PART III
ANTIBACTERIAL STUDIES

Vinod P Raphael "Physicochemical, corrosion inhibition and biological studies on schiff bases derived from heterocyclic carbonyl compounds and their metal complexes" Thesis. Department of Chemistry, St. Thomas College Thrissur, University of Calicut, 2014

PART III

ANTIBACTERIAL STUDIES

CHAPTER 1

INTRODUCTION AND REVIEW

A chemist is always curious to check the drug ability of the newly synthesized molecules in the laboratory. Many drugs are discovered accidently by the blind screening of the synthesized molecules on various diseases. Though the modern pharmaceutical chemistry is mainly interested in the development of drugs or modifying the already existing drugs by quantitative structure activity relation approaches (QSAR models), the scope for searching the drug ability of the newly synthesized molecules by random screening approach is still extant. In the present course of study it is proposed to monitor the drug ability of the newly synthesized heterocyclic azomethine class of compounds and their metal chelates against the growth of various pathogens.

Metal Chelates in Pharmacology

The modern organic and inorganic chemistry has contributed many compounds to the pharmaceutical field which have potential activities against various diseases. Medicinal inorganic chemistry can exploit the unique properties of metal ions for the design of new drugs. Many metal chelates are found to have drug abilities on various diseases. Metals are the integral part of many structural and functional components in the body, and the critical role of metals in physiological and pathological fields has always been a subject for researchers. Transition metals exhibit different oxidation states and can interact with a number of negatively charged or electron rich molecules. This ability of transition metals to coordinate with the molecules led into the development of metal based drugs with promising

pharmacological activities. This has, for instance, led to the clinical application of chemotherapeutic agents for cancer treatment, such as cisplatin (cis-dichlorodiammine platinum(II)). The development of modern medicinal inorganic chemistry is inspired very much by the discovery of cisplatin (Figure 3.1)

On administration, one of the chloride ligands is slowly substituted by water molecule and the process is termed as aquation. The aqua ligand in the resulting $[PtCl(H_2O)(NH_3)_2]^+$ is itself easily displaced, allowing the platinum atom to bind to bases of DNA. Of the bases on DNA, guanine/purine is primarily coordinate with cis-platin, leading to the formation of adduct $[PtCl(base\text{-}DNA)(NH_3)_2]^+$ (Figure 3.2). After the displacement of next chloride ligand by the water molecule, cross linking can occur with another base. Many types of cisplatin–DNA coordination complexes, or adducts, can also be formed and these complexes are referred to 1,2-intrastrand adducts.

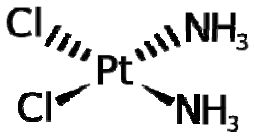


Fig. 3.1 Structure of Cisplatin

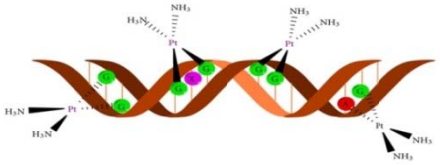


Fig. 3.2 DNA-cis platin adduct

Chelation therapy is the preferred medical treatment for reducing the toxic effects of metals in the body. Chelating agents are capable of binding with toxic metal ions to form complex structures which are easily excreted from the body and removing them from intracellular or extracellular spaces. $CaNa_2EDTA$ etc has long been the support of chelation therapy (Figure 3.3) for lead and cadmium poisoning [1]. Dimercaprol (Figure 3.4) is used for the treatment of arsenic, antimony, lead, mercury and other toxic metal poisoning. It acts as a very strong chelating agent for

various toxic metal ions. Dimercapto succinic acid [2] (Figure 3.5) is indicated for the treatment of lead poisoning in children with blood level measured above 45 µg/dL.

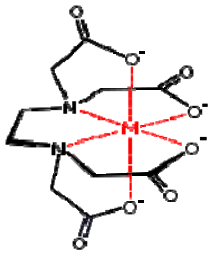


Fig. 3.3 Structure of metal-EDTA complex

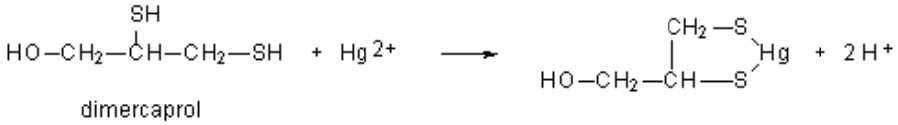


Fig. 3.4 Dimercapol chelation

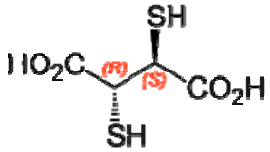


Fig. 3.5 Structure of dimercapto succinic acid

Zinc pyrithione (Figure 3.6) is best known for its use in treating dandruff and seborrhoeic dermatitis [3]. It also has antibacterial properties and iseffective against many pathogens from the *Streptococcus* and *Staphylococcus* ge nera. Its other medical applications include treatments of psoriasis, ec-

zema, ringworm, fungus, athletes foot, dry skin, atopic dermatitis, tinea, and vitiligo.

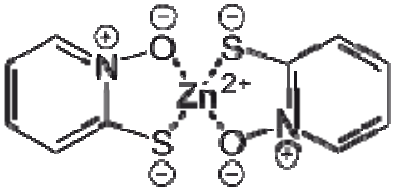
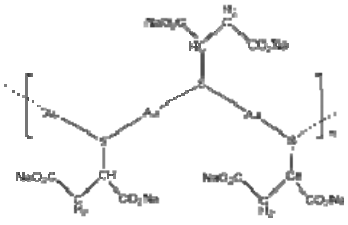


Fig. 3.6 Structure of zinc pyrithione

It was shown that Zinc pyrithione inhibits fungal growth through increased cellular levels of copper, damaging iron-sulphur clusters of proteins essential for fungal metabolism [4].

Gold salt complexes (sodium aurothiomalate) (Figure 3.7) have been used to treat rheumatoid arthritis. Even though the mechanism of action of this drug is not completely studied, it is believed to interact with albumin and eventually be taken up by immune cells, triggering anti-mitochondrial effects and eventually cell apoptosis. It was established that reactive aldehydes such as malondialdehyde, glycoaldehydes and presumably acrolein and 3-aminopropanal [5, 6] may be the ultimate mediators of cell destruction in rheumatoid arthritis joints. P. L Wood et al [7] demonstrated that thiol-containing disease-modifying antiarthritic agents both directly hide reactive aldehydes and augment intracellular thiol pools, which also can buffer increased aldehyde load and oxidative stress. These data are consistent with clinical data that penicillamine lowers synovial aldehyde levels and augments plasma thiols. In the case of D-penicillamine (Figure 3.8) and sodium aurothiomalate, the key structural feature appears to be a free thiol group.



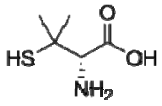


Fig. 3.7 Structure of sodium aurothiomalate **Fig. 3.8** Structure of D-penicillamine

Antibiotics

An antibiotic is an agent that kills or inhibits the growth of bacteria. There are many classes of antibiotics, with the classical ones being sulfonamides, penicillins, cephalosporins and aminoglycosides. Many antibiotics are produced naturally by microorganisms (e.g. produced by fungi in the genus *Penicillium*), while some of these natural compounds provide a building block for the manufacture of synthetic antibacterials for example, the sulfonamides, the quinolones and the oxazolidinones are produced solely by chemical synthesis. Most antibiotics from natural origin have fewer side effects than synthetic compounds and this can be largely attributed to their increased target specificity. Side-effects range from mild to very serious depending on the antibiotics used, the microbial organisms targeted, and the individual patient.

Penicillins and cephalosporins are β-lactam antibiotics [8-10] which act on the cell wall of bacteria. They share the structural feature of a β-lactam ring and inhibit the formation of peptidoglycan cross-links within the cell wall, which weakens the wall osmotically and causes cell death. Ampicillin (Figure 3.9), Penicillin-G (Figure 3.10) are the examples for β-lactam antibiotics

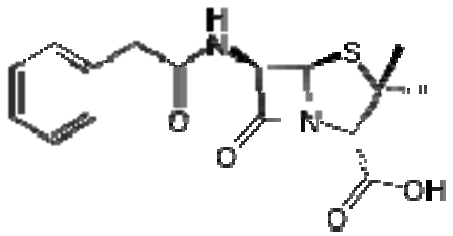


Fig. 3.9 Structure of Penicillin-G

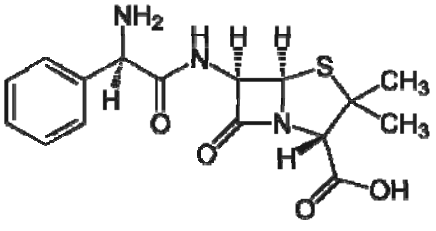


Fig. 3.10 Structure of Ampicillin

Streptomycin (Figure 3.11) is the first of a class of drugs called aminoglycosides to be discovered [11,12]. It was the first antimicrobial agent developed after penicillin and the first antibiotic effective in treating tuberculosis. It is derived from the actinobacterium *Streptomyces griseus*. Streptomycin was discovered by American biochemists Selman Waksman, Albert Schatz, and Elizabeth Bugie in 1943. The drug acts by interfering with the ability of a microorganism to synthesize certain vital proteins. It is used in combination with penicillin for treating infections of heart valves (endocarditic) and with tetracyclines in the treatment of plague, tularaemia, and brucellosis. Adverse effects of this medicine are ototoxicity, nephrotoxicity, fetal auditory toxicity, and neuromuscular paralysis.

