillin at all concentrations. All the compounds varied significantly among themselves. Similar activity was performed by Shemary *et al* 2016 against *Psuedomonas* spp.

Table 2: Effect of different Schiff bases on the growth of *Psuedomonas* sp. at different concentrations

Compounds (mg ml^{-1}) / **Compounds**	**Diameter of growth inhibition zone (mm)**				
	5.0	4.0	3.0	2.0	1.0
I	12	10	10	8	7
II	12	10	9	9	7
III	16	16	14	12	10
IV	15	14	12	11	8
V	12	11	10	9	7
VI	13	12	12	10	8
VII	12	11	10	9	7
VIII	20	18	16	14	12
IX	16	14	13	13	11
X	14	13	12	10	9
Ampicillin	44	42	40	39	38
CD (p=0.05)	0.17	0.13	0.12	0.13	0.10

MIC values of different Schiff bases against *Pseudomonas sp.* are presented in Fig. 1. Data in graph revealed that both bromo substituted Schiff bases exhibited lower MIC value as compared to other halogenated and nitro compounds. Compound VIII (having two bromo groups at para position and compound III (having one bromo group at para position) exhibited lowest MIC value of 0.22 and 0.23 mg ml^{-1} respectively.

Compounds (I, II, VI and VII) having nitro group attached to ring had higher MIC values and were least effective.

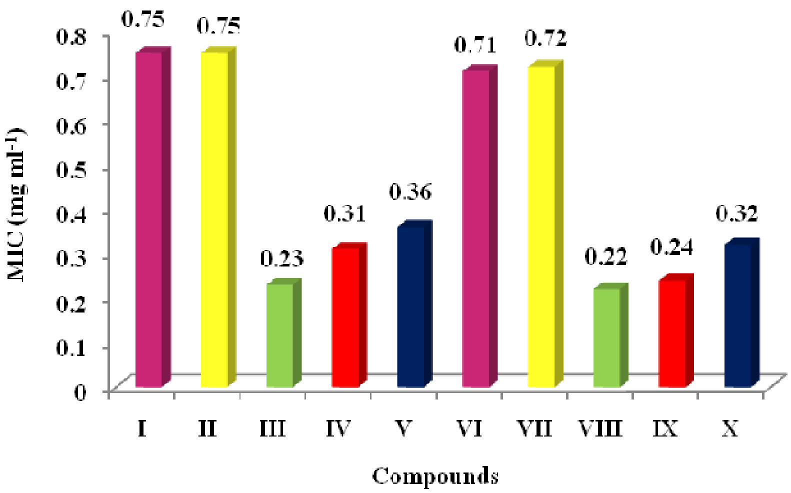


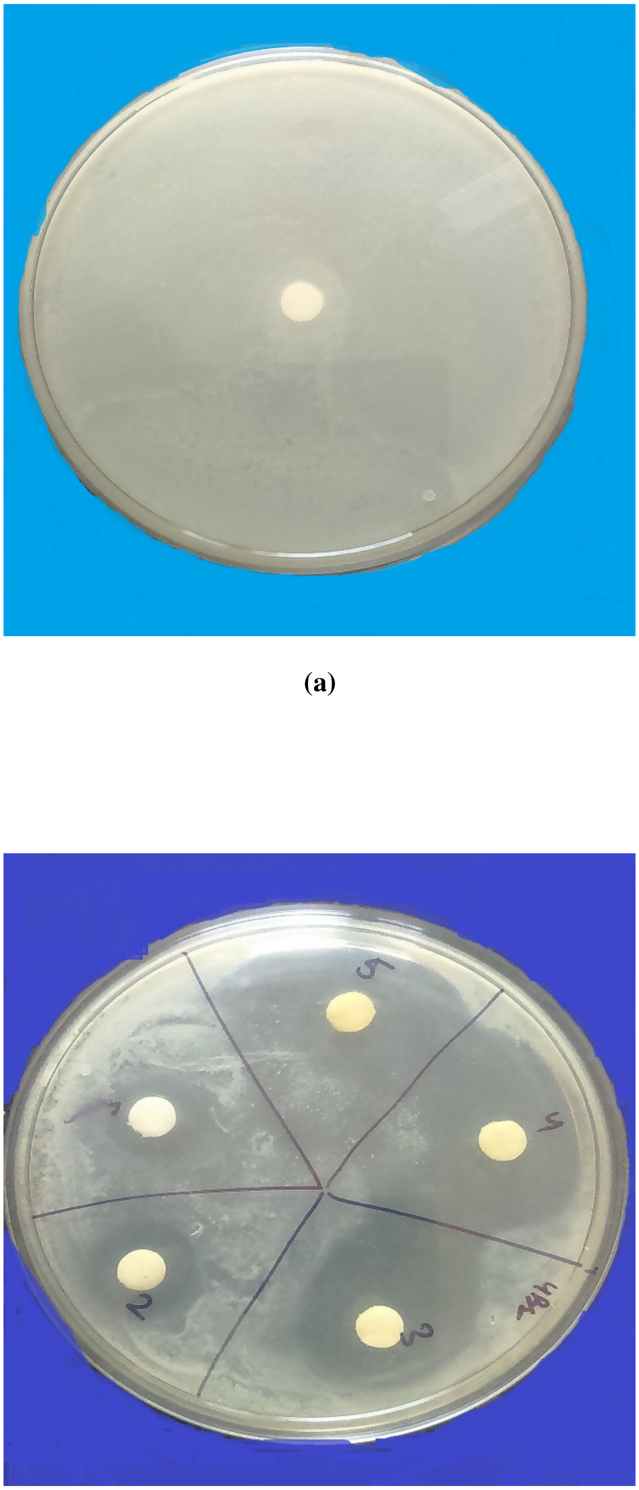
Fig. 1: Minimum Inhibitory Concentration (MIC) of different Schiff bases against *Pseudomonas* sp.

***Klebsiella* sp.**

All the synthesized Schiff bases were evaluated for their bacterial activity against *Klebsiella* sp. and the data is presented in Table 3. Schiff bases (III, IV, V, VIII, IX and X) containing halogen groups showed zones at different concentrations. The compounds containing 4-nitro and 3-nitro groups (both mono and di substituted) were recorded zero inhibition zone even at all the concentrations. So these were not effective against *Klebsiella* sp.

Table 3: Effect of different Schiff bases on the growth of *Klebsiella* sp. at different concentrations

Concentrations (mg ml^{-1}) **Compounds**	**Diameter of growth inhibition zone (mm)**				
	5.0	4.0	3.0	2.0	1.0
I	0	0	0	0	0
II	0	0	0	0	0
III	13	12	10	8	7
IV	12	11	9	7	0
V	11	10	8	7	0
VI	0	0	0	0	0
VII	0	0	0	0	0
VIII	13	12	10	9	7
IX	12	11	8	7	0
X	12	10	8	0	0
Ampicillin	44	42	40	39	38
CD (p=0.05)	0.10	0.10	0.10	0.76	0.12



(a)

(b)

Plate 1: Zones of inhibition formed by control (a) and compound VIII (b) against *Pseudomonas* at 5.0 mg ml^{-1}.

No difference in activity was observed for mono and di-substituted bromo and chloro Schiff bases. The compounds with bromo substitution were found to be more effective than those having chloro and fluoro substitution in both mono and di-substituted compounds. The compounds bearing bromo (III, VIII) and chloro groups (IV, IX) on benzene ring exhibited moderate bacterial activity at all the concentrations but were less effective than the standard *i.e.* ampicillin at all the concentrations. All Schiff bases were significantly less active than standard ampicillin at all concentrations. Significant differences were found in activity among compounds.

MIC values of different Schiff bases against *Klebsiella* sp. are presented in Fig. 2. Data presented in graph revealed that all bromo substituted Schiff bases exhibited lower MIC value as compared to other halogenated and nitro compounds. Compound III (having one bromo groups and compound VIII (having two bromo groups) exhibited lowest MIC value of 0.75 and 0.70 mg ml^{-1} respectively.

