Journal of Applied Pharmaceutical Science Vol. 2 (12), pp. 027-038, December, 2012
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2012.21206
ISSN 2231-3354

Synthesis, characterization, and biological applications of some 2-acetylpyridine and acetophenone derivatives

Nura Suleiman Gwaram[*1], Hapipah Mohd Ali[1], Siti Munirah Saharin[1], Mahmood Ameen Abdulla[2], Pouya Hassandarvish[2], Thong Kwai Lin[3], Chai Lay Ching[3] and Cher Lin Ooi[3]
[1]Chemistry Department, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[2]Molecular Medicine Department, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[3]Institute of Biological Science Faculty of science, University of Malaya, 50603 Kuala Lumpur, Malaysia.

ARTICLE INFO

Article history:
Received on: 09/12/2012
Revised on: 21/12/2012
Accepted on: 26/12/2012
Available online: 30/12/2012

Key words:
Schiff bases; Complexes; Anticancer; MCF-7; Antibacterial; MRSA

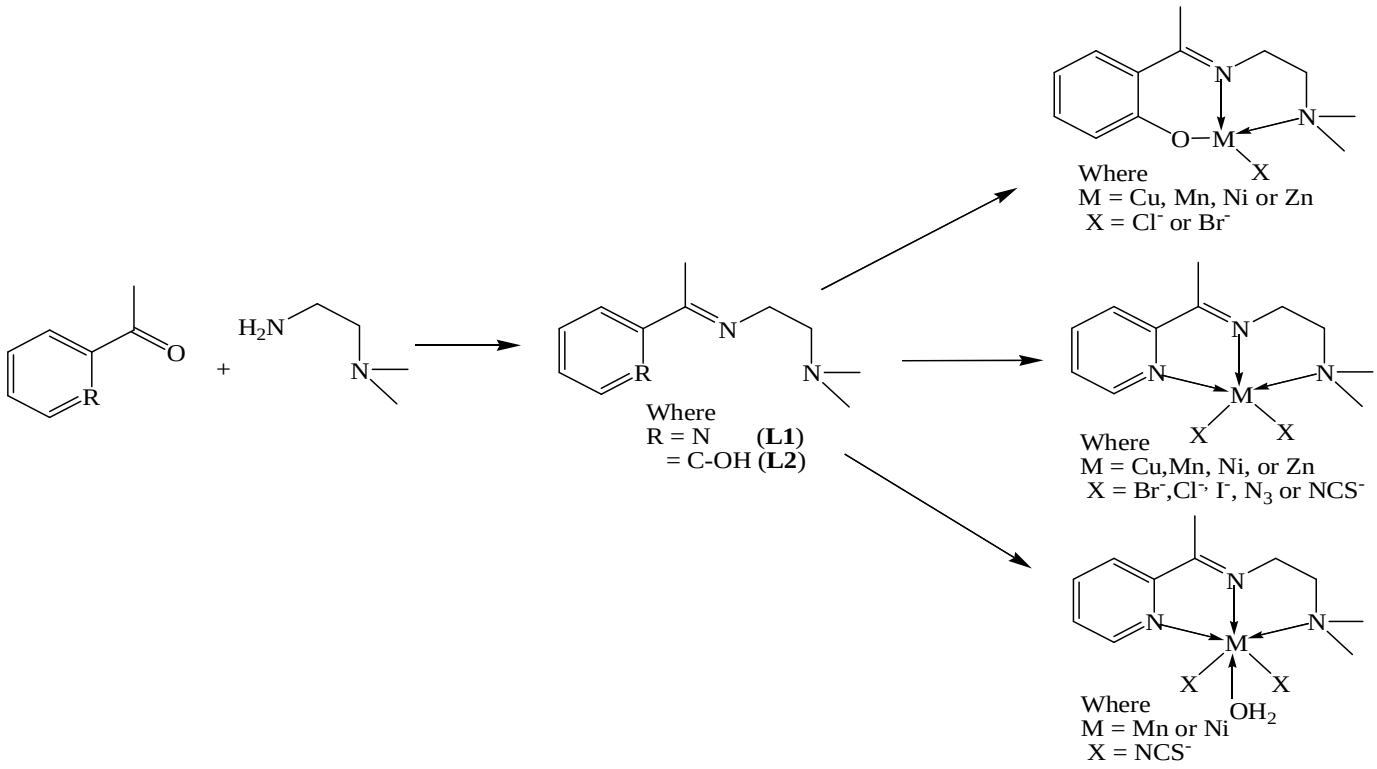
ABSTRACT

This paper comprises two series of complexes which showed different modes of coordination. The fist series involved an *N,N',N"*-donor Schiff base ligand from the reaction of 2-acetylpridine as carbonyl compound with *N,N'*-dimethylethylenediamine (**L1**), the second series involved *N,N',O*-donor Schiff base from the condensation reaction of 2-hydroxyacetophenone (ketone) as carbonyl compound with *N,N'*-dimethylethylenediamine (**L2**). The ligands and complexes were characterized by using melting point, elemental analysis, FT-IR, NMR, and UV/Visible spectroscopy. The complexes showed very low cytotoxicity towards MCF-7 breast cancer cell line. They also showed moderate zone inhibition against Gram positive bacterium *Methicillin-resistant Staphylococcus aureus, Acinetobacter baumannii* and *Pseudomonas aeruginosa*. No antimicrobial activity observed with *Klebsiella pneumonia*.

INTRODUCTION

Many pyridines find application in areas where bioactivity is important, as in medicinal drugs and in agricultural products such as herbicides, insecticides, fungicides, and plant growth regulators (Goe, 1982). 2-Acetylpyridine is used as a chemical intermediate in organic synthetic, pharmaceutical & agricultural chemical manufacture and as an analytical reagent. Also used as a food additive as a flavor enhancer, flavoring agent or adjuvant (Goe, 1982) In the area of bioinorganic chemistry the interest in the imines complexes lies in that they provide synthetic models for the metal-containing sites in metalloproteins/enzymes and also contributed enormously to the development of medicinal chemistry, radio immunotherapy, cancer diagnosis and treatment of tumor (Adoración *et al.,* 2004; Larry, 2003). In addition, some of the complexes containing N and O donor atoms are effective as stereo specific catalysts for oxidation (Kureshy *et al.,* 1999) reduction (Yasuhiro *et al.,* 1986), hydrolysis (Ross *et al.,* 1986), biocidal activity (Parbati *et al.,* 2001) and other transformations of organic and inorganic chemistry. Some of the members of the first row transition metals play major roles in various biological activities on which recently have been included in anticancer agents to exploit their various applications because of the exceptionally wide range of reactivity available and have been particularly attractive (Nenad *et al.,* 1996) these transition metal complexes offer a great diversity in their action; such as anticancer and antiviral properties (Bernadette *et al.,* 2010; Garoufis *et al.,* 2009, Bernadette *et al.,* 2010; Raman *et al.,* 2010 and Shakir *et al.,* 2011), DNA binding and DNA cleavage activities (Shahabadi *et al.,* 2010) and many other biological activities (Mladenova *et al.,* 2002). It is also known that the existence of metal ions bonded to biologically active compounds may enhance their antimicrobial activities (Prakash *et al.,* 2010). Such as anticonvulsant (Sridhar *et al.,* 2002), antifungal (Bharti *et al.,* 2010), anti-HIV (Pandeya *et al.,* 1999), antiviral and anticancer (Zhang *et al.,* 2009) and antimicrobial (Mandal *et al.,* 2011; Yusnita *et al.,* 2009; Pignatello *et al.,* 1994; Nair *et al.,* 2010; Tajudeen *et al.,* 2009 and Ling-Wei *et al.,* 2011).

* *Corresponding Author*
Department of chemistry, Faculty of Science,
University of Malaya, 50603 Kuala lumpur, Malaysia.



(Scheme 1): Synthetic pathway for the Schiff base ligands and their complexes.

Nosocomial infections caused by multidrug resistant bacteria are an increasing medical problem worldwide, particularly among immuno compromised patients and those hospitalized in intensive care units. Both gram positive and gram negative bacteria have developed high level resistance to multiple classes of antibacterial agents. These include methicillin resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli* and *vancomycin resistant enterococci* (VRE) (Guidos *et al.,* 2010). Few available drugs such as linezolid and some newer glycopeptides, tigecycline are active against MRSA and VRE but their success rates are variable (Esposito *et al.,* 2007). As such, there is a need to explore other sources of effective antibacterial compounds to augment the limited choice of drugs for therapeutic treatment.

In this paper, some Schiff base complexes of newly synthesized *N,N'N"* and *N'N"O* donor Schiff base ligands from the Reaction of *N,N*-dimethylethylenediamine with 2-acetylpyridine and 2-hydroxyacetophenone in presence of Cl^-, N_3^-, and SCN^- ions (scheme 1). All the complexes were examined for potential anti-cancer and antibacterial applications.

EXPERIMENTAL SECTION

Materials and methods

N,N-dimethylethylenediamine, 2-hydroxyacetophenone and 2-acetylpyridine were purchased from the Aldrich–Sigma

Melting Point Determination

Melting points were determined using a MEL-TEMP II melting point instrument at Department of Chemistry, University of Malaya and were not corrected. All samples are placed in the micro haemotocrit tube (soda lime glass). The melting points of the sample were recorded after they have completely melted.

Carbon, Hydrogen and Nitrogen (CHN) Analyses

Microanalyses for carbon, hydrogen and nitrogen in the compounds were carried out on a Perkin-Elmer 2400 elemental analyzer at the Department of Chemistry, Faculty of science University of Malaya. All compounds (1.75–2.00 mg) were weighted in aluminium foil capsules. The instrument was calibrated with sulfamethinezine.

Infra Red (IR) Spectroscopy

The Infrared spectra were recorded on a Perkin Elmer Spectrum 400 ATR-FT-IR spectrometer at the Department of Chemistry, Faculty of science University of Malaya. All the spectra were run in the range of 400-4000 cm^{-1} at room temperature.

Nuclear Magnetic Resonance (NMR) Spectroscopy

The ^1H and ^{13}C NMR spectra of the Schiff bases were recorded using a Bruker Apex 600MHz; FT-NMR spectrometers chemical shifts are given in δ values (ppm) using TMS as the

internal standard. Deuterated dimethylsulphoxide (DMSO) was used as solvent.

