ChemSearch Journal 9(1): 19 – 28, June, 2018
Publication of Chemical Society of Nigeria, Kano Chapter

Received: 08/02/2018 Accepted: 06/04/2018

Synthesis, Characterization and Antibacterial Assessment of some Ni(II) and Cd(II) azomethine Complexes derived from 4 (2-aminoethyl) morpholine

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ABSTRACT

Azomethine complexes derived from 4 (2-aminoethyl) morpholine and their metal(II) complexes have been synthesized. The complexes were characterized by spectroscopic techniques and X-ray crystallography. The complexes were tested for antibacterial activity against selected nosocomial pathogens of *Acinetobacter baumannii, Klebsiella pneumoniae,* methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* using the disc diffusion and broth micro-dilution. Overall results showed that the cadmium containing complex exhibited considerable antibacterial activities against *Acinetobacte baumannii* and MRSA. Results from the broth micro-dilution assay showed a much higher MIC value of the complex, ranging from 156.3 µg/mL to 625.0 µg/mL, when compared to the MIC value of antibiotics control of 2.0 µg/mL to 4.0 µg/mL. The *in -vitro* results indicate that metal complexes may be potentially utilized as an alternative to antibacterial drugs against nosocomial infections caused by both MRSA and *A. baumannii*.

Keywords: Azomethine; ccomplexes; nosocomial bacteria; antibacterial

INTRODUCTION

Azomethine compounds are more often regarded as ligands that will bind with other elements such as metallic ions in the synthesis of complexes. The presence of nitrogen (N) donor atoms in their structure resulted in its unique coordination behaviors with metal ions (Vinuelas *et al.,* 2011). The chemical coordination geometry of complexes that are bound to metallic ions can be altered in order to change its chemical properties (Shakir *et al.,* 2012). The azomethine complexes have been the focus of researchers due to simplicity in their synthesis and also the potential of the complexes to be used as antimicrobial agents (Yamada, 1999). In recent years, many studies were conducted on the efficacy of these type of metal complexes as potential antimicrobial agents, and tested against a variety of bacterial and fungal species (Valent *et al.,* 2002, Noyce *et al.,* 2006, Reiss *et al.,* 2009, Sabik *et al.,*2012, Gwaram *et al.,* 2012, Gupta *et al.,*2012, Sunitha *et al.,* 2012).

In clinical settings, *Acinetobacter baumannii, Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are among the medically important bacteria that not only cause nosocomial infections, but they are also highly resistant to multiple antibiotics used in clinical treatments (Navon *et al.,* 2005, Defres *et al.,*2009, Sikarwar *and* Batra 2011, Boon *et al.,*2011). Serious infections caused by *Staphylococcus aureus* are treated with the glycopeptide antibiotics such as vancomycin and teicoplanin. However, the methicillin-resistant *Staphylococcus aureus* (MRSA) is known to be resistant to multiple antibiotics including the antibiotic of last resort for the treatment of its infections, vancomycin (Casey *et al.,* 2007). Some of the multiple drug-resistant strains of *Klebsiella pneumoniae* are capable of producing the extended-spectrum β-lactamase enzyme that renders all β-lactam antibiotics ineffective (Won *et al.,* 2011). As for *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, both of these pathogenic bacteria are feared not only for their ability to grow and survive in unfavorable conditions, but also their highly intrinsic resistance to most of the available antibiotics (Zavascki *et al.,* 2010). More recently, *bacteria* have developed resistance to the virtually most drug of choice and this can lead to potential treats to society in general.

The objective of the study was to synthesize, characterize and assess the antibacterial activity of Nickel and Cadmium Azomethine complexes against selected nosocomial pathogens of *methicillin-resistant Staphylococcus aureus* (MRSA), *Acinetobacter baumannii* (AC),

Klebsiella pneumoniae (KB) and *Pseudomonas aeruginosa* (PA) by using the disc diffusion and broth micro-dilution.

MATERIALS AND METHODS

Materials

All chemicals used in this paper were of analytical grades and used without any further purification. Nickel(II) and Cadmium(II) acetate, Sodium azide, 4-(2-aminoethyl)morpholine and 2-acetylpyridine were purchased from the Aldrich–Sigma. Ethanol and Methanol Solvents were distilled prior to use. Melting points were determined using an MEL-TEMP II melting point instrument. Microanalyses were carried out on a Perkin-Elmer 2400 elemental analyzer. 1H-NMR and 13C-NMR spectra were determined with a JEOL Lambda 400 MHz FT-NMR (1H: 400 MHz and 13C: 100.4 MHz) spectrometer. Chemical shifts are given in δ values (ppm) using TMS as the internal standard.