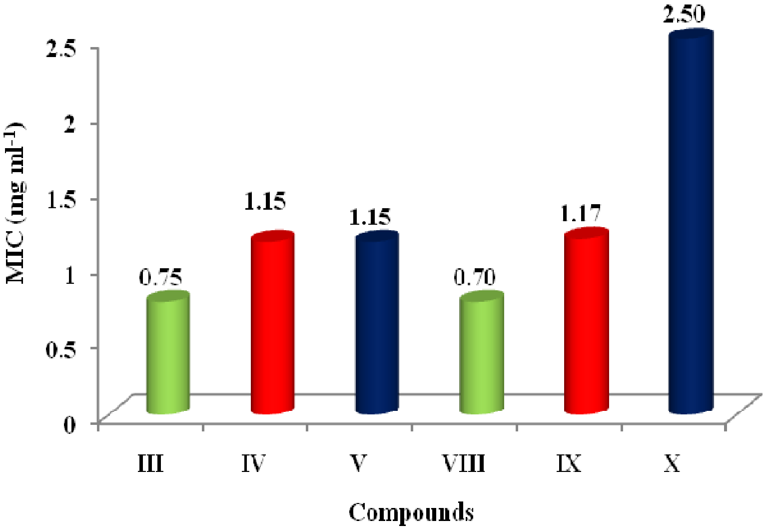
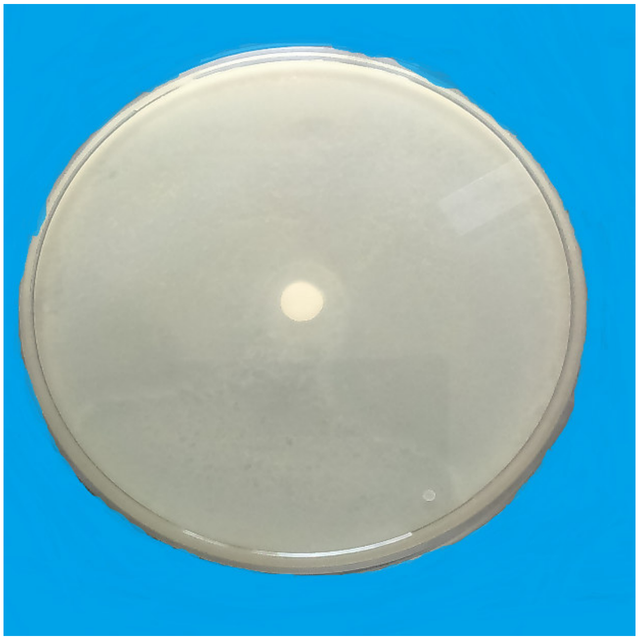


Fig. 2: Minimum Inhibitory Concentration (MIC) of different Schiff bases against *Klebsiella* sp.

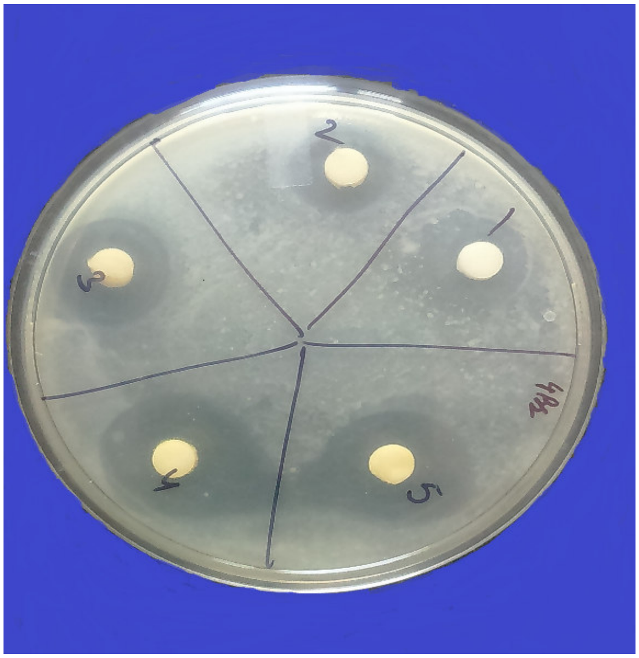
Compound (I, II, VI and VII) having nitro group attached to benzene ring were found to be inactive against *Klebsiella* sp. All the synthesized Schiff bases exhibited less activity than standard.

***Burkholderia* sp**

The data presented in Table 4 exhibited that all the compounds were active against *Burkholderia* sp. at 2.0 mg ml^{-1} concentration with inhibition zone in range of 7.0-12.0 mm. At 5.0 mg ml^{-1}, compound VIII (di-substituted compound) having bromo group at para position of benzene ring showed maximum inhibition zone of 18.0 mm which was higher than other tested compounds therefore compound VIII showed the highest activity at concentration



(a)



(b)

Plate 2: Zones of inhibition formed by control (a) and compound VIII (b) against *Klebsiella* at 5.0 mg ml^{-1}.

of 5.0 mg ml^{-1}.

At all concentrations, compounds containing halogen substitution were found to be more active than other compounds having nitro group. The compounds IX (containing 2 chloro groups) and X (containing 2 fluoro groups) were found to be less effective than compound VIII (containing 2 bromo groups). Activity was shown by compounds I (having one nitro group at para position attached to benzene ring) and II (one nitro group at ortho position) were least effective with inhibition zone of 8 mm and 0 mm respectively. Mono-halogenated compounds were less effective than compounds having di-substitution of halogen group. The compounds (III and VIII) containing bromo groups showed zone of inhibition of 10 mm even at 1.0 mg ml^{-1} thus found to be most effective among all the synthesized compounds. Significant differences were observed in activity of synthesized compounds containing halogen groups and nitro groups on benzene ring. All Schiff bases exhibited less activity than standard ampicillin at all the tested concentrations.

Table 4: Effect of different Schiff bases on the growth of *Burkholderia* sp. at different concentrations

Concentrations (mg ml^{-1}) / **Compounds**	**Diameter of growth inhibition zone (mm)**				
	5.0	4.0	3.0	2.0	1.0
I	12	11	10	9	8
II	12	10	9	7	0
III	16	14	13	11	10
IV	15	14	12	10	8
V	13	12	11	9	7
VI	13	11	10	9	7
VII	12	10	9	8	0
VIII	18	16	14	12	10
IX	15	14	12	10	8
X	14	13	12	9	7
Ampicillin	44	42	40	39	38
CD (p=0.05)	0.15	0.93	0.19	0.05	0.05

All Schiff bases were further studied for minimum inhibitory concentration (MIC) values. Compound II was having maximum MIC value (1.90 mg ml^{-1}) having nitro group on benzene ring. Compounds containing bromo groups at para position (III) and (VIII) exhibited lowest minimum inhibitory concentration (MIC) value (0.57 and 0.52 mg ml^{-1}). Synthesized compounds having chloro group (IV and IX) at para position of benzene ring registered minimum inhibitory concentration (MIC) values of 0.82 and 0.75 mg ml^{-1} respectively. All the compounds having substitution of halogen groups on benzene ring exhibited lower MIC values than compounds having substitution of nitro groups on benzene ring. Thus compound (III and VIII) containing bromo substitution showed maximum zone of inhibition with lowest MIC value.

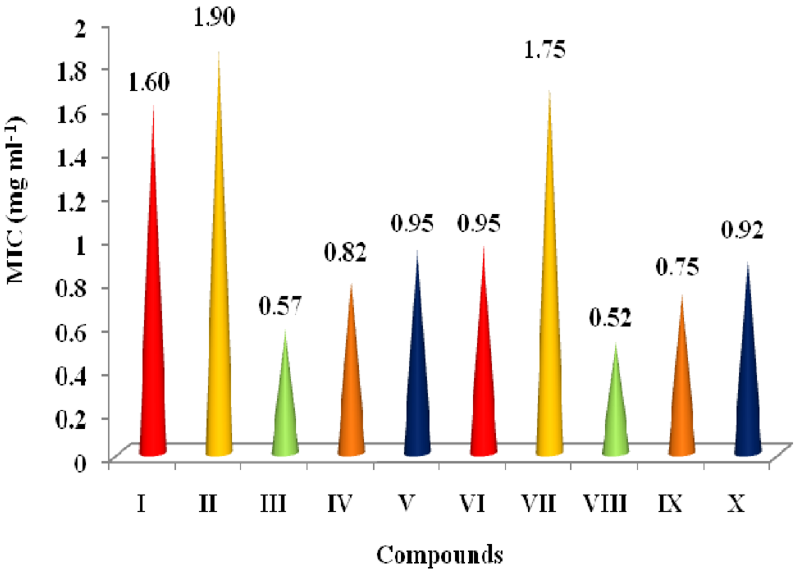
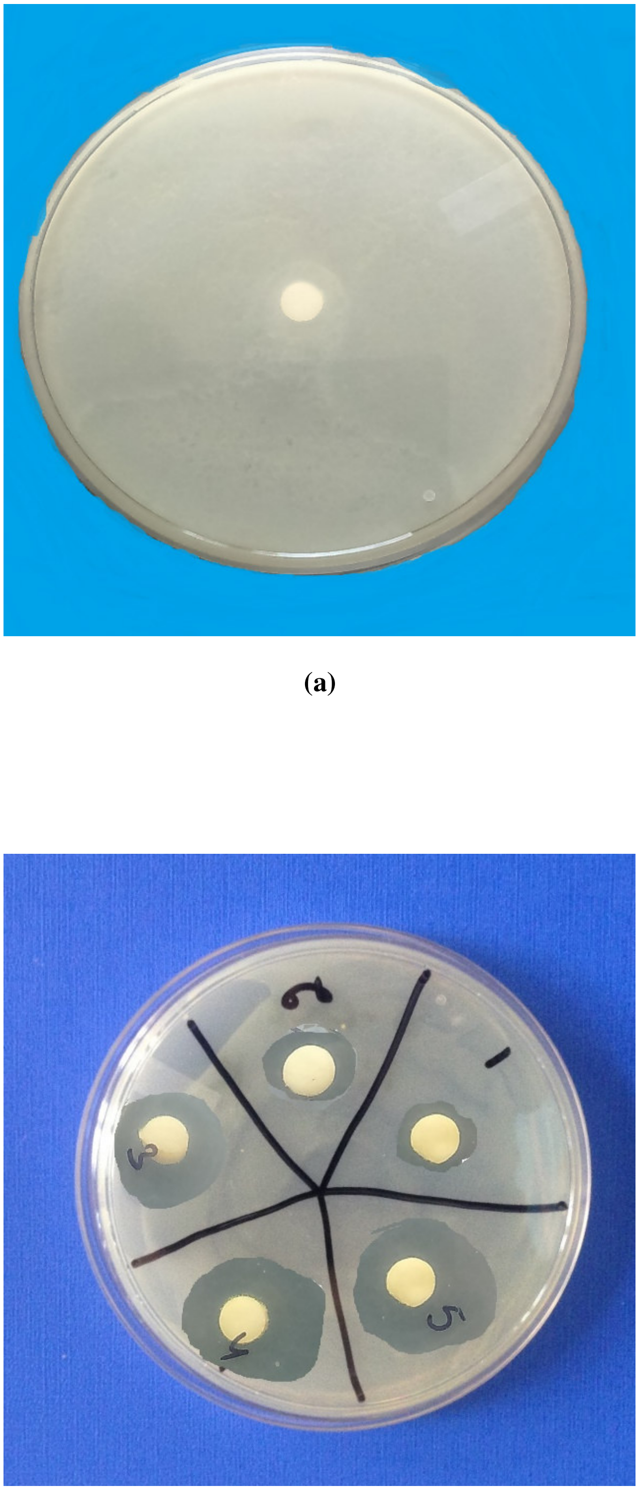