Ultraviolet-Visible (UV-Vis) Spectroscopy.

The spectra (solid) were obtained from reflectance electronic technique by using UV-3600 Shimadzu UV-Vis-NIR Spectrophotometer and were scan from 200-1000, while the spectra (DMSO) were recorded in quartz cuvettes on a Shimadzu 1601 spectrophotometer in the region of 200-1000 nm.

Single Crystal X-Ray Diffraction

The single crystals of suitable size were mounted on a glass fibre using perfluoropolyether oil and cooled rapidly to 100 K in a stream of cold N2. Diffraction data were measured using a Bruker APEX II CCD area-detector diffractometer (graphite-monochromated Mo Ka radiation, k = 0.71073 Å). The orientation matrix, unit cell refinement and data reduction were all handled by the APEX II software (SAINT integration, SADABS absorption correction). The structure was solved using direct methods and vectors in the program SHELXS-97 and was refined by the full matrix least-squares method on F2 with SHELXL-97. All the nonhydrogen atoms were refined anisotropically and all the hydrogen atoms were placed at calculated positions and refined isotropically. Drawings of the molecules were produced by using XSEED.

Cell Lines

Human cell lines will be obtained from the American Type Culture Collection ATCC, USA.

Bacterial strains

The antibacterial activities of the investigated compounds were tested against a panel of multi-drug resistant nosocomial bacterial pathogens, consisted of MRSA, *A. baumannii*, *K. pneumonia* and *P. aeruginosa*. All tested bacterial strains were of clinical origin and have shown resistance to at least 4 antibiotics based on disc diffusion assay. For each bacterial species, six to ten strains were included in the antibacterial study to capture possible drug resistance variation within species. All bacterial strains used in this study were obtained from the bacteria culture collection of Biomedical Science Laboratory, Institute of Graduate Studies University of Malaya, Malaysia.

SYNTHESIS OF THE COMPOUNDS

N,N^1-dimethyl-*N*^{2}- (1-(pyridin-2-yl) ethylidene) ethane-1,2-diamine (L1)

The ligand was obtained from the condensation reaction of 2-acetylpyridine (0.61 g, 5 mmol) and *N,N*-dimethylethyldiamine (0.44 g, 5 mmol) in ethanol (25 mL) at a temperature of 75–85 °C and refluxed for three hours. The product was oily yellowish solution, which became yellow hygroscopic solid after 12 hours at 55^{o}C in an oven. The solid product was dissolved in methanol and heated to 60 °C. After evaporating from the solvent under reduced pressure, a yellow solid was formed. The ligand is characterized by using melting point, elemental analysis, IR, NMR and UV/Vis-spectroscopy. Yield: 54%. Molecular formula: $C_{11}H_{17}N_3$ (191.27). Analytical Calculated. (Found): C, 69.87 (68.48); H, 8.80 (8.98); N, 13.58 (14.98). IR (ATR cm−1): 2946.18, 2825.46, 2781.92 ν(C-H), 1698.01, 1639.70 ν(C=N), 1585.72, 1566.34 ν(C=N)pyr, 1464.83, 1435.25 ν(C-C), 1153.71, 1103.38 ν(C-N). UV-Vis [λ_{max} (nm) (DMSO)]: 321 (n→π*); 233 (π→π*). ^{1}H-NMR (600 MHz δ, DMSO-*d*6): 8.28-7.87 (CH_2-Ar), 2.76-2.68 (NCH_2-CH_2N), 1.22 (CH_3-), 1.9 (6H, CH_3-N). ^{13}C NMR (600 MHz, δ, DMSO-*d*6): 166.59 (C=N, Schiff) 147.58 (CO-phenolic), 145.83 (C-aromatic), 139.56 (CH-aromatic), 126.82 (CH-aromatic), 122.62 (CH-aromatic), 54.43 (2C, CH_2-aliphatic), 43.44. 43.29 (2C, CH_3-n,n-dimethyl), 14.69 (CH_3, Methyl).

COMPLEXES OF LIGAND L1

A series of copper(II), iron(II), manganese(II), nickel(II), tin(II) and zinc(II) complexes of **L1** figure 1 Schiff base were prepared *in situ* by mixing 2-acetylpyridine (0.61 g, 5 mmol) and *N,N*-dimethylethyldiamine (0.44 g, 5 mmol) in ethanol (25 mL) at a temperature of 75–85 °C and refluxed. After 2 hr an ethanolic solution containing metal(II) halides and in other case hydrated metal(II) acetates in the presence of potassium bromide (KBr), sodium thiocyanate (NaSCN), sodium azide (NAN_3) and Sodium iodide (NAI_2) was then added and the mixture was refluxed for 2-5 hours (Scheme 1). The resultant precipitates were filtered off, washed with cold ethanol and dried under vacuum.

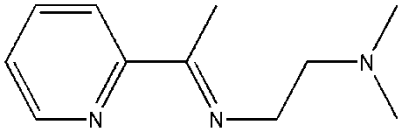


Fig. 1: Proposed structure for **L1.**

Dichlorido{*N,N*-dimethyl-N'-[1-(2-pyridyl)ethylidene] ethane-1,2-diamineκ^3 *N,N',N"*}copper(II) ($Cu(L1)Cl_2$)

Light Green solid, 94% yield, m.p. >400 °C, Analytical calculated for C11H17Cl2CuN3 (324.01) C, 40.56; H, 5.26; N, 12.90, Found C, 41.99; H, 5.62; N, 12.87. IR: ATR ν_{max}/cm^{-1} 3050.31 (CH aromatic) 2979.67-2910.48 (CH aliphatic), 1659.15 (C=N Schiff), 1446.12 (C-C), 1163.20, 1144.79 (C-N), 573.40 (M-N).

{*N,N*-Dimethyl-*N'*-[1- (2-pyridyl) ethylidene] ethane-1,2-diamine-κ^3*N,N',N"*} bis(thiocyanato-κ^N) nickel(II) [$Ni(L1)(SCN)_2(H_2O)$]

Green solid, 91% yield, m.p. >400 °C, Analytical calculated for C13H17N5NiS2 (366.13) C, 42.65; H, 4.68; N, 19.13. Found C, 42.85; H, 5.00; N, 19.82. IR: ATR ν_{max}/cm^{-1} 3090.22 (CH aromatic) 2890.11-2855.30 (CH aliphatic), 2060.45, 2082.19 (S=C=N), 1621.60 (C=N Schiff), 1449.21 (C-C), 1116.78 (C-N), 514.83 (M-N), 467.51 (M-O). UV-Vis: λ_{max}/nm DMSO

745.00 (d→d*).574.00 (LMCT); 305 (n→π*); 279.00 (π→π*). The x-ray crystal structure for the complex has been reported (Nura *et al.*, 2011).

Dichlorido{*N,N*-dimethyl-*N'*-[1-(2-pyridyl)ethylidene] ethane-1,2-diamineκ^3*N,N',N"*} manganese(II) [Mn(L1)Cl$_2$]

Brown solid, 82% yield, m.p. 345-350 °C. Analytical calculated for C11H17Cl2MnN3 (317.12) C, 41.66; H, 5.40; N, 13.25. Found C, 41.68; H, 5.41; N, 13.25. IR: ATR ν_{max}/cm^{-1} 3100.37 (CH aromatic), 2838.02-2823.55 (CH aliphatic), 1650.30 (C=N Schiff), 1463.72 (C-C), 1109.21 (C-N), 554.54 (M-N). for x-ray crystal structure of the manganese(II) complex has been reported (Nurul *et al.*, 2011).

Dibromido{*N,N*-dimethyl-*N'*-[1-(2-pyridyl)ethylidene] ethane-1,2-diamineκ^3*N,N',N"}* copper(II) [Cu(L1)Br$_2$]

Dark blue solid, 79% yield, m.p. >400, Analytical calculated for C11H17Br2CuN3 (414.63): C, 31.86; H, 4.13; N, 10.13, Found: C, 32.09; H, 4.33; N, 10.90. IR (ATR cm−1): 3020.13 ν(C-H), 2086.68, 2016.35 ν(C=N), 1656.11 ν(C-C), 144.44 ν(C-N), 1115.16 ν(M-N), 522.96 ν(M-N).

{*N,N*-dimethyl-N'-[1- (2-pyridyl) ethylidene] ethane-1,2-diamineκ^3*N,N',N"}*bis(azido-κ^N)copper(II) [Cu(L1)(N$_3$)$_2$]

Green solid, 63% yield, Melting point 390-395 ºC. Analytical calculated for C11H17CuN9 (338.86): C, 38.99; H, 5.06; N, 37.20, Found: C, 40.09; H, 5.04; N, 37.90. IR: ATR ν_{max}/cm^{-1} 3070.00 (CH aromatic), 2959.67-2810.48 (CH aliphatic), 2050.45 (N=N=N), 1650.15 (C=N Schiff), 1441.12 (C-C), 1113.20, 1144.79 (C-N), 543.40 (M-N).

Diiodido{*N,N*-dimethyl-N'-[1-(2-pyridyl)ethylidene]ethane-1,2-diamineκ^3*N,N',N"*}zinc(II) [Zn(L1)I$_2$]

Grey solid, 55% yield, Melting point >400 ºC. Analytical calculated for C11H17I2N3Zn (510.47): C, 25.88; H, 3.36; N, 8.23. Found: C, 26.26; H, 3.67; N, 7.91. IR: ATR ν_{max}/cm^{-1} 3070.77 (CH aromatic) 2956.52-2852.42 (CH aliphatic), 1652.20 (C=N Schiff), 1433.32 (C-C), 1119.13 (C-N), 557.71 (M-N). ^{1}H-NMR (600 MHz, δ, DMSO-d_6): 8.595-8.588 (d, 1H, aromatic protons), 8.27-8.20 (m, 2H, aromatic protons), 7.85-7.80 (t, 1H, aromatic protons), 3.82 (s, 4H, 2CH$_2$), 3.74-3.72 (t, 2H, 2CH$_2$), 2.82-2.80 (t, 6H, 3CH2), 2.65 (s, 3H, CH$_3$). ^{13}C-NMR (600 MHz, δ, DMSO-d_6): 167.50 (1C, C=N), 145.46 δ(C aromatic carbon), 142.63 (CH aromatic carbon), 140.41 (CH aromatic carbon), 127.66 (CH aromatic carbon), 122.81 (CH aromatic carbon), 63.45 (2C, 2CH$_2$ ethylenediamine carbons), 56.09 (2C, 2CH$_3$N,N dimethyl), 15.82 (1C, CH$_3$ methyl ketone carbon).

