SYNOPSIS

of the thesis entitled

New Synthetic Mimics of Type 3 Copper Active Site: Characterization, Dioxygen Transport and Radical Quenching

to be submitted

for the award of the degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

by

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under the supervision of

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SYNOPSIS OF THE THESIS

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Research Student	—	**Priyanka Sandesh Salunke**
Title of Thesis	—	***New Synthetic Mimics of Type 3 Copper Active Site: Characterization, Dioxygen Transport and Radical Quenching***
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The work presented in this thesis deals with developing new binuclear complexes as synthetic models for active sites in metalloenzymes, specially, those containing copper(II) ions at the active site. The newly synthesized complexes have been characterized by various techniques and various types of activities associated with them have been studied. The thesis will be presented in the form of following chapters:

Chapter 1

INTRODUCTION

Chapter 2

μ-Phenoxo bridged homometallic Cu(II)Cu(II) and heterometallic Cu(II)Zn(II) complexes of compartmental Schiff bases derived from biogenic amines

Chapter 3

μ-Phenoxo bridged dicopper (II) complexes of binucleating diimines derived from 2,6-diacetyl-4-methyl phenol

Chapter 4

μ-Alkoxo bridged dicopper (II) complexes of compartmental Schiff bases of bidentate aldehydes & naphthonimines

Chapter 5

μ-Phenoxo bridged dicopper (II) complexes of Schiff bases derived from 2,6-diaminomethyl-4-nitro phenol

Chapter 6

DNA & BSA binding and Cytotoxicity studies of compartmental ligands & complexes

Summary

The thesis contains a total of six chapters where **Chapter 1** deals with a general introduction with special emphasis on Schiff base ligand and synthetic analogues of catechol oxidase, ascorbate oxidase and SOD enzyme. Design, synthesis and reactivity of various copper complexes reported so far, their catalytic or biological activity and literature related to the present work has been discussed. **Chapter 2-6** summarize the entire research work that I have carried in my research lab. Finally, the summary of overall entire work.

Chapter 1 Molecular oxygen is considered as primary and ideal oxidant for use in bulk chemical industries from the economic and environmental point of view as it is direct availability from air. [1–4] However, due to kinetically inert nature of molecular oxygen, its application is challenging while 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 [4–10] of using catalytic amount of an activator in oxidation reactions to activate the molecular oxygen with minimum chemical waste is very attractive. Though 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. 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.[11-15]

In recent decades, the development of biomimetic oxidation catalysts, involving copper(II) ion as active metal center has received great attention due to the versatility of the copper-O2 chemistry involved in the copper containing active sites of metalloenzymes[16-21](**Figure 1.1**).

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.

