In coordination chemistry, one of the most fascinating areas is the interaction of metal ions with biological molecules. All metals will form coordination compounds. The structure of metal complexes consists of a central metal atom, bonded to a surrounding array of molecules or anions [1]. The role of coordination compounds in nature is very important. Most of the metal ions are essential to maintain human homeostasis and also play crucial roles in many biological processes by acting as cofactors in the proteins function thereby resulting in the stabilization, regulation and completion courses of cellular functions [2-5]. Chlorophyll, hemoglobin and vitamin B12 are some examples for such metal complexes. Inorganic medicinal chemistry and metal-based complexes seem to be one of the best therapeutic approaches to treat and diagnose the diseases [6-8]. On reviewing the literature, it is confirmed that metal complex exhibits greater biological property than that of free organic compounds [9-11]. Medici et al. reviewed historical development and amazing broad uses of metals, and the importance of their complexes in the medical area [12].
Compared to purely organic molecules, metal complexes have several advantages as a result of their varied reactivity pattern, structural diversity, and unique photo and electrochemical properties. To exploit these advantages, it is crucial to select good performing chelators with coordinating properties suitable for the proper stabilization of a given metal core [13-15]. Inorganic compounds mainly transition metals have played an important role in the development of new metal based drugs [16]. Transition metals exhibit various coordination geometry, oxidation states, spectral and magnetic properties and they can interact with large number of negatively charged molecules. This property of transition metals led to the recent development of drugs which are based on metals and are regarded to be potential candidates for pharmacological and therapeutic applications [17-20].

1.1 Schiff bases

Schiff bases are versatile ligands having imine or azomethine (–C=N–) functional group. They were first described by Hugo Schiff, German Chemist in 1864 and hence they are named so [21]. Schiff bases are the backbone of large number of organic compounds and have enormous applications in many fields including analytical, biological, and inorganic chemistry [22]. Schiff bases are well known for their wide range of applications and are useful intermediates in organic synthesis [23]. These compounds have intrinsic biological activities including anticancer [24-26], anti-inflammatory [27-28], antitubercular [29-30], antioxidant [31-32], antibacterial [33-35], analgesic [36-37], antifungal and antifertility [38], herbicidal [39-40], anticonvulsant [41], anthelmintic [42] and antiproliferative [43]. Moreover, Schiff bases also exhibit fluorescence [44], photoluminescence [45], a potentiometric cation caring [46] and aggregation [47] properties.
1.1.1 Structure and properties of Schiff base

The functional group of Schiff base contain a carbon-nitrogen double bond with nitrogen atom connected to an alkyl, aryl, cyclo alkyl or heterocyclic groups which may be variously substituted, other than with hydrogen [48]. Scheme 1 shows the general formula of azomethine group which is the most common structural feature of Schiff bases.

![Scheme 1. General formula of Schiff base having azomethine linkage](image)

Schiff bases are weak bases and are easily hydrolysed by dilute mineral acids, but not by aqueous alkali. They also form insoluble salts with strong acids through coordination of the electrons on nitrogen atom of azomethine group [49]. Most of the Schiff bases are stable in alkaline solutions. Aromatic aldehydes especially with an effective conjugation system, form stable Schiff bases, where as those from aliphatic aldehydes are found to be less stable. Aliphatic Schiff bases have a tendency to polymerize and are difficult to isolate [50]. Aldehydes can form Schiff base ligands more readily than ketones. This is because the reaction centres of aldehydes are sterically less hindered than ketones and the additional carbon of ketone contributes more electron density to the azomethine carbon making them less electrophilic compared to aldehydes [51].
The classical synthesis of Schiff bases reported by Schiff involves the condensation of primary amines and active carbonyl groups under azeotropic distillation [52]. The water thus formed in the system is removed using molecular sieves [53]. In the 1990s, an in situ method was developed for water elimination. Dehydrating solvents such as tetramethyl orthosilicate or trimethyl orthoformate were used for this purpose [54-55]. In 2004, Chakraborti et al. reported that the efficiency of these methods is dependent on the use of highly electrophilic carbonyl compounds and strongly nucleophilic amines. They also reported the use of substances that function as Lewis acids or Bronsted-Lowry to activate the carbonyl group of aldehydes (or ketones) catalyze the nucleophilic attack by amines, and dehydrate the system, eliminating water as the final step [56]. In the past thirteen years, a number of innovations and new techniques have been reported including solvent-free/clay/microwave irradiation [57-60]. Scheme 2 shows the general method for synthesis of Schiff bases.

**Scheme 2: Synthesis of Schiff base**

Mechanistically, two steps are involved in the formation of Schiff base (Scheme 3). In the first step, the azomethine nitrogen acts as a nucleophile, attacking the electrophilic carbonyl carbon of aldehydes or ketones. In the second step the nitrogen is deprotonated, and the electrons from this N-H bond push the oxygen off the carbon, leaving a compound with a C=N bond (an imine) and a water molecule is displaced [61-63].
Scheme 3: Mechanism of Schiff base (imine) formation

Several studies revealed that the chemical and biological importance of Schiff base is due to the presence of a lone pair of electrons in sp$^2$ hybridized orbital of nitrogen atom of the azomethine group. Ease of preparation, synthetic flexibility and the special characteristic property of C=N group makes Schiff base an excellent chelating agent [64]. The Schiff bases with functional group like (–OH), (–SH), (–NO$_2$) are considered as useful chelating agents. Schiff bases are insoluble in water and some of them are readily hydrolyzed back to amine and aldehyde [65]. Schiff bases can act as bidentate, tridentate, tetradentate or polydentate ligands (Figure 1.1) [66-68].

