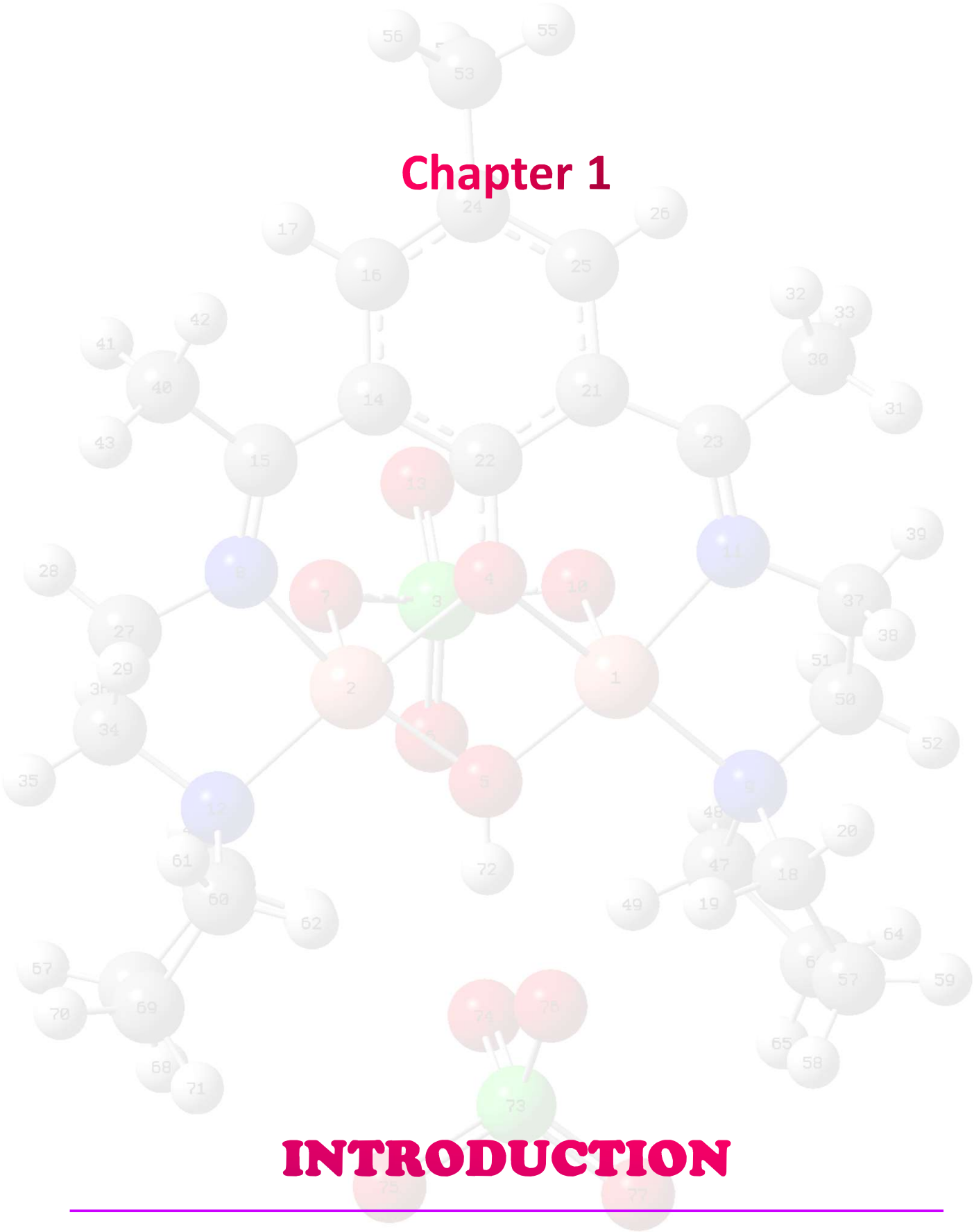
Chapter 1



INTRODUCTION

Chapter 1 Introduction

1.1 General Introduction

The primary and ideal oxidant from the economic and environment point of view for use in bulk chemical industries is molecular oxygen because of its availability directly from air rendering it inexpensive and environment friendly. [1–4] The use of various inorganic or organic oxidants in classical oxidation reactions in stoichiometric quantities is toxic and hazardous to environment. Instead, the nature inspired idea of using catalytic amount of an activator in oxidation reactions to activate the molecular oxygen with minimum chemical waste is very attractive. However, due to kinetically inert nature of molecular oxygen, many challenges are being faced for its use in oxidation reactions. Once activated, its reactivity is difficult to control and can lead to over-oxidation. Low selectivity is another major disadvantage of using oxygen in chemical transformations.

To overcome the kinetic inertness of dioxygen activation, transition metal ions are incorporated in proteins i.e. metalloenzymes. [4–10] They have metal cores at their active sites which catalyse the biological reactions with high selectivity and rate without affecting their structure. In metalloenzymes, the most utilised ions are transition metals due to their unique characteristics. Recent decades have witnessed many attempts to mimic enzymes structures and functions by inorganic chemists with the help of their knowledge of coordination chemistry, redox chemistry and electronic factors governing the reactivity of complexes. This has led to the developments in the areas of bio-inorganic chemistry. These synthetic mimics composed of two main parts - ligand and core. To probe the complexes as the structural and functional models, the enzyme donor sites are modelled with small organic molecules called as ligands which are incorporated with metals such as transition metal ions and lanthanide ions which form core part of the metalloenzyme active sites. Also, they may have one, two, three or even four cores in their structure[6,7,11–17]. During past few decades, the bio inorganic chemistry has mainly focused on the development of catalytic reactions with a motive to understand the active sites and possible mechanistic pathways of metalloenzymes, and also to develop complexes which would mimic the metalloenzymes and would be very useful catalysts. Structural-functional stability, high rate, selectivity, ability to bind to a substrate are the desired qualities of a synthetic model. These are expected to be mainly governed by the coordination environment of the metal core and hence

designing of a suitable ligand is the most difficult aspects of developing a synthetic model. For achieving these goals, it is necessary to understand the active site of metalloenzymes, mechanisms involved and structure–function relationships.

Thought the nature has developed the most efficient catalysts in the form of enzymes, their applications in industry are limited due to the sensitivity of natural enzymes to heat, pressure and pH. Thus, the development of synthetic models of these active sites which can mimic their structure and have specific and desired characteristics can help overcome this difficulty. It becomes essential to manipulate the selectivity of these structural mimics by way of functional group modifications so that the rates can be enhanced, and it can become resistant to heat, pressure and pH for its industrial applications. In spite of the efforts by researchers to mimic and develop various enzymes as model complexes, a turnover as close to native enzymes could not be attained so far, but the understanding of mechanistic pathways and any other alternative pathway has been accomplished. The use of small molecules as active site models to mimic metalloproteins in the bioinorganic chemistry of copper has provided a wealth of information through enzymatic studies, concerning structure-activity relationships and the potential role of different oxidation states of copper in biosynthetic pathway (**Figure 1.1**).

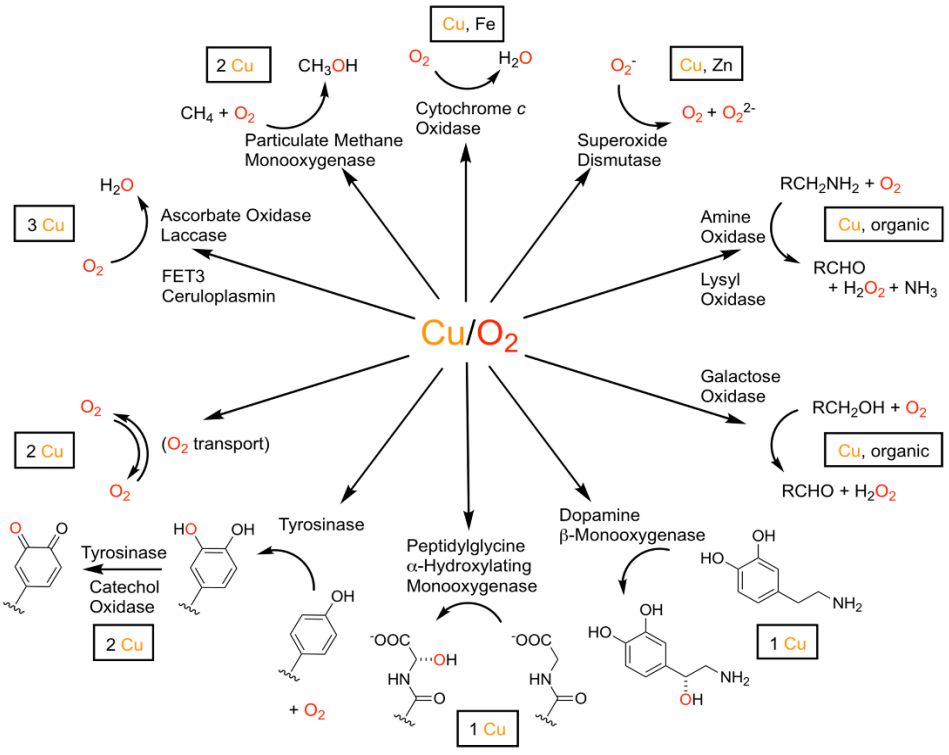


Figure 1.1 *Potential role of copper in biosynthetic pathway*

Bio inspired approach has been employed to this chemical catalysis, which consists in partially mimicking the structure of active site of enzymes to try to reproduce its activity.[18,19] First row transition elements such as iron, zinc, nickel, manganese along with copper participate in many biochemical processes and can be used as catalysts in many oxidation reactions.[20] In recent decades, the development of biomimetic oxidation catalysts, involving copper(II) ion as active metal center has received great attention.[21–26] This is because a copper(II) complex with specific coordination can match the redox potentials of reactive oxygen species and activate molecular oxygen. The main objective of these studies has been to understand the properties of an enzyme to activate molecular oxygen.

Proteins containing copper ions at their active site can be classified by the type of copper environment and their spectroscopic characteristics. Further classification is based on their biological function, by type and number of prosthetic groups and by sequence similarity. Historically, all copper proteins were divided into four different groups according to their spectroscopic features:

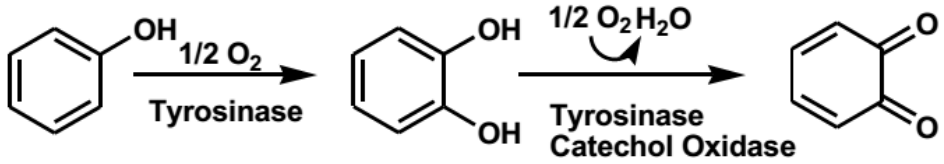
1. Blue copper - Type 1 site
2. Normal copper –Type 2 site
3. Coupled Binuclear copper – Type 3 site
4. Multicopper oxidases.

The most well-known examples of these enzymatic sites are the CuZnSOD having normal copper active site, catecholase & tyrosinase having type 3 active site and ascorbic acid oxidase, a multicopper oxidase having a combination of all.

