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Synthesis, characterization and anti-Methicillin Resistant *Staphylococcus aureus* (MRSA) evaluation of 4-bromo-2-(4,5-diphenyl-1H-imidazol-2-yl) phenol [Br-HPI] and its complexes with Co^{II} , Cu^{II} metal ions

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Abstract

Background: Methicillin-resistant Staphylococcus aureus strains have a gene that makes them resistant to nearly all beta-lactam antibiotics. 2-hydroxy phenyl imidazole derivatives are considered significant compounds due to their various uses in many fields such as antimicrobial, antiviral, anti-inflammatory and organic synthesis. Therefore, the objective of current study was to synthesize, and to evaluate antibacterial activities of, imidazole derivative and their complexes. Methods: A simple, fast and efficient one-step synthesis of 2-hydroxy phenyl imidazole derivative ligand 4-bromo-2-(4,5-diphenyl-1H-imidazol-2-yl) phenol [Br-HPI] was prepared by reaction between 5-bromo-salicylaldehyde and benzil under inert atmosphere. This ligand was synthesized and characterized by IR spectra, ^{1}H NMR and its solid complexes were characterized by IR spectra, molar conductivity and magnetic moment. Also, these complexes were screened against Methicillin resistant Staphylococcus aureus (MRSA) strain via *in vitro* utilizing the tube dilution assay.

Result: Antimicrobial assessment uncovered that a portion of these compounds showed critical antimicrobial activity against tested pathogenic agent, wherein the ligand was most intense with MIC and MBC values at 0.625 mg/ml and at 2.5 mg/ml, respectively, and after that took after by both Co II and Cu II metal ions with same MIC value (1.25 mg/ml). These outcomes were compared with the standard antibiotic Ciprofloxacin, from 0.312 to 10 mg/ml, as a positive control.

Conclusion: The present study offers some helpful data and information keeping in mind the end goal to understand the bactericidal and bacteriostatic activity also, performing the chemical design or modification of this series as a ligand compound can help with distinguishing new and powerful antimicrobial agents for extremely serious strains.

Keywords: MIC, MBC, Ligand, Co II, Cu II, metal ions, Methicillin-resistant Staphylococcus aureus, imidazole derivatives.

INTRODUCTION

The fast growing bacterial resistance to existing antibacterial agents has turned into a noteworthy enthusiasm among medicinal chemists and bacteriologists around the world. Also, currently available antimicrobials became less effective against infectious diseases; therefore, numerous scientists have focused on synthesis products as wellspring of new bioactive molecules. So it has sparked keen attention in developing the new potent drugs with low toxicity and high bioavailability. A broad utilization of antibacterial agents and their obstacles has prompted extreme medical issues in the doctor's facilities and communities particularly with **Methicillin Resistant *Staphylococcus aureus* (MRSA)** strains [1,2].

MRSA strains have a gene that makes them resistant to nearly all beta-lactam antibiotics. Protection from different anti-infection agents is additionally normal, particularly in human healthcare-associated MRSA [3]. These life forms are not kidding nosocomial pathogens and finding a viable treatment can be challenging. Community-associated MRSA (CA-MRSA) strains, which began outside doctor's facilities, are likewise prevalent in some areas. While these CA-MRSA strains have for the most part been less demanding to treat, some have moved into hospitals and have turned out to be progressively resistant to drugs other than beta-lactam antibiotics [4].

2-hydroxy phenyl imidazole derivatives are considered significant compounds due to their various uses in many fields such as antimicrobial, antiviral [5,6] anti-inflammatory [7] and organic synthesis [8]. Some natural compounds such as enzymes, nucleic acids, alkaloids and many others with biological importance [9-11] contain imidazole derivatives. Metal complexes of these derivatives are also having biological significance too [12]. In other words, the biological influence and activity of imidazole derivatives become further intense with involvement of metal ion.

