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Full Paper

Synthesis, characterisation and antimicrobial activities of vic-dioxime derivatives containing heteroaromatic hydrazone groups and their metal complexes

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Abstract: Three novel heteroaromatic hydrazone derivatives bearing vic-dioxime groups (L^1H_2: 5-methyl-2-furfural hydrazone glyoxime, L^2H_2: 3-acetylpyridine hydrazone glyoxime and L^3H_2: 4-acetylpyridine hydrazone glyoxime) and their Ni(II), Cu(II) and Co(II) complexes were prepared. They were characterised by elemental analysis, gel permeation chromatography (GPC), FT-IR, UV, ^{1}H NMR and ^{13}C NMR. The antimicrobial activities of compounds L^1H_2, L^2H_2, L^3H_2 and their Ni(II), Cu(II) and Co(II) complexes were evaluated using the disc diffusion method against 13 bacteria and 5 yeasts. The minimal inhibitory concentrations (MICs) against 3 bacteria and 3 yeasts were also determined. Among the test compounds attempted, L^1H_2, $[Co(L^1H)_2(H_2O)_2]$, $[Ni(L^2H)_2]$, $[Cu(L^2H)_2]$, L^3H_2, $[Ni(L^3H)_2$ and $[Co(L^3H)_2(H_2O)_2]$ showed some activities against certain Gram-positive bacteria and some of the yeasts tested.

Key Words: vic-dioximes, hydrazone glyoximes, metal complexes, antimicrobial activity

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INTRODUCTION

Schiff base ligands are well known for their wide range of applications in pharmaceutical and industrial fields [1-3]. Moreover, the hydrazone group plays an important role of the antimicrobial and possesses interesting antibacterial, antifungal [4-6] and anti-tubercular activities [7-12]. In addition, their varied coordinating behaviour makes them interesting candidates for metal-based drugs. Generally, the ligands act synergistically with metals towards their biological activity [11, 12].

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Hydrazones possessing an azomethine proton (-NHN=CH-) constitute an important class of compounds for new drug development. Many researchers have synthesised these compounds as well as their metal complexes as target structures and evaluated their biological activities. These observations have guided the development of new hydrazones with varied biological activities [13]. The biological activity of complexes derived from hydrazones have been studied and contrasted with regard to their antibacterial, antitumoral, antiviral, antimalarial and antitubercular properties [14]. It has also been shown that the azomethine N, which has a lone pair of electrons in an sp^2 hybridised orbital, is biologically important [15].

Oximes are becoming increasingly important as analytical, biochemical and antimicrobial reagents and they have received attention due to their use as liquid crystals and dyes [16]. Coordination compounds of oximes also receive considerable attention due to their structural features. A large amount of work has been accumulated in areas such as structural stability and reactivity, biochemical modelling and synthesis of molecules with unusual electronic properties [e.g. 17, 18]. However, detailed literature survey reveals that there is only a little investigation made so far on the synthesis of metal hydrazone-oxime chelates. In continuation of our interest in the chemistry and biology of transition metal hydrazone-oxime chelates [19], we now carry out another systematic study of their synthesis and biological activity. Herein, the synthesis of the novel 5-methyl-2-furfural hydrazone glyoxime (L^1H_2), 3-acetylpyridine hydrazone glyoxime (L^2H_2) and 4-acetylpyridine hydrazone glyoxime (L^3H_2) as well as their complexes with Ni(II), Cu(II) and Co(II) ions are described and their antimicrobial properties are evaluated.

EXPERIMENTAL

The ^1H and ^{13}C NMR spectra were recorded in DMSO-d^6 on a Bruker-400 MHz spectrophotometer using tetramethylsilane as an internal reference. The apparent resonance multiplicity was described as: s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet) and m (multiplet). Infrared spectra were recorded in the range 400-4000 cm^{-1} on Spectrum 900 by Varian. Electronic spectra were recorded on a Shimadzu UV1601 spectrophotometer. Elemental analysis was carried out using PerkinElmer CHNS/O 2400. Room temperature magnetic susceptibility measurements were carried out using a Sherwood-Scientific Gouy magnetic balance (Calibrant: $Hg[Co(SCN)_4]$).

Cobalt(II) chloride hexahydrate (Merck), nickel(II) chloride hexahydrate (Merck), copper(II) chloride dihydrate (Merck), 5-methyl-2-furfuraldehyde (Merck), 3-acetylpyridine (Merck) and 4-acetylpyridine (Merck) were used as received for the sythesis of ligands and complexes. *Anti*-glyoximehydrazine (GH_2) was prepared according to a reported procedure [20]. Commercially available pure grade solvents, dried and purified by conventional procedure were used.

Synthesis

General procedure for the synthesis of ligands

L^1H_2, L^2H_2 and L^3H_2 were synthesised from the starting materials, namely *anti*-glyoximehydrazine (GH_2) [20] (Scheme 1), 5-methyl-2-furfuraldehyde (for L^1H_2), 3-acetylpyridine (for L^2H_2) and 4-acetylpyridine (for L^3H_2), using glacial acetic acid as a catalyst (Schemes 2 and 3). A cooled (5°C) solution of ketone or aldehyde (1 mmol) in ethanol was added dropwise into a

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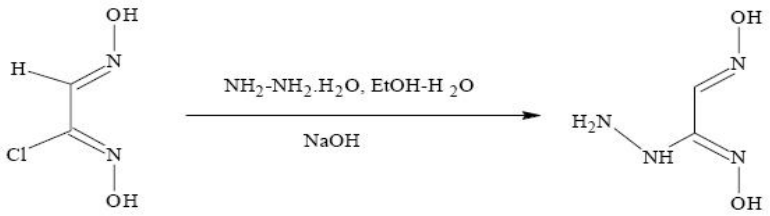
cooled solution (5°C) containing 1 mmol (0.118 g) of *anti*-glyoximehydrazine (GH_2) and 3-5 drops of acetic acid with constant stirring. After the addition of aldehyde or ketone was completed, the solution was stirred for 2-4 hours at room temperature. The resulting solid compounds were filtered off, washed with water and ethanol and dried at room temperature in a vacuum oven. The results of the spectroscopic and composition analyses are as follows.

