Preparation of Mixed ligand Complexes of Heterocyclic Azo Quinoline Ligand and Imidazole Molecule with Some of Divalent Transition Ions and their Biological Activity Against Multi Drug Resistance Pathogenic Bacteria

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Abstract. Heterocyclic azo compound 2-(8-quinolyl azo)-4,6-dimethyl phenol as a primary ligand and imidazole molecule as a secondary ligand in the basic medium were prepared with novel mixed ligand complexes of Hg(II), Mn(II), Ni(II), Co(II) and Cu(II) ions, these compounds were characterized by Mass, IHNNMR, IR, UV-Vis, Magnetic susbtibility and Molar Conductivity, which suggested octahedral conductivity. Free ligands and five mixed ligand complexes of Hg(II), Mn(II), Ni(II), Co(II) and Cu(II) metal ions with a general formula of [M(L1)(L2)2Cl] against eight pathogenic multidrug resistance bacteria, six G-ve bacteria (Pr. mirabilis, S. typhi, E. coli, P. aeroginosa, A. baumanii and K. pneumoniae) and two G+ve bacteria (E. faecalis and S. aureus) were capable of antimicrobial efficacy. The findings show that free ligands have had stronger antibacterial activity on S. Bacterial isolation of typhi and P. aeroginosa relative to other isolates. As for the effectiveness of metal complexes, compared to G+ve bacteria, they usually have a large antibacterial effect on G-ve bacteria, whereas the Hg (II) ion complex has a higher antibacterial effect on most bacterial isolates compared to other metal complexes. Compared with other metal complexes, Mn (II) ion complexes demonstrated poorer antibacterial activity.

Key words: Imidazole, azo, mixed ligand complexes, MDR bacteria, biological activity.

1. Introduction:
In recent years, researchers have seen an interesting increase in the preparation of mixed ligand complexes, especially in the biological fields[1]. These complexes contained high bioavailability ligands that prove their value, such as the imidazole molecule, known to be included in the structure of several biological systems such as histidine, in addition to its anti-bacterial and antifungal activity, imidazole was considered a monodintate ligand that coordinated through nitrogen number with various metal ions (3) [2-4]. In several areas, such as quantitative, qualitative and biological research, Azo compounds have demonstrated their potential and significance[5,6]. They were characterized by their stability and sensitivity, and also showed their skill in the field of coordination chemistry, especially heterocyclic azo ligands that are considered to be a remarkable form of quinoline compounds that act as di and tridentate ligands[2,7,8].

A global issue that threatens our ability to kill common infectious diseases is resistance to antimicrobial agents. It occurs naturally over time through genetic alterations or can occur in lower growth due to the
liveliness of unkillled bacteria [9]. The guidelines recommended by the WHO are to prevent infections and prevent the prevalence of resistance, pathway resistant bacteria, to increase the use of current antibiotics and to promote the emergence of new antibiotics and to develop new diagnostic tests for resistant bacteria in order to avoid lethal diseases due to antibiotic-resistant bacteria. While several studies have been conducted in recent years to tackle multidrug-resistant microorganisms, further advances are still required to develop new and effective molecules in order to extend the antibiotic treatment options[10].

The goal of this study is to prepare new mixed ligand complexes by primarily using azo quinoline ligand and imidazole molecule as secondary ligand with five divalent ions Mn(II), Co(II), Ni(II), Cu(II) and Hg(II) than by testing their biological activity against eight pathogenic bacteria isolated from different clinical specimens that are multi-drug resistance.

2. Instruments and chemicals:
AB SCIEX 3200 QTRAP Mass Analyzer reported mass spectrum, while Shimadzu UV-1650 UV-Vis Spectrophotometer, Japan, and IR spectra performed electronic spectra on Shimadzu FTIR8400 by KBr disk in the region (400-4000) cm-1, Costech ECS Elemental 4010 measured element analysis, and 720(WTW) measured molar conductivity, 1HNMR spectra in the DMM spectra.

2.1. Main Ligand (QADMP) Preparation and Complexes:
The primary ligand (QADMP) was prepared by diazonation of 8-amino quinoline with 4,6-dimethyl phenol in the presence of (NaNO2) and hydrochloric acid at temperatures between (0-5)oC [11] as shown in scheme (1), while the complexes of metal ions were prepared by mixing (10) mmol of each metal ion chloride with (10) mmol of (QADMP) (L1) and (20) mmol of each metal ion chloride with (10) mmol of (M:L1:L2) (1:1:2) Table 1 describes the percentage of complexes and some of their physicochemical properties.

