CHAPTER IV

RELATIVE MERITS OF THE COMPOUNDS AS ANTI-BACTERIALS

SECTION I: Method Used

SECTION II: Experimental results & conclusions derived therefrom.
SECTION I

METHOD USED

A few compounds (on a representative scale), from each of the series, mentioned below, have been systematically screened for their bactericidal action. The results of antibacterial screening and the conclusions derived from the antibacterial data regarding the relationship between antibacterial action and structural change are given in section II.

1. Thiazolo[3',2': 2,3]-as-triazino[5,6-b]indole
2. Thiazolo[2',3': 3,4]-as-triazino[5,6-b]indole
3. Thiazino[3',2': 2,3]-as-triazino[5,6-b]indole
4. Thiazino[2',3': 3,4]-as-triazino[5,6-b]indole
7. Thiazolo[3,2-b]-s-triazole
8. Thiazolo[2,3-c]-s-triazole
10. s-Triazolo[3,4-b]-1,3,4-thiadiazine
11. s-Triazolo[3',4': 2,3]-1,3,4-thiadiazino [5,6-b] quinoxaline
12. Bis[s-triazolo[3,4-b]-1,3,4-thiadiazolo[3,2-b] [imidazo [4,5-b] cyclohexane]-5a, 6a-diene]
13. s-Triazolo[3,4-b]-1,3,4-thiadiazolo[3,2-b]imidazo[4,5-b] quinoxaline
14. Imidazo[2,1-b]-1,3,4-thiadiazolo[2,3-c]-s-triazole
15. Imidazo [2,1-b]-1,3,4-thiadiazole.
17. Imidazo[2,1-b]pyrazolo [3,4-d]thiazole

21. Spiropiperidine-4',7(8H)-[6H]-pyrazolo[3,4-d] thiazolo [3,2-b]-s-tetrazine

The most widely used method for determining the antibacterial activity of drugs consists of cultivating the bacteria in a test tube or nutrient agar plate to which the drugs have been added. Factors which influence the results of any test method, include (i) species of test organism (ii) composition and pH of the medium (iii) inoculum of organism (iv) diluting fluid (v) concentration and stability of the drug solution and (vi) temperature and duration of incubation.

For studying the antibacterial properties, many methods are available but Kirby-Barr disc diffusion and plate diffusion methods as reported by Nakahara et al273 have been used by the author in the present investigations.

The test organism was a two-hour culture of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, incubated and grown in peptone-water medium (temp.37°C).

The peptone-water and nutrient agar media are prepared as follows:

**Peptone-Water medium**

A mixture of peptone (10.0g) and sodium chloride (5.0g), was dissolved in warm distilled water (1 litre) and the pH of the solution was adjusted to 7.4-7.5. It was then filtered in test tubes (measuring 2mL in each test tube) and sterilized in an autoclave at 121°C for 15 minutes.
**Nutrient agar medium**

A mixture of peptone (10.0g), beef extract (10.0g) and sodium chloride (5.0g) was dissolved in distilled water (1 litre) and the pH of the solution was adjusted to 7.4-7.5. It was then filtered and agar-agar (20.0g) was then added. The mixture was sterilized in an autoclave at 121°C for 15 minutes and poured into petridishes.

**Determination of activity in neat concentration**

10 mg of the compound was dissolved in minimum amount of the appropriate solvent. 4mm filter paper discs were soaked in these solutions and incorporated in nutrient agar plates inoculated with bacteria. These plates were incubated at 37°C for 18 hours and then examined for inhibition of bacterial growth. The dishes showing zone of inhibition (interpreted as active against the particular bacteria) were further studied for minimum inhibitory concentration (MIC) by serial plate dilution method in nutrient agar plates.

**Determination of activity at different dilutions**

The compounds were incorporated in nutrient agar in doubling dilutions, from 1024 to 1.0 mg/mL. One plate for each dilution was divided into four sectors. They were then inoculated with microorganisms (*E.Coli*, *S.aureus* and *P.aeruginosa*) followed by incubation at 37°C for 18hr. The antibacterial activity was indicated by inhibition of growth around the filter paper disc in case of the neat concentrations and in case of plate dilution method, absence of growth on the entire sector of the nutrient agar indicated antibacterial activity.
### SECTION II

