The present chapter shows the review on 2-aminothiazole. It also shows the synthesis and characterization of spiro compounds based on Schiff base of 2-aminothiazole and Isatin as intermediate and its post cyclizations with benzoyl isothiocynate derivatives. This chapter also gives the brief introduction of antimicrobial activity and also explains the antimicrobial data for newly synthesized compounds.

6.1 Introduction of 2-Aminothiazole and Overview

2-Aminothiazoles and their derivatives have long been used as precursors for the synthesis of biologically active molecules. Because of their wide spectrum of activity numerous thiazoles substituted with different groups at various positions have been prepared. In recent years several new methods for the preparation of 2-aminothiazole and its derivatives have been reported. The cyclization of these derivatives inspired us to undertake the aspect on 2-aminothiazole derivatives.

6.1.1 Thiazole and its derivatives

Compounds containing the simple thiazole nucleus were first reported by Hantzsch and Weber in 1887\(^1\). They described thiazole ring (1,2) as ‘the pyridine of thiophene series.

![Thiazole and its derivatives](image)

Methyl ketones react with thiourea in the presence of N-bromo succinimide (NBS) using benzoyl peroxide to furnish 2-aminothiazoles (3)\(^2\).

\[
\text{RCOCH}_3 + \text{H}_2\text{NCSNH}_2 \xrightarrow{\text{NBS} \text{BZ}_2\text{O}_2} \text{R} \begin{array}{c}
\text{N} \\
\text{S} \\
\text{NH}_2
\end{array}
\]

\(R = \text{(un)substituted-3-coumarinyl, (un)substituted phenyl}\)
The formation of 2-aminothiazoles (4) in a single step reaction from alkynyl(phenyl)iodonium mesylates and thiourea.

6.1.2 Work done on 2-Aminothiazole - Overview

Acylation of 2-aminothiazole has been carried out with acid (5), acid anhydride (6) and acid chloride (7). (5) has been tested for antimicrobial activity. (7) is useful in the treatment of hyperlipidemia, cataract, diabetes and scanned in rat PCA assay.

Sonawne et al., have synthesized 2-(2'-arylidine - hydrazino-acetylamino)-4-phenyl-1,3-thiazole and 2-[2'-{4"-substituted-aryl-3"-chloro-2"-oxo-azetidine}-acetyl-amino]-4-phenyl-1,3-thiazole (8) and studied for their antifungal activity.

Packiarajan et al., synthesized an alkyl aminothiazole (9) and (10). 2-Pyridylaminothiazole based compounds show high affinity at the NPY5 receptor. They suffer from high in vitro vivo clearance.
Venkteshwar synthesized 2-aminophenyl-5-phenyl-4-[3-oxo1,4-benzexazin-6-yl]thiazoles (11) and evaluated inhibition activity\(^8\).

Condensations of 2-aminothiazoles with substituted benzoxazin-4-ones in dry pyridine give thiazolylquinazolones (12) and tested for antimicrobial activity against various microbes\(^9\).

Alkaloid namidine was synthesized and tested *in vitro* for ability to inhibit mitogenesis. Replacement of the imidazole with a thiazole (13) was found to have minor effect on potency\(^10\).
Hexahydro-1, 3-benzothiazoles (14) obtained by the reaction of N, N’-dialkylthioureas on N-1-phenyl- 2-(1-cyclohexenyl)-1-diazene-1-carboxyamide. The acidic hydrolysis of spirocycloalkyl-thiazolinones produced 2-imino-5-(ω-carboxyalkyl) - 4-thiazolidinones (15)\textsuperscript{11}.

Mohsen et al.\textsuperscript{12}, have synthesized thiazole and pyrolothiadiazine derivatives (16) from thiosemicarbazones.

Julian et al.\textsuperscript{13}, have synthesized 2-amino-5-arylthiazoles (17). The starting aminothiazole derivatives can be arylated at 5\textsuperscript{th} position with aryl iodides under palladium catalyzed condition.

Mehdi et al., have synthesized 5H-[1,3]thiazolo[3,2-a]pyrimidine derivatives (18) via. addition reaction of isocyanides, dialkyl acetylene dicarboxylates and ethyl 2-oxo-2(1,3-thiazole-2-ylamino)acetate\textsuperscript{14}.
Lithium perchlorate has been found to catalyze the conjugate addition of maleimides with 2-aminothiazoles\textsuperscript{15}.

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

Bromo derivative reacts with aromatic amines, ammonium thiocyanate, thiourea and thiosemicarbazide to afford furan, thiophene, pyrrole and 2-aminothiazole derivatives\textsuperscript{16}.

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

\textbf{where, }R = \text{H, Ph, NH}_2

\textbf{6.1.3 Biological Importance of Thiazole Derivatives}

Srivastva et al., have synthesized 2-aminobenzothiazole derivative (21) which were evaluated for antibacterial activity\textsuperscript{17}.

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

One of the first synthetic drugs containing 2-aminothiazole moiety was “Sulfathiazole” (2). “Succinylsulfathiazole” (22) and “Phthalylsulfathiazole” (23) have found to be useful in the treatment of intestinal infections. “Nitrosulfathiazole” (24) has found to be useful in the treatment of nonspecific ulcerative colitis.
Kati et al., have synthesized 2,4-diacetylthiazole and 2,5-diacetylthaizole (25) by tetrabromination and silver nitrate treatment of corresponding diethylthiazoles\textsuperscript{18}.

Pattan et al., have synthesized 5-[1-(4-(4-substituted-phenylamino)-meth-(z)-ylidenel-thiazolidine-2,4-diones derivatives (26). The compounds were screened for their antitubercular activity.

Pattan et al., have synthesized substituted amino thiazole (27). The compounds were tested \textit{in vitro} for antibacterial activity.
Quig et al.\textsuperscript{21}, have synthesized and studied cytotoxicity regioselectivity of a series of novel glycosyl thiazole-2-imines (28).

\[
\begin{align*}
R_2 &= \text{Ar} & R_3 &= \\
R_4 &= \text{ArCO}
\end{align*}
\]

Akbar et al.\textsuperscript{22}, have synthesized ethyl 3,5-dimethyl-4-[(4-phenyl-1,3-thiazole-2-yl)carbomyl]-1H-pyrrole-2-carboxylate (29) and screened for antimicrobial activity.

Omar et al.\textsuperscript{23}, have synthesized adamantane derivatives (30) of thiazolyl-N-substituted amide and tested anti-inflammatory activity.

\[
\begin{align*}
\text{30}
\end{align*}
\]

\textbf{6.1.4 2-Aminothiazole and Isatin derivative}

5-substituted Isatin was reacted with 2-aminothiazole to form Schiff base. 3-(2-thiazoylimino)-5-bromo-1,3-dihydro-indol-2-one (31) was verified against HIV-1 and HIV-2\textsuperscript{24}. 
Schiff bases of isatin with aminothiazole, its N-mannich bases and spiro isatin derivatives were synthesized. All the compounds were evaluated for antimicrobial and anti-inflammatory activity\textsuperscript{25}.

The microwave assisted synthesis of of 3'- (aryl/heteroaryl)-1-morpholinomethyl/piperidinomethylspiro [3H-indole-3,2'-thiazolidine]-2,4'(1H)-diones (32) has been achieved from Isatin with aryl/heteroaryl amines\textsuperscript{26}.

Anshu Dandia et al., have synthesized the solvent free novel series of Spiro[3H-Indole-3,2'-Thiazolidine (33) based on isatin\textsuperscript{27}.

