PART IV
ANTIMICROBIAL ACTIVITY OF THE COMPOUNDS

SECTION A
INTRODUCTION, REVIEW OF LITERATURE AND PROBLEM

INTRODUCTION

The man is well aware of the existence of many chemical agents said to be effective in destroying microorganisms, particularly bacteria. The treatment of diseases with chemotherapeutic substances has been known since the 1500s but only since 1935 this therapy has been widely practiced. "Chemotherapeutic agents are chemical substances used for the treatment of infectious diseases or diseases caused by the proliferation of malignant cells." The chemotherapeutic agents attack and destroy the invading organisms without injuring or destroying the cells of the infected host. The first chemotherapeutic agents introduced by Ehrlich, in 1910, were certain organo-arsenic compounds against syphilis. Ehrlich's contributions were especially important because his was the first systematic and deliberate search for a compound that had potent parasiticidal properties, low toxicity for man and other animals, and good chemical stability.

The interaction between potent chemicals and living system contribute to the understanding of life processes and provide effective methods for the treatment, prevention and diagnosis of many diseases. Chemical substances used for this purpose are called 'drugs' and their actions on living system are referred to as 'drug effect'.

The history of drugs is as old as our ancient civilizations. However, the Greeks were the first who liberated medicine from superstition and magic. Hippocrates, (400 B.C.) a Greek Physician known as the 'Father of Medicine', condemned the use of charms and chants in medicine. He laid down a code of conduct for medical practitioners. Scientific therapy started with Hippocrates.
In 1935, Domagk showed the therapeutic value of a group of compounds known as the sulfonamides. These substances are not specific for a special group of organisms, as arsphenamine is for Treponema, but are effective against a large variety of pathogenic organisms. Sulfanilamide, the first compound in this group was made by Gelmo in 1908 and in 1913 Eisenberg studied the bactericidal properties of azo dyes with a sulfonamide grouping.

After Domagk's reports in 1935 and confirmatory work by investigators in other countries- notably England, France and later the United States - interest in chemotherapy reached an all time high. The compound on which Domagk reported was known as prontosil. French chemists at the Pasteur Institute who studied its action on bacteria and attempted to improve it discovered that its antimicrobial activity is due to the sulfanilamide moiety, previously synthesized and reported by Gelmo in 1908. This observation lighted the fuse for an extensive search for related compounds having therapeutic value. By 1945 it was estimated that 5,488 derivatives of sulfanilamide had been synthesised.

'Antibiotics' are a special kind of chemotherapeutic agents usually obtained from living organisms. The word antibiotic has come to refer to a metabolic product of one organism that is detrimental or inhibitory to other microorganisms in very small amounts. According to Waksman (1945) the term 'antibiotics' applies to those chemical substances of microbial origin which in small amounts exert antimicrobial activity. Antibiotics were known by their activities long before they were given the name by which we know them. Many years ago the Chinese used moldy soyabean curd for the treatment of boils and controlled foot infections by wearing sandals furry with mold.
In 1881, Tyndall reported that culture media cloudy with bacterial growth became clear when mold grew on the surface. Pasteur and Joubert found that pure cultures of anthrax bacilli grew well in urine but that when certain other organisms were present, the anthrax bacilli disappeared. This observation was related to that of Emmerich and Low, who demonstrated in 1901 that when liquid cultures of Pseudomonas aeruginosa were injected into rabbits, the animals were protected against anthrax. They called this material pyocyanase because they thought its activity was due to enzymes from Bacillus pyocyaneus, as Ps. aeruginosa was then called.

An early clinical application of bacterial antagonism was the use of the lactobacillus in the treatment of dysentery, as recommended by Metchnikoff in 1899. This was an example of replacement therapy; i.e. a harmless microbe was able to eliminate and replace one that could cause disease. Modern antibiosis is based not on replacement but on utilization of an active inhibitory principle obtained from the antibiotic-producing microbes.

The first systematic search for, and study of, antibiotics, made by Gratia and Dath in 1924, resulted in the discovery of actinomycetin in strains of actinomycetes, soil organisms that are representative of the group that has given us a number of antibiotics since 1940. Actinomycetin was never used for the treatment of patients but was used to lyse cultures of bacteria for the production of vaccines.

In 1929, Alexander Fleming noticed that an agar plate inoculated with staphylococcus aureus had become contaminated with a mold and that the mold colony was surrounded by a clear zone, indicating inhibition of bacterial growth or lysis of
sometimes antagonize the effects of each other. Combinations of antibiotics with sulfonamides may also give therapeutic results not possible when either is used alone.

It is very important to know the specific mechanism by which a chemotherapeutic agent inhibits or kills microorganisms. This information has wide applications. It is conducive to intelligent use of the drug. It may suggest some new chemical entity as a superior drug, e.g. a similar compound but with some modification in its configuration; it provides a better understanding of the biochemical mechanism of cells. More often than not, chemotherapeutic agents have been discovered by screening experiments, i.e. by trial and error. New chemotherapeutic agents are intensively investigated in order to establish their mode of action.

The point of attack of chemotherapeutic agents on microorganisms varies. It may be at the molecular level, e.g. interference with enzyme synthesis, or at the cellular level, e.g. inhibition of cell-wall synthesis. There are, no doubt, many vulnerable sites for attack between these extremes. A few examples of specific drugs and their inhibition mechanisms can be described as below.

Many bacteria require p-aminobenzoic acid (PABA) as a precursor to their synthesis of the essential folic acid coenzyme. PABA is a structural part of the folic acid molecule.

The selective action of sulfonamides is explained by the fact that the PABA molecule and a sulfonamide molecule are so similar that the sulfonamide may enter the reaction in place of PABA and block the synthesis of an essential cellular constituent, which in this case is folic acid. The cellular functions of the folic acid
coenzyme include amino acid synthesis, vitamin synthesis, etc. Lack of this coenzyme will disrupt normal cellular activity.

