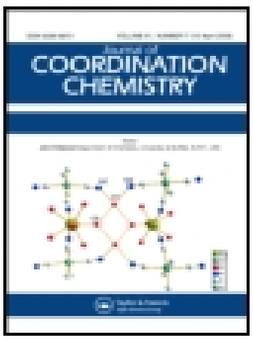

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Mononuclear copper(I) complexes with triphenylphosphine and *N,N′*-disubstituted thioureas: Synthesis, characterization and biological evaluation

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Twelve new complexes, of the general formula $CuCl(TPP)_2Tu^{1\text{-}12}$ (Tu = thiourea), were synthesized by the reaction of $CuCl(TPP)_3$ (TPP = triphenylphosphine) and various *N,N′*-disubstituted thioureas. The structures of the synthesized complexes were characterized by different techniques such as Fourier transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy (^{1}H, ^{13}C, ^{31}P and ^{19}F) and the representative complexes (**1**, **2** and **12**) were analyzed *via* single crystal X-ray diffraction. The single crystal X-ray analysis revealed that copper(I) is coordinated with chlorine, two TPP and the thiourea ligands through the sulfur atom in a mononuclear distorted tetrahedral mode. The compounds were tested for antibacterial, antifungal, cytotoxicity, antileishmanial and antioxidant activities. The results showed that the synthesized complexes are significantly more active than the free ligands and the commercial reference compounds. The high biological activities of the complexes versus free ligands can be attributed to the copper(I) chloride complexation with thiourea ligands. The synthesized complexes were also evaluated, both experimentally and theoretically, for DNA binding studies. The UV-visible spectroscopic and molecular docking studies demonstrated that the complexes are conjugating with DNA through a groove binding mode.

Keywords: Complexes; Fourier transform infrared; Biological activities; Antibacterial; DNA-Binding

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1. Introduction

Medicinal inorganic chemistry offers a wide range of opportunities for the design of therapeutic agents which are not accessible to organic compounds [1-3]. Some properties of the cationic metal ion and the ligand such as wide range of coordination numbers, geometries, various oxidation states, thermodynamic and kinetic properties, and intrinsic properties of the metal and ligand attract the medicinal chemist to exploit their reactivity for the development of new drugs. The success of cisplatin in the clinical treatment of various types of neoplasias has placed coordination chemistry in the frontline in the fight against cancer due to metal-based drugs [1, 2]. Treatment of cancer and other infectious diseases faces serious difficulties due to the development of resistance to present anticancer/antibiotic drugs. There are many anticancer and antibiotic agents having thiourea functionality of natural and synthetic origin. Polyfunctional thioureas are physiologically active substances possessing a wide range of activities such as herbicides [3], insecticides [4], plant-growth regulators [5, 6], antihypertensive agents [7], antitubercular, antimalarial, anticancer [8], antithyroid, antihelmintic and TRPV1 receptor antagonists [11-13]. The copper complexes, due to their unusual structural features, have attained considerable importance due to their application in luminescence, solar cells, supramolecular devices, biological activities and catalytic properties [14-17].

DNA is the pharmacological target for many drugs that are in clinical use or in advanced clinical trials. Small ligand molecules interact with the DNA and alter its function. These small molecules act as drugs when DNA alteration is required to cure or control the disease [18]. Since 1969 it has been found that copper has a high affinity for DNA binding [19] analogous to the phenomenon illustrated for cisplatin [20]. The crystal structure of $CuCl_2$ and DNA was published for the first time in 1991 in which it was found that copper is bound to a guanine N7 residue. This binding is dependent on the electron affinity, complex size and geometry of the adduct formed. It induces irreversible modifications in the DNA conformational structure. Due to these observations, large numbers of copper complexes for targeting DNA were tested and still progress is going on. Furthermore, it was found that the copper complexes interacted non-covalently with DNA rather than coordinating through covalent adduct formation with DNA. The copper complexes interact non-covalently with DNA through electrostatic, intercalative and groove binding, including the major or minor groove [21]. Moreover, it was found that physiochemical features such as planarity, hydrophobicity, size of the ligand, nature of co-ligand

and the geometry of metal complex all play vital roles in determining the binding/intercalating power with DNA.

A series of copper(I) complexes with *N,N'*-disubstituted thiourea ligands have been screened for *in vitro* cytotoxic activity in several human cancer cell lines (A498, EVSAT, H226, IGROV, M19, MCF-7 and WiDr), showing a moderate cytotoxicity which is comparable to that of cisplatin and etoposide [22]. The *N*-(alkyl/aryl)-*N'*-acylthiourea and the *N*-di(alkyl/aryl)-*N'*-acylthiourea act as the versatile ligands and they can coordinate through the S, N and oxygen atom [23, 24]. The thiourea can exhibit monodentate behavior with various transition metals ions such as Ag(I) [25], Au(I) [26], Pt(II) [27], Hg(II) [28] and Cu(I) [29]. Triphenylphosphine is widely used in the synthesis of complexes due to its reducing properties and nucleophilic character. In the present work we have used triphenylphosphine as supporting ligand along with thiourea as the main ligand.

In this present work we have synthesized a series of complexes with CuCl using triphenylphosphine as co-ligand and various disubstituted thioureas of the general formula ArNHCSNHCOPh, shown in figure 1. The synthesized complexes were characterized by spectroscopic techniques and elemental analyses. Complexes **1**, **2** and **12** were also analyzed by X-ray crystallography. After successful characterization, the complexes were tested for DNA-binding and selected complexes for different biological activities such as cytotoxicity, antioxidant and antimicrobial behavior. All the selected complexes have demonstrated significant activities.

2. Experimental

2.1. *Chemicals*

All chemicals, reagents and organic solvents were purchased from Sigma-Aldrich, Fluka and E. Merck. CuCl, organic acids, and triphenylphosphine (TPP) were used as received without purification. The organic solvents acetone, dichloromethane, chloroform, alcohols, petroleum ether and *n*-hexane were distilled, purified and dried according to reported methods [30], saturated with nitrogen, stored over molecular sieves 4 Å and degassed before use.

2.2. *Instrumentation*

Melting points were determined using a melting point apparatus, model Bio Cote SMP10- UK; all the values are uncorrected. NMR spectra were recorded on Bruker AV-300 MHz and Bruker AV-400 MHz spectrometers using deuterated solvents. ^{1}H NMR (300 MHz): internal standard solvent $CDCl_3$ {7.28 ppm from TMS ($Si(CH_3)_4$)}; ^{13}C NMR (75.47 MHz): internal standard solvent $CDCl_3$ (77.0 ppm from TMS). The ^{31}P chemical shift was measured relative to 83% orthophosphoric acid (H_3PO_4) in water at 121.49 MHz (operating frequency). The ^{19}F chemical shift was measured at 282.23 MHz (operating frequency). The splitting of proton resonance in the ^{1}H NMR spectra are defined as s = singlet, d = doublet, t = triplet and m = multiplet. Infrared (FT-IR) absorption measurements were carried out on an Alpha Bruker Model *10018394* in the range of 4000-400 cm^{-1} using attenuated total reflection (*ATR*). Elemental analyses were performed on a *Fisons EA1108* CHNS analyzer while the metal (copper) contents were determined using an atomic absorption spectrophotometer (AAS), Perkin Elmer A800. Single crystal analysis of the synthesized thiourea phosphine copper(I) halide complexes were conducted on Bruker Smart APEX diffractometers. A suitable crystal was selected and kept at 100 K during data collection. The structures were solved and refined using Olex2 software.

2.3. *Synthesis*

2.3.1. Synthesis and characterization of chlorotris[triphenylphosphinecopper(I)] [$Cu(TPP)_3$]Cl precursor. All the reactions were carried out in inert atmosphere. The synthesis of the precursor [$Cu(TPP)_3$]Cl according to the procedure is given below. The triphenylphosphine (TPP) was dissolved in acetonitrile and was added to the suspension of copper(I) chloride in 3:1 giving a white solid of the precursor formed after a few minutes (scheme 1 equation 1). The resulting suspension was stirred for at least 1 h. The solution was filtered and the solid was washed with ethanol several times. The white solid was dissolved in dichloromethane (DCM) and filtered to ensure the removal of insoluble salts. The resulting solution was kept for recrystallization and transparent crystalline solids formed after 24 h with very high yield. The melting point was determined and compared with the literature value. The melting point of the resulting precursor was found to be 176 °C, which falls in the melting point range given in the literature (175 °C).

The precursor was insoluble in water and the yield was 77%; molecular weight = 885.87; FT-IR (ATR, υ in cm^{-1}): 3048 aromatic (C-H), 1584 (aromatic C=C), 1431, 1090, 740 (TPP); ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 7.16-7.36 (m, 45H, Ar-*H*), ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 128.52 (d, ^{3}J=8.66 Hz, P-C coupling, Ar-C), 129.49, 133.45 (d, ^{1}J=20.36 Hz, P-C coupling, Ar-C), 133.87 (d, ^{2}J=15.95 Hz, P-C coupling, Ar-C); ^{31}P NMR (121.49 MHz, 83% aqueous H_3PO_4, δ in ppm): -3.53(s), elemental analyses for $C_{55}H_{45}ClCuP_3$ (885.87): C, 73.22; H, 5.12; Cu, 7.17. Found: C, 73.02; H, 5.06; Cu, 7.01%.

