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Full Length Research Paper

Synthesis, characterization and biological activity of *vic*-dioxime derivatives containing benzaldehydehydrazone groups and their metal complexes

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In this study, three new *vic*-dioxime derivatives containing benzaldehydehydrazone groups (L^1H_2: 4-methoxybenzaldehydehydrazone glyoxime, L^2H_2: 4-methylbenzaldehydehydrazone glyoxime and L^3H_2: 3-methylbenzaldehydehydrazone glyoxime) and their Ni(II), Cu(II) and Co(II) complexes are used. The biological activity of these aromatic hydrazone-oxime derivatives has been determined in both prokaryotic and eukaryotic systems. For evaluating the antimicrobial activity disc diffusion method has been used and for determining the antiproliferative effect on neoplastic cells, HL 60 (Human promyelocytic leukemia cells) cell line was cultured. 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 against 13 bacteria and 5 yeasts. Besides they were evaluated using the minimal inhibitory concentration (MIC) dilution method against 1 bacterium and 5 yeasts. The obtained results from disc diffusion method are assessed in sideby-side comparison with those of Chloramphenicol, Gentamycin, Tetracycline, Erytromycine, Ampicillin well-known antibacterial agents and Nystatine antifungal agent. The results from dilution procedure are compared with Streptomycine as antibacterial and Nystatine as antifungal. The antifungal activities are reported on five yeast cultures namely, *Candida utilis*, *C. albicans*, *C. glabata*, *C. trophicalis* and *Saccharomyces cerevisiae* ATCC 9763 and the results are referenced with Nystatine, a commercial antifungal agent. As a result of this study, among the test compounds attempted 1, 2, 7 and 9 showed slightly higher activities against *B. thrungiensis* and some of yeasts that are comparatively higher or equipotent to the antibiotic and antifungal agents in the comparison tests. Furthermore Co(II) complexes of these derivatives can be described as potent anti-cancer agents due to their antiproliferative effects with an I_pC_{50} between 5 to 40 µM concentrations. The strongest antiproliferative activity was determined with the Co(II) complex of L^3H_2 at 5 µM.

Key words: Vic-dioximes, hydrazone, transition metal complexes, antimicrobial activity, antiproliferative, leukemia.

INTRODUCTION

Hydrazones are a versatile class of compounds with innumerous chemical as well as pharmacological applications. In fact, hydrazones have shown to possess antimicrobial, anticonvulsant, analgesic, anti- inflammatory, and antitumoral properties (Recio Despaigne et al., 2009). Arylhydrazone complexes of transition metal ions are known to provide useful models for ellucidation of the mechanisms of enzyme inhibition by hydrazine derivatives and for their possible pharmacological applications (Ray et al., 2008). Vic-dioximes are important complexing ligands that have received considerable attention in biology and chemistry. The

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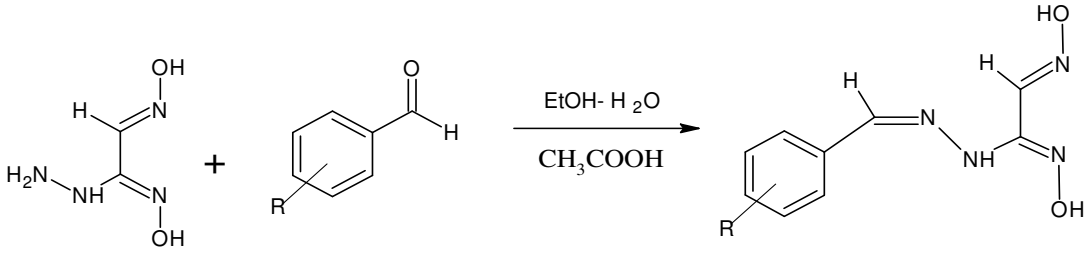

Scheme 1. Synthesis of ligands, R: 4-methoxy for L^1H_2, 4-methyl for L^2H_2 and 3-methyl for L^3H_2.

ability of oxime ligands to stabilize particular metal ion redox states is important in bioinorganic systems. In addition, some Vic-dioximes also show antimicrobial properties (Das (Karfa) et al., 2009).

Many studies of hydrazones, and mono- and dioximes have been carried out; yet, little information related to the derivatives of Vic-dioximes with hydrazone side groups was found in the literature. Herein, the new ligands, 4-methoxybenzaldehydehydrazone glyoxime (L^1H_2), 4-methylbenzaldehydehydrazone glyoxime (L^2H_2) and 3-methylbenzaldehydehydrazone glyoxime (L^3H_2), and their complexes with Ni(II), Cu(II), and Co(II) ions are described and evaluated as potential antimicrobial and anticancer agents.

EXPERIMENTAL

Materials and instrumentation

All reagents used were purchased from Merck and Fluka. Elemental analyses, ^{1}H n.m.r-^{13}C N.M.R spectra (Bruker 400 MHz), I.R spectra (Varian 900), melting points (Buchi SPM-20) and pH measurements (Orion Expandable Ion Analyzer EA 940) were used to elucidate the structures of the products. The magnetic moments of the complexes were measured by the Gouy method with a Newport type D-104 instrument magnet power supply.

Synthesis of ligands

L^1H_2, L^2H_2 and L^3H_2 were synthesized from the starting materials, namely anti-glyoximehydrazine (GH_2) (Babahan et al., 2006) (Scheme 1), 4-methoxy benzaldehyde (for L^1H_2), 4-methyl benzaldehyde (for L^2H_2), and 3-methyl benzaldehyde (for L^3H_2), using glacial acetic acid as a catalyst. A cooled (5°C) solution of ketone or aldehyde (1 mmol) in ethanol was added dropwise into a cooled solution (5°C) containing (1 mmol, 0.118 g) anti-glyoximehydrazine (GH_2) and 3-5 drops CH_3COOH with constant stirring. After the addition of aldehyde was completed, the solution was stirred for an additonal 2 to 4 h at room temperature. The resulting solid compounds were filtered off, washed with water and ethanol dried at room temperature in a vacuum oven. The chemical reaction and molecular formula are shown in Scheme 1. Results of the compositional and spectroscopic analyses are as follows.

L^1H_2: Yield; (70%), M.P.; 107°C, color; yellow, IR (KBr, cm^{-1}): 3367 (N-H), 3348 (O-H), 3059 (C-$H_{arom.}$), 2932 to 2838 (C-$H_{aliph.}$), 1605 (C=N_{oxime}), 1669 (C=$N_{hydr.}$), 983 (N-O). ^{1}H-NMR (DMSO, p.p.m.): 10.13 s, 1H (NH), 11.34 to 10.20 s, 2H (OH), 7.86 s, 1H (CH=NOH), 7.36-6.85 d, 4H, (Ar-C), 8.54 s, 1H (-CH=N-NH), 2.82 s, 3H (-CH_3). ^{13}C-NMR (DMSO, P.P.M.): 168.72 (-CH=N-NH-), 141.17 (N-NH-C=N-OH), 138.80 (C-CH=N-OH), 137.03, 135.20, 134.15, 131.79 (Ar-C), 45.37 (-CH_3). U.V.-vis. Spectrum (in DMSO) λ_{max}/nm: 224 and 342. For $C_{10}H_{12}O_3N_4$ (236.227 g.mol^{-1}) calculated: 50.84% C, 5.12% H, 23.72% N; found: 50.65% C, 5.46% H, 24.24% N.

L^2H_2: Yield; (60%), M.P.; 109°C, color; yellow, IR (KBr, cm^{-1}): 3325 (N-H), 3140 (O-H), 3055 (C-$H_{arom.}$), 2922 to 2859 (C-$H_{aliph.}$), 1608 (C=N_{oxime}), 1662 (C=$N_{hydr.}$), 971 (N-O). ^{1}H-NMR (DMSO, P.P.M.): 8.57 s, 1H (NH), 11.74 to10.25 s, 2H (OH), 7.87 s, 1H (CH=NOH), 7.54 to 7.09 s, 4H (Ar-C), 8.06 s, 1H (-CH=N-NH), 2.41 s, 3H (-CH_3). ^{13}C-NMR (DMSO, P.P.M.): 161.82 (-CH=N-NH-), 141.99 (N-NH-C=N-OH), 141.16(C-CH=N-OH), 131.90, 130.18, 128.99, 126.70 (Ar-C), 21.83 (-CH_3). U.V.-vis. Spectrum (in DMSO) λ_{max}/nm: 267 and 320. For $C_{10}H_{12}O_2N_4$ (220.228 g.mol^{-1}) calculated: 54.54% C, 5.49% H, 25.44% N; found: 54.68% C, 5.52% H, 25.98% N.

