Synthesis, cytotoxicity, antibacterial activity and molecular modeling study of new mono, homo and heterobimetallic complexes of palladium (II) with some transition metal ions containing the ligands N-phenyl-N'(2-thiazolyl)thiourea and Diphosphines Ph₂P(CH₂)nPPh₂ (where n = 1–3)

Nazk M Aziza,* & Bayazeed H Abdullah

Department of Chemistry, College of Science, University of Sulaimani, Kurdistan, Iraq
Email: nazk.aziz@univsul.edu.iq (NMA)/ bayazeed.abdullah@univsul.edu.iq (BHA)

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The ligand N-phenyl-N'(2-thiazolyl) thiourea (LH) has been prepared from reaction of phenyl isothiocyanate with 2-aminothiazol. Treatment of deporotonated ligand (LH) with sodium tetrachloro palladate(II) afforded [Pd(L)₂] complex 3. Reaction of complex 3 with [bis(diphenylphosphino) methane, dppm], [1,2-bis(diphenylphosphino)ethane, dppe] and [1,3-bis(diphenylphosphino)propane, dppp] afforded the mixed ligand complexes of the type [Pd(II)L₂(Ph₂P(CH₂)nPPh₂)], {where n = 1, dppm, n = 2, dppe or n = 3, dppp} 4-6, respectively. Further, complexes 4-6 have been treated with some transition metal salts to give homo- and heterobimetallic complexes 7-21 of the types: [(Ph₂P(CH₂)nPPh₂)Pd (II)-µ-L₂M'(II)Xm] and [(Ph₂P(CH₂)nPPh₂)Pd(II)-µ-L₂M'(II)Xm x H₂O] (where n = 1, 2, or 3 , M' = Pd(II), Ni(II), Mn(II), Co (II) or Cu (II) and X = Cl-, m = 2). The ligand and the prepared complexes have been characterized by elemental analysis, molar conductivity, magnetic susceptibility, FTIR, UV-Vis, 1H-31P{1H} NMR and mass spectroscopy. Some of these complexes have been assayed for their inhibition activity against human rhabdomyosarcoma (RMS) cell line. Interestingly, compound 19 exhibited a significant cytotoxicity inhibition activity ~90% for RMS cell line, suggesting being a new lead in the development of human muscle anticancer agent. All the compounds have been screened for their antibacterial activity against S. aureus and E. coli bacterium.

Keywords: Alkyl diphenylphosphines, Antibacterial activity, Pd(II) complexes, Rhabdomyosarcoma cell line, Thiourea derivatives ligands, Transition metal complexes

The palladium (II) as nonplatinum metal complexes highly attracted the researchers because of its significant biological activity as well as lower side effects along with higher lipophilicity or solubility compared to cisplatin7. Chelating thiourea ligands containing N, S and O donor atoms show broad biological activity and the existence of metal ions bonded to biologically active compounds may enhance their activities8-11.

There are several reports describing studies of acylthiourea palladium complexes focusing on their antimicrobial12, fungicidal13, thermal14 or mesomorphic15 properties. Pd (II) complexes are preferable metal-based anticancer drugs due to their structural and thermodynamic similarities to Pt (II) complexes along with the coordination geometry and complex forming processes of Pd (II) are closely related to those of Pt (II) metal16. Many of the prepared palladium (II) complexes showed a discrete antitumor activity In vitro compared to the platinum based drugs because of their extremely high lability in biological fluids17, such as the ionic Pd (II) alkylidiphosphine complex 1 (Fig. 1) which caused 100% tumor cell death at very low concentration (< 1.25 μM). It has been reported that palladium complexes, e.g. complex 2 (Fig. 1), exhibited remarkable antiproliferative activity against some cancer cell lines such as MT-4, CD4 human acute T-lymphoblastic leukemia (CCRF-SB) (IC₅₀ = 0.70 ± 0.05 μM), human splenic B-lymphoblastoid cells, human acute B-lymphoblastic leukemia, skin melanoma, and prostate carcinoma cell lines18. The anticancer activity of palladium complexes has

![Fig.1 — Palladium (II) complexes as anticancer agents.](image-url)
recently been subject of a detailed review\(^{19}\). On the other hand, transition metal complexes with thiourea ligands have been extensively investigated for their antibacterial, antifungal, antitubercular, antithyroid, insecticidal and anticancer properties\(^{20-24}\).

In view of varied pharmacological activities of palladium and transition metal complexes, herein we report the synthesis and characterization of some new palladium (II) complexes of the deprotonated thioureas; mononuclear, homo and heterobimetallic complexes of palladium (II) with some transition metal ions containing the ligands N-phenyl-N’-(2-thiazolyl)thiourea and diphosphines Ph\(_2\)P(CH\(_2\)\(_n\))PPh\(_2\) (where \(n = 1-3\)), as well as evaluation of their inhibition of rhabdomyosarcoma (RMS) cell line and antibacterial activity.

**Materials and Methods**

The compounds Na\(_2\)PdCl\(_4\), NiCl\(_2\) \(6\)H\(_2\)O, MnCl\(_2\) \(4\)H\(_2\)O, CoCl\(_2\) \(6\)H\(_2\)O, CuCl\(_2\) \(2\)H\(_2\)O, dppm, dppe, and dppp were obtained from Fluke and BDH Companies. The compounds 2-aminothiazol, and Phenyl isothiocyanate, were purchased from Solarbio Life Sciences Co. All the chemicals and solvents were analytically pure and used without further purification.

