Chapter-V

BIOLOGICAL ACTIVITY

The Bacteria are a large group of unicellular microorganisms. Typically a few micrometers in length, bacteria have a wide range of shapes, ranging from spheres to rods and spirals. Bacteria are ubiquitous in every habitat on Earth, growing in soil, acidic hot springs, radioactive waste\(^1\), water and deep in the Earth's crust, as well as in organic matter and the live bodies of plants and animals. There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in a milliliter of fresh water.; in all, there are approximately five nonillion (\(5 \times 10^{30}\)) bacteria on Earth\(^2\), forming much of the world’s biomass.\(^2\) Bacteria are vital in recycling nutrients, with many steps in nutrient cycles depending on these organisms, such as the fixation of nitrogen from the atmosphere and putrefaction. However, most bacteria have not been characterized, and only about half of the phyla of bacteria have species that can be grown in the laboratory.\(^3\) The study of bacteria is known as bacteriology, a branch of microbiology.

There are approximately ten times as many bacterial cells in the human flora of bacteria as there are human cells in the body, with large numbers of bacteria on the skin and as gut flora.\(^4\) The vast majority of the bacteria in the body are rendered harmless by the protective effects of the immune system, and a few are beneficial. However, a few species of bacteria are pathogenic and cause infectious diseases, including cholera, syphilis, anthrax, leprosy, and bubonic plague. The most common fatal bacterial diseases are respiratory infections, with tuberculosis alone killing about 2 million people a year, mostly in sub-Saharan Africa. In developed countries, antibiotics are used to treat bacterial infections and in agriculture, so antibiotic resistance is becoming common. In industry, bacteria are important in sewage treatment the production of cheese and yogurt through fermentation, as well as in biotechnology, and the manufacture of antibiotics and other chemicals.\(^5\)

Once regarded as plants constituting the class Schizomycetes, bacteria are now classified as prokaryotes. Unlike cells of animals and other eukaryotes, bacterial cells do not contain a nucleus and rarely harbour membrane-bound organelles. Although the term
bacteria traditionally included all prokaryotes, the scientific classification changed after the discovery in the 1990s that prokaryotes consist of two very different groups of organisms that evolved independently from an ancient common ancestor. These evolutionary domains are called Bacteria and Archaea.  

**Growth and Reproduction:**

Unlike multicellular organisms, increases in the size of bacteria (cell growth) and reproduction by cell division are tightly linked in unicellular organisms. Bacteria grow to a fixed size and then reproduce through binary fission, a form of asexual reproduction. Under optimal conditions, bacteria can grow and divide extremely rapidly, and bacterial populations can double as quickly as every 9.8 minutes. In cell division, two identical clone daughter cells are produced. Some bacteria, while still reproducing asexually, form more complex reproductive structures that help disperse the newly formed daughter cells. Examples include fruiting body formation by Myxobacteria and aerial hyphae formation by Streptomyces, or budding. Budding involves a cell forming a protrusion that breaks away and produces a daughter cell.

In the laboratory, bacteria are usually grown using solid or liquid media. Solid growth media such as agar plates are used to isolate pure cultures of a bacterial strain. However, liquid growth media are used when measurement of growth or large volumes of cells are required. Growth in stirred liquid media occurs as an even cell suspension, making the cultures easy to divide and transfer, although isolating single bacteria from liquid media is difficult. The use of selective media (media with specific nutrients added or deficient, or with antibiotics added) can help identify specific organisms.

Most laboratory techniques for growing bacteria use high levels of nutrients to produce large amounts of cells cheaply and quickly. However, in natural environments nutrients are limited, meaning that bacteria cannot continue to reproduce indefinitely. This nutrient limitation has led the evolution of different growth strategies. Some organisms can grow extremely rapidly when nutrients become available, such as the formation of algal (and cyanobacterial) blooms that often occur in lakes during the summer. Other organisms have adaptations to harsh environments, such as the
production of multiple antibiotics by Streptomyces that inhibit the growth of competing microorganisms. In nature, many organisms live in communities (e.g., biofilms) that may allow for increased supply of nutrients and protection from environmental stresses. These relationships can be essential for growth of a particular organism or group of organisms.

