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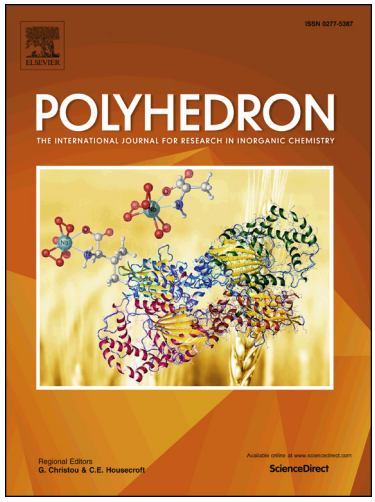

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PII: S0277-5387(17)30118-3
DOI: http://dx.doi.org/10.1016/j.poly.2017.02.008
Reference: POLY 12475

To appear in: *Polyhedron*

Received Date: 10 December 2016
Revised Date: 7 February 2017
Accepted Date: 8 February 2017

Please cite this article as: H. Keypour, M. Mahmoudabadi, A. Shooshtari, L. Hosseinzadeh, F. Mohsenzadeh, R.W. Gable, Synthesis of Mn(II) and Zn(II) complexes with new macrocyclic Schiff-base ligands containing piperazine moiety: spectroscopic, structural, cytotoxic and antibacterial properties, *Polyhedron* (2017), doi: http://dx.doi.org/10.1016/j.poly.2017.02.008

Synthesis of Mn(II) and Zn(II) complexes with new macrocyclic Schiff-base ligands containing piperazine moiety: spectroscopic, structural, cytotoxic and antibacterial properties

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Abstract

The new diamine 2,2'-(piperazine-1,4-diylbis(methylene))dianiline (A^1) was synthesized by reaction of 2-nitrobenzylchloride and piperazine. Its corresponding Mn(II) and Zn(II) macrocyclic Schiff-base complexes were prepared via the metal templated [1+1] cyclocondensation of this diamine and 2,6-diacetylpyridine or 2,6- pyridinedicarbaldehyde. The diamine and all the macrocyclic complexes were characterized by elemental analysis, mass spectrometry and spectroscopic methods such as: FT-IR, ^{1}H and ^{13}C NMR. Also, the crystal structures of $[MnL^1(ClO_4)_2]$ (1) and $[ZnL^1(H_2O)_2](ClO_4)_2$ (3) complexes were obtained by single-crystal X-ray crystallography. The cytotoxic properties of all macrocyclic complexes were studied. The results showed, some of these complexes were more potent than doxorubicin in U87 MG cell line predicating their therapeutic potential in treatment of glioblastoma. Also, the complexes were tested for *in vitro* antibacterial properties against some Gram-positive and Gram-negative bacteria. The complexes had antibacterial properties and in some cases even more than those of Tobramycin and Tetracycline as standards.

Keywords: Macrocyclic Schiff-base complexes, Piperazine moiety, X-ray structures, Antibacterial activity, Cytotoxicity.

1. Introduction

Schiff base macrocyclic ligands and their related complexes are an important group of compounds which have been extensively studied [1-4]. Macrocyclic Schiff-base complexes have many applications in several areas such as DNA binding and cleavage reagents [5], models of reaction centers of metalloenzymes [6], magnetic resonance imaging [7-8], sensors [9–11], catalyst [12–14], antibiotics [15] and can be utilized for metal biosites modeling [16-18]. They are polydentate systems whose donor atoms are parts of a cyclic hydrocarbon backbone [19]. The most important problem with the synthesis of macrocycles is the cyclization step that contains pathway in bringing two ends of a chain together. So, the best strategy involves reaction in the presence of a transition metal ion which increases the yield of the cyclization pathway and directs the strict course of the reaction towards cyclic products rather than polymeric ones. The effect has been called the metal-template effect [20-21]. The formation of macrocyclic complexes depends on the size of the internal cavity, the nature of its donor atoms, the rigidity and flexibility of the macrocycle, and stereochemical preference of metal ions [22]. Following previous reports macrocyclic complexes of manganese and zinc have antibacterial and cytotoxic properties [23-29]. Prompted by these facts and previous reports on synthesis macrocyclic complexes [30-39], in the present paper, the synthesis and characterization of Mn(II) and Zn(II) macrocyclic complexes derived from diamine A^1 and 2,6-diacetylpyridine or 2,6-pyridinedicarbaldehyde are discussed. All of the complexes were characterized by elemental analyses, mass spectrometry and spectroscopy (IR and NMR). The crystal structures of the macrocyclic complexes 1 and 3 were determined by single-crystal X-ray analyses.

2. Experimental

2.1. Materials

Piperazine, 2-nitrobenzylchloride, 2,6-diacetylpyridine, 2,6-pyridinedicarbaldehyde, metal salts and all of solvents were purchased from the Merck Company and used without further purification.

Caution! Perchlorate salts are potentially explosive. Only small amount of material should be prepared and handled with great care.

2.2. Physical measurements

Infrared spectra were measured using KBr pellets on a BIO-RAD FTS-40A spectrophotometer (4000-400 cm^{-1}). Mass spectra were recorded on an Agilent technologies (HP) 5973 mass spectrometer operating at an ionization potential of 70eV. CHN analyses were carried out using a Perkin-Elmer, CHNS/O elemental analyzer model 2400 series 2. ^{1}H and ^{13}C NMR spectra were taken in DMSO-d_6 on a Bruker Avance 400 MHz spectrometer using $Si(CH_3)_4$ as an internal standard.

2.3. X-ray crystallography

Orange crystals of $[MnL^1(ClO_4)_2]$ (1) and yellow crystals of $[ZnL^1(H_2O)_2](ClO_4)_2$(3) suitable for single-crystal X-ray analyses were grown by slow diffusion of diethyl ether vapor into their acetonitrile solution. A suitable crystal was selected and mounted on an Oxford Diffraction SuperNova, Dual, Cu at zero, Atlas diffractometer. The crystal was

kept at 130.01(10) K during data collection. With Olex2 [40], the structure was solved with the SHELXT [41] structure solution program using Direct Methods and refined with the SHELXL [42] refinement package using Least Squares minimization. Crystallographic data for the macrocyclic complexes $[MnL^1(ClO_4)_2]$ (1) and $[ZnL^1(H_2O)_2](ClO_4)_2$ (3) are listed in Table 1.

<Please insert Table 1>

2.4. Synthesis

2.4.1. Synthesis of 2,2'-(piperazine-1,4-diylbis(methylene))dianiline (A^1)

Piperazine (0.43 g, 5 mmol) was dissolved in acetonitrile (30 mL) and heated under refluxing condition, then K_2CO_3 (1.80 g, 13 mmol) and 2-nitrobenzylchloride (1.71 g, 10 mmol) were added. The mixture was refluxed for 48 h, being stirring and then allowed to cool to room temperature. Solution was dried and water was added and the product was extracted with chloroform (3×25 mL), the product is a yellow powder. A mixture of this powder (1.65 g, 4.63 mmol), NH_4Cl (6.94 g, 130 mmol) and 10 mL of H_2O in 100 mL of EtOH was heated to boil and zinc dust (12.11 g, 185 mmol) in 0.50 g portions at intervals of several minutes was added. The mixture was then refluxed for 12 h. The resulting solution was cooled to room temperature and filtered under vacuum. The solution was dried and water was added and the product was extracted with chloroform (3×25 mL). The white powder was dried and characterized as the pure compound (Scheme 1). Yield: 0.81 g (55%). Anal. Calc. for $C_{18}H_{24}N_4$ (MW: 296.42): C, 72.94; H, 8.16; N, 18.90. Found: C, 72.45; H, 8.36; N, 19.19%. EI-MS (m/z): 296 (100.0%). IR (KBr, cm^{-1}): 3267-3440, 1612 υ (NH_2)**.**

<Please insert scheme 1>

2.4.2. Synthesis of complexes

2.4.2.1. General procedure

A solution of $M(ClO_4)_2.6H_2O$ (0.50 mmol) (M: Mn, Zn) and 2,6-pyridinedicarbaldehyde (complexes:1, 3) or 2,6-diacetylpyridine (complexes: 2, 4) (0.5 mmol) (Scheme 2 and Scheme 3) was refluxed for 4 hours in absolute methanol (20 mL). After this time, A^1 (0.15 g, 0.5 mmol) dissolved in ethanol was added drop wise to hot solution over a period of 2h. After the solution was refluxed for 24 h, it was filtered. The powder was dried and characterized. Crystalline compounds were obtained by slow diffusion of diethyl ether vapor into the acetonitrile solution of the complexes 1 and 3.