Fig. 3: Minimum Inhibitory Concentration (MIC) of different Schiff bases against *Burkholderia* sp.

Fusarium oxysporum

Synthesized compounds were screened for their antifungal activity by poisoned food technique against *Fusarium oxysporum*. The results were determined in inhibition percent (I) and ED_{50} *i.e* the effective dose at which 50 per cent inhibition has occurred. All compounds showed mild to moderate antifungal activity against the test fungi.

Perusal of data in Table 5 showed that Schiff bases were moderately effective against this fungus. Compound VIII exhibited highest per cent inhibition among all the synthesized compounds. Compounds having di-substitution of bromo groups at para positions had maximum inhibition (73.75%) at 1000 µg ml^{-1}. The inhibition percent values of chloro substituted compound IX (70.41%) and fluoro substituted compound X (68.75%) were found to be quite promising. Thus di-substituted compounds (VIII, IX and X) showed more activity than mono-substituted compounds. Compounds with mono-substitution of nitro groups (I and II) having inhibition percent values 52.37% and 47.50% respectively at 1000 µg ml^{-}were found to be least effective against *F. oxysporum*. The compound (III) which contained one bromo group at para position found to have maximum inhibition (63.75%) among mono-substituted compounds. But none of the compounds was found as effective as standard carbendazim (bavistin).



(a)

(b)

Plate 3: Zones of inhibition formed by control (a) and compound VIII (b) at 5.0 mg ml^{-1} against *Burkholderia*

Table 5: Effect of different Schiff bases on per cent inhibition (I) of *F. oxysporum* at different concentrations

Compounds	**Concentrations (µg ml^{-1})**				
	50	100	250	500	1000
I	3.75	8.70	25.00	36.00	52.37
II	0.00	6.40	22.50	32.50	47.50
III	11.25	26.25	33.75	38.75	63.75
IV	8.75	25.00	31.25	37.50	61.25
V	12.50	21.25	30.00	35.50	60.00
VI	7.50	16.25	27.50	48.75	58.50
VII	7.50	8.75	26.25	46.25	56.25
VIII	13.75	30.00	38.75	56.25	73.75
IX	12.50	28.33	36.25	52.75	70.41
X	7.50	25.80	34.16	51.25	68.75
Carbendazim (Bavistin)	100	100	100	100	100
CD (p= 0.05)	0.55	0.11	0.05	0.31	0.01

ED_{50} values of all the tested compounds were given in Table 6. Compound VIII had highest ED_{50} among all the synthesized compounds. The compound VI and VII having di-substitution of nitro groups were found to be less effective with ED_{50} 780 ad 840 µg ml^{-1} respectively. Compound (II) having nitro group at ortho position did not show any ED_{50} thus was least effective. None of the compound was found effective as compared to standard fungicide carbendazim (bavistin) (ED_{50} = 10 µg ml^{-1}).

Table 6: ED_{50} values of different Schiff bases against *F. Oxysporum*

Compounds	**ED_{50} (µg ml^{-1})**
I	900
II	-
III	730
IV	790
V	800
VI	590
VII	670
VIII	380
IX	420
X	470
Carbendazim (Bavistin)	10

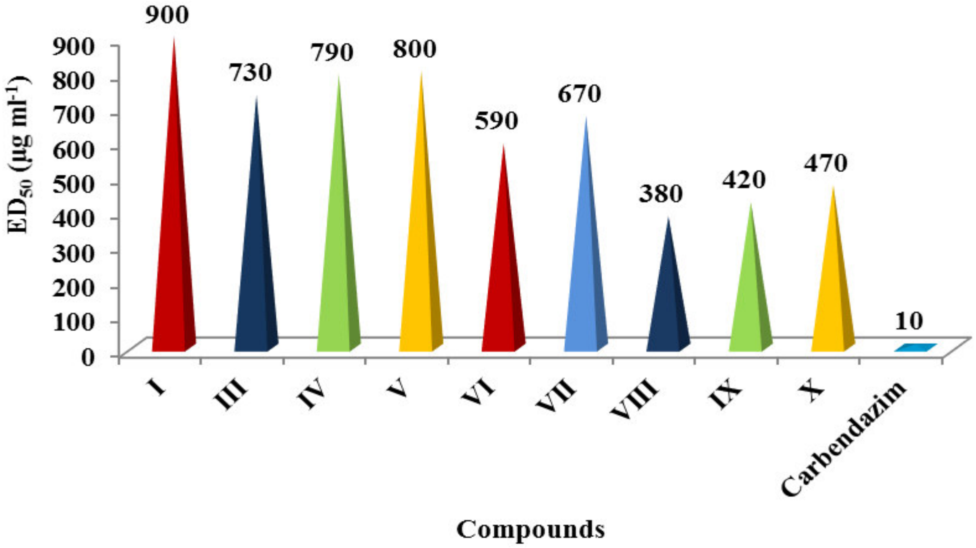
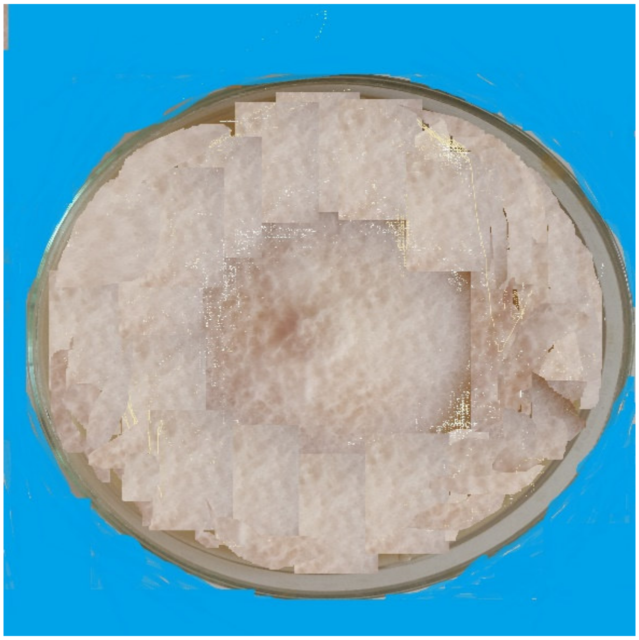


Figure 4: ED_{50} of different Schiff bases and carbendazim against *F.oxysporum*

The present study revealed that the compounds with halogen substitution were more effective than the compounds with nitro substitution against the test bacteria and fungus. The compounds with halogen substitution showed their effectiveness against the bacteria and fungus in the order of Br > Cl > F.



