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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological activity of new cycloalkylthiophene-Schiff bases and their Cr(III) and Zn(II) complexes

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Abstract A series of some novel Ethyl 2-((1-hydroxynaphthalen-2-yl)methyleneamino)-5,6-dihydro-4H-cyclopenta[b]thiopehene-3-carboxylate, Ethyl 2-((1-hydroxynaphthalen-2-yl)methyleneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate, Ethyl 2-((1-hydroxynaphtalen-2-yl)methyleneamino)-5,6,7,8-tetrahydro-4H-cyclohepta [b]thiophene-3-carboxylate and their Cr(III) and Zn(II) complexes have been synthesized. All of these substances have been examined for antibacterial activity against pathogenic strains *Listeria monocytogenes* 4b (ATCC-19115), *Staphylococcus aureus* (ATCC25923), *Proteus* OX2 Wrah (ETS.40-A-4), *Escherichia coli* (ATCC-1280), *Salmonella typhi H* (NCTC-901.8394), *Pseudomonas putida sp.*, *Brucella abortus* (A.99, UK-1995) RSKK-03026. *Sh. boydii type 11* (Pasteur51.6), *Sh. boydii type 16* (cHe 67.11), *Sh. boydii type 6* (RSKK-96043), and antifungal activity against *Candida albicans* (Y-1200-NIH, Tokyo). Some of the compounds exhibited activity comparable to ampicillin ofloxacin, nystatin, kanamycin, sulphamethoxazol, amoxycillin, and chloroamphenicol. Most of the studied compounds were found effective against bacteria studied and yeast.

Keywords Sulfur · Gewald methods · Cycloalkylthiophenes · Schiff bases · Antimicrobial activity · Drug

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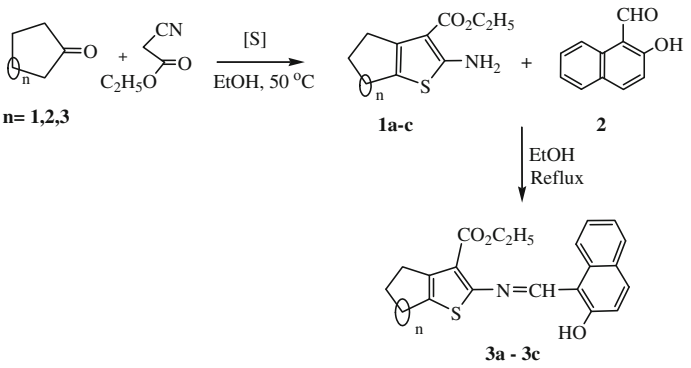
Introduction

A drug containing sulfur is an antibacterial agent because it is a competitive inhibitor of an enzyme catalyzed reaction using p-aminobenzoic acid in the synthesis of folic acid (Bermingham and Derrick, 2002). Ligands and complexes that include sulfur have wide applications for the synthesis of drugs (Jain and Singh, 2006; Angelici, 1990). Therefore, there is considerable interest in the synthesis and characterization of these compounds. However, drug resistances against antibacterial agents may pose a problem in their use for medical purpose (Singh *et al*., 2006). The problem could be overcome by the preparation of metal complexes, using a process of chelation with the coordination of transition metal ions. It is well known that N and O atoms play a key role in the coordination of metals. Schiff bases have N atoms as their basic elements. Schiff base derivatives containing donor atom can act as good chelating agents for the transition of metal ions (Avaji *et al*., 2008). Schiff bases and their metal complexes have been widely studied due to their import antiparasitic (Takeuchi *et al*., 1999), fungicidal-bactericidal (Sari *et al*., 2003), and anticancer (Modi *et al*., 1970) properties. Substituted 2-aminothiophenes have had wide applications in agrochemicals (Nawwar and Shafik, 1995), dyes and pharmacologically active compounds (Sabnis *et al*., 1999), etc. However, few studies have been performed on Schiff bases and the metal complexes derived from aminothiophenes, including cycloalcylthiophene (Mohanan *et al*. 2006). This study was designed to fill in this gap. Some new cycloalcylthiophene derivatives were investigated to determone the antibacterial properties of Schiff bases and their complexes, including cycloalcylthiophene derivatives. Cycloalkylaminothiophene derivatives were synthesized by using the Gewald methods (Gewald *et al*., 1966; Zhikuan *et al*., 2003; Hallas and Choi, 1999) (Scheme 1, 1a–c). Then, three Schiff bases (Scheme 1, 3a–c) prepared from cycloalkylaminothiophene and 2-hydroxy-1-napthaldehyde and their Cr(III) and Zn(II) complexes were synthesized and characterized.