Figure 1.1 Examples for bidendate and tridendate Schiff base ligands.

Schiff base macrocycles formed by the self condensation reaction of appropriate formyl- or keto- and primary amine precursors (Figure 1.2) have many applications in the field of macrocycles and supramolecular chemistry [69].
Figure 1.2 Example for Schiff base macrocycle.

1.2 Importance of heterocyclic Schiff’s base transition metal complexes

Schiff bases derived from heterocyclic scaffolds mainly sulfur, nitrogen and oxygen atom have great significance in many areas like biological, clinical, medicinal, analytical and pharmacological field [70]. Schiff base transition metal complexes obtained from heterocyclic molecules have been known to possess a wide range of biological and pharmacological activities. In recent years, they have received significant interest from many researchers in the area of drug research and development owing to their broad bioactivities such as antibacterial, antifungal, anti-inflammatory, anticonvulsant, antiviral and anticancer activities [71]. Heterocyclic Schiff-base metal complexes are
considered to be among the most important stereochemical models in main group and transition metal coordination chemistry due to their ease of preparation and structural variety [72].

Schiff base ligands are able to coordinate many metals and to stabilize them in different oxidation states. Multidentate Schiff bases ligands have been widely used, as they can form highly stable coordination compounds on coordination with metal ions [73]. Schiff base transition metal complexes exhibit well-defined coordination geometries and are attractive moieties for reversible recognition of nucleic acids research. Besides these, they often show distinct photophysical or electrochemical properties, thereby increasing the functionality of the binding agent. These properties of Schiff base transition metal complexes stimulated large interest for their noteworthy contributions to single molecule-based magnetism, material science [74], catalysis of many reactions like hydroformylation, carbonylation, reduction, oxidation, epoxidation [75] and their industrial applications [76] in the past two decades. Recently metal complexes with stable $d^{10}$ electronic configuration have received a lot of attention in the field of bioinorganic and environmental chemistry [77].

The treatment of Schiff base ligands with metal salts gives metal complexes of Schiff base under suitable experimental conditions. Cozzi reported different synthetic routes (Figure1.3) for the preparation of metal complexes [78]. In route 1, metal complexes are prepared by refluxing Schiff bases with the corresponding metal acetate. Route 2 employs synthesis of metal alkoxides (M(OR)$_n$). The transition metal (M = Ti, Zr) bearing alkoxides can be easily handled and is commercially available. It is
difficult to use other alkoxide derivatives, especially in the case of highly moisture sensitive lanthanide derivatives. Metal alkyl Schiff base complexes are synthesized through route 3. The route 4 represents a schematic outline for the preparation of salen-type metal complexes. It is a two-step reaction based on deprotonation of the Schiff bases and then upon reaction with halides of metal. For deprotonation in coordinating solvents, sodium hydride NaH or potassium hydride KH is used and excess of hydrides can be eliminated by filtration. Deprotonation is a rapid step and refluxing of hot reaction mixture does not cause any decomposition. Metal amides \( \text{M(NMe}_2\text{)}_4 \) (M = Ti, Zr) are also employed as precursors for the synthesis of Schiff base metal complexes (Route 5).

**Figure 1.3** Different synthetic routes for the preparation of metal complexes.
1.2.1 Applications of Schiff base and their transition metal complexes

Schiff bases are used as starting material in the synthesis of industrial and biological compounds such as lactams, used in the construction of poly vinyl chloride powder (PVC) based membrane selective sensors and also as ionophore in metal ion-selective electrodes [79-81]. Schiff bases contain phenyl or substituted phenyl group are sometimes called azo dyes. Many Schiff base transition metal complexes from this kind have been synthesized with metals such as, aluminum(III), iron(III), cobalt(II), nickel(II) and copper(II) complexes [82]. Schiff bases have been used in the preparation of a number of industrial and biologically active compounds like formazans, 4-thiazolidinines, benzoxazines, via ring closure, cyclo addition, and replacement reactions [83]. Schiff bases are very important compounds because of their wide spectrum of biological activities. Some examples for bioactive Schiff bases are shown in Figure1.4 [84].

![Figure 1.4 Examples of bioactive Schiff bases [Ref.84]](image-url)
Many transition metal Schiff base complexes exhibit high catalytic activity and play a significant role in various reactions so to enhance their yield and product selectivity. The convenient route of preparation and thermal stability of Schiff base ligands have contributed significantly as metal complexes for their possible applications in catalysis [85].

The catalytic activity of metal complexes has been reported in various reactions as given below [86-90].

- Polymerization reaction
- Reduction of thionyl chloride
- Oxidation of organic compounds
- Reduction reaction of ketones
- Aldol reaction
- Epoxidation of alkenes
- Henry reaction

1.3 Biological activities of Schiff bases and their metal complexes

Schiff bases are important compounds in medicinal and pharmaceutical fields because of their wide spectrum of biological activities. The activity of these compounds is usually increased upon complex formation with transition metals.

