Executive summary of the evaluated final report for the Major Research Scheme Submitted to UGC, New Delhi

"Evolution of metal based drug as SOD mimic and artificial metallonucleases"

By

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EXECUTIVE SUMMARY OF THE EVALUATED FINAL REPORT

Medicinal inorganic chemistry is a discipline of growing significance in both therapeutic and diagnostic medicine and it has emerged since the discovery of antitumor activity of cisplatin. Current burgeoning interest in molecules, which are capable of binding and cleaving DNA is related to their utility in the design and development of chemical nucleases, synthetic restriction enzymes, new drugs, DNA footprinting agents etc. In this regard, metal complexes have been found to be particularly useful because they can bind to DNA via different mode of interactions and cleave the duplex by virtue of their intrinsic chemical, electrochemical and photochemical reactivities.

Since the discovery of bis(phen)copper(I) complex as the first copper based chemical nuclease, many attempts have been made to study copper(II) complexes of polypyridyl ligands in combination with quinolone family drugs as antitumor, antibacterial, antimicrobial, DNA intercalating, artificial nucleases and SOD mimic agents. Palladium(II) compounds are emerging as a new class of metal being evaluated as potential antitumor agents.

In accumulation of above facts and with the aim to design artificial metallonucleases and therapeutic applicable molecules (antibacterial and antitumor), we planned to synthesize metal complexes of copper(II) and palladium(II) metal ions and tested for their diverse biological activity like DNA interaction, SOD mimic, cytotoxic and antibacterial activity.

In vitro antibacterial activity of $CuCl_2 \cdot 2H_2O$, palladium salt, norfloxacin, pefloxacin, ligands (L^1-L^{28}) and the synthesized copper(II) and palladium(II) complexes against three Gram$^{(-ve)}$ and two Gram$^{(+ve)}$ microorganisms was studied in terms of minimum inhibitory concentration (MIC), which is the minimum concentration that can effectively inhibits the bacterial growth. The result shows that complexes are more bacteriostatic than metal salt and ligands. Some copper(II) complexes are even more bacteriostatic than norfloxacin and pefloxacin. The increase in antimicrobial activity of metal complexes can be the result of chelate effects, nature of the ligands, nature of the ion neutralising the complex and nuclearity of the metal centre in the compounds. The lower MIC value of complexes is probably due to the greater lipophilic nature of complexes according to Overtone's concept and Tweedy's chelation theory.

The DNA binding activities of ruthenium(II), platinum(II), copper(II) and palladium(II) complexes were studied using viscosity measurement and UV-Vis. absorption studies. Estimation of DNA binding constant and mode of DNA interaction of the ruthenium(II), platinum(II) complexes with Haring sperm DNA (HS DNA) and copper(II), palladium(II) complexes with calf thymus DNA (CT DNA) were studied by UV-Vis. absorption spectra of complexes in presence of increasing amount of DNA. The UV-Vis. spectra of ruthenium(II), copper(II) and palladium(II) complexes show hypochromism with bathochromic shift, which indicate that complexes bind to HS DNA and CT DNA through intercalation mode. The intrinsic binding constants (K_b) for ruthenium(II) complexes are in the range of 0.89×10^4 - 4.45×10^4 M^{-1}. The intrinsic binding constants (K_b) for copper(II) and palladium(II) complexes are in the range of 1.16×10^5 - 8.71×10^5 M^{-1} and 2.72 - 9.93×10^4 M^{-1}. The absorption spectra of the platinum(II) complexes in the

absence and presence of HS DNA are shows covalent mode of binding. The intrinsic binding constants (K_b) for platinum(II) complexes are in the range of 6.05×10^4 to 3.48×10^5 M^{-1}. The highest binding affinity was observed for complexes bearing electron withdrawing substituent on bipyridine ligand.

The relative viscosity measurement of DNA was studied in presence of increasing amount of complexes. Flow time was measured with a digital stopwatch. Each sample was measured three times and an average flow time was calculated. In the viscosity measurement of ruthenium(II), copper(II) and palladium(II) complexes, increase in relative viscosity of DNA with increasing concentration of the complex was observed, which suggests classical intercalation mode of binding. A classical intercalation model demands that the DNA helix lengthens as base pairs are separated to accommodate the complex, leading to the increase of DNA viscosity. In the viscosity measurement of platinum(II) complexes, the slight depression in relative specific viscosity was observed resulting from bending or kinking of the DNA helix, which attributes to covalent binding of complexes with DNA bases.

DNA cleavage activity of ruthenium(II), platinum(II), copper(II) and palladium(II) complexes towards pUC19 DNA was performed using gel electrophoresis technique. The percentage cleavage of DNA reveals that the complexes can cleave DNA more efficiently than the metal salt. Ruthenium(II), copper(II) and palladium(II) complexes shows the highest percentage conversion of SC form into OC and L forms. This suggests hydrolytic cleavage of DNA by the complexes. The platinum(II) complexes shows unwinding of DNA. The compound that unwinds the DNA duplex reduces the number of supercoils in DNA so their number decreases. This decrease upon binding of unwinding agents causes a decrease in the rate of migration through agarose gel, beyond the point where both forms co-migrates, the migration rate begins to increase again as positive supercoils are induced. This makes it possible to observe and quantify the mean value of unwinding.

The cytotoxic activity of platinum(II), copper(II) and palladium(II) complexes were performed using brine shrimp lethality bioassay on *Artemia cysts*. Data were analyzed by simple logit method to determine the LC_{50} values, in which log of concentration of samples were plotted against percentage of mortality of nauplii. The LC_{50} value is the antilogarithm of log[complex] value corresponds to the 50% mortality. The LC_{50} value for platinum(II) complexes are obtained in the range of 8.46 – 19.15 µg/mL. The LC_{50} value for copper(II) complexes are obtained in the range of 6.88 – 17.50 µg/mL and for palladium(II) complexes are obtained in the range of 6.83 – 20.02 µg/mL. In the brine shrimp lethality bioassay, it is evident that complex 6, 9, 10, 16, 20, 21, 22, 27-29 and 37-40 displayed potent cytotoxic activity against *Artemia Cysts* owing to its less bulky nature and presence of fluorine atom. The high electronegativity increases the lipophilic nature of complexes bearing fluorine atom and subsequently increase its biological distribution. The higher activity of above complexes is also due to the greater H- bonding nature of fluorine atom with biomolecules. The lower activity of other complexes is due to bulkiness of substituent on the bipyridine ligand.

The superoxide dismutase like activity of copper(II) complexes was performed using NBT/NADH/PMS system. In the assay, the metal complexes compete with NBT for the oxidation of the generated superoxide ions. The more efficient the complex, the lower the concentration that corresponds to 50% inhibition of NBT reduction; this concentration is termed IC_{50} for comparative purposes. The IC_{50} values of the complexes are found in the range of 0.488 - 1.140 µM. The SOD mimic activity of complexes shows lower IC_{50} for complexes bearing electron withdrawing substituent on bipyridine ligand. More electronegative atoms intensify the formal positive charge on the copper(II) ion and hence facilitate the Cu- $O_2^{\cdot -}$ interaction.

Hence, we succeed primarily in developing efficient antimicrobial agents, artificial nuclease and SOD mimics by synthesizing some bioactive square pyramidal copper(II) and square planar palladium(II) complexes, which possesses good cytotoxic, DNA binding and DNA cleavage activity. In addition, we also found that the copper(II) complexes shows good antibacterial and SOD activity. All these activities are helpful in designing new metal based biological active compounds with their applications as therapeutic agents.