ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Synthesis and IR Spectral Studies of Certain Novel Schiff Bases From Para Amino Benzoic acid and 2- Aminophenol and it's In Vitro Screening Against *Mycobacterium Tuberculosis*

**Shanmugakumar SD*, Raghava R, Srinivas Y, Swamycharan, Sneha M, Vijayavardhini M, Malleswari and Yogitha P**

Jyothishmathi College of Pharmacy, Turkapally (V), Shamirpet (M), R.R.District -78

## ABSTRACT

The designation of the tuberculosis as a global public health crisis denoted by the World Health organization. In the mid 1990s has underscored the severe challenges facing the antimicrobial research community. The occurrence of the some 3 million new cases of tuberculosis per year worldwide and emergence of new resistant strains of Mycobacterium tuberculosis increases the virulence of the newer and powerful drugs to combat the situation. In order to full fill the lacuna, newer drugs were synthesized by the molecular manipulation by the intermediates. Schiff bases were been synthesized as an intermediate which on the molecular manipulation leads to various heterocyclic moieties. With the core aim in the mind, we had attempt to synthesize certain novel schiff bases by the coupling of the certain aromatic aldehydes with the para amino benzoic acid and 2- amino phenol and the functional groups were been determined by the interpretation of the IR spectra. Further, the synthesized Schiff bases were been screened for the antitubercular activity by in vitro LJ (Lowenstein-Jensen) method. It was observed that Schiff bases of para amino benzoic acid were showed the inhibitory activity at the concentration of 0.5mg/mL and the Schiff bases of 2- amino phenol showed the inhibitory activity at the concentration of 0.2mg/mL respectively.

**Keywords:** PABA; 2-Aminophenol; LJ medium; Mycobacterium tuberculosis

***Corresponding author***

ISSN: 0975-8585

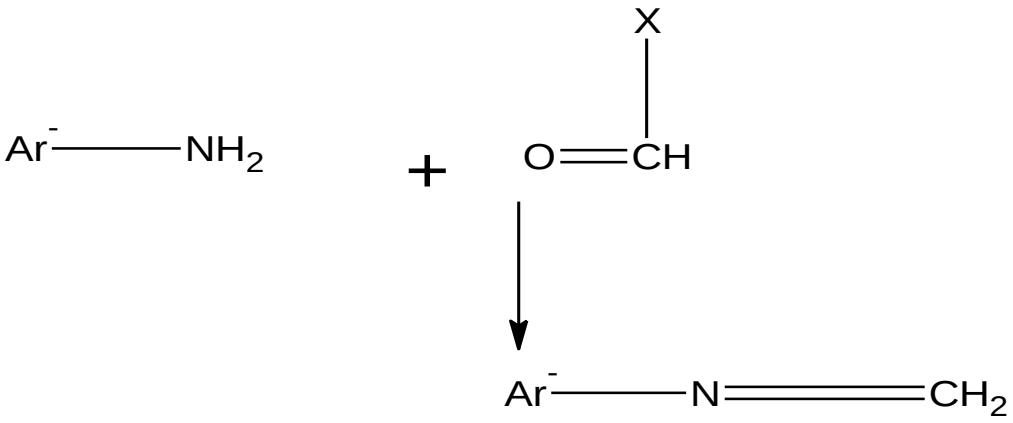
## INTRODUCTION

The discovery and development of antibiotics are among the most powerful and suceesful achievements of modern science and technology for the control of infectious diseases.Tuberculosis are a global health crisis which is prevailing from the ancient arena. World Health Organization has underscored the severe challenges in the facing the antimicrobial research community [1]. Worldwide, nearly 3 million new cases were been reported with the emergence of new drug resistant strains of *Mycobacterium tuberculosis* or increased virulence have made clear the pressing need for the evolution of newer and powerful drugs. Extensive investigations in the field of Schiff bases have been reported [2]. Their preparation, chemical and physical properties have been described by various workers [3]. Several workers have reported that Schiff bases formed from aromatic aldehydes or aromatic ketones and their derivatives quite stable. Due to the great flexibility and diverse structural aspects of Schiff bases, a wide range of these compounds have been synthesized. Sahu *et al* [4] reported fungi toxicity of some Schiff bases. Zhang HJ *et al* [5] synthesized a series of Schiff bases and illustrated the anti bacterial perspectives. In the present work, we had synthesized a series of Schiff bases and characterized its functional groups and its antitubercular activity is established.