METHODS

Synthesis of Nickel and Cadmium Complexes

The complexes were synthesized via *in situ* method as follows: A mixture of 2-acetylpyridine (0.20 g, 1.65 mmol) and 4-(2-aminoethyl)morpholine (0.21 g, 1.65 mmol) in ethanol (20 ml) was refluxed for 2 hrs followed by addition of a solution of either cadmium(II) acetate dihydrate (0.44 g, 1.65 mmol) or of nickel(II) acetate tetrahydrate (0.41 g, 1.65 mmol) and sodium azide (0.22 g, 3.30 mmol) in a minimum amount of water. The resulting solution was refluxed for 30 mins, and then left at room temperature. The crystals of the complexes (Fig. 1) were obtained in 2-3 days; the resulting pure crystals were filtered off, washed with cold ethanol and dried over silica gel (Gwaram and Hassandarvish 2014).

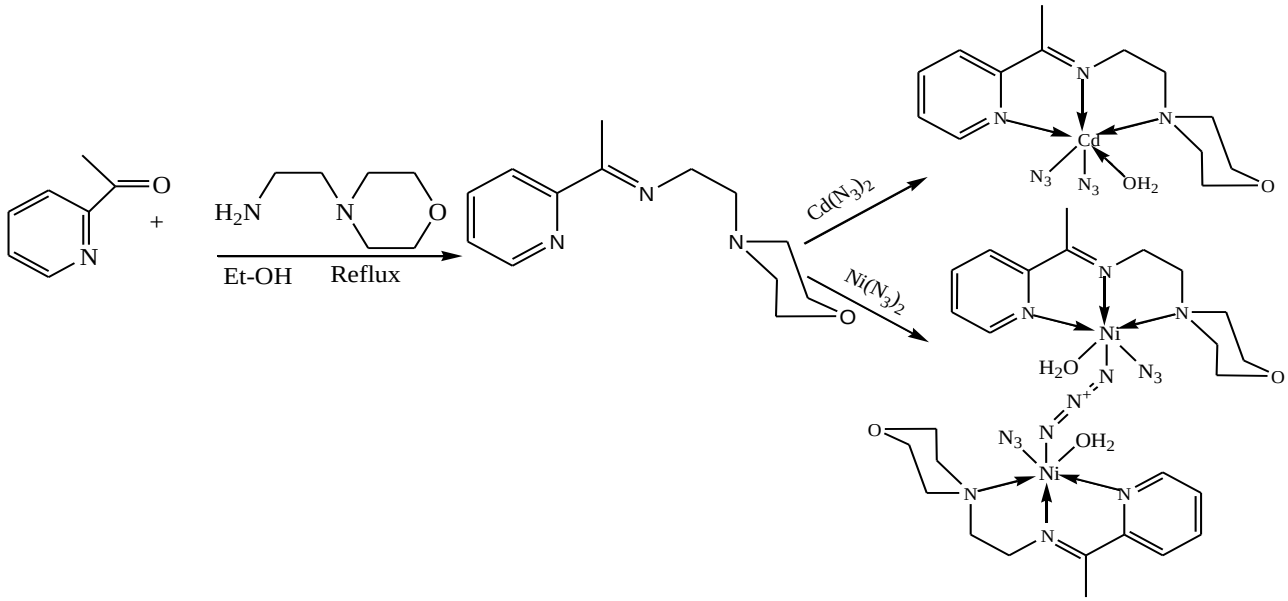

Fig. 1: Proposed synthesis scheme for the complexes

Table 1: Spectroscopic Analysis data for the Complexes characterization

Analysis	Cadmium Complex	Nickel Complex
CHN Analysis	Theory: C, 40.80; H, 5.00; N, 32.94. Found: C, 40.92; H, 5.21; N, 33.04.	Theory: C, 41.85; H, 5.67; N, 28.16. Found: C, 40.65; H, 5.60; N, 28.32.
FT-IR (ATR cm−1)	2951.03, 2848.69 ν(C-H), 2044.84 ν(N=N=N), 2018.95 ν(N=N=N), 1648.78 ν(C=N), 1437.97 ν(C-C), 1109.85 ν(C-N), 551.73 ν(M-N).	3360.55 ν(OH_2), 2943.55, 2880.00 ν(C-H), 2074.48, 2031.03 ν(N=N=N), 1662.25 ν(C=N), 1437.62 ν(C-C), 1113.15 ν(C-N), 565.26 ν(M-N), 452.82 ν(M-O).
UV-Vis [(DMSO)(nm)]	246.00 (π→π*); 281.00 (n→π*).	712.00 (d→d*), 608.00 (LMCT); 307.00 (n→π*); 279.00 (π→π*).
^{1}H-NMR (DMSO-d^6)ppm	8.681, 8.674 [d, 1H, δ(Ar-H)pyr], 8.281, 8.268, 8.255, 8.243, 8.231 [m, 2H, δ(Ar-H)pyr], 7.854 [s, 1H, δ(Ar-H)pyr], 3.791 [s, 4H, δ($2CH_2$)], 3.738, 3.731, 3.722 [t, 2H, δ(N-CH2)], 2.788, 2.779, 2.769 [t, 6H, δ($2CH_2$)], 2.533 [s, 3H, δ(CH_3)].	- No Proton NMR due to Paramagnetic nature of Nickel

