CHAPTER-2

STUDIES ON HYDRAZIDE-HYDRAZONE DERIVATIVES
PART-A

CHAPTER-2

SECTION-I

Synthesis and biological evaluation of (E)-N'-(E)-3-(4-chlorophenyl)-1-substitutedphenyl allylidene) pyrazine-2-carbohydrazides
INTRODUCTION

Hydrazones are characterized by the presence of the triatomic group > C=N-N <. Many of the physiologically active compounds find applications in the field of medicinal and physical chemistry. Hydrazones contain two connected nitrogen atoms of different nature and a C=N double bond that is conjugated with a lone electron pair of the terminal nitrogen atom. Both nitrogen atoms of the hydrazone group are nucleophilic. The carbon atom of hydrazone group has both electrophilic and nucleophilic character.

\[
R-\text{HC} = \text{N} = \text{NH} \quad R'
\]

Hydrazine-hydrazones and their derivatives are a versatile class of compounds in organic and physical chemistry. These compounds possess diversifying biological properties. Hydrazones are important class of compounds for drug design, as possible ligands for metal complexes, in organo-catalysis and also for the synthesis of heterocyclic compounds.

SYNTHETIC ASPECT

Various methods for the preparation of hydrazine-hydrazone have been cited in literature, some of them are as under.

1. D. Sriram et al. have synthesized hydrazine-hydrazone derivatives containing Isoniazide as pharmaco phore.

\[
\text{Acetic acid} \quad \text{Et.OH}
\]

2. P. Vicini et al. have studied and synthesized hydrazones of 1,2-benzisothiazole by QSAR.
3. K. Bedia\textsuperscript{5} and co-workers have synthesized and characterized novel hydrazine-hydrazone derivatives based on a series of 4-substituted benzoic acid.

4. N. P. Belskya\textsuperscript{2} \textit{et al.} have synthesized hydrazones bearing amide, thioamide and amidine functional groups.

5. S. G. Kucukguzel\textsuperscript{6} \textit{et al.} have synthesized several diflunisal hydrazide-hydrazone derivatives namely 2',4'-difluoro-4-methoxy biphenyl-3-carboxylic acid.

6. R. Maccari\textsuperscript{7} \textit{et al.} synthesized isoniazide related hydrazones and screened them as antimycobacterial agent.
THERAPEUTIC ASPECT

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

1. Antibacterial
2. Anticonvulsant
3. Antiinflammatory
4. Anti Malarial
5. Antitubercular
6. Antitumour
7. Antidepressant
8. Antimicrobial
9. Anticonvulsant
10. Antiinflammatory
11. Antitumour
12. Antidepressant
13. Antimicrobial
14. Anticonvulsant

1. N. Demibars\textsuperscript{15} \textit{et al.} synthesized new hydrazide-hyrazones containing 5-oxo-[1,2,4]triazole ring as antitumor agents against breast cancer.

\begin{align*}
\text{N-} & \text{N} \\
\text{NH} & \text{NH} \\
\text{CH} & \text{Ph} \\
\text{O} & \text{O}
\end{align*}

2. S. K. Sridhar\textsuperscript{16} \textit{et al.} have synthesized hydrazones of isatin as anticonvulsant agents.

\begin{align*}
\text{R'} & \text{N-R}_2 \\
\text{R}_1 & \text{N-R}_2 \\
\text{O} & \text{O}
\end{align*}

3. B. Chautal\textsuperscript{17} \textit{et al.} have reported Hydrazone derivatives with antiinflammatory activity.

\begin{align*}
\text{N} & \text{S} \\
\text{NH} & \text{NH} \\
\text{CH}_3 & \text{CH}_3 \\
\text{S} & \text{S}
\end{align*}

4. M. Malhotra\textsuperscript{18} \textit{et al.} have synthesized Mannich bases containing isoniazide 2-propoxybenzylidine isoniconohydrazide and screened for their potential antimicrobial activity.