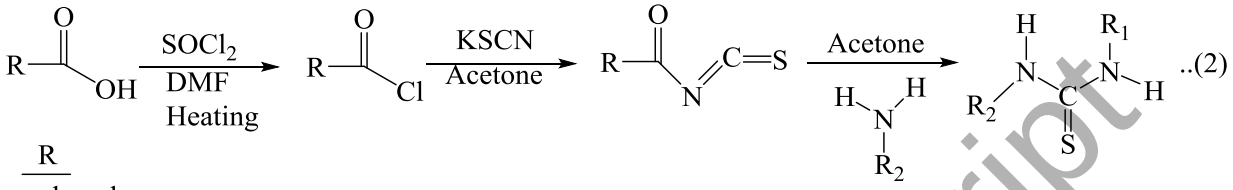
2.3.2. Synthesis and characterization of N,N'-disubstituted thioureas. The general synthetic route for the synthesis of ligands **1-12** is shown in scheme 1, while the proposed chemical structures are shown in figure 1. The ligands 1-benzoyl-3-(3-trifluoromethyl)phenyl)thiourea (Tu^1), 1-benzoyl-3-(2-methoxyphenyl)thiourea (Tu^2), 1-benzoyl-3-(2,3,5,6-tetrachlorophenyl)thiourea (Tu^3), 1-benzoyl-3-(2-trifluoromethyl)phenyl)thiourea (Tu^4), 1-benzoyl-3-(3,5-dichlorophenyl)thiourea (Tu^5), 1-benzoyl-3-(2,4,6-trichlorophenyl)thiourea (Tu^6), 1-benzoyl-3-cyclohexylthiourea (Tu^7), 1-(benzo[d]thiazol-2-yl)-3-benzoylthiourea (Tu^8), 1-(2-chlorobenzoyl)-3-phenylthiourea (Tu^9), 1-(2-chlorobenzoyl)-3-(2-chlorophenyl)thiourea (Tu^{10}), 1-(2-chlorobenzoyl)-3-(2,3-dichlorophenyl)thiourea (Tu^{11}) and 1-(2-chlorobenzoyl)-3-(3-(trifluoromethyl)phenyl)thiourea (Tu^{12}) are shown in figure 1. These ligands were synthesized according to our previous reported work, scheme 1 and equation 2 [31]. The general synthetic scheme used for the complexes is given in scheme 1 equation 3. All the ligands and complexes were insoluble in aqueous media.

2.3.3. Synthesis and characterization of $[CuCl(TPP)_2Tu^{1-12}]$ complexes. To a 100 mL double necked round bottomed flask containing the appropriate thiourea ligand (1 mmol) dissolved in 20 mL of methanol, was added the salt $[Cu(TPP)_3]Cl$ (1 mmol) dissolved in chloroform. The color of the solution changed to yellow immediately and a solid formed after a few minutes. The suspension was stirred for 1 h. The yellow solid was separated by filtration and washed twice with methanol. The product was dissolved in chloroform and filtered, from which 5 ml solution was separated to stand at 25 °C in which prism-like light yellow crystals of complexes **1**, **2** and **12** were grown.

Synthesis of Precursor $[Cu(TPP)_3]Cl$

$$CuCl + 3PPh_3\ (TPP) \xrightarrow[\text{Stirring for 1 hour}]{\text{Acetonitrile}} [Cu(TPP)_3]Cl \quad .....(1)$$

Synthesis of *N,N′*-Disubstituted Thioureas Ligands (Tu^{1-12})



..(2)

R

-phenyl

-(2-chlorophenyl)

For ligand Tu^1: $R_1 = C_6H_5$-CO- and $R_2 = 3$-(CF_3)-C_6H_4-

For ligand Tu^2: $R_1 = C_6H_5$-CO- and $R_2 = 2$-CH_3O-C_6H_4-

For ligand Tu^3: $R_1 = C_6H_5$-CO- and $R_2 = 2,3,5,6$-Cl_4-C_6H-

For ligand Tu^4: $R_1 = C_6H_5$-CO- and $R_2 = 2$-(CF_3)-C_6H_4-

For ligand Tu^5: $R_1 = C_6H_5$-CO- and $R_2 = 3,5$-Cl_2-C_6H_3-

For ligand Tu^6: $R_1 = C_6H_5$-CO- and $R_2 = 2,4,6$-Cl_3-C_6H_2-

For ligand Tu^7: $R_1 = C_6H_5$-CO- and $R_2 = C_6H_{11}$-

For ligand Tu^8: $R_1 = C_6H_5$-CO- and $R_2 = C_7H_4NS$-(benzo[d]thiazol-2-yl)

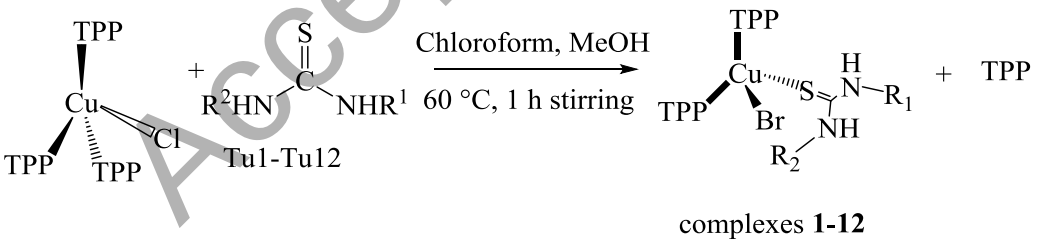
For ligand Tu^9: $R_1 = 2$-Cl-C_6H_4-CO- and $R_2 = C_6H_5$-

For ligand Tu^{10}: $R_1 = 2$-Cl-C_6H_4-CO- and $R_2 = 2$-Cl-C_6H_4-

For ligand Tu^{11}: $R_1 = 2$-Cl-C_6H_4-CO- and $R_2 = 2,3$-Cl_2-C_6H_3-

For ligand Tu^{12}: $R_1 = 2$-Cl-C_6H_4-CO- and $R_2 = 3$-(CF_3)-C_6H_4-

Synthesis of Cu(I) Complexes with *N,N′*-Disubstituted Thioureas (Tu^{1-12})



.....(3)

complexes **1-12**

Scheme 1. General synthetic route for the synthesis of $[Cu(TPP)_3]Cl$, *N,N′*-disubstituted thioureas ligands (Tu^{1-12}) and their Cu(I) complexes.

2.3.3.1. Synthesis of the complex chloro(1-benzoyl-3-(3-trifluoromethyl)phenyl)thiourea) bis(triphenylphosphine)copper(I) (**1**). Quantities used were 0.075 g of the ligand (Tu^1) (0.22 mmol) and 0.20 g (0.22 mmol) of chlorotris(triphenylphosphine)copper(I). Physical state: light yellow solid; Yield: 0.149 g (78%); M.P: 145 °C; FT-IR (ATR, υ in cm^{-1}): 3229 (N-H), 3110 (C-H), 1666.7 (C=O), 1528 and 1478 (aromatic C=C), 1454 (C-F), 1433 (C-P), 1266 (C=S), 1091 (C-N), 742 (TPP); ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 7.14-7.78 (m, 37 H, Ar-H), 8.19-8.21 (d, ^1J=7.41 Hz, 2H, Ar-H) 10.98 (s, 1H, NH protons), 11.57 (s, 1H, NH proton); ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 121.52, 123.35, 127.88, 128.31-128.42 (d, ^3J=8.38 Hz, due to splitting of P-C coupling, Ar-C), 128.56, 128.72, 129.30, 131.42-131.94 (t, ^4J=39.02 Hz, due to splitting of F-C coupling, Ar-C), 132.02-132.15 (d, ^2J=9.75 Hz, due to splitting of P-C coupling, Ar-C), 133.24, 133.2, 133.72-133.92 (d, ^1J=15 Hz, due to splitting of P-C coupling, Ar-C), 134.03, 134.29, 168.9, 184.8; ^{31}P NMR (121.49 MHz, 83% aqueous H_3PO_4, δ in ppm): (s) -3.07; ^{19}F NMR (282.23 MHz, $CFCl_3$, δ in ppm): (s) -63.99, Anal. Calcd for $C_{51}H_{41}ClCuF_3N_2OP_2S$ (947.89): C, 64.62; H, 4.36; N, 2.96; S, 3.38; Cu, 6.70. Found: C, 64.11; H, 4.06; N, 2.16; S, 3.11; Cu, 6.12%.

2.3.3.2. Synthesis of the complex chloro(1-benzoyl-3-(2-methoxyphenyl)thiourea) bis(triphenylphosphine)copper(I) (**2**). Quantities used were 0.0646 (0.22 mmol) of the ligand (Tu^2) and 0.20 g (0.22 mmol) of chlorotris(triphenylphosphine)copper(I). Physical state: light yellow solid; Yield 0.168 g (82%); M.P.: 180 °C; FT-IR (ATR, υ in cm^{-1}):3226 (N-H), 3112 (C-H), 1667(C=O), 1528 (C=C), 1434 (C-P), 1260 (C=S), 1093 (C-N), 743 (TPP); ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 3.89 (s, due to CH_3 protons), 6.88-7.62 (m, 36H, Ar-H), 8.10-8.20 (d, ^1J=8.13 Hz, 3H, Ar-H), 11.30 (s, NH proton), 11.87 (s, NH proton), ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 55.90 (s, OCH_3 carbon), 110.79, 120.00, 124.61, 126.57-127.21 (d, ^1J=48.41 Hz, due to splitting of P-C coupling, Ar-C), 128.32-128.44 (d, ^3J=9.04 Hz, due to splitting of P-C coupling, Ar-C), 129.08, 129.43, 131.63, 133.30, 133.45, 133.79, 133.85-134.04 (d, ^2J=14.80 Hz, due to splitting of P-C coupling, Ar-C), 151.47, 168.78, 177.59; ^{31}P NMR (121.49 MHz, 83% aqueous H_3PO_4, δ in ppm): (s) -3.60, Anal. Calcd for $C_{51}H_{44}ClCuN_2O_2P_2S$ (909.92): C, 67.32, H, 4.87; N, 3.08; S, 3.52; Cu, 6.98. Found: C, 66.98; H, 4.01; N, 2.91; S, 2.98; Cu, 6.04%.