<Please insert scheme 2>

<Please insert scheme 3>

2.4.2.2. $[MnL^1(ClO_4)_2](1)$

Orange crystals. Yield: 0.20 g (62%). Anal. Calc. for $C_{25}H_{25}Cl_2MnN_5O_8$ (MW: 649.34): C, 46.24; H, 3.88; N, 10.79. Found:C, 47.65; H, 4.22; N, 10.12%. EI-MS (m/z): 649. IR (KBr, cm^{-1}):1617 υ (C=N), 1585, 1491 [υ (C=C) and υ $(C=N)_{Py}$], 1121**,** 619 υ (ClO_4^-).

2.4.2.3. $[MnL^2(ClO_4)_2](2)$

Orange powder. Yield: 0.23 g (69%). Anal. Calc. for $C_{27}H_{29}Cl_2MnN_5O_8$ (MW: 678.39): C, 47.87; H, 4.32; N, 10.34. Found: C, 48.04; H, 5.02; N, 11.20; %. EI-MS (m/z): 677. IR (KBr, cm^{-1}): 1617 υ (C=N), 1589, 1432 [υ (C=C) and υ $(C=N)_{Py}$], 1095, 623 υ (ClO_4^-).

2.4.2.4. $[ZnL^1(H_2O)_2](ClO_4)_2$(3)

Yellow crystals. Yield: 0.21 g (60%). Anal. Calc. for $C_{25}H_{29}Cl_2ZnN_5O_{10}$ (MW: 695.84): C, 43.15; H, 4.20; N, 10.06. Found: C, 42.86; H, 4.62; N, 10.20%. EI-MS (m/z): 696. IR (KBr, cm^{-1}): 1637 υ (C=N), 1597, 1493 [υ (C=C) and υ $(C=N)_{Py}$], 1119, 621 υ (ClO_4^-).

2.4.2.5. $[ZnL^2(H_2O)_2](ClO_4)_2$(4)

Yellow powder. Yield: 0.22 g (61%). Anal. Calc. for $C_{27}H_{33}Cl_2ZnN_5O_{10}$ (MW: 723.89): C, 44.80; H, 4.59; N, 9.67%. Found: C, 45.11; H, 5.02; N, 9.17%. EI-MS (m/z): 724. IR (KBr, cm^{-1}): 1637 υ (C=N), 1583, 1496 [υ (C=C) and υ $(C=N)_{Py}$], 1145, 627 υ (ClO_4^-).

2.4.3. ^1H and ^{13}C NMR characterization of compounds

2.4.3.1. Diamine A^1

^1H NMR (DMSO-d_6, ppm) δ_H = 2.52 (broad, $8H_h$), 3.39 (s, $4H_g$), 5.31 (s, $4H_{(NH2)}$), 6.48 (d, H_b), 6.62 (m, H_d), 6.92 (d, H_e), 7.01(m, H_c). ^{13}C NMR (DMSO-d_6, ppm) δ_C =52.9 (C_g), 61.3 (C_h), 115.1 (C_b), 116.3 (C_d), 121.3 (C_f), 128.5 (C_c), 130.58 (C_e), 148.2 (C_a).

2.4.3.2. $[ZnL^1(H_2O)_2](ClO_4)_2$(3)

^{1}H NMR (DMSO-d_6, ppm) δ_H= 2.10 (s, $H_{(H2O)}$), 2.34 (broad, 8H_l), 3.40 (s, 4H_k), 7.48 (d, H_f), 7.52 (m, H_h), 7.60 (d, H_i), 7.70(m, H_g), 8.50 (s, H_d), 8.73(m, H_a), 8.75(d, H_b). ^{13}C NMR (DMSO-d_6, ppm) δ_C =39.4 (C_k), 58.5 (C_l), 120.4 (C_j), 129.7 (C_f), 130.4 (C_h), 131.0 (C_b), 132.0 (C_g), 132.2 (C_i), 143.8 (C_a), 147.0 (C_e), 147.3 (C_c), 158.1 (C_d).

2.4.3.3. $[ZnL^2(H_2O)_2](ClO_4)_2$(4)

^{1}H NMR (DMSO-d_6, ppm) δ_H = 2.11(s, $H_{(H2O)}$), 2.32 (s, $H_{(CH3)}$), 2.76(broad, 8H_l), 5.23 (s, 4H_k), 6.50 (d, H_f), 6.53 (d, H_i), 6.63 (m, H_g), 6.65 (m, H_h), 6.96(m, H_a), 7.01(d, H_b). ^{13}C NMR (DMSO-d_6, ppm) δ_C =25.9 (C_{CH3}), 39.4 (C_k), 49.3 (C_l), 115.2 (C_j), 116.3 (C_f), 125.1 (C_h), 128.6 (C_b), 129.7 (C_g), 130.7 (C_i), 139.6 (C_a), 148.2 (C_e), 152.8 (C_c), 199.3 (C_d).

2.5. Cytotoxic assay

H1299 (human nonsmall cell lung carcinoma) cell line was a gift from Prof. G. Storm. The cells had a homozygous partial deletion of the p53 protein and lacked p53 protein expression. A2780, human ovary carcinoma cell line, and U87 MG, human glioblastoma carcinoma, were obtained from Pasteur Institute (Tehran, Iran). In addition, the non-transformed human foreskin fibroblast cell line Hs27 was used as a comparative control. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM-F12) with 5% (v/v) fetalbovine serum, 100 UmL^{-1} penicillin, and 100 $mgmL^{-1}$ streptomycin. The medium was changed 2-3 days and sub-cultured when the cell population density reached 70–80% confluence. Cytotoxic effects of the compounds have been studied in cancer cell lines evaluated by MTT assay. Exponentially growing mammalian cells (on 96 well plate) were exposed for 24h to different concentrations of compounds. Stock solutions of the compounds and doxorubicin were prepared in dimethyl sulfoxide (DMSO). The

final concentration of the vehicle in the medium was always 0.5%. Control cells were kept in medium containing 0.1% DMSO. After 24 h of incubation, the medium was omitted and 0.1 mg of MTT was added to cells, with plates further incubated for 4 h at 37 °C. The formazan crystals were solubilized in 0.1 mL of dimethylsulfoxide and the optical density (OD570) was measured using a microplate reader (Bio Tek Instruments, USA). IC50 values were calculated by plotting the log10 of percent cell viability versus drug concentrations. In this paper, we studied the cytotoxic effects of Mn(II) and Zn(II) macrocyclic complexes and compared them to Doxorubicin.

2.6. Antibacterial assays

The antibacterial effect of four macrocyclic complexes was determined against the three Gram-positive bacteria*:* *Bacillus subtilis* (ATCC 6051), *Bacillus thuringiensis* (PTCC 1358) and *Staphylococcouss aprophyticus* (ATCC 6633), and two Gram negative bacteria: *Pectobacterium Sp*., *Pseudomonas fluorescens* (PTCC 1310). The microorganisms were prepared from Persian Type Culture Collection, Tehran, Iran. The organisms were sub-cultured in nutrient agar (Oxiod Ltd.) and nutrient broth for useing in experiments, while diagnostic sensitivity test agar (DST) (Oxoid Ltd.) was used in antibiotic sensitivity testing. For bioassays, suspensions of approximately 1.5×10^8 cells per mL in sterile normal saline were prepared [43]. The sensitivity testing was determined by using the agar-gel diffusion method [44-45]. 20 mcL of each chemical compound, containing 10 mcg in DMSO, was loaded in each disk. The minimum inhibitory concentration (MIC) of each compound was also determined using a twofold dilution method [46]. The isolated bacterial strains were first grown in nutrient broth for 18 h before being used.

The inoculums suspensions were standardized and then were tested against the test compound, with 20 mcL for each disk in DST medium. The plates were then incubated at 37.0 ± 0.5 °C for 24h after which they were observed for zones of inhibition. These effects were compared with those of Tobramycin and Tetracycline as standard antibiotics at a concentration of 1 mg/mL [47]. The MIC was also determined by tube dilution techniques in Mueller–Hinton broth (Merck) according to the procedure [48]. The tests were repeated at least three times for each organism.