1.1.1 Catecholase activity & synthetic models

Catecholase activity is the oxidation of a broad range of catechols to quinones through the four-electron reduction of molecular oxygen to water undertaken by catechol oxidase. Catechol oxidase (CO), hemocyanin and tyrosinases belong to the family of type 3 copper protein. The active site of these proteins contains dicopper core in which both copper ions are surrounded by three nitrogen donor atoms of histidine residues. The main important feature of these proteins is their ability to reversibly bind dioxygen at ambient conditions. In many living organisms, oxygen activation is an important process which is often assigned to metalloproteins

containing copper, such as hemocyanin (HC) which transports dioxygen in molluscs and arthropods[27–29], tyrosinase[30,31] (catalyzes aerial oxidation of phenol to o-diphenol) and then the catalysis of the oxidation of catechol to o-quinone (**Scheme 1.1.1.1**) by catechol oxidase enzymes (CO).[13,32–35] The latter then undergoes polymerization with the production of the pigment melanin. The two copper(II) centres show antiferromagnetic coupling and EPR silent behaviour in the *oxy* state of these proteins. The crystal structures of hemocyanin[27,36,37], tyrosinase[38,39] and catechol oxidase[40,41] have been solved. The detailed description of catechol oxidase (CO) has been presented in earlier reviews[12,13,42–44], specifically that by Reedijk *et.al.*[13].



Scheme 1.1.1.1 *Oxidation of phenol to catechol to o-quinone*

In 1998, the crystal structure of the met form of the enzyme CO was determined. The active site of the enzyme CO in its native form has a strongly antiferromagnetically coupled dinuclear copper core based on the crystal structure where each copper(II) is coordinated by three histidine nitrogens and adopts an almost trigonal pyramidal environment with one nitrogen at the apical site and a bridging OH^- ion (**Figure 1.1.1.1**)[41]. Krebs and co-workers[41] reported crystal structure of catechol oxidase isolated from ipomea batatas (sweet potato) in three catalytic forms: the native met ($Cu^{II}Cu^{II}$) state, the reduced deoxy ($Cu^{I}Cu^{I}$) form and the oxy form in the complex with inhibitor phenylthiourea. In the met state ($Cu^{II}Cu^{II}$), the Cu·····Cu distances are 2.9Å and bridged hydroxide ion is at distance of about 1.8Å from each cupric ion with each of them having coordination number four. In the deoxy or reduced state, the oxidation state of both copper atoms is +1 which increases the distance between the two copper atoms from 2.9Å (oxy) to 4.4Å with slight change in position of histidine residues without hindering the other residues. The coordination number for Cu A is four (three histidine ligands and one water molecule) and that for Cu B is three (the coordination site occupied by bridging OH^- in the oxy sate is vacant). In an adduct of CO with inhibitor

phenylthiourea, inhibitor binds to met form by replacing OH^- bridge. The distance between two copper atoms increases to 4.2Å from 2.9Å present in oxy state. There is no change in coordination number of both copper atoms but there is a conformational change in active site of protein. In order to be active, these dicopper complexes must be oxy-bridged as shown by Oishi *et. al.* (1980)[45].

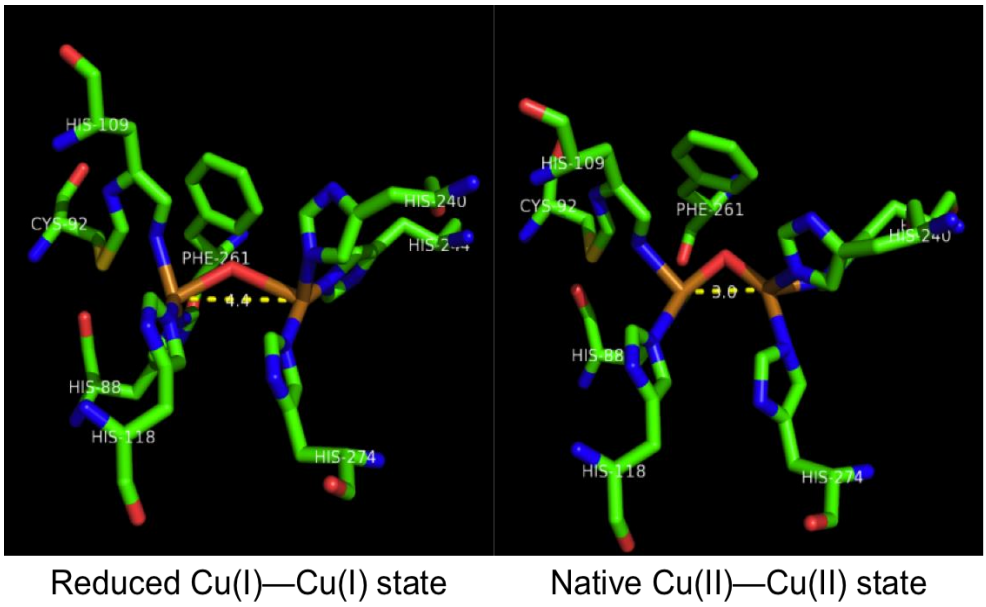


Figure 1.1.1.1 *The reduced (Cu(I)-Cu(I)) and native (Cu(II)-Cu(II)) catechol oxidase di-copper active site from the Ipomoea batata crystal structure (PDB: 1BT1, 1BT2). (PDB ID: 1BT3)*

X-Ray crystal structure analysis of CO in different catalytic states helps us to investigate the structure-function-relationship and to evaluate the functional mechanism for developing ultimately accurate structural as well as functional models of the native enzymes. The possible mode of binding of catechol to copper(II) center is shown in **Figure 1.1.1.2.**

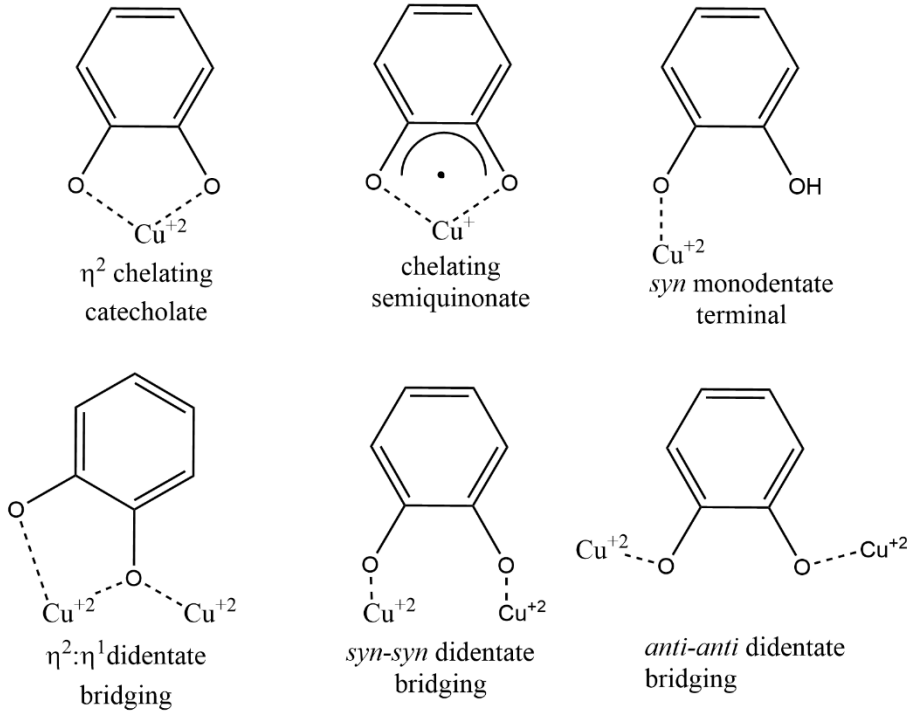
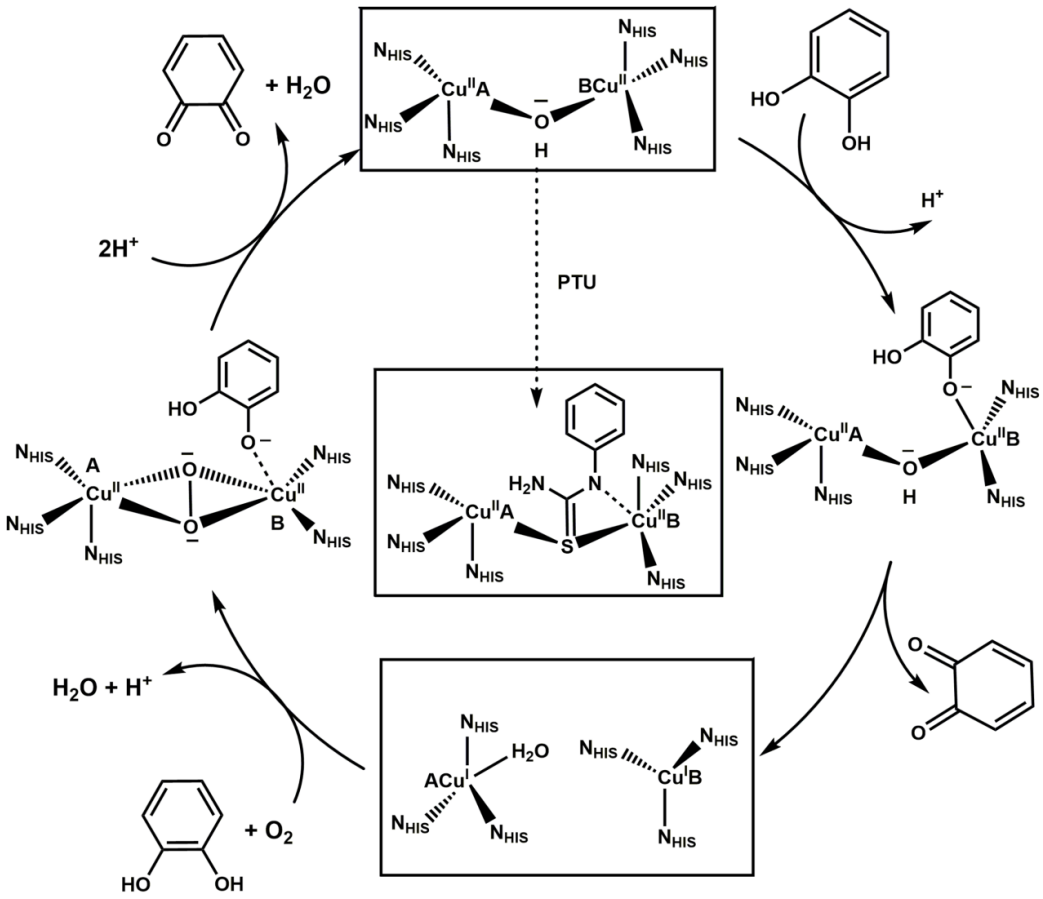
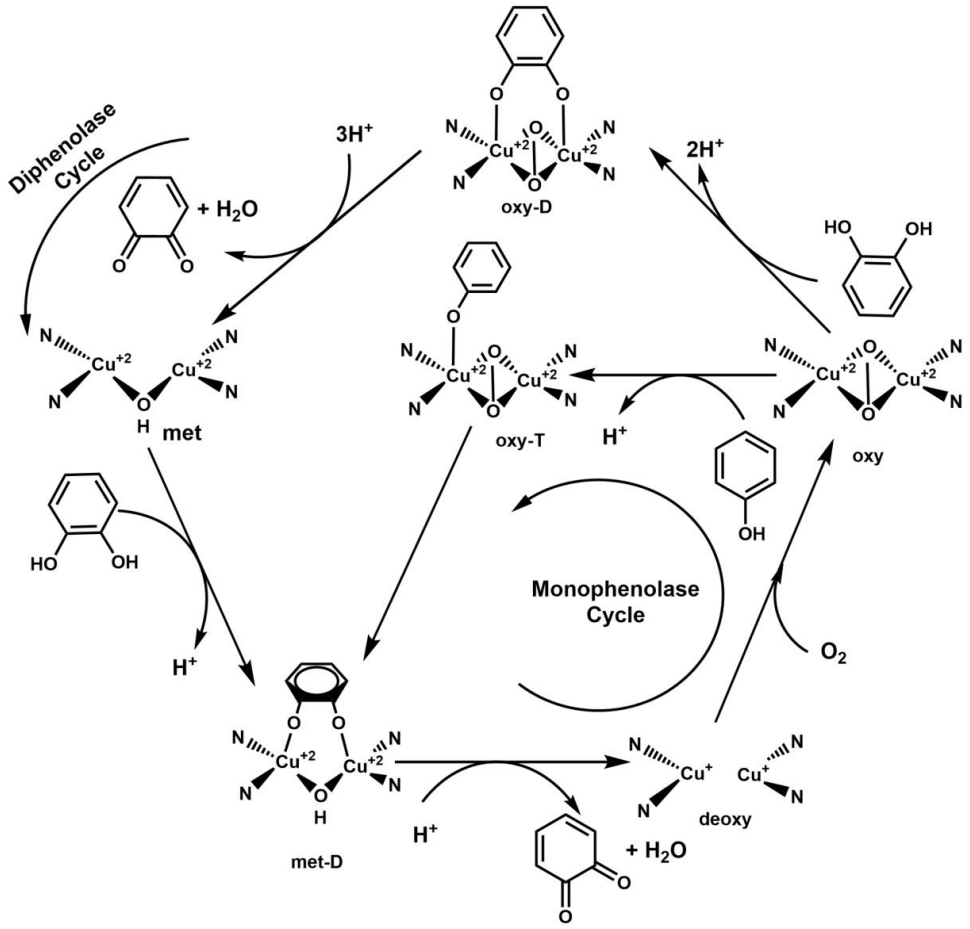


