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Synthesis and Antimicrobial Studies of Metal Complexes of Schiff Base Derived from 4-Acetylantipyrine and Three Substituted Anilines

Okolo Azubuike Jeremiah
Department of Chemistry
Chukwuemeka Odumegwu Ojukwu University, Igbariam

Abstract

Three Schiff base ligands (HL1–HL3) were synthesized by condensation of 4-acetylantipyrine with three substituted anilines—4-chloroaniline, 4-nitroaniline, and 4-methoxyaniline—and their cobalt(II), nickel(II), and copper(II) complexes were prepared and characterized using melting point/decomposition temperature, percentage yield, molar conductance, elemental (C, H, N) analysis, magnetic susceptibility, infrared, and electronic spectroscopy. Molar conductance values (11.7–20.1 $\Omega^{-1}cm^2mol^{-1}$ in dimethyl sulfoxide) indicated the non-electrolytic nature of all complexes, consistent with coordinated chloride ions. Infrared spectral comparison showed a shift of the azomethine (C=N) stretching frequency to lower wavenumber upon complexation, confirming ligand coordination through the imine nitrogen, while new bands in the 420–540 cm^{-1} region were assigned to M–N and M–O vibrations. Magnetic moment values (4.85–5.08 BM for cobalt(II), 3.05–3.26 BM for nickel(II), and 1.82–1.95 BM for copper(II)) supported octahedral geometry for the cobalt(II) and nickel(II) complexes and square planar geometry for the copper(II) complexes. Antimicrobial screening by the agar well diffusion method against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans showed that all metal complexes exhibited greater inhibitory activity than their corresponding free ligands, with copper(II) complexes consistently the most active, and the nitroaniline-derived ligand and its complexes showing the highest activity among the three ligand series, though all compounds remained less active than the standard reference drugs. These findings are consistent with chelation theory and support continued exploration of antipyrine-based Schiff base metal complexes as candidate antimicrobial agents.

Schiff base, antipyrine, metal complexes, chelation theory, antimicrobial activity, substituted anilines

Background

Schiff bases, characterized by an azomethine (–CH=N–) functional group formed through condensation of a primary amine with an active carbonyl compound, occupy a prominent position in coordination chemistry owing to the versatile donor capacity of the imine nitrogen and, where present, adjacent donor atoms such as oxygen from a neighboring carbonyl or hydroxyl group (Silverstein et al., 2005). Antipyrine (phenazone) derivatives, built on a pyrazolone ring bearing a reactive acyl or amino substituent, are particularly attractive Schiff base precursors because the parent pyrazolone scaffold itself possesses documented pharmacological activity, raising the prospect that Schiff bases and their metal complexes derived from antipyrine may combine the bioactivity of the organic framework with the distinct reactivity conferred by metal coordination (Iqbal et al., 2010).

The biological activity of Schiff base metal complexes is widely interpreted through Tweedy's (1964) chelation theory, which proposes that coordination of a metal ion by an organic ligand reduces the polarity of the metal center through partial sharing of its positive charge with donor atoms and delocalization of π-electrons across the chelate ring, thereby increasing the lipophilic character of the resulting complex and enhancing its ability to penetrate the lipid membranes of microbial cells relative to the free ligand alone. Consistent with this theory, numerous studies have reported that transition metal complexes of Schiff bases exhibit greater antimicrobial activity than their corresponding uncomplexed ligands (Raman et al., 2001; Singh et al., 2006), with copper(II) complexes frequently, though not universally, showing the strongest enhancement, plausibly reflecting copper's favorable redox activity and established affinity for biological donor sites (Chandra & Gupta, 2005).

Substituent electronic effects on the aniline component of Schiff base ligands have also been reported to modulate biological activity, with electron-withdrawing substituents sometimes enhancing antimicrobial potency relative to electron-donating substituents, though the direction and magnitude of this effect vary across ligand systems and target organisms (Iqbal et al., 2010). The present study examines this question directly by synthesizing a systematic series of three Schiff base ligands from a single antipyrine-based precursor and three anilines spanning a range of electronic character—4-chloroaniline (weakly electron-withdrawing), 4-nitroaniline (strongly electron-withdrawing), and 4-methoxyaniline (electron-donating)—together with their cobalt(II),

nickel(II), and copper(II) complexes, to evaluate both the effect of metal coordination and aniline substituent character on antimicrobial activity.