L^3H_2: Yield; (60%), M.P.; 137°C, color; yellow, IR (KBr, cm^{-1}): 3313 (N-H), 3161 (O-H), 3080 (C-$H_{aromatic}$), 2985-2850 ($CH_{aliphatic}$), 1609 (C=N_{oxime}), 1662 (C=$N_{hydrazone}$), 980 (N-O). ^{1}H-NMR (DMSO, P.P.M.): 8.34 s, 1H (NH), 11.90 to 10.02 s, 2H (OH), 6.91 s, 1H (CH=NOH), 7.85 to 7.18 d, 2H: 7.36 t, 1H: 8.05 s, 1H (Ar-C), 8.10 s, 1H (-CH=N-NH), 2.50 s, 3H (-CH_3). ^{13}C-NMR (DMSO, P.P.M.): 162.13 (-CMe=N-NH-), 156.10 (N-NHC=N-OH), 138.85 (C-CH=N-OH), 134.46, 132.71, 129.47, 126.40, 124.58, 121.01 (Ar-C), 21.75 (-CH_3). U.V.-vis. Spectrum (in DMSO) λ_{max}/nm: 260 and 330. For $C_{10}H_{12}O_2N_4$ (220.228 g.mol^{-1}) calculated: 54.54% C, 5.49% H, 25.44% N; found: 54.42% C, 5.52% H, 25.12% N.

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 $NiCl_2.6H_2O$ or 1 mmol, 0.170 g of $CuCl_2.2H_2O$ or 1 mmol 0.237 g $CoCl_2.6H_2O$ in 20 mLL of water) was added to 2 mmol of the ligand solution (0.472 g for L^1H_2, 0.442 g for L^2H_2 and 0.442 g for L^3H_2 in 15 mLL of ethanol) with stirring. An initial sharp decrease in the pH of the solution from 5.5 to about 3 to 3.5 was observed. After raising the pH to 5 to 5.5 using a 1% aqueous NaOH solution, the reaction mixture was kept in a hot water bath (60°C) for 2 h 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 compositional and spectroscopic analyses are shown as follows. Proposed structures of complexes are shown in Figures 1a to 1b.

[Ni(L^1H)$_2$]; yield; (60%), M.P.; >400°C, color; red, IR (KBr, cm^{-1}): 3434 (N-H), 3093 (C-$H_{aromatic}$), 2929 to 2838 (C-$H_{aliphatic}$), 1574 (C=N_{oxime}), 1622 (C=$N_{hydrazone}$), 1789 (H...OH), 967 (N-O). U.V.-vis. Spectrum (in DMSO) λ_{max}/nm: 276, 340 and 484. For $C_{20}H_{22}O_6N_8Ni$ (529.132 g.mol^{-1}), calculated: 45.40% C, 4.19% H, 21.18% N; found: 45.48% C, 4.66% H, 21.36% N.

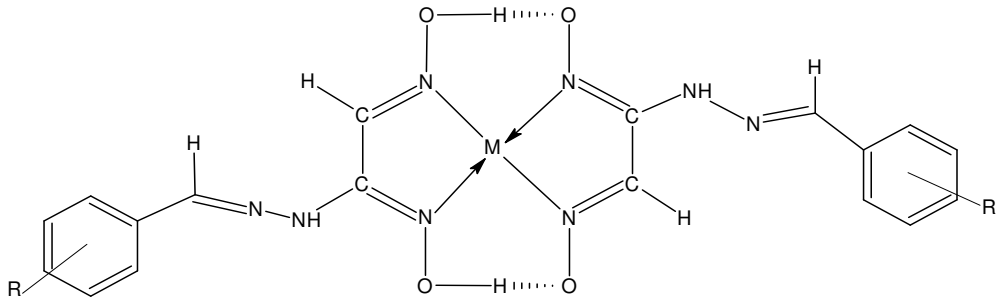

Figure 1. A trans- Suggested structure of the Co(II)·2H_2O, Ni(II), and Cu(II) complexes for L^1H_2, L^2H_2 and L^3H_2. R: 4-methoxy for L^1H_2, 4-methyl for L^2H_2 and 3-methyl for L^3H_2).

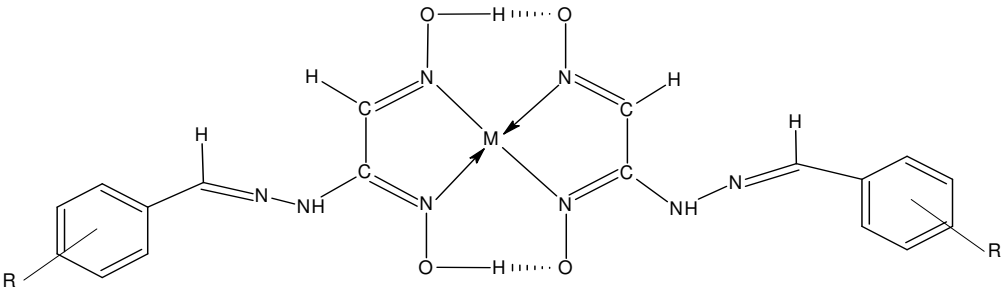

Figure 1b. cis- Suggested structure of the Co(II)·2H_2O, Ni(II), and Cu(II) complexes for L^1H_2, L^2H_2 and L^3H_2. R: 4-methoxy for L^1H_2, 4-methyl for L^2H_2 and 3-methyl for L^3H_2).

[Cu(L^1H)$_2$]; yield; (60%), M.P.; >400°C, Color; Brown, IR (KBr, cm^{-1}): 3345 (N-H), 3070 (C-$H_{aromatic}$), 2908 to 2850 (C-$H_{aliphatic}$), 1570 (C=N_{oxime}), 1620 (C=$N_{hydrazone}$), 1782 (H...OH), 940 (N-O). U.V.-vis. Spectrum (in DMSO) λ_{max}/nm: 283, 331 and 700. For $C_{20}H_{22}O_6N_8Cu$ (533.985 $g.mol^{-1}$), calculated: 44.99% C, 4.15% H, 20.98% N; found: 45.03% C, 3.87% H, 20.62% N.

[Co(L^1H)$_2$(H_2O)$_2$]; yield; (60%), M.P.; >400°C, color; brown, IR (KBr, cm^{-1}): 3376 (N-H), 3203 (OH/H_2O), 3109 (C-$H_{aromatic}$), 2933 to 2836 (C-$H_{aliphatic}$), 1574 (C=N_{oxime}), 1623 (C=$N_{hydrazone}$), 1735 (H...OH), 967 (N-O). U.V.-vis. Spectrum (in DMSO) λ_{max}/nm: 279, 389 and 705. For $C_{20}H_{26}O_8N_8Co$ (565.403 $g.mol^{-1}$), calculated: 42.49% C, 4.64% H, 19.82% N; found: 42.67% C, 4.91% H, 18.76% N.

[Ni(L^2H)$_2$]; yield; (60%), M.P.; >400°C, Color; Red, IR (KBr, cm^{-1}): 3482 (N-H), 3020 (C-$H_{aromatic}$), 2916 to 2858 (C-$H_{aliphatic}$), 1570 (C=N_{oxime}), 1620 (C=$N_{hydrazone}$), 1732 (H...OH), 963 (N-O). U.V.-vis. Spectrum (in DMSO) λ_{max}/nm: 274, 333 and 482. For $C_{20}H_{22}O_4N_8Ni$ (497.134 $g.mol^{-1}$), calculated: 48.32% C, 4.46% H, 22.54% N; found: 48.08% C, 4.34 % H, 22.44% N.

[Cu(L^2H)$_2$]; yield; (60%), M.P.; >400°C, color; brown, IR (KBr, cm^{-1}): 3432 (N-H), 3023 (C-$H_{aromatic}$), 2917 to 2854 (C-$H_{aliphatic}$), 1571 (C=N_{oxime}), 1623 (C=$N_{hydrazone}$), 1735 (H...OH), 967 (N-O). U.V.-vis. Spectrum (in DMSO) λ_{max}/nm: 260, 320 and 705. For $C_{20}H_{22}O_4N_8Cu$ (501.986 $g.mol^{-1}$), calculated: 47.85% C, 4.42% H, 22.32% N; found: 48.34% C, 5.01% H, 22.53% N.