Scheme 1 Synthesis of Schiff bases (3a–c)

Materials and methods

All chemicals investigated in the study were reagent grade and were purified when it was necessary. All organic solvents used in this study were purified according to standard methods. Elemental analyses were performed with a LECO-CHNS-9320 instrument. Metal contents were determined by using a Philips PU 9285 atomic absorption instrument. ^{1}H and ^{13}C-n.m.r spectra were recorded with a Bruker DPX-300 MHz and 100 MHz using TMS as an internal standard and $CDCl_3$ as solvent. Mass spectra were recorded on a Micro Mass-UK Platform II mass spectrometer at Tubitak, Ankara, Turkey. Electronic spectra were recorded on a Unicam-UV2-100 spectrophotometer in ethanol. IR spectra were recorded on a Mattson-5000 FT-IR instrument in KBr pellets. Melting points were determined with a Gallenkamp melting point apparatus. The molar conductivities were measured with a Siemens WPACM 35 conductivity meter (10^{-3} mol L^{-1} in DMF solution). Magnetic measurements were performed with a Sherwood Scientific magnetic susceptibility balance (Model No: MK 1) at 21°C with $Hg[Co(NCS)_4]$ as a calibration.

Syntheses of 2-aminothiophenes: general procedure

2-Aminothiophene-3-carboxylic acid ethyl esters (1a–c) were prepared according to the procedure described by Gewald (Peet *et al*., 1986). A mixture of 5.66 g (0.05 mol), ethyl cyanoacetate, 0.05 mol of carbonyl compound (4.20 g cyclopentanone, 4.91 g cyclohexanone, and 5.60 g cycloheptanone, respectively for 1a, 1b, and 1c) 1.60 g, (0.05 mol) of sulfur, 4.91 g (0.05 mol) of morpholine, and 15 ml of ethanol was mixed at room temperature. Once an exothermal reaction was observed and the reaction temperature was increased 50°C, after 1 hour, a thick precipitate was obtained. This mixture was poured into the water. The resulting solid was collected and recrystallized from ethanol.

Syntheses of imine compounds: general procedure

A mixture of 2-hydroxy-1-napthaldehyde (1.72 g, 0.01 mol) and amine compound (2.11 g of 1a, 2.25 g of 1b, 2.39 g of 1c, 0.01 mol) in 20 ml of ethanol was refluxed for 2 hours and then cooled at room temperature. The precipitated solid was collected, washed with cold ethanol, and recrystallized from ethanol. All imine compounds were prepared by using the same procedure (Scheme 1, 3a–c).

Syntheses of complexes

All complexes were prepared by following a general method: methanolic solutions of the ligand (0.912 g of HNAP-pT, 0.947 g of HNAP-tT, 0.983 g of HNAP-hT) 2.5 10^{-4} mol in 25 ml and metal salt (0.013 g of $CrCl_36H_2O$ and 0.068 g $ZnCl_2$) 5 10^{-4} mol in 25 ml were mixed and refluxed for 2 hours. The reaction mixture was, after refluxing for 2 hours, concentrated through evaporation until half of the volume. After keeping it another 2 days, the solid complexes formed were collected by filtration and then dried in a desiccator over $CaCl_2$.