## MATERIALS AND METHODS

All the aldehydes required were obtained from SD fine chem limited. para amino benzoic acid and 2-aminophenol were procured from Merck chemicals (Germany).The solvents and chemicals were obtained from Lobachemie, Bombay. All the procured compounds were purified and dried whenever required.

**Chemistry of Schiff base formation** [6]: Aldehydes and ketones react with primary amine to form azomethines which are usually known as “Schiff bases” or sometimes if the amine is aromatic as “ Anils”



ISSN: 0975-8585

**EXPERIMENTAL WORK:**

1. **Synthesis of Schiff bases from para amino benzoic Acid (PABA)** : A mixture of equimolar quantities of (0.1Mole) of para amino benzoic acid and the appropriate aromatic aldehydes (0.1Mole) in 1,4 dioxan (100ml) was refluxed on a steam bath for 1hr . After refluxation, excess of the solvent was removed and cooled the solid mass that separated out was filtered and recrystallized from 95% ethanol. The percentage of yield is 82%. The purity of the compound was checked by employing TLC method (silica gelG). In all cases the product were obtained showed single spot on T.L.C. plate .Schiff bases prepared from para amino benzoic acid are given in Table I.

**Table No I:Compound Code, Molecular formula, Molecular weight, Melting point, Percentage yield and $R_f$ Values for the stated solvent system**

| Compound code | Molecular formula | Molecular weight | Melting point °C | Percentage of Yield | $R_f$ Value* |
|---|---|---|---|---|---|
| JCP-1 | $C_{14}H_{11}NO_3$ | 241.24 | 189°C | 85% | 0.75 |
| JCP-2 | $C_{16}H_{16}N_2O_2$ | 268.31 | 197°C | 80% | 0.83 |
| JCP-3 | $C_{15}H_{13}NO_3$ | 255.26 | 195°C | 75% | 0.80 |
| JCP-4 | $C_{13}H_{11}NO_2$ | 213.23 | 156°C | 67% | 0.43 |
| JCP-5 | $C_{14}H_{13}NO_2$ | 227.25 | 159°C | 65% | 0.42 |

***METHANOL : CHLOROFORM (7:3)**

2. **Synthesis of Schiff bases from 2- amino phenol:** 0.1 mole of 2-amino phenol and 0.1 mole of appropriate aromatic aldehydes in 1, 4 dioxan (100mL) was refluxed on a steam bath for 1hr. After refluxation, excess of the solvent was removed and cooled the solid mass that separated out was filtered and recrystallized from 95% ethanol. The percentage of yield is 67%. The purity of the compound was checked by employing TLC method (silica gelG). In all cases the product were obtained showed single spot on T.L.C. plate. Schiff bases prepared from 2-amino phenol are given in Table I.

3. **Preparation of the test compounds:** The compounds were dissolved at a concentration of 10mg/mL in dimethyl formamide (DMF) in order to obtain a final concentration of 1mg/mL. In all, four different concentrations of the schiff bases were prepared (0.8mg/mL, 0.6mg/mL,0.4mg/mL and 0.2mg/mL) for the antitubercular assay. About 0.5mL of schiff bases of each concentration were been transferred in to LJ (Lowenstein – Jensen) medium slants. They were incubated at 37° C for about 6-8 weeks.

4. **Test Organism:** Drug resistant *Mycobacterium* strains were procured from the Institute of Tuberculosis Research, Trivandrum Medical College, Trivandrum, Kerala. The clinical specimen (sputum from the XDR –TB patients (Extreme Drug resistant) – drugs treated) were been identified by AFB staining method (Fluorescence stain) in order to identify the positive growth of *Mycobacterium tuberculosis* in the sputum. The specimens were

been dissolved in the saline water and incubated in the BOD (Biological Oxygen Demand) incubator at 37° +/-1° C for 48 hrs prior to incorporation in the LJ medium.

