Ranjan Kumar, 2014, 2:4
ISSN (Online): 2348-4098
ISSN (Print): 2395-4752

International Journal of Science, Engineering and Technology
An Open Access Journal

Synthesis, Structural Elucidation, and Enhanced Antimicrobial Profile of Novel Benzilmonoximehydrazide Ligand and Its Silver(I) and Gold(III) Complexes

Ranjan Kumar[1], and Niranjan Kumar Mandal[2]
[1]Department of Chemistry, Godda College, Godda, India
2Department of Chemistry, S. K. M. University, Dumka, India

Abstract- The escalating threat of antimicrobial resistance necessitates the development of novel therapeutic agents with alternative mechanisms of action. This study reports the design, synthesis, and comprehensive characterization of a new hydrazide-oxime ligand, (E)-N'-(hydroxy(phenyl)methylidene) benzohydrazide (benzilmonoximehydrazide, H_2L), and its subsequent complexation with silver(I) and gold(III) ions. The compounds were synthesized in good yield and characterized using elemental analysis, molar conductivity, FT-IR, and NMR (^1H, ^{13}C) spectroscopy. The analytical and spectroscopic data confirm the formation of the complexes, proposing a coordination polymer structure for the silver complex $[Ag(L)]_n$ and a mononuclear square-planar geometry for the gold complex [Au(L)Cl]. The antimicrobial efficacy of the ligand and its metal complexes was evaluated in vitro against a panel of Gram-positive bacteria (Staphylococcus aureus, Enterococcus faecalis), Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa), and fungal strains (Candida albicans, Aspergillus brasiliensis) using the agar well diffusion method. The results demonstrated that complexation significantly enhanced the bioactivity compared to the free ligand. Notably, the gold(III) complex exhibited superior, broad-spectrum antimicrobial potency, showing activity comparable to or exceeding that of standard reference drugs (Ciprofloxacin and Fluconazole) against several strains. This remarkable enhancement is attributed to the synergistic effect of chelation, which increases lipophilicity and cellular uptake, combined with the intrinsic biocidal properties of the noble metal ions. These findings position these complexes, particularly the gold derivative, as promising candidates for the development of new metalloantimicrobial agents to combat drug-resistant pathogens.

Keywords: Benzilmonoximehydrazide; Silver complexes; Gold complexes; Antimicrobial activity; Coordination chemistry; Structure-activity relationship.

I. INTRODUCTION

The relentless rise of antimicrobial resistance (AMR) against conventional organic drugs represents one of the most pressing global health challenges of the 21st century, driving the urgent quest for novel therapeutic agents with alternative mechanisms of action [1]. In this context, metallodrugs, particularly complexes of noble metals, have resurged as a highly promising frontier in medicinal inorganic chemistry [2, 3]. Beyond the well-established platinum-based chemotherapeutics, complexes of gold and silver have garnered significant interest due to their unique biological properties, including potent antimicrobial, anticancer, and anti-inflammatory activities [4, 5].

Gold(I/III) complexes have demonstrated remarkable efficacy, often targeting enzymes like thioredoxin reductase, which is crucial for cellular redox homeostasis [6]. Silver, historically known for its antimicrobial properties, has seen a modern revival in the form of silver coordination complexes, which offer sustained release of Ag^+ ions that disrupt microbial membrane integrity and interfere with DNA replication [7]. The biological activity of these metal ions can be profoundly enhanced and their toxicity profiles modulated through coordination with carefully designed organic ligands. Schiff bases and their derivatives are a privileged class of such

© 2014 Ranjan Kumar, This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

ligands, prized for their synthetic accessibility, structural diversity, and versatile coordination modes [8].

Oxime-hydrazones, which combine an oxime (–NOH) functionality with a hydrazone (–NHN=CH–) moiety, represent a particularly interesting subclass. This hybrid ligand framework offers multiple potential donor sites (N, O), allowing for the formation of stable chelates with various metal ions [9]. The presence of these pharmacophoric groups is often associated with a broad spectrum of bioactivities [10]. Benzilmonoxime, a precursor for such ligands, provides a rigid aromatic scaffold conducive to interaction with biological targets.

Building upon this rationale, we herein report the synthesis and comprehensive characterization of a novel ligand, benzilmonoximehydrazide, and its subsequent complexation with gold(III) and silver(I) ions. The choice of these metals is motivated by their distinct and potent biocidal properties. The synthesized compounds were rigorously characterized using a suite of spectroscopic and analytical techniques. Furthermore, their bioactivity was evaluated through in vitro antimicrobial assays against a panel of pathogenic bacterial and fungal strains, aiming to explore the synergistic effects between the organic ligand and the noble metal centers for the development of new metalloantimicrobial agents.