Detection of antimicrobial activity

The bacterial subcultures chosen were *Listeria monocytogenes 4b* ATCC-19115, *Staphylococcus aureus* ATCC25923, *Proteus OX2 Wrah* (ETS.40-A-4), *Escherichia coli* ATCC-1280, *Salmonella typhi H* (NCTC-901.8394), *Pseudomonas putida sp., Brucella abortus* (A.99, UK-1995) RSKK-03026. *Sh. boydii type 11* (Pasteur 51.6), *Sh. boydii type 16* (cHe 67.11), *Sh. boydii type 6* (RSKK-96043). An antifungal susceptibility test was used by *Candida albicans* Y-1200-NIH, Tokyo. The ligands and the complexes were tested for their antimicrobial activity by the well-diffusion method (Arslan *et al*., 2006; Logoglu *et al*., 2006). Each ligand and complex was kept dry at room temperature and dissolved (0.25 μg/μl) in DMSO. DMSO was used as solvent and also for control. It was found to have no antimicrobial activity against any of the tested organisms. 1% (v/v) of a 24-hour broth culture containing 10^6 CFU/ml was placed in sterile Petri dishes. Muller-Hilton Agar (15 ml) kept at 45°C was then poured in to the Petri dishes and allowed to solidity. Then 6-mm diameter wells were punched carefully by using a sterile cork borer and were entirely filled with the test solutions. The plates were incubated for 24 hours at 37°C. On completion of the incubation period, the mean value obtained for the two holes was used to the calculate the zone of growth inhibition of each sample. Bacterial subcultures and antifungal were tested for resistance to seven antibiotics (produced by Oxoid Lt., Basingstoke, UK): ampicillin (preventing the growth of gram-negative bacteria), ofloxacin (entering the bacterial cell and inhibiting DNA-gyrase), nystatin (binding to sterols in the fungal cellular membrane, altering the permeability and allowing leakage of the cellular contents), kanamycin (used in molecular biology as agent to isolate bacteria), sulphamethoxazol (bacteriostatic antibacterial agent that interferes with folic acid synthesis in susceptible bacteria), amoxycillin (β-lactam antibiotic used to treat bacterial infections caused by susceptible microorganisms), chloroamphenicol (effective against a wide variety of microorganisms).

Results and discussion

Analytical data and some of the physical properties of the Schiff bases and their complexes are summarized in Table 1. The complexes are only soluble in DMF and DMSO but insoluble in other common organic solvents. Molar conductance values of the Cr(III) complexes were found to be ca. 60. Ω^{-1} cm^2 mol^{-1}. This state indicated that the 1:1 electrolytic behaviour for Cr(III) (Sari and Gürkan 2004). Zn(II) complexes were nonelectrolytic.

$$\left[\mathrm{M(NAP\text{-}T)_2}\right]\mathrm{Cl} \leftrightarrow \left[\mathrm{M(NAP\text{-}T)_2}\right]^{+} + \mathrm{Cl^{-}M{:}Cr(III)}$$

$$\left[\mathrm{M(NAP\text{-}T)_2}\right] \leftrightarrow \left[\mathrm{M(NAP\text{-}T)_2}\right]\mathrm{M{:}\,Zn(II)}$$

Table 1 Analytical and physical data for Schiff bases derivatives and their complexes

Compound empirical formula	Formula weight color, yield (%)	m.p.(°C), μ_{eff} (BM) Λ (ohm^1 cm^{-1} mol^{-1})	Elemental analysis found (Calcd) %				
			C	H	N	M	Cl
(HNAP-pT)	365	170–173, –	69.14	5.60	3.02	–	–
$C_{21}H_{19}NO_3S$	White, 81	–	(69.04)	(5.21)	(3.84)		
(HNAP-tT)	379	201–202, –	69.42	5.23	3.54	–	–
$C_{22}H_{21}NO_3S$	Yellow, 78	–	(69.66)	(5.54)	(3.69)		
(HNAP-hT)	393	152–154, –	69.97	5.73	3.07	–	–
$C_{23}H_{23}NO_3S$	Orange, 73	–	(70.23)	(5.85)	(3.56)		
$[Cr(NAP\text{-}pT)_2]Cl$	815.4	208.0, 4.12	61.21	4.18	3.41	6.13	4.27
$C_{42}H_{36}N_2O_6S_2ClCr$	Dark violet, 29	61	(61.84)	(4.66)	(3.43)	(6.37)	(4.35)
$[Cr(NAP\text{-}tT)_2]Cl$	843.4	250.5, 4.27	62.79	4.51	3.71	5.76	3.81
$C_{44}H_{40}N_2O_6S_2ClCr$	Dark violet, 40	62	(62.59)	(4.98)	(3.32)	(6.16)	(4.21)
$[Cr(NAP\text{-}hT)_2]Cl$	871.4	199.2, 4.23	63.29	6.31	2.87	5.49	3.56
$C_{46}H_{44}N_2O_6S_2ClCr$	Dark violet, 23	67	(63.33)	(5.28)	(3.21)	(5.97)	(4.03)
$[Zn(NAP\text{-}pT)_2]H_2O$	811.4	194.0, D	62.48	4.17	3.23	8.02	–
$C_{42}H_{38}N_2O_7S_2Zn$	White, 78	25	(62.11)	(4.68)	(3.45)	(8.06)	
$Zn(NAP\text{-}tT)_2]$	821.4	201.2, D	63.94	4.71	3.11	7.48	–
$C_{44}H_{40}N_2O_6S_2Zn$	Yellow, 72	14	(64.28)	(4.88)	(3.41)	(7.96)	
$[Zn(NAP\text{-}hT)_2]H_2O$	867.4	194.0, D	63.48	5.21	3.81	7.19	–
$C_{46}H_{46}N_2O_7S_2Zn$	Orange, 75	21	(63.64)	(5.30)	(3.23)	(7.54)	

