Synthesis, Characterization, and Antimicrobial Properties of a New Ligand and Its Pd(II), Ru(II), Fe(II), Co(II) Complexes

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ABSTRACT

Schiff bases and their complexes are versatile compounds synthesized from the condensation of an amino compound with carbonyl compounds and widely used for industrial purposes and also exhibit a broad range of biological activities including antifungal, antibacterial, antimalarial, antiproliferative, antiviral, and antipyretic properties. In this study, the complexes of (E)-2-(3,5-di-tert-buty1-2-hydroxybenzylideneamino)-6-methyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (L) with Co(II), Fe(II), Pd(II) and Ru(II) were successfully prepared. Structures of Schiff base and its metal complexes obtained were confirmed by FT-IR, 1H-NMR, 13C-NMR, UV–vis, magnetic susceptibility, mass spectroscopy and elemental analysis. Antimicrobial activity of the synthesized Schiff base and its metal complexes have been studied. When the antimicrobial activities of the compounds were examined, it was observed that the lowest activity was exhibited by the ligand, and the highest activity was exhibited by the Co(II) and Pd(II) complexes. None of our compounds were been found to exhibit antifungal activity.

Keywords: Antimicrobial activity, Metal complexes, Schiff base

Yeni Bir Ligand ve Pd(II), Ru(II), Fe(II), Co(II) Komplekslerinin Sentezi, Karakterizasyonu ve Antimikrobiyel Özellikleri

ÖZET

Schiff bazları ve kompleksleri, karbonil bileşikleri ile bir amino bileşinin kondensasyonundan sentezlenen ve endüstriyel amaçlı yaygın olarak kullanılan ve ayrıca geniş bir aralıktaki antifungal, antibakteriyel, antimalarial, antiproliferatif, antiviral ve antipiretik özellikler sergileyen çok yönlü bileşiklerdir. Bu çalışmada, (E)-2-(3,5-di-tert-bütyl-2-hidroksibenzilidenamino)-6-metil-4,5,6,7-tetrahidrobencoz[b]tiyofen-3-karbonitril (L) ile Co(II), Fe(II), Pd(II) ve Ru(II) kompleksleri başarıyla hazırlanmıştır. Elde edilen Schiff bazi ve metal komplekslerinin yapıları FT-IR, 1H-NMR, 13C-NMR, UV-vis, manayetik süsceptibilite, kütü spektroskopisi ve element analiz yöntemleri ile belirlendi. Sentezlenen Schiff bazi ve metal komplekslerinin antimikrobiyal aktiviteleri çalışıldır.
Antimikrobiyal aktivitelere bakıldığında en düşük aktiviteyi ligandın gösterdiği, en yüksek aktiviteyi ise Co(II) ve Pd(II) komplekslerinin gösterdiği görüldü. Maddelerimizin hiçbirinin antifungal aktivite göstermediğini tespit edilmişdir.

Anahtar Kelimeler: Antimikrobiyal aktivite, Metal kompleksler, Schiff bazı

I. INTRODUCTION

The design, synthesis and structural characterization of salicylaldimine complexes are a subject of current interest due to their interesting structural, magnetic, spectral, and catalytic and redox properties, use as models for enzymes and various theoretical interests [1,2]. A variety of transition metal complexes with bi-, tri- and tetradentate Schiff bases containing oxygen and nitrogen donor atoms are of particular interest, because of their remarkable biomedical activities [3,4]. It is also known that N and S atoms play a key role in the coordination of metals at the active sites of numerous biomolecules. Metalo-organic chemistry is becoming an emerging area of research due to the demand for new metal based antibacterial and antifungal compounds [5]. In particular, complexes of metal ions with tetrahedral (e.g. Ag(I)) or octahedral (e.g. Cu(II), Co(II), Fe(II) and Zn(II)) coordination preferences have been found to display important physicochemical properties as well as biological activity [6].

The half-sandwich arene ruthenium(II) complexes with nitrogen-donor ligands have received a lot of attention from researchers due to their potential applications in photochemical devices, catalysis and applications as cytotoxic agents for anticancer therapy [7-9]. Ru(II)–arene compounds have also been reported as antifungal and antibacterial agents [10]. Cobalt is found in the coenzyme of vitamin B12, which is used as vitamin supplement, and at least eight cobalt-dependent proteins have been reported. Antimicrobial, antifungal, antiviral and antioxidant activities have been reported in cobalt complexes of different structural properties, showing antitumor antiproliferative activity, since the first reported study of the biological activity of Co complexes in 1952 [11-13].

