

Synthesis and Antibacterial Studies of Novel Phosphorus Ligand 5-(2-diphenylphosphino) phenyl-1,2-dihydro-1,2,4-triazole-3-thione and its Metal Complexes with some Transition Metals

تحضير ودراسة الفعالية الحيوية لمركب الفوسفور الجديد 5-(2-ثنائي فنييل فوسفينو) فنييل-2,1-ثنائي هيدرو-4,2,1-ترايازول-3-ثايون ومعقداته مع بعض العناصر الإنتقالية

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Abstract

This study involves the chemical synthesis of the novel ligand 5-(2-diphenylphosphino) phenyl-1,2-dihydro-1,2,4-triazole-3-thione (DPD TT) by the reaction of 2-diphenylphosphino benzoic acid with absolute ethanol that yield ethyl 2-diphenylphosphino benzoate and by cyclization of this compound with thiosemicarbazide, DPD TT will be produced. The chelating complexes of this ligand with Cr(III), Co(II), Ni(II), Cu(II) and Cd(II) were also prepared and studied. The new complexes were characterized by FT-IR, UV/visible spectra, and room temperature magnetic susceptibility. The stability for the prepared complexes was also measured using the density function theory and it was found that the cadmium complex is the most stable and the chromium complex is the least stable. Free ligand and its metal complexes have been tested *in vitro* against a number of microorganisms, like gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *E. coli*, *Proteus vulgaris*, *Pseudomonas* and *Klebsiella* in order to assess their antimicrobial properties. All complexes showed considerable activity against all the studied bacteria.

المستخلص

تم تحضير معقدات جديدة للمشتق 5-(2-ثنائي فنييل فوسفينو) فنييل-2,1-ثنائي هيدرو-4,2,1-ترايازول-3-ثايون (DPD TT) مع ايونات الفلزات: الكروم الثلاثي، الكوبالت، النيكل، النحاس، والكاديوم الثنائية. شخّصت المركبات المحضرة بالطرق الطيفية المتوفرة (طيف الأشعة تحت الحمراء، وطيف الأشعة فوق البنفسجية والمرئية)، فضلاً عن قياس العزم المغناطيسي المؤثر للمعقدات الصلبة. تم دراسة الاستقرار للمعقدات المحضرة حيث وجد ان معقد الكاديوم هو الأكثر استقراراً ومعقد الكروم الأقل استقراراً. درست الفعالية المضادة للجراثيم لكل من الليكاند ومعقداته على خمسة أنواع من البكتيريا هي:

(*Staphylococcus aureus*, *E. coli*, *Proteus Vulgaris*, *Pseudomonas*, and *Klebsiella*).

Introduction

The structural element 1,2,4-triazole system is a one of many drugs that have antimycotic activity such as fluconazol, itraconazol, voriconazol [1,2]. One of the methods of preparing derivatives of 1,2,4-triazole is the cyclization reaction of acyl derivatives of thiosemicarbazide in alkaline medium [3,5]. The coordination chemistry of triazole and benzotriazole derivatives was studied due to their importance in industry, agriculture and their biological activity. The mercapto group

often coordinated to metal ions in many biological molecules [6] and information about the relative reactivity of the coordinated mercapto group might give insight into the specific reactivity of active sites in some metalloproteins. On the other hand, some of the transition metals present in trace quantities are essential elements for biological systems. Tremendous interest has been shown in the study of complexes of substituted phosphines. This stems from the ability of phosphorus donor, in addition to usual sigma bonds, by $d\pi$ - $d\pi$ interaction of their empty orbitals with non-bonding electrons of metal ion [7].

The above facts and in continuation of our interest in studying the behavior of such compounds, we aim to (i) synthesize and characterize the solid complexes of the newly ligand containing both the triazole and phosphorus moieties, (DPDPTT), with Cr(III), Co(II), Ni(II), Cu(II) and Cd(II), (ii) investigate their antimicrobial effects towards some Gram-positive and Gram-negative bacteria.