D diamagnetic

IR, UV-visible and NMR spectra of ligands and their complexes

Table 2 summarizes the main IR and UV-visible bands of the compounds. IR bands in the 1612–1625 cm^{-1}, 1697–1712 cm^{-1}, 3409–3416 cm^{-1}, and 2925–2912/2952–2956 cm^{-1} regions are characteristic of ν(CH=N), ν(C=O), ν(OH), and $\nu(CH)_{arom}$, respectively. In the Schiff bases, the bands ca. 430 nm are attributed to the azomethine chromophore $\pi \rightarrow \pi^*$ transition (Basak *et al*., 2007). The bands at higher energies (270–320 nm) are associated with the benzene $\pi \rightarrow \pi^*$ transition (Silverstein *et al*., 1981). The ^{1}H NMR and ^{13}C NMR data of the Schiff bases are presented in Table 3. In general, the duplets or triplets observed at 8.22–7.11 ppm are assigned to aldehyde ring protons. The singlets at 9.30–9.32 ppm and 14.30–14.83 ppm are assigned to imine and aromatic hydroxyl protons respectively. The protons of $-CH_3$ and $-CH_2$ of $-CO_2C_2H_5$ group in the Schiff bases also are observed as expected. The ^{13}C-NMR spectra data of the Schiff bases (Table 3) are in accord with the proposed structures.

The IR assignments for the important bands of the complexes are given in Table 2. The bands in the 1697–1701 cm^{-1} and 1611–1621 cm^{-1} region may be, respectively, ascribed to ν(C=O) and ν(–CH=N) vibrations (Joseph and Mehta, 2007). A shift at ν(C=O) bands is not seen in the complexes, lending further support

Table 2 Key IR bands (cm^{-1}) and electronic spectral data (nm) (ε_{max}, mol^{-1} $cm^{-1}L$) of Schiff bases and their metal complexes

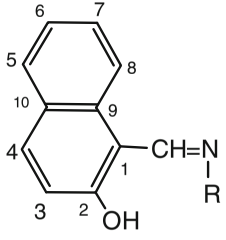
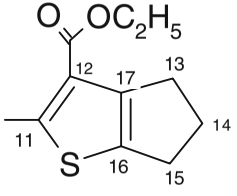
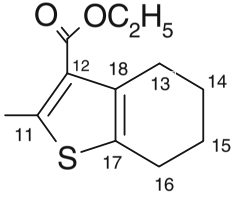
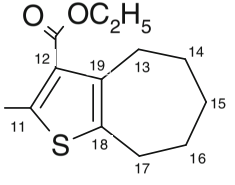
Compound	$\nu_{(CH=N)}$ $\nu_{(C=O)}$	$\nu_{(M-O)/(M-N)}$	λ_{max} ($\varepsilon \times 10^4$)
(HNAP-pT)	1625, 1702		275 (14), 327 (7.5), 446 (27)
(HNAP-tT)	1625, 1696		270 (12), 325 (7.1), 436 (21)
(HNAP-hT)	1612, 1697		271 (26), 322 (12), 429 (32)
$[Cr(NAP\text{-}pT)_2]Cl$	1618, 1701	491/510	275 (3.1), 325 (0.19)
			434 (0.14), 453 (0.10), 600 (0.09)
$[Cr(NAP\text{-}tT)_2]Cl$	1621, 1698	472/510	275 (3.5), 318 (0.42)
			431 (0.09), 457 (0.19), 606 (0.06)
$[Cr(NAP\text{-}hT)_2]Cl$	1621, 1698	492/523	275 (3.9), 318 (0.42)
			434 (0.07), 458 (0.18) 604 (0.08)
$[Zn(NAP\text{-}pT)_2] \cdot H_2O$	1618, 1701	479/514	261 (0.5), 320 (3.3), 442 (0.78)
$[Zn(NAP\text{-}tT)_2]$	1621, 1698	472/516	261 (21), 318 (0.19), 452(0.09)
$[Zn(NAP\text{-}hT)_2] \cdot H_2O$	1620, 1706	452/518	261 (21), 320 (0.08), 444 (0.02)