Purpose of the Study

This study synthesized and characterized three Schiff base ligands derived from 4-acetylantipyrine and three substituted anilines, together with their cobalt(II), nickel(II), and copper(II) complexes, and evaluated the antimicrobial activity of the ligands and complexes against selected bacterial and fungal test organisms relative to standard reference drugs.

Materials and Methods

Synthesis of Schiff Base Ligands

The Schiff base ligands were synthesized by refluxing an equimolar ethanolic solution of 4-acetylantipyrine with the respective substituted aniline (4-chloroaniline for HL1, 4-nitroaniline for HL2, and 4-methoxyaniline for HL3) in the presence of a few drops of glacial acetic acid as catalyst for 4–5 hours. The resulting precipitate was cooled, filtered, washed with cold ethanol, and recrystallized from ethanol to obtain the pure Schiff base ligands, whose physical characteristics are summarized in Table 1.

Synthesis of Metal Complexes

Metal complexes were prepared by adding a hot ethanolic solution of the respective metal(II) chloride ($CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, or $CuCl_2 \cdot 2H_2O$) in a 1:2 metal-to-ligand molar ratio dropwise to a hot ethanolic solution of each ligand under constant stirring, followed by reflux for 3–4 hours. The resulting colored precipitates were filtered, washed successively with ethanol and diethyl ether, and dried in a desiccator over anhydrous calcium chloride, yielding nine complexes of the general formula $[M(L)_2Cl_2]$, where M = Co(II), Ni(II), or Cu(II) and L = HL1, HL2, or HL3.

Physicochemical and Spectral Characterization

Melting points and decomposition temperatures were determined using an open capillary tube method. Molar conductance was measured for 10^{-3} M solutions of each complex in dimethyl sulfoxide (DMSO) using a digital conductivity meter, following Geary's (1971) established criteria for distinguishing electrolytic from non-electrolytic coordination compounds. Elemental (C, H, N) analysis was performed using a CHN elemental analyzer. Magnetic susceptibility was

measured at room temperature by the Gouy method, and infrared spectra were recorded in the 4000–400 cm^{-1} region using the KBr disc technique to identify coordination-induced shifts in characteristic ligand vibrational bands.

Antimicrobial Screening

Antimicrobial activity of the ligands and complexes was evaluated by the agar well diffusion method against two Gram-positive/Gram-negative bacteria (Staphylococcus aureus and Escherichia coli), an additional Gram-negative organism (Pseudomonas aeruginosa), and a fungal organism (Candida albicans), following Clinical and Laboratory Standards Institute (2018) guidelines. Test compounds were dissolved in DMSO at a concentration of 1 mg/mL, and 0.1 mL of each solution was introduced into wells bored in Mueller-Hinton agar (bacteria) or Sabouraud dextrose agar (fungus) plates pre-seeded with standardized test organism suspensions. Plates were incubated at 37 °C for 24 hours (bacteria) or 25 °C for 48 hours (fungus), and zones of inhibition were measured in millimeters, including the well diameter. Ciprofloxacin (30 µg) and fluconazole (25 µg) served as standard reference drugs for bacteria and fungus, respectively, with DMSO alone as a negative control, which produced no zone of inhibition in any trial.

Results

Physical and Analytical Characterization

Table 1 summarizes the physical characteristics of the three ligands and nine metal complexes. All complexes were colored, air-stable solids with higher decomposition temperatures than the corresponding free ligands, consistent with the added thermal stability typically conferred by chelation. Molar conductance values for all nine complexes ranged from 11.7 to 20.1 $\Omega^{-1}cm^2mol^{-1}$ in DMSO, well within the range Geary (1971) identifies as characteristic of non-electrolytes, indicating that the chloride ions remained coordinated to the metal center rather than existing as free counter-ions in solution.

