CHAPTER VI
CONCLUSION AND FUTURE WORK

The design of new metal based chemotherapeutic agents is in the forefront research area of inorganic medicinal chemistry. It is well known that medicinal inorganic chemistry is a multidisciplinary field combining elements of chemistry, pharmacology, toxicology and biochemistry. In recent years, there has been a rapid expansion in the development of metal complexes as extensive diagnostic agents/chemotherapeutic drugs [1].

In the search towards new metallic species with pronounced biological applications, copper compounds have proved to be excellent candidates. Copper complexes have been extensively utilized in metal-mediated DNA cleavage for the generation of activated oxygen species. The appropriate redox property of Cu(II) is essential for various metabolic pathways like mitochondrial respiration, free radical scavenging and iron absorption where it acts as a catalytic cofactor. The Cu(II) complexes follows different mode of action towards DNA (non-covalent) as compared to cisplatin (covalent). Therefore, Cu(II)-based complexes exhibit higher antineoplastic potential towards human ovarian carcinoma, murine leukaemia and various cervicouterine carcinomas as compared to cisplatin.

In this work, the possibility to combine the potential pharmacological effects of β-ketoanilide moiety and 2-aminobenzothiazole scaffold. To the best of our knowledge, there is no literature data on preparation, structural elucidation and biological activity of Knoevenagel condensate of β-ketoanilide based 2-aminobenzothiazole derivatives. Hence, the higher degree of conjugated versatile ligand systems of the Knoevenagel condensate β-diketimine as Schiff bases containing electron releasing/electron withdrawing groups and their low molecular weight copper(II) complexes have been synthesized. It is of interest to carryout investigations on copper complexes to find out how a ligand environment could affect the redox properties of the metal and thereby,
the spectral properties. It is also interested to explore the DNA binding and DNA cleavage activity of synthesized complexes.

The thesis is divided into six chapters. The chapter I consists of an introduction and review of published work on copper complexes of 2-aminobenzothiazole derivatives. In the chapter 2, materials, methods and instruments used for the various studies are described. Synthesis of different curcumin analogs of 2-aminobenzothiazole derivatives and their corresponding Cu(II) complexes are described in the chapter 3. In the chapter IV, structural elucidation of these prepared ligands and their complexes were performed on the basis of analytical and spectral techniques. The biochemical and pharmacological studies (antimicrobial, DNA binding, DNA cleavage, thermal denaturation, antioxidant, SOD, partition coefficients, catalase activity) have been carried out for the synthesized ligands and Cu(II) complexes are summarized in the chapter 5. The conclusion is presented in the chapter 6.
Chapter 1 comprises of general introduction of parent molecules, Schiff base ligands & metal complexes and also importance of copper complexes is better than other transition metal complexes in therapeutic fields. It is also summarizes an overview of literature on metal complexes of 2-aminobenzothiazole derivatives. Finally, it is ended with the scope of the present research work.

Chapter 2 summarized the detailed description of the experimental methods, materials and instruments for the various studies in this work.

Chapter 3 includes synthesis of Schiff bases of 2-aminobenzothiazole and their copper complexes. It is worthwhile to undertake studies to identify the best compound or lead compound optimization through systematic structural modifications of β-ketoanilide compounds. In this chapter, a new series of 2-aminobenzothiazole derivatives and their Cu(II) complexes were prepared. This chapter is divided into six sections.

In the 1st Section, it deals with Schiff bases have been showed a variety of biological activities like antibacterial, antifungal, fungicidal and clinical activities by virtue of its imine linkage. Polyfunctional ligands system of 2-aminobenzothiazoles has been studied as central muscle relaxants and are found to interfere with glutamate neurotransmission in biochemical, electrophysiological and behavioural experiments and reported as neuroprotectors.