to the suggestion that the atoms O in the -C=O do not coordinate with the metal ion (Herrera *et al*., 2007) (Scheme 2). The azomethine and carbonyl bands in the IR spectra of the complexes are in the 1697–1701 cm^{-1} range, somewhat lower than observed for the free ligands. These indicate that the azomethine nitrogen is coordinated to metal ion (Sari *et al*., 2008; Burlov *et al*., 2006).

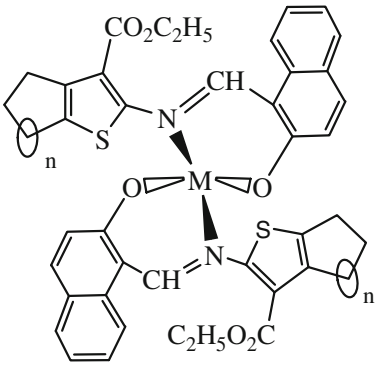
The IR spectra of the Zn(II) complexes exhibit brought bands in 3400 cm^{-1} and new band ca. 1600 cm^{-1} (H–O–H bending mode), which are bonds because of lattice water absorptions (Nakamoto, 1986). The IR spectra of all of the Cr(III) complexes and $[Zn(NAP\text{-}tT)_2]$ complexes do not contain the ν(OH) bands. Furthermore, the appearance of new bands in the 508–587 and 472–493 cm^{-1} regions is due to ν(M-O) and ν(M-N), respectively (AbouEl-Enein *et al*., 2007).

Because Cr(III) complexes are paramagnetic, the ^{1}H-NMR spectra could not be obtained. ^{1}H-NMR spectra of Zn(II) complexes were obtained due to the diamagnetic. The Zn(II) complexes exhibit signals in the range of 9.44–9.53 ppm due to –CH=N protons. These signals are observed in the higher field than ligands. The signals (^{1}H-NMR and IR spectra) of Zn(II) complexes are different from those of the corresponding ligands, suggesting the coordination through oxygen atoms in a phenol ring and azomethine groups. The imine proton signal showed a downfield shift of approximately 0.25 ppm in Zn(II) complexes. The ligands showed broad signals at 14.77–14.26 ppm but the complexes do not contain OH signals because the ligands can coordinate Zn(II) ions with deprotonated phenolic oxygen except $[Zn(NAP\text{-}tT)_2]$ (Scheme 2). –OH signal is observed in different field than ligand for $[Zn(NAP\text{-}tT)_2]$, which state may coordinate Zn(II) ions with protonated phenolic oxygen (Table 4). More detailed information about the structure of the ligands and their Zn(II) complexes were provided by the ^{13}C-NMR spectra data. ^{13}C-NMR spectra of the ligands were assigned by comparison with those of their Zn(II) complexes. The signals of carbon atoms that neighbor to an OH group were different. This can be attributed to the coordination of the phenolic oxygen atom