2.3.3.3. Synthesis of the complex chloro(1-benzoyl-3-(2,3,5,6-tetrachlorophenyl)thiourea) bis(triphenylphosphine)copper(I) (**3**). Quantities used were 0.0895 g (0.22 mmol) of the ligand (Tu^3) and 0.20 g (0.22 mmol) of chlorotris(triphenylphosphine)copper(I). Physical state: light yellow solid; Yield 0.181 g (79%); M.P.: 200 °C; FT-IR (ATR, υ in cm^{-1}): 3229 (N-H), 3114 (C-H), 1664.8 (C=O), 1526 (C=C), 1433 (C-P), 1265 (C=S), 1092 (C-N), 741.9 (TPP), 692 (C-Cl); ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 7.11-7.66 (m, 34H, Ar-H), 8.16-8.32 (d, ^1J=8.29 Hz, 2H, Ar-H), 12.05 (s, NH proton), 12.62 (s, NH proton; ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 128.31-128.43 (d, ^3J=9.00 Hz, due to splitting of P-C coupling, Ar-C), 128.55, 129.47, 130.47, 130.97, 132.02, 132.10, 133.28-133.62 (d, ^1J=24.00 Hz, due to splitting of P-C coupling, Ar-C), 133.82-134.02 (d, ^2J=15 Hz, due to splitting of P-C coupling, Ar-C), 135.61, 169.76, 181.12; ^{31}P NMR (121.49 MHz, 83% aqueous H_3PO_4, δ in ppm): (s) -3.51, Anal. Calcd for $C_{50}H_{38}Cl_5CuN_2OP_2S$ (1017.67): C, 59.01; H, 3.76; N, 2.75; S, 3.15; Cu, 6.24. Found: C, 58.76; H, 3.08; N, 2.12; S, 2.79; Cu, 6.02%.

2.3.3.4. Synthesis of the complex chloro(1-benzoyl-3-(2-trifluoromethyl)phenyl)thiourea) bis(triphenylphosphine)copper(I) (**4**). Quantities used were 0.091 (0.28 mmol) of the ligand (Tu^4) and 0.25 g (0.28 mmol) of chlorotris(triphenylphosphine)copper(I). Physical state: light yellow solid; Yield 0.164 g (77%); M.P.: 165 °C; FT-IR (ATR, υ in cm^{-1}): 3223 (N-H), 3116 (C-H), 1669.8 (C=O), 1523.6 (C=C), 1433 (C-P), 1320 (C-F, 1262 (C=S), 1092(C-N), 741(TPP); ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 6.99-8.25 (m, 37H, Ar-H) 8.16-8.19 (d, ^1J=7.26 Hz, 2H, Ar-H), 11.88 (s, due to NH proton); ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 126.27, 127.42, 128.36-128.42 (d, ^2J=8.63 Hz, due to splitting of P-C coupling, Ar-C), 129.43, 130.20, 131.17-131.94 (d, ^1J=58.39 Hz, due to splitting of P-C coupling, Ar-C), 132.04, 132.17, 133.60, 133.82-134.02 (d, ^3J=15.02 Hz, due to splitting of P-C coupling, Ar-C), 135.20, 169.57, 181.02; ^{31}P NMR (121.49 MHz, 83% aqueous H_3PO_4, δ in ppm): (s) -3.75; ^{19}F NMR (282.23 MHz, $CFCl_3$, δ in ppm): (s) -62.61, Anal. Calcd for $C_{51}H_{41}ClCuF_3N_2OP_2S$ (947.89): C, 64.62; H, 4.36; N, 2.96; S, 3.38; Cu, 6.70. Found: C, 63.99; H, 4.09; N, 2.49; S, 3.19; Cu, 6.22%.

2.3.3.5. Synthesis of the complex chloro(1-benzoyl-3-(3,5-dichlorophenyl)thiourea) bis(triphenylphosphine)copper(I) (**5**). Quantities used were 0.0734 (0.22 mmol) of the ligand (Tu^5) and 0.20 g (0.22 mmol) of chlorotris(triphenylphosphine)copper(I). Physical state: light

yellow solid; Yield 0.171 g (80%), M.P.: 165 °C; FT-IR (ATR, υ in cm^{-1}):3221(N-H), 3121(C-H), 1663.6 (C=O), 1522 (C=S), 1433 (C-P), 1263 (C=S), 1092 (C-N), 741.5 (TPP), 691.6 (C-Cl); ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 7.00-8.29 (m, 36H, Ar-H), 8.10-8.34 (d, ^{1}J=7.20 Hz, 2H, Ar-H), 11.74 (s, 1H, NH proton), 12.54 (s, 1H, NH proton); ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 123.44, 126.98, 128.36-128.48 (d, ^{2}J=9.00 Hz, due to splitting of P-C coupling, Ar-C), 128.63, 129.27, 129.50, 131.06, 133.33, 133.68, 133.79-133.98 (d, ^{1}J=14.25 Hz, due to splitting of P-C coupling, Ar-C), 134.87, 138.92, 169.57, 179.40; ^{31}P NMR (121.49 MHz, 83% aqueous H_3PO_4, δ in ppm): (s) -3.23, Anal. Calcd for $C_{50}H_{40}Cl_3CuN_2OP_2S$ (948.78): C, 63.30; H, 4.25; N, 2.95; S, 3.38; Cu, 6.70. Found: C, 63.29; H, 4.02; N, 2.39; S, 3.11; Cu, 6.24%.

2.3.3.6. Synthesis of the complex chloro(1-benzoyl-3-(2,4,6-trichlorophenyl)thiourea)bis(triphenylphosphine)copper(I) (**6**). Quantities used were 0.0811 (0.22 mmol) of the ligand (Tu^6) and 0.20 g (0.22 mmol) of chlorotris(triphenylphosphine)copper(I). Physical state: light yellow solid; Yield 0.179 g (81%), M.P.: 180 °C; FT-IR (ATR, υ in cm^{-1}): 3228 (N-H), 3119 (C-H), 1673.7 (C=O), 1519 (C=C), 1434 (C-P), 1254 (C=S), 1070 (C-N), 742.3 (TPP), 694.9 (C-Cl); ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 7.19-7.75 (m, 39H, Ar-H), 11.67 ((s, 1H, NH proton), 12,52 (s, 1H, NH proton); ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 127.73, 128.60-128.73 (d, ^{2}J=9.75 Hz due to splitting of P-C coupling, Ar-C), 129.67, 129.83, 130.02, 131.95, 132.34, 132.76, 133.80-133.87 (d, ^{1}J=12.75 Hz due to splitting of P-C coupling, Ar-C), 134.00, 134.21, 134.81, 168.91, 181.41; ^{31}P NMR (121.49 MHz, 83% aqueous H_3PO_4, δ in ppm): (s) -1.36, Anal. Calcd for $C_{50}H_{39}Cl_4CuN_2OP_2S$: C, 61.08; H, 4.00; N, 2.85; S, 3.26; Cu, 6.46. Found: C, 52.75; H, 3.09; N, 2.26; S, 3.98; Cu, 8.41%.

2.3.3.7. Synthesis of the complex chloro(1-benzoyl-3-cyclohexylthiourea)bis(triphenylphosphine)copper(I) (**7**). Quantities used were 0.059 (0.22 mmol) of the ligand (Tu^7) and 0.20 g (0.22 mmol) of chlorotris(triphenylphosphine)copper(I). Physical state: light yellow solid; Yield 0.148 g (74%), M.P.: 140 °C; FT-IR (ATR, υ in cm^{-1}): 3312 (N-H), 3126 (C-H), 1664 (C=O), 1525.8 (C=C), 1433.6 (C-P), 1262 (C=S), 1093 (C-N), 741 (TPP); ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 1.14-1.83 (m, 10H, cyclohexylproton), 5.24

(m, 1H, cyclohexylproton), 7.15-7.96 (m, 35H, Ar-H), 9.08 (s, 1H, NH proton), 11.35 (s, 1H, NH proton); ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 25.4 (s,C_6H_{11}), 25.7 (s, C_6H_{11}), 32.5 (s, C_6H_{11}), 58.70 (s, C_6H_{11}), 127.52-127.54 (d, ^3J=9.00 Hz, due to splitting of P-C coupling, Ar-C), 128.41-128.66 (d, ^1J=18.00 Hz, due to splitting of P-C coupling, Ar-C), 130.70, 131.10-131.21 (d, ^2J=8.25 Hz due to splitting of P-C coupling, Ar-C), 132.1, 133.2, 134.0, 168.5, 183.1; ^{31}P NMR (121.49 MHz, 83% aqueous H_3PO_4, δ in ppm): (s) -3.47, Anal. Calcd for $C_{50}H_{48}ClCuN_2OP_2S$ (885.94): C, 67.79; H, 5.46; N, 3.16; S, 3.62; Cu, 7.17. Found: C, 67.13; H, 5.17; N, 2.19; S, 3.10; Cu, 7.01%.