^{13}C-NMR (DMSO-d^6) ppm	164.66 [1C, δ(C=N)], 149.02 δ(C), 140.37 δ(CH), 127.36 δ(CH), 124.31 δ(CH), δ(CH) [5C, δ(Ar-pyr)], 65.43 [2C, δ($2CH_2$)], 58.06 [2C, δ($2CH_2$)], 53.44 [1C, δ(CH_2)], 44.46 [1C, δ(CH_2)], 15.29 [1C, δ(CH_3)].	- No Carbon NMR due to Paramagnetic nature of Nickel

PHARMACOLOGY

Bacterial Strains

The nosocomial bacterial pathogens originated from clinical settings and were resistant to multiple antibiotics. Eight clinical strains from four different bacterial pathogens were tested: *Acinetobacter baumannii* (AC) (AC06127, AC08121), *Klebsiella pneumoniae* (KB) (KB88, KB198); methicillin-resistant *Staphylococus aureus* (MRSA) (MRSA080425, MRSA08071) and *Pseudomonas aeruginosa* (PA) (PA30, PA42). All bacterial strains were obtained from the cultures collection of Laboratory of Biomedical Science and Molecular Microbiology, Institute of Graduate Studies University of Malaya 50603 Kuala Lumpur Malaysia.

Kirby-Bauer Disk Diffusion Assay

Potential antibacterial activity for the synthesized compounds were initially investigated using the Kirby-Bauer disc diffusion assay (CLSI, 2006) against two (2) selected strains from each of the bacterial species, namely; MRSA080425 and MRSA08071 for MRSA; KB88 and KB198 for *K.pneumoniae*; AC06127 and AC08121 for *A.baumannii*; and PA30 and PA42 for *P.aeruginosa*. For inoculum preparation, overnight bacterial culture were suspended in saline solution containing 0.85% NaCl (w/v) and diluted to match the 0.5 McFarland turbidity standards of approximately '1 x 10^8' CFU/mL. The prepared inoculum was streaked with sterile swab onto the surface of cation-adjusted Mueller Hinton II agar (CAMHA; Oxoid) to obtain bacterial lawn. Respective antibacterial compounds of 10,000 µg/mL were transferred onto sterile paper disks (Thermo Fisher, 6.0 mm diameter). Dimethyl Sulfoxide (DMSO) was used as the solvent and was transferred onto disk as negative control. The commercial antibiotics disks (Oxoid) of polymyxin B sulfate (300.0 units) and vancomycin (30.0 µg) were included as positive controls. The disks were placed onto agar surfaces of respective bacterial lawn of inocula. Inhibition zones (in mm) around disks were observed and measured after 18 hours of incubation of the plates at 37 ^{o}C. Larger inhibition zones after incubation indicated more potent antibacterial activity against the tested bacterial strains. The compound containing the metal cadmium had shown the largest inhibition zones and was selected for MIC determination in broth micro-dilution against susceptible bacterial strains.

MIC determination

The minimum inhibitory concentrations (MIC) of the compound were determined through the broth micro-dilution assay (CLSI 2006) with 96-wells microtiter plates. Selected susceptible overnight bacterial cultures were suspended in cation-adjusted Mueller Hinton II broth (CAMHB; BBL) before being diluted in similar broth to the concentration of approximately 1 x 10^8 CFU/mL (equivalent to 0.5 McFarland turbidity standards) for the inoculum preparation. Two-fold serial dilution of the tested Schiff base compound; the Cd(II) complex was prepared in sterile distilled water in the 96-wells microtiter plate with the highest concentration starting from 2,500.0 µg/mL in duplicate rows. The commercial antibiotics powder of polymyxin B sulfate (Sigma) and vancomycin (Sigma) were prepared beforehand and included as positive controls with the highest concentration starting from 16.0 µg/mL. Wells containing bacterial suspensions and wells without bacterial suspensions and compound were used as positive growth control and broth sterility control, respectively. The prepared inoculum was transferred to all wells except the broth sterility control wells in 1:1 dilution ratio. The inoculated plates were incubated at 37 ^{o}C for 18 hours. After incubation, the turbidity of wells containing the compound was visually compared to the turbidity in wells without compound to determine the growth end points. The MIC is the lowest concentration of the compound that completely inhibits the growth of organism in wells.

