PART-A

STUDIES ON PYRAZINE 2-CARBOXYLIC ACID DERIVATIVES
INTRODUCTION

The treatment of tuberculosis (TB) infections has become an important and challenging problem due to the emergence of multiple-drug-resistant organisms. Different factors are responsible for the resurgence of TB, such as people infected with HIV virus. According to WHO global report in 2011 there were an estimated 9.2 million worldwide new cases of TB with 0.5 million cases of multidrug-resistant TB (MDR-TB) and estimated 1.4 million deaths from TB in HIV-negative people and 0.2 million among people infected with HIV. This is despite the availability of treatment that will cure most cases of TB. Pyrazinamide is one of the frontline agents that played a significant role in shortening the duration of treatment of MDR-TB.

Pyrazinamide (PZA), a first-line sterilizing drug in tuberculosis chemotherapy is the prodrug of the pharmacologically active agent pyrazinoic acid (POA).

Pyrazinoic acid is released by amide hydrolysis of pyrazinamide as shown in figure below.

Pyrazinamide (PZA) is a first line agent for the treatment of tuberculosis and is itself a prodrug. Pyrazinamide, along with isoniazid and rifampicin, forms the cornerstone of modern TB therapy. Pyrazinamide plays a unique role in shortening the therapy from previously 9–12 months to 6 months, because it kills a population of semi-dormant tubercle bacilli in acidic pH. The mechanism of action of pyrazinamide (PZA) is poorly understood: pyrazinoic acid (POA), the active moiety of PZA, has been shown to inhibit various functions at acid pH in Mycobacterium tuberculosis. Experimental
evidence suggests that PZA diffuses into M. tuberculosis and is converted into POA by pyrazinamidase (PZAase); the in vitro susceptibility of a given strain of the organism corresponds to its PZAase activity\textsuperscript{5-7}.

PZA analogs, such as pyrazinoic acid esters are potent antimycobacterial drugs than PZA and also potent against Mycobacterium tuberculosis fatty acid synthase type I(FASI). In fact POA esters showed good antitubercular activity against \textit{M. tuberculosis}. In addition to alkyl esters, other POA derivatives also presented activity against PZA-susceptible and PZA-resistant isolates of \textit{M.tuberculosis}. Examples are pyrazine thiocarboxamide and pyrazinoic acid pivaloxymethyl esters. In fact most of the reported compounds showed an activity greater or comparable to that of PZA demonstrating the feasibility of the approach\textsuperscript{8-12}.

Since pyrazinoic acid esters and other derivatives showed good activities. It was interesting to study pyrazine 2-carboxylic acid derivatives. So we have undertaken this research problem. This part is divided in to three chapters as shown below:

\textbf{CHAPTER-1: STUDIES ON 1,2,4 TRIAZOLE DERIVATIVES}

\textbf{CHAPTER-2: STUDIES ON HYDRAZIDE-HYDRAZONE DERIVATIVES}

\textbf{CHAPTER-3: STUDIES ON OXADIAZOLE DERIVATIVES}
REFERENCES

CHAPTER-1

STUDIES ON 1,2,4 TRIAZOLE DERIVATIVES
INTRODUCTION

The heteroaromatic triazole ring system is composed of five atoms, two carbons, and the three nitrogens, which can be rearranged in two combinations to give either 1,2,3-triazole or 1,2,4-triazoles.

1,2,4-Triazoles have proved to be most useful framework for biological activities among nitrogen containing five membered heterocycles. In five membered heterocyclic ring system 4-aryl triazole have three nitrogen atoms at 1,2 and 4 positions, an aryl group at 4-position and free mercapto group at 3-position.

SYNTHETIC ASPECT

Several methods have been reported in the literature for the preparation of 1,2,4-aryl triazoles. Few of them are as under.

1. The pellizari reaction between hydrazides and amides at high temperature in the absence of solvent produced 1,2,4-triazoles, which proceed through acylhydrazidines as intermediates\(^1\).

2. S. Svetik\(^2\) \textit{et al.} have reported that the interaction of ethyl N-cynoformimidate with benzimidazole-2-hydrazine in triethyl amine gave the corresponding 5-amino triazole.
3. A. Narender et al. have reported the synthesis of 1,2,4-aryl triazoles via reaction of 2N’-(2-cyclopropyl-[1,8]naphthyridine-3-carbonyl)-hydrazinecarbodithioic acid potassium salt with hydrazine hydrate.

4. R. J. Singh et al. have synthesized some novel 1,2,4- triazoles as potent bacteriocidal agents with the reaction of 4- methyl phenyl amine, carbon disulphide and ammonia in the methanol as a solvent and lead nitrate as a catalyst.

5. G. Naganagowda et al. have synthesized 5-substituted-4-aryl-3-mercapto-4H-1,2,4-triazoles by the reaction of 3-chloro-1-benzothiophene-2-carbohydrazide and various isothiocynobenzenes.
SOME MARKETED DRUGS HAVING PYRAZINE NUCLEUS ALONG WITH ITS BIOLOGICAL ACTIVITY.

