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Metalloantibiotics: Synthesis, characterization and *in-vitro* antibacterial studies on cobalt (II), copper (II), nickel (II) and zinc (II) complexes with cloxacillin

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Abstract

The synthesis and characterization of cloxacillin (clox) complexes with divalent metal ions [Co (II), Cu (II), Ni (II) and Zn (II)] is described. The nature of bonding of the chelated cloxacillin and the structures of the metal complexes have been elucidated on the basis of their physical and spectroscopic data. In all the complexes, the cloxacillin acts as a uninegatively charged bidentate ligand with coordination involving the carboxylate-O and endocyclic-N of the β-lactam ring. The new compounds have been screened for *in-vitro* antibacterial activity against *Escherichia coli (a)*, *Klebsiella pneumonae (b)*, *Proteus mirabilis (c)*, *Pseudomonas aeruginosa (d)*, *Salmonella typhi (e)*, *Shigella dysentriae (f)*, *Bacillus cereus (g)*, *Corynebacterium diphtheriae (h)*, *Staphylococcus aureus (j)* and *Streptococcus pyogenes (k)* bacterial strains. The brine shrimp bioassay was also carried out to study their *in-vitro* cytotoxic properties. All compounds, respectively, showed a promising activity (90%) against five bacterial species at 10 μg/ml concentration and a significant activity (52%) against the same test bacteria at 25 μg/ml concentration.

Keywords: *Cloxacillin, metal complexes, antibacterial, cytotoxicity*

Introduction

Cloxacillin (clox) is a commonly used biologically important drug which has been shown to exert pronounced biological effects on various bacterial strains. Most living systems contain metal ions essential for their proper functioning [1–4]. Much research has indicated [5–11] that antibacterial or antifungal drugs when used as chelates of different metals have enhanced activity. Similar studies of the effect of metal ions on the cloxacillin nucleus could possibly lead to a better understanding of those complex biological processes that occur in living systems. The present work is a continuation of our ongoing research [12–24] directed towards perceiving the nature of metal binding, behavior of cloxacillin as a potential ligand and its biological evaluation upon coordination with different metal ions [Co (II), Cu (II), Ni (II) & Zn (II)]. For this purpose, different analytical tools have been used. The prepared metal chelates of cloxacillin have been found to have *in-vitro* antibacterial activity against *E. coli*, *K. pneumonae*, *P. mirabilis*, *P. aeruginosa*, *S. typhi*, *S. dysentriae*, *B. cereus*, *C. diphtheriae*, *S. aureous* and *S. pyogenes* bacterial strains. The brine shrimp bioassay was also carried out to study their *in-vitro* cytotoxic properties.

Material and methods

Solvents used were of analytical grade and all metal (II) were used as chloride salts. IR spectra were recorded on a Philips Analytical PU 9800 FTIR spectrophotometer, NMR spectra on a Perkin–Elmer

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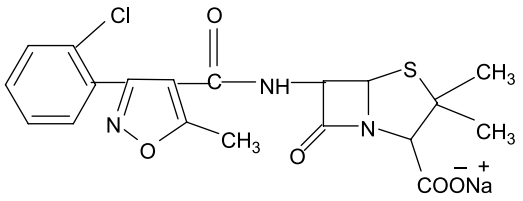


Figure 1. Structure of cloxacillin.

283B spectrometer and UV–Visible spectra in DMF on a Hitachi U-2000 double-beam spectrophotometer. Butterworth Laboratories Ltd (U.K.) carried out C, H and N analyses. Conductance of the metal complexes was determined in DMF on a Hitachi (Japan) YSI-32 model conduct meter. Magnetic measurements were carried out on solid complexes using Gouy's method. Melting points were recorded on a Gallenkamp (U.K.) apparatus and are not corrected. The complexes were analyzed for their metal content by EDTA titration. Antibacterial and cytotoxic screening was done at HEJ Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Pakistan.