Melting points were measured on Electrothermal digital melting point apparatus Model 1102D. Micro analytical data were obtained with EA 3000 from Euro Vector and Perkin Elmer-2400 CHNS analyzer\(^{31}\). P and \(^1\)H-NMR spectra were recorded on Bruker Avance-III 400 MHz, and \(^{13}\)C NMR spectra were recorded on Bruker, Germany instrument. I.R. spectra were recorded on a Shimadzu FT-IR 8400 spectrophotometer using CsI discs. Mass spectrum was recorded on Shimadzu GCMS- spectrometer. Magnetic measurements were recorded on a Bruker BM6 instrument at room temperature using the Faraday method. The conductivities of the complexes were measured in DMF using Fisher Scientific Multimeter Model XL600 and Electronic spectra of the ligand and the complexes were measured in DMF using a Jenway 6485 spectrophotometer.

**Synthesis of N-phenyl-N’-(2-thiazolyl) thiourea (LH)**

This ligand was prepared using a modified literature method\(^{25}\). This ligand was prepared by adding Phenyl isothiocyanate (1.35 mL, \(0.1 \times 10^{-6}\) mmol) to a solution of 2-aminothiazol (1 g, \(0.1 \times 10^{-4}\) mmol) in ethanol (10 mL). The mixture was heated under reflux for 2 h, and then cooled in an ice bath. The formed off-white solid was filtered off and washed with ethanol and it was recrystallized from ethanol and purified using column chromatography (flash chromatography), (silica gel 60) and dichloromethane as a solvent. Yield 88% as off-white crystal; M.p.: 184 °C. Anal.(%) calcd. for C\(_{10}\)H\(_9\)N\(_8\)S\(_2\) (235.33): C, 50.99; H, 3.55; N, 4.76. Found: C, 50.99; H, 3.55; N, 4.75. IR (\(\nu\), cm\(^{-1}\)): 3188, 3325 (NH); 3080 (CH arom.); 1562 (C=N); 1068 (C=S). UV-Vis (\(\nu'\), cm\(^{-1}\)): 29411; 31847 and 38461 (C.T.). \(^1\)H NMR (CDCl\(_3\) ppm) \(\delta\) : 11.03, 12.83 (s, 2H, NH), 6.9–7.7 (m, 7H, H arom.). \(^{13}\)C NMR (CDCl\(_3\) ppm) \(\delta\) : 176.91 (s, (C=S)), 162.17(s, (C=N)), 111.70–137.93 (m, C arom.).

**Synthesis of [Pd (L)\(_2\)] (3)**

This complex was prepared following a modified literature method\(^{25}\). To a warm solution of sodium salt of the ligand (NaL) [from LH, (200 mg, 0.87 mmol) and NaOH, (243 mg, 0.87 mmol)] in EtOH (5 mL) a solution of Na\(_2\)PdCl\(_4\) (126 mg, 0.43 mmol) in EtOH (5 mL) was added and the solution mixture was stirred for 1.5 h. After cooling, a yellow solid was formed, filtered, washed with H\(_2\)O, and recrystallized from DMF-EtOH (1:1) and finally dried to give 3. Yield (229 mg, 93%) as a yellow solid; M.p.: 255–257 °C. Anal.(%) calcd. for C\(_{20}\)H\(_{16}\)N\(_6\)PdS\(_4\) (575.08): C, 41.73; H, 2.78; N, 14.61. Found: C, 41.62; H, 2.90; N, 14.71. IR (\(\nu\), cm\(^{-1}\)): 3299 (NH); 3122, 3057 (CH arom.); 1595 (C=N); 1030 (C=S); 457 (Pd-S); 540 (Pd-N). UV-Vis (\(\nu'\), cm\(^{-1}\)): 30487 (C.T.) and 38461 (C.T.). \(^1\)H NMR ([D\(_6\)]DMSO ppm) \(\delta\) : 10.31 (s, 2H, NH), 7.67–7.05 (m, 14H, Harom.+ 4-Hthiazole + 5-Hthiazole).

**General procedure for the preparation of Pd (II) complexes 4-6**

A solution of bis-(diphenylphosphine)alkane Ph\(_2\)P(CH\(_2\)\(_n\))PPh\(_2\) (where \(n = 1, 2 \) or \(3 \)) (0.21 mmol) in CHCl\(_3\) (5 mL) was heated and added to a warm suspension solution of [Pd (L)\(_2\)] (0.21 mmol) in CHCl\(_3\) (5 mL), with the exception for the complex 4 which was prepared in a mole ratio (2:2) 0.42 mmol. The colored mixture stirred for 1.5 h. The solvent was evaporated and a mixture of (CH\(_2\)Cl\(_2\)-ether) (5 mL) was added to the residue. The mixture was stirred for another hour. A colored solid was formed, filtered, and recrystallized from CH\(_2\)Cl\(_2\)-EtOH (2:1) and dried to give the desired complex.
solid product was obtained. The solid was extracted with CH2Cl2 (10 mL) and the solvent was evaporated to dryness to give a colored solid product which was recrystallized from CH2Cl2-EtOH (2:1) and dried to afford the desired complex.

