Studies on 1,2,4-Triazine Derivatives VI

Synthesis and Reactions of Some 1,2,4-Triazine Derivatives with Potential Antimicrobial Activities

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Triazines, Antimicrobial activities

Treatment each of 3-mercapto-1,2,4-triazine derivatives (1a) and (2b) with alkyl (or aryl) halides afforded the corresponding 3-alkyl(or aryl)mercapto-1,2,4-triazine derivatives (1b-e) and (2b-e) respectively. Treatment each of compounds (1b-e) and (2b-e) with different amines effected the formation of the corresponding 3-substituted-amino-1,2,4-triazine derivatives (1g-k) and (2g-k). The 4,5-dihydro-derivatives (3a, c, e, d) were obtained by reduction of the corresponding triazines (1a, f, g) with different reducing agents. The structure of compound 3d was confirmed via synthesis through another route. The triazine (1f) reacted with Grignard reagents to give the corresponding S-alkyl(benzyl)-4,5-dihydro-1,2,4-triazine derivatives (4a-e). The antagonistic properties of the triazines (1a-k) and (2a-k) were tested against some Gram positive and Gram negative bacteria as well as the fungus Candida albicans.

Much attention has been directed to the study of the chemistry of 1,2,4-triazines because of the pronounced biological activity of this class of compounds. In continuation of the work of one of the authors1-2, the action of alkylating agents, amines, reducing agents and Grignard reagents on some 1,2,4-triazine derivatives has been undertaken. Thus when 3-mercapto-5,6-di-p-methoxyphenyl-1,2,4-triazine (1a) and 3-mercapto-5-hydroxy-6-p-methoxystyryl-1,2,4-triazine (2a) were treated with methyl iodide (or dimethyl sulfate), ethyl monobromoacetate, phenacyl chloride and 2,4-dinitrochlorobenzene in alkaline medium, the corresponding 3-alkyl(aryl)mercapto-derivatives (1b-e) and (2b-e) were obtained respectively. The treatment of compound 1a with ethereal diazomethane effected also S-methylation to give compound 1b.

\[
\begin{align*}
\text{1a} & \quad \text{1b} \\
\text{R} = & \quad \text{SH} \\
\text{R} = & \quad \text{SCH}_3 \\
\text{R} = & \quad \text{SCH}_2 \text{CO}_2 \text{Et} \\
\text{R} = & \quad \text{SCH}_2 \text{COC}_6 \text{H}_5 \\
\text{R} = & \quad \text{S}-2,4-(\text{NO}_2)_2 \text{C}_6 \text{H}_3 \\
\text{R} = & \quad \text{OH} \\
\text{R} = & \quad \text{NHC}_6 \text{H}_5 \\
\text{R} = & \quad \text{NHC}_6 \text{H}_4 \text{Cl-p} \\
\text{R} = & \quad \text{NHC}_6 \text{H}_4 \text{OMe-p} \\
\text{R} = & \quad \text{morpholino} \\
\end{align*}
\]

Hydrolysis of compound 1b with hydrochloric acid gave 3-hydroxy-5,6-di-p-methoxyphenyl-1,2,4-triazine (1f). On the other hand, acid hydrolysis of compound 2c under the same experimental conditions gave the semicarbazone of p-methoxybenzylidenepyruvic acid. It is believed that the latter acid is obtained from hydrolysis of the intermediatly formed, but not isolated, 3,5-dihydroxy-6-p-methoxystyryl-1,2,4-triazine (2f). This was confirmed by cyclising the isolated semicarbazone derivative in alkaline medium to give the triazine 2f.

Treatment each of 3-methylmercapto-1,2,4-triazine-derivatives (1b) and (2b) with the amines namely, aniline, p-chloroaniline, p-anisidine, morpholine and piperidine effected the formation of the corresponding 3-substituted-amino-5,6-di-p-methoxyphenyl-1,2,4-triazines (1g-k) and 3-substituted-amino-5-hydroxy-6-p-methoxystyryl-1,2,4-triazines (2g-k). In each case methyl mercaptan evolved was identified as its 2,4-dinitrophenyl
derivative. On the other hand, treatment each of the other alkyl(aryl)mercapto-derivatives (1c-e) and (2c-e) with aniline afforded the 3-phenylamino-1,2,4-triazines (1g) and (2g) respectively.