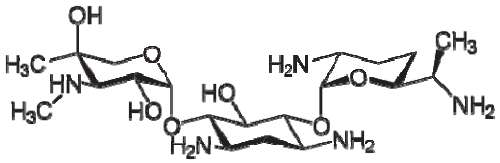


Fig. 3.11 Structure of Streptomycin

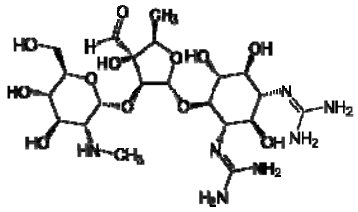
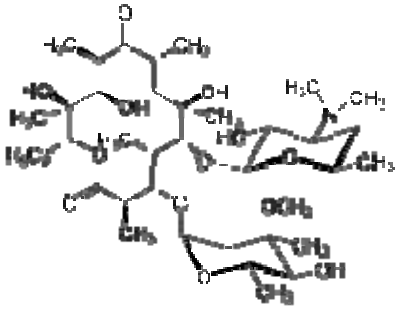


Fig. 3.12 Structure of Gentamicin

Gentamicin (Figure 3.12) is an aminoglycoside antibacterial consists of a linked ring system composed of aminosugars and an aminosubstituted cyclic polyalcohol [13]. They bind to proteins in the ribosome of the bacteria and prevent DNA replication. Aminoglycosides are poorly absorbed when given orally and so are administered intravenously. They also exhibit high toxicity, affecting the ear and kidney. Gentamicin is used to treat many types of bacterial infections, particularly those caused by Gram-negative organisms.

Gentamicin is also ototoxic and nephrotoxic, with this toxicity remaining a major problem in clinical use. It is synthesized by *Micromonospora*, a genus of Gram-positive bacteria widely present in the environment (water and soil). It was discovered in 1963 by Weinstein, Wagman et al [14-16].

Erythromycin (Figure 3.13) is an antibiotic useful for the treatment of a number of bacterial infections. Erythromycin is a macrolide antibiotic produced by *Streptomyces erythreus* and has an antimicrobial spectrum similar to or slightly wider than that of penicillin, and is often prescribed for people who have an allergy to penicillins. It inhibits bacterial protein synthesis by binding to bacterial 50S ribosomal subunits; binding inhibits peptidyl transferase activity and interferes with translocation of amino acids during translation and assembly of protein [17,18]. Cefotaxime (Figure 3.14) is a β-lactam antibiotic (which refers to the structural components of the drug molecule itself). As a class, β-lactams inhibit bacterial cell wall synthesis by binding to one or more of the penicillin-binding proteins (PBPs). This inhibits the final transpeptidation step of peptidoglycan synthesis in bacterial cell walls, thus inhibiting cell wall biosynthesis [19].



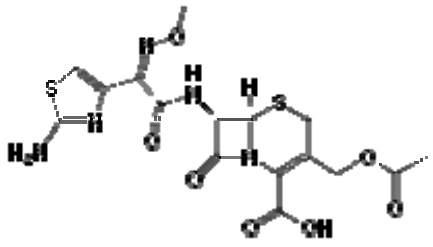


Fig. 3.13 Structure of Erythromycin **Fig. 3.14** Structure Cefotaxime

Over many years, some bacterial strains have developed resistance to commercial antibacterial compounds. Resistance usually arises either after long

periods of exposure to the drug or in conditions that may support the gradual stepwise development of bacteria. Resistance can arise through either a modification of the target site or enzyme, prevention of access for the antibiotics or production of enzymes that destroy or inactivate the antibiotic.

A combination of mutations and alterations can lead to the occurrence of resistant. The problem of bacterial resistance to antibiotics has preoccupied many scientists for years. Aside from bacteria that have undergone mutations, making them resistant to antibiotics, another kind of bacterium exists as well, which is inherently unaffected by antibiotic treatment, called "persistent bacteria".

Important Pathogens

Bacteria are prokaryotic cells that are essential for life on earth. They are present in every inhabitant on the planet, from soil to hot springs and even deep within the Earth's crust [20,21]. They have various roles in both the environment and in the bodies of humans and animals. They decompose matter from dead organism and return vital nutrients to the earth. Bacteria have a vital role in balancing several constructive and destructive processes in the environment. But some bacteria are harmful to life of humans, animals and plants by causing diseases. Based on the structural characteristics of the cell walls of bacteria, Hans Christian Gram divided the bacteria into two types using gram stain test. Crystal violet was used for the experiment and he divided the bacteria as Gram-positive and Gram-negative. Gram-positive organisms are able to retain the crystal violet stain because of their thick peptidoglycan layer, which is superficial to the cell membrane. This is in contrast to Gram-negative bacteria, which may have a thick or thin peptidoglycan layer that is located between two cell membranes [22].

Escherichia coli

Escherichia coli (abbreviated *E. coli*), is a Gram-negative rod shaped bacterium (Figure 3.15) that is commonly found in the lower intestine of humans and warm-blooded animals. They are part of the normal flora of the gastrointestinal tract and are the dominant species in the aerobic faecal flora of humans. In 1885, a German paediatrician, Theodor Escherich, first discovered this species in the feces of healthy individuals and called it *Bacterium coli commune* due to the fact that it was found in the colon and early classifications of prokaryotes placed these in a handful of genera based on their shape and motility. They are actively mobile due to the presence of flagella.

Fig. 3.15 Micrograph of *E. coli*

Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in humans and are occasionally responsible for product recalls due to food contamination. The harmless bacteria are part of the normal flora of the gut and can benefit their hosts by producing vitamin K [23] and by hindering the establishment of pathogenic bacteria within the intestine [24-25].

The bacterium can also be grown effortlessly and inexpensively in a laboratory setting and has been intensively investigated for over 60 years. *E. coli* is the most widely studied prokaryotic model organism and an important species in the areas of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA.

Transmission of *E. coli* is by faecal-oral contact (usually by ingestion of food and water) and the presence of *E. coli* in water or soil is an indicator of faecal contamination. The bacterium produces toxins, known as Shiga toxins, which damage the lining of the intestines and other target organs such as the kidneys. They can cause diarrhoea, urinary tract infections, meningitis, wound infections and pneumonia. Strains of *E. coli* resistant to many broad spectrum antibiotics have come out over the last number of years.

Staphylococcus aureus

Staphylococcus aureus is a Gram-positive spherical bacterium, a member of the Firmicutes and is commonly found in the human respiratory tract [23] and on our skin (Figure 3.16). Methicillin-resistant *Staphylococcus* (MRSA) is a strain of *S. aureus* that is resistant to the β-lactam class of antibiotics, including the penicillins and cephalosporins. Emerging of antibiotic-resistant forms of *S. aureus* (e.g. MRSA) is a worldwide problem in clinical medicine [27].

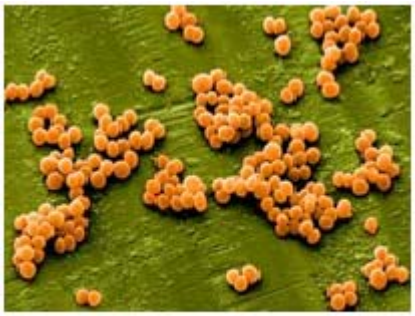


Fig. 3.16 Micrograph of *S. aureus*

It can be transmitted by touch alone, and due to this, hospitals and nursing homes, containing patients which are more susceptible to infection, are ideal breeding grounds for it. MRSA becomes particularly problematic if it enters the body and treatment is generally by administration of glycopeptide antibiotics like vancomycin and teicoplanin. These antibiotics inhibit the growth of bacterial cells by binding to the

amino acids in the cell walls and preventing peptidoglycan synthesis. *S. aureus* was discovered in Scotland in 1880 by Sir Alexander Ogston [28].

S. aureus can cause a range of illnesses, from minor skin infections, suchas pimples,impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome and abscesses to life threatening diseases such as pneumoni a, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia and sepsis. It is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections.

Bacillus subtilis

Bacillus subtilis is a Gram-positive, rod shaped bacteria, commonly found in soil. It was originally named "*Vibrio subtilis"* when it was discovered in 1835 by Christian Gottfried Ehrenberg. It was renamed as "*Bacillus subtilis"* in 1872 by Ferdinand Cohn. This bacterium is besides known by the names *hay bacillus*, *grass bacillus* or *Bacillus globigii.* Unlike several other well known species, *B. subtilis* has been historically classified as an obligate aerobe, though recent research has demonstrated that this is not strictly correct [29,30].

Bacillus subtilis (Figure 3.17) is an endospore forming bacteria, and the endospore that it forms allows it to withstand extreme temperatures as well as dry environments. *B. subtilis* is considered as obligate aerobe, but can also function anaerobically in the presence of nitrates or glucose. It is not considered pathogenic or toxic and is not a disease

Fig. 3.17 Micrograph of *B. subtilis*

causing agent. *Bacillus subtilis* has a flagellum which makes motility faster. Since this bacterium is resistant to extreme temperatures, it can withstand high cooking temperatures. This is not to cause alarm, as it does not cause sickness, if ingested. This bacterium can cause a stringy consistency in spoiled bread dough, if dough is exposed.