### Preparation of [(dpdp) Pd (µ- L)2 CuCl2(H2O)2] (10)

From CuCl2.2H2O (60 mg) and complex 4 (337 mg): Yield: (287 mg, 65%) as a greenish brown powder. M.p.: 220 °C. Anal. (%) calcd. for C38H34Cl2CuN5O2P2PdS4: C, 47.5; H, 3.49; N, 7.19. Found: C, 47.15; H, 3.50; N, 7.17. 1H NMR ([D6]DMSO ppm) δ: 10.12 (s, 2H, NH), 8.04–7.00 (m, 34H, H arom. + 4-Hthiazole + 5-Hthiazole), 1.53 (br, 2H, P-CH2-P). UV-Vis (ν’, cm–1): 26954 (νA’g (F)→νT1g(p)), 27173 (νA2g (F)→νT1g (p)) and 29095 (C.T.).

### Preparation of [(dpdp) Pd (µ- L)2 NiCl2(H2O)2] (9)

From Na2PdCl4 (103 mg) and complex 4 (336 mg): Yield: (322 mg, 81%) as an orange powder. M.p.: 230 °C. Anal. (%) calcd. for C40H34Cl2NiN5O2P2PdS4: C, 47.53; H, 3.45; N, 7.16. Found: C, 47.19; H, 3.39; N, 7.17. 1H NMR ([D6]DMSO ppm) δ: 10.12 (s, 2H, NH), 8.04–7.00 (m, 34H, H arom. + 4-Hthiazole + 5-Hthiazole), 1.53 (br, 2H, P-CH2-P). UV-Vis (ν’, cm–1): 26954 (νA’g (F)→νT1g(p)), 27173 (νA2g (F)→νT1g (p)) and 29095 (C.T.).
Preparation of [(dppe) Pd (µ- L)2 PdCl2] (I4)

From NaN3PdCl4 (103 mg) and complex 5 (341 mg). Yield: (306 mg, 76%) as a brown powder. M.p.: 220 °C dec. Anal. (%) calc'd. for C46H44Cl2MnN6O2P2PdS4 (1150.92): C, 47.96; H, 3.48; N, 7.30. Found: C, 47.82; H, 3.21; N, 7.18. IR (ν, cm–1): 3365 (NH); 3126, 3056 (CH arom.); 2950 (CH aliph.); 1558 (C=N); 1028 (C=S); 1074 (C-P); 488 (Pd-P); 338 (Pd-S); 530 (Pd-N); 280 (Pd-Cl). 1H NMR ([D6]DMSO ppm) δ: 10.51 (s, 2H, NH), 8.14–6.99 (m, 34H, H arom. + 4-Hthiazole + 5-Hthiazole), 2.77, 2.62 (2xbs, 4H, dppe). 13P NMR ([D6] DMSO) δp: 66.6 (s, P-Pd) S-bonded isomer. UV-Vis (ν', cm–1): 28212 (1A1g→1Eg), 37878, and 41000 are attributed to C.T. respectively.

Preparation of [(dppp) Pd (µ- L)2 CuCl2(H2O)] (15)

From CuCl2.2H2O (60 mg) and complex 5 (343 mg). Yield: (248 mg, 62%) as a green solid. M.p.: 146 °C. Anal. (%) calc'd. for C46H44Cl2CuN6O2P2PdS4 (1144.05): C, 48.25; H, 3.85; N, 7.34. Found: C, 48.13; H, 3.61; N, 7.17. IR (ν, cm–1): 3490 b (OH); 3350 (NH); 3055 (CH arom.); 2914 (CH aliph.); 1560(C=N); 1029 (C=S); 1073 (C-P); 484 (Pd-P); 343 (Pd-S); 530 (Pd-N); 270 (Cu-Cl). UV-Vis (ν', cm–1): 27110 (1A1g→1Eg), 28571 (1A1g→1B1g), 32787 (C.T.), and 43860 (C.T.).

Preparation of [(dppe) Pd (µ- L)2 CoCl2(H2O)] (16)

From CoCl2.6H2O (83 mg) and complex 5 (334 mg). Yield: (271 mg, 68%) as a yellow powder. M.p.: 231 °C dec. Anal. (%) calc'd. for C46H44Cl2MnN6O2P2PdS4 (1139.19): C, 48.46; H, 3.86; N, 7.37. Found: C, 48.23; H, 3.60; N, 7.08. IR (ν, cm–1): 3427 (OH); 3360 (NH); 3098, 3058 (CH arom.); 2928, 2858 (CH aliph.); 1586 (C=N); 1026 (C=S); 1074 (C-P); 482 (Pd-P); 368 (Pd-S); 529 (Pd-N); 278 (Ni-Cl). UV-Vis (ν', cm–1): 25000 (1A1g(F)→1T1g(p)), 29411 (1A1g→1B1g), 39683 (C.T.).

