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Original article

Synthesis, spectroscopic characterization and antibacterial activity of new cobalt(II) complexes of unsymmetrical tetradentate (OSN_2) Schiff base ligands

Lotf Ali Saghatforoush [a,*], Firoozeh Chalabian [b], Ali Aminkhani [c], Ghasem Karimnezhad [a], Sohrab Ershad [d]

[a] Department of Chemistry, Payame Noor University, Khoy 58168-45164, Iran
[b] Department of Biology, Islamic Azad University, Tehran North Campus, Tehran, Iran
[c] Department of Chemistry, Azad Islamic University, Khoy Branch, Khoy, Iran
[d] Department of Chemistry, Payame Noor University, Marand, Iran

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ABSTRACT

Cobalt ion complexes with the Schiff bases, (4-X-2-{[2-(2-pyridine-2-yl-ethylsulfanyl)ethylimino]methyl} phenol (X = methoxy (OMe), phenylazo (N_2Ph), bromo (Br), nitro (NO_2)),were synthesized and investigated by several techniques using elemental analysis (C, H, N), FTIR, electronic spectra and molar conductivity. The thermal stability of free ligands and related cobalt complexes were studied by using differential scanning calorimetry (DSC) and thermogravimetric analyses (TGA). Cyclic voltammetry indicates that the investigated cobalt complexes, under the experimental conditions, have irreversible redox behavior. The synthesized compounds have antibacterial activity against the four Gram-positive bacteria: *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Staphylococcus aureus* and *Bacillus anthracis* and also against the two Gram-negative bacteria: *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The activity data show that the parent Schiff bases are more potent antibacterials than the cobalt complexes.

1. Introduction

Schiff bases are considered as a very important class of organic compounds which have wide applications in many biological aspects. Some Schiff bases were reported to possess antibacterial, antifungal and antitumor activities [1,2]. Due to their multiple implications, the transition metal complexes with Schiff bases, as ligands, are of paramount scientific interest [3]. Schiff bases with donors (N, O, S, etc.) have structure similarities with natural biological systems and due to the presence of imine group, are utilized in elucidating the mechanism of transformation and rasemination reaction in biological systems [4–6]. Schiff base complexes have been used as drugs. Moreover, it is well known that some drug activities, when administered as metal complexes, are being increased [7], and several Schiff base complexes have also been shown to inhibit tumor growth [8]. The effect of the presence of various substituents in the phenyl rings of aromatic Schiff bases on their antimicrobial activity has been reported [9]. It was also reported that salicylaldehyde derivatives with halo atoms in the aromatic ring, showed variety of biological activities, like antibacterial activities [2,10]. This work deals with the synthesis and characteristics of four new cobalt(II) complexes of [Co(Xpesei)]Cl, where Xpesei is =(4-X-2-{[2-(2-pyridine-2-yl-ethylsulfanyl)ethylimino]methyl}phenolato (X= methoxy (OMe), phenylazo (N_2Ph), bromo (Br), nitro (NO_2)). The coordination behavior of Schiff base towards cobalt(II) ion was investigated via the IR, molar conductivity and thermal studies. The antibacterial activity of Schiff bases and their metal chelates are reported against the four Gram-positive bacteria: *Streptococcus pyogenes* (RITCC 1940), *Streptococcus agalactiae* (RITCC 1913), *Staphylococcus aureus* (RITCC 1885) and *Bacillus anthracis* (RITCC 1036) and also against the two Gram-negative bacteria: *Klebsiella pneumonia* (RITCC 1249) and *Pseudomonas aeruginosa* (RITCC 1547).

2. Chemistry

In this study, four Co(II) complexes with the unsymmetrical tetradentate Schiff bases, derived from aminothioether pyridine and salicylaldehyde derivatives, (4-X-2-{[2-(2-pyridine-2-yl-ethylsulfanyl)ethylimino]methyl}phenol (X = OMe, N_2Ph, Br, NO_2), were synthesized and investigated by several techniques using elemental analysis (C, H, N), FTIR, electronic spectra and molar conductivity measurements. The elemental analyses data suggest that the stoichiometry be 1:1 [M:L] ratio formation. All the complexes were found to be 1:1 electrolyte systems in acetonitrile.

* Corresponding author. Fax: +98 4612332556.
E-mail address: saghatforoush@gmail.com (L.A. Saghatforoush).

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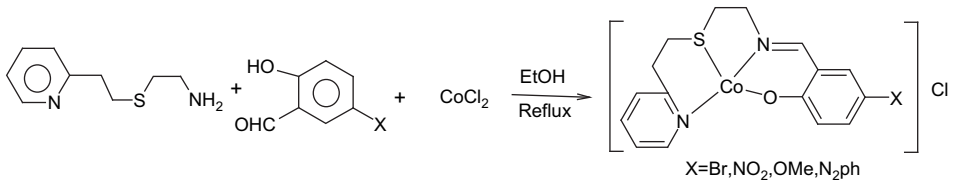

Fig. 1. Representative synthesis of cobalt(II) Schiff base complexes.