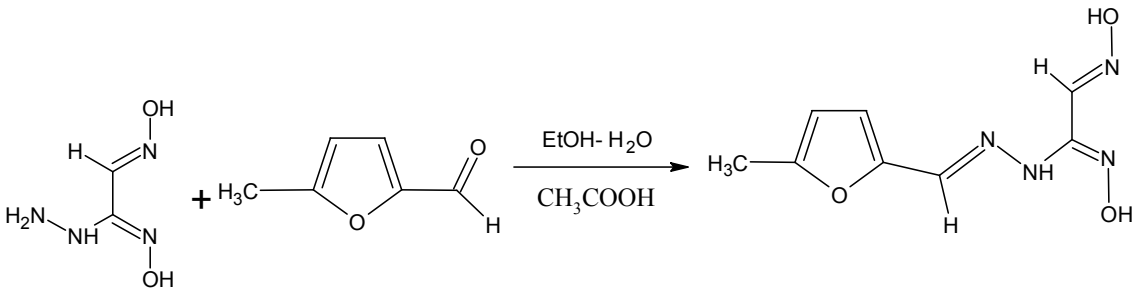
L^1H_2: yield 70 %; m.p. 118°C; colour yellow; IR (KBr, cm^{-1}) 3256 (N-H), 3124 (O-H), 3068 (C-$H_{arom.}$), 1608 (C=N_{oxime}), 1644 (C=$N_{hydr.}$), 953 (N-O); ^{1}H-NMR (DMSO, ppm) 10.18 s, 1H (NH), 11.50–10.65 s, 2H (OH), 6.70 s, 1H (CH=NOH), 6.60 d (J 4.66 Hz), 2H (Ar-H), 7.80 s, 2H (-CH=N-NH), 2.20 s, 3H (-CH_3); ^{13}C-NMR (DMSO, ppm) 158.80 (-CH=N-NH-), 154.64 (N-NH-C=N-OH), 150.34 (C-CH=N-OH), 148.48, 147.67, 141.13, 126.33 (Ar-C), 14.14 (-CH_3); UV-Vis (DMSO, λ_{max}/nm) 262, 394. For $C_8H_{10}O_3N_4$ (210.190 $g.mol^{-1}$), calculated: 45.71 % C, 4.80 % H, 26.66 % N; found: 45.65 % C, 4.46 % H, 26.24 % N.

L^2H_2: yield 60 %; m.p. 146.5°C; colour yellow; IR (KBr, cm^{-1}) 3364 (N-H), 3180 (O-H), 3066 (C-$H_{arom.}$), 1605 (C=N_{oxime}), 1656 (C=$N_{hydr.}$), 962 (N-O); ^{1}H-NMR (DMSO, ppm): 8.82 s, 1H (NH), 11.50–10.70 s, 2H (OH), 7.82 s, 1H (CH=NOH), 8.70 s, 1H (Ar-H), 8.50 d (J 6.0 Hz), 1H, 7.94 d (J 7.0 Hz), 1H, 7.53 t (J 7.6 Hz), 1H (Ar-H), 2.13 s, 3H (-CH_3); ^{13}C-NMR (DMSO, ppm) 154.06 (-CMe=N-NH-), 149.83 (N-NH-C=N-OH), 147.58 (C-CH=N-OH), 146.47, 141.86, 134.45, 133.60, 124.08 (Ar-C), 12.69 (-CH_3); UV-Vis (DMSO, λ_{max}/nm) 260. For $C_9H_{11}O_2N_5$ (221.216 $g.mol^{-1}$), calculated: 48.86 % C, 5.01 % H, 31.66 % N; found: 48.68 % C, 5.52 % H, 30.98 % N.

L^3H_2: yield 60 %; m.p. 136°C; colour yellow; IR (KBr, cm^{-1}) 3340 (N-H), 3161 (O-H), 3073 (C-$H_{arom.}$), 1605 (C=N_{oxime}), 1640 (C=$N_{hydr.}$), 960 (N-O); ^{1}H-NMR (DMSO, ppm) 8.92 s, 1H (NH), 11.60–10.87 s, 2H (OH), 7.61 s, 1H (CH=NOH), 8.27 d (J 6.8 Hz) 4H (Ar-H), 2.19 s, 3H (-CH_3); ^{13}C-NMR (DMSO, ppm) 151.45 (-CMe=N-NH-), 150.50 (N-NH-C=N-OH), 146.17 (C-CH=N-OH), 143.34, 141.68, 121.96 (Ar-C), 12.20 (-CH_3); UV-Vis (DMSO, λ_{max}/nm) 262. For $C_9H_{11}O_2N_5$ (221.216 $g.mol^{-1}$), calculated: 48.86 % C, 5.01 % H, 31.66 % N; found: 48.69 % C, 5.34 % H, 31.37 % N.