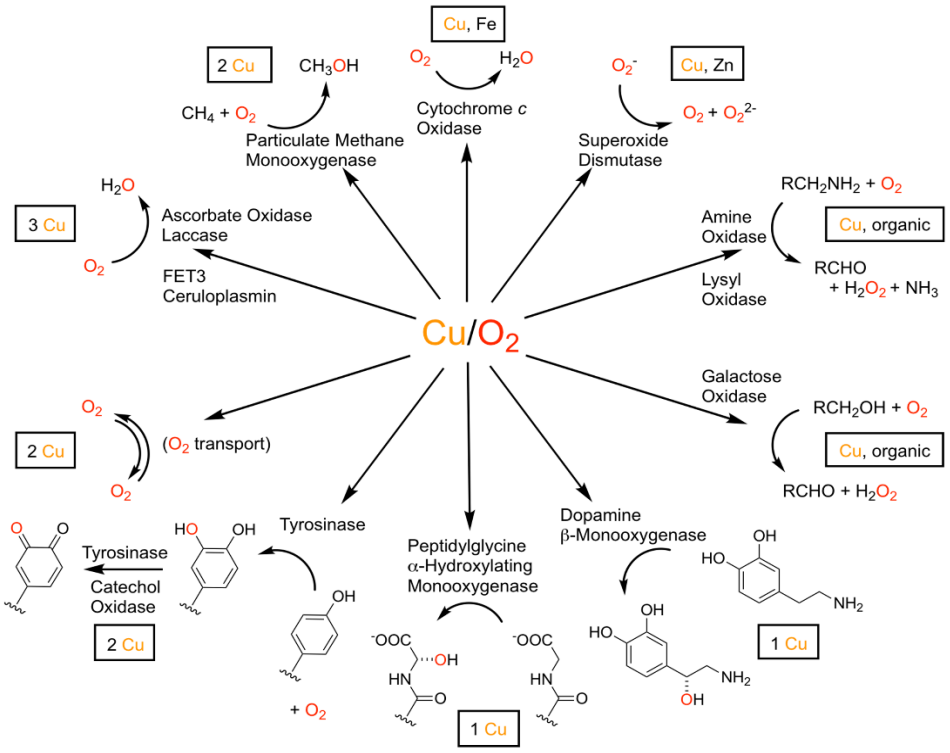


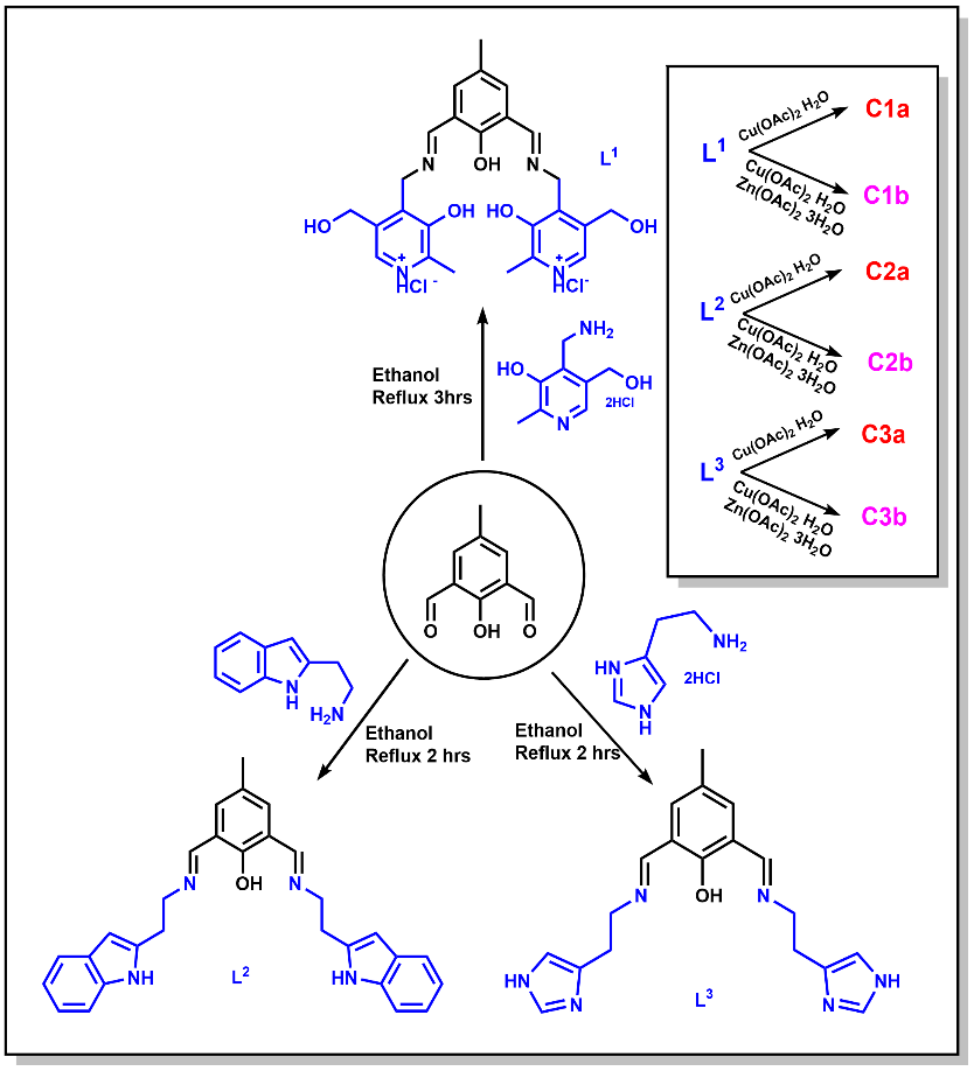
Figure 1.1 *Potential role of copper in biosynthetic pathway*

Several attempts have been made to understand the mechanism[13-15,22-24] involved in the catechol oxidase activity and efforts have been put in developing synthetic structural as well as functional models for the active site of catecholase[13-15, 25-27]. Many efforts have been made for the synthesis of low molecular weight copper (II) complexes having SOD activity[28-35]. However, the reports on ascorbate oxidase mimics are scanty. There is one with copper-binding compound from bacteria[36] and another with copper nanoclusters[37].