(a)

(b)

Plate 4: Mycelial growth inhibition of control (a) and compound VIII (b) against *Fusarium oxysporum* at 1000 µg ml^{-1}

CHAPTER-V

SUMMARY

Schiff bases were synthesized by condensation of carbonyl compounds (aldehydes and ketones) with primary amines. These were first reported by a German chemist Hugo Schiff in 1864 (Dhar and Taploo 1982). Schiff bases have characterstic feature of possessing azomethine group with general formula $RHC=N-R_1$, where R and R_1 can be various alkyl, aryl, cyclo alkyl or heterocyclic groups which may also have substitution at various positions. These compounds were often termed as anils, imines or azomethines. Several Schiff bases are considerably chemically and biologically important and this can be attributed to the presence of lone pair of electrons in sp^2 hybridised orbital of nitrogen atom of the azomethine group (Cimerman *et al* 2000). Schiff bases have been found to plethora of biological activities *viz* antimicrobial (Sharma *et al* 2008), antibacterial (Karthikeyan *et al* 2006), antifungal (Pandeya *et al* 1999) and anticancer potential (Cozzi 2014). These were used as substrate in the preparation of a large number of bioactive and industrial compounds such as azo Schiff bases (Zarei and Jarrahpour 2011). Imine formation was one of the major importance in biological processes as many of these involve the initial binding of carbonyl compounds to an enzyme through imine formation.

o-Phenylenediamine is an aromatic diamine which is a precursor for the synthesis of many heterocyclic compounds. Ajaily *et al* (2007) reported the antibacterial activity of Schiff bases that are synthesized by reacting *o*-phenylenediamine with different aldehydes. The work incorporated in the present thesis reports the synthesis of both mono and di-substituted Schiff bases from different substituted aromatic aldehydes and *o*-phenylenediamine. N-substituted benzylidene benzene-1,2-diamines (I-V) were prepared using equimolar quantities of *o*-phenylenediamine and different substituted aldehydes *viz* 4-nitro, 3-nitro, 4-bromo, 3-chloro and 4-fluoro whereas N, N'-bis substituted benzylidene benzene-1,2-diamines (VI-X) were synthesized by taking *o*-phenylenediamine and different substituted aldehydes namely 4-nitro, 3-nitro, 4-bromo, 3-chloro and 4-fluoro in 1:2 ratio. All the reactions were carried out in basic medium *i.e.* in the presence of catalytic amount of 10% NaOH solution using ethanol as a solvent. The progress of reaction was monitored by thin layer chromatography. The crude product obtained was recrystallized with ethanol. A single spot on the thin layer chromatographic plate revealed the purity of synthesized compound. The mono and di-substituted Schiff bases formed are also known as unsymmetrical and symmetrical Schiff bases respectively. Physical data such as yield, melting point, state and color of synthesized compounds were also determined. All the synthesized compounds were characterized by UV-visible, IR and ^1H NMR spectroscopic techniques.

All the synthesized compounds were also evaluated for their antimicrobial potential against *Psuedomonas* sp., *Klebsiella* sp., *Burkholderia* sp. and *Fusarium oxysporum.* Di-substituted compounds showed more activity than the mono-substituted compounds against all the bacteria and fungus. Among di-substituted, the compounds having halogen group exhibited more activity than the compounds having nitro groups. Moreover, di-substituted compounds with halogen substitution follows the order Br > Cl > F against *Psuedomonas* sp. and also against all other bacteria the order of activity was same. However, all the synthesized compounds exhibited less activity than the standard (ampillicin and bavistin). Also the di-substituted compounds followed the same order against *F. oxysporum i.e* the synthesized Schiff bases (VIII-X) were in the order of Br > Cl > F. Hence the compound (VIII) which contains bromo groups at para position was found to be more effective than other synthesized compounds but less than the standard ampicillin and carbendazim (bavistin).

CONCLUSION

N- substituted benzylidene benzene-1,2-diamines (I-V) and N, N'-bis substituted benzylidene benzene-1,2-diamines (VI-X) were synthesized by reacting different aldehydes with *o*-phenylenediamine in the presence of catalytic amount of sodium hydroxide (10%) by taking 1:1 and 2:1 molar ratio respectively. These reactions were carried out by conventional method. The synthesized compounds were screened for microbial activity against *Psuedomonas* sp., *Klebsiella* sp., *Burkholderia* sp. and *Fusarium oxysporum*. The di-substituted compounds exhibited more activity than the mono-substituted compounds. Also the compounds containing halogen groups showed more activity than the compounds with nitro groups. Among the di-substituted compounds, the compound (VIII) with bromo substitution exhibited more activity than the compounds with chloro and fluoro substitution. The synthesized compounds were found to be effective against all the bacteria and fungus and these follows the order of Br > Cl > F followed by di-substituted nitro Schiff bases and then mono-substituted halogen and nitro containing groups. But none of the compound showed more activity than the standard ampicillin and carbendazim (bavistin).

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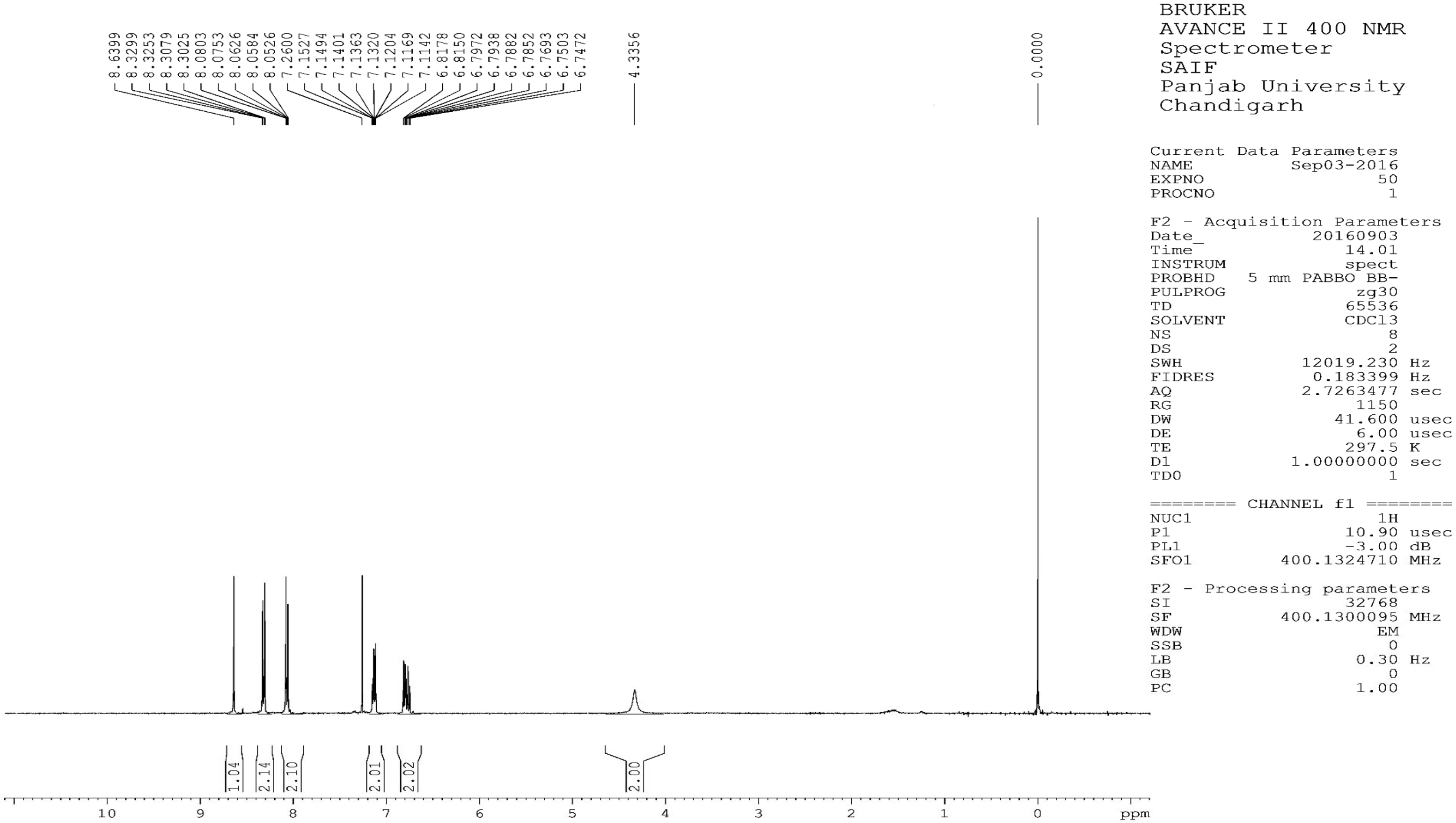
ANNEXURE



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Spectrometer
SAIF
Panjab University
Chandigarh

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NS                      8
DS                      2
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FIDRES           0.183399 Hz
AQ              2.7263477 sec
RG                   1150
DW                 41.600 usec
DE                   6.00 usec
TE                  297.5 K
D1             1.00000000 sec
TD0                     1

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PL1                 -3.00 dB
SFO1          400.1324710 MHz