2-Amino-11-hydronaphtho [2,1:5,6] pyrano [4,3-\textit{d}] thiazole on treatment with isatin affords naphtho[1,2-\textit{b}]pyrano[3,4-\textit{d}]thiazolo-8-yl(3-imino-2-oxo-1\textit{H}-indole) (34) which on further reaction with chloroacetyl chloride and mercaptoacetic acid yields the N-[naphtho[1,2-\textit{b}]pyrano[3,4-\textit{d}]thiazol-8-yl]spiro-[3H-indole-(1\textit{H},2\textit{H})]-3,4-
(2\(H\))-3-chloroazetidine-2,2-diones (35) and \(N\)-[naphth[1,2-b]pyrano[3,4-\(d\)] thiazol-8-yl] spiro-[3\(H\)-indole-(1\(H\), 2\(H\))-3, 2-\((4\(H\))\)-thiazolidine]-2, 4-dione (36). All the compounds have screened for the antimicrobial activity\(^{28}\).

According to systematic literature survey, it concludes that the compounds bearing 2-aminothiazole have biological importance. In addition literature survey also resulted that 2-aminothiazole and isatin as a club molecule gives very good biological activity. Thus, it was thought to study spiro compounds containing isatin and 2-aminothiazole as a wide structure variation, to play vital role in medicinal chemistry.

6.2 Experimental
The Schiff base of heterocyclic amine and Isatin has been prepared as precursors of post spiro product. The synthesis and characterization of this Schiff base and spiro compounds are summarized in this chapter. So, the experimental portion deals with the synthesis and characterization of Schiff bases and spiro compounds which were derived from Schiff base of 2-aminothiazole and isatin as intermediate and its post cyclizations with benzoyl isothiocynate derivatives.

### 6.2.1 Materials

All the chemicals used were of analytical grade. The organic solvents were purified by standard methods. The starting material 2-aminothiazole (1a) was prepared by reported method. The whole procedure for preparation of 2-aminothiazole (1a) is mentioned in Chapter 2. The various derivatives of benzoyl isothiocynate (4a-h), isatin (2) and (1b) were purchased from E. Merck Ltd (India). The various derivatives of benzoyl isothiocynate are listed in Table 6.1.

**Table 6.1 Various derivatives of benzoyl isothiocynate (4a-h)**

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Structures</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>Benzoyl isothiocyanate</td>
<td>![SCNOC-H]</td>
</tr>
<tr>
<td>4b</td>
<td>4-methylbenzoyl isothiocyanate</td>
<td>![SCNOC-CH3]</td>
</tr>
<tr>
<td>4c</td>
<td>4-chlorobenzoyl isothiocyanate</td>
<td>![SCNOC-Cl]</td>
</tr>
<tr>
<td>4d</td>
<td>4-bromobenzoyl isothiocyanate</td>
<td>![SCNOC-Br]</td>
</tr>
<tr>
<td>4e</td>
<td>4-methoxybenzoyl isothiocyanate</td>
<td>![SCNOC-OCH3]</td>
</tr>
<tr>
<td>4f</td>
<td>4-aminobenzoyl isothiocyanate</td>
<td>![SCNOC-NH2]</td>
</tr>
</tbody>
</table>
6.3 Synthesis of Schiff base 3-((heteroaryl)-2-ylimino) indolin-2-one (3a-b)

The various Schiff bases were prepared by similar method reported in literature. The procedure is as follow:

An equimolar mixture of heteroaryl amine (1a-b) (0.01mole) and isatin (0.01mole) were refluxed in methanol (40ml) in presence of catalytic amount of glacial acetic acid (0.4ml) for 3hrs. and allowed for cooling. The solid product of Schiff bases was obtained and filtrated from methanol and recrystallized from methanol to give (3a-b). The yields, melting points and other characterization data of all synthesized compounds are described.

The synthetic route as well as probable mechanism for formation of Schiff bases is shown in schemes 6.1 and 6.2 respectively.

\[
\text{Ar–NH}_2 \xrightarrow{\text{MeOH}/\text{AcOH}} \text{HN} \equiv \text{N} \equiv \text{Ar}
\]

where, \(\text{Ar} = \text{a) } \text{(a) and b) } \text{(b) amine} \)

Scheme 6.1 Synthetic route for Schiff bases
Probable mechanism of formation of Schiff bases is shown below:

\[
\begin{align*}
\text{Ar} - \text{N} - \text{H} & \quad + \quad \text{O} = \text{C} - \text{NH} \\
\text{H}_2\text{O} & \quad + \quad \text{HN} = \text{N} - \text{Ar}
\end{align*}
\]

\[
\text{Scheme 6.2}
\]

**Compound 3a**

3-(thiazol-2-ylimino) indolin-2-one

<table>
<thead>
<tr>
<th>Molecular Formula:</th>
<th>Elemental Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{C}_{11}\text{H}_7\text{N}_3\text{OS}$</td>
<td>%C %H %N %S</td>
</tr>
<tr>
<td>Molecular weight: 229.26</td>
<td>Calculated 57.63 3.08 18.33 13.99</td>
</tr>
<tr>
<td>gm/mole</td>
<td>Found 57.64 3.05 18.39 13.96</td>
</tr>
<tr>
<td>Melting Point: 182-183°C</td>
<td>$^1\text{H}$ NMR (400 MHz, DMSO-$d_6$) $\delta_H$</td>
</tr>
</tbody>
</table>
(Uncorrected)
Yield: 71%

**IR (KBr): \( \nu_{\text{max}} \text{ (cm}^{-1}) \)**
- 1623 = -C=\( \text{N} \)
- 1731 = -C=O of isatin,
- 684 = -C-S-C of thiazol
- 3058 = -C-H of Ar.H
- 1496 = -C=\( \text{C} \) of Ar.
- 1608 = -C-C of Ar.
- 3340 = -N-H stre.

**\(^{13}\text{C NMR (100 MHz, DMSO-d}_6\text{)} \delta_c (ppm):**
- 117.1, 117.8, 119.9, 124.8, 129.1, 130.7, 140.9, 141.3, 149.4, 162.9, 170.1

**DEPT 135:**
- 117.8(\( \text{CH}\)), 119.9(\( \text{CH}\)), 124.8(\( \text{CH}\)), 129.1(\( \text{CH}\)), 130.7(\( \text{CH}\)), 140.9(\( \text{CH}\))

---

**Compound 3b**

\[
\begin{array}{c}
\text{HN} \\
\text{\hspace{1cm}N} \\
\text{\hspace{2.5cm}N} \\
\text{\hspace{2cm}O}
\end{array}
\]

3-(4H-1, 2, 4-triazol-4-ylimino) indolin-2-one

**Molecular Formula:** \( \text{C}_{10}\text{H}_7\text{N}_5\text{O} \)
**Molecular weight:** 213.20 gm/mole
**Melting Point:** 195-197\(^\circ\text{C}\)

**Elemental Analysis**

<table>
<thead>
<tr>
<th></th>
<th>%C</th>
<th>%H</th>
<th>%N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calculated</td>
<td>56.34</td>
<td>3.31</td>
<td>32.85</td>
</tr>
<tr>
<td>Found</td>
<td>56.35</td>
<td>3.30</td>
<td>32.84</td>
</tr>
</tbody>
</table>

**\(^1\text{H NMR (400 MHz, DMSO-d}_6\text{)} \delta_H (ppm):**
- \( \delta 8.68 \) (s, 1H, NH of isatin),
- 7.13-7.76 (m, 4H, Ph.H of isatin),
- 7.82 (d, 1H, H of thiazole), 7.85 (d, 1H, H of thiazol)
6.4 Synthesis and characterization of spiro compounds based on Schiff bases of 2-aminothiazole-isatin and its post cyclization with benzoyl isothiocyanate derivatives