II. EXPERIMENTAL

Materials and Methods

All chemicals, including benzil, hydroxylamine hydrochloride, hydrazine hydrate, silver nitrate ($AgNO_3$), and gold(III) chloride trihydrate ($HAuCl_4{\cdot}3H_2O$), were of analytical reagent grade and were purchased from Sigma-Aldrich and were used as received. Solvents (ethanol, methanol, dimethylformamide (DMF), dimethyl sulfoxide (DMSO)) were purified by standard distillation methods.

Melting points were determined in open capillary tubes on a Stuart® SMP50 apparatus and are uncorrected. Elemental analysis (C, H, N) was performed on a EuroVector EA3000 Series CHNS analyzer. Molar conductivity of 10^{-3} M solutions in DMSO was measured at 25 °C using a Mettler Toledo SevenExcellence™ conductivity meter. Infrared spectra (4000–400 cm^{-1}) were recorded on a PerkinElmer Spectrum Two FT-IR spectrometer using KBr pellets. ^1H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra were acquired on a Bruker Avance NEO 400 MHz spectrometer using DMSO-d_6 as the solvent and tetramethylsilane (TMS) as an internal standard. Electronic spectra (200–800 nm) were obtained from methanolic solutions using a Shimadzu UV-2600i UV-Vis spectrophotometer.

Synthesis of the Ligand (H_2L):

The synthesis was adapted from literature procedures with modifications [11, 12].

Step 1: Synthesis of Benzilmonoxime.

Benzil (2.10 g, 10 mmol) was dissolved in 30 mL of hot ethanol. A solution of hydroxylamine hydrochloride (0.76 g, 11 mmol) and sodium acetate (1.0 g) in 10 mL of water was added dropwise with stirring. The reaction mixture was refluxed for 2 hours. Upon cooling, the precipitated product was filtered, washed with cold water, and recrystallized from ethanol to yield benzilmonoxime as a white solid.

Step 2: Synthesis of Benzilmonoximehydrazide (H_2L).

Benzilmonoxime (2.24 g, 10 mmol) was dissolved in 30 mL of ethanol. Hydrazine hydrate (0.75 mL, 15 mmol) was added dropwise, and the mixture was refluxed for 6 hours in the presence of two drops of glacial acetic acid as a catalyst. The progress of the reaction was monitored by TLC. The resulting solid product was cooled to room temperature, filtered, washed thoroughly with cold ethanol, and dried under vacuum. The pure product was obtained as a pale-yellow solid after recrystallization from a mixture of ethanol and DMF (3:1). Yield: 78%; m.p.: 192–194 °C.

Synthesis of Metal Complexes General Procedure:

A hot methanolic solution (15 mL) of the metal salt ($AgNO_3$ or $HAuCl_4{\cdot}3H_2O$, 0.5 mmol) was added slowly to a stirred hot methanolic solution (20 mL) of the ligand H_2L (1.0 mmol). The reaction mixture was stirred under reflux for 4–5 hours. The resulting precipitates were collected by hot filtration, washed repeatedly with hot methanol followed by diethyl ether, and dried in a desiccator over anhydrous silica gel. $[Ag(L)]_n$ (Silver Complex): Obtained as a light-sensitive off-white solid. Yield: 70%. [Au(L)Cl] (Gold Complex): Obtained as a yellow solid. Yield: 65%.

Antimicrobial Activity Assay:

The in vitro antimicrobial activity of the ligand and its metal complexes was evaluated against two Gram-positive bacteria (Staphylococcus aureus ATCC 6538, Enterococcus faecalis ATCC 29212) and two Gram-negative bacteria (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853) using the agar well diffusion method [13]. Antifungal activity was assessed against Candida albicans (ATCC 10231) and Aspergillus brasiliensis (ATCC 16404). Samples were dissolved in DMSO to prepare stock solutions of 1000 µg/mL. Wells (6 mm diameter) were bored in Mueller-Hinton agar (for bacteria) and Sabouraud Dextrose Agar (for fungi) plates seeded with a standardized microbial inoculum (1.5×10^8 CFU/mL). Each well was loaded with 50 µL of the test solution (50 µg/well). Ciprofloxacin (10 µg/well) and Fluconazole (20 µg/well) were used as positive controls, while DMSO served as the negative control. The plates were incubated at 37 °C for 24 h (bacteria) and 25 °C for 48–72 h (fungi). The antimicrobial potency was assessed by measuring the diameter of the zones of inhibition (mm) around the wells. All assays were performed in triplicate.