Experimental

All chemical used were of reagent grade (supplied by Either Merck or Fluka) and used as supplied. The FT-IR spectra in the range $(4000 - 200) \text{ cm}^{-1}$ were recorded as cesium iodide disc on FT-IR 8300 Shimadzu Spectrophotometer. The UV-Visible spectra were measured in ethanol using Shimadzu UV-Vis. 160 A spectrophotometer in the range (200-1000) nm. Magnetic susceptibility measurement for complexes were obtained at room temperature using (Magnetic susceptibility Balance), Jhonson Matthey catalytic systems division. Gallencamp M.F.B.600 F melting point apparatus was used to measure the melting point of all the prepared compounds.

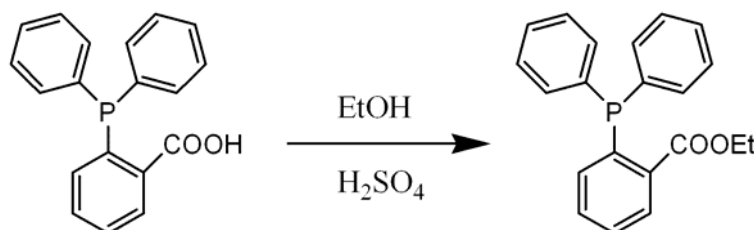
1) Synthesis of ligand

1. Synthesis of 2-diphenylphosphino benzoic acid

This compound was prepared according to the method reported by [8] by phosphorylation of *o*-chlorobenzoic acid with sodium diphenylphosphine (NaPPh_2). It was recrystallized from methanol, m.p = 170°C and the yield was 52% (lit: m.p = 175°C , yield 49%).

2. Synthesis of ethyl 2-diphenylphosphino benzoate

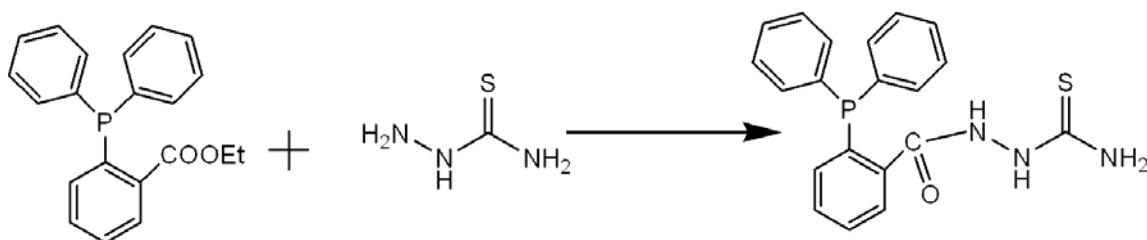
The mixture of 2-diphenylphosphino benzoic acid (75.34 gm, 0.246 mole) with 50 ml absolute ethanol, and 2.5ml concentrated sulfuric acid was refluxed for 4hrs, yield 55% of oily yellow (ethyl 2-diphenylphosphino benzoate) according to the following reaction:



3. Synthesis of 2-(2-diphenylphosphino benzoyl) hydrazine carbothioamide

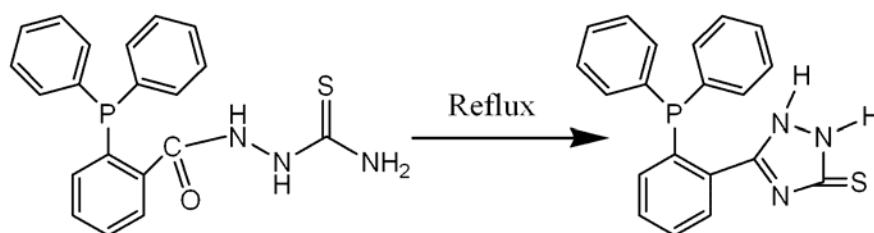
Hot ethanolic solution of thiosemicarbazide (1.82 g, 0.02 mol) and ethanolic solution of ethyl 2-diphenylphosphino benzoate (6.68gm, 0.02 mol) were mixed with constant stirring and refluxed for 1 hour [9]. After cooling, the reaction mass was evaporated

on a rotatory evaporator where 2-(2-diphenylphosphino benzoyl) hydrazine carbothio amide was separated, filtered, washed with cold ethanol, and dried under vacuum over P_4O_{10} , yield 53%.



