CENTRAL ASIAN JOURNAL OF THEORETICAL AND APPLIED SCIENCE

https://cajotas.casjournal.org/index.php/cajotas
Volume: 07 Issue: 04 | October 2026 ISSN: 2660-5317

Article

Synthesis and Characterization of Novel Phosphine-Zinc Complexes and Evaluation of Their Antibacterial Activity

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Abstract: This manuscript discusses the synthesis of two types of complexes: [Zn(L)$_2$(dppm)] and [Zn(L)$_2$(dppe)] using 2-thiouracil as a ligand, which reacts with zinc chloride, sodium hydroxide and the phosphines dppm and dppe, each separately. This was confirmed by spectroscopic measurements, including FT-IR spectroscopy, which revealed new bands indicating the formation of complex bonds, and ^{1}H -NMR, which confirmed the incorporation of the phosphine group into the complex, and ^{31}P-NMR, which indicated that the complex has a single conformational state. The efficacy of the complexes was also evaluated against two types of bacterial isolates, one Gram-positive (*Staphylococcus aureus*) and the other Gram-negative (*Escherichia coli*), and these were then compared with the antibiotic ampicillin. The complexes demonstrated very similar efficacy in inhibiting the bacteria; however, they did not match the level of the antibiotic, which showed clear superiority. Nevertheless, this does not mean that they will not be potential future antibiotics if combined with other agents.

Keywords: Coordination Chemistry, 2-Thioracil, Biological Activity

Citation: Mohammed, S. F. Synthesis and Characterization of Novel Phosphine-Zinc Complexes and Evaluation of Their Antibacterial Activity. Central Asian Journal of Theoretical and Applied Science 2026, 7(4), 100-107.

Received: 30th Jun 2026
Revised: 20th Jul 2026
Accepted: 10th Aug 2026
Published: 28th Aug 2026

1. Introduction

It is clear that minerals play a crucial role in the proper development of plants and animals. One of the essential microelements is zinc, which was identified as pure metal in 1746 in Germany by Andreas Marggraf [1]. Zinc is widely recognized as the element of health. Zinc is supplied to the body mostly through food of plant and animal origin, less often through the respiration process and skin, while zinc excretion takes place in the form of feces and partly as urine [2]. Zinc absorption occurs in the small intestine, colon, and stomach; and its absorption depends on environmental contamination, stress, improper nutrition, physical activity, age, too much sugar, protein deficiency and excess of iron consumption and phytates derived from plants [3,4]. Zinc is an essential microelement for regular operation of the organism of the plants and animals. Its insufficiency or excessive amount in the organism can lead to numerous diseases. Insufficient level of zinc can hinder the development of children because it is essential in the process of bone metabolism [5-7]. It influences the work of the immune system by blocking inflammatory processes, but zinc deficiency was observed among the children having pneumonia and bronchitis [8,9]. Consequences that follow from an excessively low content of zinc include hormonal imbalances, high glucose tolerance [10], aggravation of depression symptoms [11], disorders of libido [12] and problems with nerve conductivity [13]. The uracil is a pyrimidine that has been found in the nucleic acids. This pyrimidine base has got four different binding sites, for example, N1, N3, O2, and O4 molecules. The uracil is one of the pyrimidines that have great importance in some enzymes and pharmaceutical products [14]. Inhibition of biosynthesis of thyroid hormones was shown for 2-thiouracil. In 1942 Prof. Edwin W. Astwood applied 2-thiouracil to the therapy of the patients suffering from Graves' disease [15]. Thyroid gland (Lat. Glandula thyr(e)oidea) is one of the most significant glands in the human organism. This gland reproduses hormones T3, T4 and calcitonin, which function is stimulation of metabolic processes of the cells and several organ's growth. It is necessary to mention, that possible methods of treatment of the patients with the Graves' disease include conservative therapy, based on using antithyroid medicines—thiamazole (methizol, tyrozole) and propylthiouracil (propicillin), surgical therapy and treatment with radioactive iodine. Methylthiouracil was defined as an antithyroid medicine. This medicine stimulates growth and reproduction, wound and ulcer healing, burns healing and the organism resistance to infections [16]. It should be mentioned that one of the characteristic features of this medicine is the capability to activate hematopoiesis (reproduction and intensification of leukocytes and erythrocytes in the bone marrow). The similar mechanisms and side effects are typical of this drug, like for propylthiouracil. The drug blocks synthesis of the stored hormones, for example, thyroglobulin in thyroid gland.