{*N,N*-dimethyl-N'-[1- (2-pyridyl) ethylidene] ethane-1,2-diamineκ^3*N,N',N"*}- bis (thiocyanato-κ^N) manganese(II) [Mn(L1)(SCN)$_2$(H$_2$O)]

Brown solid, 86% yield, Melting point 375-380 ºC. Analytical calculated for C13H17MnN5S2 (362.38): C, 43.09; H, 4.73; N, 19.33. Found: C, 45.64; H, 5.05; N, 19.49. IR: ATR ν_{max}/cm^{-1} 3043.53 (CH aromatic), 2984.21-2855.19 (CH aliphatic), 2081.01 ν(S=C=N), 1651.08 ν(C=N), 1442.00 (C-C), 1165.11 (C-N), 461.09 (M-N).

2-(1-(2-(dimethylamino)ethylimino)ethyl)phenol (L2)

The ligand was obtained from the condensation reaction of 22-hydroxyacetophenone (0.61 g, 5 mmol) and *N,N*-dimethylethyldiamine (0.44 g, 5 mmol) in ethanol (25 mL) at a temperature of 75–85 °C and refluxed for three hours. The product is oily orange solution was formed, which became orange colored hygroscopic solid after 12 hours at 55°C in an oven. The solid product was dissolved in methanol and heated to 60 °C. After evaporating from the solvent under reduced pressure, an orange colored solid was formed. This ligand was characterized by using melting point, elemental analysis, IR, NMR and UV/Vis-spectroscopy. Yield: 45%. Molecular formula: $C_{12}H_{18}N_2O$ (206.28)**.** Analytical Calculated. (Found): C, 69.87 (68.48); H, 8.80 (8.98); N, 13.58 (14.98). IR (ATR cm−1): 3422.23 ν(O-H) 2944.14 ν(C-H), 1642.32 ν(C=N), 1445.43 ν(C-C), 1182.71 ν(C-N). UV-Vis [λ_{max} (nm) (DMSO)]: 322 (n→π*); 223 (π→π*). ^{1}H-NMR (600 MHz δ, DMSO-*d*6): 11.09 (1H, OH), 8.23-7.89 (CH$_2$-Ar), 2.74-2.69 (NCH$_2$-CH$_2$N), 2.22 (CH$_3$-), 1.9 (6H, CH$_3$-N). ^{13}C-NMR (600 MHz δ, DMSO-*d*6): 169.60 (C=N) 150.59 (1C, CO-phenolic), 148.84 (1C, C-aromatic), 142.56 (1C, CH-aromatic), 129.83 (1C, CH.aromatic), 125.62 (1C, CH-aromatic), 57.43 (2C, CH$_2$-aliphatic), 46.44, 46.30 (2C, CH$_3$-N,N-dimethyl) 17.69 (1C, CH$_3$-methyl).

COORDINATED COMPLEXES FOR L2

A series of copper(II), manganese(II), nickel(II) and zinc(II) complexes of **L2** figure 2 Schiff base were prepared *in situ* by mixing 2-hydroxyacetophenone (0.20 g, 1.65 mmol) and 4-(2-aminoethyl)morpholine (0.21 g, 1.65 mmol) in ethanol (20 ml) and refluxed. After 2 hr an ethanolic solution containing metal(II) halides was then added and the mixture was refluxed for 2-5 hours (Scheme 1). The resultant precipitates were filtered off, washed with cold ethanol and dried under vacuum. The crystals of the complexes were obtained at different conditions.

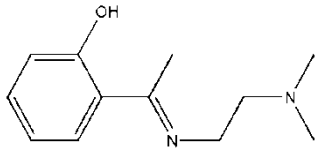


Fig. 2: Proposed structure for **L2**

Chlorido[2-(1-(2 (dimethylamino)ethylimino)ethyl) phenol] copper(II) [Cu(L2)Cl]

Green solid Melting point 355-360 °C. Analytical calculated for C12H17ClCuN2O (304.28): C, 47.37; H, 5.63; N, 9.21. Found: C, 48.19; H, 6.11; N, 9.39. IR: ATR ν_{max}/cm^{-1} 3105.05 (CH aromatic), 2957.60-2869.67 (CH aliphatic), 1579.98 (C=N Schiff), 1432.88 (C-C), 1113.24 (C-N), 526.00 (M-N) 492.67 (M-O).

Bromido[2-(1-(2-(dimethylamino)ethylimino) ethyl) phenol] copper(II) [Cu(L2)Br]

Brown solid Melting point 355-360 °C. Analytical calculated for C12H17BrCuN2O (348.73): C, 41.33; H, 4.91; N, 8.03. Found: C, 42.19; H, 4.91; N, 8.29. IR (ATR cm−1) 3102.43 (CH aromatic), 2980.76 (CH aliphatic), 1654.00 ν(C=N), 1433.12 (C-C), 1121.01 (C-N), 531.00 (M-N), 423.98 (M-O).

Chlorido[2-(1-(2-(dimethylamino)ethylimino) ethyl) phenol] zinc(II) [Zn(L2)Cl]

Yellow solid, 82% yield, Melting point >400 ºC. Analytical calculated for C12H17ClN2OZn (306.12): C, 47.08; H, 5.60; N, 9.15. Found: C, 48.48; H, 5.79; N, 9.98. IR (ATR cm−1) 3105.64 (CH aromatic), 2981.16, 2850.01 (CH aliphatic), 1654.08 ν(C=N), 1435.91 (C-C), 1119.87 (C-N), 531.12 (M-N), 433.53 (M-O). ^{1}H-NMR (600 MHz, δ, DMSO-d_6): 8.40-7.86 (4H, aromatic protons), 3.98-3.69 (4H, NCH_2-CH_2N ethylenediamine protons), 2.95-2.70 (6H, CH_3-N,N-dimethyl protons), 2.60 (3H, CH_3 methyl ketone protons). ^{13}C-NMR (600 MHz, δ, DMSO-d_6): 168.90 (C aromatic carbon), 148.01 (CH aromatic carbon), 146.64 (CH aromatic carbon), 140.50 (CH aromatic carbon), 137.22 (CH aromatic carbon), 134.40 (CH aromatic carbon), 65.60 (2C, $2CH_2$ ethylenediamine carbons), 56.45 (2C, CH_3-N,N-dimethyl), 18.90 (1C, CH_3 methyl ketone carbon).

Bromido[2-(1-(2-(dimethylamino)ethylimino) ethyl) phenol] zinc(II) [Zn(L2)Br]

White solid, 87% yield. Melting point 345-355 ºC. Analytical calculated for C12H17BrN2OZn (350.57): C, 41.11; H, 4.89; N, 7.99. Found: C, 42.94; H, 4.98; N, 7.99. IR (ATR cm−1) 3070.60 (CH aromatic), 2983.21 (CH aliphatic), 1650.01 (C=N Schiff), 1428.51 (C-C), 1121.41 (C-N), 557.29 (M-N), 451.01 (M-O). ^{1}H-NMR (600 MHz, δ, DMSO-d_6): 7.58-6.98 (4H, aromatic protons), 3.10-2.80 (2H, NCH_2-CH_2N), 3.98-3.66 (6H, CH_3-N,N-dimethyl), 2.24 (3H, CH_3, methyl ketone protons). ^{13}C-NMR (600 MHz, δ, DMSO-d_6): 169.52 (1C, C=N), 148.46 (C aromatic carbon), 145.60 (CH aromatic carbon), 141.40 (CH aromatic carbon), 126.90 (CH aromatic carbon), 120.80 (CH aromatic carbon), 60.40 (2C, $2CH_2$ ethylenediamine carbons), 53.09 (2C, CH_3-N,N-dimethyl), 15.81 (1C, CH_3 methyl ketone carbon).

Chlorido[2-(1-(2-(dimethylamino)ethylimino) ethyl) phenol] nickel(II) [Ni(L2)Cl]

Green solid, 76% yield, 360-365 ºC. Analytical calculated for C12H17ClN2NiO (299.42): C, 48.14; H, 5.72; N, 9.36. Found: C, 49.25; H, 5.73; N, 9.50. IR (ATR cm−1) 3050.05 (CH aromatic), 2743.53-2700.66 (CH aliphatic), 1605.42 (C=N Schiff), 1474.35-1436.35 (C-C), 1112.37 (C-N), 508.09 (M-N), 423.95 (M-O).

Bromido[2-(1-(2-(dimethylamino)ethylimino) ethyl) phenol] nickel(II) [Ni(L2)Br].

Brown solid, 68% yield, Melting point >400 ºC. Analytical calculated for C12H17BrN2NiO: (343.87): C, 41.91; H, 4.98; N, 8.15. Found: C, 42.00; H, 5.01; N, 8.28. IR (ATR cm−1) 3058.77 (CH aromatic), 2780.11-2745.30 (CH aliphatic), 1597.05 (C=N Schiff), 1471.21 (C-C), 1116.78 (C-N), 505.83 (M-N), 422 (M-O).

Chlorido[2-(1-(2-(dimethylamino)ethylimino) ethyl) phenol] manganese(II) [Mn(L2)Cl]

Brown solid, 73% yield, Melting point 380-385 ºC. Analytical calculated for C12H17ClMnN2O (295.67): C, 48.75; H, 5.80; N, 9.47. Found: C, 48.99; H, 5.84; N, 9.50. IR(ATR cm−1) 3103.63(CH aromatic), 2834.23-2815.08(CH aliphatic), 1651.50ν(C=N), 1449.24 (C-C), 1105.55 (C-N), 528.23 (M-N), 461.77(M-O).