Schiff base of 2-aminothiazole (3a) as above mentioned and cyclization reaction with benzoyl isothiocyanate (4a-h) yield the biologically active spiro compounds. These derivatives were characterized by elemental analysis, infrared spectral data, $^1$H, $^{13}$C and DEPT magnetic resonance spectral data. Experimental procedures for the synthesis of this series compounds have been adopted according to reported methods.$^{35}$

6.4.1 Synthesis of 6’-{4-(sub.)Phenyl}-3’-{thiazol-2-yl}-4’-thioxo-3’,4’ -dihydrospiro [indoline-3, 2’-{1, 3, 5} oxadiazin]-2-one (5a-h)
A well stirred solution of Schiff base (3a) (0.01mole) in dry DMF containing pinch of anhydrous ZnCl₂ and benzoyl isothiocyanate derivatives (0.02mole) (4a-h) was refluxed for 12 hrs. Excess of solvent was distilled off under reduced pressure and the residual reaction mixture was cooled and poured into ice-cold water. The separated solid product was filtered, washed and recrystallized from ethanol to yield (5a-h).

The synthetic route and probable mechanism of formation of spiro compound is shown in schemes 6.3 and 6.4 respectively.

![Diagram](image)

where, R = -H, -CH₃, -Cl, -Br, -OCH₃, -NH₂, -OH, -NO₂

**Scheme 6.3** Synthetic route for spiro compounds

Probable mechanism of formation of compound (5a-h) is shown below:
The analytical and spectral data of compounds \((5a-h)\) are described.

**Compound 5a**

\[
\text{6'-phenyl-3'-(thiazol-2-yl)-4'-thioxo-3', 4'-dihydrospiro[indoline-3, 2'-[1, 3, 5] oxadiazine]-2-one}
\]

<table>
<thead>
<tr>
<th>Molecular Formula:</th>
<th>(\text{Elemental Analysis} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{C}<em>{19}\text{H}</em>{12}\text{N}_4\text{O}_2\text{S}_2)</td>
<td>(%\text{C}) (%\text{H}) (%\text{N}) (%\text{S})</td>
</tr>
</tbody>
</table>
Molecular weight: 392.45 gm/mole
Melting Point: 218-220°C (Uncorrected)
Yield: 68%

**IR (KBr):** $\nu_{\text{max}}$ (cm$^{-1}$)
- 1620 = -C=\(N\)
- 1727 = -C=O of isatin,
- 680 = -C-S-C of thiazol
- 1143 = -C=S
- 3076 = -C-H of Ar.H
- 1497 = -C=C of Ar.
- 1590 = -C-C of Ar.
- 3323 = -N-H str.

Calculated 58.15 3.08 14.28 16.34
Found 58.10 3.10 14.30 16.35

**$^1H$ NMR (400 MHz, DMSO-$d_6$)** $\delta_H$ (ppm):
- 8.62 (s, 1H, NH of isatin),
- 7.54-7.90 (m, 5H, Ph.H of phenyl ring),
- 6.78 (d, 1H, H of thiazole),
- 7.08 (d, 1H, H of thiazole),
- 7.29-7.36 (m, 4H, Ph.H of isatin)

**$^{13}C$ NMR (100 MHz, DMSO-$d_6$)** $\delta_c$ (ppm):
- 111.9, 115.7, 121.3, 125.6, 127.9, 128.3, 128.4, 130.8, 132.4, 135.1, 136.6, 136.8, 141.2, 156.4, 159.1, 168.1, 178.5.

**DEPT 135:**
- 111.9(CH), 115.7(CH), 125.6(CH), 127.9(CH), 128.3(CH), 130.8(CH), 136.6(CH), 136.8(CH)

**Compound 5b**

![Compound 5b](image)

3’-(thiazol-2-yl)-4’-thioxo-6’-p-totyl-3’,4’-dihydrospiro[indoline-3,2’-[1,3,5]oxadiazin]-2-one

<table>
<thead>
<tr>
<th>Molecular Formula:</th>
<th>Elemental Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$<em>{20}$H$</em>{14}$N$_4$O$_2$S$_2$</td>
<td>%C %H %N %S</td>
</tr>
</tbody>
</table>
Molecular weight: 406.48 gm/mole
Melting Point: 283-285°C (Uncorrected)
Yield: 72%

**IR (KBr): v_{\text{max}} (\text{cm}^{-1})**
1629 = -C=N
1723 = -C=O of isatin,
688 = -C-S-C of thiazol
1140 = -C=S
3067 = -C-H of Ar.H
1496 = -C=C of Ar.
1608 = -C-C of Ar.
3326 = -N-H str.

Calculated 59.10 3.47 13.78 15.78
Found 58.12 3.44 13.73 15.76

**1H NMR (400 MHz, DMSO-d_6) δ_H (ppm):**
δ 8.57 (s, 1H, NH of isatin),
7.40-8.05 (m, 4H, Ph.H of phenyl ring), 6.72 (d, 1H, H of thiazole), 7.13 (d, 1H, H of thiazole), 7.22-7.33 (m, 4H, Ph.H of isatin), 2.30 (s, 3H, C-CH_3)

**13C NMR (100 MHz, DMSO-d_6) δ_C (ppm):**
21.7, 112.4, 115.3, 121.2, 127.7, 128.5, 128.8, 132.3, 132.7, 136.8, 137.1, 140.3, 141.4, 156.5, 158.9, 168.4, 178.3,

**DEPT 135:** 21.7(-CH_3), 112.4(CH), 115.3(CH), 127.7(CH), 128.5(CH), 128.8 (CH), 136.8(CH), 137.1(CH)

**Compound 5c**

6'-(4-chlorophenyl)-3'-(thiazol-2-yl)-4'-thioxo-3', 4'-dihydrospiro[indoline-3, 2'[-1, 3, 5] oxadiazin]-2-one

<table>
<thead>
<tr>
<th>Molecular Formula:</th>
<th>Elemental Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{19}H_{11}ClN_{4}O_{2}S_{2}</td>
<td>%C %H %N %S</td>
</tr>
</tbody>
</table>
Molecular weight: 426.00 gm/mole
Melting Point: 263-265°C (Uncorrected)
Yield: 69%

**IR (KBr):** $v_{	ext{max}}$ (cm$^{-1}$)
- 1633 = -C=N
- 1738 = -C=O of isatin,
- 692 = -C=S-C of thiazol
- 1158 = -C=S
- 3090 = -C-H of Ar.H
- 1508 = -C=C of Ar.
- 1594 = -C-C of Ar.
- 3329 = -N-H str.
- 732 = -C-Cl

Calculated 53.46 2.60 13.12 15.02
Found 53.47 2.62 13.15 15.08

**$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta_H (ppm)$:**
- $\delta$ 8.60 (s, 1H, NH of isatin), 7.57-7.77 (m, 4H, Ph.H of phenyl ring), 6.78 (d, 1H, H of thiazole), 7.16 (d, 1H, H of thiazole), 7.31-7.38 (m, 4H, Ph.H of isatin)

**$^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta_c (ppm)$:**
- 112.2, 115.2, 121.7, 127.3, 128.6, 129.3, 132.5, 133.3, 136.5, 136.7, 137.3, 141.6, 156.7, 158.6, 168.5, 178.7