2. Materials and Method

2.1. Chemicals and Instruments

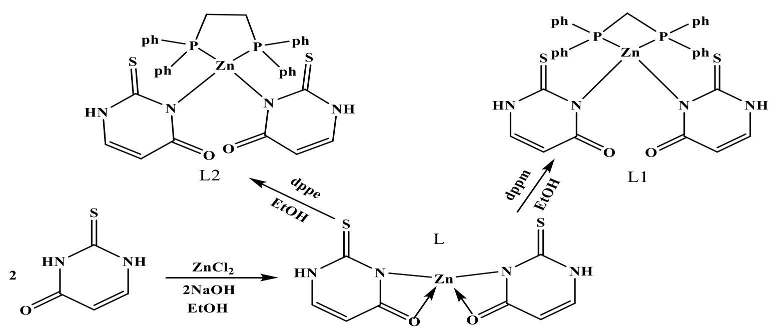
Based on the experience of famous companies like Sigma-Aldrich, BDH, Fluka, and Merctem, pure chemicals and solvents were provided. The measuring of melting points was done using the Stuart MP-200D-120 Col-Paramer digital melting point spectrometer. Fourier Transform Infrared Spectroscopy analysis was carried out using a Shimadzu FT-IR-8400S instrument. For the ^{1}H-NMR and ^{31}P-NMR spectra measurement, a Varian-Innova USA 500 MHz spectrometer was used, working at 400 and 162 MHz, respectively, with DMSO-D^{6} and TMS as the solvent and internal standard, respectively.

2.2. Preparation of Complex [Zn(L)$_2$(dppm)][17]:

In 15 ml of ethanol, inside a round-bottomed glass flask, an equal amount (1 mol) of ligand 2 –thio-uracil with sodium hydroxide for half an hour. Then, 0.5 mol of zinc chloride and 0.5 mol of dppm were added, and the mixture was left unheated for 3 hours until a white precipitate formed; It was then filtered, dried and 101ecrystallized, resulting in a product yield of 75 per cent.

2.3. Preparation of Complex [Zn(L)$_2$(dppe)] [17]:

In 15 ml of ethanol, under a round bottomed flask, the same molar concentration of the ligand, 2-thio-uracil, along with sodium hydroxide is mixed for thirty minutes. Following that half mole of zinc chloride and half mole of dppe are put together with the solution kept unheated for three hours till white precipitate was obtained. This was then filtered, dried and recrystallized yielding 70 percent of output.



Scheme 1. Preparation of complexes (L$_1$-L2)

2.4. Biological activity evaluation

Due to the fact that they are prevalent and easy to acquire via the Biology Laboratories of Tikrit University, we have taken two of the most common bacteria types, which grow mostly in soil and water, such as *Staphylococcus aureus*, which is a type of positive bacteria, and *Escherichia coli*, which is a type of negative bacteria. Firstly, the growth medium of these bacteria, Mueller-Hinton agar, was made by dissolving 40 grams of this solution in one liter of distilled water [18]. This medium was sterilized by heating it up to the temperature of 121°C under the pressure of 1.5 bar in an autoclave; after that, the solution was cooled down and poured in Petri dishes at normal temperature. These dishes were then contaminated with bacteria that we acquired in three different ways in order to make sure that the bacteria are evenly distributed, and then the dish was pricked in three holes, which are 1mm in diameter [19,20].The chemical solutions were made by continuously adding DMSO solvent at three different concentrations (0.01, 0.0001, and 0.001) mg/ml. After that, each concentration was added to a well, and three distinct concentrations were added to one plate. After that, the plates were incubated for a whole day at 37°C in a dedicated incubator. The inhibition's diameter was measured in millimeters [21].