4. Synthesis of 5-(2-diphenylphosphino phenyl)-1,2-dihydro-1,2,4-triazole-3-thione (L)

Hot ethanolic solution of 2-(2-diphenylphosphino benzoyl) hydrazine carbothioamide (1.89 g, 0.005 mol) was refluxed for 8 hrs. On cooling, the product separated out, filtered, washed with cold ethanol, and dried under vacuum over P_4O_{10} . Yield 50% of oily brown 5-(2-diphenylphosphino phenyl)-1,2-dihydro-1,2,4-triazole-3-thione (L) according to the following reaction:



2) Synthesis of complexes

Hot ethanolic solution 20 mL of corresponding metal salts 0.01 mol was mixed with hot ethanolic solution of the ligand 0.02 mol. The mixture was heated for 3-4 hours at 50–60°C. On cooling the contents, the complex separated out in each case. It was filtered and washed with 50% ethanol- H_2O and dried under vacuum over P_4O_{10} . The physical properties for the prepared complexes are shown in Table (1).

Table (1): Physical data for the metal complexes.

No.	Compound	Color	MP ($^{\circ}C$)
C_1	Bis(5-(2-(diphenylphosphino)phenyl)-1,2-dihydro-1,2,4-triazole-3-thione)chromium(III)Chloride	Dark brown	Oily
C_2	Bis(5-(2-(diphenylphosphino)phenyl)-1,2-dihydro-1,2,4-triazole-3-thione)Cobalt(II) Chloride	Dark green	300d
C_3	Bis(5-(2-(diphenylphosphino)phenyl)-1,2-dihydro-1,2,4-triazole-3-thione)Nickel(II)Chloride	Light green	180d
C_4	Bis(5-(5-(2-(diphenylphosphino)phenyl)-1,2-dihydro-1,2,4-triazole-3-thione)Copper(II)Chloride	Green	235d
C_5	Bis(5-(2-(diphenylphosphino)phenyl)-1,2-dihydro-1,2,4-triazole-3-thione)Cadmium(II).	White	277-280

3) Study of biological activity for ligand L and C₁-C₅ complexes

The biological activity of the newly prepared ligand (DPDPTT) and its metal complexes were studied against selected types of bacteria which included gram positive bacteria (*Staphylococcus aureus*), and gram negative bacteria (*E. Coli*, *Proteus Vulgaris*, *Pseudomonas*, and *Klebsiella*), in brain heart infusion (broth, agar, media), which is used DMF as a solvent and as a control for the disc sensitivity test [10]. This method involves the exposure of the zone of inhibition toward the diffusion of micro-organism on agar plate. The plates were incubated for 24 hrs, at 37°C. The antimicrobial activity was recorded as any area of microbial growth inhibition that occurred in the diffusion area. The quantitative antimicrobial activity assay was performed by the nutrient broth for bacterial [11, 12].

Results and Discussion

The complexes were synthesized by the reaction of the ligand (DPDPTT) with the metal ions in 1:2 molar ratios in ethanolic medium. The ligand behaves as bidentate coordinate through phosphorous and nitrogen donor atoms Figure (1).