Although this species is commonly found in soil, more evidence suggests that *B. subtilis* is a normal gut commensal in humans. A study in 2009 compared the density of spores found in soil (~10^6 spores per gram) to that found in human feces (~10^4 spores per gram). *Bacillus subtilis* is readily present everywhere; the air, soil and in plant compost. Along with enzymes, *B. subtilis* also produces a toxin called subtilisin. Subtilisin can cause allergic reactions if there is repeated exposure in high concentrations. This only poses a risk to fermentation plants that use high quantities of subtilisin.

Bacillus thuringiensis

B. thuringiensis (Bt) is a Gram-positive, soil-dwelling bacterium, commonly used as a biological pesticide; alternatively, the Cry toxin may be extracted and used as a pesticide. *B. thuringiensis* (Bt) is a gram-positive, soil-dwelling, spore-forming, rod-shaped bacteria. It is approximately 1 μm in width and 5 μm in length. It grows at body temperature and produces a diamond-shaped crystal from its crystal proteins and uses it to fend off insects, predators, and other pathogens [31,32].

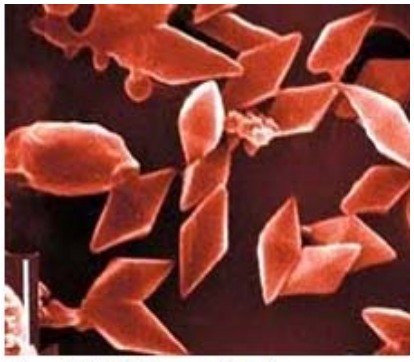


Fig. 3.18 Micrograph of *B. thuringiensis*

B. thuringiensis (Figure 3.18) occurs naturally in the gut of caterpillars of various types of moths and butterflies, as well as on leaf surfaces, aquatic environments, animal feces, insect rich environments, flour mills and grain storage facilities [33]. *B. thuringiensis* was first discovered in 1901 by Japanese biologist Ishiwata Shigetane. In 1911, *B. thuringiensis* was rediscovered in Germany by Ernst Berliner, who isolated it as the cause of a disease called *Schlaffsucht* in flour moth caterpillars. During sporulation, many bacterial strains produce crystal proteins (proteinaceous inclusions), called δ-endotoxins, that have insecticidal action. This has led to their use as insecticides and more recently to genetically modified crops using Bt genes. Many crystal-producing Bt strains, though, do not have insecticidal properties [34].

It was first used as a commercial insecticide in France 1938, and then in USA in 1950s. However, these early products were replaced by more effective ones in the 1960s, when various highly pathogenic strains were discovered with particular activity against different types of insect. *B. thuringiensis* subspecies are neither toxic nor pathogenic to mammals, including humans [35,36]. Animal experimentation, however, has shown that intraperitoneal injection of *B. thuringiensis* can cause death in guinea pigs and that pulmonary infection can result in the deaths of immune compromised.

Proteus vulgaris

P. vulgaris is a Gram-negative rod-shaped bacterium (Figure 3.19) inhabits in the intestinal tract of humans. It can be found in soil, water and fecal matter. It belongs to the family entero bacteriaceae and is an opportunistic pathogen

Fig. 3.19 Micrograph of *P. vulgaris*

of humans. Urinary tract infections and wound infections are mainly caused by this bacterium.

The first use of the term "*Proteus*" (meaning: God of rivers in Greek) in bacteriological nomenclature was made by Gustav Hauser (1885) who described under this term three types of organisms which he isolated from putrefied meat. One of the three species, Hauser identified was *P. vulgaris* and this microbe has a long history in microbiology and pathology.

Major taxonomic revisions have been undergone over the past two decades for the genus *Proteus*. On the basis of production of indole from tryptotphan, these bacteria were classified into three biogroups in 1982. Biogroup one was indole negative bacteria and represented a new species *P. penneri*; while biogroup two and three clubbed together as *P. vulgaris* which satisfactorily answered the indole test [37].

Enterobacter aerogenes

E. aerogen is a nosocomial and pathogenic bacterium, that causes opportunistic infections including most types of infections. *E. aerogenes* are (Figure 3.20) rod shaped Gram- negative bacteria which do not answer indole test. *E. aerogenes* are commonly found in the human gastrointestinal tract and does not generally cause disease in healthy individuals. It has been found to live in various wastes, hygienic chemicals

Fig. 3.20 Micrograph of *E. aerogenes*

and soil. Majority of *E. aerogenes* are sensitive to most antibiotics designed for this bacteria group, but this is entangled by their inducible opposition mechanisms, particularly lactamase which means that they rapidly become resistant to standard antibiotics during treatment. This needs change of antibiotics to avoid worsening of the disease like sepsis [38].

Some of the infections caused by *E. aerogenes* result from specific antibiotic treatments, venous catheter insertions and/or surgical procedures. This bacterium has a definite role commercially, in producing hydrogen gas during the fermentation of molasses.

Metal Chelates as Antibacterial Agents- A Review

Many of the metal chelates have been screened for their antimicrobial activity by various scientists and researchers. Among these active metal complexes found, many Schiff base metal chelates have got amazing antimicrobial activity against various bacteria and fungi. Furthermore it has been generally observed that metal chelates were demonstrated higher activity than their respective Schiff bases. The probable mechanism of inhibition of the growth of the microbes by the metal chelates suggested by various researchers is discussed in the third chapter. Reviews of the literature containing the antimicrobial activity of metal chelates of Schiff base are given below.

A series of Schiff bases derived from 2-acetylpyridne and 4-(2-aminoethyl)morpholine, and 4-(2-aminoethyl)piperazine and their metal complexes were synthesized and characterized by N. S. Gwaram et al [39]. The complexes were screened for anti-bacterial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumanni* (AC), *Klebsiella pneumonie* (KB) and

Pseudomonas aeruginosa (PA) using the disc diffusion and micro broth dilution assays. Based on the overall results, the complexes showed the highest activities against MRSA while a weak antibacterial activity was observed against *A. baumanii* and *P. aeruginosa*.

Metal complexes of Ni(II), Co(II), Cu(II), Mn(II), Zn(II) and VO(IV) with a Schiff base derived from 3-ethoxy salicylaldehyde and 2-(2-amino-phenyl) 1-*H*-benzimidazol(2-[(Z)-{(2-(1*H*-benzimidazole-2yl)phenyl]imino}methyl]-6-ethoxy phenol-BMEP) were synthesized successfully by M. Sunitha et al [40]. Antimicrobial activity of the ligand and its metal complexes were studied against two Gram-negative bacteria: *Escherichia coli, Pseudomonas fluorescence* and two Gram-positive bacteria: *Bacillus subtilis, Staphylococcus aureus*. The activity data show that the metal complexes are more potent than the free ligand. Among the studied complexes, Cu(II) complex displayed higher inhibitory effect on the growth of *E. coli*. Zn(II) chelate also demonstrated elevated inhibitory action against the growth of all pathogens.

Recently E. Yousif et al [41] have synthesized five new metal complex derivatives of 2N-salicylidene-5-(*p*-nitro phenyl)-1,3,4-thiadiazole, HL with the metal ions VO(II), Co(II), Rh(III), Pd(II) and Au(III) in alcoholic medium. The preliminary *in vitro* antibacterial screening activity revealed that all complexes showed moderate activity against tested bacterial strains such as *S. aureus*, *S. typhi* and *E. coli* bacterial than the ligand. Agar diffusion method was employed for the determination of inhibition zone. Rhodium complex showed poor antibacterial activity against the growth of *S. aureus*. The activity of the gold

complex was comparatively higher than all other complexes against the growth of *S. typhi*. All metal chelates equally exhibited good antimicrobial activity against *E. Coli*. Authors claim that it is due to the large size of the metal chelates they exhibit moderate antibacterial capacity since the cell penetration power decreases with increase in the molecular size.

Novel transition metal [Co(II), Cu(II), Ni(II) and Zn(II)] complexes of substituted pyridine Schiff-bases (derived from substituted aminopyridine and salicylaldehyde) have been prepared and characterized by physical, spectral and analytical data by Z. H. Chohan et al [42]. In order to evaluate the effect of metal ions upon chelation, the Schiff bases and their complexes have been screened for antibacterial activity against the strains such as *E. coli*, *S. aureus*, and *P. aeruginosa*. The complexed Schiff bases have shown to be more antibacterial against one more bacterial species as compared to uncomplexed Schiff-bases. Paper disc diffusion method was used for the antibacterial screening. All metal ions have varying antibacterial influence on bacterial species. The Co(II) complex of hydroxyl substituted Schiff base was more antibacterial against one species and less against the other as compared to the Co(II) complex of the other Schiff bases (bromo, nitro and methoxy substituted). Same results were found for other metal complexes. They assure that metal ions do play a significant role in enhancing the antibacterial activity of antibacterial agents on chelation. They suggest that in the chelated complex, the positive charge of the metal ion is partially shared with the donor atoms and there is electron delocalization over the whole chelate ring. This increases

the lipophilic character of the metal chelate and favours its permeation through lipid layers of the bacterial membranes.