Sulfonamides will inhibit growth of those cells which synthesize their own folic acid and will not interfere with the growth of those cells (including mammalian host cells) which require preformed folic acid. This accounts for the selective antibacterial action of sulfonamides and makes them useful in the treatment of many infectious diseases.

There are four ways in which antibiotics can inhibit or kill microorganisms.

1. Inhibit cell-wall formation
2. Damage the cell membrane
3. Interfere with protein synthesis
4. Inhibit nucleic acid metabolism.

Penicillin inhibits cell-wall formation by preventing the incorporation of N-acetylmuramic acid peptide from a "carrier" within the cell to its position in the mucopentide structure that normally constitutes the rigid bacterial cell wall. This type of action is consistent with the fact that penicillin acts only upon actively growing bacteria. Sensitive cells grown in the presence of penicillin are abnormally large and have unusual shapes. Bacilli exposed to penicillin produce extrusions in the cell wall into which the cytoplasm flows. The cell loses its cytoplasm by lysis and empty cytoplasmic membranes are left as "ghosts".

The nitrofurans are antimicrobial drugs which differ from the antibiotics in that they do not occur naturally. The prototype of the nitrofuran derivatives is
furfural which can be prepared from corncobs and cornstalks, oat hulls, beet pulp, peanut hulls and other vegetable by-products. Furfural, an aldehyde derivative of furan also known as 2-furfuraldehyde, was identified in 1832 as an accidental finding during sugar-distillation studies. However, it was not until 1944 that the American investigators Dodd and Stillman reported on the discovery of the antimicrobial properties of nitrated furan derivatives. They found that a surprisingly high antibacterial effect was conferred upon furans by the addition of a nitro group in the 5-position of the furan ring. Variations in the side chain in the 2-position of 5-nitrofuran provide a wide spectrum of compounds in this group and over 1000 compounds have been synthesized and studied. As a class, the nitrofurans are generally effective against a broad spectrum of both gram-positive and gram-negative bacteria, several pathogenic protozoa and some fungi which cause superficial infections in both man and other animals.

In general it may be said that agents that damage the cell wall or the cell membrane are bactericidal while those which interfere with an enzyme action are bacteriostatic.
REVIEW OF LITERATURE

The world is endowed with many systems of medicine, Allopathy, Homeopathy, Ayurveda, the Arabic, the Egyptian, the Graeco-Roman etc. While the Western system has entrenched itself with multifarious growth, there is a growing awareness of the distinctive efficacy of Eastern systems like the Ayurveda.

All ancient civilizations - Egyptian, Babylonian, Indian and Chinese - developed their own systems of medicine. Egyptian system seems to have been the first and the best in the field. It had a fully developed medical system by the third millennium B.C.

We know very little of the Babylonian system and much less, almost nothing of the Indus Valley System. The Indian System, as we know it, starts with the Rigveda (2000 B.C.). The earliest known medical treatise in China appeared around 450 B.C. However, scientific therapy started with hippocrates (400 B.C.) a Greek Physician.

The modern pharmacology as a science had originated recently and probably took the shape following the introduction of experimental procedure by Francois Magendie (1783-1855) which was later expanded by Claude Bernard (1813-1878). The names like Oswald Schmiedeberg (1838-1921) from Germany and John Jacob Abel (1857-1938) from the United States are commonly associated with the development of experimental pharmacology.

Rapid developments in physiology, biochemistry and organic chemistry
during the recent years have greatly accelerated and revolutionised the progress in pharmacology.

From the literature, it appears that much work has been done on several heterocyclic compounds for their antimicrobial activities including both gram positive and gram negative bacteria.

Chalcones and their substituted derivatives are reported to have antibacterial\(^1\), antifungal, antiparasitic, antitubercular, antiinflammatory and insect repellent properties\(^2,3\). Bhatt et al\(^4\) synthesised quinoline derivatives of chalcones and screened the products for antibacterial activities, while Ahluwalia and coworkers\(^5\) screened in vitro dihydrochalcones and their derivatives against some microorganisms.

Flavones play vital role in plant life\(^6\) and are reported to be antibacterial\(^7,8\). Pyrimidine derivatives are reported to possess various biological\(^9\) and antibacterial properties\(^10\).

Isoxazolines and pyrazolines\(^11-18\) revealed that these compounds are not only used in textiles and cinematographic films but they also show widely differing bacteriological activity. Ozawa and co-workers\(^19\) worked on pyrazolines and found them effective in killing houseflies on contact. The insecticidal properties of pyrazolines were studied in 1982 by Van-Hes and co-workers\(^20\). Pyrazolines and fluorinated heteroarylpyrazolines also possess antibacterial activity\(^21-22\). Roda and co-workers\(^23\) synthesised some new 2-pyrazoline derivatives and tested them for antimicrobial activity.
Like pyrazolines and isoxazolines, pyrazoles and isoxazoles were also synthesised and tested for antibacterial activity. Sharma and co-workers reported that hydroxyarylpazoles were found to be effective antimicrobials.

Giri reported the antifungal nature of 1-substituted-3-(2-hydroxyphenyl)-5-(4-nitrophenyl) pyrazoles while trifluoromethyl-1-arylpazoles were reported to be analgesic, antipyretic and antiinflammatory agents. Anderson and Paolella showed that 1-phenylpyrazole derivatives are effective antidiabetic while Faucher reported that (phosphanadithioacetamido)-phenylpyrazoles are good insecticides. Chlorosubstituted isoxazoles also possess antibacterial activity.