Scheme 1. Synthesis of *anti*-glyoximehydrazine [20]



Scheme 2. Synthesis of 5-methyl-2-furfuraldehyde hydrazone glyoxime (L^1H_2)

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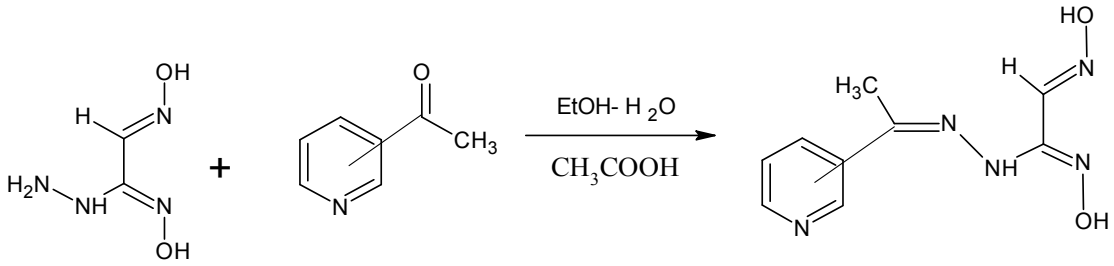

Scheme 3. Synthesis of 3-acetylpyridine hydrazone glyoxime (L^2H_2) and 4-acetylpyridine hydrazone glyoxime (L^3H_2)

Synthesis of the Ni(II), Cu(II) and Co(II) complexes of ligands

A solution of a metal salt (1 mmol: 0.237 g of $NiCl_2.6H_2O$, 0.170 g of $CuCl_2.2H_2O$ or 0.237 g of $CoCl_2.6H_2O$ in 20 mL of water) was added to 2 mmol of the ligand solution (0.420 g of L^1H_2, 0.442 g of L^2H_2 or 0.442 g of L^3H_2 in 15 mL of ethanol) with stirring. An initial sharp decrease in the pH of the solution from 5.5 to 3-3.5 was observed. After raising the pH to 5-5.5 using a 1% aqueous NaOH solution, the reaction mixture was kept in a hot water bath (60°C) for 2 hours to complete the precipitation. Then the precipitated complexes were filtered, washed with water and dried at room temperature in a vacuum oven. Results of the spectroscopic and composition analyses are shown as follows. Proposed structures of complexes are shown in Figures 1 and 2.

$[Ni(L^1H)_2]$: yield 60 %; m.p. >400°C; colour red; IR (KBr, cm^{-1}) 3437 (N-H), 3105 (C-$H_{arom.}$), 1578 (C=N_{oxime}), 1633 (C=$N_{hydr.}$), 1882 (H...OH), 947 (N-O); UV-Vis (DMSO, λ_{max}/nm) 221, 350, 490. For $C_{16}H_{18}O_6N_8Ni$ (477.058 $g.mol^{-1}$), calculated: 40.28 % C, 3.80 % H, 23.49 % N; found: 40.48 % C, 3.66 % H, 23.36 % N.

$[Cu(L^1H)_2]$: yield 60 %; m.p. >400°C; colour brown; IR (KBr, cm^{-1}) 3433 (N- H), 3120 (C-$H_{arom.}$), 1567 (C=N_{oxime}), 1622 (C=$N_{hydr.}$), 1735 (H...OH), 956 (N-O); UV-Vis (DMSO, λ_{max}/nm) 266, 322, 390. For $C_{16}H_{18}O_6N_8Cu$ (481.910 $g.mol^{-1}$), calculated: 39.88 % C, 3.76 % H, 23.25 % N; found: 39.03 % C, 3.87 % H, 23.68 % N.

$[Co(L^1H)_2(H_2O)_2]$: yield 60 %; m.p. >400°C; colour brown; IR (KBr, cm^{-1}) 3398 (N-H), 3121 (OH/H_2O), 3109 (C-$H_{arom.}$), 1573 (C=N_{oxime}), 1623 (C=$N_{hydr.}$), 1762 (H...OH), 949 (N-O); UV-Vis (DMSO, λ_{max}/nm) 255, 310, 390. For $C_{16}H_{22}O_8N_8Co$ (513.328 $g.mol^{-1}$), calculated: 37.44 % C, 4.32 % H, 21.83 % N; found: 37.20 % C, 3.94 % H, 22.32 % N.

$[Ni(L^2H)_2]$: yield 60 %; m.p. >400°C; colour red; IR (KBr, cm^{-1}) 3444 (N-H), 3109 (C-$H_{arom.}$), 1578 (C=N_{oxime}), 1652 (C=$N_{hydr.}$), 1782 (H...OH), 942 (N-O); UV-Vis (DMSO, λ_{max}/nm) 273, 388, 482. For $C_{18}H_{20}O_4N_{10}Ni$ (499.110 $g.mol^{-1}$), calculated: 43.32 % C, 4.04 % H, 28.06 % N; found: 43.08 % C, 4.34 % H, 27.44 % N.

$[Cu(L^2H)_2]$: yield 60 %; m.p. >400°C; colour brown; IR (KBr, cm^{-1}) 3393 (N-H), 3066 (C-$H_{arom.}$), 1574 (C=N_{oxime}), 1648 (C=$N_{hydr.}$), 1782 (H...OH), 941 (N-O); UV-Vis (DMSO, λ_{max}/nm) 267, 300, 390. For $C_{18}H_{20}O_4N_{10}Cu$ (503.962 $g.mol^{-1}$), calculated: 42.90 % C, 4.00 % H, 27.79 % N; found: 43.58 % C, 4.51 % H, 27.68 % N.

$[Co(L^2H)_2(H_2O)_2]$: yield (60 %); m.p. >400°C; colour brown; IR (KBr, cm^{-1}) 3380 (N-H), 3194 (OH/H_2O), 3072 (C-$H_{arom.}$), 1562 (C=N_{oxime}), 1636 (C=$N_{hydr.}$), 1749 (H...OH), 941 (N-O);

UV-Vis (DMSO, λ_{max}/nm) 264, 294, 404. For $C_{18}H_{24}O_6N_{10}Co$ (535.380 g.mol^{-1}), calculated: 40.38 % C, 4.52 % H, 26.16 % N; found: 40.54 % C, 4.51 % H, 26.86 % N.