[Co(L^2H)$_2$(H_2O)$_2$]; yield; (60%), M.P.; >400°C, color; brown, IR (KBr, cm^{-1}): 3422 (N-H), 3236 (OH/H_2O), 3024 (C-$H_{aromatic}$), 2919 to 2866 (C-$H_{aliphatic}$), 1570 (C=N_{oxime}), 1616 (C=$N_{hydrazone}$), 1789 (H...OH), 967 (N-O). U.V.-vis. Spectrum (in DMSO) λ_{max}/nm: 260, 318 and 704. For $C_{20}H_{26}O_6N_8Co$ (533.404$g.mol^{-1}$), calculated: 45.03% C, 4.91% H, 21.01% N; found: 44.79% C, 4.72% H, 21.39% N.

[Ni(L^3H)$_2$]; yield; (60%), M.P.; >400°C, color; red, IR (KBr, cm^{-1}): 3448 (N-H), 3041 (C-$H_{aromatic}$), 2914-2846 (C-$H_{aliphatic}$), 1581 (C=N_{oxime}), 1624 (C=$N_{hydrazone}$), 1746 (H...OH), 959 (N-O). U.V.-vis. Spectrum (in DMSO) λ_{max}/nm: 271, 335 and 477. For $C_{18}H_{20}O_4N_{10}Ni$ (499.110 $g.mol^{-1}$), calculated: 48.32% C, 4.46% H, 22.54% N; found: 48.62% C, 4.10% H, 22.38% N.

[Cu(L^3H)$_2$]; yield; (60%), M.P.; >400°C, color; brown, IR (KBr, cm^{-1}): 3386 (N-H), 3047 (C-$H_{aromatic}$), 2924 to 2862 (C-$H_{aliphatic}$), 1562 (C=N_{oxime}), 1624 (C=$N_{hydrazone}$), 1824 (H...OH), 972 (N-O). U.V.-vis. Spectrum (in DMSO) λ_{max}/nm: 260, 320 and 750. For $C_{20}H_{22}O_4N_8Cu$ (501.986 $g.mol^{-1}$), calculated: 47.85% C, 4.42% H, 22.32% N; found: 47.58% C, 4.51% H, 22.68% N.

[Co(L^3H)$_2$(H_2O)$_2$]; yield; (60%), M.P.; >400°C, color; brown, IR (KBr, cm^{-1}): 3369 (N-H), 3172 (OH/H_2O), 3059 (C-$H_{aromatic}$), 2920 to 2850 (C-$H_{aliphatic}$), 1577 (C=N_{oxime}), 1644 (C=$N_{hydrazone}$), 1755 (H...OH), 970 (N-O). U.V vis. Spectrum (in DMSO) λ_{max}/nm: 259, 342 and 750. For $C_{20}H_{26}O_6N_8Co$ (533.404 $g.mol^{-1}$), calculated: 45.03% C, 4.91% H, 21.01% N; found: 45.15% C, 4.34% H, 20.05% N.

Pharmacology

Micro-organisms

Nine microorganism strains were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). Other

microorganism strains were obtained from Adnan Menderes University Faculty of Medicine. They were gram negative (G-): *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Proteus sp., Serratia marcescens*, *Enterobacter sp*. and gram positive (G+): *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Bacilllus cereus* ATCC 11778, *Bacillus thrungiensis, Enterococcus faecalis* ATCC 29212*, Streptococcus pneumoniae* ATCC 49617, *Listeria sp.* The following five yeast strains were also tested, *Candida utilis*, *Candida albicans*, *Candida glabata*, *Candida trophicalis*, *Saccharomyces cerevisiae* ATCC 9763 using both disc diffusion method (NCCLS, 1993; Collins et al., 1989) and measuring the MIC determined by the broth dilution method (Jones et al., 1984).

Methods

Disc diffusion method

Screening for antibacterial and antifungal activities are carried out using sterilized antibiotic discs (6 mm), following the procedure performance standards for Antimicrobial Disc Susceptibility Tests, outlined by the National Committee for Clinical Laboratory Standards-NCCLS (NCCLS, 1993; Collins et al., 1989). Fresh stock solutions of the ligands and complexes are prepared in DMSO according to the needed concentrations (0.1 M) for experiments.

The inoculum suspensions of each group of bacteria and yeast were prepared from 18 to 24 h broth cultures and adjusted to obtain a turbidity equivalent to 0.5 McFarland standard tube to give a concentration of $1x10^8$ bacteria and $1x10^6$ yeast per milliliter. In order to test the antimicrobial activity of aromatic hydrazone derivatives bearing Vic-dioxime groups and their Ni(II), Cu(II) and Co(II) complexes, 15 mLL of Mueller Hinton Agar were poured in petri dishes which were then inoculated with strains of bacteria and yeast by taking 0.1 mLL from cell culture media. It was kept to solidify at room temperature for a while and then holes were made on top with a sterile stick. These holes were filled with 10 µl of pyridyl hydrazone derivatives containing Vic-dioxime groups and their Ni(II), Cu(II) and Co(II) complexes.

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*, *Enterobacter sp*. were incubated at 37°C for 24 h and with *M. luteus* ATCC 9341, *B. cereus* ATCC 11778, *B. thrungiensis, S. cerevisiae* ATCC 9763, *C. albicans* ATCC 90028, *C. glabata*, *C. utilis* and *C. trophicalis* were incubated at 30°C for 24 h. At the end of incubation time, the diameters of the inhibition zones formed on the MHA were evaluated in millimetres. Discs of Chloramphenicol (C30), Gentamycine (CN10), Tetracycline (TE30), Erytromycine (E15), Ampicillin (AMP10) and Nystatine (NS100) were used as positive controls. The developing inhibition zones were compared with those of the reference discs.

Dilution method

Screening for antibacterial and antifungal activities was carried out by preparing a broth micro-dilution, following the procedure outlined in Manual of Clinical Microbial (Jones et al., 1984). All the bacteria were incubated and activated at 37 to 30°C for 24 h inoculation into nutrient broth, and the yeasts were incubated in Malt Extract Broth for 48 h. The compounds were dissolved in DMSO (2 mg $mLLL^{-1}$) and then diluted using caution adjusted Mueller Hinton Broth. Two-fold serial concentrations of the compounds were employed to determine the (MIC) ranging from 256 to 1.0 µg $mLLL^{-1}$. Cultures were grown at 37 to 30°C (18 to 20 h) and the final inoculation (inoculums) was approximately 10^6 cfu $mLLL^{-1}$. Test cultures were incubated at 37°C (24 h). The lowest concentrations of antimicrobial agents that result in complete inhibition of the micro-organisms were represented as MIC (µg $mLLL^{-1}$). In each case triplicate tests were performed and the results are expressed as means.

Biological data

Standardised samples of Chloramphenicol is effective against a wide variety of gram-positive and gram-negative bacteria, including most anaerobic organisms (exerting their antimicrobial effect the inhibition of protein synthesis), Gentamycine is an aminoglycoside antibiotic, used to treat many types of bacterial infections, particularly those caused by gram-negative bacteria, Ampicillin (penetrating and preventing the growth of gram-negative bacteria), Tetracyline (exerting their antimicrobial effect the inhibition of protein synthesis), Erytromycine (exerting their antimicrobial effect the inhibition of protein synthesis) and Nystatin (binding to sterols in the fungal cellular membrane, altering the permeability and allowing leakage of the cellular contents). Mueller Hinton Media, Nutrient Broth and Malt Extract Broth are purchased from Merck and yeast extracts is obtained from Oxoid.

Cell culture

HL-60 promyeloic leukaemia cells were purchased from ATCC. Cells were grown in RPMI-1640 medium supplemented with 10% heat inactivated fetal calf serum, 1% L-glutamine and 1% penicillin/ streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. All media and supplements were obtained from Life Technologies.

Proliferation inhibition analysis

HL 60 cells were seeded in T-25 tissue culture flasks at a concentration of $1x10^5$/mLL and incubated with increasing concentrations of agents (corresponding to 5, 10, 20 and 40 µM of the drug). Cell counts and IC_{50} values were determined at 24 and 72 h using a Thoma slide. Experiments were done in triplicate. The percent of cell divisions compared to the untreated control were calculated as follows:

[(C72 h + drug - C24 h + drug) / (C72 h - drug - C24 h - drug)] x 100 = % cell division,

where C72 h + drug is the cell number after 72 h of drug treatment, C24 h + drug, is the cell number after 24 h of drug treatment, C72 h - drug is the cell number after 72 h without drug treatment, and C24 h-drug, is the cell number after 24 h without drug treatment.