Other similar hydroxy phenyls such as 2(2-hydroxyphenyl) benzimidazoles have their own application in different areas. For example, they are used as analytical reagents to determine mercury [13]. The coordination occurs through pyridine nitrogen and hydroxy group in the [Br-HPI] ligand. There is no indication, in the literature, for the involvement of pyrrole nitrogen in the complex formation [14] · Intra-hydrogen bonding has been proved to take place, in 2-hydroxy phenyl imidazoles between hydrogen of the hydroxy group in the phenol moiety and the pyridine nitrogen in the imidazole moiety [15-16]. Studies in the literature [17-18] showed that imidazole derivatives can form hydrogen bonding in the solution as well. Imidazole derivatives may form hydrogen bonding in the aqueous phase too [19-20]. Intra-hydrogen bonding which might occur in the solid phase of ligand could have no effect on the process of coordination comparing with the strong coordination bonds [21]. The coordination yields five-member chelate ring which leads to dissociation of the hydroxy group to end up with hydrolyzed (de-protonated) complexes

Imidazole and its derivatives have been reported to be the bioactive molecules in numerous essential biological systems with an expansive scope of pharmacological activity. They were recognised as proton donors and/or acceptors in enzymatic reactions, coordination framework ligands and as the basis of charge– exchange processes and as antibacterial, anti-parasitic, anti-inflammatory and anticancer agents [22]. Some imidazole-containing drugs are able to damage membranes directly when utilized at a high concentration for a brief period, autonomous of the nutritious medium and growth rate [23]. In addition, imidazoles cooperate specifically with the lipid bilayer of the cell membrane, most likely by binding to the unsaturated fats, part of the phospholipid components of the plasma membrane [24].

The aims of current study were to (1) synthesize, and to evaluate antibacterial activities of, imidazole derivative and their complexes, and (2) to assess bacterial responsive for 2-hydroxy phenyl imidazole derivative ligand 4-bromo-2-(4,5-diphenyl-1H-imidazol-2-yl) phenol [Br-HPI] and its complexes.

MATERIALS AND METHODS

Bacterial strains used in this study

The reference strains used in this study were chosen according to their pathological effects on human and multiple resistance to antibiotics. They included the Gram-positive bacteria Methicillin–Resistant *Staphylococcus aureus* (MRSA) strains were obtained from the Lab of Microbiology/ Colle OF Sciencesge /University of Wasit, Iraq.

The isolates were diagnosed by utilizing VITEK 2 system (Healthcare, biomerieux) and the growth of the *S. aureus* was confirmed after incubation by observing the colony characteristics under a microscope and by using strips of API 20 Staph (biomerieux).

Ligands and complexes synthesis

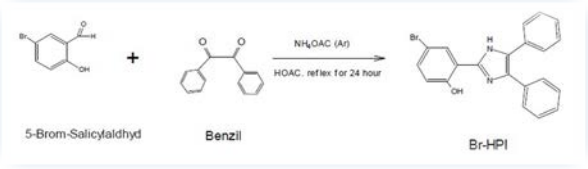
All the chemicals and solvents used for the synthesis of the ligands and complexes were purchased from Aldrich and used without further purification. The Infrared spectra were recorded on a (FT-IR-8400S Fourier Transform infrared spectrophotometer–SHIMADZU) in the range (600-4000) cm^{-1} using (KBr) discs. The TLC technique was used for ligand synthesis (Gelman sciences LTD. U.K.) device was used for measurement in the range (253.7 nm). Melting points were determined in an open glass capillary tube using a (Gallenkamp) apparatus. ^1H NMR spectra were recorded using a (1H-NMR 300 MHz, using DMSO, ppm). Magnetic susceptibilities were determined by Sherwood Scientifics'Magnetic Susceptibility Balances. Molar conductance of the transition metal complexes were determined in DMSO using conductivity meter Alpha-800 at 25°C, the concentration of the solutions was (10^{-3} mol L^{-1}).

Phenotypic screening for methicillin-resistant Staphylococci

Detection of MRSA was carried out using oxacillin screen agar and cefoxitin disc diffusion test in a previous study by the researcher AL-Saad [4]

Preparation of the Ligand 4-bromo-2-(4,5-diphenyl-1H-imidazol-2-yl) phenol [Br-HPI].

