

Biologically Active Transition Metal Chelates with a 2-Thiophenecarboxaldehyde-Derived Schiff Base: Synthesis, Characterization, and Antibacterial Properties

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Metal complexes, ML_2Cl_2 , where M is Fe(II), Co(II), Ni(II), Cu(II), Zn(II), or Cd(II), and L is the Schiff base formed by condensation of 2-thiophenecarboxaldehyde with 2-aminopyridine, N-(2-thienylmethylidene)-2-aminopyridine (TNAPY) have been prepared and characterized by elemental analysis, and magnetic and spectroscopic measurements. Elemental analysis of the chelates suggests the stoichiometry is 1:2 (metal-ligand). Infrared and NMR spectra of the complexes agree with the coordination to the central metal atom through the nitrogen of the azomethine (-HC=N-) group and the sulfur atom of the thiophene ring. Magnetic susceptibility data coupled with electronic and ESR spectra suggest a distorted octahedral structure for the Fe(II), Co(II), Ni(II), and Cu(II) complexes, and a tetrahedral geometry for the Zn(II) and Cd(II) complexes. The Schiff base and its metal chelates have been screened for their in vitro antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The metal chelates were shown to possess more antibacterial activity than the uncomplexed Schiff-base.

Key Words: Schiff base, transition metal complex, biological activity.

Introduction

A large number of Schiff bases and their complexes have been studied for their interesting and important properties, e.g., catalytic activity¹ and transfer of the amino group,² photochromic properties,³ and complexing ability towards some toxic metals.⁴

In continuation of our work on metal complexes of Schiff bases^{5–8} we report herein the results of our studies on the metal complexes of a Schiff base derived from 2-thiophenecarboxaldehyde (TFCA) and 2-

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aminopyridine (APy), N-(2-thienylmethylidene)-2-aminopyridine (TNAPY). Tentative structures have been proposed on the basis of analytical, spectral, magnetic, and conductance data.

In order to establish the biological role of metals, this prepared Schiff base and its metal chelates have been screened for biological activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

Experimental

Reagents: FeCl₂·4H₂O (Merck, 99%), CoCl₂·6H₂O (Merck, 99.99%), NiCl₂·6H₂O (Merck, 99.99%), CuCl₂·2H₂O (Merck, 99.99%), ZnCl₂ (Aldrich, 99.99%), CdCl₂ (Aldrich, 99.99%), 2-thiophenecarboxaldehyde (Merck, 98%), and 2-aminopyridine (Merck, 98%).

Synthesis of the bidentate schiff base

The Schiff base was prepared according to the following procedure: An ethanolic solution of TFCA (0.001 mol, 25 mL) was added to an ethanolic solution of APy (0.001 mol, 25 mL) and refluxed for 4-6 h in a water bath. After concentration of the solution, the precipitate was separated, filtered, washed with ethanol, and dried over calcium chloride in vacuum. *Anal.* Calculated for TNAPY: C, 63.83; H, 4.25; N, 14.89; S, 17.02. Found: C, 63.79; H, 4.23; N, 14.96; S, 17.43. ¹H-NMR: δ_x 8.7, gδ_y 7.4; ¹³C-NMR: δ_x 162.7, δ_y 132.5, gδ_z 111.2.