3. Results and Discussion

3.1. Characterization of complexes [Zn(L)$_2$(dppm)]

The complex [Zn(L)(dppm)] was characterized using IR spectroscopy, which revealed a difference in the spectrum due to the appearance of new bands following the introduction of the phosphine ligand dppm into the complex and the removal of one of the NH groups in 2 –thio-uracil. and the appearance of an aromatic (CH) band at (3047) cm^{-1}, in addition to two bands indicative of the aromatic (C=C) bond at (1521, 1461) cm^{-1}, whilst the presence of two bands at (2962, 2914) cm^{-1} was attributed to aliphatic (CH) groups. A new band not previously observed also appeared at (1425) cm^{-1}, the presence of a (P-Ph) group was inferred, as well as a band at (1018) cm^{-1} indicating the presence of a (C-P) group. The spectrum showed that the band appearing at (453) cm^{-1} indicated the presence of a (Zn-n) group [22]. As shown in Figure 1.

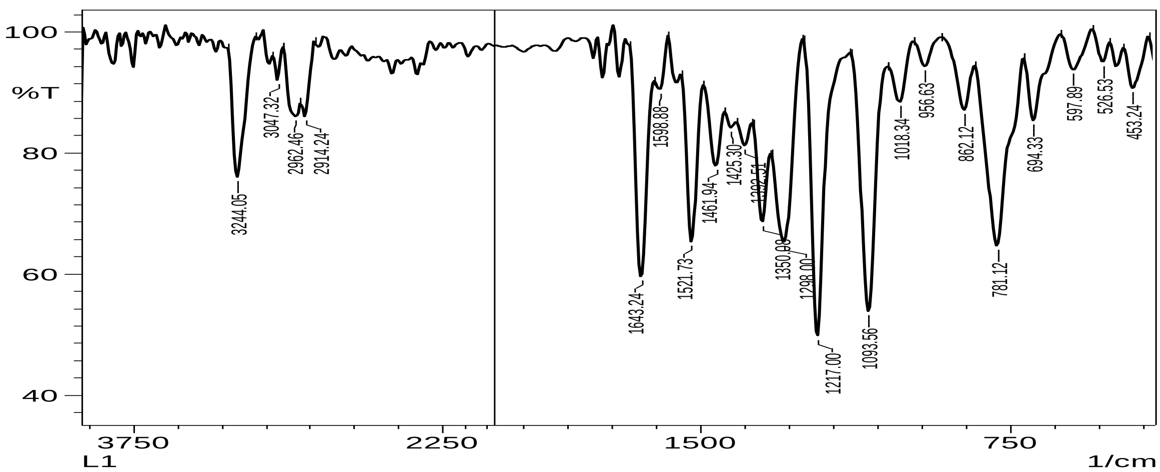


Figure 1. FT-IR of [Zn(L)$_2$(dppm)]

As for the ^{1}H-NMR spectrum, it showed that the (NH) group located between the carbonyl and thionyl groups had disappeared, whilst the presence of signals with an integration of 20 protons indicated the presence of phosphine at (7.35–7.52) ppm; As for the (NH) group adjacent to the double bond, it was present at (7.96) ppm with only one proton, As for the double bond, it appeared as a double peak for each proton and a single peak at each end at (5.84–7.31) ppm, as well as a characteristic signal indicating the presence of (CH_2) in the complex at (1.61) ppm, with two protons[23]. As shown in Figure 2