![Scheme 1. Preparation reaction of primary ligand (QADMP)](image)

2.2. Biological Activity
2.2.1. Collection and diagnosis of bacterial isolates:
The following multidrug resistance pathogenic bacterial isolates (MDR): six grams of bacteria (Proteus mirabilis, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumnnii and Klebsiella pneumoniae) and two grams of bacteria (Staphylococcus aureus and Enterococcus faecalis) were isolated from various clinical samples, such as burns, stools, synovial fluids, wounds, vomit, A variety of morphological as well as biochemical tests were used to diagnose isolates [12], which were later recently verified using the Vitek-2 compact system GP and GN card automatic bacterial recognition instrument. BHI broth supplemented with (15 percent) glycerol at (-20 °C) was stored on all bacterial isolates. On BHIA, the isolates were sub-cultivated and incubated for 24 hours at 37 ° C before use.

2.2.2. Preparation of ligands (L1) , (L2), and the Complexes Concentrations:
The concentrations below were utilized in the biological activity test:
1- Free ligands concentrations: (0.02) gram of the powder of every ligands (L1) and (L2) has been dissolved in (1) ml of DMSO to yield (20) mg/ml concentration.
2- Mixed ligand complexes concentrations: (0.02) gram of powder of every mixed ligand complexes of the studied ions were dissolved in (1) ml of DMSO to yield concentration (20) mg/ml.
2.2.3. **Antibacterial activity experimental**

The preparation of bacterial suspensions was as Ramalivhana, *et al.* [13] explained. Agar well diffusion method was utilized to evaluate free ligand and mixed ligand ion complexes antibacterial activity versus bacterial isolates [14]. MHA medium was utilized to evaluate the biological activity of free ligand and mixed ligand complexes of ions versus bacterial isolates.

2.2.4. **Agar well diffusion assay**

Bacterial isolate suspensions were prepared to match the normal 0.5 McFarland. Distribution of 100 μl of the BHIB bacterial suspension using the micropipette on the surfaces of the MHA layer. Wells were punctured using a sterile cork borer on all the culture plates. One of the wells was perforation in the center of the plate, adding 100 μl of Gentamicin as a positive control; adding 100μl of DMSO as a negative control in the other well; adding 100μl of free ligand and mixed ligand complexes alone, adding five wells to the [M(L1)(L2)2]Cl complexes in the residual wells. Then the cultivation plates were incubated at 37 °C for 24 h. The clear inhibition zone around wells has been measured in mm. Testing was performed in triplicate [15].

| Table 1. Some of the physicochemical properties of ligands and their complexes |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|
| **Empirical Formula** | **M.wt.** | **Yield(%)** | **C%** | **H%** | **N%** | **M%** | **m.p (°C)** |
| C17H15ON (L1) | 277 | 85 | 73.63 (73.61) | 5.45 (5.47) | 15.15 (15.13) | ---- | 56-58 |
| C13H15N2 (L2) | 68.07 | - | 52.93 (52.91) | 5.92 (5.90) | 41.15 (41.14) | ---- | 90 - 92 |
| C17H14ClMnN7O5 | 504.88 | 502.87 | 54.72 (54.71) | 4.79 (4.81) | 19.42 (19.45) | 10.88 (10.86) | 245-247 |
| C17H14ClCoN7O5 | 508.88 | 84 | 54.29 (54.30) | 4.75 (4.73) | 19.27 (19.25) | 11.58 (11.61) | 259-261 |
| C17H14ClNiN7O5 | 508.64 | 71 | 54.31 (54.35) | 4.76 (4.78) | 19.28 (19.26) | 11.54 (11.54) | 265-267 |
| C17H14ClCuN7O5 | 513.49 | 81 | 53.80 (53.82) | 4.71 (4.70) | 19.09 (19.05) | 12.38 (12.40) | 271-273 |
| C17H14ClHgN7O5 | 651.14 | 76 | 42.47 (42.50) | 3.72 (3.74) | 15.07 (15.10) | 30.83 (30.85) | 284-286 |

