CHAPTER 6

ANTIBACTERIAL STUDIES ON SOME INDOLE DERIVATIVES

6.1 ANTIBACTERIAL STUDIES ON SOME INDOLE DERIVATIVES

6.1.1 Introduction

The biological activities of indole and their derivatives have been known for long time. Indole and its derivatives have been shown to exhibit good antibacterial properties (Wang and Ng 2002, Singh et al 2000, Quetin-Leclercq et al 1995). The halogenated indole derivatives exhibit marked antibacterial activity against Gram-positive, Gram-negative bacteria and fungi (Piscopo et al 1990).

The synthesis of cell wall, capsule and extracellular enzymes are membrane-associated phenomena in bacteria. Any specific drug affecting this structure is a potential antibacterial agent and its biochemical mechanism of action may be expressed in the inhibition of any of the cell’s vital function. The heterocyclic compounds particularly five and six-membered ring systems have occupied a prominent place among various classes of organic compounds in view of their diverse biological activities. The Indole ring system acquired a special place in the heterocyclic compounds owing to its specific structural motif in many pharmacological applications. In view of the above properties, some of the indole derivatives, which were screened against
human pathogenic bacteria namely *Escherichia coli* are presented and obtained results are discussed in this chapter in detail.

The following indole derivatives presented in chapter 3 to 5 were subjected to anti-bacterial study;

1. 3-Bromo-2-(2-bromo-4,5-dimethoxybenzyl)-1-phenylsulfonyl-1H-indol, C$_{23}$H$_{19}$Br$_2$NO$_4$S (BPSI-BD)
2. 3-Bromo-2-(4-bromo-2,5-dimethoxybenzyl)-1-phenylsulfonyl-1H indole, C$_{23}$H$_{19}$Br$_2$NO$_4$S (BPSI-DB)
3. N-[4-Bromo-2-[3-bromo-1-phenyl-sulfonyl-1H-indol-2-yl methyl]-5-methoxyphenyl] acetamide, C$_{24}$H$_{20}$Br$_2$N$_2$O$_4$S (BPSI-BMA)
4. 1-(3-Bromo-1-phenylsulfonyl-1H-indol-2-ylmethyl) pyrrolidine-2,5-dione, C$_{19}$H$_{15}$BrN$_2$O$_4$S (BPSI-PY)
5. 2-(3-bromo-1-phenylsulfonyl)-1H-indole-2ylmethyl-sulfanyl)-6-methyl-1 H-benzimidazole, C$_{23}$H$_{18}$BrN$_3$O$_2$S$_2$ (BPSI-BZ)

### 6.1.2 Introduction to antibacterial activity testing

The susceptibility of microorganisms to any chromotherapeutic agents can be determined by either the paper disc-plate technique (also called Kirby-Baur disk diffusion method) or the broth dilution technique (or the tube dilution technique) (Enest et al 1984). Each method had its own merits and demerits.
The paper-disc-plate technique is a highly standardized method, approved by the FDA (Federal Drug Administration) of USA for determining the susceptibility of bacteria to any antimicrobial agent. The limitations of the method are (1) the diffusion of certain compounds are known to be affected by the agar incorporated in the medium, (2) the exact minimum inhibitory concentration (MIC) cannot be determined, and (3) quality control is difficult to adhere in the experimental aspect. In order to avoid these discrepancies, the broth dilution method is often preferred for anti-bactericidal assays (Jenkins et al 1985).

6.1.3 Well diffusion method

Twenty milliliter of nutrient agar was poured into the sterile petri dishes and allowed to solidify. Wells were made on the agar using a sterile cork (8 mm in diameter). About 24 hours old bacterial medium from peptone broth was swabbed on the agar surface using a sterile cotton swab. The compounds were dissolved separately in 10% of DMSO solvent to set. The solution of dissolved compounds was of 50 µl and 100 µl added separately into the wells. In order to check the activity of the solvent, DMSO (10%) was added into the control wells. The antibiotic, streptomycin (10 mg/ml) as the drug control. The plates were incubated at 37 ºC for 24 hours and the inhibition was observed. The percentage (%) of inhibition was calculated using the formula (World Health Organization 1977).

\[
\text{Percentage of inhibition} = \frac{\text{Diameter of the inhibition zone}}{\text{Diameter of the Petri-plate in mm}} \times 100
\]

6.1.4 Broth Dilution Method

This method is considered to be the most accurate for the determination of susceptibility to measured amounts of any antimicrobial
agent or one can determine the smallest amount of agent required to inhibit the growth of the organism \textit{in vitro}. This is referred as the minimum inhibitory concentration (MIC).