1.3.1 Schiff base as antioxidant

Schiff base and their metal complexes are identified as having high capacity in scavenging free radicals [91-95]. Free radicals are causative agents of several oxidative damages such as cancer, liver cirrhosis, diabetes,
Atherosclerosis and ageing [96]. Antioxidants are compounds which slow down or defense against free radical damage. The need of antioxidants become even more critical with increased exposure to free radicals pollution caused by drugs, cigarette smoke, stress, illness and even exercise can increase free radical production. Mruthyunjayaswamy et al. evaluated the antioxidant capacity of a new Schiff base ligand N-(4-phenylthiazol-2-yl)-2-(thiophen-2-ylmethylene) hydrazinecarboxamide and its Cu(II), Co(II), Ni(II) and Zn(II) complexes by DPPH method. The Schiff base ligand and its Cu(II), Co(II) complexes have exhibited a good antioxidant activity, whereas Ni(II) and Zn(II) complexes have shown moderate activity. The scavenging activity is concentration dependent [97].

Antioxidant property of (3E)-3-[(2-((E)-[1(2,4dihydroxyphenyl) ethylidene]amino)ethyl]imino]-1-phenylbutan-1-one (DEPH2) and its metal [Co(II), Ni(II), Zn(II), Cu(II)] complexes [Figure 1.5] were screened by Ikechukwu et al. using DPPH and ABTS method. Compared to free Schiff base DEPH2, metal complexes showed higher antioxidant activity. The increased antioxidant activity of the metal complexes can be attributed to the electron withdrawing effect of the metal ions which facilitates the release of hydrogen to reduce the DPPH radical. The order of radical scavenging activity is Vitamin C > Cu (DEP) > Ni(DEP) = Rutin > Co(DEP) > Zn(DEP) > Schiff base (DEPH2) and that of the ABTS radical is: BHT > DEPH2 > Cu(DEP) > Zn(DEP) > Rutin > Ni(DEP) > Co(DEP). All the compounds exhibited moderate to higher % inhibition scavenging activity than the standard BHT and rutin at the lowest concentration (100 μg/mL). Cu(DEP) possessing the highest potency (IC₅₀ = 2.11 ± 1.69 μM). Schiff base and their metal complexes can be used as therapeutic agents for the treatment of pathological diseases and conditions caused as a result of excessive radicals or stress [98].
1.3.2 Antimicrobial activity of Schiff base and its metal complexes

Antimicrobial and antifungal activities of various Schiff bases and their transition metal complexes have been reported [99-102]. Thamarai Selvi et al. synthesized a new heterocyclic Schiff base ligands and their complexes from 4-aminoantipyrine, thiophene-2-carboxaldehyde and 2-aminobenzoic acid. Antibacterial and antifungal activity evaluation of the metal complexes against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Candida albicans* exhibited that the complexes have potent biological activity than the ligands [103] (Figure 1.6).

El-Sonbati *et al.* reported synthesis, structure characterization, antimicrobial, antioxidant and antitumor activity of 3-[(2-hydroxy-3-methoxy-
benzylidene)-hydrazono]-1,3-dihydro-indol-2-one and its Cu(II), Co(II), Ni(II) and Cd(II) metal complexes. The ligand and its metal complexes showed antimicrobial activity against bacterial species, gram positive (Staphylococcus aureus), gram negative (Escherichia coli) bacteria and yeast (Candida albicans). The ligand exhibited higher activity than the complexes. Molecular docking studies was used to predict the binding between ligand and the receptors of crystal structure of E. coli (3T88), crystal structure of S. aureus (3q8u) and crystal structure of C. albicans [104].

3.3 Analgesic, Anti-inflammatory activity

Chinnasamy et al. reported the synthesis and analgesic activity of novel Schiff base Isatin 3-(4-(4-Hydroxy-3-methoxybenzylideneamino) phenylamino) indoline-2-one [105]. Analgesic and anti-inflammatory activity of N-(acridin-9-yl)-4 (benzo[d]imidazol/oxazol-2-yl) benzamides Schiff base (Figure 1.7) was reported by Sondhi et al. [106]. Schiff bases derived from 2-[(2,6-dichloroanilino) phenyl] acetic acid (Diclofenac acid) was synthesized and studied for their anti-inflammatory, analgesic and ulcerogenic activities [107].

Figure 1.7 N-(acridin-9-yl)-4 (benzo[d]imidazol/oxazol-2-yl) benzamides Schiff bases [Ref.106].
1.3.4 Anticancer activity

Metal complexes of Schiff bases with heterocyclic compounds also find applications as potential anticancer drugs, due to the presence of multifunctional groups [108-111]. Zinc complex of Schiff base synthesised from 2-amino-4-phenyl-5-methyl thiazole (Figure 1.8) shows potent anticancer activity when studied against human tumour cells such as breast cancer MCF-7, liver cancer HepG2, lung carcinoma A549 and colorectal cancer HCT116 [112]. A series of sulfapyridine-polyhydroxyalkylidene (or arylidene)-imino derivatives have been prepared and reported for antitumor activity [113].

Figure 1.8 Example for Schiff base metal complexes showing anticancer activity [Ref. 112].