RESULTS AND DISCUSSION

In this study, three new *vic*-dioxime compounds containing hydrazone side groups and their transition metal complexes Ni(II), Cu(II) and Co(II) were synthesized and evaluated as potential antimicrobial agents. The new ligands were synthesized by reacting anti-glyoximehydrazine (GH_2) (Babahan et al., 2006) with 4-methoxybenzaldehyde for L^1H_2, 4-methylbenzaldehyde for L^2H_2 and 3-methylbenzaldehyde for L^3H_2. The Ni(II), Cu(II) and Co(II) complexes of ligands were prepared in ethanol by using $MCl_2.xH_2O$ as metal salts. The

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

Compounds Formula	M.p.(d)[b]	Color	μ_{eff}	Calculated (Found) % of		
	(ºC)		(BM)[a]	C	H	N
L^1H_2	107	Yellow	-	50.84 (50.65)	5.12 (5.46)	23.72 (24.24)
$[Ni(L^1H)_2]$	> 400	Red	Dia.	45.40 (45.48)	4.19 (4.66)	21.18 (21.36)
$[Cu(L^1H)_2]$	> 400	Brown	1.72	44.99 (45.03)	4.15 (3.87)	20.98 (20.68)
$[Co(L^1H)_2(H_2O)_2]$	> 400	Brown	4.30	42.49 (42.20)	4.64 (4.94)	19.82 (19.32)
L^2H_2	109	Yellow	-	54.54 (54.68)	5.49 (5.52)	25.44 (25.98)
$[Ni(L^2H)_2]$	> 400	Red	Dia.	48.32 (48.08)	4.46 (4.34)	22.54 (22.44)
$[Cu(L^2H)_2]$	> 400	Brown	1.72	47.85 (48.34)	4.42 (5.01)	22.32 (22.53)
$[Co(L^2H)_2(H_2O)_2]$	> 400	Brown	4.10	45.03 (44.79)	4.91 (4.72)	21.01 (21.39)
L^3H_2	137	Yellow	-	54.54 (54.42)	5.49 (5.52)	25.44 (25.12)
$[Ni(L^3H)_2]$	> 400	Red	Dia.	48.32 (48.62)	4.46 (4.10)	22.54 (22.38)
$[Cu(L^3H)_2]$	> 400	Brown	1.70	47.85 (47.58)	4.42 (4.51)	22.32 (22.68)
$[Co(L^3H)_2(H_2O)_2]$	> 400	Brown	4.20	45.03 (45.15)	4.91 (4.34)	21.01 (20.05)

[a]μ_{eff} : magnetic moment, Dia. : diamagnetic, [b]d : decomposition,

antimicrobial activities of ligands and their metal complexes were evaluated using disc diffusion method against 13 bacteria and 5 yeast. The obtained results from disc diffusion method were assessed in side by side comparison with those of Chloramphenicol (C30), Gentamycine (CN10), Tetracycline (TE30), Erytromycine (E15), Ampicillin (AMP10) and Nystatine (NS100), well-known antibacterial and antifungal agents. Total 18 microorganims were used in MIC and disc diffusion methods. But 1 bacterium and 5 yeasts in MIC and disc diffusion methods show activity in our study. The other 12 bacteria show did not activity.

Furthermore, HL 60 (Human promyelocytic leukemia cells) cell line was used for determining the antiproliferative effect on neoplastic cells. Ligands form mononuclear complexes $[(LH)_2M]$ with a metal to ligand ratio of 1:2 with M=Co(II)$(H_2O)_2$, Ni(II), and Cu(II). The Co(II) complexes of the ligands are proposed to be octahedral with water molecules as axial ligands, the Ni(II) and Cu(II) complexes are proposed to be square planar.

New ligands were characterized by a combination of ^{1}H-NMR, ^{13}C-NMR, F-TIR, UV and elemental analytical techniques. Attempts to isolate crystals suitable for X-ray diffraction were unsuccessful for ligands and complexes. FT-IR, UV, elemental analysis and magnetic susceptibility techniques were employed in order to determine the structural characteristics of the 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. Some physical properties, elemental, analytical, and magnetic susceptibility data of the ligands and complexes are given 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. Antimicrobial activities of ligands and their metal complexes are given Tables 4 and 5. Antiproliferative effects of Co(II) complexes are given in Figures 2 to 4.

IR spectra

In the IR spectrum of the new hydrazone-oxime compounds (L^1H_2, L^2H_2 and L^3H_2), an O-H strecthing vibration was observed at 3348 cm^{-1} for L^1H_2, 3140 cm^{-1} for L^2H_2 and 3161 cm^{-1} for L^3H_2 as a broad absorption (Bielsa et al., 1987; Bilgin and Gök, 2001; Brian, 1984; Canpolat and Kaya, 2005; Canpolat and Kaya, 2005; Canpolat et al., 2004; Choi et bal., 2010; Collins et al., 1989; Collins et al., 1995; Cuong et al., 2010; Damgaard et al., 1997; Das (Karfa) et al., 2009; Dolaz et al., 1991). The characteristic bands of hydrazone are 1669 cm^{-1} for L^1H_2, 1662 cm^{-1} for L^2H_2 and 1662 cm^{-1} for L^3H_2 (Bilgin and Gök, 2001; Canpolat and Kaya, 2005). The other characteristic bands of oxime are 1605 cm^{-1} for L^1H_2, 1608 cm^{-1} for L^2H_2 and 1609 cm^{-1} for L^3H_2 (Babahan et al., 2006; Güp, 2006; Macit et al., 2000; Kiliç et al., 2006; Canpolat et al., 2005). N-H and N-O strecthing vibration bands of the ligands were shown at 3367 cm^{-1} and 983 cm^{-1} for L^1H_2, 3325 cm^{-1} and 971 cm^{-1} for L^2H_2 and 3313 cm^{-1} and 980 cm^{-1} for L^3H_2. These values are in accord with the previously reported oxime derivatives (Bielsa et al.,1987; Bilgin and Gök, 2001; Brian, 1984; Canpolat and Kaya, 2005; Canpolat and Kaya, 2005; Canpolat et al., 2004; Choi et al., 2010; Collins et al., 1989; Collins et al., 1995; Cuong et al., 2010; Damgaard et al., 1997; Das (Karfa) et al., 2009; Dolaz et al., 1991; Drobniewski, 1993; Durmuş et al.,2004)CH strecthing vibrations were shown between 2932 and 2838 cm^{-1} for L^1H_2, between 2922 and 2859 cm^{-1} for L^2H_2, and between 2985 and 2850 cm^{-1} for L^3H_2 (Babahan et al., 2006; Güp, 2006; Macit et al., 2000; Kiliç et al., 2006; Canpolat et al., 2005).

In the IR spectrum of Co(II) complexes, the weak deformation vibration band assigned to the intramolecular hydrogen bond O-H....O bending vibration is observed around 1755-1789 cm^{-1} (Güp, 2006; Macit et al., 2000;

Table 2. Characteristic IR bands of the Vic-dioxime ligand and its metal complexes (cm^{-1}, KBr).

Compounds	υ(N-H) (b)	υ(O-H) (OH/H_2O) (b)	$υ(C=N)_{oxime}$ (s)	$υ(C=N)_{hydr.}$ (s)	$υ(C-H)_{arom.}$ (w)	$υ(C-H)_{aliph.}$ (w)	υ(N-O) (m)	υ(OH...O) (w)
L^1H_2	3367	3348	1605	1669	3059	2932-2838	983	-
$[Ni(L^1H)_2]$	3434	-	1574	1622	3093	2929-2838	967	1789
$[Cu(L^1H)_2]$	3345	-	1570	1620	3070	2908-2850	940	1782
$[Co(L^1H)_2(H_2O)_2]$	3376	3203	1574	1623	3109	2933-2836	967	1735
L^2H_2	3325	3140	1608	1662	3055	2922-2859	971	-
$[Ni(L^2H)_2]$	3482	-	1570	1620	3020	2916-2858	963	1732
$[Cu(L^2H)_2]$	3432	-	1571	1623	3023	2917-2854	967	1735
$[Co(L^2H)_2(H_2O)_2]$	3422	3236	1570	1616	3024	2919-2866	967	1789
L^3H_2	3313	3161	1609	1662	3080	2985-2850	980	-
$[Ni(L^3H)_2]$	3448	-	1581	1624	3041	2914-2846	959	1746
$[Cu(L^3H)_2]$	3386	-	1562	1624	3047	2924-2862	972	1824
$[Co(L^3H)_2(H_2O)_2]$	3369	3172	1577	1644	3059	2920-2850	970	1755

s: Strong, m: medium, w: weak , b: broad.