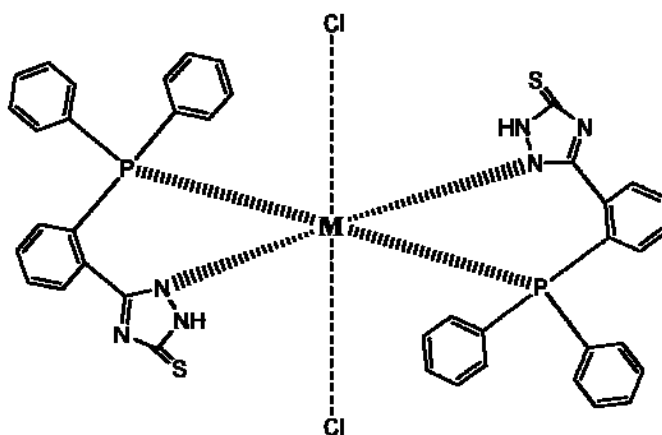


Fig (1): The proposed structure for the prepared complexes

Infrared Spectroscopy

The IR spectra provide valuable information regarding the nature of functional group attached to the metal atom. The ligand and metal complexes were characterized mainly using the secondary amine band (NH). The main infrared bands and their assignments are listed in Table (2). The appearance of a broad strong band in the IR spectra of the ligand in 3424cm^{-1} is assigned to N-H stretching vibrations of the secondary amine group. In the complexes, this band is shifted to lower frequency indicating that the ligand was coordinated to metal ions through the nitrogen atoms of the NH group [13]. The bands attributed to (M-N) and (M-Cl) were observed in the region $(450-520)$ and $(300-390)\text{cm}^{-1}$ respectively in all complexes [14].

Table (2): Characteristic IR bands (cm^{-1}) of the compounds studied.

No.	NH	C=N Sym., Asym	C=S	M-N	M-Cl
<i>L</i>	3424	1641 _s , 1624 _m .	831 _b	-	-
<i>C₁</i>	3389	1616 _s , 1603 _s	810 _b	451 _b	335 _w
<i>C₂</i>	3412	1610 _s , 1594 _m	801 _b	449 _b	297 _w
<i>C₃</i>	3394	1627 _s , 1602 _s	791 _b	438 _b	299 _m
<i>C₄</i>	3401	1620 _m , 1599 _b	831 _b	489 _w	389 _w
<i>C₅</i>	3385	1625 _s , 1607 _m	808 _b	465 _b	287 _w

S=strong, b=broad, m=medium, w=weak.

UV/visible spectra:

The ultraviolet spectra of the synthesized ligand in DMF showed two absorption bands, the position of the first band at 305nm (32787 cm^{-1}) which represents the ($\pi - \pi^*$) transition while the position of the second band (which has higher intensity than the first band due to conjugated system) at 345 nm (28985 cm^{-1}) which represents the ($n - \pi^*$) transition. Generally, the bands of the newly synthesized complexes are either shifted to shorter or longer wavelengths than that of ligands, but the high intensity bands is an indication for complex formation [15].

The peaks are classified into two distinct groups: those that belong to ligand transitions appeared in the UV region while d-d transitions appeared in the visible region; these transitions are assigned in relevance to the structures of complexes Table (3).

Cr-complex (*C₁*)

At room temperature the magnetic susceptibility measurement after diamagnetic corrections yielded a magnetic moment of 5.0 BM which is close to that expected for octahedral Cr (III) complexes [16] as shown in Figure (2). The UV-visible spectrum of the green solution of chromium the Cr(III) complex recorded in DMF showed three bands with the absorbance maxima at 14981 cm^{-1} , 19926 cm^{-1} and 31667 cm^{-1} which were considered as ν_1 , ν_2 , and ν_3 absorption bands respectively.

Co-complex (*C₂*)

The magnetic susceptibility measurement after diamagnetic corrections yielded a magnetic moment of 4.40BM which is close to that expected for an octahedral Co(II) complexes [17] as shown in Figure (2). The electronic absorption spectrum in DMF showed two absorption bands at (14111 and 19422) cm^{-1} which are considered as ν_2 and ν_3 respectively. These transitions may be assigned as:

$$\nu_2 = {}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$$

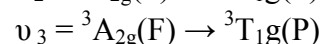
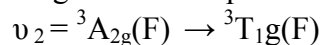
$$\nu_3 = {}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$$

Experimental ν_2 and ν_3 values have been employed to calculate the position of ν_1 [${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$] band from [18].