Synthesis and anticancer activity (on MDA-MB-231 breast cancer cells) of novel ternary Cu(II) complex (shown in Figure 1.9) with Schiff base derived from 2-amino-4-fluorobenzoic acid and salicylaldehyde have been reported by Xin Li and coworkers. They identified that the tumor proteasome is a target of the complex. They have shown that the inhibition of the proteasomal activity (especially, chymotrypsinlike activity) by the complex can strongly induce apoptosis in the cultured breast cancer MDA-MB-231 cells. Their study reinforces the idea that proteasome targeted copper complexes have great potential to be developed into novel anticancer drugs [114].
1.3.5 Antiviral activity

Schiff bases derived from salicylaldehyde and 1-amino-3-hydroxyguanidine tosylate can act as antiviral agents. Compound shown in (Figure 1.10) is very effective against mouse hepatitis virus [115]. A series of 3-(benzylideneamino)-2-phenylquinazoline-4(3H)-one was synthesized and evaluated for their cytotoxicity and antiviral activity [116]. Compounds having –OH group in 2nd position showed better antiviral activity. Thiazolines and azetidinones synthesized by the reaction of Schiff base with thioglycolic acid and chloral acetyl chloride were evaluated for antibacterial and antiviral (against HIV-I) potential. All the compounds were found to be good HIV inhibitors except those with electro withdrawing group [117].
Kumar et al. reported antiviral activity of new series of 3-(benzyldieneamino)-2-phenylquinazoline-4(3H)-ones. These compounds were prepared through Schiff base formation of 3-amino-2-phenylquinazoline-4(3H)-one with various substituted carbonyl compounds. Cytotoxicity and antiviral activity were screened against a series of virus including herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), vaccinia virus, vesicular stomatitis virus, herpes simplex virus-1 TK-KOS ACVr, Sindbis virus, para influenza-3 virus, reovirus-1, Punta Toro virus, Coxsackie virus B4, feline corona virus (FIPV), feline herpes virus, respiratory syncytial virus, influenza A H1N1 subtype, influenza A H3N2 subtype, and influenza B virus [118].

### 1.3.6 Antimalarial Activity

Harpstrite et al. reported the mixed ligand complexes of Ni(II), Cu(II) and Fe(III) with Schiff-base-phenol and naphthalene-amine as antimalarial agents [119]. In vitro activity of Schiff base-functionalised 5-nitroisooquinolines prepared by reacting 1-formyl-5-nitroisooquinoline with amines were investigated against an ACC Niger chloroquine resistant P. falciparum strain. Compound shown in Figure 1.11 was the most effective antimalarial agent.

**Figure 1.10** Example for Schiff base showing antiviral activity [Ref. 115].
1.3.7 Anticonvulsant activity

Ragavendran et al. synthesized 4-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the mammalian brain. GABA hydrazones were synthesized and evaluated for their anticonvulsant properties in different animal models [120]. Anti convulsing activity of new erindolylthiadiazoles and their thiazolidionones and formazans have been reported by Srivastava et al. [121]. A series of Schiff bases of phthalimide, 4-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-N-(substitutedphenyl) methylene/ethylidene benzohydrazide, was synthesized and evaluated for anticonvulsant and neurotoxic activities. All the compounds were found to be active and less toxic than phenytoin which was employed as a standard drug [122]. Schiff base of 3-aryl-4(3H)-quinazolinones-2-carboxaldehydes and thiosemicarbazone derivatives showed anticonvulsant potential due to thiosemicarbazone side chain at position ending with a free amino group and fluorine atom.

1.4 Antioxidant and its importance

1.4.1 Free radicals

A free radical may defined as a molecule or molecular fragments containing one or more unpaired electrons in its outermost atomic or molecular orbital and are capable of independent existence [123]. Free radicals are formed by the homolytic cleavage of a chemical bond. Once
formed these highly reactive radicals initiates a rapid destructive chain reaction [124]. The most commonly formed free radicals in biological system are superoxide (O$_2^-$), hydroxyl (OH$^-$), peroxy (RO$_2^-$), hydroperoxyl (HO$_2^-$), alkoxyl (RO$^-$), peroxy (ROO$^-$), nitric oxide (NO$^-$), nitrogen dioxide (NO$_2^-$) and lipid peroxyl (LOO$^-$) [125-126]. Three important steps in free radical reaction are 1. Initiation step: Formation of radicals, 2. Propagation step: In this step, required free radical is regenerated repeatedly as a result of chain reaction, which would take the reaction to completion, 3. Termination step: Destruction of radicals [127].

Generation and sources of free radicals - Free radicals can be formed either endogenously or exogenously. Important sources of free radicals are [128]

Endogenous sources of free radicals
- Oxidative metabolic transformation
- Mitochondrial respiratory chain
- Oxygen burst (respiratory burst) during phagocytosis
- Eicosanoid synthesis
- Enzymatic reactions (oxygenases, oxidases)

Exogenous sources of free radicals
- Ionizing radiation
- Ultraviolet radiation, X-rays, gamma rays and microwave radiation
- Chemicals, tobacco smoke, etc.
- Oxygen free radicals in the atmosphere considered as pollutants

Free radical production occurs continuously in all cells as a part of cellular function. Excessive amounts of ROS may be harmful because they can initiate bimolecular oxidations which lead to cell injury and death, and
create oxidative damage or oxidative stress (refers to the situation of serious imbalance between production of reactive species and antioxidant defense) to biomolecules. Their presence in the biological system is very harmful and these free radical species are responsible for the damage of biomolecules such as nucleic acid, proteins, lipids, DNA and carbohydrates and this may cause many diseases such as cancer, atherosclerosis, ageing, hair loss, inflammation, immunosupression, diabetes and neurodegenerated disorders (such as Alzheimer’s and Parkinson’s diseases) (Figure 1.12)[129-130].

**Figure 1.12** Sources and diseases of free radical

1.4.2 Antioxidants

Antioxidants are compounds which slow down or prevent the oxidation of other target molecules. They mop up free radicals and prevent them from causing cell damage. The human body uses an antioxidant
defense system to neutralize the excessive levels of reactive oxygen species. Major antioxidant defense enzymes are superoxide dismutase, catalase and glutathion peroxidase [Figure 1.13].