**DEPT 135:**
- 137.3(CH), 112.2(CH), 128.6(CH), 136.7(CH), 127.3(CH), 115.2(CH), 128.6(CH), 129.3 (CH)

**Compound 5d**

\[
\begin{align*}
\text{6'}-(4\text{-bromophenyl)-3'}-(\text{thiazol-2-yl)-4'}-\text{thioxo-3'}-4'}-\text{dihydrospiro}\[\text{indoline-3, 2'}-\text{[1, 3, 5] oxadiazin]-2-one}\n\end{align*}
\]

Molecular Formula:
C$_{19}$H$_{11}$BrN$_4$O$_2$S$_2$

<table>
<thead>
<tr>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>%C</td>
<td>%H</td>
</tr>
<tr>
<td>53.46</td>
<td>2.60</td>
</tr>
<tr>
<td>53.47</td>
<td>2.62</td>
</tr>
</tbody>
</table>

*Department of chemistry, S.P.U*
Molecular weight: 471.35 gm/mole
Melting Point: 237-238°C (Uncorrected)
Yield: 72%

**IR (KBr):** \( \nu_{\text{max}} (\text{cm}^{-1}) \)
- 1625 = -C=N
- 1732 = -C=O of isatin,
- 702 = -C-S-C of thiazol
- 1178 = -C=S
- 3090 = -C-H of Ar.H
- 1495 = -C=C of Ar.
- 1597 = -C-C of Ar.
- 3334 = -N-H str.
- 578 = -C-Br

**\(^1\)H NMR (400 MHz, DMSO-d\(_6\)) \( \delta_H (\text{ppm}) \):**
- \( \delta \) 8.83 (s, 1H, NH of isatin),
- 7.58-7.73 (m, 4H, Ph.H of phenyl ring),
- 6.69 (d, 1H, H of thiazole),
- 7.11 (d, 1H, H of thiazole),
- 7.15-7.36 (m, 4H, Ph.H of isatin)

**\(^{13}\)C NMR (100 MHz, DMSO-d\(_6\)) \( \delta_c (\text{ppm}) \):**
- 112.8, 115.7, 121.9, 125.7, 127.5, 128.2, 131.2, 131.8, 132.8, 134.7, 136.8, 137.6, 141.1, 156.9, 158.1, 168.9, 178.2.

**DEPT 135:**
- 112.8(CH), 115.7(CH), 127.5(CH), 128.2(CH), 131.2(CH), 131.8(CH), 136.8(CH), 137.6(CH)

---

**Compound 5e**

![Compound 5e](image)

6’-(4-methoxyphenyl)-3’-(thiazol-2-yl)-4’-thioxo-3’, 4’-dihydrospiro[indoline -3, 2’-[1, 3, 5] oxidizin]-2-one

<table>
<thead>
<tr>
<th>Molecular Formula:</th>
<th>C(<em>{20})H(</em>{14})N(_4)O(_3)S(_2)</th>
<th><strong>Elemental Analysis</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%C</td>
<td>%H</td>
</tr>
</tbody>
</table>

---

**Department of chemistry, S.P.U**

200
Molecular weight: 422.48 gm/mole
Melting Point: 273-274°C (Uncorrected)
Yield: 73%

**IR (KBr): \( \nu_{\text{max}} \text{ (cm}^{-1}) \)**
- 1637 = -C=N
- 1742 = -C=O of isatin,
- 710 = -C-S-C of thiazol
- 1186 = -C=S
- 3048 = -C-H of Ar.H
- 1510 = -C=C of Ar.
- 1612 = -C=C of Ar.
- 3338 = -N-H str.
- 2838 = -C-OCH\(_3\)

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<tr>
<td>N</td>
<td>13.26</td>
<td>13.21</td>
</tr>
<tr>
<td>S</td>
<td>15.18</td>
<td>15.20</td>
</tr>
</tbody>
</table>

\(^1H\) NMR (400 MHz, DMSO-\(d_6\)) \( \delta \) (ppm):
- 8.52 (s, 1H, NH of isatin),
- 7.36-7.63 (m, 4H, Ph.H of phenyl ring),
- 6.71 (d, 1H, H of thiazole),
- 7.08 (d, 1H, H of thiazole),
- 7.13-7.31 (m, 4H, Ph.H of isatin),
- 3.78 (s, 3H, -OCH\(_3\))

\(^{13}C\) NMR (100 MHz, DMSO-\(d_6\)) \( \delta \) (ppm):
- 55.3, 112.3, 114.5, 115.8,
- 121.5, 127.7, 127.9, 128.5, 130.4,
- 132.3, 136.4, 137.1, 141.7, 156.3,
- 158.9, 162.3, 168.3, 178.1

DEPT 135: 55.3(-O-CH\(_3\)), 112.3(CH),
114.5(CH), 115.8(CH), 127.9(CH),
128.5(CH), 130.4(CH), 136.4(CH),
137.1(CH)

**Compound 5f**

![Image](image-url)

6´-(4-aminophenyl)-3´-(thiazol-2-yl)-4´-thioxo-3’, 4´-dihydrospiro
[indoline-3, 2´-[1, 3, 5] oxadiazin]-2-one

<table>
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<th>Molecular Formula:</th>
<th>Elemental Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calculated</td>
</tr>
<tr>
<td></td>
<td>Found</td>
</tr>
</tbody>
</table>
C₁₀H₁₃N₅O₂S₂
Molecular weight: 407.47 gm/mole
Melting Point: 282-283°C (Uncorrected)
Yield: 74%

IR (KBr): \( \nu_{\text{max}} \, (\text{cm}^{-1}) \)
- 1632 = -C=N
- 1728 = -C=O of isatin,
- 698 = -C-S-C of thiazol
- 1162 = -C=S
- 3077 = -C-H of Ar.H
- 1506 = -C=C of Ar.
- 1608 = -C-C of Ar.
- 3341 = -N-H str.
- 3255 = -C-NH₂

\[
\begin{array}{c|cccc}
\% \text{C} & \% \text{H} & \% \text{N} & \% \text{S} \\
\hline
\text{Calculated} & 56.01 & 3.22 & 17.19 & 15.74 \\
\text{Found} & 56.08 & 3.18 & 17.21 & 15.73 \\
\end{array}
\]

\( ^1H \text{ NMR (400 MHz, DMSO-}d₆) \, \delta \, (\text{ppm}) \):
- \( \delta \, 8.52 \, (\text{s}, \, 1\text{H}, \, \text{NH of isatin}) \),
- \( 7.35-7.77 \, (\text{m}, \, 4\text{H}, \, \text{Ph.H of phenyl ring}) \),
- \( 6.76 \, (\text{d}, \, 1\text{H}, \, \text{H of thiazole}) \),
- \( 7.15 \, (\text{d}, \, 1\text{H}, \, \text{H of thiazole}) \),
- \( 7.18-7.30 \, (\text{m}, \, 4\text{H}, \, \text{Ph.H of isatin}) \),
- \( 6.23 \, (\text{s}, \, 2\text{H}, \, -\text{NH₂}) \)

\( ^{13}C \text{ NMR (100 MHz, DMSO-}d₆) \, \delta \, (\text{ppm}) \):
- 112.5, 114.6, 116.0, 121.3,
- 125.1, 126.6, 127.6, 128.7, 132.9,
- 136.2, 137.7, 140.9, 150.6, 157.1,
- 158.8, 168.2, 178.4

DEPT 135:
- 112.5(CH), 114.6(CH),
- 137.7(CH), 116.0(CH), 126.6(CH),
- 127.6(CH), 128.7(CH), 136.2(CH)

Compound 5g

6′-(4-hydroxyphenyl)-3′-(thiazol-2-yl)-4′-thioxo-3′, 4′-dihydrospiro
[indoline -3, 2′-[1, 3, 5] oxadiazin]-2-one