Bromido[2-(1-(2-(dimethylamino)ethylimino) ethyl) phenol] manganese(II) [Mn(L2)Br]

Brown solid, 65% yield, Melting point >400 ºC. Analytical calculated for C12H17BrMnN2O (340.12): C, 42.38; H, 5.04; N, 8.24. Found: C, 42.97; H, 5.17; N, 8.40. IR (ATR cm−1) 3107.01 (CH aromatic), 2838.02-2813.55 (CH aliphatic), 1650.80 (C=N Schiff), 1443.72 (C-C), 1109.21 (C-N), 524.54 (M-N) 461.59 (M-O).

Iodo[2-(1-(2-(dimethylamino)ethylimino)ethyl)phenol] zinc(II) [Zn(L2)I]

Yellow solid, 82% yield, Melting point >400 ºC. Analytical calculated for C12H17ClN2OZn (359.44): C, 40.10; H, 4.77; N, 7.79. Found: C, 41.08; H, 4.79; N, 7.98. IR: ATR ν_{max}/cm^{-1} 3110.07 (CH aromatic), 2974.47 (CH aliphatic), 1656.07 ν(C=N), 1421.82 (C-C), 1115.53 (C-N), 521.12 (M-N), 423.92 (M-O). ^{1}H-NMR (DMSO-d_6,): δ 8.60-7.85 (4H, aromatic protons), 3.88-3.69 (8H, CH_2 morpholine), 2.85-2.65 (4H, NCH_2-CH_2N ethylenediamine protons), 2.55 (3H, CH_3 methyl ketone protons). ^{13}C-NMR (DMSO-d_6,) 166.50 (1C, C=N), 145.46 (C aromatic carbon), 142.60 (CH aromatic carbon), 140.40 (CH aromatic carbon), 124.90 (CH aromatic carbon), 120.20 (CH aromatic carbon), 70.40 (2C, $2CH_2$ ethylenediamine carbons), 60.29 (2C, 2CH3N ethylene carbons), 18.00 (1C, CH_3 methyl ketone carbon).

MTT-CULTURE OF CELLS AND CYTOTOXICITY ASSAY

The MCF-7 cells (human breast cancer cells) were seeded into 96 well plates at an initial cell density of approximately $5x10^5$ cells cm^{-3}. After 24 hours incubation for cell attachment and growth, the medium was removed and replaced with fresh medium containing varying concentrations of the compounds. The compounds added were first dissolved in DMSO at the required concentration. Subsequent 6 desirable concentrations was prepared using growth medium.

Control wells received only DMSO. Each concentration of the compound under study was assayed in six replicates. The assay was terminated after 48 hours incubation period. Again, the medium was removed and cell viability was determined after further 4 hours with 5 mg cm^{-3} MTT [3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium] bromide; also named thiazol blue.

DMSO was then added per well and the dissolving formazan precipitate was read by using elisa plate reader, Dynatech MR5000 at 570 nm. And comparison was made with positive control cisplatin.

ANTIMICROBIAL TESTING

The synthesized compounds were screened for potential antibacterial activity by testing against two randomly selected strains from each bacterial species, using disc diffusion assay (Jeffrey *et al.*, 2008). Briefly, a loopful of an overnight bacterial culture of each strain was suspended in sterile 0.85% saline (BDH) to the concentration of approximately "10^8" cfu/ml (equivalent to 0.5 Mc Farland units) before inoculated evenly on the entire surface of Mueller Hinton agar (Oxoid) with a sterile cotton swab. Sterilized paper discs (Thermo Fisher, 6mm diameter), impregnated with 30 µg of respective synthetic Schiff compound, and was placed on the inoculated agar plate. DMSO disc was used as a negative control in the test. The diameter of inhibition zone around the impregnated paper disc was measured after 18 hour of incubation at 37˚C. All tests were performed in duplicate. Synthetic Schiff compounds, in which inhibition zone was observed in any one of the four species tested, were selected for MIC with broth micro-dilution assay. Only compounds with MIC value lesser than "10 µg/mL," is considered potentially active against the corresponded bacterial species; and further tested against the entire collection of the corresponded bacterial species with disc diffusion assay. This was to determine the variation in the susceptibility responses to the tested compound within the bacterial species tested.

The two selected strains for each species in the initial screening were: MRSA 0804-25 and MRSA 0807-7 for MRSA; AC 0612-7 and AC 0903-21 for *A. baumannii*; KB 71 and KB 83 for *K. pneumonia*; and PA45 and PA104 for *P. aeruginosa*.

MIC determination

Minimum inhibition concentration (MIC) was determined with 96-well broth micro-dilution assay. The inoculum was prepared by suspending an overnight culture in a sterile 0.85% saline (BDH) to approximate 10^8 cfu/ml (equivalent to 0.5 McFarland units) and further diluted in cation adjusted Mueller Hinton broth (CMHB; BD) to the concentration of 10^6 cfu/ml. Two-fold serial dilutions of the tested compound were prepared in CMHB across the 96-well microtiter plate with the highest concentration starting from 750 ppm (750 µl/ml) in duplicate rows. The prepared suspension of the bacteria was added to each well except the negative control wells of the microtitre plate in a 1:1 ratio with final bacteria concentration of approximately "5 x 10^5" cfu/ml. The inoculated microtitre plates were incubated at 37˚C for 24 hour.

The MIC was determined as the lowest concentration of the test compound that exhibits no visible growth. A further confirmative test was done by adding 30 µl of MTT dye (Sigma) to each well. Wells with color changes within 30 - 60 minutes incubation at room temperature were scored as positive growth. The lowest concentration of the tested compound that exhibits no color changes (yellow) is determined as the MIC value.

RESULTS AND DISCUSSION

Synthetic Chemistry

The reaction of *N,N*-dimethylethylenediamine with 2-acetylpyridine resulted in the formation of corresponding Schiff base ligand; *N,N'*-dimethyl-N2-(1-(pyridin-2-yl)ethylidene)ethane-1,2-diamine (**L1**). The prepared Schiff bases further reacted copper(II), manganese(II), nickel(II) and zinc(II) ions in the presence of either Cl^-, Br^-, I^-, SCN^- and N_3^- ions giving rise to different coordination complexes (Scheme 1). The compounds exhibited NMR, IR and UV-Visible spectra consistent with the proposed structures which allowed the synthesized complexes to be recognized as Dichlorido{*N,N*-dimethyl-*N'*-[1-(2-pyridyl)ethylidene]ethane-1,2-diamineκ^3*N,N',N"*} manganese(II) [Mn(L1)Cl_2], Aqua {*N,N*-dimethyl -*N'*-[1-(2-pyridyl)-ethylidene] ethane-1,2-diamineκ^3*N,N',N"*}bis(thiocyanato-κ^N) nickel (II) [Ni(L1) $(SCN)_2(H_2O)$], Dibromido{*N,N*-dimethyl-*N'*-[1-(2-pyridyl)ethylidene]ethane-1,2-diamineκ^3*N,N',N"*}zinc [Zn(L1) Br_2], Dichlorido {N,N-dimethyl- N'-[1-(2-pyridyl)ethylidene] ethane-1,2-diamineκ^3 *N,N',N"*}copper(II) [Cu(L1)Cl_2], Dibromido {N,N-dimethyl-N'-[1-(2-pyridyl)ethylidene]ethane- 1,2-diamineκ^3 N,N',N"}copper(II) [Cu(L1)Br_2], [N,N-dimethyl-N'-[1-(2-pyridyl) ethylidene]ethane-1,2-diamineκ^3N,N',N"]bis(azido-κN)copper(II), [N,N-dimethyl-N'-[1-(2-pyridyl)ethylidene]ethane-1,2- diamineκ^3 N,N',N"]-bis (thiocyanato-κN)manganese(II)[Mn(L1)$(SCN)_2$ (H_2O)], Diiodido{N,N-dimethyl-N'-[1-(2-pyridyl)ethylidene] ethane-1,2-diamineκ^3N,N',N"} zinc(II) [Zn(L1)I_2]. Also the reaction of *N,N*-dimethylethylenediamine with 2-hydroxy-acetophenone resulted in the formation of corresponding Schiff base ligands 2-(1-(2-(dimethylamino) ethylimino)ethyl)phenol (**L2**) further reacted copper(II), manganese(II), nickel(II) and zinc(II) ions in the presence of Cl^-, Br^-, I^-, SCN^- and N_3^- ions giving rise to different coordination complexes (Scheme 1) namely: Chlorido[2-(1-(2-(dimethylamino)ethylimino)ethyl) phenol] copper(II) [Cu(L2)Cl], Bromido[2-(1-(2-(dimethylamino) ethylimino)ethyl)phenol] copper(II) [Cu(L2)Br], Chlorido[2-(1-(2-(dimethylamino) ethylimino)ethyl)phenol]zinc(II) [Zn(L2)Cl], Bromido[2-(1-(2-(dimethylamino) ethylimino) ethyl) phenol] zinc(II)[Zn(L2)Br],Chlorido[2-(1-(2-(dimethylamino) ethylimino) ethyl) phenol]nickel (II) [Ni(L2)Cl], Bromido[2-(1-(2-(dimethylamino) ethylimino) ethyl)phenol]nickel(II) [N1(L2)Br], Chlorido [2-(1-(2-(dimethylamino)ethylimino)ethyl)phenol] manganese (II) [Mn(L2)Cl], Bromido[2-(1-(2-(dimethylamino) ethylimino)ethyl) phenol]manganese(II) [Mn(L2)Br], Iodo[2-(1-(2-(dimethylamino) ethylimino)ethyl)phenol]zinc(II) [Zn(L2)I].

