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SYNTHESIS, SPECTRAL, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF Ni^{2+}, Co^{2+}, Cu^{2+} AND Zn^{2+} COMPLEXES WITH A NOVEL LIGAND; METHYL 2-NITRO-3-(((E)-(2-OXO-1,DIPHENYLETHYLIDENE) HYDRAZONO)METHYL)BENZOATE

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ABSTRACT

In this paper, we attempted to prepare a novel Schiff base methyl 2-nitro-3-(((E)-(2-oxo-1,diphenylethylidene)hydrazono)methyl)benzoate *via* condensation between benzilmonohydrazone with methyl 3-formyl-2-nitrobenzoate, which was further subjected to synthesize the complexes of Ni^{2+}, Co^{2+}, Cu^{2+}, and Zn^{2+} and underlining the biological importance. A variety of spectral processes, including UV-visible, FT-IR, and ^{1}H NMR, were used to evaluate the newly synthesized Schiff's base and associated metal complexes. The Schiff base derivative (BO-8) and the associated complexes have been assessed in vitro for their antibacterial and antioxidant properties using disc diffusion method, they demonstrated notable activities for the bacterial and fungal strains *Escherichia coli* (TM: ATCC 25922), *Pseudomonas aeruginosa* (TM: ATCC 27852), *Staphylococcus aureus* (TM: ATCC 25923), *Aspergillus niger* (TM: MTCC 282), and *Candida albicans* (TM: ATCC 14053).

Keywords: Benzilmonohydrazone, Methyl-3-Formyl-2-Nitrobenzoate, Antimicrobials, Metal Complexes.
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INTRODUCTION

Globally, a sharp increase in infectious disease cases poses a serious hazard in the modern period. Therefore, it is crucial to produce more advanced and technical pharmacological compounds or their derivatives. The use of Schiff base derivatives in pharmaceuticals is noteworthy in this context. Schiff bases, often known as "privileged ligands" due to the way they interact and stabilize a range of metals, are frequently made by condensation of aldehyde and primary amine.[1] They are bi- or tri-dentate and are favored because of their easy synthesis and complex coordination chemistry with metal ions.[2] The different Schiff bases can be produced from the various methods by combining with different alkyl or aryl substituents.[3] The azomethine linkage in these types of ligands has biological actions like pharmaceutical and medicinal, antibacterial, antimicrobial, anticancer, antituberculosis, anti-inflammatory, and antioxidant.[4-10] Besides their biological uses, they are used in industry as pigments, photochromic properties, complexing capacity towards certain harmful metals, removable oxygen binding, polymer stabilizers, and catalytic activity in the hydrogenation of olefins.[11] The generation of Schiff base-metal complexes with different donor atom sets and molecular topologies, particularly those incorporating transition metal ions, is a fast-expanding field of research. Its lipophilicity may be modulated to achieve extraordinary stability, selectivity, and sensitivity for a particular metal ion. Regarding this, Sunjuk *et al*. reported on the Schiff base (HB, CB, and NB) ligands that have been created with 2,4-dihydroxybenzaldehyde, 2-nitrobenzaldehyde, and 2-chlorobenzaldehyde, respectively, for the 2-amino-6-methoxybenzothiazole reaction. These reactions were then examined for Fe(III), Cr(III), Cd(II), Cu(II), Ni(II), and Co(II) chlorides at a 2:1 molar ratio.[12] In light of the aforementioned information, and the continued related work in our laboratory aimed at exploring the new Schiff base ligand by the reaction of benzilmonohydrazone with methyl 3-formyl-2-nitrobenzoate.[13-20] Then, its coordination behavior with Ni^{2+}, Co^{2+}, Cu^{2+}, and Zn^{2+} transition metal ions was examined. The FT-IR, ^{1}H NMR, and UV-visible techniques were used to analyze the newly synthesized ligand and complexes of metal ions. Antibacterial, using the microdilution broth method, antibacterial, antifungal, and antioxidant activities have also been measured as MIC values against strains of *Aspergillus niger* (MTCC282), *Candida albicans*

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(ATCC14053), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC27852), and *Staphylococcus aureus* (ATCC25923).

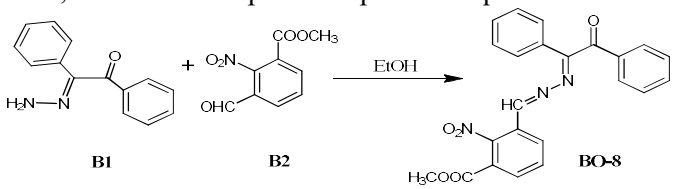
EXPERIMENTAL

Materials and Methods

Glasswares were oven-dried for one hour at 100 °C to facilitate all reactions. All reagents and solvents, including benzilmonohydrazone, methyl 3-formyl-2-nitrobenzoate, nickel chloride hexahydrate ($NiCl_2.6H_2O$), cobaltous chloride ($CoCl_2.6H_2O$), sodium hydroxide (NaOH), copper chloride dihydrate ($CuCl_2.2H_2O$), anhydrous zinc chloride ($ZnCl_2$), hydrochloric acid (HCl) and ethanol, were acquired analytical grade from Sigma Aldrich (Merck KGaA) which were used as received. The 60 F254 silica gel was pre-coated on aluminium plates and subjected to UV light (λmax = 254 nm) for Thin Layer Chromatography. Using KBr pellets, FT-IR spectra in the 400–4000 cm^{-1} range were recorded using a Perkin Elmer FT-IR spectrophotometer. The ^1H NMR spectra in deuterated DMSO-d6 have been collected at 400 MHz using the JEOL-400 NMR spectrometer. A SHIMADZU 1800 spectrophotometer was used to record the absorption spectra between 200 and 1100 nm using an appropriate solvent. At Centre for Innovation, Research and Development of Dr. B. Lal Clinical Laboratory Pvt. Ltd., Jaipur, the antibacterial and antifungal activities of newly synthesized metal (II) complexes against strains of *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC27852), *Staphylococcus aureus* (ATCC 25923), *Candida albicans* (ATCC14053), and *Aspergillus niger* (MTCC282) were assessed in vitro using the Muller Hinton Agar well diffusion method.

Preparation of Schiff Base

Methyl 3-formyl-2-nitrobenzoate (0.978 g, 4.68 mmol) and benzilmonohydrazone (1.051 g, 4.69 mmol) were condensed in 20 mL of absolute ethanol to create the ligand (BO-8). This reaction was conducted under reflux conditions for 3 hrs at 60 °C. After being separated by filtration, the precipitate was extracted with ethanol and allowed to dry overnight in a vacuum desiccator (Scheme-1). Then checked the purity of the ligand was checked, and it was recrystallized further as required using ethanol as solvent to afford a yellow-colored solid powder, which was stored for further use. To confirm the ligand synthesis, it was studied using UV, FT-IR, and ^1H NMR spectroscopic techniques.



Scheme 1: Synthesis of BO-8

Ligand (BO-8)

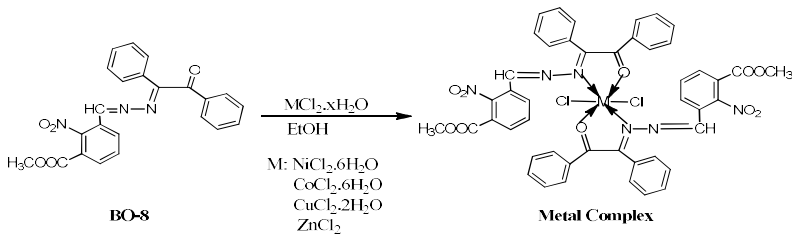
Methyl 2-nitro-3-(((E)-(2-oxo-1,diphenylethylidene)hydrazono)methyl)benzoate (BO-8), yellow solid powder (70% yield), mp = 160 °C. IR (KBr), $\nu_{max.}$: 1681 υ(C=O), 1608 υ(C=N), 1536 υ(CH=N), 1008 υ (N-N); UV-Vis in DMSO λ_{max}: 226, 316, 402 nm; ^1H NMR (400 MHz, in DMSO - d6): δ 8.61 (s, 1H), 8.04 (s, J = 7.8 Hz, 1H), 8.02 (s, 1H), 7.79 (d, 2H), 7.72 (d, 2H), 7.67 (s, 1H), 7.55 (m, J = 1.1 Hz, 3H), 7.49 (m, J = 2.9, 1.3 Hz, 3H).

Synthesis of Divalent Metal Complexes [BO-8(I) to BO-8(IV)]

The ethanolic solution of nickel chloride hexahydrate (0.28 g, 1.20 mmol) was mixed with 2-nitro-3-(((E)-(2-oxo-1,2-diphenylethylidene)hydrazono)methyl)benzoate (BO-8) (1 g, 2.40 mmol) and refluxed for 3 hrs. The complex formed a bright green precipitate when the reaction mixtures pH was kept at 7.0 using a diluted aqueous sodium hydroxide solution. It was then filtered, cleaned with 15-20 mL ethanol, and placed in a vacuum desiccator to dry overnight (Scheme-2). Copper, cobalt, and zinc complexes were synthesized using a similar process.

Ni(II) Complex (BO-8(I))

Dark Green powder (75% yield), mp = <300 °C; IR (KBr), 3634 υ(OH/H_2O), 1612 υ(C=O), 1534 υ(C=N), 513 υ(M-O), 448 υ(M-N); UV-Vis. (DMSO) λ_{max}: 346, 468, 520, 604 nm, ^1H NMR (400 MHz, DMSO-d_6): δ 8.36 (s, 1H), 7.81 (d, 2H), 7.72 (s, 1H), 7.61 (d, 2H), 7.46 (d, 2H), 7.30 (m, 6H).



Scheme 2: Synthesis of Metal complexes

Co(II) Complex (BO-8(II))

Light Green Solid (65% yield), mp: <300 °C, IR (KBr), 3625 υ(OH/H_2O), 1675 υ(C=O), 1574 υ(C=N), 474 υ(M-O), 435 υ(M-N); UV-Vis. (DMSO) λ_{max}: 320, 472, 590, 614 nm, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.63 (s, 1H), 7.82 (m, 3H), 7.71 (m, 3H), 7.59 (d, 2H), 7.51 (s, 1H), 7.37 (m, 4H).

Cu(II) Complex (BO-8(III))

Brown Solid (70% yield), mp = <300 °C; IR (KBr), 3330 υ(OH/H_2O), 1534 υ(C=O), 1482 υ(C=N), 518 υ(M-O), 427 υ(M-N); UV-Vis. (DMSO) λ_{max}: 312, 496, 566, 662 nm, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35 (s, 1H), 7.88 (d, 2H), 7.81 (m, 3H), 7.71 (d, 2H), 7.61 (s, 1H), 7.53 (d, 2H), 7.47 (s, 1H), 7.34 (d, 2H).

Zn(II) Complex (BO-8(IV))

Pale Yellow Solid (85% yield), mp = <300 °C; IR (KBr), 3494 υ(OH/H_2O), 1670 υ(C=O), 1586 υ(C=N), 551 υ(M-O), 456 υ(M-N); UV-Vis. (DMSO) λ_{max}: 316, 430, 546, 634 nm, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (s, 1H), 7.83 (d, 2H), 7.81 (d, 2H), 7.72 (s, 1H), 7.61 (s, 2H), 7.34 (m, 6H).

Microbiological Investigations

To evaluate the potential of produced metal (II) complexes against bacterial and fungal strains, three types of antimicrobial tests were conducted.

Antibacterial Activity

Dimethyl sulphoxide (DMSO) was utilized to dissolve the ligand and its complexes to attain dosages of 10 mg/mL, 50 mg/mL, and 100 mg/mL. The Muller Hinton agar medium and the disc diffusion procedure, the antibacterial activity of the identified products against *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27852), and *S. aureus* (ATCC 25923) was ascertained. The mixture was sterilized for about 30 min at 121 °C. After homogenizing the nutritional agar with peptone water, the corresponding well was filled with a 100 µL solution of the relevant compounds. One by one, the mixture was put on distinct filter paper discs, and it was maintained at 37 °C for 24 hrs. For *Pseudomonas aeruginosa* (ATCC27853), a disc containing 30 µg of streptomycin was utilized as a positive control, and DMSO as a negative control. The ligand and its complexes antibacterial activities produced evident circular zones of inhibition. Amoxicillin is used as the standard antibacterial drug. Each complex's inhibitory zones were measured in millimeters. The compounds actions were compared to those of the ligand.

Antifungal Activity

The method of disc diffusion and the Sabouraud dextrose-agar medium were utilized to assess the compounds antifungal activity at doses of 10 mg/mL, 50 mg/mL, and 100 mg/mL, similar to the antibacterial tests. *Candida albicans* (ATCC 14053) and *Aspergillus niger* (MTCC 282) were the fungal strains chosen for this investigation. For thirty minutes, the medium was sterilized at 121 °C. One by one, each compound was placed on a corresponding filter paper disc, and it was kept at 37 °C for 24 hrs. The development of *Aspergillus niger* at 37 °C (2–3 days) and *Candida albicans* at 28 °C (24 hrs). Ketoconazole served as the positive control, whereas the negative control was distilled water. The ligands and complexes created basic circular zones of inhibition through their antifungal activities. Each compounds inhibition zones were measured in millimeters. The compounds actions were compared to the ligand moiety.

Antioxidant Activity

The DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate) appraises the antioxidant efficacy of the ligand BO-8 and divalent metal-ligand products [BO-8(I) to BO-8(IV)]. The test compounds have demonstrated their

radical scavenging activity spectrophotometrically against DPPH, a stable free radical. The attack of the antioxidant moiety that donates hydrogen caused DPPH reduction, changing the dark purple color to yellow in methanol. An aliquot of 100µg from compounds in a range of concentrations (20–100 µg/mL) was mixed with 3.9 mL of 0.1 mM DPPH (methanolic) solution. After being well mixed with a vortex, the mixture was put in an incubation chamber for 30 – 35 min. The optical density (OD) was determined at 517 nm using methanol as a blank. The % radical scavenging potency was determined by the ratio = (Abcontrol – Absample/ Abcontrol) × 100, where Abcontrol and Absample represent the absorbance of the DPPH solution and sample, respectively.

RESULTS AND DISCUSSION

The new ligand (BO-8) interacts with the metal (II) ions through the 2 nitrogen atoms of the azomethine and oxime moieties, acting as a di-dentate ligand. The complexes dissolve entirely in polar coordinating solvents like DMF and DMSO but partially in ethanol. Table-1 and Fig.-1 display their physical and analytical data.

Table-1: Ligand and Metal (II) Complexes Analytical and Physical Data

S. No.	Sample name	Empirical formula	Color	%Yield* (g)	MP (°C)	Solubility
1.	BO-8	$C_{23}H_{17}N_3O_5$	Bright yellow	70 (1.35)	160	DMSO
2.	BO-8(I)	$C_{46}H_{38}N_6O_{12}Cl_2Ni$	Olive Green	75 (0.45)	<300	DMSO
3.	BO-8(II)	$C_{46}H_{38}N_6O_{12}Cl_2Co$	Light Green	65 (0.41)	<300	DMSO
4.	BO-8(III)	$C_{46}H_{38}N_6O_{12}Cl_2Cu$	Brown	70 (0.42	<300	DMSO
5.	BO-8(IV)	$C_{46}H_{38}N_6O_{12}Cl_2Zn$	Pale Yellow	85 (0.59)	<300	DMSO

*wt%, BO-8; Ligand, BO-8(I); Ligand+$NiCl_2.6H_2O$, BO-8(II); Ligand+$CoCl_2.6H_2O$, BO-8(III); Ligand+$CuCl_2.2H_2O$, BO-8(IV); Ligand+$ZnCl_2$.

^{1}H NMR Spectral Studies

The metal complexes and ligand ^{1}H NMR spectra were collected in DMSO-d6. The newly synthesized Schiff base ligand features peaks appearing at 8.61 ppm, which may be detected due to the presence of azomethine protons (CH=N).[21] After complexation, there was no change in the azomethine proton signals, which shows that the azomethine nitrogen is not participating in the bond formation. The range of 8.03-7.34 ppm may result from the aromatic protons bound to the phenyl ring.

FT-IR Spectral Studies

The stretching vibrations C=N, H_2O, C=O, M-N, and M-O have been identified to structurally define the ligand moiety (BO-8) and its associated metal products [BO-8(I) to BO-8(IV)]. The ligand FT-IR spectrum shows two major frequencies, 1681 cm^{-1} and 1608 cm^{-1}, which have been mapped to ν(-C=O) and ν(-C=N).[22] On the other hand, every complex demonstrates the emergence of a wide region in the 3634–3494 cm^{-1} that is ascribed to the non-coordination of a water molecule. A band appeared at 1681 cm^{-1} as a result of the ligand's ν(-C=O) group. The values moved to a lower frequency as a result of oxygen donating electrons to the metal's empty orbitals, confirming the bond within the oxygen of the carbonyl group and the metal-ion. After complexation, the ligand BO-8 displays a band at 1608 cm^{-1} that has been moved to a lower frequency. As a result, it confirmed that metal ions coordinate with the N atom of -C=N. The metal-oxygen (M-O) and metal-nitrogen (M-N) bonding interactions are represented by the medium-intensity bands in the regions 551-474 cm^{-1} and 427-456 cm^{-1} (Fig.-1).[23]

Microbiological Investigations

The newly synthesized ligand BO-8 along with its associated metal complexes were subjected to antimicrobial screening using the disc diffusion method towards two gram -ve (*E. coli and Pseudomonas aeruginosa*) and one gram +ve (*Staphylococcus aureus*) bacteria, as well as two fungi (*Candida albicans*

and *Aspergillus niger*). Although the Schiff base ligand inhibitory effect on the growth of different strains varied, its metal complexes showed moderate to considerable inhibition on the development of a variety of fungal and bacterial strains. Chelation theory can be used to understand the rise in activity. The metal ion's polarity is decreased by chelation.[24-25]

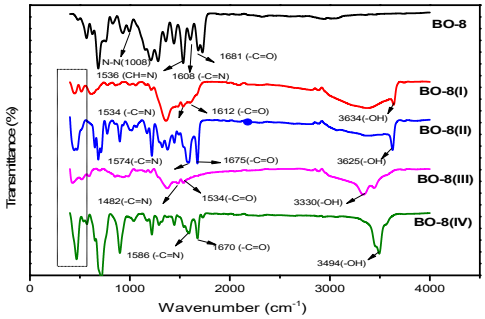


Fig.-1: FT-IR Spectra of Ligand (BO-8) and Metal (II) Complexes

This gives the complex a lipophilic quality that facilitates the interaction of metal ions with lipids. This is the reason to degrade the cells permeability barrier, affecting normal cellular functions. Additionally, it promotes electron delocalization within the chelate ring, increasing the complex's lipophilicity. The results of this investigation show that all complexes have greater biological activities than the free ligand against most of the bacteria and fungi that were examined, suggesting that complexation increases the activity of compounds against microbes (Table-2). At 100 mg/mL, the Co (II) complex had the strongest antibacterial activity against *P. aeruginosa* and *S. aureus*, while the Ni (II) complex demonstrated the strongest antibacterial activity against *E. coli*. Conversely, at 100 mg/mL, the Cu (II) complex had the greatest level of activity against the fungal strains *A. niger* and *C. albicans*. On the other hand, Cu (II) and Zn (II) complexes showed no activity against *Pseudomonas Aeruginosa* (Fig.-2). The compounds will show higher antioxidant potential when the IC50 value is small. It has been noticed that the divalent metal complexes of Ni^{2+} and Cu^{2+} ions exhibit the highest DPPH free radical scavenging activity with IC50 = 238.59 µg/mL and 330.74 µg/mL obtained at 50 µg/mL, respectively. According to the findings, the produced metal complexes have high antioxidant properties and may be used as a component in food packaging to shield food from oxidative damage (Fig.-3).

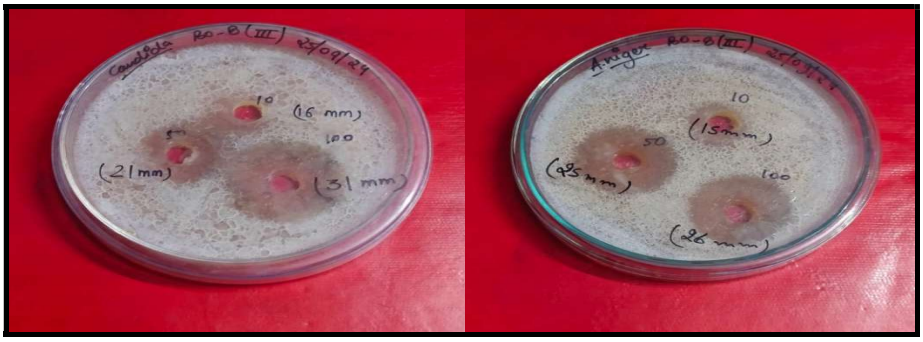


Fig.-2: Antifungal Activity of Metal (II) Complexes

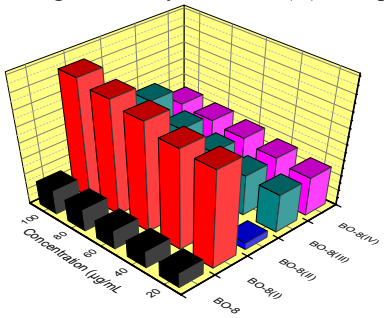


Fig.-3: Antioxidant Activity of Ligand and Metal (II) Complexes

Table-2: Antimicrobial Activity of Ligand and Metal (II) Complexes

S. No	Strain Type	Metal (II) Complex Loading (mg/ml)	Zone of Inhibition (ZoI) (mm)				
			BO-8	BO-8(I)	BO-8(II)	BO-8(III)	BO-8(IV)
1.	*Pseudomonas Aeruginosa*	10	0	7	12	0	0
		50	12	10	12	0	0
		100	14	15	18	0	0
2.	*Staphylococcus aureus*	10	12	11	15	12	8
		50	13	14	17	17	9
		100	14	20	18	19	12
3.	*Escherichia coli*	10	9	12	11	8	8
		50	13	14	13	9	9
		100	15	16	15	11	10
4.	*Candida albicans*	10	0	7	0	16	0
		50	0	8	0	21	0
		100	7	20	7	31	7
5.	*Aspergillus niger*	10	0	0	0	15	7
		50	10	7	9	25	9
		100	15	16	10	26	13

CONCLUSION

The production of the novel ligand 2-nitro-3-(((E)-(2-oxo-1,2-diphenylethylidene)hydrazono)methyl)benzoate and its complexes with Ni^{2+}, Co^{2+}, Cu^{2+}, and Zn^{2+} was followed by spectral characterization and screening using bioassays such as antioxidant, antifungal, and antibacterial tests. The results of each spectral characterisation technique proposed the structures of the ligand BO-8 and metal (II) complexes [BO-8(I) to BO-8(IV)]. Bidentate is the nature of the ligand. According to the analysis, the newly prepared Schiff base BO-8 coordinates to the metal (II) ion through the ligand's azomethine group nitrogen and oxime group oxygen, giving the metal (II) complexes an octahedral geometry. The antioxidant potential of each extract indicates that the highest DPPH free radical scavenging activity is exhibited by divalent metal complexes of Ni^{2+} and Cu^{2+} ions. Although both metal and ligand complexes demonstrated antibacterial qualities, the metal complex, especially the $Co^{+2,}$ showed stronger antimicrobial effects. Compared to *Candida albican*, all of the compounds had stronger antifungal activity against *Aspergillus niger*.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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