Synthesis, characterization, electrochemical and antibacterial studies of new water soluble cobalt (III) Schiff base complexes derived from meso-1,2-diphenyl-1,2-ethylenediamine

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Abstract: A new N₂O₂ type water soluble Schiff base ligand and three new Cobalt (III) complexes of this ligand were synthesized and characterized by means of ¹H-NMR, IR and UV-Vis spectroscopies as well as elemental analyses. The complexes have general formula [Co(L)(L')₂]PF₆₃, in which L is the Schiff base ligand and L’s are pyridine (CoL₁), Imidazole (CoL₂) or N-methylimidazol (CoL₃). The in vitro antimicrobial activity of the ligand and the corresponding complexes was studied against four human pathogenic bacteria. The complexes showed moderate antibacterial activity against both Gram positive and Gram negative bacteria. The Antibacterial activity of the complexes was higher than that of the ligand due to complexation and CoL₁ had better antibacterial activity compared to the others.

Keywords: Schiff base; Water soluble; Cobalt; Antibacterial.

INTRODUCTION

The problem of bacterial resistance is a serious global issue and many researches are directed towards finding new solutions to this problem⁶⁻¹⁰. Transition metals, and especially their chelate complexes are well studied in this regard, and it has been found that chelation has increased the antibacterial activity of such complexes⁶⁻¹². Recently, considerable attention has been paid to the study of the antibacterial activity of Schiff base ligands and their transition metal complexes¹³⁻²⁰. But poor aqueous solubility of such compounds precludes their potential development for clinical uses. In fact, aqueous solubility is considered as a key factor in the designation of actual antibacterial drugs²¹⁻²³. One strategy to increase aqueous solubility of Schiff base complexes is to introduce especial substituents to their structure. It is previously shown that the presence of SO₃Na or PR₃X groups (R = alkyl or aryl and X = Cl or other anions) on the salicylaldehyde part of the Schiff base
ligands results in increased aqueous solubility of their metal complexes\(^2\)\(^3\)\(^4\). Considering this, and in continuation of our previous studies, herein we report the synthesis, characterization and antibacterial activity of a new water soluble Schiff base ligand and its cobalt (III) complexes. The Schiff base ligand is derived from the condensation of meso-1,2-diphenyl-1,2-ethylenediamine with 3-formyl-4-hydroxy-benzyltriphenylphosphonium chloride. The complexes have general formula \([\text{Co(L)(L')}_2](\text{PF}_6)_3\) in which L is the Schiff base ligand and L’ is pyridine (py), Imidazole (Im) or 1-methylimidazole (MeIm). The antibacterial activity of the ligand and the cobalt complexes were studied against four human pathogenic bacteria. The complexes showed moderate antibacterial activity against both Gram positive and Gram negative bacteria. Besides, the antibacterial activity of the metal complexes was higher than that of the free ligand.

**EXPERIMENTAL**

**Reagents and instruments**

All chemicals and solvents were of the highest purity and were used as received. All syntheses and purifications were performed in aerobic conditions. Meso-1,2-diphenyle-1,2-ethylenediamine\(^2\)\(^6\) and 3-formyl-4-hydroxy-benzyltriphenylphosphonium chloride were synthesized as described elsewhere\(^2\)\(^7\)\(^8\). The cobalt (III) complexes were also synthesized following a routine procedure\(^1\)\(^7\). Elemental analyses were performed using a Perkin-Elmer 2400II CHNS-O elemental analyzer. \(^1\)HNMR spectra were recorded on a 500MHz Bruker FT-NMR spectrometer using DMSO-d\(^6\) as solvent; chemical shifts (\(\delta\)) are given in ppm; s = singlet, dt = doublet of triplets, m = multiplets. IR spectra were obtained as KBr plates using a Bruker FT-IR instrument. UV-Vis spectra were obtained on a Shimadzu UV-1650PC spectrophotometer. A Metrohm 757 VA computer instrument was employed to obtain cyclic voltammograms in DMSO at room temperature (25°C) using 0.1 M tetra-n-butylammonium hexafluorophosphate solution as supporting electrolyte.

