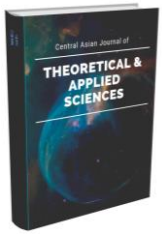

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Article

Study of the Bacterial Efficacy of Some Hg(II) Complexes with S-(thiophene-2-yl)2-((2-hydroxyphenyl)amino) Ethanethiol and Phosphines as Ligands

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Abstract: This research involved the use of thiophene-2-thiol as a nucleus to create the ligand S-(thiophene-2-yl)2-((2-hydroxyphenyl)amino)ethanethiol (AZ2), a transition compound in the creation of many complexes. The complexes [[Hg(AZ2)(dppp)]Cl2] and [Hg(AZ2)(dppm)]Cl2] were created by combining the ligand with mercuric chloride and phosphine compounds. The structures of the intended compounds were confirmed by physical and spectroscopic methods such as infrared spectra and proton and carbon nuclear magnetic resonance (1H, 13C, 13P-NMR) spectra. The biological activity was evaluated using antibiotic-resistant bacteria, E. coli, Gram-negative (G-negative), and S. aureus, Gram-positive (G-positive). These organisms were compared with the antibiotic as a control (Controls), amoxicillin. The results showed a practical inhibitory effect against two different types of bacteria and a high degree of efficacy and specificity.

Keywords: coordination chemistry, Ternary phosphines, biological activity

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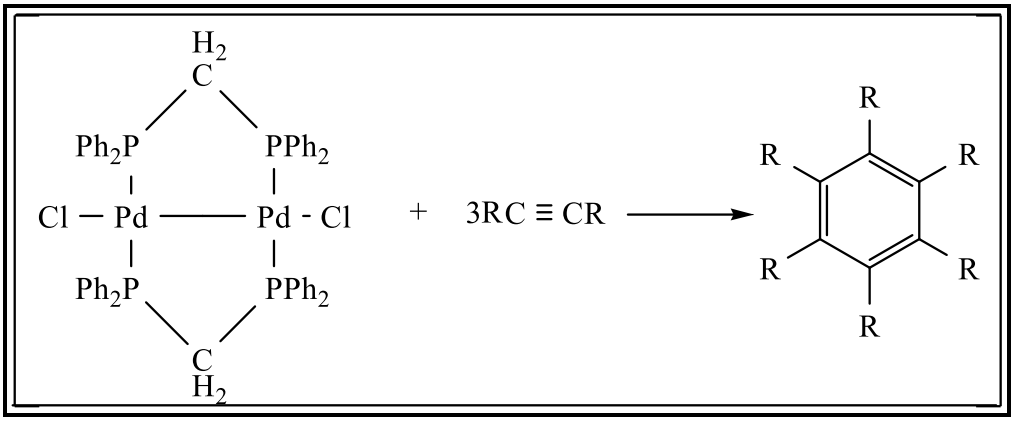
1. Introduction

Coordination chemistry is the chemical study of the types, preparations, and reactions of coordination compounds or complexes and the theory of interactions between them. Coordination complexes consist of a metal atom or ion surrounded by some ions or molecules (called ligands), linked by coordination bonds if the number exceeds the valence (oxidation state) [1]. Complexes can be positively, negatively, or neutrally charged depending on the charge groups of the central metal atom and its ligands [2]. Ternary phosphines are compounds in which phosphorus is linked to three organic groups that may be similar or different and are considered essential bonds in coordination chemistry and organometallic chemistry [3]. In the Skma bond, the triple phosphine bond acts as a Lewis base by donating a pair of electrons to an empty orbital (dz2, dx2y2), for example, in the metal. As for the pi bond, the triple phosphine bond acts as a Lewis base in it. A Lewis acid, which accepts a pair of electrons from the metal to the empty d orbital, shows that the photon is a substantial donor, forming SMA bonds, and a suitable acceptor, forming Pi bonds Triphosphate ligands are used in many fields because they can improve the stability of intermediates they form during reactions , for example, was found to be used as a catalyst in the alkyl cycle of acetylene to form hexacylbenzene [4].



2. Materials and Methods

2.1. Chemical Used

Chemical prepared from Aldrich, BDH Thomas, Fluka, and Merck, were used.

2.2. Devices Used

Metling points were measured with a thermoelectric melter 9300. KBr disk at 400-4000 cm-1 scale, Shimadzu FT-IR 8400S spectrophotometer; Bruker equipment running at 400 MHz for 1H-NMR spectra. Fluka silica gel plates, with a thickness of 0.2 mm, were used in thin-layer chromatography (TLC).

2.3. Preparation of the ligand S-(thiophene-2-yl) 2-((2-hydroxyphenyl)amino)ethanethiol (AZ2) [5]

Equal moles of thiophene-2-thiol (2.32g, 0.017mol) dissolved in (10ml) of THF were mixed with 2-chloroacetyl chloride (2ml, 0.017mol) in a round-bottomed flask, then (2ml) of Et3N was added to it and the mixture was stirred for 1 hour in an ice bath, then the flask was placed in a water bath at (50°C) with stirring for 1 hour. The medium was neutralized by adding sodium bicarbonate, then (2g, 0.017mol) of 2-amino phenol was added to the solution, and the mixture was stirred at (50°C) for 6 hours. The mixture turned yellow; then, the solution was concentrated, filtered, and recrystallized in ethanol. The result was brown with a product percentage of 80% and a melting point of 117-118°C.