3. Results and Discussion
Mass spectra of the (QADMP) \((L)\) show molecular peak at \(m/z= 277.26\) which is agreed with the molecular weight of the free ligand, mass fragmentation take two paths the first one started by losing (N\(_2\)) molecule at (250.24) that represented the mother peak, while the second path started by cleavage (-N=N-) bond to give two peaks at (m/z = 144.19) and (m/z=137.17) that due to the fragments which have molecular formula (C\(_9\)H\(_8\)N\(_2\)) and (C\(_8\)H\(_{11}\)NO) respectively, molecular peak in mass spectrum of Ni(II) complex appeared at m/z = 508.46 which was concerted with molecular weight of this complex the initial fragmentation was started by berk Cl\(^-\) ion from it, as shown in Figures 1, 2 and schemes 2, 3:

**Figure 1.** Mass spectrum of (QADMP) ligand
Scheme 2. Mass fragmentation of (QADMP) ligand
Figure 2. Mass Spectrum of Nickel (II) Complex

Scheme 3. Mass fragmentation of Ni(II) Complex
1H NMR spectra of primary ligand (QADMP) in DMSO solvent showed two singlet peaks at (2.46) ppm, and (2.62) ppm due to the protons of methyl groups (-CH₃), singlet peak at (5.58) ppm of (-OH) [11], while the peaks of aromatic protons appeared between (6.87-7.64) ppm [16], Some changes found in 1H NMR spectra of Hg(II) complex a singlet peaks at (1.36) and (12.36) ppm for (H₂) , and (H₁) protons of imidazole ring appeared and absence of (-OH) peak as a result of it's coordination with metal ion after deprotonation, as shown in Figures (3 , 4').

![1HNMR Spectra of primary ligand (QAMPD) in DMSO](image1)

![1HNMR Spectra of Hg (II) complex in DMSO](image2)

IR spectrum of free ligand (QADMP) (L₁) showed the ν(C-O) [17] of phenolic group at (1265) cm⁻¹ which was shifted to lower wave numbers in the complexes, also the change in position and intensity of this bond in plane and out of plane was appeared in the complexes, these changes give an indicating of participation of (-OH) group after deprotonation in coordination process. The wave number in (1498) cm⁻¹ in (L₁) spectra due to ν(C-N=N-C) group [18] also shifted to lower wave numbers in the complexes which was indicted the coordination of this ligand through one of nitrogen atoms of azo group with each of metal ions, also the ν(C=N) of imidazole (L₂) [19] at (1541) cm⁻¹ showed red shift in the complexes that considered as an evidence for coordination of this ligand by (N₃) atom, New peaks were appeared in the complexes refers to ν(M-O) and ν(M-N) considered as additional proof of the coordination of the two ligands, as shown in Table 2.
Table 2. IR wavenumber (cm⁻¹) values of free ligands and their complexes

| Compound                  | 𝜈(C=N)  | 𝜈(C=N)  | 𝜈(C=N=N-C) | 𝜈(C-O)   | 𝜈(C-O)   | 𝜈(M-O)   | 𝜈(M-N)   |
|---------------------------|---------|---------|-------------|-----------|-----------|-----------|-----------|
|                           |         | imidazole| Quinoline   | Phenolic  | In plane  | Phenolic  | Out of plane |
| (QADMP) (L₁)              | -----   | 1506 m  | 1498 m      | 1265 m    | 684 m     | 856 m     | -----      |
| Imidazole (L₂)            | 1541 m  | -----   | -----       | 1213 m    | 638 m     | 867 m     | 509 w      |
| [Mn(L₁) (L₂)₂Cl]          | 1524 m  | 1454 s  | 1409 m      | 1294 m    | 659 m     | 894 m     | 528 w      |
| [Co(L₁) (L₂)₂Cl]          | 1530 m  | 1471 s  | 1433 m      | 1255 m    | 672 m     | 869 m     | 584 w      |
| [Ni(L₁) (L₂)₂Cl]          | 1533 m  | 1469 s  | 1440 m      | 1236 m    | 667 m     | 879 m     | 549 w      |
| [Cu(L₁) (L₂)₂Cl]          | 1530 m  | 1467 m  | 1444 m      | 1257 m    | 661 m     | 879 m     | 549 w      |
| [Hg(L₁) (L₂)₂Cl]          | 1525 m  | 1473 s  | 1442 m      | 1257 m    | 661 m     | 879 m     | 549 w      |

UV-Vis. Spectra of (QADMP) (L₁) in ethanol shows three bands at (219, 226, 266) nm due to (π – π*) transitions of the aromatic rings, Intra charge transfer, and one band in (375) nm for (n-π*) transitions all of these bands appears red shift in the complexes spectra as a result of coordination, the values are explained in the ‘Figures 5-7 ’ and Table 3.

