Antimicrobial activity

The work incorporated in this chapter is on the antimicrobial activity of various compounds synthesized in chapters 2, 3, 4 and 5. The compounds were tested on variety of micro-organisms viz. gram positive and negative bacteria and on fungi. The compounds synthesized were tested on two Gram-positive bacteria *viz.* *Bacillus subtilis* and *Staphylococcus aureus*, two Gram-negative bacteria *viz.* *Salmonella typhi* and *Escherichia coli* and two fungi *viz.* *Aspergillus niger* and *Candida albicans*.

6.1 Introduction

Invasion of host defense mechanism by micro-organisms leads to the onset of infections and diseases. In order to combat infection, skilled management of antimicrobial drugs is of utmost importance. The term chemotherapy is used for the drug treatment of parasitic infections in which the parasites (viruses, bacteria, protozoa, fungi, and worms) are destroyed or removed without injuring the host. Fundamental to antimicrobial therapy is an appreciation that individual species of bacteria are associated with particular infectious diseases and that specific antimicrobials are more likely to be useful for killing them.

All of our internal fluids, organs and body structures are sterile under normal circumstances and the presence of bacteria, fungi, virus, etc. in these parts is diagnostic evidence of infection. Micro-organisms are harmful to mankind in many ways either when they come in contact and invade the tissues and cause diseases or if they find suitable conditions for their growth\(^1-3\). Therefore, one must constructively do for prevention and cure of such infectious diseases. Protection against such infection can be achieved by inhibition of microbial growth or by killing them. This can be done by using various physical agents, physical processes or chemical agents. The major physical agents or processes used for the control of microorganisms are temperature, desiccation, osmotic pressure, radiation and
A large number of chemical compounds have the ability to inhibit the growth of metabolism of microorganisms or to kill them. Research and development in different areas of chemistry have shown that several classes of chemical substrates are used to reduce the microbial flora. In nature, so many types of microorganisms are found, out of which some of the pathogenic microorganisms causing infectious diseases are shown in the following tree. (Fig 1)
6.1.1 Pathogens:

The microorganism, or infectious agent or more commonly germ, is a biological agent capable of producing diseases in host are known as pathogen. There are several substrates and pathways whereby pathogens can invade a host; the principal pathways have different episodic time frames, but soil contamination has the longest or most persistent potential for harboring a pathogen.

Pathogens have certain characteristics that they need and use, to cause disease. These so-called virulence factors have specific functions in the successive steps that result in an infection. An infection can be seen as a miniature battle between pathogen and host, the first trying to remain present and to feed and multiply, while the host is trying to prevent this. The resulting infection is a process with three possible outcomes: the host wins and the pathogen are removed (possibly with the help of medication) so that the host can recover; the pathogen win the ultimate battle and kill their host; or an equilibrium is reached in which host and pathogen live involuntarily together and damage is minimized.

6.1.2 Bacterial pathogens

Bacteria that cause disease are called pathogenic bacteria. Bacteria can cause diseases in humans, in other animals and also in plants. Some bacteria can only make one particular host ill; others cause trouble in a number of hosts, depending on the host specificity of the bacteria. The diseases caused by bacteria are almost as diverse as the bugs themselves and include infectious diseases such as pneumonia, food borne illnesses, tetanus, typhoid fever, diphtheria, syphilis and leprosy and even certain forms of cancer. Bacterial cells grow and divide, replicating repeatedly to form large numbers, present during an infection or on the surfaces of the body. To grow and divide, organisms must synthesize or take up many types of biomolecules.

In 1828, a German scientist Christian Gottfried Ehrenberg first used the term “bacterium” to denote small microscopic organism with a relatively simple and primitive form of the cellular organization known as “prokaryotic”. The Danish physician Christian Gram in 1884 discovered a stain known as Gram stain, which can divide all
bacteria into two classes “Gram positive” and “Gram negative”. The Gram-positive bacteria resist discoloration with acetone, alcohol and remain stained (methyl violet) as dark blue color, while Gram-negative bacteria are decolorized. We have used following listed bacterial pathogens for antibacterial study of synthesized compounds.

