CHAPTER-4

MICROWAVE ASSISTED SYNTHESIS
AND SPECTRAL STUDIES OF
PHENOTHIAZINES
This chapter highlights a new environmental benign method for synthesis of phenothiazine derivatives using microwave irradiation. A mixture of o-substituted aromatic amine and p-substituted phenol converted into biphenyl amine derivative using microwave irradiation. Further, thionation of biphenyl amines under same irradiation had provided the title phenothiazine derivatives.

All the synthesized title compounds have screened for their antimicrobial activity and their structure confirmed by spectral as well as other analytical data.
The subject of drugs is as old as disease. Drug designing is a continuous and iterative process, which starts with some lead molecule of interesting biological profile and ends with the optimization of this lead, leading for the selection of candidate molecule for drug development. In last few decades a considerable amount of attention has been focused on synthesis of nitrogen and sulfur containing phenothiazines. The investigation of substituted 10H-phenothiazines has steadily flourished because they exhibit a large scope of applications. These moieties are widely employed as antibacterial, antiviral, anti-inflammatory, anticancer, sedatives, tranquilizers agents etc. Slight change in substitution pattern in phenothiazine nucleus causes distinguishable difference in their biological activities\(^1\)\(^-\)\(^4\).

Phenothiazine is an aromatic electron-rich amine which has long been used as an insecticides\(^5\). Phenothiazine ring system shows various pharmacological activities and generally these derivatives are known as active adrenergic blocking agents. They are especially used as
antipsychotic drugs. In veterinary medicine, these are effective against a wide range of parasitic insects in animals which provides food for human.

Depending on the substitutions, phenothiazine and its derivatives show different biological and pharmacological activities such as antiinflammatory, analgesic, anti-tubercular, anti-HIV, antipsychotropic, anticonvulsant, cardiovascular, antiviral, antimicrobial, antifungal, anti-tumor, multidrug resistance, anti-depressant, tranquilizer, antihistanimics, anthelmintics, local anesthetics, antiseptic, CNS depressant, ulcer inhibitors, antiparasitic, antiparkinson, anticarcinogenic and antioxidant.

The mechanism for the variety of therapeutic activity believed due to the presence of a fold along the nitrogen-sulfur axis.

Various methods reported in the literature for the preparation of phenothiazines are discussed here.

(1) **THIONATION OF DIPHENYLAMINES**

Phenothiazines have been synthesized by refluxing substituted diarylamines with sulfur in xylene or o-dicholobenzene in presence of catalyst (iodine/aluminium chloride) (scheme 4.1).
When thionation was carried out by using thionyl chloride in place of sulfur, polychlorophenothiazines were obtained. The thionation with sulfur monochloride was accompanied by nuclear chlorination.

This method suffers from some limitations. The thionation of ortho/para substituted diarylamines provides 1/3-substituted phenothiazine respectively but meta substituted diarlylamines yield a mixture of 2- and 4-substituted phenothiazines (scheme 4.2).
Moreover diarylamines having halo group undergo dehalogenation during the course of thionation. In addition, some diarylamines failed to undergo thionation\textsuperscript{64-66}.

(2) CYCLIZATION OF DIPHENYLSULFIDES

Synthesis of phenothiazines was carried out by cyclization of diphenylsulfides using Ulmann reaction which involves dehydrohalogenation of substituted 2-amino-2-halodiphenyl sulfides in the presence of copper as catalyst (scheme 4.3). During the course of cyclization, the halogen bearing ring may invert itself to yield phenol-thiazines\textsuperscript{67-68}. Therefore, such precursors were selected which avoids anomalous consequences (scheme 4.3).

\[
\begin{align*}
\text{NH}_2 & \quad \text{Cu} \\
\text{X} & \quad \Delta
\end{align*}
\]

(Scheme: 4.3)

(3) SMILES REARRANGEMENT

This method has been widely used for the synthesis of phenothiazines\textsuperscript{69-71}. It involves the migration of an aromatic ring form one hetero atom to another by intramolecular nucleophilic aromatic substitution reaction\textsuperscript{72} (scheme 4.4).
Smiles rearrangement is a base catalyzed reaction which involves the removal of proton from -YH converting it to \(-Y^-\) followed by the nucleophilic attack of the resulting \((-Y^-)\) on electrophilic carbon atom (*) which then displaces \(Z^-\) from the ring B in its anionic form. The mechanism of Smiles rearrangement is governed by the following factors\(^{73-75}\).