Preparation of metal (II) complex (1)

For the preparation of metal (II) complexes with cloxacillin (Figure 1), two different molar ratios of metal: cloxacillin as 1:1 and 1:2 were used. The complexes having a molar ratio of metal: cloxacillin as 1:1 were prepared adding a solution of the corresponding metal (II) chloride (0.01 mol) in ethanol (25 mL) to a solution of the sodium salt of cloxacillin (0.01 mol) in a mixture of water-ethanol (20 mL, 1:1 v/v). The mixture was refluxed for 1 h and concentrated to half volume. Then, on cooling to room temperature the precipitated complex formed was filtered, washed with water-ethanol, then with ether and dried *in vacuo*. Crystallization from hot aqueous-ethanol (30:70) gave the desired metal complex. The same method was used for the preparation of the other complexes (**2**–**8**) using their corresponding molar ratios.

Biological activity

Antibacterial bioassay (in-vitro)

All the synthesized metal (II) complexes (**1**–**8**) were screened *in-vitro* for their antibacterial activity against *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *S. typhi*, *S. dysenteriae*, *B. cereus*, *C. diphtheriae*, *S. aureous* and *S. pyogenes* using the agar well diffusion method [25]. Two to eight hours old bacterial inoculums containing approximately 10^4–10^6 colony forming units (CFU)/ml were used in these assays. The wells were dug in the media with the help of a sterile metallic borer with centers at least 24 mm. Recommended concentration (100 μl) of the test sample (1 mg/ml in DMSO) was introduced into the respective wells. Other wells supplemented with DMSO and reference antibacterial drug, imipenum served as negative and positive controls respectively. The plates were incubated immediately at 37°C for 20 h. Activity was determined by measuring the diameter of zones (mm) showing complete inhibition. Growth inhibition was compared with the standard drug. In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions of DMSO alone which showed no activity against any bacterial strains.

Minimum inhibitory concentration (MIC)

Compounds showing promising antibacterial activity were selected for minimum inhibitory concentration studies. The minimum inhibitory concentration was determined using the disc diffusion technique by preparing discs containing 10, 25, 50 and 100 μg/ml of the compounds and applying the reported protocol [26].

*Cytotoxicity (*in-vitro*)*

Brine shrimp (*Artemia salina* leach) eggs were hatched in a shallow rectangular plastic dish (22x32 cm) filled with artificial seawater, which was prepared with commercial salt mixture and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the minor compartment was opened to ordinary light. After two days nauplii were collected by a pipette from the lighted side. A sample of the test compound was prepared by dissolving 20 mg of each compound in 2 ml of DMF. From these stock solutions 500, 50 and 5 μg/ml were transferred to 9 vials (three for each dilutions were used for each test sample and the LD_{50} is the mean of three values) and one vial was kept as control having 2 mL of DMF only. The solvent was allowed to evaporate overnight. After two days, when shrimp larvae were ready, 1 mL of seawater and 10 shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with seawater to 5 mL per vial. After 24 h the number of survivors was counted. Data were analyzed by a Finney computer program to determine the LD_{50} values [26].

Results and discussion

Chemistry

The sodium salt of cloxacillin was used as ligand to prepare its cobalt (II), copper (II), nickel (II) and zinc (II) metal complexes (1-8) which were all characterized

Table I. Physical and spectral data of the metal (II) complexes.