Similarly, 1,3-thiazines are also reported to possess antimicrobial and pesticidal properties. Khadse has synthesised a series of 4-(5-nitro-2-furyl)-2-(substituted phenylamino) thiazoles, 4-(2,4,5-trichlorophenyl)-2-(substituted phenylamino) thiazoles and 4-(di and trihydroxyphenyl)-2-(substituted phenylamino) thiazoles and studied their antitubercular activity. Many of these compounds showed antitubercular activity at 1.56 mg/ml concentration. 6-Amino-2-alkylthiobenzothiazoles were found to possess bacteriostatic activity against Mycobacterium tuberculosis. Zawadzka et al synthesised and studied pharmacological action of thiazole derivatives. Gudadhe has reported antitubercular activity of iodo substituted-4,6-diaryl-2-imino-6H-2,3-dihydro-1,3-thiazines and 5-(2-hydroxy-3-iodo-5-methylphenyl)-4-anisoyl-2-amino-1,3-thiazole. Ramekar reported antitubercular activity of 3,5-diaryl-4-aroyl isoxazolines, pyrazolines and 4,6-diaryl-5-aroyl-2-amino-6H-2,3-dihydro-1,3-thiazines. Raghuwanshi reported the synthesis and antimicrobial as well as antifungal activity of 1,3-thiazines obtained from nitrochalcones. Modi and Naik have reported synthesis of some new 1-H-3-(2'-hydroxy-4'-methoxy-5'
bromophen-1"-yl)-5-substituted phenyl-2-pyrazolines and related compounds and their antibacterial activity. Ankhiwala has synthesised some 1-phenyl-3,5-diaryl-1,2-pyrazolines and 3,5-diaryl-1,2-isoxazolines and reported their antibacterial activity. Similarly, Thore et al. have reported synthesis of some new pyrazolines and isoxazolines and their antibacterial activity. Ahmad Roshan reported synthesis and antimicrobial activity of 3-(2-hydroxyphenyl)-5-(4-substitutedphenyl)-1-pyrazoleacetic acids. Newly synthesised 1,3-thiazines from chloro and chlorobromosubstituted chalcones have been reported to show antimicrobial activity. Shingare et al. have reported antimicrobial activity of some dihydropyridino pyrazoles. Raut and Doshi have reported synthesis and antimicrobial activity of 1-H-3-(2-hydroxy-3-bromo-5-chlorophenyl)-5-substituted phenyl-2-pyrazolines and their acetyl and benzoyl derivatives. Recently, Deshmukh reported synthesis and antimicrobial activity of dichlorosubstituted 3,5-diarylisoxazolines, isoxazoles, pyrazolines, pyrazoles and 4,6-diaryl-1,3-thiazines.

Antimicrobial susceptibility test is carried out on a large scale in clinical laboratories as a guide to antibiotic therapy when one adds to these clinical tests those done in pharmaceutical and research laboratories in screening and evaluation of new compounds. They can be carried out by many different methods with variable degrees of accuracy and relevance. In the management of a specific infection in a patient, the test is performed to determine whether or not the infecting organism is susceptible to a series of antibiotics that might be relevant in the treatment. Antibiotic sensitivity tests are also often used as an aid in the identification of infecting organisms. All are helpful in indicating the identity of isolates from clinical material.

The disk-diffusion method provides a simple, convenient and reliable test specially applicable in routine clinical bacteriology laboratory. It consists of
impregnating small disks of a standard filter paper with given amounts of a chosen range of antibiotics. These are placed on plates of culture medium previously spread uniformly with an inoculum of the bacterial isolate to be tested. After incubation, the degree of sensitivity is determined by measuring the easily visible areas of inhibition of growth produced by the diffusion of the antibiotic from the disks into the surrounding medium.

Paper discs as reservoirs of antibiotics for sensitivity testing were introduced in the 1940s (Vincent and Vincent, 1944; Morley, 1945; Bondi et al, 1947) and are now more commonly used than others such as cups (Rose and Miller, 1939), tablets (Lund et al, 1951) or Wells cut in the medium. Although it is possible to produce dry filter-paper discs in the laboratory (Fair-brother and Sherris, 1959; Leach and Willis, 1971) most laboratories consider this to be too time consuming and consequently rely on commercial sources.
PROBLEM

From the review of literature it appears that most of the heterocyclic compounds show antibacterial, antifungal, antiparasitic, herbicidal and insecticidal activity. The work presented in this part of the thesis deals with the antimicrobial study of newly synthesised heterocyclic compounds. These compounds have been subjected to antimicrobial test against some common pathogenic micro-organisms such as Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumoniae, Salmonella typhii, Shigella dysentery and Proteus mirabilis in order to know their antimicrobial activity.

The following compounds were tested.