FTIR spectral data supported that the cobalt ion in all complexes has N_2OS coordination sphere, bounded by deprotonated phenolic oxygen, imine and pyridine type nitrogens and the thioether sulfur atoms.

3. Results and discussion

XpesesiH (X = NO_2, Br, OMe, N_2Ph) was prepared by the condensation of Pyta with salicylaldehyde derivative in absolute ethanol in good yield and purity. The tetra coordinated mononuclear cobalt(II) complexes were prepared by the reaction of equimolar quantities of Pyta, required salicylaldehyde, Co(II) chloride hexahydrate, and methanolic NaOH in absolute ethanol (Fig. 1). The cobalt complexes were characterized by elemental analysis, molar conductivity, FTIR and electronic spectroscopy. These complexes were stable at room temperature in air in the solid state. Solution conductivity measurements were performed to establish the electrolyte type of the complexes. The molar conductivities at 10^{-3} M concentration for the complexes in acetonitrile were in the expecting range of their formulations as 1:1 electrolytes [11]. The FTIR spectra of all complexes compared with those of the ligands, indicates that the ν(C=N) band at 1634–1655 cm^{-1} is shifted to lower frequency by 7–39 cm^{-1} in the complexes, indicating that the ligands are coordinated to the metal ions through the nitrogen atom of the azomethine group [1]. On the other hand, the disappearance of the OH bands of the free ligands in the complexes indicates that the OH group has been deprotonated and bounded to metal ions as O^-. The intense band at 1243–1315 cm^{-1}, assigned to phenolic C–O linkage, shifted towards higher wave number of 1300–1340 cm^{-1} confirming the involvement of OH group in bond formation with metal ion [10,12–14]. The UV–vis absorption spectra, performed on the complexes dissolved in acetonitrile, show the modification of the absorption bands characteristic of the ligands as well as the occurrence of some new bands, characteristic of the formation of the coordinative compounds. All of the complexes show three closely spaced bands in the visible region and very intense bands in the UV region. In the regular tetrahedral and and near-tetrahedral Co(II) complexes, only one d–d transition ($^4A_2$ (F) → $^4T_1$ (P)) is observed in the visible region [15]. Three closely spaced bands in the spectrum of complexes are because of the distortion in tetrahedral symmetry around the metal center. This splitting originates from the reduction of the orbital degeneracy, due to the difference in the ligand field strength of imine and pyridine N donor atoms, thiol S and phenoxide O donor atoms [2,16]. Therefore the appearance of broad intense absorption band at 508–515 nm in the spectra of the complexes of [Co(Xpesesi)] Cl, (X = OMe, N_2Ph, Br, NO_2) respectively, seems to be little influenced by different substitutions on the salicylaldehyde moiety, and suggests that the coordination geometry at the metal ion could be distorted from tetrahedral [18]. The broad intense and poorly resolved bands between 320 and 450 nm may be assigned to LMCT or MLCT [8,15,17,18]. The high intensity band below 320 nm is of ligand origin assignable to intraligand n–π^*/π–π^* transitions [10,19].

The obtained cyclic voltammetric parameters for the complexes in acetonitrile solution are listed in Table 1. It is noted that in the region of potentials in which the complexes are studied, the ligands are electroinactive. In this condition the ΔE ($\Delta E = E_c - E_a$) value for the reversible redox couple Fc^+/Fc^-, as internal standard, is equal to 91 mV. On the basis of voltammetric data, all four complexes under experimental condition, underwent irreversible reduction processes in potential range of −0.9 to +0.20 V. All the complexes exhibited one oxidation and one reduction peak related to the Co^{III}/Co^{II} couple. As shown from the obtained data in Table 1, E_{Pa} become less positive in the sequence of $NO_2 < N_2ph < Br < OMe$. This is due to the electron withdrawing effect of the substituent at the para position of salicylaldehyde moiety. One of the voltammograms of the cobalt complexes is shown in Fig. 2.

Thermal studies over the some Schiff base ligands and their cobalt(II) complexes, through the differential scanning calorimetric (DSC) and thermogravimetric (TGA) techniques, were performed. Transition temperatures, enthalpy changes, decomposition temperatures of Schiff base ligands, and related cobalt(II) complexes are tabulated in Table 2. DSC studies presented a melting process at 54.70, 68.2, 96.3 and 140.8 for OMepesesiH, BrpesesiH, NO_2pesesiH and N_2PhpesesiH, respectively, followed by decomposition

Table 1
Cyclic voltammetry data for the cobalt(II) Schiff base complexes.