No	Complex	M.P (°C)	Yield (%)	B.M (μ_{eff})	M. Conductance ($ohm^{-1}\,cm^{-2}\,mol^{-1}$)	Calc. (Found) % C	H	N
1.	$[Co(clox)_2(OH_2)_2]Cl_2$ [1034.85] $C_{38}H_{38}CoCl_4N_6O_{12}S_2$	〉300	64	4.1	95	44.06(44.23)	3.67(3.44)	8.11(8.50)
2.	$[Cu(clox)_2]Cl_2$ [1003.46] $C_{38}H_{34}CuCl_4N_6O_{10}S_2$	〉300	67	1.6	93	45.44(41.81)	3.38(3.62)	8.37(8.49)
3.	$[Ni(clox)_2(OH_2)_2]Cl_2$ [1034.61] $C_{38}H_{38}NiCl_4N_6O_{12}S_2$	〉300	65	3.3	92	44.07(44.35)	3.67(3.81)	8.11(8.40)
4.	$[Zn(clox)_2(OH_2)_2]Cl_2$ [1041.30] $C_{38}H_{38}ZnCl_4N_6O_{12}S_2$	〉300	64	Dia	93	43.79(44.03)	3.64(3.37)	8.06(8.32)
5.	$[Co(clox)(OH_2)_3Cl]Cl$ [618.34] $C_{19}H_{23}CoCl_3N_3O_8S$	〉300	66	3.9	86	36.87(35.49)	3.71(3.56)	6.79(6.27)
6.	$[Cu(clox)(OH_2)_2]Cl$ [569.50] 〉 $C_{19}H_{21}CuCl_2N_3O_7S$	300	68	1.5	72	40.03(40.42)	3.68(3.28)	7.37(7.52)
7.	$[Ni(clox)(OH_2)_3Cl]Cl$ [618.10] $C_{19}H_{23}NiCl_3N_3O_8S$	〉300	65	3.1	84	36.88(36.12)	3.72(3.49)	6.79(6.34)
8.	$[Zn(clox)(OH_2)_3Cl]Cl$ [624.79] $C_{19}H_{23}ZnCl_3N_3O_8S$	〉300	65	Dia	78	36.49(36.83)	3.68(3.51)	6.72(6.54)

by IR, NMR, UV-Visible, molar conductance, magnetic moment and elemental analyses data. All metal complexes (Table I) were prepared by the stoichiometric reaction of the corresponding metal (II) salt as chloride with the ligand having molar ratios M:L of 1:2 and 1:1. They are all air and moisture stable and are intensely colored amorphous solids which decompose without melting. They are insoluble in common organic solvents and only soluble in water, DMF and DMSO. Molar conductance values of the Co (II), Ni (II) and Zn (II) complexes having molar ratio 1:2 (M:L), in DMF (10^{-3} M solution at 25°C) indicated higher values (92–95 $ohm^{-1}\,cm^{-2}\,mol^{-1}$) suggesting that they are electrolytic in nature [27]. However, other complexes with a molar ratio of M:L (1:1) showed their molar conductance values lower (72–86 $ohm^{-1}\,cm^{-2}\,mol^{-1}$) than the complexes having molar ratio 1:2 also indicating their electrolytic behavior [27]. All the metal complexes decomposed at more than 300°C.

The elemental analyses data (Table I) agree well with the proposed formulae for the metal (II) complexes. Efforts to grow good crystals of the ligands and their metal complexes for X-ray diffraction studies were unsuccessful due to their poor solubility in common organic solvents.

IR spectra. IR spectra of cloxacillin and its metal complexes are reported in Table II. Cloxacillin has various potential donor sites. A comparison between the IR spectra of cloxacillin and those of its transition metal complexes provides evidence regarding the