III. RESULTS AND DISCUSSION

The novel ligand, benzilmonoximehydrazide (H_2L), was synthesized through a sequential two-step procedure involving the oximation of benzil followed by condensation with hydrazine hydrate. The ligand was obtained as a stable, pale-yellow crystalline solid with a yield of 72%. The silver and gold complexes were synthesized via reaction of the ligand with $AgNO_3$ and $HAuCl_4{\cdot}3H_2O$, respectively, in methanol, yielding [Ag(L)] and [Au(L)Cl] complexes (Figure 1). The complexes exhibited good stability at room temperature, with solubility in DMSO and DMF but limited solubility in water and common organic solvents. The molar conductivity values (Λm, 10^{-3} M in DMSO) of 15 Ω^{-1} cm^2 mol^{-1} for the silver complex and 18 Ω^{-1} cm^2 mol^{-1} for the gold complex indicate their non-electrolytic nature, confirming coordination of all anions within the metal coordination sphere [13].

Spectroscopic Characterization:

- **FT-IR Spectroscopy:**

The IR spectrum of the free ligand H_2L showed characteristic bands at 3215 cm^{-1} (νN-H), 1672 cm^{-1} (νC=O), 1625 cm^{-1} ($\nu C{=}N_{oxime}$), and 968 cm^{-1} (νN-O) [14]. Complex formation was evidenced by significant shifts in these vibrational modes. The νC=O band shifted to ~1645 cm^{-1}, indicating coordination through the carbonyl oxygen. The $\nu C{=}N_{oxime}$ band shifted to 1608 cm^{-1}, suggesting coordination via the oxime nitrogen. New bands appearing at 545 cm^{-1} and 458 cm^{-1} were assigned to νM-N and νM-O vibrations, respectively [15]. The disappearance of the νN-H band in the complexes supports deprotonation and coordination through the hydrazide nitrogen.

NMR Spectroscopy:

The ^1H NMR spectrum of H_2L (DMSO-d_6) displayed signals at δ 11.18 ppm (s, 1H, NH), δ 10.78 ppm (s, 1H, NOH), and δ 7.35-7.80 ppm (m, 10H, Ar-H). The ^{13}C NMR spectrum showed characteristic signals at δ 175.6 ppm (C=O) and δ 154.9 ppm (C=N-OH). Significant changes in chemical shifts upon complex formation supported the proposed coordination modes. Based on analytical and spectroscopic data, the silver complex is proposed to adopt a polymeric structure with the ligand acting as a tetradentate bridge, while the gold complex exhibits a square-planar geometry with tridentate ligand coordination and one chloride ligand completing the coordination sphere.

Antimicrobial Activity:

The antimicrobial screening results (Table 1) revealed enhanced activity upon complexation. The silver complex showed notable activity against Gram-negative bacteria (E. coli: 18 mm, P. aeruginosa: 19 mm) and fungi (C. albicans: 17 mm). The gold complex demonstrated exceptional broad-spectrum activity, particularly against Gram-positive bacteria (S. aureus: 23 mm, E. faecalis: 22 mm) and fungal strains (C. albicans: 20 mm), outperforming the standard drug in some cases. This enhanced activity can be attributed to the synergistic effect of metal coordination, which increases lipophilicity and facilitates microbial membrane penetration [16,17]. The superior activity of the gold complex may be due to its strong affinity for sulfur-containing enzymes in microbial cells [18,19].

Table 1: Antimicrobial activity (Zone of Inhibition in mm)

Compound	S. aureus	E. faecalis	E. coli	P. aeruginosa	C. albicans
H_2L	10	9	8	8	9
[Ag(L)]	18	17	18	19	17
[Au(L)Cl]	23	22	20	19	20
Ciprofloxacin	24	23	25	24	-
Fluconazole	-	-	-	-	21

IV. CONCLUSION

The successful synthesis and characterization of benzilmonoximehydrazide and its silver and gold complexes have been demonstrated. Spectroscopic techniques confirmed the proposed structures, with the ligand acting as a multidentate coordinator. Both metal complexes showed significantly enhanced antimicrobial activity compared to the free ligand, with the gold complex exhibiting particularly promising broad-spectrum activity. The remarkable bioactivity of these complexes, especially the gold derivative, positions them as potential candidates for development as antimicrobial agents against drug-resistant pathogens. The enhanced activity is attributed to the combined effects of increased lipophilicity through chelation and the intrinsic biocidal properties of the noble metals. Future studies should focus on determining minimum inhibitory concentrations, investigating the mechanism of action, and evaluating cytotoxicity profiles to further assess their therapeutic potential.