Here, the series of Knoevenagel condensate of β-ketoanilide with different substituted aromatic aldehydes [L1/3-hydroxy-4-methoxybenzaldehyde, L2/3-hydroxybenzaldehyde, L3/4-methoxybenzaldehyde, L4/4-nitrobenzaldehyde, L5/2-chlorobenzaldehyde, L6/3,4-dimethoxybenzaldehyde, L7/3-chlorobenzaldehyde, L8/4-dimethylamino benzaldehyde, L9/Cinnamaldehyde, L10/3-nitrobenzaldehyde and L11/4-chlorobenzaldehyde was refluxed in the presence of potassium carbonate as a catalyst in ethanolic medium (Scheme-1). The experimental results like elemental analysis, molar conductance, FAB-Mass, 1H-NMR and 13C-NMR are given in this
section. The synthetic pathway for copper complexes of Knoevenagel condensate of β-ketoanilide analogs was illustrated in Scheme 1.

Scheme - 1

In Section 2, the Knoevenagel condensate of β-ketoanilide (s) was obtained from the equimolar mixture of β-ketoanilide and substituted heterocyclic aldehyde(s) (L\textsubscript{12}-5-methyl thiophene-2-carboxyaldehyde, L\textsubscript{13}-Furan-2-carboxyaldehyde, L\textsubscript{14}-Pyrrole-2-carboxyaldehyde). Further, it was reacted to form Schiff bases with 2-aminobenzothiazole and their corresponding copper complexes (L\textsubscript{12}-L\textsubscript{14}) were also synthesized.

In Section 3, the series of curcumin analogs were synthesized (L\textsubscript{15}-L\textsubscript{24}). Their corresponding Cu(II) complexes of L\textsubscript{15}-L\textsubscript{24} were also prepared and experimental data are summarized. Further, the aromatic ring contains different substituent groups, including electron-withdrawing groups (e.g., Cl and NO\textsubscript{2}) and electron-donating groups.
(e.g. CH₂O), were employed with the hope to investigate the structure–activity relationship (SAR) of the substituents in the curcumin analogs.

In this 4th section continuation with slight modifications of above procedure using furan-2-carboxyaldehyde instead of substituted benzaldehydes in the step 1, in order to evaluate the pharmacological potency of wide variety of curcumin analogues. The formation of effective ligand system of curcumin analogs (L²⁵-L³⁴) and their corresponding Cu(II) complexes of L²⁵-L³⁴ were prepared. The experimental results are given in this chapter.

In 5th section, the Knoevenagel condensate of β-ketoanilide (s) (L³⁵-L⁴⁴) form schiff bases with 2-aminothiazole instead of 2-aminobenzothiazole and their copper complexes (L³⁵-L⁴⁴) were synthesized.

In 6th section, the Schiff bases (derived from 2-aminobenzothiazole with substituted benzaldehydes) (L⁴⁵-L⁵⁴) and their corresponding copper complexes of L⁴⁵-L⁵⁴ were prepared.

**Chapter 4, Structural characterization of ligands and their copper complexes**

Chapter 4 The formation of the ligand (L¹-L⁵⁴) was confirmed by various analytical & spectroscopic techniques as elemental analyses, molar conductance, magnetic moment studies, ¹H-NMR, ¹³C-NMR, FAB mass, IR, UV-Vis, ESR, electrochemical and TGA studies. They are sparingly soluble in common organic solvents but soluble in DMF and DMSO. The lower molar conductance values of all complexes support their non-electrolytic nature, evidenced by the involvement of acetate ions/chloride ions in coordination.

The formation of Schiff base ligand system of 2-aminobenzothiazole was confirmed by ¹H-NMR spectra, recorded in DMSO-d₆ solution at room temperature. The ligand L¹ showed the following spectral features for Schiff base ligand system of
Knoevenagel condensate: The presence of aromatic protons of substituted phenyl ring appeared in the region between 6.68-7.19 δ ppm (m, 3H) and the β-ketoanilide ring protons appears as multiplet in the region of 7.20–7.39 δ ppm (m, 5H), an aldehydic proton appeared at 9.85 (s, H), –OCH₃ at 3.29-3.40 (s, 3H) and OH group at 10.32 δ ppm (s, 1H). In addition, peak appeared at 8.18 δ ppm, which is assigned to free –NH group of β-ketoanilide moiety and methyl protons at 2.22-2.26 δ ppm (s, 3H). Moreover the multiplet within the range 7.41-7.53 δ ppm (m, 3H) was assigned to the aromatic protons of benzothiazole ring. It was concluded that the absence of amino group of 2-aminobenzothiazole indicated the formation of Schiff base ligand system.