Table 3. ^{1}H-NMR and ^{13}C-NMR spectrum of ligands[a,b] in DMSO-d_6 in δ (PPM).

^{1}H-NMR spectrum of the ligands						
	-OH[c]	-NH[c]	Ar-H	CH=NOH	CH=NNH	$-CH_3$
L^1H_2	11.34-10.20, s, 2H	10.13, s, 1H	7.36-6.85, d, 4H	7.86, s, 1H	8.54, s, 1H	2.82, s, 3H
L^2H_2	11.74-10.25, s, 2H	8.57, s, 1H	7.54-7.09, d, 2H	7.87, s, 1H	8.06, s, 1H	2.41, s, 3H
L^3H_2	11.90-10.02, s, 2H	8.34, s, 1H	7.85-7.18, d, 2H, 7.36, t, 1H, 8.05, s, 1H	6.91, s, 1H	8.10, s, 1H	2.50, s, 3H

^{13}C-NMR spectrum of the ligands					
	HNC=NOH	HC=NOH	(H)C=NNH	Ar-C	$-CH_3$
L^1H_2	141.17	138.80	168.72	137.03-131.79	45.31
L^2H_2	141.99	141.16	161.82	131.90-126.70	21.83
L^3H_2	156.10	138.85	162.13	134.46-121.01	21.75

[a]Chemical shifts(δ) are reported inp pm relative to $SiMe_4$ at 30°C, s: singlet, d: doublet, [b]inDMSO-d_6 [c]Disappears on D_2O exchange.

Kiliç et al., 2006; Canpolat et al., 2005).

The $C=N_{oxime}$ stretch decreases from 1605 to 1609 cm^{-1} in the free ligands to 1577 to 1571 cm^{-1} in Co(II) complexes (Güp, 2006; Macit et al., 2000; Kiliç et al., 2006; Canpolat et al., 2005). For $[(L^1H)_2Co(H_2O)_2]$ and $[(L^2H)_2Co(H_2O)_2]$, coordinated H_2O molecules are identified by a broad OH absorption around 3236 to 3203 cm^{-1}, with constant intensities after heating above 110 °C for 24 h. The IR spectrum of Ni(II) and Cu(II) complexes exhibit a $C=N_{oxime}$ stretching vibration around 1581 to 1562 cm^{-1}. These vibrations are at a lower frequency than for the free ligands, which is attributable to N,N-chelation (Güp, 2006; Macit et al., 2000; Kiliç et al., 2006; Canpolat et al., 2005).

A weak band around 1824 to 1732 cm^{-1} can be assigned to the intramolecular hydrogen bond O-H....O bending vibration (Güp, 2006; Macit et al., 2000; Kiliç et al., 2006; Canpolat et al., 2005). The intensity of characteristic stretching and bending vibrations of the free ligands were shifted and lowered on complex formation, and new vibrational bands characteristic of the Ni(II) and Cu(II) complexes were observed.

The dioxime ligand is a neutral compound; in the complexes it is a monoanion formed 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 (Bilgin and Gök, 2001). The band (O-H...O) is absent in the FT-IR spectra of the ligand but appears in FT-IR spectrum of the complexes showing that the complexes of the ligand Ni(II) and Cu(II) have square-planar structures (Figures 1a and 1b).

^{1}H and ^{13}C-NMR spectrum of the ligands

When the ^{1}H-NMR spectrum of the ligands in DMSO were examined, peaks corresponding N-OH protons were

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

Test Microorg.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
a	-	-	-	-	-	-	-	-	-	-	-	-	24	21	-	22	12	NT
b	-	-	-	-	-	-	-	-	-	-	-	-	16	17	21	26	23	NT
c	-	-	-	-	-	-	-	-	-	-	-	-	17	24	21	-	-	NT
d	-	-	-	-	-	-	-	-	-	-	-	-	23	19	14	16	11	NT
e	-	-	-	-	-	-	-	-	-	-	-	-	25	15	35	29	36	NT
f	-	-	-	-	-	-	-	-	-	-	-	-	19	20	-	21	10	NT
g	-	-	-	-	-	-	-	-	-	-	-	-	23	20	19	26	29	NT
h	-	-	-	-	-	-	-	-	-	-	-	-	22	17	19	29	28	NT
i	-	-	-	-	-	-	-	-	-	-	-	-	23	24	16	30	26	NT
j	-	11	-	-	-	-	12	-	-	-	-	-	26	21	-	25	30	NT
k	-	-	-	-	-	-	-	-	-	-	-	-	16	11	22	21	22	NT
l	-	-	-	-	-	-	-	-	-	-	-	-	24	20	-	21	29	NT
m	-	-	-	-	-	-	-	-	-	-	-	-	16	11	-	21	-	NT
n	-	-	-	-	-	-	-	-	19	-	-	-	NT	NT	NT	NT	NT	21
o	12	-	-	-	-	-	14	13	15	-	12	-	NT	NT	NT	NT	NT	21
p	-	-	-	-	-	-	-	-	20	-	-	-	NT	NT	NT	NT	NT	21
r	13	12	-	-	14	-	-	-	15	-	-	-	NT	NT	NT	NT	NT	21
s	-	-	-	-	-	-	-	-	19	-	-	-	NT	NT	NT	NT	NT	21

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: $[Co(L^3H)_2(H_2O)_2]$, 13: Cloramphenicol (C30), 14: Gentamycin (CN10), 15: Ampicillin (AMP10), 16:Tetracycline (TE30), 17: Erytromycine (E15), 18: Nystatine (NS100) *a. Escherichia coli* ATCC 25922, b. *Salmonella typhimurium* ATCC 14028, c. *Proteus sp.**, d. *Serratia marcescens**, e. *Micrococcus luteus* ATCC 9341, f. *Enterobacter sp.**, g. *Stapylococcus aureus* ATCC 25923, h. *Stapylococcus epidermidis* ATCC 12228, i. *Bacilllus cereus* ATCC 11778, j. *Bacillus thrungiensis**, k. *Entereococcus faecalis* 29212, l. *Streptococcus pneumoniae* ATCC 49617, m. *Listeria sp**, n *Candida utilis**, o. *Candida albicans**, p. *Candida glabrata**, r. *Candida trophicalis**, s. *Saccharomyces cerevisiae* ATCC 9763.(-): No zone, NT: Not tested.*Special gift from Adnan Menderes University Faculty of Medicine.

Table 5. Antimicrobial activities of ligands and their metal complexes (MIC, µg mLL^{-1}).

Test Microorganisms	1	2	5	7	8	9	11	Str	NS100
*Bacillus thrungiensis**		16		16				64	
*Candida utilis**						4			64
*Candida albicans**	16			8	8	8	16		64
*Candida glabata**						4			64
*Candida trophicalis**	16	8	8			8			64
Saccharomyces cerevisiae ATCC 9763						4			128

1: L^1H_2, 2:$[Ni(L^1H)_2]$, 5: L^2H_2, 7:$[Cu(L^2H)_2]$, 8: $[Co(L^2H)_2(H_2O)_2]$, 9: L^3H_2, 11: $[Cu(L^3H)_2]$. Str: Streptomycine. NS: Nystatine.

observed at 11.34 to 10.20 PPM (s, 2H) for L^1H_2, 11.74 to 10.25 PPM (s, 2H) for L^2H_2, and 11.90 to 10.02 PPM (s, 2H) for L^3H_2, (Babahan et al., 2006; Güp, 2006; Macit et al., 2000; Kiliç et al., 2006; Canpolat et al., 2005).

The peaks of NH proton of ligands appear at 10.13 PPM (s, 1H) for L^1H_2, 8.57 PPM (s, 1H) for L^2H_2, and 8.34 PPM (s, 1H) for L^3H_2 (Babahan et al., 2006; Güp, 2006; Macit et al., 2000; Kiliç et al., 2006; Canpolat et al., 2005). The vanishing of these peaks by addition of D_2O to the ligand solution indicates that the observed resonances are those of the protons of O-H and N-H groups. These values are in accord with the previously reported oxime derivatives (Babahan et al., 2006; Güp, 2006; Macit et al., 2000; Kiliç et al., 2006; Canpolat et al., 2005).

C-H protons neighbouring to oxime groups were observed at 7.86 PPM (s, 1H) for L^1H_2, 7.87 PPM (s, 1H) for L^2H_2 and 6.91 PPM (s, 1H) for L^3H_2 (Babahan et al., 2006; Güp, 2006; Macit et al., 2000; Kiliç et al., 2006; Canpolat et al., 2005). The peaks of –CH=N-NH proton of aldehydes appear at 8.54 PPM (s, 1H) for L^1H_2, 8.06 PPM (s, 1H) for L^2H_2 and 8.10 PPM (s, 1H) for L^3H_2.