N. Raman et al [43] have synthesized novel tetradentate N_2O_2 type Schiff base, from 1-phenyl-2,3-dimethyl-4-aminopyrazol-5-one(4-aminoantipyrine) and 3-salicylidene-acetylacetone and the stable complexes with transition metal ions such as Cu(II), Ni(II), Co(II) and Zn(II) in ethanol. The *in vitro* antimicrobial activities of the investigated compounds were tested against bacteria such as *K. pneumoniae, S. aureus, B. subtilis* and *E. coli* and fungi like *A. niger* and *R. bataicola*. All the metal chelates showed higher antimicrobial activity for the above microorganisms than that of the free ligand.

The synthesis, characterization, spectroscopic and biological properties of *trans*-$[Co^{III}(L^1)(Py)_2]ClO_4$ and *trans*-$[Co^{III}(L^2)(Py)_2]ClO_4$ complexes, where H_2L^1 = N,N′-bis(5-chloro-2-hydroxybenzylidene)-1,3-propylenediamine and H_2L^2 = N,N′-bis(5-bromo-2-hydroxybenzylidene)-1,3-propylenediamine, have been done by M. Salehi et al [44]. The *in vitro* antimicrobial activity of the Schiff base ligands and their corresponding complexes have been tested against human pathogenic bacteria such as *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *E. coli*. The cobalt(III) complexes showed lower antimicrobial activity than the free Schiff base ligands.

Mixed ligand complexes of type ML'B (M(II)=Mn(II), Co(II), Ni(II), Cu(II) and Zn(II); HL'=o-vanillidene-2-aminobenzothiazole; B=1,10-phenanthroline) and Schiff base metal complexes of types (ML_2") and (M_2L") (HL"= *o*-vanillidene-2-amino-N-(2-pyridyl)-benzene sulfonamide) were synthesized and characterized by M. A. Neelakantan et al [45]. The newly synthesized ligands and their metal

complexes were screened *in vitro* for their antibacterial activity against bacteria: *E. coli, P. aeruginosa, S. Typhi and V. parahaemolyticus* by well diffusion method using agar nutrient. The antifungal activities were tested against fungus: *A. Niger, Penicillium, T. virida* and yeast: *S. cerevisiae* by well diffusion method using potato dextrose agar as the medium. Ampicillin and nystatin are used as control for bacteria and fungi, respectively. They found that majority of all complexes are found to be active against various microorganism. Some of them showed the inhibitive action very near to the standard antibiotic. But the metal chelates of the Schiff base o-vanillidene-2-amino-2-N(2-pyridyl)-benzene sulfonamide was totally inactive against the growth of *E. coli* and *P. aeruginosa* whereas the nickel complex of Schiff base o-vanillidene-2-aminobenzothiazole exhibited escalated antibacterial activity against the growth of *E. coli* and *P. aeruginosa* than the standard antibiotic. Also the antifungal acivity of the metal chelates were appreciable when compared to the activity of standard drug.

The coordination complexes of VO(II), Co(II), Ni(II) and Cu(II) with the Schiff bases derived from isatin with 3-chloro-4-fluoroaniline and 2-pyridinecarboxaldehyde with 4-aminoantipyrine have been synthesized by conventional as well as microwave methods by A. P. Mishra et al [46]. The Schiff base and metal complexes show a good activity against the bacteria; *Staphylococcus aureus, Escherichia coli* and *S. fecalis* and fungi *A. niger, T. polysporum, C. albicans* and *A. flavus*. The antimicrobial results also indicate that the metal complexes are better antimicrobial agents as compared to the Schiff bases. The minimum inhibitory concentrations of the metal complexes were found in the range

10~40 μg/mL. Streptomycin and nystatin were used as the standard antibiotics and miconazole was taken as the standard antifungal.

Mechanism of Growth Inhibition

The complete mechanism of the inhibitory action of the metal chelates against the growth of several micro organisms has not yet widely studied. In the present chapter an attempt was made to explain the mechanism of inhibition in tandem with the mechanistic pathways of the growth inhibition of the standard antibiotics. By learning the structural features of microorganisms, microbiologists could reach into possible conclusions regarding the obstruction of the growth of microorganism by antibiotics. Antimicrobial therapy is based on the selective toxicity of the agents on the microbial cells. Antibacterial drugs may be either bacteriostatic or bactericidal. Bacteriostatic agents will inhibit the growth of bacteria while bactericidal drugs will kill bacteria. The activity of penicillins, streptomycin and gentamicin are bacteriocidal in nature, but antibiotics erythromycin, cindamycin, chloramphenicol etc act as bacteriostatic agents.

Based on the cellular structure of bacteria or the function of the affecting antibiotics, the role of the antibiotics may be generally one among the following.

They may 1) inhibit nucleic acid synthesis

2) inhibit protein synthesis

3) inhibit cell wall biosynthesis

4) alter or inhibit cell membrane permeability or transport

5) inhibit folate metabolism

6) antimetabolites

Pictorial representation of sites of the action of antibiotics inside and outside the cell of bacteria is given Figure 3.21.

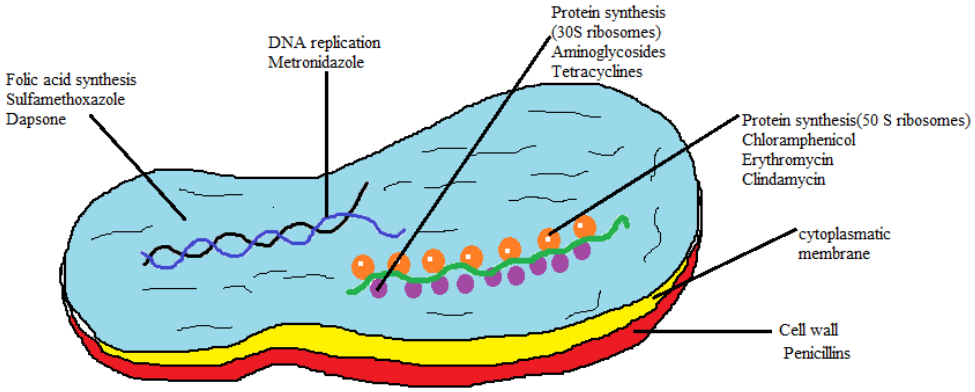


Fig 3.21 Mechanism of action of antibiotics in microbial cells

The first class of antimicrobial drugs that interfere with cell wall synthesis is the beta-lactam antibiotics). This includes penicillin derivatives. The second class of antimicrobial drugs that interfere with cell wall synthesis are the glycopeptide antibiotics. Significant glycopeptide antibiotics include vancomycin, teicoplanin etc.

Antifolate drugs will inhibit the synthesis of folic acid (vitamin B9). A well known example is Trimethoprim. It is a bacteriostatic antibiotic used mainly in the prophylaxis and treatment of urinary tract infections [47].

The production of the nucleic acid (DNA and RNA) is inhibited by particular antibiotics. (e.g. metronidiazole). These act by generating metabolites that are incorporated into DNA strands, which then are more prone to breakage. These drugs are selectively toxic to anaerobic organisms, but can affect human cells [48]. Protein synthesis inhibitors in general work at different stages of prokaryotic mRNA translation into proteins like initiation, elongation and termination.

Tetracyclines block the A site on the ribosome, preventing the binding of aminoacyl tRNAs. Aminoglycosides interfere with the proofreading process, causing an increased rate of error in synthesis with premature termination. Chloramphenicol blocks the peptidyl transfer step of elongation on the 50S ribosomal subunit in both bacteria and mitochondria. Antimetabolites are structural analogs of normal metabolites that inhibit the action of specific enzymes. They include bacteriostatic (sulfonamide, trimethoprim, para-aminosalicylic acid) and bactericidal (isoniazid) drugs [49].

Microbial organisms attain resistance to certain drugs. They gain the resistance through different means but primarily based on the chemical structure of the antimicrobial agent and the mechanisms through which the agents acted [50].

Resistance can be described in two ways:

a) Non genetical pathways: arises due to the alteration or loss of the specific receptors, e.g. cell wall membrane.
b) Genetical reason: results by the mutation of the bacterial cell. This mechanism may be either chromosomal or extra chromosomal resistance. A chromosomal mutation alters the structure of the receptor of the drug or the permeability of the drug. In extra chromosomal mechanism, plasmid enzymes degrade or modify the drug. The different ways in which a bacterium achieves resistance to a drug was effectively studied by Fluit et al [51]. The mechanism is summarised in Figure 3.22.

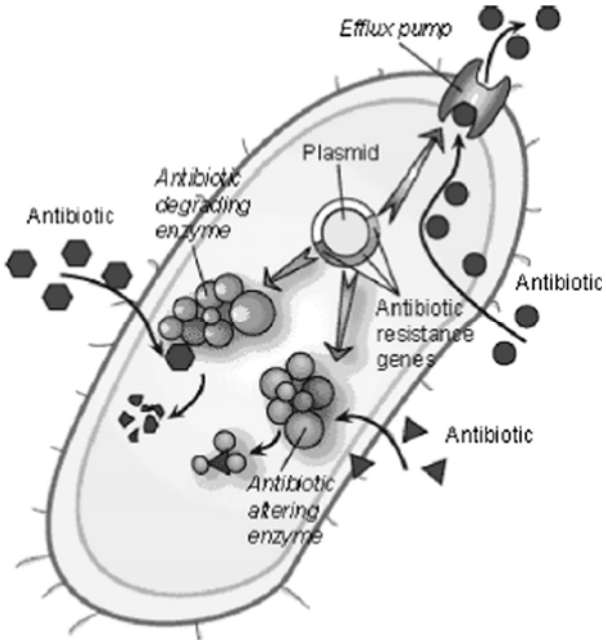


Fig. 3.22 Illustration of how some antimicrobial agents are rendered ineffective (Fluit et al., 2001)

Scope and Objectives of the Present Investigation

Previous researchers have established that many Schiff base and their metal chelates show antimicrobial activities and other pharmaceutical abilities. But thorough investigations are going on in this area to find out effective antimicrobial agents which can yield prolonged action without affecting the normal cells of living beings adversely.