5. **Preparation of the LJ(Lowenstein –Jensen) slants and antitubercular assays:**

**Composition and preparation of Lowenstein –Jensen (LJ) Medium slants [7]** : Monopotassium phosphate, anhydrous -4g, Magnesium sulphate -0.4g, Magnesium citrate -1.0g , Glycerol – 20mL, Distilled water to 1000mL, Whole fresh eggs -1600mL, Malachite green 1% W/V aqueous solution – 50mL. Dissolve the salts and glycerol in the distilled water and steam in autoclave or steamer at atmospheric pressure for 2 hours. This is the mineral salt solution which may be prepared in bulk and stored. Wash the eggs with soap water, rinse with 70% alcohol and wipe dry. Break the eggs in to a screw capped jar (10mm diameter) glass beads and shake to break yolks and to homogenize the material with the aid of homogenizer. Add the mineral salt solution and Malachite green to the yolks. The mixture was well homogenized and dispensed in 30mL screw capped bottles. The bottles on sloped racks and inspissate at 80-85° C until the medium is solidified. This process takes around 50-90 minutes.

6. **Recovery test procedure** : About 0.5mL of test strain were been transferred in to LJ medium slants .They were incubated at 37° +/-1° C for about 6-8 weeks in the BOD incubator. Visible appearance of colonies indicates the drug is resistant and No appearance of colonies indicates the drug is sensitive. Minimum Inhibitory concentration (MIC) was determined after the growth of *Mycobacterium tuberculosis* in the solid media.

## RESULTS AND DISCUSSION

In all, five compounds were synthesized (Table I) and the UV and IR spectral data confirmed their molecular functional groups. The UV and IR analysis data are given below:

**JCP-1**: IR (KBr $cm^{-1}$): -OH (Str):3345, C=O (Str) : 1661 N=C (Str) :1608, -OH (bend) :1317.
UV spectral data: Solvent: 1, 4 dioxan; $\lambda_{max}$: 432 nm

**JCP-2**: IR (KBr $cm^{-1}$): C=O (Str): 1672, N=C (Str): 1598, Ar-H (bend): 796.
UV spectral data: Solvent: 1, 4 dioxan ; $\lambda_{max}$ : 446 nm

**JCP-3 :** IR (KBr $cm^{-1}$) : C=O (Str) : 1674, N=C (Str) :1601.2, C-O-C(Str) : 1396,
UV spectral data: Solvent: 1, 4 dioxan; $\lambda_{max}$: 423 nm

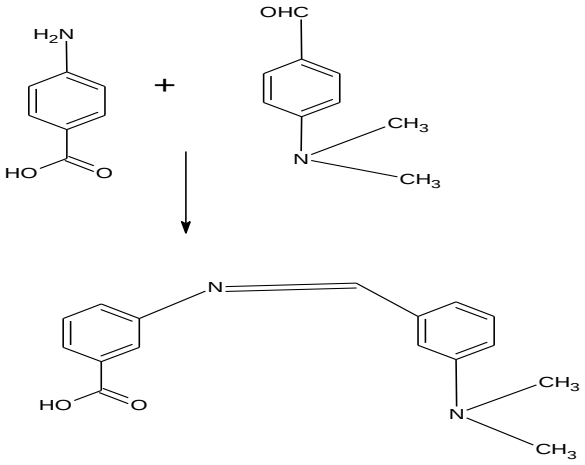
**JCP-4 :** IR (KBr $cm^{-1}$) : -OH (Str) :3417 : C=O(Str) : 1629.85, Ar-H (bend) :804.32, N=C (Str) :1581, C-N (Str) : 1174.65
UV spectral data: Solvent: 1, 4 dioxan; $\lambda_{max}$: 417 nm