In the ^{1}H-NMR spectrum, two peaks are present for the O-H protons of the oxime groups. These two deuterium

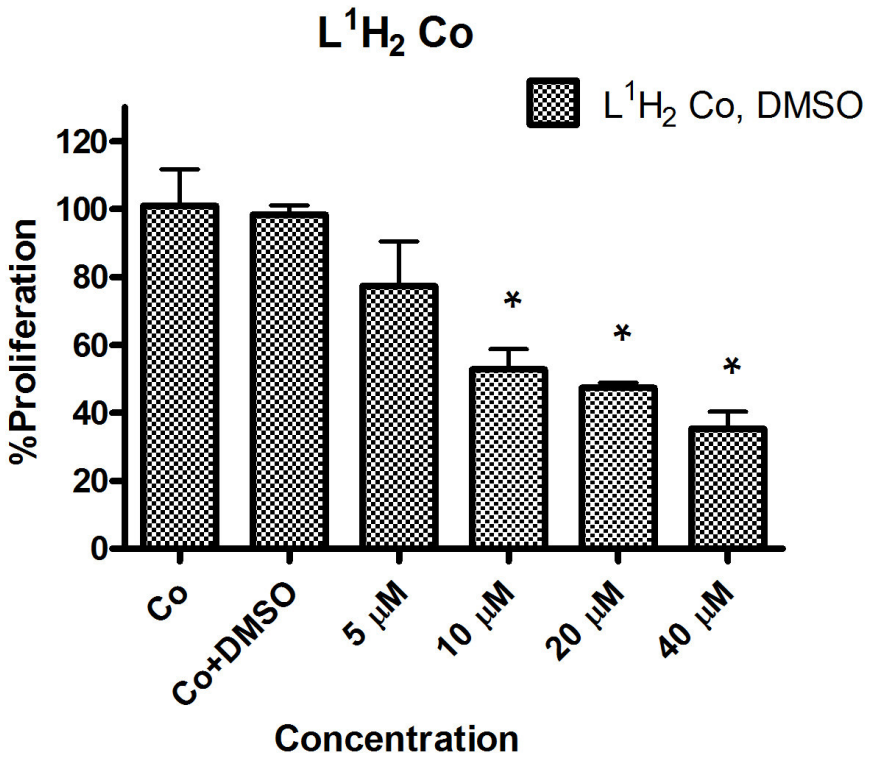

Figure 2. Antiproliferative effect of Co(II) complex of L^1H_2..*p<0.05, one way ANOVA.

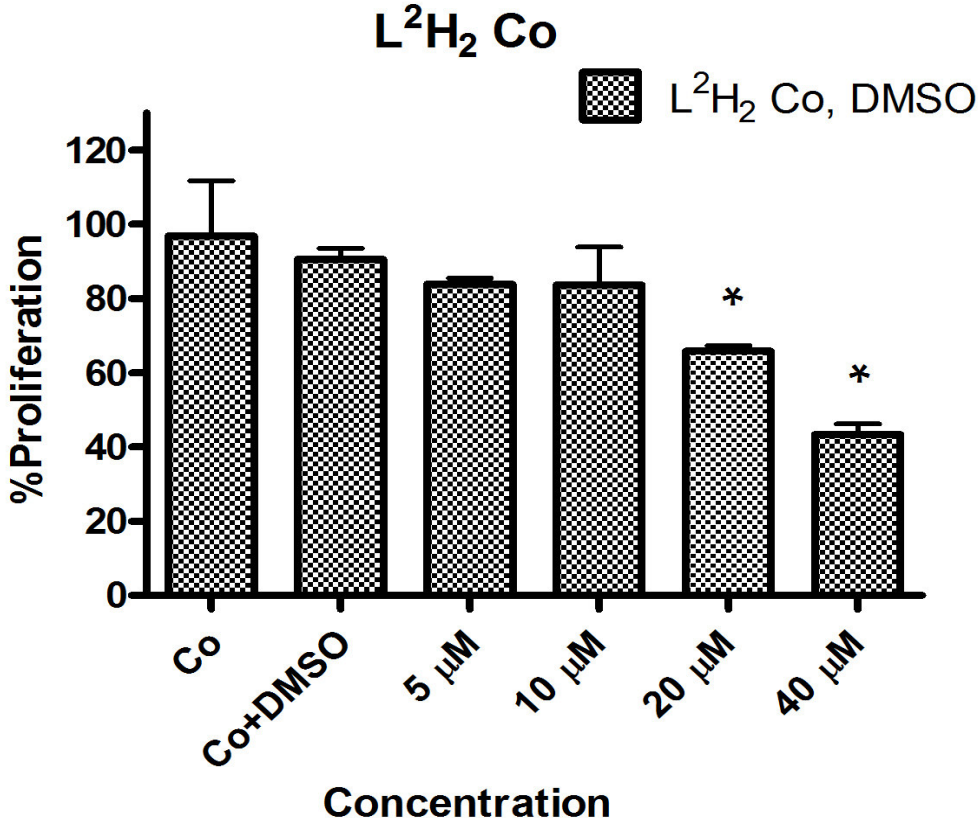

Figure 3. Antiproliferative effect of Co(II) complex of L^2H_2. .*p<0.05, one way ANOVA.

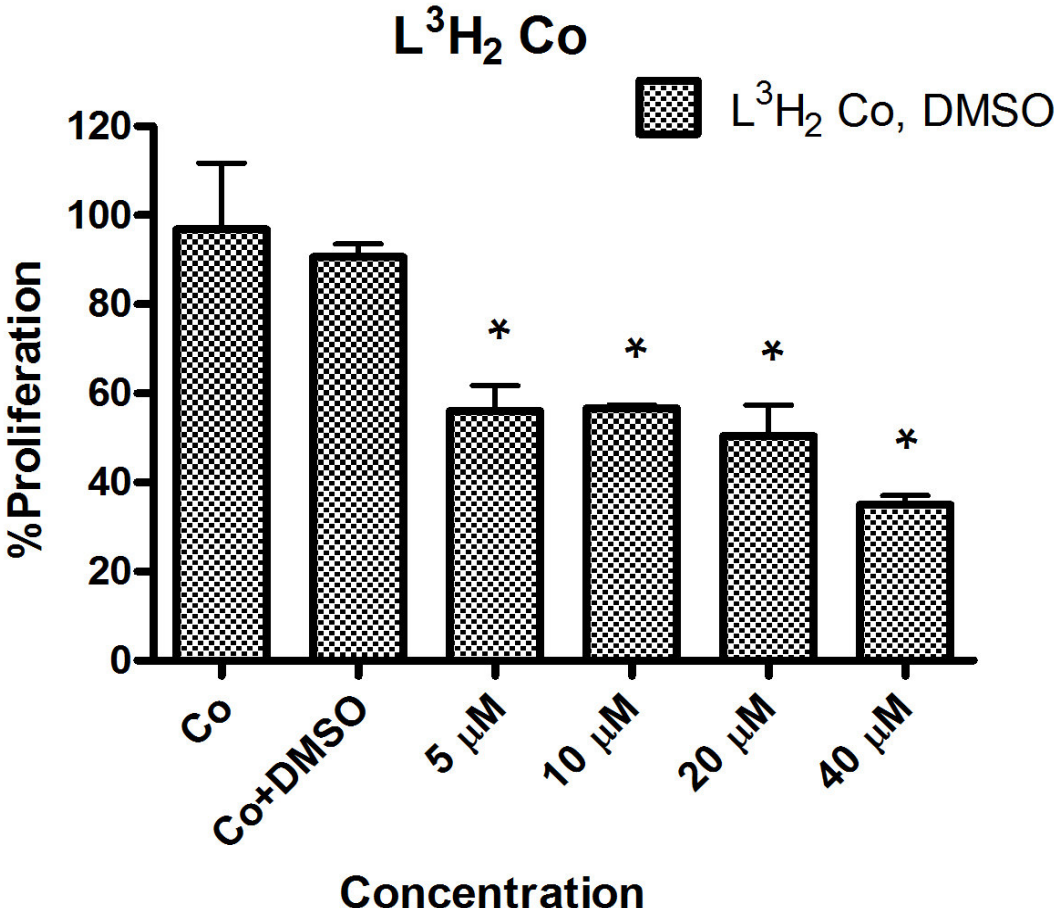

Figure 4. Antiproliferative effect of Co(II) complex of L^3H_2. *p<0.05, one way ANOVA.

exchangeable singlets correspond to two inequivalent O-H protons that also indicate the anti-configuration of the O-H groups relative to each other (Babahan et al., 2006; Güp, 2006; Macit et al., 2000; Kiliç et al., 2006; Canpolat et al., 2005). The aromatic protons of compounds appear at 7.36 (d, 1H)-6.85 (d, 1H) PPM for L^1H_2, 7.54 (d, 1H)-7.09 (d, 1H) PPM for L^2H_2 and 7.85 (d, 1H)-7.18 (d, 1H) PPM for L^3H_2, as 2 different doublets (Bilgin and Gök, 2001; Dolaz, 2001) and 7.36 (t, 1H) PPM for L^3H_2 as a different triplet and 8.05 (s, 1H) PPM for L^3H_2 as a different singlet.