F2 - Processing parameters
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GB                      0
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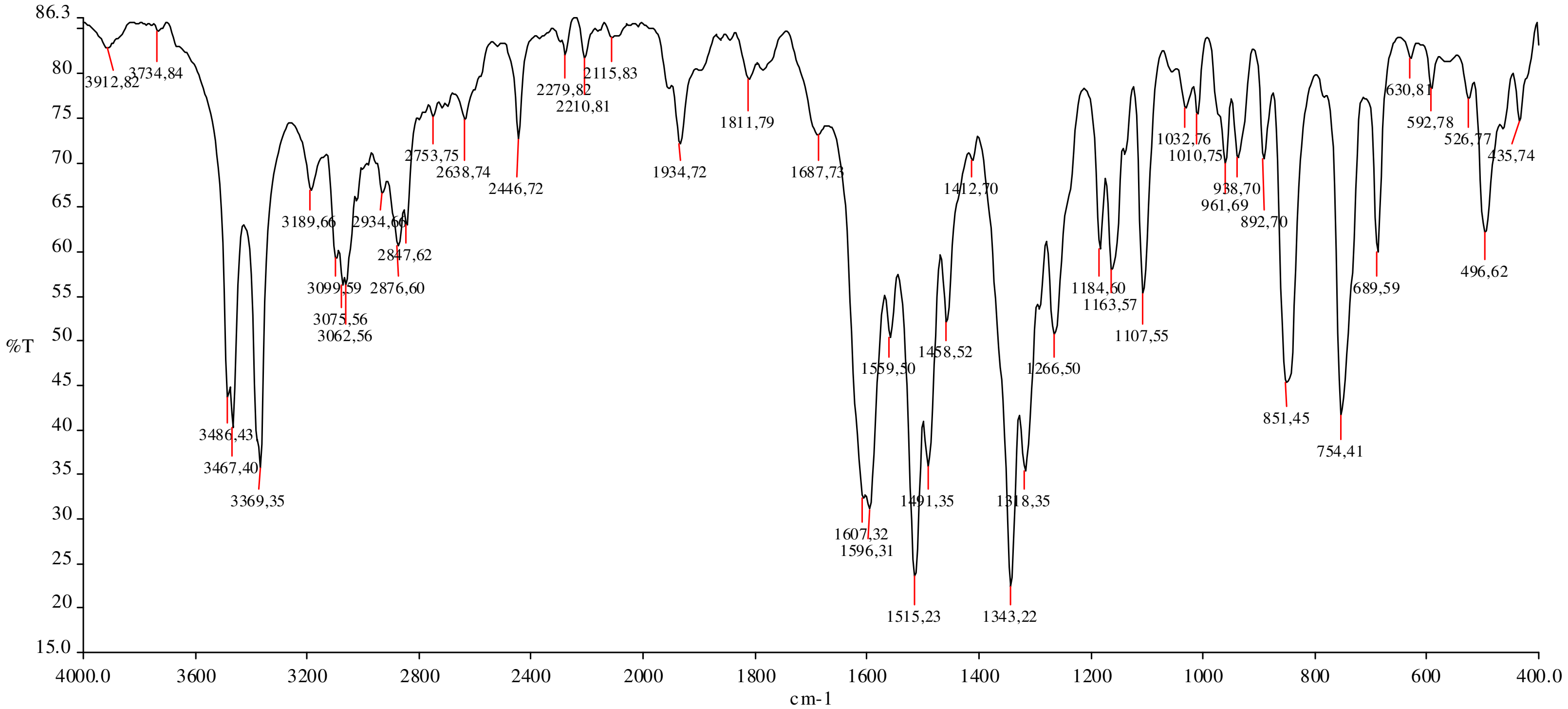
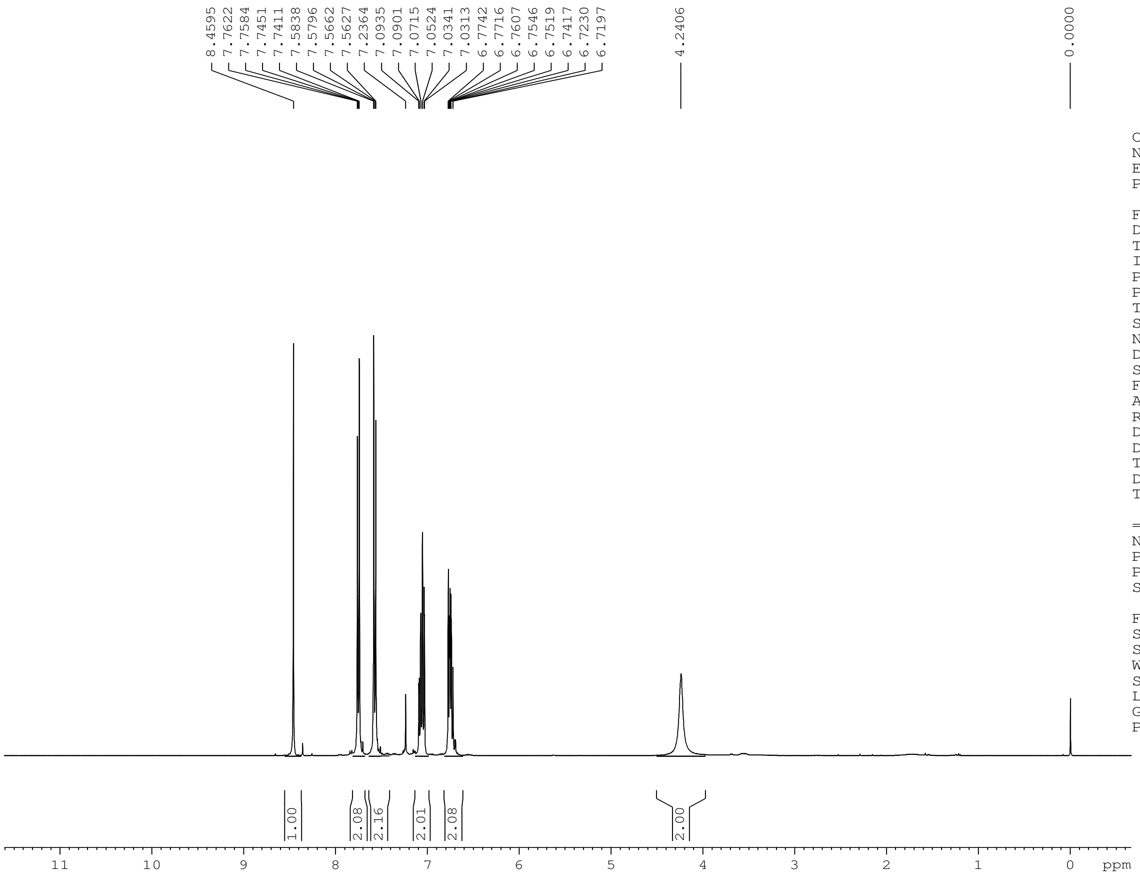
^{1}H NMR spectrum of Compound I

ii:



FT-IR spectrum of Compound I



BRUKER
AVANCE II 400 NMR
Spectrometer
SAIF
Panjab University
Chandigarh

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Current Data Parameters
NAME          Jan18-2017
EXPNO                 80
PROCNO                 1