- Terconazole
- Fluconazole (Anti fungal)
- Itraconazole (Anti fungal)
- Triazolam (Anti fungal)
- Etizolam (sedative)
- Alprazolam (anxiety disorder)
- Diaryl thiazolotriazole (COX-1 & COX-4 Inhibitor)
- Deferasirox (chronic anemia)
- Ribavirin (Antiviral)
THERAPEUTIC ASPECT

1,2,4-Triazoles are the broad investigated molecules. They have proved to be most useful framework for biological activities among nitrogen containing five membered heterocycles. Some of them are listed below.

1. Antimicrobial\textsuperscript{6-7} 
2. CNS depressant\textsuperscript{8} 
3. Antiviral\textsuperscript{9} 
4. Sedative\textsuperscript{10} 
5. Insecticides\textsuperscript{11} 
6. Antiasthamatics\textsuperscript{12} 
7. Anticonvulsants\textsuperscript{13} 
8. Plant growth regulators\textsuperscript{14}

1. A. M. Abdel-Megged\textsuperscript{15} \textit{et al.} carried out molecular modeling study and reported acylated 1,2,4-aryl triazoles-3-acetates as potential anti-inflammatory activity.

\begin{center}
\includegraphics[width=0.5\textwidth]{image1.png}
\end{center}

2. A. Alanine\textsuperscript{16} \textit{et al.} have synthesized and evaluated 1,2,4-triazoles as A\textsubscript{2A} receptor antagonists.

\begin{center}
\includegraphics[width=0.5\textwidth]{image2.png}
\end{center}

3. N. Raghav and M.Singh\textsuperscript{17} presented facile microwave assisted synthesis of triazoles and evaluated them as protease inhibitors and carried out inhibitory studies on cathepsin B and cathepsin H, two significant lysosomal cysteine proteases.

\begin{center}
\includegraphics[width=0.5\textwidth]{image3.png}
\end{center}

4. M. D. Grandi\textsuperscript{18} \textit{et al.} have synthesized 3,4,5- substituted triazoles derivatives as inhibitors of HIV RT Ribonuclease H.
5. A.A. Siddiqui et al. have designed, synthesized and screened 1,2,4-triazole incorporated with pyridazinones as a new class of antihypertensive agents.

M. Takaoka et al. have reported that some 3-amino-1,2,4-triazole (ATZ), 3-mercapto-1,2,4-triazole (MTZ), and 3-nitro-1,2,4-triazole (NTZ) derivatives showed antithyroid activity. S. W. Schneller et al. have reported that some 1,2,4-triazole C-nucleosides lacked antiviral properties against herpes simplex virus 1 and 2 (HSV-1 and -2) along with other viruses. S.L. Vasoya have synthesized some new thiosemicarbazide and 1,2,4-triazoles heterocycles bearing the benzo[b]thiophene nucleus as potent antitubercular and antimicrobial agents.

Furthermore, it had been reported that many compounds having a 1,2,4-triazole skeleton possessed significant biological activity. On the basis of the above mentioned reports, the present work is concerned with the synthesis of different 1,2,4-triazole derivatives with the objective of discovering new and potent anti-microbial/antitubercular agents. This chapter is divided into four sections which are mentioned below:

**SECTION-I:** Synthesis and antitubercular evaluation of (E)-4-(substituted benzylideneamino)-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiols.

**SECTION-II:** UV studies of some (E)-4-(substituted benzylidene amino)-5(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiols.

**SECTION-III:** Synthesis and antimicrobial evaluation of 6-(substituted phenyl)-3-(pyrazin-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles.

**SECTION-IV:** Synthesis and antimicrobial evaluation of 6-(substituted phenyl)-3-(pyrazin-2-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines
REFERENCES


PART-A

CHAPTER-I

SECTION-I

Synthesis and antitubercular evaluation of (E)-4- (substituted benzylideneamino)-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiols.
INTRODUCTION

Schiff bases, named after Hugo Schiff\(^1\), are formed when any primary amine reacts with an aldehyde or a ketone under specific conditions. Structurally, a Schiff base (also known as imine or azomethine) (Fig. 1) is a nitrogen analogue of an aldehyde or ketone in which the carbonyl group (C=O) has been replaced by an imine or azomethine group(C=N-R).

\[
\begin{array}{c}
R_1 \hspace{1cm} C \hspace{1cm} N \hspace{1cm} R_3 \\
\text{R}_1, \text{R}_2 \text{and/or } R_3 = \text{alkyl or aryl}
\end{array}
\]

(Fig.1)

Schiff bases are some of the most widely used organic compounds. They are well known intermediate for the preparation of azetidinone, thiazolidinone, formazone, aryl acetamide and many other derivatives. These are the compounds contain characteristic C=N group. They are used as pigments and dyes, catalysts, intermediates in organic synthesis, and as polymer stabilisers\(^2\). Schiff bases have also been shown to exhibit a broad range of biological activities.

SYNTHETIC ASPECT

Various methods for the preparation of Schiff bases derivatives have been cited in literature, some of them are as under.