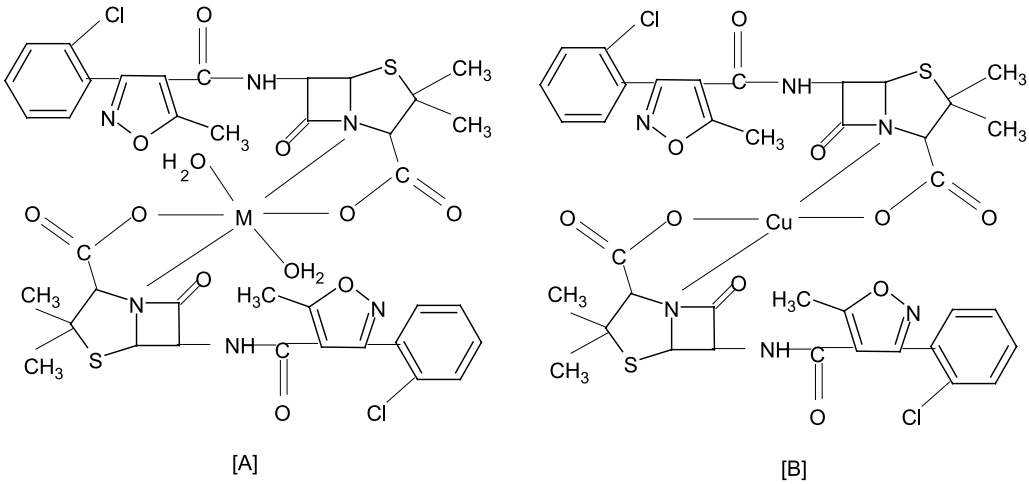


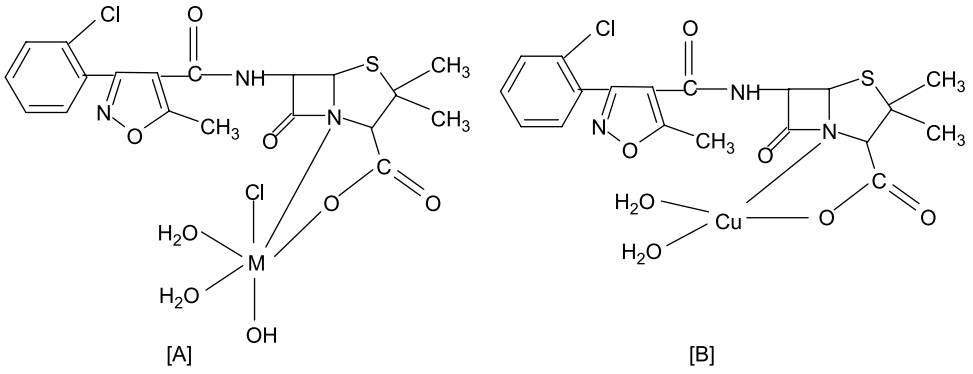
Figure 2. Proposed structure of the metal (II) complexes.

Table II. Selected IR and UV–Visible spectral data of cloxacillin and its complexes.

No	IR (cm^{-1})	λ_{max} (cm^{-1})
Cloxacillin-Na	3520 (OH), 3370 (NH), 1760 (C=O of β-lactam), 1665 (CONH), 1610 & 1415 (COO), 1495 $(C=N)_{cyclic}$, 1355 (C–N of β-lactam).	
1	3520 (OH), 1600, 1395 (COO), 1345 (M–N of β-lactam), 1765 (C=O of β-lactam),1765 1660 (C0NH), 1335 (C–N of β-lactam), 1495 $(C=N)_{cyclic}$, 850, 525 (M–N), 410 (M–O), 320 (M–Cl).	7,225, 17,165, 20,380, 27,285
2	1600, 1395 (COO), 1345 (M–N of β-lactam), 1765 (C=O of β-lactam), 1660 (C0NH), 1335 (C–N of β-lactam), 1495 $(C=N)_{cyclic}$, 525 (M–N), 410 (M–O).	14,655, 19,105, 30,110
3	3500 (OH), 1600, 1395 (COO), 1345 (M–N of β-lactam), 1765 (C=O of β-lactam),1660 (C0NH), 1335 (C–N of β-lactam), 1495 $(C=N)_{cyclic}$, 850, 525 (M–N), 410 (M–O), 320 (M–Cl).	10,185, 15,480, 26,215, 29,980
4	3500 (OH), 1600, 1395 (COO), 1345 (M–N of β-lactam), 1765 (C=O of β-lactam), 1660 (C0NH), 1335 (C–N of β-lactam), 1495 $(C=N)_{cyclic}$, 850, 525 (M–N), 410 (M–O), 320 (M–Cl).	29,115
5	3500 (OH), 1600, 1395 (COO), 1345 (M–N of β-lactam), 1765 (C=O of β-lactam), 1660 (C0NH), 1335 (C–N of β-lactam), 1495 $(C=N)_{cyclic}$, 850, 525 (M–N), 410 (M–O), 320 (M–Cl).	7,375, 17,280, 20,595, 27,560
6	1600, 1395 (COO), 1345 (M–N of of β-lactam), 1765, (C=O of β-lactam), 1660 (C0NH), 1335 (C–N of β-lactam), 1495 $(C=N)_{cyclic}$, 525 (M–N), 410 (M–O).	15,150, 19,375, 30,280
7	3500 (OH), 1600, 1395 (COO), 1345 (M–N of β-lactam), 1765 (C=O of β-lactam), 1660 (C0NH), 1335 (C–N of β-lactam), 1495 $(C=N)_{cyclic}$, 850, 525 (M–N), 410 (M–O), 320 (M–Cl).	10,215, 15,535, 26,280, 30,115
8	3500 (OH), 1600, 1395 (COO), 1345 (M–N of β-lactam), 1765 (C=O of β-lactam), 1660 (C0NH), 1335 (C–N of β-lactam), 1495 $(C=N)_{cyclic}$, 850, 525 (M–N), 410 (M–O), 320 (M–Cl).	29,350