F2 - Acquisition Parameters
Date_           20170118
Time               17.58
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                     8
DS                     2
SWH            12019.230 Hz
FIDRES          0.183399 Hz
AQ             2.7263477 sec
RG                   161
DW                41.600 usec
DE                  6.00 usec
TE                 293.2 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 10.90 usec
PL1                -3.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF           400.1300189 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
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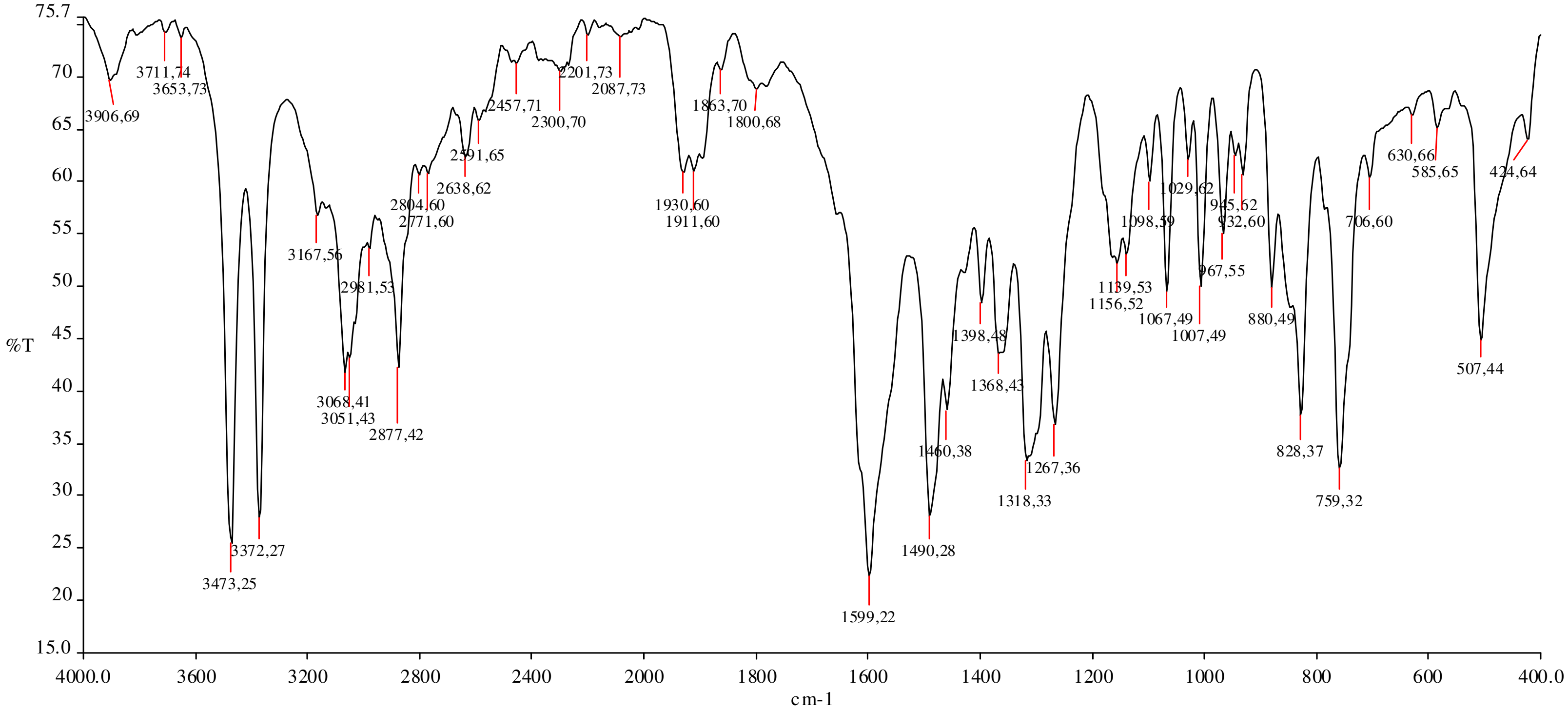
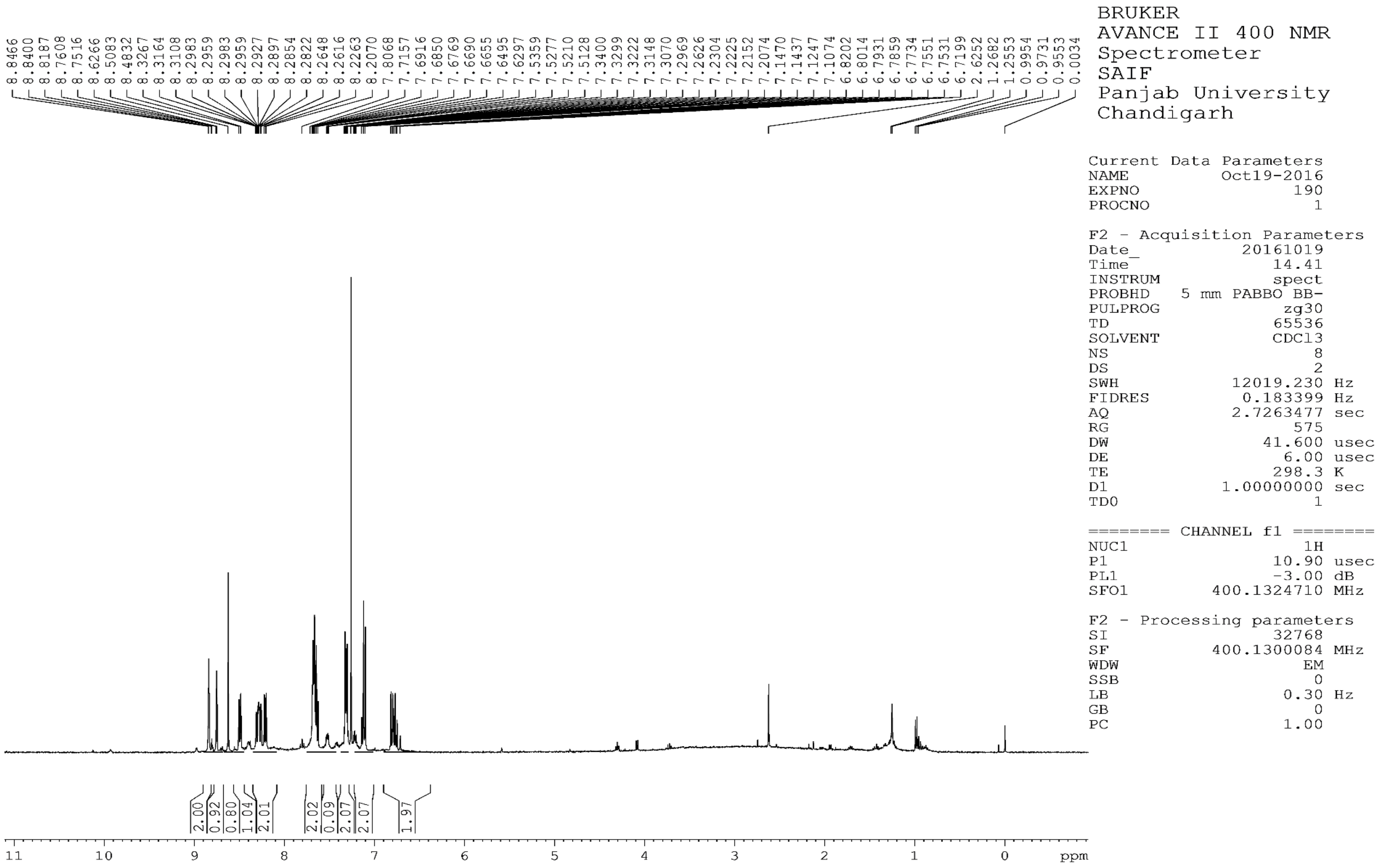
^{1}H NMR spectrum of Compound III



FT-IR spectrum of Compound III



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BRUKER
AVANCE II 400 NMR
Spectrometer
SAIF
Panjab University
Chandigarh

Current Data Parameters
NAME          Oct19-2016
EXPNO                190
PROCNO                 1