Contemporary medicines that are widely used as antibiotics are seriously suffering from the potential threat of resistance by various bacteria. Any use of antibiotics can increase selective pressure in a population of bacteria to allow the resistant bacteria to thrive and the susceptible bacteria to die off. The overuse and misuse of antibiotics are considered as the two important reasons for resistance to the microbes. Since the resistance towards antibiotics becomes more common, a serious need for alternative antibiotics arises.

The present investigation which explores the potential activity of the newly synthesized Schiff bases and their metal chelates is very relevant in this scenario. By examining the *in vitro* drug abilities of these newly synthesized molecules, a class of compounds with high potential antimicrobial activities may emerge.

Antimicrobial Testing Method

Kirby-Bauer or disc diffusion [52] method was employed for the antibacterial screening. Mueller-Hintor agar was chosen for the preparation of the medium [53-56]. Appreciable batch-to-batch reproducibility can be obtained using this agar. It also offers a satisfactory growth of many microbes. Moreover many of the antimicrobial experiments has been done and reported in the media prepared from Mueller-Hintor agar [57-60]. It typically contains (w/v) 30.0% beef extract, 1.75% casein hydrolysate, 0.15% starch and 1.7% agar. The pH of the medium was adjusted to neutral at 25 °C.

Preparation of Agar Plates

After preparing the agar medium, it was autoclaved and allowed to cool in a water bath kept at 45-50^{0}C. After cooling this medium, it was poured into petri dishes having 150mm diameter by keeping inside the Laminar flow hood chamber. Approximately 4-4.5 mm of depth was achieved by the careful transferring of the medium. 60-75ml of the agar medium was used for the preparation of one plate. The plate was allowed for solidification and each batch of the plates was examined for sterility by incubating for 24 hours at 30-35^{0}C.

Nutrient Broth (NB)

Nutrient broth for the preparation of inoculum was prepared by dissolving and heating the following ingredients in distilled water. The NB was distributed in 25ml quantities in 250ml conical flask, plugged with non absorbing cotton and autoclaved at 100^{0}C for 15 minutes.

Peptone	10g
Beef Extract	5g

NaCl	5g
Agar	20g
Distilled Water	1L
pH (25^0C)	7.5 ±0.02

Preparation of Inoculums

The growth method was employed for the preparation of inoculums. Three to five isolated colonies of the bacteria having the same morphologies was first selected from an agar plate culture. The growth is then transferred into a tube containing 4-5ml of nutrient broth. The broth culture was then incubated at 35^0C until it gain or exceeds the turbidity of 0.5 McFarland standards. Around 2-6 hours was required to achieve this turbidity.

Preparation of Dried Paper Discs

Stock solutions of the synthesized ligands, complexes and standard antibiotics were prepared in DMSO. It was then dissolved and further diluted to obtain different concentrations ranging from 100µgdisc^{-1} to 500µgdisc^{-1}. Paper disc method was employed for drug inoculation. These samples were applied to paper discs having 5mm diameter (Whatman No:1) with the help of a micropipette. Also paper disc containing the solvent DMSO was tested. The discs were placed in a petri dish and kept in an incubator for 24 hours at 37^0C.

Test Plate Inoculation

After adjusting the turbidity of the inoculum suspension (using sterile saline solution or broth), a sterile cotton swab is dipped into the suspension. The cotton swab was rotated many times in the suspension and pressed strongly on the inner

wall of the tube above the liquid level. The pressing removed the excess of inoculums from the cotton swab. Using cotton swab, the inoculum was gently swabbed over the entire surface uniformly of the dried Mueller-Hinton agar plate. To ensure an even distribution of inoculums, this procedure was repeated by streaking two more times, rotating the plate approximately 60^0. After completing the swabbing process, the inoculated agar plates were allowed to dry for 15-20 minutes.

Application of Sample Discs to Inoculated Agar Plates

Predetermined battery of dried (and previously impregnated by the drug) paper discs were dispensed on the surface of the inoculated agar plate using forceps. To get uniform contact, each disc was gently pressed down on the agar. At least a minimum of 30 mm distance was maintained between the discs. After this, the petri dishes were inverted and kept in an incubator for 24 hours at 35^0C in air ambience.

As soon as the disc comes in contact on the wet agar surface, the water will start to enter into the disc through the capillaries of the paper, simultaneously the drug will start to leach into the agar medium. The rate of extraction of the drug from the paper disc is higher than the outward diffusion through the agar medium and the concentration of the drug very near to the disc will be higher than the concentration of the drug present in the disc. However a logarithmic fall of concentration of the drug will occur as we move from the circumference of the disc to the diffused portion of the drug in agar plate. When the concentration of the diffused drug reaches a critical value, the growth of bacteria in the premises of the paper disc is inhibited. The zone of inhibition was measured (in mm) using sliding callipers.

Apparatus and Equipments Used

1) Petri dishes
2) Sample dishes
3) Pipette (1ml to 10ml capacity)
4) Test Tubes- 10ml, 25ml, 50ml capacity
5) Durham's tubes
6) Flasks – 100ml, 250ml, 500ml and 1 Litre
7) Microscopic slides
8) Microscope (Olympus Model)
9) Incubator maintaining 37^0C
10) Electronic balance
11) Serological water bath
12) Air oven
13) Autoclave
14) Platinum loop
15) Sterilized swabs
16) Spirit lamp
17) Laminar flow (Lab India)

CHAPTER 3

ANTIBACTERIAL INVESTIGATIONS ON THE SCHIFF BASES AND THEIR METAL CHELATES

The antibacterial studies on various metal chelates have been performed by the researchers and they repeatedly affirmed that the coordination compounds of Schiff bases show marked activity against the growth of several microorganism. In quest for finding out new potential microbial agents, they compare the activity of the complexes with the activity of standard antibiotics. In the present chapter, the details of the inhibitory power of the Schiff bases APSC, APTSC, APPH, CPFASC, CPTASC, CPFAPH and FAABA and their transition metal chelates on the growth of *S. aureus, B. subtilis, B. thuringiensis, E. aerogenes, E. coli* and *P. vulgaris* are listed and compared with the activity of standard drugs such as erythromycin, streptomycin, gentamicin, ampicillin, penicillin, cefotaxime, benzylpenicillin and cloxacillin. Different concentrations of the compounds used for the entire study were 100µgdisc^{-1}, 200µgdisc^{-1} and 500µgdisc^{-1} in DMSO solvent. The role of the solvent in the microbial screening was also studied and necessary correction factors were applied in the results obtained by disc diffusion method.

Results and Discussion

All the newly synthesized Schiff bases and the metal chelates were screened for the antibacterial activity on six different bacterial cell lines using disc diffusion method. Zone of inhibition of bacterial growth in mm was measured after 24h in each case. For comparison the inhibitive action of standard antibiotics was also screened. The results are given in this section and explanations based on the data are presented in the subsequent paragraphs.

Antibacterial Studies on APTSC and its Transition Metal Chelates

The Schiff base, 3-acetlypyridine thiosemicarbazone (APTSC) and its complexes were screened for antimicrobial studies. Table 3.1 shows the zone of inhibition values of the ligand and metal chelates against the growth of six microbes described earlier. These data was compared with the results obtained for the standard antibiotics (Table 3.8). It is obvious from the data that the Schiff base and the metal chelates displayed moderate antimicrobial activities. An appreciable activity was shown by the ligand on the Gram-positive bacteria like *E. coli* and *P. vulgaris* which was comparable to standard drugs such as benzylpenicillin, cloxacillin and gentamicin. This can be attributed to the appreciable hydrophilic nature of the Schiff base and this might have caused the entering of drug through the hydrophilic cell membrane of the bacteria. On increasing concentration beyond 200µgdisc^{-1}, this Schiff base didn't display any enhancement in the inhibition against the growth of *E. coli* and *P. vulgaris*. At this concentration the drug reached into a saturation level for the growth inhibition of the microbe. The Cd(II) complex of this ligand was significantly active against the growth of *B. thuringiensis* which was comparable with the inhibition of gentamicin, but displayed poor or moderate inhibition on the growth of other microbes. This metal chelate was totally inactive against *E. aerogenes*. The Cr(III) complex showed marked zone of inhibition of 19mm at a concentration of 500µgdisc^{-1}, against the growth of *E. aerogenes*. But this chelate was totally inactive against *P. vulgaris*.