Structural-functional stability, high rate, selectivity, ability to bind to a substrate are the desired qualities of synthetic models. 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. Specially designed Schiff base ligands[38,39] may be used as compartmental ligands[40]. They are privileged ligands as they can be designed to have large variations in their structures, flexibility and auxiliary groups.

A detailed account of these will be presented in this chapter.

Chapter 2 deals with three end off compartmental Schiff base ligands of biogenic amines, namely, Tryptamine, Histamine and pyridoxamine with 2,6-diformyl-4-methylphenol **(L^1-L^3)**. The reactions of these ligands have been carried out with 2 eq of copper(II) acetate monohydrate to form its homometallic Cu(II)Cu(II) complexes **(C1a-C3a)** & with 1 eq each of zinc(II) acetate hexahydrate and copper(II) acetate monohydrate to form heterometallic Cu(II)Zn(II) complexes **(C1b-C3b)**.



Scheme 2.1 End-off Compartmental Schiff bases ligands (L^1-L^3) and its Cu(II)Cu(II) and Cu(II)Zn(II) complexes

All synthesized ligands and complexes have been characterized by various spectroscopic methods (such as IR, UV-Vis, NMR, Mass, Elemental analysis and single crystal analysis), ESR analysis and Magnetic measurements.

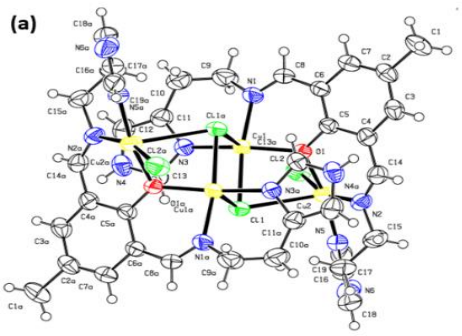


Figure 2.1 *ORTEP (50% probability) of complex C3a*

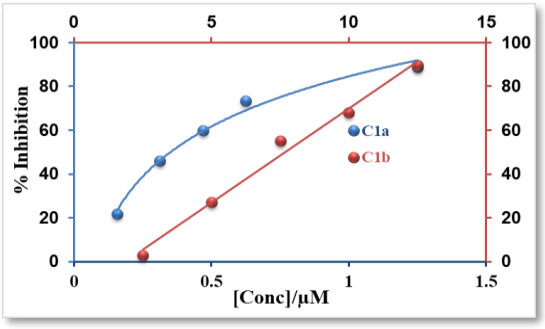


Figure 2.2. *Plot of % Inhibition vs Conc. of complex*

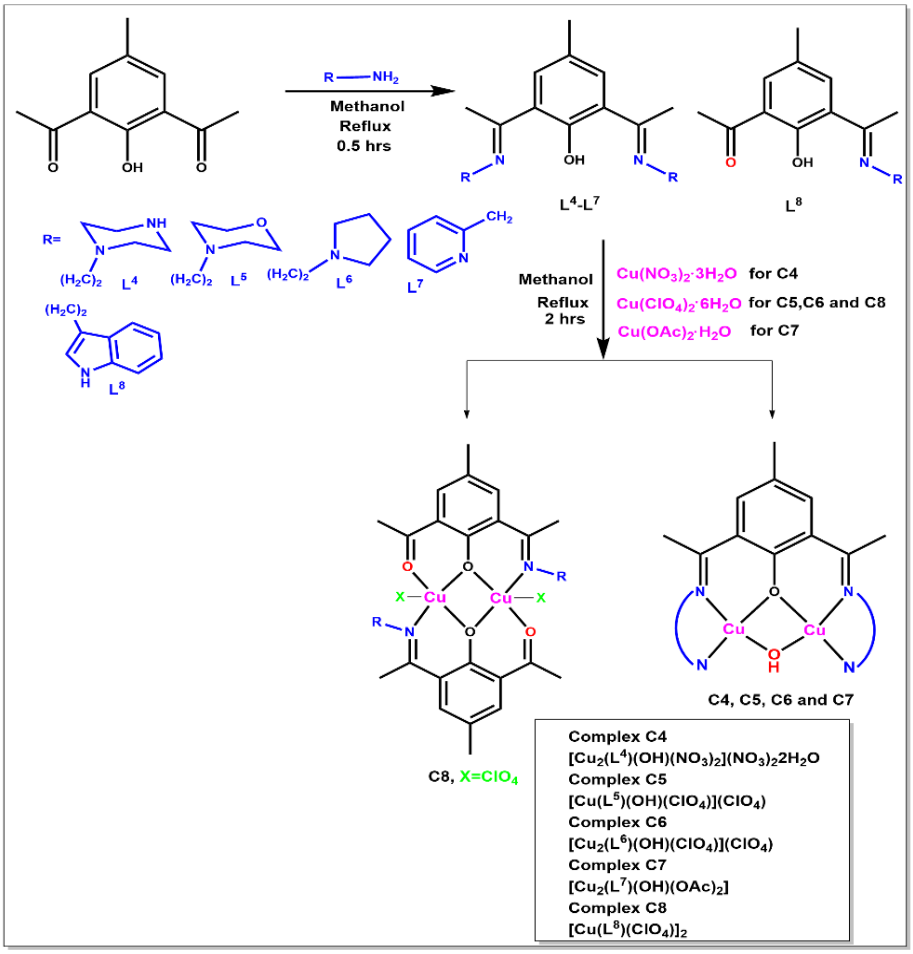
Enzyme mimic activities such as SOD, catecholase and Ascorbate oxidase activities have been carried out by using UV-Vis spectroscopy. Superoxide anion ($O_2^{\bullet-}$) was generated in nonenzymatic i.e. PMS (phenazine methosulphate)/ NADH (Nicotinamide adenine dinucleotide reduced) systems in the presence or absence of test compounds, and scavenging of $O_2^{\bullet-}$ was determined by monitoring reduction in rate of NBT to monoformazan dye formation monitored at 560 nm in UV-vis spectra. The IC_{50} values of all synthesized complexes