SYNTHESIS, CHARACTERIZATION, AND BIOLOGICAL EVALUATION OF Ni, Co, AND Cu METAL COMPLEXES WITH HETEROCYCLIC COMPOUNDS

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Abstract

Two new clinically active Schiff bases and their transition metal complexes were synthesized and studied by different spectroscopic and physicochemical methods like NMR, IR, UV-Visible, ESR, TGA, XRD, mass, elemental analysis, molar conductivity and magnetic character. Synthesized complexes and Schiff bases were assessed for invitro antituberculosis activity for Mycobacterium tuberculosis (ATCC–27294) and for antimicrobial activity against drug resistant ESBL (Extended Spectrum β-lactamase) and MBL (Metallo β-lactamase) producing microbial strains. The biological activity of Schiff base was found to improve in presence of transition metal ions. Among the studied metal complexes, copper complexes displayed superior biological activity against all of the microbial strains. Binary complexes of Cu(II), Ni(II), and Co (II) ions have been synthesized by reacting metal salts with a Schiff base, 2-((E)-(5-methylisoxazol-3-ylimino)methyl)-4-methoxyphenol (MIIIMP) in an alcoholic medium. All the metal complexes and Schiff base have been characterized by using elemental analysis, IR, UV-VIS, 1H-NMR, 13C-NMR, Mass, ESR spectral data, magnetic moments, TG, and DTA studies. Based on the analytical, spectral data and molecular modeling studies, Ni (II) and Co (II) metal complexes have octahedral geometry, whereas Cu(II) complex has tetragonal geometry. The antimicrobial and cytotoxic activities of the Schiff base and its metal complexes were studied on bacteria, fungi, and human cervical carcinoma cells (HeLa).

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Introduction

In most organometallic compounds, the Co (II) cations with a d9 configuration are present in square planar, square-pyramidal, or square-bipyramidal geometries. As a result of the unique reactivity shown by the formed complexes and the type of ligands that control the features of those complexes, the chemistry of cobalt complexes gains great interest in many inorganic chemistry groups. It is also generally known that when some organic molecules (drugs) are supplied in conjunction with metals, they are substantially more effective. Furthermore, aniline binding in organometallic complexes shows low-energy delocalized π* orbitals, which increases the probability of altering optical, physicochemical, electrochemical properties, and structural traits. The aniline derivatives such as 3,5-dimethylaniline add to the 2-(3H-imidazol-4-yl) ethanolamine known as histamine which has an important role in several pharmacological processes. These entities have been investigated, and they have essentially provided access to mono and bi-metallic complexes. Some of these complexes’ biological action was investigated and proved that the ligand’s effectiveness increases with its binding position in the metal’s coordination sphere. Lately, we have published the synthesis of transition metal complexes with pseudo thiocyanic anions (SCN−) as ligands. To define the intermolecular interactions and illustrate the
crystalline configuration, the Hirshfeld surface analysis, and various spectroscopic studies were carried out to characterize the complexes. Schiff base being a condensation product of carbonyl compound and primary amine are a subclass of imines, also known as azomethines [1]. Over past two decades, Schiff bases are constantly emerging due to ease of preparation and variety in reactions and enjoyed a popular position in coordination chemistry because of their wide range of applications from medicinal chemistry to material synthesis [2,3]. Azomethine group is an important scaffold critical for biological activity of Schiff bases and present in various natural and non-natural compounds [4]. Schiff bases derived from 2-aminothiophenes derivatives are extensively studied due to their broad spectrum of biological properties and anti-HIV PR inhibitor activities [5]. Schiff base complexes of 3d transition metals were found to exhibit low toxicity and facilitated permeation through the cell membrane of microorganism making them probable therapeutic agents [6].