Elemental analysis data and physical properties of ligands and complexes are summarized in the experimental part; ligands were obtained in a liquid form. The reaction of ligands with transition metals produced a series of metal complexes with different colors. The data obtained from the melting point

apparatus showed that the complexes' melting point were higher than 300ºC. The percentages of C, H and N obtained are in agreement with calculated values. The IR spectra of transition metal complexes were carried out in 4000–400 cm^{-1} range. The characteristic IR stretching frequencies of the metal complexes along with their proposed assignments are summarized in experimental part. There are similarities in the IR spectrum of the metal complexes to each other, except for some slight variations in the shifts and intensities of few vibration peaks caused by different metal (II) ions, indicating that the metal complexes had similar structure. However, there were some significant differences between the metal (II) halides complexes and that of the azides and thiocyanate complexes, as expected. The IR spectra of all the complexes possess very strong characteristic absorption bands in the region of 1670.00-1650.00cm^{-1} which is attributed to the C=N stretching vibration of the Schiff base imino functional group (Gwaram *et al.,* 2012; Khan *et al.,* 2011; Nakamoto *et al.,* 1978; Laskar *et al.,* 2001 and Banerjee *et al.,* 2005). For thiocyanates and azide complexes a chemical shifts were observed at a region of 2110-2020 attributable to metal coordination (Pallab *et al.,* 2010). The spectra for the complex showed M–N bands at a lower wavelength in the range of 477-575 cm^{-1} (Raman *et al.,* 2011; Khan *et al.,* 2011). Electronic spectra and magnetic properties of complexes were recorded in DMSO and are summarized in table 1. The electronic spectra of Mn^{2+} ion $Mn(L1)Cl_2$ exhibited bands at 272, 603 and 813 corresponding to $^6A_1 \rightarrow {}^4E(D)$ in distorted square bipyramidal geometry (Syamal *et al.,* 2002). $Mn(LMA)(NCS)_2(H_2O)$ with a transitions of $^6A_1 \rightarrow {}^4E(D)$ and $^6A_1 \rightarrow {}^4T_2(D)$ can attributed to the band at 203, 486, 327, and 641 nm indicates that they have tetrahedral geometries. The electronic spectra of Mn(L2)Br and Mn(L2)Cl indicate that they have tetrahedral geometries. The $^6A_1 \rightarrow {}^4E_{(D)}$ and $^6A_1 \rightarrow {}^4T_{2(D)}$ can attributed to the band at 290, 606, 813 and 364, 601 nm respectively (Syamal *et al.,* 2002; Lever 1968). Moreover, the magnetic moments of the complexes were 5.30-6.50 BM which was within the expected spin-only value. The electronic spectra of Ni(II) complexes $Ni(LMA)(NCS)_2(H_2O)$ showed d-d transitions in the region of 270, 364 and 807. These are assigned to the transitions $^3A_2g_{(F)} \rightarrow {}^3T_1g_{(P)}$, $^3A_2g_{(F)} \rightarrow {}^3T_1g_{(F)}$ and $^3A_2g_{(F)} \rightarrow {}^3T_2g_{(F)}$ consistent with distorted octahedral geometry (Tansir *et al.,* 2008; Shayma *et al.,* 2009; Cotton *et al.,* 1998 and Selbin *et al.,* 1983). The magnetic moments of nickel(II) complexes are in the range of 2.90-3.00 BM as expected for the complexes having monomeric structures. The higher value of the magnetic moment for Ni^{2+} d^8 complex was 3.00 BM which was probably due to the orbital contribution. The spectrum of Cu(II) complex $Cu(L1)(NCS)_2$ complex exhibits broad bands in the region of 28190 and 643 corresponding to the d-d transition $^2e_2g_{(D)} \rightarrow {}^2b_1g_{(D)}$. Therefore, these transitions confirmed that the Cu(II) complex has a distorted square pyramidal geometry (Shayma *et al.,* 2010). The magnetic moment ($\mu_{eff.}$) values in the range of 1.85-2.30 BM for the copper(II) complexes are within expected region for the high spin system table 1.

Lastly the electronic spectra for Zn(II) and Cd(II) complexes with an electronic configuration of d^{10} did not presented any (d-d) transitions. Instead the absorption bands observed were due to charge transfer transitions which suffered from blue shift with hyper chromic effect (Shayma *et al.,* 2009). Coordination of the free ligand to Zn(II) and Cd(II) ions, is supported by the appearance of three main bands. Both $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition bands experience bathochromic shift and appear at the shorter wavelength of 220-250 nm and 280-370 nm respectively (Mohamed *et al.,* 2009). The exhibition of the new bands at the region between 300-630 nm can be associated with the (LMCT) charge transfer in the spectra of metal complexes supported the formation of the Zinc(II) and Cadmium(II) complexes (Yusnita *et al.,* 2009; Gwaram *et al.,* 2012). All these absorptions for the free ligands and their corresponding metal complexes have been fully assigned. The chemical shift in the region 8.70-7.80 ppm, were observed for zinc and tin complexes respectively and they were assigned to the aromatic ring protons (Ceyhan *et al.,* 2011). The other single peaks appeared in the region 2.60 ppm-2.90ppm respectively were attributed to $\delta(CH_3)$ indicating the methyl on the carbonyl group in the complexes (Ceyhan *et al.,* 2011). In the ^{13}C NMR spectra of the metal complexes, the signal at region of 165.00 ppm-170.00 can be assigned to the azomethine (C=N) carbon atoms for complexes (Gwaram *et al.,* 2012). Aromatic ring carbon atoms of the ligands were determined in the region of 120.00-160.00 ppm (Gwaram *et al.,* 2012).

X-ray crystallography

The compound crystallizes with two molecules per asymmetric unit into triclinic crystal system a = 9.70890(10) Å, b = 10.16870(10) Å, c = 15.8397(2) Å, α = 76.3130(10)°, β = 82.1760(10)°, γ = 77.0200(10)° with a space group of P-1. It is clear that the two molecules in the asymmetric unit are almost identical. The asymmetric unit consists of two geometrically slightly different molecules; the weighted r.m.s. fit for the superposition of the non-H atoms in both molecules (after inversion) being 0.078 Å. The zinc(II) ion in $Zn(L1)Br_2$ is penta-coordinated by the *N,N',N''*-tridentate Schiff base ligand and two Br atoms in a distorted square-pyramidal geometry. The molecular structure with atom-numbering scheme is shown in figure 3, the τ value being 0.17 which is in accordance with (Addison *et al.,*. 1984). The crystal parameters are reported in table 2 and selected bond lengths and angles are given in table 3 and table 4 respectively. This arrangement is similar to what was observed in the structure of the closest analogous zinc complex (Gwaram *et al.,* 2011). In the crystal, pairs of the molecules, related by symmetry 1+ *x*, *y*, *z*, are bonded into centrosymmetric dimers *via* C7-H20B···Br_2 interaction figure 4. The Zn-Br and Zn—N bond lengths (Zn2-Br1 = 2.4356(5), Zn1-Br2 = 2.4152(6), Zn2-Br3 = 2.4187, Zn1-Br4 = 2.4201 and Zn2-N1 = 2.183(3), Zn2-N2 = 2.112(3), Zn2-N3 = 2.223(3), Zn1-N4 = 2.201(3), Zn1-N5 = 2.106(3), Zn1-N6 = 2.217) are comparable to those reported for similar complexes (Gwaram *et al.,* 2011).

Table. 1: Electronic spectra and magnetic properties of complexes.

Compound	Parameters				
	λ_{max}	Absorb.	ε_{max} ($Lmol^{-1}cm^{-1}$)	Assignment	μ_{eff} (B.M)
L1	487	0.058	116.00	n→ π*	-
$[Mn(L1)Cl_2]$	272	2.591	5182.00	$^6A_1g \rightarrow {}^4E_1g_{(G)}$	5.34
	603	0.065	130.00	$^6A_1g_{(F)} \rightarrow {}^4T_2g_{(G)}$	
	813	0.094	188.00	$^6A_1g_{(F)} \rightarrow {}^4T_1g_{(G)}$	
$[Ni(L1)(NCS)_2H_2O]$	270	2.061	4042.00	$^3A_2g_{(F)} \rightarrow {}^3T_1g_{(P)}$	2.98
	364	0.900	1800.00	$^3A_2g_{(F)} \rightarrow {}^3T_1g_{(F)}$	
	807	0.109	218.00	$^3A_2g_{(F)} \rightarrow {}^3T_2g_{(F)}$	
$[Cu(L1)(NCS)_2]$	281	2.696	5392.00	C.T. M → L	1.89
	643	0.125	250.00	$^2E_{2g(D)} \rightarrow {}^2B_{1g(D)}$	
$[Zn(L1)Cl_2]$	284	3.675	7350.00	C.T. M → L	Zero
	603	0.084	168.00	C.T. M → L	
$[Zn(L1)(NCS)_2]$	280	2.285	4570.00	π→ π*	Zero
	606	0.064	128.00	C.T. M → L	
$[Cd(L1)Cl_2]$	279	0.285	570.00	C.T. M → L	Zero
	407	0.089	178.00	C.T. M → L	
$[Cd(L1)(NCS)_2]$	280	2.767	5534.00	π→ π*	-
	467	0.055	110.00	C.T. M → L	
L2	272	3.612	7224.00	π→ π*	Zero
	364	1.823	3646.00	n→ π*	
[Mn(L2)Br]	290	2.285	4570.00	C.T. M → L	6.40
	606	0.064	128.00	$^6A_1 \rightarrow {}^4E_{(D)}$	
	813	0.094	188.00	$^6A_1 \rightarrow {}^4T_{2(D)}$	
[Mn(L2)Cl]	364	0.750	1500.00	$^6A_1 \rightarrow {}^4E_{(D)}$	6.10
	601	0.124	248.00	$^6A_1 \rightarrow {}^4T_{2(D)}$	
[Zn(L2)Br]	279	0.241	482.00	n→ π*	Zero
	307	1.068	2138	C.T. M → L	
[Zn(L2)Cl]	277	2.672	5344.00	n→ π*	Zero
	626	0.114	228.00	C.T. M → L	