In the ^{13}C-NMR spectrum of ligands, different signals which were observed at 141.17 PPM for L^1H_2, 141.99 PPM for L^2H_2, 150.50 PPM for L^3H_2 (HN<u>C</u>=N-OH) and 138.80 PPM for L^1H_2, 141.16 PPM for L^2H_2, 138.85 ppm for L^3H_2 (H-<u>C</u>=N-OH) show asymetrically substituted Vic-dioximes (Babahan et al., 2006; Güp, 2006; Serin, 2001).

^{13}C-NMR spectra at two different frequencies in each case indicate that the Vic-dioxime has anti- structure (Taş et al., 2004; Uçan and Mercimek, 2005). Spectrum of H<u>C</u>=N-N appear at 168.72 PPM for L^1H_2, 161.82 PPM for L^2H_2 and 162.13 ppm for L^3H_2 (Babahan et al., 2006; Güp, 2006; Taş et al., 2004).

The signals of the $C_{aromatic}$ were observed at 137.03, 135.20, 134.15, 131.79 PPM for L^1H_2 and 131.90, 130.18, 128.99, 126.70 PPM for L^2H_2, as 4 peaks and 134.46, 132.71, 129.47, 126.40, 124.58, 121.01 PPM for L^3H_2, as 5 peaks. The signals of CH_3 were shown at 45.37 PPM for L^1H_2, 21.83 PPM for L^2H_2, and 21.75 PPM for L^3H_2 (Güp, 2006; Taş et al., 2004; Uçan and Mercimek, 2005).

Magnetic susceptibility

The magnetic susceptibility measurements of the nickel(II) complexes indicate that these complexes are diamagnetic. The cobalt(II) and copper(II) complexes are paramagnetic. The copper complexes show 1.72 BM for L^1H_2, 1.72 BM for L^2H_2 and 1.70 BM for L^3H_2. These results indicate square-planar structures for the Cu(II) complexes (Babahan et al., 2006; Güp, 2006; Taş et al., 2004; Uçan and Mercimek, 2005). The cobalt complexes show 4.30 BM for L^1H_2, 4.10 BM for L^2H_2 and 4.20 BM for L^3H_2.

These data obtained from the microanalyses show that

the complexes of Co(II) can be octahedral (Güp, 2006; Durmuş et al., 2004). According to above results, square-planar geometries for Nickel(II) and Copper(II) complexes, and a octahedral geometry for the cobalt (II) complexes are proposed. On the basis of above analyses, Figures 1a-1b may be suggested for the complexes.

UV spectra

The electronic spectra of soluble complexes in DMSO are given in "experimental". The electronic spectra of the Ni(II), Cu(II) and Co(II) complexes of the ligands exhibit two bands with λ_{max} situated at 224 and 342 nm for L^1H_2, three bands between 276 to 283, 331 to 389 and 484 to 705 nm for metal complexes of L^1H_2, two bands at 267 and 320 nm for L^2H_2, three bands between 260 to 274, 318 to 333 and 482 to 705 nm for metal complexes of L^2H_2, two bands 260 and 330 nm for L^3H_2, three bands between 259 to 271, 320 to 342 and 477 to 750 nm for nickel(II), copper(II) and cobalt(II) complexes for L^3H_2. These bands were assigned to both a charge transfer transition from the metal to 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, NiII exhibits a preference for square planar geometry with dioxime complexes. The decrease in the intensities of the transitions indicates coordination with the nitrogen atoms (Canpolat et al., 2004; Kurtoğlu et al., 2008).

Antimicrobial assays

The antimicrobial activities of three new vic-dioxime derivatives containing benzaldehydehydrazone groups (L^1H_2: 4-methoxybenzaldehydehydrazone glyoxime, L^2H_2: 4-methylbenzaldehyde hydrazone glyoxime and L^3H_2: 3-methylbenzaldehydehydrazone glyoxime) and their Ni(II), Cu(II) and Co(II) complexes were analysed by the disc diffusion method and MIC (Collins et al., 1995; Murray et al., 1995).

The results concerning *in vitro* antimicrobial activities of the water soluble dendrimers together with the inhibition zone (mm) and (MIC) values of compared antibiotic and antifungal reagents are listed in Tables 4 and 5. All the compounds tested exhibit moderate antimicrobial activities. Among the test compounds attempted, 1, 2, 7 and 9 showed slightly higher activities against some bacteria and yeasts (Table 4). The MIC values in Table 5 also indicate that some of the compounds tested exhibit moderate antimicrobial activity on the tested microorganisms. Once again the data indicate that 1, 2, 7 and 9 compounds have stronger activity against some bacteria such as *B. thrungiensis* (2 = 16 µg mLL^{-1}, 7 = 16 µg mLL^{-1}) compared with Streptomycine on these microorga-nisms 64 and 128 µg mLL^{-1}, respectively. These compounds also have strong activity against the yeast cultures such as *Candida utilis* (9 = 4 µg mLL^{-1}), *Candida albicans* (1 = 16 µg mLL^{-1}, 7 = 8 µg mLL^{-1}, 9=8 µg mLL^{-1}), *Candida glabata* (9 = 4 µg mLL^{-1}), *Candida trophicalis* (1 = 16 µg mLL^{-1}, 2 = 8 µg mLL^{-1} , 9 = 8 µg mLL^{-1}), *Saccharomyces cerevisiae* ATCC 9763 (9 = 4 µg mLL^{-1}) compared with Nystatine antifungal agent on these microorganisms which are 64 and 128 µg mLL^{-1}, respectively (Table 5).

$[(L^2H)_2Cu]$, $[(L^2H)_2Co]$, L^3H_2 and $[(L^3H)_2Cu]$ have antimicrobial effect against *C. albicans*. L^1H_2, $[(L^1H)_2Ni]$, L^2H_2 and L^3H_2 have antimicrobial effect against *C. trophicalis*. L^3H_2 has antimicrobial effect against *C. albicans*, *C. trophicalis*, *C. utilis, C. glabata* and *S. cerevisiae* ATCC 9763. 9763, *C. trophicalis*, *C. albicans, C. glabata* and *C. utilis*.

L^3H_2 showed antimicrobial effect against *S. cerevisiae* ATCC 9763, *C. trophicalis*, *C. albicans, C. glabata* and *C. utilis* as Nystatine (NS100) was used as positive control. The developing inhibition zones of L^3H_2 compared with Nystatine (NS100). L^3H_2 has more inhibition zone than Nystatine (NS100) for *C. glabata*.

None of the ligands and their metal complexes showed antimicrobial effect against *E. coli* ATCC 25922, *S. typhimurium* ATCC 14028, *S. marcescens*, *M. luteus* ATCC 9341, *Enterobacter sp., S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *B. cereus* ATCC 11778, *E. faecalis* 29212, *S. pneumoniae* ATCC 49617 and *Listeria sp*.

In general, the ligands and their metal complexes have antimicrobial activities on Gram positive bacteria and yeasts, especially *B. thrungiensis*, *S. cerevisiae* ATCC 9763, *C. trophicalis*, *C. albicans, C. glabata* and *C. utilis*.

Members of the genus *Bacillus* are aerobic spore-forming rods which are ubiquitous in nature (Tuazon et al., 1995). Despite their widespread distribution, even as normal skin flora, *Bacillus* spp. rarely causes infections. The exception is *Bacillus cereus*, which is a well-known cause of food poisoning and a dreaded cause of posttraumatic endophthalmitis (Tuazon et al., 1995). Despite *B. cereus* can also cause opportunistic infections, mainly in the immunocompromised host (Tuazon et al., 1995; Drobniewski et al., 1993). Despite the fact that *B. anthracis* and *B. cereus* behave as human pathogens and *B. thuringiensis* is a common insect pathogen, robust genetic evidence indicates that these microorganisms should be regarded as a unique species (Helgason et al., 2000). *B. thuringiensis* has been used worldwide as a biopesticide in forestry and agriculture (Schnepf et al., 1998), 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 diarrheas (Jackson et al., 1995), corneal ulcer (Samples and Beuttner, 1983), periodontitis

(Tuazon et al., 1995), burn (Damgaard et al., 1997), and wound (Hernandez et al., 1998), infections.