Preparation of [(dppe) Pd (µ- L)2 NiCl2(H2O)] (17)

From NiCl2.6H2O (84 mg) and complex 5 (344 mg). Yield: (283 mg, 71%) as a pale green powder. M.p.: 248 °C. Anal. (%) calc'd. for C46H44Cl2NiN6O2P2PdS4 (1139.43): C, 48.45; H, 3.86; N, 7.37. Found: C, 48.48; H, 3.86; N, 7.37. IR (ν, cm–1): 3408–3466 (OH); 3345 (NH); 3056 (CH arom.); 2960 (CH aliph.); 1568 (C=N); 1028 (C=S); 1074 (C-P); 482 (Pd-P); 361 (Pd-S); 530 (Co-N); 235 (Co-Cl). UV-Vis (ν', cm–1): 14880 (1T1g(F)→1T1g(p)), 20408 (1A1g→1B1g), 28571 (1A1g→1Eg) and 33560 (C.T.).
C₄₇H₄₆Cl₂MnN₆O₂P₂PdS₄ (1149.46): C, 49.07; H, 4.00; N, 7.31. Found: C, 48.97; H, 4.05; N, 7.25. IR (ν, cm⁻¹): 3400–3550 (OH); 3363 (NH); 3055 (C-H arom); 2950 (C-H aliph); 1540 (C=N); 1028 (C=S); 1072 (C-P); 444 (Pd-P); 343 (Pd-S); 509 (Pd-N); 235 (Mn-Cl). UV-Vis (ν', cm⁻¹): 26526 (6A₁g→4T₂g (4D)), 27286 (6A₁g→4T₂g (4D)), and 30959 (C.T).

Preparation of [(dppp) Pd (µ-L)₂ CoCl₂(H₂O)₂] (21)
From Na₂PdCl₄ (103 mg) and complex 6 (346 mg). Yield: (294 mg, 72%) as a reddish orange. M.p.: 198 °C dec. Anal.(% calcd. for C₄₇H₄₂Cl₂N₆P₂Pd₂S₄ (1164.94): C, 48.41; H, 3.61; N, 7.11. Found: C, 48.59; H, 3.82; N, 7.11. IR (ν, cm⁻¹): 3410 (NH); 3078 (CH arom); 2900 (CH aliph); 1562 (C=N); 1030 (C=S); 1072 (C-P); 451 (Pd-P); 363 (Pd-S); 513 (Pd-N); 241 (Pd-Cl). 1H NMR ([D₆] DMSO ppm) δ: 10.52 (s, 2H, NH), 8.14-7.00 (m, 34H, H arom + 4-Hthiazolee + 5-Hthiazolee), 2.82, 2.68 (2xq, 4H, dppp), 1.48 (bs, 2H, dppp). 31P NMR ([D₆] DMSO) δₚ: 12.53 (s, P-Pd) S-bonded isomer. UV-Vis (ν', cm⁻¹): 22727 (1B₁g→1Eg), 27473 (1A₁g→1B₁g), 28329 (C.T.) and 30581 (C.T.).

Preparation of [(dppp) Pd (µ-L)₂ CuCl₂(H₂O)₂] (20)
From CuCl₂H₂O (60 mg) and complex 6 (348 mg). Yield: (324 mg, 80%) as a deep green solid. M.p.: 148–150 °C. Anal.(%) calcd. for C₄₇H₄₂Cl₂N₆P₂Pd₂S₄ (1164.94): C, 49.08; H, 3.97; N, 7.25. Found: C, 48.97; H, 4.02; N, 7.42. IR (ν, cm⁻¹): 3450 (OH); 3350 (NH); 3120, 3055 (C-H arom); 2952 (C-H aliph); 1543 (C=N); 1026 (C=S); 1069 (C-P); 430 (Pd-P); 359 (Pd-S); 511 (Pd-N); 235 (Cu-Cl). 1H NMR ([D₆] DMSO ppm) δ: 10.22 (s, 2H, NH), 7.88–6.11 (m, 34H, H arom + 4-Hthiazolee + 5-Hthiazolee), 2.82, 2.68 (2xq, 4H, dppp), 1.77 (bs, 2H, dppp). UV-Vis (ν', cm⁻¹): 22727 (6B₁g→6Eg), 27473 (6A₁g→6B₁g), 28329 (C.T.) and 30581 (C.T.).

Preparation of [(dppp) Pd (µ-L)₂ CuCl₂(H₂O)₂] (21)
From CuCl₂H₂O (83 mg) and complex 6 (346 mg). Yield: (283 mg, 70%) as a deep green solid. M.p.: 169 °C, dec. Anal.(%) calcd. for C₄₇H₄₆Cl₂CuN₆O₂P₂PdS₄ (1158.07): C, 48.70; H, 3.89; N, 7.25. Found: C, 48.53; H, 3.89; N, 7.17. IR (ν, cm⁻¹): 3492–3500 (OH); 3350 (NH); 3055 (C-H arom); 2952 (C-H aliph); 1543 (C=N); 1026 (C=S); 1069 (C-P); 430 (Pd-P); 359 (Pd-S); 511 (Pd-N); 235 (Cu-Cl). 1H NMR ([D₆] DMSO ppm) δ: 10.62 (s, 2H, NH), 8.14-7.00 (m, 34H, H arom + 4-Hthiazolee + 5-Hthiazolee), 2.82, 2.68 (2xq, 4H, dppp), 1.77 (bs, 2H, dppp). UV-Vis (ν', cm⁻¹): 26526 (6A₁g→4T₂g (4D)), 27286 (6A₁g→4T₂g (4D)), and 30959 (C.T.).