ISSN: 0975-8585

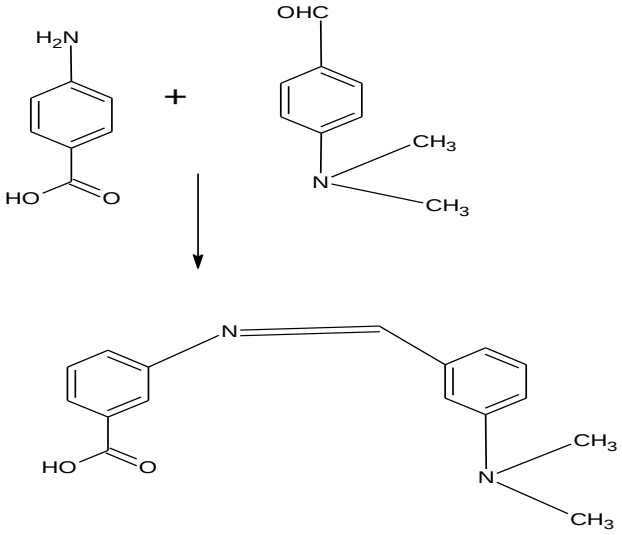
**JCP-5:** IR (KBr $cm^{-1}$) : C=O (Str) :1685.2, N=C(Str) :1600.8, C-N (Str) : 1176.73
UV spectral data : Solvent : 1,4 dioxan ; $\lambda_{max}$ : 408 nm

