


**ANTIBACTERIAL ACTIVITY OF &-TRIAZINYL ARYL/ALKYL SULPHONES**

*p*-Aminobenzoic acid derivatives have been found to be powerful local anaesthetics and possess bacteriostatic properties. *S*-Triazine derivatives also possess therapeutic activity against malaria, cancer and viral diseases. We have prepared 4'-((2-p-chlorophenylsulphonyl-4-aryl/alkylamino-s-triazine-6-yl)-aminobenzoic acids of the type (1) and tested for antibacterial activity.

![Chemical Structure](image)

where R = arylamino, alkylamino, etc.

The first chlorine of the cyanuric chloride was reacted with *p*-chlorothiophenol at 0°C, leading to the formation of 2, 4-dichloro-*s*-triazine-6-yl *p*-chlorophenyl sulphide. The second chlorine was condensed with *p*-aminobenzoic acid at 30°-35° and the third chlorine at 80°-90° with different bases using dioxane as the solvent. The product was then oxidised to the corresponding sulphones.

**Antibacterial Testing.**—The following strains were used for testing the antibacterial activity:

A. Gram-positive bacterial strains like *Bacillus subtilis* and *Staphylococcus aureus*.

B. Gram-negative bacterial strains like *Escherichia coli*, *Xanthomonas citri*, *Salmonella typhosa*, *Shigella shiga* and *Pseudomonas aeruginosa*.

Thirty-one sulphones were tested using dilution broth method *in vitro*. Loopful suspensions prepared from the above actively growing microorganisms were inoculated into the nutrient broth containing different concentrations (800, 500, 400, 250, 200 and 100 µg/ml) of the sulphones and incubated for 24 hours at 37°C. The minimum inhibitory concentrations (MIC) were determined in µg/ml.

*p*-Bromoanilino, *p*-idoanilino and 2, 4, 6-tri-bromoanilino derivatives were found to be most active as they inhibited the growth of Gram-positive bacteria at 200 µg/ml and Gram-negative bacteria at 400 µg/ml. The other substituents in the benzene ring of arylamino group like methyl, *m*-chloro, methoxy, ethoxy, nitro, hydroxy, sulpho, other heterocyclic derivatives like pyridylamino, morpholino, piperidino and alkylamino, arylalkylamino derivatives inhibited the growth of *B. subtilis* and *S. aureus* at 250 µg/ml; *E. coli*, *X. citri*, *P. aeruginosa* at 400 µg/ml; *S. typhosa* and *S. shiga* at 500 µg/ml.

**DRUGS ON PROTEIN BINDING OF TOLBUTAMIDE**

Sulphonyl ureas have been reported to bind extensively to plasma proteins. Displacement of sulphonyl ureas by number of acidic drugs has been well demonstrated in human serum and in solutions of purified albumin. The studies reported herein explore the influence of some drugs not reported earlier on protein binding of tolbutamide.
TABLE I

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration of drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>2.34</td>
</tr>
<tr>
<td>Sulphathiazole</td>
<td>2.34</td>
</tr>
<tr>
<td>Sulphanilamide</td>
<td>2.36</td>
</tr>
<tr>
<td>Sulphadimidine</td>
<td>2.34</td>
</tr>
<tr>
<td>Analgin</td>
<td>2.34</td>
</tr>
<tr>
<td>Salicylcamide</td>
<td>2.35</td>
</tr>
<tr>
<td>Phenobarbitone sodium</td>
<td>2.35</td>
</tr>
<tr>
<td>Chlorpheniramine maleate</td>
<td>2.35</td>
</tr>
</tbody>
</table>

Each value is an average of five readings. The dialysis system had the total volume of 12 ml and consisted of $24 \times 10^{-6}$ M of albumin and $77 \times 10^{-6}$ M of tolbutamide.

The binding studies were carried out by the technique of equilibrium dialysis. In a dialysis bag 1 ml of 2% Bovine serum albumin (Sigma Co.), 1 ml of tolbutamide (Hochst, Bombay) solution of appropriate concentration and 1 ml of phosphate buffer ($0.067$ M, pH 7.4) were placed, thus adjusting the volume to 3 ml. While testing the materials for their influence on protein binding of tolbutamide, 1 ml of the solution of the test drug of various concentrations was added to the bag instead of phosphate buffer. The solutions of albumin and drugs were all prepared in $0.067$ M phosphate buffer of pH 7.4. The mixture in the dialysis bag was dialysed against 9 ml of phosphate buffer ($0.067$ M, pH 7.4) for 24 hours at $37^\circ$ C. The bags were removed after 24 hours and the concentration of tolbutamide in the dialysate was estimated by the method of Spingler. Control experiments using phosphate buffer instead of albumin solution were also performed. The total amount of drug bound to the protein was calculated from the difference between total amount of drug added and free drug concentration in the entire dialysis system. The binding of tolbutamide to protein is expressed in mole/mole of protein.

Of the compounds tested, paracetamol, sulphathiazole, sulphanilamide, sulphadimidine were found to interfere with protein binding of tolbutamide (Table I). The binding of tolbutamide with albumin was inhibited markedly with increasing concentration of these drugs. Sulphathiazole had the maximum inhibitory effect on protein binding of tolbutamide. It has been demonstrated that tolbutamide involves binding to 2 classes of sites on albumin. The interference produced by the above drugs in binding of tolbutamide with protein suggests involvement of the common binding sites and relatively stronger affinities of the drugs than tolbutamide for the binding sites on protein. Especially, the sulpha drugs by virtue of possessing structural resemblance with tolbutamide can compete with tolbutamide for common binding sites. Sulphadiazine, however, failed to have any effect on protein binding of tolbutamide, presumably due to lower affinity than tolbutamide for binding with protein.

The interactions of the drugs with protein binding of tolbutamide bear clinical relevance since the displacement of the hypoglycemic agent from the carrier protein in presence of the competitor could aggravate hypoglycemic response and also reduce biological half life of the drug.

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