RESULTS AND DISCUSSION

The spectroscopic analysis data of complexes are summarized in (Table 1). The result percentages of C, H and N obtained are in agreement with calculated values. The IR spectra of all the complexes possess a characteristic absorption bands in the region of 1648.78 and 1662.25cm^{-1} for Cd(II) and Ni(II) respectively which is attributed to the C=N functional group stretching vibration (Gwaram and Hassandarvish 2014). Another very strong absorption at the region of 2044.84, 2018.95 and 2074.48, 2031.03 corresponding to ν(N=N=N) functional group

frequencies for Cd(II) and Ni(II) respectively (Gwaram 2017).

Fourier transform-Nuclear Magnetic Resonance (FT-NMR)

The proton Nuclear Magnetic Resonance (NMR) characteristic chemical frequencies observed in the region 8.681-7.854 ppm, were assigned to the aromatic ring protons for Cd(II) complex (Gwaram *et al.,* 2012). The other single peak appeared at 2.533 ppm was attributed to $\delta(CH_3)$ representing the methyl on the carbonyl group (Mustafa *et al.,* 2009). In the ^{13}C Nuclear Magnetic Resonance (NMR) spectra the signal at region of 164.66ppm is assigned to the azomethine (C=N) carbon atoms for Cd(II) complex (Mustafa *et al.,* 2009). Subsequent signals for the aromatic ring carbon atoms were determined in the region of 149.02 -124.31 ppm (Gwaram *et al.,* 2012).

X-ray crystallography

Aqua {2-Morpholino-*N*-[1-(2-pyridyl) ethylidene] ethanamine-κ^3*N,N',N"*} bis (azido-κ^N) cadmium (II)

The single crystal of this compound has been obtained *via* the complexation of cadmium(II) azide by the *N,N',N"*-tridentate ligand, 2-morpholino-*N*-[1-(2-pyridyl)ethylidene]ethanamine, which had itself been prepared from the condensation of 4-(2-aminoethyl)morpholine and 2-acetylpyridine. In the compound, the Cd(II) atom is octahedrally coordinated by the *N,N',N"*-tridentate Schiff base ligand and two terminal azide *N* atom. The adjacent Cd(II) ions are coordinated by one additional water ligand (Fig. 2). The geometry of the complex can be defined as distorted octahedral geometry with one of the water molecule act as coordinated ligand to have complete the geometry. The cadmium complex crystal system is monoclinic with space group $P2_1/c$ and a/Å, b/Å, c/Å of 17.2330(8), 6.6786(3), 30.1578(14) whereas 90.00, 91.8670(10) and 90.00 corresponding to α/°, β/° and γ/° respectively (Table 2).