F2 - Acquisition Parameters
Date_           20161019
Time               14.41
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                     8
DS                     2
SWH            12019.230 Hz
FIDRES          0.183399 Hz
AQ             2.7263477 sec
RG                   575
DW                41.600 usec
DE                  6.00 usec
TE                 298.3 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 10.90 usec
PL1                -3.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF           400.1300084 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
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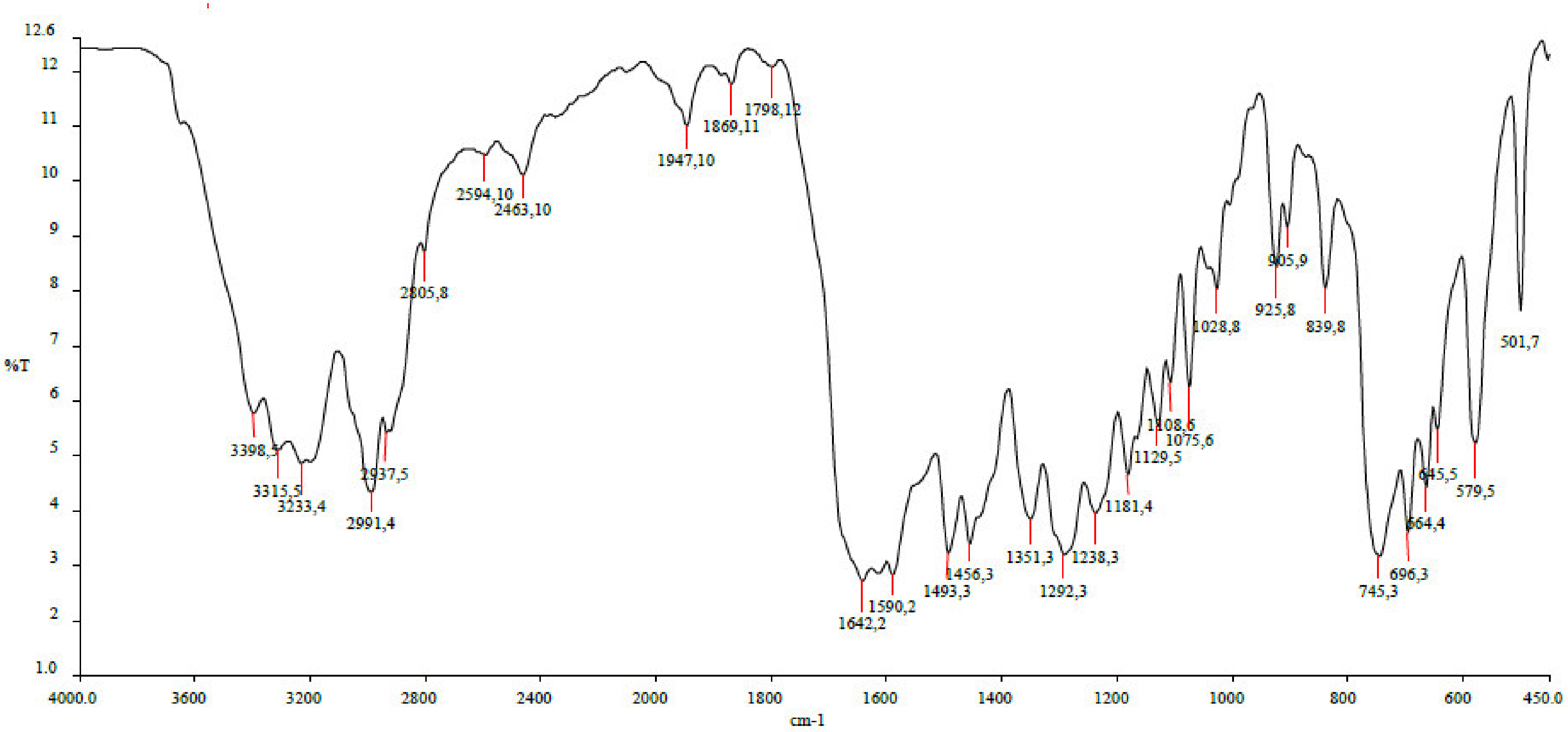
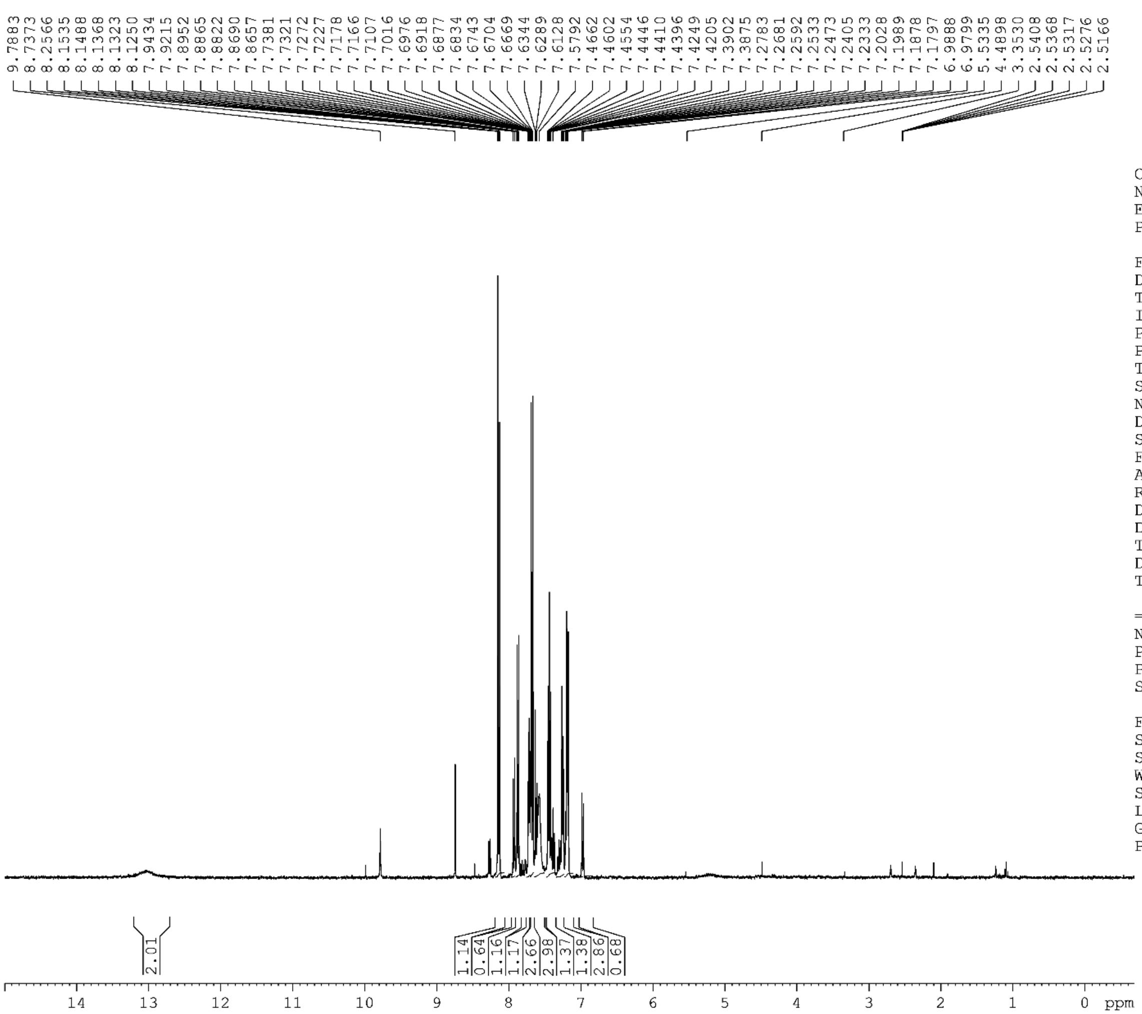
^{1}H NMR spectrum of compound VI

VI



FT-IR spectrum of Compound VI



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BRUKER
AVANCE II 400 NMR
Spectrometer
SAIF
Panjab University
Chandigarh

Current Data Parameters
NAME          May15-2017
EXPNO                360
PROCNO                 1