In vitro cytotoxicity assessment
The cytotoxic activity of the NCD was evaluated against RMS cell line using the MTT assay [49]. These cells were maintained in Dulbecco’s Modified Eagle Medium (DMEM) supplement with 10% heat inactivated fetal bovine serum (FBS). In order to maintain the cells in an exponential phase cellular suspension, aliquots were replenished with fresh DMEM two or three times per week. Cells were inoculated in 96-well microtiter plate (105 cells/well) for 48 h to allow growth of cell monolayer to the wall of the microtiter plate. The optimization protocol was achieved for the toxicity screening of compounds to the cell monolayer. The tested compounds were freshly resolved in DMSO and diluted in DMEM consequently. The final concentration of the solvent never exceeded than 0.1%. Triplicates were prepared for each individual dose. Monolayer cells were incubated with the target compounds for 24 h, at 37 °C, 5% CO₂ and incubated at 37 °C in a humidified atmosphere. Color intensity was measured in an ELISA reader. The viability and inhibition % of cancer cell line after the specified time were then detected.

Results and Discussion
The ligand N-phenyl-N’-2-thiazolyl thiourea (LH) was prepared by reaction of phenyl isothiocyanate with 2- aminothiazol in 86% yield, following our modified literature method [25]. Sodium salt of deprotonated ligand (LH) was reacted with sodium tetrachloro palladate(II) (Na₂PdCl₄) (2:1 molar ratio) to give PdL₂ in 93% yield. (Scheme1). The 1H and 13C NMR spectroscopy and other physical properties of the ligand (LH) and complex PdL₂ were identical with the authentic samples prepared previously in our laboratory [25]. Treatment of 3 with Ph₂P(CH₂)nPPh₂ (n = 1, 2, or 3) afforded the mixed ligand complexes 4-6 in 65, 61 and 55 % yield, respectively.

The structures of 4-6 was identified using elemental analysis, IR, 31P-{1H} and 1H-{31P} NMR spectra, and also from spectral comparison with the ligand (LH) as well as with complex 3 which have been synthesized previously [25] and other complexes [26,27]. The 31P-{1H} NMR data have been used effectively to identify the produced linkage isomers (Scheme1). The IR spectra of 4-6 showed bands between 1028–1030 cm⁻¹ 1558–1595 cm⁻¹ assigned to ν (C=S) and ν(C=N) of thiazole backbone (L), respectively [27]. The strong bands at the range 322–351 cm-1 were attributed to ν (C=S) and ν(C=N), respectively [28] which confirmed coordination of the complexes26,27. The 31P NMR data have been used effectively to identify the produced linkage isomers (Scheme1). The IR spectra of 4-6 showed bands between 1028–1030 cm⁻¹ 1558–1595 cm⁻¹ assigned to ν (C=S) and ν(C=N) of thiazole backbone (L), respectively [27]. The strong bands at the range 322–351 cm-1 were attributed to ν (C=S) and ν(C=N), respectively [28].
Syntheses of the ligand (LH) and some Pd (II) mononuclear, homo and heterobimetallic mixed ligand complexes 3-21

Scheme 1
atoms. Further, the two bands appeared at the ranges 1070–1074 cm\(^{-1}\), and 428–484 cm\(^{-1}\) were assigned to \(\nu(C-P)\) and \(\nu(Pd-P)\), respectively\(^{29-31}\), which still confirmed the coordination of diphosphine ligands to the Pd(II) ions. The \(^{31}P\{^1H\}\) NMR spectrum of 4 showed a signal at \(\delta_p = 16.93\) ppm. The positive chemical shift value of this signal indicated that the dpdm ligand behaved as a bidentate bridging ligand\(^{32}\), while a single peak denoted to one isomeric form of this complex with indication that the ligands \(\text{L}\) are S-bonded to the Pd(II) ion. The \(^{31}P\{^1H\}\) NMR spectra of 5 and 6 showed two doublets at \(\delta_p = 62.64, 54.29\) ppm (\(\Gamma_J = 40\) Hz) and \(\delta_p = 15.45, 4.18\) ppm (\(\Gamma_J_{P-P} = 140\) Hz). The coupling between the two doublets indicated the presence of a single isomer with two different phosphorus atoms; one was \textit{trans} to sulfur atom while the other was \textit{trans} to nitrogen atom\(^{29,30}\). The \(^1H\{^{31}P\}\) NMR spectra of 4, 5 and 6 showed broad signals at the regions \(\delta = 8.42-6.32\) ppm assigned to the two aromatic protons of the ligands, while the singlet at \(\delta = 10.18\) ppm assigned to the ligand NH. The resonance at \(\delta = 3.60\) ppm was attributed to methylene protons of the dpdm group of 4, while the two triplets at \(\delta = 2.70\) and 2.90 ppm were assigned to two methylene protons of the dppe moiety of 5. The multiplet and quartet at \(\delta = 1.20\) (2H) and 2.90 ppm (4H) were assigned to three methylene protons of the dppe group of complex 6. Elemental analyses, molar conductivity and magnetic susceptibility measurement are additional support for the formation of the complexes 4-6.