Complex	E_{pa} (V)	E_{pc} (V)	ΔE (V)	i_{pc}/i_{pa}
[Co(NO_2pesesi)]Cl	−0.224	−0.366	−0.142	0.30
[Co(OMepesesi)]Cl	−0.064	−0.073	−0.137	0.49
[Co(N_2phpesesi)]Cl	−0.105	−0.275	−0.175	0.65
[Co(Brpesesi)]Cl	−0.096	−0.032	−0.128	0.57

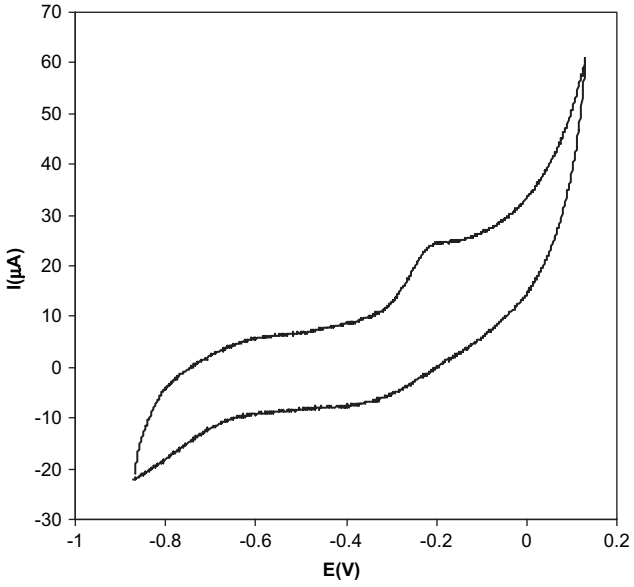

Fig. 2. Cyclic voltammogram of [Co(NO_2pesesi)]Cl.

Table 2
Thermoanalytical (transition temperatures, enthalpy changes and decomposition temperatures) results of free Schiff base ligands and related Co(II) complexes.

Compound	Transition[a]	$T^{b,c}$ (°C)	ΔH^b (J g^{-1})	T_d^d (°C)
BrpesesiH		68.2	−97.67	270
OMepesesiH		54.7	−84.34	279
NO_2pesesiH		96.3	−128.30	238
N_2PhpesesiH		140.8	−69.38	253
[Co(Brpesesi)]Cl		243.4 (dec.)	186.38	243
[Co(OMepesesi)]Cl		196.5 (mp)	−61.88	218
[Co(NO_2pesesi)]Cl	Cr. to I. (105)	255.4 (dec.)	192.70	255
[Co(N_2phpesesi)]Cl		170.4 (mp)	−68.46	234

[a] Cr: crystal, I: isotropic liquid.
[b] Data obtained from first DSC cycle.
[c] dec.: decomposed, mp: melting point.
[d] Data obtained from TGA; 10 °C min^{-1} under N_2 gas.

represented by exothermic processes. The TGA data indicate that the ligands BrpesesiH, OMepesesiH, NO_2pesesiH and N_2PhpesesiH start decomposition at 270, 279, 238 and 253 °C, respectively. Among free Schiff base ligands, OMepesesiH has the greatest stability. DSC data of the cobalt(II) complexes show that the [Co(NO_2pesesi)]Cl complex has one solid–solid transition at 105 °C and before melting, is being decomposed. [Co(OMepesesi)]Cl and [Co(N_2phpesesi)]Cl are melted first at 196.5 °C and 170.4 °C, respectively, then during a exothermic process they are being decomposed. [Co(Brpesesi)]Cl is being decomposed at 243 °C. The TGA data indicate that the cobalt complexes: [Co(Brpesesi)]Cl, [Co(OMepesesi)]Cl, [Co(NO_2pesesi)]Cl and [Co(N_2phpesesi)]Cl start decomposition at 243, 218, 255 and 234 °C, respectively. There is no mass loss up to 200 °C, indicating that either water or solvent molecules are absent in these complexes [20]. Among cobalt complexes, [Co(NO_2pesesi)]Cl has higher thermal stability and the thermal stability order of cobalt complexes is not the same, with the order resulted for free ligands. Final decomposition product was cobalt chloride as confirmed by qualitative analysis. The typical thermograms for N_2PhpesesiH and related cobalt complex are presented in Figs. 3 and 4.