\begin{align*}
\text{N} & \text{N} \\
\text{NH} & \text{NH} \\
\text{CH} & \text{Ph} \\
\text{O} & \text{O}
\end{align*}
5. S. Gamma\textsuperscript{19} \textit{et al.} have synthesized and evaluated hydrazones of N'-arylidine-N\textsuperscript{2}-quinolyl and N\textsuperscript{2}-acrydinyln hydrazones as antiplasmodial activity.

\[ \text{NH-N} \equiv \text{CH} \quad \text{Ar} \]

6. P. C. Lama\textsuperscript{20} \textit{et al.} have reported a new series of compounds N-acryl hydrazones as analgesic agents.

\[ \text{O} \quad \text{NH-N} \equiv \text{CH} \quad \text{Ar} \]

7. A. G. Silva\textsuperscript{21} \textit{et al.} reported bioactive compound of the N-acyl hydrazine class of 3,4-methylene dioxybenzoyl-2-thienyl hydrazones as vasodialatory activities.

\[ \text{O} \quad \text{NH-N} \equiv \text{CH} \quad \text{Ar} \]

The easy preparation, versatile biological activity and tendency toward crystallinity are all looked for features of hydrazones. Due to these special features, hydrazones have been under study for a long time. Thus the important role displayed by hydrazide-hydrazone inspired us to synthesize Hydrazones of pyrazine 2-carbohydrazide in order to achieve compounds having better therapeutic activities described as the following.

\textbf{SECTION-I: SYNTHESIS AND BIOLOGICAL EVALUATION OF (E)-N'-(\textbf{(E)}-3-(4-CHLOROPHENYL)-1-SUBSTITUTEDPHENYL ALLYLIDENE) PYRAZINE-2-CARBOHYDRAZIDES}

\textbf{SECTION II: X-RAY CRYSTALLOGRAPHIC STUDY OF (E)-N'-(\textbf{(E)}-1-(4-BROMOPHENYL)-3-(4CHLOROPHENYL)ALLYLIDENE) PYRAZINE-2-CARBOHYDRAZIDE}
SECTION-I: SYNTHESIS AND BIOLOGICAL EVALUATION OF (E)-
N’-((E)-3-(4-CHLOROPHENYL)-1-SUBSTITUTED
PHENYLALLYLIDENE)PYRAZINE-2-
CARBOHYDRAZIDES

Hydrazide-hydrazones have been attracted prevalent attention due to their diverse pharmacological properties. Looking to this, the synthesis of hydrazones has been undertaken by the condensation of pyrazine 2-carbohydrazides with various chalcones in the presence of acetic acid.

**Reaction scheme**

Where \( R = H, 3,4-O(CH_3)_2, 4-Br, 4-Me, 4-Cl \) etc..
EXPERIMENTAL SECTION

[A] **Synthesis of Methyl pyrazine-2-carboxylate (2)**
As per Part-A, Chapter-1(Section-I) page no.26

[B] **Synthesis of Pyrazine-2-carbohydrazide (3)**
As per Part-A, Chapter-1(Section-I) page no.26

[C] **Synthesis of Chalcones 6(a-j)**

4-Chloro benzaldehyde (4) and various substituted acetophenones 5(a-j) in
the presence of ethanolic solution of sodium hydroxide yielded chalcones 6(a-j) as
per literature procedure\(^2\).

[D] **Synthesis of (E)-N'-(E)-3-(4-chlorophenyl)-1-substitutedphenyl allylidene)pyrazine-2-carbohydrazides (7(a-j))**