Table 3 ^{1}H- and ^{13}C-NMR chemical shift (ppm) of the Schiff bases

Compound	R		
	(HNAP-pT)	(HNAP-tT)	(HNAP-hT)
	***^{1}H NMR*** (400 MHz, *DMSO/TMS*): *δ(ppm)* 1.33 (t, 3H, $\underline{CH_3}$), 2.22 (m, 2H, H-14), 2.82 (q, 4H, H-13, H-15), 4.33 (q, 2H, $\underline{CH_2}$), 7.22 (d, 1H, H-3), 7.34 (t, 1H, H-6), 7.43 (t, 1H, H-7), 7.89 (d, 1H, H-8), 8.01 (d, 1H, H-5), 8.13 (d, 1H, H-4), 9.38 (s, 1H, C$\underline{H}$=N), 14.77 (s, 1H, Ar-O$\underline{H}$).	***^{1}H NMR*** (400 MHz, *DMSO/TMS*): *δ(ppm)* 1.34 (t, 3H, $\underline{CH_3}$), 1.75 (m, 4H, H-14, H-15), 2.70 (m, 4H, H-13, H-16), 4.33 (q, 2H, $\underline{CH_2}$), 7.17 (d, 1H, H-3), 7.42 (t, 1H, H-6), 7.59 (t, 1H, H-7), 7.87 (d, 1H, H-8), 8.00 (d, 1H, H-5), 8.40 (d, 1H, H-4), 9.39 (s, 1H, C$\underline{H}$=N), 14.48 (s, 1H, Ar–O$\underline{H}$).	***^{1}H NMR*** (400 MHz, *DMSO /TMS*): *δ(ppm)* 1.35 (t, 3H, $\underline{CH_3}$), 1.62 (m, 4H, H-14, H-16), 1.85 (m, 2H H-15), 2.84 (m, 4H, H-13, H-17), 4.35 (q, 2H, $\underline{CH_2}$), 7.20 (d, 1H, H-3), 7.43 (t, 1H, H-6), 7.60 (t, 1H, H-7), 7.89 (d, 1H, H-8), 8.02 (d, 1H, H-5), 8.46 (d, 1H, H-4), 9.43 (s, 1H, C$\underline{H}$=N), 14.26 (s, 1H, Ar-O$\underline{H}$).
	^{13}C NMR (100 MHz, *DMSO*):15, 27, 29, 31, 61, 112, 119, 120, 121, 124, 128, 129, 130, 134, 135, 137, 146, 153, 159, 164, 165	***^{13}C NMR*** (100 MHz, *DMSO*): 14, 22, 23, 26, 28, 61, 113, 118, 119, 124, 127, 129, 130, 132, 135, 136, 141, 142, 154, 147, 163, 164	***^{13}C NMR*** (100 MHz, *DMSO*): 14, 27, 28, 29, 31, 32,62, 112, 120, 122, 125, 127, 128, 129, 131, 133, 136,138, 141, 150, 157, 164, 165
			

Scheme 2 The proposed structures of the complexes (M: Cr(III) and Zn(II), $n = 1,2,3$)



(AbouEl-Enein *et al*., 2007) (Table 4). C_1, C_2 and C_{11} carbon atoms in the free Schiff bases were showed a significant shift after complexation. This case may be due to the coordination of the ligand to the metal atom by the azomethine nitrogen and phenolic oxygen.

Electronic spectra and magnetic studies

In the UV-*vis*. spectra of the complexes, the azomethine chromophore $\pi \rightarrow \pi^*$ transition is shifted to 429–452 nm, which indicates that the imino nitrogen is involved in the coordination of the metal ion. The absorption frequencies ascribed to the benzene $\pi \rightarrow \pi^*$ transition are slightly changed as a result of the influence of the benzene ring on the coordination interaction (Silverstein *et al*., 1981).

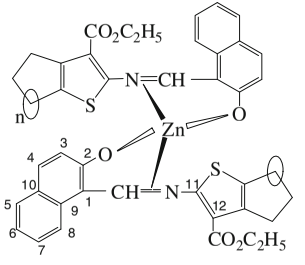
The magnetic moments for the Cr(III) complexes are 4.7 BM, indicating the presence of three unpaired electrons. The electronic spectrum of the Cr(III) complex exhibits band at 604 nm and 450 nm, which may be assigned to $^4B_{1g} \rightarrow {}^4E_g$, and $^4B_{1g} \rightarrow {}^4E_g$ transitions, respectively (Farkas *et al*., 2007; Cotton and Wilkinson, 1972). The position of these bands is consistent with a distorted octahedral geometry with D_{4h} symmetry. Because Zn(II) has no unpaired d-electrons, no absorption peak is observed in the visible region for these complexes. A tetrahedral arrangement of the donor atoms around Zn(II) is the probable structure of the Zn(II) complexes.