Antibacterial activities (zone of growth inhibition and minimal inhibitory concentrations) of three Schiff base ligands, their related cobalt complexes and gentamicine (as a standard compound) are shown in Tables 3 and 4. The organisms used in the present investigation included *S. pyogenes* (RITCC 1940), *S. agalactiae* (RITCC 1913), *S. aureus* (RITCC 1885) and *B. anthracis* (RITCC 1036) as Gram-positive bacteria and *K. pneumoniae* (RITCC 1249) and *P. aeruginosa* (RITCC 1547) as Gram-negative bacteria. Obtained data indicate high activity of BrpesesiH Schiff base against Gram-positive bacteria, *S. agalactiae*, *B. anthracis*, *S. pyogenes* and *S. aureus*, and moderate activity of it towards Gram-negative bacteria, *K. pneumoniae* and *P. aeruginosa*. Two other Schiff ligands: NO_2pesesiH and OMepesesiH, have strong activity against *S. pyogenes* and moderate one towards *S. agalactiae*, *B. anthracis* and *K. pneumoniae*. While NO_2pesesiH ligand has moderate activity against *S. aureus* and *P. aeruginosa*, OMepesesiH ligand against these bacteria has weak activity. Considerable activity of Schiff base ligands may be arisen from the presense of imine group which imports in elucidating the mechanism of transformation reaction in biological system and also from the presense of the hydroxyl and N-pyridyl groups, which may play an important role in the antibacterial activity [2,21–23]. BrpesesiH ligand was found to be the most potent antibacterial agent, indicating that bromine atom played an important role in the antibacterial activity [2]. All the three Co(II) complexes have moderate activity (inhibitory zones >15 mm) against all four Gram-positive bacteria, except [Co(NO_2pesesi)]Cl that has strong activity towards *B. anthracis* [24]. The activity of [Co(NO_2pesesi)]Cl complex may be explained on the basis of chelation theory; chelation reduces the polarity of the metal atom mainly because of partial sharing of its positive charge with the donor groups and possible π electron delocalization within the whole chelation. Also, chelation increases the lipophilic nature of the central atom which subsequently favors its permeation through the lipid layer of the cell membrane [1,25]. The collected results in Table 3, indicated that the all three complexes are weakly active against two Gram-negative bacteria (inhibitory zones < 15 mm), except [Co(Brpesesi)]Cl complex that shows moderate activity towards *K. pneumoniae* [24]. The antibacterial activity values for the complexes are lower than those found for the free Schiff base ligands, except for [Co(NO_2pesesi)]Cl complex that shows strong activity against *B. anthracis* with respect to free NO_2pesesiH Schiff base ligand. It appears that the coordination of cobalt ion to tetradentate Schiff base ligands diminishes the antibacterial activities of the corresponding Schiff bases. The quantitative assays gave MIC values in the range 3.125–100 mg ml^{-1} (Table 4), that confirmed the above obtained results.

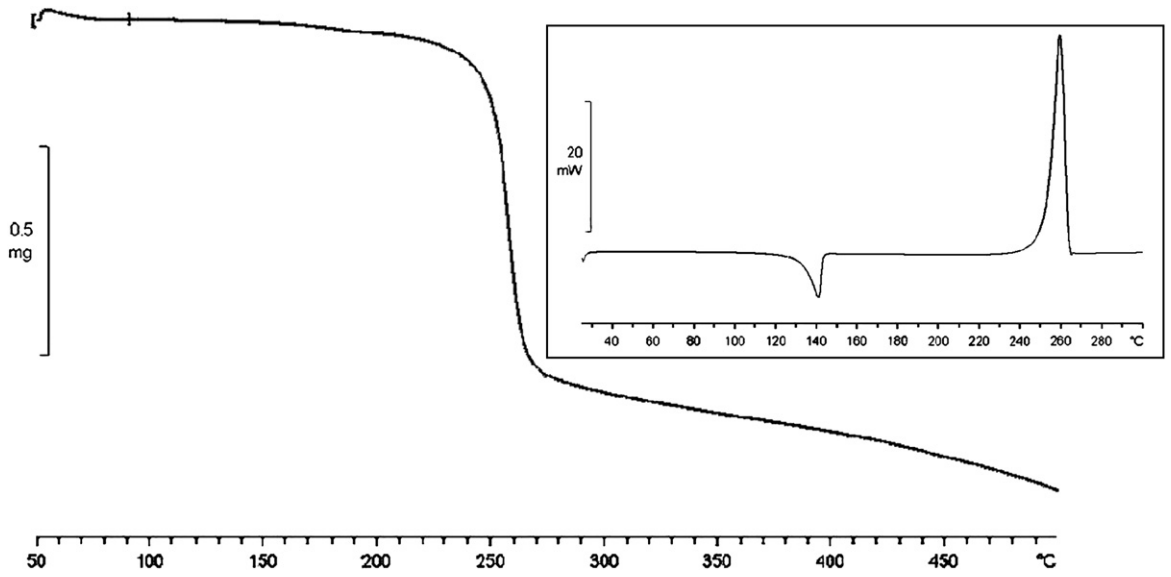

Fig. 3. DSC (above) and TGA (below) curves of N_2PhpesesiH Schiff base ligand.