Next, the complexes 4-6 were treated with salts of Ni (II), Cu (II), Mn (II), Co (II) and Pd (II) in refluxing EtOH to give the homo- and heterobimetallic complexes 7-21 in 60–81% yield (Scheme 1). Characterization of these complexes was carried out using elemental analysis, molar conductivity, magnetic susceptibility measurements UV-Vis, FT-IR, \(^{31}P\{^1H\}\) NMR and \(^1H\{^{31}P\}\) NMR spectroscopy. The UV-Vis spectrum of complex 3 showed two bands at 30487, and 38461 cm\(^{-1}\) assigned to \(\nu(C-P)\) and \(\nu(Pd-P)\), respectively. In the IR spectra, the \(\nu(C=\text{N})\) of the ligand at 1627 cm\(^{-1}\) is found to be shifted to a lower energies between 32–34 cm\(^{-1}\) in the spectra of the complexes 8, 11, 12 and 21, indicating coordination via the azomethine nitrogen of the thiazole backbone. Major shifts of the \(\nu(C=\text{N})\), around 32–94 cm\(^{-1}\), were observed as well in the IR spectra of 7, 9, 10, 11 and 13-21 indicative of chelation of N atoms of the thiazole ligand to the metal ions. Additionally, the complexes 7-21 (except 9, 14 and 19) exhibited a broad band in the range of 3400–3500 cm\(^{-1}\) assigned to the lattice water, whereas the absence of such IR broad band in Pd(II) complexes 9, 14 and 19 is in agreement with the analytical data, where water molecules do not exist. The position of the \((C=S)\) band in the complexes 3-21 is around 1028 cm\(^{-1}\) which is changed significantly in comparison to the free ligand LH (~1068 cm\(^{-1}\)) indicating coordination of the thioamide group to the metal ions through its S atom. A medium intensity band in the range 1072–1099 cm\(^{-1}\) was attributed to \(\nu(C-P)\), while a strong band at 444–503 cm\(^{-1}\) assigned to \(\nu\) (Pd-P). Furthermore, the IR spectra of 7-21 showed a medium intensity band at around 500–550 cm\(^{-1}\) assigned to \(\nu\) (M-N), while a band in the range 308–385 cm\(^{-1}\) referred to the \(\nu\) (Pd-S) of the prepared complexes. The IR spectra of the new complexes (except 9, 14 and 19) showed strong intensity bands in the range 240–293 cm\(^{-1}\) assigned to \(\nu\) (M-Cl, M = Ni, Mn, Cu, Co) suggesting a \textit{cis} arrangement of the chlorine atoms\(^{32}\). Complexes 9, 14 and 19 exhibited medium intensity bands between 235–293 cm\(^{-1}\) assigned to \(\nu\)(Pd-Cl) in a \textit{cis} arrangement, which are in agreement with the reported values\(^{27,18,33}\). The \(^1H\) NMR spectra of 7-21 showed multiplets in the regions \(\delta = 6.11–8.14\) ppm assigned to the aromatic protons together with 4-H and 5-H of the thiazole rings. The methylene groups of diphenylphosphine residues were fully analyzed (c.f. Experimental section).

The \(^{31}P\{^1H\}\)NMR spectra of 9, 14 and 19 showed a sharp singlet at \(\delta_p = –52.3, 66.6\) and 12.53 ppm respectively, indicated the formation of single isomers where the ligands coordinated to Pd(II) ion through the thioamide and thiazole sulfur atoms (S-bonded isomers), and are in a good agreement with the reported values\(^{34}\).

**Magnetic susceptibility measurements**

The electronic spectral measurements were used for assigning the stereochemistry of metal ions in the complexes based on the positions and number of d-d transition peak. Magnetic susceptibility measurements obtained at room temperature for complexes 7, 8, 10-13, 15-18, 20 and 21 are listed in Table 1, and they were found to be paramagnetic in nature, while, 9, 14 and 19 were diamagnetic. For Ni(II) in complexes 7, 12 and 17, the observed magnetic moment values (\(\mu_{eff} = 3.65, 3.18\) and 3.16 BM) respectively\(^{35}\), which are in well agreement with the expected range for
Ni(II) complexes with octahedral stereochemistries. The observed magnetic moment for Mn(II) in complexes 8, 13, and 18 are 5.67, 5.63 and 6.10 BM respectively, suggested octahedral arrangements around Mn(II) ions in these complexes (theoretical \( \mu_{\text{eff}} = 5.92 \text{ BM} \)). The observed magnetic moment for the Cu (II) in complexes 10, 15 and 20 are 1.93, 2.02 and 2.10 BM, respectively. The observed values (1.73 BM) are slightly higher than the spin-only value due to one unpaired electron and suggesting octahedral geometry. Thus, the present Cu(II) complex is devoid of any spin interaction with distorted octahedral geometry. In the present investigation the observed magnetic moment values of the Co(II) in complexes 11, 16 and 21 are 3.99, 4.04 and 4.51 BM, which indicates octahedral geometry for the Co(II) in these complexes (theoretical \( \mu_{\text{eff}} = 3.88 \text{ BM} \)). Magnetic susceptibility values for the complexes 9, 14 and 19 are zero which suggests a square planar geometry around Pd (II) ions. In conclusion, in all the prepared complexes 3-21, Pd (II) ion has a square planar geometry while the metal ions Ni (II), Mn(II), Co(II) and Cu(II) have octahedral geometries. The complexes 3-21 are considered as non-electrolytes since their molar conductivity values fall in the range 0–2.702 \( \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1} \) in dimethyl formamide (DMF), which are below 30 \( \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1} \) (Table 1).