**Antioxidant Defense**

- **Superoxide dismutases -** It catalyze the rapid dismutation of superoxide radicals to hydrogen peroxide and oxygen
  \[ \text{SOD} \quad \text{O}_2^{-} + \text{O}_2^{-} + 2\text{H}^{+} \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]

- **Catalase -** It reduces peroxide to water
  \[ \text{H}_2\text{O}_2 + \text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \]

- **Glutathione peroxidase**
  \[ \text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow \text{H}_2\text{O} + \text{GSSG} \]

**Figure 1.13** Antioxidant defense

Human antioxidant system is divided into enzymatic and non-enzymatic antioxidants. They are further classified into different groups as shown in the Figure 1.14. Non enzymatic molecules include glutathione, tocopherol (vitamin E), vitamin C, β-carotene, and selenium [131-132]. Kinetically antioxidants are classified into different categories:

- Antioxidants that break chains by reacting with peroxyl radicals having weak O-H or N-H bonds: phenol, naphthol, hydroquinone, aromatic amines and amino phenols.
Biological Importance of Schiff Bases and Its Transition Metal Complexes

- Antioxidants that break chains by reacting with alkyl radicals: quinones, nitrones, iminoquinones.
- Hydroperoxide decomposing antioxidants: sulphide, phosphide, thiophosphate.
- Metal deactivating antioxidants: diamines, hydroxyl acids and bifunctional compounds.
- Cyclic chain termination by antioxidants: aromatic amines, nitroxy radical, variable valence metal compounds.
- Synergism of action of several antioxidants: phenol sulphide in which phenolic group reacts with peroxyl radical and sulphide group with hydro peroxide [133-134].

Figure 1.14 Classification of antioxidant.
The treatment with antioxidants is potentially a way to overcome the oxidative stress or oxidative damage. Antioxidant molecule can react with single free radicals and are capable of neutralizing free radicals by donating one of their own electrons (Figure 1.15), ending the carbon-stealing reaction [135]. Because of this, there is a great interest in the discovery of natural and synthetic antioxidants that can serve as protective agents against diseases caused by free radicals.

![Antioxidants prevent free radical damage](image)

**Figure 1.15** Prevention of free radical damage by antioxidant

### 1.4.3 Natural and synthetic antioxidants

Based on the origin antioxidants are classified into natural, synthetic and nature-identical antioxidants. Natural antioxidants are synthesized by microorganisms, fungi, animals and plants. Antioxidants identical to natural antioxidants but synthesized in the industry are called nature identical antioxidants. Antioxidants synthesized or biosynthesized by human are called synthetic antioxidants. Synthetic antioxidants have been developed for the stabilization of bulk fats and oils or foods rich in lipids. They are substantially more efficient than α-tocopherol and other natural antioxidants.
which are usually less liposoluble. Natural antioxidants are not pure substance as their active fraction is much lower than the actual addition while synthetic antioxidants are nearly 100% pure. BHT (butylated hydroxyl toluene) and BHA (butylated hydroxyl anisole) are the most widely used synthetic antioxidants [136-138]. Examples for some synthetic antioxidants are given below.

**Figure 1.16** Synthetic antioxidants.

### 1.4.4 Phenolic antioxidants

Phenolic compounds are known as powerful chain breaking antioxidants. Radical scavenging activities of phenolic antioxidants are highly influenced by their chemical structure. Bond dissociation energy (BDE) of OH bond is one of the factors for the highest radical scavenging activity of phenolic antioxidants. Lower the BDE of OH bond, the more
active the antioxidant. Presence of intramolecular hydrogen bonding also stabilizes the radical and hence increases the radical scavenging activity [139-143]. Phenol acts as antioxidant by breaking the free radical chain reaction through the donation of its hydrogen atom to free radicals. Phenoxy radical thus formed can be reduced to its parent compound by enzymatic or non-enzymatic reaction. The phenoxy radical formed is stabilized by the delocalization of unpaired electron on the aromatic ring. Figure1.17 depicts the conjugated resonance stabilization of phenoxy radicals [144-146].

Figure 1.17 Resonance stabilization of phenoxy radical.

Antioxidant potential of phenolic compounds depends on the different substituent groups present and the extent of structure conjugation. Electron donating group enhances radical scavenging activity and electron withdrawing group reduces it. Structurally phenols may be divided into phenolic acid and flavonoids. Phenolic antioxidant, such as caffeic acid and its analogues showed antiviral and anti inflammatory property [147]. Resveratrol, a phenolic antioxidant is known for its anticancer and heart protecting effect [148]. Olive oil phenols inhibit human low density lipoprotein oxidation [149]. Some phenolic antioxidants are shown in Figure1.18.
1.5 DNA interactions

DNA, deoxyribonucleic acid is the critical therapeutic target of most antitumor drugs as well as many antiviral and antibacterial agents. In humans and almost all other organisms, the primary genetic material is double strand DNA. The important function of DNA is to store and transmit genetic information. To accomplish this function DNA must have two properties. Firstly, it must be chemically stable so as to reduce the possibility of damage. Secondly, DNA must also be capable of copying the information it contains. The two-stranded structure of DNA gives it both of these properties. Double helix structure of DNA molecule is proposed by Watson and Crick in 1953 [150].
DNA is made up of subunits called nucleotides. Each nucleotide is made up of 5-carbon sugar (deoxyribose), a phosphate and nitrogen containing heterocyclic base. Adenine, guanine, (purine) cytosine and thymine (pyrimidine) are the bases found in a DNA molecule [Figure 1.19]. The deoxyribose sugar of the DNA backbone has five carbons and three oxygens. The hydroxyl groups on the 5'- and 3'- carbons link to the phosphate groups to form the DNA backbone. In DNA double helix structure, the nucleotides connect the two strands through hydrogen bonds [Figure 1.20]. The nucleotide sequence contains the information found in DNA. As each nucleotide has a unique complimentary nucleotide, each strand contains all the information required to synthesize a new DNA molecule. The double stranded structure also makes the molecule more stable.