2.4. Preparation of the complex AZ26[Hg(AZ2)(dppp)]Cl_2] and AZ25[Hg(AZ2)(dppm)]Cl_2] [6]

A solution of (AZ2) (0.00037mol, 0.1g) in (10ml) of absolute ethanol) was added to a solution of mercuric chloride (HgCl2) (0.00037mol, 0.117g) in (10ml) of distilled water and (0.00037mol) of (dppp) or (dppm) each dissolved in (10ml) of chloroform was added gradually. The mixture was stirred for 3 hours at laboratory temperature until a light yellow precipitate was formed. The precipitate was filtered, washed with absolute ethanol, dried, and recrystallized in ethanol. The product percentage was 64%, and the melting point was 122-124.

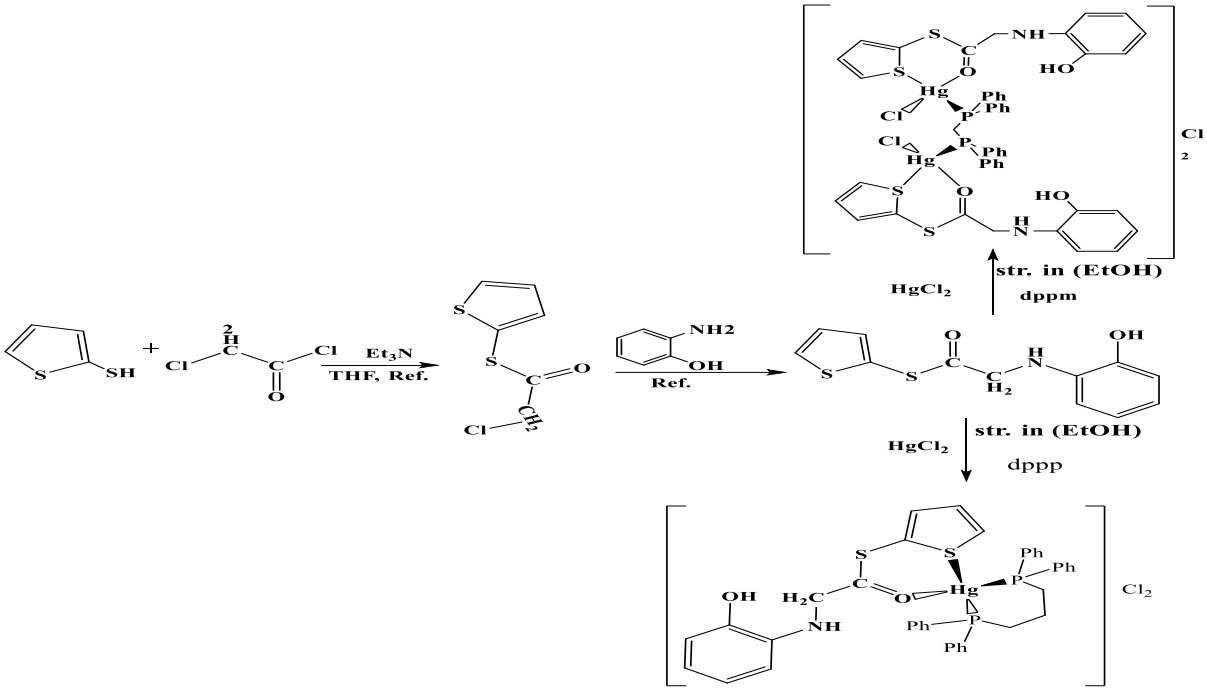
2.5. Biological Activity Study

Use DMSO solvent to prepare a complex solution at a concentration of ($1*10^{-2}.10^{-3}.10^{-4}$M), then prepare 38 g of culture medium for biological activity testing according to the supplier's instructions. Dissolve it in distilled water using a hot plate with a magnetic

stirrer [8-13]. After dissolving, the culture medium was sterilized in the same manner at 121°C and 1 atm for 15 min. The tools used were sterilized to avoid any bacterial contamination outside the experiment. Once sterilization was complete, the culture medium was cooled and poured into Petri dishes until solid [14-19]. Before testing with liquid nutrient broth, bacteria were re-seeded in liquid nutrient broth cultures and grown in a laboratory incubator at 37°C for 24 h in preparation for inoculum preparation for efficacy measurement. The bacterial inoculum was prepared by diluting a portion of the active bacterial culture with sterile distilled water to obtain a bacterial inoculum concentration of 1.5 × 108 bacterial cells, calculated by comparing the inoculum with the standard McFarland solution [20-25].

3. Results and Discussion

The Scheme shows the series of prepared compounds.



Scheme 1. Complex preparation process

3.1. Characterization of the ligand S-(thiophene-2-yl) 2-((2-hydroxyphenyl) amino) ethanethiol (AZ2)

The infrared spectrum of the prepared compound (L) shows a broad band belonging to the OH group stretching at a frequency (3411 cm-1) and a moderate single peak at a frequency (3180 cm-1). This is attributed to the NH group stretching, and the spectrum also shows a strong band at (1666 cm-1), which can be attributed to the vibrational frequency of the C=O group. The band at a frequency (1552 cm-1) can be attributed to the C=C groups stretching, and it is found that the band at a frequency (1330 cm-1) is attributed to the CO-O stretching, the band at a frequency (1166 cm-1) is due to the C-N stretching, and the strong band at a frequency (750 cm-1) is due to the CS stretching, in addition to the two intermediate bands at shifts (3029 cm-1) and (2923 cm-1)[26,27], which are due to the stretching of aromatic and aliphatic groups (CH), respectively, as shown in Figure (1).