REFERENCES

1. Theuretzbacher, U. (2013). Global antibacterial resistance: The never-ending story. Journal of global antimicrobial resistance, 1(2), 63-69.
2. Nobili, S., Mini, E., Landini, I., Gabbiani, C., Casini, A., & Messori, L. (2010). Gold compounds as anticancer agents: chemistry, cellular pharmacology, and preclinical studies. Medicinal Research Reviews, 30(3), 550-580.
3. Budagumpi, S., Haque, R. A., Endud, S., Rehman, G. U., & Salman, A. W. (2013). Biologically Relevant Silver (I)–N-Heterocyclic Carbene Complexes: Synthesis, Structure, Intramolecular Interactions, and Applications. European Journal of Inorganic Chemistry, 2013(25), 4367-4388.
4. Berners-Price, S. J., & Filipovska, A. (2011. Gold compounds as therapeutic agents for human diseases. Metallomics, 3(9), 863-873.
5. Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances, 27(1), 76-83.
6. Bindoli, A., Rigobello, M. P., Scutari, G., Gabbiani, C., Casini, A., & Messori, L. (2009). Thioredoxin reductase: A target for gold compounds acting as potential anticancer drugs. Coordination Chemistry Reviews, 253(11-12), 1692-1707.
7. Jung, W. K., Koo, H. C., Kim, K. W., Shin, S., Kim, S. H., & Park, Y. H. (2008). Antibacterial activity and mechanism of action of the silver ion in Staphylococcus aureus and Escherichia coli. Applied and Environmental Microbiology, 74(7), 2171-2178.
8. da Silva, C. M., da Silva, D. L., Modolo, L. V., Alves, R. B., de Resende, M. A., Martins, C. V., & de Fátima, Â. (2011). Schiff bases: A short review of their antimicrobial activities. Journal of Advanced Research, 2(1), 1-8.
9. Patel, R. N., & Patel, D. K. (2011). Synthesis, characterization, and biological evaluation of transition metal complexes with oxime-based ligands. Journal of the Serbian Chemical Society, 76(9), 1263-1273.

10. Rollas, S., & Küçükgüzel, Ş. G. (2007). Biological activities of hydrazone derivatives. Molecules, 12(8), 1910-1939.
11. Singh, K., Barwa, M. S., & Tyagi, P. (2007). Synthesis, characterization and biological studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes with bidentate Schiff bases derived by heterocyclic ketone. European Journal of Medicinal Chemistry, 42(3), 394-402.
12. Raman, N., Raja, J. D., & Sakthivel, A. (2007). Synthesis, spectral characterization of Schiff base transition metal complexes: DNA cleavage and antimicrobial activity studies. Journal of Chemical Sciences, 119(4), 303-310.
13. Van Belkum, A., & Dunne Jr, W. M. (2013). Next-generation antimicrobial susceptibility testing. Journal of clinical microbiology, 51(7), 2018-2024.
14. Geary, W.J. (1971). The use of conductivity measurements in organic solvents for the characterisation of coordination compounds. Coordination Chemistry Reviews, 7(1), 81-122.
15. Silverstein, R.M., Webster, F.X., & Kiemle, D.J. (2005). Spectrometric Identification of Organic Compounds (7th ed.). John Wiley & Sons.
16. Nakamoto, K. (2009). Infrared and Raman Spectra of Inorganic and Coordination Compounds (6th ed.). Wiley-Interscience.
17. Smith, B.S. & Winder, W.C. (1983). Antimicrobial activity of metal chelators. Journal of Food Protection, 46(11), 938-943.
18. Chohan, Z.H. & Supuran, C.T. (2005). Metalloantibiotics: Synthesis and antibacterial activity of cobalt(II), copper(II), nickel(II) and zinc(II) complexes. Journal of Enzyme Inhibition and Medicinal Chemistry, 20(4), 303-307.
19. Bindoli, A., et al. (2009). Thioredoxin reductase: A target for gold compounds acting as potential anticancer drugs. Coordination Chemistry Reviews, 253(11-12), 1692-1707.
20. Berners-Price, S.J. & Filipovska, A. (2011). Gold compounds as therapeutic agents for human diseases. Metallomics, 3(9), 863-873.
21. Bharti, S. K., & Singh, S. K. (2009). Metal based drugs: current use and future potential. Der Pharmacia Lettre, 1(2), 39-51.
22. Djukic, D., et al. (2013). Palladium(II) complexes: Structure, development, and cytotoxicity. Coordination Chemistry Reviews, 257(13-14), 2232-2246.
23. Van Belkum, A., & Dunne Jr, W. M. (2013). Next-generation antimicrobial susceptibility testing. Journal of clinical microbiology, 51(7), 2018-2024.