(Scheme: 4.4)

\(-YH= -OH, -SH, -NHR, -CONHR\) or \(SO_2NHR\)

\(-Z = O, S, SO\) or \(SO_2\)

(a) The convenience of rearrangement depends upon the tendency of \(-YH\) to lose a proton to the medium. By increasing the alkalinitity, the rate of the reaction accelerates. The speed of the rearrangement in alcoholic solvents follow the order:

\(NaOH > CH_3ONa > C_2H_5ONa > (CH_3)_2-CHONa\)
(b) The electron donating capability of $Y^-$ also affect the Smiles rearrangement. The ease of rearrangement is decreased when $YH$ is varied in the following order.

$\text{NHAc} > \text{NH}_2 > \text{OH} > \text{SH}$

(c) The presence of electron withdrawing groups such as nitro at ortho or para-position to the electrophilic carbon atom (*) in ring B enhance its electrophilic character and facilitates the attack of nucleophile (-$Y^-$).

(d) The replacibility of –Z from ring B depends upon the relative positive character (electropositivity) of Z as compared to -$YH$ or -$Y^-$ affects the rearrangement, e.g. compound having -$YH$ as $\text{-SO}_2$, $\text{-SO}$ or $\text{-S}$ undergoes Smiles rearrangement easily while the compounds with -$YH$ as $\text{-OH}$ (phenolic), the rearrangement occurs only when -$Z^-$ is $\text{-SO}_2$ or $\text{-SO}$ but not when -$Z^-$ is $\text{-S}$.

For the rearrangement to be facile, it is necessary that the group Z should be more electropositive and $YH$ should be sufficiently acidic to yield strong nucleophile. The substituents as $\text{-NHCHO}$, $\text{-NHCOC}_6\text{H}_4\text{NO}_2$-$2$, $\text{-NHC}_6\text{H}_2(\text{NO}_2)_3$-$2,4,6$, $\text{-NHSO}_2\text{C}_6\text{H}_5$, $\text{-NHCH}_3$ which enhance the acidity of -$YH$ group may decrease the nucleophilicity of
the anion -Y and the net effect on the rearrangement must be the resultant of these two counter balancing effects. The diphenyl sulfides with -NHCHO, -NHCOCH$_3$, -NHCOC$_6$H$_4$NO$_2$-2, undergo Smiles rearrangement whereas the diphenyl sulfides with -NHC$_6$H$_2$(NO$_2$)$_3$, 2,4,6, -NHSO$_2$C$_6$H$_5$ fail to do so. Although they are sufficiently acidic to ionize, Smiles rearrangement does not take place because the resulting anions are too weak nucleophiles to affect the rearrangement. Due to insufficient acidic character of methyl group, the diphenyl sulfides with -NHCH$_3$ also do not rearrange under Smiles conditions.

Under present investigation some known phenothiazine derivatives were synthesized by conventional method involving Smiles rearrangement. Further, we have made efforts to synthesize these compounds by microwave assisted operations in varying experimental conditions to optimize the method.

Generally, phenothiazine derivatives are being synthesized in six steps through conventional method involving Smiles rearrangement. In first three steps we get 2-aminobenzenethiol from aromatic amines and further three steps are required for synthesis of phenothiazines. Therefore, the conventional method is laborious, lengthy, time consuming and low yield providers with respect to starting material, aromatic amines.
Therefore, efforts were made to design a new environmentally benign method for synthesis of bioactive title compounds to make available these compounds for pharmacological aspects. Finally, we get a new microwave assisted method for synthesis of substituted phenothiazine derivatives. The reaction yield of final product improved drastically with respect to the initially used amines as compared to the conventional Smiles rearrangement and the reaction time lowered.

**EXPERIMENTAL**

The melting point of synthesized compounds has not been corrected and their purity was checked by TLC (thin layer of silica gel) in various non-aqueous solvent systems. Infrared (IR) spectra of all the synthesized compounds recorded using KBr from Shimadzu FTIR, Affinity-1. The NMR spectra has taken by Varian Gemini 400 spectrometer (300MHz) using TMS as an internal standard. The reactions carried out in domestic microwave oven. Elemental data obtained from all the synthesized compounds found satisfactory

1. **Synthesis of Biphenyl Derivatives**

An equimolar mixture of o-substituted aniline (I) and p-substituted phenol (II) taken in a flat bottomed flask fitted with a suitable reflux condenser was dissolved in minimum quantity of absolute ethanol and a
catalytic amount of zinc chloride added. The reaction mixture so obtained in flask irradiated in microwave oven intermittently at 30 seconds for 5-6 minutes. After completion of reaction as monitored by TLC, the solid separated out was filtered and washed with distilled water. The crude product dried in vacuum and recrystallized from ethanol (scheme 4.5).

