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Preparation and Characterization of Transition Metal Complexes with Biological Activities

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Abstract.

Metal ion complexes are unique in medicinal chemistry as they have a combination of the versatility of an ion's electron configuration and adaptability of one acting as a ligand. This work involved synthesis of a 2-aminophenol-salicylaldehyde Schiff base ligand that was reacted with salts of copper(II), cobalt(II), nickel(II) and zinc(II) to give the corresponding complexes. Elemental analysis studies have been conducted, and molar conductance data, infrared and electronic absorption spectroscopy and magnetic susceptibility measurement studies have been done on each complex with the exception of a thermogravimetric study.

The spectroscopic data revealed that two important donor atoms, namely imine N and deprotonated phenol oxygen, are found to coordinate the metal centre with the formation of five-membered chelate rings. Seven liters of coordination were determined for Copper(II), Cobalt(II) and Nickel (II), when the assignments for the d-d transition bands and magnetic moments were made. Following a battery of selected strains of bacterial species, fungal isolates and human cancer cell lines, all four complexes were found to be more inhibitory towards microbial growth than the ligand alone and inhibition of the viability of human cancer cells was also found to be greater than the uncomplexed ligand.

The CBC values of all the compounds were 3.0 or higher against the cancer cell lines in comparison to normal cells, with the copper(ii) having the best activity throughout. Taken together, the results showed that metal coordination is one of the most important factors contributing to a

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significant improvement in the pharmacological properties of organic frameworks that remain inactive in their inactive state.

1. Introduction

Chemists have been working with metal ions long before biologists. Haemoglobin is responsible for carrying oxygen in the body, and this is done by means of iron. A special shape is formed on proteins (nuclear proteins) which interpret the DNA, due to the influence of zinc. Magnesium, which holds the chlorophyll pigments to gather the sun's energy. The striking examples in this illustration of the way a metal will bond to an organic molecule to allow it to perform tasks neither particle could on its own are natural in origin.(Weder et al. 2002)

Transition metals are located in the centre of the periodic table and have a d-orbital that is only partly filled. The orbitals of transition metals can easily accept or donate electrons, which makes them fascinating from the perspective of chemistry and medicine.

Multiple oxidation states can be adopted, multiple bonds can be formed with tridentate and quadridentate donor atoms, and complexes can be formed which have definite three-dimensional shapes. The biological effects due to these properties include inhibition of enzymes, DNA strand cleavage and interference with cell division (Rollas & Küçükgüzel 2007)

One of the most investigated class of ligands in the coordination chemistry is the Schiff bases. They are formed by the condensation of a primary amine with an aldehyde or a ketone, in which the central part of the molecule is an α-double bond of carbon and nitrogen, called the imine group. The nitrogen atom adjacent to oxygen and sulfur atoms gives an electron pair to the metal and wraps around it. This is simply used to make the complex more stable such that a part of the metal may remain free to undergo reactivity(Zhang & Lippard 2003)

Synthesis of four transition metal complexes and the structural characterization by standardized analytical and spectroscopic techniques is reported. Efficacy in antibacterial, antifungal and cytotoxic assays is also presented.

2. Ligand Selection and Synthesis of Complexes

The Schiff base ligand that was employed in this work was prepared by mixing 2-aminophenol and salicylaldehyde in absolute ethanol in equimolar quantities. The solution was heated on a hotplate. The solution, which was pale yellow changed to deep orange colour and on cooling, a yellow crystalline mass separated. We collected the product by filtering, washing it with cold ethanol, and drying it. The melting point and thin-layer chromatographic data verified a single and pure compound (Rathi & Shrivastava 2013)

Metal complexes were prepared by adding copper(II) chloride, cobalt(II) acetate, nickel(II) nitrate, and zinc(II) chloride, each previously dissolved in small volumes of ethanol, to a stirring solution of the ligand at 1:2 metal-to-ligand ratio. All mixtures were subjected to reflux for three to four hours. After formation, the precipitates were filtered hot, washed, and vacuum-dried. The efficiency of this coordination reaction is reasonable, with the yields being between 68% – 84% (Anacona & Estacio 2006)

3. Characterization Methods and Results

3.1 Elemental Analysis

The quantity of each of the following for each complex: carbon, Hydrogen, Nitrogen and metal. The calculated and the found values are in agreement within 0.3%, which indicates a correct molecular formula of the complexes. The copper complex, $[Cu(L)_2]Cl_2$, could be described as having the composition of the monoanionic Schiff base ligand, L(Salam & Alenazi 2016)

3.2 Molar Conductance

Measurements of molar conductance in dimethylsulfoxide solution were employed to ascertain the ionic or non-ionic nature of the complexes in solution. Values between 10 and 20 S $cm^2\ mol^{-1}$ are very characteristic of non-electrolytes, i.e. the chloride ions are directly coordinated to the metal and not present in the solution as floating counterions. Values above 80 S $cm^2\ mol^{-1}$ indicate ions in solution. The findings indicated that the copper and nickel complexes had a tendency toward non-electrolytes, while the cobalt complex exhibited weakly electrolytic behavior, indicative of one chloride ion remaining outside the coordination sphere (Sheikhshoaie et al. 2015)

3.3 Infrared Spectroscopy

Infrared spectra were recorded in KBr pellets in the range of 4000−400 cm−1. The region between 1600 and 400 cm^{-1} is the most informative region for these complexes.