Candida is a yeast and the most common cause of opportunistic mycoses worldwide. It is also a frequent colonizer of human skin and mucous membranes. *Candida* is a member of normal flora of skin, mouth, vagina, and stool. Infections caused by *Candida* spp. are in general referred to as candidiasis. The clinical spectrum of candidiasis is extremely diverse. Almost any organ or system in the body can be affected. Candidiasis may be superficial and local or deep-seated and disseminated. Disseminated infections arise from hematogenous spread from the primarily infected locus. *Candida albicans* is the most pathogenic and most commonly encountered species among all (Bielsa et al., 1987). The term candidiasis often is used to describe an infection caused by the yeastlike fungus *Candida albicans*. Species of *Candida* other than *C. albicans*, however, also have the potential to cause infection, particularly in patients who are immunologically or physiologically compromised (Rippon et al., 1982; Wingard et al., 1979).

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. In one study, *C. tropicalis* was the most frequent opportunistic fungus isolated from specimens from patients in a critical care unit (Morganti et al., 1982). *C. tropicalis* also has been reported to be a frequent opportunistic pathogen in a cancer hospital (Horn et al., 1985) and has been identified as the etiologic agent in a variety of infections including pyelonephritis (Seidenfeld et al., 1982) lower urinary tract infections, thrombophlebitis, arthritis, bursitis, meningitis, multiple organ infection, pericarditis, and candidia vulvovaginitis (Seidenfeld et al., 1982; Finberg et al., 2004). The point of the treatment of nosocomial infections, it was a consequential decision. Therefore, this result may suggest that the ligands and their metal complexes with antimicrobial properties which can be used as antimicrobial agents in new drugs for therapy of infectious diseases in human.

Suggestions are made that the negative inductive effect plays a significant role, dimerization of oxime involves the formation of a pair of H bonds (Ling, 1986; Hania, 2009). This feature will cause a decrease of electronic density in oximes compared with phenylhydrazones, thereby facilitating entry of the oxime into the cell. This is likely to increase the antibacterial potency. Most of ligands and complexes were found to possess moderate antibacterial activity at concentration 200 µg except those free ligands which has electron donating groups. This means that compounds with high electron density gave poor antibacterial activity which makes the diffusion of these compounds more difficult throw the body of the bacteria cell (Hania, 2009).

Antiproliferative activity

The antiproliferative activities of three novel aromatic hydrazone derivatives containing Vic-dioxime groups (L^1H_2: 4-methoxy-benzaldehydehydrazone-glyoxime, L^2H_2: 4-methylbenzaldehydehydrazone glyoxime and L^3H_2: 3-methylbenzaldehyde hydrazone glyoxime) and their Ni(II), Cu(II) and Co(II) complexes were analysed by culturing HL-60 cell line. The HL-60 (*Human promyelocytic leukemia cells*) cell line is a leukemic cell line that has been used for laboratory research. The HL-60 cultured cell line provides a continuous source of human cells for studying the molecular events of myeloid differentiation and the effects of physiologic, pharmacologic, and virologic elements on this process. Among the tested compounds Co(II) complexes of this derivatives can be described as potent anti-cancer agents due to their antiproliferative effects with an I_pC_{50} between 5 to 40 µM concentrations (Figures 2, 3 and 4). The strongest antiproliferative activity was determined with the Co(II) complex of L^3H_2 (Figure 4). The other complexes of these derivatives have shown weak antiproliferative effects against used cancer cell line.

It is evident in the literature that hydrazone and oxime derivatives and their metal complexes possess antiproliferative properties against tumour cells. The search for antitumoral drugs led to discovery of several hydrazones having antitumoral activity. Some hydrazones have potent antitumor activities against human malignant breast cell lines, ovarian cancer cell lines, renal cancer cell lines, haematological tumors and prostate cancer cell line. They exhibit their potential as antiproliferative, cytotoxic, cell cycle arrest at G2/M, inducing apoptosis, caspase activation and by inhibiting tubulin polymerization (Seidenfeld et al., 1982). It has shown that 2,6-dichloro benzaldehydehydrazone 29 inhibits 60 tumour cell lines with nanomolar potency and did not show animal toxicity (Finberg et al., 2004). A novel ribavirin hydrazone derivative inhibits the growth of A549 lung cancer cells at 20 µM (Ling, 1986).

Some novel 2-substituted-6-bromo-3-methylthiazolo[3,2-a]benzimidazole derivatives has shown strong cytotoxicity against both colon carcinoma cells (HCT 116) and hepatocellular carcinoma cells (Hep-G2) (Rollas and Küçükgüzel, 2007). Pyrazole-5-carbohydrazide hydrazone derivatives showed inhibitory effects on the growth of A549 lung cancer cell and induced apoptosis (Zheng et al., 2009). INNO-206, the 6-maleimidocaproyl hydrazone derivative of doxorubicin has shown more potent antitumor efficacy than free doxorubicin in tested three cell lines (breast carcinoma, ovarian carcinoma and small cell lung cancer) (Graeser et al., 2010) Aryl hydrazones of 2-phenylindole-3-carbaldehydes inhibited the growth of MDA-MB 231 and

MCF-7 breast cancer cells with IC(50) values of 20-30 nM. They did not inhibit tubulin polymerization as the aldehydes but were capable of blocking the cell cycle in G(2)/M phase (Vogel et al., 2008).

Diarylmethyloxime and hydrazone derivatives showed potent tubulin polymerization inhibitory action as well as cytotoxic activity against tested cancer cell lines (Alvarez et al., 2008). 5,5'-substituted indirubin-3'-oxime derivatives displayed potent inhibtory activity against CDK2, with IC(50) values of 1.9 and 1.7 nM (Serin, 2001). Indirubin-3'-oxime inhibited the growth of HL-60 cells with a GI50 value of 36.6 microM. It can be suggested that indirubin derivatives might be useful candidate agents for exploring potential antileukemic drugs (Cuong et al., 2010).

Cancer is the second reason leading mortality in USA. In 2007, 1.44 million people incurred cancer and 559.650 of it concluded with mortality (Jemal et al., 2007). Otherwise during the year 2002, 1.28 million individuals incurred cancer and the mortality rate was 38%. When the mortality rates were investigated, it could be seen that there were not any changes in the mortality of cancer between 2002 and 2007 (Brian et al., 1984). Because of the unfavourable effects of the cancer on the population, investigation of new anticancer drugs is important to avoid the high costs on therapy and to improve the living qualities of the patients. The aims on investigation of the anticancer drugs is to discover new structures which are possessing specific action of mechanisms. In this frame three new aromatic hydrazone derivatives containing Vic-dioxime groups synthesized in this work can be considered as potential anticancer agents for further invesitagations.

Conclusion

Three new Vic-dioxime derivatives containing hydrazone side groups and their transition metal complexes with Ni(II), Cu(II) and Co(II) were synthesized and evaluated, their antimicrobial activities using disk diffusion method against 13 bacteria and 5 yeasts, their antiproliferative effect on neoplastic cells were determinated, HL 60 (Human promyelocytic leukemia cells) cell. Besides they were evaluated using the minimal inhibitory concentration (MIC) dilution method against 1 bacterium and 5 yeasts.

As a result of this study, among the test compounds attempted 1, 2, 7 and 9 showed slightly higher activities against *B. thrungiensis* and some of yeasts are comparatively higher or equipotent to the antibiotic and antifungal agents in the comparison tests. In general, the ligands and their metal complexes have antimicrobial activities on gram positive bacteria and yeasts, especially *B. thrungiensis*, *S. cerevisiae* ATCC 9763, *C. trophicalis*, *C. albicans, C. glabata* and *C. utilis*. The variation in the activity of oxime-hydrazone derivatives and their metal complexes against different microorganisms depends either on the impermeability of the cells of the microbes or differences in ribosomes in microbial cells.

Furthermore Co(II) complexes of these derivatives can be described as potent anti-cancer agents due to their antiproliferative effects with an I_pC_{50} between 5 to 40 µM concentrations. The strongest antiproliferative activity was determined with the Co(II) complex of L^3H_2 at 5 µM.

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