To a stirred solution of chalcones 6(a-j) in ethanol, pyrazine 2-
carbohydrazide (3) and glacial acetic acid was added as solvent. The resulting
reaction mass was refluxed in oil bath for 24-48 hours. After completion of the
reaction on TLC, reaction mass poured onto crushed ice and precipitates were
filtered off, washed with water, dried and crystalized from appropriate solvent to
give analytically pure (E)-N'-(E)-3-(4-chlorophenyl)-1-
substitutedphenylallylidene)pyrazine-2-carbohydrazides. Physical
constants of newly synthesized (E)-N'-(E)-3-(4-chlorophenyl)-1-
substitutedphenylallylidene)pyrazine-2-carbohydrazides 7(a-j) are
recorded in Table I.
TABLE I: PHYSICAL CONSTANTS OF (E)-N’-((E)-3-(4-CHLOROPHENYL)-1-SUBSTITUTEDPHENYL ALLYLIDENE) PYRAZINE-2-CARBOHYDRAZIDES

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Substitution R</th>
<th>M. F.</th>
<th>Melting range(°C)</th>
<th>M. W.</th>
<th>Yield (%)</th>
</tr>
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<tbody>
<tr>
<td>7a</td>
<td>4-F</td>
<td>C_{20}H_{14}ClFN_4O</td>
<td>135-137°C</td>
<td>380.80</td>
<td>68</td>
</tr>
<tr>
<td>7b</td>
<td>4-Cl</td>
<td>C_{20}H_{14}Cl_2N_4O</td>
<td>148-150°C</td>
<td>397.25</td>
<td>69</td>
</tr>
<tr>
<td>7c</td>
<td>4-Me</td>
<td>C_{21}H_{17}CIN_4O</td>
<td>151-153°C</td>
<td>376.83</td>
<td>65</td>
</tr>
<tr>
<td>7d</td>
<td>3,4-diCl</td>
<td>C_{20}H_{13}Cl_3N_4O</td>
<td>124-126°C</td>
<td>431.70</td>
<td>70</td>
</tr>
<tr>
<td>7e</td>
<td>4-NO_2</td>
<td>C_{20}H_{14}CIN_5O_3</td>
<td>158-160°C</td>
<td>407.80</td>
<td>62</td>
</tr>
<tr>
<td>7f</td>
<td>2,4-diCl</td>
<td>C_{20}H_{13}Cl_3N_4O</td>
<td>145-147°C</td>
<td>431.70</td>
<td>72</td>
</tr>
<tr>
<td>7g</td>
<td>3,4-(OCH_3)_2</td>
<td>C_{22}H_{19}CIN_4O_3</td>
<td>126-128°C</td>
<td>422.86</td>
<td>68</td>
</tr>
<tr>
<td>7h</td>
<td>4-Br</td>
<td>C_{20}H_{14}BrCIN_4O</td>
<td>153-155°C</td>
<td>440.00</td>
<td>70</td>
</tr>
<tr>
<td>7i</td>
<td>H</td>
<td>C_{20}H_{15}CIN_4O</td>
<td>145-147°C</td>
<td>261.29</td>
<td>75</td>
</tr>
<tr>
<td>7j</td>
<td>3-Br</td>
<td>C_{20}H_{14}BrCIN_4O</td>
<td>149-151°C</td>
<td>440.00</td>
<td>65</td>
</tr>
</tbody>
</table>
ANALYTICAL DATA

(E)-N\textsuperscript{2}-(E)-3-(4-chlorophenyl)-1-(4-fluorophenyl)allylidene)pyrazine-2-carbohydrazide(7a) IR (ν\textsubscript{max} cm\textsuperscript{-1}, KBr): 3322(-NH str.), 3021 (Ar-C-H str.), 2987 (C-H str.), 1685(C=O str.), 1624 (C=N str.), 1562(-NH bend.) 1484 (C=C str.), 1434(C-H bend.), 816(para substituted), 771(C-Cl str.) EI\textsuperscript{+} m/z: 380.80 Anal. Calcd for C\textsubscript{20}H\textsubscript{14}ClFN\textsubscript{4}O.C, 63.08%; H, 3.71%; Cl, 9.31%; F, 4.99%; N, 14.71%; O, 4.20%.