3. Results and discussion

The new symmetric diamine A^1 was prepared from condensation of 2-nitrobenzylchloride and piperazine in acetonitrile solution. A^1 was characterized by elemental analysis, mass spectrometry, IR and NMR spectroscopy. The mass spectrum of A^1 showed the molecular peak at m/z = 296, consistent with the molar mass of this compound. Four new Schiff base macrocyclic complexes, $[MnL^1(ClO_4)_2]$ (1), $[MnL^2(ClO_4)_2]$ (2), $[ZnL^1(H_2O)_2](ClO_4)_2$(3) and $[ZnL^2(H_2O)_2](ClO_4)_2$(4) were prepared by template condensation reactions starting from A^1 and 2,6-pyridinedicarbaldehyde or 2,6-diacetylpyridine and metal salts (mole ratio1:1:1), in mixture of methanol and ethanol solvents (scheme. 2). All of complexes were characterized by elemental analysis, mass spectrometry and IR spectroscopy, and Zinc complexes were characterized by NMR spectroscopy. Also the crystal structures of macrocyclic complexes (1) and (3) were determined by single-crystal X-ray crystallography. The infrared spectra of complexes confirmed the formation of the macrocyclic compounds by the absence of bands of carbonyl and amine groups of the starting materials. The IR spectra of all complexes exhibited a υ(C=N) vibration in the range1617–1637 cm^{-1} and for the perchlorate anions, absorptions were found at

approximately 1100 and 620 cm^{-1}. The cytotoxic and antibacterial activities of the complexes were studied.

3.1. X-ray structures

Orange crystals of $[MnL^1(ClO_4)_2]$ (1) and yellow crystals of $[ZnL^1(H_2O)_2](ClO_4)_2$(3) suitable for single-crystal X-ray analyses were grown by slow diffusion of diethyl ether vapor into acetonitrile solution. For complex(1), the Mn atom was in a distorted pentagonal bipyramidal environment, surrounded by five nitrogen atoms from the ligand, with two monodentate perchlorate anions coordinating to Mn in the axial positions. The pyridyl nitrogen (N2) and the imine nitrogen atoms (N1 and N3) are co-planar with Mn atom, with the tertiary amine nitrogens, N4 and N5, lying above and below this plane (N4: 0.211(3) Å and N5 -0.849(3) Å) respectively. The rms deviation of the five nitrogen atoms from being planar is 0.312 Å, with Mn lying 0.0771(8) Å out of the least-squares plane. All Mn-O and Mn-N distances were between 2.201 and 2.425Å. The 10 atoms of the pyridine and imine nitrogen system were coplanar to within 0.0803(13) Å, while the dihedral angles between this plane and the imine aromatic rings were 27.59(7)° and 37.89°(5), respectively. Weak C-H...Cl and C-H...O interactions (Table 3) link the molecules into a 2D network lying in the ac plane.

For complex 3**,** the coordination environment of the Zn atom, and the conformation of the ligand, are similar to the one found for Mn complex. Zn atom is in a distorted pentagonal bipyramidal environment, surrounded by five nitrogen atoms from the ligand, with two water molecules coordinating to Zn in the axial positions. The pyridine nitrogen (N2) and the imine nitrogen atoms, (N1 and N3) are co-planar with the Zn atom, with the tertiary amine nitrogens, N4 and

N5, lying above and below the plane (N4: 0.292(3) Å and N5 -0.754(3) Å). The rms deviation of the five nitrogen atoms from being planar is 0.311 Å, with the Zn lying 0.0350(8) Å out of the least-squares plane. All Zn-O and Zn-N distances are between 2.141 & 2.486 Å. The 10 atoms of the pyridine and imine nitrogen system are coplanar to within 0.0742(14) Å, while the dihedral angles between this plane and the imine aromatic rings are 25.82(7)° and 40.92°(5), respectively. Both perchlorate anions are non-coordinating, however both are involved in hydrogen bonds with the coordinating water molecules linking the moieties into a 2D network parallel to the plane (Table 4). Additional weak C-H...O interactions, link the ions into a 3D network. The ORTEP views of complexes 1 and 3 are shown in Figs. 1 and 2, respectively. Crystallographic data and structure refinement parameters are given in Table 1 and selected bond distances and angles are given in Table 2.

Seven coordination, pentagonal bypyramidal, is unusual for first row transition elements, where octahedral coordination tends to predominate. A search of the Cambridge Structural Database [49] found 114 seven coordinate Mn(II) complexes, compared to 986 six coordinate complexes, while seven-coordinate Zn(II) complexes were even rarer, with only 31 entries, compared to 1509 six coordinate complexes. The present complexes **1** and **3** can be compared to the seven coordinates Mn(II) and Zn(II) complexes of 2,6-diacetylpyridine bis(thiosemicarbazone) ($[Mn(daptsc)(H_20)_2](ClO_4)_2$ (A) and $[Zn(H_2daptsc)(H_20)_2](N0_3)_2$ (B)) [50- 51]; in both cases the ligand adopts a pentagonal planar arrangement of the five donor atoms, with two water molecules coordinating in the apical positions. The maximum deviations from the least squares plane of the five donor atoms involve the imine N atoms, 0.14 and -0.14 Å for the complex A, and 0.166 and -0.205 Å for the complex B, indicating that the 2,6-diacetylpyridine bis(thiosemicarbazone) ligand is coordinating to the metal in a more planar arrangement than the

ligand, L^1, used here for **1** and **3.** The Mn-O bond length of 2.229(3) Å and the Mn-N bond lengths of 2.268(3) and 2.364(2) Å, are similar to those found here for **1**, while the Zn-O bond lengths of 2.128(9) and 2.13(1) Å, and the Zn-N bond lengths of 2.213(7) -2.348(9) Å, are similar to those found here for **3**. Slight differences in the geometry around the metal atom are presumably due to the 2,6-diacetylpyridine bis(thiosemicarbazone) being an open-ended macrocycle, with N_3S_2 donor atoms, while L^1 is a fully enclosed macrocycle, with five nitrogen donor atoms. The views of complexes A and B are shown in Fig. 3.

The structures of two complexes with similar ligands are known ([MnL(CH_3CN)] (C) and [CdL(CH_3OH)] (D)) [52-53], where the amine nitrogens (corresponding to N4 and N5 here) are secondary amines, with the two amine nitrogen atoms being linked by only one ethyl group, rather than two ethyl groups as for the present ligands. The differences between the ligands result in a major change in the conformation of the ligand and in the coordination environment of the metal ions. Whereas the metal in the present complexes are in a seven coordinate, distorted pentagonal planar environment, the metals in the structures [MnL(CH_3CN)] (C) and [CdL(CH_3OH)] (D) are six coordinate, in a coordination environment that is between distorted pentagonal pyramidal and distorted octahedral. The rms deviations of the five nitrogens from the least squares planes for [MnL(CH_3CN)] (C) and [CdL(CH_3OH)] (D) are 0.653 Å and 0.677 Å, respectively, with Mn atom lying 0.265 Å and the Cd atom lying 0.213 Å out of the respective least squares planes. 10 atoms of the pyridine and imine nitrogen system are coplanar within 0.114 Å, for A, and 0.165 Å for C, while the dihedral angles between this plane and the imine aromatic rings are 46.42° and 55.70° for A, and 45.95° and 51.43°, for C, respectively. The present ligand, with tertiary amines, results in the conformation of the ligand being more planar than the ligand with secondary amines. The five nitrogen atoms are closer to being planar,

allowing a more planar coordination of the ligand to the metal, and less steric hindrance, permitting a seven coordinate geometry around the metal. Space fill models (Mercury) for A and B show that the conformation of the ligand does not permit 7-coordination due to steric hindrance, while for the present complexes, the conformation of the ligand easily allows seven coordination. The macrocycle in C and D, with secondary amines, is flexible enough to allow the metal to achieve a 6-coordination environment, with an additional donor molecule, even if the coordination environment is highly distorted. The present ligand, with tertiary amines, is less flexible, which results in the five nitrogen donor atoms being in a, somewhat distorted, pentagonal planar arrangement, thus permitting 7-coordination, with 2 additional donor atoms. The views of complexes C and D are shown in Fig. 4. The Space Fill diagrams for the complexes 1, 3, C and D are shown in Fig. 5.