**SCHEME-I**

**JCP-1 : N' –(4-Carboxy phenyl) -4-hydroxy phenyl azomethine**

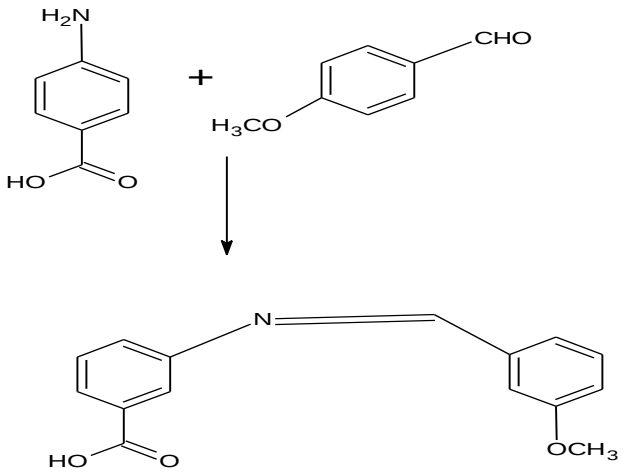



**JCP-2: N'- (4-carboxyphenyl)- N,N –dimethyl phenyl azomethine**

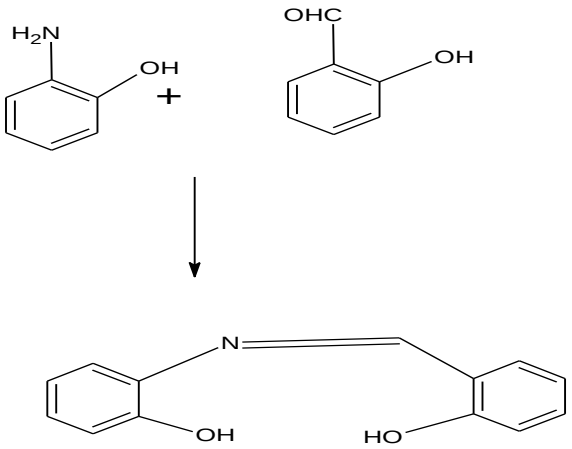



**JCP-3: N'-(4- Carboxy phenyl) –N- P-methoxy Phenyl azomethine**

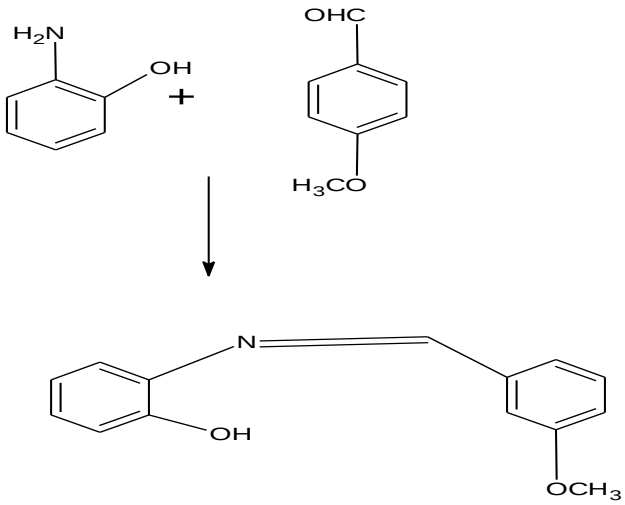
ISSN: 0975-8585



**JCP-4: N'-(2-hydroxy Phenyl)-2-hydroxyphenyl azomethine**



**JCP-5: N'-(4-methoxy Phenyl)-2-hydroxy phenyl azomethine**



The five synthetic compounds were been subjected to antitubercular assay in the LJ (Lowenstein –Jensen) medium with the concentrations ranging from 0.2mg/mL – 1mg/mL.

Rifampicin (0.2mg/mL) were been used as a positive control. The *in vitro* antimycobacterial activity of the five schiff bases against *Mycobacterium tuberculosis* (**XDR-Strain**).

It was observed that schiff bases of para amino benzoic acid (**JCP 1-3**) showed inhibitory activity against *Mycobacterium tuberculosis* at the concentration of 0.2 & 0.4mg/mL respectively. On contrary, schiff bases procured from 2- aminophenol (**JCP 4-5**) showed inhibitory activity at the concentration of 0.2mg/mL.

It can be deduced from these results that the different response of the synthesized schiff bases arise because of their structural differences and also solvent dependent, *i.e* the polarity of the solvent is also responsible for the inhibition process of the *Mycobacteria* which is under further investigation.

From the present investigation, it can be concluded that it cannot be assumed that one solvent is better than the other in the respective of yield of schiff bases. It varies with the different solvents. It is dependent on the molecular structure and the particular bacterial concerned. In the present study, we had confirmed the antitubercular perspectives of the schiff bases derived from the 4- amino benzoic acid and 2- aminophenol which possessed the prodrug concept for the antibacterial properties. From the present study, it was concluded that Schiff bases were been able to possess an additional perspective such as antimycobacterial activity. Further studies can be postulated for the preparation of certain novel heterocyclic compounds by the molecular manipulation of the intermediate anils.

## CONCLUSION

From this study, it can be postulated for the preparation of certain novel heterocyclic compounds by the molecular manipulation of the intermediate anils.

## ACKNOWLEDGEMENTS

Authors would express their gratitude and profound thanks to chairman Shri. J.Sagar rao and secretary Shri.J.Sumith sai for supporting us by providing the necessary facilities for carry out the project work. Further, authors would express our gratitude to Prof. M.Sainarayana for giving us encouragement in the project work. Authors would express our profound thanks to the Mr. Bhaktha Singh Lazarus for helping us in getting the clinical sample of *Mycobacterium tuberculosis* from the Govt.Medical College, Trivandrum, Kerala.

## REFERENCES

[1] World Health Organization. TB: A Global Emergency WHO Report No.14977.WHO, Geneva, Switzerland 1994; 97-98.
[2] Michael J Hearn, Michael H Cynamon. J Antimicrobial Chem 2004; 53:185-191.

[3] Jyh-Jia Jiang, Tsu-Chung Chang, Wen-lin Hsu, Jing –Min Hwang, Ling-Yih Hsu. Chem Pharm Bull 2003; 51(11) :1307-1310.
[4] Sahu K, Behera K, Pathaik R C, Nayak A, Behera G B. Indian J Chem 1979 ; 18B :557.
[5] Zhang HJ, Qin X, Liu K, Zhu DD, Wang XM, Zhu HL. Bio Org Med Chem 2011; 15:19(18): 570-577.
[6] Tim Soderbig. Organic Chemistry with Biological Emphasis. University of Minnosta, Morris 1985; 156-157.
[7] Anandi Martin. Procedure Manual: Assay in LJ medium. Institute of Tropical Medicine, Mycobacteriology Unit, Anwerp, Belgium, 1987; 75-82.