(E)-N\textsuperscript{2}-(E)-1,3-bis(4-chlorophenyl)allylidene)pyrazine-2-carbohydrazide(7b) IR (ν\textsubscript{max} cm\textsuperscript{-1}, KBr): 3303(-NH str.), 3073 (Ar-C-H str.), 1679(C=O str.), 1633 (C=N str.), 1560(-NH bend.) 1482 (C=C str.), 1413(C-H bend.), 821(para substituted), 771(C-Cl str.) EI\textsuperscript{+} m/z: 396.25 Anal. Calcd for C\textsubscript{20}H\textsubscript{14}Cl\textsubscript{2}N\textsubscript{4}O.C, 60.47%; H, 3.55%; Cl, 17.85%; N, 14.10%; O, 4.10%.

(E)-N\textsuperscript{2}-(E)-3-(4-chlorophenyl)-1-(p-tolyl)allylidene)pyrazine-2-carbohydrazide(7c) IR (ν\textsubscript{max} cm\textsuperscript{-1}, KBr): 3307(-NH str.), 3064 (Ar-C-H str.), 1695(C=O str.), 1616 (C=N str.), 1558(-NH bend.) 1489 (C=C str.), 1430(C-H bend.), 810(para substituted), 771(C-Cl str.). \textsuperscript{1}H NMR 400 MHz (DMSO-d\textsubscript{6}): 10.54(-NH, s), 9.25(1H,s), 8.86(1H,s), 8.55(1H,s) 7.64-7.62(2H, dd), 7.51-7.49(2H,dd), 7.43-7.39(1H, dd), 7.39-7.38(1H,dd), 7.34-7.32(2H,dd) 6.46-6.41(1H,dd). \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): 158(O),157(=C-N), 148.2(CH, pyrazine ring), 146(C, pyrazine ring) , 143.7(CH, pyrazine ring), 143.3(CH, pyrazine ring), 136.4(C=CH), 134.5(C, phenyl ring), 133.3(C, phenyl ring), 128.9(2CH, phenyl ring), 128.7(2CH, phenyl ring), 128.0(2CH, phenyl ring), 127.0(2CH, phenyl ring), 127.0, 21.03(-CH\textsubscript{3})(HC=C). EI\textsuperscript{+} m/z: 376 Anal. Calcd for C\textsubscript{21}H\textsubscript{17}ClN\textsubscript{4}O.C, 66.93%; H, 4.55%; Br, Cl, 9.41%; N, 14.87%; O, 4.25%.

(E)-N\textsuperscript{2}-(E)-3-(4-chlorophenyl)-1-(3,4-dichlorophenyl)allylidene)pyrazine-2-carbohydrazide(7d) IR (ν\textsubscript{max} cm\textsuperscript{-1}, KBr): 3301(-NH str.), 3028 (Ar-C-H str.), 2912 (C-H str.), 1693(C=O str.), 1611 (C=N str.), 1555(-NH bend.) 1452 (C=C str.), 1403(C-H bend.), 771(C-Cl str.) EI\textsuperscript{+} m/z: 431.70 Anal. Calcd for C\textsubscript{20}H\textsubscript{13}Cl\textsubscript{2}N\textsubscript{3}O.C, 55.64%; H, 3.04%; Cl, 24.64%; N, 12.98%; O, 3.71%.

(E)-N\textsuperscript{2}-(E)-3-(4-chlorophenyl)-1-(4-nitrophenyl)allylidene)pyrazine-2-carbohydrazide(7e) IR (ν\textsubscript{max} cm\textsuperscript{-1}, KBr): 3313(-NH str.), 3009(Ar-C-H str.), 2918(C-H str.), 1687(C=O str.), 1658 (C=N str.), 1558(N-O str.), 1539(-NH bend.) 1464 (C=C str.), 1419(C-H bend.), 1367(N-O str.), 814(1,-disubstituted) EI\textsuperscript{+} m/z: 407.80 Anal. Calcd for C\textsubscript{20}H\textsubscript{14}Cl\textsubscript{2}N\textsubscript{3}O.C, 58.90%; H, 3.46%; Cl, 8.69%; N, 17.17%; O, 11.77%.