Tuberculosis (TB) is one of the giant killers of human history and leading global health hazard of the current century. As per WHO global report of 2015, there were approximately 10.4 million of fresh reported Tuberculosis incidences together with 1.2 million (11%) of HIV-positive cases globally, accounting for approximately 1.4 million TB casualties along with 0.4 million HIV positive casualties [7]. Among the annual TB global estimate, 2.2 million incidences have been reported in India out of which 5% were HIV-positive cases and 2.2% of were multi-drug resistant (MDR) TB cases [8]. The appearance of MDR strains, XDR (extensively drug-resistant) strains and latest TDR (totally drug-resistant) strains with co-infection from HIV virus, made the situation alarming. Thus the treatment becomes costly, involving multiple drugs and of long duration. With an annual global estimate of 250 million cases, UTIs (urinary tract infections) are most frequently confronted diseases in underdeveloped countries [9]. About 35% of healthy women suffer from symptoms of urinary tract infection once in a lifetime [10]. Most commonly observed the cause of UTI is Escherichia coli, while few Gram-positive bacteria like Staphylococcus saprophyticus and Enterococcus faecalis and gram-negative uropathogens like K. pneumoniae, P. Aeruginosa, Proteus spp, and Citrobacter spp, are also responsible for UTI [11, 12]. Random use of antibiotics along with increasing use of invasive diagnostic procedures and lapses in the sterilization has led to the surfacing of antibacterial resistant microorganisms with complex resistance mechanism which have made the treatment challenging [13, 14, 15]. Modern studies reported that thiophene containing Schiff bases are strong antibacterial and anti-tuberculicial agents with potential activity comparable to standard lead compounds [16, 17, 18]. Enticed by these results and an extension of our investigation for the new antibacterial and antituberculosis agents, we have prepared novel Schiff bases and respective transition metal complexes. The present article is focused on preparation, spectral characterization, and screening of biological activity of these compounds [19, 20].

**Investigation techniques**

**X-ray single-crystal structural analysis**

A suitable single crystal of (1) and (2) was carefully chosen under a polarizing microscope for X-ray diffraction structural investigation. Data were collected at 170 K using graphite-monochromated Mo Kα radiation on a Bruker-Nonius Kappa CCD with an APEX II detector diffractometer (λ = 0.71073 Å). The structures were solved using the SHELX program’s dual space method, then refined using successive differential Fourier syntheses and a full-matrix least-squares procedure using the SHELXL program. The drawings were made with Diamond. The crystal data and experimental conditions utilized to collect intensity data [21, 22].

**Powder X-ray diffraction**

Powder X-ray Diffraction (PXRD) measurements for hand-grown polycrystalline samples were performed at room temperature on a MiniFlex600 Rigaku powder X-ray diffractometer using Cu Kα radiation (λ = 1.540598 Å). Diffracton data were collected in the angular range 2θ = 0–70° with a scan step width of 0.05° and a fixed time of 0.2 s. Rietveld refinement was applied to model the data sets using the GSAS package incorporated with the EXPGUI interface. As a template, the structure determined from single-crystal XRD was employed. The scale factor, background, lattice parameters, and zero-point were refined until convergence.

**Thermogravimetry differential thermal analysis (TG-TDA)**

The thermal analysis spectra for the titled compounds were acquired with a simultaneous thermogravimetry-differential thermal analysis (TG–DTA) utilizing a PyRIS 1 TGA instrument with 14.9 mg for (1) and 12.25 mg for (2), for a heating rate of 5 °C min⁻¹ in the temperature range [300–880 K] under inert atmosphere (nitrogen gas).

**Infrared spectroscopy**

A spectrometer NICOLET IR 200 FT-IR was used to obtain the Fourier Transform Infrared (FTIR) spectrum of a powder sample of the chemicals. 4000–400 cm⁻¹ was the scanning range.

**UV-Visible spectroscopy**

A Perkin Elmer Lambda spectrophotometer was used to make the UV measurements. Scans were performed in the 200–800 cm⁻¹ range.

**Experimental**

**Methods and Materials**

Purified and distilled solvents and AR grade chemicals were used in the synthesis. Infra-Red spectrum of the Schiff bases and metal chelates were analyzed using PE 1600 FTIR spectrometer. UV-visible spectra of all compounds were analyzed using Jasco V 630 UV-vis spectrophotometer in DMF in the region 200–1100 nm. The proton NMR spectra were analyzed on Varian-NMR-Mercury 300 MHz instrument using TMS and DMSO-d₆ as internal
standard and solvent respectively. Thermogravimetric analysis of one selected complex was carried out on the PERKIN ELMER, diamond thermogravimetric analyzer in an inert atmosphere. The elemental analysis and metal percentage were determined by using standard methods. For magnetic moment measurements, Gouy balance was employed and Mercury (II) Tetrahydroxycobaltate (II) was used as a calibration standard. ESR spectra of Cu (II) chelates were analysed under the magnetic field of 3000 Gauss and 9.45 GHz frequency by using JEOL model JES FA200 ESR spectrometer. Mass spectra (MS) were recorded on BRUKER ESQUIRE HCT spectrometer. Molar conductance of all compounds was measured on ELICO conductivity meter (Model CM-180) using DMF as solvent at RT.