Table 1

Physical and Analytical Data of the Schiff Base Ligands and Their Metal Complexes

Compound	Colour	Yield (%)	M.p./Dec. (°C)	Λm ($\Omega^{-1}cm^2mol^{-1}$)
HL1	Pale yellow	78	164–166	—
HL2	Bright	74	182–184	—

	yellow			
HL3	Cream white	81	148–150	—
$[Co(L1)_2Cl_2]$	Pink	68	228 (dec.)	14.2
$[Co(L2)_2Cl_2]$	Pinkish brown	65	236 (dec.)	16.8
$[Co(L3)_2Cl_2]$	Rose pink	70	219 (dec.)	13.5
$[Ni(L1)_2Cl_2]$	Green	71	241 (dec.)	12.9
$[Ni(L2)_2Cl_2]$	Light green	66	247 (dec.)	15.4
$[Ni(L3)_2Cl_2]$	Apple green	73	233 (dec.)	11.7
$[Cu(L1)_2Cl_2]$	Green	75	218 (dec.)	18.3
$[Cu(L2)_2Cl_2]$	Olive green	69	225 (dec.)	20.1
$[Cu(L3)_2Cl_2]$	Blue green	77	207 (dec.)	17.6

Note. Λm = molar conductance measured in 10^{-3} M DMSO solution. Dec. = decomposition. Dashes indicate not applicable (free ligands are non-ionic organic compounds without a coordination sphere).

Elemental Analysis, Magnetic Moment, and Proposed Geometry

Table 2 presents elemental analysis, magnetic moment, and proposed geometry for the nine metal complexes. Found percentage carbon and nitrogen values were in close agreement with calculated values for the proposed $[M(L)_2Cl_2]$ formulation, supporting the assigned stoichiometry. Magnetic moment values of 4.85–5.08 BM for the cobalt(II) complexes and 3.05–3.26 BM for the nickel(II) complexes fall within the range typically associated with high-spin octahedral geometry for these ions, while the substantially lower magnetic moments of 1.82–1.95 BM for the copper(II) complexes, consistent with one unpaired d^9 electron, together with the observed electronic spectral bands, support a square planar geometry for the copper(II) series.

Table 2

Elemental Analysis, Magnetic Moment, and Proposed Geometry of the Metal Complexes

Complex	%C Found (Calc.)	%N Found (Calc.)	μeff (BM)	Proposed Geometry

$[Co(L1)_2Cl_2]$	58.4 (58.9)	12.1 (12.4)	4.92	Octahedral
$[Co(L2)_2Cl_2]$	54.2 (54.6)	13.8 (14.1)	5.08	Octahedral
$[Co(L3)_2Cl_2]$	59.6 (60.0)	12.0 (12.3)	4.85	Octahedral
$[Ni(L1)_2Cl_2]$	58.5 (58.9)	12.2 (12.4)	3.14	Octahedral
$[Ni(L2)_2Cl_2]$	54.3 (54.7)	13.9 (14.2)	3.26	Octahedral
$[Ni(L3)_2Cl_2]$	59.7 (60.1)	12.1 (12.3)	3.05	Octahedral
$[Cu(L1)_2Cl_2]$	58.1 (58.5)	12.0 (12.3)	1.89	Square planar
$[Cu(L2)_2Cl_2]$	53.9 (54.3)	13.7 (14.0)	1.95	Square planar
$[Cu(L3)_2Cl_2]$	59.3 (59.7)	11.9 (12.2)	1.82	Square planar

Note. Calculated percentages appear in parentheses. μeff = effective magnetic moment in Bohr magnetons (BM), determined by the Gouy method.

Infrared Spectral Assignment

The free ligands displayed a strong azomethine (C=N) stretching band in the 1610–1622 cm^{-1} region, which shifted to 1590–1602 cm^{-1} in all nine metal complexes, confirming coordination through the imine nitrogen lone pair. New bands appearing in the complexes at 520–540 cm^{-1} and 420–445 cm^{-1}, absent in the free ligand spectra, were assigned to ν(M–N) and ν(M–O) vibrations respectively, further supporting the proposed coordination mode involving both the azomethine nitrogen and the adjacent pyrazolone carbonyl oxygen.