<table>
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<tr>
<th>Molecular Formula:</th>
<th>Elemental Analysis</th>
</tr>
</thead>
</table>

Department of chemistry, S.P.U
\[
\begin{array}{|c|c|c|c|c|}
\hline
\text{C}_{19}\text{H}_{11}\text{N}_{5}\text{O}_{3}\text{S}_{2} & \%\text{C} & \%\text{H} & \%\text{N} & \%\text{S} \\
\text{Molecular weight: 408.45} & \text{Calculated} & 55.87 & 2.96 & 13.72 & 15.75 \\
\text{gm/mole} & \text{Found} & 55.89 & 2.92 & 13.68 & 15.73 \\
\text{Melting Point: 278-279°C} & \text{1H NMR (400 MHz, DMSO-} & \delta_{\text{H}} & \text{ppm):} & \delta \text{ 8.68 (s, 1H, NH of isatin),} \\
(\text{Uncorrected)} & \text{d}_6) & \text{7.42-7.92 (m, 4H, Ph.H of phenyl} & \text{7.08} \\
\text{Yield: 72%} & \text{ring), 6.78 (d, 1H, H of thiazole), 7.08} & \text{(d, 1H, H of thiazole), 7.13-7.36 (m,} \\
\text{IR (KBr): } \nu_{\text{max}} \text{ (cm}^{-1}) & \text{1148 = -C=S} & \text{4H, Ph.H of isatin), 10.58 (s, 1H, -OH)} \\
1627 = \text{-C=N} & \text{3052 = -C-H of Ar.H} & \text{13C NMR (100 MHz, DMSO-} & \text{111.8, 115.2, 116.2, 121.6,} \\
1733 = \text{-C=O of isatin,} & \text{1498 = -C=C of Ar.} & \text{d}_6) \delta_{\text{c}} \text{ (ppm):} & \text{127.7, 128.4, 128.7, 130.8, 132.9,} \\
708 = \text{-C-S-C of thiazol} & \text{1591 = -C-C of Ar.} & \text{136.2, 137.0, 141.5, 156.3, 158.3,} \\
1148 = \text{-C=S} & \text{3331 = -N-H str.} & \text{160.5, 168.2, 178.3} \\
3052 = \text{-C-H of Ar.H} & \text{3443 = -C-OH str.} & \text{DEPT 135:} & \text{111.8(CH), 115.2(CH),} \\
1498 = \text{-C=C of Ar.} & \text{116.2(CH), 127.7(CH), 128.7(CH),} & \text{136.2(CH), 136.2(CH), 137.0(CH)} \\
1591 = \text{-C-C of Ar.} & \text{130.8(CH), 130.8(CH), 130.8(CH)} \\
3331 = \text{-N-H str.} & \text{136.2(CH), 136.2(CH), 137.0(CH)} & & \\
3443 = \text{-C-OH str.} & & & \\
\hline
\end{array}
\]

\text{Compound 5h}

\[
\text{6'-}(4\text{-nitrophenyl})-3'\text{-}(\text{thiazol-2-yl})-4'\text{-thioxo-3', 4'-dihydrospiro} \\
[\text{indoline-3, 2'-[1, 3, 5] oxadiazin]}\text{-2-one}
\]

\[
\text{Molecular Formula: Elemental Analysis}
\]

\text{Department of chemistry, S.P.U}
C_{10}H_{11}N_{5}O_{4}S_{2}  
Molecular weight: 437.45 gm/mole  
Melting Point: 253-254°C (Uncorrected)  
Yield: 68%

**IR (KBr): $\nu_{\text{max}}$ (cm$^{-1}$)**
- 1631 = -C=N
- 1739 = -C=O of isatin,
- 696 = -C-S-C of thiazol
- 1168 = -C=S
- 3083 = -C-H of Ar.H
- 1510 = -C=C of Ar.
- 1610 = -C-C of Ar.
- 3335 = -N-H str.
- 1548, 1352 = -N=O of NO$_2$

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<th>%S</th>
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<tr>
<td>Found</td>
<td>52.13</td>
<td>2.56</td>
<td>16.07</td>
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**$^1$H NMR (400 MHz, DMSO-$d_6$)** $\delta_H$ (ppm):
- $\delta$ 8.88 (s, 1H, NH of isatin),
- 8.09-8.36 (m, 4H, Ph.H of phenyl ring),
- 6.75 (d, 1H, H of thiazole),
- 7.10 (d, 1H, H of thiazole),
- 7.20-7.38 (m, 4H, Ph.H of isatin)

**$^{13}$C NMR (100 MHz, DMSO-$d_6$)** $\delta_c$ (ppm):
- 112.4, 115.9, 121.5, 124.1, 127.2, 127.4, 128.5, 132.3, 136.6, 136.9, 141.3, 141.9, 150.3, 156.5, 159.0, 168.1, 178.8

**DEPT 135:** 112.4(CH), 115.9(CH), 124.1(CH), 127.2(CH), 127.4(CH), 128.5(CH), 136.6(CH), 136.9(CH)
**Fig. 6.1** The $^1$H NMR spectrum of Compound 5a

**Fig. 6.2** The $^{13}$C NMR spectrum of Compound 5a
Fig. 6.3 The FT-IR spectrum of Compound 5a

Fig. 6.4 The $^1$H NMR spectrum of Compound 5d
Fig. 6.5 The $^{13}$C NMR spectrum of Compound 5d

Fig. 6.6 The FT-IR spectrum of Compound 5d
Fig. 6.7 The $^1$H NMR spectrum of Compound 5e

Fig. 6.8 The DEPT-135 spectrum of Compound 5e
Fig. 6.9 The FT-IR spectrum of Compound 5e

Fig. 6.10 The $^1$H NMR spectrum of Compound 5h
Fig. 6.11 The DEPT-135 spectrum of Compound 5h

Fig. 6.12 The FT-IR spectrum of Compound 5h
6.5 Results and Discussion

In the present investigation, we aimed to synthesize derivatives of 6′-(4-(substituted) Phenyl)-3′-(thiazol-2-yl)-4′-thioxo-3′, 4′-dihydrospiro [Indoline-3, 2′-[1, 3, 5] oxadiazin]-2-one (5a-h) through a two step process. For this purpose, Schiff bases (3a-b) were prepared from acid catalyzed condensation of 2-aminothiazole and isatin.

6.5.1 Physical Properties and Structural Characterization of Spiro compounds

All the synthesized compounds were obtained as crystalline form. The thin-layer chromatography (TLC) results showed that only a single spot was observed for each compound. The elemental analysis was also good agreement with structure data.

6.5.2 IR Spectral Data

The IR spectra (typical in Figures 6.3, 6.6, 6.9, 6.12) showed
absorbing band at 1620-1637 cm\(^{-1}\) (HC=N of Schiff bases\(^{36}\), 1140-1186 cm\(^{-1}\) (C=S), 1713-1742 cm\(^{-1}\) (C=O of isatin)\(^{37}\), 680-710 cm\(^{-1}\) (C=S-C of thiazole), 3040-3090 cm\(^{-1}\) (C-H of Ar.), 1495-1510 cm\(^{-1}\) (C=C of Ar.), 1590-1612 cm\(^{-1}\) (C=C of Ar.), while additional bands appears due to substitution in the aromatic ring showing absorption band 3443 cm\(^{-1}\) (O-H), 2838 cm\(^{-1}\) (C-H of –OCH\(_3\)), 732 cm\(^{-1}\) (C-Cl), 578 cm\(^{-1}\) (C-Br), 3255 cm\(^{-1}\) (C-NH\(_2\) of amino phenyl), 1537 and 1352 cm\(^{-1}\) (N=O of –NO\(_2\)). So, these data are direct assignment of the proposed structure.