**ANTIBACTERIAL TESTING**

*(Experimental results and conclusions derived therefrom)*

<table>
<thead>
<tr>
<th>Incubation period</th>
<th>18 hr.</th>
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<tbody>
<tr>
<td>Temperature</td>
<td>37°C</td>
</tr>
<tr>
<td>M.I.C.</td>
<td>Minimum Inhibitory Concentration</td>
</tr>
<tr>
<td><em>E. Coli</em></td>
<td><em>Escherichia coli</em> (NCTC 10418)</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td><em>Staphylococcus aureus</em> (NCTC 6571)</td>
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<tr>
<td><em>P. aeruginosa</em></td>
<td><em>Pseudomonas aeruginosa</em> (NCTC 10662)</td>
</tr>
<tr>
<td>S. No.</td>
<td>Name</td>
</tr>
<tr>
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<td>----------------------------------------------------------------------</td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>7,8-Dichloro-3-$p$-bromophenyl thiazolo[3',2': 2,3]-as-triazino</td>
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<tr>
<td></td>
<td>[5,6- $b$]indole. IXa</td>
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<td>2.</td>
<td>7,8-Dichloro-1-$p$-bromophenyl thiazolo[2',3': 3,4]-as-triazino</td>
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<td></td>
<td>[5,6- $b$] indole. VIA</td>
</tr>
<tr>
<td>3.</td>
<td>7,8-Dichloro-2, 3-dihydrothiazolo [3',2':2,3]-as-triazino [5,6- $b$]</td>
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<td>indole. X</td>
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<tr>
<td>4.</td>
<td>7,8-Dichloro-2,3-dihydrothiazolo [2',3':3,4]-as-triazino[5,6- $b$]</td>
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<td>indole. XI</td>
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<tr>
<td>5.</td>
<td>8,9-Dichloro-4$H$-2, 3-dihydro[1,3] thiazino[3',2': 2,3]-as-triazino</td>
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<td>[5,6- $b$]indole. XII</td>
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<tr>
<td>6.</td>
<td>8,9-Dichloro-1$H$-2,3-dihydro[1,3] thiazino[2',3': 3,4]-as-triazino</td>
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<td>[5,6- $b$]indole. XIII</td>
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<tr>
<td>7.</td>
<td>9,10-Dichloroquinoxalino[2',3':4,5] thiazolo[3,2-$b$]indolo[2,3-$e$]-as-triazine.XIV</td>
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<tr>
<td>8.</td>
<td>2,3-Dichloroquinoxalino[2',3': 4,5] thiazolo[2,3-$c$]indolo[2,3-$e$]-as-triazine. XV</td>
</tr>
<tr>
<td></td>
<td>Chemical Formula</td>
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<td>----------------------------------------------------------------------------------</td>
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<tr>
<td>9</td>
<td>2-m-Hydroxyphenyl-5-p-bromophenyl thiazolo[3,2-b]-s-triazole. XIXa</td>
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<tr>
<td>10</td>
<td>3-m-Hydroxyphenyl-5-p-bromophenyl[2,3-c]-s-triazole. XXIa</td>
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<tr>
<td>11</td>
<td>6-o-Nitrophenyl-2-(2, 4-dinitrophenyl)-3-(p-chlorophenyl) -3,3α-dihydropyrazolo[3',4':4,5] thiazolo[3,2-b]-s-triazole.XXIVa</td>
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<td>12</td>
<td>6-o-Nitrophenyl-2-(2,4-dinitrophenyl)-3-(p-dimethylamino phenyl)-3,3α-dihydropyrazolo [3',4': 4,5]thiazolo [3,2-b]-s-triazole. XXIVa</td>
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<td>14</td>
<td>6-p-Bromophenyl-3-m-hydroxyphenyl-7H-s-triazolo[3,4-b] [1,3,4]thiadiazine. XXVI</td>
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<tr>
<td>15</td>
<td>3-m-Hydroxyphenyl-6,7-diphenyl-5H-s-triazolo[3,4-b][1,3,4]thiadiazine. XXVII</td>
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<tr>
<td>16</td>
<td>3-o-Chlorophenyl-s-triazolo[3,4-b]-1,3,4-thiadiazolo[3,2-b]imidazo [4,5-b]quinoxaline. XXXII</td>
</tr>
</tbody>
</table>
17. 6-Bromo-7-p-bromophenyl-3-o-chlorophenyl imidazo[2,1-b]-1,3,4-thiadiazolo [2,3-c]-s-triazole. XXXIVa

18. 6-Bromo-7-p-chlorophenyl-3-o-chlorophenyl imidazo[2,1-b]-1,3,4-thiadiazolo [2,3-c]-s-triazole. XXXIVb