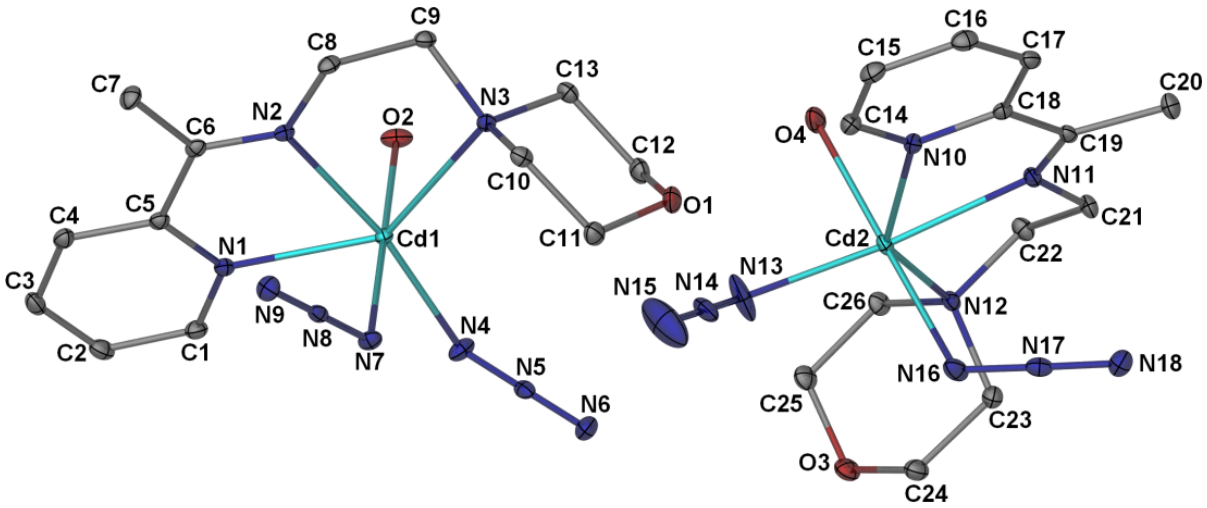

Fig. 2: Crystal structure for $[Cd(N_3)_2(C_{13}H_{19}N_3O)]$-$H_2O$

Nickel(II)poly(azido-κ^N){2-Morpholino-N-[1-(2-pyridyl)ethylidene] ethanamine-κ^3N,N',N"}

In this complex crystal system is triclinic with space group PI and 90.00, 90 and 90.00 corresponding to α/°, β/° and γ/° respectively (Table 2). The atom is octahedrally coordinated by the *N,N',N"*-tridentate Schiff base ligand and one terminal azide N atom. In the crystal, adjacent Ni(II) ions are linked by the azide *N:N*-bridges into polymeric chains along the c axis. The azide ions act as either bridging or terminal ligands. However, different from the doubly bridged dimeric structure of the former, in the present structure the bridging azide ligands singly bridge the adjacent metal centers into infinite chains along the *c* axis (Fig. 3). Within this coordination polymer, Two azide *N:N*-bridges, one terminal azide *N* atom and the Ni(II) ion is coordinated by one additional water ligand to have an octahedral geometry.