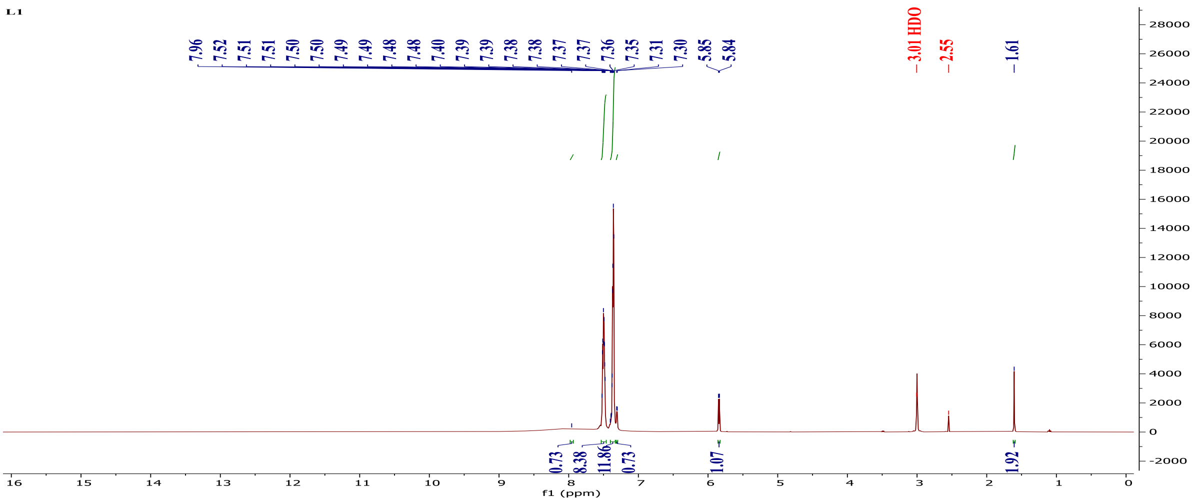


Figure 2. ^{1}H-NMR of [Zn(L)$_2$(dppm)]

As for the ^{31}P-NMR spectrum, it was found that the complex contains a single isomer—that is, it has a single conformation—as indicated by a signal appearing at (24.59) ppm, where the two phosphorus atoms are symmetrical [24]. As shown in Figure 3

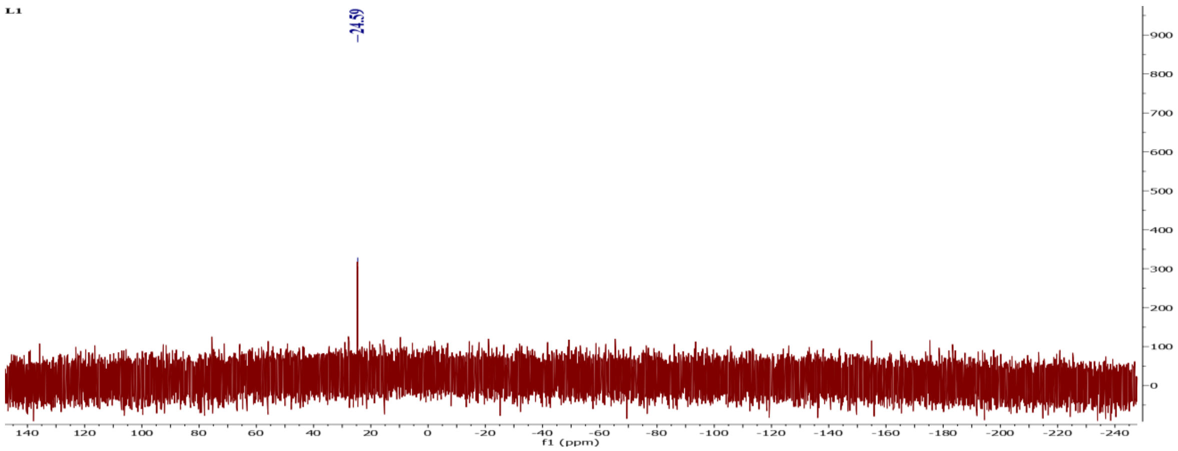


Figure 3. ^{31}P-NMR of [Zn(L)$_2$(dppm)]

3.2. Characterization of complexes [Zn(L)$_2$(dppe)]

The complex [Zn(L)(dppe)] using IR spectroscopy, which revealed a difference in the spectrum due to the appearance of new bands resulting from the introduction of the phosphine ligand dppe into the complex, along with the removal of one of the NH groups in 2-thiouracil and the appearance of a band attributed to the aromatic (CH) group at (3051) cm^{-1}, in addition to two bands indicating the presence of an aromatic (C=C) bond at 1541 and 1469 cm^{-1}, alongside two bands attributed to aliphatic (CH) groups at 2939 and 2883 cm^{-1}. A new band not previously observed also appeared at 1407 cm^{-1}, the presence of a (P-Ph) group was inferred, as well as a band at (1012) cm^{-1} indicating the presence of a (C-P) group. The spectrum showed that the band appearing at (445) cm^{-1} indicated the presence of a (Zn-n) group. As shown in Figure 4