2.3.3.8. Synthesis of the complex chloro(1-(benzo[d]thiazol-2-yl)-3-benzoylthiourea)bis(triphenylphosphine)copper(I) (**8**). Quantities used were 0.0707 g (0.22 mmol) of the ligand (Tu^8) and 0.20 g (0.22 mmol) of chlorotris(triphenylphosphine)copper(I). Physical state: light yellow solid; Yield 0.154 g (73%), M.P.: 165 °C; FT-IR (ATR, υ in cm^{-1}): 3227 (N-H), 3137 (C-H), 1666 (C=O), 1525.5 (C=C), 1434 (C-P), 1260 (C=S), 1091 (C-N), 742 (TPP); ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 7.17-8.24 (m, 37H, Ar-H), 8.19-8.26 (d, ^1J=7.53 Hz, 2H, Ar-H), 11.63 (s, 1H, NH proton), 12.08 (s, 1H, NH proton); ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 121.04, 121.72, 124.55, 126.45, 128.42-128.53 (d, ^3J=8.87 Hz, due to splitting of P-C coupling, Ar-C), 128.65, 129.29, 129.60, 130.88, 132.04, 132.25, 133.10-133.45 (d, ^1J=26.10 Hz, due to splitting of P-C coupling, Ar-C), 133.81-134.00 (d, ^2J=14.65 Hz, due to splitting of P-C coupling, Ar-C), 148.42, 158.16, 168.82, 176.39; ^{31}P NMR (121.49 MHz, 83% aqueous H_3PO_4, δ in ppm): (s) -3.05, Anal. Calcd for $C_{51}H_{41}ClCuN_3OP_2S_2$ (936.97): C, 65.38; H, 4.41; N, 4.48; S, 6.84; Cu, 6.78. Found: C, 64.43; H, 3.98; N, 4.19; S, 6.30; Cu, 6.01%.

2.3.3.9. Synthesis of the complex chloro(1-(2-chlorobenzoyl)-3-phenylthiourea) bis(triphenylphosphine)copper(I) (**9**). Quantities used were 0.0065 g (0.22 mmol) of the ligand (Tu^9) and 0.20 g (0.22 mmol) of chlorotris(triphenylphosphine)copper(I). Physical state: light yellow solid; Yield 0.152 g (74%), M.P.: 180 °C; FT-IR (ATR, υ in cm^{-1}): 3240 (N-H), 3138 (C-H), 1669 (C=S), 1592 (C=C), 1433 (C-P), 1253 (C=S), 1070 (C-N), 740.6 (TPP), 690 (C-Cl); ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 7.17-7.60 (m, 37H, Ar-H), 7.65-7.73 (d, ^1J=6.24 Hz, 2H, Ar-H), 12.08 (s, 1H, NH), 12.52 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 125.04, 126.82, 127.17, 128.33-128.45 (d, ^3J=8.94 Hz, due to splitting of P-C coupling, Ar-C), 128.84,

129.48, 130.57-136.94 (d, ^{1}J=240.24 Hz, due to splitting of P-C coupling, Ar-C), 132.10, 132.19, 132.77, 133.38, 133.73, 133.85-134.04,(d, ^{2}J=14.65 Hz, due to splitting of P-C coupling, Ar-C), 136.94, 168.50, 178.74; ^{31}P NMR (121.49 MHz, 83% aqueous H_3PO_4, δ in ppm): (s) -3.48, Anal. Calcd for $C_{50}H_{41}Cl_2CuN_2OP_2S$ (914.34): C, 65.68; H, 4.52; N, 3.06; S, 3.51; Cu, 6.95. Found: C, 65.13; H, 4.01; N, 2.89; S, 3.10; Cu, 6.21%.

2.3.3.10. Synthesis of the complex chloro(1-(2-chlorobenzoyl)-3-(2-chlorophenyl)thiourea)bis(triphenylphosphine)copper(I) (**10**). Quantities used were 0.0734 (0.22 mmol) of the ligand (Tu^{10}) and 0.20 g (0.22 mmol) of chlorotris(triphenylphosphine)copper(I). Physical state: light yellow solid; Yield 0.162 g (76%), M.P.: 190 °C; FT-IR (ATR, υ in cm^{-1}): 3237 (N-H), 3129 (C-H), 1669.9 (C=O), 1528 (C=S), 1433 (C-P), 1250 (C=S), 1091 (C-N), 741.6 (TPP), 691 (C-Cl); ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 7.18-7.93 (m, 36H, Ar-H), 7.87-7.90 (d, ^{1}J=9.60 Hz, 2H, Ar-H), 11.67 (s, NH proton), 12.64 (s, NH proton); ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 126.79, 126.92, 127.14, 128.05, 128.40-128.52 (d, ^{3}J=8.97 Hz, due to splitting of P-C coupling, Ar-C), 128.71, 129.56, 129.73, 130.61, 130.73, 131.95, 132.02, 132.93, 133.16-133.51 (d, ^{1}J=26.60 Hz, due to splitting of P-C coupling, Ar-C), 133.81-134.00 (d, ^{2}J=14.84 Hz, due to splitting of P-C coupling, Ar-C), 134.49, 167.89, 178.94; ^{31}P NMR (121.49 MHz, 83% aqueous H_3PO_4, δ in ppm): (s) -3.46, Anal. Calcd for $C_{50}H_{40}Cl_3CuN_2OP_2S$ (948.78): C, 63.30; H, 4.25; N, 2.95; S, 3.38; Cu, 6.70. Found: C, 65.13; H, 4.01; N, 2.89; S, 3.10; Cu, 6.21%.

2.3.3.11. Synthesis of the complex chloro(1-(2-chlorobenzoyl)-3-(2,3-dichlorophenyl)thiourea)bis(triphenylphosphine)copper(I) (**11**). Quantities used were 0.0811 (0.22 mmol) of the ligand (Tu^{11}) and 0.20 g (0.22 mmol) of chlorotris(triphenylphosphine)copper(I). Physical state: light yellow solid; Yield 0.168 g (71%), M.P: 197 °C; FT-IR (ATR, υ in cm^{-1}): 3218 (N-H), 3048.6 (C-H), 1669.9 (C=O), 1523 (C=C), 1432.6 (C-P), 1265.6 (C=S), 1091.4 (C-N), 748.8 (C-N), 691 (C-Cl); ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 7.13-7.74 (m, 37H, Ar-H), 12.03 (s, NH proton), 12.14 (s, NH proton); ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 125.60, 126.79-126.87 (d, ^{3}J=6.29 Hz, due to splitting of P-C coupling, Ar-C), 127.86, 128.37-128.49 (d, ^{2}J=8.39 Hz, due to splitting of P-C coupling, Ar-C), 128.71, 130.61, 130.76, 131.89, 132.07, 132.02, 132.96, 133.25, 133.46, 133.60, 133.82-134.01

(d, ^{1}J=14.65 Hz, due to splitting of P-C coupling, Ar-C), 136.17, 168.20, 179.30; ^{31}P NMR (121.49 MHz, 83% aqueous H_3PO_4, δ in ppm): (s) -3.42, Anal. Calcd for $C_{50}H_{39}Cl_4CuN_2OP_2S$ (983.23): C, 61.08; H, 4.00; N, 2.85; S, 3.26; Cu, 6.46. Found: C, 60.91; H, 3.90; N, 2.49; S, 3.13; Cu, 6.31%.

2.3.3.12. Synthesis of the complex chloro(1-(2-chlorobenzoyl)-3-(3-(trifluoromethyl)phenyl)thiourea)bis(triphenylphosphine)copper(I) (**12**). Quantities used were 0.0809 (0.22 mmol) of the ligand (Tu^{12}) and 0.20 g (0.22 mmol) of chlorotris(triphenylphosphine)copper(I). Physical state: light yellow solid; Yield 0.157 g (71%), M.P.: 158 °C; FT-IR (ATR, υ in cm^{-1}): 3198 (N-H), 3044.4 (C-H), 1663.7 (C=O), 1531.9 (C=C), 1434 (C-P), 1327 (C-F), 1279.6 (C=S), 1092.6 (C-N), 744 (TPP); ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 7.17-7.76 (m, 38H, Ar-H), 12.34 (s, NH proton), 12.64 (s, NH proton); ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm): 122.03, 123.61, 126.89, 128.20, 128.36-128.48 (d, ^{3}J=8.89 Hz, due to splitting of P-C coupling, Ar-C), 129.35-137.49 (t, ^{1}J=306.72 Hz, due to splitting of F-C coupling), 129.48, 130.65, 130.98, 131.41, 131.41, 132.01, 132.18, 132.92, 133.38, 133.79-133.99 (d, ^{2}J=14.78 Hz, due to splitting of P-C coupling, Ar-C), 137.49, 168.77, 179.07; ^{31}P NMR (121.49 MHz, 83% aqueous H_3PO_4, δ in ppm): (s) -3.35; ^{19}F NMR (282.23 MHz, $CFCl_3$, δ in ppm): (s) -63.98, Anal. Calcd for $C_{51}H_{40}Cl_2CuF_3N_2OP_2S$ (982.34): C, 62.36; H, 4.10; N, 2.85; S, 3.26; Cu, 6.47. Found: C, 62.10; H, 4.01; N, 2.59; S, 3.04; Cu, 6.25%.