Figure 1.1.1.2 *Possible binding modes of the catechol substrate to copper centres by Reedjik et al* [13]

Till now, two mechanisms have been proposed for the catalytic cycle of catechol oxidase. One by Krebs and by his co-workers[46] and the other by Solomon *et. al.*[31]. Krebs and his co-workers proposed that catechol binds to one copper centre through asymmetric monodentate coordination (**scheme 1.1.1.2**) whereas Solomon and his co-workers proposed that catechol binds to both copper centres through symmetric coordination (**Scheme 1.1.1.3**). Rest of the mechanisms are not significantly different.

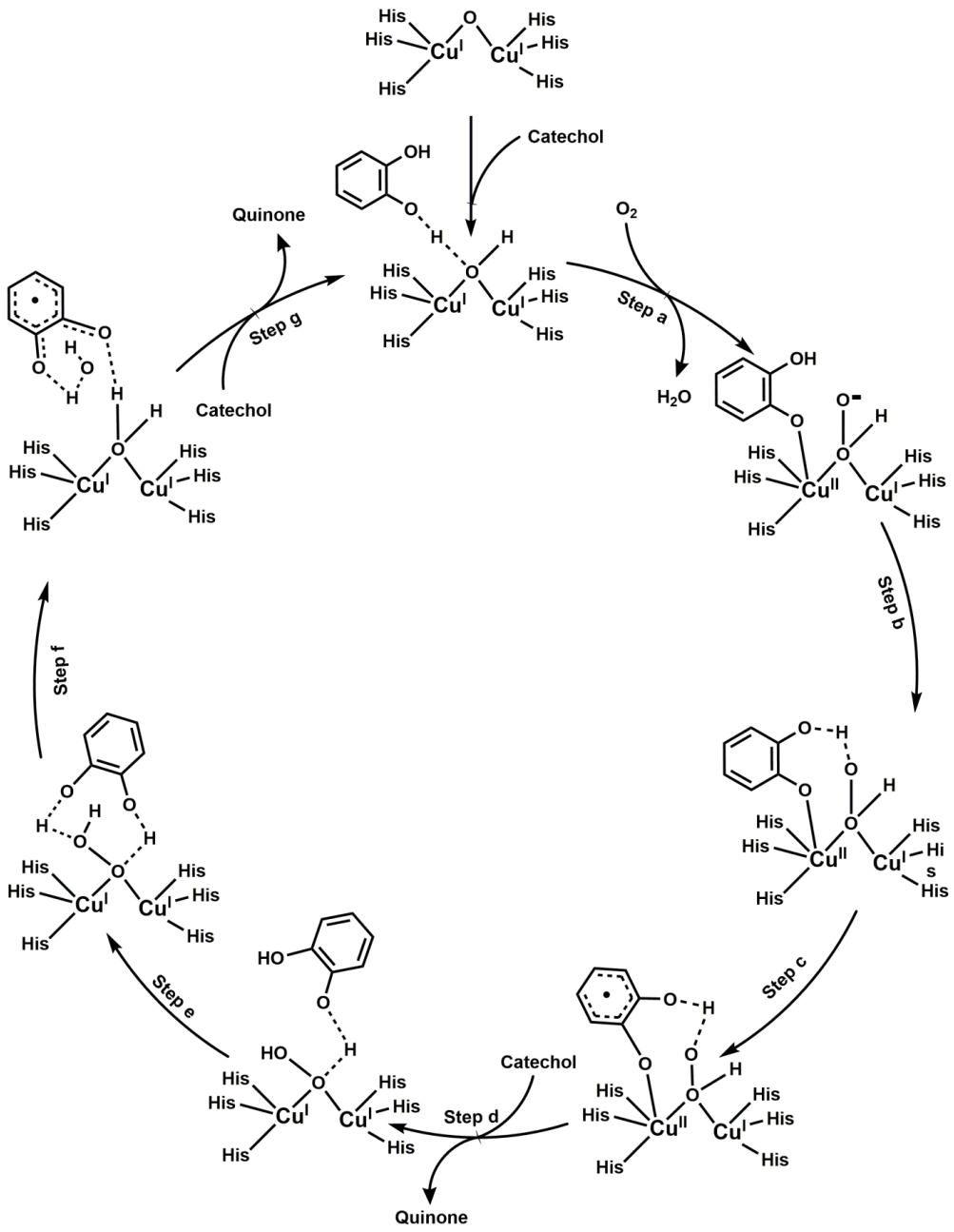


Scheme 1.1.1.2 *Catalytic cycle of CO from Ipomoea batatas, as proposed by Krebs et al* [46] *on the basis of structural, spectroscopic and biochemical data (Redrawn from ref* [46]*)*



Scheme 1.1.1.3 *Catalytic cycle for monooxygenation of monophenols and the oxidation of o-diphenols to o-quinones by tyrosinase proposed by Solomon et al [31]. Axial ligands at Cu not included for clarity. T = tyrosinase and D = DOPA bound forms (Redrawn from reference [31])*

Siegbahn[47] proposed different mechanisms (radical) of catalytic cycle (**scheme 1.1.1.4**) based on hybrid DFT for a quantum chemical study of the catalytic cycle. The active site of enzyme should not change its charge during catalytic cycle and the reaction cycle starts from deoxy dicopper (I) form.



Scheme 1.1.1.4 *The mechanism of the catalytic cycle of CO proposed by Siegbahn*[47] *based on hybrid DFT calculations (Redrawn from reference*[47]*)*

The k_{cat}, K_M and k_{cat}/ K_M values of catechol as substrate by catechol oxidase enzyme from *Lycopus europaeus* are 5.7 x 10^5 h^{-1}, 0.005 M and 31.67 $mM^{-1}s^{-1}$, respectively[48]. The k_{cat}, K_M and k_{cat}/ K_M values of catechol as substrate by catechol oxidase enzyme from *Ipomoea batatas* are 8.25 x 10^6 h^{-1}, 0.0025 M and 916.67 $mM^{-1}s^{-1}$, respectively[49]. The k_{cat}, K_M and k_{cat}/ K_M values of catechol as substrate from GriF (tyrosinase homolog) are 4.0 x 10^4 h^{-1}, 0.0025 M and 4.44 $mM^{-1}s^{-1}$ respectively[50]. The k_{cat}, K_M and k_{cat}/ K_M values of catechol as substrate from

mushroom tyrosinase are 3.15 x 10^6 h^{-1}, 0.00016 M and 5463.13 $mM^{-1}s^{-1}$ respectively[51].

In the mechanistic studies on the synthetic models compounds for studying catecholase activity, four approaches have been used by Reedjik *et al* [13] which includes substrate-binding studies, structure-activity relationship, kinetic studies on catalytic reactions and stoichiometric oxidation of catechol substrates by peroxo- and oxo- dicopper complexes. The approach of correlating structure with activity has been more frequently employed by various researchers. This includes relationship between metal-metal distance, exogenous bridging ligand, ligand structure, solvent nature and pH of the solution with catecholase activity that have been explored. Depending upon these, there are many literature reports on various transition metal containing synthetic model compounds which act as artificial enzymes.

To propose possible mechanistic pathways and the influencing factors of catecholase activity, several biosynthetic model compounds are synthesized. 3,5-DTBC is the most commonly used substrate for the study of catecholase activity due to its low reduction potential and also the presence of t-butyl group on substrate inhibit the auto polymerisation of 3,5-DTBQ. Other substrates which have been used are pyrocatechol and tetrachlorocatechol (TCC) but they are hard to oxidize.

K D. Karlin *et al* [52] has first structurally characterized tetrachloro-o-catecholate-bridged dicopper(II) complex (**Figure 1.1.1.3**) as a model for intermediates in copper-catalyzed oxidation of catechols.