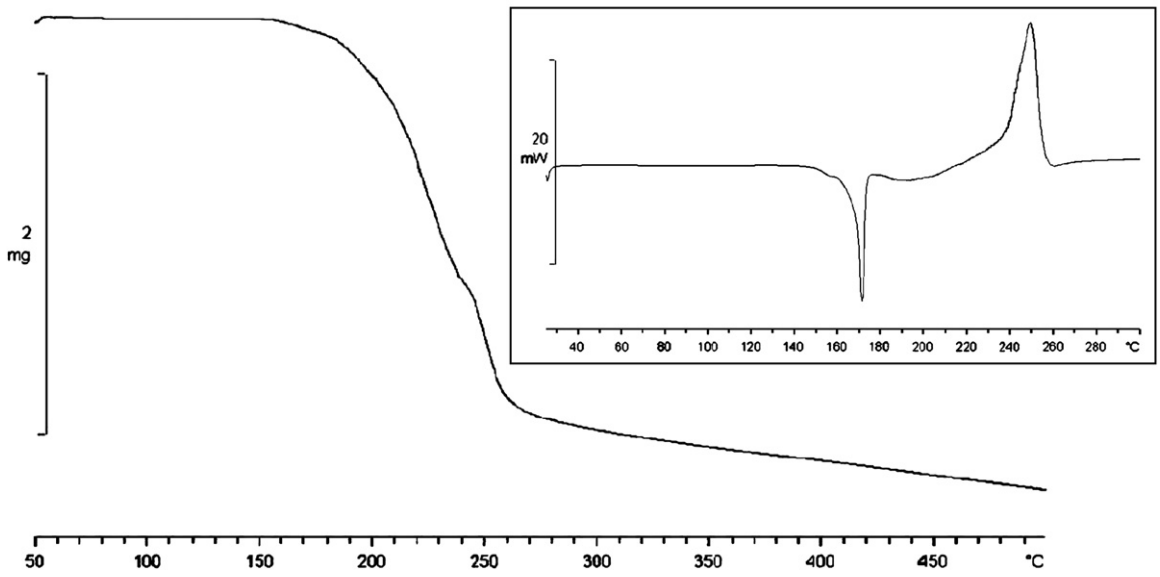

Fig. 4. DSC (above) and TGA (below) curves of [Co(N_2Phpesesi)]Cl complex.

Table 3
Quantitative antimicrobial assay results (zone of growth inhibition) of the free Schiff base ligands and related cobalt complexes.

Microorganism	Standard	Growth inhibitory zone [mm]					
		Main compounds					
	Gentamicine	BrpesesiH	NO_2pesesiH	OMepesesiH	[Co(Brpesesi)]Cl	[Co(NO_2pesesi)]Cl	[Co(OMepesesi)]Cl
Stereptococcus pyogenes (+)	20	40	40	40	20	20	15
Stereptococcus agalactiae (+)	–	50	30	20	25	20	15
Staphylococcus aureus (+)	20	40	20	10	30	20	15
Bacillus anthracis (+)	32	50	20	20	30	50	25
Klebsiella pneumonia (−)	20	30	20	20	15	10	10
Pseudomonas aeruginosa (−)	16	20	20	10	5	5	10

Table 4
Quantitative antimicrobial assay results (minimal inhibitory concentrations) of the free Schiff base ligands and related cobalt complexes.

Microorganism	Minimum inhibitory concentration(mg/ml)					
	Main compounds					
	BrpesesiH	NO_2pesesiH	OMepesesiH	[Co(Brpesesi)]Cl	[Co(NO_2pesesi)]Cl	[Co(OMepesesi)]Cl
Stereptococcus pyogenes (+)	6.25	6.25	6.25	25	25	37.5
Stereptococcus agalactiae (+)	3.125	12.5	25	18.75	25	37.5
Staphylococcus aureus (+)	6.25	25	50	12.5	25	37.5
Bacillus anthracis (+)	3.125	25	25	12.5	3.125	18.75
Klebsiella pneumonia (−)	12.5	25	25	37.5	50	50
Pseudomonas aeruginosa (−)	25	25	50	100	100	50

4. Conclusion

Cobalt(II) complexes of the tetradentate Schiff base ligands (XpesesiH) have been synthesized and characterized by various physicochemical studies. Conductivity measurements show that all complexes have electrolytic nature (1:1 type) and contain one Cl anion out of the coordination sphere. The proposed structures of the complexes are shown in Fig. 1. The antibacterial activity results evidently show that the cobalt(II) complexes, except [Co(N-O_2pesesi)]Cl which has strong activity against *B. anthracis*, have moderate activity against Gram-positive bacteria. They have weak activity against Gram-negative bacteria, except [Co(Brpesesi)]Cl which has moderate activity against *K. pneumoniae*. Generally, the antibacterial activities of free Schiff base ligands are more than cobalt(II) complexes. The BrpesesiH exhibits most activity against tested bacteria compared with standard antibiotic.