Conductivity measurements of the complexes in (DMSO) solvent showed the non-ionic character [20], also it was observed that the silver chloride salt was not deposited when added when adding drops of silver nitrate solution to solution of soluble complexes in ethanol and (DMSO) solvents confirming the absence of chloride ion outside the coordination sphere. Magnetic susceptibility showed that the complexes of Mn(II), Co(II), Ni(II), and Cu(II) have octahedral geometry with high spin values while the complex of Hg(II) is diamagnetic due to absence of single electrons in the electronic configuration as shown in Table 3.

![Figure 5. UV-Vis. Spectra of primary ligand (QAMPD)](image-url)
Figure 6. UV-Vis. Spectra of Mn(II) Mixed ligand complex

Figure 7. UV-Vis. Spectra of Cu(II) Mixed ligand complex

Table 3. Electronic transitions, and conductivity measurements of free ligands and their complexes

| Compound                | $\lambda_{\text{max}}$ (nm) | Transitions | Molar Conductivity | $\mu_{\text{eff.}}$ (B.M.) | Geometry   |
|-------------------------|-----------------------------|-------------|-------------------|-----------------------------|------------|
| (QADMP) (L1)            | 375                         | n-\(\pi^*\) |                   |                             |            |
|                         | 266                         | Intra C.T   |                   |                             |            |
|                         | 226                         | $\pi-\pi^*$ |                   |                             | Octahedral |
|                         | 219                         | $\pi-\pi^*$ |                   |                             | Octahedral |
| Imidazole(L2)           | 278                         | n-\(\pi^*\) |                   |                             |            |
|                         | 227                         | $\pi-\pi^*$ |                   |                             |            |
|                         | 498                         | MLCT        |                   |                             | Octahedral |
| [Mn(L1) (L2)2Cl]       | 346                         | C.T         | 19.2              | 5.74                        | Octahedral |
|                         | 268                         | $\pi-\pi^*$ |                   |                             | Octahedral |
From the results presented, it is suggested that the complexes have octahedral geometry where the primary ligand (QADMP) coordinated with each metal ion as a tridentate from oxygen atom of hydroxyl group after deprotonation, one nitrogen atom of azo group, and nitrogen atom of quinoline ring while the secondary ligand (imidazole) coordinated through nitrogen atom number (3) of heterocyclic ring, as shown in ‘Figure 8’.

**Figure 8.** Suggested general formula of the mix ligand complexes
3.1. Antibacterial efficiency of free ligand and ligand complexes:

The ability of antibacterial efficiency of free ligand (QADMP ) (L1) and five mixed ligand complexes of studied ions which have a general structure [M(L1)(L2)2Cl] to each ion toward eight MDR bacteria, two G +ve bacteria (E. faecalis and S. aureus) and six G –ve bacteria (Pr. mirabilis, S. typhi, E. coli, P. aeroginosa, A. baumanii and K. pneumoniae) were evaluated by the present or absence of inhibition zone around the holes. The results of the antibacterial effectiveness of various ingredients in solutions are listed in Table, and Figure. The study proves that free ligand is best at killing the bacteria Staphylococcus. typhi and P. aeroginosa isolates were better than the other isolates.

As for the efficacy of the metal complexes, they typically have a strong antibacterial efficacy on G-ve bacteria comparison to G +ve bacteria, whereas Hg (II) ion complexes had greater biological efficacy on most bacterial isolates than other metal complexes while Mn (II) ion complex had weaker antibacterial efficacy comparing to other metal complexes Table (5). The results in this paper conflict with the result of [2] that found the complexes to have strong antibacterial activity against gram-negative bacteria. The free ligand (L1) showed high antibacterial efficiency on S. typhi and P. aeroginosa than other mixed ligand complexes of ions. Hg (II) ion complexes had higher biological efficacy on E. faecalis, S. aureus, Pr. mirabilis, E. coli, A. baumanii and K. pneumonia.