The 1H NMR spectrum of the ligand measured in DMSO-d6 solvent showed a single signal at the shift (δH = 10.12 ppm) and with one proton integration attributed to the proton of the (OH) group, a double signal at (δH = 7.48 ppm) and coupling constant (3JH-H = 7.72

Hz) and with one proton integration attributed to the ((Hg) protons, as well as a triple signal at the chemical shift (δH = 7.42 ppm) and coupling constant (3JH-H = 7.78 Hz) and with one proton integration attributed to the (Hd) proton, in addition to a triple signal at (δH = 7.15 ppm) and with one proton integration and coupling constant (3JH-H = 7.45 Hz) attributed to the (He) proton, and another triple signal at the shift (δH = 7.01 ppm) and coupling constant (3JH-H= 7.50 Hz) and the integration of one proton attributed to proton (Hf). The spectrum also showed a binary signal at (δH= 6.96 ppm) and with the integration of one proton and the integration of one proton attributed to (Ha), as well as a binary signal at (δH= 6.88 ppm) and the integration of (3JH-H= 7.84 Hz) and the integration of one proton attributed to proton (Hc), and the presence of a triple signal at the shift (δH= 6.74 ppm) and with the coupling constant (3JH-H= 7.46 Hz) and the integration of one proton attributed to proton (Hb), in addition to the presence of a broad single signal at the shift (δH= 5.73 ppm) and with the integration of one proton attributed to (NH), and the signal Monoatomic at displacement (δH= 4.12 ppm) and integration of two protons back to protons (CH_2)[28,29].as shown in Figure (2).

The 13C-NMR spectrum of the ligand (L) showed an intermediate signal at the shift (**δ**13C=170.73 ppm) attributed to carbon (1), and also showed a signal at the shift (**δ**13C=149.69 ppm) attributed to carbon (2), as well as a signal at the shift (**δ**13C=148.05 ppm) attributed to carbon (3), in addition to the appearance of another strong signal at (**δ**13C=133.39 ppm) attributed to carbon (4), and the carbon spectrum showed an intermediate signal at the shift (**δ**13C=132.19 ppm) attributed to group (5), and an intermediate signal at the shift (**δ**13C=129.61 ppm) It was attributed to carbon (6), and also showed the presence of a signal at the displacement (**δ**13C = 126.64 ppm) attributed to carbon (7), in addition to the appearance of another signal at (**δ**13C = 122.32 ppm) attributed to carbon (8), and showed a signal at the displacement (**δ**13C = 119.82 ppm) attributed to carbon atom (9), in addition to the appearance of a signal at the displacement (**δ**13C = 116.21 ppm) attributed to carbon (10), and also showed an intermediate signal at the displacement (**δ**13C = 65.58 ppm) attributed to carbon (11)[30,31], as shown in Figure (3).

3.2. Characterization of the complex AZ26[Hg(AZ2)(dppp)]Cl_2] and A Z25[Hg(AZ2)(dppm)]Cl_2]

The infrared spectrum showed strong bands in the range (1425-1433 cm-1) attributed to the stretching of the υ(P-Ph) group [4], and other strong bands in the range (1091-1095 cm-1) and the range (692-694 cm-1) attributed to the stretching and bending of the (P-C) group. The spectra also showed the appearance of broad bands in the range (3348-3411 cm-1) attributed to the (O-H) group , in addition to the presence of medium bands in the range (3197-3203 cm-1) attributed to the stretching of the (N-H) group, and the presence of strong bands in the range (1670-1676 cm-1) attributed to the stretching group (C=O), in addition to the presence of strong bands in the range (1525-1562 cm-1) attributed to the stretching group (C=C). The spectra showed bands in the range (1325-1338 cm-1) due to the (C-O) stretch, as well as the presence of strong bands in the range (1163 cm-1) attributed to the (C-N) group, with the presence of other strong bands in the range (742-750 cm-1) which are attributed to the (C-S) stretch, in addition to the appearance of medium bands in the range (3020-3041 cm-1) and the range (2914-2935 cm-1) due to the vibrational frequencies of the aromatic and aliphatic (C-H) stretch, respectively. In addition, medium bands were shown in the range (455-580 cm-1) and the range (435-513 cm-1) due to the (Hg-S) and (Hg-O) groups, respectively [32,33]. as shown in Table 1 and Figure (4,5).