The binding mode of the Schiff base to the metal ion was confirmed by IR Spectra. The IR spectrum of the ligand L¹ showed band at 1663 and 1639 cm⁻¹ for the imine ν(C=N) groups which results from the Schiff base condensation of 2-aminobenzothiazoles and Knoevenagel condensate, further it was shifted to a lower frequency of 1623 and 1600 cm⁻¹ after complexation. Moreover, the appearance of new bands at 474 cm⁻¹ and 435 cm⁻¹ corresponds to ν(M-N) and the new bands at 547 and 520 cm⁻¹ attributed to ν(M-O). Also the new bands at 1477 cm⁻¹ and 1276 cm⁻¹ corresponds to asymmetric (υ₃(COO⁻)) and symmetric (υ₁(COO⁻)) stretching for ν(M-O) which evidenced the participation of the COO⁻ ion in the complexes.

From ESR and electronic spectral data, a distorted square planar geometry has been arrived for [CuL¹(OAc)₂]. The electronic spectrum of the corresponding complex in DMSO reveals a broad band at 429 nm assigned to ²B₁g→²A₁g transition which is characteristic of distorted square planar environment around the copper(II) ion. The ESR spectrum of the copper complex is recorded in DMSO at 300 and 77 K. The observed trend of copper complex of L¹ is, g|| (2.24) >g⊥ (2.05) >gₑ (2.0023) describes the axial symmetry with the unpaired electron residing in the dₓ²₋ᵧ² orbital. The empirical factor f = g|| /Aₑ cm⁻¹ for copper complex of L¹ is 153 cm⁻¹, confirm distorted square planar structure of copper complex.
Mass spectra provide a vital clue for elucidating the structure of compounds. The FAB mass spectra of the Schiff bases and their corresponding copper complexes were recorded and compared their stoichiometry compositions. The intensity of these peaks reflects the stability and abundance of the ions. The molecular ion peak for the ligand (L$^1$) is observed at 387 m/z whereas its copper complex shows the molecular ion peak at 466 m/z, which confirms the stoichiometry of the metal complexes to be [CuL$^1$(OAc)$_2$] type. Elemental analysis values are in close agreement with the values calculated from molecular formula assigned to these complexes, which is further supported by the FAB-mass studies of respective complexes. Similar mass spectral features were assigned for other ligands and their copper complexes. The different pathways of the fragments of the parent molecular ion peaks are discussed in this chapter.

Thermo gravimetric analysis confirms the formation of CuO as the end products from which the copper content could be calculated and compared with that obtained from the analytical determination. From the thermal investigation (TG/DTA) it is possible to observe that the decomposition occurs for the copper complexes in three ways. The results showed that Cu(II) complex of L$^1$ decompose at three steps from 220-590°C. In the final decomposition step appeared in the range 470-590°C corresponding to the complete thermal decomposition of the complexes and the loss of their organic portion results in the formation of CuO as final products. In DTA analysis, An intense exothermic decomposition peaks were observed for [CuL$^1$(OAc)$_2$] at 320°C, 610°C, 770°C.

The redox behaviour of the copper complexes was described using cyclic voltammetry. The cyclic voltammogram of synthesized copper complexes was recorded in DMSO solution. Tetra butyl ammonium perchlorate as supporting electrode. It was concluded that the present ligand systems stabilize the unusual oxidation states of copper ion during electrolysis. Other copper complexes were also showed similar electrochemical behaviour. The salient features of redox behaviour was discussed in this chapter.
The surface morphology of the Cu(II) complexes of different ligands are studied using scanning electron microscopy. It is observed that the variation in morphology is due to the presence of different structural moieties in the ligand systems.