<Please insert Fig. 1>

<Please insert Fig. 2>

<Please insert Fig. 3>

<Please insert Fig. 4>

<Please insert Fig. 5>

<Please insert Table 2>

<Please insert Table 3>

<Please insert Table 4>

3.2. Cytotoxic activity

Cytotoxic activity of the four macrocyclic complexes was evaluated against three cell lines of human cancer including H1299, A2780 and U37 MG and a normal cell line Hs27 using MTT method, and the IC50 values for each compound were determined. As shown in Figures 6, complex 3 efficiently inhibited all cell growth tested in a dose dependent manner. The IC50 of complex 3 was 40 ±3.28 mcM, 25 ± 4.02 mcM, and 20±1.52 mcM in H1299, A2780 and U87 MG cells, respectively. Also, all complexes had a potent cytotoxic effect on U87 MG human glioblastoma carcinoma cells. The IC50 values for doxorubicin were 4.2±0.89, 27.3±3.9, 23.3±1.7 in A2780, H1299 and U87 MG cell lines, respectively. According to results, complex 3 was more potent than doxorubicin in U87 MG cell line predicting their therapeutic potential in treatment of glioblastoma. In contrast to what was observed for the cancer cell lines, no cytotoxicity was observed in the non-transformed Hs27 cell line at concentration up to 75 mcM (Fig 6). Therefore, the apparent absence of cytotoxicity of all compounds to the non-transformed Hs27 cell line in vitro is interesting, but more studies are required on other non-transformed cell lines.

<Please insert Fig. 6>

3.3. Antibacterial properties

Antibacterial properties of four macrocyclic complexes were studied against a number of Gram-positive and Gram-negative bacterial strains (Table 5). All of these complexes inhibited the growth of bacterial strains, producing a zone of inhibition of diameter 7.0–32.0 mm. The most effective compounds were 1 and 2 against *B. Thuringiensis* and *Pectobacterium SP*. In some cases, these compounds were even more effective than the standard antibiotics Tobramycin and Tetracycline. Since the comparison of the size of inhibition zones is generally not trustworthy,

the MIC values of the compounds were also determined. The results indicated that the MIC values against the tested organisms varied between 4 against *Pectobacterium Sp.* to 12 against *B. thuringiensis* $lgmL^{-1}$. The standard antibiotic tobramycin showed MIC values 4 lg mL^{-1} and Tetracycline 8 $lgmL^{-1}$. Hence, these compounds have stronger properties than the standard antibiotics against some bacterial strains.

<Please insert Table 5>

4. Conclusion

This paper involved the synthesis and spectroscopic characterization of new symmetric diamine A^1 that was prepared from condensation of 2-nitrobenzylchloride and piperazine in acetonitrile solution. Four macrocyclic Schiff-base complexes of Mn(II) and Zn(II) were prepared via the metal template [1+1] cyclocondensation of 2,6-pyridinedicarbaldehydeor 2,6-diacetylpyridine with a new diamine A^1. All of products were characterized by using different physical techniques. Molecular structures of complexes 1 and 3 were revealed by single crystal X-ray analysis. The crystal structure of 1 and 3 shows distorted pentagonal bipyramidal geometry. All products were investigated for Cytotoxic and antibacterial properties. The results of cytotoxic activity studies indicated complex 3 was more potent than doxorubicin as standard in U87 MG cell line predicating their therapeutic potential in treatment of glioblastoma. The results of antibacterial studies indicate that complexes 1 and 2 against *B. thuringiensis*, *S. saprophyticus* and *Pectobacterium SP.* exhibit more activity than Tobramycin and Tetracycline as standards.

Acknowledgements

We are grateful to the Faculty of Chemistry of Bu-Ali Sina University, for financial supports.

Appendix A. Supplementary data

CCDC 1491879 and 1491880 contain the supplementary crystallographic data for $[MnL^1(ClO_4)_2]$ and $[ZnL^1(H_2O)_2](ClO_4)_2$ compounds, respectively. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e303 mail: deposit@ccdc.cam.ac.uk

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Table 1.
Crystal data and structure refinement for macrocyclic complexes $[MnL^1(ClO_4)_2]$ (1) and $[ZnL^1(H_2O)_2](ClO_4)_2$ (3).

Identification code	$[MnL^1(ClO_4)_2]$ (1)	$[ZnL^1(H_2O)_2](ClO_4)_2$ (3)
Empirical formula	$C_{25}H_{25}Cl_2MnN_5O_8$	$C_{25}H_{29}Cl_2N_5O_{10}Zn$
Formula weight	649.34	695.80
T (K)	130.01(10)	130.00(10)
Crystal system	triclinic	monoclinic
Space group	$P\bar{1}$	$P2_1/c$
Unit cell dimensions		
a (Å)	9.4526(5)	14.0988(6)
b (Å)	10.9939(7)	10.7745(4)
c (Å)	14.3110(9)	18.3415(6)
α (°)	83.879(5)	90
β (°)	72.528(5)	92.392(4)
γ (°)	66.272(6)	90
Volume ($Å^3$)	1298.52(15)	2783.79(17)
Z	2	4
D_{calc} (mg/m^3)	1.661	1.660
Absorption coefficient (mm^{-1})	0.776	1.142
F(000)	666.0	1432.0
Crystal size (mm^3)	0.296 × 0.221 × 0.141	0.277 × 0.219 × 0.172
Radiation	MoK_α (λ = 0.71073)	MoK_α (λ = 0.71073)
2Θ range for data collection (°)	6.57 to 60.842	6.596 to 60.99
Index ranges	$-11 \le h \le 13$	$-19 \le h \le 14$
	$-13 \le k \le 15$	$-15 \le k \le 14$
	$-18 \le l \le 18$	$-25 \le l \le 25$
Reflections collected	13427	29112
Independent reflections	6761 [R_{int} = 0.0306, R_{sigma} = 0.0478]	7705 [R_{int} = 0.0340, R_{sigma} = 0.0339]
Data/restraints/parameters	6761/0/370	7705/0/404
Goodness-of-fit on F^2	1.051	1.046
Final R indexes [I>=2σ (I)]	R_1 = 0.0412	R1 = 0.0399
	wR_2 = 0.0963	wR2 = 0.0928
Final R indexes [all data]	R_1 = 0.0522	R1 = 0.0506
	wR_2 = 0.1070	wR2 = 0.1013
Largest differences in peak and hole (e $Å^{-3}$)	0.44 and -0.68	0.92 and -0.81

Table 2.
Bond Lengths and Bond Angles for complexes $[MnL^1(ClO_4)_2]$ (1)and $[ZnL^1(H_2O)_2](ClO_4)_2$ (3)

$[MnL^1(ClO_4)_2]$ (1)		$[ZnL^1(H_2O)_2](ClO_4)_2$ (3)	
Bond	Length (Å)	Bond	Length (Å)
Mn1- O1	2.2871(15)	Zn1- O1	2.1684(16)
Mn1- O5	2.2488(15)	Zn1- O2	2.1407(17)
Mn1- N1	2.3920(17)	Zn1- N1	2.3637(17)
Mn1- N2	2.2007(16)	Zn1- N2	2.1640(17)
Mn1- N3	2.4247(17)	Zn1- N3	2.4859(18)
Mn1- N4	2.2786(16)	Zn1- N4	2.2234(17)
Mn1- N5	2.3199(16)	Zn1- N5	2.2397(18)
Bond	Angle (˚)	Bond	Angle (˚)
O1- Mn1- N1	92.11(6)	O1- Zn1- N1	92.97(6)
O1- Mn1- N3	83.26(6)	O1- Zn1- N3	81.96(6)
O1- Mn1- N5	78.62(6)	O1- Zn1- N4	105.98(6)
O5- Mn1-O1	174.69(6)	O1- Zn1- N5	83.84(6)
O5- Mn1-N1	89.61(6)	O2- Zn1- O1	169.15(7)
O5- Mn1-N3	92.31(6)	O2- Zn1- N1	80.84(7)
O5- Mn1-N4	80.39(6)	O2- Zn1- N2	88.13(7)
O5- Mn1-N5	106.65(6)	O2- Zn1- N3	97.51(7)
N1- Mn1-N3	142.23(6)	O2- Zn1- N4	84.37(7)
N2- Mn1-O1	85.21(6)	O2- Zn1- N5	103.56(7)
N2- Mn1-O5	90.58(6)	N1- Zn1- N3	142.56(6)
N2- Mn1-N1	71.31(6)	N2- Zn1- O1	81.45(6)
N2- Mn1-N3	70.96(6)	N2- Zn1- N1	71.86(6)
N2- Mn1-N4	148.67(6)	N2- Zn1- N3	70.70(6)
N2- Mn1-N5	145.42(6)	N2- Zn1- N4	145.92(7)
N4- Mn1-O1	101.59(6)	N2- Zn1- N5	146.58(7)
N4- Mn1-N1	137.84(6)	N4- Zn1- N1	138.74(6)
N4- Mn1-N3	79.44(6)	N4- Zn1- N3	77.40(6)
N4- Mn1-N5	65.39(6)	N4- Zn1- N5	67.25(7)
N5- Mn1-N1	78.90(6)	N5- Zn1- N1	79.16(6)
N5- Mn1-N3	135.70(6)	N5- Zn1- N3	136.27(6)

Table 3.
Hydrogen bonds for complex (1).