The 1H NMR spectrum of the complex [Hg2(L)2(μ-dppm)Cl2]Cl2 showed a single signal at the shift (δH= 9.63 ppm) and a single proton integration due to the OH proton, a double signal at the shift (δH= 7.59 ppm) and a coupling constant (4JH-H= 1.36 Hz, 3JH-H= 7.76 Hz) and a single proton integration due to the Hg proton, a double signal at the

chemical shift (δH= 7.50 ppm) and a coupling constant (3JH-H= 7.82 Hz) and a single proton integration due to the Hd proton, and a new broad signal at the shift (δH= 7.35 ppm) and a four-proton integration due to some protons of the phenylphosphine rings dppm (Hph), and it showed a triple signal at the displacement (δH = 7.28 ppm) and a coupling constant (3JH-H = 7.50 Hz) and with the integration of one proton attributed to the proton (He), in addition to a broad signal at (δH = 7.17 ppm) and with the integration of six protons attributed to the protons of the remaining phenylphosphine rings (Hph). The spectrum also showed the presence of a triple signal at the displacement (δH = 7.12 ppm) and with a coupling constant (3JH-H = 7.46 Hz) and the integration of one proton attributed to the proton (Hf), in addition to the presence of a binary signal at the displacement (δH = 7.03 ppm) and with a coupling constant (3JH-H = 7.88 Hz) and the integration of one proton attributed to the proton (Ha). The spectrum also showed the appearance of a binary signal at (δH = 6.94 ppm). (3JH-H= 7.70 Hz) and the integration of one proton is attributed to proton (Hc), in addition to the appearance of a triple signal at (δH= 6.86 ppm) and the comparison (3JH-H= 7.46 Hz) and the integration of one proton is attributed to proton (Hb), also between the spectrum there is a wide single signal at the shift (δH= 5.95 ppm) and the integration of one proton is attributed to proton (NH), in addition to a single signal at the shift (δH= 4.03 ppm) and the integration of two protons is attributed to protons (CH_2) of the ligand, and the appearance of a new triple signal at the chemical shift (δH= 2.79 ppm) and the integration of two protons is attributed to protons (CH2 of phosphine dppm) as shown in Figure (6).

The NMR spectrum {1H} 31P- showed the presence of an isomer, as it showed a single signal at the chemical shift (δP= 24.43 ppm), indicating that the dppm ligand is linked in a bridged manner due to the positive signal since the dppm ligand is linked in a chelated manner, the signal is negative due to the ring tension forces, as in the square planar complex with the formula [Hg2(L)2(μ-dppm)Cl2]Cl2, in addition to the presence of another isomer in the sample solution, as the spectrum showed two double signals at the positive shift (δP= 31.53 ppm) and the negative shift (δP= -24.65 ppm) and with a coupling constant (2JP-P= 25.76 Hz) and (2JP-P= 25.72 Hz), respectively, where the two positive signals indicate the linkage of one of the phosphorus atoms (PA) to the metal ion The other two negative signs indicate that the other phosphorus atom (PX) is free and not bound, as in the tetrahedral complex [11] with the formula [Hg(L)(κ1-dppm)Cl]Cl, shown in Figure (7).

The 1H NMR spectrum of the complex [Hg(L)(dppp)]Cl2 showed a single signal at the shift (δH= 10.23 ppm) and with the integration of one proton attributed to the ((OH) proton, and showed a double signal at (δH= 7.51 ppm) and a coupling constant (3JH-H= 7.78 Hz) and the integration of one proton attributed to the ((Hg) proton. The spectrum also showed the presence of a double signal at the shift (δH= 7.51 ppm) and the integration of one proton and the coupling (4JH-H= 1.28 Hz, 3JH-H= 7.74 Hz) attributed to the ((Hd) proton. The spectrum also showed the appearance of a new broad signal at the shift (δH= 7.33 ppm) and with the integration of eight protons attributed to some protons of the phenylphosphine rings dppp (Hph), in addition to the appearance of a broad integrated signal at (δH = 7.15 ppm) and with an integration of approximately thirteen protons due to the protons of the remaining phenylphosphine rings (Hph) and a proton (He), and showed a triple signal at the chemical shift (δH = 7.05 ppm) and with a coupling constant (4JH-H = 1.22 Hz, 3JH-H = 7.53 Hz) and with an integration of one proton due to the proton (Hf), and also showed a binary signal at the shift (δH = 6.92 ppm) and with a coupling constant (3JH-H = 7.74 Hz) and an integration of one proton due to the proton (Ha), in addition to the presence of a binary signal also at (δH = 6.82 ppm) and with a coupling (3JH-H = 7.69 Hz) and an integration of one proton due to the proton (Hc), as well as It showed a triple signal at the displacement (δH = 6.71 ppm) and with the integration of one proton it goes back to the proton (Hb), it also showed a wide single signal at the displacement (δH = 6.01 ppm) and with the integration of one proton it goes back to the

proton (NH), and it showed the presence of a single signal at the displacement (δH = 4.15 ppm) and the integration of two protons it goes back to the protons (CH2) of the ligand, as well as the presence of a triple signal at the displacement (δH = 2.32 ppm) and with the integration of four protons it was attributed to the terminal protons (CH2) of phosphine, and it also showed a five-proton signal at the displacement (δH = 1.83 ppm) and with the integration of two protons it goes back to the two middle protons (CH2) of phosphine [34,35], as shown in Figure (8).

The NMR spectrum {1H} 31P- showed the presence of two isomers in the sample solution, as it showed the presence of a positive single signal at the shift (δP = 30.09 ppm). The positive signal indicates that the two phosphorus atoms are equivalent [4], as in the tetrahedral complex [Hg(L)(dppp)]Cl2, in addition to another isomer, as the spectrum showed the presence of two double signals at the positive chemical shift (δP = 35.19 ppm) and the coupling (2JP-P = 45.90 Hz) and the negative chemical shift (δP = -9.12 ppm) and the coupling (2JP-P = 45.86 Hz), as the positive double signal indicates the association of one of the two phosphorus atoms with the metal, and the other negative double signal indicates that the other phosphorus atom is free and not bound in the tetrahedral complex [36,37] with the formula [Hg(L)(κ1-dppp)Cl]Cl. as shown in Figure (9).