[Ni(L^3H)$_2$]: yield 60 %; m.p. >400°C; colour red; IR (KBr, cm^{-1}) 3437 (N-H), 3047 (C-$H_{arom.}$), 1588 (C=N_{oxime}), 1629 (C=$N_{hydr.}$), 1855 (H...OH), 950 (N-O); UV-Vis (DMSO, λ_{max}/nm) 270, 335, 498. For $C_{18}H_{20}O_4N_{10}Ni$ (499.110 g.mol^{-1}), calculated: 43.32 % C, 4.04 % H, 28.06 % N; found: 43.62 % C, 4.34 % H, 27.38 % N.

[Cu(L^3H)$_2$]: yield 60 %; m.p. >400°C; colour brown; IR (KBr, cm^{-1}) 3422 (-NH), 3086 (C-$H_{arom.}$), 1562 (C=N_{oxime}), 1634 (C=$N_{hydr.}$), 1782 (H...OH), 949 (N-O); UV-Vis (DMSO, λ_{max}/nm) 258, 270, 394. For $C_{18}H_{20}O_4N_{10}Cu$ (503.962 g.mol^{-1}), calculated: 42.90 % C, 4.00 % H, 27.79 % N; found: 42.64 % C, 3.58 % H, 27.35 % N.

[Co(L^3H)$_2$(H_2O)$_2$]: yield 60 %; m.p. >400°C; colour brown; IR (KBr, cm^{-1}) 3397 (N-H), 3225 (OH/H_2O), 3050 (C-$H_{arom.}$), 1589 (C=N_{oxime}), 1616 (C=$N_{hydr.}$), 1776 (H...OH), 946 (N-O); UV-Vis (DMSO, λ_{max}/nm) 258, 279, 395. For $C_{18}H_{24}O_6N_{10}Co$ (535.380 g.mol^{-1}), calculated: 40.38 % C, 4.52 % H, 26.16 % N; found: 40.85 % C, 5.05 % H, 25.94 % N.

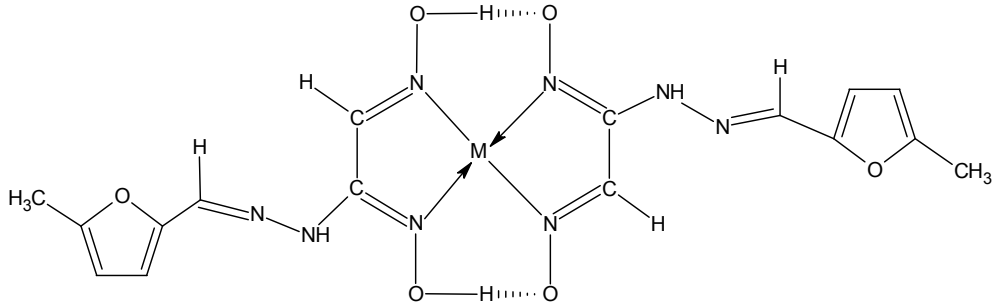

Figure 1. Suggested structure of Co(II)·2H_2O, Ni(II) and Cu(II) complexes of 5-methyl-2-furfuraldehyde hydrazone glyoxime

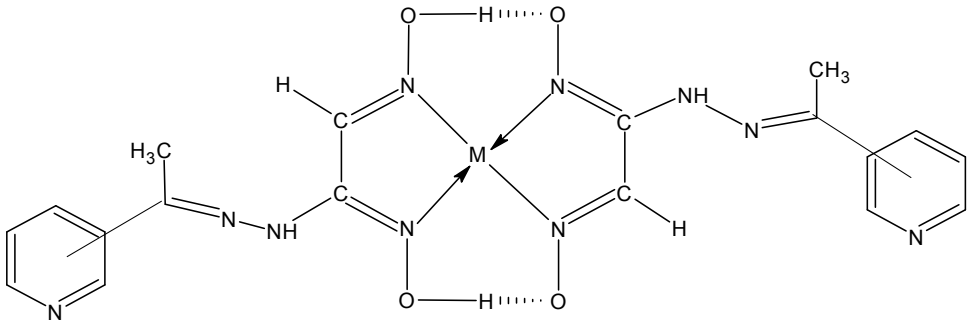

Figure 2. Suggested structure of the Co(II)·2H_2O, Ni(II) and Cu(II) complexes of L^2H_2 and L^3H_2 (3-acetylpyridine for L^2H_2 and 4-acetylpyridine for L^3H_2)

Biological Studies

Eight bacterial strains and one yeast strain were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Other strains were obtained from Faculty of Medicine, Adnan Menderes University. The Gram-negative (G-) were: *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Proteus sp*., *Serratia marcescens* and *Enterobacter sp*., and

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the Gram-positive (G+) were: *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Bacilllus cereus* ATCC 11778, *Bacillus thuringiensis*, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 49617 and *Listeria sp*. The following five yeast strains, i.e. *Candida utilis*, *C. albicans*, *C. glabrata*, *C. Tropicalis* and *Saccharomyces cerevisiae* ATCC 9763, were also tested using disc diffusion method [21, 22] and the minumum inhitory concentration (MIC) was determined by broth dilution method [23].

Disc diffusion method

Screening for antibacterial and antifungal activities were carried out using sterile antibiotic discs (6 mm), following the standard procedure of Antimicrobial Disc Susceptibility Tests outlined by the National Committee for Clinical Laboratory Standards-NCCLS [21, 22]. Fresh stock solutions (30 $\mu g.mL^{-1}$) of the ligands were prepared in DMSO according to the needed concentrations for the experiments.