D H A	d(D-H) (Å)	d(H-A) (Å)	d(D-A) (Å)	D-H-A (°)
C3 H3 Cl2[1]	0.95	2.95	3.558(2)	123.0
C3 H3 O7[1]	0.95	2.62	3.488(3)	151.8
C5 H5 O3[2]	0.95	2.50	3.319(3)	144.2
C14 H14A O2[3]	0.99	2.58	3.451(3)	147.0

[1]1-X, 1-Y, 2-Z; [2]2-X, 1-Y, 1-Z; [3]-1+X, +Y, +Z

Table 4.
Hydrogen bonds for complex (3).

D H A	d(D-H) (Å)	d(H-A) (Å)	d(D-A) (Å)	D-H-A (°)
O1 H1A O7[1]	0.77(3)	2.06(3)	2.808(2)	164(3)
O1 H1B O7	0.82(4)	1.99(4)	2.775(2)	160(3)
O2 H2A O4[2]	0.74(4)	2.17(4)	2.882(3)	164(4)
O2 H2B O3	0.72(4)	2.07(4)	2.787(3)	174(4)
C14 H14B O3	0.99	2.65	3.592(3)	159.5
C14 H14B O4	0.99	2.65	3.334(3)	126.4
C15 H15A O6[3]	0.99	2.38	3.346(3)	164.5
C15 H15B O8	0.99	2.65	3.546(3)	151.3

[1]-X, 1-Y, 1-Z; [2]1-X, 1/2+Y, 3/2-Z; [3]1-X, -1/2+Y, 3/2-Z

Table 5.
Antibacterial activity of macrocyclic complexes that was expressed as diameter of inhabitation zone (mm) and minimum inhibitory concentration (MIC)

Microorganisms	standard		Zone of inhabitation (mm) Main compounds			
	Tobramycin (10 mg mL^{-1})	Tetracyclin (10 mg mL^{-1})	(1)	(2)	(3)	(4)
MIC (mcg/ml)	4	8	4	4	12	12
Gram (+)						
B. Thuringiensis	6	8	30	32	8	7
S. Saprophyticus	22	15	20	21	0	0
B. Subtilis	13	15	0	0	15	8
Gram (-)						
P. Fluorescens	5	0	0	0	0	0
Pectobacterium SP	4	6	28	29	7	7

Figure caption:

Scheme 1. The process for the synthesis of diamine (A^1).

Scheme 2. The process for the Synthesis of macrocyclic complexes $[MnL^1(ClO_4)_2]$ (1), $[MnL^2(ClO_4)_2]$ (2), $[ZnL^1(H_2O)_2](ClO_4)_2$ (3) and $[ZnL^2(H_2O)_2](ClO_4)_2$ (4)

Scheme 3. Schematic structures of macrocyclic complexes (1), (2), (3) and (4)

Fig. 1. ORTEP representation of $[MnL^1(ClO_4)_2]$ (1). Displacement ellipsoids are drawn at the 50 % probability level. The H atomsare omitted for clarity.

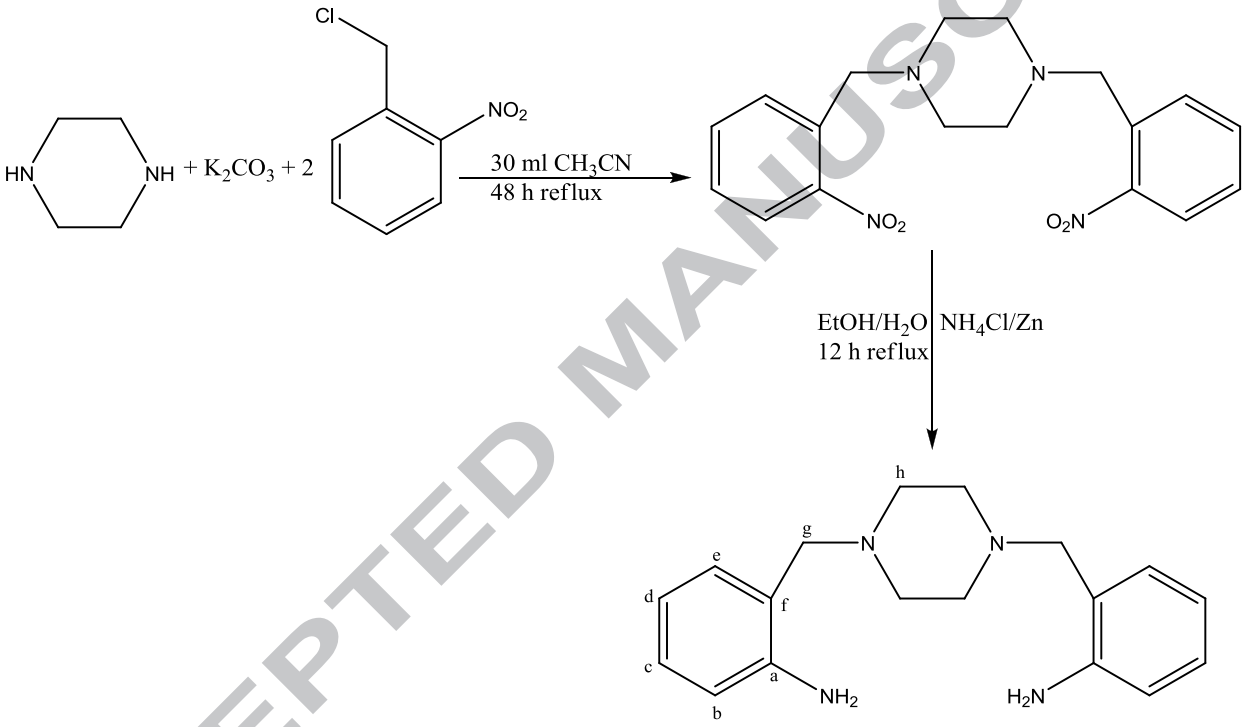
Fig. 2. ORTEP representation of $[ZnL^1(H_2O)_2](ClO_4)_2$ (3). Displacement ellipsoids are drawn at the 50 % probability level. The H atoms and counter ions (ClO_4^-) are omitted for clarity

Fig. 3. ORTEP representation of $[Mn(daptsc)(H_20)_2](ClO_4)_2$ (A) and$[Zn(H_2daptsc)(H_20)_2](N0_3)_2$ (B) [50-51].

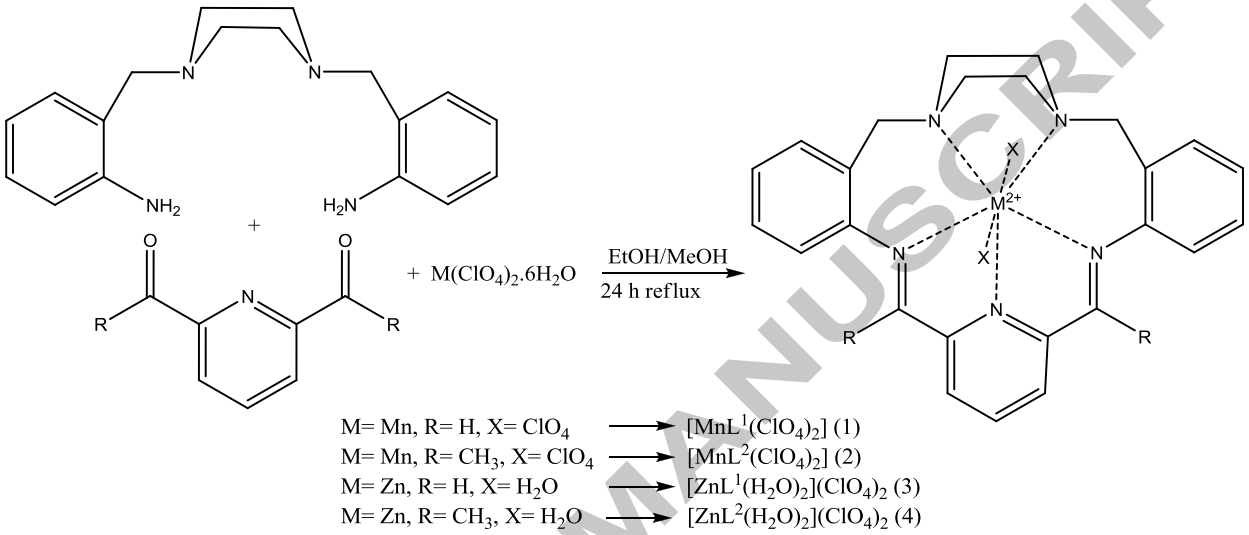
Fig. 4. The structures of $[MnL(CH_3CN)]$ (C) and $[CdL(CH_3OH)]$ (D) [52-53].