Ni-complex (*C₃*)

Nickel complex is paramagnetic with a room temperature magnetic moment of 2.86 B.M. which is consistent with an octahedral field as shown in Figure (2). The electronic absorption spectrum in DMF showed two absorption bands at (18649 and

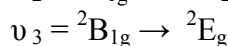
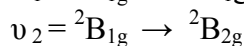
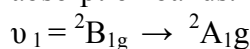
30015) cm^{-1} which are considered as ν_2 and ν_3 respectively. These bands may be assigned as two spin-allowed transitions:



Experimental ν_2 and ν_3 values have been employed to calculate the position of ν_1 [${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{2g}(\text{F})$] band from [18]. The position of bands indicates that the complex have six coordinate octahedral geometry [19].

Cu-complex (C_4)

The complex has a room temperature magnetic moment of 1.98 B.M. which corresponds to octahedral structure for the Cu(II) ion [19,20] as shown in Figure (2). The electronic absorption spectrum of Cu(II) complex in DMF shows three bands at 11138, 14367, and 19356 cm^{-1} respectively which were considered as ν_1 , ν_2 , and ν_3 absorption bands.



Cd-complex (C_5)

The prepared white Cd(II) complex exhibited a band which appeared at 30664 cm^{-1} which may be related to the ligand ($\pi \rightarrow \pi^*$) transition. The filled d-orbitals show no ligand field transitions and the magnetic moment measurement show that this complex is diamagnetic. The proposed structure for this complex is square planar as shown in Figure (2).

Table (3): Electronic spectral data, magnetic moments, and suggested geometries for the ligand L and its complexes.

Compound	Absorption Bands (cm^{-1}) in DMF	Assigned transition	Magnetic moment (B.M.)
L	32787	$\pi \rightarrow \pi^*$	-
	28985	$n \rightarrow \pi^*$	
	14981	${}^4\text{A}_{2g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{F})$	
C_1	19926	${}^4\text{A}_{2g}(\text{F}) \rightarrow {}^4\text{T}_{1g}(\text{F})$	5.0
	31667	${}^4\text{A}_{2g}(\text{F}) \rightarrow {}^4\text{T}_{1g}(\text{P})$	
	7625 (calc.)	${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{F})$	
C_2	14111	${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}(\text{F})$	4.4
	19422	${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{1g}(\text{P})$	
	11707 (calc.)	${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{2g}(\text{F})$	
C_3	18649	${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{F})$	2.86
	30015	${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{P})$	
	11138	${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}$	
C_4	14367	${}^2\text{B}_{1g} \rightarrow {}^2\text{B}_{2g}$	1.98
	19356	${}^2\text{B}_{1g} \rightarrow {}^2\text{E}_g$	
C_5	30664	$\pi \rightarrow \pi^*$	-