2.4. *Biological assays*

2.4.1. Brine shrimp lethality assay. For the selected ligands and their complexes in a 96 well plate the lethality test was performed against brine shrimp (*Artemia salina*) larvae as previously reported [32] with slight changes. For 24-48 h *A.salina* eggs (Ocean star, USA) were incubated under light at 30-32 °C in a specially designed two-compartment plastic tray in simulated sea water (38 g/L supplemented with 6 mg/L dried yeast). With the help of Pasteur pipettes, ten mature phototropic nauplii were harvested and transferred to each well of the plate. A corresponding volume of each extract containing ≤ 1% DMSO in sea water at final concentrations of 500 and 200 µg/mL was transferred to the wells containing sea water and shrimp larvae. The final volume in each well was kept at 300 µL. Positive and negative control wells included serial concentrations of doxorubicin and 1% DMSO, respectively. After 24 h

incubation, live shrimps were counted and percentage of deaths was determined. Median lethal concentration (LC_{50}) was calculated using TableCurve 2D v5.01 software.

2.4.2. Antibacterial assay. Susceptibility of extracts against bacterial species was tested for the selected ligands and their complexes according to a formerly described procedure [33]. Three Gram negative strains (*Bordetella bronchiseptica*, ATCC # 4617, *Salmonella typhimurium*, ATCC # 14028 and *Enterobacter aerogenes*, ATCC # 13048) were tested. The strains were cultured in nutrient broth media and incubated for 24 h at 37 °C. Sterilized deionized water was used to adjust the turbidity to 10^4 CFU/mL by comparing with McFarland 0.5 $BaSO_4$ turbidity standards. The refreshed inoculums (100 µL) were then swabbed onto Petri plates containing 20 mL of nutrient agar. Test samples (5 µL of 20 mg/mL DMSO; 100 µg/disc) were infused on sterile filter paper discs (6 mm) and placed on seeded nutrient agar plates. Cefixime-USP at a concentration of 20 µg/disc and DMSO impregnated discs were included as positive and negative controls, respectively. After 24 h of incubation, clear zones of growth inhibition were measured.

2.4.3. Antifungal assay. Antifungal activity of extracts for selected ligands and their complexes was evaluated following the reported literature standard protocol [33]. The fungal strains (*Aspergillus fumigatus* FCBP# 66, *Fusarium solani* FCBP# 0291 and *Mucor specie* FCBP# 0300) were purchased from the fungal culture bank of Pakistan and cultured on SDA. Prior to the sensitivity determination, the spores were harvested in 0.02% Tween 20 solution and turbidity was adjusted according to the McFarland 0.5 turbidity standard. 100 µL of each harvested spore type was swabbed on plates containing 25 mL sterilized SDA. Filter paper discs loaded with 5 µL of test sample (20 mg/mL DMSO; 100 µg/disc) as well as standard antifungal terbinafine (50 µg/disc) and DMSO were placed on seeded SDA plates. The plates were incubated at 28 °C for 24-48 h. Thereafter, clear zones of inhibition around discs were measured using a Vernier caliper.

2.4.4. Antileishmanial assays. *In vitro* antileishmanial activity for selected ligands and their complexes was performed with *Leishmania tropica* khw23 strain with the previously reported standard protocol [33] with slight modification. The parasite was cultured in M199 media

supplemented with 10% Fetal Bovine Serum at 24 °C. The culture (promastigotes) was ingathered at a concentration of 1×10^6 cells/mL. Stock solutions of each test sample were prepared in DMSO (10 mg/mL) and serially diluted in 96 well plates. The antileishmanial activity of each sample was determined at concentrations ranging from 500, 250, 125 and 31.25 µg/mL. The cultured plates, inoculated with parasite and test samples, were incubated at 24 °C for 72 h. Amphotericin-B was employed as positive control and media as a negative control. Subsequent to the incubation, the live promastigotes were counted under a light microscope using an improved Neubauer chamber. The data obtained was then statistically analyzed and LC_{50} was estimated using TableCurve 2D Ver.4 software.

2.4.5. Antioxidant assays. The antioxidant activity of the phosphine thiourea copper(I) halide complexes was conducted using the following assays.

DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity: Copper(I) complex (400 µg L^{-1}) up to 20 µL (from 400 µg L^{-1}) was taken in 96 well-plates and 180 µL of DPPH from stock was added. Ascorbic acid and DMSO were used as positive and negative control, respectively. The plates were incubated for 1 h. Readings were taken at 515 nm using a microplate ELISA reader [34]. DPPH free radical scavenging activity of the complexes was calculated as a percent (%) of radical inhibition by the following equation:

$$\text{Scavenging (\%)} = [1 - \left(\frac{\text{Sample OD}}{\text{Control OD}}\right)] \times 100$$

Reducing power: The reducing power of the complexes was estimated using vitamin C as standard reference. In the procedure, 40 µL of the sample and 50 µL of phosphate buffer (0.2 $molL^{-1}$; pH 6.6) were taken in a lmate tube followed by the addition of 50 µL of potassium ferricyanide (1%). After incubation, at 50 °C for 20 minutes, 50 µL of trichloroacetic acid (10%) was added to the mixture and it was centrifuged (3000 rpm) for 10 minutes. After layer separation, the upper-layer 166.66 µL was transferred to 96 well microplates and 33.33 µL was added to the isolate. The reducing power of the synthesized complex was calculated as ascorbic acid equivalents; µg of AE per mg of complex.

Total antioxidant capacity: The phosphomolybdenum method was used to estimate the total antioxidant capacity of the synthesized complexes. The synthesized complex sample (20 µL from 4 mgL^{-1}) and ascorbic acid (20 µL), as standard, were taken in separate tubes. This was

followed by the addition of reagent solution (H_2SO_4; 0.6 $molL^{-1}$, Na_3PO_4; 28 $mmolL^{-1}$, $(NH_4)_6Mo_7O_{24}$; 4 $mmolL^{-1}$). The tubes were properly capped and incubated at 95 °C in a thermal block for 90 minutes. After heat incubation, the samples were cooled to room temperature and transferred to 96-well microplates. The assurance of the solution was measured versus blank at 630 nm using a Microplate ELISA reader. The blank was prepared using the same procedures *i.e.* having 200 µL of reagent solution and appropriate volume of the same solvent. Water soluble antioxidant capacities were expressed as equivalents of ascorbic acid for the synthesized complexes. Ascorbic acid equivalents were calculated using a standard graph of ascorbic acid. The experiments were carried out in triplicate and values were expressed as ascorbic acid equivalents (AAE) μgmg^{-1} of complex.

2.4.6. DNA Binding study by UV-Vis spectroscopy. For the DNA binding study, UV-Vis spectroscopy was used to determine the DNA binding constant. For this purpose the spectrum of the free complex without the addition of DNA was taken using the solvent in the reference cell. The spectra were recorded by adding different concentrations of DNA to the solution of the investigated complexes having constant concentration. The concentration and volume of the compound were kept constant during evaluation while the concentration of the DNA was changed [35].

The equilibrium constants (binding constant) were calculated by fitting data in the *Benesi-Hildebrand* equation (2). $A_o/A\text{-}A_o = (\varepsilon_G/\varepsilon_{H\text{-}G}\text{-}\varepsilon_G) + (\varepsilon_G/\ \varepsilon_{H\text{-}G}\text{-}\varepsilon_G)\ (1/\ K[DNA])$ (2), where A_o and A are the absorbance of the free compound and of the compound–DNA complex, and ε_G and $\varepsilon_{H\text{-}G}$ are the molar extinction coefficients of free compound and of the compound–DNA complex, respectively.

2.4.7. Molecular docking method. Molecular Operating Environment (MOE) from Chemical Computing Inc. 2015.08 was used to draw and optimize Cu complexes **1-12** on MOE window using MOE builder. Optimized molecular structures were entered into the MOE database. The X-ray crystallographic structure of the DNA with PDB ID 5JLT was obtained through the protein data bank (PDB) and imported to the MOE window. 5JLT fragment was protonated with their standard geometry using MOPAC 7.0. All water molecules were removed from the complex with 11 base pairs running in 5'-3' direction. The resulting DNA model was subjected

to systematic conformational search analysis at default parameters with RMS gradient of 0.01 kcal mol^{-1} using Site Finder. In order to perform docking simulations, a number of conformational runs were carried out to get a final binding docking pose with the lowest energy as accurate as possible. The best conformation was selected based on energetic ground and the minimum Final Docking Energy (ΔG) [36, 37].

3. Results and discussion

3.1. *Elemental analyses*

For elemental analyses, CHNS analyzer was used to determine the percentages of carbon, hydrogen, nitrogen and sulfur while an atomic absorption spectrophotometer (AAS) was used to find the copper content in the synthesized complexes. The results are given in the Experimental section and have close agreement with those of calculated values that confirmed the complexes formation.

3.2. *FT-IR Results*

The FT-IR analysis of the synthesized complexes was carried out for structural elucidation and functional group identification. All the significant recorded bands of the complexes are listed in the Experimental section. The observed bands in all the complexes are at (ν in cm^{-1}): 3150-3350 (N-H), 2910-3050 (Ph; C-H), 1660-1685 (C=O), 1475-1600 (Ph; C=C), 1433 and 1477 (C-P), 1320 and 1327 (C-F), 1220-1270 (C=S), 1146 (C-O), 1035-1090 (C-N), 690-695 (C-Cl), 320-337 (Cu-S) and 263-297 (Cu-X). The FT-IR results clearly showed the addition of secondary amine to C=N bond resulting in the formation of thiourea [22]. Band shifting for the NH-group (3400 cm^{-1}) and carbonyl C=O (1750 cm^{-1}) to lower wavelength is attributed to hydrogen bonding between H of one NH-group and the O of carbonyl on one side and between H of another NH-group and halogen attached to Cu on another side, as can be seen in the data of single crystal analysis. An IR band for SH (2500-2600 cm^{-1}) has not been observed in the complexes, confirming non-tautomeric behavior of the ligand [22, 38, 39].