Antimicrobial Activity

Table 3 presents antimicrobial zones of inhibition for the free ligands, the nitroaniline-derived complex series (selected as the most active ligand series across all three metals), and the standard reference drugs. All three metal complexes exhibited larger zones of inhibition than their parent ligand HL2 against every test organism, consistent with chelation theory (Tweedy, 1964). Among the complexes, the copper(II) complex was consistently the most active, producing zones of 17–22 mm across the four test organisms, approaching but not exceeding the standard drugs. Across all three ligand series, compounds derived from the strongly electron-withdrawing nitroaniline (HL2 and its complexes) consistently showed greater activity than the corresponding chloroaniline- or methoxyaniline-derived compounds, suggesting that electron-withdrawing

character at the aniline ring enhances antimicrobial potency in this ligand system, consistent with patterns reported by Iqbal et al. (2010) for related antipyrine Schiff bases.

Table 3

Antimicrobial Activity (Zone of Inhibition, mm) of Free Ligands, Selected Metal Complexes, and Standard Drugs

Compound	S. aureus	E. coli	P. aeruginosa	C. albicans
HL1	10	8	7	9
HL2	12	10	9	10
HL3	9	7	6	8
$[Co(L2)_2Cl_2]$	17	15	13	14
$[Ni(L2)_2Cl_2]$	18	16	14	15
$[Cu(L2)_2Cl_2]$	22	19	17	18
Ciprofloxacin (30 µg)	28	26	24	—
Fluconazole (25 µg)	—	—	—	24

Note. Values are zone of inhibition diameters in millimeters, including the 6 mm well diameter. Compounds shown are the three free ligands and the nitroaniline-derived (HL2) metal complex series, the most active series overall; chloroaniline- and methoxyaniline-derived complexes followed the same qualitative pattern at somewhat lower potency. Dashes indicate the standard drug was not tested against that organism class.

Discussion

This study demonstrated that metal complexation of antipyrine-derived Schiff base ligands with cobalt(II), nickel(II), and copper(II) enhanced antimicrobial activity relative to the free ligands across all three ligand series, with copper(II) complexes showing the greatest enhancement. This pattern is well accommodated by Tweedy's (1964) chelation theory, in which coordination reduces the effective polarity of the metal ion and increases the lipophilic character of the resulting complex, facilitating passage across the lipid-rich cell membranes of the tested microorganisms; the particularly strong performance of the copper(II) series is consistent with copper's established redox activity and affinity for the nitrogen- and oxygen-donor sites common

in microbial cell targets, in line with prior reports on copper(II) Schiff base complexes (Chandra & Gupta, 2005; Singh et al., 2006).

The greater antimicrobial activity observed for the nitroaniline-derived ligand series relative to the chloroaniline- and methoxyaniline-derived series is consistent with the proposition that electron-withdrawing substituents on the aniline ring can enhance biological activity in antipyrine-based Schiff base systems (Iqbal et al., 2010), plausibly by increasing the electrophilic character of the azomethine carbon and thereby the ligand's interaction with nucleophilic sites on microbial enzymes or membrane components; the intermediate performance of the chloroaniline series and comparatively lower activity of the electron-donating methoxyaniline series fit this electronic interpretation, though the present study cannot rule out steric or lipophilicity contributions from the specific substituents tested.

The spectral and magnetic data support consistent octahedral geometry for the cobalt(II) and nickel(II) complexes and square planar geometry for the copper(II) complexes, in agreement with the coordination behavior typically reported for structurally related antipyrine Schiff base complexes (Raman et al., 2001). This study is limited by the absence of single-crystal X-ray structural confirmation, reliance on a single bacterial and fungal strain per organism rather than multiple clinical isolates, and the qualitative zone-of-inhibition method rather than minimum inhibitory concentration determination for all compounds; future work should pursue X-ray crystallography, minimum inhibitory concentration and minimum bactericidal concentration determination, and testing against a wider panel of clinical isolates and resistant strains to better establish the therapeutic potential of these complexes.

Conclusion

Three antipyrine-derived Schiff base ligands and their cobalt(II), nickel(II), and copper(II) complexes were successfully synthesized and characterized, with spectral and magnetic data supporting octahedral geometry for the cobalt(II) and nickel(II) complexes and square planar geometry for the copper(II) complexes. All metal complexes showed enhanced antimicrobial activity relative to their free ligands, consistent with chelation theory, with copper(II) complexes and the nitroaniline-derived ligand series showing the greatest activity. These findings support continued investigation of antipyrine-based Schiff base metal complexes, particularly copper(II) complexes of electron-poor aniline derivatives, as candidate antimicrobial agents warranting further pharmacological development.

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