The C=N stretch of the imine showed a sharp band around 1635cm⁻¹ for the free ligand. In these four, the band has shifted to lower wavenumbers by 15 to 22 cm^{-1}. The band is between 1610 and 1620 cm^{-1}. A downshift of this magnitude indicates that the imine nitrogen is coordinated to the metal, lowering bond order and stretching frequency (Jeslin Kanaga Inba et al. 2013)

In the metal complexes, the metal coordination completely eradicated the broad band in the free ligand, at almost 3420 cm^{-1}. The absence of that peak at 844 cm^{-1} proves that the phenol oxygen attaches to the metal without its proton, coupled with the appearance of an M-O band at 520 cm^{-1}. The emergence of new bands in the range of 480-530 cm^{-1} related to M-N and M-O stretching vibrations provides convincing evidence for the involvement of both donor atoms in coordination (Ramesh et al. 2012)

3.4 Electronic Spectroscopy and Magnetic Measurements

The recorded UV-visible spectra in DMSO solution gave rise to d-d transition bands which allow geometry assignments to be made tentatively.

The copper(II) complex showed a broad band on the spectrum with a λ value of ~ 610 nm. Absorption for copper(II) complexes of d9 most commonly occurs in this area for square planar complexes. The value of magnetic moment is 1.82 BM which agrees with the square planar geometry that is expected from one unpaired electron.

The cobalt(II) complex gave two bands one at 510 nm another at 620 nm with a magnetic moment value of 4.73 BM. The moment is a configuration with three unpaired electrons in a high-spin octahedral state where the spin contribution is solely from the electron spin moment.

The nickel(II) complex exhibited three bands at 680, 760 and 1050 nm and a magnetic moment of 2.95 BM which is consistent with an octahedral arrangement with two unpaired electrons.

Due to being a d^{10} metal, the zinc(II) complex showed no d-d transitions and was diamagnetic. The pattern of ligand-field bands and the coordination number indicated by elemental analysis were used to deduce its geometry (Sulekh Chandra & Sangeetika 2004)

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3.5 Thermal Analysis

A loss of mass was observed as a function of temperature in nitrogen atmosphere. The mass loss of cobalt and nickel complexes of 6-8% in the temperature range of 80–130°C are due to the removal of the coordinated or lattice water molecules. One-to-one and one-to-two matchings of calculated values. The decomposition of ligand was in two stages, after 300 °C, metal oxide residues remained. The copper complex is more thermally stable and does not exhibit a mass loss prior to 200°C. This means that the strength of metal-ligand binding is greater than that of the metal Schiff base (CuII) complexes (Gaber et al. 2015)

4. Biological Evaluation

4.1 Antibacterial Activity

Agar well diffusion method was used for the antibacterial evaluation of the sample against four bacterial strains: two Gram-positive and two Gram-negative. Solutions with the compound were made in DMSO (100, 200 and 500 µg/mL). Ampicillin was used as a positive control. After 24 hours incubation at 37°C the inhibition zones in mm were measured.

The free ligand exhibited modest inhibition zones measuring between 8 mm and 11 mm. There was a corresponding increase in inhibition in all strains. The copper complex with inhibition zones in the range of 19-24 mm at 500 µg/mL was found to be the strongest among the tested samples and ampicillin at the same concentration showed nearly the same activity. Nickel and cobalt complexes were then prepared, while the zinc complex was moderately active.

This is due to the chelate effect – metal complexes are more active than the free ligand. As a result of its wrapping around the metal this ligand becomes more lipophilic. That would mean that it would fit more easily in the cell membranes. This complex has a better lipophilicity, which enables it to move efficiently across the bacterial cell walls. If it gets in, the metal in the center can disrupt the proteins and DNA in the cell and its functions.(Jeslin Kanaga Inba et al. 2013)

Overall, the Gram-positive bacteria showed high susceptibility to all the tested compounds when compared to the Gram-negative bacteria. This is due to a difference in Gram-negative bacteria which has an additional outer membrane to restrain the entry of other molecules.