1. 3-(2-Hydroxy-5-chlorophenyl)-5-(4-dimethylaminophenyl) isoxazoline.
2. 3-(2-hydroxy-5-chlorophenyl)-5-(4-dimethylaminophenyl)-Δ2-pyrazoline
3. 4-(2-hydroxy-5-chlorophenyl)-6-(4-dimethylaminophenyl)-2-amino-6H-2,3-dihydro-1,3-thiazine.
4. 1-Carboxamido-3-(2-hydroxy-5-chlorophenyl)-5-(4-dimethylaminophenyl)-Δ3-pyrazoline.
5. 3-(2-Hydroxyphenyl)-5-(4-dimethylaminophenyl) isoxazoline.
6. 3-(2-Hydroxyphenyl)-5-(4-dimethylaminophenyl)-Δ2-pyrazoline.
7. 4-(2-Hydroxyphenyl)-6-(4-dimethylaminophenyl)-2-imino-6H-2,3-dihydro-1,3-thiazine.
8. 1-Carboxamido-3-(2-hydroxyphenyl)-5-(4-dimethylaminophenyl)-Δ2-pyrazoline
9. 3-(2-Hydroxy-5-methylphenyl)-5-(4-dimethylaminophenyl) isoxazoline.
10. 3-(2-Hydroxy-5-methylphenyl)-5-(4-dimethylaminophenyl)-Δ2-pyrazoline.
11. 4-(2-Hydroxy-5-methylphenyl)-6-(4-dimethylaminophenyl)-2-imino-6H-2,3-dihydro-1,3-thiazine.
12. 1-Carboxamido-3-(2-hydroxy-5-methylphenyl)-5-(4-dimethylaminophenyl)-Δ²-pyrazoline
13. 3-Phenyl-5-(4-dimethylaminophenyl) isoxazoline.
14. 3-Phenyl-5-(4-dimethylaminophenyl)-Δ²-pyrazoline.
15. 4-Phenyl-6-(4-dimethylaminophenyl)-2-imino-6H-2,3-dihydro-1,3-thiazine.
16. 1-Carboxamido-3-phenyl-5-(4-dimethylaminophenyl)-Δ²-pyrazoline.
17. Chalcone
18. 4-Methoxychalcone
19. 3,5-Diphenyl isoxazoline
20. 3,5-Diphenyl-Δ²-pyrazoline
21. 4,6-Diphenyl-2-imino-6H-2,3-dihydro-1,3-thiazine.
22. 1-Carboxamido-3-5-diphenyl-Δ²-pyrazoline
23. 3-Phenyl-5-(4-methoxyphenyl)-isoxazoline
24. 3-Phenyl-5-(4-methoxyphenyl)-Δ²-pyrazoline
25. 4-Phenyl-6-(4-methoxyphenyl)-2-imino-6H-2,3-dihydro-1,3-thiazine
26. 1-Carboxamido-3-phenyl-5-(4-methoxyphenyl)-Δ²-pyrazoline.
27. 1-Carboxamido-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-(4-dimethyl aminophenyl)-Δ²-pyrazoline
28. 3-(2-Hydroxy-5-chlorophenyl)-4-benzoyl-5-(4-dimethylaminophenyl)-Δ²-pyrazoline
29. 1-Carboxamido-3-(2-hydroxy-5-methylphenyl-4-benzoyl-5-(4-dimethylaminophenyl)-Δ²-pyrazoline
30. 3-(2-Hydroxy-5-methylphenyl)-4-benzoyl-5-(4-dimethylaminophenyl)-Δ²-pyrazoline
31. 1-Carboxamido-3-(2-hydroxyphenyl)-4-benzoyl-5-(4-dimethylaminophenyl)-Δ²-pyrazoline
32. 3-(2-Hydroxyphenyl)-4-benzoyl-5-(4-dimethylaminophenyl)-Δ²-pyrazoline
33. 1-Carboxamido-3-(2-hydroxy-chlorophenyl)-4-benzoyl-5-phenyl-Δ²-pyrazoline
34. 3-(2-Hydroxy-5-chlorophenyl)-4-benzoyl-5-phenyl-Δ²-pyrazoline
35. 1-Carboxamido-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-(4-methoxyphenyl)-Δ²-pyrazoline
36. 3-(2-Hydroxy-5-chlorophenyl)-4-benzoyl-5-(4-methoxyphenyl)-Δ²-pyrazoline
37. 3-(2-Hydroxy-5-chlorophenyl)-4-benzoyl-5-(4-dimethylaminophenyl) isoxazoline.
38. 3-(2-Hydroxy-5-methylphenyl)-4-benzoyl-5-(4-dimethylaminophenyl) isoxazoline.
39. 3-(2-Hydroxyphenyl)-4-benzoyl-5-(4-dimethylaminophenyl) isoxazoline.
40. 3-(2-Hydroxy-5-chlorophenyl)-4-benzoyl-5-phenyl isoxazoline.
41. 3-(2-Hydroxy-5-chlorophenyl)-4-benzoyl-5-(4-methoxyphenyl) isoxazoline.
42. 4-(2-Hydroxy-5-chlorophenyl)-5-benzoyl-6-(4-dimethylaminophenyl)-2-imino-6H-2,3-dihydro-1,3-thiazine.
43. 4-(2-Hydroxy-5-methylphenyl)-5-benzoyl-6-(4-dimethylaminophenyl)-2-imino-6H-2,3-dihydro-1,3-thiazine.
44. 4-(2-Hydroxyphenyl)-5-benzoyl-6-(4-dimethylaminophenyl)-2-imino-6H-2,3-dihydro-1,3-thiazine.
45. 4-(2-Hydroxy-5-chlorophenyl)-5-benzoyl-6-phenyl-2-imino-6H-2,3-dihydro-1,3-thiazine.
46. 4-(2-Hydroxy-5-chlorophenyl)-5-benzoyl-6-(4-methoxyphenyl)-2-imino-6H-2,3-dihydro-1,3-thiazine.
47. 3-(2-Hydroxy-5-chlorophenyl)-5-(2-furyl) isoxazoline
48. 3-(2-Hydroxy-4-methylphenyl)-5-(4-methoxyphenyl) isoxazoline.
49. 3-(2-Hydroxy-5-chlorophenyl)-5-phenylisoxazole
50. 3-(2-Hydroxy-5-chlorophenyl)-1-H-5-phenylpyrazole.
These compounds have been tested against the following bacteria which are known human pathogens.

1. **Klebsiella pneumoniae**
   
   It is generally found in the mucosa of upper respiratory tract, intestine and genitourinary tract and appears in the form of nonmotile, capsulated, Gram-negative rods forming large mucoid colonies with oil paint like appearance on MCA. It is responsible for causing Friedlander's Pneumonia (destructive pneumonia), urinary tract infections and suppurative lesions elsewhere in the body (biliary tract, peritoneum, mastoids, paranasal sinuses, meninges).

2. **Escherichia coli**
   
   It is found in human or animal intestine as commensal in gastrointestinal tract. However, it has also been detected in drinking water which is the direct evidence of water pollution due to human or animal excreta. They are Gram-negative, noncapsulated, non-sporing, motile with peritrichous flagella, producing lactose fermenting colonies on Mac-Conkey medium and are responsible for causing diarrhoea or gastroenteritis, urinary tract infections, peritoneal and biliary infections, neonatal infections and pyogenic infections etc.

3. **Proteus mirabilis**
   
   These are Gram-negative, non-sporeforming, rodshaped, motile bacteria. They are usually found in faeces, water, sewage and decayed matter. They can cause urinary tract infection, pyelonephritis, pyogenic lesions of various types such as
abscesses and infection of wounds, ear or respiratory tract. They are also responsible for infantile diarrhoea.