Table. 2: Crystal data and structure refinement for $Zn(L1)Br_2$

Identification code	a
Empirical formula	$C_{11}H_{17}Br_2N_3Zn$
Formula weight	416.47
Temperature/K	569(2)
Crystal system	triclinic
Space group	P-1
a/Å	9.70890(10)
b/Å	10.16870(10)
c/Å	15.8397(2)
α/°	76.3130(10)
β/°	82.1760(10)
γ/°	77.0200(10)
Volume/$Å^3$	1474.88(3)
Z	4
$\rho_{calc}mg/mm^3$	1.876
m/mm^{-1}	7.067
F(000)	816.0
Crystal size/mm^3	0.4 × 0.2 × 0.1
2Θ range for data collection	2.66 to 50.5°
Index ranges	$-11 \le h \le 11$, $-11 \le k \le 12$, $-19 \le l \le 19$
Reflections collected	11871
Independent reflections	5314[R(int) = 0.0269]
Data/restraints/parameters	5314/0/308
Goodness-of-fit on F^2	0.994
Final R indexes [I>=2σ (I)]	$R_1 = 0.0270$, $wR_2 = 0.0674$
Final R indexes [all data]	$R_1 = 0.0350$, $wR_2 = 0.0714$
Largest diff. peak/hole / e $Å^{-3}$	1.12/-0.64

Table. 3: Bond Lengths for $Zn(L1)Br_2$

Table 2: Bond Lengths for $Zn(L1)Br_2$

Atom	Atom	*Length/Å*	Zn2	Br1	2.4356(5)	Zn2
Zn1	Br2	*2.4152(6)*	Zn2	Br3	2.4187(6)	Zn2
Zn1	Br4	*2.4201(6)*	Zn2	N1	2.183(3)	Zn2
Zn1	N4	*2.201(3)*	Zn2	N2	2.112(3)	Zn2
Zn1	N5	*2.106(3)*	Zn2	N3	2.223(3)	Zn2
Zn1	N6	*2.217(3)*	Zn2	Br1	2.4356(5)	Zn2

Table. 4: Selected Bond lengths for $Zn(L1)Br_2$

Table 3: Selected Bond lengths for $Zn(L1)Br_2$									
Br2	Zn1	Br4	113.98(2)	N3	Zn2	Br1	102.10(8)	N3	Zn2
N4	Zn1	Br2	98.56(8)	N3	Zn2	Br3	97.06(8)	N3	Zn2
N4	Zn1	Br4	94.57(8)	C1	N1	Zn2	114.6(2)	C1	N1
N4	Zn1	N6	150.60(12)	C5	N1	Zn2	126.2(2)	C5	N1
N5	Zn1	Br2	109.33(9)	C5	N1	C1	119.1(3)	C5	N1
N5	Zn1	Br4	136.43(9)	C6	N2	Zn2	119.9(3)	C6	N2
N5	Zn1	N4	74.27(11)	C6	N2	C8	122.9(3)	C6	N2
N5	Zn1	N6	78.20(12)	C8	N2	Zn2	116.7(2)	C8	N2
N6	Zn1	Br2	100.09(8)	C9	N3	Zn2	104.6(2)	C9	N3
N6	Zn1	Br4	98.33(9)	C9	N3	C10	110.8(3)	C9	N3
Br3	Zn2	Br1	111.98(2)	C9	N3	C11	109.9(3)	C9	N3
N1	Zn2	Br1	97.39(8)	C10	N3	Zn2	111.4(2)	C10	N3
N1	Zn2	Br3	95.85(8)	C10	N3	C11	109.2(3)	C10	N3
N1	Zn2	N3	150.54(11)	C11	N3	Zn2	110.9(2)	C11	N3
N2	Zn2	Br1	111.45(8)	C12	N4	Zn1	114.8(2)	C12	N4
N2	Zn2	Br3	136.38(8)	C16	N4	Zn1	126.1(3)	C16	N4
N2	Zn2	N1	74.76(11)	N2	Zn2	N3	77.63(11)		N2

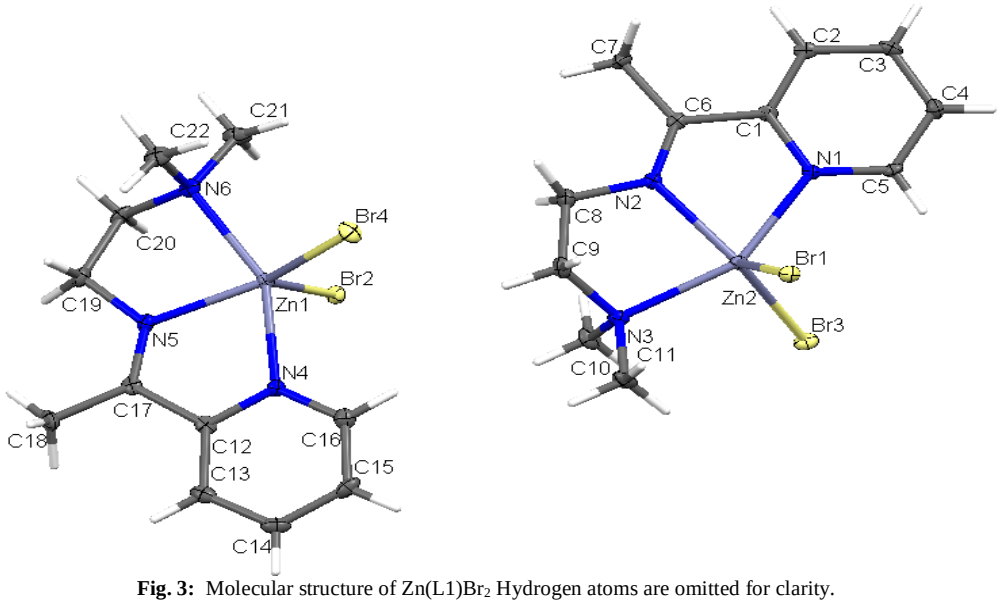


Fig. 3: Molecular structure of $Zn(L1)Br_2$ Hydrogen atoms are omitted for clarity.

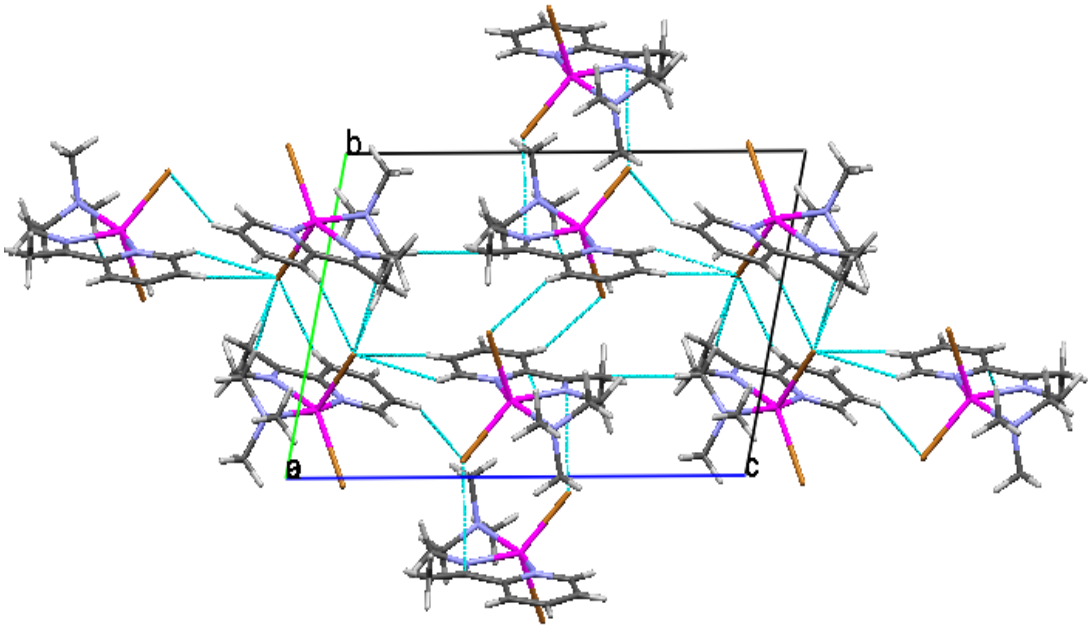


Fig. 4: Packing structure along a-axis of $Zn(L1)Br_2$

MTT assay

MTT assay was used to determine the metal complexes cytotoxicity against human breast cancer cells MCF-7. This assay served as an index used to determine cytotoxicity of the metal complex to stimulate or inhibit cell viability and growth by detecting the reduction of tetrazolium salt to blue formazan by mitochondrial enzyme activity of succinate dehydrogenase in living cells. MCF-7 cells were treated with varying concentrations of the complexes for 48 h, and the cells viabilities were measured by MTT assay. The metal complexes were found to have showed a very weak activity on MCF-7 breast cancer cell line table 5, as compared to the current anticancer drugs.

Antibacterial activity

A series of Schiff-bases compounds were synthesized and screened for potential antibacterial activity against the emerging multi-drug resistance nosocomial bacterial pathogens in hospital settings, namely, MRSA, *A. baumanii*, *K. pneumonia* and *P. aeroginosa*. In the first level antibacterial screening with disc diffusion assay, all compounds under study were tested against eight clinical strains, with two from each bacterial species. Inhibition zones were observed in metal-bonded Schiff base compounds. However, no inhibition zone was observed in any of the compounds tested against *K. pneumonia*.

All of the copper (II) and nickel(II) metal-bonded Schiff-base compounds were moderately inhibitive against MRSA, while showed weak antibacterial activity against *A. baumanii* and *P. aeroginosa* in the disc diffusion test table 6. The compounds were then analyzed further for the MIC value using broth micro-dilution assay. The result from broth micro-dilution assay was in total analogue to the disk diffusion screening. The test showed that, all of the copper and Nickel compounds were active against MRSA, with MIC value ranged from 2.0 to 3.4 µg/mL table 6. Both *A. baumanii* and *P. aeroginosa* were resistant to the tested compounds. Therefore, it was concluded that compounds were inhibitive only against the gram positive bacterium, MRSA. A comparative study of minimum inhibitory concentration (MIC) values of the Schiff base L and the two complexes indicate that the zinc complexes have better activity than the free Schiff base ligand. This higher antimicrobial activity of the metal complexes, compared with that of Schiff base ligands, is perhaps due to the change in structure as a result of coordination, and chelating tends to make metal complexes act more powerful and potent bacteriostatic agents, thus inhibiting the growth of the microorganisms (Zahid *et al.*, 2006; Chohan *et al.*, 2003). On chelating, the polarity of the metal atoms will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of positive charge of the metal atoms with donor atoms.