19. 2-(α-Naphthyl)-6-(p-bromophenyl) imidazo[2,1-b]-1,3,4-thiadiazole. XXXVIa

20. 2-(α-Naphthyl)-6-(p-chlorophenyl) imidazo[2,1-b]-1,3,4-thiadiazole. XXXVIb

21. 5-Bromo-2-(α-naphthyl)-6-(p-bromophenyl)imidazo[2,1-b]-1,3,4-thiadiazole.XXXVIIa

22. 3-p-Chlorophenyl-2H-7-methyl-3,3a,6,7-tetrahydro-2H-imidazo [2,1-b]pyrazolo [3,4-d]thiazole XLIa

23. 3-p-Chlorophenyl-2-(2,4-dinitrophenyl)-7-methyl-3,3a,6,7-tetrahydroimidazo[2,1-b]pyrazolo [3,4-d]thiazole.XLIb

24. 3-(p-dimethylaminophenyl)-2-(p-nitrophenyl)-3,3a,6,7-tetrahydro-5H-pyrazolo[3',4':4,5]thiazolo [3,2-a]pyrimidine. XLIVa

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25. 2,3-di-(p-nitrophenyl)-trans-3,3a, 6,7-tetrahydro-5H-pyrazolo 
XLIVb

26. 6'-(p-chlorophenyl)-2'H-3'(p- 
dimethylaminophenyl)-3',3'α 
dihydrospiro [3H-5,6-dichloro- 
indole-3,5'-pyrazolo [3',4':4,5] 
thiazolo-2(1H)-one] XLVIIIa 

27. 6'-(p-chlorophenyl)-2'(2"',4"'- 
dinitrophenyl)-3'- (p-dimethyl 
aminophenyl)3',3'α-dihydrospiro 
[3H-5,6-dichloro indole-3,5'- 
pyrazolo [3',4':4,5]thiazolo-2(1H) 
one] XLVIIIb

28. 3',6'-di-(p-chlorophenyl)-2' (2"',4"'- 
dinitrophenyl)-trans-3',3'α- 
dihydrospiro [3H-5,6-dichloro 
indole-3,5'-pyrazolo[3',4':4,5] 
thiazolo-2(1H)-one] XLVIIIb₁

29. 3,3α-Dihydro-2-(p-nitrophenyl) 
-3-(p-anisyl)spiro[cyclododecane-1', 7(8H)-[6H]pyrazolo [3',4':4,5] 
thiazolo[3,2-β]-s-tetrazine. LIIa

30. 3,3α-Dihydro-2-(p-nitrophenyl)-3 
-(p-chlorophenyl)spiro[cyclodo-
decane-1',7(8H)-[6H]pyrazolo 
[3',4':4,5]thiazolo[3,2-β]-s-
tetrazine LIIb
31. 7'-p-chlorobenzylidene-6'(7'H)-
oxospiro [2,6-di(o-hydroxyphenyl)
piperidine-3',4(4'H)-[2H]thiazolo
[3,2-b]-s-tetrazine LV

32. 3,3a-Dihydro-2-(2'',4''-dinitrophenyl) -3-(p-chlorophenyl)-2',6'-di
(o-hydroxyphenyl)spiropiperidine-
4',7(8H)-[6H]pyrazolo[3,4-d]thiazolo
[3,2-b]-s-tetrazine. LVI
DISCUSSION (CONCLUSION)

The results of evaluation of antibacterial screening of 32 samples against gram positive bacterium *Staphylococcus aureus* and two gram negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* are recorded in the beginning of this section. A few generation have been made, regarding the antibacterial activity and chemical structure, as described below:

(i) The linear isomer thiazolo [3',2':2,3]-as-triazino [5,6-b]indole and angular isomer thiazolo [2',3';:3,4]-as-triazino [5,6-b] indole exhibit same antibacterial activity and the replacement of thiazole ring with quinoxaline ring does not affect the antibacterial activity (compare compounds 3 & 4 with 7 & 8).

(ii) Thiazolo [3,2-fa]-s-triazole system and its isomeric system thiazolo [2,3-c]-s-triazole display similar antibacterial activity (compare 9 & 10).

(iii) Date of 11, 12 & 13 reveals that fusion of pyrazole ring to thiazolo-s-triazole system leads to enhancement of antibacterial activity.

(iv) Imidazo [2,1-b]-1,3,4-thiadiazoles exhibit more activity but the introduction of bromine at 5 position decreases the antibacterial activity of the compound (compare 19 & 21).

(v) Fusion of pyrazole ring with spiro piperidine-thiazolo [3,2-b]-s-tetrazine enhanced the antimicrobial activity of the resulting system (compare 31 & 32).