6.1.2(A) Gram positive bacterial pathogens

*Bacillus subtilis:*

They are rod-shaped with rounded ends, more or less strictly aerobic, found in soil and vegetation. They are motile and sporulating. They are small in size, occurring single or in short chains. *B. subtilis* produces the proteolytic enzyme subtilisin. *B. subtilis* grow in the mesophilic temperature range. The optimal temperature is 25-35 °C and a basic pH of 8. In 1835, the bacterium was originally named Vibrio subtilis by Christian Gottfried Ehrenberg and renamed Bacillus subtilis by Ferdinand Cohn in 1872. They can contaminate food; however, they seldom result in food poisoning. *B. subtilis* spores can survive the extreme heating that is often used to cook food and it is responsible for causing ropiness which is a sticky, stringy consistency caused by bacterial production of long-chain polysaccharides as in spoiled bread dough.

*Staphylococcus aureus:*

Staphylococcus aureus are bacteria that produce toxin (a poisonous chemical substance produced and released by the bacteria during its normal life and growth), which causes illness by causing inflammation of the intestine wall. The individual cells of *S. aureus* are 0.8 to 0.9 micro in diameter. They are ovoid or spherical, non motile, non capsulated, typically arranged in groups of irregular clusters like branches of groups found in pus, singly or in pairs, grows best in the presence of oxygen but can grow anaerobically (absence of oxygen). The optimum temperature for the growth is 37° C; optimum pH is 7.4 to 7.6.

It is a true food poisoning organism that causes nausea, vomiting and abdominal cramps which may be followed by diarrhea. In severe cases, headaches, sweating and fever may occur. Changes in blood pressure and pulse rate may also occur. It also causes toxic
shock syndrome, pyoregenic of pus forming conditions, mastitis of women and cows, boils, carbuncles infantile impetigo and internal abscesss. Some strains are capable of producing a highly heat stable protein toxin in food that can cause the illness (staphyloenterotoxaemia) when ingested.

### 6.1.2(B) Gram negative bacterial pathogens

**Salmonella typhi:**

This rod-shaped food born pathogen has adapted to grow under both, aerobic and anaerobic conditions. It grows best between 35 and 37 °C and pH range of 3.8 to 9.5. It was discovered by Karl J. Erberth in 1880. Its infections cause systemic infections and typhoid fever in humans. It gets killed by heating at 70 °C for 1 min or less. Transmission of disease is mainly through food, water or human carriers. *S. typhi* usually invades the surface of the intestine in humans, but have developed and adapted to grow into the deeper tissues of the spleen, liver and the bone marrow. Symptoms most characterized by this disease often include a sudden onset of a high fever, headache and nausea. Other common symptoms include loss of appetite, diarrhea and enlargement of the spleen (depending on where it is located).

**Escherichia coli:**

They are rods, 2 to 4 µm by 0.4 µm in size, commonly seen in coccobacillary form and rarely in filamentous form. Colonies are circular, raised and smooth and emit a faecal odor. It grows best at 37 °C, through a pH range of 4.4 to 9.0, in the presence or absence of oxygen. Escherichia was discovered by Theodor Escherich in 1885. They are normally present in the intestine without causing problems, but a few types cause illness after consuming contaminated food or water, when the bacteria produces toxin in the intestine causing diarrhea. It causes infantile diarrhea, gastroenteritis, traveller’s diarrhea, bacillary dysentery, hemorrhagic colitis, hemolytic uraemic syndrome (HUS) or thrombocytopenic purpura. It does not form toxin in food but in the intestine of infected people. Illness is caused after ingestion of a sufficient number of *E. coli* when the bacteria travels through the stomach and small intestine, attaches itself to the inside
surface of the large intestine and causes inflammation of the intestinal wall.

### 6.1.3 Fungal Pathogens

Fungi are one of the five kingdoms of life. They are plant-like organisms that lack chlorophyll. Since they do not have chlorophyll, fungi absorb food from surroundings. Since they don't use light to make food, they can live in damp and dark places. Fungi are saprophytic organism, as they grow on dead organic matter such as soil or dead plant material. Fungi are non photosynthetic eukaryotes growing either as colonies of single cells (yeasts) or as filamentous multicellular aggregate (molds). Fungi comprise a eukaryotic kingdom of microbes that are usually saprophyles but can cause diseases in humans, animals and plants.