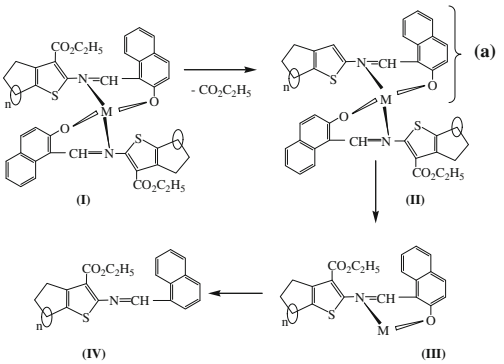
LC-mass spectra for complexes

The results obtained showed that the structure of complexes is the ML_2 complexes according to the LC-mass spectroscopy $[M\text{-}C_2H_5]^+$ 738.4 (m/z: %11.2), $[M\text{-}C_2H_5]^+$ 748.4 (m/z: %9.8), $[M\text{-}C_2H_5]^+$ 794.7 (m/z: %18.2), $[M\text{-}C_2H_5]^+$ 742.2 (m/z: %32.1), $[M\text{-}C_2H_5]^+$ 770.1 (m/z: %7.1), $[M\text{-}C_2H_5]^+$ 798.7 (m/z: %7.1); for $[Zn(NAP\text{-}pT)_2]H_2O$, $[Zn(NAP\text{-}tT)_2]$, $[Zn(NAP\text{-}hT)_2]H_2O$ and $[Cr(NAP\text{-}pT)_2]Cl$, $[Cr(NAP\text{-}tT)_2]Cl$, $[Cr(NAP\text{-}hT)_2]Cl$, respectively. The major fragmentation pathways are followed by the molecular ion of complexes (Scheme 3).

Table 4 ^{1}H- and ^{13}C-NMR spectral data (δ ppm) of Zn(II) complexes

Labeling number	$[Zn(NAP\text{-}pT)_2] \cdot H_2O$		$[Zn(NAP\text{-}tT)_2]$		$[Zn(NAP\text{-}hT)_2] \cdot H_2O$	
	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
1	–	116	–	115	–	116
2	–	157	–	155	–	158
11	–	134	–	133	–	134
12	–	137	–	136	–	136
–CH=N	9.58	165	9.50	167	9.61	165
$-OH_{phenolic}$	–		14.48	–	–	–





	$[I-(CO_2C_2H_5)]^+$	% m/z	$[II-(a)]^+$	% m/z	$[III-MO]^+$	% m/z
$[Zn(NAP\text{-}pT)_2]$	738.4	11.2	446.4	36	365	78
$[Zn(NAP\text{-}tT)_2]$	748.4	9.8	442.4	41	379	73
$[Zn(NAP\text{-}hT)_2]$	794.7	18.2	474.7	38	393	77
$[Cr(NAP\text{-}pT)_2]$	742.2	6.3	450.2	31	365	58
$[Cr(NAP\text{-}tT)_2]$	770.1	7.1	464.1	43	379	62
$[Cr(NAP\text{-}hT)_2]$	798.7	7.3	478.7	32	393	44

Scheme 3 Mass fragmentation of the complexes

Biological activity of ligands and their complexes

The ligands and their complexes were screened for antimicrobial activity in DMSO solvent as a control substance. The compounds were tested with the same concentrations in DMSO solution (0.25 μg/μl).

All synthesized compounds and antibiotics exhibited varying degrees of inhibitory effects on the growth of different tested strains (Table 5). All of the compounds were active against S. *aureus, Sh. Boydii type 11, Sh. Boydii type 16*, and *Sh. Boydii type 6*. All synthesized compounds showed moderate activity against *S. typhi H*, except Cr(III) and Zn(II) complexes of (HNAP-hT). (HNAP-hT) and its complexes were inactive in *L. monocytogenes 4b*. As shown in Table 5, the compound (HNAP-pT) showed a significant activity against S. aureus and C. albicans; however, *Proteus OX*$_2$ and *Br. Abortus* did not display any activity against them.

