CHAPTER
FOURTH
SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 4-AMINO-5-ARYL-1,2,4-TRIAZOLES

1,2,4-triazoles and its derivatives are widely used in the field of medicine, agriculture and industry. Several derivatives of 1,2,4-triazole have been reported as bactericides, fungicides, anticomulants and anti-inflammatory activities.

The scientific literature also states that the antibacterial activities of thiourea derivatives are due to the presence of the \(-\text{NH-C(S)-NH-}\) function in the molecule and the changes in this activity depend on the nature of its substituent’s. These observations prompted us to synthesize some new triazoles and to investigate their antibacterial activities.

EXPERIMENTAL

General

The melting point of synthesized compounds was determined in open glass capillaries containing liquid paraffine and is uncorrected. The IR spectra of compounds were recorded in KBR and FTIR spectrophotometer.
The $^1$HNMR was recorded on Brucker 300 MHz instrument in DMSO/CDCl$_3$ using TMS as an internal standard.

**Synthesis of derivatives**

(i) **Synthesis of methyl esters of acids (1a-f)**

There were synthesized by esterification of isonicotinic acid, benzoic acid, 1-naphthyl acetic acid, trichloro acetic acid, phenyl acetic acid and salicylic acid respectively using excess methanol is the presence of sulphuric acid$^{89}$. 

(ii) **Synthesis of hydrazides of acids (2a-f)**

These were prepared by the reaction of the corresponding methyl esters (1a-f) with hydrazine hydrate$^{90-92}$. 

(iii) **Synthesis of potassium salts of substituted dithio carbazinic acid (3a-f)**

A mixture of (2a-f) ) (0.01 mol), CS$_2$ (0.15 mol) and KOH (0.15 mol) in absolute ethanol (350 ml) was heated under the reflux for 10 hrs, cooled to room temperature and diluted with dry ether (200 ml). The precipitate, that appeared was filtered, washed with 2×50 ml of ether and vacuum dried.
(iv) Synthesis of 4-Amino-5-Aryl-1,2,4-triazoles (4a-f)

To a suspension of (3a-f) (0.002 mol), hydrazine hydrate (0.04 mol) and water (4 ml) were added and the mixture was refluxed with stirring for several hours, until the evolution of H₂S has ceased. After dilution with water (100 ml) and the acidification with HCl, the precipitates were filtered, washed with 2×30 ml of water and re-crystallized from ethanol water.

The reaction scheme is given in fig.1. The melting points, yields and elemental analysis of these compounds are given in table-1.

(4a) IR (cm⁻¹) : 1240 (C=S), 1558 (C=N), 1160 (C=N), 730 (C-H).

¹HNMR (δ) : 7.2-7.28 (4H, s, aromatic), 8.9-8.92 (1H, s, NH), 2.9-2.92 (2H, s, NH₂).

(4b) IR (cm⁻¹) : 1244 (C=S), 1521 (C=N), 1140 (C=N), 2925 (NH₂ Str).

¹HNMR (δ) : 7.8-7.9 (5H, s, aromatic), 7.7-7.72 (1H, s, NH), 4.32-4.34 (2H, s, NH₂).

(4c) IR (cm⁻¹) : 1240 (C=S), 1519 (C=N), 1130 (C=N), 3053 (NH₂), 2920 (CH₂).

¹HNMR (δ) : 8.0-8.02 (7H, s, aromatic), 7.7-7.72 (H, s, NH), 4.32-4.34 (2H, s, NH₂), 2.40-2.42 (2H, m, CH₂).
(4d) IR (cm⁻¹) : 778.81 (C–Cl), 1268 (C=S), 1598 (C=N), 1138 (C–N), 3057 (NH₂), 2916 (NH).

¹H NMR (δ) : 7.9–7.92 (1H, s, NH), 4.02-4.1 (2H, s, NH₂).

(4e) IR (cm⁻¹) : 1250 (C=S), 1499 (C=N), 1120 (C–N), 3250 (NH₂), 2900 (CH₂).