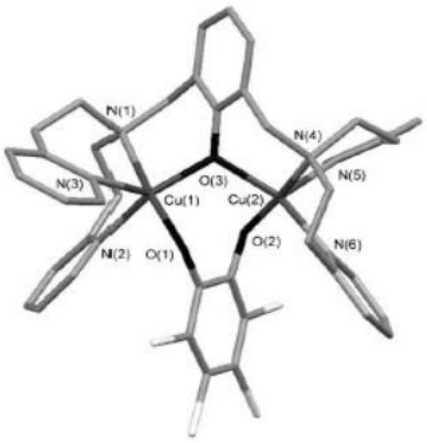


Figure 1.1.1.3 *First report of TTC adduct with dicopper(II) complex (didentate mode) by Karlin et al*[52]

Later structures of four different copper-TCC adducts (**Figure 1.1.1.4**) with different modes of binding to copper centers were reported by Borzel *et al.* [53]

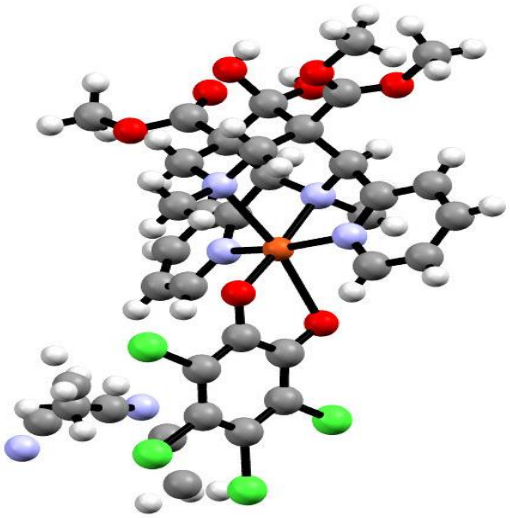


Figure 1.1.1.4 *Crystal structure of Copper(II) complex-TCC adduct reported by Borzel et al*[53]

Kao *et al* [54] reports an investigation of the structural correlation between catecholase activity and the Cu----Cu distance of a series of oxy-bridged binuclear copper(II) complexes (**Figure 1.1.1.5**) with HL^1 (=1-diethylaminopropan-2-ol), HL^2(=N-ethyl-2-hydroxy benzylidenimine), and HL^3(=N-(salicylidene)-N'-(2-pyridylaldene)propanediamine). This short metal-metal distance enables strong magnetic coupling which can be achieved by dimeric ligands with bridging alcoholate or phenolate oxygen atoms.

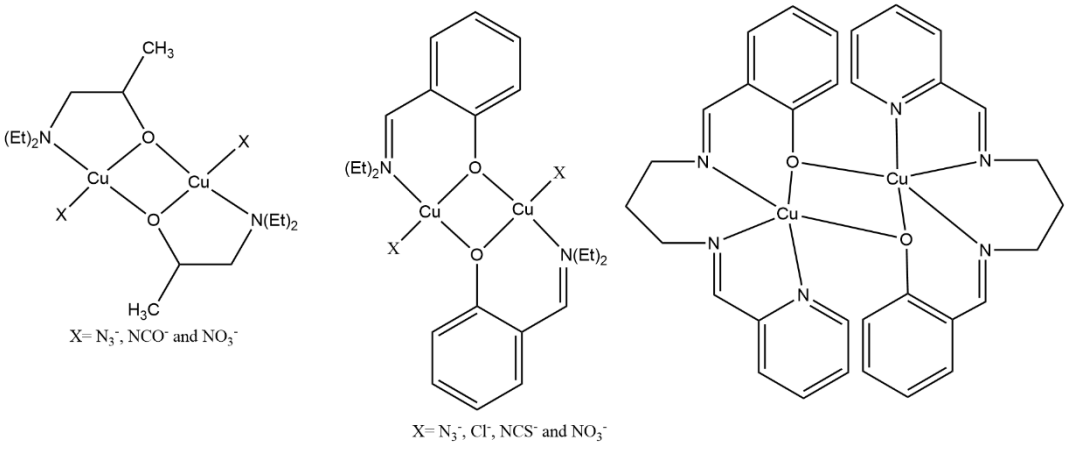


Figure 1.1.1.5 *Scheme of oxy-bridged binuclear copper(II) complexes reported by Kao et al*[54]

Ackermann *et al*[55] synthesized a series of copper(II) complexes (**Figure 1.1.1.6**) and structurally characterized with one major difference *i.e.* metal-metal separation that is enforced by pyrazolate based ligand scaffold: it ranges from 3.45 to 4.53 Å. The structure of adducts of L^3Cu_2 and L^4Cu_2 with substrate analogue suggests bidentate binding to one of the copper ions for those catalysts that feature short ligand side arms which increases metal-metal separations which may be reason of low catalytic activity.

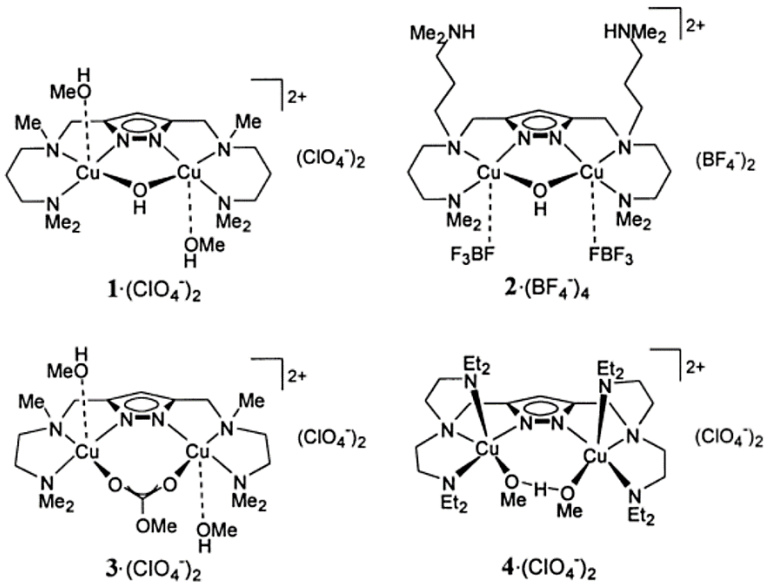


Figure 1.1.1.6 *Dinuclear copper(II) complexes of various pyrazolate ligands. (Adapted from Ackermann et al* [55]*)*

Laura Gasque *et al* [56] have reported a very efficient diazecine based dicopper(II) catalyst (**Figure 1.1.1.7**) for oxidation of 3,5-DTBC where Cu-Cu separation is more than 7 Å.

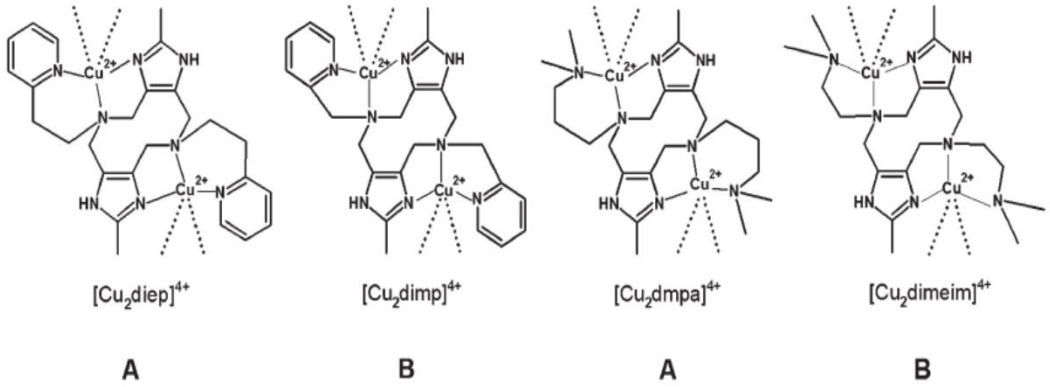


Figure 1.1.1.7 *Diazecine based dinuclear copper complexes (A) and their related previously described analogues (B). (Adapted from Laure Gasque et al* [56]*)*

The presence of substituents on the ligand backbone has a profound effect on the catecholase activity. Dasgupta *et al* [57] synthesized four dinuclear copper(II) complexes (**Figure 1.1.1.8**) by varying the auxiliary part of ligand back bone and modification of auxiliary part of ligand influences had a profound effect on catecholase activity. The presence of electron donating group on ligand enhances the activity whereas the electron withdrawing group, the reverse effect is observed.

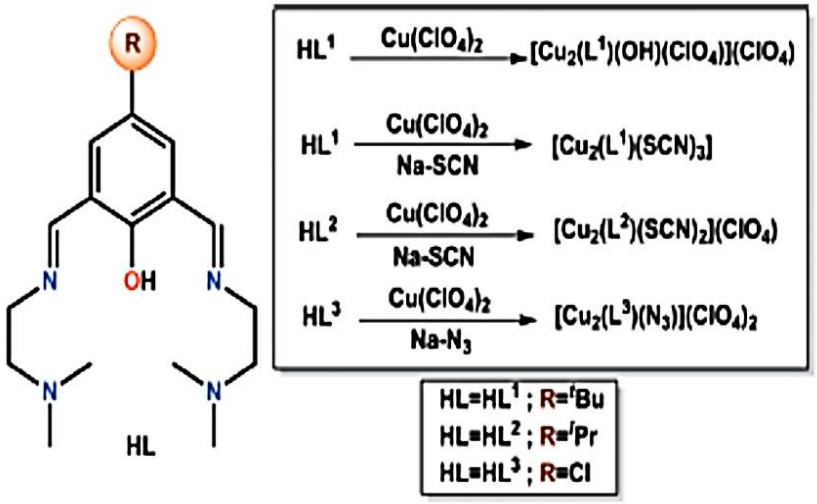


Figure 1.1.1.8 *Schematic representation of dinuclear copper(II) complexes of Schiff bases of aliphatic amines (Adapted from Dasgupta et al* [57]*)*

The change in pH is observed to influence to change the geometry of copper(II) centers which in turns affect its catalytic efficiency.[58,59] Krebs *et al* [60] investigated catecholase activity of copper(II) complexes with both symmetric and asymmetric ligand systems (**Figure 1.1.1.9**) which concludes that geometric factors affects the activity of complexes. The highest activity was observed by the most strained structure in their system.