Ag(I) chelate exhibited good inhibition on the growth of *E. aerogenes*, while it was inactive against *B. subtilis*, *E. coli* and *P. vulgaris*. Generally the VO(II)

complex demonstrated moderate antimicrobial activities. It was significantly active against the growth of *B. thuringiensis.* No activity was shown by this metal chelate against S. aureus and *P. vulgaris*. The antimicrobial ability of the Cu(II) and Ni(II) chelates was appreciable for the inhibition of Gram-positive bacteria when compared to Gram-negative bacteria. This may be accounted due to the enhanced lipophilicity of the chelate on complexation as suggested by Tweedy [61]. A comparable antimicrobial activity to that of standard antibiotics was achieved by these metal chelates against the growth of *E. coli* and *E. aerogenes*. For *P. vulgaris* growth, Cu(II) chelate displayed a saturation value for inhibition of 14mm at a concentration of 200μgdisc^{-1}.

Antibacterial Studies on APSC and its Transition Metal Chelates

The Schiff base, 3-acetylpyridine semicarbazone (APSC) and its transition metal chelates of VO(II), Cr(III), Ni(II), Cu(II), Cd(II) and Ag(I) ions were screened for their antimicrobial activity. The results obtained by the disc diffusion method are summarized in Table 3.2. It is evident from the table that all the metal chelates and the Schiff base were moderately active against the growth of the microorganisms. Majority of the chelates exhibited higher inhibition than the Schiff base APSC. But this ligand was equally active as the standard drugs such as gentamicin, erythromycin and streptomycin against the growth of *E. coli* and showed lesser activity than cefotaxime and ampicillin. The Schiff base showed least activity on the growth of Gram-negative bacterium *B. subtilis*. On close examination of the zone of inhibition values, one can say that the Schiff base APSC possess better activity against the growth of Gram-positive bacteria. A saturation value of

CHAPTER 2

MATERIALS AND METHODS

The newly synthesized ligands and their transition metal chelates were screened for antimicrobial studies using disc diffusion technique. The details of synthesis of the Schiff base ligands and chelates are given in part I. All chemicals used for the antimicrobial studies were purchased from Glaxo or E. merck. To prepare the medium, Muller- Hinter agar was used.

Compounds Taken For Antibacterial Screening

VO(II), Cr(III), Ni(II), Cu(II), Cd(II) and Ag(I) complexes of Schiff bases APSC, APTSC and APPH and the three ligands were dissolved in DMSO in various concentrations and screened for the antibacterial activity. The Schiff base CPFASC and its for metal chelates of Cr(III), Ni(II), Cu(II) and Ag(I) were also taken for the studies. Similarly Cr(III), Fe(III), Ni(II), Cu(II), Zn(II) and Cd(II) complexes of the Schiff base CPTASC and Co(II), Cu(II) and Zn(II) complexes of CPFAPH and the respective ligands were screened for antimicrobial studies. The antibacterial studies on ten transition metal chelates of the Schiff base FAABA such as VO(II), Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Ag(I) were also performed.

Pathogens Taken For the Study

1) *Staphylococcus aureus*
2) *Bacillus subtilis*
3) *Bacillus thuringiensis*
4) *Enterobacter aerogenes*
5) *Escherichia coli*
6) *Proteus vulgaris*

Standard Antibiotics Used

1) Erythromycin
2) Streptomycin
3) Gentamicin
4) Ampicillin
5) Penicillin-G
6) Cefotaxime

zone of inhibition was noted for this Schiff base on the growth of *P. vulgaris* at a concentration of 200μgdisc^{-1}. A further increase in the concentration of APSC was not resulted in the enhancement of growth inhibition. At this level, the concentration of the drug was quite enough to stop the growth of the microbial growth by any of the mechanism discussed earlier. The enhanced activity of this Schiff base can be attributed to the high hydrohililicity of this molecule. The highly soluble nature of this molecule in aqueous medium and in DMSO helps it to interact the living cells by any of the mechanism mentioned above. The enhanced hydrophilicity may reduce the penetration of this molecule into Gram-negative bacteria, since the primary lipid layer on the cell wall of Gram-negative bacteria exists as the barrier for this molecule which is absent in Gram-positive bacteria. The ample power of the standard drugs can be explained by the suitable binding of molecule with the receptor molecules. Since the standard antibiotics display hydrophilicity and at the same time lipophilicity, which will help them either to bind on the cellular membrane or to penetrate into the cell cytoplasm and thus to achieve proper drug ability by blocking certain biosynthesis, such as protein synthesis and nucleic acid synthesis, or replication of DNA or the cell divisions.

When compared to the standard antibiotics, Cu(II) complex showed moderate or lower activities against the growth of all bacteria except *P. vulgaris* and *E. coli*. Among the metal chelates studied for antimicrobial activities, the Cu(II) complex exhibited very high activity against *E. coli.*

The increased biological activity of the Schiff bases upon chelation can be explained with the help of Tweedy's chelation theory. On chelation the positive

charge on the metal ion considerably reduces. The partial sharing of the delocalized electrons from the ligand side to the metal will decrease the positive charge on the metal ion significantly and hence the total polarity of the metal decreased considerably. This scenario cause to improve the lipophilicity or hydrophobicity of the molecule when compared to the bare ligand. At this state the metal chelates will get an ample opportunity to enter into the cell cytoplasm by crossing the lipid membrane. This enhances increased uptake or the entrance of the metal chelates into the cell and to improve the cytotoxicity [61].

Although metal chelation enhances the inhibition response of molecules, a serious retarding factor, which causes to reduce the growth inhibitive power, is the bulky nature of the complex. The large sized molecules restrict the motion inside and outside of the microbial cells.

The Cd(II) chelate of APSC displayed appreciable activities against the growth of pathogens. This complex was as active as the standard drugs cloxicillin and erythromycin against the growth of *S. aureus*. It also displayed better antimicrobial activity against *E. coli*, but showed low inhibition on the growth of *E. aerogenes* and *P. vulgaris*. In general, this chelate exhibited better antimicrobial efficiency on the Gram-negative bacteria.

Ni(II) and VO(II) metal complexes generally showed comparatively low or moderate antimicrobial activities. But these metal chelates unusually exhibited significant activity against the growth of *E. coli* than the other chelates. Ni(II) displayed a saturation level for inhibition against the growth of *S. aureus* at a concentration of 200μgdisc^{-1}. Cr(III) chelate showed comparatively low zone of

inhibition. Ag(I) exhibited 5-10 mm of zone of inhibition for 500μgdisc^{-1}. To sum up, all the six studied metal chelates and the ligand displayed appreciable antibacterial properties. All showed better activity especially against the growth of *E. coli.*

Antibacterial Studies on APPH and its Transition Metal Chelates

Antimicrobial activity of the Schiff base, 3-acetylpyridine phenylhydrazone (APPH) and its transition metal chelates were determined by disc diffusion method and reported in Table 3.3. From the data of growth inhibition it is understandable that the complexes of Cu(II), VO(II) and Ni(II) have relatively moderate antimicrobial activity against the growth of Gram-positive bacteria such as *E. coli, E. aerogenes* and *P. vulgaris* and this was slightly higher than the activity of the ligand APPH. The Ni(II) chelate demonstrated a zone of inhibition of 11mm at 200μgdisc^{-1}, which was not increased further with increasing of the concentration. These chelates were generally inactive or little active against the growth of other studied Gram-negative bacteria. The vanadyl complex displayed considerable activity against the growth of *B. subtilis* but not higher than that of ligand. Generally the activity of the Cd(II) complex was poor against the growth of both Gram-positive and Gram-negative bacteria, compared to that of standard antibiotics. The Cr(III) chelate was inactive against the growth of *E. aerogenes*. Also this complex showed good zone of inhibition for *S. aureus*. A pronounced antimicrobial activity was shown by Ag(I) complex of APPH against *E. coli,* which was comparable to the activity of the standard antibiotic gentamicin. This complex displayed a zone of inhibition of 15mm towards the growth of *E. coli* at the concentration 200μgdisc^{-1},

which was slightly increased to 16mm at 500µgdisc^{-1}. This shows that the drug achieved a saturation value at 200µgdisc^{-1}. Compared to the antimicrobial activity of the metal chelates of APSC and APTSC, the transition metal complexes of APPH generally possess lower activities which may be due to the increased size of the molecule which in turn to enhance the steric hindrance to interact with the bacterial cell membrane.

Antibacterial Studies on CPFASC and its Transition Metal Chelates

The Schiff base, carboxyphenyl furan-2-aldehyde semicarbazone (CPFASC) showed good inhibition against the growth of *B. thuringiensis, E. aerogenes and E. coli*. The activity against *E. coli* was comparable to that of the standard antibiotics. Generally the metal chelates Cr(III) and Ni(II) showed very poor or no antimicrobial activity. This may be due to the bulky nature of the molecule, since these metal ions are coordinated to two ligand molecules, which was confirmed by the structural analysis given in part I. The Cu(II) complex displayed potential inhibitive strength against the growth of *B. thuringiensis*. In general, Ag(I) chelate showed better growth inhibitory power and displayed a saturation value of 14mm zone of inhibition against the growth of *P. vulgaris* at the concentration 200µgdisc^{-1}. The details of zone of inhibition data of the ligand CPFASC and its chelates are listed in Table 3.4.