Genetics:

Most bacteria have a single circular chromosome that can range in size from only 160,000 base pairs in the endosymbiotic bacteria Candidatus Carsonella ruddii to 12,200,000 base pairs in the soil-dwelling bacteria, Sorangium cellulosum. Spirochaetes of the genus Borrelia are a notable exception to this arrangement, with bacteria such as Borrelia burgdorferi, the cause of Lyme disease, containing a single linear chromosome. The genes in bacterial genomes are usually a single continuous stretch of DNA and although several different types of introns do exist in bacteria, these are much more rare than in eukaryotes.

Bacteria may also contain plasmids, which are small extra-chromosomal DNAs that may contain genes for antibiotic resistance or virulence factors.

Bacteriophages:

Bacteriophages are viruses that change the bacterial DNA. Many types of bacteriophage exist, some simply infect and lyse their host bacteria, while others insert into the bacterial chromosome. A bacteriophage can contain genes that contribute to its host's phenotype: for example, in the evolution of Escherichia coli and Clostridium botulinum, the toxin genes in an integrated phage converted a harmless ancestral bacterium into a lethal pathogen. Bacteria resist phage infection through restriction modification systems that degrade foreign DNA, and a system that uses CRISPR sequences to retain fragments of the genomes of phage that the bacteria have come into contact with in the past, which allows them to block virus replication through a form of RNA interference. This CRISPR system provides bacteria with acquired immunity to infection.
Movement:

Motile bacteria can move using flagella, bacterial gliding, twitching motility or changes of buoyancy. In twitching motility, bacterial use their type IV pili as a grappling hook, repeatedly extending it, anchoring it and then retracting it with remarkable force (<80 pN).

Significance in Technology:

Bacteria, often lactic acid bacteria such as Lactobacillus and Lactococcus, in combination with yeasts and molds, have been used for thousands of years in the preparation of fermented foods such as cheese, pickles, vinegar, wine and yoghurt.

The ability of bacteria to degrade a variety of organic compounds is remarkable and has been used in waste processing and bioremediation. Bacteria capable of digesting the hydrocarbons in petroleum are often used to clean up oil spills. Fertilizer was added to some of the beaches in Prince William Sound in an attempt to promote the growth of these naturally occurring bacteria after the 1989 Exxon Valdez oil spill. These efforts were effective on beaches that were not too thickly covered in oil. Bacteria are also used for the bioremediation of industrial toxic wastes. In the chemical industry, bacteria are most important in the production of enantiomerically pure chemicals for use as pharmaceuticals or agrichemicals.

Bacteria can also be used in the place of pesticides in the biological pest control. This commonly involves Bacillus Thuringiensis (also called BT), a Gram-positive, soil dwelling bacterium. Subspecies of this bacteria are used as a Lepidopteran-specific insecticides under trade names such as Dipel and Thuricide. Because of their specificity, these pesticides are regarded as environmentally friendly, with little or no effect on humans, wildlife, pollinators and most other beneficial insects.

Because of their ability to quickly grow and the relative ease with which they can be manipulated, bacteria are the workhorses for the fields of molecular biology, genetics and biochemistry. By making mutations in bacterial DNA and examining the resulting
phenotypes, scientists can determine the function of genes, enzymes and metabolic pathways in bacteria, then apply this knowledge to more complex organisms.\textsuperscript{31} This aim of understanding the biochemistry of a cell reaches its most complex expression in the synthesis of huge amounts of enzyme kinetic and gene expression data into mathematical models of entire organisms. This is achievable in some well-studied bacteria, with models of Escherichia coli metabolism now being produced and tested.\textsuperscript{32-33} This understanding of bacterial metabolism and genetics allows the use of biotechnology to bioengineer bacteria for the production of therapeutic proteins, such as insulin, growth factors, or antibodies.\textsuperscript{34-35}

A biocide is a chemical substance capable of killing living organism, usually in a selective way. Biocides are commonly used in medicine, agriculture, forestry, and in industry where they prevent the fouling of water and oil pipelines. Some substances used as biocides are also employed as anti-fouling agents or disinfectants under other circumstances: chlorine, for example, is used as a short-life biocide in industrial water treatment but as a disinfectant in swimming pools. Many biocides are synthetic, but a class of natural biocides, derived from e.g. bacteria and plants, includes brassica oleracea, brassica oleracea gemmifera, and clostridium botulinum bacteria.