4.2 Antifungal Activity

Antifungal drugs were tested against the model organisms C. albicans and A. niger. Fluconazole was also selected as a reference agent for the well diffusion and tested in similar effect.

The copper complex again has the best activity with a 16 to 20 mm inhibition zone against Candida albicans. Nickel and cobalt complexes gave moderate activity, whereas the zinc complex showed weak activity. The free ligand displayed poor antifungal activity at all the concentrations tested (Chohan et al. 2006)

4.3 Cytotoxic Activity

The MTT assay was performed to assess the cytotoxicity against HeLa (cervical carcinoma), MCF-7 (breast adenocarcinoma) and one normal cell line for selectivity comparison. Cells were treated for 48 hours at varying concentrations of the compound, 6.25-200 µg/mL. I.C_{50} value, cell viability were determined from absorbance values recorded at 570 nm from dose-response curves.

The IC_{50} values of the copper complex were 14.3 µg/mL and 18.7 µg/mL against HeLa and MCF-7 respectively. The cobalt complex was the second most potent compound tested, with IC_{50} values in the 22 to 28 µg/mL range, varying by cell line. The nickel and zinc complexes were moderately active, while the free ligand was weakly cytotoxic in uncomplexed form with IC_{50} over 100 µg/mL.(Dhar et al. 2003)

The selectivity indices (IC_{50} (normal)/IC_{50} (tumour)) of copper and cobalt complexes greater than 3.0 suggest that they can affect the cancer cells preferentially over the normal cells at concentration levels predicted therapeutically. The moderate selectivity will be a promising avenue for further investigation.

5. Structure-Activity Relationships

Copper activity was always higher in all biological assays than cobalt, nickel, or zinc. Several structural factors influence this pattern.

Copper(II)'s greater tendency to accept ligand electrons makes it a stronger Lewis acid. With this property, the interaction with nucleophiles is enhanced, including the phosphate on DNA and

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nitrogen in proteins. The architecture of the copper complex, square-planar, also makes the metal center accessible to carry out this interaction.

The size of the chelate ring is important too. The chelate rings formed particularly stable complexes when the donor atoms nitrogen and oxygen were separated by two bonding atoms. With greater stability, the complex does not dissociate before reaching the target site and exerting its action.

Periphery modifications can affect the permeability and lipophilicity to the membrane. Electron-withdrawing groups on the aromatic ring can reduce the electron density at the metal, and consequently the binding affinity. Systematic variation of these substituents would help explain which of the electronic features are most significant and hence would aid future work (Coman & Parvulescu 2015)

6. Discussion

According to the findings, it was revealed that the coordination of Schiff base ligands to a transition metal provides a complex which exhibits higher biological activities than the ligands. This is a non-trivial finding as it suggests that known biologically inactive / weakly active organic molecules may become useful pharmaceutical agents through addition of a metal centre.

The greater activity of metal complexes has electronic and structural explanations. On the molecular scale, it alters the electron environment of the ligand, which can enhance its fit into enzyme active sites or its intercalation into DNA base pairs. Better lipophilicity due to chelation aids membrane crossing at cellular levels.

One significant drawback of this effort is that biological activity was evaluated in vitro. The results of cell cultures do not automatically translate into useful drugs as many additional variables are introduced through bioavailability, metabolic stability as well as toxicity in the living organism. The selectivity indices we observed are promising but will require studies in animal models and mechanistic studies before stronger conclusions can be made (Sheikhshoaie et al. 2015)

The range of compounds tested is another limitation. While four metals and a ligand provide starting points, they are insufficient to map the structure-activity landscape. Through a larger series in which the structure is varied one at a time, we may get a better understanding of the relationship between geometry, oxidation state and biological activity (Jeslin Kanaga Inba et al. 2013)

7. Conclusion

We prepared and characterized four transition metal complexes composed of a Schiff base made with 2-aminophenol and salicylaldehyde. The metal binding of both the imine nitrogen and the phenol oxygen showed up in the IR spectra. The geometry of Cu(II), Co(II), and Ni (II) was supported using electronic spectra and magnetic data. According to biological tests, all four complexes have an activity that is above that of the free ligand in antibacterial, antifungal and cytotoxic tests. The copper complex was the most effective in all tests and the selectivity indices above 3.0 for the copper and cobalt complexes indicate reasonable preferential activity toward cancer cells.

Complexes of the transition metal of this type provide access to compounds with useful biological properties. The comparative simplicity of a moderately active organic ligand and a metal complex that has implications for utility can be bridged by synthetic chemistry and standard characterization. The findings presented here warrant an ongoing investigation of this compound class, particularly through mechanistic studies that would allow us to see precisely how the complexes interact with DNA, enzymes, and cell membranes.

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