Fig. 5. Space Fill diagrams for the complexes 1, 3, $[MnL(CH_3CN)]$ (A) and $[CdL(CH_3OH)]$ (B).

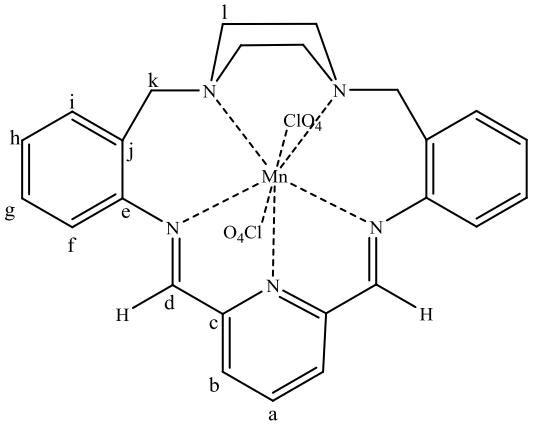
Fig 6. Cell viability of H1299, A2780, U87 MG and Hs27 cell lines after 24 hr treatment with different concentrations of 1, 2, 3, 4 Data are expressed as Mean ± S.E.M three experiments.



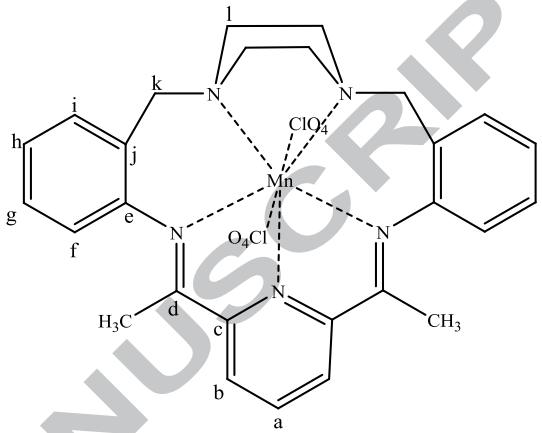
Scheme 1. The process for the Synthesis of diamine (A^1).



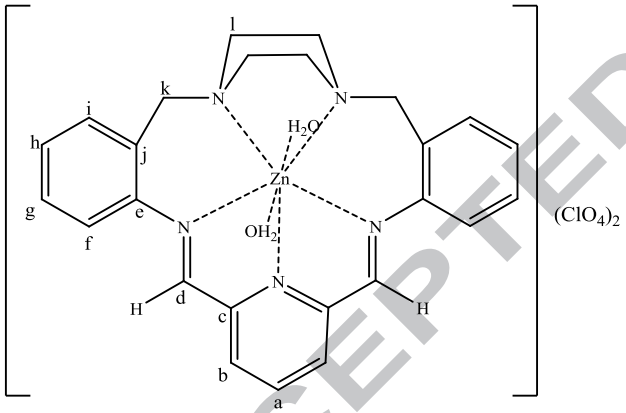
Scheme 2. The process for the Synthesis of macrocyclic complexes $[MnL^1(ClO_4)_2]$ (1), $[MnL^2(ClO_4)_2]$, $[ZnL^1(H_2O)_2](ClO_4)_2$ (3) and $[ZnL^2(H_2O)_2](ClO_4)_2$ (4)



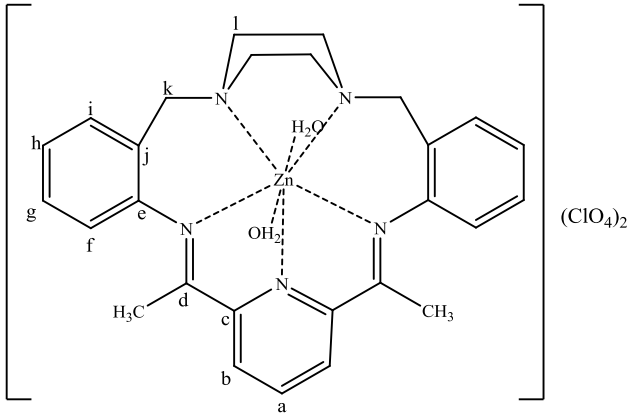
$[MnL^1(ClO_4)_2]$ (1)



$[MnL^2(ClO_4)_2]$ (2)



$[ZnL^1(H_2O)_2](ClO_4)_2$ (3)



$[ZnL^2(H_2O)_2](ClO_4)_2$ (4)

Scheme 3. Schematic structures of macrocyclic complexes (1), (2), (3) and (4)

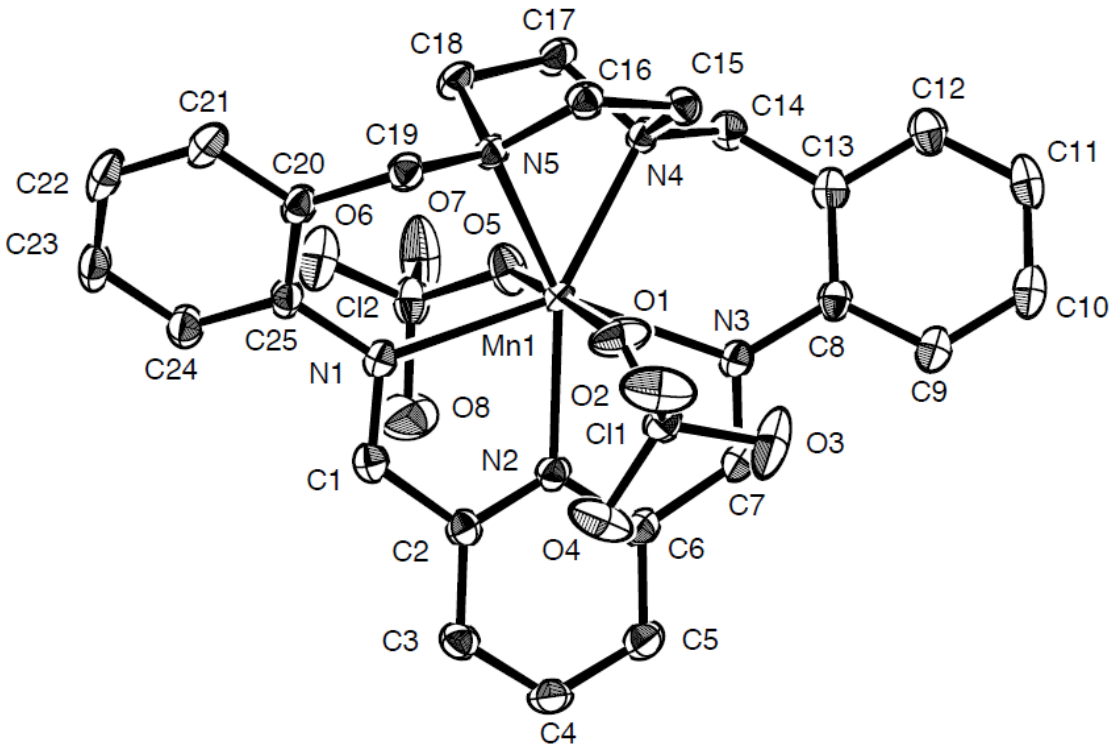


Fig. 1. ORTEP representation of $[MnL^{1}(ClO_4)_2]$ (1). Displacement ellipsoids are drawn at the 50 % probability level. The H atoms are omitted for clarity.