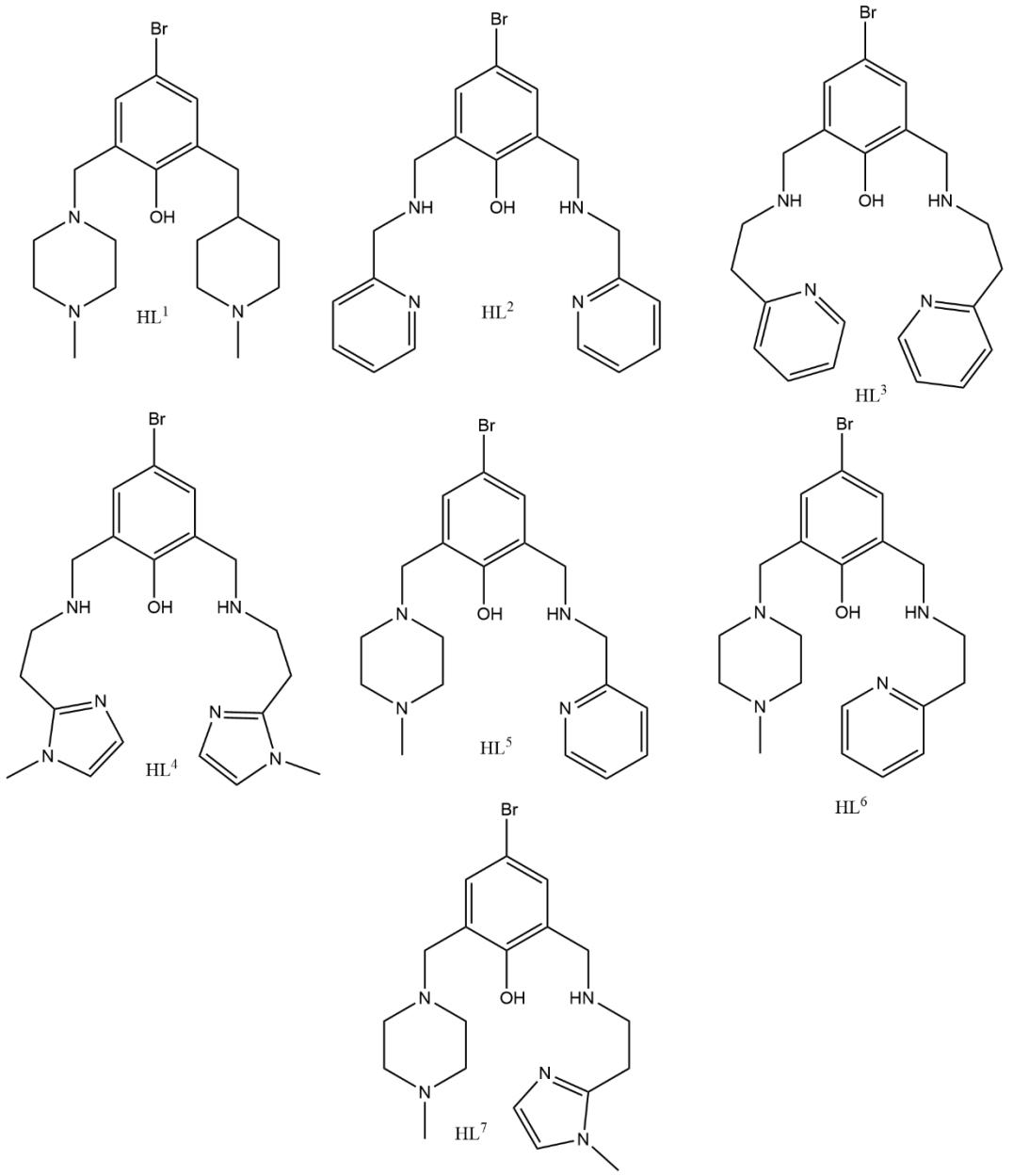


Figure 1.1.1.9 *Pentadentate dinucleating ligands employed for the synthesis of copper(II) complexes reported by Krebs et al* [60]

Mukherjee *et al* [61] synthesized series of doubly bridged dicopper(II) complexes derived from endogenous and exogenous bridges (**Figure 1.1.1.10**) and correlated their electrochemical property and catecholase activity. They found that there was no direct relationship between these two properties and also best activity was achieved with dicopper(II) compounds having Cu—Cu separation around 3Å.

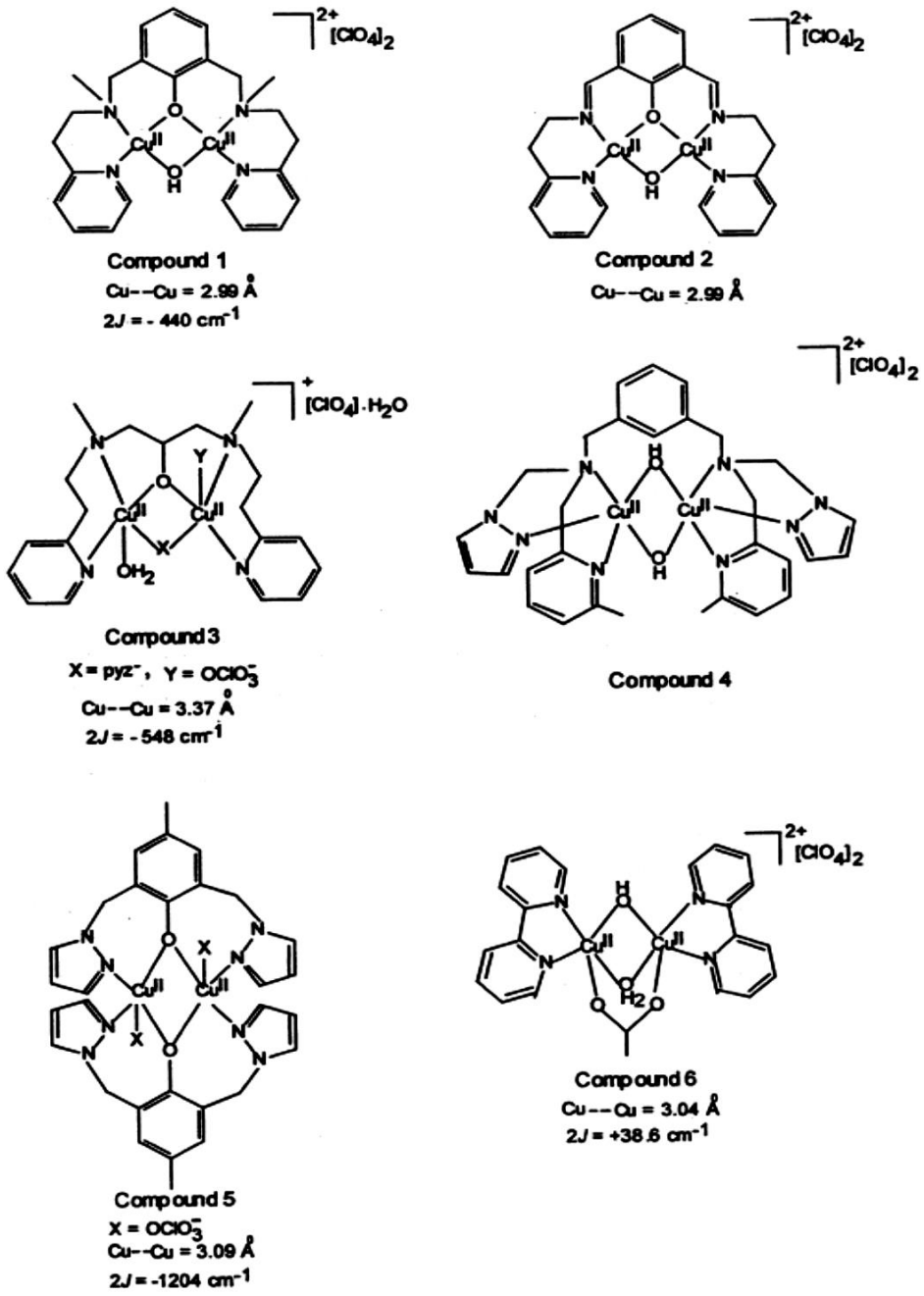


Figure 1.1.1.10 *Structure of dicopper(II) complexes reported by Mukherjee et al*[61]

Vittal *et al* [62] synthesized copper(II) complexes of Schiff base and reduced Schiff base ligands (**Figure 1.1.1.11)** to understand the role of flexibility of ligand towards catecholase activity. They reported that the reduced analogue provided more insight into the understanding of their catalytic activity.

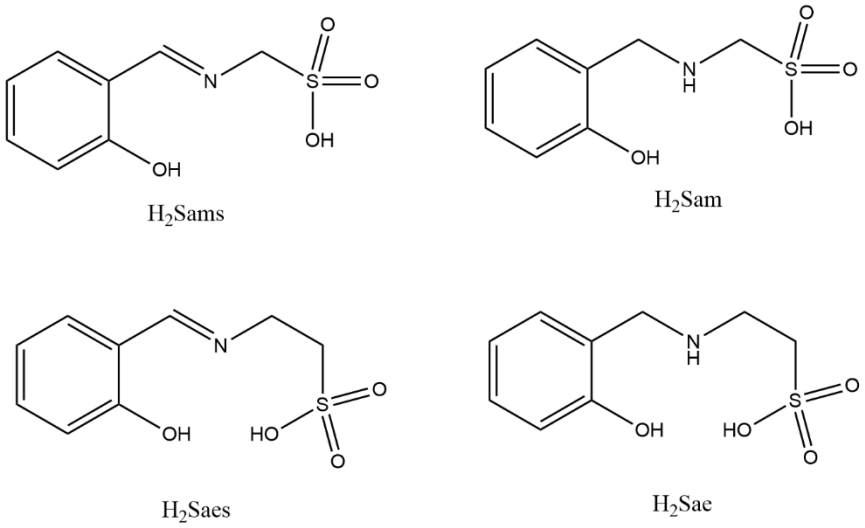


Figure 1.1.1.11 *Schiff base and reduced Schiff base ligands employed for the synthesis of copper(II) complexes reported by Vittal et al*[62]

Anitha *et al* [63] syntheized a mononuclear copper(II) complexes (**Figure 1.1.1.12**) as a functional models for catechol oxidase enzymes and correlated with ligand stereo-electronic factor of the metal complexes determine the catecholase activity.

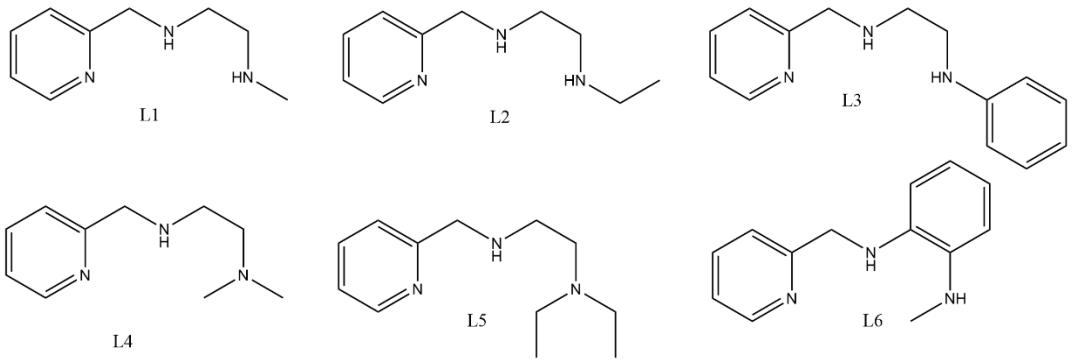


Figure 1.1.1.12 *Ligands used for the synthesis of mononuclear copper(II) complexes reported by Anitha et al*[63]

Hung *et al* [64] synthesized a series of six coordinated copper(II) complexes (**Figure 1.1.1.13**) and correlated its catecholase activity. Heteroleptic complex which have two aquo-ligands oriented in cis positions have better catecholase activity than other copper(II) complexes reported here within, which suggests the essential role of liable *cis*-aquo ligands to promote the catalytic reaction.