The incidence of fungal infections has increased dramatically in the past 20 years. Accordingly, the increase in rates of morbidity and mortality because of fungal infections has been now recognized as a major problem. Most fungal infections are due to opportunistic pathogens; these affect people who are already ill or have a suppressed immune system (e.g. in patients who have been given an organ transplant or AIDS patients), although fungi are common problems in the immunocompetent population as the causative agents of skin, nail or yeast infections. Most commonly, fungi grow as pathogen on the skin of animals or people. This is sometimes called Ringworm symptom. Fungi also cause a number of plant and animal diseases e.g. in humans, ringworm, athlete's foot and several more serious diseases are caused by fungi. Because fungi are more chemically and genetically similar to animals than other organisms, this makes fungal diseases very difficult to treat. Plant diseases caused by fungi include rusts, smuts and rottening in leaf, root and stem and may also cause severe damage to crops. Most antibiotics that function on bacterial pathogens cannot be used to treat fungal infections due to the fact that fungi and their hosts both have eukaryotic cells. The typical fungal spore size is 1-40 µm in length. We have used following listed fungal pathogens for antifungal study of synthesized compounds.
**Candida albicans:**

It is a dimorphic fungus i.e. it grows both as mycelium and yeasts. This is one reason why there were so many names given to this fungus. This fungus is found among the normal flora of the mouth, digestive tract and vagina of perfectly healthy people, but under some circumstances and for unknown reasons, it may cause severe and even fatal infections, with lesions and eruptions of the skin, nails, mouth, bronchial tubes and lungs. The reason for this outbreak is difficult to pinpoint since the fungus is generally present on and within the body of healthy individuals. There are suggestions that there are special strains of this species that are pathogenic. This is suggested by the fact that this disease can be contagious and epidemics have occurred. Predisposition may also play a role in infection. An oral infection known as thrush is relatively common which causes various infections on the body.

**Aspergillus niger:**

Aspergillus niger is fungus and one of the most common species of the genus Aspergillus. It causes black mould on certain types of fruits and vegetables and is a common contaminant of food. Aspergillus includes a set of fungi that are generally considered asexual although the perfect forms have been found. Aspergillus is ubiquitous in nature. They are geographically widely distributed and have been observed in a broad range of habitats because they can colonize a wide variety of substrates. *A. niger* is commonly found as a saprophyte growing on dead leaves, stored grain, compost piles and other decaying vegetation. The spores are widespread and are often associated with organic materials and soil. The primary uses of *A. niger* are for the production of enzymes and organic acids by fermentation. *A. niger* is also used to produce organic acids such as citric acid and gluconic acid and the enzymes glucoamylase and α-galactosidase. Aspergillus niger is less likely to cause disease than some other Aspergillus species, but if large amounts of the spores are breathed in, the serious lung disease aspergillosis can occur. Aspergillosis is particularly frequent among horticultural workers.
breathing in peat dust, which can be rich in Aspergillus spores. It is less harmful, though not entirely free from risks, if eaten and digested.

6.1.4 Antimicrobial agents

The modern era of antimicrobial chemotherapy began following Alexander Fleming’s discovery in 1928 of the powerful bactericidal substance penicillin and Gerhard Domagk’s discovery in 1935 of synthetic chemicals (sulfonamides) with broad antimicrobial activity. In 1939 Gerhard Domagk, a German bacteriologist and pathologist was awarded the Nobel Prize for discovery of the first synthetic antibacterial compound “Prontosil”.

Antimicrobial agents may be either bactericidal, killing the target bacterium or fungus or bacteriostatic, inhibiting its growth. Bactericidal agents are more effective, but bacteriostatic agents can be extremely beneficial since they permit the normal defenses of the host to destroy microorganisms. Antimicrobial agents may be classified according to the type of organism against which they are active i.e. antibacterial, antiviral, antifungal, antiprotozoal and anthelmintic drugs. It can also be useful to combine various antimicrobial agents for broadening the activity spectrums and to minimize the possibility of the development of bacterial resistance. Some antibiotic combinations are more effective together than the combine effectiveness of the single agent. This is termed as Synergism. Combination therapy has proved its value as latest therapy for antimicrobials. Some bacteriostatic agents on novel combination give bactericidal activity. Sulphamethoxazole is bacteriostatic and Trimethoprim is also bacteriostatic but combination of both the drugs is now widely used as a bactericidal combination. Two such bactericidal drugs are also used in combination therapy. Refampin along with Dapsone is used in leprosy, Refampin with Isoniazide in tuberculosis. WHO has also approved this type of combination.