### Bioactivities

**In vitro inhibition activity of rhabdomyosarcoma (RMS)**

Rhabdomyosarcoma (RMS) is a malignancy that arises from skeletal muscle that have failed to fully differentiate. It is the most common type of soft tissue sarcoma in children and adolescents, less than 20 years old. Rhabdomyosarcomas are highly chemosensitive, with approximately 80% of cases responding to chemotherapy and the combination of anticancer drugs: vincristin, actinomycin D and cyclophosphamide (VAC) remained the standard chemotherapy in North America for nonmetastatic RMS. Few laboratories have reported the synthesis of various analogs aiming to evaluate their cytotoxicity against Rhabdomyosarcoma cell line. Al-Asady et al. have reported the cytotoxicity and cytogenetic effects of crude extract of Nicotiana on RMS and LB20B cell lines at different concentrations. Some synthesized complexes been selected to evaluate their inhibitory activity against rhabdomyosarcoma (RMS) cell line at different concentrations (500, 250, 125 \( \mu \text{g/mL} \)) for 48 h, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method. The cytotoxicity of these complexes are devoid of anticancer activity since their (% of Table 2 — Magnetic susceptibility and molar conductivity of the complexes 3-21 in DMF

<table>
<thead>
<tr>
<th>Compd.</th>
<th>( \Lambda m \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1} )</th>
<th>( \mu_{\text{eff}} \text{ (B.M)} ) prac. (theor.)</th>
<th>Compd.</th>
<th>( \Lambda m \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1} )</th>
<th>( \mu_{\text{eff}} \text{ (B.M)} ) prac. (theor.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>27.02</td>
<td>5.63(5.92)</td>
<td>13</td>
<td>27.02</td>
<td>5.63(5.92)</td>
</tr>
<tr>
<td>4</td>
<td>9.98</td>
<td>0</td>
<td>14</td>
<td>9.98</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>23.90</td>
<td>2.02(1.73)</td>
<td>15</td>
<td>23.90</td>
<td>2.02(1.73)</td>
</tr>
<tr>
<td>6</td>
<td>8.45</td>
<td>4.04(3.88)</td>
<td>16</td>
<td>8.45</td>
<td>4.04(3.88)</td>
</tr>
<tr>
<td>7</td>
<td>3.07</td>
<td>3.65</td>
<td>17</td>
<td>2.70</td>
<td>3.16(2.83)</td>
</tr>
<tr>
<td>8</td>
<td>26.97</td>
<td>5.67(5.92)</td>
<td>18</td>
<td>26.98</td>
<td>6.10(5.92)</td>
</tr>
<tr>
<td>9</td>
<td>9.23</td>
<td>0</td>
<td>19</td>
<td>9.49</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>22.06</td>
<td>1.93(1.73)</td>
<td>20</td>
<td>25.1</td>
<td>2.10(1.73)</td>
</tr>
<tr>
<td>11</td>
<td>8.32</td>
<td>3.99(3.88)</td>
<td>21</td>
<td>8.10</td>
<td>4.51(3.88)</td>
</tr>
<tr>
<td>12</td>
<td>2.80</td>
<td>3.18(2.83)</td>
<td></td>
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</table>

### Table 2 — Cytotoxicity effect of some Pd(II) and metal ions complexes on RMS cells line

<table>
<thead>
<tr>
<th>Compd.</th>
<th>% of RMS growth inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500 µg/mL</td>
</tr>
<tr>
<td>LH</td>
<td>12.0</td>
</tr>
<tr>
<td>3</td>
<td>30.0</td>
</tr>
<tr>
<td>4</td>
<td>32.0</td>
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<tr>
<td>5</td>
<td>18.0</td>
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<td>10</td>
<td>40.0</td>
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<td>13</td>
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<td>15</td>
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<td>16</td>
<td>38.0</td>
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<td>19</td>
<td>25.0</td>
</tr>
<tr>
<td>20</td>
<td>12.0</td>
</tr>
<tr>
<td>21</td>
<td>29.0</td>
</tr>
</tbody>
</table>

![Fig. 2 — The growth inhibition of RMS cancer cell line by some Pd(II) and metal ion complexes determined by MTT test after 48 h at different concentrations.](image-url)
RMS growth inhibition is less than 50%, except Pd(II) complex 19 which exhibited significant inhibition activity for RMS cell line (90%) at 250 µg/mL.

In conclusion, the structure-activity relationship (SAR) suggested that the potency of complex 19 could be attributed to the presence of the two palladium (II) atoms coordinated with the thioamide and thiazole moieties via their sulfur atoms. Therefore, complex 19 can be considered as a promising agent for treatment of human rhabdomyosarcoma (RMS) waiting for further structural modification.

**Antimicrobial investigations**

In this work, N-phenyl-N'-(2-thiazolyl) thiourea (LH) and its complexes were screened for their *In vitro* antimicrobial activity against the two microbial isolates. The antimicrobial activities of the compounds were tested by the agar disc-diffusion method. The starting ligand N-phenyl-N'-(2-thiazolyl) thiourea (LH) as well as its some metal complexes showed no antimicrobial activity. However, complexes 5, 10, 13, 18 and 20 were highly sensitive against *Staphylococcus aureus* bacterium while the same complexes showed resistance against *Escherichia coli* bacterium. On the other hand, complexes 3, 8 and 9 showed resistance against both bacteria.