Preliminary studies of antimicrobial activity showed which the azo dye containing 1,3,4-benzothiazole moiety had a possible antibacterial efficacy [21]. The improvement of antimicrobial efficacy of the free ligand might be related to transient metal chelation along with it. Complicated reduction of metal ion polarity by ligand coordination and increased lipophilicity of the metals [22]. This makes it easy for the novel prepared complex to penetrate the bacterial lipid cell membrane and inhibit its growing.

Some studies showed the potential of methyl nitro imidazole to reduce the growth of G-ve bacteria including K. pneumoniae, Proteus merabilis, E. coli and P. auroginosa in addition to reducing effects on G+ve bacteria. This ligand could release free radicals which destroy and kill bacteria [23]. In addition, the azo compounds are reported to have a variety of biological functions, including nucleic acid inhibition, protein synthesis, nitrogen fixation and carcinogenesis [24]. Azo derivatives and their metal combinations are too extremely essential pigments for artificial leather and vinyl polymers [25]. They are the strong pharmaceutical agents with adaptable therapeutic efficiency such as antimicrobial [26-29] efficiency, DNA, RNA, and protein synthesis, nitrogen fixation, and cancer [30].

![Comparison of inhibition zone measurement (mm) of free ligand (QADMP ) (L1) against multidrug resistance bacteria.](image-url)
Table 4 Inhibition zone measurements (antibacterial activity) of free ligand against multidrug resistance bacteria

| Type of bacteria                  | Inhibition zone(mm) [Free ligand (L₁)] |
|----------------------------------|----------------------------------------|
| Staphylococcus aureus            | 22                                     |
| Enterococcus faecalis            | 12                                     |
| Escherichia coli                 | 13                                     |
| Klebsiella pneumoniae            | 15                                     |
| Proteus mirabilis                | 12                                     |
| Pseudomonas aeruginosa           | 31                                     |
| Salmonella typhi                 | 35                                     |
| Acinetobacter baumannii          | 15                                     |

Table 5. Inhibition zone measurements (antibacterial activity) of mixed ligand complexes of Hg(II), Mn(II), Ni(II), Co(II) and Cu(II) ions against multidrug resistance bacteria

| Compound                  | Inhibition zone (mm) |
|---------------------------|-----------------------|
|                           | S. aureus | E. faecalis | E. coli | A. baumannii | S. typhi | K. pneumoniae | P. mirabilis | P. aeruginosa |
| Mn (L₁)(L₂)₂Cl            | 12        | 0           | 15      | 18          | 18       | 12           | 0           | 15           |
| Co (L₁)(L₂)₂Cl            | 24        | 13          | 14      | 15          | 15       | 14           | 17          | 25           |
| Ni (L₁)(L₂)₂Cl            | 18        | 0           | 20      | 12          | 13       | 17           | 13          | 20           |
| Cu (L₁)(L₂)₂Cl            | 18        | 13          | 14      | 13          | 12       | 15           | 14          | 13           |
| Hg (L₁)(L₂)₂Cl            | 25        | 20          | 24      | 22          | 26       | 23           | 16          | 17           |

Figure 10. Antibiogram pattern of free ligand and five mixed ligand complexes of Hg(II), Mn(II), Ni(II), Co(II) and Cu(II) ions against Acinetobacter baumannii.
4. Conclusions:
For primary azo ligand (QADMP) (L1) and imidazole molecule (L2) as a secondary molecule with a
general formula [M(L1) (L2)2Cl] and octahedral geometry, new mixed ligand complexes of Mn(II),
Co(II), Ni(II), Cu(II) and Hg(II) have been prepared. Biologically active molecules possessing
antimicrobial properties are proposed in this study. These new complexes of free and mixed ligands show
essential antimicrobial activities. A strong antimicrobial efficacy of azo dye with 1,3,4-thiadiazole moiety
was shown in preparatory antimicrobial activity studies. Therefore, it can be concluded that this new class
of compounds certainly provides a greater promise to discover a potent antimicrobial agent.

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