3.3. Evaluation of the bacterial activity of the prepared complexes

Biological activity was measured by the agar-well diffusion method according to the literature [38-43]. This method involved placing the bacterial inoculum in the middle of the culture medium, using a bacterial diffuser, and then creating wells in the agar medium using a sterile 6 mm diameter drill to load solutions in a volume of 100 µl of each concentration [44-50]. This method allowed three different concentrations of the same solution to be loaded onto a single plate, all populated with a single bacterial species. After filling the plates with solutions, they were left for 15–20 min at room temperature. They were then placed in the incubator and the results were read after 24 h. The activity of the compounds was inferred from measuring the diameter of the inhibition zone around each hole for all samples [50-57].

Table 1. Infrared absorption values in (cm-1) for the prepared ligand and mercury complexes

Complexes	O-H	N-H	C=O	C=C	Ph-P	C-O	C-N C-S	υ(P-C) δ(P-C)	(C-H)Aro (C-H)Alip	Hg-S Hg-O
L	3411s	3180m	1656s	1562s	---	1330s	1166m 750s	---	3029m 2923m	---
$[Hg_2(L)_2(\mu\text{-}dppm)Cl_2]Cl_2$	3411s	3197m	1676s	1525s	1425s	1325m	1163s 742s	1091s 692s	3020m 2914m	580m 455m
$[Hg(L)(dppp)]Cl_2$	3348s	3203s	1670s	1562s	1433s	1338m	1163s 750s	1095s 694s	3041s 2935m	513m 435m

Table 2. Biological efficacy of produced subtances and control methods (measured in mm of inhibition)

Comp. No.	E. Coil Conc. mg/ml			Staph. Aureus Conc. mg/ml		
	0.01	0.001	0.0001	0.01	0.001	0.0001
L	18	13	5	16	10	10
dppm	17	13	10	19	15	11

dppp	20	10	10	20	15	10
Amoxicillin	27	20	18	25	23	19

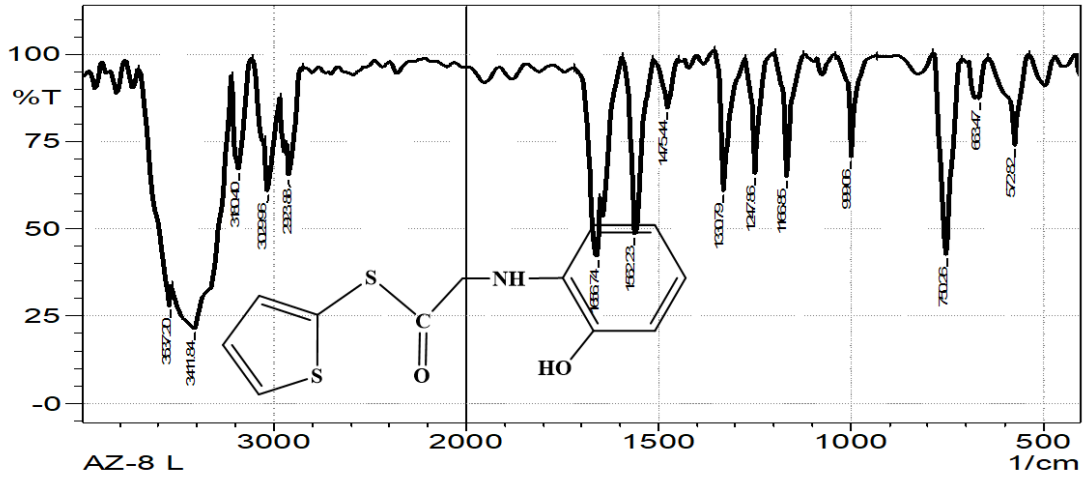


Figure 1. Infrered spectrum of the ligand used (L)

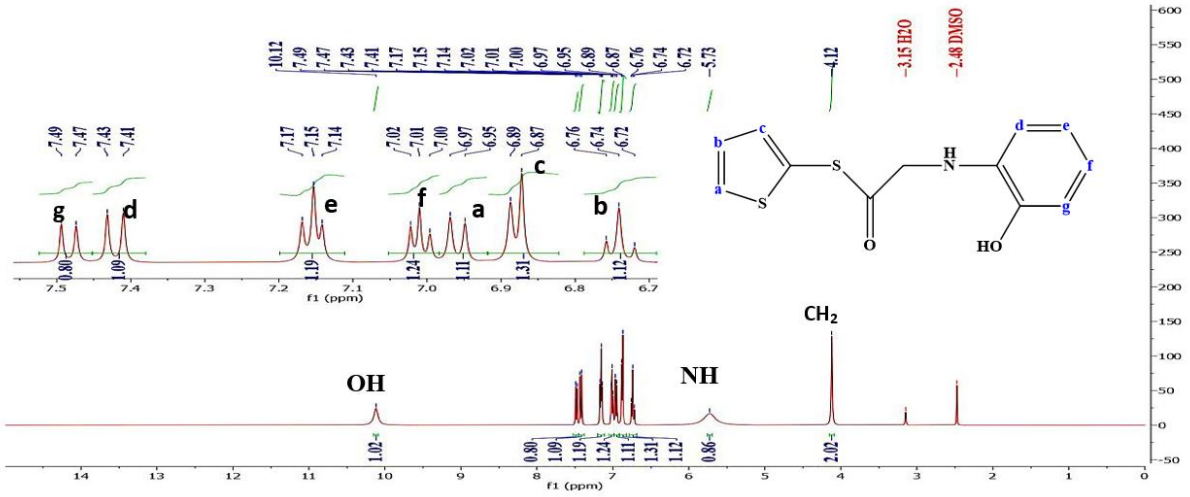


Figure 2. 1H NMR spectrum of ligand (L)

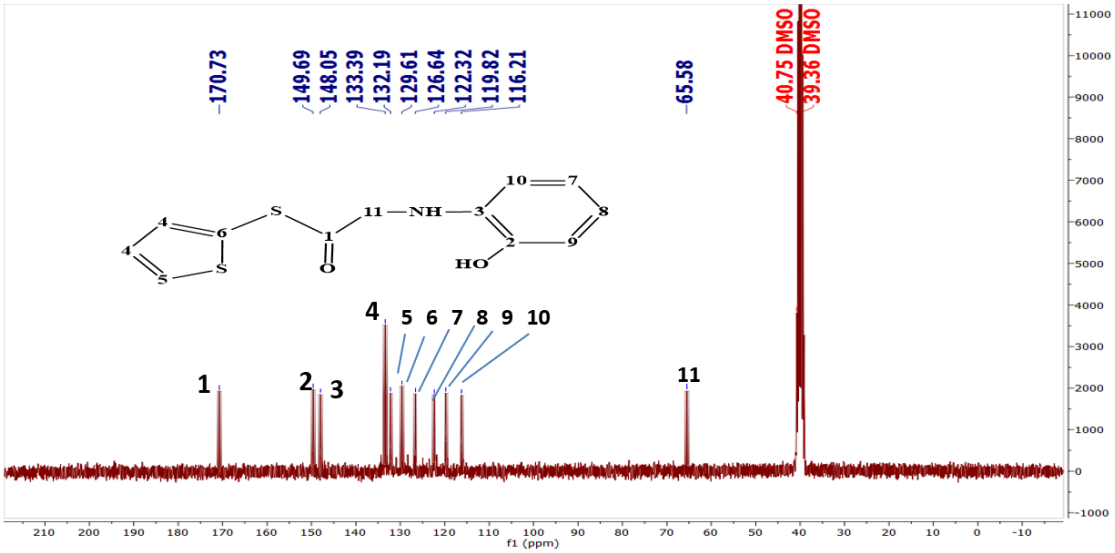


Figure 3. 13C-NMR spectrum of ligand (L)

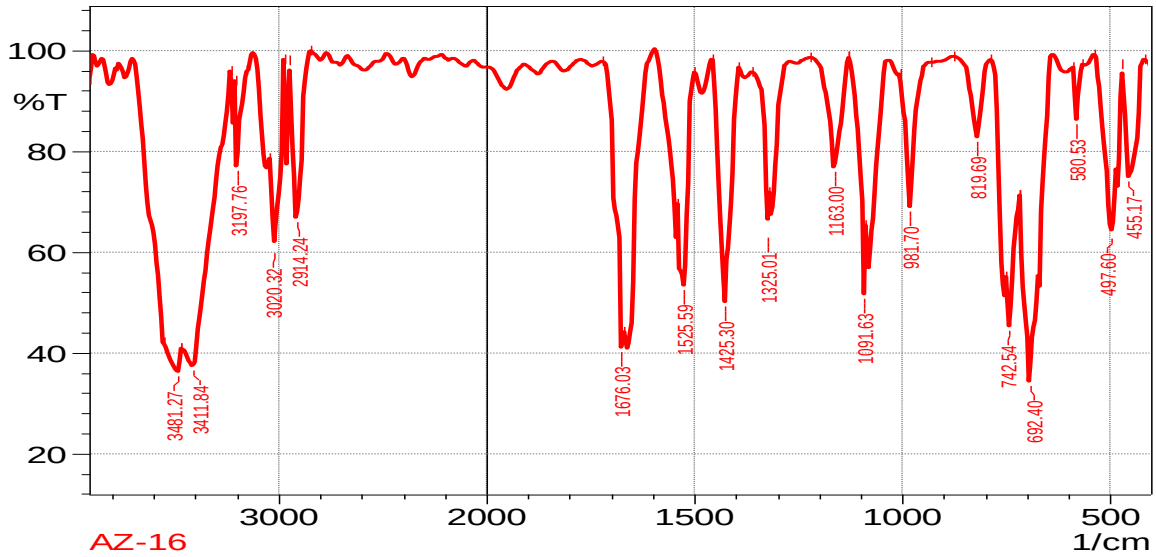


Figure 4. Infrared spectrum of the complex [Hg2(L)2(μ-dppm)Cl2]Cl2

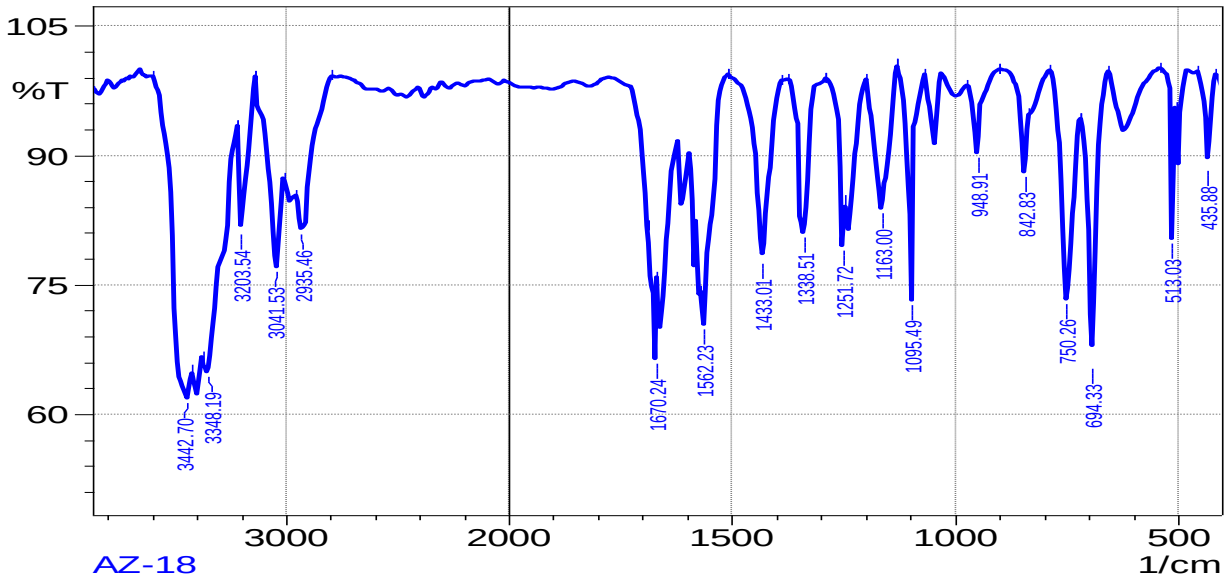


Figure 5. Infrared spectrum of the complex [Hg(L)(dppp)]Cl2

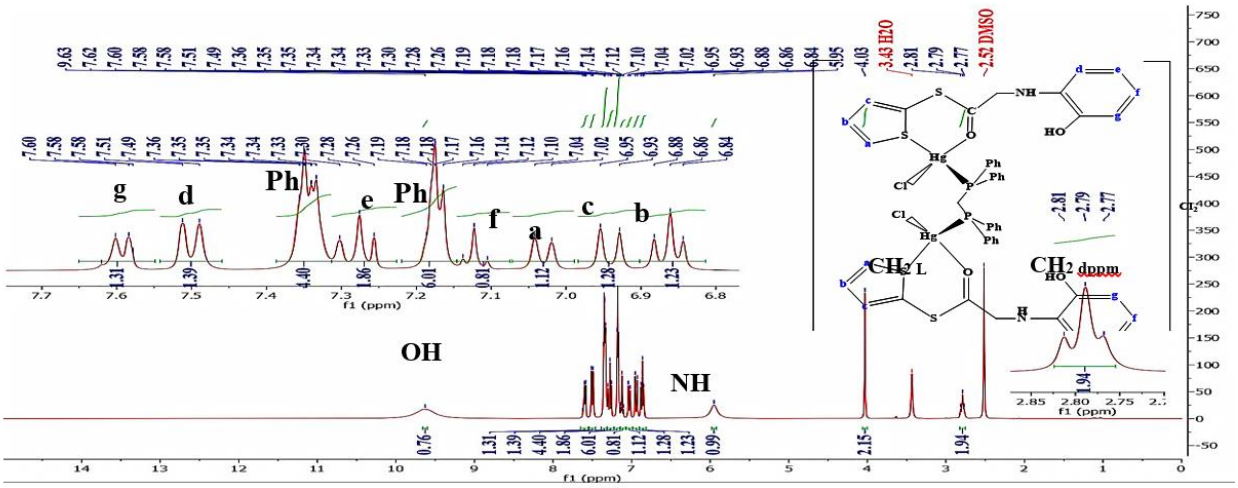


Figure 6. 1H NMR spectrum of the complex [Hg2(L)2(μ-dppm)Cl2]Cl2

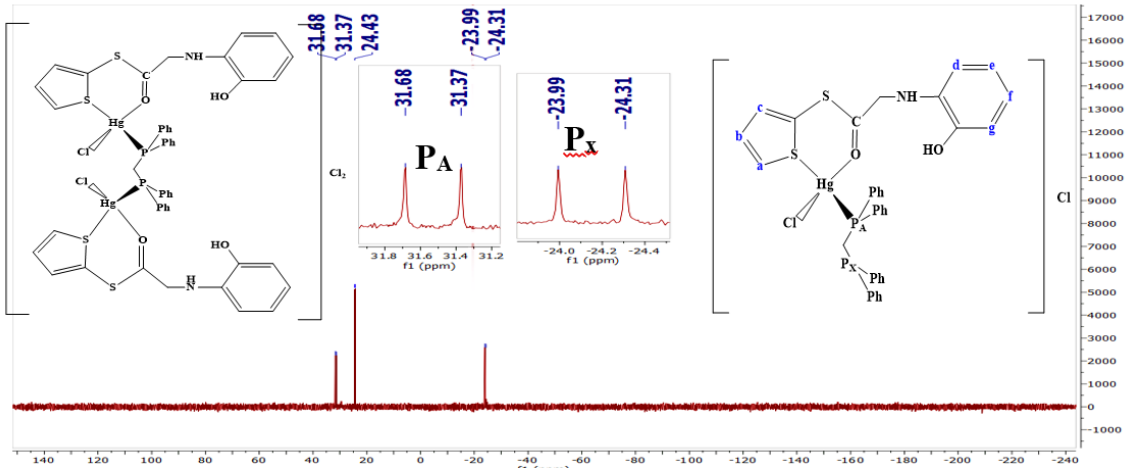


Figure 7. NMR Spectrum [1H] 31P- for the complex [Hg2(L)2(μ-dppm)Cl2]Cl2

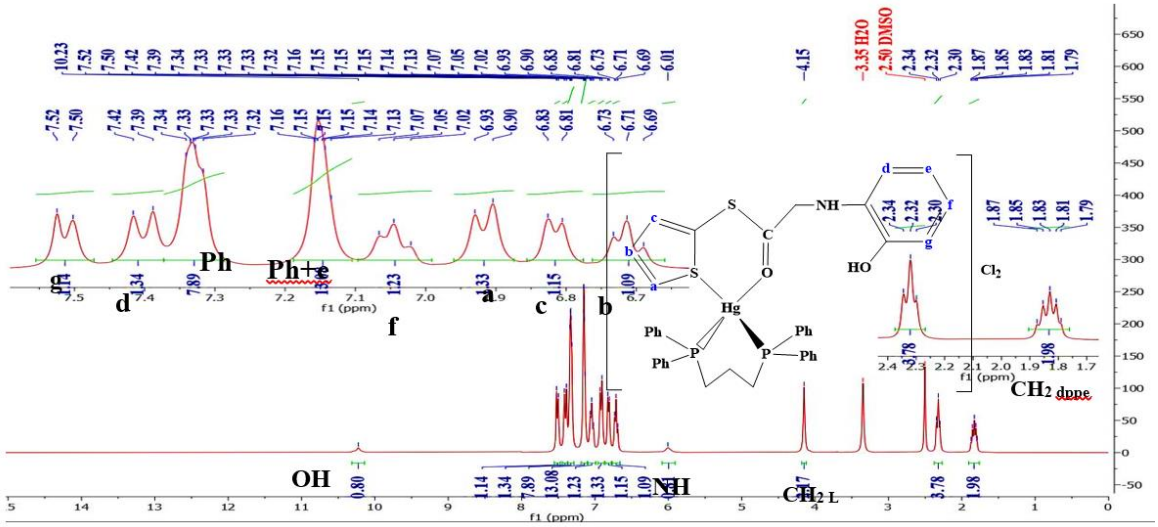


Figure 8. 1H NMR spectrum of the complex [Hg(L)(dppp)]Cl2