The aim of this study is to obtain new Schiff base ligand (L) and its complexes to investigate the biological properties. All the metal complexes are stable to air and moisture. The synthesized ligand and its complexes were tested for antimicrobial activity against various microorganisms. When comparing the antimicrobial activities of the ligand and its metal complexes, the metal complexes were more antimicrobial on the growth of the microorganisms than the ligand.

II. MATERIAL AND METHOD

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. The metal chlorides were used in their hydrated form. The reference antibiotic discs were obtained from Oxoid, Thermo Fisher Scientific (Wade Road, Basingstoke, Hampshire RG24 8PW, United Kingdom).
Elemental analysis (C, H, and N) was carried out on a Leco CHNS-O model 932 elemental analyzer. Fourrier-transform infrared (FT-IR) spectra were collected with KBr pellets (typically 2 mg sample in 200 mg KBr) using Perkin Elmer Precisely Spectrum One spectrometer (4000-400 cm⁻¹ range). ¹H-NMR and ¹³C-NMR were recorded on Bruker GmbH DPX-400 MHz FT spectrometer using CDCl₃ as solvent and tetramethylsilane (TMS) as an internal standard. UV-Vis. spectra of the complexes were recorded on a Shimadzu model UV-1800 Spectrophotometer in the range 200-1100 nm in dimethylformamide (DMF) solution (1×10⁻³ M). Magnetic susceptibility measurements were performed using the standard Gouy tube technique using Hg[Co(SCN)₄] as a calibrate. LC/MS-API-ES mass spectra were recorded using an AGILENT model 1100 MSD mass spectrophotometer.

A. SYNTHESIS of SCHIFF BASE LIGAND (L)

The ligand was synthesized as at Fig. 1 by refluxing reaction mixture of hot ethanol solution (30 mL) of 2-amino-6-methyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (0.01 mol) and hot ethanol solution (30 mL) of 3,5-di-tert-butyl-2-hydroxybenzaldehyde (0.01 mol) for 7 h with addition of 3-4 drops of glacial acetic acid. Precipitate that formed during reflux was filtered, washed with cold ethanol, and recrystallized from hot ethanol, m.p.: 190-192 °C (Yield: 90%) of ligand.

B. CHARACTERIZATION of (E)-2-(3,5-DI-TERT-BUTYL-2-HYDROXY BENZYLIDENEAMINO)-6-METHYL-4,5,6,7-TETRAHYDROBENZO[b]THIOPHENE-3-CARBONITRILE (L)

Anal. Calcd. for (C₂₃H₂₂N₂OS) (FW: 408.60 g/mol) (%): C, 73.42; H, 7.83; N, 6.85; S, 7.80. Found: C, 73.50; H, 7.85; N, 6.92; S, 7.84. FT-IR (KBr, v max (cm⁻¹)): 3445, 3381 (OH), 1158, 1558 (ar-C=C), 1400 (C=O), 772 (C=S=C). ¹H-NMR (400 Mz, CDCl₃): δ (ppm): δ 12.04 (s, 1H, OH), 8.70 (s, 1H, CH=N), 7.50-7.40 (s, 2H, Ar-CH). 2.90-2.30 (m, 6H, cyclohexyl-CH₂), 2.04 (s, H, cyclohexyl-CH). 1.30 (s, 18H, tert-butyl-CH₃), 0.96 (d, 3H, cyclohexyl-CH₃). ¹³C NMR (DMSO-d₆, 400 MHz) δ 160.00 (CH=N), 158.00, 140.60 (C=S-Cthiophene), 154.00 (C=OH), 117.70-138.60 (Ar-C), 115.30 (C≡N), 31.60-34.50 (tert-butyl-CH₃), 21.70 (cyclohexyl-CH₃), 20.04-32.02 (cyclohexyl-CH₂). UV–vis bands (λ max, nm: π→π*, 229, 267; n→π*, 316, 343, 374.

![Figure 1. Synthesis schema of Schiff base ligand (L)](image)