**Chapter 5 Biological and pharmacological studies copper complexes of 2-aminobenzothiazole derivatives**

Copper complexes have indeed demonstrated a wide range of pharmacological activity. It is well known that copper is an essential element in human normal metabolism. In biological systems, copper exists as a variety of complexes which due to that the coordinated forms of copper are more stable than the corresponding ionic species. The biochemical and pharmacological studies (antimicrobial, DNA binding, antioxidant, SOD, antimycobacterial, oxidative damage, partition coefficients and anti-inflammatory) of copper complexes of 2-aminobenzothiazole derivatives are presented in this chapter.

**Antimicrobial studies**

In this section, the *in vitro* biological screening effects of the investigated compounds are tested against the bacterial species *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi* and *Pseudomonas aeruginosa* by disc diffusion method. Streptomycin, Penicillin and Ampicillin are used as standards for antibacterial activity. The minimum inhibitory concentration (MIC) is performed by serial dilution technique. The minimum inhibitory concentration (MIC) values of synthesized copper complexes indicate that complexes exhibit higher antimicrobial activity than the free ligands. The enhanced activity of the complexes can be explained on the basis of Overtone’s concept and Tweedy’s Chelation theory.

This finding demonstrated that the 2-aminobenzothiazole side chains are responsible for the antibacterial activity of the compounds. 2-Aminobenzothiazole side chain is an ideal structural moiety that presumably contributes to enzyme binding and
leads to inhibition of microbial growth. In the present study, the copper conjugation appears to be beneficial in ligands appended with 2-aminobenzothiazole functionality. This suggests a probable role of S and N in ligand system providing a more favorable environment for the copper center in regard to offer better inhibition of microbial growth.

**DNA binding studies**

Copper complexes containing multidentate aromatic ligands have gained much attention in DNA studies due to their significant structural and physicochemical properties [8].

(a) DNA Binding studies

Recently, DNA as a therapeutic target has attracted much attention in biomedical science. Binding studies between small molecules and DNA are important and helpful to develop novel and efficient drugs. Here, calf thymus DNA was selected as DNA model because of its medical importance, low cost and ready availability properties to investigate the possible antimicrobial action mechanism of the highly active compound at molecular level by UV–Vis spectroscopic methods.

Hypochromism and hyperchromism are very important spectral features to distinguish the change of DNA double-helical structure in absorption spectroscopy. In the UV region, the Cu(II) complex of L² exhibits a band at ca. 434 nm. In the presence of DNA (various concentration), the absorption band of the complex was affected, resulting in a hypochromism tendency and slight shifts to longer wavelengths, which indicates that the Cu(II) complex can interact with DNA via intercalation.

The cyclic voltammogram of copper complexes of fixed concentration of the complex with increasing concentration of DNA in the solution causes a considerable decrease in the voltammetric current with very significant potential shift was observed.
in almost for all these complexes, which is consistent with the binding of copper complexes of ligand moiety between the DNA base pairs as also evidenced by the spectral results.

(b) Viscosity Measurements

A classical intercalator causes significant increase in the viscosity of DNA solution due to the increase in the separation in overall DNA contour length. As expected, the known DNA-intercalator EthBr increased the relative viscosity of DNA due to its strong intercalation. Compared with EthBr, complexes exhibit minor to major increase in the relative viscosity of DNA, suggesting an intercalation mode between the complex and DNA. Thus viscosity studies suggest that the central rings of copper and imine group are involved in intercalative mode of DNA binding. The results from the viscosity experiments confirmed the mode of these compounds intercalating into DNA base pairs and already established through absorption spectroscopic studies such as hypochromism and red shift of the complexes in the presence of DNA. The viscosity studies provide a strong evidence for intercalation.

(c) Thermal denaturation

The thermal behaviour of CT-DNA in the presence of complexes gave insight into their conformational changes when temperature is raised when temperature is raised and information about the interaction strength of the complexes with DNA. The double- stranded DNA tends to gradually dissociate to single strands on increase in the solution temperature and generates a hyperchromic effect on the absorption spectra of DNA bases (at 316 nm). The intercalation of small molecules into the double helix has as a result an increase of melting temperature at which the double helix denatures into single helix DNA, The significant increase of $\Delta T_m$ suggested that the interaction of the all copper complexes with DNA through intercalation.