bonding sites in the cloxacillin complexes. The IR spectra of all cloxacillin complexes show bands at 3500 and 850 cm^{-1} characteristic of coordinated water molecules, respectively [28]. The IR spectra of $[M(clox)_2(OH_2)_2]Cl_2$ complexes where M=Co (II), Ni (II) and Zn (II) reveal bands at 1610–1600 and 1415–1395 cm^{-1} due to $\nu_{asym}(COO^-)$ and $\nu_{sym}(COO^-)$, these bands are decreased in intensity and shifted to negative frequency on complexation suggesting coordination via the carboxylate group. However, the band due to ν(C–N) of the β-lactam ring (1355 cm^{-1}) in the free ligand is shifted to negative frequency and decreased in intensity, thus indicating [29] coordination through the β-lactam nitrogen group. In addition, the ligand exhibits bands at 1765, 1660 and 1495 cm^{-1} assigned to the ν(C=O) of β-lactam, amide and C=O stretch and $\nu(C=N)_{cyclic}$ respectively, and they remain unchanged on complexation. In the far IR spectra, the evidence of bonding of nitrogen and oxygen is provided by the presence of bands at 525 cm^{-1} (M – N) and 420–410 cm^{-1} (M–O) [30]. The metal complexes of Co (II), Ni (II) and Zn (II) possess chlorides attached to the metal ions which are supported by the presence of ν(M–Cl) at 315–320 cm^{-1}. The presence of chloride ions in the structural configuration of these complexes suggests that cloxacillin acts as a bidentate monoanionic molecule during complexation with the metal ions by possibly forming octahedral geometry (Figure 2A). However, this band at 320 cm^{-1} due to ν(M–Cl) was absent in the spectra of the Cu (II) complex (Figure 2B) indicating non-involvement of chloride ions bonding with the Cu (II) metal ion thus giving a square-planar geometry, $[Cu(L)_2]Cl_2$. The Co (II), Ni (II) and Zn (II) complexes having 1:1 molar ratio of M:L showed bands at 3500 and 850 cm^{-1} due to the coordinated water molecules, 1610 and 1415 cm^{-1} assigned [30] to the coordination of $\nu_{asym+sym}(COO^-)$, 1345, 525, 410 and 320 cm^{-1} assigned to ν(C–N) of the β-lactam ring, ν(M–N), ν(M–O) and ν(M–Cl), respectively (Figure 3A) possessing an octahedral geometry. The Cu (II) complex, having 1:1 molar ratio of Cu:L showed all the expected bands however, that of the ν(M–Cl) at 315 cm^{-1} was absent showing a square-planar geometry (Figure 3B).

NMR spectra. The ^{1}H NMR spectra of the free ligand and its diamagnetic Zn (II) chelates were taken in



M = Co (II), Ni (II) or Zn (II)

Figure 3. Proposed structure of the metal (II) complexes.

DMSO-d_6. The ^{1}H NMR spectral data is reported along with the possible assignments in Table III. All the protons were found to be in their expected region [31]. The conclusions drawn from these studies lend further support to the mode of bonding discussed above from their IR spectra. In the spectra of diamagnetic Zn (II) complexes, these protons shifted downfield due to the increased conjugation and coordination to the metal atoms [32]. The number of protons calculated from the integration curves, agree with obtained from the values of the expected CHN analyses. It was also observed that DMSO did not have any coordinating effect either on the spectra of the ligands or on their metal complexes.