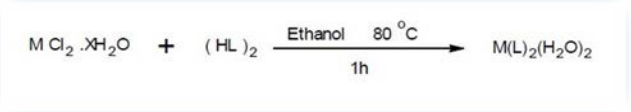
The [Br-HP] ligand (Scheme 1) was synthesized according to the procedure described in [25]. By the reaction between 5-bromo-salicylaldehyde (20.102 gm) (0.1 mole) and benzil (21.023 gm) (0.1 mole) together with 53 gm of ammonium acetate and 300 ml of glacial acetic acid. The reaction mixture was arranged to reflux for a 24 h in (500 ml) round flask, under inert atmosphere, Argon gas with high purity was used to maintain such atmosphere. Yield was a white solid, which was filtered and washed with hot distilled water several times to remove the glacial acetic acid. Litmus paper was used to check the full removal of the acid from the precipitate. The precipitate then re-crystallized by a mixture of 20% of water and 80% ethanol, and the crystals left overnight in the room temperature, then dried in oven at (60 °C) for several hours. The yield was 93% and the melting point is 178 -180 °C. TLC technique was used in order to control and assess the product, the eluent was (1:19) Ethanol and benzene. ^1H NMR (300 MHz, DMSO, TMS): δ = 13.10 (s, 1H, NH), 8.24 (s, 1H, OH), 7.52–6.94 (m, 13H, ArH) ppm. IR (KBr, cm^{-1}): [3201 (O – H), 3406 (N – H), 1581 (C = N), 696 (Ar – Br).



Scheme 1 Preparation of the [Br-HP] ligand

Synthesis of complexes

Complexes of Br-HPI with Cobalt (II), Copper (II) metal (Scheme 2) were prepared from Cobalt (II) chloride hexahydrate and Copper (II) chloride dihydrate using the molar ratio (1:2) of metal and ligand in both complexes.



Scheme 2 Complexes with [Br-HPI] ligand; M= Co(II), Cu(II). X= 2, 6. HL= [Br-HPI].

Preparation of copper (II) complex Br-HPI.

The ligand [Br-HPI] (2 mmol, 0.782 gm) was dissolved in ethanol (15 ml). $CuCl_2.2H_2O$ (1 mmol, 0.17 gm) in ethanol (10 ml) was added gradually. Gray precipitate was formed and after refluxing for 1 h, the precipitate filtered and washed with 3 × 30 ml of ethanol. Yield 1.342 gm (79 %).

Preparation of Cobalt (II) complex Br-HPI.

The ligand [Br-HPI] (2 mmol, 0.782 gm) was dissolved in ethanol (15 ml). $CoCl_2.6H_2O$ (1 mmol, 0.238 gm) in ethanol (10 ml) was added gradually. Deep orange precipitate was formed and after refluxing for 1 h, the precipitate filtered and washed with 3 × 30 ml of ethanol. Yield 1.242 gm (73 %).

In Vitro Antimicrobial Studies

Antibacterial activity of the samples (ligand and metal complexes) were determined using tube dilution method as described by the National Committee for Clinical Laboratory Standards [26]. Laboratory strains of (MRSA) were investigated. Ciprofloxacin was used as the standard antibacterial agents (positive control). The bacterial strains were grown at 37 °C overnight and maintained on nutrient agar. Inoculums of the test organisms were prepared in normal saline compared with 0.5 McFarland standard to attain 5 × 10^5(cfu /ml). The suspension was then used to inoculate tubes with Mueller Hinton broth in which the test organisms were grown. A stock solution of the compounds were prepared in DMSO (Sigma) and further diluted in Mueller Hinton broth to give a final concentration ranging from 0.312- 0.625- 1.25 - 2.5 -5 -10 mg/mL. After inoculation, tubes were incubated at 37 °C for 24 h under aseptic conditions. The MIC was recorded as the lowest concentration at which no visible bacterial growth (non-turbid tube) was observed, and further MBC was determined, the dilution representing the MIC and at least two of the more concentrated test product dilutions were plated and enumerated to determine viable cells cfu/ml. The MBC is the lowest concentration that demonstrates a pre-determined reduction (such as 99.9%) in cfu/ml when compared to the MIC value. All assays were performed in triplicate and MIC's and MBC's values are given in mg/mL.