4. **Staphylococcus aureus**:  
They are Gram-positive cocci existing in grape like irregular clusters, nonmotile, nonsporing, noncapsulated, coagulase positive, toxigenic mannitol fermenting. Pathogenic staphylococci produce golden yellow colonies on nutrient agar. It may cause the majority of acute pyogenic lesions in man. Staphylococcal lesions are characteristically localized. The pyogenic infections include boils, furuncles, abscesses, carbuncles. Staphylococcus aureus specifically produces pus in wounds and burns. The food contaminated with enterotoxin, produced by staphylococcus is responsible for diarrhoea and vomiting. It is also responsible for cross-infection in hospitals.

5. **Shigella dysentery**:  
They are Gram-negative rods, slender, nonmotile, nonflagellate, noncapsulate, nonlactose fermenting pale colonies on MCA. They are responsible for bacillary dysentery, loose motion accompanied with blood and mucous. Shigella dysentery type I usually causes complications like arthritis, toxic neuritis, conjunctivitis, parotitis, intussusception and myocarditis.

6. **Salmonella typhi**:  
They are Gram-negative rods, motile with peritrichous flagella, noncapsulate, mostly fimbriate showing non-lactose fermenter and colourless colonies on Macconkey's medium. They are caustive agents for acute gastroenteritis, enteric fever (typhoid or paratyphoid fever), food poisoning, septicaemia
(Bacteriæmia), asymptomatic intestinal infection, osteomyelitis, endocarditis, abscess etc.

7. **Bacillus subtilis**

   They are Gram-positive, rod-shaped, sporulating, generally motile and found in the soil. They can cause skin diseases in man.

8. **Proteus vulgaris**

   These are Gram-negative, non-sporeforming, generally motile, rod-shaped bacteria. They are found in soil, sewage and decaying matter. They can produce H₂S gas from sulphur containing amino acids, but do not ferment lactose. They may inhabit intestinal tract of man and animals and are responsible for infection of urinary tract, septicaemia etc.
SECTION B
EXPERIMENTAL AND DISCUSSION OF THE RESULTS

During the 19th century, a number of natural products were isolated and subjected to detailed investigations of their structure and pharmacological action. Some of the compounds were found to possess a definite physiological activity and later on it was observed that the physiological activity of a compound is associated with a particular structural unit or group and hence if this structural unit is present in other compounds the latter also becomes biologically active. Such a part of the drug which is responsible for the actual physiological activity is known as pharmacophore group. The pharmacophoric group is then somewhat modified by the common and simple unit processes to give more active compounds with low toxicity. It is believed that some physiological effect is often associated with a particular group and hence the biological activity.

The work presented here deals with the antimicrobial activity of synthesised compounds (1-52). These compounds were characterised on the basis of chemical properties, elemental analysis and spectral data. Their experimental details have been discussed in their respective chapters in Part I and II of the thesis.

Antimicrobial Activity of the Compounds:

These compounds were assayed for their antimicrobial activities against the test organisms, such as, *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, *Shigella dysentery* and *Salmonella typhi* at a concentration of 1000 µg/ml by agar well technique. Further their Minimal Inhibitory
Concentration values (MIC values) against these organisms were determined by serial dilution method$^*$ using DMF as a solvent.

Method:

The medium used for the antimicrobial study was HI-media, India make nutrient agar having the following composition.

Composition of Nutrient Agar:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
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<tbody>
<tr>
<td>Peptone</td>
<td>5 gms/litre</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5 gms/litre</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.5 gms/litre</td>
</tr>
<tr>
<td>Beef extract</td>
<td>1.5 gms/litre</td>
</tr>
<tr>
<td>Agar</td>
<td>15 gms/litre</td>
</tr>
<tr>
<td>pH</td>
<td>7.4 ± 0.2</td>
</tr>
</tbody>
</table>

The medium was prepared by dissolving 28 gms of ingredients in one litre of distilled water and was sterilized at 121°C temp and 15 lbs/inch$^2$ pressure in an autoclave for fifteen minutes. After sterilization the medium was cooled to about 50°C and poured into sterile petriplates and allowed to solidify. The media plates were then seeded with 24 hrs old active nutrient broth cultures of the test organism in order to obtain lawn culture. Standard 6 mm size wells (cups) were then prepared in the solidified medium with the help of standard quality cork boarers. For aseptic conditions the operations were carried out under microfilt, India make ultraclean air Laminar flow system.

The test compounds were dissolved in dimethylformamide keeping the initial concentration of solutions 1000 μgm/ml. From this successive dilutions were made by twice diluting the previous solutions. The wells were then filled by the
Antimicrobial activity of compounds (Sr. Nos.: 7, 8, 9, 10, 11, 12)

Antimicrobial activity of compounds (Sr. Nos.: 13, 14, 15, 16, 17, 18)
solutions of each concentration of synthesised compounds. Plane DMF solvent was used as a control. The plates were then kept at 37°C for 24 hours for incubation. After 24 hours (incubation period), the plates were observed for inhibitory action of test compounds for each concentration as a clear zone around the wells (shown in the two photographic plates for the compounds with Sample Nos. 7,8,9,10,11,12 and 13,14,15,16,17,18). Thus, MIC values were determined by critically examining the inhibitory action of test compounds of various concentrations. The results are shown in the following Tables.