(E)-N\textsuperscript{2}-(E)-3-(4-chlorophenyl)-1-(2,4-dichlorophenyl)allylidene)pyrazine-2-carbohydrazide(7f) IR (ν\textsubscript{max} cm\textsuperscript{-1}, KBr): 3313(-NH str.), 3026 (Ar-C-H str.), 2916 (C-H
str.), 1688(C=O str.), 1624 (C=N str.), 1559(-NH bend.) 1461 (C=C str.), 1428(C-H bend.), 771(C-Cl str.) EI⁺ m/z: 431.70 Anal. Calcd for C₂₀H₁₃Cl₂N₄O: C, 55.64%; H, 3.04%; Cl, 24.64%; N, 12.98%; O, 3.71%.

**(E)**-N’-((**E**)-3-(4-chlorophenyl)-1-(3,4-dimethoxy)allylidene)pyrazine-2-carbohydrazide(7g) IR (ν_{max} cm⁻¹, KBr): 3346(-NH str.), 3071 (Ar-C-H str.), 2939 (C-H str.), 1658(C=O str.), 1621 (C=N str.), 1547(-NH bend.) 1458 (C=C str.), 1404(C-H bend.), 771(C-Cl str.) EI⁺ m/z: 422.86 Anal. Calcd for C₂₂H₁₉ClN₄O₃: C, 62.49%; H, 4.53%; Cl, 8.38%; N, 13.25%; O, 11.35%.

**(E)-N’-((E)-1-(4-bromophenyl)-3-(4-chlorophenyl)allylidene)pyrazine-2-carbohydrazide**(7h) IR (ν_{max} cm⁻¹, KBr): 3302(-NH str.), 3064 (Ar-C-H str.), 2926 (C-H str.), 1691(C=O str.), 1625 (C=N str.), 1550(-NH bend.) 1489 (C=C str.), 1402(C-H bend.), 812(para substituted), 771(C-Cl str.). 

**¹H** NMR 400 MHz (DMSO-d₆): 10.48(-NH, s), 9.25(1H, s), 8.87(1H, s), 8.56(1H, s) 7.91-7.88(2H, dd), 7.67-7.65(2H, dd), 7.44-7.42(4H, m), 7.46-7.42(1H, dd), 6.48-6.44(1H, dd). 

**¹³C** NMR (100 MHz, CDCl₃): 158.6(C=O), 156.4(=C=N), 147.7(CH, pyrazine ring), 144.9(C, pyrazine ring), 143.6(CH, pyrazine ring), 142.6(CH, pyrazine ring), 137.6(C=CH), 134.9(C, phenyl ring), 133.6(C, phenyl ring), 131.1(C, phenyl ring), 130.4(2CH, phenyl ring), 129.9(2CH, phenyl ring), 129.3(2CH, phenyl ring), 129.0(2CH, phenyl ring), 128.6(C, phenyl ring) 124.7(HC=C). 

EI⁺ m/z: 441 Anal. Calcd for C₂₀H₁₄BrClN₄O: C, 54.38%; H, 3.019%; Br, 18.09%; Cl, 8.99%; N, 12.68%; O, 3.62%.

**(E)-N’-((**E**) 1-(3-bromophenyl)-3-(4-chlorophenyl)allylidene)pyrazine-2-carbohydrazide**(7j) IR (ν_{max} cm⁻¹, KBr): 3298(-NH str.), 3078(AR-C-H str.), 2907 (C-H str.), 1689(C=O str.), 1648 (C=N str.), 1545(-NH bend.) 1464 (C=C str.), 1419(C-H bend.), 768(mono substitution) EI⁺ m/z: 362.41 Anal. Calcd for C₂₀H₁₅Cl₄O: C, 66.21%; H, 4.17%; Cl, 9.77%; N, 15.44%; O, 4.41%.