Most microbiologists distinguish two groups of antimicrobial agents used in the treatment of infectious disease: antibiotics, which are natural substances produced by certain groups of microorganisms and chemotherapeutic agents, which are chemically synthesized. A hybrid substance is a semisynthetic antibiotic, wherein a molecular
version produced by the microbe is subsequently modified by the chemist to achieve desired properties. Furthermore, some antimicrobial compounds, originally discovered as products of microorganisms, can be synthesized entirely by chemical means. In the medical and pharmaceutical worlds, all these antimicrobial agents used in the treatment of disease are referred to as antibiotics, chemicals that are produced by living organisms which, even in minute amounts, inhibit the growth of or kill another organism.

6.1.5 Characteristics of antimicrobial agent

a) It should have a wide spectrum of activity with the ability to destroy or inhibit many different species of pathogenic organisms.
b) It should be non allergenic and non toxic to the host and without undesirable side effects.
c) It should not eliminate the normal flora of the host.
d) It should be able to reach the part of the human body where the infection is occurring.
e) It should be inexpensive and easy to produce.
f) It should be chemically stable (have a long shelf life).
g) Microbial resistance is uncommon and unlikely to develop.
h) It must have solubility in body fluids to be active and can rapidly penetrate body tissues.

There is not a single chemical agent which is best for the control of microorganisms for any and all purposes. According to the application of antimicrobial agents they are classified in different groups.

6.1.6 Classification of antimicrobial agents

Antimicrobial agents may be classified in several ways as follows:

1. Type of organism against which antimicrobial agents are active:
   - Antibacterial agents: active against bacterial organisms.
   - Antiviral agents: active against viral organisms.
   - Antifungal agents: active against fungal organisms.
   - Antiprotozoal agents: active against protozoa.
(2) According to mode of action of antimicrobial agents:

- **Bacteriostatic**: act primarily by arresting bacterial multiplication.
- **Bactericidal**: act primarily by killing bacteria.

(3) According to activity of antimicrobial agents against the range of bacteria or other organisms:

- **Broad spectrum**: Effective against prokaryotes which kill or inhibit a wide range of Gram positive and Gram negative bacteria.
- **Narrow spectrum**: Effective against Gram positive or Gram negative bacteria.
- **Limited spectrum**: Effective against single organism or disease.

The microorganisms are controlled by various physical agents, physical processes or chemical agents. A variety of techniques and agents are available; which act in many different ways and each has its own limits of application. As we have used chemical agents for inhibition of growth of bacteria and fungi, these two have been discussed in the following topic.

### 6.1.7 Antibacterial and Antifungal Activity

During the last twenty years a very large number of organic compounds have been tested for their possible fungicidal and bactericidal activity (the ability to kill or inhibit their growth). A very few of these compounds were found to be useful as plant protectants and out of which a very few are universally accepted chemicals for disease control. In earlier days due to the lack of reliable testing methods for the fungicidal activity, the progress in this field was slow. The bioassay technique should be such that (i) the laboratory trials must be reproducible (ii) the laboratory bioassay and the field performance of the test chemical must produce uniform and congruent results.

The modern methods give reliable and reproducible results regarding protective values of a test fungicide or bactericide under field condition. The present method of the laboratory bioassay requires only a few milligrams of the test chemicals and screen out unsuccessful candidates by trials against specific phytopathogens.
The following few conditions must be met for the screening of antimicrobial activity by modern method:

- Aseptic/sterile environment should be maintained.
- Mandatory conditions should be provided for the growth of microorganisms.
- There should be an intimate contact between test organisms and the substance to be evaluated for its activity.
- Same conditions should be maintained throughout the study.

Various methods have been used from time to time by several workers to evaluate the antimicrobial activity either in the form of a zone size or minimum inhibitory concentration (MIC). They are as follows:

1. Turbidometric method,
2. Agar streak dilution method,
3. Serial dilution method,
4. Agar diffusion method,
5. Stokes diffusion method,
6. Kirby-Bauer diffusion method,
7. Broth Dilution Method, and
8. E-Test dilution and diffusion method.

### 6.2 Present work

Scientific interest for antimicrobial activity of coumarins has been continuously grown from the mid 20th century until today. As a result, a few coumarin antibiotics became candidates for human and veterinary medicine applications. The most important representative is the 3-amino coumarin derivative novobiocin, the antibiotic that has been relatively recently approved for medical use in the U.S. for SA infection treatment. Besides novobiocin, other coumarin derivatives like eskuletin, umbelliferon and related compounds possess antibacterial properties as well. Antifungal activity has been attributed to some of the coumarin derivatives, including coumarin (1,2-benzopyranone) itself. During last two decades many researchers have synthesized large number of
coumarin derivatives and screened them for their antibacterial and antifungal activity.