C. PREPARATION OF THE COMPLEXES

The metal complexes were prepared by adding stoichiometric quantities to a hot methanol solution (30 mL) containing 0.01 mol ligand and methanol solution (20 mL) of a metal chlorides ([RuCl₂(p-cymene)] (0.005 mol), [PdCl₂(C₂H₅CN)₂], FeCl₃-H₂O and CoCl₃·6H₂O (0.01 mol). The mixtures were stirred under reflux for 4 hours, whereupon the complexes precipitated. They were collected by filtration, washed with hot ethanol and dried at room temperature. m.p.: >300 °C (Yield: 80-90%).
Co(II) Complex: FW: 663.84 g/mol. FT-IR (KBr, v max (cm\(^{-1}\))): 3025, 3400 (−OH), 3195 (ar−CH), 2951 (aliph.–CH), 2212 (C≡N), 1622 (CH=N), 1578, 1556 (ar−C=C), 1272 (C-O), 773 (C=S–C), 537 (M–O), 486, 468 (M–N). Anal. Calcd. for (C\(_{63}H_{58}N_2SO_{10}CoCl\): C; 45.22, H; 7.38, N; 4.22, S; 4.82. Found: C; 45.15, H; 7.40, N; 4.24, S; 4.78. UV–vis bands (\(\lambda_{max}\), nm): \(\pi \rightarrow \pi^*\), 225; \(n \rightarrow \pi^*\), 267, 293, 378, 408, 577, 656, 751. MS [ESI]: m/z 592.73 (calcd.), 592.38 (found) [M+4H\(^2\)+H\(^+\)]. \(\mu_{eff}\) (B.M.): 4.57. Color: Orange.

Fe(II) Complex: FW: 588.90 g/mol. FT-IR (KBr, v max (cm\(^{-1}\))): 3392, 3449 (−OH), 3001 (ar–CH), 2951, 2910 (aliph.–CH), 2212 (C≡N), 1610 (CH=N), 1578, 1558 (ar–C=C), 773 (C=S–C), 508 (M–O), 487 (M–N). Anal. Calcd. for (C\(_{58}H_{44}N_2SO_{8}FeCl\): C; 50.90, H; 6.96, N; 4.75, S; 5.43. Found: C; 50.91, H; 6.89, N; 4.72, S; 5.41. UV–vis bands (\(\lambda_{max}\), nm): \(\pi \rightarrow \pi^*\), 268, 293; \(n \rightarrow \pi^*\), 315, 379, 407, 985. MS [ESI]: m/z 590.88 (calcd.), 590.91 (found) [M+2H\(^2\)+]. \(\mu_{eff}\) (B.M.): 4.70. Color: Black.

Ru(II) Complex: FW: 713.97 g/mol. FT-IR (KBr, v max (cm\(^{-1}\))): 3449, 3380 (−OH), 3036, 3005 (ar–CH), 2952 (aliph.–CH), 2211 (C≡N), 1608 (CH=N), 1578, 1558 (ar–C=C), 1270 (C-O), 772 (C=S–C), 486, 458 (M–N). Anal. Calcd. for (C\(_{38}H_{24}N_2OSRuCl\): C; 58.82, H; 6.44, N; 3.92, S; 4.48. Found: C; 58.66, H; 6.36, N; 3.90, S; 4.50. UV–vis bands (\(\lambda_{max}\), nm): \(\pi \rightarrow \pi^*\), 269, 293; \(n \rightarrow \pi^*\), 316, 366, 379, 407, 963. Mass Spectra [ESI]: m/z 715.97 (calcd.), 715.82 (found) [M+2H\(^2\)+]. \(\mu_{eff}\) (B.M.): 4.70. Color: Black.

Pd(II) Complex: FW: 548.85 g/mol. FT-IR (KBr, v max (cm\(^{-1}\))): 3012 (ar–CH), 2975, 2956 (aliph.–CH), 2213 (C≡N), 1603 (CH=N), 1573-1562 (ar–C=C), 1276 (C-O), 769 (C=S–C), 518 (M–O), 491 (M–N). Anal. Calcd. for (C\(_{38}H_{24}N_2OSPdCl\): C; 54.66, H; 5.64, N; 5.10, S; 5.83. Found: C; 54.70, H; 5.68, N; 4.99, S; 5.72. UV–vis bands (\(\lambda_{max}\), nm): \(\pi \rightarrow \pi^*\), 220, 236 n → \(\pi^*\), 330, 384, 407, 919. Mass Spectra [ESI]: m/z 513.40 (calcd.), 513.09 (found) [M-Cl\(^+\)]. Color: Dark Brown.

\[\text{Figure 2. Suggested structures of the Co(II) and Fe(II) complexes}\]

\[\text{Figure 3. Suggested structures of the Ru(II) and Pd(II) complexes}\]

**D. MICROORGANISMS and ANTIMICROBIAL ASSAYS**

Ligand, Ru(II), Co(II), Fe(II) and Pd(II) samples were separately tested against *Enterobacter aerogenes* ATCC 27859, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* 6538, *Bacillus megaterium* DSM 32, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumoniae* RSKK 574,
Escherichia coli, Candida albicans ATCC 10231, Yarrowia lipolytica and Saccharomyces cerevisiae. All the bacterial and fungal strains examined in this work were supplied by Muş Alparslan University, Microbiology Laboratory (Turkey). Ampicillin/sulbactam (SAM-20), Rifampicin (RD-5), Erythromycin (E-15), Amikacin (AK-30) and Fluconazole (FCA-25) were used as positive controls.