Comparison between the FT-IR data of free ligands [40] and complexes have revealed that ligand bonding to metal has not modified NH, CN, CO and CS stretching, probably due to hydrogen bonding. All the FT-IR spectra of the complexes have characteristic bands of phosphine: 1433 cm^{-1}, 1477 cm^{-1}, 1092 cm^{-1} and 741-747 cm^{-1} [29].

3.3. *NMR Results*

NMR spectroscopy was conducted for the structural elucidation of the synthesized copper(I) complexes. The ^{1}H NMR data of the complexes have peaks for the aromatic protons in the range of 7.14-8.20 ppm [41, 42]. The other peaks at around 10 ppm and 12 ppm are assigned to the N-H protons of CONH and CSNH, respectively. Two separate peaks for the NH protons in the ^{1}H NMR spectra confirm that none of the N is bonded with copper metal.

All the ^{13}C NMR signals, aromatic ring, of the synthesized complexes are in close agreement to the reported values [43]. The carbon signals at 160-168 ppm and 173-183 ppm were assigned to the carbon of CONH and CSNH, respectively. The ^{31}P NMR data of the complexes have singlet peaks in the range of -1.36 to -3.75 ppm. The ^{19}F NMR of complexes **1**, **4** and **12** give singlet peaks in the range of -62.61 to -63.99 ppm.

3.4. *Single crystal X-ray diffraction study*

Recrystallizations of copper(I) complexes of thiourea were carried out in chloroform and pure yellow crystals were collected for solid state X-ray studies after 24 h. Suitable crystals of complexes **1**, **2**, and **12** were analyzed on a Bruker Smart diffractometer equipped with an APEX II CCD Detector, an Incoatec IMuS source and a Quazar MX mirror. The crystal-to-detector distance was 4.0 cm, and the data collection was carried out in 512 × 512 pixel mode.

The initial unit cell parameters were determined by a least-squares fit of the angular setting of strong reflections, collected by a 180.0 degree scan in 180 frames over three different parts of the reciprocal space. Suitable crystals of small sizes were selected and mounted on a Bruker Smart APEX diffractometer. The crystals were kept at 100 K during data collection. The Cu Kα radiations with wavelength 1.54178 Å were used. For each reflection, integrated intensity information was obtained by reduction of data frames using APEX2 [44]. Using Olex2 [45], the structure was solved with the SHELXT [46] structure solution program using intrinsic Phasing and refined with the XL [47] refinement package using full matrix least squares minimization on F^2 and using all the unique data [45, 46]. Atoms other than hydrogen were refined with anisotropic thermal parameters and all hydrogens were at calculated positions and were refined isotropically. The fluorine atoms in CF3 group of complex **12** were disordered and had the occupancies F1A 0.52(13), F1B 0.48(13), F2A 0.52(13), F2B 0.48(13), F3A 0.52(13), F3B

0.48(13). The additional systematic absences and thermal voids were verified with Platon [48]. For the absorption corrections SADABS was used [49]. The crystallographic data and the results of the refinements are summarized in table 1.

The complex ORTEP structures are given in figures 2-4, crystal data and refinement parameters in table 1, selected bond lengths and angles given in table S1, and hydrogen-bond lengths in table S2. The X-ray analysis of complexes **1**, **2** and **12** (figures 2-4) show the coordination of triphenylphosphine, thiourea and chloride with copper(I) in a distorted tetrahedral-fashion. Complex **1** has space group P-1 with Z = 2 in the triclinic crystal system, while complexes **2** and **12** have space group $P2_1/c$ in the monoclinic crystal system with Z = 4, as shown in table 1. The 3D crystal packing diagrams of complexes **1**, **2** and **12** are given in figures S1-S3, which confirm the Z values. The Cu–S bond lengths lie in the range of 2.3425(4) Å to 2.4243(6) Å, the Cu–P bond lengths lie in the range of 2.2456(6) to 2.2762(4) Å, and the Cu–Cl bond lengths lie in the range of 2.3166(6) to 2.3415(4) Å (table S1). The average value of bond angles of Cl–Cu–S lies in the range of 104.729(13) to 109.628(15)°, P–Cu–Cl bond angles lie in the range of 105.87(8) to 114.52(2)°, P–Cu–S bond angles lie in the range of 102.85(2) to 109.972(14)°, and P–Cu–P bond angles lie in the range of 117.15(2) to 124.616(15)°, which are nearly close to the perfect tetrahedral geometry (109.5 Å), table S1. The values of the torsion angles showed that the carbonyl groups and thiocarbonyl of the thiourea ligand are coplanar. The structural analysis further confirmed that there are two types of intramolecular hydrogen-bonding, between O of C=O and H of –NH group (O1⋯HN2) and between Cl and H of –NH group (Cl1⋯ HN1) presenting the *trans*-configuration of thiourea N protons (table S2).

3.5. *Biological activity results*

3.5.1. Antibacterial activity. In antibacterial tests, clear zones of growth inhibition were measured after 24 h incubation. From the data in table S3, it is clear that complex **11** is more active against *E. Aerogens* while against *B. Bronchiseptaca* it is complex **8** and against *S. Typhimurium* it is complexes **2** and **3** with ZOI values comparable to the reference drugs. From the data it is clear that the ligands and precursor are also active while complex activities are higher than precursor and the ligands which mean complex formation further enhances activity.

3.5.2. Antifungal activity. Fungal zone of inhibition values of the selected ligands and their complexes are given in table S4 and it has been found that almost all the complexes showed higher activities against the tested species than the ligands and precursor.

3.5.3. Brine shrimp assay. Live shrimps were counted and percentage of deaths was determined after 24 h incubation. Median lethal concentration (LC_{50}) was calculated using TableCurve 2D v5.01 software. It is clear from table S5 that the tested compounds are active in damaging the cells. The values are in the range of 52.6 to 88.6 µg/mL for the complexes while the ligands and the precursor are less active in damaging the cells as compared to the complexes.

3.5.4. Antileishmanial activity. The data in table S6 shows that the precursor and the ligands are active, but showed low activities compared to their copper(I) complexes. From the data it is clear that complex **2** shows high activity with LC_{50} value of 21.25 as compared to the standard drug while complexes **1** and **8** show moderate activities and complex **5** shows low activity.

3.5.5. Antioxidant activity. In antioxidant activities, it was observed that all the complexes showed higher activity than the precursor and ligands toward DPPH. The antioxidant activities for the complexes were found in the range of 54.96 to 62.67%. This high activity can be attributed to the high electron-withdrawing power of chlorine which facilitates the hydrogen release and can reduce the DPPH [50]. Likewise all the tested complexes have shown significantly high reducing power toward Fe^{3+} to Fe^{2} (table S7) and significantly high total antioxidant activity compared to the precursor and the ligands, which means the complex formation further increase the activity.

3.5.6. DNA Interaction studies by UV-visible spectroscopy and molecular docking. The tested complexes showed strong hypochromicity which was observed in the spectral order by the addition of DNA concentration (figure 5). All the complexes showed DNA interactions which can be attributed to the presence of chloride and phenyl rings in their structures. It has been reported previously that copper(I) tetrahedral complexes show DNA binding either by partial interaction or by the phenyl ring binding to one of the grooves [51, 52]. The plot of 1/[DNA] *vs*. $A_o/A\text{-}A_o$ also supports the groove binding of the complexes with DNA (figure 6). Complexes of

current studies have demonstrated groove binding with DNA, which is in close agreement with molecular docking studies.

The optimized molecular geometries of Cu complexes **1-12** were fully optimized with the PM3 semi-empirical quantum mechanical method and the two representative optimized geometries of complexes **1** and **2** with electronic charge distribution are presented in figure 7. Optimized structures show that all Cu-complexes have puckered conformations with crescent shaped geometry, which is the characteristic property of groove binders [53]. In order to have an understanding of the molecular mechanism of interaction between Cu compounds and double stranded DNA, different results of docking poses were obtained. Lowest energy docked poses of Cu compounds with double stranded DNA showed that all compounds interact with DNA through mixed mode of interaction for groove binding with the major groove of DNA, except for complexes **1**, **2**, **4** and **5**, which interact with the major groove of DNA via groove binding. Ligplots of **1**, **2**, **4** and **5** (figure 7 left) show that these compounds develop weak van der Waal's forces of attractions with the sugar phosphate backbone in the groove and fewer interactions with DNA base pairs.

The most important parameter obtained from docking simulations is the binding constant (K_b) calculated by scoring function free energy using the equation $\Delta G = -RT\ln K_b$, where K_b is measure of binding strength of a ligand with receptor. The highest value of K_b was found to be 1.08×10^5 for "complex **8**" (table S8) which is attributed to the intrastrand cross linking of benzo[*d*]thiazol-2-ylmoiety of complex **8** with DA17 and DT6 base pairs of DNA in addition to groove binding interactions. Following "complex **8**", **6** and **7** are the other complexes having higher values of binding strength, which is also attributed to their mixed mode of interactions with DNA. The lowest value of K_b was found for "**4**" and "**5**" (table S8). This fact is also manifested in figures of molecular docked complexes and the ligplot DNA base pairs with complexes **4** and **5** that these complexes are significantly distant from DNA and not cohesively attached to backbone. Negative values of free energies (ΔG) in the case of all Cu-complexes with DNA showed that they interact with DNA spontaneously.