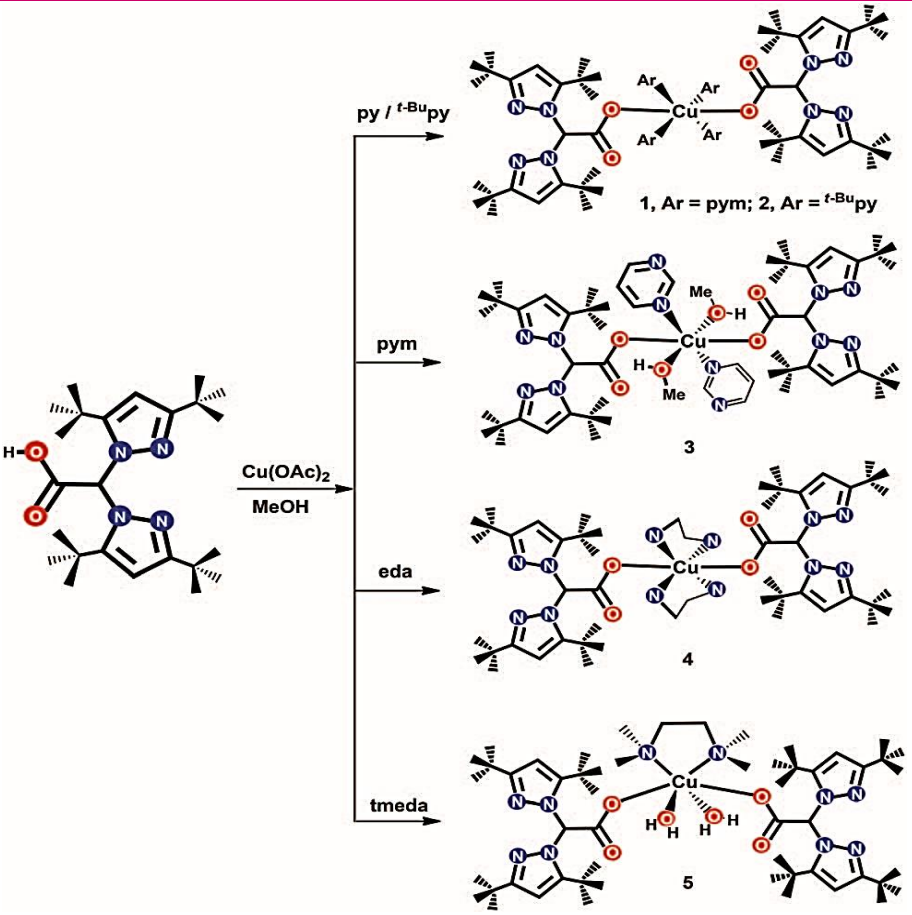


Figure 1.1.1.13 *Ligands used for the synthesis of mononuclear copper(II) complexes reported by Hung et al* [64]

1.1.2 Superoxide Dismutase: activity & synthetic models

In cellular redox processes, excessive production of electrons in solutions occurs by oxidative phosphorylation. Superoxide can rapidly be formed with the attachment of one electron as cells have reasonable oxygen. Superoxide Dismutase (SODs) are enzymes that catalytically convert superoxide ($O_2^{\cdot-}$) to oxygen (O_2) and hydrogen peroxide (H_2O_2).[65,66] Based on the metal co factor present, they are classified into four groups: FeSOD, MnSOD, CuZnSOD and NiSOD (**Figure 1.1.2.1**). They are found in prokaryotic organisms. In chloroplasts, Eukaryotes FeSOD can be found. Mn SOD are found in mitochondria and in peroxisomes. The most abundant SOD is CuZnSOD and are found in chloroplast, cytosol and in extracellular spaces.

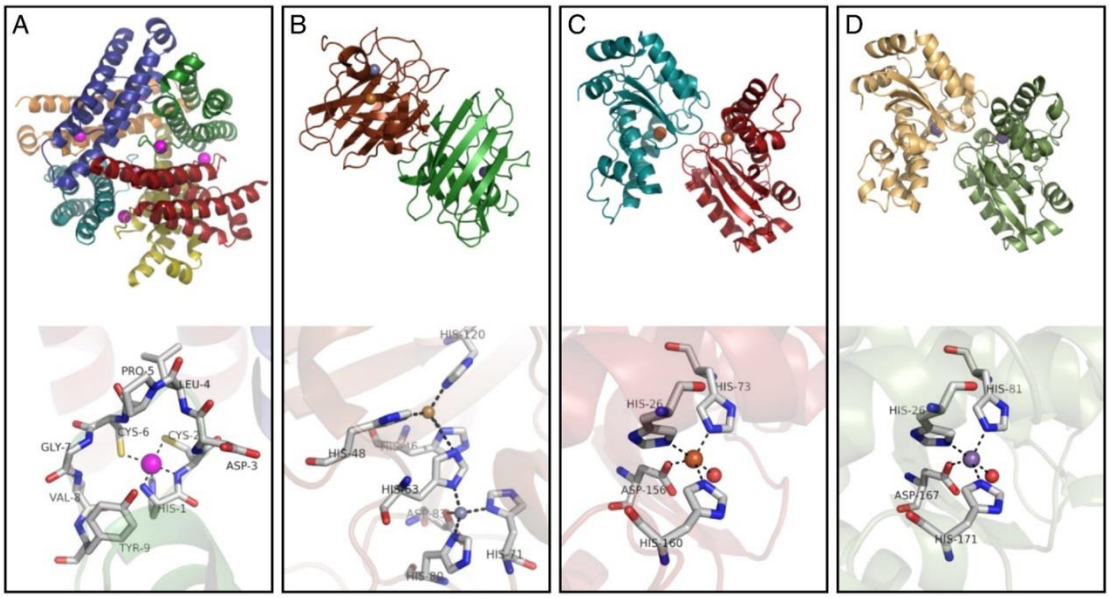


Figure 1.1.2.1 *A comparison of the enzyme structures and active sites for the four SODs, (A) Streptomyces coelicolor NiSOD (pdb ref.: 1t6u), (B) human CuZnSOD (pdb ref.: 1pu0), (C) E. coli FeSOD (pdb ref.: 1isa) and (D) MnSOD (pdb ref.: 1vew). Picture created with PyMOL. (Adapted from Arbeu et al* [67]*)*

The most fascinating features of this class of enzymes are that the catalytically active metal of SODs can be copper, iron, manganese or nickel. The overall mechanisms by which these SODs function has been called "ping pong" mechanism as it involves reduction and oxidation of the metal center in sequential manner with concomitant oxidation and reduction of superoxide radicals (**Scheme 1.1.2.1**)

$$M^{ox} + O_2^{-} \rightarrow M^{red} + O_2 \text{ ---------- (i)}$$

$$M^{red} + O_2 + 2H^{+} \rightarrow M^{ox} + H_2O_2 \text{ ---------- (ii)}$$

Scheme 1.1.2.1 *Ping pong mechanism of SODs*

Deficiency of SOD concentration in human body leads to various diseases and disorders like diabetes, cataract, ischemia, Parkinson's disease, cancer, etc. to overcome such harmful consequences, all oxygen metabolizing organisms possesses metalloenzymes known as superoxide dismutase (SODs). Native SODs behaves as efficient scavengers of free radicals generated in the body besides dismutating O_2^{-} radical to O_2 and H_2O_2. But if there arises an imbalance between generation of toxic free radicals and concentration of dismutation enzymes, a need of supplement arises and antioxidant enzymes are thought beneficial in treating such disorders. However,

the administration of such supplements is restricted because of high molecular weight that impedes their cell permeability and also rapid degradation and short life span of SODs in the biological systems affects their clinal use. Hence, there is a need for synthetic mimics of low molecular weight compounds with good radical scavenging activities. Synthetic superoxide distmutase (SOD) mimetics have emerged as a potential novel class of drugs for the treatment of the oxidative stress related diseases. Many efforts have been made by bio-inorganic chemists for the synthesis of low molecular weight copper(II) complexes showing good SOD activity[68–74].

Patel *et al*[75] synthesized two copper(II) complexes and characterized by various spectroscopic techniques. Biological activity of these complexes in terms of superoxide dismutase were evaluated and it reveals that these two complexes catalyze the fast disproportionation of superoxide in DMSO solution.

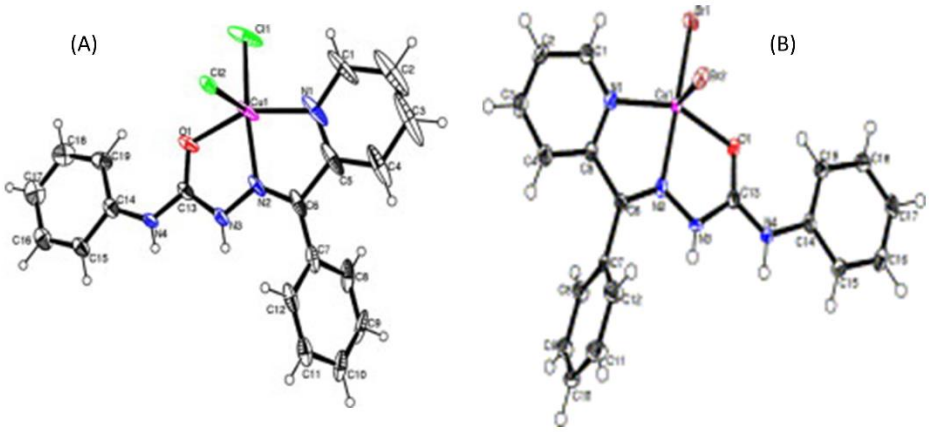
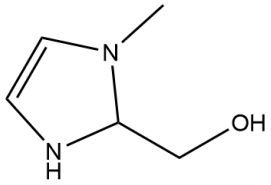


Figure 1.1.2.2 *Crystal structure of two mononuclear copper(II) complexes reported by Patel et al*[75]

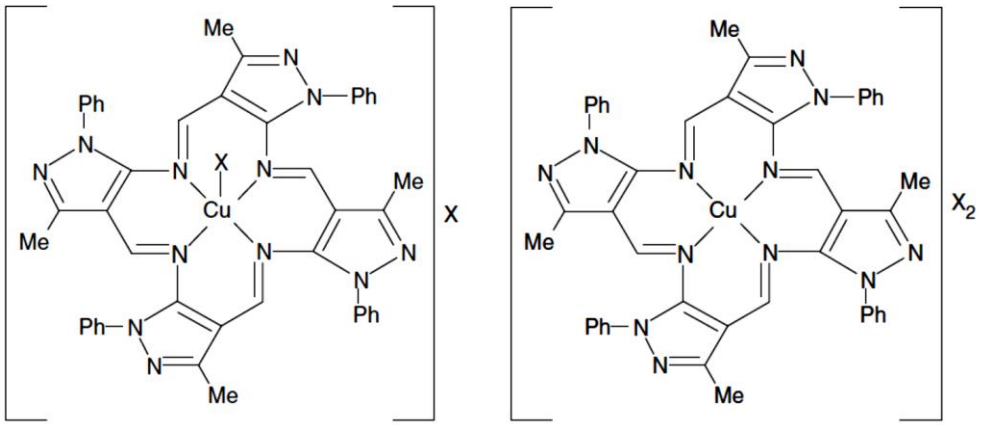
Zhou *et al* [76] have synthesized new copper(II) complexes, $[Cu_2(HL)_2(L)_2](ClO_4)_2$, $[Cu(HL)_2(phen)](ClO_4)_2$, where HL = (*N*-methyl-2-methylol)imidazole, phen = 1,10-phenanthroline) (Figure 1.1.2.3) to mimic the active site of Cu,Zn-SOD. These complexes were tested for their SOD mimic activity which reveals that higher SOD activity of imidazole related complex could be due to the coordination configuration and the labile hydroxymethyl pendants.



(N-methyl-1H-imidazol-2-yl)methanol

Figure 1.1.2.3 *Ligand employed for the synthesis of copper(II) complexes reported by Zhou et al*[76]

Ramadan *et al*[77] synthesized a series of macrocyclic complexes, $[Cu(TAAP)]X_2$, $X = ClO_4$ and CH_3COO^-; $[Cu(TAAP)X]X$, $X = NO_3$, Cl, and Br by self-condensation of 5-amino-3-methyl-1-phenylpyrazole-4-carbaldehyde (AMPC) in the presence of copper(II) (**Figure 1.1.2.4**). Mimetics of antioxidant enzymes such as superoxide dismutase (SOD) and catalase demonstrated that there is a correlation between the observed redox properties and the SOD and catalase biomimetic activities of the copper(II) complexes.