RESULTS AND DISCUSSION

Preparation of the ligand is represented in (Scheme 1). The synthesized ligand Br-HPI gave satisfactory analysis for the proposed structures which were confirmed on the basis of their elemental analysis and spectral (FT-IR and ^1H NMR) data. Two complexes copper, Cobalt with Br-HPI ligand were synthesized by the reaction of 1:2 ratio of Metal with ligand. The complexes were obtained in excellent yields (79-73%) insoluble in methanol and water, but soluble in dimethyl sulfoxide (DMSO). The results of molar conductivity measurements (Table 1) suggest that all compounds were non-electrolytes in DMSO. Their melting points and magnetic moments (μeff) are listed in Table 1.

Infrared spectra

In general, the ligand exhibited very similar IR features (Table 2). The symmetrical stretching band of (OH) group appeared at (3201cm^{-1}) (Figrure 1). The stretching band of (NH) located at (3406 cm^{-1}). A Ar-Br group were observed at (696 cm^{-1}). The band at (1581 cm^{-1}) due to (C=N) of the (3N) imidazole nitrogen [27]. The (C=C) ring stretch, (1487, 1433 cm^{-1}) [28].

Coordination of the Copper, Cobalt atoms with the functional groups of the ligand was established from the IR spectra (Table 2). In general, the spectra of complexes show a change in frequency (Figures 2 and 3). In addition, disappearance of the hydroxyl group stretching vibration (O-H) of Br-HPI at (3201cm^{-1}) was observed. The Copper, Cobalt complexes new weak bands (Table 2) appear that assign to bending vibrations of (M-O), (M-N) bonds [29-30]. The phenolic C-O stretching vibrations appeared at (1267 cm^{-1}) in free ligand and under a shift towards lower frequencies in the complexes (Table 2). This shift conforms to the participation of oxygen in the C-O-M bond [31,32]. In complexes C=N bands are shifted by (45-50cm^{-1}) to lower wave numbers. These indicated the coordination of the (3N) imidazole nitrogen to metal ions. important bands has been observed in the bending vibration at (1606, 1641 cm^{-1}) and stretching vibration at (3735, 3732 cm^{-1}) in Copper, Cobalt complexes, respectively. These bands provide evidence of the existence of water molecules within the complexes structure [33].

Table 2 IR spectra of the free Br-HPI ligand and the Cu (II), Co(II) coordination complexes

Compound	υ O-H	υ N-H	υ C=N	υAr-Br	υ (phenolic C-O)	υ(M-N)	υ(M-O)
Br-HPI	3201	3406	1581	696	1267	----	----
Cu complex	----	3420	1535	696	1252	515	447
Co complex	----	3417	1531	696	1228	507	439

Table 1 Analytical data and some physical properties of the Br-HPI ligand and complexes

Compound	Chemical Formula	Formula Weight (g·mol^{-1})	Color	Melting Point(°C)	Yield (%)	μeff (B.M.)	Conductivity Cohm^{-1}.cm^{2}.mol^{-1}
Br-HPI	$C_{12}H_{15}N_2OBr$	391	white	178-180	93	____	
[Cu (Br-HPI)$_2$(H$_2$O)$_2$]	$C_{42}H_{32}N_4O_4Cu$	719.5	Gray	221-223	79	1.81	3.8
[Co(Br-HPI)$_2$(H$_2$O)$_2$]	$C_{42}H_{32}N_4O_4Co$	715	Deep orange	217-220	73	3.86	3.6