In the present work, all the synthesized compounds have been screened for their antibacterial and antifungal activity. The antibacterial activity of the synthesized compounds has been screened against *Bacillus subtillis* and *Staphylococcus aureus* (Gram +ve bacteria) and *Escherichia coli* and *Salmonella typhi* (Gram -ve bacteria) and antifungal activity has been screened against *Aspergillus niger* and *Candida albicans* (Fungi). The evaluation of antimicrobial activity has been carried out using **Broth Dilution method** for antimicrobial study recommended by the National Committee for Clinical Laboratory Standards (NCCLS)\(^25\).

NCCLS is an international, interdisciplinary, non-profit, non-governmental organization composed of medical professionals, government, industry, healthcare providers, educators, etc. It promotes accurate antimicrobial susceptibility testing (AST) and appropriate reporting by developing standard reference methods, interpretative criteria for the results of standard AST methods, establishing quality control parameters for standard test methods, provides testing and reporting strategies that are clinically relevant and cost effective. Interpretative criteria of NCCLS are developed based on international collaborative studies and well correlated with MIC’s and the results have corroborated with clinical data. Based on study results, NCCLS interpretative criteria are revised frequently. NCCLS is approved by FDA-USA and recommended by WHO.

**6.2.1 Broth dilution method**

Dilution susceptibility testing methods are used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganism i.e. aim of broth dilution methods is to determine the lowest concentration of the assayed antimicrobial agent (MIC) that, under defined test conditions, inhibits the visible growth of the pathogen being investigated. MIC values are used to determine susceptibilities of pathogen to drugs and also to evaluate the activity of new antimicrobial agents.
This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms. The tube dilution test is the standard method for determining levels of resistance to an antibiotic.

### 6.3 Experimental
1) The *in vitro* antimicrobial activity of the synthesized compounds and standard drugs were assessed against two representative of Gram-positive bacteria *viz.* *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 96), two Gram-negative bacteria *viz.* *Escherichia coli* (MTCC 443) and *Salmonella typhi* (MTCC 98) and two fungi *viz.* *Aspergillus niger* (MTCC 282) and *Candida albicans* (MTCC 227) and the strains employed for the activity were procured from (MTCC – Micro Type Culture Collection) Institute of Microbial Technology, Chandigarh.

2) Inoculum size for test strain was adjusted to $10^8$ CFU/mL (Colony Forming Unit per milliliter) by comparing the turbidity (turbidimetric method).

3) Mueller Hinton Broth was used as nutrient medium to grow and dilute the compound suspension for the test bacteria and Sabouraud Dextrose Broth was used for fungal nutrition.

4) Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamicin and Norfloxacin were used as standard antibacterial drugs, whereas griseofulvin and nystatin was used as standard antifungal drugs.

5) DMSO was used as diluents/vehicle to get desired concentration of synthesized compounds and standard drugs to test upon standard microbial strains.

6) Serial dilutions were prepared in primary and secondary screening. Each synthesized compound and standard drugs were diluted obtaining $2000 \ \mu\text{g/mL}$ concentration, as a stock solution. In primary screening 1000, 500 and 250 $\mu\text{g/mL}$ concentrations of the synthesized drugs were taken. The active synthesized compounds found in this primary screening were further diluted to obtain 200, 125, 100, 62.5, 50, 25, 12.5 and 6.250 $\mu\text{g/mL}$ concentrations for
secondary screening to test in a second set of dilution against all microorganisms.

7) The control tube containing no antibiotic is immediately sub cultured (before incubation) by spreading a loopful evenly over a quarter of the plate on a medium suitable for the growth of the test organism. The tubes are then put for incubation at 37 °C for 24 hour for bacteria and 48 hour for fungi. The highest dilution (lowest concentration) showing at least 99 % inhibition or preventing appearance of turbidity is considered as Minimal Inhibitory Concentration (μgmL⁻¹) i.e. the amount of growth from the control tube before incubation (which represents the original inoculum) is compared. A set of tubes containing only seeded broth and the solvent controls were maintained under identical conditions so as to make sure that the solvent had no influence on strain growth. The result of this is much affected by size of the inoculum. The test mixture should contain 10⁸ CFUmL⁻¹ organisms. The protocols were summarized and compared with standard drugs as the Minimal Inhibitory Concentration (μgmL⁻¹).