5. Experimental

5.1. Material

All reagents were used as supplied by Merck and Fluka without further purification. Solvents used for reactions were purified and dried by conventional method [26]. 5-Phenylazo-salicylaldehyde,

1-(2-pyridyl)-3-thia-5-amino pentane (pyta) were synthesized according to the known procedures [27,28]. 2-Vinylpyridine was distilled in vacuum before using.

5.2. Physical measurements

The elemental analyses for the compounds were carried out using Elementar CHN Analyzer Vario El III. Melting points were determined using an electrothermal apparatus and were uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker spectrospin Avance 400 MHz in $CDCl_3$ and chemical shifts were indicated in ppm relative to tetramethylsilane. The electronic spectra in 200–900 nm range were obtained in acetonitrile on a Perkin–Elmer lambda 25 spectrophotometer. Infrared (FTIR) spectra were recorded using KBr discs on a Shimadzu FTIR model Prestige21 spectrometer. The conductivity measurements were carried out in acetonitrile in room temperature using a Jenway 4510 conductometer instrument. The DSC thermograms of the compounds were obtained on a Mettler-Toledo DSC 822e module, which was calibrated with indium metal ($T = 156.6 \pm 0.3$, $\Delta H = 28.45 \pm 0.61$ J g^{-1}). Samples of 2–5.8 mg in solid form were placed in aluminium pans (40 μl) with a pierced lid, and heated or cooled at a scan rate of 10 °C min^{-1} under nitrogen flow. TGA were carried out on a Mettler-Toledo TGA 851e at a heating rate of 10 °C min^{-1} under a nitrogen atmosphere. Cyclic voltammograms (CVs) were obtained using an Autolab modular electrochemical system (Eco chimie, Ulterecht, The Netherlands) equipped with a PGSTAT 20 module and driven by GPES (Eco chimie) in conjunction with a three-electrode system and a personal computer for data storage and processing. An Ag/AgCl (saturated KCl)/3 M KCl reference electrode, a Pt wire (counter electrode) and a glassy carbon working electrode, (Metrohm 0.0314 cm^2) were employed for the electrochemical studies. Voltammetric measurements were performed at room temperature in acteonitrile solution with 0.1 M tetrabutylammonium perchlorate as the supporting electrolyte.

5.3. Synthesis of ligands

All unsymmetrical tetradentate Schiff base ligands were prepared in a similar manner [17]. A solution of 1 mmol of Pyta in 5 ml absolute ethanol was added to a solution of 1 mmol of required salicylaldehyde in 5 ml absolute ethanol to give clear yellow or light orange solutions which were gently refluxed for about 1 h. Evaporation of the solution in vacuum gave viscous liquids. The ligands, (4-X-2-{[2-(2-pyridine-2-yl-ethylsulfanyl) ethylimino]methyl}phenol which [X = methoxy, nitro, bromo, phenylazo] will be abbreviated as (OMe)pesesiH, (NO_2)pesesiH, (Br)pesesiH, (N_2Ph)pesesiH, respectively, were obtained as microcrystals. The microcrystals were filtered off, washed with 5 ml cooled absolute ethanol and then recrystallized from ethanol–chloroform (2:1, v/v).

5.3.1. (4-Nitro-2-{[2-(2-pyridine-2-yl-ethylsulfanyl)ethylimino] methyl}phenol (**1**), (NO_2)pesesiH

Yield 78%, Anal. Calcd for $C_{16}H_{17}N_3O_3S$: C 57.99, H 5.17, N 12.67. Found: C 57.70, H 5.20, and N 12.50. ^{1}H NMR (400 MHz $CDCl_3$) δ 14.55 (br s, 1H, OH), 8.35 (s, 1H, iminic), [8.55 (d, 1H), 8.16-8.22 (m, 2H), 7.65 (t, 1H), 7.19 (m, 2H), 6.96 (d, 1H) (total 7H ArH)], 3.84–2.88 (t, 8H, $4 \times CH_2$). ^{13}C NMR (400 MHz $CDCl_3$) δ 31.70, 32.72, 38.21, 57.04, 116.65, 119.02, 121.69, 123.35, 128.21, 128.41,136.62, 138.72, 149.25, 159.48, 164.98, 169.22 (16 C). FTIR (KBr) ν 3447, 3053, 2922–2940, 1655,1325–1557 cm^{-1}. Mp 96 °C. Orange microcrystal.