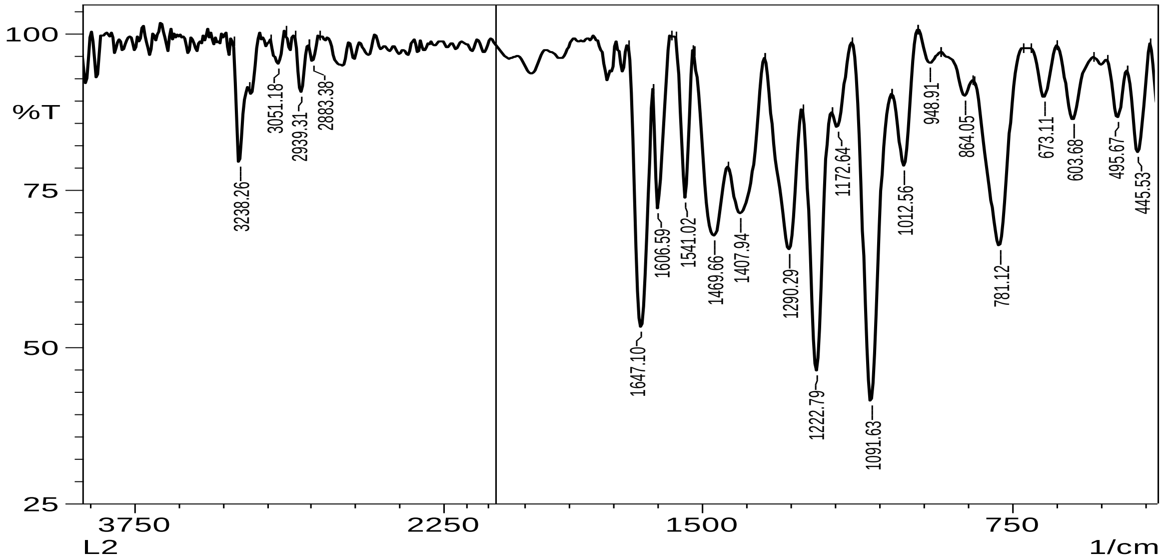


Figure 4. FT-IR of $[Zn(L)_2(dppe)]$

As for the ^{1}H-NMR spectrum, it showed that the (NH) group located between the carbonyl and thionyl groups had disappeared, whilst the presence of signals with an integration of 20 protons indicated the presence of phosphine at (7.23–7.32) ppm; As for the (NH) group adjacent to the double bond, it was present at (7.62) ppm with only one proton, As for the double bond, it appeared as a double peak for each proton and a single peak at each end at (5.97–7.15) ppm, as well as a characteristic signal indicating the presence of ($2CH_2$) in the complex at (1.99-2.02) ppm, with 4 protons. As shown in Figure 5

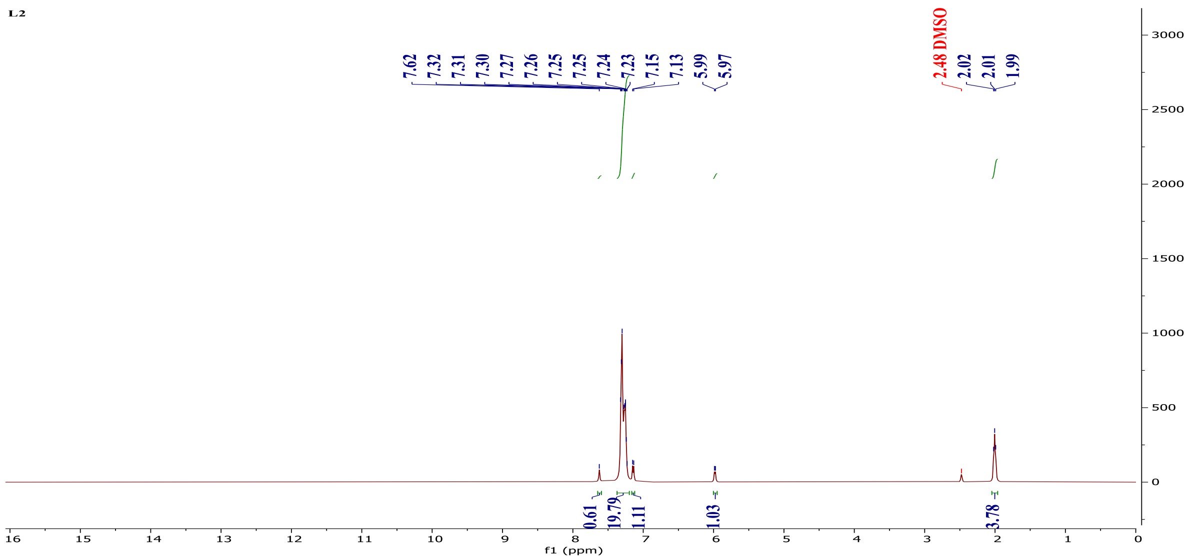


Figure 5. ^{1}H-NMR of $[Zn(L)_2(dppe)]$

As for the ^{31}P-NMR spectrum, it was found that the complex contains a single isomer—that is, it has a single conformation—as indicated by a signal appearing at (27.84) ppm, where the two phosphorus atoms are symmetrical. As shown in Figure 6