The compounds given in Table 1 (Sr.No. 1 to 16), when tested for their antimicrobial activity against *K. pneumoniae, E. coli, P. mirabilis, S. aureus, S. dysentery* and *S. typhi* [at a temperature of 37°C (±1°C)], most of them showed positive results. Many of these compounds have been found to be moderately active against above mentioned organisms. Statistically, it can be said that 77% of the total samples tested showed antimicrobial activity. Among these, the compounds synthesised from 2'-hydroxy-5'-chloro-4-dimethylamino chalcone possess very high antimicrobial activity which can be attributed to the presence of -Cl and -N(CH₃)₂ groups as substituents in these compounds (Sr.No. 1-4). Except two, these were found to be active in all the cases. If comparison is made between compounds 5,6,7,8 and 9,10,11,12 obtained from 2'-hydroxy-4-dimethylaminochalcone and 2'-hydroxy-5'-methyl-4-dimethylamino chalcone respectively, it appears that introduction of methyl group in the latter has not contributed substantially to the activity of these compounds since both of them show similar activity (seventeen each). However, in the last category (i.e. compounds obtained from 4-methoxychalcone), where there is only N(CH₃)₂ group as a substituent, antimicrobial activity appears to have increased as it showed positive results in eighteen cases.
Table 1: Antimicrobial activity of compounds.

<table>
<thead>
<tr>
<th>S No</th>
<th>Compound</th>
<th>MIC values in (in mg/ml) against test organism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>K.pneumoniae</td>
</tr>
<tr>
<td>1.</td>
<td>3-(2-Hydroxy-5-chlorophenyl)-5-(4-dimethylaminophenyl)-isoxazole</td>
<td>62</td>
</tr>
<tr>
<td>2.</td>
<td>3-(2-Hydroxy-5-chlorophenyl)-5-(dimethylaminophenyl)-$\Delta^2$-pyrazoline</td>
<td>62</td>
</tr>
<tr>
<td>3.</td>
<td>4-(2-Hydroxy-5-chlorophenyl)-6-(4-dimethylaminophenyl)-2-imino-6H-2,3-dihydro-1,3-thiazine</td>
<td>16</td>
</tr>
<tr>
<td>4.</td>
<td>1-Carboxamido-3-(2-hydroxy-5-chlorophenyl)-5-(4-dimethylaminophenyl)-$\Delta^2$-pyrazoline.</td>
<td>31</td>
</tr>
<tr>
<td>5.</td>
<td>3-(2-hydroxyphenyl)-5-(4-dimethylaminophenyl) isoxazoline.</td>
<td>31</td>
</tr>
<tr>
<td>6.</td>
<td>3-(2-hydroxyphenyl)-5-(4-dimethylaminophenyl) $\Delta^1$-pyrazoline.</td>
<td>62</td>
</tr>
<tr>
<td>7.</td>
<td>4-(2-hydroxyphenyl)-6-(4-dimethylaminophenyl) 2-imino-6H-2,3-dihydro-1,3-thiazine.</td>
<td>62</td>
</tr>
<tr>
<td>S No.</td>
<td>Compound</td>
<td>K.pneumoniae</td>
</tr>
<tr>
<td>-------</td>
<td>-------------------------------------------------------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>8.</td>
<td>1-Carboxamido-3-(2-hydroxyphenyl)-5-(4-dimethylaminophenyl)-Δ²-pyrazoline.</td>
<td>62</td>
</tr>
<tr>
<td>9.</td>
<td>3-(2-Hydroxy-5-methylphenyl)-5-(4-dimethylaminophenyl)-isoxazoline</td>
<td>62</td>
</tr>
<tr>
<td>10.</td>
<td>3-(2-Hydroxy-5-methylphenyl)-5-(4-dimethylaminophenyl)-Δ²-pyrazoline.</td>
<td>62</td>
</tr>
<tr>
<td>11.</td>
<td>4-(2-hydroxy-5-methylphenyl)-6-(4-dimethylaminophenyl)-2-imino-6H,2,3-dihydro-1,3-thiazine</td>
<td>62</td>
</tr>
<tr>
<td>12.</td>
<td>1-Carboxamido-3-(2-hydroxy-5-methylphenyl)-5-(4-dimethylaminophenyl)-Δ²-pyrazoline.</td>
<td>62</td>
</tr>
<tr>
<td>13.</td>
<td>3-Phenyl-5-(4-dimethylaminophenyl)-isoxazoline</td>
<td>62</td>
</tr>
<tr>
<td>14.</td>
<td>3-Phenyl-5-(4-dimethylaminophenyl)-Δ²-pyrazoline.</td>
<td>31</td>
</tr>
<tr>
<td>15.</td>
<td>4-Phenyl-6-(4-dimethylaminophenyl)-2-imino-6H,2,3-dihydro-1,3-thiazine.</td>
<td>62</td>
</tr>
<tr>
<td>16.</td>
<td>1-Carboxamido-3-phenyl-5-(4-dimethylaminophenyl)-Δ²-pyrazoline.</td>
<td>62</td>
</tr>
</tbody>
</table>
It can, therefore, be concluded that dimethylamino (-N(CH$_3$)$_2$) group possesses distinct antimicrobial activity which is independent of -OH and -CH$_3$ groups. Heterocyclics having N(CH$_3$)$_2$ and -Cl groups can effectively act as antimicrobial agents. The general trend in antimicrobial activity of these heterocyclics in decreasing order can be given as - Pyrazolines> Isoxazolines > Carboxamidopyrazolines > Thiazines.