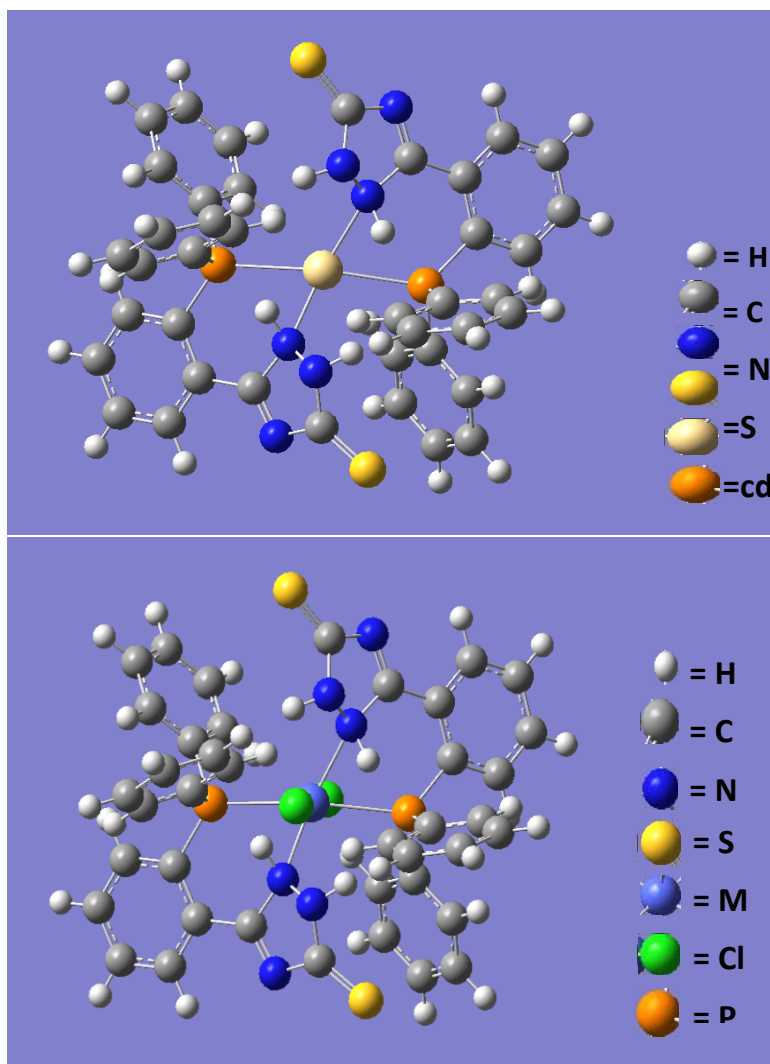


Fig (2): Expected structure for complexes

Antimicrobial activity assays

Biological Activity

As a result from the study of antimicrobial of prepared ligand and it metal complexes are shown in Table (5). The following points were concluded:

1. The results of antibacterial activity study for the 5-(2-(diphenylphosphino) phenyl)-1,2-dihydro-1,2,4-triazole-3-thione(DPDTT) indicated that the new ligand exhibited antibacterial activity against the studied bacteria at low and high concentration .
2. Generally, the result of prepared complexes exhibited antibacterial activity toward *E. Coli*, *Staphylococcus aureu*, and *Klebsiella* was more than the complexes inhibition on *Pseudomonas* and *Proteus vulgaris* , bacteria.

Table (5): The effect of Test organism toward Ligand and it complexes (C₁-C₅).

Test organism	Compound	Diameter (mm)					Concentration (mg\ml)				
<i>Staphylococcus aureus</i>	L	++	++	+++	+++	+++					
	C ₁	-	+	+	+	++					
	C ₂	-	+	+	+	++	0.1	0.30	0.5	0.7	1.0
	C ₃	+	+	++	++	+++	5			5	
	C ₄	+	+	+	++	+++					
	C ₅	-	-	+	++	++					
<i>E. Coli</i>	L	++	++	+++	+++	+++					
	C ₁	+	+	+	+	++					
	C ₂	+	+	+	++	++	0.1	0.30	0.5	0.7	1.0
	C ₃	+	+	+	+	++	5			5	
	C ₄	+	+	+	+	+					
	C ₅	+	+	++	++	++					
<i>Proteus Vulgaris</i>	L	++	++	+++	+++	+++					
	C ₁	-	-	-	+	+					
	C ₂	-	-	-	+	+	0.1	0.30	0.5	0.7	1.0
	C ₃	-	-	-	+	+	5			5	
	C ₄	+	+	+	++	++					
	C ₅	+	+	+	+	++					
<i>Pseudomonas</i>	L	++	++	+++	+++	+++					
	C ₁	+	+	+	+	++					
	C ₂	+	+	+	+	++	0.1	0.30	0.5	0.7	1.0
	C ₃	-	-	+	+	+++	5			5	
	C ₄	-	-	-	+	++					
	C ₅	-	-	-	-	+					
<i>Klebsiella</i>	L	++	++	+++	+++	+++					
	C ₁	+	+	+	++	++					
	C ₂	+	+	++	+++	+++	0.1	0.30	0.5	0.7	1.0
	C ₃	+	+	+	+	+++	5			5	
	C ₄	-	-	+	+	++					
	C ₅	-	-	-	-	++					

Results were interpreted in terms of the diameter of the inhibition zone: (-) < 0.5, (+)=0.5-0.9, (++)= 1.0-1.4,(++) => 1.5

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