Biocides can be added to other materials (typically liquids) to protect them against biological infestation and growth. For example, certain types of quaternary ammonium compounds (quats) are added to pool water or industrial water systems to act as an algicide, protecting the water from infestation and growth of algae. It is often impractical to store and use poisonous chlorine gas for water treatment, so alternative methods of adding chlorine are used. These include hypochlorite solutions, which gradually release chlorine into the water, and compounds like sodium dichloro-s-triazinetrione (dehydrate or anhydrous), sometimes referred to as “dichlor”, and trichloro-s-triazinetrione, sometimes referred to as “trichlor”. These compounds are stable while solid and may be used in powdered, granular, or tablet form. When added in small amounts to pool water or industrial water systems, the chlorine atoms hydrolyze from the rest of the molecule forming hypochlorous acid (HOCl) which acts as a general biocide killing germs, micro-
organisms, algae, and so on. Halogenated hydantoin compounds are also used as biocides.

Because biocides are intended to kill living organisms, many biocidal products pose significant risk to human health and welfare. Great care is required when handling biocides and appropriate protective clothing and equipment should be used. The use of biocides can also have significant adverse effects on the natural environment. Anti-fouling paints, especially those utilizing organic in compounds such as TBT, have been shown to have severe and long-lasting impacts on marine eco-systems and such materials are now banned in many countries for commercial and recreational vessels (though sometimes still used for naval vessels).

**PREPARATION OF SAMPLE SOLUTIONS:**

Solutions of all test compounds were prepared by dissolving 1mg/ml. of the substance in propylene glycol. All the solutions were sterilized by moist heat sterilization method in an autoclave.

**CULTIVATION OF ORGANISMS FOR BIOCIDAL STUDIES:**

For most satisfactory growth of micro organisms, a proper temperature, pH, necessary nutrients and growth media free from other contamination (micro organisms) was provided for the preparation of culture of pathogenic bacteria and fungi using aseptic techniques the culture media used for slant and broth was sterilized by moist heat sterilization method (by autoclaving at 121ºc using 15 lbs pressure for 15 minutes). All the utensils used were also sterilized by their usual methods. The incubation period for bacteria was kept 24 hours at 37ºc temperature and for fungi 96 hours at 28ºc

**Serial dilution method and its importance:**

This method has been employed during the course of present investigation. This method to study the comparative antibacterial and antifungal activity of synthesized, dihydrazides and their macrocyclic complexes was conducted in the following steps:-
A. Sterilization of glassware.

B. Preparation of culture media.

C. Sterilization of culture media.

D. Preparation of solutions of ligand fragments (esters and dihydrazides) and the macro cyclic complex.

E. Determination of average percentage inhibition.

A. Sterilization of glassware:

The glassware made up of pyrex glass was used for the purpose of biocidal study. The cleaned glassware such as test tubes, petridishes, pipettes and flasks were sterilized in an autoclave at 121º pound pressure; however, some glassware was also sterilized in hot air oven at 170ºC for one two hours.

B. Preparation of culture media:

The material in which micro-organisms is itself a culture. Following are the salient features of satisfactory culture media for the growth of a living micro-organism whether fungi or bacteria.

1. Culture media must be sterilized completely and be handled in such a way a to prevent all the atmospheric contaminations.

2. The pH of the culture media must be equal to 7.1 it should be Natural in nature.

3. Culture media must be free from chemicals that would hinder the growth of desired organisms.

4. All the ingredients (viz. protein, sugar of starch, minerals and water) Must be present in proper amount in the culture media.

To study the biological activity of the ligand fragments and their metal complexes, the culture media was always prepared in double distilled water.
Following in gradients were used to prepare culture media.