The inoculum suspensions of the tested bacteria and yeasts were prepared from the broth cultures (18-24 hr) and the turbidity equivalent adjusted to 0.5 McFarland standard tube to give a concentration of $1x10^8$ bacterial cells and $1x10^6$ yeast cells/mL. To test the antimicrobial activity of each aromatic hydrazone derivative bearing vic-dioxime group or its complex, a Mueller Hinton agar plate was inoculated with 0.1 mL broth culture of bacteria or yeast. Then a hole of 6 mm in diameter and depth was made on top with a sterile stick and filled with 10 µL of the hydrazone derivative or its complex containing vic-dioxime group.

Plates inoculated with *E. coli* ATCC 25922, *S. typhimurium* ATCC 14028, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *E. faecalis* ATCC 29212, *S. pneumoniae* ATCC 49617, *Listeria sp., Proteus sp., S. Marcescens* and *Enterobacter sp*. were incubated at 37°C for 24 hr and those inoculated with *M. luteus* ATCC 9341, *B. cereus* ATCC 11778, *B. thuringiensis, S. cerevisiae* ATCC 9763, *C. albicans* ATCC 90028, *C. glabata*, *C. utilis* and *C. tropicalis* were incubated at 30°C for 24 hr. The diameter of the inhibition zone was then measured. Discs of chloramphenicol (C30, Oxoid), gentamycin (GN10 Oxoid), nystatin (NS100 Oxoid) were used as positive controls. The inhibition zones were compared with those of the reference discs.

Dilution method

Screening for antibacterial and antifungal activities were carried out by preparing a micro-dilution broth, following the procedure outlined in Manual of Clinical Microbiology [23]. All the bacteria were inoculated in the nutrient broth and incubated at 30-37°C for 24 hr while the yeasts were inoculated in malt extract broth and incubated at 30°C for 48 hr. The compounds were dissolved in DMSO (2 mg mL^{-1}) and then diluted in Mueller Hinton broth. Twofold serial dilution of the compounds were employed to determine the MIC ranging from 256 to 1.0 µg mL^{-1}. Cultures were grown at 30-37°C (18-20 hr) and the final inoculum was approximately 10^6 cfu mL^{-1}. Test cultures were incubated at 37°C (24 hr). The lowest concentration of antimicrobial agent that resulted in complete inhibition of the microorganisms was represented as MIC (µg mL^{-1}). As positive controls, streptomycin (I. E. Ulagay) for bacteria and nystatin (NS100, Oxoid) for yeast were used in the dilution method. In each case, the test was performed in triplicate and the results were expressed as means.

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RESULTS AND DISCUSSION

Some physical properties, elemental analysis results and magnetic susceptibility data of the ligands and complexes are summarised in Table 1. FT-IR data of the ligands and their complexes are given in Table 2. ^{1}H-NMR and ^{13}C-NMR data of the ligands are given in Table 3. Attempts to isolate crystals suitable for X-ray diffraction were unsuccessful for the ligands and complexes. ^{1}H-NMR and ^{13}C-NMR spectra of these complexes could not be taken because of their very low solubility in organic solvents. FT-IR, UV, elemental analysis and magnetic susceptibility techniques were employed in order to determine the structural characteristics of the complexes. Antimicrobial activities of the ligands and their metal complexes are given Tables 4 and 5.

IR Spectra

The IR spectra of the new hydrazone-oxime compounds (L^1H_2, L^2H_2 and L^3H_2) were in accord with the previously reported oxime derivatives [16, 20, 24-27]. In the IR spectra of Co(II) complexes, the weak deformation vibration band assigned to the intramolecular hydrogen bond O-H....O bending vibration was observed around 1749-1776 cm^{-1} [16, 20, 27]. The $C=N_{oxime}$ stretch decreased from 1608-1605 cm^{-1} in the free ligands to 1589-1562 cm^{-1} in the Co(II) complexes [22, 25, 28]. The coordinated H_2O molecules of $(L^1H)_2Co(H_2O)_2$ and $(L^2H)_2Co(H_2O)_2$ were identified by a broad OH absorption around 3225-3194 cm^{-1} with constant intensities after heating above 110°C for 24 hr. The IR spectra of Ni(II) and Cu(II) complexes exhibited a $C=N_{oxime}$ stretching vibration around 1588-1562 cm^{-1}. These vibrations were at a lower frequency than for the free ligands, which were attributed to N,N- chelation [16, 20, 26-31].

A weak band around 1882-1735 cm^{-1} can be assigned to the intramolecular hydrogen bond O-H....O bending vibration [16-20, 31]. The intensity of the characteristic stretching and bending vibrations of the free ligands shifted and lowered on complex formation and new vibrational bands characteristic of the Ni(II) and Cu(II) complexes were observed.

The dioxime ligand, a neutral compound, forms, when complexed, a monoanion by the loss of an oxime proton with concomitant formation of an intra-molecular hydrogen bond. The cobalt ion coordinates with the ligand through its nitrogen donors in the equatorial positions [31]. The band O-H...O is absent in the ligand but appears in the spectrum of the complexes, showing that the Ni(II) and Cu(II) complexes have a square-planar structure (Figure 1).

NMR Spectra

The signals in the ^{1}H-NMR spectra of the ligands were in accord with the previously reported oxime derivatives [24, 31]. Two peaks were observed for the O-H protons of the oxime groups. These two deuterium-exchangeable singlets correspond to two inequivalent O-H protons that also indicate the *anti*-configuration of the O-H groups relative to each other [29-32].

In the ^{13}C-NMR spectra of the ligands, different signals which were observed at 154.64 ppm for L^1H_2, 149.83 ppm for L^2H_2 and 150.50 ppm for L^3H_2 (HN<u>C</u>=N-OH), together with 150.34 ppm for L^1H_2, 147.58 ppm for L^2H_2 and 146.17 ppm for L^3H_2 (H-<u>C</u>=N-OH) showed that the vic-dioximes are asymetrically substituted [25, 33]. The two different frequencies in each case also indicate that the vic-dioxime has *anti* structure [25].