F2 - Acquisition Parameters
Date_           20170516
Time               10.34
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                     8
DS                     2
SWH            12019.230 Hz
FIDRES          0.183399 Hz
AQ             2.7263477 sec
RG                   812
DW                41.600 usec
DE                  6.00 usec
TE                 298.0 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 10.90 usec
PL1                -3.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF           400.1299905 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
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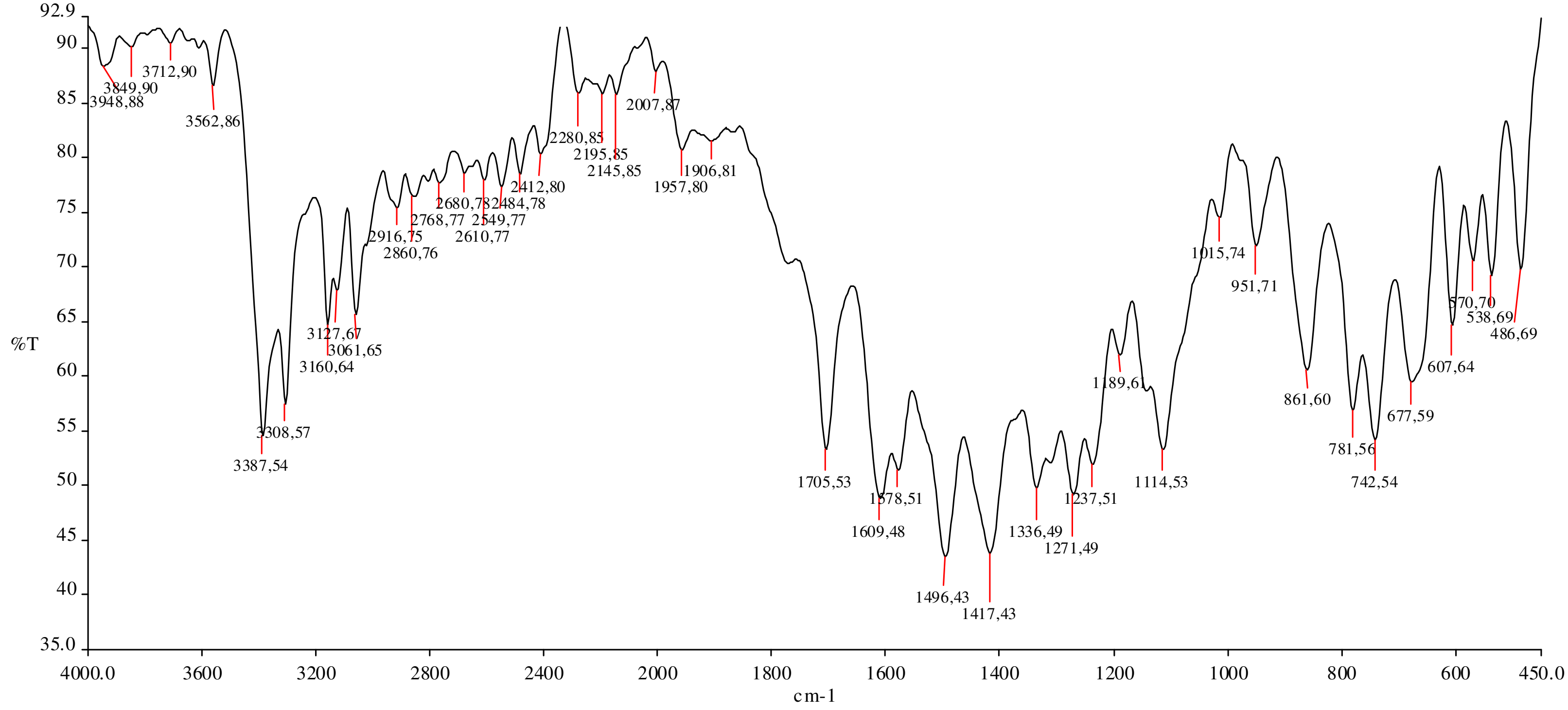
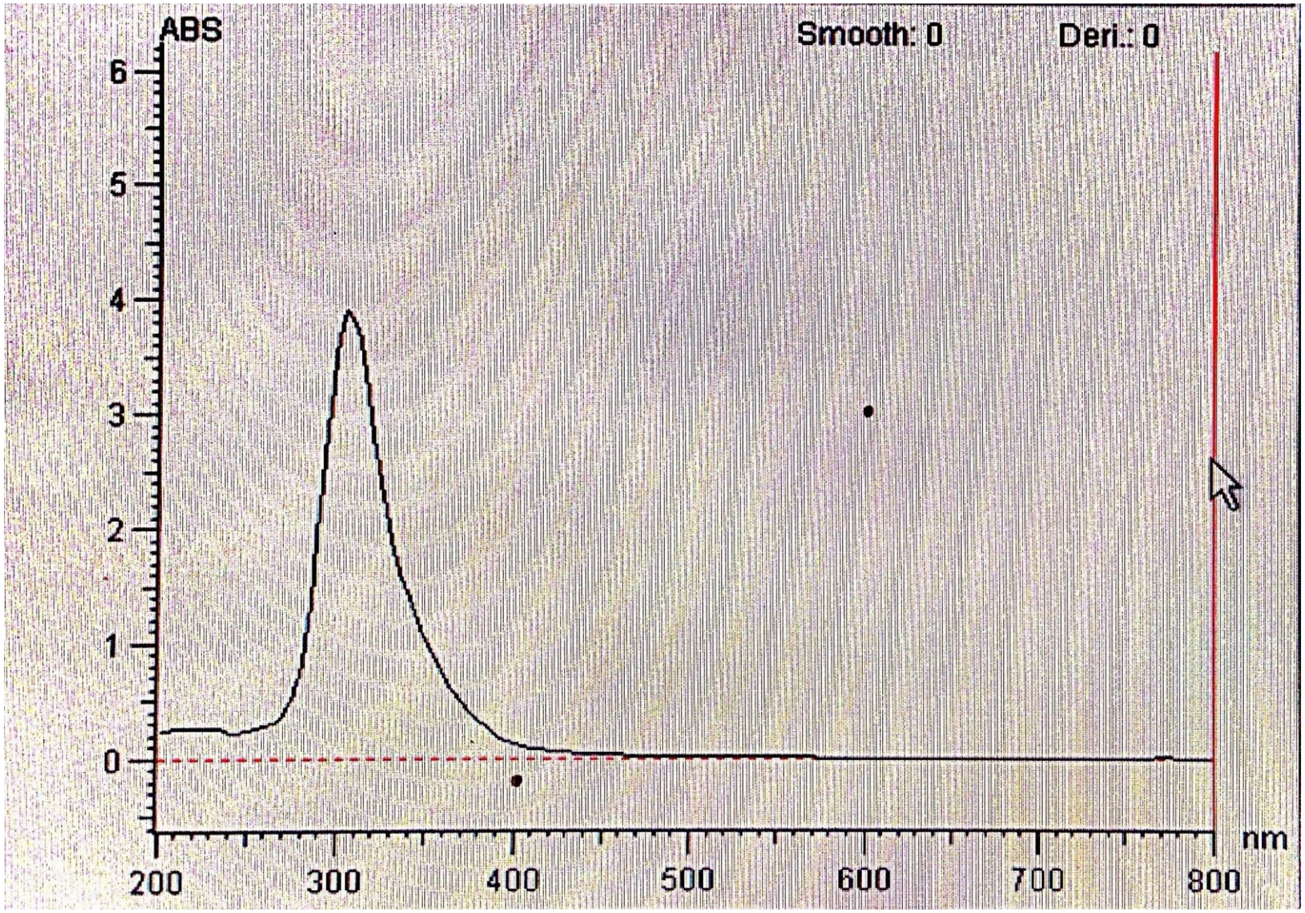
1H NMR spectrum of Compound X

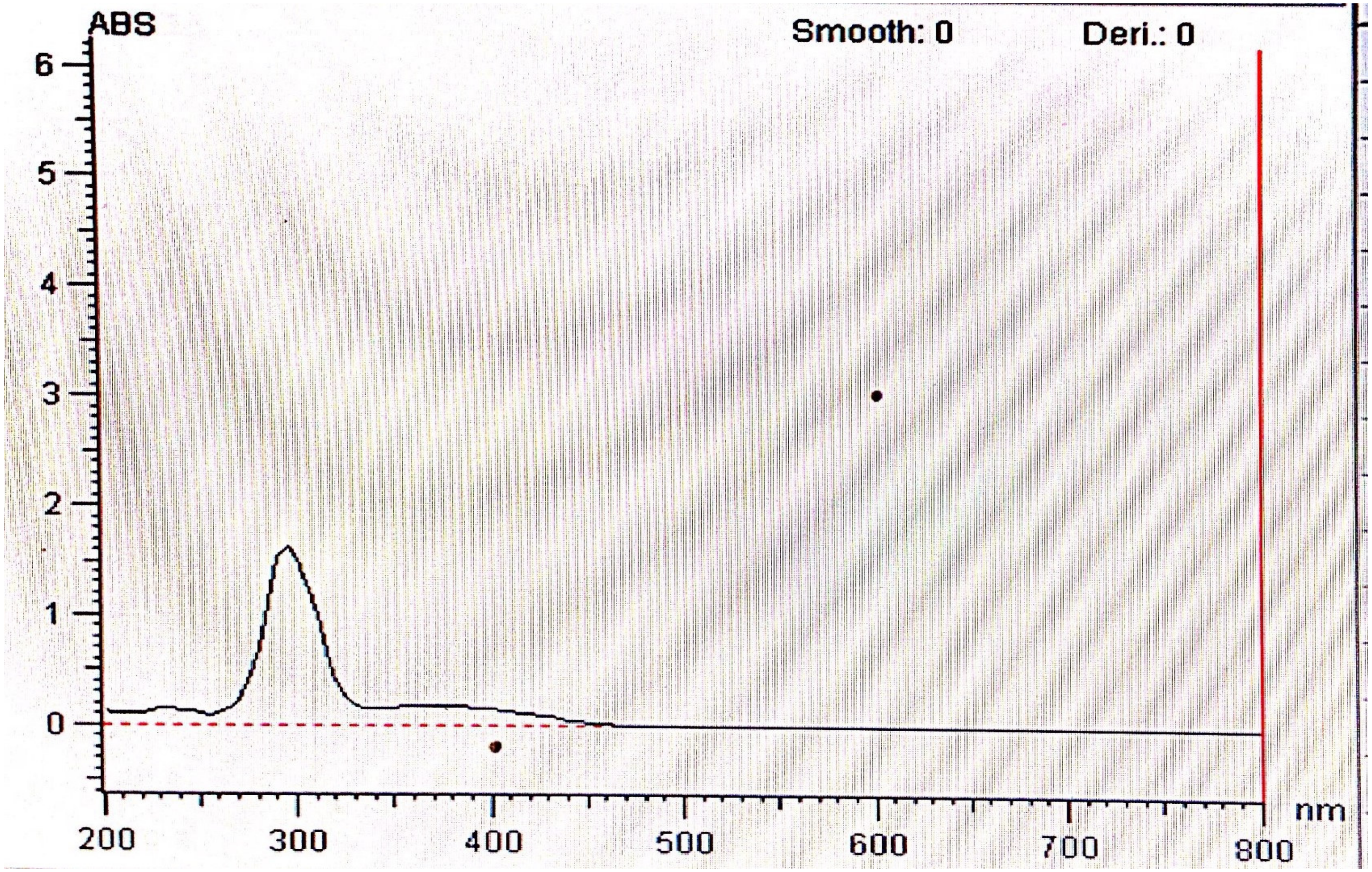


FT-IR spectrum of Compound X



UV-visible spectrum of Compound II

x



UV-visible spectrum of Compound VIII

VITA

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EDUCATIONAL QUALIFICATION

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University : Punjab Agricultural University, Ludhiana

Year of Award : 2017

OCPA : 7.30/10.00

Title of Master's Thesis : Synthesis, characterization and microbial activity of novel schiff bases of *o*-phenylenediamine

Awards/Distinctions/Fellowships/ Scholarship : First in Youth Festival-2016 (Cultural Event Giddha)
Second in Inter Varsity-2017 (Cultural Event Giddha)