were found to be in the range of 0.351-208.1µM. **Complex C1a** was found to be more active as compared to other complexes but less active as compared to the native enzyme. The homometallic Cu(II)Cu(II) complexes were found to be more active as compared to heterometallic Cu(II)Zn(II) complexes. Ascorbate oxidase activity of all synthesized complexes has been studied using UV-Vis spectroscopy. Kinetics of the reaction have been studied wherein three parameters, concentration of ascorbic acid, concentration of complex and temperature, have been varied. Rates were calculated by initial rate method. Michealis Menten model was applied for enzyme kinetics. The Lineweaver-Burk plot was used to calculate V_{max} and K_m values Arrhenius plot was used to calculate the activation energy.

Catecholase activity of the complexes has been studied using UV-Vis spectroscopy. Two substrates have been employed in this chapter, namely, 3,5-Ditert-butylcatechol and 4-methyl catechol. The concentration of substrate, concentration of complex and temperature have been varied. Complex 1a and 1b-3b were inactive.

Complex 2a was found to have more ascorbate oxidase and catecholase activity than the other complexes. The homometallic Cu(II)Cu(II) complexes were found to be more active as compared to heterometallic Cu(II)Zn(II) complexes.

Chapter 3 deals with the synthesis of dicopper(II) complexes with end-off compartmental diimine ligands of nitrogen rich amines (such as N-aminoethyl

piperazine, N-aminoethyl morpholine, N-aminoethyl pyrrolidine, 2-picoyl amine and tryptamine) with 2,6-diacetyl-4-methylphenol ($\mathbf{L^4}$-$\mathbf{L^8}$).



Scheme3.1 *Schematic representation of ligand and its copper(II) complexes*

The ligands were used *in-situ* with 2 eq of copper(II) acetate monohydrate to get the corresponding dicopper(II) complexes (**C4-C8**). They have been characterized by various spectroscopic methods (such as IR, UV-Vis, NMR, mass spectrometry and elemental analysis) and ESR analysis. The crystal structure of complexes **C4** and **C6** was determined.