Complex **1**, X = Cl; **2**, X = Br; **3**, X = NO_3 Complex **4**, X = AcO; **5**, X = ClO_4

Figure 1.1.2.3 *Structure of copper(II) tetraazamacrocyclic complexes reported by Ramadan et al*[77]

Barik *et al* [78] synthesized two copper(II) curcumin complexes (**Figure 1.1.2.4**) for comparative study as superoxide dismutase and free radical scavengers. Depending on their structures, these two complexes possess different SOD activities and free radical neutralising abilities. Cu(II)-curcumin complex (1:1) having large distortion from square planar structure shows higher SOD activity.

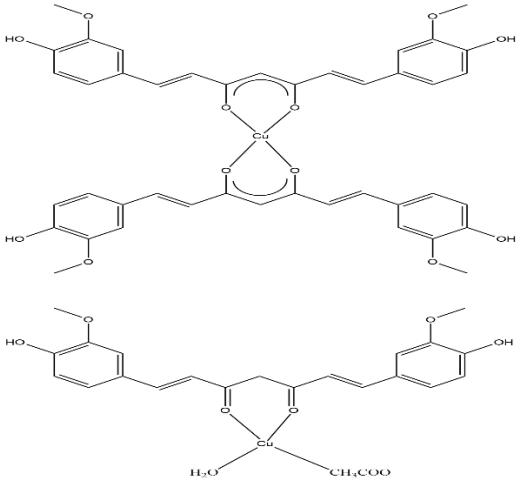


Figure 1.1.2.4 *Structure of copper(II)-curcumin complexes reported by Barik et al*[78]

Labadi *et al*[79] synthesized several imidazole bridged copper(II)zinc(II) complexes for mimicking the superoxide dismutase enzyme. Different coordinating ligands such as 2,2'-bipyridine, 2,2':6'2''-terpyridine and tris(2-aminoethyl)amine were used for the synthesis of these complexes. Lowest SOD activity was observed by complex containing 2,2':6'2''-terpyridine ligand which indicates the importance of the rigidity of the copper complex in SOD activity.

Diószegi *et al*[80] synthesized copper(II) complexes (**Figure 1.1.2.4**) of pyridine based ligands functionalized with alanine (PydiAla) and tyrosine (PydiTyr) moieties as novel SOD mimics. They exhibit high SOD activity. PydiTyr complex exhibit higher SOD activity than PydiAla complex probably due to the presence of the phenolic OH group in the former species, which promotes the binding of the superoxide anion radical to the metal center.

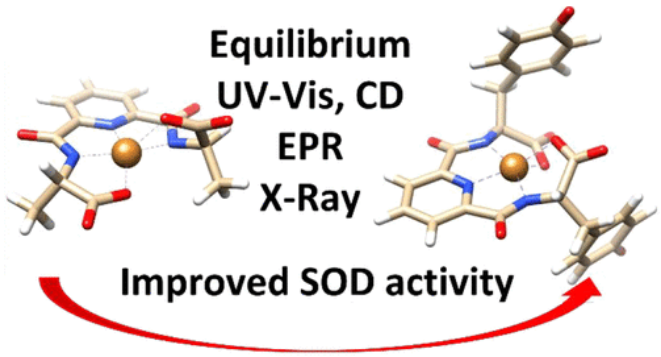


Figure 1.1.2.4 *Structure of copper(II) complexes reported by Doiszegi et al*[78]

Joanna *et al*[81] synthesized five new copper(II) complexes, ([Cu(2-(HOCH$_2$)py)$_3$](ClO$_4$)$_2$, [Cu(2-(HOCH$_2$)py)$_2$(H$_2$O)$_2$]SiF$_6$, [Cu$_2$(2-(HOCH$_2$CH$_2$)py)$_2$(2-(OCH$_2$CH$_2$)py)$_2$](ClO$_4$)$_2$, [Cu(pyBIm)$_3$](BF$_4$)$_2$·1.5H$_2$O and [Cu(py$_2$C(OH)$_2$)$_2$](ClO$_4$)$_2$) to evaluate the antioxidant activity of potential synthetic enzyme mimetics. Water soluble complexes were selected for biological testing. Results reveal that complex [Cu(2-(HOCH$_2$)py)$_3$](ClO$_4$)$_2$ shows better radical scavenging activity. Based on TAS, SOD and CAT activity, this complex was considered as a functional mimetic of the enzyme.

Silva *et al*[82] synthesized copper(II) complexes with ligands derived from 8-hydroxy quinoline (8-HQ). SOD activity of the complexes was evaluated. Results reveal that insertion of electron withdrawing substituents in the structure have increased mimetic activity of the complex.

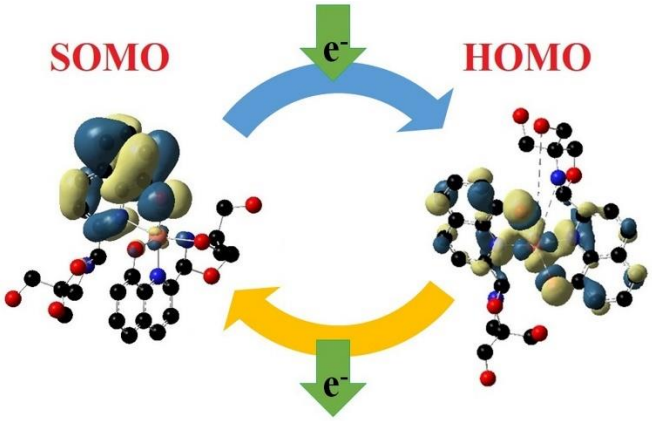


Figure 1.1.5 *Diagrammatic representation of copper(II) complexes reported by Silva et al*[82]

Bhatt *et al*[83] studied cytotoxicity, SOD mimic and antibacterial studies of ciprofloxacin based copper(II) complexes with isatin derivatives (**Figure 1.1.2.6**). The SOD-mimic activity showed that metal complexes could serve as potent antioxidants due to the presence of vacant coordination sites.

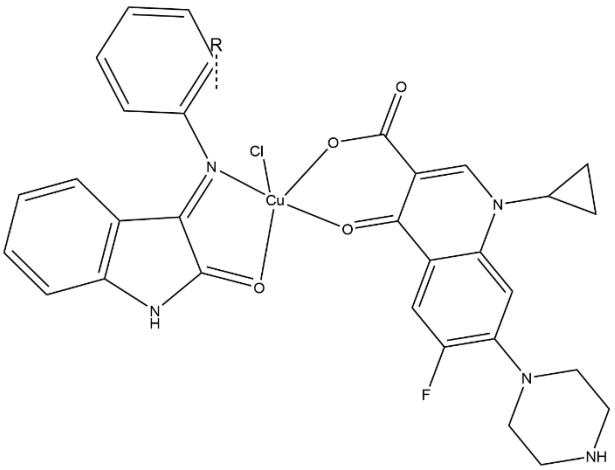


Figure 1.1.6 *Different copper(II) complexes with ciprofloxacin reported by Bhatt et al*[82]

Siqueria *et al*[84] synthesized six copper(II) complexes with ligands derived from pyridoxal to find the potential of these compounds to mimic the catalytic activity of the enzyme SOD (**Figure 1.1.2.7**).

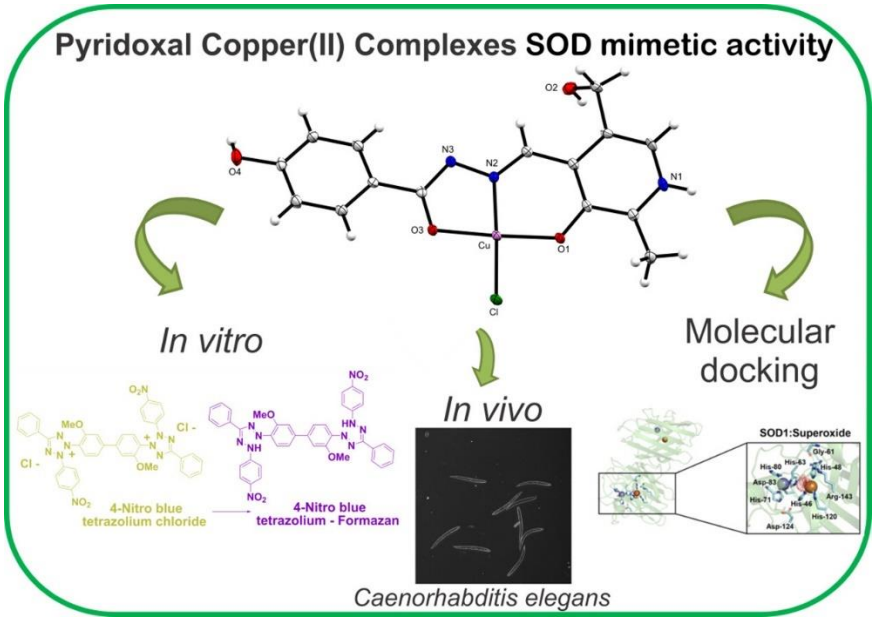


Figure 1.1.2.7 *Diagrammatic representation of pyridoxal copper(II) complexes along with SOD mimetic activity, Molecular docking and in vivo studies reported by Siqueria et al*[84]

1.1.3 Ascorbate Oxidase enzyme: Activity and Modelling

Multicopper oxidases containing a combination of type 1, type 2 and type 3 copper centres are also known as blue oxidases.[85] Laccase, ascorbate oxidase and

ceruloplasmin are the classical examples of these oxidase. Ascorbate oxidase was the first structurally characterized blue oxidase from zucchini squash in oxidized native, type-2 depleted, reduced, peroxide and azide forms. It is a homodimeric enzyme with molecular mass of 70kDa and 552 amino acid residues per subunit (zucchini). **Figure 1.1.3.1** shows the three-domain structure and the location of type 1 and trinuclear copper (type 2 + type 3) centres in the ascorbate oxidase monomer derived from crystal structure.

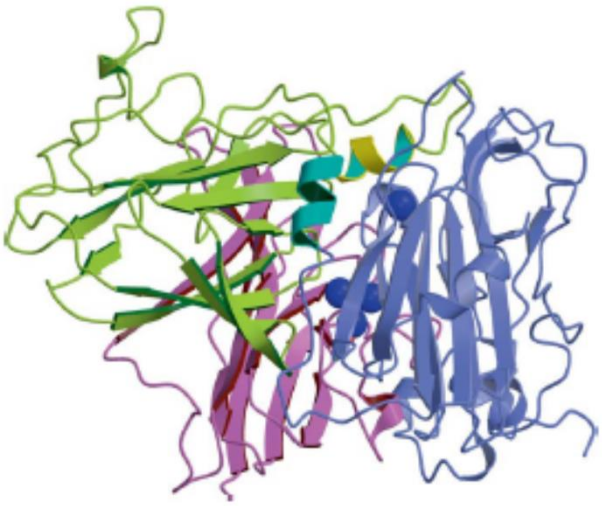


Figure 1.1.3.1 *Ribbon diagram of the monomer structure of ascorbate oxidase (pDB: 1AOZ); prepared with MOLSCRIPT*[85] *(Adapted from book section Copper Metalloenzymes*[85] *of Comprehensive Natural products II)*

A 'two-site ping pong bi bi' mechanism has been deduced for free laccase from steady-state kinetics. This is considered to be valid for ascorbate oxidase as well because both enzymes are structurally and mechanistically closely related.