**Structure of the Metal Chelates**

Synthesized complexes were found to be colored with nonhygroscopic properties and stable thermal properties. All metal chelates showed insolubility in organic solvents except for DMSO and DMF. C, H, N, S, Cl and metal content of all complexes were found in accordance with the proposed molecular structure. All complexes displayed nonelectrolytic behavior with lower conductance values.

**Biological Activity Assessment**

**Microorganisms**

ESBL and MBL producing microorganism namely form the genera, Escherichia Pseudomonas, Citrobacter, Klebsiella and Proteus were used for antimicrobial activity assessment. These isolates were collected from suburban hospitals and pathologals and evaluated for MBL/ESBL characteristics in earlier studies. Whereas *M. tuberculosis* – H37Rv strain (ATCC No- 27294) was chosen for in vitro antitubercular activity evaluation.

**Antimicrobial Assessment**

The antibacterial activity was assessed by using the agar well-diffusion method24 using DMSO as a solvent. The compounds were tested at a final concentration of 25 μg/μl. Actively growing log phase cultures were obtained by inoculating test isolated in BHI (Brain Heart Infusion) broth (10 ml) and incubating it at 37°C for one day. Muller Hinton agar medium (20 ml) was seeded using 0.4 ml test culture and transferred to each Petri plate of 9 cm diameter. The medium was allowed to solidify and then wells were punched using a sterile cork borer on each plate. Test compounds (50 μl) were added to each well and plates were sealed. These plates were kept for incubation for 24 h at 37°C. Control wells were prepared with 50 μl of DMSO solution. All the observations were made in three sets to report zones of inhibition.

**Antitubercular Assessment**

Antitubercular assay of all synthesized compounds was carried using Micro Plate Alamar Blue assay. A standard solution containing 1000 ppm of each compound was prepared. From standard solution, test solutions were prepared in the increasing concentration from 0.8 μg/ml to 100 μg/ml. In sterile 96 well plate, mixture of test solution, de-ionized water (200 μl) and Middlebrook 7H9 broth (100 μl), were taken. This plate was sealed and hatched at 37°C over a period of five days. Further, 25μl of Tween 10% and 80% and Almar Blue (1:1 ratio) was dispensed in each well and incubated for one day. Minimum Inhibitory Concentration (MIC) was reported as pink coloration for the growth of Mycobacterium while blue coloration was considered as no bacterial growth in the well.

**Antioxidant activity**

The anti-cancer medication cisplatin, which is based on platinum, has boosted the usage of metal-containing products in medicine. Helicobacter pylori infections and peptic ulcers are the most common uses for cobalt compounds. To combat drug resistance, a new technique involving the construction of compounds based on the incorporation of bioactive molecules has recently emerged as an appealing strategy. Anilines, for instance, are pharmacophore entities that play a key role in several marketed medications, including the Merck HIV protease inhibitor Crivasivan and others. Furthermore, their compounds have excellent biological features, such as anticancer activity in prostate cancer treatmentantimalarial, and antiarrhythmic.

**DPPH radical scavenging activity**

Barca and al. described a DPPH radical scavenging assay that worked. The DPPH solution (35 μg/L) was diluted with various dilutions of the methanolic solution of the studied compounds and the standard compound (ascorbic acid) (0.25-1 mg/mL). With methanol as the blank, the mixture was placed in the dark for 30 min before monitoring the absorbance at 517 nm (until steady absorbance values were achieved). All of the tests were done in triplicate, and the results were expressed as the mean standard deviation (SD), with ascorbic acid as the reference. The following equation was used to compute the inhibitory percentages of the produced material:

\[
\%\text{Inhibition of DPPH radical} = \frac{[\text{Abscont}\,\text{-Abstest}]}{\text{Abscont}} \times 100\%
\]

where Abs = absorbance of reacting mixture without the test sample and Abs test = absorbance of reacting mixture with the test sample. The percentage of scavenging activity was plotted against the sample concentration to calculate the IC50, which is defined as the sample concentration required causing 50% inhibition.