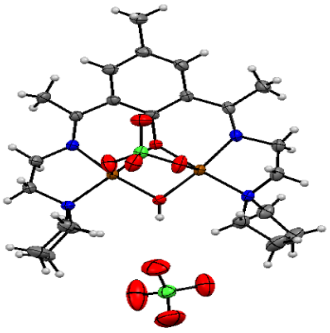


Figure 3.1 ORTEP (50% probability) of complex C6

Enzyme mimic activities such as SOD, catecholase and Ascorbate oxidase activities have been studied using UV-Vis Spectroscopy. SOD mimic activity was studied by formazan reduction method while ascorbate oxidase and catecholase activity of all complexes was studied by UV-Vis spectroscopy.

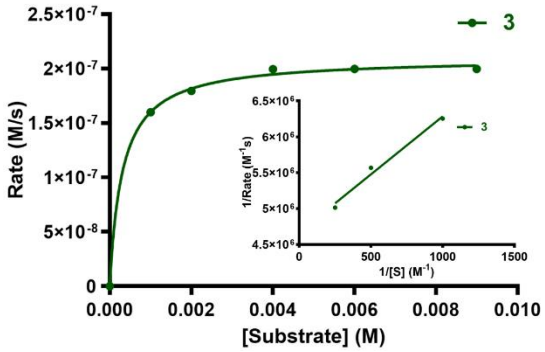
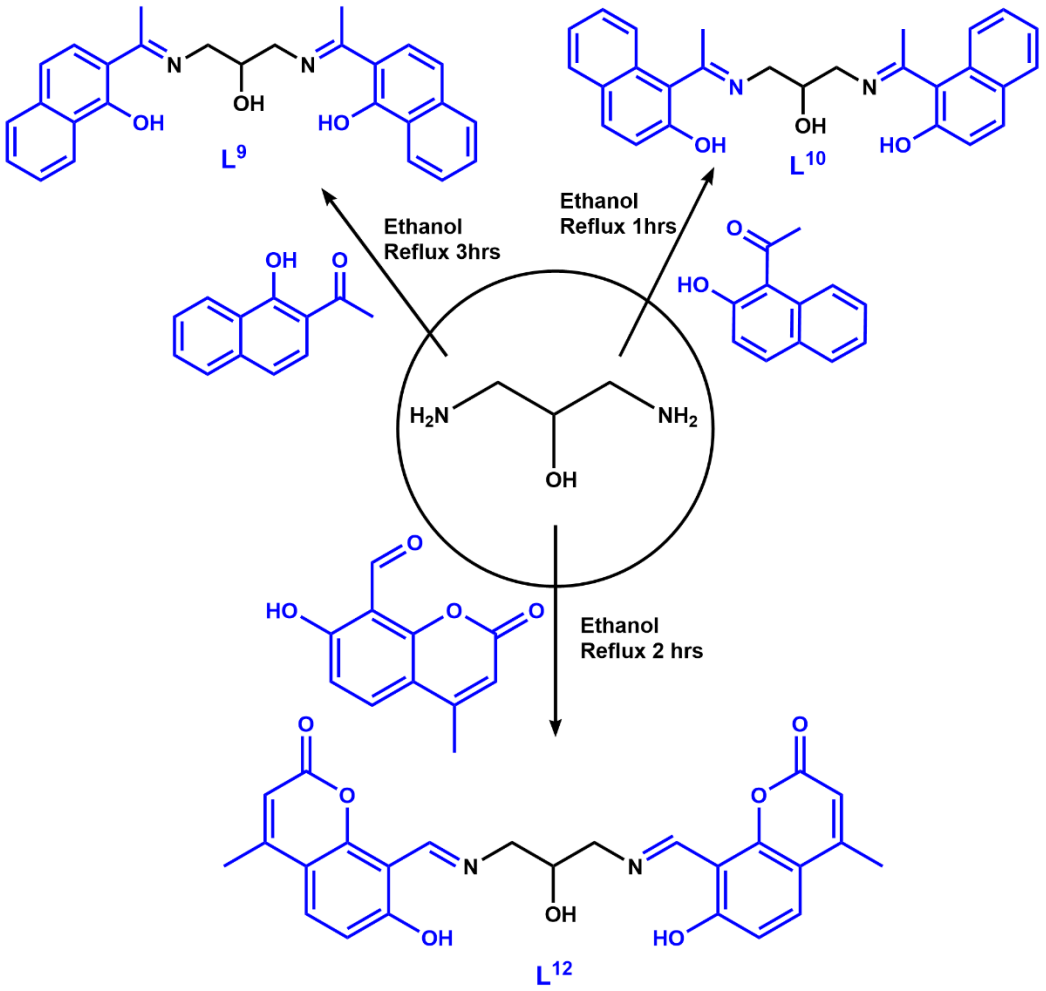