Xia *et al*[86] synthesized peptide-Cu(II) complexes for oxidase mimicking activities for oxygen reduction and ascorbate oxidation. In the structure of peptide, a histidine residue (His) is in first position with respect to the free N-terminal amine. To explore the analytical performance of the peptide-Cu(II) complexes in biosensing, the complexes are easily integrated with gold nanoparticles to form nanocatalysts or nanozymes and sandwich electrochemical and fluorescent immunoassays of prostate specific antigen (PSA) (**Figure 1.1.3.2**) were performed. For the fluorescent assays of PSA, the nanolabels coined as nanozymes catalyzed the oxidation of ascorbic acid (AA), and the resulting oxidation product dehydroascorbic acid (DHA) was

reacted with o-phenylenediamine (OPD) to form fluorescent 3-(dihydroxyethyl)furo [3,4-b]quinoxaline-1-one (DFQ). The low detection limits of the methods (0.40 and 1.00 pg/mL) enabled clinical detection of PSA in serum samples. The findings help in understanding the biological functions of peptide-Cu^{2+} complexes and give a new insight to develop optical and electrochemical sensing platforms for clinical diagnosis.

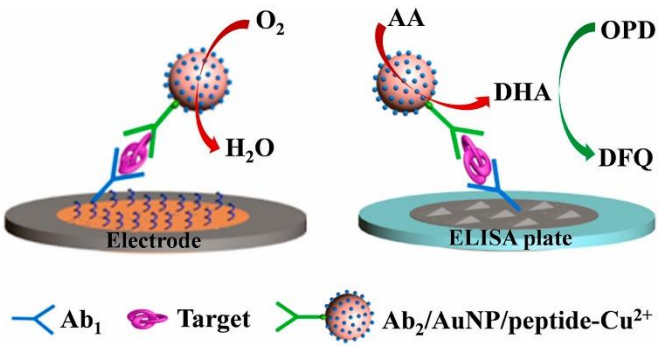


Figure 1.1.3.2 *Pictorial representation of nanozymes and sandwich electrochemical and fluorescent immunoassays of prostate specific antigen (PSA) reported by Xia et al*[86]

Avdeeva *et al*[87] studied the oxidation of L-ascorbic acid in the presence of the copper-binding compound (cbc) from Methanotrophic bacteria *Methylococcus capsulatus* (M). It was assumed that cbc can be involved in a multilevel system of antioxidant protection and can protect a bacterial cell from oxidation stress. Liu *et al*[88] designed a facile and green method for preparing monodisperse, homogenous copper nanoclusters (Cu NCs) with smaller size. They exhibited excellent tetraenzyme-like activities, including peroxidase, catalase, superoxide and ascorbic acid oxidase mimic activities. A novel fluorimetric ascorbic acid (AA) sensor was developed on the basis of the principle that AA is oxidized to dehydroascorbic acid (DHAA) by AAO-like activity while DHAA further reacts with o-phenylene diamine (OPDA) to form highly fluorescent quinoxaline (DFQ) derivative. Thus, the Cu NCs-based multienzyme mimic is a promising candidate for biocatalysis and biosensing.

1.1.4 Brief literature survey on synthesis of Schiff bases

Robson[89] in 1970 first introduced the concept of binucleating ligands so that it can simultaneously bind to two metal ions in close proximity. After this discovery, there has been steady increase in the synthesis of this type of ligands. Basically,

binucleating ligands are broadly classified into two categories (a) compartmental ligands and (b) ligands with isolated donor sets (**Figure 1.1.4.1**)

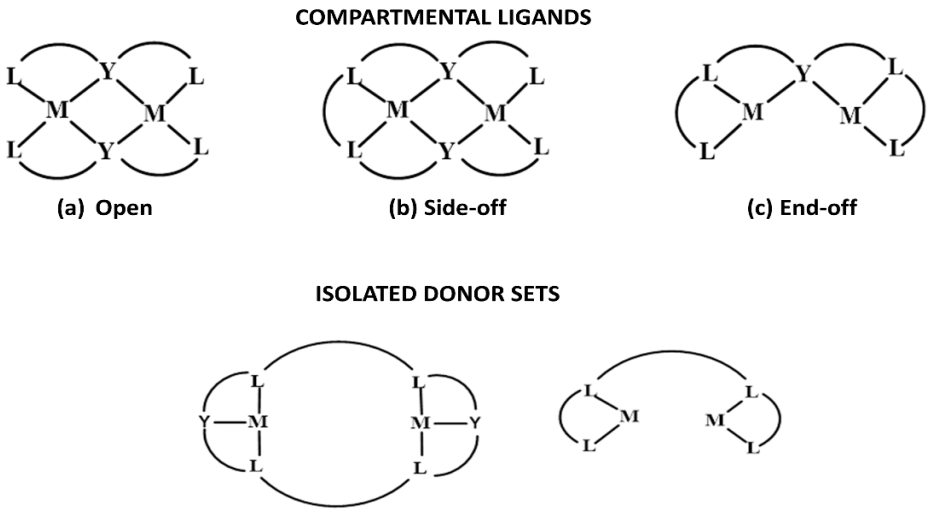


Figure 1.1.4.1 *Schematic representation of types of binucleating ligands*

Specially designed Schiff base ligands[90,91] may be used as compartmental ligands[92]. They are privileged ligands as they can be formed by condensation between formyl and amine precursors. These ligands lead to planar or three dimensional complexes in a single step. Further advantages include, (i) extra donor sets can be introduced for better complexation (ii) controlling steric and electronic factors becomes possible based on the selection of starting materials (iii) template synthesis may be employed as alternative method for complex formation (iv) can be doped on the surface of nanomaterials to functionalize the surface of the material which may enhance the catalytic activity (v) stereogenic centres or chiral centers can be incorporated while designing the ligands.

1.2 Aim and scope of the present work

The present work has been carried out, keeping all the above considerations in mind and to find answers to some of the questions such as:

- Can the change in the flexibility of ligands or change in the auxiliary part of the ligands can influence activity of the complexes?
- Can all structural models of enzyme active sites, particularly of catechol oxidases, behave as functional models also?
- Do the complexes with good enzyme mimic activity have good cytotoxic activity and can bind with DNA and BSA also?

The work involves synthesis of new binucleating ligands and binuclear complexes derived from the Schiff bases of (a) 2,6-diformyl-4-methylphenol, (b) 2,6-diacetyl-4-methylphenol (c) 1,3-diaminopropan-2-ol and (d) 2,6-bis(aminomethyl)-4-nitrophenol, with appropriate amines and carbonyl compounds, their characterization and study of their ability to bind and activate various substrates and biomolecules. An attempt is made to evolve a structure activity relationship. The work is presented in the next five chapters.

Chapter 2 deals with three end off compartmental Schiff base ligands of biogenic amines, namely, tryptamine, histamine and pyridoxamine formed by condensation with 2,6-diformyl-4-methylphenol **(L^1-L^3).** Homometallic Cu(II)Cu(II) complexes **(C1a-C3a)** and the heterometallic Cu(II)Zn(II) complexes **(C1b-C3b)** have been synthesized using these ligands. All synthesized ligands and complexes have been characterized by various spectroscopic methods (IR, UV-Vis, NMR, mass spectrometry), X-ray crystallography (representative compounds), elemental analysis, ESR spectroscopy and magnetic measurements. Enzyme mimic activities such as SOD, catecholase and ascorbate oxidase activities have been studied. Superoxide anion ($O_2^{\bullet-}$) generated in nonenzymatic i.e. PMS (Phenazine methosulphate)/ NADH (Nicotinamide adenine dinucleotide reduced) systems in the presence or absence of test compounds, and scavenging of $O_2^{\bullet-}$ was determined by monitoring reduction in rate of NBT to monoformazan dye formation. Kinetics of the catecholase and ascorbate oxidase activity of the complexes has been studied by initial rate method and the activation energy determined. Michealis Menten model for enzyme kinetics was applied and the V_{max} and K_m values were calculated. The homometallic Cu(II)Cu(II) complexes were found to be more active as compared to heterometallic Cu(II)Zn(II) complexes. The details of all these findings have been discussed in this chapter.

Chapter 3 deals with the synthesis of dicopper(II) complexes with end-off compartmental Schiff base ligands of nitrogen rich amines (such as N-aminoethyl piperazine, N-aminoethyl morpholine, N-aminoethyl pyrrolidine, 2-picoyl amine and tryptamine) formed by condensation with 2,6-diacetyl-4-methylphenol (**L^4-L^8**) *in situ*. All complexes, (**C4-C8**) have been characterized by various spectroscopic methods (IR, UV-Vis, NMR, mass spectrometry), X-ray crystallography (representative compounds), elemental analysis and ESR spectroscopy. Enzyme

mimic activities such as SOD, catecholase and ascorbate oxidase activities have been studied using UV-Vis Spectroscopy. The activities have been compared based on the structures of the complexes.

Chapter 4 deals with the synthesis of dicopper(II) complexes (**C9-C12**) of end-off compartmental ligands, (**L^9-L^{12}**), derived from isomeric acetonaphthones and 7-hydroxy-8-formyl-4-methyl coumarin by condensation with 1,3-diaminopropan-2-ol All synthesized ligands and complexes have been characterized by various spectroscopic methods (IR, UV-Vis, NMR, mass spectrometry), X-ray crystallography (representative compounds), elemental analysis, ESR spectroscopy and variable temperature magnetic measurements. Enzyme mimic activities such as SOD, catecholase and ascorbate oxidase activities have been studied using UV-Vis Spectroscopy. An interesting observation was made about the selectivity of these complexes towards substrates. The results of these studies are presented in this chapter.

Chapter 5 deals with the synthesis of dicopper(II) complexes (**C13-C16**) of end-off compartmental diimine ligands (**L^{13}-L^{16}**), derived from isomeric acetonaphthones, pyrrole-2-carboxaldehyde and 7-hydroxy-8-formyl-4-methyl coumarin by condensation with 2,6-bis(aminomethyl)-4-nitrophenol. All synthesized ligands and complexes have been characterized by various spectroscopic methods (IR, UV-Vis, NMR, mass spectrometry), elemental analysis and ESR spectroscopy. Enzyme mimic activities such as SOD, catecholase and Ascorbate oxidase activities have been studied using UV-Vis spectroscopy.

Chapter 6 deals with the binding interactions of the synthesized copper(II) complexes with two important biomolecules, DNA and Serum albumin by UV-Vis and fluorescence spectroscopy. The anticancer activity of a few selected dicopper(II) complexes from cytotoxicity (MTT) assay on human hepatoma (HepG2) cancer cell line has been determined. An attempt has been made to evolve a structure-activity relationship.

Finally, a summary and a cumulative discussion of the results is presented in this thesis.

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