The reactivity of the C=N in position 4.5 in compound 1 has been illustrated by:
i) The ready reduction of compounds 1a,1 with lithium aluminium hydride, p-thiocresol and sodium borohydride to give 3-mercapto-6-di-p-methoxyphenyl-4,5-dihydro-1,2,4-triazine (3a) and 3-hydroxy-5,6-di-p-methoxyphenyl-4,5-dihydro-1,2,4-triazine (3c) respectively. On the other hand, treatment of compound 1g with lithium aluminium hydride afforded 3-phenylamino-5,6-di-p-methoxyphenyl-4,5-dihydro-1,2,4-triazine (3d). The structure of the latter compound 3d was confirmed by its synthesis via the interaction of 3-methylmercapto-5,6-di-p-methoxyphenyl-4,5-dihydro-1,2,4-triazine (3b) with aniline. Compound 3b was prepared either by reduction of compound 1b with lithium aluminium hydride or by treating compound 3a with methyl iodide.

\[ \text{R} = \text{SH}, \quad \text{b) } \text{R} = \text{SCH}_3, \quad \text{c) } \text{R} = \text{OH}, \quad \text{d) } \text{R} = \text{NHC}_6\text{H}_5. \]

ii) Reaction of compound 1f with Grignard reagents namely, n-propyl, n-butyl and benzyl-magnesium halides to give the corresponding 3-hydroxy-5-alkyl(benzyl)-5,6-di-p-methoxyphenyl-4,5-dihydro-1,2,4-triazines (4a-c). The structure of the Grignard products finds support by the results previously obtained by Mustafa et al.\(^3\) and M'Packo et al.\(^4\).

The antagonistic properties of compounds 1a-k and 2a-k were tested against some Gram positive and Gram negative bacteria as well as the fungus Candida albicans. They showed different activities toward bacteria (Table II). Compounds 1b,e and 2,d,e,k were found to be the most active compounds. All compounds showed no activity when tested against the fungus Candida albicans.

**Experimental**

*Action of alkyl (or aryl) halides on the triazines (1a) and (2a)*

A 0.005 mole of each of methyl iodide, ethyl monobromoacetate, phenacyl chloride or 2,4-dinitrochlorobenzene was added to a solution of the triazine (1a) or (2a) (0.005 mole) in methanolic sodium methoxide (0.005 atom sodium and 100 ml methanol). Each mixture was refluxed (water-bath) for 30 min. It was then, poured on ice-water and the product was collected and crystallised from ethanol to give the corresponding 3-alkyl(or aryl)-mercapto-derivatives (1b-e) and (2b-e) respectively. (cf. Table I).