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Table 1. Physical properties and elemental analyses of the ligands and complexes

Compound Formula	m.p. (ºC)	(%) Yield	μ_{eff} [a] Colour	(BM)	Calculated (Found) % C	H	N
L^1H_2	118	65	Yellow	-	45.71(45.65)	4.80 (4.46)	26.66 (26.24)
$[Ni(L^1H)_2]$	> 400	60	Red	Dia.	40.28 (40.48)	3.80 (3.66)	23.49 (23.36)
$[Cu(L^1H)_2]$	> 400	47	Brown	1.75	39.88 (39.03)	3.76 (3.87)	23.25 (23.68)
$[Co(L^1H)_2(H_2O)_2]$	> 400	60	Brown	4.40	37.44 (37.20)	4.32 (3.94)	21.83(22.32)
L^2H_2	146.5	64	Yellow	-	48.86 (48.65)	5.01 (4.46)	31.66 (31.24)
$[Ni(L^2H)_2]$	> 400	60	Red	Dia.	43.32 (43.08)	4.04 (4.34)	28.06 (27.44)
$[Cu(L^2H)_2]$	> 400	55	Brown	1.75	42.90 (43.58)	4.00 (4.51)	27.79 (27.68)
$[Co(L^2H)_2(H_2O)_2]$	> 400	57	Brown	4.10	40.38 (40.54)	4.52 (4.51)	26.16 (26.86)
L^3H_2	136	68	Yellow	-	48.86 (48.69)	5.01 (5.34)	31.66 (31.37)
$[Ni(L^3H)_2]$	> 400	60	Red	Dia.	43.32 (43.62)	4.04 (4.34)	28.06 (27.38)
$[Cu(L^3H)_2]$	> 400	57	Brown	1.73	42.90 (42.64)	4.00 (3.58)	27.79 (27.35)
$[Co(L^3H)_2(H_2O)_2]$	> 400	50	Brown	4.21	40.38 (40.85)	4.52 (5.05)	26.16 (25.94)

[a] magnetic moment (Dia. = diamagnetic)

Table 2. Characteristic IR bands (cm^{-1}) of the vic-dioxime ligands and their metal complexes

Compound	(N-H)	(O-H) (OH/H_2O)	$(C=N)_{oxime}$	$(C=N)_{hydr.}$	$(C-H)_{arom.}$	$(C-H)_{aliph.}$	(N-O)	(OH...O)
L^1H_2	3256 (b)	3124 (b)	1608 (s)	1644 (s)	3068 (w)	2935-2820 (w)	953 (m)	-
$[Ni(L^1H)_2]$	3437 (b)	-	1578 (s)	1633 (s)	3105 (w)	2920- 2898 (w)	947 (m)	1882 (w)
$[Cu(L^1H)_2]$	3433 (b)	-	1567 (s)	1622 (s)	3120 (w)	2912-2850 (w)	956 (m)	1735 (w)
$[Co(L^1H)_2(H_2O)_2]$	3398 (b)	3121 (b)	1573 (s)	1623 (s)	3109 (w)	2916-2835 (w)	949 (m)	1762 (w)
L^2H_2	3364 (b)	3180 (b)	1605 (s)	1656 (s)	3066 (w)	2942-2808 (w)	962 (m)	-
$[Ni(L^2H)_2]$	3444 (b)	-	1578 (s)	1652 (s)	3109 (w)	2912-2850 (w)	942 (m)	1782 (w)
$[Cu(L^2H)_2]$	3393 (b)	-	1574 (s)	1648 (s)	3066 (w)	2908-2850 (w)	942 (m)	1782 (w)
$[Co(L^2H)_2(H_2O)_2]$	3380 (b)	3194 (b)	1562 (s)	1636 (s)	3072 (w)	2919-2851 (w)	941 (m)	1749 (w)
L^3H_2	3340 (b)	3161 (b)	1605 (s)	1640 (s)	3073 (w)	2957-2844(w)	960 (m)	-
$[Ni(L^3H)_2]$	3437 (b)	-	1588 (s)	1629 (s)	3047 (w)	2920-2843 (w)	950 (m)	1855 (w)
$[Cu(L^3H)_2]$	3422 (b)	-	1562 (s)	1634 (s)	3086 (w)	2924-2850 (w)	949 (m)	1782 (w)
$[Co(L^3H)_2(H_2O)_2]$	3397 (b)	3225 (b)	1589 (s)	1616 (s)	3050 (w)	2912-2858 (w)	946 (m)	1776 (w)

Note: s = strong, m = medium, w = weak, b = broad

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Table 3. Important ^{1}H-NMR and ^{13}C-NMR signals (ppm) of ligands in DMSO-d_6

	^{1}H-NMR					
	-OH[a]	-NH[a]	Ar-H	CH=NOH	CH=NNH	-CH_3
L^1H_2	11.50-10.65, s, 2H	10.18, s, 1H	7.00, 6.20, d, 2H	6.70, s, 1H	7.80, s, 1H	2.20, s, 3H
L^2H_2	11.50-10.70, s, 2H	8.82, s, 1H	8.50, 7.94, d, 2H 8.70 s, 1H, 7.53, t, 1H	7.82, s, 1H	-	2.13, s, 3H
L^3H_2	11.60-10.87, s, 2H	8.92, s, 1H	8.54, 7.99, d, 4H	7.61, s, 1H	-	2.19, s, 3H

	^{13}C-NMR				
	HNC=NOH	HC=NOH	Me(H)C=NNH	Ar-C	-CH_3
L^1H_2	154.64	150.34	158.80	148.48-126.33	14.14
L^2H_2	149.83	147.58	154.06	146.47-124.08	12.69
L^3H_2	150.50	146.17	151.45	143.34-121.96	12.20

[a] Disappears on D_2O exchange.