Table 2: Antimicrobial activity of compounds.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compound</th>
<th>MIC values (in μgm/ml) against test organisms -</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>17.</td>
<td>Chalcone</td>
<td>80</td>
</tr>
<tr>
<td>18.</td>
<td>4-Methoxychalcone</td>
<td>100</td>
</tr>
<tr>
<td>19.</td>
<td>3,5-Diphenylisoxazoline</td>
<td>80</td>
</tr>
<tr>
<td>20.</td>
<td>3,5-Diphenyl-Δ$^2$-pyrazoline</td>
<td>50</td>
</tr>
<tr>
<td>21.</td>
<td>4,6-Diphenyl-2-imino-6H-2,3-dihydro-1,3-thiazine</td>
<td>100</td>
</tr>
<tr>
<td>22.</td>
<td>1-Carboxamido-3,5-diphenyl-Δ$^2$-pyrazoline</td>
<td>100</td>
</tr>
<tr>
<td>23.</td>
<td>3-Phenyl-5-(4-methoxyphenyl) isoxazoline</td>
<td>80</td>
</tr>
<tr>
<td>24.</td>
<td>3-Phenyl-5-(4-methoxyphenyl)-Δ$^2$-pyrazoline</td>
<td>100</td>
</tr>
<tr>
<td>25.</td>
<td>4-Phenyl-6-(4-methoxyphenyl)-2-imino-6H-2,3-dihydro-1,3-thiazine</td>
<td>-</td>
</tr>
<tr>
<td>26.</td>
<td>1-Carboxamido-3-phenyl-5-(4-methoxyphenyl)-Δ$^2$-pyrazoline</td>
<td>-</td>
</tr>
</tbody>
</table>
The compounds in Table 2 (Sr.No. 17-26) were tested for antimicrobial activity against Gram-negative bacillus (*Escherichia coli*), Gram-positive bacillus (*Bacillus subtilis*) and Gram-positive cocci (*Staphylococcus aureus*) at an initial concentration of 100 μgm/ml. From the Table, it follows that Compound No. 17 is more active than Compound No. 18. Likewise the heterocyclic compounds obtained from Compound No. 17 show greater antimicrobial activity than those obtained from Compound No. 18. Among these compounds, only compound No. 21 showed antimicrobial activity against *B. subtilis*. Compound Nos. 20, 17, 19, 23, 18, 21, 24 were found to possess antimicrobial activity in decreasing order against *E. coli*. Compound Nos. 17, 23, 24, 19, 21 and 25 showed activity in decreasing order against *Staphylococcus aureus*. Thus, it can be concluded that the compounds having N-N and O-N atoms on the heterocyclic ring enhance the antimicrobial activity.

The compounds given in Table 3 (Sr.No. 27 to 36) when assayed for their antimicrobial activity against the test organisms, *K. pneumoniae*, *E. coli*, *S. aureus*, *P. vulgaris*, *S. typhi*, *S. dysentery* and *P. mirabilis* [at a temperature of 37°C (±1°C)], a large number of them showed moderate activity. It can be observed that 84% of the total samples tested showed antimicrobial activity. Most of these compounds showed antimicrobial activity in the concentration range of 250-500 μgm/ml. All compounds showed their activity against the test organisms *K. pneumoniae*, *E. coli*, and *Shigella dysentery*. The compound Nos. 29, 30, 33 and 34 did not show activity against *S. aureus*. Similarly the compound Nos. 27, 29, 30, 31 and 35 did not show activity against *S. typhi*. It is noteworthy to mention that the compounds with serial numbers 28, 32 and 36 have been found to be active against all the test organisms. Among these, both compound numbers 28 and 32 contain dimethylamino group as one of the substituents but-Cl is absent in the latter. The Compound No. 36 contains methoxy and chloro groups as substituents and hence its activity is enhanced.
<table>
<thead>
<tr>
<th>S. No</th>
<th>Compound</th>
<th>MIC values in (in mg/ml) against test organism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>K. pneumoniae</td>
</tr>
<tr>
<td>27</td>
<td>1-Carboxamido-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-(4-dimethylanilinephenyl)-△³-pyrazoline.</td>
<td>250</td>
</tr>
<tr>
<td>28</td>
<td>3-(2-Hydroxy-5-chlorophenyl)-4-benzoyl-5-(4-dimethylanilinephenyl)-△³-pyrazoline.</td>
<td>500</td>
</tr>
<tr>
<td>29</td>
<td>1-Carboxamido-3-(2-hydroxy-5-methylphenyl)-4-benzoyl-5-(4-dimethylanilinephenyl)-△³-pyrazoline.</td>
<td>63</td>
</tr>
<tr>
<td>30</td>
<td>3-(2-Hydroxy-5-methylphenyl)-4-benzoyl-5-(4-dimethylanilinephenyl)-△³-pyrazoline.</td>
<td>125</td>
</tr>
<tr>
<td>31</td>
<td>1-Carboxamido-3-(2-hydroxyphenyl)-4-benzoyl-5-(4-dimethylanilinephenyl)-△³-pyrazoline.</td>
<td>125</td>
</tr>
<tr>
<td>32</td>
<td>3-(2-Hydroxyphenyl)-4-benzoyl-5-(4-dimethylanilinephenyl)-△³-pyrazoline.</td>
<td>250</td>
</tr>
</tbody>
</table>
Table 3...contd...

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>MIC values in (in mg/ml) against test organism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>K.pneumoniae</td>
</tr>
<tr>
<td>33</td>
<td>1-Carboxamido-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-phenyl-Δ²-pyrazoline</td>
<td>500</td>
</tr>
<tr>
<td>34</td>
<td>3-(2-Hydroxy-5-chlorophenyl)-4-benzoyl-5-phenyl-Δ²-pyrazoline</td>
<td>250</td>
</tr>
<tr>
<td>35</td>
<td>1-Carboxamido-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-(4-methoxyphenyl)-Δ²-pyrazoline</td>
<td>250</td>
</tr>
<tr>
<td>36</td>
<td>3-(2-Hydroxy-5-chlorophenyl)-4-benzoyl-5-(4-methoxyphenyl)-Δ²-pyrazoline</td>
<td>500</td>
</tr>
</tbody>
</table>
Therefore, it can be said that dimethylamino group possesses distinct antimicrobial activity which is independent of presence of other substituents.

On the basis of MIC values, the general trend in antimicrobial activity of these compounds in decreasing order can be given as, Compound No. 32 > 36 > 28.