1. L. B. Pierre\(^3\) and coworkers have synthesized (E)-N-phenyl methyleneglycine ethyl ester by the cyclocondensation of glycine ethyl ester hydrochloride, \(t\)-butylmethyl ether (TBME), benzaldehyde was added followed by anhydrous \(\text{Na}_2\text{SO}_4\) and triethylamine.
2. J. G. Amanda\textsuperscript{4} \textit{et al.} have prepared Schiff bases by condensation of equimolar quantity of 3,6-diformylcatechol and substituted o-phenylenediamine.

\begin{center}
\begin{tikzpicture}

\node (a) at (0,0) {\text{O}};
\node (b) at (0,-1) {\text{OH}};
\node (c) at (2,0) {\text{H}_2\text{N}};
\node (d) at (2,-1) {\text{OR}};
\node (e) at (4,0) {\text{OH}};
\node (f) at (4,-1) {\text{H}_2\text{N}};
\node (g) at (6,0) {\text{OR}};
\node (h) at (6,-1) {\text{OR}};
\node (i) at (8,0) {\text{N}};
\node (j) at (8,-1) {\text{N}};
\node (k) at (10,0) {\text{O}};
\node (l) at (10,-1) {\text{OH}};

\draw (a) -- (b) -- (c) -- (d) -- (e) -- (f) -- (g) -- (h) -- (i) -- (j) -- (k) -- (l);
\end{tikzpicture}
\end{center}

3. A. Nayar\textsuperscript{5} \textit{et al.} have studied QSAR and designed ring substituted -2/4-quinolene carbaldehyde derivatives by short, convenient and high yielding protocol.

\begin{center}
\begin{tikzpicture}

\node (a) at (0,0) {\text{N}};
\node (b) at (0,-1) {\text{O}};
\node (c) at (2,0) {\text{NH}_2\text{NHR}};
\node (d) at (2,-1) {\text{abs.} \text{EtOH}};
\node (e) at (4,0) {\text{N}};
\node (f) at (4,-1) {\text{N}-\text{NH-R}};

\draw (a) -- (b) -- (c) -- (d) -- (e) -- (f);
\end{tikzpicture}
\end{center}

4. R. Somani\textsuperscript{6} \textit{et al.} have synthesized Schiff bases having benzimidazole by green chemistry approach using microwave.

\begin{center}
\begin{tikzpicture}

\node (a) at (0,0) {\text{N}};
\node (b) at (0,-1) {\text{CH}_3};
\node (c) at (2,0) {\text{O}};
\node (d) at (2,-1) {\text{NH}};
\node (e) at (3,-1) {\text{NH}_2};
\node (f) at (4,0) {\text{N}};
\node (g) at (4,-1) {\text{CH}_3};
\node (h) at (6,0) {\text{N}};
\node (i) at (6,-1) {\text{N}};
\node (j) at (8,0) {\text{R-CHO}};
\node (k) at (8,-1) {\text{R}};

\draw (a) -- (b) -- (c) -- (d) -- (e) -- (f) -- (g) -- (h) -- (i) -- (j) -- (k);
\end{tikzpicture}
\end{center}

5. A. K. Chakrabarti\textsuperscript{7} \textit{et al.} have synthesized imines and phenyl hydrazones in high yield by using magnesium perchlorate as efficient catalyst.

\begin{center}
\begin{tikzpicture}

\node (a) at (0,0) {\text{R}_1\text{O}};
\node (b) at (1,0) {\text{R}_2\text{O}};
\node (c) at (2,0) {\text{R}_3\text{NH}_2};
\node (d) at (2,0) {\text{Mg(ClO}_4)_2};
\node (e) at (2,-1) {\text{5mol\%}};
\node (f) at (3,0) {\text{R}_1\text{N-R}_3};

\draw (a) -- (b) -- (c) -- (d) -- (e) -- (f);
\end{tikzpicture}
\end{center}

6. K. Guzen\textsuperscript{8} \textit{et al.} have carried out ecofriendly synthesis of imines by ultra sound irradiation.
H. Naeimi\textsuperscript{9} et al. have carried out mild and convenient one pot synthesis of Schiff bases in the presence of P\textsubscript{2}O\textsubscript{5}/Al\textsubscript{2}O\textsubscript{3} catalyst under solvent free conditions. Gopalkrishnan\textsuperscript{10} et al. have synthesized imines by using silicagel supported sodium hydrogen phosphate as a catalyst by microwave irradiation.

**THERAPEUTIC ASPECT**

Schiff bases, derived mostly from variety of heterocyclic rings, were reported to possess a broad spectrum and a wide variety of biological activities, such as:

1. Antimicrobial\textsuperscript{11}
2. Antibacterial\textsuperscript{12,13}
3. Anticancer\textsuperscript{14}
4. Antiviral\textsuperscript{15}
5. Anticonvulsant\textsuperscript{16}
6. FabH inhibitors\textsuperscript{17}
7. Cytotoxic activity\textsuperscript{18}
8. Anti proliferatives\textsuperscript{19}
9. Anti malerial\textsuperscript{20}

1. Pandeya\textsuperscript{21} and coworkers have reported isatin derived Schiff base as potent antibacterial agent.

2. M.J. Hearn\textsuperscript{22} et al. have designed and synthesized Schiff bases of isoniazide as antitubercular agents.
3. Sriram\textsuperscript{23} and coworkers reported the synthesis of abacavir derived Schiff bases as antiviral agents.