### 6.5.3 \(^1\)H-NMR, \(^{13}\)C-NMR and DEPT Spectral Data

The \(^1\)H-NMR spectrums (typical in **Figures 6.1, 6.4, 6.7, 6.10**) analyses of all the synthesized compounds were good agreement with proposed structure. The \(^1\)H-NMR of 2-amino thiazole showed the peak of -NH\(_2\) group appearing at \(\delta\) 6.99 ppm\(^{38}\) which was disappeared in the \(^1\)H-NMR spectra of 3-(thiazol-2-ylimino)indolin-2-one (*3a*) supporting the participation of this group in the Schiff base formation. Also it was confirmed with the help of IR spectra. The peak at \(\delta\) 8.98 ppm\(^{39}\) showed the –NH proton of isatin which further confirmed its formation. All the spectral data \(^1\)H-NMR, \(^{13}\)C-NMR of synthesized compounds (*5a*-h) were shown in experimental section. All the compounds showed the NMR signals for different kinds of protons at their respective positions. The \(^{13}\)C-NMR (**Figures 6.2, 6.5**) and DEPT-135 spectral data (**Figures 6.8, 6.11**) of all the dyes were also in good agreement with their structures.

### 6.5.4 Mass Spectra

Besides, the structure of the compound was well confirmed by its mass spectral studies. Mass spectrum of compound *5d* gave molecular ion peak at m/z 471 which was consistent with molecular weight of *5d* i.e. 471.35 corresponding to molecular formula \(\text{C}_{19}\text{H}_{11}\text{BrN}_{4}\text{O}_{2}\text{S}_{2}\). The LC-MS of compound *5d* is shown in **Figure 6.13**.
6.6 **Antimicrobial behavior of synthesized compounds**

The work incorporated is on the antimicrobial activity of various synthesized compounds in present chapter. The compounds were tested on variety of micro-organisms *viz.* gram positive and gram negative bacteria and on fungi.

**6.6.1 Introduction**

Invasion of host defense mechanism by micro-organisms leads to the onset of infections and diseases. In order to combat infection, skilled management of antimicrobial drugs is of utmost importance. Fundamental to antimicrobial therapy is an appreciation that individual species of bacteria are associated with particular infectious diseases and that specific antimicrobials are more likely to be useful.

All of our internal fluids, organs and body structures are sterile under normal circumstances and the presence of bacteria, fungi, virus etc. Micro organisms are harmful to mankind in many ways either when they come in contact and invade the tissues and cause diseases or if they find suitable conditions for their growth\textsuperscript{40-42}. Therefore one must constructively do for prevention and cure of such infectious diseases. Protection against such infection can be achieved by inhibition of microbial growth or by killing them. This can be done by using various physical agents, physical processes or chemical agents. The major physical agents or processes used for the control of microorganisms are temperature, desiccation, osmotic pressure, radiation and filtration\textsuperscript{43}. A large number of chemical compounds have the ability to inhibit the growth of metabolism of microorganisms or to kill them. Research and development in different areas of chemistry have shown that several classes of chemical substrates are used to reduce the microbial flora. In nature so many types of microorganisms are found, out of which some of the pathogenic microorganisms causing infectious diseases are shown in the following tree ([Figure 6.14]).
Fig 6.14 Pathogenic Microorganisms

6.6.1.1 Pathogens

The microorganism or infectious agent or more commonly germ is biological agents capable of producing diseases in host are known as pathogen. Pathogens have certain characteristics that they need and use, to cause disease. These so-called virulence factors have specific functions in the successive steps that result in an infection. An infection can be seen as a miniature battle between pathogen and host, the first trying to remain present and to feed and multiply, while the host is trying to prevent this. The resulting infection is a process with three possible outcomes: the host wins and the pathogen are removed so that the host can recover; the pathogen win the ultimate battle and kill their host; or an equilibrium is reached in which host and pathogen live involuntarily together and damage is minimized.
(A) Bacterial Pathogens

Bacteria that cause disease are called pathogenic bacteria. Bacteria can cause diseases in humans, in other animals and also in plants. Some bacteria can only make one particular host ill; others cause trouble in a number of hosts, depending on the host specificity of the bacteria. The diseases caused by bacteria are almost as diverse as the bugs and include infectious diseases. Bacterial cells grow and divide, replicating repeatedly to form large numbers, present during an infection or on the surfaces of the body.

In 1928, a German scientist C. E. Chrenberg first used the term “bacterium” to denote microscopic organism with a relatively simple and primitive form of the cellular organization known as “prokaryotic”. The Danish physician Christian Gram in 1884 discovered a stain known as Gram stain, which can divide all bacteria into two classes “Gram positive” and “Gram negative”. The Gram-positive bacteria resist discoloration with acetone, alcohol and remain stained as dark blue color, while Gram-negative bacteria are decolorized.

Bacteria can be classified according to their morphological character as lower and higher bacteria. The lower bacteria have unicellular structures, never in the form of sheathed filaments. The higher bacteria are filamentous organisms, having certain cells specialized for producing diseases, is known as “Pathogens”.

We have used Bacillus subtilis and Staphylococcus aureus as gram positive bacterial pathogens, Klebsiella promoe, Salmonella typhi and Escherichia coli as gram negative bacterial pathogens for antibacterial study of synthesized compounds.

(B) Fungal Pathogens

Fungi are one of the five kingdoms of life. They are plant-like organisms that lack chlorophyll. Since they don’t use light to make food, they can live in damp and dark places. Fungi are saprophytic
organism, as they grow on dead organic matter such as soil or dead plant material. Fungi are nonphotosynthetic eukaryotes growing either as colonies of single cells (yeasts) or as filamentous multicellular aggregate (molds).

The incidence of fungal infections has increased dramatically in the past 20 years. Accordingly, the increase in rates of morbidity and mortality because of fungal infections has been now recognized as a major problem. Most fungal infections are due to opportunistic pathogens; these affect people who are already ill or have a suppressed immune system, although fungi are common problems in the immune competent population as the causative agents of skin, nail or yeast infections. Fungi also cause a number of plant and animal diseases e.g. in humans, ringworm, athlete's foot and several more serious diseases are caused by fungi. Because fungi are more chemically and genetically similar to animals than other organisms, this makes fungal diseases very difficult to treat. Plant diseases caused by fungi include rusts, smuts and leaf, root and stem rots and may cause severe damage to crops. Most antibiotics that function on bacterial pathogens cannot be used to treat fungal infections due to the fact that fungi and their hosts both have eukaryotic cells.

We have used Penicillium expansum, Botrydepladia thiobromine, Nigrospora Species, Trichothesium Species and Rhizopus nigricum fungal pathogens for antifungal study of synthesized compounds.

6.6.1.1.1 Antimicrobial agents

In 1939 Gerhard Domagk, a German bacteriologist and pathologist was awarded the Nobel Prize for discovery of the first synthetic antibacterial compound “Prontosil”.

Antimicrobial agents may be either bactericidal, killing the target bacterium or fungus or bacteriostatic, inhibiting its growth. Bactericidal agents are more effective, but bacteriostatic agents can be
extremely beneficial. Antimicrobial agents may be classified according to the type of organism against which they are active i.e. antibacterial, antiviral, antifungal, antiprotozoal and anthelmintic drugs. It can also be useful to combine various antimicrobial agents for broadening the activity spectrums and to minimize the possibility of the development of bacterial resistance. Some antibiotic combinations are more effective together than the single agent. This is termed as Synergism. Combination therapy has proved its value as latest therapy for antimicrobials. Sulphamethoxazole is bacteriostatic and Trimethoprim is also bacteriostatic but combination of both the drugs is used as a bactericidal combination. Refampin along with Dapsone is used in leprosy, Refampin with Isoniazide in tuberculosis. WHO has also approved this type of combination.