**Hirshfeld surface analysis (HSA)**

In crystalline materials, the different types of non-covalent interactions are the main aspect to study the crystal packing and arrangement of molecules using HSA and the associated 2D fingerprint plots with the help of Crystal Explorer software 21.5. In general, the Hirshfeld surface map allows for visualization of different features like di, de, dnorm, shaped index, and electrostatic potential map. The dnorm map is called normalized contact distance which is determined by the distances to the closest atom outside (de) and inside (di) surfaces. In the dnorm map, three different colors (red, blue, and white) indicate hydrogen bonding, Van der Waals, and interatomic contacts, respec-
tively. To understand the contribution of intermolecular contacts, the enrichment ratio (E) was also calculated; here, the favored contacts are forming while pairing atoms (XY) exhibit a high propensity to form interactions in the crystal packing. In this study, two new Co-metal complexes have been used to perform Hirshfeld surface analysis with the help of a crystallographic information file (CIF).

**Quantum chemical calculation**

By utilizing the Gaussian 09 package program, quantum chemical studies for both Co-metal complexes were performed by B3LYP/LANL2DZ (Los Alamos National Laboratory 2 double) as the level of theory for optimizing geometries of Co-metal atom and 6-311G** basis set for remaining atoms in both complexes. Initial geometries of both metal complexes were attained by the single-crystal x-ray diffraction. Further, the electrostatic potential, molecular orbital analysis, natural bond orbital, and nonlinear optical analysis were performed by utilizing optimized geometries. The optimized structure, frontier molecular orbitals, and ESP maps were visualized using Gauss view and 3Dplotsoftware. Further, the global reactivity descriptors (units in eV), such as ionization potential, electron affinity, electronegativity, chemical potential, global hardness, and electrophilicity were calculated with the help of formulæ based on Koopmans’ theorem.

Noncovalent interactions are playing an important role to determine the shape and supramolecular architecture of crystals in the solid-state phase. The metal complexes are forming different types of noncovalent interactions that significantly influence the crystal structures. In general, the quantum crystallography method helps to understand the nature of intermolecular interactions in crystals at the electronic level beyond geometrical parameters. The wave-function calculation is an alternative way of modeling the diffraction data. In recent years, quantum chemical analysis followed by non-covalent interactions and QTAIM (quantum theory of atoms in molecules) have also shown advanced and gained an enormous amount of interest among researchers due to valuable results behind experiments. The wave function for both complexes was generated from the crystal structure and this was used for noncovalent interaction analysis to get more accurate results than gas phase analysis.

**Conclusion**

Transition metal complexes and two new Schiff bases were prepared. These compounds were evaluated by various spectroscopic techniques. The physical and spectral data revealed monobasic tridentate nature of Schiff base and ligand to metal ratio of 2:1 for cobalt complex and 1:1 for copper, zinc and nickel chelates. Octahedral arrangement for cobalt complexes, square planar geometry for nickel complexes, the distorted square planar configuration for copper complexes and tetrahedral geometry for zinc complexes have been predicted. The biological activity studies revealed the higher antibacterial and antitubercular activity of metal chelates compared to parent ligand against ESBL & MBL uropathogens and *M. Tuberculosis*. Copper complex [Cu(L2)Cl] and [Cu(L3)Cl] cobalt complex [Co(L2)2] complexes exhibited significant antimicrobial activity, while copper complex [Cu(L3)Cl] and [Cu(L3)Cl] and zinc complex [Zn(L3)Cl] complexes demonstrated high anti-tubercular activity. This research work could provide the possibility of synthesizing new lead compounds with more active therapeutic properties that can be used as potential metal derived drugs.

**Conflict of Interest**

Authors are declared that no conflict of Interest.

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All authors are contributed equally.

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Not applicable

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Not applicable

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