Generally, from antibacterial data of these complexes, we have observed that the synthesized complexes are sensitive or resistant due to the lipophilic character of the metal ion in the complexes which can be increased or decreased upon chelation with the free ligands and make the bacterial membrane to be permeable or non-permeable respectively for these complexes through the lipid layer of the bacteria organisms. In addition, stereochemistry of these complexes plays important role in the antimicrobial activity which could improve their binding with amino acid of *S. aureus*.

Factors such as solubility, conductivity, electron density, the molecular size and stereochemistry of the complexes, permeability of organism membrane and the concentration have influence on the activity progress of the synthesized complexes.

**Molecular modeling analysis**

Seitz *et al.*, reported that inhibition of glutathione-S-transferase (GST) would lead for a treatment strategy for multidrug resistance in childhood rhabdomyosarcoma (RMS), since the highest induction of GST activity was found in embryonal RMS (up to 12-fold). In accordance to Seitz and co-workers, we decided to study the modeling analysis of one of the GST protein codes with the most active inhibitors of RMS cell lines (compound 19). Our molecular docking analysis is based on the modeling studies to understand the binding mode of this analogue with the GST binding pockets.

Glutathione S-transferases (GSTs) comprise a family of detoxification enzymes that catalyze the conjugation of glutathione with carcinogens, drugs, toxins and products of oxidative stress. It is believed that the function of these enzymes is to reduce the incidence of deleterious interactions between reactive toxic species and cellular components. Glutathione S-transferases have been implicated in the development of resistance to cancer chemotherapeutic agents. High levels of GST expression have been reported in a number of tumors compared to normal tissues. GST has many PDB codes like: 5YVN, 6ATO, 6EZY, 6AP9, 6ATP. The binding energy score (kcal/mol) and root mean square deviation (RMSD) are important parameters in prediction of the selective and potency profiles of the ligand to bind the active site of GST pocket. Generally, RMSD values representation mainly for analysing the stability of protein and predicting conformational changes of protein RMSD values depends upon the binding interaction and energy between protein and ligand. The optimized protein has lowest RMSD values (ideally less than 1.5 Å, or even better, less than 1 Å). In this case a low RMSD with respect to the true binding pose is good. This represents good reproduction of the correct pose. GST with (PDB ID 5YVN) has a lowest binding energy score (−7.589 kcal/mol) in comparison to the other codes (as shows in Table 3), with acceptable RMSD value (1.296 Å), which is under the expected range of < 2 Å therefore PBD ID 5YVN of GST has been selected for our molecular docking study.

The molecular docking was performed using the Molecular Operating Environment 2016 (MOE 2016)

<table>
<thead>
<tr>
<th>Protein</th>
<th>S</th>
<th>RMSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>5YVN</td>
<td>−7.589</td>
<td>1.296</td>
</tr>
<tr>
<td>6ATO</td>
<td>−6.923</td>
<td>1.246</td>
</tr>
<tr>
<td>6EZY</td>
<td>−5.765</td>
<td>2.517</td>
</tr>
<tr>
<td>6AP9</td>
<td>−6.538</td>
<td>1.951</td>
</tr>
<tr>
<td>6ATP</td>
<td>−6.534</td>
<td>1.519</td>
</tr>
</tbody>
</table>

Table 3 — The S and RMSD values of GTS PBSs docking with 19
software and the docking results were also shown by MOE53. Compound 19 has been selected to show its binding to the enzyme pocket (Fig. 3). As shown in Fig. 3a, the aromatic ring of thiourea group of 19 fitted into an arene-rich subpocket surrounded by the aromatic side chain of Phe31. Detailed analysis of the binding mode showed that the aromatic rings point toward the aromatic ring Phe31 residue apparently developing π-π stacking interaction with 19 phenyl group. The thiazole backbones as well as the triphenylphosphine groups are located in the middle of the binding pocket, anchoring the N-phenylthiourea substituent at thiazole moiety in a favorable position for hydrophobic interactions with other substituents of 19. Overall, the combination of hydrophobic interaction and π-π stacking appears to govern the binding of 19 with GST amino acid residues. In conclusion, the presence of two Pd(II) atoms and bis(diphenylphosphino) propane, dppp gives flexibility to molecule to locate properly in the active site of the amino acids pocket of GST (PBD ID 5YVN). The 3D interaction of 19 with amino acid residue (Phe31) of GST is shown in Fig. 3b.

Conclusions
A new series of the Pd(II) complexes with some transition metals containing the ligands N-phenyl-N’-(2-thiazolyl)thiourea and diphenylphosphines have been prepared 4-21. In the complex 4, dppm behaved as a bridged ligand while in other complexes its behaved as a chelate ligand but the dppe and dppp behaved as chelate ligands in their complexes.

Some new complexes were assayed for their inhibition activity against human rhabdomyosarcoma (RMS) cell line and found that 19 exhibited a significant cytotoxicity inhibition activity ~90% for RMS cell line, suggesting to be a new lead in the development of human muscle anticancer agent. All complexes have been assayed for their antibacterial activity and complexes 5, 10, 13, 18 and 20 exhibited a highly sensitive inhibition activity against S. aureus bacterium while revealed a resistance against E. coli bacterium. The other complexes showed no activity. The Modeling calculations of 19 have given significant information for improving the biological activity of the new synthesized complexes. The docking study of 19 showed a pi-pi interaction with Phe31 of GST.

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References