**Synthesis of the ligand: N,N’-bis{5-[(triphenylphosphonium)-methyl]salicylidine}-meso-1,2-diphenyl-1,2-ethylenediamine chloride (L)**

In a typical experiment, to a solution of 1.06 g (5 mmol) of meso-1,2-diphenyl-1,2-ethylenediamine in 40 mL of ethanol was added a solution of 3.98 g (10 mmol) of 3-formyl-4-hydroxy-benzyltriphenylphosphonium chloride in 40 mL of ethanol. The reaction mixture was stirred and refluxed for 60 min and was then left undisturbed to cool down to room temperature. The solution was evaporated to about 20 mL with a rotary evaporator in order to obtain the yellow powder of the Schiff base ligand, then it was washed with a minimum amount of ethanol and dried in vacuum. Recrystallization from ethanol yielded the desired ligand in high yield and purity (3.93 g, 95 %). The schematic representation of the structure of Schiff base ligand is shown in figure 1. M.p. = 228 °C. \(^1\)HNMR: 4.99 (s, 2H, CHPh); 5.06 (d, 4H, CH\(_2\)PPh\(_3\)); 6.69-7.89 (m, 46H, CH\(_2\)Ar); 7.99 (s, 2H, HC=N); 13.12 (s, 2H, O-H). IR (\(\text{í}, \text{cm}^{-1}\)): 3409 (\(\text{í}\)O-H); 1627 (\(\text{í}\)C=N); 1589, 1488 (\(\text{í}\)C=C); 1110 (\(\text{í}\)C-O). UV-Vis in methanol 775 nm (\(\epsilon_\text{M}, \text{M}^{-1}\)\text{cm}^{-1}\)): 278 (22700); 325 (8600). Anal. Calcd. (found) for C\(_{66}\)H\(_{56}\)Cl\(_2\)N\(_2\)O\(_2\)P\(_2\).C\(_2\)H\(_5\)OH: C: 75.03 (74.97); H 5.70 (5.75); N 2.57 (2.65).

**Figure 1**: Schematic representation of the structure of cationic part of the Schiff base ligand, axial ligands and the cationic part of the complexes.
Synthesis of the complexes

(a) Synthesis of [Co(L)(py)]2(PF3)3, (CoL3)

0.97 g (1 mmol) of the Schiff base ligand was dissolved in 20 mL of methanol and was constantly stirred. To this solution was added a solution of 0.25 g (1 mmol) of Co(OAc)2-H2O in 20 mL of methanol followed by the addition of 1.0 mL of pyridine. The reaction mixture was stirred at room temperature for 4 h while air was being bubbled through it. The color of this solution gradually changed to dark brown. Then, Excess NH4PF6 (6 mmol, 1.0 g) was added and the reaction mixture was stirred for a further 5 min. The resulting brown precipitate was filtered off, washed with 5 mL of cold methanol and then 20 mL of diethyl ether and then was air-dried. Recrystallization from CH3CN/Et2O (1:1) gave 1.22 g (73 %) of the target complex. 1H NMR: 4.64, 4.80 (dt, 4H, CH2PPh); 6.35 (s, 2H, CHPh); 7.00-8.06 (m, 58H, H; and HC=N). IR (υ, cm-1): 1620 (υC=O); 1527, 1434 (υp,p); 840 (υp,p). UV-Vis in CH2Cl2, λ, nm (ε, M-1 cm-1): 268 (78400); 310 (51500); 537 (8100). Anal. Calcd. (found) for C27H21F2N6O2.5P Co: C 53.27 (53.32); H 4.15 (4.09); N 5.08 (5.16).

(b) Synthesis of [Co(L)(Im)]2(PF3)3, (CoL3)

This complex was synthesized following a similar procedure as described for CoL3 except imidazole was used instead of pyridine. Recrystallization from CH2Cl2 gave 1.44 g (86.2 %) of the target complex. 1H NMR: δ (ppm): 3.62 (s, 6H, MeIm N-CH3); 4.83, 4.98 (dt, 4H, CH2PPh); 5.78 (s, 2H, CHPh); 6.20-7.87 (m, 56H, H; and HC=N). IR (υ, cm-1): 1620 (υC=O); 1527, 1465 (υC=O); 840 (υp,p). UV-Vis in CH2Cl2, λ, nm (ε, M-1 cm-1): 268 (78400); 383 (6100); 419 (3900); 517 (600). Anal. Calcd. (found) for C76H71F2N6O2.5PCo: C 53.27 (53.32); H 4.15 (4.09); N 4.90 (4.97).