**Figure 1.19** DNA bases.
The three different forms of DNA double helix are A, B and Z. (Figure 1.21). These conformations are distinguished by the handedness of the helix, distance between consecutive bases, their pitch (the distance between base and the base obtained after a full 360° turn) and the number of nucleotides within one pitch [152]. A and B-DNA forms are right-handed while Z-DNA form is left-handed. DNA adopts mainly the B-conformation, with both forming right-handed helices. However, DNA double strands are able to take up the A-conformation in some protein-DNA complexes and under dehydrated conditions. Both B-helix and A-form helix have two grooves, the major and the minor grooves [153]. The major and minor groove differs in shape, size, hydration, electrostatic potential and position of hydrogen bonding sites [154]. In B form, two grooves differ in their
width but are equally deep. In contrast, the A-form helix possesses a small deep major groove, which is accessible only to water and metal ions. The term Z-DNA stems from the observed zig-zag conformation of the phosphate backbone of a left-handed helix taken up by alternating purine-pyrimidine DNA sequences (GC repeats) under high salt conditions. The distance between consecutive base-pairs and the degree of rotation of the helix per residue results from the changes in the sugar pucker from a C3'-endo to C2'-endo. Base pair sequence, relative humidity, and the presence of DNA-binding compounds influence the sugar puckering and conformation of DNA [155-156].

![A, B and Z. form of DNA double helix.](image)

**Figure 1.21** A, B and Z. form of DNA double helix.

### 1.5.1 Drug–DNA interactions

Transcription and replication are the major determinant of the gene expression that allows cells to proliferate, differentiate and maintain proper homeostasis. This also helps in the smooth functioning of all body processes [158]. Transcription machinery regulation is one of the ways to control gene expression. This has been achieved either at the transcription initiation stage
or at the elongation stage. DNA transcription or replication occurs only when DNA receives a signal in the form of a regulatory protein binding to a particular region of the DNA. DNA - small molecules interaction plays a significant role towards inhibition, modulation and activation of the transcription machinery. Thus synthetic/natural small molecule can act as therapeutic agents when activation or inhibition of DNA function is required to cure or control a disease [159].

Small molecules bind to double-stranded DNA either covalently or non-covalently (intercalation and groove binding, Figure 1.22).

![Figure 1.22 Modes of Binding in DNA.](image)

Covalent binding is a common method of DNA interaction for anticancer drugs [160]. A major advantage of covalent binders is the high binding strength. Three modes of covalent binding to DNA are: Inter and intra-strand cross linking, replacement of nitrogenous bases, and alkylation of nitrogenous bases. Cisplatin is the most clinically successful DNA covalent binder that and makes an intra/interstrand cross-link through the
chloro groups with the nitrogen on the DNA bases (Figure 1.23) although it reacts with a diverse range of other biomolecules [161]. Such binding results in the unwinding of the double helix and subsequent inhibition of transcription, thereby resulting in subsequent cell death [162].

**Figure 1.23 Inter and intra-strand cross linking of cisplatin.**

### 1.5.2 Non covalent binding

Intercalation is defined as the insertion of a positively charged planar heterocyclic aromatic molecule between two adjacent base pairs of DNA double helix [163]. Intercalation stabilizes, lengthens, stiffens and unwinds the DNA double helix [164]. This effect dependent upon the “depth of insertion”[165]. Intercalation is reversible, and is stabilised by a combination of electrostatic, hydrogen bonding, entropic, van der waals and hydrophobic interactions. The two major types of intercalation-binding modes are: (1) classical intercalation and (2) threading intercalation. Binding by the classical mode is typified by the much-studied DNA stain
ethidium bromide and the antimalarial quinacrine [166]. Molecules that bind to double-stranded DNA (DNA) by intercalative mode have been significantly used as drugs. Intercalators can cause more significant distortion of the native conformation of DNA, indicating that intercalators produce strong structural perturbations in DNA.

Groove binding – Groove binders are another important class of small molecules with crescent shaped that bind to DNA and play major role in drug development [167]. In this type, molecules can bind to both the major and minor groove of DNA. These grooves are vastly different in size, shape and properties. The major groove is much wider than the minor groove and is the site for binding of many DNA interacting proteins [168-169]. Minor-groove binding usually involves greater binding affinity and higher sequence specificity than that of intercalator binding. Minor-groove binding has been demonstrated for neutral, mono-charged and multicharged ligands [170]. Netropsin is an example for minor groove binder. Major groove of DNA is an enthalpy-driven process, while minor groove interactions are dominated by entropic effects. Considerably small number of molecules is reported to bind to major groove. The reason for this probably lies in the fact that nitrogen and oxygen atoms in base pairs of wide and deep major groove are oriented towards the axis of the helix, making them accessible for proteins. Some antitumor agents with acridine carboxamide skeleton were reported as major groove binders [171]. Groove binding is based upon intermolecular interactions such as electrostatic and van der Waals attractions [172].