Antibacterial Studies on CPTASC and its Transition Metal Chelates

The ligand carboxyphenyl thiophene-2-aldehyde semicarbazone (CPTASC) and its transition metal chelates were subjected to antimicrobial activity analysis and the results are represented in Table 3.5. In general, the ligand and the metal

complexes showed moderate to high antibacterial activities. The molecule CPTASC was appreciably active against *E. aerogenes* while it was completely inactive against the growth of *E. coli*. The copper complex was significantly obscured the growth of the Gram–negative bacterium *B. thuringiensis* and Gram-positive bacterium *E. coli*. It is worthwhile to mention that the total inactive character of the Schiff base against *E. coli* was reverted on complexation through the Cu(II) metal ion (12mm inhibition zone for 500µgdisc^{-1}). Surprisingly, this chelate didn't exhibit any activity against *B. subtilis* while its ligand showed 8mm of zone of inhibition for 500µgdisc^{-1} concentration. These results emphasizes that not only the chelation but other factors such as the electronic structure of the molecule or chelate, geometry, size, ability to bind on enzymes, DNAs, cell wall etc also influence the antimicrobial activities. Though we can make general conclusions regarding the active or inactive behaviour of molecules, mechanism of interaction of each molecule with each bacterial cell lines are different.

The Cd(II) and Fe(III) chelates showed moderate activities. Fe(III) chelate exhibited better inhibitive power against *P. vulgaris* (12mm inhibition zone for 500µgdisc^{-1}). This chelate was inactive against *E. coli*. The Ni(II) chelate also displayed enhanced activity against *P. vulgaris* (14mm for 500µgdisc^{-1}) which was comparable to that of the standard antibiotic cefotaxime. An appreciable antimicrobial activity was shown by zinc complex of CPTASC against the pathogens *B. subtilis, E. coli* and *P. vulgaris*, which was comparable to the activities of standard antibiotics cloxacillin, gentamicin, benzylpencillin and cefotaxime.

Antibacterial Studies on CPFAPH and its Transition Metal Chelates

Carboxyphenyl furan-2-aldehyde phenylhydrazone (CPFAPH) exhibited comparatively low inhibition zones against the growth of all microbes except *P. vulgaris*. This may be due to the low chelating power and large size of the Schiff base. Co(II) complex showed marked activity against *E. aerogens* only, while Cu(II) chelate behaved as a good microbial growth inhibitor for all microbes except *E. coli* and *P. vulgaris*. In general, the Zn(II) chelate displayed poor antimicrobial activity. Table 3.6 portraits the details of antimicrobial data of the Schiff base CPFAPH and its transition metal chelates.

Antibacterial Studies on FAABA and its Transition Metal Chelates

The antibacterial screening on the Schiff base, furan-2-aldehyde-3-aminobenzoic acid (FAABA) and its metal chelates revealed that complexes were considerably more active than the Schiff base against the growth of all microbes. This is clearly in accordance with the Tweedy's theory for explaining the enhanced antimicrobial activity of the ligands upon chelation. The ligand along with ten metal complexes of VO(II), Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Ag(I) were screened for their antibacterial activity and the results are reported in Table 3.7. The zone of inhibition obtained by the disc diffusion method of Cr(III) and Fe(III) complexes followed almost same trends. Both were inactive against *S. aureus* and *E. coli* but displayed moderate or pronounced activities against other microbes. A marked activity was shown by these complexes against the growth of *P. vulgaris*. At a concentration of 500μgdisc^{-1} Cr(III) and Fe(III) chelates exhibited 17mm and 19mm of inhibition zones respectively. These values are very much

comparable with the zone of inhibitions of the standard antibiotics such as cefotaxime and erythromycin. The Cr(III) metal chelates exhibited saturation values of 17mm and 13mm of zone of inhibition towards *P. vulgaris* and *B. thuringiensis* respectively at 200μgdisc^{-1}. The activity of Mn(II) chelate was generally poor but the same chelate displayed enhanced activity against the Gram–negative bacteria *B. subtilis* (15mm for 500μgdisc^{-1}). Moreover this chelate was totally inactive against *E. coli*. The Ni(II), Co(II) and Cu(II) chelates exhibited significant antimicrobial activities. Cu(II) chelate showed very high activity against *E. coli* and *P. vulgaris*. Generally these chelates found to have enhanced activity against the growth of Gram -positive bacteria. Though Zn(II) complex demonstrated poor inhibitory response on the Gram-negative bacteria, it showed better activities against two Gram-positive bacteria namely *E. coli* and *E. aerogenes*. The growth of *S. aureus* was significantly hindered by the Ag(I) complex of FAABA (18mm for 200 μgdisc^{-1}). This value was higher or comparable with the inhibition zones of standard antibiotics such as erythromycin, cefotaxime and cloxacillin at this concentration. This chelate achieved a saturation value at this concentration and no enhancement in the inhibitive power was noted on further rise in concentration. Similar behaviour was noted for the Fe(III) chelate for the growth of *P. vulgaris*. Silver complex showed appreciable activity against *S. aureus* (Figure 3.23) which was comparable with the activity of standard antibiotic streptomycin (Figure 3.24). The geometry of this molecule might have benefited to penetrate into the bacterial cell lines effectively. The Cd(II) chelate was generally less or inactive against the growth of Gram–negative bacteria but showed moderate growth inhibition on Gram-positive bacteria.

Fig. 3.23 Antibacterial activity of Ag(I)-FAABA complex at 200µgdisc^{-1}against *S. aureus*

Fig. 3.24 Antibacterial activity of streptomycin at 100µgdisc^{-1} against *S. aureus*

Table 3.1 Antibacterial activity of the Schiff base, 3-acetylpyridine thiosemicarbazone (APTSC) and its transition metal complexes

Compound	Diameter of zone of inhibition (mm) at different concentrations ($\mu g disc^{-1}$)																	
	S. aureus			*B. subtilis*			*B. thuringiensis*			*E. aerogenes*			*E. coli*			*P. vulgaris*		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
APTSC (LH)	2	5	6	7	8	10	2	5	7	3	7	9	5	14	15	9	15	15
$[(VO)L_2]$	0	0	0	1	2	5	9	12	15	4	5	10	6	10	13	0	0	0
$[CrLAc_2(H_2O)]_2$	2	6	11	5	8	14	6	13	14	11	17	19	8	12	15	0	0	0
$[NiLAc(H_2O)_3]$	3	5	7	2	2	4	10	11	13	9	13	15	8	13	15	4	10	13
$[CuLAc]_2$	5	8	10	3	7	10	2	6	9	3	9	12	7	12	17	8	14	14
$[CdL_2]$	5	7	8	4	7	8	11	14	17	0	0	0	12	13	15	7	10	12
$[AgL(H_2O)_2]$	3	5	7	0	0	0	9	12	13	9	11	15	0	0	0	0	0	0

Table 3.2 Antibacterial activity of the Schiff base, 3-acetylpyridine semicarbazone (APSC) and its transition metal complexes

Compound	Diameter of zone of inhibition (mm) at different concentrations (μgdisc^{-1})																	
	S. aureus			*B. subtilis*			*B. thuringiensis*			*E. aerogenes*			*E. coli*			*P. vulgaris*		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
APSC (LH)	4	7	8	1	3	4	4	5	8	6	10	11	14	16	18	11	13	13
$[(VO)L_2]$	6	8	10	7	9	10	5	7	9	5	8	10	8	11	14	4	8	10
$[CrLAc_2(H_2O)]_2$	5	6	7	7	8	10	4	6	9	5	5	6	7	10	11	8	9	10
$[NiLAc(H_2O)_3]$	6	9	9	9	10	12	1	3	6	4	7	10	5	7	14	6	11	12
$[CuLAc]_2$	6	9	10	3	6	7	2	7	10	2	9	10	11	17	21	10	12	16
$[CdL_2]$	5	11	18	9	11	14	9	11	15	2	6	7	7	12	14	2	6	8
$[AgL(H_2O)_2]$	2	6	7	5	7	8	5	5	6	2	7	9	2	7	10	0	0	0

Table 3.3 Antibacterial activity of the Schiff base, 3-acetylpyridine phenylhydrazone (APPH) and its transition metal complexes

Compound	Diameter of zone of inhibition (mm) at different concentrations (μgdisc^{-1})																	
	S. aureus			*B. subtilis*			*B. thuringiensis*			*E. aerogenes*			*E. coli*			*P. vulgaris*		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
APPH (L)	2	7	8	4	6	10	3	5	7	5	10	11	3	6	8	4	5	8
$[(VO)L_2]SO_4$	3	4	7	5	7	9	0	0	1	4	10	12	5	9	11	5	7	9
$[CrLAc_3]_2$	13	16	19	4	7	10	5	7	8	0	0	0	6	11	13	4	7	9
$[NiL_2Ac_2]$	1	1	2	2	3	4	0	0	0	2	7	9	9	11	11	3	7	10
$[CuLAc_2]$	1	2	7	1	3	5	2	3	7	4	11	13	3	8	9	4	10	12
$[CdL(NO_3)_2]$	0	0	0	3	4	6	2	3	4	3	6	7	2	4	5	2	4	6
$[AgLNO_3]$	0	0	0	3	6	9	0	0	0	7	9	11	10	15	16	8	10	11

Table 3.4 Antibacterial activity of the Schiff base, carboxyphenyl furan-2-aldehyde semicarbazone (CPFASC) and its transition metal complexes