Electrochemical studies

Cyclic voltammetry was used to study the electrochemical properties of the complexes. The voltammograms were obtained at room temperature, from approximately 10^-3 M DMSO solutions, under nitrogen atmosphere, with a working Pt electrode, a Pt auxiliary electrode and Ag/AgCl reference electrode, in the range of -1.5 to +0.7 volt. 0.1 M Tetra-n-butylammonium hexafluorophosphate (TBAHFP) was used as supporting electrolyte and Ferrocene was used as internal standard to reference the data vs. Fc+/0 couple.

Biological studies

(a) Bacterial strains

The ligand and the cobalt (III) complexes were individually tested against a panel of microorganisms (Gram negative and Gram positive), namely Bacillus subtilis (B. subtilis; Gram positive, ATCC 6633); Staphylococcus aureus (S. aureus; Gram positive, ATCC 25923), Salmonella Typhi (S. Typhi; Gram negative, ATCC 19430), and Escherichia coli (E. coli; Gram negative, ATCC 25922. The organisms were purchased from Iranian Research Organization for Science and Technology (IROST).

(b) Minimum inhibitory concentrations (MICs)

Minimum inhibitory concentrations of the chemicals were determined by the broth twofold dilution method as a quantitative assay[28]. Briefly, serial diluted chemical compounds in the range of 0.05-0.1 mg/mL were added to a final inoculum of approximately 1.5×10^6 organisms per mL in log-phase growth. The cultures were incubated on a rotary shaker at 37 °C for 24 h. MIC (μg/mL) of each tested compound was defined as the lowest concentration exhibiting no visible growth compared to the drug-free control wells. All the tests were performed in duplicates on different days to measure the reproducibility of the tests.
(c) Disc diffusion assay

Single-disc diffusion as a qualitative assay was performed according to Bauer et al. [29]. Briefly, four to five colonies of each organism were inoculated into 4 mL of broth and incubated for 4 to 6 h at 37°C. A suspension of each organism was then standardized against a turbidity standard of 0.5 McFarland. Bacteria were cultured on agar plates using sterile absorbent cotton swabs. Then plates were incubated at 37°C and the inhibition zone diameters (IZD) were measured after 24h. Each organism was tested in duplicate on different days to measure the reproducibility of the test. Kanamycine (30μg/disc) and chloramphenicol (30μg/disc), purchased from Himedia Laboratories (India), were used as reference antibacterial agents. A set of assay tubes containing only inoculated medium was kept as negative control and likewise solvent controls were also done simultaneously.

RESULTS AND DISCUSSIONS

Spectroscopic characterization of the ligand and the complexes

$^1$HNMR, IR and UV-Vis spectroscopies were used to characterize the ligand and the complexes. In the IR spectrum of 3-formyl-4-hydroxy-benzyltriphenylphosphonium chloride, an intense band at 1674 cm$^{-1}$ was assigned to the stretching vibration of the carbonyl group [27]. This band was removed in the IR spectrum of the Schiff base ligand and was replaced with a new band at 1627 cm$^{-1}$ which was assigned to the stretching vibration of the azomethine (HCP%N) group. In the IR spectra of the complexes, this band was shifted to lower wave numbers and appeared at around 1620 cm$^{-1}$. This observation was indicative of the coordination of the nitrogen atoms of the Schiff base ligand to the metal center. Another important band in the IR spectra of the complexes was the band at around 840 cm$^{-1}$ which was due to the presence of uncoordinated PF$_6^-$ ions. In the $^1$HNMR spectra of the free ligand, the signals due to the phenolic OH protons were observed at 13.12 ppm. This signal was absent in the $^1$HNMR spectra of the diamagnetic cobalt (III) complexes which indicated that the Schiff base ligand had acted as a dianionic ligand. The observation of a sharp signal at 7.99 ppm in the $^1$HNMR spectrum of the ligand was assigned to the presence of the azomethine protons. This signal was shifted down field upon complexation. Unfortunately, the azomethine $^1$HNMR signals were masked with the signals of the aromatic protons and it was not possible for us to assign an exact signal to these protons. The observation of downfield shift of the $^1$HNMR signals of the azomethine protons confirmed the coordination of the nitrogen atoms of the azomethine groups, therefore, it was clearly concluded that the Schiff base ligand had acted as a tetradeutate N$_2$O$_2$ type ligand. Actually, this mode of coordination is the most commonly observed mode in such ligands. Another interesting feature in the $^1$HNMR spectra of the complexes was the observation of doublet of triplets which was assigned to the CH$_2$ protons attached to the Pph$_3$ group. This splitting pattern was due to the coupling with the phosphorous atom ($I = 1/2$) and most probably coupling to the two aromatic protons of the phenyl ring in ortho position to the CH$_2$Pph$_3$ group. Other signals are also in good agreement with the proposed structures. Figure 2 shows the $^1$HNMR spectrum of CoL$^3$ as an example. In the UV-Vis spectra of the ligand, the two bands at 278 and 325 nm are assignable to intraligand π→π* transitions. These bands are observed in the UV-Vis spectra of the complexes with a moderate blue shift due to complexation. Another band at around 400 nm could be assigned to the LMCT [30,31]. The d-d transitions of the octahedrally surrounded cobalt (III) complexes are usually observed in the visible region at around 500-600 nm. The same bands are also observed in our complexes, confirming the proposed structures. Analytical data also further confirmed the synthesis of the complexes.