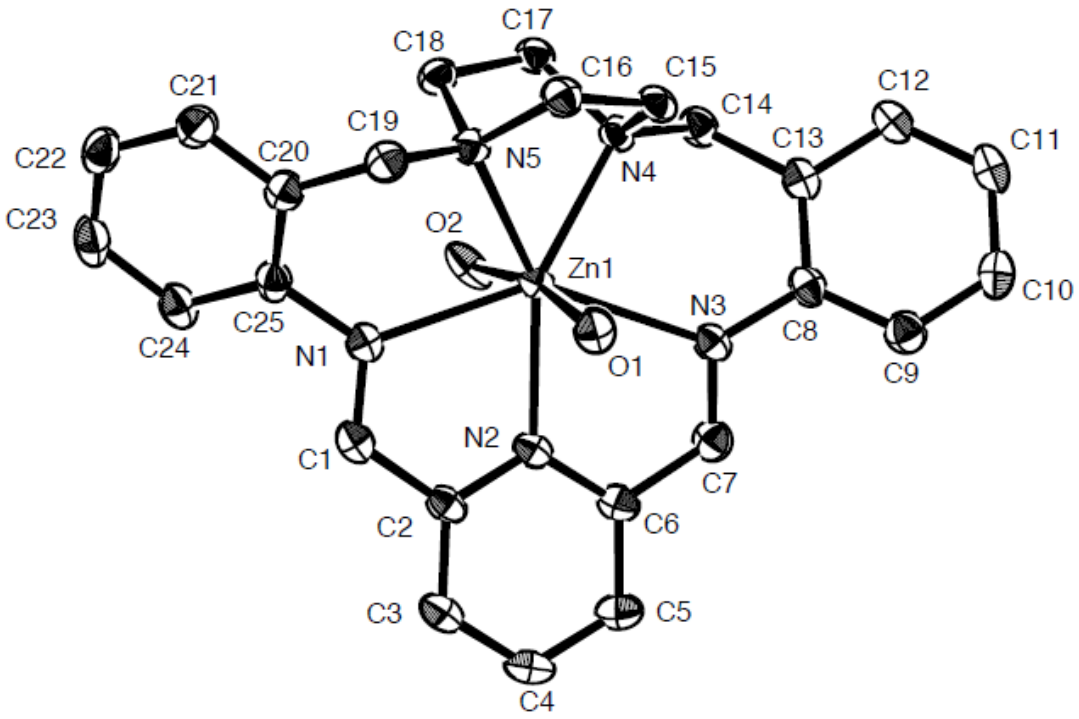
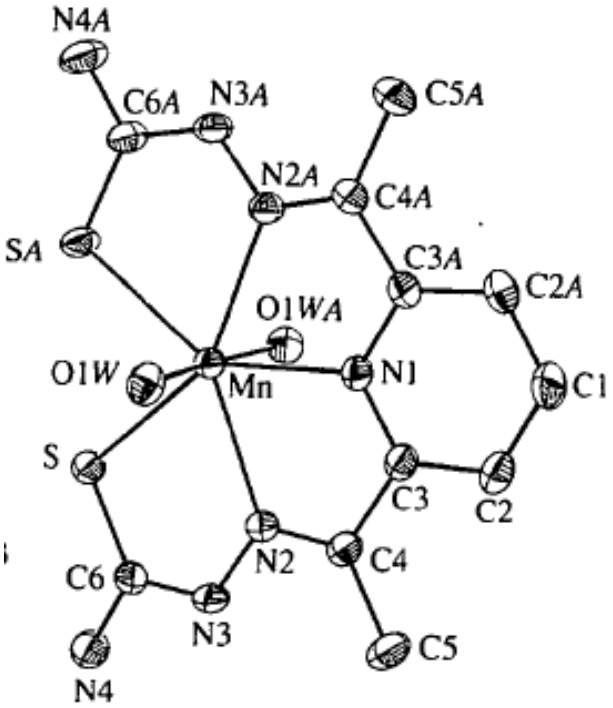
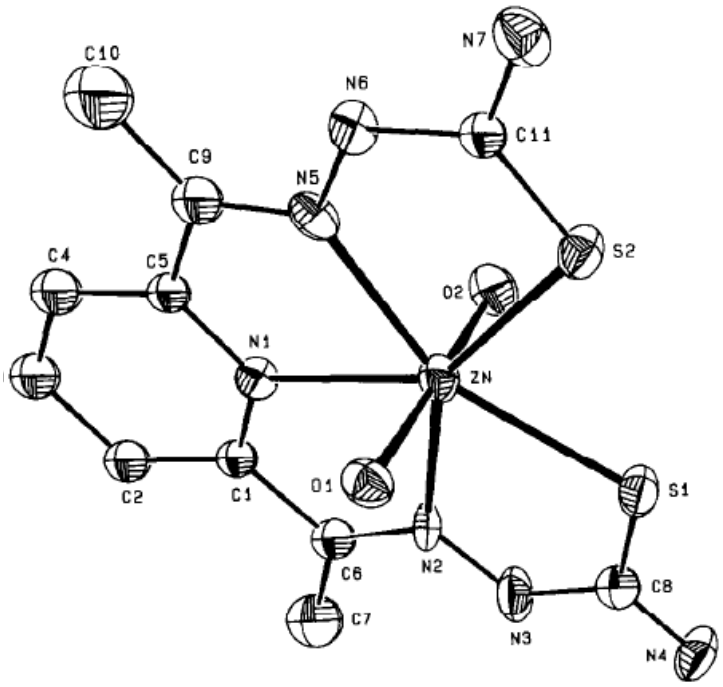


Fig. 2. ORTEP representation of $[ZnL^{1}(H_2O)_2](ClO_4)_2$ (3). Displacement ellipsoids are drawn at the 50 % probability level. The H atoms and counter ions (ClO_4^-) are omitted for clarity.

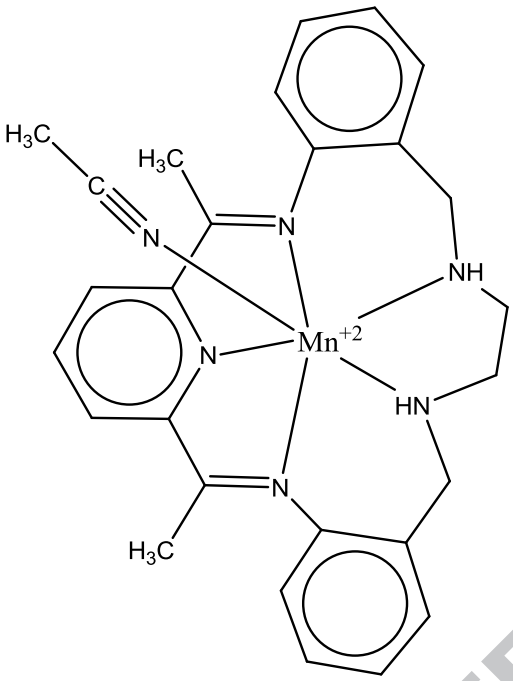




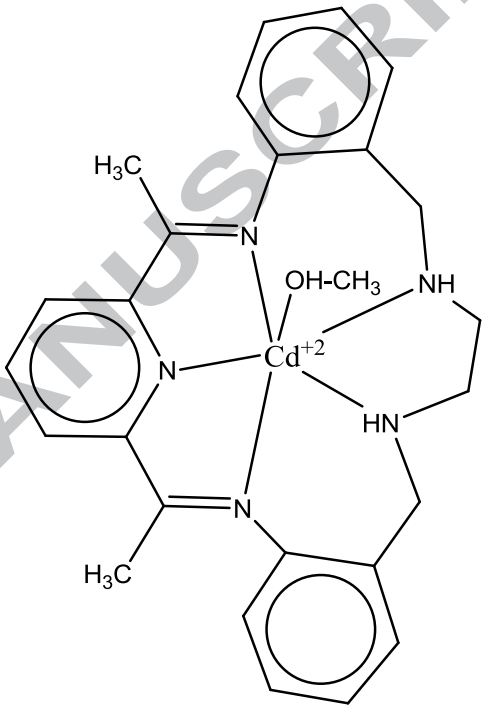
[Mn(daptsc)(H_20)$_2$](ClO_4)$_2$ (A)

[Zn(H_2daptsc)(H_20)$_2$]($N0_3$)$_2$ (B)

Fig. 3. ORTEP representation of [Mn(daptsc)(H_20)$_2$](ClO_4)$_2$ (A) and[Zn(H_2daptsc)(H_20)$_2$]($N0_3$)$_2$ (B)

