CHAPTER 5
ANTIMICROBIAL PROPERTIES OF AMINOIMIDAZOLINONES

Introduction

Microorganisms are universally associated with the lives of humans, other animals and plants. Some of them are beneficial and others are detrimental. Microorganisms play an important role in the food and pharmaceutical industry. They are involved in the making of yogurt, cheese, wine, buttermilk and in the production of antibiotics. Besides their role as a beneficiary, microorganisms can cause diseases, spoil food and deteriorate materials like iron pipes, glass lenses and wood pilings. There is no field of human endeavour, whether it be in industry or agriculture or in the preparation of food and the combating of diseases, where the microbes do not play an important and often dominant role.

Each kind of microorganism has specific growth requirements. Many of them can be grown in the laboratory culture medium containing necessary nutrients for their growth and multiplication. Some of them require a supply of inorganic salts, particularly the anions, phosphate and sulphate and the cations sodium, potassium, iron, etc. whereas others can grow in a medium containing organic compounds (amino acids, vitamins or coenzymes) in
minute quantities. Some other require complex natural substances (peptone, blood, serum, etc.) and microorganisms like rickettsias cannot be grown in an artificial laboratory medium. On solid culture media microbial cells can grow and form visible masses called colonies.

**Bacteria**

The bacterium is a single celled organism that does not have intracellular membrane bound organelles such as nucleus, golgi bodies, endoplasmic reticulum or mitochondria. Therefore, the bacterium's essential metabolic and biosynthetic activities must be carried out within the cytoplasm and the cell envelope. Bacteria lack a true nucleus and are classified as prokaryotes. One of the most important cytological features of bacteria is their reaction to a simple staining procedure, called the Gram-stain. The procedure involves staining the cells with crystal violet and a mordant known as Lugol's solution (3% I₂/KI) is added to set the stain. The bacteria are next decolourised with alcohol. Finally the bacteria are counterstained with Safranin. Gram-positive bacteria retain the crystal violet, whereas Gram-negative bacteria, which lose the crystal violet on counter staining by Safranin, appear red colour. The most possible explanation for this difference in behaviour lies in the relative differences between the cell walls of the above two types of bacteria.
Bacterial cell wall is made up of peptidoglycan, an insoluble, porous, heteropolymer of alternating N-acetylglucosamine and N-acetylmuramic acid units. The cell wall in Gram-positive bacteria has a relatively thick layer of peptidoglycan 20 to 80 mm across. The peptidoglycan layer is closely attached to outer surface of the cell membrane. Chemical analysis shows that 60 to 90 percent of the cell wall of a Gram-positive bacterium is peptidoglycan. The thick cell walls of Gram-positive bacteria retain such stains as crystal violet-iodine dye in the cytoplasm.

The cell wall of a Gram-negative bacterium is thinner but more complex than that of a Gram-positive bacterium. Only 10 to 20 percent of the cell wall is peptidoglycan, the remainder consists of various polysaccharides, proteins and lipids. Gram-negative bacteria fail to retain the crystal violet-iodine dye during the decolourising procedure partly because of their thin cell walls and partly because of the relatively large quantities of lipoproteins and lipopolysaccharides in the wall.145

**Fungi**

Unlike bacteria, which are prokaryotes, fungi are eukaryotes. Each fungus has a golgi apparatus, mitochondria, nucleus, ribosomes, endoplasmic reticulum and a cell membrane, making it difficult to develop antibiotics that are selectively toxic for fungi. A large number of fungi are parasites of
terrestrial plants. Fungi cause the majority of economically significant diseases of crop plants.

Fungal cell walls resemble plant cell walls architecturally, but not chemically. Although cellulose is present in the walls of certain fungi, many fungi have non cellulosic walls.

The cell wall is composed of cross linked polysaccharides, proteins and glycoproteins and it provides the fungus with osmotic stability and acid rigidity. In nature fungi are important decomposers. Trees and leaves that fall in the forest are decomposed in large part by fungi. Many fungi produce enzymes that attack plant polymers such as cellulose and lignin. They also can grow in relatively dry locations. This enables them to decompose complex materials that are difficult for bacteria to attack.

Viruses which traditionally are considered as microorganisms, lack the fundamental structure of living organism. No functioning cytoplasmic membrane separates the virus from its surroundings and viruses have no means of independent life support activities. They have a genetic molecule which may be DNA or RNA and a protein coat.

**Modes of Action of Antimicrobial Agents**

Micro organisms can be inhibited or killed by various physical and chemical agents. The agents that kill or destroy the organisms are referred to
as 'cidal', whereas the one that merely halts the growth of the micro organism is called 'static'. If a static agent is removed from a culture, the organism will resume growth but the effects of cidal agents are irreversible. The manner in which antimicrobial agents inhibit or kill can be attributed to the following kinds of actions.146

Several types of chemical agents damage the cell wall by blocking its synthesis. Some of them will disrupt the cell membrane, so that the cell loses its selective permeability and can neither prevent the loss of vital molecules nor bar the entry of damaging chemicals. Some others will inhibit the enzyme action and will damage the microbial life. Chemicals such as strong solvents (alcohols, acid and phenolics) coagulate bacterial proteins; some agents disrupt or denature protein. Such losses in normal protein function can arrest bacterial metabolism, thereby inhibit the growth or kill them.