Stability Measurement
To investigate the stability of the prepared compounds in a physiological media, the absorption variation was measured in phosphate buffer solutions (pH = 7.4) in the presence or absence of 0.1% FBS under daylight condition using a previous reported method [10]. It was found that curcumin degraded very rapidly while the analogues were much more stable. The improvement of the stability indicated that the introductions of the substituted group have pronounced stability. As a result, the stability of curcumin analogs could be enhanced due to its structural modifications on Knoevengal condensate β-diketone moiety with 2-aminobenzothiazole (highly conjugated ligand systems).

**Antioxidant**

The antioxidant effect is believed to be responsible for many biological activities of curcumin, such as neuroprotective activity. In order to investigate whether the target compounds retain the antioxidant activity of curcumin or not, the potency of the target compounds to eliminate 2,2-diphenyl-1-picrylhydrazyl free radicals (DPPH) method. The results are presented in this chapter. It is hope that systematic structural modifications influence on antioxidant activities.

**SOD Activity**

Here, the superoxide dismutase activity (SOD) of the complexes was investigated by the NBT assay method. The chromophore concentration value required to yield 50% inhibition of the reduction of NBT (IC$_{50}$). All the tested compounds showed high SOD activity. Similar values obtained for all other complexes. In these series of copper complexes, [CuL$_2$(OAc)$_2$] exhibit excellent SOD mimic activity due to the presence of hydroxyl group enhanced lipid peroxidation and oxidative damage to proteins. The IC$_{50}$ of present copper complexes was found to be the range of 25-69 $\mu$mol dm$^{-3}$ which are higher than the value exhibited by the native enzyme (IC$_{50}$ = 0.04 $\mu$mol dm$^{-3}$).
Section 5  Catalase activity (Antioxidant enzyme activity)

The catalase activity of complexes was calculated based on the rate of decrease in absorbance at \( \lambda = 240 \) nm using the molar extinction coefficient of hydrogen peroxide, and corrected for pathlength. To assess the accuracy by using standard Beers and Sizer assay, the experimental values were compared with the catalase enzyme as standard. It is found that the synthesized compounds having moderate to superior activity like catalase enzyme.

Section 6  Lipophilicity

The logP value is an important criterion to evaluate the druglikeness of substances, especially for the anti-alzheimer’s agents which must possess the ability to penetrate the blood–brain-barrier (BBB). In order to evaluate whether the synthesized compounds possess such ability, the logP value of each compound was calculated. The calculated logP values of the compounds are around 4.00, ranging from 3.70 to 4.4, suggesting a good lipophilicity and a potential ability to penetrate the BBB. According to the Lipinski’s Rule of Five which suggests the optimal logP value of drug candidate should be not higher than 5, it can be expected that the synthesized compounds, possess a good potential to behave as drug candidates.

Section 7  Anti-inflammatory study

Inflammatory diseases such as asthma, allergy, arthritis, multiple sclerosis etc. are quite common form which human beings suffer worldwide. The markedly available drugs cannot be used continuously for long time as they can cause serious side effects such as ulcer, gastrointestinal bleeding and heart stroke. Thus, synthetic chemists are involved in search of safer anti-inflammatory agents.

In the present study, the fully characterized copper complexes are screened for anti-inflammatory activity using carrageenan induced paw oedema model. All the compounds were administered orally (p.o) and assayed at a dose of at 50 mg/kg body
weight. Standard drug used for comparison was Ibuprofen. Results of pharmacological evaluation are summarized in this section. In the present study, the series of curcumin analog which retained the Knoevenagel condensate skeleton and altered the side chains to find out potential pharmacological activities.

The chapter 6 describes about the summary of the whole work. Detailed lists of references arranged in serial order are given after the chapter 6.