Table. 5: EC_{50} values in µg/ml of the tested compounds on WRL68 and MCF-7 cell lines.

	Cpd.	EC50 µg/ml			Cpd.	EC50 ug/ml	
		WRL68	MCF-7			WRL68	MCF-7
1	L1	n.d.	n.d.	**11**	L2	n.d.	n.d.
2	$Cu(L1)Cl_2$	>100	>100	**12**	Cu(L2)Cl	>100	>100
3	$Cu(L1)Br_2$	>100	>100	**13**	Cu(L2)Br	>100	>100
4	$Cu(L1)(N_3)$	65.5	53.37	**14**	Zn(L2)Br	>100	>100
5	$Ni(L1)(SCN)_2(H_2O)$	>100	>100	**15**	Zn(L2)Cl	>100	>100
6	$Mn(L1)Cl_2$	59.81	87.93	**16**	Ni(L2)Cl	>100	>100
7	$Mn(L1)(SCN)_2(H_2O)$	>100	>100	**17**	Ni(L2)Br	>100	>100
8	Zn(L1)I2	>100	>100	**18**	Mn(L2)Cl	45.06	61.53
9	$Zn(L1)Br_2$	23.94	>100	**19**	Mn(L2)Br	57.01	>100
10	Cisplatin	-	2.50	**20**	Zn(L2)I	>100	>100

Table. 6: The Summary of antimicrobial activity of the synthesis compound for 2 selected strains of Methicillin-resistant Staphylococcus aureus (MRSA, Acinetobacter baumannii (AC), pneumonia (KB) and Pseudomonas aeruginosa (PA).

Compound	MRSA 0804-25	MRSA 0807-7	AC 0612-7	AC 0903-21	KB 71	KB 83	PA 45	PA 104
Zone of inhibition in mm (MIC in µg/ml)								
L1	0	0	0	0	0	0	0	0
Cu(L1)Cl2	0	0	0	0	0	0	0	0
$Ni(L1)(NCS)_2$	20 (2.2)	18 (2.0)	10 (>90.0)	10 (>90.0)	0	0	8 (>90.0)	8 (>90.0)
Mn(L1)Cl2	0	0	0	0	0	0	0	0
Cu(L1)Br2	19 (2.6)	17 (2.8)	7 (>90.0)	7 (>90.0)	0	0	8 (>90.0)	8 (>90.0)
Cu(L1)N3	17 (3.0)	16 (3.0)	9 (>90.0)	8 (>90.0)	0	0	10	10
Zn(L1)I2	0	0	0	0	0	0	0	0
Zn(L1)Br2	0	0	0	0	0	0	0	0
$Mn(L1)(NCS)_2$	0	0	0	0	0	0	0	0
L2	0	0	0	0	0	0	0	0
Cu(L2)Cl	16 (3.2)	12 (3.4)	0	0	0	0	0	0
Cu(L2)Br	18 (3.0)	15 (3.0)	9 (>90.0)	8 (>90.0)	0	0	17 (6.0)	19 (4.0)
Zn(L2)Br	0	0	0	0	0	0	0	0
Zn(L2)Cl	0	0	0	0	0	0	0	0
Ni(L2)Cl	19 (2.6)	17 (2.6)	9 (>90.0)	8 (>90.0)	0	0	10 (>90.0)	9 (>90.0)
Ni(L2)Br	21 (2.2)	20 (2.8)	10	9	0	0	9 (>90.0)	9 (>90.0)
Mn(L2)Cl	0	0	0	0	0	0	0	0
Mn(L2)Br	0	0	0	0	0	0	0	0
Control (DMSO)	0	0	0	0	0	0	0	0

Further, it increases the delocalization of *p*-electrons over the whole chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity enhances the penetration of the complexes into lipid membrane and blocks the metal binding sites on enzymes of micro-organisms.

CONCLUSION

In this paper we presented synthesis, characterization and cytotoxicity mediated by synthesized metal complexes on MCF-7 cells. The Schiff-based complexes showed a moderate inhibitive activity only against the Gram positive bacterium (*Methicillin-resistant Staphylococcus aureus).* Very weak antimicrobial activity was observed with *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. No antimicrobial activity observed with *Klebsiella pneumonia.* Further study on time-kill assay is needed to confirm if the compound are bactericidal. In advanced, the study of mechanism of action (MOA) of the compound is suggested to further expand the knowledge on its target organism and application.

ACKNOWLEDGMENTS

The authors would like to thank the Department of Chemistry and the Institute of Biological Science, Faculty of Science, University of Malaya for the provision of laboratory facilities. The authors also wish to acknowledge the UMRG grant (RG066/12BIO) and CENAR (TA019-2012A) as well as flagship project (FL011-2012) provided by the University of Malaya to conduct this study.

REFERENCES

Addison A.W., Rao T.N., Reedijk, J., Rijn, V.J., Verschoor G.C. Synthesis, structure, and spectroscopic properties of copper(II) compounds containing nitrogen-sulphur donor ligands; the crystal and molecular structure of aqua[1,7-bis(*N*-methylbenzimidazol-2′-yl)-2,6-dithiaheptane]copper(II) perchlorate. J. Chem. Soc. Dalton Trans. 1984; *7:* 1349–1356.

Adoración G.Q., Carmen N.R. Contribution to the SAR field of metallated and coordination complexes: Studies of the palladium and platinum derivatives with selected thiosemicarbazones as antitumoral drugs. Coord. Chem. Rev. 2004; 248: 119-133.

Larry Y., Chemistry and Biology of Salicylihalamide A and Related Compounds. Chem. Rev. 2003; 103: 4283–4306

Banerjee S., Wu B., Lassahn P.-G., C. Janiak C., Ghosh A., Synthesis, structure and bonding of cadmium(II) thiocyanate systems featuring nitrogen based ligands of different denticity; Inorg. Chim. Acta 2005; 358: 535-544.

Bernadette S.C., Brian D., Denise A.E., Kevin K., Georgina R., Venkat R. T., Maureen W. Anticancer and antifungal activity of copper(II) complexes of quinolin-2(1*H*)-one-derived Schiff bases. Inorg. Chim. Acta. 2010; 363: 4048-4058.

Bernadette S.C., Michael D., Agnieszka F., Siobhán M., Georgina R., Venkat R.T., Maureen W. Quinolin-2(1H)-one-triazole derived Schiff bases and their Cu(II) and Zn(II) complexes: Possible new therapeutic agents. Polyhedron. 2010; 29: 813-822.

Bharti S.K., Nath G., Tilak R., Singh S.K. Synthesis, anti-bacterial and anti-fungal activities of some novel Schiff bases containing 2,4-disubstituted thiazole ring. Eur. J. Med. Chem. 2010; 45: 651-660.

Ceyhan G., Çelik, C. Antioxidant, electrochemical, thermal, antimicrobial and alkane oxidation properties of tridentate Schiff base ligands and their metal complexes. Spectrochim. Acta. 2011; 81: 184–198.

Chohan Z.H., Scozzafava A., Supuran C.T. Zinc complexes of benzothiazole-derived Schiff bases with antibacterial activity. J. Enz. Inhib. Med. Chem. 2003; 18: 259-263.

Cotton F.A., Wilkinson G. Advanced Inorganic Chemistry. Wiley-Interscience, New York 1998.

Selbin J., Marion C., and MC D: Theoretical Inorganic Chemistry Transition Elements. Reinhold, New York, 1983.

Garoufis A., Hadjikakou S.K., Hadjiliadis N. Palladium coordination compounds as anti-viral, anti-fungal, anti-microbial and anti-tumor agents. Coord. Chem. Rev. 2009; 253: 1384-1397, 2009.

Goe G.L. (1982) Pyridine and pyridine derivatives. In: Grayson, M. & Eckroth, D. Kirk- Othmer Encyclopedia of Chemical Technology, 3rd ed., New York, John Wiley & Sons, Vol. 19, p. 474.

Guidos R.J., The 10×'20 Initiative: Pursuing a Global Commitment to Develop 10 New Antibacterial Drugs by 2020. *Am. Clin. Infect. Dis.* 2010; *50*: 1081-1083.

Esposito S., Leone S. Antimicrobial treatment for Intensive Care Unit (ICU) infections including the role of the infectious disease specialist. Int. J. Antimicrob. Agents. 2007; *29*: 494-500.

Gwaram N.S., Ali H.M., Abdulla M.A., Buckle M.J.C., Sukumaran S.D., Chung L.Y., Othman R., Alhadi A.A., Yehye W.A., Hadi A.H.A., Hassandarvish P., Khaledi H., Abdelwahab S.I. Synthesis, Characterization, X-ray Crystallography, Acetyl Cholinesterase Inhibition and Antioxidant Activities of Some Novel Ketone Derivatives of Gallic Hydrazide-Derived Schiff Bases. Molecules. 2012; 17:2408-2427.

Gwaram N.S., Ali H.M., Khaledi H., Dichlorido{*N*,*N*-dimethyl-*N*'-[1-(2-pyridyl)ethylidene]ethane-1,2-diamine- ^{3}*N*,*N*',*N*"}zinc. Acta Cryst. 2011; E67: m1027.

Zahid H.C., Arif M., Muhammad A.A., Supuran C.T. Metal-Based Antibacterial and Antifungal Agents: Synthesis, Characterization, and In Vitro Biological Evaluation of Co(II), Cu(II), Ni(II), and Zn(II) Complexes with Amino Acid-Derived Compounds. Bioinorg. chem. Appl. 2006; 13: 2006.

Gwaram N.S., Ali H.M., Khaledi H., Abdulla M.A., Hadi A.H.A., Lin T.K., Ching C.L., Ooi C.L. Antibacterial Evaluation of Some Schiff Bases Derived from 2-Acetylpyridine and Their Metal Complexes. Molecules. 2012; 17:5952-5971.

Jeffrey L.W., Thomas R.S., Michael A., Donald J.B., Steven D.B., Jeffrey T.G., Henry H., Rob P.H., Dik J.M., Mark G.P., Peter S., Zurenko G.E. Performance Standards for Antimicrobial Disc and Dilution Susceptivity Tests for Bacteria Isolated from Animals; Approved Standards. In: A standard for global application developed through the clinical and laboratory standards institute concensus process. Clinical and Laboratory Standards Institute, USA, 2008; (pp M31-A3).