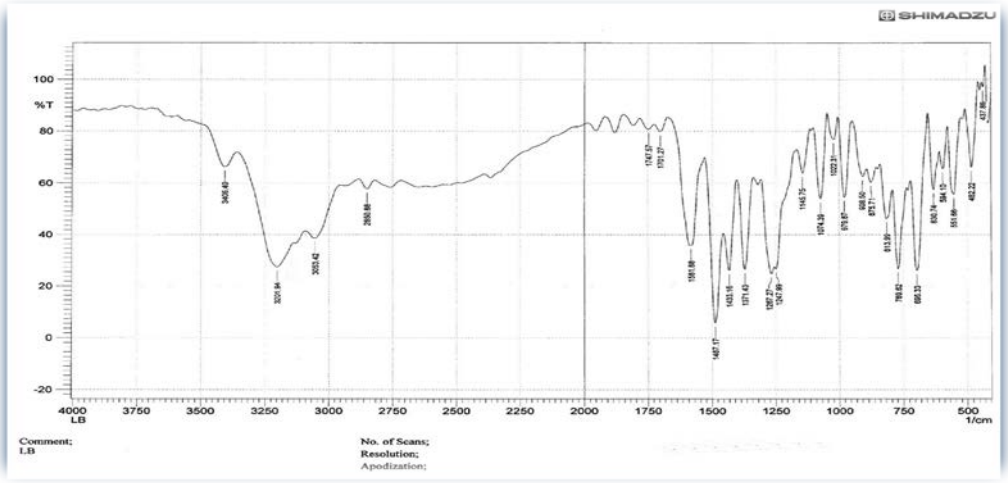


Figure 1 FTIR for Br-HPI ligand.

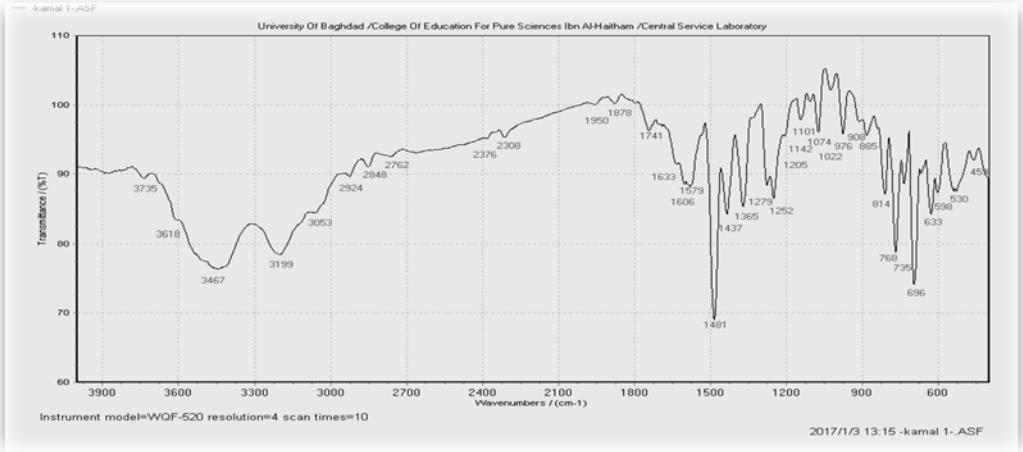


Figure 2 FTIR for copper complex.

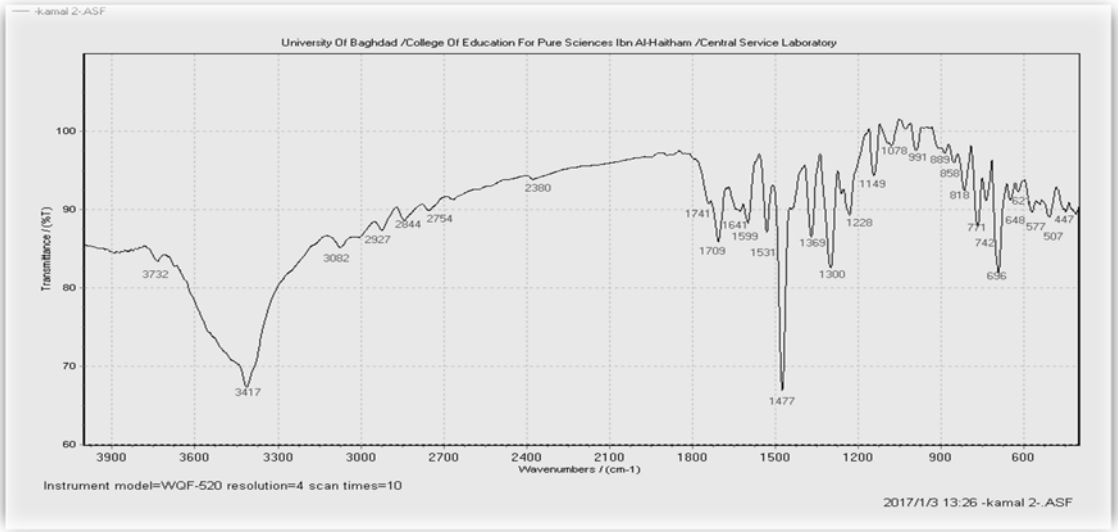


Figure 3 FTIR for Cobalt complex.