The test organism was cultured in Mueller Hinton Broth overnight. (A 1:1000 dilution of the overnight culture gives \(10^5\)–\(10^6\) organisms/ml). To 1ml of the culture, various concentrations of the compound (indole derivatives) to be tested were added. Subsequently, the tubes were incubated overnight at 37°C and examined macroscopically for growth. The lowest concentration of the compound that showed no growth was considered as the MIC. Minimum bactericidal concentration (MBC) (the concentration of the compound required for the complete clearance of the bacteria) was determined by comparing the colony count using automated micropipette or quantitative loop (Bauer et al 1966).

6.1.5 Study of antibacterial activity

Overnight culture of \textit{Escherichia coli} with known number of cells was inoculated on sterile Muller Hinton broth. The drug was dissolved in ethyl acetate to get a final concentration of 4096 \(\mu\)g/ml. Colony forming units (cfu/ml) were calculated using the spread plate method.

The method of Makane and Kandal was followed to determine the MIC. The components were diluted to obtain concentrations ranging from 32 \(\mu\)g/ml to 2048 \(\mu\)g/ml. To carry out doubling dilution, Muller Hinton broth was prepared and sterilized. To a tube containing 3 ml of Muller Hinton broth, 0.1 ml bacterial suspension and the different concentrations of the test samples were added. Appropriate controls were used. Later, 0.1 ml of the test samples form each of the tubes were spread on Muller Hinton agar using an L-rod and incubated at 37°C for 24 hrs. Simultaneously, they were inoculated
on Mueller Hinton agar and incubated at 37°C for 4 hrs and observed for growth. The number of colonies in each plate was calculated and the MIC of the drug was determined accordingly.

### 6.1.6 Result and Discussion

The results indicate that the phenyl sulfonyl containing indole derivatives exhibit good antibacterial properties. The results are given in the Table 6.1.

The compound BPSI-BD shows more activity than other compounds even at 120 µg/mol. The compound BPSI-PY shows very less activity than other compounds and this may be due to the different substituent at C2 position. The compound BPSI-DB, BPSI-BMA and BPSI-PZ show moderate activity. The BPSI-DB and BPSI-BMA having bromo phenyl benzyl moiety and BPSI-PZ having imadazole moiety at 2\textsuperscript{nd} position. The BPSI-BD, BPSI-DB compounds containing bromo benzyl at 2\textsuperscript{nd} position. The antibacterial activity of the compounds may be differing due to Br groups attached at different position. The \textit{meta} substituent BPSI-BD compound shows more activity. The dihedral angle between phenyl and indole moieties is 86.9, 89.2, 89.1, 71.0 and 88.5° for BPSI-BD, BPSI-DB, BPSI-BMA, BPSI-PY and BPSI-PZ respectively. Since all of them are nearly orthogonal, the dihedral angle, hence the geometry of indole/sulfonyl phenyl conformation, it may only be attributed to other substituent may not be involve in the antibacterial activity of the compounds.
Table 6.1  Anti-bacterial actions of the some indole derivatives

<table>
<thead>
<tr>
<th>Component</th>
<th>2048 µg/ml</th>
<th>1024 µg/ml</th>
<th>512 µg/ml</th>
<th>256 µg/ml</th>
<th>128 µg/ml</th>
<th>64 µg/ml</th>
<th>32 µg/ml</th>
</tr>
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<td>NG</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
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<td>NG</td>
<td>NG</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
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<td>+</td>
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<td>++</td>
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</tr>
<tr>
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<td>NG</td>
<td>NG</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

NG Denotes inhibition (no growth)

+ Denotes growth (no inhibition)