1. Culture media for growing bacteria

1. Peptone 0.6%
2. Yeast extract 0.3%
3. Beef extract 0.15%
4. Dextrose 0.1%
5. Agar (only for slant) 1.5%
6. Water added to make total volume 250ml.
7. pH adjusted to 6.5-6.6

2. Culture media for growing fungi

1. Peptone 1.6%
2. Dextrose 2.0%
3. Agar (only for slant)
4. Water added to make total volume 250ml
5. pH adjusted to 5.4%

C. Sterilization of culture media:

The culture media was sterilization in an autoclave equipped with thermometer, as the temperature is more reliable than the pressure. Secondly, minimum heat should be used, because overheating may destroy growth stimulating substances as well as caramelizing and hydrolyzing sugars. The required temperature (121º at 15 pound pressure) for sterilization of culture media in an autoclave was maintained according to the following chart,
<table>
<thead>
<tr>
<th>Pressure in pounds</th>
<th>Equivalent Temp° C</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>108.4</td>
</tr>
<tr>
<td>10</td>
<td>115.2</td>
</tr>
<tr>
<td>15</td>
<td>212.0</td>
</tr>
<tr>
<td>20</td>
<td>130.4</td>
</tr>
<tr>
<td>30</td>
<td>134.5</td>
</tr>
</tbody>
</table>

D. Preparation of solutions of ligand fragments (esters and dihydrazides) and the macrocyclic complexes:

The calculated weight of all the test compounds (i.e. free ling and fragments and their metal complexes) were dissolved in a know volume of propylene glycol to get the solution of know concentration for the study of biocidal properties of potentially active compounds in vitro. Following method are generally employed.

1. Evaluation of minimum inhibitory concentration (MIC) by agar diffusion method.

2. The end point or extinction determination

3. Determination of mean death time

4. Turbidimeric method

Following general and necessary condition are to be maintained for successful results.

1. The substances under examination should be brought in intimate contact with the micro–organism against which the activity is to be studied.

2. Favorable conditions have to be maintained for the optimum growth of the test micro-organism in the absence of other antimicrobial agents except the substances under examination in suitable growth media.
3. To avoid the environmental contaminations, all the operations have to be conducted using aseptic techniques.

The serial dilution method is treated as one of the best result oriented and convenient methods for the evaluation of MIC values.

**SERIAL DILUTION METHOD:**

In this method, graded dilutions of the test compounds in a suitable nutrient medium are inoculated with the organism under examination using aseptic techniques and incubated under suitable condition in an incubator.

The minimum concentration of the compound preventing detectable growth (MIC), is taken as a measure of biocidal activity.

**TEST ORGANISMS:**

**BACTERIA:**

1. *Staphylococcus aureus* (gram positive)

   - *Staphylococcus aureus* is a gram positive coccus bacterium that is a member of the Firmicutes, and is frequently found in the human respiratory tract and on the skin.
• It is positive for catalase and nitrate reduction. Although S. aureus is not always pathogenic, it is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning.

• Disease-associated strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant forms of pathogenic S. aureus (e.g. MRSA) is a worldwide problem in clinical medicine.

• Staphylococcus was first identified in 1880 in Aberdeen, United Kingdom, by the surgeon Sir Alexander Ogston in pus from a surgical abscess in a knee joint.36 It is estimated that 20% of the human population are long-term carriers of S. aureus37 which can be found as part of the normal skin flora and in anterior nares of the nasal passages.37-38

• S. aureus is the most common species of staphylococcus to cause Staph infections and is a successful pathogen due to a combination of nasal carriage and bacterial immunoevasive strategies.37-38 S. aureus can cause a range of illnesses, from minor skin infections, such pimples, boils and scalded skin. It is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections.
2. *Escherichia coli* (gram negative)

- *Escherichia coli* is a gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms).\(^{39}\)

- Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination.\(^{40-41}\)

- The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K2,\(^{42}\) and preventing colonization of the intestine with pathogenic bacteria.\(^{43-44}\)

- *E*-coli and other facultative anaerobes constitute about 0.1% of gut flora,\(^{45}\) and fecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them ideal indicator organisms to test environmental samples for fecal contamination.\(^{46-47}\)

- *E*-coli is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA.
Fungi:

1. Aspergillus niger

- Aspergillus niger or A. niger is a fungus and one of the most common species of the genus Aspergillus.

- It causes a disease called black mold on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food.