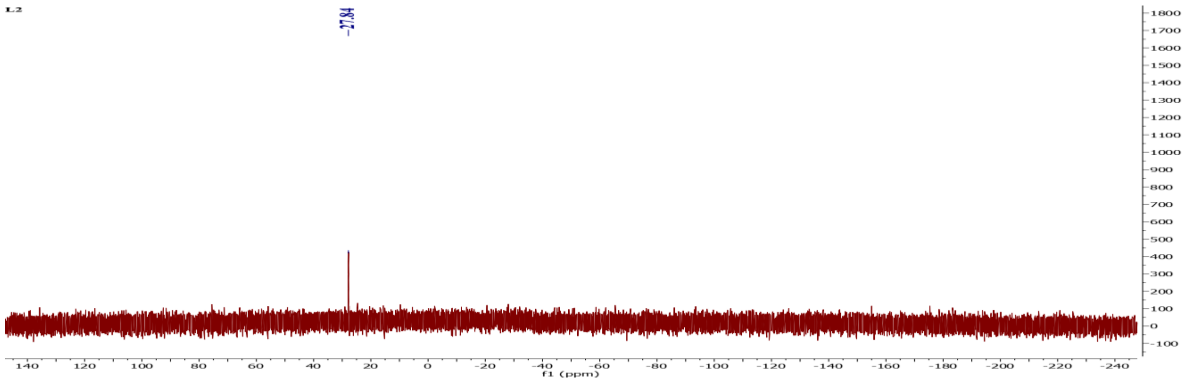


Figure 6. ^{31}P-NMR of [Zn(L)$_2$(dppe)]

3.2. Biological activity test results

The results of the biological activity of the synthesized compounds were based on a direct proportionality between the concentration of the substances and the degree of their inhibitory activity in relation to microorganisms. With increasing concentration, the size of the inhibition zone increased. The same level of inhibiting effectiveness in the size of about 10 mm was demonstrated by both complexes against Gram-negative microorganisms; however, only the [Zn(L)$_2$(dppe)] complex proved to be superior in activity at the lowest concentration in the size of 5 mm, whereas the other complex did not possess any activity [25, 26]. At the concentration of 0.01 mg/ml against Staphylococcus aureus, the inhibition zone of the [Zn(L)$_2$(dppm)] complex had a size of 16 mm and surpassed the [Zn(L)$_2$(dppe)] complex, whose inhibition zone was slightly smaller and amounted to 15 mm. Generally, the complexes demonstrated higher efficiency against Gram-positive microorganisms than Gram-negative microorganisms. The reason for such activity lies in the simplicity of binding of the compounds with the cells of the bacteria compared to antibiotic amoxicillin, which remains effective against the studied microorganisms [27–29]. As it can be seen from Table 1.

Table 1. Antibacterial effect of the synthesized substances (zone of inhibition in mm).

Comp No.	*E.coli*.mg/ml			*Staph. aureus* mg/ml		
	0.01	**0.001**	**0.0001**	**0.01**	**0.001**	**0.0001**
[Zn(L)$_2$(dppm)]	10	5	5	16	10	5
[Zn(L)$_2$(dppe)]	10	5	0	15	15	10
Amoxicillin.	27	20	15	30	20	15

4. Conclusion

Several phosphine complexes were synthesized with 2-Thiouracil glycanide. The accuracy of results obtained has been checked through the spectroscopic analysis, as it was found that there were spectral bands of (Zn-n) , suggesting that there was an interaction between the phosphine and ligand while the presence of additional spectral bands like (P-Ph) and (C-P) suggests an interaction between the metal phosphine. There is a single peak in ^{31}P- NMR spectra, which proves stereosymmetric binding, with the occurrence of only one stereoisomer in the phosphine complex. The complexes show considerable activity towards the bacteria Gram-positive, Gram-negative; however, there is no significant activity difference between these two complexes.

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