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<tbody>
<tr>
<td>1c</td>
<td>139</td>
<td>C(_2)H(_2)(_3)N(_4)O(_2)S</td>
<td>61.3</td>
<td>61.4</td>
<td>5.1</td>
<td>5.2</td>
<td>10.2</td>
<td>10.0</td>
<td>7.8</td>
<td>8.0</td>
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<tr>
<td>1d</td>
<td>151</td>
<td>C(_2)H(_2)(_3)N(_4)O(_2)S</td>
<td>67.7</td>
<td>67.6</td>
<td>4.7</td>
<td>4.9</td>
<td>9.4</td>
<td>9.1</td>
<td>7.2</td>
<td>7.4</td>
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<tr>
<td>1e</td>
<td>145</td>
<td>C(_2)H(_2)(_3)N(_4)O(_2)S</td>
<td>56.2</td>
<td>56.0</td>
<td>3.4</td>
<td>3.6</td>
<td>14.2</td>
<td>14.5</td>
<td>6.5</td>
<td>6.8</td>
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<tr>
<td>1g</td>
<td>218</td>
<td>C(_2)H(_2)(_3)N(_4)O(_2)S</td>
<td>71.8</td>
<td>71.9</td>
<td>5.2</td>
<td>5.0</td>
<td>14.5</td>
<td>14.8</td>
<td>7.2</td>
<td>7.4</td>
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<tr>
<td>1h</td>
<td>243</td>
<td>C(_2)H(_2)(_3)N(_4)O(_2)S</td>
<td>65.9</td>
<td>65.8</td>
<td>4.5</td>
<td>4.3</td>
<td>13.3</td>
<td>13.5</td>
<td>6.5</td>
<td>6.8</td>
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<tr>
<td>1i</td>
<td>200</td>
<td>C(_2)H(_2)(_3)N(_4)O(_2)S</td>
<td>69.5</td>
<td>69.2</td>
<td>5.3</td>
<td>5.7</td>
<td>13.5</td>
<td>13.8</td>
<td>7.2</td>
<td>7.4</td>
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<tr>
<td>1j</td>
<td>128</td>
<td>C(_2)H(_2)(_3)N(_4)O(_2)S</td>
<td>66.6</td>
<td>66.8</td>
<td>5.8</td>
<td>6.0</td>
<td>14.8</td>
<td>14.5</td>
<td>7.2</td>
<td>7.4</td>
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<tr>
<td>1k</td>
<td>135</td>
<td>C(_2)H(_2)(_3)N(_4)O(_2)S</td>
<td>70.2</td>
<td>70.1</td>
<td>6.3</td>
<td>6.3</td>
<td>14.8</td>
<td>15.0</td>
<td>7.2</td>
<td>7.4</td>
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</table>

Crystallisation solvent is ethanol. Cl: Caled. 8.5, Found 8.4%.
Action of diazomethane on 1a

When the triazine (1a) (1 g) was treated with ethereal diazomethane (prepared from 7 g nitrosomethyleurea) and then left overnight in the refrigerator, it gave the 3-methylmercapto-derivative (1b) in 95% yield, m.p. 152°C. Mixed m.p. with an authentic sample showed no depression.

Action of hydrochloric acid on the triazines (1c) and (2e)

When each solution of the triazines (1c) and (2e) (0.5 g) dissolved in 15 ml ethanol containing concentrated hydrochloric acid (3 ml) was refluxed for 7 h, then cooled, diluted with water and the product was crystallised from ethanol; the triazine (1c) gave 3-hydroxy-5,6-di-p-methoxyphenyl-1,2,4-triazine (0.25 g), m.p. and mixed m.p. 202°C and the triazine (2e) gave the semicarbazone of p-methoxybenzylidene pyruvic acid (0.25 g), m.p. and mixed m.p. with an authentic sample 183°C. On refluxing the latter acid (0.25 g) in solution of sodium carbonate (10%, 30 ml) for 1 h followed by acidification, it gave 3,5-dihydroxy-6-p-methoxyphenyl-1,2,4-triazine (0.15 g) m.p. and mixed m.p. 271°C.

Action of amines on 3-alkyl(aryl)-1,2,4-triazine derivatives (1b) and (2b)

A mixture of each of the triazines (1b) and (2b) (0.5 g) and each of the amines namely, aniline, p-chloroaniline, p-anisidine, morpholine and piperidine (2 g) was heated at 130-140°C for 3 h. The reaction mixture was cooled, treated with water (in case of morpholine and piperidine), filtered and crystallised from the proper solvent (cf. Table I and II). The methylmercaptan evolved during the reaction was absorbed and identified as its 2,4-dinitrophenyl derivative, m.p. and mixed m.p. 128°C.

When each of the triazines (1c-e) and (2c-e) were similarly treated with aniline, they gave the corresponding 3-phenylamino-derivatives (1g) and (2g) respectively.