- It is ubiquitous in soil and is commonly reported from indoor environments, where its black colonies can be confused with those of Stachybotrys (species of which have also been called “black mould”).

- Some strains of A. niger have been reported to produce potent mycotoxins called ochratoxins, other sources disagree, claiming this report is based upon misidentification of the fungal species. Recent evidence suggests some true A. niger strains do produce ochratoxin A. It also produces the isoflavone orobol.
3. Candida albicans

- Candida albicans is a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in humans, and candidal onychomycosis, an infection of the nail plate. Systemic fungal infections (fungemias) including those by C. albicans have emerged as important causes of morbidity and mortality in immunocompromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation).

- C. albicans biofilms may form on the surface of implantable medical devices. In addition, hospital-acquired infections by C. albicans have become a cause of major health concerns.

- C. albicans is commensal and a constituent of the normal gut flora comprising microorganisms that live in the human mouth and gastrointestinal tract.

- C. albicans lives in 80% of the human population without causing harmful effects, although overgrowth of the fungus results in candidiasis (candidosis). Candidiasis is often observed in immunocompromised individuals such as HIV-infected patients.
A common form of candidiasis restricted to the mucosal membranes in mouth or vagina is thrush, which is usually easily cured in people who are not immunocompromised. For example, higher prevalence of colonization of C. albicans was reported in young individuals with tongue piercing, in comparison to unpierced matched individuals.

To infect host tissue, the usual unicellular yeast-like form of C. albicans reacts to environmental cues and switches into an invasive, multicellular filamentous form, a phenomenon called dimorphism.

Subculture of the above mentioned micro organisms was prepared monthly from the principal culture and both culture weekly from the sub culture. All culture were stored at 4ºC. The inculcation process was carrier out in a well cleaned inoculation chamber having UV lamp. Seeded broth for the test to be conducted was prepared by diluting the broth culture of the desired organisms 1 : 100 times and already kept overnight at their optimum temperature.

DETERMINATION OF MIC VALUES:

The sets of two fold serial dilution of the test compounds were prepared as follows
1 ml of the seeded broth (obtained by 1:100 dilution of the indicated micro-organism broth culture in both) was taken in 10 well-sterilized tubes (3x100 size), keeping the first tube empty. 2 ml of each of the seeded broth was prepared having 100 µg/ml and 150 µg/ml of test compound in tubes A and B respectively [prepared by dissolving 0.2 ml and 0.3 ml of the stock solutions (1 mg/ml) in 1.8 ml and 1.7 ml of broth respectively.

Contents of A tube were placed in the first empty tube using a fresh sterilized pipette. 1 ml contents from the B tube were withdrawn and added to second tube and mixed well. Similarly, 1 ml contents from the first tube were withdrawn and added into the third tube and mixed well. 1 ml contents from the third tube pipetted out with another fresh sterilized pipette and added into the fourth tube shaken well. This gradient dilution process is continued for all ten tubes using a fresh pipette each time 1 ml contents were taken out from the 10th tube and rejected. All the tubes were labeled with 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.50 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.56 µg/ml, 0.78 µg/ml, and 3.39 µg/ml respectively.

1 ml of each of the seeded broth and the broth was placed in two separate tubes for the control of culture and control of broth media respectively in each set of above experiments simultaneously. All the above sets of tubes were incubated BOD incubator at given temperature and time for the respective indicated micro-organism. The tube having the highest dilution showing no visible turbidity is chosen. The amount of the test compound in this tube is the “minimum inhibitory concentration” (bactericidal concentration). The sub-culture from the tube showing no visible growth on agar slants having respective nutrient media and appropriate amount of agar is developed.
RESULT AND DISCUSSION:

The observed MIC values of the esters, dihydrazides and the macrocyclic complexes have been presented in tables.

1. On complexation, the liposoluble nature of the biologically active ligand is increased.

2. The metal ion present in metal enzyme of the biological system is displaced by foreign macrocyclic ring of more liposoluble metal complexes. This is only possible when the foreign metal has a stronger affinity for the apoprotein molecule of the metal enzyme in biological system.

3. Due to the combined activity effect of both (metal molecule/metal atom) the metal and ligand the biocidal effect may have multiplied.