Electronic spectra. The Co(II) complexes exhibited well-resolved, low-energy bands at 7,225–7,375 cm^{-1}, 17,165–17,280 cm^{-1} and a strong high-energy band at 20,380–20,595 cm^{-1} (Table II) which are assigned [33] to the transitions $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$ for a high-spin octahedral geometry. A high intensity band at 27,285–27,560 cm^{-1} was assigned to the metal → ligand charge transfer. The magnetic susceptibility measurements (3.9–4.1 B.M) for the solid Co (II) complexes are also indicative of three unpaired electrons per Co (II) ion suggesting [34] consistency with their octahedral environment. The electronic spectra of the Cu (II) complexes (Table II) showed two low-energy weak bands at 14,655–15,150 cm^{-1} and 19,105–19,375 cm^{-1} and a strong high-energy band at 30,110–30,280 cm^{-1} which was assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions, respectively [35]. The strong high-energy band, in turn, is assigned to metal → ligand charge transfer. Also, the magnetic moment values (1.5–1.6 B.M) (Table I) for the copper (II) complexes are indicative of anti-ferromagnetic spin-spin interaction through molecular association [36]. The electronic spectra of the Ni (II) complexes showed d–d bands in the region 10,185–10,215, 15,480–15,535 and 26,215–26,280 cm^{-1}. These are assigned [36] to the transitions $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{2g}(P)$, respectively, consistent with their well-defined octahedral configuration. The band at 29,880–30,115 cm^{-1} was assigned to metal → ligand charge transfer. The magnetic measurements (3.1–3.3 B.M) showed two unpaired electrons per Ni (II) ion suggesting [37] also an octahedral geometry for the Ni (II) complexes. The electronic spectra of the Zn (II) complexes exhibited only a high-intensity band at

Table III. ^{1}H-NMR data of cloxacillin and its Zn (II) complexes.

Compound/Metal complex	^{1}H-NMR (ppm)
Cloxacillin-Na	7.1–7.55 (4H, m, aromatic), 5.62 (1H, d, β-lactam), 5.48 (1H d, β-lactam), 4.83 (1H, s, NH), 2.63 (3H, s, CH_3-β-lactam), 1.45 (3H, s, CH_3), 1.35 (3H, s, CH_3).
4	9.63 (4H, s, OH_2), 7.3–7.81 (8H, m, aromatic), 5.76 (2H, d, β-lactam), 5.52 (2H d, β-lactam), 4.97 (2H, s, NH), 2.81 (6H, s, CH_3-β-lactam), 1.53 (6H, s, CH_3), 1.47 (6H, s, CH_3).
8	9.88 (6H, s, OH_2), 7.42-7.98 (4H, m, aromatic), 5.94 (1H, d, β-lactam), 5.75 (1H d, β-lactam), 5.17 (1H, s, NH), 2.93 (3H, s, CH_3-β-lactam), 1.67 (3H, s, CH_3), 1.64 (3H, s, CH_3).

Table IV. *In-vitro* antibacterial activity data of cloxacillin and its metal (II) complexes.

	Diameter (mm) of zones showing complete inhibition of growth									
Compound	*(a)*	*(b)*	*(c)*	*(d)*	*(e)*	*(f)*	*(g)*	*(h)*	*(j)*	*(k)*
Cloxacillin	18	16	10	18	10	14	5	4	16	17
1	18	16	11	18	11	14	6	5	16	16
2	19	17	12	19	12	15	7	6	17	17
3	18	15	10	17	11	13	5	5	16	15
4	20	18	13	20	10	14	6	7	18	18
5	18	17	11	19	11	13	6	5	17	16
6	20	17	12	20	10	15	7	7	17	18
7	18	16	11	18	11	13	6	5	17	16
8	19	20	13	21	12	15	7	6	17	19

Cloxacillin: >15 mm = significant activity; 7–14 mm = moderate activity; <7 mm = weak activity.
(a) = *Escherichia coli*, *(b)* = *Klebsiella pneumonae*, *(c)* = *Proteus mirabilis*, *(d)* = *Pseudomonas aeruginosa*, *(e)* = *Salmonella typhi*, *(f)* = *Shigella dysentriae*, *(g)* = *Bacillus cereus*, *(h)* = *Corynebacterium diphtheriae*, *(j)* = *Streptococcus pyogenes*, *(k)* = *Staphylococcus aureous*.