Synthesis of the ML₂Cl₂ complexes

A mixture of TFCA (0.002 mol, 50 mL), and APy (0.002 mol, 50 mL) in ethanol was added to an ethanolic solution of metal chlorides (0.001 mol, 50 mL) at pH 7-7.5. The mixture of reaction was refluxed for 6-10 h and then excess solvent was distilled. The precipitated compounds that separated were filtered, washed with ethanol, and dried over CaCl₂ in vacuum. *Anal.* Calculated for [Fe(TNAPY)₂Cl₂]: Fe, 11.08; C, 47.64; N, 11.11; S, 12.70; Cl, 14.27. Found: Fe, 11.64; C, 47.98; N, 11.03; S, 12.92; Cl, 14.11. Calculated for [Co(TNAPY)₂Cl₂]: Co, 11.63; C, 47.35; N, 11.05; S, 12.63; Cl, 14.18. Found: Co, 11.71; C, 47.29; N, 11.28; S, 12.60; Cl, 14.67. Calculated for [Ni(TNAPY)₂Cl₂]: Ni, 11.59; C, 47.37; N, 11.05; S, 12.63; Cl, 14.19. Found: Ni, 11.31; C, 47.05; N, 10.86; S, 12.76; Cl, 14.38. Calculated for [Cu(TNAPY)₂Cl₂]: Cu, 12.42; C, 41.92; N, 10.95; S, 12.51; Cl, 14.06. Found: Cu, 12.11; C, 41.72; N, 11.15; S, 12.52; Cl, 14.11. Calculated for [Zn(TNAPY)₂Cl₂]: Zn, 12.47; C, 46.76; N, 10.91; S, 12.47; Cl, 14.00. Found: Zn, 12.44; C, 46.58; N, 10.66; S, 12.45; Cl, 13.56; ¹H-NMR: δ_x 9.1, gδ_y 7.5; ¹³C-NMR: δ_x 165.7, δ_y 137.2, gδ_z 116.7. Calculated for [Cd(TNAPY)₂Cl₂]: Cd, 20.06; C, 42.83; N, 9.99; S, 11.42; Cl, 12.83. Found: Cd, 20.41; C, 42.56; N, 9.40; S, 11.73; Cl, 13.30; ¹H-NMR: δ_x 9.1, gδ_y 7.6; ¹³C-NMR: δ_x 166.6, δ_y 138.7, gδ_z 114.8.

Instruments

The ligand and complexes were analyzed for M by a conventional method,⁹ while C, H, N, S, and Cl were analyzed with a Thermo Electron elemental analyzer. The IR spectra were obtained in a KBr disk using a BIO-RAD FTS 135 spectrophotometer. The UV-VIS spectra were recorded on a Varian Cary 50 spectrophotometer in DMF solution. The ¹H-NMR spectra (in CDCl₃) were recorded on a Varian T60 and the ¹³C-NMR spectra were obtained using a Bruker WH 270 spectrophotometer. The ESR spectra were

recorded on an ART 5 spectrophotometer in a polycrystalline sample at room temperature. The magnetic moments were determined by the Faraday method. A K 612 digital conductivity meter was used to measure molar conductivity in DMF solution.

Antibacterial studies

The synthesized metal complexes and the uncomplexed Schiff base ligand were screened for antibacterial activity against pathogenic bacterial species (*E. coli*, *S. aureus*, and *P. aeruginosa*) and comparisons were made. The paper disk diffusion method¹⁰ was adopted for the determination of antibacterial activity.

Preparation of disks

The ligand/complex (30 μg) in DMF (0.01 mL) was placed on a paper disk [prepared from blotting paper (3 mm in diameter)] with the help of a micropipette. The disks were left in an incubator for 48 h at 37 °C and then were applied to the bacteria grown on agar plates.

Preparation of agar plates

Minimal agar was used for the growth of specific bacterial species. For the preparation of agar plates for *E. coli*, MacConkey agar (50 g), obtained from Merck, was suspended in freshly distilled water (1 L). It was allowed to soak for 15 min and then was boiled in a water bath until the agar was completely dissolved. The mixture was autoclaved for 15 min at 120 °C, poured into previously washed and sterilized petri dishes, and then stored at 40 °C for inoculation.

Inoculation procedure

Inoculation was performed with a platinum wire loop that was made red hot in a flame, cooled, and then used for the application of bacterial strains.

Application of disks

Sterilized forceps were used to place each paper disk on an already inoculated agar plate, which was then incubated at 37 °C for 24 h. The zone of inhibition was then measured (diameter) around the disk.

Results and Discussion

The complex combinations of Fe(II), Co(II), Ni(II), Cu(II), Zn(II), and Cd(II) with the Schiff base TNAPY (Figure 1) appeared as powders with high melting points. They are not soluble in ethanol, ethyl ether, or chloroform, but are soluble in acetone and are more soluble in DMF.

Some important bands of TNAPY and its complexes, along with their assignments, are presented in Table 2.