6.3.1 Factors influencing antimicrobial susceptibility testing
a) Choice of media: Consistent and reproducible results are obtained in media prepared especially for sensitivity testing.

b) Size of inoculums: Although large numbers of organisms do not markedly affect many antibiotics, the ideal inoculum is one, which gives an even dense growth without being confluent. Overnight broth cultures of organisms and suitable suspensions from solid media can be diluted appropriately to give optimum inoculum for sensitivity testing.

c) pH: The medium used should have a pH between 7.2 and 7.4 at room temperature after gelling. If the pH is too low, certain drugs will appear to lose potency (e.g., aminoglycosides, quinolones and macrolides), while other agents may appear to have excessive activity (e.g., tetracyclines). If the pH is too high, the opposite effects can be expected.
d) **Moisture:** The surface should be moist, but no droplets of moisture should be apparent on the surface of the medium or on the tubes and plates at the time of inoculation.

e) **Effects of variation in divalent cations:** Variations in divalent cations affect results. Excessive cation content or low cation content may result in unacceptable results.

f) **Testing strains that fail to grow satisfactorily:** Only aerobic or facultative bacteria that grow well on unsupplemented media should be tested on that medium. Certain fastidious bacteria do not grow sufficiently on unsupplemented media. These organisms require supplements or different media to grow and they should be tested on the media.

### 6.4 Results

(1) Antimicrobial activity results of various 3-(4-aryl-5H-indeno[1,2-b]pyridin-2-yl)coumarins (3a-r). (Chapter 2)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Minimum Inhibitory Concentration (MIC, μg mL⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td>Gram +ve bacteria</td>
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<tr>
<td>3a</td>
<td>250</td>
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<tr>
<td>3b</td>
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<td>250</td>
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</table>

**Ampicillin**

|   | 250 | 250 | 100 | 100 | -   | -   |

**Chloramphenicol**

|   | 50  | 50  | 50  | 50  | -   | -   |

**Ciprofloxacin**

|   | 50  | 50  | 25  | 25  | -   | -   |

**Norfloxacin**

|   | 100 | 10  | 10  | 10  | -   | -   |

**Gentamycin**

|   | 1   | 0.25| 0.05| 5   | -   | -   |

**Griseofulvin**

|   | -   | -   | -   | -   | 100 | 500 |

**Nystatin**

|   | -   | -   | -   | -   | 100 | 100 |

**B.s.**: Bacillus subtilis, **S.a.**: Staphylococcus aureus, **E.c.**: Escherichia coli, **S.t.**: Salmonella typhi, **A.n.**: Aspergillus niger, **C.a.**: Candida albicans

From the results it can be seen that, compounds 3a-r show activity against *B. subtilis, S. aureus, E. coli* and *S. typhi*. The compounds 3a-r also show activity against *A. niger* and *C. albicans* except compound 3h which is inactive against *A. niger*. Compounds 3m and 3q are inactive against *C. albicans*. Compounds 3b, 3f, 3h, 3j, 3l, 3n, 3o, 3p and 3r show better activity compared to Ampicillin and Griseofulvin against particular bacteria or fungi.

(2) Antimicrobial activity results of various 6-ary-2-(coumarin-3-yl)-4-(7-methoxy coumarin-4-yl)pyridines (8a-l). (Chapter 3, Section-1)
From the results it can be seen that, compounds 8a-l show activity against *B. subtilis*, *S. aureus*, *E. coli* and *S. typhi*. The compounds 8a-l also show activity against *A. niger* and *C. albicans* except compounds 8c, 8h, 8i, 8j and 8l that are inactive against *A. niger* and compounds 8c, 8i and 8j that are inactive against *C. albicans*. Compounds 8a-l, except 8i show better activity compared to Ampicillin and Griseofulvin against particular bacteria or fungi.