![Schiff base structure](image1)

4. P. Rathecot\textsuperscript{24} \textit{et al.} have synthesized novel 5-nitro isoquinolines as antimalarial agents.

![Nitro isoquinoline structure](image2)

5. M. S. Karthikeyan\textsuperscript{25} \textit{et al.} have synthesized Schiff bases as antiinflammatory agents.

![Schiff base structure](image3)

6. K. M. Thaker\textsuperscript{26} \textit{et al.} and S.L. Vasoya\textsuperscript{27} \textit{et al.} have reported facile synthesis of new Schiff bases possessing benzo[b] thiophene as potent biologically active agents.

![Schiff base structures](image4)

K. N. Venugopal\textsuperscript{28} \textit{et al.} have synthesized Schiff bases of 4-hydroxy-6-carboxyhydrazino benzothiophene analog with different substituted aldehydes and determined pharmacological study. Ergenc\textsuperscript{29} and coworkers have synthesized
azomethine derivatives having antifungal activity. B. Yadav and S. S. Sangapure\textsuperscript{30} have synthesized some azomethines and tested for their biological activity. B. S. Holla\textsuperscript{31} \textit{et al.} have prepared some new Schiff bases having anticancer activity.

The design and synthesis of hybrid molecules encompassing two pharmacophores in one molecular scaffold is a well-established approach to the synthesis of more potent drugs with dual activity. With this aspect, 1, 2, 4-triazoles possessing pyrazine and Schiff base found as a promising target for the current research project.

Thus the important role displayed by the Schiff base derivatives for various therapeutic and biological activities prompted us to synthesize some Schiff bases derivatives bearing pyrazine moiety in order to achieve compounds having better therapeutic activities described as in the following.

\textbf{SECTION-I: SYNTHESIS AND ANTITUBERCULAR EVALUATION OF (E)-4-(SUBSTITUTEDBENZYLIDENEAMINO)-5-(PYRAZIN-2-YL)-4H-1,2,4-TRIAZOLE-3-TIOLS.}
SECTION-I: SYNTHESIS AND ANTITUBERCULAR EVALUATION OF (E)-4-(SUBSTITUTEDBENZYLIDENEAMINO)-5-(PYRAZIN-2-YL)-4H-1,2,4-TRIAZOLE-3-TIOLS.

Schiff bases constitute an important class of biologically active drug molecules which has attracted attention of medicinal chemists due to their wide range of pharmacological properties. These compounds are being synthesized as drugs by many researchers in order to combat diseases with minimal toxicity and maximal effects. These predictions has provided therapeutic pathway to develop new effective biologically active Schiff base derivatives.

Reaction scheme

Where R= 4-OCH₃, 4-Cl, 3-Br, 2,5-(OCH₃)₂, 3,4,5-(OCH₃)₃ etc.
Plausible mechanism
EXPERIMENTAL SECTION

[A] Synthesis of methyl pyrazine-2-carboxylate (2)

To a stirred solution of pyrazine 2-carboxylic acid (1) (10 mmol) in methanol concentrated H$_2$SO$_4$ (1 mmol) was added at RT. The reaction mixture was allowed to reflux for 10 hours. Completion of the reaction was monitored by TLC. After completion of the reaction, solvent was removed in vacuo and resulting mass was poured into water. The aqueous layer was extracted with ethyl acetate. The organic layer was neutralized with saturated NaHCO$_3$ solution. The organic layer was dried over sodium sulphate and evaporated under reduced pressure to give methyl pyrazine 2-carboxylate (2) in sufficient purity. Yield: 80%

[B] Synthesis of pyrazine-2-carbohydrazide

To a stirred solution of methyl pyrazine 2-carboxylate (2) in absolute ethanol hydrazine hydrate (99%) was added at (0-5) °C temp and reaction mixture was allowed to stir for 30 mins. Completion of the reaction was monitored by TLC. After the completion of reaction solid residue was filtered, washed with cold ethanol and dried to afford pyrazine-2-carbohydrazide (3). Yield: 85%

[C] Synthesis of potassium-2-(pyrazine-2-carbonyl)hydrazinecarbodithioate (4)

Potassium hydroxide (15 mmol) was dissolved in absolute ethanol (10V/V). To the above solution, pyrazine-2-carbohydrazide (3) (10 mmol) was added and cooled the solution in ice. To this, carbon disulfide (15 mmol) was added in small portions with constant stirring. The reaction mixture was agitated continuously for a period of 12 hours. It was then diluted with anhydrous ether. The precipitated potassium dithiocarbazinate (4) was collected by filtration. The precipitate was further washed with anhydrous ether and dried under vacuum. The potassium salt thus obtained was in quantitative yield and was used in the next step without further purification. Yield: 88%