2. Synthesis of Substituted Phenothiazines

A well grinded mixture of biphenyl derivatives (III) (10 mmol), sulphur (20 mmol) and iodine (1% weight of reaction mixture) was subjected to microwave irradiation intermittently at 30 seconds for 8-10 minutes. The colored solid separated out was washed repeatedly with distilled water followed by small amount of alcohol. The crude product was dried and recrystallized from methanol (scheme 4.5). These compounds have also been synthesized by conventional method as described by Gupta et al. to compare the physicochemical data.
In present investigation following Phenothiazines have been synthesized:

(1) 7-Bromo-1-methylphenothiazine
(2) 7-Carboxy-1-methylphenothiazine
(3) 1-Methyl-7-nitrophenothenothiazine
(4) 7-Trifluromethyl-1-methylphenothiazine
(5) 1-Chloro-7-nitrophenothenothiazine
(6) 7-Carboxy-1-chlorophenothenothiazine
(7) 1-Chloro-7-trifluromethylphenothiazine
(1) **7-Bromo-1-methylphenothiazine**

Molecular Formula : $\text{C}_{13}\text{H}_{10}\text{N} \text{SBr}$

Yield : 71.5%, M.P. 214°C

IR (KBr, $v_{\text{max}}$, cm$^{-1}$): 3380 (N-H), 1490, 1380 (C-H), 750 (C-Br)

$^1\text{H NMR (CDCl}_3)$ $\delta$: 8.10 (1H, s, NH), 2.00 (3H, s, CH$_3$), 7.25 (6H, m, Ar-H).

Anal. Calculated (%) for: C$_{13}$H$_{10}$NSBr: C, 53.89; H, 3.41; N, 4.83. Found (%): C,53.42; H , 3.42; N, 4.79.

(2) **7-Carboxy-1-methylphenothiazine**

Molecular Formula : $\text{C}_{14}\text{H}_{11}\text{N} \text{SO}_2$

Yield : 62%, M.P. 153°C

IR (KBr, $v_{\text{max}}$, cm$^{-1}$): 3395 (N-H), 1470, 1375 (C-H), 1690 (C=O).

$^1\text{H NMR (CDCl}_3)$ $\delta$: 8.15 (1H, s, NH), 1.90 (3H, s, CH$_3$), 9.80 (1H, s, COOH), 7.10 (6H, m, Ar-H).
Anal. Calculated (%) for C_{14}H_{11}NSO_2: C, 65.45; H, 4.30; N, 5.46.

Found (%): C, 65.36; H, 4.28; N, 5.44.

(3) **1-Methyl-7-nitrophenothiazine**

Molecular Formula: C_{13}H_{10}N_{2}SO_2

![Molecular Structure of 1-Methyl-7-nitrophenothiazine](image)

Yield: 58%, M.P. 172°C

IR (KBr, v_max, cm^{-1}): 3340 (N-H), 1530, 1520 (NO_2), 1485, 1375 (C-H).

^1H NMR (CDCl_3) δ: 8.50 (1H, s, NH), 2.10 (3H, s, CH_3), 7.40 (6H, m, Ar-H).

Anal. Calculated (%) for C_{13}H_{10}N_{2}SO_2: C, 60.20; H, 3.88; N, 10.82. Found (%): C, 60.46; H, 3.87; N, 10.85.

(4) **7-Trifluoromethyl-1-methylphenothiazine**

Molecular Formula: C_{14}H_{10}NSF_3

![Molecular Structure of 7-Trifluoromethyl-1-methylphenothiazine](image)

Yield: 54.5%, M.P. 167°C
IR (KBr, $\nu_{\text{max}}$, cm$^{-1}$): 3370 (N-H), 1460, 1370 (C-H), 1330, 1115 (C-F).

$^1$H NMR (CDCl$_3$) $\delta$: 8.3 (1H, s, NH), 2.5 (3H, s, CH$_3$), 7.3 (6H, m, Ar-H).