(3) Antimicrobial activity results of various 2,3-diarylpyrido[3,2-c]coumarins 3a-l. (Chapter 3, Section 2)
<table>
<thead>
<tr>
<th>Compound</th>
<th>Minimum Inhibitory Concentration (MIC, μg/mL)</th>
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<tbody>
<tr>
<td></td>
<td>Gram +ve bacteria</td>
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<tr>
<td>3a</td>
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<tr>
<td>3k</td>
<td>250</td>
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<tr>
<td>3l</td>
<td>200</td>
</tr>
</tbody>
</table>

Ampicillin | 250 | 250 | 100 | 100 | - | - |
Chloramphenicol | 50 | 50 | 50 | 50 | - | - |
Ciprofloxacin | 50 | 50 | 25 | 25 | - | - |
Norfloxacin | 100 | 10 | 10 | 10 | - | - |
Gentamycin | 1 | 0.25 | 0.05 | 5 | - | - |
Griseofulvin | - | - | - | - | 100 | 500 |
Nystatin | - | - | - | - | 100 | 100 |

* B.s.: Bacillus subtilis, S.a.: Staphylococcus aureus, E.c.: Escherichia coli, S.t.: Salmonella typhi, A.n.: Aspergillus niger, C.a.: Candida albicans

From the results it can be seen that, compounds 3a-l show activity against *B* subtilis, *S* aureus, *E* coli and *S* typhi. The compounds 3a-l also show activity against *A* niger and *C* albicans except compounds 3h, 3i and 3l that is inactive against *A* niger and compounds 3d, 3e and 3l that are inactive against *C* albicans. Compounds 3d, 3g, 3h, 3i and 3l show better activity compared to Ampicillin and Griseofulvin against particular bacteria or fungi.

(4) Antimicrobial activity results of various 3-(6'-aryl-2'',4''-bipyridin-2'-yl)coumarins (5a-i), 3-[(2',4'';2'',2'')terpyridin-6''-yl]coumarins (9a-c), 3-[(2',4'';2'',3'')terpyridin-6''-yl]coumarins (10a-c) and 3-[(2',4'';2'',4''')terpyridin-6''-yl]coumarins (11a-c). (Chapter 4)
The table below shows the Minimum Inhibitory Concentration (MIC, μg/mL⁻¹) of various compounds against different bacterial and fungal strains.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Gram +ve bacteria</th>
<th>Gram –ve bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>250</td>
<td>125</td>
<td>250</td>
</tr>
<tr>
<td>5b</td>
<td>100</td>
<td>200</td>
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* B.s.: Bacillus subtilis, S.a.: Staphylococcus aureus, E.c.: Escherichia coli, S.t.: Salmonella typhi, A.n.: Aspergillus niger, C.a.: Candida albicans

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From the results it can be seen that, compounds 5a-i, 9a-c, 10a-c and 11a-c show activity against *B. subtilis*, *S. aureus*, *E. coli* and *S. typhi*. The compounds 5a-i, 9a-c, 10a-c and 11a-c also show activity against *A. niger* and *C. albicans* except compounds 9c and 10b that are inactive against *A. niger* and compounds 5d and 11b that are inactive against *C. albicans*. Compounds 5a-i, 9a-c, 10a-c and 11a-c expect 9b, 9c and 10b show better activity compared to Ampicillin and Griseofulvin against particular bacteria or fungi.

(5) Antimicrobial activity results of various 3-[1-acetyl/propionyl-5-(1,3-diaryl-1H-pyrazol-4-yl)-4,5-dihydro-1H-pyrazol-3-yl]coumarins (2a-h). (Chapter 5)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Minimum Inhibitory Concentration (MIC, μg mL⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td>Gram +ve bacteria</td>
</tr>
<tr>
<td>2a</td>
<td>200</td>
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<td>Griseofulvin</td>
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<tr>
<td>Nystatin</td>
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</table>

From the results it can be seen that, compounds 2a-h show activity against *B subtilis, S aureus, E coli* and *S typhi*. The compounds 2a-h also show activity against *A niger* and *C albicans* except compounds 2e and 2f that are inactive against both *A niger* and *C albicans*. Compounds 2a-h, except 3g show better activity compared to Ampicillin and Griseofulvin against particular bacteria or fungi.

(6) Antimicrobial activity results of various 3-[5-(1,3-diaryl-1H-pyrazol-4-yl)-1-aryl-4,5-dihydro-1H-pyrazol-3-yl]coumarins (4a-l). (Chapter 5)
From the results it can be seen that, compounds 4a-l show activity against *B subtilis*, *S aureus*, *E coli* and *S typhi*. The compounds 4a-l also show activity against fungi *A niger* and *C albicans* except compounds 4a, 4e and 4l that are inactive against *A niger* and compounds 4e and 4l that are inactive against *C albicans*. All the compounds 4a-l, except 4d show better activity compared to Ampicillin and Griseofulvin against particular bacteria or fungi.
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