5.3.2. (4-Methoxy-2-{[2-(2-pyridine-2-yl-ethylsulfanyl)ethylimino] methyl}phenol (**2**), (OMe)pesesiH

Yield 75%, Anal. Calcd for $C_{17}H_{20}N_2O_2S$: C 64.50, H 6.37, N 8.85. Found: C 64.00, H 6.30, and N 8.70. ^{1}H NMR (400 MHz $CDCl_3$) δ 12.75 (br s, 1H, OH), 8.29 (s, 1H, iminic), [8.52 (d, 1H), 7.58 (t, 1H), 7.11–7.20 (m, 4H), 6.76(d, 1H) (total 7H ArH)], 3.77–2.83 (m, t, CH3 and $4 \times CH_2$). ^{13}C NMR (400 MHz $CDCl_3$) δ 31.90, 33.09, 38.44, 55.93, 59.33, 114.95, 117.23, 118.30, 119.38, 121.54, 123.27, 136.48, 149.29, 152.00, 155.21, 159.77, 165.55 (17 C). FTIR (KBr) ν 3447, 3053, 2850–2937, 1641 cm^{-1}. Mp 55 °C. Orange microcrystal.

5.3.3. (4-Bromo-2-{[2-(2-pyridine-2-yl-ethylsulfanyl)ethylimino] methyl}phenol (**3**), (Br)pesesiH

Yield 83%, Anal. Calcd for $C_{16}H_{17}BrN_2OS$: C 52.50, H 4.70, N 7.60. Found: C 52.60, H 4.69, and N 7.66. ^{1}H NMR (400 MHz CDCl3) δ 13.20 (br s, 1H, OH), 8.55 (d, 1H), 8.26 (s, 1H, iminic), 7.67 (t, 1H), 7.39–7.21 (m, 4H), 6.85 (d, 1H) (total 7H ArH), 3.80–2.86 (t, 8H, $4 \times CH_2$), ^{13}C NMR (400 MHz $CDCl_3$) δ 31.82, 32.94, 38.36, 58.93, 109.95, 119.02, 119.96, 121.56, 123.25, 133.46,134.92, 136.45, 149.32, 159.66, 160.16, 164.61 (16 C). FTIR (KBr) ν 3447, 3015–3050, 2850–2930, 1634 cm^{-1}. Mp 69 °C. Yellow microcrystal.

5.3.4. (4-Phenylazo-2-{[2-(2-pyridine-2-yl-ethylsulfanyl) ethylimino]methyl}phenol (**4**), (N_2ph)pesesiH

Yield 65%, Anal. Calcd for $C_{22}H_{22}N_4OS$: C 67.70, H 5.67, N 14.35. Found: C 67.35, H 5.85, N 14.50. ^{1}H NMR (400 MHz $CDCl_3$) δ 13.80 (br s, 1H, OH), 8.39 (s, 1H, iminic), [8.54 (d, 1H), 7.98 (d, 1H), 7.86–7.89 (m, 3H), 7.61(t, 1H), 7.41–7.51 (m, 3H), 7.13–7.18 (m, 2H), 7.05 (d, 1H) (total 12H ArH)], 3.80–2.85 (t, 8H, $4 \times CH_2$). ^{13}C NMR (400 MHz CDCl3) δ 31.83, 32.94, 38.24, 58.38, 118.05, 118.27, 121.71, 122.53, 122.53, 123.45, 126.96, 127.50, 129.09, 129.09, 130.45, 136.77, 145.02, 149.12, 152.56, 159.54, 164.94, 165.67 (22 C). FTIR (KBr) ν 3400, 3069, 2850–2930, 1641 cm^{-1}. Mp 141 °C. Red brown crystal.

5.4. Synthesis of cobalt complexes

All mononuclear type complexes were prepared from chloride salt of cobalt(II) using 1:1:1 mol ratio of the Pyta, required salicylaldehyde and metal salt in ethanol [17] (Fig. 1).

General procedure. A solution of 1 mmol of Pyta in 5 ml absolute ethanol was added to solution of 1 mmol of the required salicylaldehyde in 5 ml absolute ethanol and the mixture was refluxed for 40 min and then 1 ml of methanolic NaOH was added and refluxed and stirring was continued for a further 5 min. Then 1 mmol of $CoCl_2 \cdot 6H_2O$ in 5 ml absolute ethanol was added to the ligand solution with stirring and the reaction mixture was stirred under reflux for 60 min. The resultant colored solution was left at room temperature. The product was removed by filtration, washed with cooled absolute ethanol and recrystallized from methanol or acetonitrile and dried in vacuo.