[D] Synthesis of 4-amino-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol (5)
A suspension of potassium dithiocarbazinate (4), (10mmol) in water (5v/v) and hydrazine hydrate (30 mmol) was refluxed for 6–7 hours with occasional shaking. The color of the reaction mixture changed to green with the evolution of hydrogen sulfide. The completion of the reaction was monitored by TLC. The reaction mixture was cooled to RT and diluted with water. On acidification with concentrated hydrochloric acid, the required triazole was precipitated. It was filtered, washed thoroughly with cold water and recrystallized from ethanol to give compound (5). Yield: 82%

**[E] Synthesis of (E)-4-(substitutedbenzylideneamino)-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiols. 7(a-j)**

To a stirred solution of 4-amino-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol(5) (10 mmol) in methanol (10 v/v) various aromatic aldehydes 6(a-j)(10 mmol) were added. After addition of Catalytic amount of con.HCL, the reaction mixture was allowed to stir for 2 hours. The completion of reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured on to ice, precipitates were filtered, dried and recrystallized from ethanol to give Schiff bases in analytical pure form. Physical constants of newly synthesized 4-(substitutedbenzylideneamino)-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiols 7a-7j are recorded in Table I
### TABLE I: PHYSICAL CONSTANTS OF (E)-4-(SUBSTITUTEDBENZYLIDENEAMINO)-5-(PYRAZIN-2-YL)-4H-1,2,4-TRIAZOLE-3-THIOLS.7(a-j)

- **Structure:** ![Structure](image)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Substitution R</th>
<th>M. F.</th>
<th>M. W.</th>
<th>Melting range (°C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>3-Cl</td>
<td>C₁₃H₉ClN₆S</td>
<td>316.76</td>
<td>238-240</td>
<td>90</td>
</tr>
<tr>
<td>7b</td>
<td>4-Cl</td>
<td>C₁₃H₉ClN₆S</td>
<td>316.76</td>
<td>225-227</td>
<td>89</td>
</tr>
<tr>
<td>7c</td>
<td>3-Br</td>
<td>C₁₃H₉BrN₆S</td>
<td>361.2</td>
<td>192-194</td>
<td>91</td>
</tr>
<tr>
<td>7d</td>
<td>2,5-(OCH₃)₂</td>
<td>C₁₅H₁₄N₆O₂S</td>
<td>342.37</td>
<td>200-202</td>
<td>88</td>
</tr>
<tr>
<td>7e</td>
<td>3,4,5-(OCH₃)₃</td>
<td>C₁₆H₁₆N₆O₃S</td>
<td>372.40</td>
<td>189-191</td>
<td>92</td>
</tr>
<tr>
<td>7f</td>
<td>4-F</td>
<td>C₁₃H₉FN₆S</td>
<td>300.31</td>
<td>179-181</td>
<td>93</td>
</tr>
<tr>
<td>7g</td>
<td>3,4-(OCH₃)₂</td>
<td>C₁₅H₁₄N₆O₂S</td>
<td>342.37</td>
<td>194-196</td>
<td>90</td>
</tr>
<tr>
<td>7h</td>
<td>4-NO₂</td>
<td>C₁₃H₉N₇O₂S</td>
<td>327.32</td>
<td>279-281</td>
<td>90</td>
</tr>
<tr>
<td>7i</td>
<td>2-OCH₃</td>
<td>C₁₄H₁₂N₆OS</td>
<td>312.34</td>
<td>210-212</td>
<td>87</td>
</tr>
<tr>
<td>7j</td>
<td>2-Cl</td>
<td>C₁₃H₉ClN₆S</td>
<td>316.76</td>
<td>185-187</td>
<td>82</td>
</tr>
</tbody>
</table>
ANALYTICAL DATA

(E)-4-((3-chlorobenzylidene)amino)-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol (7a)
IR (ν max cm⁻¹, KBr): 3023 (C-H str.), 2988(Alkane-C-H str.), 2521(-S-H str.) 1590(C=N str.), 1533(C=C str.), 1361 (C-N str.), 1285(=C-N bend.), 831(C-Cl str.), 690(1,3-di substituted). El⁺ m/z: 316.76 Anal. Calcd for C₁₃H₉ClN₆S, C, 49.23%; H, 2.86%; Cl, 11.19%; N, 26.53%; S, 10.12%.

(E)-4-((4-chlorobenzylidene)amino)-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol (7b) IR (ν max cm⁻¹, KBr): 3034(C-H str.), 2963(Alkane-C-H str.), 2499(-S-H str.) 1610(C=N str.), 1545(C=C str.), 1361 (C-N str.), 1268(=C-N bend.), 808(C-Cl str.) 840(1,4-di substitution). El⁺ m/z: 316.76 Anal. Calcd for C₁₃H₉ClN₆S, C, 49.23%; H, 2.86%; Cl, 11.19%; N, 26.53%; S, 10.12%.