Figure 3.3. *Plot of rate vs [S] (Inset: Lineweaver Burk plot)*

Complex C5 was found to be more active towards superoxide dismutation while **Complex C8** was found to be more active towards the oxidation of ascorbic acid and catechol derivatives.

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 with 1,3-diaminopropan-2-ol by reaction with 2 eq of copper(II) acetate monohydrate. All synthesized ligands and complexes have been characterized by various spectroscopic methods (such as IR, UV-Vis, NMR, Mass, Elemental analysis and single crystal analysis), ESR analysis and Magnetic measurements.

Enzyme mimic activities such as SOD, catecholase and Ascorbate oxidase activities was studied using UV-Vis Spectroscopy. **Complex C9** was found to be more active as compared to other synthesized complexes but was less active as compared to native enzyme for SOD activity. **Complex C10** was found to more active as compared to other complexes with ascorbic acid, 3,5-DTBC and 4-methyl catechol. **Complex C9** was found to be more active for dopamine as compared to other complexes. This shows the selectivity of substrate towards the complex**. Complex C12** was found to be inactive for all substrates employed.



Scheme 4.1 *Schematic representation of synthesized ligands (**L**[9], **L**[10] and **L**[12])*

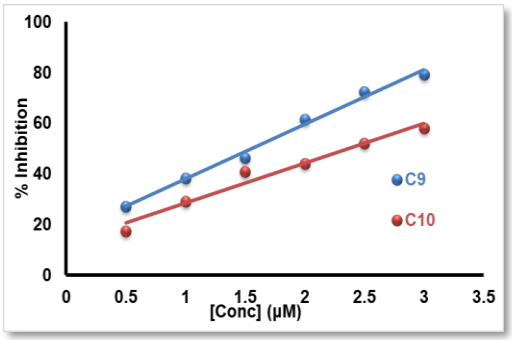


Figure 4.1 Plot of % Inhibition vs Conc. of complex

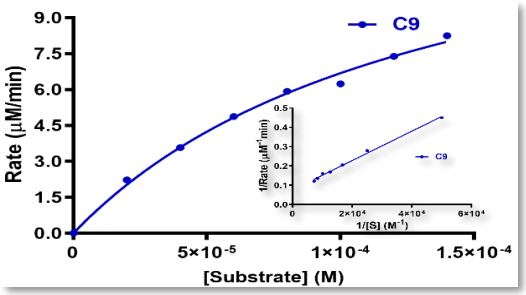
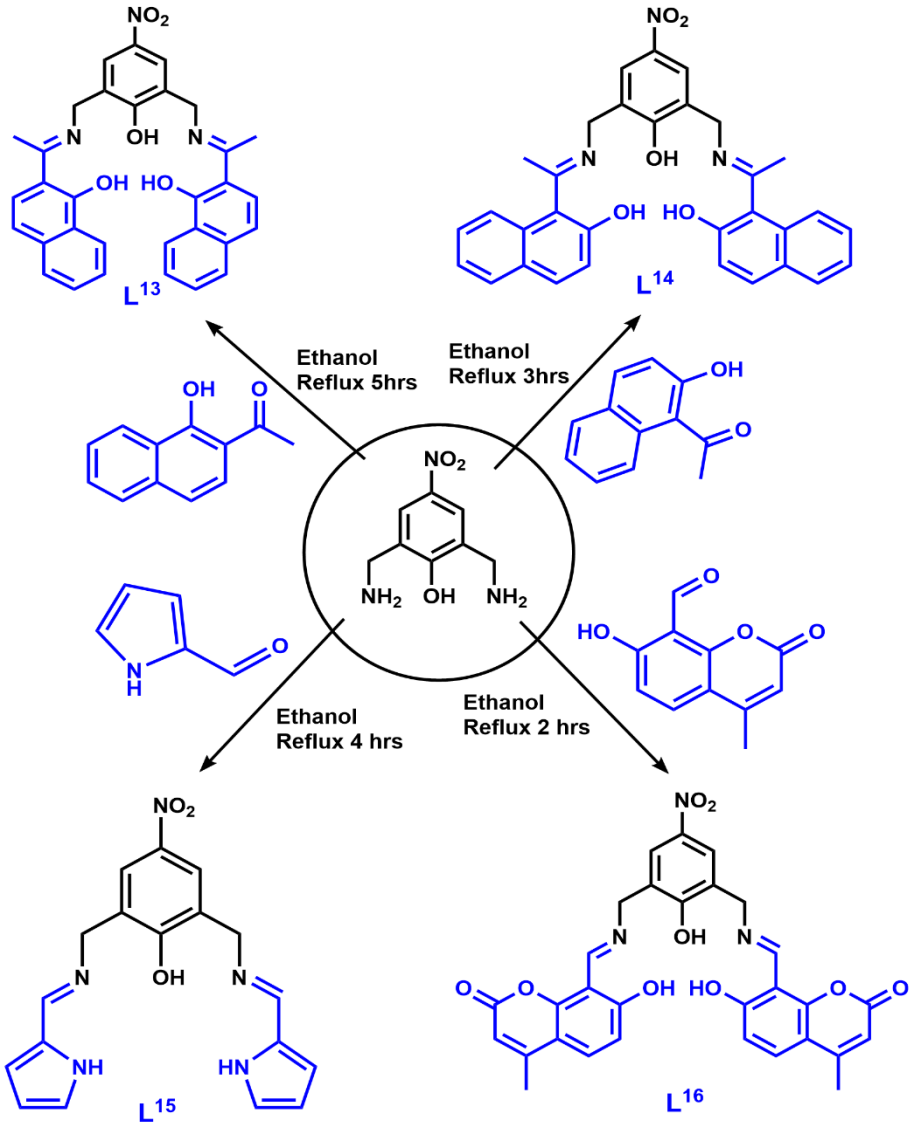


Figure 4.2 Plot of rate vs [S] (Inset: Lineweaver Burk plot)

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 with 2,6-bis(aminomethyl)-4-nitrophenol. All ligands were reacted *in-situ* with 2 equivalents of copper(II) acetate monohydrate to get the desired complexes. All synthesized ligand

and complexes have been characterized by various spectroscopic methods (such as IR, UV-Vis, NMR, Mass, Elemental analysis), and ESR analysis.



Scheme 5.1 *Schematic representation of synthesized ligands (**L**[13]-**L**[16])*

Enzyme mimic activities such as SOD, catecholase and Ascorbate oxidase activities have been carried out by using UV-Vis Spectroscopy. **Complex C13** was found to be more active as compared to other synthesized complexes but was higher as compared to native enzyme for SOD mimic activity. **Complex C13-C15** was found to have significantly same activity as compared to **complex C16** in ascorbate oxidase activity. **Complex C13** have better catecholase activity as compared to other complexes with 3,5-DTBC and only **C13** is active for 4-methyl catechol.