The antimicrobial activities of the samples were determined by well diffusion method [8, 9]. For this purpose, bacterial and fungal strains were cultured overnight at 37 °C in nutrient agar and at 25 °C in Sabouraud dextrose agar medium, respectively. 100 µL of suspension of test microorganisms, containing 1×10^8 colony-forming units CFU/mL of bacteria cells and 1×10^4 CFU/mL spores of fungal strains spreaded on Nutrient agar and Sabouraud dextrose agar medium, respectively. Then, the medium was poured into a petri dish on a horizontally leveled surface. After the medium was solidified, 9 mm diameter wells per dish were made in the agar medium. Then 20µL, 40 µL and 80 µL aliquots of ligand, Co(II), Fe(II), Pd(II) and Ru(II) suspensions (dispersed in DMSO as 10 wt. %/V) were loaded into the wells separately. The petri dishes were incubated at 37 °C for 24 h for bacteria and at 25 °C for 24-48 h for fungal strains. The average zone diameters were measured after repeating the experiment for at least three times.

The synthesized complexes of (E)-2-(3,5-di-tert-butyl-2-hydroxybenzylideneamino)-6-methyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile) with Co(II), Fe(II), Pd(II) and Ru(II) ions in the stoichiometry ratio of 1:1 are partly soluble in ethanol, methanol and insoluble in common organic solvents like benzene and petroleum ether but soluble in DMSO, DMF. Analytical data and various physic-chemical properties such as yield and melting point of the synthesized complexes are given in material and method section.

III. RESULTS AND DISCUSSION

The infrared (IR) spectra have an important role for identifying the functional groups of the ligand alongside its significant evidence for the interaction between those groups and the central metal ions of the prepared complexes. The characteristic absorption band at 1615 cm\(^{-1}\) was due to azomethine linkage. The band of the azomethine linkage of the Schiff base ligand was shifted to different frequencies (1622-1603 cm\(^{-1}\)) due to complexation with the metal ion via azomethine nitrogen. Two new weak bands appeared at lower frequencies at 1622–1603 cm\(^{-1}\), which associated with metal to nitrogen and metal to oxygen bonds, respectively [14].

The IR spectrum of Fe(II), Co(II) complexes confirms that these complexes bind to the azomethine and hydroxyl group oxygen. A sharp band at 772 cm\(^{-1}\) was assigned to the characteristic \(\nu(C=\text{S}C)\) thiophene ring. The asymmetric and symmetric stretching of \(\nu(C=S)\) was appeared as sharp band at 770 and 739 cm\(^{-1}\) in the Pd(II) complex, respectively [17,18]. Ru(II) complexes of only ligand azomethine group was bonded to the nitrogen atom. Whereas the IR spectra of Pd(II) complex confirmed the coordination mode of the participation of oxygen atom of the ligand for OH group, Pd(II) ion via the azomethine nitrogen, thiophene sulfur. The infrared spectra for ligand and its metal complexes were summarized in material and method section.

The UV-vis. spectra were recorded in a DMF solution in the wavelength range 200-1100 nm. The bands in the UV-vis. spectra of ligand and its complexes were assigned upon comparison with
spectrum of the ligand. Bands between 315-384 and 220-293 nm $\rightarrow \pi^*$ and $\pi \rightarrow \pi$ assigned to (LC) ligand-centered [19]. The ligand metal charge transfer bands of metal complexes appeared as a shoulder in the range of 407-408 nm [20]. Based on the pattern of the electronic spectra of all the complexes, indicated that Ru(II) octahedral geometries similar to that for Co(II), Fe(II) complexes, as expected Pd(II) complex tetrahedral geometry [21].

$^1$H-NMR spectra of ligand and synthesized complexes were recorded in CDCl$_3$. OH proton peak, in the spectra of ligand and complexes, was characteristic for identification of complexation. This proton peak in the region of 12.04 ppm in ligand was absent in all complexes except Ru(II) complex [19]. This observation led to conclusion that metal to oxygen bonds have been formed. The signal of azomethine CH=N linkage was found at 8.70 ppm. Multiple signals appeared in the range of 7.50-7.40 ppm were assigned to the proton of the phenyl group. Strong sharp singlet signals appeared at 0.96 and 1.30 ppm were assigned to the C(CH$_3$)$_3$ protons [22]. The protons of isopropyl group of the Ru(p-cymene) complex appeared as two sets of doublets around 0.8-1.0 ppm. The methyl protons appeared as singlet at 1.4-1.7 ppm. The isopropyl CH proton appeared as a septet in the range of 2.4-2.6 ppm. The aromatic hydrogens of the p-cymene appeared as four sets of doublets in the range of $\delta$ 3.7-5.6 ppm. Furthermore methoxy protons were observed as singlet for complex 3.6 ppm.