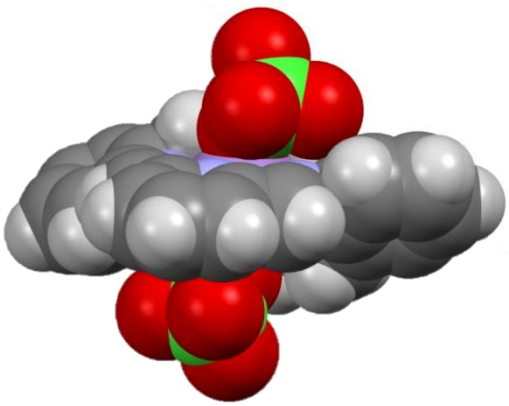


[MnL(CH_3CN)] (C)

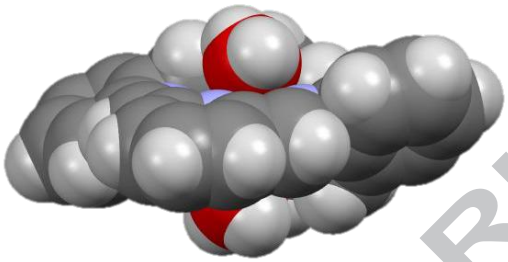


[CdL(CH_3OH)] (D)

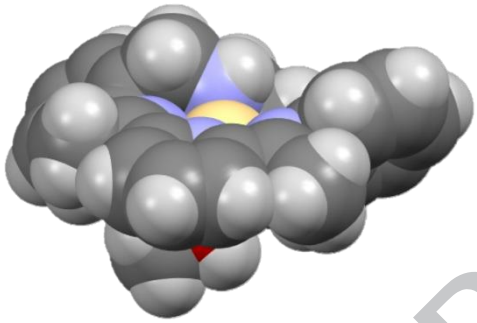
Fig. 4. The structures of [MnL(CH_3CN)] (C) and [CdL(CH_3OH)] (D).



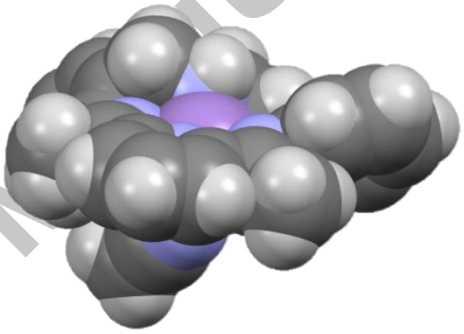
(1)



(3)



C



D

Fig. 5. Space Fill diagrams for the complexes 1, 3, C and D.

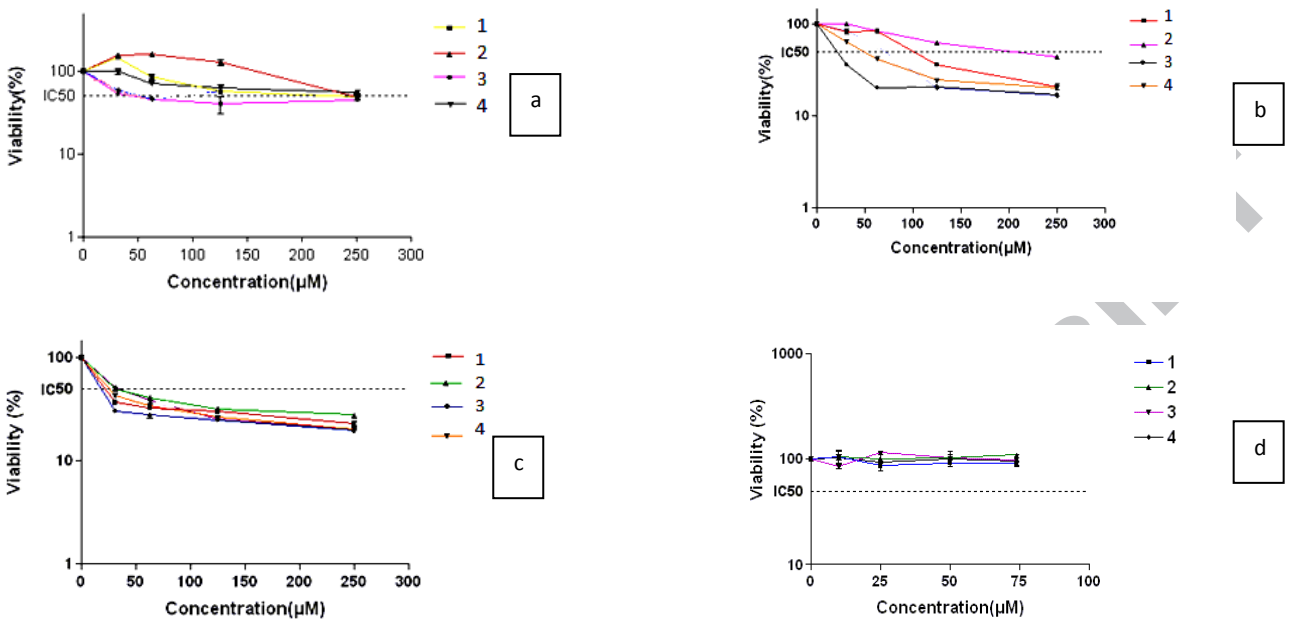
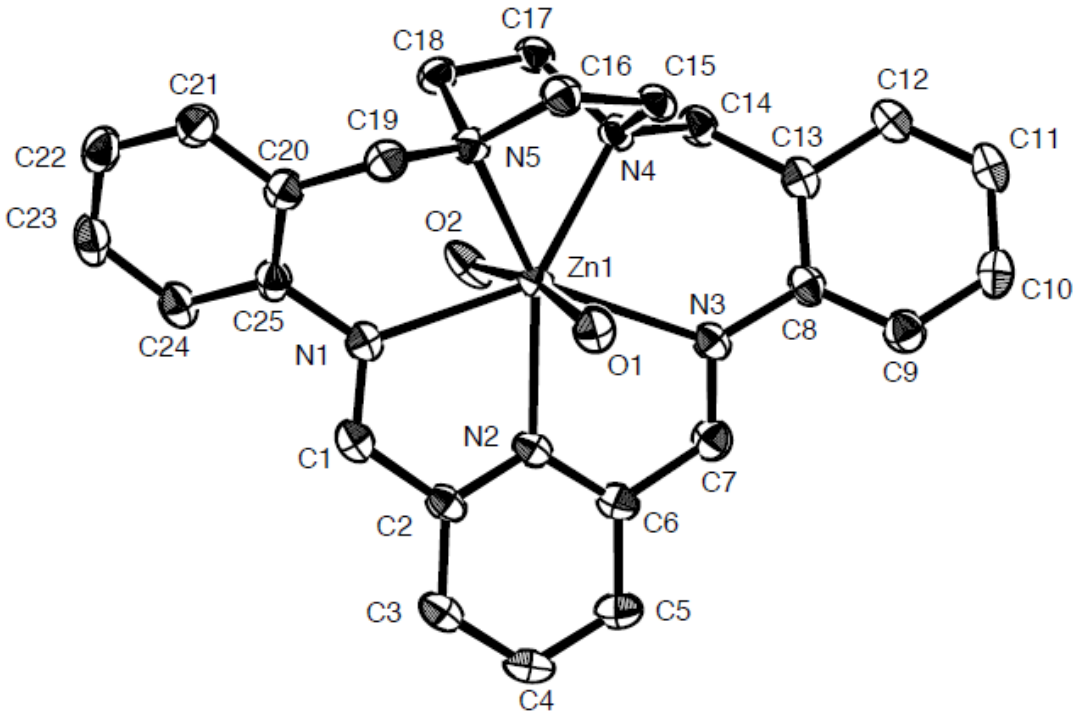


Fig. 6. Cell viability of H1299, A2780, U87 MG and Hs27 (a-d) cell lines after 24 h treatment with different concentrations of 1, 2, 3, 4 Data are expressed as Mean ± S.E.M three experiments.



Four new Mn(II) and Zn(II) macrocyclic Schiff-base complexes have been prepared via the metal templated [1+1] cyclocondensation of 2,6-diacetylpyridine or 2,6- pyridinedicarbaldehyde with new diamine A^1 . The crystal structures of macrocyclic complexes $[MnL^1(ClO_4)_2]$ (1) and $[ZnL^1(H_2O)_2](ClO_4)_2$ (3) were determined by single-crystal X-ray crystallography. The cytotoxic and antibacterial properties of the isolated macrocyclic Shiff-base complexes have been studied.