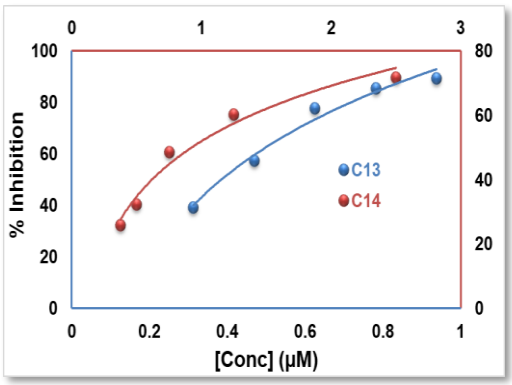


Figure 5.3. *Plot of % Inhibition vs Conc. of complex*

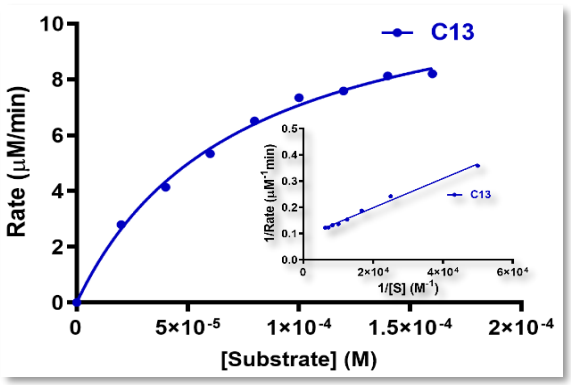


Figure 5.4. *Plot of rate vs [S] (Inset: Lineweaver Burk plot)*

Chapter 6 deals with the binding interactions of the synthesized copper(II) complexes with two important biomolecules; DNA and Serum albumin. The strength of binding i.e. the binding constants have been determined using UV-Vis spectroscopy and fluorescence spectroscopic methods.

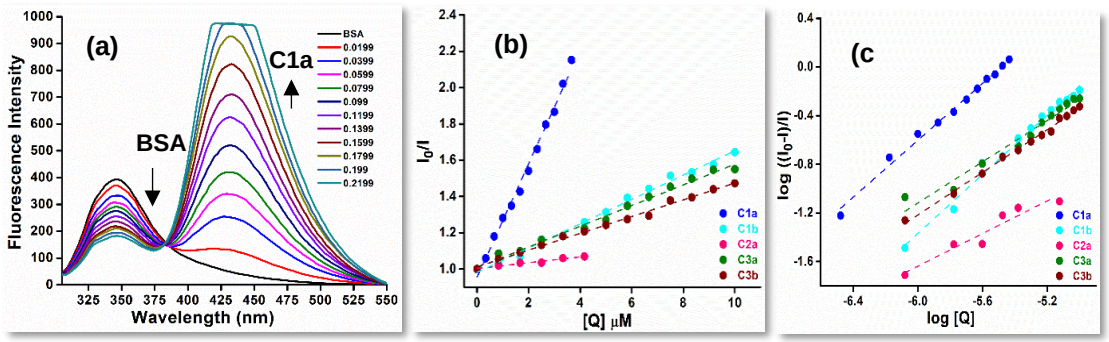


Figure 6.1 *(a) The emission spectra of BSA (1 x 10^{-6} M; λ_{ex} = 296 nm, λ_{em} = 344 nm) in presence of increasing amounts of C1a (b) Stern-Volmer plots and (c) Scatchard plots of fluorescence quenching with different concentrations of complexes*

Anticancer activities of selected copper(II) complexes from *in-vitro* cytotoxicity (MTT) assay on human hepatoma (HepG2) cancer cell line have been determined. The cell viability values (%) were obtained with continuous exposure of the cells to the said compounds for 24 h. The cytotoxicity of the complexes was found to be dose dependent, that is, the cell viability decreased with increasing concentrations of the complexes (**Figure 6.2**).

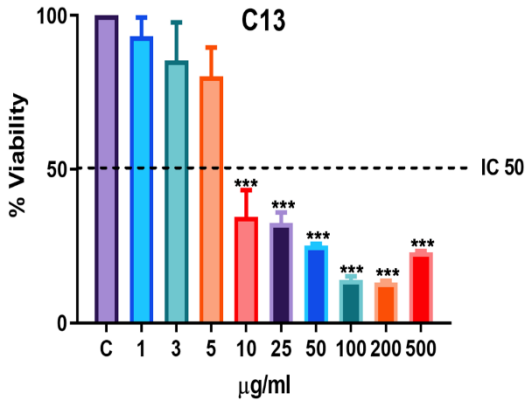


Figure 6.2 *Cell viability verses concentration plots of* ***C13*** *on human hepatoma (HepG2) cell line. Each point is the mean ± standard error obtained from three independent experiments*

It was observed that complex **C13 (IC_{50} = <10 µg/mL)** is the most cytotoxic among all selected complexes for this activity and also when compared to its isomeric copper(II) complex **C14 (IC_{50} = 25-50 µg/mL)** . All other complexes have cytotoxicity in the range of **50-200 µg/mL.**

Finally, a **summary** and a cumulative discussion of the results obtained in this study has been presented.

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