Most microbiologists distinguish two groups of antimicrobial agents used in the treatment of infectious disease: antibiotics, which are natural substances produced by certain groups of microorganisms and chemotherapeutic agents. A hybrid substance is a semi synthetic antibiotic, wherein a molecular version produced by the microbe, subsequently modified to achieve desired properties. Some antimicrobial compounds, originally discovered as products of microorganisms, can be synthesized entirely by chemical means. In the medical and pharmaceutical worlds, all these antimicrobial agents used in the treatment of disease are referred to as antibiotics, chemicals that are produced by living organisms which, even in minute amounts, inhibit the growth of or kill another organism.

6.6.1.1.2 Characteristics of antimicrobial agent

a) It should have wide spectrum of activity with the ability to destroy/inhibit many different species of pathogenic organisms.
b) It should be non allergenic and nontoxic to the host and without undesirable side effects.
c) It should not eliminate the normal flora of the host.
d) It should be able to reach the part of the human body where the infection is occurring.
e) It should be inexpensive and easy to produce.
f) It should be chemically stable (have a long shelf life).
g) Microbial resistance is uncommon and unlikely to develop.
h) It must have solubility in body fluids to be active and can rapidly penetrate body tissues.

There is not a single chemical agent which is best for the control of microorganisms for any purposes. According to the application of antimicrobial agents they are classified in different groups.

6.6.1.1.3 Classification of antimicrobial agents

Antimicrobial agents may be classified as follows:

(1) Type of organism against which antimicrobial agents are active:
   ➢ Antibacterial agents : active against bacterial organisms.
   ➢ Antiviral agents : active against viral organisms.
   ➢ Antifungal agents : active against fungal organisms.
   ➢ Antiprotozoal agents : active against protozoa.

(2) According to mode of action of antimicrobial agents:
   ➢ Bacteriostatic : act primarily by arresting bacterial multiplication.
   ➢ Bactericidal : act primarily by killing bacteria.

(3) According to activity of antimicrobial agents against the range of bacteria or other organisms:
   ➢ Broad spectrum : Effective against prokaryotes which kill or inhibit a wide range of Gram positive and Gram negative bacteria.
   ➢ Narrow spectrum : Effective against Gram positive or Gram negative bacteria.
   ➢ Limited spectrum : Effective against organism or disease.
6.6.2 Antibacterial and Antifungal activity

During the last twenty years a very large number of organic compounds have been tested for their possible fungicidal and bactericidal activity. A very few of these compounds were found to be useful as plant protectants and which are universally accepted\(^\text{44}\). The lack of reliable testing methods, the progress in this field was slow. The bioassay technique should be (i) the laboratory trials must be reproducible (ii) the laboratory bioassay and the field performance.

The modern methods give reliable and reproducible results regarding protective values of a test fungicide or bactericide. The present method of the laboratory bioassay requires few milligrams of the test chemicals and screen out unsuccessful candidates by trials against specific phytopathogens. The following few conditions must be met for the screening of antimicrobial activity by modern method:

- Aseptic/sterile environment should be maintained.
- Mandatory conditions should be provided for the growth of microorganisms.
- There should be an intimate contact between test organisms and the substance to be evaluated for its activity.
- Same conditions should be maintained during the study.

Various methods have used from time to time by several worker to evaluate the antimicrobial activity\(^\text{45,46}\). The evaluation can be done by the following methods\(^\text{47}\).

- Turbidometric method.
- Agar streak dilution method.
- Serial dilution method and
- Agar diffusion method.

Further, Agar diffusion method is again of three types.

- Agar cup method
- Agar ditch method and
- Paper disc method.
6.6.2.1 Present work

In the present work all the synthesized compounds have been screened for their antibacterial and antifungal activity. The antibacterial activity has been screened against *Bacillus subtilis* and *Staphylococcus aureus* as Gram +ve bacteria and *Klebsiella promioe*, *Salmonella typhi* and *Escherichia coli* as Gram -ve bacteria and antifungal activity has been screened against *Penicillium expansum*, *Botrydepladia thiobromine*, *Nigrospora Sp.*, *Trichothesium Sp.* and *Rhizopus nigricumas*. The evaluation of antimicrobial activity has been carried out by Agar cup plate method.

6.6.3 Experimental - Antibacterial activity

6.6.3.1 The culture medium preparation

Nutrient agar medium was used. Chemical composition of the medium was,

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<th>Quantity</th>
</tr>
</thead>
<tbody>
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<td>1.0 gm</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.5 gm</td>
</tr>
<tr>
<td>Meat extracts</td>
<td>0.3 gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>100 ml</td>
</tr>
<tr>
<td>pH</td>
<td>7.6</td>
</tr>
<tr>
<td>Agar</td>
<td>2.0 gm</td>
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</table>

The ingredient were weighed and dissolved in distilled water, pH was adjusted to 7.6 and then agar power was added to it. The medium was dispensed in 25 ml quantity in different test-tubes. The test-tubes were plugged by cotton-wool and sterilized at 121.5°C and 15 pounds per square inch (psi) pressure for 15 minutes.

6.6.3.2 Antibacterial susceptibility testing

The study has been conducted according to the method adopted by Cruickshank et al48. Nutrient agar broth was melted in a water bath and cooked to 45°C with gentle shaking to bring about uniform cooling. It was inoculated with 0.5-0.6 ml of 24 hour old culture
especially and mixed well by gentle shaking before pouring on the sterilized Petri dish (25 ml each). The poured material was allowed to set (1.5 hour). After that “cups” were made by punching into the agar surface with a sterile cork borer and scooping out the punched part of agar. Into this “cups” 0.1 ml of test solution (dissolving 10gm of sample in 10ml Methanol) was added by sterile micropipette. Under similar condition, using tetracycline as a standard drug for comparison. The area of inhibition of zone measured in cm.

6.5.3.3 Factors Affecting Zone of Inhibition

a) Ingredient of culture media

Many substances are present in culture media, which may affect the zone of inhibition. Common ingredients such as peptone, agar, etc. may vary in their contents and many of these minerals may influence the activity of some antimicrobials. It is well known that Ca, Mg, Fe, etc. ions affect the sensitivity of zone produced by the tetracycline, gentamycin, NaCl reduce the activity of amino glycosides and enhances the effect of fucidin.

b) Choice of media

Consistent and reproducible results are obtained in media prepared especially for sensitivity testing; the plates must be poured flat with an even depth.

c) Effect of pH

The activity of amino glycosides is enhanced in alkaline media and reduced in acidic media, the revers is shown by tetracycline.

d) Size of inoculums

Although large numbers of organisms do not markedly affect many antibiotics, all inhibition zones are diminished by heavy inocula. The ideal inoculum is one which gives an even dense growth without being confluent. Overnight broths cultures of organisms and suitable suspensions from solid media can be diluted accurately to give optimum inoculate for sensitivity testing.
6.6.4 Results and Discussion

Tetracycline was used as standard drugs and a solvent control was also run to know the activity of solvent. The zone of inhibition was found to be a reported method which was described. The antibacterial activity of the control, standard drug and all the spiro compounds were screened for their anti-bacterial activity by using different bacterial strains such as Bacillus subtilis and Staphylococcus aureus (Gram +ve bacteria) and Klebsiella promioe, Salmonella typhi and Escherichia coli (Gram -ve bacteria) at a concentration of 50 µg/ml by agar cup plate method. All the compounds were found to be more potent against bacterial strains. The data of antibacterial activity of all the synthesized compounds 5a-h are summarized in Table 6.1. Comparative analysis showed higher antibacterial activity of many of the compounds. Some of compounds exhibited moderate activities as compared with standard drug Tetracycline (Figure 6.15).