Table 4. Antimicrobial activities of ligands and their metal complexes (Inhibition zone in mm)

Test Microorganism	1	2	3	4	5	6	7	8	9	10	11	12	GN10	C30	NS100
Escherichia coli ATCC 25922	-	-	-	-	-	-	-	-	-	-	-	-	21	24	NT
Salmonella typhimurium ATCC 14028	-	-	-	-	-	-	-	-	-	-	-	-	16	17	NT
*Proteus sp.**	-	-	-	-	-	-	-	-	-	-	-	-	24	17	NT
*Serratia marcescens**	-	-	-	-	-	-	-	-	-	-	-	-	19	23	NT
*Micrococcus luteus,*ATCC 9341	-	-	-	-	-	-	-	-	-	-	-	-	15	25	NT
*Enterobacter sp.**	-	-	-	-	-	-	-	-	-	-	-	-	20	19	NT
Staphylococcus aureus ATCC 25923	-	-	-	-	-	-	-	-	-	-	-	12	20	23	NT
Staphylococcus epidermidis ATCC 12228	-	-	-	-	-	-	-	-	-	-	-	11	17	22	NT
Bacilllus cereus ATCC 11778	10	-	-	-	-	11	12	-	-	11	11	18	24	23	NT
*Bacillus thuringiensis**	-	-	-	13	-	-	-	-	13	-	-	12	21	26	NT

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Table 4. (Continued)

Test Microorganism	1	2	3	4	5	6	7	8	9	10	11	12	GN10	C30	NS100
Enterococcus faecalis 29212	-	-	-	-	-	-	-	-	-	-	-	-	11	16	NT
Streptococcus pneumoniae ATCC 49617	9	-	-	-	-	-	-	-	-	-	-	13	20	24	NT
*Listeria sp**	-	-	-	-	-	-	-	-	-	-	-	-	11	16	NT
*Candida utilis**	-	-	-	10	-	-	-	-	-	-	-	17	NT	NT	21
*Candida albicans**	-	-	-	-	-	-	-	-	-	-	-	-	NT	NT	21
*Candida glabata**	-	-	-	-	-	-	-	-	-	-	-	-	NT	NT	15
*Candida tropicalis**	-	-	-	-	13	-	-	-	14	12	-	-	NT	NT	15
Saccharomyces cerevisiae ATCC 9763	11	-	-	-	-	11	10	-	10	-	-	-	NT	NT	15

Note: 1= L^1H_2, 2= $[Ni(L^1H)_2]$, 3= $[Cu(L^1H)_2]$, 4= $[Co(L^1H)_2(H_2O)_2]$, 5= L^2H_2, 6= $[Ni(L^2H)_2]$, 7= $[Cu(L^2H)_2]$, 8= $[Co(L^2H)_2(H_2O)_2]$, 9= L^3H_2, 10= $[Ni(L^3H)_2]$, 11= $[Cu(L^3H)_2]$, 12= $[Co(L^3H)_2(H_2O)_2]$
C30= chloramphenicol, GN10= gentamycin, NS100= nystatin
(-)= No zone, NT= Not tested
* from Faculty of Medicine, Adnan Menderes University

Table 5. Antimicrobial activities of ligands and their metal complexes (MIC, $\mu g.mL^{-1}$)

Test Microorganism	1	4	5	6	7	9	10	11	12	Str	NS100
Bacilllus cereus ATCC 11778	8	–	–	8	16	–	128	8	–	64	–
*Bacillus thuringiensis**	–	16	–	–	–	64	–	–	32	64	–
Streptococcus pneumoniae ATCC 49617	16	–	–	–	–	–	–	–	128	128	–
*Candida utilis**	–	4	–	–	–	–	–	–	4	–	64
*Candida tropicalis**	–	–	8	–	–	16	8	–	–	–	64
Saccharomyces cerevisiae ATCC 9763	16	–	–	16	–	32	–	–	–	–	128

Note: 1= L^1H_2, 2= $[Ni(L^1H)_2]$, 3= $[Cu(L^1H)_2]$, 4= $[Co(L^1H)_2(H_2O)_2]$, 5= L^2H_2, 6= $[Ni(L^2H)_2]$, 7= $[Cu(L^2H)_2]$, 8= $[Co(L^2H)_2(H_2O)_2]$, 9= L^3H_2, 10= $[Ni(L^3H)_2]$, 11= $[Cu(L^3H)_2]$, 12= $[Co(L^3H)_2(H_2O)_2]$
Compounds 2,3,8 did not show antibacterial activity.
Str= streptomycin, NS100= nystatin
(-)= no effect
* from Faculty of Medicine, Adnan Menderes University

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Magnetic Susceptibility

The magnetic susceptibility measurements of the Ni(II) complexes indicate that these complexes are diamagnetic while the Co(II) and Cu(II) complexes are paramagnetic. The copper complexes showed 1.75 BM for L^1H_2, 1.75 BM for L^2H_2 and 1.73 BM for L^3H_2. These results indicate square-planar structures for the Cu(II) complexes [20, 25, 33-36]. The cobalt complexes showed 4.40 BM for L^1H_2, 4.10 BM for L^2H_2 and 4.21 BM for L^3H_2. The microanalysis shows that the complexes of Co(II) can be octahedral [20, 25, 33]. Therefore, square-planar geometry for the Ni(II) and Cu(II) complexes and octahedral geometry for the Co(II) complexes are proposed (Figures 1 and 2).