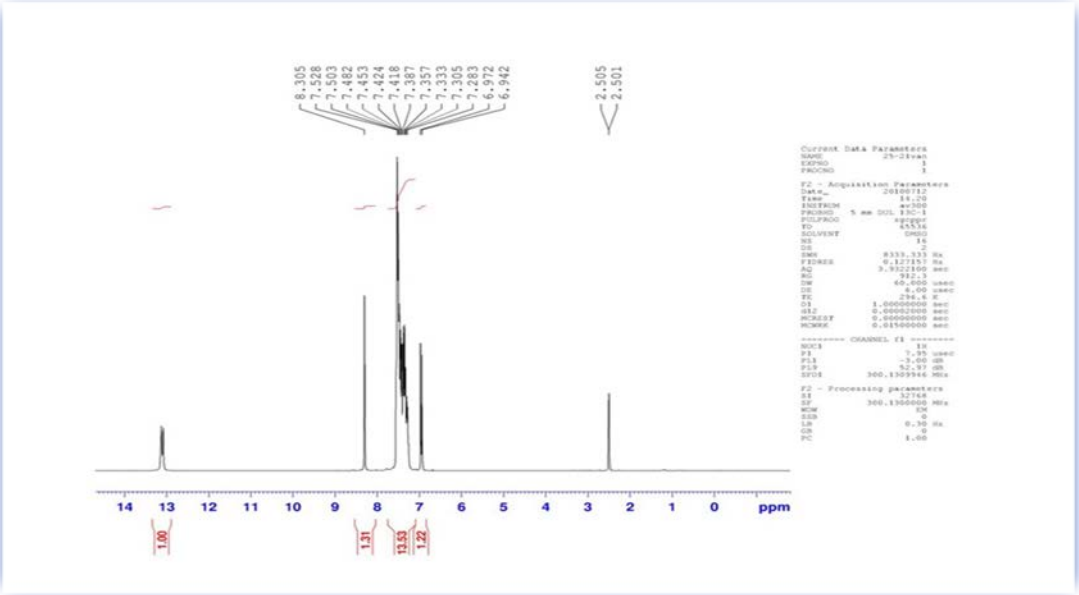


Figure 4 ^{1}H NMR of Br-HPI.

^{1}H NMR spectra of the compounds

The ^{1}H NMR spectra of the ligand Br-HPI were obtained in d_6 - DMSO at room temperature using TMS as an internal standard (Figure 4). The ^{1}H NMR data Showed δ (ppm): The chemical shift observed for the (OH) proton in the ligand 8.24 (s, 1H). The 7.52–6.94 (m, 13H) for aromatic protons while (NH) proton appeared in 13.10 (s, 1H).

Evaluation of anti-MRSA activity

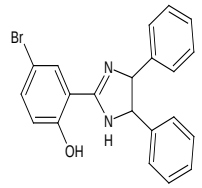
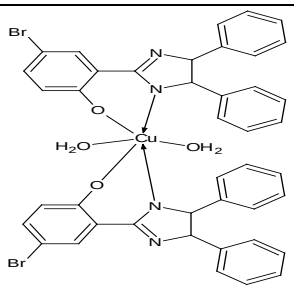
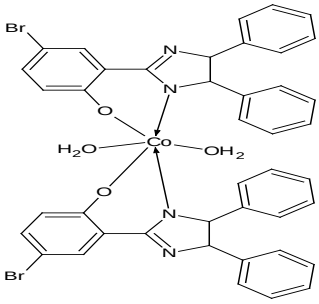
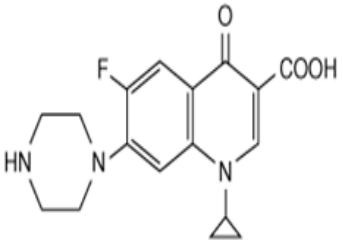
The anti-MRSA activity was assessed in terms of both minimum inhibitory concentrations (MICs) by using tube dilution assays and minimum bactericidal concentrations (MBCs) according to the CLSI guidelines [26, 24]. All the tested compounds were dissolved in dimethylsulfoxide (DMSO) which was used as a negative control with concentrations ranged from 0.312-10 mg/ml. The synthetic antibiotic, ciprofloxacin in the same range of concentrations, was used as a positive control.