Anal. Calculated (%) for C$_{14}$H$_{10}$NSF$_3$: C, 59.40; H, 3.59; N, 4.95. Found (%): C, 59.78; H, 3.56; N, 4.98.

(5) 1-Chloro-7-nitrophenothiazine

Molecular Formula: C$_{12}$H$_7$N$_2$SClO$_2$

Yield: 68%, M.P. 202$^0$C

IR (KBr, $\nu_{\text{max}}$, cm$^{-1}$): 3365 (N-H), 1530, 1525 (NO$_2$), 725 (C-Cl).

$^1$H NMR (CDCl$_3$) $\delta$: 8.5 (1H, s, NH), 7.4 (6H, m, Ar-H).

Anal. Calculated (%) for C$_{12}$H$_7$N$_2$SClO$_2$: C, 51.22; H, 2.53; N, 9.96. Found (%): C, 51.70; H, 2.51; N, 10.05.

(6) 7-Carboxy-1-chlorophenothiazine

Molecular Formula: C$_{13}$H$_8$NSClO$_2$
Yield: 69.5%, M.P. 195°C

IR (KBr, $\nu_{\text{max}}, \text{cm}^{-1}$): 3370 (N-H), 1685 (C=O), 735 (C-Cl).

$^1$H NMR (CDCl$_3$) $\delta$: 8.30 (1H, s, NH), 9.60 (1H, s, COOH) 7.35 (6H, m, Ar-H).

Anal. Calculated (%) for C$_{13}$H$_8$NSClO$_2$: C, 55.80; H, 2.86; N, 5.01. Found (%): C, 56.21; H, 2.88; N, 5.04.

(7) 1-Chloro-7-trifluoromethylphenothiazine

Molecular Formula: C$_{13}$H$_7$NSClF$_3$

Yield: 56%, M.P. 159°C

IR (KBr, $\nu_{\text{max}}, \text{cm}^{-1}$): 3365 (N-H), 1320, 1110 (C-F), 720 (C-Cl).

$^1$H NMR (CDCl$_3$) $\delta$: 8.5 (1H, s, NH), 7.30 (6H, m, Ar-H).

Anal. Calculated (%) for C$_{13}$H$_7$NSClF$_3$: C, 51.43; H, 2.31; N, 4.65. Found (%): C, 51.74; H, 2.32; N, 4.64.

Spectral Analysis

The IR spectra of all the synthesized phenothiazine derivatives showed a sharp peak in the region 3325-3395 cm$^{-1}$ corresponding to the NH stretching vibrations. Two bands observed in the region 1530-1535
cm⁻¹ in case of compound 3 and 5 have ascribed to the asymmetric and symmetric valence vibrations of nitro group at position-7. Phenothiazines (5, 7) having chloro group at position -1 have exhibited a sharp intense peak corresponding to chloro group in the region 740-720 cm⁻¹. Spectra of 1-methyl phenothiazines (1 to 4) have sharp bands in the region 1490-1450 cm⁻¹ and 1380-1375 cm⁻¹ have assigned to C-H deformation vibrations of CH₃ group. Peaks corresponding to deformation vibrations of CF₃ in compounds 4, 7 observed in the region 1330-1320 and 1140-1125 cm⁻¹. Sharp band corresponding to carbonyl group in compound 2 and 6 has also been observed in the region 1690-1685 cm⁻¹.

NMR spectral studies have also extended to synthesized title compounds. These studies reveal that NMR spectra of the compounds are in accordance with their structures. A singlet peak observed in the region 8.6-8.2 δ ascribed to N-H proton at position-10 in all the phenothiazines. Peaks corresponding to aromatic protons in all compounds and methyl protons in case of compound (1 to 4) have observed in the region 7.6-7.2δ and 2.7-2.2 δ respectively.
THIN LAYER CHROMATOGRAPHIC STUDIES OF PHENOTHIAZINES

For the separation and identification, we have extended thin layer chromatographic studies to substituted phenothiazines in various non-aqueous solvent system.

Experimental

Standard thin layer chromatographic equipment (plates, atomizer, applicators with fixed thickness and developing tanks) obtained from M/s Adair Dutt and Co., New Delhi. Solvents were dried over sodium wire.

Chromatographic Procedure

The same type of procedure has been adopted as discussed in chapter 3rd A.