4. The more rapid penetration of the metal complexes as a whole through the wall of cells of the micro-organism may be one of the other important factors. The active constituents may also act on protein synthesis and nucleic acids (DNA or RNA)

The comparative results of biological activity indicate that the macrocyclic complexes of Sc(II) with the ligand fragments show much enhanced activity against both the bacterial and fungal species as contrasted with the activity shown by the ligand fragments.
# TABLE 5.1

MINIMUM INHIBITORY CONCENTRATION (MIC) IN MOLAR CONCENTRATION ($10^{-4}$) OF SYNTHESIZED DIHYDRAZIDES AND THEIR METAL COMPLEXES.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compounds</th>
<th>Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E.Coli</td>
<td>S.aureus</td>
</tr>
<tr>
<td>1.</td>
<td>[PDADH]</td>
<td>0.6450</td>
<td>0.6200</td>
</tr>
<tr>
<td>2.</td>
<td>[ODADH]</td>
<td>0.6290</td>
<td>0.6180</td>
</tr>
<tr>
<td>3.</td>
<td>[DTDADH]</td>
<td>0.6150</td>
<td>0.6140</td>
</tr>
<tr>
<td>4.</td>
<td>[TDPDH]</td>
<td>0.5890</td>
<td>0.5900</td>
</tr>
<tr>
<td>5.</td>
<td>[TBADH]</td>
<td>0.6100</td>
<td>0.6120</td>
</tr>
<tr>
<td>6.</td>
<td>Sc (II) [DPPDH] (BF$_4$)$_2$</td>
<td>0.230</td>
<td>0.240</td>
</tr>
<tr>
<td>7.</td>
<td>Sc (II) [DCPDH] (BF$_4$)$_2$</td>
<td>0.210</td>
<td>0.220</td>
</tr>
<tr>
<td>8.</td>
<td>Sc (II) [DPODH] (BF$_4$)$_2$</td>
<td>0.250</td>
<td>0.270</td>
</tr>
<tr>
<td>9.</td>
<td>Sc (II) [DCODH] (BF$_4$)$_2$</td>
<td>0.215</td>
<td>0.220</td>
</tr>
<tr>
<td>10.</td>
<td>Sc(II) [DPDTADH] (BF$_4$)$_2$</td>
<td>0.290</td>
<td>0.245</td>
</tr>
<tr>
<td>11.</td>
<td>Sc(II) [DCDTADH] (BF$_4$)$_2$</td>
<td>0.210</td>
<td>0.190</td>
</tr>
<tr>
<td>12.</td>
<td>Sc (II) [DTPPDH] (BF$_4$)$_2$</td>
<td>0.190</td>
<td>0.180</td>
</tr>
<tr>
<td>13.</td>
<td>Sc (II) [DCTPDH] (BF$_4$)$_2$</td>
<td>0.195</td>
<td>0.190</td>
</tr>
<tr>
<td>14.</td>
<td>Sc (II) [DPTBDH] (BF$_4$)$_2$</td>
<td>0.200</td>
<td>0.205</td>
</tr>
<tr>
<td>15.</td>
<td>Sc (II) [DCTBDH] (BF$_4$)$_2$</td>
<td>0.215</td>
<td>0.210</td>
</tr>
</tbody>
</table>
Activity order of Antimicrobial activities of the ligand and their complexes is as follows:

1. **Pyridine-2,6-dicarboxylic acid**
   
   PDADH <<<< Sc (II) [DPPDH] (BF4)2 < Sc (II) [DCPDH] (BF4)2

2. **Oxydiacetic acid**
   
   ODADH <<< Sc (II)[DPODH](BF4)2 ≈ Sc(II) [DCODH](BF4)2

3. **Thio diacetic acid**
   
   TDADH <<< Sc (II)[DPTDH] (BF4)2 < Sc(II) [ DCTDH] (BF4)2

4. **Di thio di propionic acid**
   
   DTDPDH <<< Sc(II) [DPDTPH] (BF4)2 ≈ Sc(II) [DCDTPH] (BF4)2

5. **Thiodibutanoic acid**
   
   TDBDH <<< Sc(II) [DPTBH] (BF4)2 < Sc(II) [DCTBH] (BF4)2
References:


