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Synthesis, characterization of N-(1-(4-nitrophenyl)ethylidene)-1*H*-benzo[d]imidazol-2-amine and amino acid alanine and it's metal complexes

Sriramoju Shamili
Department Of Chemistry, Chaitanya (Deemed to be University), Hanumakonda 506 001, Telangana, India
E-mail: shamoct02@gmail.com

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Transition metal ion complexes that include a variety of ligands have distinct biological effects. Schiff bases that comprise amino acids and azomethine may be used as building blocks for ternary complexes. In an effort to produce a novel metal(II) complexes, the conventional reflux method has been used to the interaction of two ligands, N-(1-(4-nitrophenyl)ethylidene)-1*H*-benzo[d]imidazol-2-amine as L_1, and an amino acid alanine as L_2, with freshly produced cuprous chloride solution in a 1:1:1 molar ratio. The synthesized ligand and metal(II) complexes are characterized using elemental analysis, molecular weight, magnetic moment, thermal analysis, and spectroscopic techniques. *Bacillus subtilis*, *Escherichia coli*, and *Candida albicans* have been used as test organisms to evaluate the compounds' antibacterial activities. These metal(II) complexes have been observed to have powerful antibacterial and antifungal activities.

Keywords: Azomethine group, Ligand, Metal complex, Antimicrobial activity

Schiff bases are a particularly significant class of molecules[1]. Many of these ligands have been the focus of intensive research over the last few years due to the interest raised by their desirable chemical and physical features[2,3]. These adaptable Schiff base ligands can be employed as photochromic commercial catalysts in the hydrogenation and oxidation of olefins, as well as fluorescence sensors for dangerous metal ions[4-8]. The emergence of Schiff base compounds was also essential to the growth of coordination chemistry[9]. Furthermore, we can see that there has been a persistent interest in studying Schiff bases over the last decade owing to their usefulness in a wide range of biological, biochemical, analytical and industrial contexts[10-13]. Some Schiff bases have been shown to have potent antiviral, antimicrobial, antifungal, antitumor, anticancer, and antibacterial properties[14-19]. Antibacterial materials are in high demand due to rising raising attention of the pathogenic effects, malodors, and stain formations produced by germs in a variety of applications including medical devices, healthcare, sanitary applications, water purification systems, hospital and dental surgical equipment, textiles, food packaging, and storage[20-31]. It is now evident that the Schiff bases' biological activity merits additional study. Zn, Mg, Sn(II) complexes with Schiff base ligands have been recently synthesized, and their spectroscopic, structural, and cyclic voltammetric characteristics have been described[32,33]. Our focus in this study has been on the synthesis, structural characterisation, and computational analyses of two novel Schiff bases with promising antibacterial characteristics[34] (Scheme 1). In addition to their role as ligands, the Schiff bases listed above have also been used in the past to create NCN complexes[35].

Results and Discussion

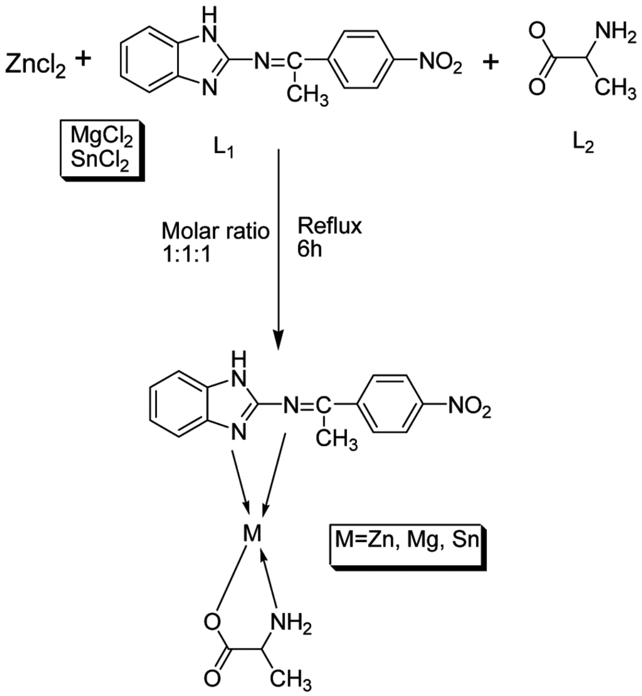
Synthesis of N-(1-(4-nitrophenyl)ethylidene)-1*H*-benzo[d]imidazol-2-amine, L_1

1.34g of 4-nitroacetophenone (0.1 M) was combined with 0.1 g of 2-aminobenzimidazole to generate the ligand. We used a Silica Gel-G TLC plate to monitor the reaction for five to six hours while heating the reaction mixture on a heating plate in the presence of condensing reagent and glacial acetic acid. The result of the reaction was recrystallized in alcohol, cooled and then vacuum dried.

Synthesis of metal complex $[L_1L_2]$

A 0.98 g (0.01 M) metal solution was prepared by adding pure HCl to a small volume of distilled water, yielding the metal complex. To make the complex, we mixed 10 mL of an aqueous solution of L-alanine (0.89 g, 0.01 mm) with 10 mL of an ethanolic solution of 4-(1-(1*H*-benzo[d]imidazol-2-

ylimino)ethyl)benzonitrile (2.49 g, 0.01 mm), and then added 10 mL of an aqueous acidic solution of metal while There are twice as many of these ligands as there is metal. Even after continuously swirling the reaction mixture, no precipitation was seen. TLC was used to monitor the development of the reaction while the mixture was refluxing for 6 hours. The result of the reaction was washed, recrystallized, dried, and collected under vacuum (Fig. 1). These primary procedures are shown in Scheme 1. IR (KBr)υ: 3304–3415, 1345–1402 cm^{-1}. The physical and analytical characterization data of ligands and complexes are shown in Table 1.



Scheme 1 — Synthetic strategy proposed for mixed ligand complex

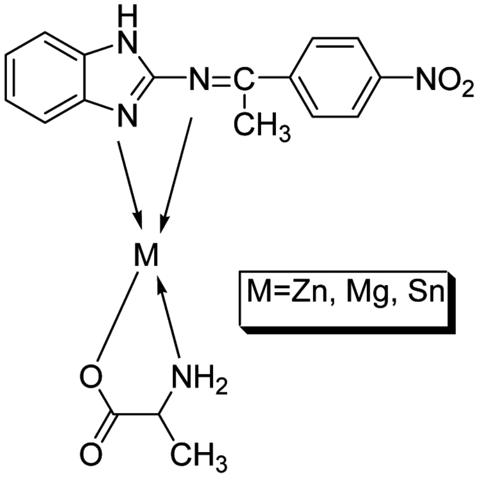


Fig. 1 — [M L_1L_2] Metal complex

In vitro anti-microbial activity of [Metal L_1L_2]

Using the well diffusion technique, the antimicrobial activity of extracts was assessed. The material was solubilized with specified doses of dimethyl sulfoxide (1 mg/mL) for sample preparation. The anti-microbial activity (antibacterial and antifungal) was then investigated using media. The newly synthesized compounds were tested *in vitro* against gram-positive bacteria such as *Staphylococcus aureus* and gram-negative bacteria such as *Escherichia coli*. The lowest concentration (maximum dilution) necessary to halt the growth of bacteria was considered as the minimum inhibitory concentration (MIC, μg/mL), was determined and compared with the Ciprofloxacin conventional antibiotics the MIC values of the substances tested are provided in Table 2.

A study was conducted to test the antifungal properties of the newly synthesized compounds against *Aspergillus fumigatus*. The antifungal activity of each compound was compared to that of the standard medication Ketocanazole. The MIC (μg/mL) was calculated and compared to that of controls the MIC values of the compounds tested are included in Table 2. The antifungal screening findings revealed that the test compounds were active.

The data for the antimicrobial activity have been summarized in Table 2. Results suggest that the complex is more effective against E. coli but less effective for *Staphylococcus aureus* compared to the synthesized ligand in terms of antimicrobial assessment of selected bacterial and fungus strains. In addition, the combination exhibits potent antifungal

Table 1 — Physical and analytical data of ligands and complexes

Compd/ Empirical Formula	Colour	Formula Wt.	Yield (%)	m.p./Decomposition Temp. (°C)	Elemental analysis (CHN) (%)
Ligand $C_{15}H_{12}N_4O_2$	Pale Yellow	280.58	80	243.32-245.35	C, 64.28; H, 4.32; N, 19.99
[Zn $C_{18}H_{18}O_4N_5$]	Silver white	432.77	78	> 300	C, 49.84; H, 4.18; N, 16.15
[Mg $C_{18}H_{18}O_4N_5$]	Metallic	392.71	81	> 300	C, 55.06; H, 4.62; N, 17.84
[Sn $C_{18}H_{18}O_4N_5$]	White	487.60	80	18202-184.02	C, 44.39; H, 3.72; N, 14.38

Table 2 — *In vitro* anti-microbial activity of Ligand Metal complexes

Sample		Bacteria		Fungi
		Escherichia coli (Gram negative)	*Staphylococcus aureus* (Gram positive)	*Aspergillus fumigatus*
Ligand	80	26	26	25
	60	35	32	35
ZnL_1L_2	80	37	30	30
	60	35	32	35
MgL_1L_2	80	34	30	33
	60	30	30	26
SnL_1L_2	80	30	29	31
	60	29	25	30

action against *Aspergillus fumigatus* the complex has showed remarkable biological activity. The Zn(I) combination elicited a stronger reaction from the bacteria than the ligand generated.

Experimental Section

Analytical grade solvents and chemicals were used in their pure form. The chemicals were manufactured and stored in such a way that they were not contaminated by air or moisture. A measurement of the sample's conductivity was made using the WTW Conductometer Bridge. The separations were performed using thin-layer chromatography plates that had been treated with silica gel. The FTIR spectra of a molecule and its complexes were acquired in anhydrous KBr pellets that used a Perkin-Elmer Series 2000 instrument. A Shimadzu 160 spectrophotometer was employed to monitor electronic transitions in the UV-visible band as nujol mulls and in DMSO solutions with concentrations ranging from 105 to 103 M.

More than 0.3% of the expected results for elemental analyses agreed with data from an LECO CHNS 932 model micro analytical instrument. After dissolving the complexes in a mineral acid mixture, the metal concentration was measured using a Shimadzu 680 G atomic absorption spectrophotometer. The JEOL MS Route Instrument was used to collect mass spectra from electron collisions (EIMS). ^{1}H NMR spectra in DMSO-d_6 solvent were acquired using a Varian-Mercury 300 MHz spectrometer with TMS as an internal standard.

Synthesis of N-(1-(4-nitrophenyl)ethylidene)-1*H*-benzo[d]imidazol-2-amine (L_1)

1.34g of 4-nitroacetophenone (0.1 M) was combined with 0.1 g of 2-aminobenzimidazole to generate the ligand. We used a Silica Gel-G TLC plate to monitor the reaction for five to six hours while heating the reaction mixture on a heating plate in the presence of condensing reagent and glacial acetic acid. The result of the reaction was recrystallized in alcohol, cooled, and then vacuum dried. Transparent crystals. Yield 57.4%. m.p.257.30-259.32°C. IR (KBr): 3300–3400, 1500–1550 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.54 (s, 3H, CH_3), 7.28 (d, *J*=2.9Hz, 2H, Ar-H), 7.63 (m, 2H, Ar-H), 7.94 (m, 2H, Ar-H), 8.34 (m, 2H, Ar-H), 10.62 (s, 1H-NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 27.17, 112.82, 114.87, 119.75, 122,41, 124.31, 129.38, 135.40, 137.95, 142.35, 146.68, 148.63, 180.77; MS: *m/z* (M+H) 260.29 Found: 260.30. Anal. Cacld for $C_{16}H_{12}N_4$: C, 73.83; H, 4.65; N, 21.52. Found: C, 73.85; H, 4.68; N, 21.55%.

Synthesis of metal complex [L_1L_2]

A 0.98 g (0.01 M) metal solution was prepared by adding pure HCl to a small volume of distilled water, yielding the metal complex. To make the complex, we mixed 10 mL of an aqueous solution of L-alanine (0.89 g, 0.01 mm) with 10 mL of an ethanolic solution of 4-(1-(1*H*-benzo[d]imidazol-2-ylimino)-ethyl)-benzonitrile (2.49 g, 0.01 mm), and then added 10 mL of an aqueous acidic solution of metal while There are twice as many of these ligands as there is metal. Even after continuously swirling the reaction mixture, no precipitation was seen. TLC was used to monitor the development of the reaction while the mixture was refluxing for 6 hours. The result of the reaction was washed, recrystallized, dried and collected under vacuum. These primary procedures are shown. IR (KBr)υ: 3304–3415, 1345–1402 cm^{-1}.

In the FTIR spectrum, an absorption band shifts to lower frequencies when C=N is coordinated to a metal ion. Also, the usual carboxylate (C=O) absorption band is located at 1730 cm^{-1}, while the L-alanine complex occurs at 1620 cm^{-1}. A new absorption band appears in the spectrum at 609 cm^{-1}, with the M-O band occupying 650 cm^{-1} and the M-N band occupying 450 cm^{-1} and 430 cm^{-1}, respectively.

The spectrum of the synthesized compound was obtained using ^{1}H NMR spectroscopy after being dissolved in DMSO-d_6. In ^{1}H NMR the metal complexes of Zn(L_1L_2), Mg(L_1L_2) shows aromatic peaks at 6.05–7.05(m), 7.25–7.77(m)ppm, which is slightly higher than the ligand. The molecular ion peaks shows at *m/z* 432.77, 392.71 and shows ^{13}C NMR at the range 115.30–132.72, 115.42–132.52 ppm. Sn(L_1L_2) shows ^{1}H at 6.02–7.02(m)ppm and

Table 3 — ^{1}H and ^{13}C NMR data of HL and complexes 1–3 in DMSO-d_6

Compd	Chemical shift δ (ppm)				
	^{1}H NMR		^{13}C NMR		
	-N=CH-	Aromatic	-N=CH-	Benzimidazole	Aromatic
HL	10.53 (s)	7.28–7.76(m)	138.90	141.05	115.33–138.44
Zn(L_1L_2), 1	2.50 (s)	6.05–7.05(m)	138.32	137.09	115.30–132.72
Mg(L_1L_2),2	2.52 (s)	7.25–7.77(m)	138.82	121.09	115.42–132.52
Sn(L_1L_2), 3	2.55 (s)	6.02–7.02(m)	138.65	145.02	115.48–132.42

^{13}C NMR at the range 115.48–132.42 ppm and the molecular ion peaks shows at *m/z* 487.60 (Table 3). The thermogram of Mg(II) 250°C and the last decomposition at 1020°C and Sn(II) at 450°C and Zn(II) at 200°C.

All of the investigated metal chelates may be seen in the mass spectrum of the resulting complex. The formula of the complex's molecular substance, [$ZnC_{18}H_{18}N_5O_4$], was found to be well supported by the mass spectrum of the substance (MW 432.76). In the ESI-MS spectra of the synthetic mixed ligand complex [$ZnC_{18}H_{18}N_5O_4$], the molecular ion peak can be detected at *m/z* 432.76. The calculated molecular weight of the complex is 432.76, therefore this result is in close accord with that reflected in the mass spectrograph (Fig. S5). The synthetic mixed ligand complex [Mg $C_{18}H_{18}N_5O_4$] shows MW-432.77, and [Sn $C_{18}H_{18}O_4N_5$] shows 487.60.

Conclusion

Physicochemical and spectroscopic studies suggest that the mixed ligand complex has a four-coordinated geometry, with the synthesized ligand serving as a bidentate chelating agent and the amino acid acting as a mono-ionic bidentate moiety. The low conductance of the synthesized complex is indicative of its non-electrolytic, diamagnetic nature, and tetrahedral form has been proposed for it. FTIR analysis did not reveal any indication of a water molecule bound to a metal ion. Furthermore, the metal(II) complex's antibacterial action against certain bacteria and fungi is typically more strong than that of the free ligand. The azomethine (C=N) link and the donor atoms (nitrogen and oxygen) provide the metal(II) complex a greater penetration into bacteria, killing or inhibiting their active sites. Meanwhile, we're still working to determine the pharmacophore of the active compound. In this work, we highlight our ongoing attempts to discover further substituted analogues with outstanding biological activity.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

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Compliance with Ethical Standards

None of the author’s experiments reported in this article involve animal or human subjects.

Conflict of Interests

The author states that there are no conflicts of interest.

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