(E)-4-((3-bromobenzylidene)amino)-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol (7c) IR (ν max cm⁻¹, KBr): 3112 (C-H str.), 2989(Alkane-C-H str.), 2358(-S-H str.) 1600(C=N str.), 1541(C=C str.), 1490 (C-N str.), 1375(=C-N bend.), 701(1,3-di substituted). El⁺ m/z: 361.21 Anal. Calcd for C₁₃H₉BrN₆S, C, 43.23%; H, 2.51%; Br, 22.12%; N, 23.27%; S, 10.89%.

(E)-4-((2,5-dimethoxybenzylidene)amino)-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol (7d) IR (ν max cm⁻¹, KBr): 3008 (C-H str.), 2973 (C-H str.), 2461(-S-H str.) 1605 (C=N str.), 1600 (Alkene C=C str.), 1465(C-H str.), 1410(C-H bend.), 1016(C-O-C str.) El⁺ m/z: 342.37 Anal. Calcd for C₁₃H₁₄N₆O₂S, C, 52.62%; H, 4.12%; N, 24.55%; O, 9.35%; S, 10.89%.

(E)-5-(pyrazin-2-yl)-4-((3,4,5-trimethoxybenzylidene)amino)-4H-1,2,4-triazole-3-thiol (7e) IR (ν max cm⁻¹, KBr): 3012 (C-H str.), 2947(Alkane-C-H str.), 2538(-S-H str.) 1620(C=N str.), 1575(C=C str.), 1359 (C-N str.), 1275(=C-N bend.), 1014(C-O-C str.), 821(sp²C-H str.). ¹H NMR 400 MHz (DMSO-d₆): 14.52(1H, s (-SH)) 9.47 (1H,s), 9.17(1H, s), 8.80(2H,s), 7.21(2H, s), 3.82(6H,s), 3.75(3H, s). ¹³C NMR (100 MHz, CDCl₃): 166.99 (C, ), 150.3(C, triazole ring), 145.1(C, triazole ring), 144.8(CH, pyrazine ring), 144.4(CH,pyrazine ring), 143.9(C, pyrazine ring), 141.8(CH, pyrazine ring), 133.1(C, phenyl ring), 132.3(CH, phenyl ring), 129.2(CH, phenyl ring), 127.5(CH,phenyl ring), 23.1(CH₂). El⁺ m/z: 372.40 Anal. Calcd for C₁₉H₁₆N₆O₃S, C, 51.60%; H, 4.33%; N, 22.57%; O, 12.89%; S, 10.89%.

(E)-4-((4-fluorobenzylidene)amino)-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol (7f) IR (ν max cm⁻¹, KBr): 3093 (C-H str.), 2952 (C-H str.), 2480(-S-H str.) 1600 (C=N str.),
1595 (Alkene C=C str.), 1465 (C-H str.), 1349 (C-N str.), 1289 (=C-N bend.), 820 (1,4-di substituted). EI+ m/z: 300.40 Anal. Calcd for C_{13}H_{9}FN_{6}S; C, 51.99%; H, 3.02%; F, 6.33%; N, 22.57%; S, 10.89%.

(E)-4-((3,4-dimethoxybenzylidene)amino)-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol(7g)

IR (ν\text{max} cm\text{^{-1}}, \text{KBr}): 3008 (C-H str.), 2973 (C-H str.), 2461(-S-H str.) 1605 (C=N str.), 1600 (Alkene C=C str.), 1465(C-H str.), 1410(C-H bend.), 1016(C-O-C str.). ^1H NMR 400 MHz (DMSO-d$_6$): 14.49(1H, s (-SH)) 9.38 (1H,s(pyrarazine)), 9.17(1H, s(N=CH)), 8.80-8.79(2H,d(Pyrarazine)), 7.46-7.44(2H, dd(phenyl ring)),7.14-7.12(1H,d(phenyl ring)) 3.82(6H,s(OMe)), 3.75(3H, s(OMe)). ^13C NMR (100 MHz, CDCl$_3$): 167.45(C, thiadiazine ring),163.09(C=N(schiff base)), 152.93(C=Nr.), 129.1(6H, s(OMe)), 128.9(1,4-O str. asymm), 111.5(CH, phenyl ring), 109.5(C, phenyl ring), 108.7(1H, dd(phenyl ring)) 14.49(1H, s (N=CH)).

Anal. Calcd for C_{13}H_{14}N_{6}O_{2}S; C, 52.62%; H, 4.12%; N, 24.55%; O, 9.35%; S, 10.89%.

(E)-4-((4-nitrobenzylidene)amino)-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol(7h)

IR (ν\text{max} cm\text{^{-1}}, \text{KBr}): 3064 (C-H str.), 2930 (C-H str.), 2493(-S-H str.) 1621 (C=N str.), 1555(N-O str. asymm)1537 (Alkene C=C str.), 1433(C-H bend.), 1310(N-O str.symm), 838(1,4-di substituted). EI+ m/z: 357.32 Anal. Calcd for C_{13}H_{9}N_{5}O_{2}S; C, 47.70%; H, 2.77%; N, 29.95%; O, 9.78%; S, 9.80%.