29,115–29,350 cm^{-1} and are assigned [37] to a ligand → metal charge transfer.

Biological activity

Antibacterial bioassay. All compounds were tested against *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *S. typhi*, *S. dysenteriae*, *B. cereus*, *C. diphtheriae*, *S. aureous* and *S. pyogenes* bacterial strains (Table IV) according to literature protocol [25,26]. The results were compared with the uncoordinated cloxacillin. It was evident that overall potency of the cloxacillin was enhanced on coordination with the metal ions.

Cytotoxic bioassay. All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Meyer et al. [38]. From the data, it is evident that only complexes, 3 and 6 displayed cytotoxic activity against *Artemia salina*, while the others gave values of $LD_{50} > 1000$ and, therefore, are considered to be inactive in this assay.

Minimum inhibitory concentration (MIC). The minimum inhibitory concentration (MIC) of some selected compounds, which showed significant activity against selected bacterial species, was determined in comparison to the standard uncoordinated cloxacillin using the disc diffusion method [21,22]. MIC of these compounds varies from 10–100 μg/ml. The results as shown in Table V and indicated that compound 1 showed a promising activity (90%) at a concentration of 10 μg/ml against four bacterial strains *(a)*, *(b)*, *(d)* and *(k)* and, a significant activity (52%) against bacterial strain *(k)* at a concentration of 25 μg/ml. Compound 2 similarly, showed a promising activity (90%) at a concentration of 10 μg/ml against two bacterial strains *(a)* and *(j)* and, a significant activity (52%) against *(b)* and *(d)* at concentration 25 μg/ml. A promising activity (90%) for compound 3 was shown against *(b)* and *(d)* strains at a concentration of 10 μg/ml and a significant activity (52%) against bacterial strains *(a)* and *(k)* at a concentration of 25 μg/ml. A promising activity for compounds 4, 5, 6, 7 and 8 was respectively shown at a concentration of 10 μg/ml against *(a) (k)*, *(a) (d) (j)*, *(b) (k)*, *(a)* and *(b) (d)* bacterial strains and, a significant activity (52%) against *(d) (j)*, *(b) (k)*, *(d)*, *(b) (j)* and *(j) (k)* at a concentration of 25 μg/ml. The rest of all other compounds showed activity (40%) at concentration 100 μg/ml against test strains.

The biological activity data exhibited enhancement of activity on coordination with the metal ions against one or more test bacterial strains. This enhancement in the activity may be rationalized on the basis of their structures. It has been suggested that the ligands with nitrogen and oxygen donor systems inhibit enzyme activity, since the enzymes which require these groups for their activity appear to be especially more susceptible to deactivation by the metal ions on coordination. Moreover, coordination reduces the polarity [39–43] of the metal ion mainly because of

Table V. Minimum inhibitory concentration (μg/ml) of cloxacillin and its metal (II) complexes against selected bacteria.

Compound No.	Cloxacillin	1	2	3	4	5	6	7	8
E. coli	10	10	10	25	10	10	>100	10	25
K. pneumonae	10	10	25	10	>100	25	10	25	10
P. aeruginosa	10	10	25	10	25	10	25	>100	10
S. pyogenes	>100	>100	10	>100	25	10	>100	25	25
S. aureous	25	10	>100	25	10	25	10	>100	25

the partial sharing of its positive charge with the donor groups within the chelate ring system thus, formed during coordination. This process increases the lipophilic nature of the central metal atom, which in turn, favors more and efficient permeation [44–47] through the lipoid layer of the micro-organism thus destroying them more aggressively.

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