Result and Discussion

The major part of the chromatographic studies is an attempt to obtain the standard conditions for their resolution and identification. Those solvents, which gave the best shaped, have been selected. Selected solvent systems are: \( S_1 = \text{Acetone: carbon tetrachloride (25:75 V/V)} \), \( S_2 = \text{Acetone: petroleum ether (40-60^0C) (25:75 V/V)} \), \( S_3 = \text{Acetone: Benzene (20:80 V/V)} \) and \( S_4 = \text{Acetone: petroleum ether (40-60^0C) (30:70 V/V)} \) In acetone with or without benzene the spots run along
with the solvent front. It was observed that silica gel G washed with chloroform gave much better and reproducible Rf values than when used unwashed. The saturation of developing tank with solvent vapours is essential in order to get reproducible results and tailless spots. The results are tabulated in table-3.

The Rf values of phenothiazines are summarized in table-3.

![Phenothiazine structure](image)

**Table No. 3**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compounds</th>
<th>M.P. °C</th>
<th>Rf X 100</th>
<th>Spot Color</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>R&lt;sub&gt;1&lt;/sub&gt;</td>
<td>System S&lt;sub&gt;1&lt;/sub&gt;</td>
<td>System S&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>1</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Br</td>
<td>214</td>
<td>67.5</td>
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<tr>
<td>2</td>
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<td>COOH</td>
<td>153</td>
<td>68.5</td>
</tr>
<tr>
<td>3</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>NO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>172</td>
<td>72.3</td>
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<tr>
<td>4</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>CF&lt;sub&gt;3&lt;/sub&gt;</td>
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</tr>
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<td>5</td>
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<td>202</td>
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<tr>
<td>6</td>
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<tr>
<td>7</td>
<td>Cl</td>
<td>CF&lt;sub&gt;3&lt;/sub&gt;</td>
<td>159</td>
<td>71.2</td>
</tr>
</tbody>
</table>

S<sub>1</sub> = Acetone: carbon tetrachloride (25:75 V/V)
S<sub>2</sub> = Acetone: petroleum ether (40-60ºC) (25:75 V/V)
S<sub>3</sub> = Acetone: Benzene (20:80 V/V)
S<sub>4</sub> = Acetone: petroleum ether (40-60ºC) (20:80 V/V)
ANTIMICROBIAL ACTIVITIES

All the synthesized phenothiazine derivatives were screened in vitro applying agar plate diffusion technique for antibacterial activity against Escherichia coli (Gram negative), and Bacillus subtilis (Gram positive) at both 25 µg/ml, 50 µg/ml concentration. Under same conditions, the Chloramphenicol with 25µg/ml has shown a zone of inhibition 26 mm for gram-negative organisms and Vancomycin with 25µg/ml showed a zone of inhibition 24 mm for gram-positive organism. The antifungal screening of the synthesized phenothiazine derivatives were carried out in vitro by paper disc method against Aspergillus niger and Canadida albicans at 25 µg/ml and 50 µg/ml concentrations by using Griseofulvin at 25 µg/m concentrations as the positive control which has shown zone of inhibition 27 mm and 25.5 mm respectively under same conditions. Antimicrobial activities of synthesized compounds have summarized in table No. 4.
Table No. 4: Antimicrobial activities

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. Coli</em> (Gram Negative)</td>
<td><em>Bacillus subtilis</em> (Gram positive)</td>
</tr>
<tr>
<td></td>
<td>25 µg/mL</td>
<td>50 µg/mL</td>
</tr>
<tr>
<td>1.</td>
<td>08.60</td>
<td>12.48</td>
</tr>
<tr>
<td>2.</td>
<td>06.98</td>
<td>11.01</td>
</tr>
<tr>
<td>3.</td>
<td>07.12</td>
<td>12.90</td>
</tr>
<tr>
<td>4.</td>
<td>13.72</td>
<td>16.89</td>
</tr>
<tr>
<td>5.</td>
<td>08.68</td>
<td>11.96</td>
</tr>
<tr>
<td>6.</td>
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</tr>
<tr>
<td>7.</td>
<td>14.73</td>
<td>17.49</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>25.99</td>
<td>-</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>-</td>
<td>-</td>
</tr>
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</table>
Fig. 7: IR Spectra of Phenothiazine
REFERENCES


49. C. S. Weil, Biometrics, 8, 249 (1952).
60. F. Ackermann, Ger. Pat., 222, 879, Frdl., 10, 144 (1911).