Electrochemistry

The cyclic voltammograms of the ligand and the complexes were obtained at room temperature under nitrogen atmosphere. The ligand was electro-inactive over a range of +0.7 to -1.5 V. The reduction potential data for the complexes are given in TABLE 1. The redox properties of the cobalt (III) complexes exhibited grossly similar features at the scan rates of 20–200 mV/s consisting of one electrochemically irreversible and one electrochemically quasi-reversible reduction. The first irreversible redox process at about -400 mV was assigned to [Co$^{III}$(L)(amine)$_2$]$^{3+} + e \rightarrow [Co^{II}(L)(L')]^{2+} + L'$ pro-
The antibacterial activity of the complexes

Potent antibacterial activity of transition metal complexes of Schiff base ligands have been reported in the literature. Among these complexes, Co(II) and Co(III) have received greater attention. But low aqueous solubility of such compounds has limited their clinical tests. The synthesized water soluble Schiff base ligand and the corresponding cobalt (III) complexes were screened for in vitro antibacterial activity against two Gram-positive and two Gram-negative bacteria. TABLE 2 and 3 show the MICs and the IZD values for the studied ligand and complexes against the bacterial strains, respectively, as well as two standard compounds. As it could be seen from these two tables, the ligand had low antibacterial activity against the studied microorganisms but the metal complexes had higher activity. According to the Clinical and Laboratory Standards Institute interpretive criteria, the three studied complexes had high activity against Gram-negative and Gram-positive bacteria. This increased activity was arisen from coordination and the results could be explained by Overtone’s concept and Tweedy’s theory; Co(OAc)₂ had low inhibitory properties. Accordingly, the polarity of the metal ions is greatly reduced within the chelated metal complexes due to overlapping with the ligand orbitals. The delocalization of the electrons over the whole complex is also observed as well. These complexes\textsuperscript{30,31}. The addition of one electron to the antibonding $d_{xy}$ orbital of the metal center is responsible for the loss of the axial ligand. The second reduction process was also a metal centered reduction which could be assigned to $[\text{Co}^\text{III}(L)(L')]^{2+} + e \rightarrow [\text{Co}^\text{II}(L)(L')]^{+}$ process. This later process is sensitive to the $\sigma$-donating properties of the axial ligands and shifts to more negative values upon increasing the strength of the ligand\textsuperscript{30}.

\begin{table}[h]
\centering
\begin{tabular}{lccccccc}
\hline
Complex & $E_{pc}$ & $E_{pa}$ & $\Delta E_p$ & $E^\circ$ & $E_{pc}$ & $E_{pa}$ & $\Delta E_p$ & $E^\circ$ \\
\hline
CoL\textsuperscript{1} & -581 & -319 & 262 & -450 & -932 & -819 & 113 & -875 \\
CoL\textsuperscript{2} & -568 & -371 & 197 & -469 & -943 & -818 & 125 & -880 \\
CoL\textsuperscript{3} & -483 & -237 & 246 & -360 & -1030 & -846 & 184 & -938 \\
\hline
\end{tabular}
\caption{Redox potentials (mV) for cobalt complexes in DMSO.}
\end{table}