UV Spectra

The bands in the electronic spectra of the Ni(II), Cu(II) and Co(II) complexes were assigned to both a charge transfer transition from the metal to the anti-bonding orbital of the ligand and to a spin-allowed transition of the ligand. The general character of the spectra was very similar to that of the corresponding complexes of symmetrically disubstituted dioximate ligands. The d^8 metal ion, Ni(II), exhibited a preference for square planar geometry with the dioxime complexes. The decrease in the intensities of the transitions indicates coordination with the nitrogen atoms [37, 38].

Antimicrobial Assays

The three novel aromatic hydrazone derivatives containing vic-dioxime groups and their Ni(II), Cu(II) and Co(II) complexes exhibited moderate antimicrobial activity (Tables 4 and 5). Among the test compounds attempted, compounds 1, 4, 6, 7, 9, 10 and 12 showed slightly higher activity against some bacteria and yeasts (Table 4). The MIC values (Table 5) also indicate that some of the compounds tested exhibited moderate antimicrobial activity. Compounds 1, 4, 6, 7, 9, 10 and 12 showed stronger activity against some bacteria (*B. thuringiensis*, *B. cereus* ATCC 11778 and *Streptococcus pneumoniae* ATCC 49617) compared with streptomycin. These compounds also had strong activity against the yeasts (*Saccharomyces cerevisiae* ATCC 9763, *Candida utilis* and *Candida tropicalis*) compared with nystatin.

All the ligands and their metal complexes studied had no effect on the Gram-negative bacteria. In general, the ligands and their metal complexes have antimicrobial activities against Gram-positive bacteria, especially *B. cereus* ATCC 11778, *B. thuringiensis*, *S. pneumoniae* ATCC 49617 and yeasts, *S. cerevisiae* ATCC 9763 and *C. tropicalis C. utilis*.

Members of the genus *Bacillus* are aerobic spore-forming rods which are ubiquitous in nature [39]. Despite their widespread distribution, even as normal skin flora, *Bacillus* spp. rarely cause infections. An exception is *Bacillus cereus*, which is a well-known cause of food poisoning and dreaded post-traumatic endophthalmitis [39]. *B. cereus* can also cause opportunistic infections, mainly in the immuno-compromised host [39, 40]. Although *B. anthracis* and *B. cereus* behave as human pathogens and *B. thuringiensis* is a common insect pathogen, genetic evidence indicates that these microorganisms should be regarded as unique species [41]. *B. thuringiensis* has been used worldwide as a biopesticide in forestry and agriculture [41, 42], being non-pathogenic to humans and able to produce potent species-specific insecticidal activities. More recently, however, repeated observations are documenting the association of this microorganism with various infectious diseases in humans such as food-poisoning associated diarrhoea [43], corneal ulcer [44], periodontitis [39], and burn [45] and wound [46] infections.

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The term candidiasis is often used to describe an infection caused by a yeast-like fungus *Candida albicans*. Species of *Candida* other than *C. albicans*, however, have the potential to cause infection, particularly in patients who are immunologically or physiologically compromised [44, 45]. *Candida tropicalis* has emerged as a potentially dangerous opportunistic fungus. This may be due both to an increased awareness and specific identification of *C. tropicalis* as an etiologic agent of infection and to an increase in the number of compromised patients susceptible to opportunistic fungi. *C. tropicalis* has been shown to be the most frequent opportunistic fungus isolated from specimens from patients in a critical care unit [45, 46]. *C. tropicalis* has also been reported to be a frequent opportunistic pathogen in a cancer hospital [47] and has been identified as the etiologic agent in a variety of infections including pyelonephritis [48], lower urinary tract infection, thrombophlebitis, arthritis, bursitis, meningitis, multiple organ infection, pericarditis and candida vulvovaginitis [47, 49].

Suggestions are made that the negative inductive effect plays a significant role. Dimerisation of oxime involves the formation of a pair of H bonds [19, 50]. This feature causes a decrease in electronic density of oximes compared with phenylhydrazones, thereby facilitating entry of the oxime into the cell. This is likely to increase the antibacterial potency [19, 50].

A comparative study of the ligands and their complexes as antibacterial agents indicates that the metal complexes are more active than the free ligands [19, 50]. Such increased activity of the metal chelates can be explained by the reduced polarity of the ligand due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with electron releasing groups. It is obvious that reducing the total electron density on free ligands makes the diffusion proceed faster through the bacterial cells [51].

It is generally observed that metal chelates have higher antibacterial activity than the free ligand due to an increase in cell permeability. The lipid membrane which surrounds the cell favours only the passage of lipid soluble materials and it is known that liposolubility is an important factor controlling antimicrobial activity [52-54]. Such screening of various organic compounds and identifying the active agents are essential because the successful prediction of a lead molecule and the drug-like properties at the onset of drug design will pay off later in drug development.

CONCLUSIONS

Three novel vic-dioxime derivatives containing hydrazone side groups and their transition metal complexes with Ni(II), Cu(II) and Co(II) were synthesised. The antimicrobial activities of compounds (L^1H_2, L^2H_2, L^3H_2 and their Ni(II), Cu(II) and Co(II) complexes) were evaluated using disc diffusion method against 13 bacteria and 5 yeasts. Minimal inhibitory concentration (MIC) dilution against 3 bacteria and 3 yeasts were also determined. Among the test compounds attempted, L^1H_2, $[Co(L^1H)_2(H_2O)_2]$, $[Ni(L^2H)_2]$, $[Cu(L^2H)_2]$, L^3H_2, $Ni(L^3H)_2$ and $[Co(L^3H)_2(H_2O)_2]$ showed activities against certain Gram-positive bacteria and certain yeasts. Some of them were comparatively higher or equipotent to the antibiotic and antifungal agents in the comparison tests. These compounds appeared to have moderate antibacterial and antifungal activity.

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