The compounds given in Table 4 when assayed for their antimicrobial activity against the test organisms *E. coli*, *S. aureus*, *P. vulgaris*, *K. pneumoniae*, *S. typhi*, *S. dysentery* and *P. mirabilis* [at a temperature of 37°C (±1°C)], many of them were found to possess moderate activity. It is observed that 78% of the total samples tested showed antimicrobial activity. Most of these compounds were found to be active in the concentration range of 125 to 500 µgm/ml. All compounds showed their activity against the test organism *Klebsiella pneumoniae*. Similarly, except compound Nos. 45 and 50, all compounds were active against *Salmonella typhi*. Among isoxazolines (Compound Nos. 37 to 41), compounds with serial numbers 37, 40 and 41 were found to be active against all the organisms. Both compound No. 40 and 41 contain chloro group but compound No. 41 has methoxy group as another substituent and hence its activity is enhanced. However, compound No. 37, which contains dimethylamino group along with chloro is found to be the most active.

In case of thiazines (Compound Nos. 42 to 46), which seem to be lesser active than isoxazolines, compound No. 42 showed antimicrobial activity against maximum number of organisms followed by compound No. 46 which showed activity against five organisms. Both of them contain chloro group but the former has dimethylamino group as another substituent and hence it showed more activity than compound No. 46, which has methoxy group as another substituent. It is observed that compound No. 47, which was obtained from 2'-hydroxy-5'-chloro-2-furylchalcone
<table>
<thead>
<tr>
<th>S No.</th>
<th>Compound</th>
<th>MIC values in (in mgm/ml) against test organism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>37</td>
<td>3-(2-Hydroxy-5-chlorophenyl)-4-benzoyl-5-(4-dimethylaminophenyl) isoxazoline</td>
<td>125</td>
</tr>
<tr>
<td>38</td>
<td>3-(2-Hydroxy-5-methylphenyl)-4-benzoyl-5-(4-dimethylaminophenyl) isoxazoline</td>
<td>125</td>
</tr>
<tr>
<td>39</td>
<td>3-(2-Hydroxyphenyl)-4-benzoyl-5-(4-dimethylaminophenyl)-isoxazoline</td>
<td>1000</td>
</tr>
<tr>
<td>40</td>
<td>3-(2-Hydroxy-5-chlorophenyl)-4-benzoyl-5-phenyl isoxazoline</td>
<td>500</td>
</tr>
<tr>
<td>41</td>
<td>3-(2-Hydroxy-5-chlorophenyl)-4-benzoyl-5-(4-methoxyphenyl) isoxazoline</td>
<td>250</td>
</tr>
<tr>
<td>42</td>
<td>4-(2-hydroxy-5-chlorophenyl)-5-benzoyl-6-(4-dimethylaminophenyl)-2-imino-6H-2,3-dihydro-1,3-thiazine</td>
<td>125</td>
</tr>
<tr>
<td>S.No.</td>
<td>Compound</td>
<td>E.coli</td>
</tr>
<tr>
<td>------</td>
<td>--------------------------------------------------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>43</td>
<td>4-(2-hydroxy-5-methylphenyl)-5-benzoyl-6-(4-dimethylaminophenyl)-2-imino-6H-2,3-dihydro-1,3-thiazine.</td>
<td>-</td>
</tr>
<tr>
<td>44</td>
<td>4-(2-hydroxyphenyl)-5-benzoyl-6-(4-dimethylaminophenyl)-2-imino-6H-2,3-dihydro-1,3-thiazine.</td>
<td>-</td>
</tr>
<tr>
<td>45</td>
<td>4-(2-hydroxy-5-chlorophenyl)-5-benzoyl-6-(phenyl)-2-imino-6H-2,3-dihydro-1,3-thiazine.</td>
<td>-</td>
</tr>
<tr>
<td>46</td>
<td>4-(2-hydroxy-5-chlorophenyl)-5-benzoyl-6-(4-methoxyphenyl)-2-imino-6H-2,3-dihydro-1,3-thiazine.</td>
<td>125</td>
</tr>
<tr>
<td>47</td>
<td>3-(2-hydroxy-4-chlorophenyl)-5-(2-furyl) isoxazoline</td>
<td>63</td>
</tr>
<tr>
<td>48</td>
<td>3-(2-hydroxy-4-methylphenyl)-5-(4-methoxyphenyl) isoxazole</td>
<td>500</td>
</tr>
<tr>
<td>49</td>
<td>3-(2-hydroxy-5-chlorophenyl)-5-phenylisoxazole</td>
<td>250</td>
</tr>
<tr>
<td>50</td>
<td>3-(2-hydroxy-5-chlorophenyl)-1-H-5-phenylpyrazole</td>
<td>500</td>
</tr>
<tr>
<td>51</td>
<td>5-(2-hydroxy-5-chlorophenyl)-4-benzoyl-2-amino-1,3-thiazole</td>
<td>-</td>
</tr>
<tr>
<td>52</td>
<td>1-Carboxamido-3-(2-hydroxy-5-chlorophenyl)-5-phenylpyrazole.</td>
<td>500</td>
</tr>
</tbody>
</table>
possesses marked antimicrobial activity which can be attributed to the presence of chloro and furyl groups as substituents.

In case of compounds with serial numbers 49, 50, 51 and 52 which have been synthesised from 1-(2-hydroxy-5-chlorophenyl)-3-phenyl-1,3-propane dione, moderate activity is observed against most of the test organisms. This activity can be attributed to the presence of chloro group in these compounds. From the MIC values, it follows that among these compounds compound No. 49 shows greater antimicrobial activity than compound Nos 50 and 52 followed by 51. The comparison between the MIC values of 48 and 49 shows that a compound containing chloro group (49) can act as a more powerful antibacterial agent than those containing methyl and methoxy groups (48).

It can, therefore, be concluded that (i) the isoxazolines possess more antimicrobial activity than thiazines. (ii) Both the combinations -N(CH₃)₂, -Cl and -OCH₃, -Cl enhance the activity of the compounds but former is more effective than the latter. (iii) The dimethylamino group possesses distinct antimicrobial activity which may be independent of other substituents. In general, it can be said that the pyrazolines possess maximum antimicrobial activity followed by isoxazolines and thiazines. The general trend can be given as pyrazolines>isoxazolines>thiazines.
REFERENCES