<table>
<thead>
<tr>
<th>Compound</th>
<th>m.p. [°C]</th>
<th>Analysis [%]</th>
<th>Formula</th>
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<tr>
<td></td>
<td></td>
<td>Carbon</td>
<td>Hydrogen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caled Found</td>
<td>Caled Found</td>
</tr>
<tr>
<td>2e</td>
<td>160</td>
<td>55.3 55.1</td>
<td>3.8 3.6</td>
</tr>
<tr>
<td>2d</td>
<td>212</td>
<td>63.3 63.7</td>
<td>3.5 3.2</td>
</tr>
<tr>
<td>2e</td>
<td>285</td>
<td>50.5 50.4</td>
<td>3.0 3.1</td>
</tr>
<tr>
<td>2g</td>
<td>321</td>
<td>67.5 67.6</td>
<td>5.0 5.0</td>
</tr>
<tr>
<td>2h</td>
<td>330</td>
<td>60.9 61.2</td>
<td>4.2 4.5</td>
</tr>
<tr>
<td>2i</td>
<td>324</td>
<td>65.1 65.3</td>
<td>5.1 5.2</td>
</tr>
<tr>
<td>2j</td>
<td>321</td>
<td>61.1 61.4</td>
<td>5.7 5.4</td>
</tr>
<tr>
<td>2k</td>
<td>272</td>
<td>65.3 65.7</td>
<td>6.4 6.4</td>
</tr>
</tbody>
</table>

Crystallisation solvent for 2c, d (ethanol); 2e (acetone); 2g, k (dimethylformamide); 2h, i (pyridine).
Cl: Caled. 10.9, Found 10.6%.
methoxide (10 ml methanol and 0.03 g sodium) and the mixture was refluxed (water-bath) for 1 h. It was then cooled and diluted with water and the product was crystallised from ethanol to give compound 3b, m.p. 165 °C (yield 85%) (cf. Table III).

Reduction of 3-phenylamino-5,6-di-p-methoxyphenyl-1,2,4-triazine (1g)

When compound 1g (0.50 g) was similarly reduced with lithium aluminium hydride (0.2 g) and treated as above, it gave the corresponding 4,5-dihydro-derivative (3d) (ethanol), m.p. 190 °C (yield ca. 80%).

Action of aniline on 3-methylmercapto-,5-6di-p-methoxyphenyl-4,5-dihydro-1,2,4-triazine (4a-c)

A solution of compound 3b (1 g) in aniline (4 ml) was refluxed for 3 hr. It was cooled, treated with cold ethanol and the precipitate obtained was crystallised from ethanol to give colorless crystals of compound 3d with m.p. and mixed m.p. 190 °C (yield ca. 85%).

Action of alkyl(benzyl)magnesium halides on 3-hydroxy-5,6-di-p-methoxyphenyl-1,2,4-triazine (1f)

To each of the Grignard solutions (prepared from 0.005 mole n-propyl iodide, n-butyl iodine or benzyl chloride, 0.005 mole magnesium and 30 ml ether), the triazine (1f) (0.001 mole) was added. The reaction mixture was refluxed (water-bath) for 30 min and then left overnight at room temperature. It was then cooled, decomposed with aqueous ammonium chloride. Ether was separated, dried and recovered. The residue was crystallised from ethanol to give the corresponding 3-alkyl(or benzyl)-5,6-di-p-methoxyphenyl-4,5-dihydro-1,2,4-triazines (1a-c) (yield ca. 75%) (cf. Table III).

Action of ferric chloride on 3-mercapto-5,6-di-p-methoxyphenyl-1,2,4-triazine (1a)

An ethanolic mixture of the triazine (1a) (1 g) and ferric chloride (2 g) was refluxed (water-bath) for 30 min. It was then cooled and the precipitate was filtered off and crystallised from benzene to give yellow crystals of bis-[5,6-di-p-methoxyphenyl-1,2,4-triazine]-3,3'-disulfide with m.p. 206 °C which showed no depression when mixed with an authentic sample9 (yield 95%).

All the above compounds showed no activity when tested against the fungus Candida albicans NRRL-Y-477.


The MIC was determined using agar disc technique11,12

Assaying media = nutrient-agar and Czapekdox-agar for bacteria and fungus respectively.

Incubation = 24 h for bacteria at 37 °C and 48 h at 26 °C for Candida albicans.

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