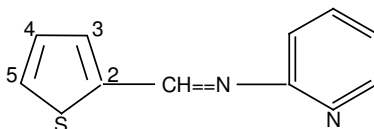


Figure 1. Structure of the ligand.

Elemental analysis suggests that the complexes have 1:2 (metal-ligand) stoichiometry. Based on the elementary chemical analysis the formula, ML_2Cl_2 , was suggested for all compounds

Table 1. Analytical and physical data for the complexes.*

Compounds	mp ($^{\circ}C$)	color	μ_{eff} (BM)	Λ_M^{**} ($\Omega^{-1}cm^2mol^{-1}$)
Fe(TNAPY) ₂ Cl ₂	230	red	5.20	9.5
Co(TNAPY) ₂ Cl ₂	180	pink	4.88	5.2
Ni(TNAPY) ₂ Cl ₂	175	greenish	3.10	19.8
Cu(TNAPY) ₂ Cl ₂	220	blue	1.88	13.5
Zn(TNAPY) ₂ Cl ₂	240	white	-	126.9
Cd(TNAPY) ₂ Cl ₂	210	yellowish	-	133.2

*All the complexes gave satisfactory metal, C, H, S, N, and Cl analyses; ** in 10^{-3} DMF solution.

Table 2. Characteristic infrared absorption frequencies (cm^{-1}) of the ligand and complexes.

Compound	$\nu_{C=N}$	$\nu_{C=Npy}$	ν_{C-S-C}	$\nu_{C-Sasym.}$	ν_{M-N}
TNAPY	1675	1456	920	673	-
[Fe(TNAPY) ₂ Cl ₂]	1635	1452	843	640	423
[Co(TNAPY) ₂ Cl ₂]	1621	1455	841	654	419
[Ni(TNAPY) ₂ Cl ₂]	1626	1456	859	650	430
[Cu(TNAPY) ₂ Cl ₂]	1625	1450	851	645	420
[Zn(TNAPY) ₂ Cl ₂]	1640	1451	860	635	418
[Cd(TNAPY) ₂ Cl ₂]	1630	1453	835	630	415

The IR spectrum of the ligand exhibits a band at 1675 cm^{-1} assignable to $\nu_{C=N}$ of the azomethine group and an intense band at 1456 cm^{-1} corresponding to the C=N stretching of the pyridine ring. The comparison of the positions of these bands with those observed in the IR spectra of the complexes indicated that the band at 1675 cm^{-1} shifted to a lower region by about $35\text{-}55\text{ cm}^{-1}$, while that at 1456 cm^{-1} did not show a marked shift. This behavior suggests the coordination of TNAPY through the azomethine N. Proof of coordination to the N atom is provided by the occurrence of the new bands at $415\text{-}430\text{ cm}^{-1}$ in the IR spectra of the compounds.

The observed medium intensity band at 920 cm^{-1} in the free ligand, which is ascribed to $\nu_{CSC(ring)}$ stretching vibration,¹¹ shifted to lower values ($60\text{-}85\text{ cm}^{-1}$) for all compounds, suggesting the involvement of the sulfur atom in the bonding with the metal's ions. The band assigned to the asymmetric ν_{C-S} is similarly shifted. This also confirms that the sulfur atom is taking part in the complex formation.¹²

In the 1H -NMR spectra of the ligands the formation of the Schiff base is supported by the presence of a singlet at $\delta g.7$ and $\delta g.4$ ppm, corresponding to the azomethine proton ($-CH=N-$) and to the H-5 proton of the thiophen's ring, respectively. These signals register downfield shifts (1-2 ppm) in the Zn(II) and Cd(II) complexes. These observations support the bonding of TNAPY through nitrogen and sulfur atoms.

The ^{13}C -NMR spectra provide further support for the mode of coordination of the ligand. In the complexes' spectra the signals due to the azomethine carbon, C-2 and C-5 (thiophene ring), show a distinct

downfield shift by nearly 3-6 ppm, clearly demonstrating the coordination of the ligand via the nitrogen and sulfur atoms.