¹H NMR (δ) : 7.9–7.92 (5H, s, aromatic), 4.30-4.32 (2H, s, NH₂), 9.0-9.02 (1H, s, NH), 2.22-2.24 (2H, m, CH₂).

(4f) IR (cm⁻¹) : 1252 (C=S), 1500 (C=N), 1125 (C–N), 3250 (NH₂), 2910 (OH, NH str).

¹H NMR (δ) : 8.02–8.04 (4H, s, aromatic), 9.0-9.02 (1H, s, OH), 9.4-9.42 (s, NH), 4.02-4.08 (2H, s, NH₂).

**Biological evaluation**

The cup-plate method was performed using nutrient agar both. The agar media was inoculated with 0.5mL of the 24 hrs liquid culture containing 10⁷ micro organisms/mL. Plates discs saturated with solution of each compound (conc. 10 mg/mL in DMSO) were placed on the indicated agar medium. The incubation time was 24 hrs at 36°C to 37°C for *Staphylococcus aureus*, *Bacillus subtilus*, *Echerichia coli* and *Salmonella enteritidis*. Inhibitory activity was measured (in mm) as the diameter of the observed
inhibition zones. The tests were repeated to confirm the findings and the average of the reading was taken into consideration.

**Antibacterial activity**

The cup plate method was employed for the in vitro study of antibacterial effects against *S. aureus, B. Subtilus, E. coli and S. enteridis*. The method was based on diffusion of antibacterial compound from reservoir nutrient agar medium such that the growth of micro-organism is inhibited as circular zone around the bore. The inhibitory effects of compounds (4a-f) against these organisms are given in table-2. The screening results indicate that not all compounds exhibited antibacterial activities. It can be noted that (4b) and (4e) showed the greatest inhibitory effect against one or more types of bacteria as compared to other aryl derivatives, and also show average effect on *S. aureus* along with (4e) and (4f) showed poor effect against *E. coli* (4a) exhibited similar action against *E. coli, S. enteridis* and *S. aureus* but not showed any effect against *B. subtilus*. 4(b) showed poor inhibitory zone against *E. coli* and *B. subtilus*. 4(b) showed poor inhibitory zone against *B. subtilus* and *S. aureus* but not shown any inhibitory zone against *E. coli* and *S. enteridis*. 4(d) exhibits the average effect against *E. coli* and good effect against other organism but exhibits no inhibitory zone against *S. enteridis* and *S. aureus*. 4(e) was found to effective against all bacteria taken for study. This may be due to the
phenyl ring and NH₂ group at 4-position. 4(e) showed the similar effect with E. coli, B. Subtilus and S. aureus. 4(f) showed the similar effect with poor inhibition zone against E. Coli and S. enteritidis and no inhibition against B. subtilus and S. aureus.

RESULTS AND DISCUSSION

The aim of this work was to synthesize 4-amino-5-aryl-1,2,4-triazole (as per reaction scheme given in fig.-1). In order to achieve this aim, it was necessary to first synthesize esters (1a-f) of some acids like isonicotinic acid, benzoic acid, 1-naphthyl acetic acid, trichloroacetic acid, phenyl acetic acid and salicylic acid, respectively (a-f). Esters were prepared by the reaction of methyl alcohol in presence of sulphuric acid. After esterifications, hydrazides (2a-f) were prepared. The next step was the conversion of the derivatives (2a-f) into the corresponding 4-amino-5-aryl-1,2,4-triazoles. The purity of the isolated compound was checked by T.L.C. in different solvents at different stages.

When (2a-f) was refluxed in ethanol with CS₂ and KOH the corresponding potassium salts of the substituted dithiocarbazinic acid (3a-f) were obtained. The structures of compounds (3a-f) were established by their IR and ¹H NMR spectra. The IR absorption due to the C=O and C=S functions appeared at 1660-1600 cm⁻¹ and 1280–1240 cm⁻¹, respectively. The absorption bands associated with other functions group appeared to be at
the expected region. The $^1$HNMR spectra of compounds (3a-f) (in DMSO- $d_6$) exhibited a multiplet in the aromatic region at 6.83-7.91 ppm.