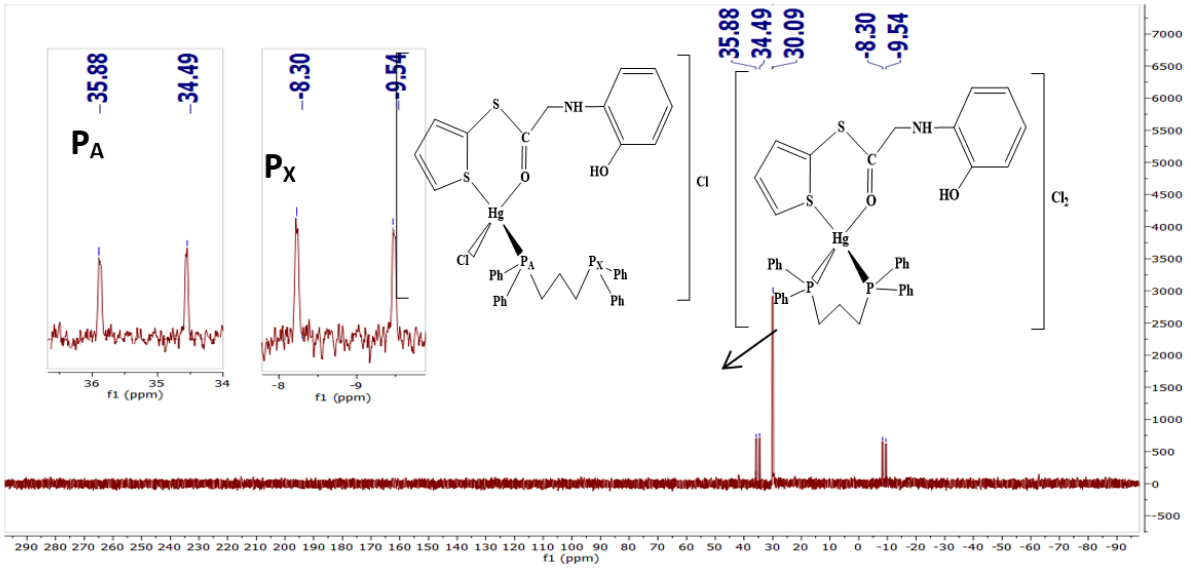


Figure 9. NMR Spectrum [1H] 31P- for the complex {Hg(L)(dppp)]Cl2

4. Conclusion

The reaction of phosphines with ligands and metals always gives complexes according to the phosphine involved in the reaction. The accuracy and validity of the results were proven by infrared spectra and (^{1}H, ^{13}C, ^{13}P-NMR) spectra. It also proved its biological effectiveness against the two types of bacteria used in the study compared to the antibiotic.

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