The results of antifungal and antibacterial screening indicated that all of the complexes of (HNAP-pT) showed more activity than the other complexes. Generally, the metal complexes are more potent bactericides than the ligand, except *S. Aureus*. *Br. abortus* is a gram-negative bacterium that causes premature abortion of a cattle fetus. What makes this bacterium so dangerous is that it is zoonotic, and it can be transferred from an animal to a human host and still remain

Table 5 Biological activity of Schiff bases, complexes (0.25 μg/μl) and standard reagents (Diameter of zone of inhibition (mm))

Compound	Gram (+)		Gram (−)								Yeast
	S. aureus	*L. monocytogenes* 4b	*P. putida*	*E. coli*	*Proteus OX2*	*S. typhi H*	*Sh. boydii*			*Br. abortus*	*C. albicans*
							Type 11	*Type 16*	*Type 6*		
(HNAP-pT)	14	7	8	6	5	6	8	7	6	–	14
(HNAP-tT)	9	9	7	6	–	7	7	8	7	–	–
(HNAP-hT)	9	–	–	9	–	8	8	8	7	5	11
$[Cr(NAP\text{-}pT)_2]Cl$	7	9	10	8	6	6	6	7	6	–	15
$[Cr(NAP\text{-}tT)_2]Cl$	7	8	6	–	–	6	6	7	6	–	13
$[Cr(NAP\text{-}hT)_2]Cl$	9	–	8	8	–	–	12	7	6	7	8
$[Zn(NAP\text{-}pT)_2] \cdot H_2O$	6	8	10	7	8	6	6	8	7	–	13
$[Zn(NAP\text{-}tT)_2]$	7	8	9	8	–	6	7	9	7	–	–
$[Zn(NAP\text{-}hT)_2] \cdot H_2O$	6	–	10	7	–	–	6	7	7	8	12
Standard reagents name											
K30	25	15	14	25	25	20	14	16	15	–	–
SXT25	24	11	18	18	20	17	19	20	20	–	–
AMP10	30	16	8	10	9	11	20	16	6	–	–
C30	25	18	12	30	27	19	18	22	18	–	–
CIP5	25	17	20	25	27	23	25	20	30	–	–
AMC30	30	22	15	14	17	19	24	14	19	–	–
NYS100	–	–	–	–	–	–	–	–	–	–	20

K30 kanamycin 30 μg, *SXT25* sulphamethoxazol 25 μg, *AMP10* ampicillin 10 μg, *C30* chloroamphenicol 30 μg, *CIP5* ciprofloxacin 5 μg, *AMC30* amoxicillin 30 μg, *NYS100* nystatin 100 μg

Results were obtained using the agar well-diffusion method; the well diameter (6 mm) was subtracted from the total zone diameter

pathogenic (Halling *et al*., 2005). In humans this disease causes both acute and chronic symptoms but can be treated with antibiotics.This research indicates that (HNAP-hT), [Cr(HNAP-hT)$_2$]Cl, and [Zn(HNAP-hT)$_2$]H_2O are active against *Br. abortus* (Table 5). This enhancement in activity may be explained on the basis of chelation theory (Kurtoglu *et al*., 2008). Chelation reduces the polarity of the metal ion, so a complex has lipophilic character and increases the interaction between metal ion and the lipid is favored. This leads to the breakdown of the permeability barrier of the cell, resulting in interference with the normal cellular processes (Murukan and Mohanan, 2007). Chelation is not the only criterion for antibacterial activity. Some important factors, such as the nature of the metal ion, nature of the ligant, geometry of the complexes, hydrophilic, and lipophilicity, have influence on antibacterial activity (Lippard and Berg, 1994). The antibacterial activity of these compounds was compared with seven commercial antibiotics: kanamycin, sulphamethoxazol, ampicillin, chloroamphenicol, ciprofloxacin, amoxycillin, and nystatin. The synthesized compounds were as effective as the antibiotics mentioned.

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