Khan N-uH., Pandya N., Prathap K.J., Kureshy R.I., Abdi S.H.R., Mishra S., Bajaj H.C., Chiral discrimination asserted by enantiomers of Ni (II), Cu (II) and Zn (II) Schiff base complexes in DNA binding, antioxidant and antibacterial activities. Spectrochim. Acta. 2011; 81: 199-208.

Nakamoto K. Infrared and Raman Spectra of Inorganic and Coordination Compounds. John Wiley & Sons Inc, Atlanta, GA, U.S.A, 1978.

Kureshy R.I., Khan N.H., Abdi, S.T. Synthesis, physico-chemical studies and solvent dependent enantio-selective epoxidation of 1,2-dihydronaphthalene catalyzed by chiral ruthenium(II) Schiff base complexes. II, J. Mol. Catal., 1999; 150: 175-183.

Laskar I.R., Maji T.K., Das D., Lu T.H., Wong W.T., Okamoto Ki., Ray C. N. Syntheses, characterisation and solid state thermal studies of 1-(2-aminoethyl)piperidine (L), 1-(2-aminoethyl)pyrrolidine (L') and 4-(2-aminoethyl)morpholine (L") complexes of nickel(II): X-ray single crystal structure analyses of trans-[NiL2(CH3CN)2](ClO4)2, trans-[NiL2(NCS)2] and trans-[NiL"2(NCS)2]. Polyhedron. 2001; 20: 2073-2082.

Lever A.B.P: Inorganic Electronic Spectroscopy. Elsevier publishing, Amsterdam, 1968. Tansir A., Nahid N., Parveen S. Synthesis, characterization and anti-microbial studies of a newlydeveloped polymeric

Schiff base and its metal-polychelates. J. Coord. Chem. 2008; 61: 1963–1972.

Ling-Wei X., Gan-Qing Z., Yong-Jun H., Yun-Xiao F. Synthesis, Structures, and Antimicrobial Activity of Schiff Base Zinc Complexes with Thiocyanate and Iodide. Synth. React. Inorg. Met. -Org. Nan. Met. Chem. 2011; 41: 141-146.

Mandal S., Karmakar T.K., Ghosh A., Fleck M., Bandyopadhyay D. Synthesis, crystal structure and antibacterial activity of a group of mononuclear manganese(II) Schiff base complexes. Polyhedron. 2011; 30: 790-795.

Mladenova R., Ignatova M., Manolova N., Petrova T., I. R. Preparation, characterization and biological activity of Schiff base compounds derived from 8-hydroxyquinoline-2-carboxaldehyde and Jeffamines ED. Eur. Polym. J. 2002; 38: 989-99.

Mohamed M., Hapipah M.A., Mahmood, A.A., Robinson T.W. Synthesis, structural characterization, and anti-ulcerogenic activity of Schiff base ligands derived from tryptamine and 5-chloro, 5-nitro, 3,5-ditertiarybutyl salicylaldehyde and their nickel(II), copper(II), and zinc(II) complexes. Polyhedron. 2009; *28:* 3993–3998.

Nair M.S., Arish D.Synthesis, characterization and antimicrobial studies of CoII, NiII, CuII and ZnII complexes involving a bidentate Schiff base ligand J. Ind. Chem. Soc. 2010; 88: 265-270.

Tajudeen S.S., Radha E., Synthesis, characterization and antimicrobial activity of transition metal complexes of schiff base derivatives from isonicotinic acid hydrazide. Asian J. Chem. 2009; 21: 313–316.

Nenad P., Ann C., Murray J.E. Identification of an Apo-Superoxide Dismutase (Cu,Zn) Pool in Human Lymphoblasts. J. Biol. Chem. 1996; 271: 28331-28334.

Nura S.G., Siti M.S., Hamid K., Hapipah M. Aqua{*N*,*N*-dimethyl-*N*'-[1-(2-pyridyl)ethylidene]ethane-1,2-diamine-κ^3*N*,*N*',*N*"}bis(thiocyanato-κ*N*)nickel(II). Acta Cryst. 2011; E67: m513.

Nurul A.I.H., Nura S.G., Hamid K., Hapipah M. Dichlorido{*N*,*N*-dimethyl-*N*'-[1-(2-pyridyl)ethylidene]ethane-1,2-diamine-κ^3*N*,*N*',*N*"}manganese(II). Acta Cryst. 2011; E67: m229.

Pallab B., Shouvik C., Michael G.B.D., Carmen D., Ashutosh G. Synthesis, structure and magnetic properties of mono- and di-nuclear nickel(II) thiocyanate complexes with tridentate N_3 donor Schiff bases. Polyhedron. 2010; 29: 2637-2642.

Pandeya S.N., Sriram D., Nath G., DeClercq E. Synthesis, antibacterial, antifungal and anti-HIV activities of Schiff and Mannich bases derived from isatin derivatives and N-[4-(4'-chlorophenyl)thiazol-2-yl] thiosemicarbazide. Eur. J. Pharm. Sci.1999; 9: 25-31.

Parbati S., Saktiprosad G., Thomas C.W. Mak. A new route for the synthesis of bis(pyridine dicarboxylato)bis(triphenylphosphine) complexes of ruthenium(II) and X-ray structural characterisation of the biologically active trans-[$Ru(PPh_3)_2(L^1H)_2$] (L^1H_2=pyridine 2,3-dicarboxylic acid) Polyhedron. 2001; 20: 975-980.

Pignatello R., Panico A., Mazzone P., Pinizzotto M.R., Garozzo A., Fumeri P.M. Schiff bases of N-hydroxy-N′-aminoguanidines as antiviral, antibacterial and anticancer agents. Eur. J. .Med. Chem. 1994; 29: 787-794.

Prakash A., Singh B.K., Bhojak N., Adhikari D. Synthesis and characterization of bioactive zinc(II) and cadmium(II) complexes with new Schiff bases derived from 4-nitrobenzaldehyde and acetophenone with ethylenediamine. Spectrochim. Acta. 2010; 76: 356-362.

Raman N., Jeyamurugan R., Senthilkumar R., Rajkapoor B., Franzblau S.G. In vivo and in vitro evaluation of highly specific thiolate carrier group copper(II) and zinc(II) complexes on Ehrlich ascites carcinoma tumor model. Eur. J. Med. Chem.2010; 45: 5438-5451.

Raman N., Selvan A., Sudharsan S. Metallation of ethylenediamine based Schiff base with biologically active Cu(II), Ni(II) and Zn(II) ions: Synthesis, spectroscopic characterization, electrochemical behaviour, DNA binding, photonuclease activity and in vitro antimicrobial efficacy. Spectrochim. Acta. 2011; 79: 873-883.

Ross T.K., Andrew W., Nizal S.C., Rationally designed, chiral Lewis acid for the asymmetric induction of some Diels-Alder reactions. *J. Am. Chem. Soc.* 1986; 108: 3510–3512.

Shakir M., Azam M., Ullah M.F., Hadi S.M. Synthesis, spectroscopic and electrochemical studies of N,N-bis[(E)-2-thienylmethylidene]-1,8-naphthalenediamine and its Cu(II) complex: DNA cleavage and generation of superoxide anion. J. Photochem. Photobiol. 2011; 104: 449-456.

Shahabadi N., Kashanian S., Darabi F. DNA binding and DNA cleavage studies of a water soluble cobalt(II) complex containing dinitrogen Schiff base ligand: The effect of metal on the mode of binding. Eur. J. Med. Chem. 2010; 45: 4239-4245.

Shayma A.S., Yang F., Abbas A.S. Synthesis and Characterization of Mixed Ligand Complexes of 8-Hydroxyquinoline and o-hydroxybenzylidene-1-phenyl-2,3-dimethyl-4-amino-3-pyrazolin-5-on with Fe(II), Co(II), Ni(II) and Cu(II) ions. Eur. J. Sci. Res. 2009; 33: 702-709.

Shayma A.S.,, Yang F., Sadia M., Eskender M. Synthesis and Characterization of Mixed Ligand Complexes of Caffeine, Adenine and Thiocyanate with Some Transition Metal Ions. Sains Malays. 2010; 39: 957–962.

Sridhar S.K., Pandeya S.N., Stables J.P., Ramesh A. Anticonvulsant activity of hydrazones, Schiff and Mannich bases of isatin derivatives. Eur. J. Pharm. Sci. 2002; 16: 129-132.

Syamal A., Kumar D., Singh A.K., Gupta P.K., Jaipal., Sharma L.K. Synthesis and characterization of a chelating resin containing ONO donor tridentate Schiff base and its coordination compounds with copper(II), nickel(II), cobalt(II), iron(III), zinc(II), cadmium(II), manganese(II), molyb-denum(VI), zirconium(IV) and uranium(VI). Indian J. Chem. Sect A. 2002; 41: 1385.

Yasuhiro A., Takamichi F., Hiroo T., Hisanobu O. Catalytic reactions of metalloporphyrins. 1. Catalytic modification of borane reduction of ketone with rhodium(III) porphyrin as catalyst. J. Am. Chem. Soc. 1986; 108: 943–947.

Yusnita J., Puvaneswary S., Mohd. A.H., Robinson W.T., Kwai-Lin T. Synthesis, structural characterization and antibacterial activity of 2,6-diacetylpyridine bis(benzenesulfonohydrazide) Schiff bases and their copper(II) complexes. Polyhedron. 2009; 28: 3050-3054.

Zhang J-A., Pan M., Zhang J-Y., Kang B-S., Su C-Y. Syntheses, structures and bioactivities of cadmium(II) complexes with a tridentate heterocyclic N- and S-ligand. Inorg. Chim. Acta. 2009; 362: 3519-3525.

How to cite this article:

Nura Suleiman Gwaram, Hapipah Mohd Ali, Siti Munirah Saharin, Mahmood Ameen Abdulla, Pouya Hassandarvish, Thong Kwai Lin, Chai Lay Ching and Cher Lin Ooi., Synthesis, characterization, and biological applications of some 2-acetylpyridine and acetophenone derivatives. J App Pharm Sci. 2012; 2 (10): 027-038