Several techniques have been employed to study the binding of small molecules to DNA including, UV-Visible spectroscopy, fluorescence spectroscopy, voltammetry, circular dichroism (CD)) and linear dichroism (LD).
DNA–small molecule interaction can be detected by UV-Vis absorption spectroscopy by measuring the changes in the absorption properties of the drug molecules or the DNA molecules. The UV–Vis absorption spectrum of DNA exhibits a broad band (200–350 nm) in the UV region with a maximum at 260 nm. This is a consequence of the chromophoric groups in pyrimidine and purine moieties responsible for the electronic transitions. Slight changes in the absorption maximum and the molar absorptivity can occur with the variations in pH or ionic strength of the media. DNA–drug interactions can be studied by comparison of UV–Vis absorption spectra of the free drug molecule and DNA–drug complexes, which are usually different. The binding with DNA through intercalation usually results in hypochromism and hypsochromism (blue shift) or bathochromism (red shift) [173-174].

Fluorescence spectroscopy is probably one of the most commonly used techniques to study interactions between small molecules (ligand and complexes) and DNA. The advantages of molecular fluorescence over other techniques are its high sensitivity, large linear concentration range and selectivity. The most intense and the most useful fluorescence are found in compounds containing aromatic functional groups with low-energy $\pi\rightarrow\pi$ transition levels. Compounds containing aliphatic and alicyclic carbonyl structures or highly conjugated double-bond structures may also show fluorescence, but the number of these transitions is small compared with those in aromatic systems [175].

CD and LD spectroscopies are useful techniques for the assessment of non-covalent drug-DNA interactions, which affect the electronic structure of the molecules. LD use polarized light and provides structural information in terms of the relative orientation between the bound drug molecule and the
DNA molecular long axis. LD spectroscopy involves measuring the difference in absorption of two linear polarizations of light, which usually are parallel and perpendicular to a sample orientation direction. In contrast to LD which depends only on the electric field vector, CD depends on both electric and magnetic interactions and provides additional structural information of DNA [176-177]. Electrochemical methods are another important techniques used to the study of metallointeraction and coordination of transition metal complexes to DNA [178].

1.6 Enzymes

Enzymes are very effective biological catalysts that accelerate or catalyze almost all metabolic reactions in living organisms. In other words, they either start chemical reactions or enhance the rate of reaction between biomolecules. Enzymes have two portions, a protein portion called the apoenzyme and a nonprotein portion, either a cofactor (inorganic) or coenzyme (organic). The proteins in enzymes are usually globular. The inter and intramolecular bonds holding proteins in their secondary and tertiary structures are disrupted by temperature and pH change. This causes structural changes indicating that the catalytic activity of an enzyme is pH and temperature sensitive. Enzymes are present in every cell in both plants and animals; and are responsible for regulating the biochemical reactions necessary to sustain life. Enzymes are highly specific, both in the substrate they affect, and in the reactions they catalyze. Enzymes are biodegradeable and work at low temperature and moderate pH. These are more stable catalysts than other chemicals or biological molecules, making them the most environment friendly solution for industrial manufacturing. Enzymes are classified according to the reactions they catalyze.
Six main groups of enzymes are hydrolases, ligases, isomerases, oxidoreductases, lyases, and transferases. Hydrolases, oxidoreductases and transferases are the most numerous forms of enzymes, while the other enzymes are less common [179].

- Hydrolases break down carbohydrates, proteins, and fats such as during the process of digestion. This is achieved by adding a water molecule, thus the name hydrolases.
- By using an energy source, the ligases catalyze the formation of a bond between two substrate molecules.
- Isomerases catalyze the rearrangement of chemical groups within the same molecule.
- Oxidoreductases make oxidation-reduction.
- Lyases catalyze the formation of double bonds between atoms by adding or subtracting chemical groups.
- Transferases transfer chemical groups from one molecule to another.

1.6.1 Factors affecting catalytic activity of enzymes

- Temperature - As the temperature increases, reacting molecules gain more kinetic energy. This enhances the chances of a successful collision and so the rate of the reaction increases.

- pH - Each enzyme works within quite a small pH range. There is a pH at which enzyme activity is maximum (the optimal pH). pH change can make and break intra- and intermolecular bonds, changing the shape of the enzyme and, therefore, its effectiveness.
Concentration of enzyme and substrate - concentrations of enzyme and substrate also affect the rate of an enzyme-catalysed reaction. As the concentration of either is increased the rate of reaction increases. For a given enzyme concentration, the rate of reaction increases with increasing substrate concentration up to a point, above which any further increase in substrate concentration produces no significant change in reaction rate. This is because the active sites of the enzyme molecules at any given point are virtually saturated with substrate.

Enzymes have a small region (typically only about 20 amino acids), known as the active site, that has the right shape and functional groups to bind to one of the reacting molecules. The reacting molecule that binds to the enzyme is called the substrate. The enzyme and substrate form a reaction intermediate (enzyme-substrate complex). This binding changes the distribution of electrons in the chemical bonds of the substrate(s), lowering the activation energy of the reaction and enabling generation of the final product [180].

Enzyme controlled reactions are very fast compared to the reactions without any enzymes. In a chemical reaction large amount of heat energy is required to make the reaction occur at a faster rate and this is called activation energy. Enzymes are very efficient catalysts for biochemical
reactions. They speed up reactions by providing an alternative reaction pathway of lower activation energy. The half way point in a reaction is called as transition state and is represented as the top of the curve representing a chemical reaction (Figure 1.24) [181].