Compound	Diameter of zone of inhibition (mm) at different concentrations ($\mu g disc^{-1}$)																	
	S. aureus			*B. subtilis*			*B. thuringiensis*			*E. aerogenes*			*E. coli*			*P. vulgaris*		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
CPFASC (LH)	3	7	10	0	0	0	8	11	17	2	6	13	12	15	19	0	0	1
$[CrL_2Ac(H_2O)]$	0	0	0	2	3	4	1	2	2	3	6	7	3	5	6	1	2	2
$[NiL_2(H_2O)_2]$	1	1	2	0	0	0	2	3	5	3	6	8	0	0	0	2	4	5
$[CuLAc(H_2O)]$	4	9	13	7	9	13	8	10	17	0	0	0	2	9	11	2	4	6
$[AgL(H_2O)_2]$	2	7	9	3	6	9	1	2	2	8	10	16	3	5	10	12	14	14

Table 3.5 Antibacterial activity of the Schiff base, carboxyphenyl thiophene-2-aldehyde semicarbazone (CPTASC) and its transition metal complexes

Compound	Diameter of zone of inhibition (mm) at different concentrations (μgdisc^{-1})																	
	S. aureus			*B. subtilis*			*B. thuringiensis*			*E. aerogenes*			*E. coli*			*P. vulgaris*		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
CPTASC (LH)	9	10	12	3	5	8	2	7	9	8	9	13	0	0	0	2	5	7
$[CrL_2Ac(H_2O)]$	7	8	8	0	0	0	1	2	2	3	5	7	0	0	0	2	4	6
$[FeL_2Ac(H_2O)]$	3	6	10	2	3	4	2	4	5	3	5	6	0	0	0	2	10	12
$[NiL_2(H_2O)_2]$	2	3	5	3	5	7	2	4	9	8	11	11	2	6	9	3	10	14
$[CuLAc(H_2O)]$	3	7	9	0	0	0	4	10	12	5	10	11	4	7	12	1	3	5
$[ZnLAc(H_2O)]$	3	6	10	3	5	12	4	5	7	1	3	7	3	12	14	4	7	14
$[CdLAc(H_2O)]$	2	4	9	1	2	5	2	8	9	3	5	9	3	8	10	2	5	6

Table 3.6 Antibacterial activity of the Schiff base, carboxyphenyl furan-2-aldehyde phenylhydrazone (CPFAPH) and its transition metal complexes

Compound	Diameter of zone of inhibition (mm) at different concentrations (μgdisc^{-1})																	
	S. aureus			*B. subtilis*			*B. thuringiensis*			*E. aerogenes*			*E. coli*			*P. vulgaris*		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
CPFAPH (L)	0	0	0	1	3	5	2	7	9	0	0	0	3	4	6	7	10	15
$[CoLAc_2(H_2O)_2]$	3	5	5	2	4	5	0	0	0	7	12	15	3	4	9	2	3	3
$[CuLAc_2(H_2O)_2]$	2	7	11	8	13	14	3	8	12	4	6	12	1	2	2	4	5	5
$[ZnLAc_2]$	0	0	0	6	7	8	1	2	2	0	0	0	1	2	2	0	0	0

Table 3.7 Antibacterial activity of the Schiff base, furan-2-aldehyde-3- aminobenzoic acid (FAABA) and its transition metal complexes

Compound	Diameter of zone of inhibition (mm) at different concentrations ($\mu g disc^{-1}$)																	
	S. aureus			*B. subtilis*			*B. thuringiensis*			*E. aerogenes*			*E. coli*			*P. vulgaris*		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
FAABA (LH)	1	2	4	2	5	7	4	7	9	5	7	9	2	5	8	2	3	5
[VOLAc]	0	0	0	2	4	7	0	0	0	7	9	11	4	9	13	0	0	0
$[CrLAc_2]_2$	0	0	0	5	7	11	10	13	13	6	7	11	0	0	0	7	17	17
$[MnLAc(H_2O)_2]$	2	6	8	6	10	15	2	9	10	2	6	9	2	5	7	1	3	6
$[FeLAc_2]_2$	0	0	0	7	9	12	4	7	11	7	8	12	0	0	0	10	18	19
$[CoLAc(H_2O)_2]$	5	8	10	3	7	10	2	6	9	2	4	8	4	6	11	4	6	7
$[NiLAc(H_2O)_2]$	5	7	9	3	6	8	2	5	9	2	7	7	0	0	0	1	2	5
[CuLAc]	2	3	9	4	9	12	5	10	11	1	2	4	4	10	15	7	11	13
[ZnLAc]	2	5	8	3	4	6	0	0	0	7	8	14	6	7	15	7	8	10
[CdLAc]	1	1	2	0	0	0	2	4	4	6	7	9	5	6	7	0	2	2
$[AgL(H_2O)]$	12	18	18	7	10	14	2	5	9	3	4	6	4	7	8	2	5	6

Table 3.8 Antibacterial activity of the standard antibiotics and the solvent DMSO

Compound	Diameter of zone of inhibition (mm) at different concentrations (μgdisc^{-1})																	
	S. aureus			*B. subtilis*			*B. thuringiensis*			*E. aerogenes*			*E. coli*			*P. vulgaris*		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
DMSO	2	2	4	2	4	5	3	3	4	3	4	4	2	3	4	0	0	0
Erythromycin	15	19	24	11	14	15	28	32	34	10	11	11	15	17	20	22	25	28
Streptomycin	22	24	26	24	28	30	20	26	30	7	9	9	15	17	20	19	27	29
Gentamicin	22	24	25	24	25	27	25	25	25	14	15	17	14	17	19	22	25	27
Ampicillin	0	0	0	0	0	0	28	32	34	0	0	0	0	0	0	0	0	0
Penicillin-G	0	0	0	0	0	0	20	21	24	0	0	0	0	0	0	0	0	0
Cefotaxime	10	14	16	12	16	18	19	27	33	15	19	22	20	28	31	10	17	18
Cloxacillin	10	12	19	20	25	27	29	37	42	15	17	19	6	10	11	21	25	28

SUMMARY

Antibacterial studies on newly synthesized heterocyclic Schiff bases and their transition metal complexes in DMSO were determined using disc diffusion method. Important pathogens like *S. aureus, B. subtilis, B. thuringiensis, E. aerogenes, E. coli* and *P. vulgaris* were taken for antimicrobial screening and standard antibiotics such as erythromycin, streptomycin, gentamicin, ampicillin, penicillin, cefotaxime, benzylpencillin and cloxacillin was selected for the comparison with the activity of newly synthesized compounds. Different concentrations of the compounds in DMSO such as 100µg $disc^{-1}$, 200µg $disc^{-1}$, 500µg $disc^{-1}$ were employed for the anti microbial investigation. The zone of inhibition was measured in millimetre and compared with the growth inhibition of standard antibiotics. The results of antibacterial studies of Schiff bases and their metal complexes are tabulated and explanation was made on the basis of the molecular structures and physical properties.

The Schiff base, 3-acetylpyridine thiosemicarbazone (APTSC) and their transition metal chelates exhibited moderate antibacterial activity. Appreciable activity was shown by the ligand on the Gram-positive bacteria like *E. coli* and *P. vulgaris* which was comparable to that of standard drugs such as benzylpenicillin, cloxacillin and gentamicin. This can be attributed to the appreciable hydrophilic nature of the Schiff base. The Cd(II) complex of this ligand was significantly active against the growth of *B. thuringiensis* which was comparable with the inhibition of gentamicin. Certain metal chelates of APSC demonstrated higher inhibition than the Schiff base. But this ligand was equally active as the standard drugs such as gentamicin,

erythromycin and streptomycin against the growth of *E. coli* and showed lesser activity than cefotaxime and ampicillin. Generally Schiff base APSC showed better activity against the growth of Gram-positive bacteria. Transition metal complexes of APPH generally displayed lower activities compared to the antimicrobial activity of the metal chelates of APSC and APTSC, which may be due to the increased size of the molecule. Cu(II), VO(II) and Ni(II) were displayed moderate antimicrobial activity against the growth of Gram-positive bacteria such as *E. coli , E. aerogenes* and *P. vulgaris* and this was slightly higher than the activity of the ligand APPH.

The Schiff base CPFASC showed good antibacterial activity against the growth of *B. thuringiensis, E. aerogenes* and *E. coli*. and was comparable to that of the standard antibiotics. Cr(III) and Ni(II) showed very poor or little antimicrobial activity which may be due to the bulky nature of the molecules. The molecule CPTASC, was appreciably active against *E. aerogenes* and was inactive against the growth of *E. coli*. The copper complex was significantly obscured the growth of the Gram–negative bacterium *B. thuringiensis* and Gram-positive bacterium *E. coli*. CPFAPH ligand displayed low inhibition zones against the growth of all microbes except *P. vulgaris*. This may be due to the low chelating power and large size of the Schiff base. The antibacterial screening on the Schiff base FAABA and its metal chelates revealed that complexes were considerably active than the Schiff base against the growth of all microbes. A marked activity was shown by Cr(III) and Fe(III) complexes against the growth of *P. vulgaris* at a concentration of 500µgdisc^{-1}. These values are very much comparable with the zone of inhibitions of the standard antibiotics such as cefotaxime and erythromycin.

To sum up, the general antibacterial response of the metal chelates of the heterocyclic chelates were found to be higher than that of Schiff bases. Some of them showed moderate activities, while few displayed great inhibition zones, comparable to that of standard antibiotics. At the same time, very few complexes were totally inactive against the growth of microbes.

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