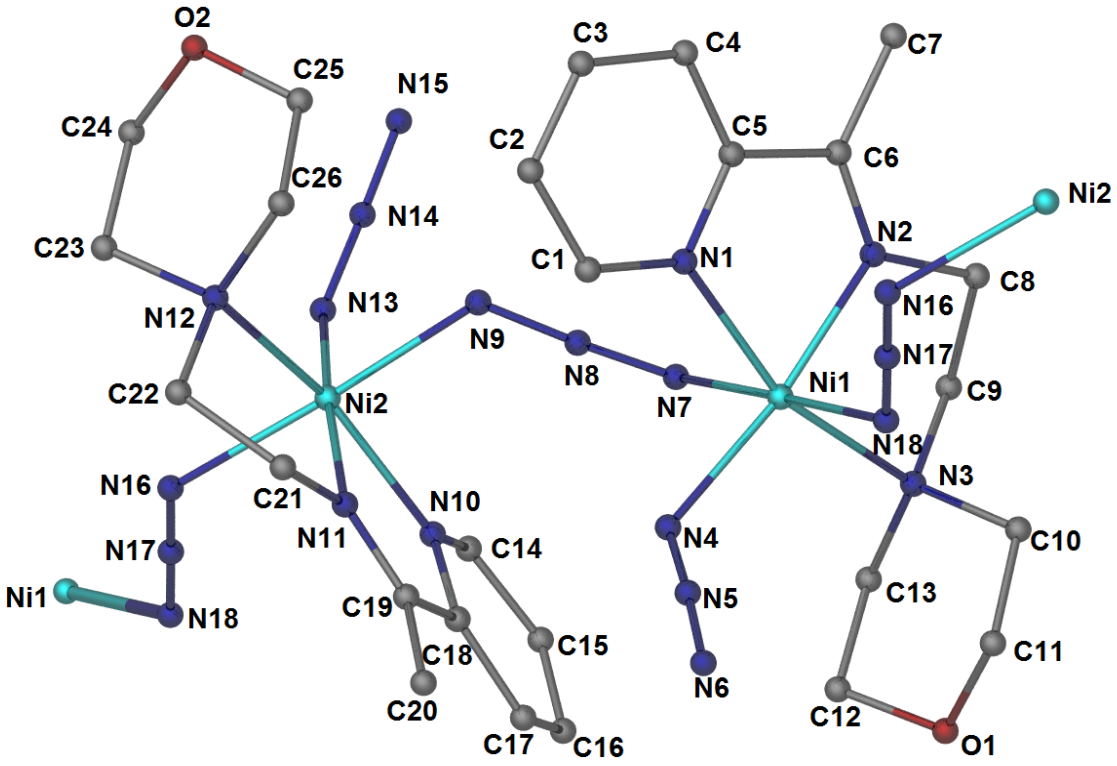

Fig. 3: crystal structure for $[Ni_2(LMA)_2(N_3)_2(H_2O)_2]$

Table 2: Crystal Data and refinement parameters for the synthesized complexes

Identification code	**$[Cd(N_3)_2(C_{13}H_{19}N_3O)]$-$H_2O$**	**$[Ni_2(LMA)_2(N_3)_2(H_2O)_2]$**
Empirical formula	$C_{13}H_{19}CdNO$	$C_{13}HN_9ONi$
Formula weight	317.69	357.94
Temperature/K	373(2)	373(2)
Crystal system	monoclinic	triclinic
Space group	$P2_1/c$	P1
a/Å	6.6786(3)	10.4169(4)
b/Å	17.2330(8)	15.0746(6)
c/Å	30.1578(14)	20.7751(9)
α/°	90.00	90.00
β/°	91.8670(10)	90.00
γ/°	90.00	90.00
Volume/$Å^3$	3469.1(3)	3262.3(2)
Z	12	8
$\rho_{calc}mg/mm^3$	1.825	1.458
m/mm^{-1}	1.866	1.209
F(000)	1920.0	1424.0

Crystal size/mm^3	0.28 × 0.11 × 0.08	0.19 × 0.11 × 0.05
2Θ range for data collection	2.7 to 53.98°	1.96 to 60.08°
Index ranges	$-8 \le h \le 8, -18 \le k \le 22, -38 \le l \le 38$	$-14 \le h \le 14, -20 \le k \le 20, -29 \le l \le 28$
Reflections collected	27005	34671
Independent reflections	7584[R(int) = 0.0343]	30044[R(int) = 0.0553]
Data/restraints/parameters	7584/0/453	30044/3/753
Goodness-of-fit on F^2	0.810	1.828
Final R indexes [I>=2σ (I)]	R_1 = 0.0305, wR_2 = 0.0854	R_1 = 0.1362, wR_2 = 0.3318
Final R indexes [all data]	R_1 = 0.0377, wR_2 = 0.1021	R_1 = 0.1667, wR_2 = 0.3515
Largest diff. peak/hole /e Å^{-3}	0.75/-0.94	8.48/-2.13

UV-Visible Spectroscopy

Nickel(II), which has d^8 configuration, commonly exhibits octahedral, square planar and tetrahedral coordination geometries. Octahedral geometry generally occurs for nickel(II) with a coordination number of six. The electronic spectra of Ni(II) complexes [Ni(LMA)$(N_3)_3$] showed d-d transitions in the region of 279, 307, 628 and 712 (Table 1, Fig. 4). These are assigned to the transitions $^3T_{1(F)} \rightarrow {}^3A_{2(F)}$, $^3T_{1(F)} \rightarrow {}^3T_{1(P)}$ and $^3T_{1(F)} \rightarrow {}^3T_{2(F)}$ consistent with distorted octahedral geometry.