**(E)-N’-((**E**) 1-(3-bromophenyl)-3-(4-chlorophenyl)allylidene)pyrazine-2-carbohydrazide**(7j) IR (ν_{max} cm⁻¹, KBr): 3330(-NH str.), 3071 (Ar-C-H str.), 2921 (C-H str.), 1688(C=O str.), 1653(C=N str.), 1545(-NH bend.) 1481 (C=C str.), 1414(C-H bend.), 753(1,3-disubstituted), 771(C-Cl str.) EI⁺ m/z: 441 Anal. Calcd for C₂₀H₁₄BrClN₄O: C, 54.38%; H, 3.019%; Br, 18.09%; Cl, 8.09%; N, 12.68%; O, 3.62%.
Mass spectrum of (E)-N’-((E)-3-(4-chlorophenyl)-1-(3,4-dimehoxyphenyl)allyl)ide)
pyrazine-2-carbohydrazide (7g)

Mass spectrum of (E)-N’-((E)-3-(4-chlorophenyl)-1-(4-methylphenyl)allyl)ide)
pyrazine-2-carbohydrazide (7c)
IR spectrum of (E)-N’-((E)-1-(4-bromophenyl)-3-(4-chlorophenyl)allylidene)pyrazine-2-carbohydrazide (7e)

IR spectrum of (E)-N’-((E)-3-(4-chlorophenyl)-1-(4-methylphenyl)allylidyne)pyrazine-2-carbohydrazide (7c)
$^1$H NMR spectrum of (E)-N'$-(E)-1-(4-bromophenyl)-3-(4-chlorophenyl)allylidene) pyrazine-2-carbohydrazide (7e)

$^1$H NMR spectrum of (E)-N'$-(E)-3-(4-chlorophenyl)-1-(4-methylphenyl)allylide) pyrazine-2-carbohydrazide (7c)
$^{13}$C NMR spectrum of (E)-N'-(E)-1-(4-bromophenyl)-3-(4-chlorophenyl)allylidene) pyrazine-2-carbohydrazide (7e)

$^{13}$C NMR spectrum of (E)-N'-(E)-3-(4-chlorophenyl)-1-(4-methylphenyl)allylide) pyrazine-2-carbohydrazide (7c)
TABLE II: ANTIMICROBIAL ACTIVITY OF (E)-N’-((E)-3-(4-CHLOROPHENYL)-1-SUBSTITUTEDPHENYL ALLYLIDENE) PYRAZINE-2-CARBOHYDRAZIDES. 7(a-j)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Antibacterial activity MIC(μg/ml)</th>
<th>Antifungal activity MIC(μg/ml)</th>
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<td></td>
<td>S.aureus</td>
<td>B.subtilius</td>
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<tr>
<td>7a</td>
<td>125</td>
<td>50</td>
</tr>
<tr>
<td>7b</td>
<td>250</td>
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<td>250</td>
<td>200</td>
</tr>
<tr>
<td>7d</td>
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<td>250</td>
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<tr>
<td>7e</td>
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<td>50</td>
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<tr>
<td>7f</td>
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<td>7g</td>
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</tr>
<tr>
<td>7i</td>
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<td>50</td>
</tr>
<tr>
<td>7h</td>
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<td>200</td>
</tr>
<tr>
<td>7j</td>
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<td>125</td>
</tr>
<tr>
<td>A.</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>B.</td>
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</tr>
<tr>
<td>C.</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>N.</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Cl.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T.</td>
<td>-</td>
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</table>

A: Ampiciliin, C: Ciprofloxacin, N: Norfloxacin, Cl: Clotrimazole, T: Terbinafeine
### TABLE III: ANTITUBERCULAR ACTIVITY OF (E)-N'-(E)-3-(4-CHLOROPHENYL)-1-SUBSTITUTEDPHENYL ALLYL IDENE)PYRAZINE-2-CARBOHYDRAZIDES7(a-j)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Code No.</th>
<th>% inhibition at 30 (μg/ml)</th>
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<tbody>
<tr>
<td>1</td>
<td>7a</td>
<td>80.4</td>
</tr>
<tr>
<td>2</td>
<td>7b</td>
<td>82.6</td>
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<tr>
<td>3</td>
<td>7c</td>
<td>82.0</td>
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<tr>
<td>4</td>
<td>7d</td>
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<tr>
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<tr>
<td>10</td>
<td>7j</td>
<td>72.6</td>
</tr>
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</table>