Electronic and ESR spectra

Within the UV spectrum of the ligand we observed the existence of 2 absorption bands assigned to the transitions $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ at 41,518 and 37,340 cm^{-1} , respectively. These transitions were also in the spectra of the complexes, but they shifted to lower frequencies ($\Delta\nu = 2000\text{-}2500 \text{ cm}^{-1}$), confirming the coordination of the ligand to the metal ions.

Information concerning the geometry of these compounds was obtained from the electronic spectra (Table 3) and from magnetic moment values.

Table 3. Electronic spectra of the complexes.

Compound	Absorption maxima (cm^{-1})				
[Fe(TNAPY) ₂ Cl ₂]	12,800	11,200			
[Co(TNAPY) ₂ Cl ₂]	21,270	19,040	15,600	9210	8330
[Ni(TNAPY) ₂ Cl ₂]	22,320	17,000	10,000	9150	
[Cu(TNAPY) ₂ Cl ₂]	16,500				
[Zn(TNAPY) ₂ Cl ₂]	26,000				
[Cd(TNAPY) ₂ Cl ₂]	24,500				

The electronic spectrum of the Fe(II) complex consists of a pair of low intensity bands at 12,800 and 11,200 cm^{-1} , arising from a ${}^5T_{2g} \rightarrow {}^5E_g$ transition, similar to those found for distorted octahedral complexes. The doublet is attributed to Jahn-Teller distortion in the excited state.¹³ The room temperature magnetic moment (9.5 BM) corresponds to octahedral symmetry.

The electronic spectrum of the Co(II) compound exhibits 5 bands (Table 3). The bands at 21,270 and 19,040 cm^{-1} arise from the ${}^4T_{1g} \rightarrow {}^4T_{1g}(P)$ transition, which is split in complexes of D_{4h} symmetry. The band at 15,600 cm^{-1} arises from the ${}^4T_{1g} \rightarrow {}^4A_{2g}$ transition and those at 9210 and 8330 cm^{-1} are from the ${}^4T_{1g} \rightarrow {}^4T_{2g}$ transition. These transitions correspond to a distorted octahedral geometry, which is also supported by the magnetic moment value (5.2 BM).

The electronic spectrum of the Ni(TNAPY)₂Cl₂ complex could be assigned assuming the pseudo-octahedral stereochemistry. The energies represent the next electronic transitions from the ${}^3A_{2g}(F)$ ground state to ${}^3T_{2g}(F)$ (ν_1), ${}^3T_{1g}(F)$ (ν_2), and ${}^3T_{1g}(P)$ (ν_3) excited states for Ni(II). The low energy band of this complex is broad and split into 2 components (at 10,000 and 9150 cm^{-1}), indicating tetragonal distortion. The magnetic moment (3.1 MB) lies within the region expected for octahedral complexes.

The Cu(TNAPY)₂Cl₂ complex shows only 1 broad absorption band at 16,500 cm^{-1} , indicating a probable distorted octahedral configuration. The ESR spectrum of the complex measured in a polycrystalline sample at room temperature gives the following values:

$g_{II} = 2.067$ and $g_{\perp} = 2.046$. The value $g_{II} < g_{\perp}$ is in good agreement with a primarily d_{z^2} ground state and the spectrum is characteristic of axial (compressed octahedral) symmetry. The G parameter determined as $G = (g_{II} - 2) / (g_{\perp} - 2)$ was much less than 4, suggesting considerable interaction in the solid state.¹⁴

Electronic spectra of the Zn(II) and Cd(II) complexes exhibited a sharp band of high intensity at 26,000 and 24,500 cm^{-1} , respectively, which may have been due to ligand-metal charge transfer. The preference of TNAPY for bidentate chelation in the Zn(II) and Cd(II) complexes may have been due to the preference of these ions for tetrahedral coordination.

The molar conductance of the complexes in DMF (10^{-3} M) are in the range 5.2-19.8 $\Omega^{-1}\text{cm}^2\text{mol}^{-1}$, indicating their non-electrolytic nature, with the exception of the Zn(II) and Cd(II) complexes, which are 1:2 electrolytes.

Antibacterial activity

The antibacterial activity of the Schiff base and its complexes against *E. coli*, *S. aureus*, and *P. aeruginosa* were studied.