5.4.1. (4-Nitro-2-{[2-(2-pyridine-2-yl-ethylsulfanyl)ethylimino] methyl}phenolato cobalt(II) chloride (**5**)

Yield 58%, Anal. Calcd for $C_{16}H_{16}ClCoN_3O_3S$: C 45.23, H 3.79, N 9.89. Found: C 45.05, H 3.8, and N 9.80. FTIR (KBr) ν 3020–3100, 2920, 1616, 1541, 1325 cm^{-1}. UV (CH_3CN) λ_{max} (nm) (log ε l mol^{-1} cm^{-1}) 675 (180), 620 (180), 510 (386). Mp 255 °C dec. Mol. conductivity 142 μS. Brown crystal.

5.4.2. (4-Methoxy-2-{[2-(2-pyridine-2-yl-ethylsulfanyl)ethylimino] methyl}phenolato cobalt(II) chloride (**6**)

Yield 62%, Anal. Calcd for $C_{17}H_{19}ClCoN_2O_2S$: C 49.82, H 4.67, N 6.83. Found: C 50.15, H 4.62, and N 6.85. FTIR (KBr) ν 3050, 2820–2980, 1629 cm^{-1}. UV (CH_3CN) λ_{max} (nm) (log ε l mol^{-1} cm^{-1}) 665

(174), 590 (165), 507 (435). Mp 196 °C. Mol. conductivity 144 μS. Green-brown crystal.

*5.4.3. (4-Bromo-2-{[2-(2-pyridine-2-yl-ethylsulfanyl) ethylimino]methyl}phenolato cobalt(II) chloride (**7**)*

Yield 60%, Anal. Calcd for $C_{16}H_{16}BrClCoN_2OS$: C 41.9, H 3.51, N 6.1. Found: C 41.48, H 3.46, and N 5.88. FTIR (KBr) ν 3020–3070, 2850–2937, 1626 cm^{-1}. UV (CH_3CN) λ_{max} (nm) (log ε l mol^{-1} cm^{-1}) 670 (230), 590 (202), 508 (357). Mp 243 °C dec. Mol. conductivity 148 μS. Dark brown crystal.

*5.4.4. (4-X-2-{[2-(2-pyridine-2-yl-ethylsulfanyl)ethylimino]methyl} phenolato cobalt(II) chloride (**8**)*

Yield 59%, Anal. Calcd for $C_{22}H_{21}ClCoN_4OS$: C 54.60, H 4.37, N 11.57. Found: C 54.45, H 4.30, and N 11.42. FTIR (KBr) ν 3030–3080, 2800–2950, 1628 cm^{-1}. UV (CH_3CN) λ_{max} (nm) (log ε l mol^{-1} cm^{-1}) 665 (210), 583 (197), 515 (sh). Mp 170 °C dec. Mol. conductivity 135 μS. Reddish brown crystal.

5.5. Antibacterial activity test

The in vitro activity test was carried out using the growth inhibitory zone (well method) [29,30]. The potency of components was determined against the four Gram-positive bacteria: *S. pyogenes*, *S. agalactiae*, *S. aureus* and *B. anthracis* (RITCC 1036) and also against the two Gram-negative bacteria: *K. pneumoniae* (RITCC 1249) and *P. aeruginosa* (RITCC 1547).

Microorganisms (obtained from enrichment culture of the microorganisms in 1 ml Muller–Hinton broth, incubated at 37 °C for 12 h) were cultured on Muller–Hinton agar medium. The inhibitory activity was compared with that of standard antibiotics, such as gentamicine (10 μg). After drilling wells on medium using a 6 mm cork borer, 100 μL of solution from different compounds were poured into each well. The plates were incubated at 37 °C overnight. The diameter of the inhibition zone was measured to the nearest. Each test was carried out in triplicate and the average was calculated for inhibition zone diameters. A blank containing only methanol showed no inhibition in a preliminary test. The macro-dilution broth susceptibility assay was used for the evaluation of minimal inhibitory concentration (MIC). Uses of 12 test tubes are required by macro-dilution method. By including 1 ml Muller–Hinton broth in each test, and then adding 1 ml extract with concentration 100 mg/ml in the first tube, we made serial dilution of this extract from first tube to last tube. Bacterial suspension prepared to match the turbidity of 0.5 Mcfarland turbidity standards. Matching this turbidity provides a bacterial inoculum concentration of 1.5×10^8 cfu/ml. Then 1 ml of bacterial suspension was added to each test tube. After incubation at 37 °C for 24 h, the last tube was determined as the minimal inhibitory concentration (MIC) without turbidity.

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