\textsuperscript{*}Potentials are vs. Fc\textsuperscript{+}/0 in 0.1 M TBAHFP, T = 298 K. Scan rate, 100 mV/s. Approximate concentrations: 10\textsuperscript{-3} M.
two factors result in the enhanced penetration of the metal complexes into the lipid membranes. They also block the metal binding sites in the enzymes of microorganisms\[^{38}\]. The complexes showed broad spectrum antibacterial activity against both Gram positive and Gram negative bacteria. Comparing the MIC values of these complexes with those similar ones in the literature showed that the studied complexes were rather good antibacterial agents\[^{18}\]. Although the antibacterial activity of the complexes against both Gram positive and Gram negative bacteria was almost similar, CoL\(^{3}\) had to some extent better antibacterial activity since it had the lowest MIC values compared to the other ones, especially for Gram negative bacteria. This judgment was further confirmed with the comparison of the IZD values, where, CoL\(^{3}\) had the highest IZD values. In the disc diffusion method, the diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium\[^{39}\]. The mechanisms of the antibacterial activity of transition metal complexes have been scarcely reported. Three possible mechanisms for inhibition have been proposed: (i) interference with electron transport; (ii) binding to DNA; and (iii) interaction with the cell membrane\[^{40}\]. Nomiyama and coworkers have also suggested that the ease of axial ligand replacement could result in further ligand replacement with biological ones and increased antibacterial activity\[^{12}\]. The same suggestion may also apply for our studied complexes, since the imidazole derived ligands are better σ-donors/π-acceptors than pyridine\[^{30}\] and are therefore more strongly bound to the metal centers. Besides, the presence of electron donating methyl group on MeIm ligand could result in better σ-donating properties and hence, increased binding. This suggestion is consistent with the IZD values, where CoL\(^{3}\) showed the highest and CoL\(^{1}\) the lowest IZD values, for Gram negative bacteria. These findings are also in good agreement with the findings of Manus and coworkers which have confirmed the same trend in axial ligand displacement\[^{41}\]. Of course, other factors are also important and more work is necessary to be done to establish a full understanding of the antibacterial activities.

**TABLE 2**: MIC values of synthesized compounds against growth of bacteria (\(\mu\)g mL\(^{-1}\))

<table>
<thead>
<tr>
<th>Compound</th>
<th>Gram Negative Bacteria</th>
<th>Gram Positive Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Escherichia coli</td>
<td>Salmonella Typhi</td>
</tr>
<tr>
<td>Ligand</td>
<td>60</td>
<td>55</td>
</tr>
<tr>
<td>CoL(^{1})</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>CoL(^{2})</td>
<td>40</td>
<td>35</td>
</tr>
<tr>
<td>CoL(^{3})</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Kanamycine</td>
<td>3.8</td>
<td>3.2</td>
</tr>
<tr>
<td>chloramphenicol</td>
<td>4.2</td>
<td>3.5</td>
</tr>
</tbody>
</table>

**TABLE 3**: IZD values (mm) of antibacterial compounds against pathogenic bacteria.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Gram Negative Bacteria</th>
<th>Gram Positive Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Escherichia coli</td>
<td>Salmonella Typhi</td>
</tr>
<tr>
<td>Ligand</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CoL(^{1})</td>
<td>23±1</td>
<td>20±1</td>
</tr>
<tr>
<td>CoL(^{2})</td>
<td>18±1</td>
<td>19±1</td>
</tr>
<tr>
<td>CoL(^{3})</td>
<td>10±1</td>
<td>8±1</td>
</tr>
<tr>
<td>Kanamycine</td>
<td>25±1</td>
<td>20±1</td>
</tr>
<tr>
<td>chloramphenicol</td>
<td>31±1</td>
<td>25±1</td>
</tr>
</tbody>
</table>

**CONCLUSION**

A new water soluble Schiff base ligand and three water soluble cobalt (III) Schiff base complexes were synthesized and characterized. Antibacterial activity of the ligand and the metal complexes were studied against two Gram negative and two Gram positive bacteria. The antibacterial activity of the complexes was higher than that of the ligand which was suggested to be due...
to the coordination to the metal center. Besides, CoL had higher activity especially against Gram negative bacteria.

REFERENCES