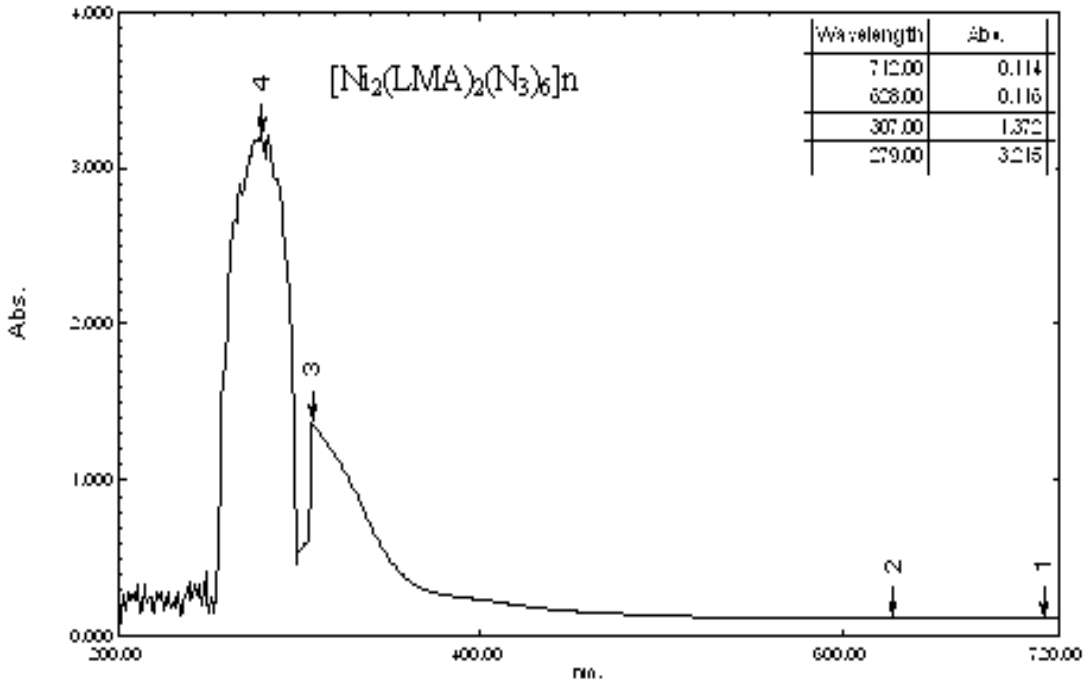

Fig. 4: electronic spectra for $Ni_2(LMA)_2(N3)_6$

The formation of the metal nitrogen bond stabilizes the electron pair on the nitrogen atoms, i.e., the energy of the nonbonding n orbital is lowered and the transition occurs at a lower wavelength. The absorption peak from approximately 400 nm to 600 nm means that the compound absorbs light in the violet-blue-green range. The sum of the non-absorbed, or reflected, wavelengths gives the products different colors.

Antibacterial Activity Results

Azomethine complexes were screened for antibacterial activity against multiple drug-resistant nosocomial bacterial pathogens, namely; *Acinetobacter baumannii* (AC), *Klebsiella pneumoniae* (KB), methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* (PA). The objective of the study was to determine the antibacterial activity of the

complexes by three approaches; namely the disc diffusion and broth micro-dilution.

In the initial screening, the synthesized compounds were tested against bacterial strains of *A. baumannii*, *K. pneumoniae*, MRSA and *P. aeruginosa*, with two randomly selected isolates from each bacterial species. Distinct clear zones indicating growth inhibition were observed from the complexes containing metal elements of nickel (Ni) and cadmium (Cd) (Table 3). Susceptible bacterial strains to those complexes include KB88, KB198, MRSA080425, MRSA08071, AC06127, and AC08121. However, there was no inhibition zone observed for *P. aeruginosa* for all of the compounds tested (Figure 5a-b). The antibiotics polymyxin B and vancomycin were included as control. Based on the results, the Cd(II) metal complex was more potent than the other compounds tested as it inhibited the growth of six bacterial strains (AC06127, AC08121, KB88, KB198, MRSA080425 and MRSA08071), resulting in 100% clear inhibition zones (no bacterial growth within the clear zones) (Figure 5c-d).

The metal complex showed antibacterial effect comparable to the antibiotics used. The enhanced antimicrobial activity of metal complexes was extensively studied (Mohamed *et al.*, 2011, Aiyelabola *et al.*, 2012). Based on chelation theory, the enhancement is due to the increased lipophilic nature of the metal complex, achieved by the overlapping of ligand orbital with metal orbital in the complex, which causes partial sharing of the positive charge of metals with the donor groups on ligands. This coordination chemistry reduces the polarity of metal and thus increasing the lipophilic nature of the metal to the lipid layer of bacterial cell membrane (Nishat *et al.*, 2011). Due to the highly negative-charged LPS on the cell walls of Gram-negative bacteria and the opposite charges of both the cationic metal ions and cationic peptides, metal ions will be adsorbed to bacterial cell surfaces through passive biosorption (Chakravarty and Banerjee, 2012). The heavy metal cadmium was described to disrupt normal cellular processes of living organisms by binding to different cellular target sites (Wang *et al.*, 2010) and the damaging effect towards membrane structure when cadmium binds to phosphate ligands present on the membrane (Vig *et al.*, 2003). Based on the screening result, the most active compound, which was the cadmium-containing complex, LMA Cd-N_3 was chosen to determine its activity on the isolates of bacteria that were sensitive to the mentioned compound. The minimum inhibitory concentrations (MICs), of the metal complex were determined in broth micro-dilution assay

Bacterial strains of MRSA (MRSA080425), *K. pneumoniae* (KB88) and *A.* (AC08121) that were susceptible to the metal complex LMA Cd-N_3 were selected for further testing in broth micro-dilution assay. Results from the broth micro-dilution assay showed a much higher MIC value of LMA Cd-N_3, ranging from 156.3 µg/mL to 625.0 µg/mL, when compared to the MIC value of antibiotics control of 2.0 µg/mL to 4.0 µg/mL (Table 4).

Table 3. Zones of inhibition from antimicrobial disc diffusion assay. Readings of 6.0 mm represents disk size, no inhibition zone observed. Schiff base complexes were tested at 10,000 µg/mL. Disc concentration of polymyxin B tested was 300.0 units; concentration of vancomycin was 30.0 µg. Polymyxin B and vancomycin were not determined on *P. aeruginosa*, represented by 'n.d'.