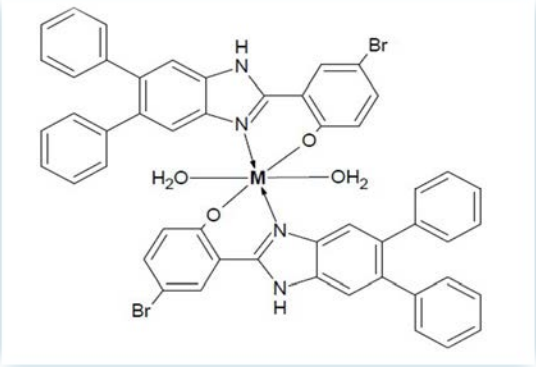
The compounds studied possessed significant antibacterial activity towards the selected microorganism (MRSA). The highest activities were observed for compound Ligand [Br-HPI], followed by $CoCl_2.6H_2O$ and then $CuCl_2.2H_2O$. Ciprofloxacin showed the least antibacterial activity for the selected concentration range (Table 3).

Thus, comparing the results of both the synthesized compounds and ciprofloxacin against a *β*-lactam-resistant Gram-positive bacteria (*Staphylococcus aureus)* demonstrated interesting antibacterial inhibitory values for most of the synthesized compounds. The MIC values were between 0.625 mg/mL (for ligand [Br-HPI]) to 1.25 mg/mL (for both $CuCl_2.2H_2O$ and $CoCl_2.6H_2O$) comparing with low activity of the fluoroquinolone drug against this strain of methicillin- resistant bacteria.

Table 3 *In vitro* antibacterial activities of the synthesized compounds (MIC & MBC)

* Positive control. ** Negative control.

Chemicals and their structures / **Serial dilution Conc. (mg/ml)**	**MIC (** mg/ml)	**MBC (** mg/ml)
 4-bromo-2-(4,5-diphenyl-4,5-dihydro-1*H*-imidazol-2-yl)phenol **[Br-HPI]** Ligand[Br-HPI]	0.625	2.5
 $CuCl_2.2H_2O$	1.25	5
 $CoCl_2.6H_2O$	1.25	2.5
 Ciprofloxacin *	2.5	5
DMSO **	-	-



Scheme 3 Complexes with [Br-HPI] ligand; M=Cu (II), Co (II).

CONCLUSIONS

Therefore, two of easily accessible metal complexes bearing Br-HPI ligand were synthesized and characterized. They were obtained from the reaction of the bidentate (N, O) ligand with the transition metals, Cu (II), Co (II). On the basis of the physical and spectral data of the ligand and the complexes discussed above, the Br-HPI ligands are bonded to the metals via pyridine nitrogen and hydroxy group. The solid complexes with the ratio [M:L] as [1:2] gave a neutral octahedral geometry (Scheme 3).

Moreover, we have identified the ligand 4-bromo-2-(4,5-diphenyl-1H-imidazol-2-yl) phenol [Br-HPI] compounds and their two complexes as potent anti-MRSA agents. It is predicted that the mechanisms of actions of these compounds could be different from that of existing antibiotics like ciprofloxacin, because of the differences in chemical structures. Further investigation including *in vivo* studies are needed to establish the mechanisms of actions and their uses as ligands and their complexes.

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Ethical Clearance: It was obtained from the Research Ethics Committee in the College of Sciences/ University of Wasit, Iraq.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

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