(E)-4-((2-methoxybenzylidene)amino)-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol(7i)

IR (ν\text{max} cm\text{^{-1}}, \text{KBr}): 3064 (C-H str.), 2930 (C-H str.), 2493(-S-H str.) 1621 (C=N str.), 1585 (Alkene C=C str.), 1405(C-H str.), 1421(C-H bend.), 1006(C-O-C str.). EI+ m/z: 312.34 Anal. Calcd for C_{14}H_{12}N_{6}O_{2}S; C, 53.83%; H, 3.87%; N, 26.91%; O, 5.12%; S, 10.27%.

(E)-4-((2-chlorobenzylidene)amino)-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol(7j)

IR (ν\text{max} cm\text{^{-1}}, \text{KBr}): 3068 (C-H str.), 2913(Alkene-C-H str.), 2521(-S-H str.) 1640(C=N str.), 1588(C=C str.), 1358 (C-N str.), 1291(=C-N bend.), 841(C-Cl str.), 770(1,2-di substituted) . EI+ m/z: 316.76 Anal. Calcd for C_{13}H_{9}ClN_{6}S; C, 49.23%; H, 2.86%; Cl, 11.19%; N, 26.53%; S, 10.12%.
IR spectrum of (E)-5-(pyrazin-2-yl)-4-((3,4,5-trimethoxybenzylidene)amino)-4H-1,2,4-triazole-3-thiol (7e)

IR spectrum of (E)-4-((3,4-dimethoxybenzylidene)amino)-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol (7g).
Mass spectrum of (E)-5-(pyrazin-2-yl)-4-((3,4,5-trimethoxybenzylidene)amino)-4H-1,2,4-triazole-3-thiol (7e)

Mass spectrum of (E)-4-((3,4-dimethoxybenzylidene)amino)-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol (7g).
\[ ^1H \text{NMR spectrum of (E)-5-(pyrazin-2-yl)-4-((3,4,5-trimethoxybenzylidene)amino)-4H-1,2,4-triazole-3-thiol (7e)} \]

![H NMR spectrum of (E)-5-(pyrazin-2-yl)-4-((3,4,5-trimethoxybenzylidene)amino)-4H-1,2,4-triazole-3-thiol (7e).](image1)

\[ ^1H \text{NMR spectrum of (E)-4-((3,4-dimethoxybenzylidene)amino)-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol (7g).} \]

![H NMR spectrum of (E)-4-((3,4-dimethoxybenzylidene)amino)-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol (7g).](image2)
$^{13}$C NMR spectrum of (E)-5-(pyrazin-2-yl)-4-((3,4,5-trimethoxybenzylidene)amino)-4H-1,2,4-triazole-3-thiol (7e)

$^{13}$C NMR spectrum of (E)-4-((3,4-dimethoxybenzylidene)amino)-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol (7g).
ANTITUBERCULAR ACTIVITY OF 6 (E)-(SUBSTITUTEDBENZYLIDENEAMINO)-5-(PYRAZIN-2-YL)-4H-1,2,4-TRIAZOLE-3-THIOLS.

All the synthesized compounds were evaluated for antitubercular activity against *M. Tuberculosis H*₃⁷*Ra* in dormant and active stage at µg/ml concentration using XTT reduction menadione (XRMA) method. Isoniazide and Rifampicin were used as standard drug for the comparison of activity. Antitubercular activity was carried out by Dr. Dhiman Sarkar, Scientist, National Chemical Laboratory, Pune.

Protocol for the antitubercular activity

*Mycobacterium tuberculosis H*₃⁷*Ra* (ATCC 25177) were grown to logarithmic phase (O.D. 595~1.0) in a defined medium (M.Pheili medium). The stock culture was maintained at -70°C and sub-cultured once in M. Pheli medium before inoculation into experimental culture. Isoniazide and Rifampicin were taken as the standard drugs. Drugs were solubilized in dimethyl sulfoxide (DMSO) and stored in aliquots at -20°C XTT sodium salt powder (sigma) was prepared as a 1.25 mM stock solution in sterile Ix PBS and used immediately. Menadione (Sigma) was always freshly prepared as 6 mM solution in DMSO before use. Compounds were screened for their inhibitory effect on MTB by following XTT reduction Menadione assay (XRMA) protocol published earlier. Briefly, 2.5 µl of these inhibitor solutions were added in a total volume of 250 µl of M.pheli medium consisting of Ix 106 bacilli. The incubation was terminated on the 8th day for MTB cultures, The XRMA was then carried out to estimate viable cells present in different wells of the assay Plate. For that, in all wells of assay plate 200µM XTT was added as a final concentration and incubated at 37°C for 20 minutes. Then 60µM Menadione was added as a final concentration and incubated at 37°C for 40 minutes. The optical density was read on a micro plate reader (Spectramax plus 384 plate reader, Molecular Devices Inc.) at 490nm filter against a blank prepared from cell-free wells. Absorbance given by cells treated with the vehicle alone was taken as a 100% cell growth. All experiments were performed in triplicates and the quantitative value was expressed as the percentage average of inhibition. Results of the anti-tubercular activity are shown in Table II.
TABLE II: ANTITUBERCULAR ACTIVITY OF 6 (E)-4-(SUBSTITUTEDBENZYLIDENEAMINO)-5-(PYRAZIN-2-YL)-4H-1,2,4-TRAIAZOLE-3-ThIOLS. 7(a-j)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>R</th>
<th>% inhibition at 30 (μg/ml)</th>
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<tbody>
<tr>
<td>7a</td>
<td>3-Cl</td>
<td>95.8</td>
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<tr>
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<td>2,5-(OCH$_3$)$_2$</td>
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<td>7e</td>
<td>3,4,5-(OCH$_3$)$_3$</td>
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<tr>
<td>7f</td>
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<td>7g</td>
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<td>7j</td>
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Graphs for the determination of MIC and IC$_{50}$