Graphs for the determination of MIC and IC$_{50}$ of 7e
RESULT AND DISCUSSION

In the present study a series of hydrazones of pyrazine 2-carbohydrazide 7a-7j have been synthesized by pyrazine 2-carbohydrazide and chalcones in the presence of acetic acid. The structures of all new synthesized compounds were established by \(^1\)H, \(^{13}\)CNMR, FT-IR, and mass spectroscopy. The mass spectrum of 7c displayed the molecular ion peak (M\(^+\)) peak at m/z 376, which was consistent with the product structure. The \(^1\)H NMR spectrum of 7h exhibited singlet of proton at \(\delta\) 14.52 which indicated the presence of –SH group. Another singlet of one proton at \(\delta\) 9.17 confirms the formation of Schiff base (–N=C-H) proton. Two singlets of 6 protons and 3 protons at \(\delta\) = 3.85 and 3.75 are of three methoxy groups. The \(^{13}\)C NMR spectrum of 7e showed C=S peak at \(\delta\) 166.99 ppm and N=C-H peak at \(\delta\) 163.05 ppm are the characteristics peaks for the compound. C-O peak at \(\delta\) 153.24 and peak at \(\delta\) 56.01 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.

All the synthesized compounds (7a-7j) were tested in vitro for their antibacterial and antifungal activity. All the glass apparatus used were sterilized before use. The broth dilution technique was used to determine the minimum inhibitory concentration (MIC) of the synthesized compounds. Bacterial strain of Staphylococcus aureus MTCC87, Bacillus subtillius MTCC 441 as a gram positive, Escherichia coli MTCC1302, shigella MTCC 11947 as a gram negative used in a present study. Fungal strains of Aspergillus niger MTCC 1344 and Candida albicans MTCC 227 were taken. DMSO was used as the solvent for the compounds. A blank test was carried out to check the antimicrobial activity of DMSO. Ampicillin, Ciprofloxacin and Norfloxacin were used as the standard drugs for antibacterial activity and Clotrimazole and Terbinafine were used as the standard drug for antifungal activity.

From the results of antimicrobial compounds 7a, 7b, 7e and 7h are most active with MIC of 50\(\mu\)g/ml against Bacillus subtillius with respect to standard Ampicillin, Norfloxacin and Ciprofloxacin having same MIC. Compounds 7b, 7d, 7e and 7h are most active with MIC of 50\(\mu\)g/ml against Escherichia coli with respect to standard.
Looking at the antifungal results compound 7c and 7e are equally active with the MIC of 100µg/ml against *Candida albicans* with respect to standard antifungal drugs like Clotrimazole and Terbinafine. Compounds 7b, 7d, 7e and 7h are more active with respect to standard having MIC of 100µg/ml against *Aspergillus niger*. Some of the compounds show significant activity while all the synthesized compounds are not promising against microorganisms like *Staphylococcus aureus and shigella*.

Overall from the results we can say that 7b, 7d, 7e and 7h are most active as antibacterial agents (gram negative) and antifungal agents.

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 hydrazine herein, we describe 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.

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, 7b, 7c, 7e and 7h are showing high percentage of inhibition 80.4, 82.6, 82.0, 84.6 and 85.2 respectively. Compound 7d, 7f, 7g and 7j are moderately active. Compounds 7e (4-bromo) and 7h (4-nitro) are showing more activity. While 7e is also selected for the studies of MIC and IC 50 against *Mycobacterium Tuberculosis H37Ra* using various dilutions at dormant stage. MIC and IC 50 values of 7e are 93.06 and 3.06 respectively.

As Compound 7e was having promising results, cytotoxicity studies of compound 7e was 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 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