**Antibiotics**

These are chemical substances produced by certain microorganisms that inhibit or kill other microorganisms. An antibiotic that acts on both Gram-positive and Gram-negative bacteria is called a broad-spectrum antibiotic, whereas narrow spectrum antibiotics, acts on only a specific group of organism. The widespread use of antibiotics has increased the number of pathogenic microorganisms that display antibiotic resistance.
Results and Discussion

The antimicrobial activity of aminoimidazoliones containing phenyl groups was studied by Shafi and Basheer\textsuperscript{113} and they found these compounds to be moderately active against bacteria. Therefore we expected better results for the aminoimidazoliones containing pyridyl groups.

Two aminoimidazoliones 4-[amino,(2-pyridyl) methylene]-2-(2-pyridyl)-2-imidazolin-5-one (C\textsubscript{1}) and 4-[amino,(4-pyridyl) methylene]-2-(4-pyridyl)-2-imidazolin-5-one (C\textsubscript{2}) and their hydrochloride (C\textsubscript{3} & C\textsubscript{4}) were tested for antimicrobial activity. Four Gram-positive bacterial strains (\textit{Staphylococcus aureus}, \textit{Bacillus subtilis}, \textit{Streptococcus faecalis} and \textit{Staphylococcus albus}) four Gram-negative bacterial strains (\textit{Escherichia coli}, \textit{Pseudomonas aeruginosa}, \textit{Proteus vulgaris} and \textit{Klebsiella aerogenes}) and two fungi (\textit{Candida albicans} and \textit{Aspergillus niger}) were the microorganisms tested against the compounds under study.

The experimental results revealed that the maximum activity was shown by the hydrochloride of 4-[amino(2-pyridyl) methylene]-2-(2-pyridyl)-2-imidazolin-5-one. But this compound was not active against the Gram-negative bacteria, \textit{Escherichia coli}. All the compounds were active against one Gram-positive bacteria, \textit{Staphylococcus albus} and one Gram-negative bacteria \textit{Pseudomonas aeruginosa} at all concentrations tried (1 mg/ml, 2.5
mg/ml and 5 mg/ml). But the extent of activity of the aminoimidazolinones depends on their concentrations.

The results obtained for the studies of the compounds $C_1$, $C_2$, $C_3$ and $C_4$ at different concentrations on different bacteria are tabulated in Tables V.1, V.2, V.3 and V.4 respectively and the results obtained for these compounds at different concentrations on different fungi are tabulated in Tables V.5, V.6, V.7 and V.8. The activity was measured in terms of the diameter of the zone of inhibition.

Ciprofloxacin (2µg/disc) was used as standard for bacteria and clotrimazole (10 µg/disc) was used as standard for fungi. DMSO was used as the solvent and it showed no effect against the microorganisms under test (No inhibitory effect is indicated by NI).
### Table V.1

**Antibacterial activity of C₁ at different concentrations**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of Bacteria</th>
<th>Diameter of zone of inhibition (mm) at different concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 mg/ml</td>
</tr>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td>NI</td>
</tr>
<tr>
<td>2</td>
<td><em>Bacillus subtilis</em></td>
<td>NI</td>
</tr>
<tr>
<td>3</td>
<td><em>Streptococcus faecalis</em></td>
<td>NI</td>
</tr>
<tr>
<td>4</td>
<td><em>Staphylococcus albus</em></td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td><em>Escherichia coli</em></td>
<td>NI</td>
</tr>
<tr>
<td>6</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td><em>Klebsiella aerogenes</em></td>
<td>NI</td>
</tr>
<tr>
<td>8</td>
<td><em>Proteus vulgaris</em></td>
<td>NI</td>
</tr>
</tbody>
</table>
Table V.2
Antibacterial activity of C\textsubscript{2} at different concentrations

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of Bacteria</th>
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</tr>
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<td></td>
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<td><em>Bacillus subtilis</em></td>
<td>NI</td>
</tr>
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<td>3</td>
<td><em>Streptococcus faecalis</em></td>
<td>NI</td>
</tr>
<tr>
<td>4</td>
<td><em>Staphylococcus albus</em></td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td><em>Escherichia coli</em></td>
<td>NI</td>
</tr>
<tr>
<td>6</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td><em>Klebsiella aerogenes</em></td>
<td>NI</td>
</tr>
<tr>
<td>8</td>
<td><em>Proteus vulgaris</em></td>
<td>NI</td>
</tr>
</tbody>
</table>
Table V.3
Antibacterial activity of C₃ at different concentrations

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of Bacteria</th>
<th>Diameter of zone of inhibition (mm) at different concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 mg/ml</td>
</tr>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td><em>Bacillus subtilis</em></td>
<td>NI</td>
</tr>
<tr>
<td>3</td>
<td><em>Streptococcus faecalis</em></td>
<td>NI</td>
</tr>
<tr>
<td>4</td>
<td><em>Staphylococcus albus</em></td>
<td>12</td>
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<td>5</td>
<td><em>Escherichia coli</em></td>
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<td>6</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td><em>Klebsiella aerogenes</em></td>
<td>NI</td>
</tr>
<tr>
<td>8</td>
<td><em>Proteus vulgaris</em></td>
<td>NI</td>
</tr>
</tbody>
</table>
Table V.4