$^{13}$C-NMR spectra of ligand and its complexes were recorded in CDCl$_3$. Ligand NMR data was compared with those of complexes. This comparative study confirms formation of metal to ligand bonds. $^{13}$C-NMR spectrum of the ligand showed characteristic signals at 160.00 and 154.00 ppm are due to HC=N and C–O carbon, respectively. In case of complexes, azomethine carbon peak shifts down field, and appeared in the range of 163-168 ppm. This downfield shift was due to electron donation to metal centre.

Magnetic susceptibilities of complexes were measured at room temperature. The results in material and method section supported that both Fe(II) and Co(II) have values of effective magnetic moment sufficient for octahedral geometry. The Fe(II) and Co(II) complexes reported herein are high spin at room temperature magnetic moment values of 4.70, 4.57 B.M respectively. These results indicate that, the complexes of Co(II), Fe(II) have a six coordinate and probably octahedral geometry [14, 23]. The Ru(II) and Pd(II) complexes were diamagnetic as expected and their geometries are probably octahedral, tetrahedral geometry, respectively.

The mass spectrum of the complexes was given in material and method section. The spectrum confirmed the proposed formula by showing peaks at m/z values.

To compare the antimicrobial activity of ligand, Co(II), Fe(II), Ru(II) and Pd(II) complexes the samples were tested against three different Gram-positive bacteria (Bacillus subtilis ATCC 6633, Bacillus megaterium DSM 32, Staphylococcus aureus ATCC 6538), four different Gram-negative bacteria (Escherichia coli, Enterobacter aerogenes ATCC 27859, Klebsiella pneumoniae RSKK 574, Pseudomonas aeroginosa ATCC 9027) and three fungi species (Yarrowia lipolytica, Saccharomyces cerevisiae and Candida albicans ATCC 10231). To determine the inhibition of bacteria growth, the inhibition zones were calculated for each sample concentration. To evaluate the value of the sample, the reference antibiotics were also analyzed for comparison (Table1, 2). The ligand showed activity against only P. aeroginosa and K. pneumonia bacteria (14±0.57-12±1.00 mm in diameter). While the Ru(II) complex showed the highest antibacterial activity against B. subtilis, it did not show any activity against B. megaterium. The highest antimicrobial activity was demonstrated against the Co(II) complex B. megaterium and E. aerogenes while the lowest antimicrobial activity was detected against
*P. aeruginosa*. Antibacterial activity against *E. aerogenes* and *K. pneumoniae* in the 16 mm zone was shown by the presence of Fe(II) complex while the least activity was found in the 12 mm zone against *B. subtilis*. Pd(II) complex showed the best antimicrobial activity against *P. aeruginosa* at all 4 concentrations and the lowest activity against *K. pneumonia* and *E. aerogenes*. When the antimicrobial activities of the compounds are generally examined, it is observed that the lowest activity is exhibited by the ligand, and the highest activity is exhibited by the Co(II) and Pd(II) complexes. The Co(II) and Pd(II) complexes were found to exhibit the highest antibacterial activity against *B. megaterium*, *E. aerogenes* and *P. aeruginosa*. None of our compounds have been found to exhibit antifungal activity.

When we compared our compounds with the antibiotics we used for control, Co(II) and Pd(II) complexes were found to have a similar action to erythromycin and more effective than the other three (Ampicillin/sulbactam, Rifampicin and Amikacin) antibiotics.

![Figure 1](image1.png)

*Figure 1. Figure Name (a), (b)*
Table 1. Antimicrobial activity of ligand and its metal complexes (10 mg/mL DMSO)

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Table 2. Antimicrobial activity results of reference antibiotics

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IV. CONCLUSION

The development in the field of bio-inorganic chemistry has increased the interest in Schiff base complexes, since it has been recognized that many of these complexes may serve as models for biologically important species and potential applications. But still there is need to explore the biological properties of these already synthesized transition metal complexes and to synthesize new complexes with more properties. The results of this study can be evaluated in different areas and can contribute to similar studies.

V. REFERENCES


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