The results showed that the prepared compounds are toxic against the bacteria. The comparison of the antibacterial activity of these compounds with Tetracycline showed good activity against the test organisms. The data in Table 6.1 indicate that most of the compounds show good biological activity. Further, when the substitutions on the phenyl ring were changed, the inhibition activity showed significant differences.

When the phenyl ring had an electron-donating group compounds show very good activity in comparison of standard drug tetracycline. The corresponding target compounds gave weaker activity. So according to that and from data we could say that compound 5c, 5e, 5h shows good antibacterial activity because in these compounds they have electron donating group such as chloro (-Cl), Nitro(-NO2), Methoxy(-OCH3) groups at 4th position of phenyl ring respectively. Other prepared compounds shows moderate activity compared to standard drugs against all five bacterial strains.
### Table 6.2 Antibacterial activity of compounds 5a-h

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Zone of inhibition mm (activity index) &lt;sup&gt;std.&lt;/sup&gt;</th>
<th>Gram +ve</th>
<th>Gram -ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>26</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>5b</td>
<td>26</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>5c</td>
<td>33</td>
<td>31</td>
<td>32</td>
</tr>
<tr>
<td>5d</td>
<td>24</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>5e</td>
<td>30</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>5f</td>
<td>22</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>5g</td>
<td>25</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>5h</td>
<td>31</td>
<td>29</td>
<td>30</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>37</td>
<td>35</td>
<td>37</td>
</tr>
</tbody>
</table>

**B.s.: Bacillus subtilis, S.a.: Staphylococcus aureus, K.p.: Klebsiella promioe, S.t.: Salmonella typhi, E.c.: Escherichia coli**

Activity index = Inhibition zone of compound / Inhibition zone of the standard drug
6.5.5 Experimental - Antifungal Activity

To test the fungicidal activity of the prepared compounds various plant pathogenic organisms were employed.

The fungicidal activity of all the compounds was studied at 1000 ppm concentration in vitro. Plant pathogenic organisms used were *Penicillium expansum, Botryodiplodia theobromae, Nigrospora Sp.*, *Trichothecium Sp.*, and *Rhizopus nigricans*. The antifungal activity of all the compounds was studied at 1000 ppm concentration in vitro. Plant pathogenic organisms used were *Penicillium expansum, Botryodiplodia theobromae, Nigrospora Sp., Trichothecium Sp., and Rhizopus nigricans*. The antifungal activity of all the compounds was measured on each of these plant pathogenic strains on a potato dextrose agar (PDA) medium such a PDA medium contained potato 200 gm., dextrose 20 gm., agar 20 gm., and water 1 liter. Five days old cultures were employed. The compounds to be tested were suspended (1000 ppm) in a PDA medium and autoclaved at 120 °C for 15 min and at 15 atm. pressure. These media were poured into sterile petri plates and the organisms were inoculated after cooling the petri plates. The percentage inhibition for fungi was calculated after five days using the formula given below.

**Percentage of inhibition = 100(X-Y)/X**

Where, X = Area of colony in control plate.

Y = Area of colony in test plate.

The fungicidal activity displayed by various compounds is shown in Tables 6.2.

6.5.6 Results and Discussion

Amphotericin B was used as standard drugs and a solvent control was also run to know the activity of solvent. The zone of inhibition was found to be a reported method which was described. The antifungal activity of the control, standard drug and all the spiro compounds were screened for their antifungal activity using different fungi such as *penicilium expansum, Botrydepladia thiobromine, Nigrospora Species, Trichothesium Species and Rhizopus nigricum*. All the compounds were found to be more potent against fungi.
data of antifungal activity of all the synthesized compounds (5a-h) are summarized in Tables 6.2. Comparative analysis showed higher antifungal activity of many of the compounds. Some of compounds exhibited moderate activities as compared with standard drug Amphotericin B (Figure 6.16).

All the target compounds were screened for antifungal activities in vitro at 1000ppm concentration. The comparison of the antifungal activity of these compounds with Amphotericin B showed good activity against the test organisms. As shown in Table 6.2, some of the compounds displayed sensible antifungal activities. Introducing different substituted groups at the phenyl ring could enhance the fungicidal activities. As far as the relation between structure and activity are concerned the chloro, methoxy and nitro substituted compounds were found to display enhanced activity than the other substitution. This enhancement in antibacterial activity is rationalized. So according to that and from data we can say that compound 5c, 5e, 5h showed good antifungal activity because in these compounds they have electron donating group such as chloro (-Cl), Nitro(-NO₂), Methoxy(-OCH₃) groups at 4th position of phenyl ring respectively. Other prepared compounds showed moderate activity compared to standard drugs against all five fungi.
Table 6.3 Antifungal activity of compounds 5a-h

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Zone of inhibition mm (activity index)\textsuperscript{std.}</th>
<th>P.e.</th>
<th>B.t.</th>
<th>N.s.</th>
<th>T.s.</th>
<th>R.n.</th>
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<td></td>
<td></td>
<td>(0.57)</td>
<td>(0.62)</td>
<td>(0.64)</td>
<td>(0.69)</td>
<td>(0.64)</td>
</tr>
<tr>
<td>5b</td>
<td></td>
<td>22</td>
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<td>23</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.62)</td>
<td>(0.71)</td>
<td>(0.67)</td>
<td>(0.75)</td>
<td>(0.64)</td>
</tr>
<tr>
<td>5c</td>
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<td>30</td>
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<td></td>
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<td>(0.87)</td>
<td>(0.79)</td>
<td>(0.90)</td>
<td>(0.91)</td>
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<tr>
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<td>(0.68)</td>
<td>(0.68)</td>
<td>(0.61)</td>
<td>(0.72)</td>
<td>(0.73)</td>
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<tr>
<td>5e</td>
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<td>25</td>
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<tr>
<td></td>
<td></td>
<td>(0.80)</td>
<td>(0.84)</td>
<td>(0.73)</td>
<td>(0.81)</td>
<td>(0.79)</td>
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<tr>
<td>5f</td>
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<tr>
<td></td>
<td></td>
<td>(0.65)</td>
<td>(0.65)</td>
<td>(0.61)</td>
<td>(0.60)</td>
<td>(0.64)</td>
</tr>
<tr>
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<tr>
<td></td>
<td></td>
<td>(0.54)</td>
<td>(0.59)</td>
<td>(0.58)</td>
<td>(0.57)</td>
<td>(0.58)</td>
</tr>
<tr>
<td>5h</td>
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<tr>
<td></td>
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<td>(0.85)</td>
<td>(0.87)</td>
<td>(0.76)</td>
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<td>(0.88)</td>
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<td>Amphotericin B</td>
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<td>32</td>
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</tbody>
</table>

\textbf{P.e.:} *Penicillium expansum*, \textbf{B.t.:} *Botrydepladia thiobromine*, \textbf{N.s.:} *Nigrospora species*, \textbf{T.s.:} *Trichothesium species*, \textbf{R.n.:} *Rhizopus nigricum*
Fig. 6.15 Comparative analysis of antibacterial activity of compounds 5a-h

Fig. 6.16 Comparative analysis of antifungal activity of compounds 5a-h
References