Three or four fields singlets were observed at the 8.11-8.96 ppm region representing the protons of the OH group and the NH (thiosemicarbazide moiety), due to strong deshieldings effect of the aromatic ring system and the thio carbonyl group. The $^1$HNMR spectra of (3a-f) also exhibited the CH$_2$ and CH-signals of all the allyl group of multiplets and doublets between 4.9 and 5.83 ppm.

Further, the potassium salts upon reaction with hydrazine hydrate yielded the corresponding 4-Amino-5-Aryl-1,2,4-triazoles (4a-f). All the compounds prepared were novel. The melting points, yields and elemental analysis of these compounds are given in table-1. The structures of (4a-f) were established by their IR and $^1$HNMR spectra. IR spectra also showed a band in the 1266-1249 cm$^{-1}$ region due to C=S function, further supporting the predominance of the thione form in the solid state and the polar solvents.$^{50,91}$

The fact that the compound exists in thion-thiol tautomeric equilibrium is supported by the absence of characteristics (SH) absorption bands in the IR spectra. The IR spectra of the compound (4a-f) showed characteristics bands around 3306-3152 cm$^{-1}$ (OH and NH-stretch), 3103-2955 cm$^{-1}$ (C–H from Ar–H stretch), 2972-2788 cm$^{-1}$ (C–H from CH$_2$
stretch) 1626-1599 cm\(^{-1}\) (C=C), 1583-1514 cm\(^{-1}\) (C=N), 1534-1480 cm\(^{-1}\) (N–H).

Table-1

<table>
<thead>
<tr>
<th>Comp</th>
<th>R</th>
<th>Mol. Formula (Mol. wt)</th>
<th>M.P. (°C)</th>
<th>Yield</th>
<th>Elemental Analysis Calcd./Found</th>
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<tr>
<td>(4a)</td>
<td>C(_5)H(_4)N</td>
<td>C(_2)H(_2)N(_2)S (193.2)</td>
<td>198-9</td>
<td>56.17</td>
<td>C 43.47  H 3.62  N 36.23</td>
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<tr>
<td>(4b)</td>
<td>C(_6)H(_5)</td>
<td>C(_8)H(_8)N(_4)S (192.2)</td>
<td>202-3</td>
<td>69.60</td>
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<tr>
<td>(4c)</td>
<td>C(_{13})H(_9)</td>
<td>C(<em>{13})H(</em>{12})N(_4)S (256.3)</td>
<td>209</td>
<td>72.02</td>
<td>C 60.86  H 4.68  N 21.84</td>
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<td>(4d)</td>
<td>CCl(_3)</td>
<td>C(_3)H(_3)N(_4)Cl(_3)S (233.5)</td>
<td>223</td>
<td>30.3</td>
<td>C 15.41  H 1.28  N 23.98</td>
</tr>
<tr>
<td>(4e)</td>
<td>C(_7)H(_7)</td>
<td>C(_9)H(_10)N(_4)S (206.12)</td>
<td>205-6</td>
<td>52.4</td>
<td>C 52.39  H 4.85  N 27.16</td>
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<tr>
<td>(4f)</td>
<td>C(_6)H(_5)O</td>
<td>C(_8)H(_8)N(_4)OS (208.17)</td>
<td>218</td>
<td>35.42</td>
<td>C 46.11  H 3.84  N 26.90</td>
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Fig. 1: Reaction Scheme
<table>
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<tr>
<th>Compound</th>
<th>E. Coli</th>
<th>B. Subtilus</th>
<th>S. enteridis</th>
<th>S. aureus</th>
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<tbody>
<tr>
<td>(4a)</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(4b)</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>(4c)</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>(4d)</td>
<td>++</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>(4f)</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
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</table>

Concentration = 10 mg/mL
Greatest inhibition zone = ++++
Good inhibition zone = +++
Average inhibition zone = ++
Poor inhibition = +
No inhibition zone = –