**Figure 1.24** The energy variation as a function of reaction coordinate Enzyme kinetics.

Kinetic parameters: Michaelis-Menten constant ($K_m$) and the maximum reaction velocity ($V_{max}$) are two important enzyme parameters in a simple enzyme catalyzed reaction. The Lineweaver -Burk plot [182] depicting the enzyme kinetics is given as Figure 1.25.

**Figure 1.25** Lineweaver-Burk plot.
$K_m$ is the substrate concentration, $[S]$ at which half maximum velocity of reaction is observed under given set of conditions. Generally a lower $K_m$ value signifies a higher affinity for the substrate. Value of $K_m$ is dependent upon pH, temperature and other reaction conditions. $V_{max}$ is the maximal activity of the enzyme when all of the active sites are saturated [183-184].

The Michaelis-Menten equation:

$$v = \frac{V_{max} [S]}{[S] + K_M}$$

To obtain $V_{max}$ and $K_m$, the enzyme activity must be recorded and then plotted on a double reciprocal plot, a Lineweaver-Burk plot, and the Michaelis Menten equation is then rearranged to

$$\frac{1}{v} = \frac{K_M}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

**Enzyme Inhibitors and Activators**

Enzyme inhibitors and activators that modulate the velocity of enzymatic reactions play an important role in the regulation of metabolism. Enzyme inhibitors act as a useful tool for the study of enzymatic reactions as well as for the design of new medicinal drugs. The enzyme inhibitors are low molecular weight chemical compounds. They can reduce or completely inhibit the enzyme catalytic activity either reversibly or permanently (irreversibly). Inhibitor can modify one amino acid, or several side chain(s) required in enzyme catalytic activity. Enzyme activators are chemical...
compounds that increase a velocity of enzymatic reaction [185]. Their actions are opposite to the effect of enzyme inhibitors. Inhibitors can be split into the following categories:

- **Irreversible Inhibitors**: Molecules that permanently bind to the enzyme's active site or specific side chain,

- **Competitive Inhibitors**: These are competing molecules that will have a very similar structure to that of the natural substrate and thus will be complementary to the enzyme active site (Figure 1.26). In competitive inhibition, $V_{\text{max}}$ remains the same but $K_m$ increases. Competitive inhibition can be overcome by an increase in substrate concentration. They are therefore useful therapeutic agents and unlike irreversible inhibitors (like aspirin) their effect isn't long lasting [186].

\[
E + S \rightarrow [E-S] \rightarrow P \\
E + I \rightarrow [E-I] 
\]

**Figure 1.26** Equation and the effect of the competitive inhibitor on the double reciprocal plot of the substrate-reaction rate relationship.
Non-competitive inhibitors: This type of inhibitors bind to the allosteric site on the enzyme other than the active site, causing changes to enzyme shape resulting in disruption of the active site (Figure 1.27). This decreases the turnover number of the enzyme rather than preventing substrate binding- $V_{\text{max}}$ decreases but $k_m$ stays the same. This cannot be overcome with an increase in substrate concentration.

\[
E + S \rightleftharpoons [E-S] \rightarrow P
\]

\[
E + I \rightleftharpoons [E-I] \rightarrow S
\]

**Figure 1.27** Equation and the effect of the non competitive inhibitor on the double reciprocal plot of the substrate-reaction rate relationship.

- Uncompetitive inhibitors only bind to an enzyme-substrate complex; so both $K_m$ and $V_{\text{max}}$ decrease as it takes longer for the substrate to leave the active site. This inhibition works when the concentration of enzyme-substrate complex is high.
1.7 Scope and objectives of the present study:

Biological activities of transition metal complexes derived from heterocyclic Schiff base ligands are one of the most exhaustively studied topics in coordination chemistry due to their enhanced activities compared to non-Schiff base complexes. The complexes of Schiff bases have wide applications in food industry, dye industry, analytical chemistry, catalysis, fungicidal, agrochemical, anti-inflammatory activity, antiradical activities and other biological activities. Metal chelates of Schiff bases hold exciting possibilities for the future, particularly in designing novel corrosion inhibitors, epoxy curing agents, semi-conducting materials, catalytic systems, in formulating new synthetic routes and in developing new antifungal, antibacterial, antiviral, anticancer, antioxidant and antidiabetic agents. From the survey of existing literature, it appears that heterocyclic Schiff base and their complexes have a variety of applications in biological, clinical and analytical fields. Keeping the pronounced biological properties of Schiff bases and their transition metal complexes, it was thought worthwhile to synthesize some new heterocyclic Schiff base complexes and study their biological properties.

The main objectives of the work can be summarized as follows:

- Synthesis and characterization of a new class of heterocyclic Schiff bases.
- Synthesis and characterization of Ni(II), Cu(II) and Zn(II) complexes of synthesised heterocyclic Schiff bases by spectroscopic data such as IR, UV-Visible, thermogravimetry, EPR, and elemental analysis.
Biological studies of heterocyclic Schiff base and their Ni(II), Cu(II) and Zn(II) complexes. Present work has been focused on the following biological properties.

- Anti-oxidant activity studies - solvent effect, structure activity relation and mechanism of action.
- DNA binding studies
- Anti-HIV studies
- Anti-bacterial studies
- Anti-Diabetics studies
- Cytotoxicity studies
Chapter 1

References


Chapter 1


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