A number of steric descriptors were calculated for the comprehension of macroscopic interactions. Electronic descriptors cannot be calculated because of the presence of Cu -- a heavy metal. An important steric descriptor is molar refractivity (M_R) which is an extent of the total polarizability of a mole of a substance. As evident from figure 8 direct correlation of the binding

strength with the M_R was observed implying increase of binding strength with an increase in the molar refractivity of the compound.

Another important steric descriptor calculated was hydrophobic surface area (V_{surf}). It is clear from figure 9 that a reasonably good inverse correlation of K_b with V_{surf} was observed depicting that compounds with higher V_{surf} have greater ability to overlap electronic clouds of DNA base pairs rendering a stronger association with DNA molecules.

4. Conclusion

Copper(I) complexes **1**-**12** of chlorine, triphenylphosphine (TPP) and different *N,N'*-disubstituted thioureas were synthesized for biological applications. The crystal structure investigation revealed that the complexes were distorted tetrahedral with copper as central metal ion coordinated with ligands. All the complexes were screened for different biological activities. In antibacterial studies complex **11** was the most active against *E. Aerogens*, complex **8** was the most active against *B. Bronchiseptaca* and complexes **2** and **3** were active against *S. Typhimurium* with ZOI comparable to the reference drug. The antileishmanial activities showed that complex **2** was the most active while complexes **3** and **8** were moderately active, and complex **5** showed low activity. The antioxidant activities showed that all the complexes have high total antioxidant activity and high reducing power toward Fe^{3+} to Fe^{2+}. During DNA interaction studies, through UV/Vis, a hypochromicity in the spectral order was observed with increasing concentration of DNA which was attributed to the groove binding. This type of interaction was further supported by molecular docking studies.

Supplementary data

Supplementary information is available containing data of bond lengths, bond angles, hydrogen bonds, crystals packs and tables of biological activates. Author ORIC ID is 0000-0002-0377-0356 and CCDC Nos. 1834952-1834953 and 1834957-1834958 contain the supplementary crystallographic data for this article. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif on request.

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Conflicts of interest

The authors declare no conflict of interest.

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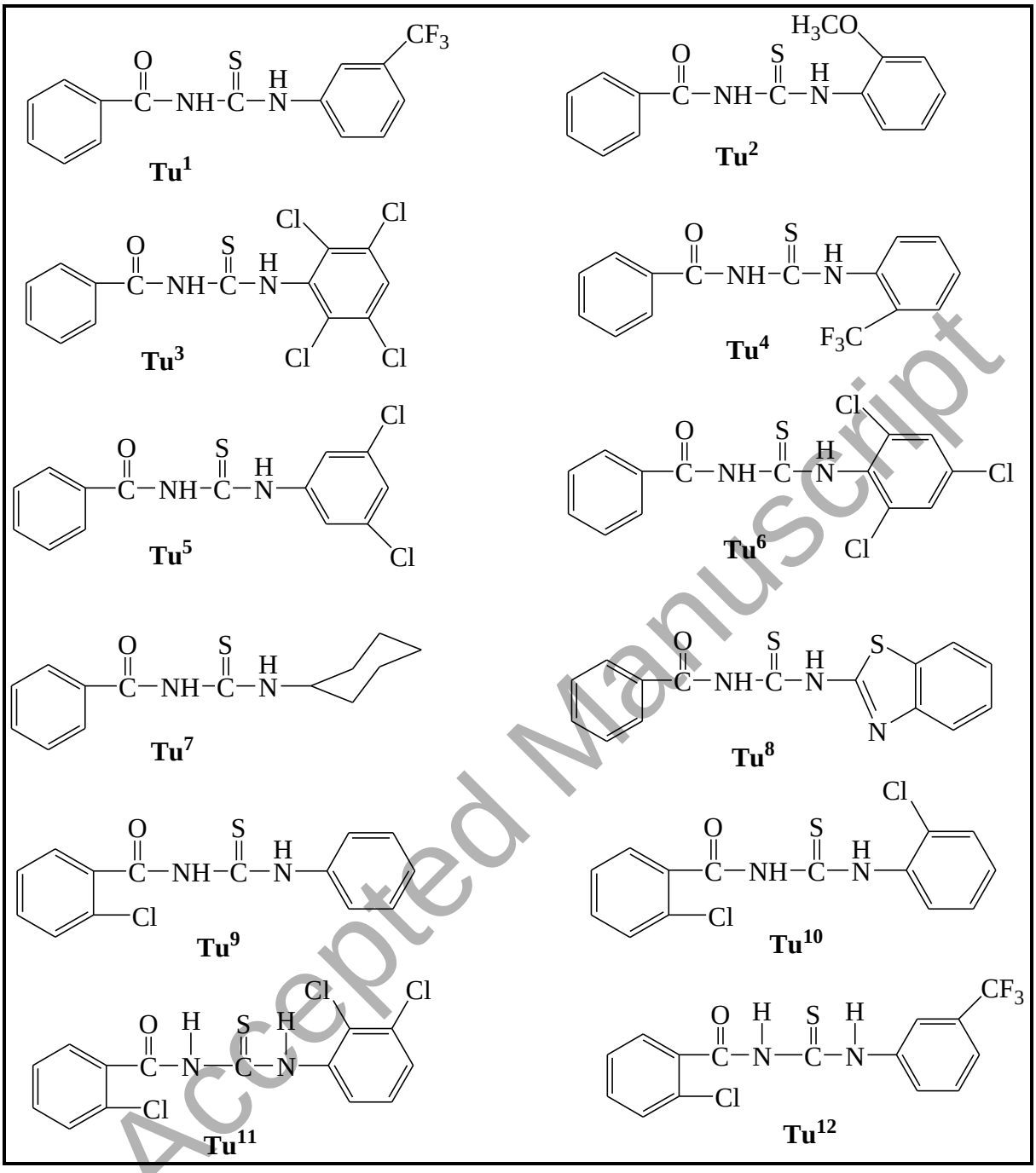

Figure 1. The chemical structure of the ligands (Tu^{1} to Tu^{12}) used in this work.

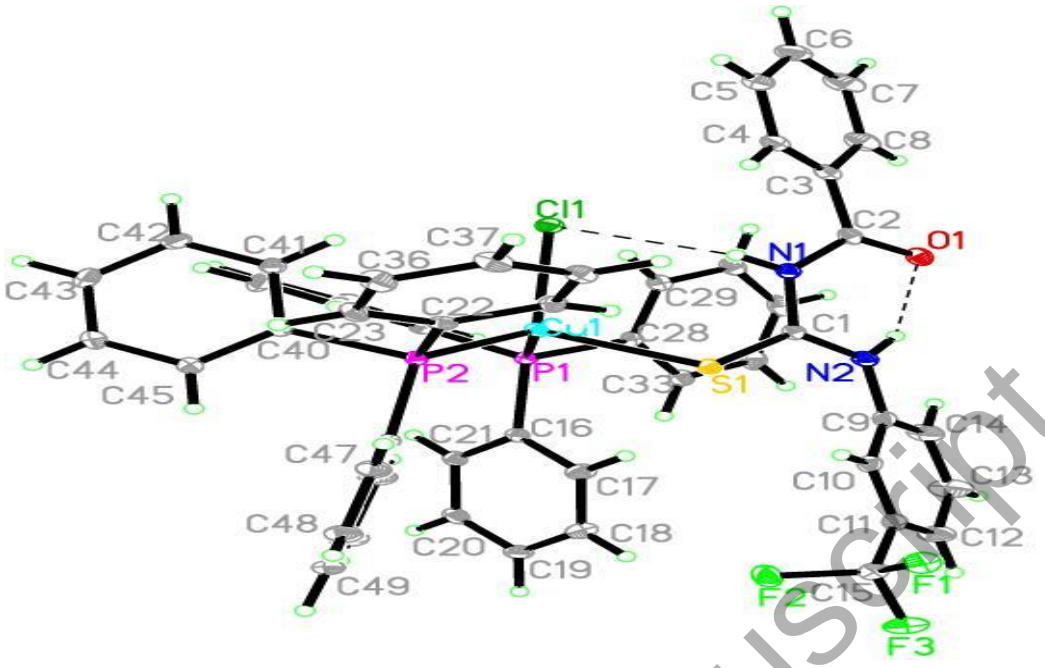

Figure 2. ORTEP drawing of complex **1** with atomic numbering scheme.