The antibacterial results are shown in Table 4.

Table 4. Antibacterial activity data.

Ligand/Chelate*	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
TNAPY	+	+	+
[Fe(TNAPY) ₂ Cl ₂]	++++	++++	+++
[Co(TNAPY) ₂ Cl ₂]	++++	+++	+++
[Ni(TNAPY) ₂ Cl ₂]	+++	++	++
[Cu(TNAPY) ₂ Cl ₂]	+++	++	++
[Zn(TNAPY) ₂ Cl ₂]	+++	++	++
[Cd(TNAPY) ₂ Cl ₂]	++	+	+

*C = 5 mg/L.

Inhibition zone diameter mm(% inhibition): +, 6-10 (27-45%); ++, 10-14 (45-64%); + + +, 14-18 (64-82%); + + + +, 18-22 (82-100%).

Percent inhibition values are relative to the inhibition zone (22 mm) of the most active compound with 100% inhibition.

All the Schiff base complexes individually exhibited varying degrees of inhibitory effect on the growth of the tested bacterial species. Table 4 shows that the activity of the Schiff base complexes became more pronounced when coordinated with the metal ions. The biological activity of the complexes follow the order: Co(II) = Fe(II) > Ni(II) = Zn(II) = Cu(II) > Cd(II). Furthermore, the data in Table 4 show that *E. coli* was inhibited to a greater degree by the Co(II) and Fe(II) complexes. The importance of this lies in the fact that these complexes could reasonably be used for the treatment of some common diseases caused by *E. coli*.

Conclusions

In this paper we reported the preparation, isolation, and characterization of a new bidentate Schiff base derived from 2-thiophenecarboxaldehyde, and its complexes with Fe(II), Co(II), Ni(II), Cu(II), Zn(II), and Cd(II). It is tentatively proposed that the Schiff base ligand coordinates through the nitrogen of the azomethine group and the sulfur of the thiophene ring, forming a stable chelate ring structure

In the light of the above discussion, distorted octahedral structures for Fe(II), Co(II), Ni(II), and Cu(II) complexes, and tetrahedral structures for Zn(II) and Cd(II) compounds are proposed (Figure 2).

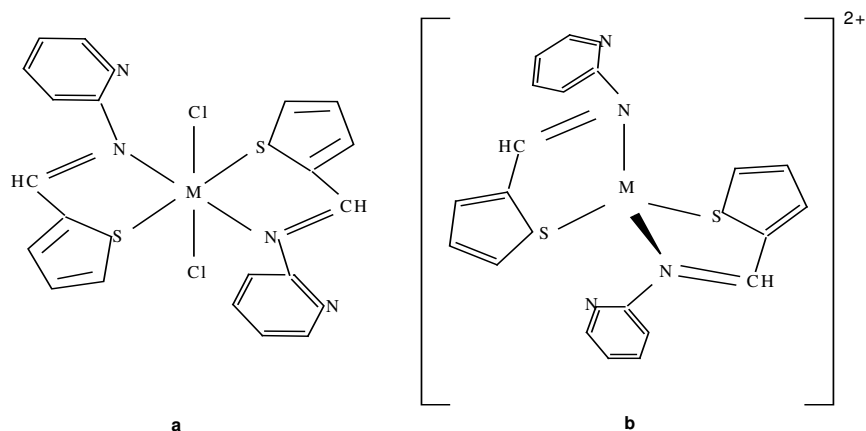


Figure 2. The proposed structure for the complexes.

a $[M(MNAPY)_2Cl_2]$, $M = Fe^{II}$, Co^{II} , Ni^{II} , and Cu^{II} .

b $[M(MNAPY)_2]Cl_2$, $M = Zn^{II}$, and Cd^{II} .

The synthesized metal complexes, in comparison to the uncomplexed Schiff base ligand, were screened for their antibacterial activity against pathogenic bacterial species (*E. coli*, *S. aureus*, and *P. aeruginosa*). The activity of the Schiff base complexes became more pronounced when coordinated with the metal ions. The biological activity of the complexes follow the order: $Co(II) = Fe(II) > Ni(II) = Zn(II) = Cu(II) > Cd(II)$.

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