		Zones of inhibition (mean value to the nearest mm)							
		Gram-positive				**Gram-negative**			
Code	**Compound**	**MRSA 080425**	**MRSA 08071**	**KB 88**	**KB 198**	**AC 06127**	**AC 08121**	**PA 30**	**PA 42**
Ctrl	0.85% saline	6	6	6	6	6	6	6	6
Ctrl	DMSO	6	6	6	6	6	6	6	6
Ctrl	Polymyxin B	6	6	15	15	16	15	n.d	n.d
Ctrl	Vancomycin	16	17	6	6	6	6	n.d	n.d
S_1	LMA Ni-N_3	6	9	10	12	6	6	6	6
S_2	LMA Cd-N_3	20	10	10	12	14	12	6	6

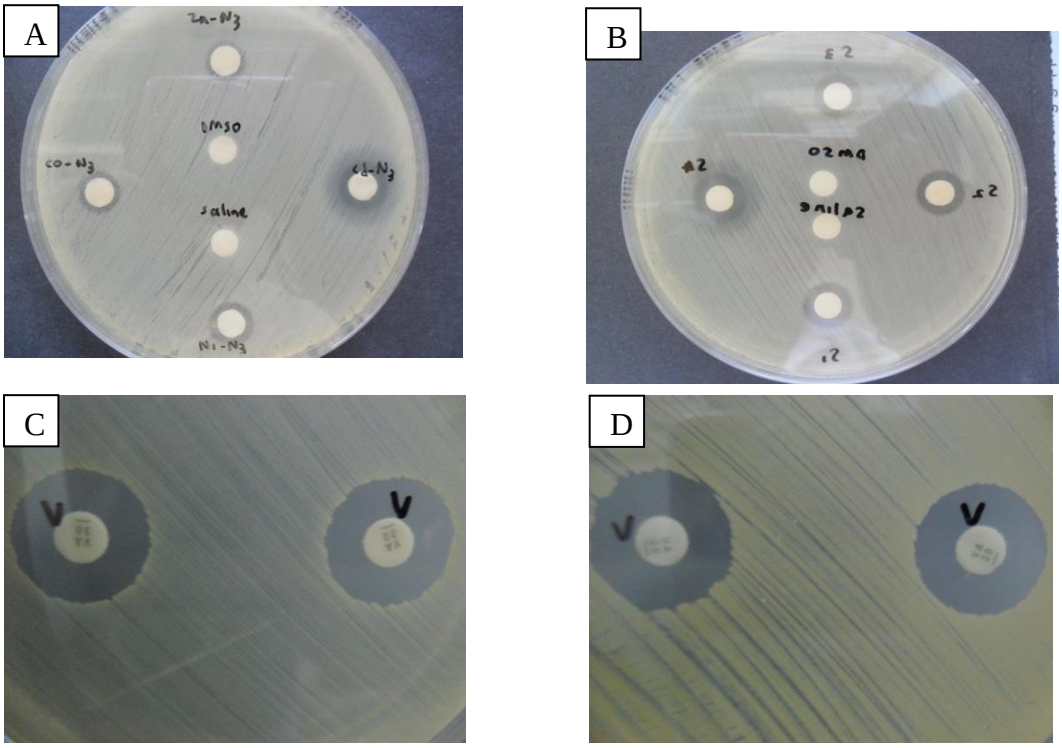

Fig. 5. Images showing the Sample of antibacterial inhibition zones of compounds of Azomethine complex tested against bacterial strains of KB88 (**A**); KB198 (**B**); MRSA080425 (**C**) and MRSA08071 (**D**);

Table 4. Minimum inhibitory concentration (MIC) of antibacterial compounds tested in the broth micro-dilution assay

Bacterial strains	**Minimum inhibitory concentration (MIC) (µg/mL)** **Antibacterial compounds**		
	LMA Cd-N_3	**Vancomycin**	**Polymyxin B**
MRSA	625.0	4.0	-
KB	312.5	-	2.0
AC	156.3	-	2.0

CONCLUSION

The results obtained from the assays showed that the synthesized compounds of Schiff base complex coupled with Ni(II) and Cd(II) ions exhibited antibacterial activity against both clinical strains of Gram-positive (represented by MRSA) and Gram-negative bacteria (represented by *A. baumanii, P. aeruginoa, K. pnumioniae*) tested in the study. The findings implied that the cadmium-containing Schiff base complex represents a good candidate for future research leading to the development of novel antibacterial drugs for the treatment of diseases caused by both the multiple drug-resistant nosocomial pathogens, MRSA and *A. baumannii*.

ACKNOWLEDGMENTS

The authors would like to thank the Institute of Biological Science, UM for the provision of laboratory facilities and support in whatsoever way. The authors also wish to acknowledge the support provided by the Department of Chemistry Faculty of Science, University of Malaya 50603 Kuala Lumpur Malaysia to conduct some part of the study.

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