Antibacterial activity of C₄ at different concentrations

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of Bacteria</th>
<th>Diameter of zone of inhibition (mm) at different concentrations</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1 mg/ml</td>
</tr>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td>NI</td>
</tr>
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<td>2</td>
<td><em>Bacillus subtilis</em></td>
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<td>3</td>
<td><em>Streptococcus faecalis</em></td>
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<td>6</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td><em>Klebsiella aerogenes</em></td>
<td>NI</td>
</tr>
<tr>
<td>8</td>
<td><em>Proteus vulgaris</em></td>
<td>NI</td>
</tr>
</tbody>
</table>

Table V.5

Antifungal activity of C₁ at different concentrations

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of Fungi</th>
<th>Diameter of zone of inhibition (mm) at different concentrations</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 mg/ml</td>
</tr>
<tr>
<td>1</td>
<td><em>Candida albicans</em></td>
<td>NI</td>
</tr>
<tr>
<td>2</td>
<td><em>Aspergillus niger</em></td>
<td>NI</td>
</tr>
</tbody>
</table>
### Table V.6
**Antifungal activity of C\textsubscript{2} at different concentrations**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of Fungi</th>
<th>Diameter of zone of inhibition (mm) at different concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 mg/ml</td>
</tr>
<tr>
<td>1</td>
<td><em>Candida albicans</em></td>
<td>NI</td>
</tr>
<tr>
<td>2</td>
<td><em>Aspergillus niger</em></td>
<td>NI</td>
</tr>
</tbody>
</table>

### Table V.7
**Antifungal activity of C\textsubscript{3} at different concentrations**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of Fungi</th>
<th>Diameter of zone of inhibition (mm) at different concentrations</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 mg/ml</td>
</tr>
<tr>
<td>1</td>
<td><em>Candida albicans</em></td>
<td>NI</td>
</tr>
<tr>
<td>2</td>
<td><em>Aspergillus niger</em></td>
<td>NI</td>
</tr>
</tbody>
</table>

### Table V.8
**Antifungal activity of C\textsubscript{4} at different concentrations**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of Fungi</th>
<th>Diameter of zone of inhibition (mm) at different concentrations</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1 mg/ml</td>
</tr>
<tr>
<td>1</td>
<td><em>Candida albicans</em></td>
<td>NI</td>
</tr>
<tr>
<td>2</td>
<td><em>Aspergillus niger</em></td>
<td>NI</td>
</tr>
</tbody>
</table>
The hydrochlorides of the aminoimidazolinones were more active on both bacteria and fungi when compared with the activity of the aminoimidazolinones as such. This can be due to the greater solubility of the hydrochlorides in aqueous medium. Gram-positive bacteria were found to be more susceptible to the action of these compounds than Gram-negative bacteria.

The biological activity of this type of compounds has been established from the study of Maneesh Kumar and Silpee. They carried out a dose dependent antiproliferative activity study of 4-[amino,(2-pyridyl) methylene]-2-(2-pyridyl)-2-imidazolin-5-one and its hydrochloride on mitogen induced human peripheral lymphocyte culture. The compound showed promising potential in arresting lymphocyte reproduction.

Experimental

Materials and Methods

Microorganisms

The test microorganisms of Gram-positive bacteria namely Staphylococcus aureus, Bacillus subtilis, Streptococcus faecalis and Staphylococcus aureus and Gram-negative bacteria Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella aerogenes and fungi Candida albicans, Aspergillus niger were obtained from National Chemical Laboratory (NCL), Pune and maintained by periodical sub culturing on
nutrient agar and dextrose medium for bacteria and fungi respectively.

The antimicrobial activity of the compounds under study against the selected microorganisms were done by disc diffusion technique.¹⁴⁸⁻¹⁵⁰

**Preparation of chemical extracts**

The amino imidazolinones under investigation were dissolved in DMSO and solutions of different concentrations (1 mg/ml, 2.5 mg/ml and 5 mg/ml) were used to check the antibacterial and antifungal activity.

**Detection of Antimicrobial activity**

The petri plates were streaked with the microbes. For this bacterial strains grown on nutrient agar slants and fungi grown on dextrose were used. As soon as the filter paper disc impregnated with the antimicrobial agent comes in contact with the moist agar surface, water is absorbed into the filter paper and the antibiotic diffuses into the surrounding medium. The rate of extraction of the antibiotic out of the disc is greater than its outward diffusion into the medium, so that the concentration immediately adjacent to the disc may exceed that in the disc itself. As the distance from the disc increased, there was a reduction in the antibiotic concentration. Sensitive organisms were inhibited by the antibiotic diffused into the plate and no growth was seen at the points of inoculation. Resistant organisms appeared as distinct colonies of microbial growth. The edges of the inhibitory zones were clear and easy to measure. The zone diameter was measured in mm.