**IC$_{50}$**: The concentration of sample required to inhibit 50% of the cell proliferation

**MIC**: The concentration of sample required to inhibit 90% of the cell proliferation
RESULT AND DISCUSSION

As part of our efforts on the development of new route for the preparation of biologically active molecules and considering the important biological properties of Schiff base herein, we described an efficient and simple synthesis of the nuclei. All the synthesized compounds were evaluated for antitubercular activity against *M. Tuberculosis* *H₃₇Ra* in dormant and active stage at 30µg/ml concentration using XTT reduction menadione (XRMA) method. Isoniazide and Rifampicin were used as standard drug for the comparison of activity.

In the present study a series of Schiff base of 1,2,4-triazole 3-thiol possessing pyrazine nucleus 7a-7j have been synthesized by 4-amino-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol, substituted benzaldehyde in the presence of acid. The structures of all new synthesized compounds were established by ¹H, ¹³CNMR, FT-IR and mass spectroscopy. The mass spectrum of 7e displayed the molecular ion peak (M⁺) peak at m/z 372, which was consistent with the product structure. The ¹H NMR spectrum of 7e exhibited singlet of proton at δ 14.52 which indicated the presence of –SH group. Another singlet of one proton at δ 9.17 confirms the formation of Schiff base (-N=C-H) proton. Two singlets of 6 protons and 3 protons at δ= 3.85 and 3.75 are of three mehoxy groups. The ¹³C NMR spectrum of 7e showed C-S peak at δ 166.99 ppm and N=C-H peak at δ 163.05 ppm are the characteristics peaks for the compound. C-O peak at δ 153.24 and peak at δ 56.01 ppm indicates the presence of methoxy group. The IR spectrum of 7e exhibited characteristic absorption band at 2538 cm⁻¹ for (-SH linkage) and all other general frequency band are in good agreement with the structure.

From the results of antitubercular activity, data reveals that all synthesized compounds shows high to moderate percentage of inhibition at 30µg/ml against *Mycobacterium Tuberculosis H₃₇Ra*.

Compounds 7a, 7e, 7f and 7h are showing highest percentage of inhibition 95.8, 102.5, 92.5and 92.0 respectively. Compounds 7b, 7c, 7i, 7j are moderately active and showed percentage of inhibition at 30µg/ml are 76.0, 73.8, 64.4 and 77.0 respectively.
From the results compounds 7d and 7g are not showing significant percentage of inhibition at 30µg/ml against *Mycobacterium Tuberculosis H37Ra*.

3-Cl, 4-F and 4-NO₂ substituted molecules are showing more activity. These three molecules are also selected for the studies of MIC and IC50 against *Mycobacterium Tuberculosis H37Ra* using various dilutions at dormant stage.

Dose dependent inhibition was performed at the concentration of 100, 50, 25.5, 12.5, 6.25, 3.125, 1.5625 and 0.7813 (µg/ml) to determine IC50 and MIC. Rifampicin and isoniazide were used as the standard. MIC and IC50 values of rifampicin and isoniazide are 0.0014, 0.0043 and 0.00023 and 0.075 respectively.

From the graph of compound 7h, MIC And IC50 values are 5.86 and 12.13µg/ml respectively. MIC And IC50 values for compound 7a are 9.28 and 95.31µg/ml and for compound 7f the values are 9 and 23.88 respectively.

From the results we can say that MIC and IC50 values of compound 7h are most prominent than that of compounds 7a and 7f, but there is less significance if we consider the MIC and IC50 values of standard drugs which are currently used for the treatment in TB.

As these three compounds showed better inhibition at 30 µg/ml against *M. Tuberculosis H37Ra*, Cytotoxicity studies of these compounds 7e, 7a and 7f were also done against THP-1(*Human acute monocytic leukemia cell line*), A549 (*Human lung adenocarcinoma epithelial cell line*), PANC 1 (*Human pancreas carcinoma cell line*) and MCF7 (*Human mammary gland/breast epithelial carcinoma cell line*) but no significant results obtained. But with the help of this studies and results of SAR, QSAR and target based drug designing; some structural modifications can be done and those new molecule may come out as a more potent molecules and show some significant activity against *Mycobacterium Tuberculosis*. 
REFERENCES