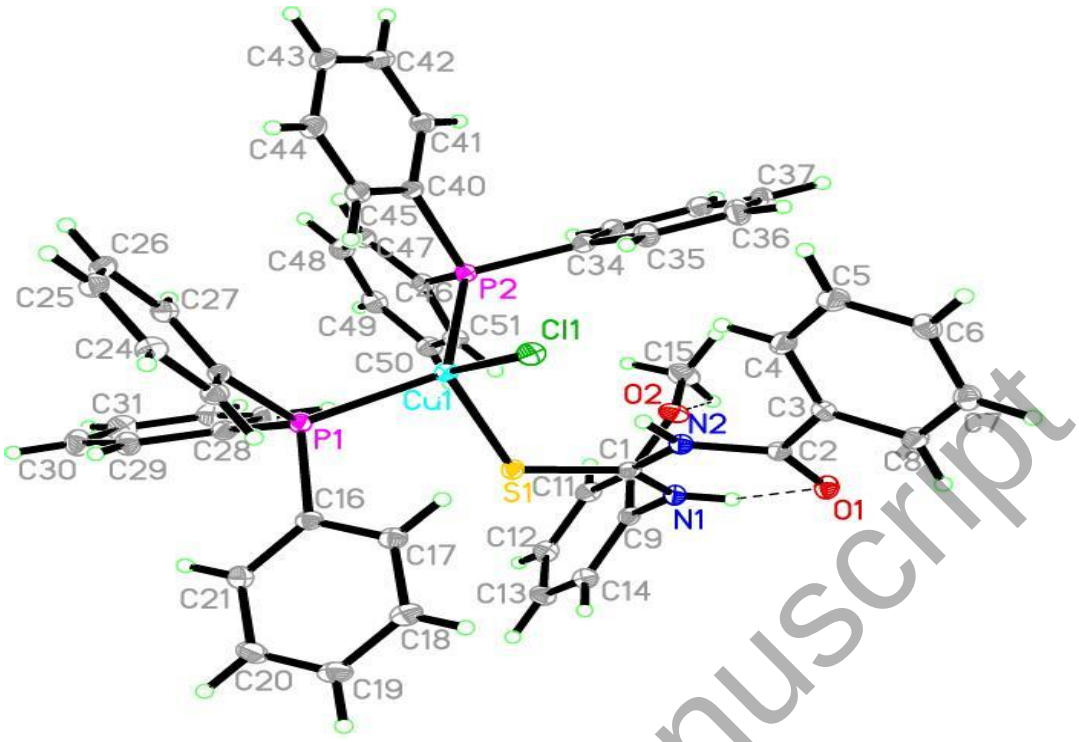

Figure 3. ORTEP drawing of complex 2 with atomic numbering scheme.

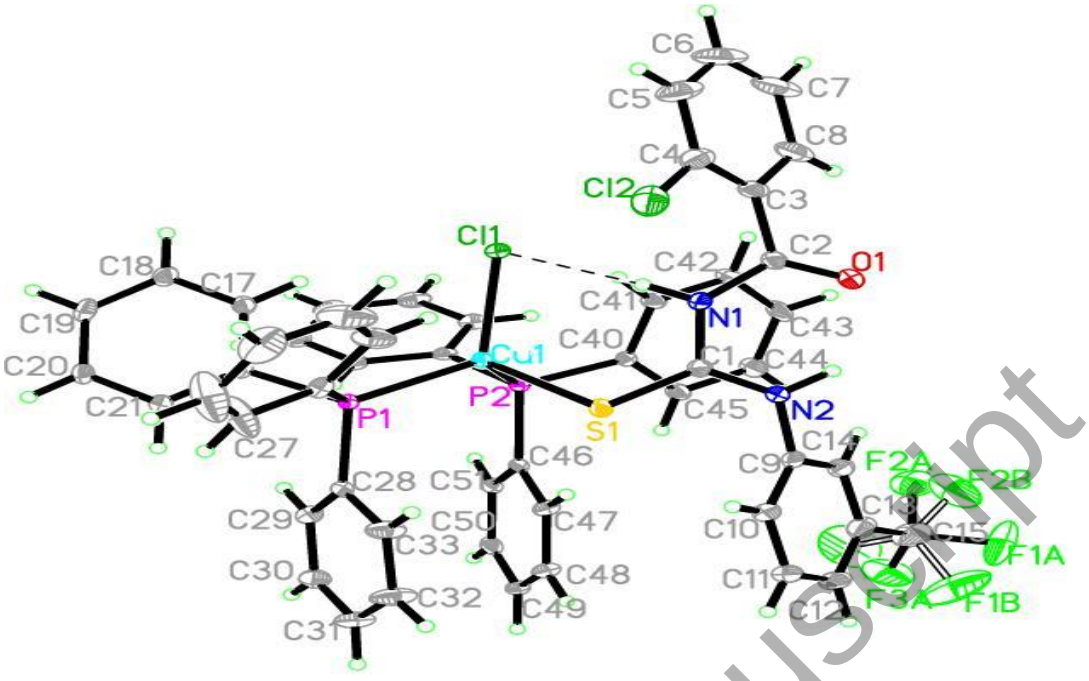

Figure 4. ORTEP drawing of complex **12** with atomic numbering scheme showing disorder in the CF_3 group.

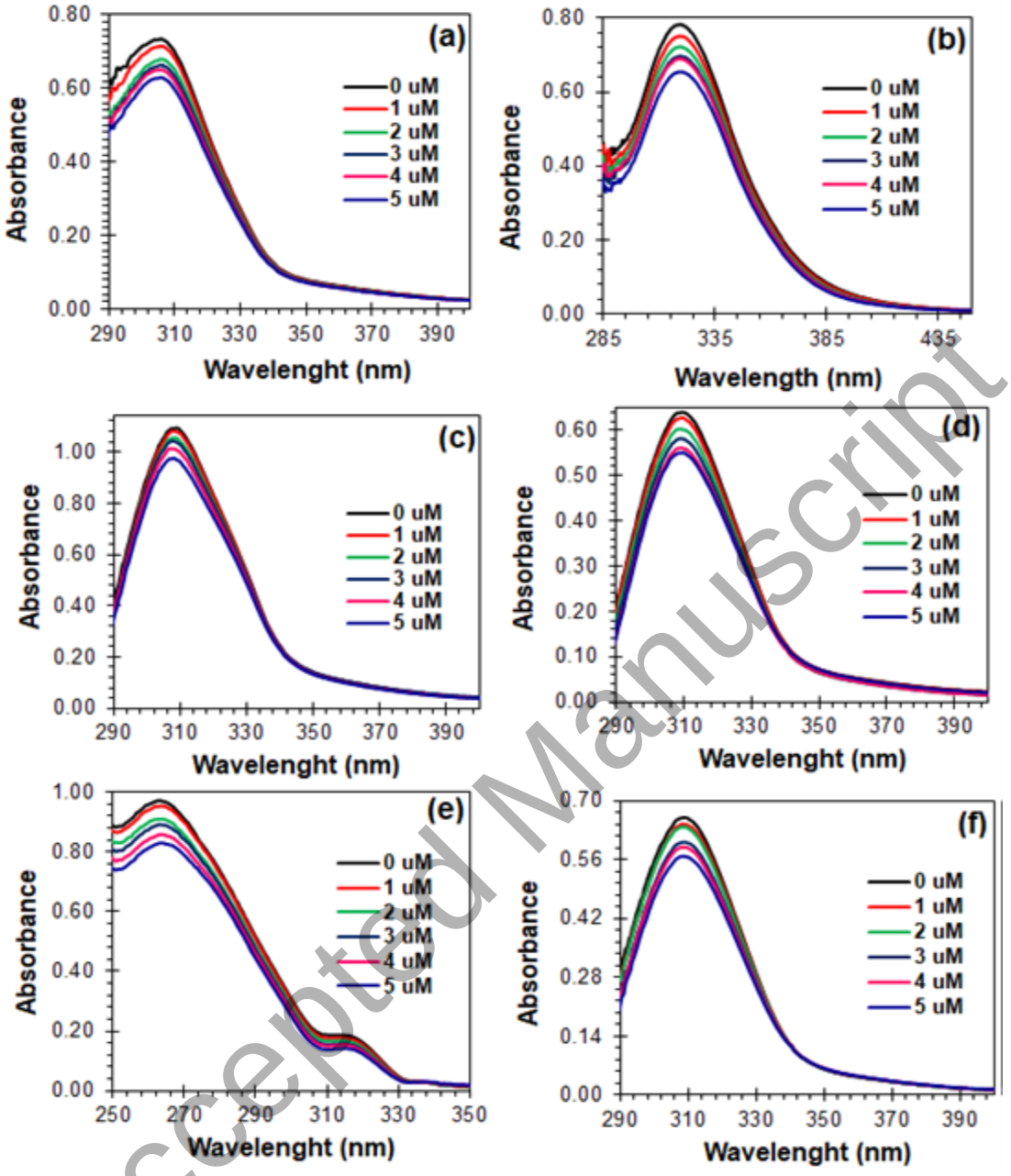

Figure 5. DNA binding studies by UV/Vis graphs for complexes **1** (a), **2** (b), **3** (c), **4** (d), **5** (e) and **6** (f).

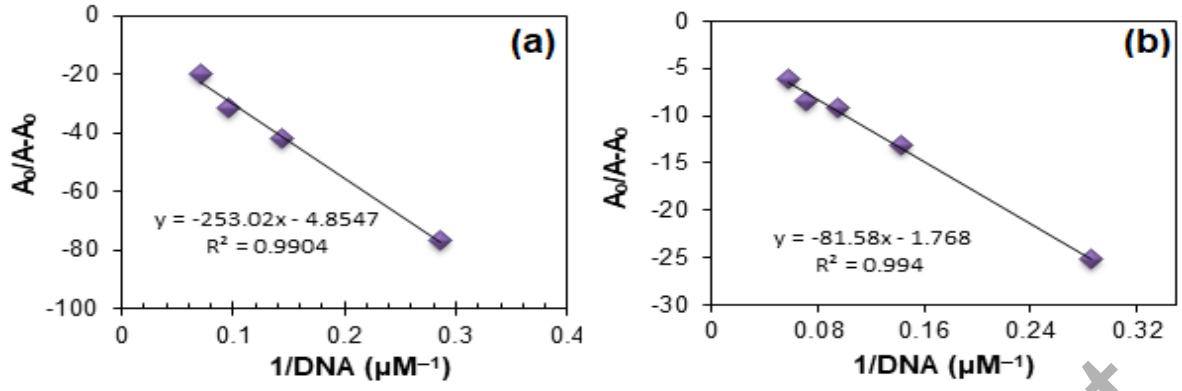

Figure 6. Plot of relative absorbance and reciprocal DNA concentration for complexes **1** (a) and **2** (b).

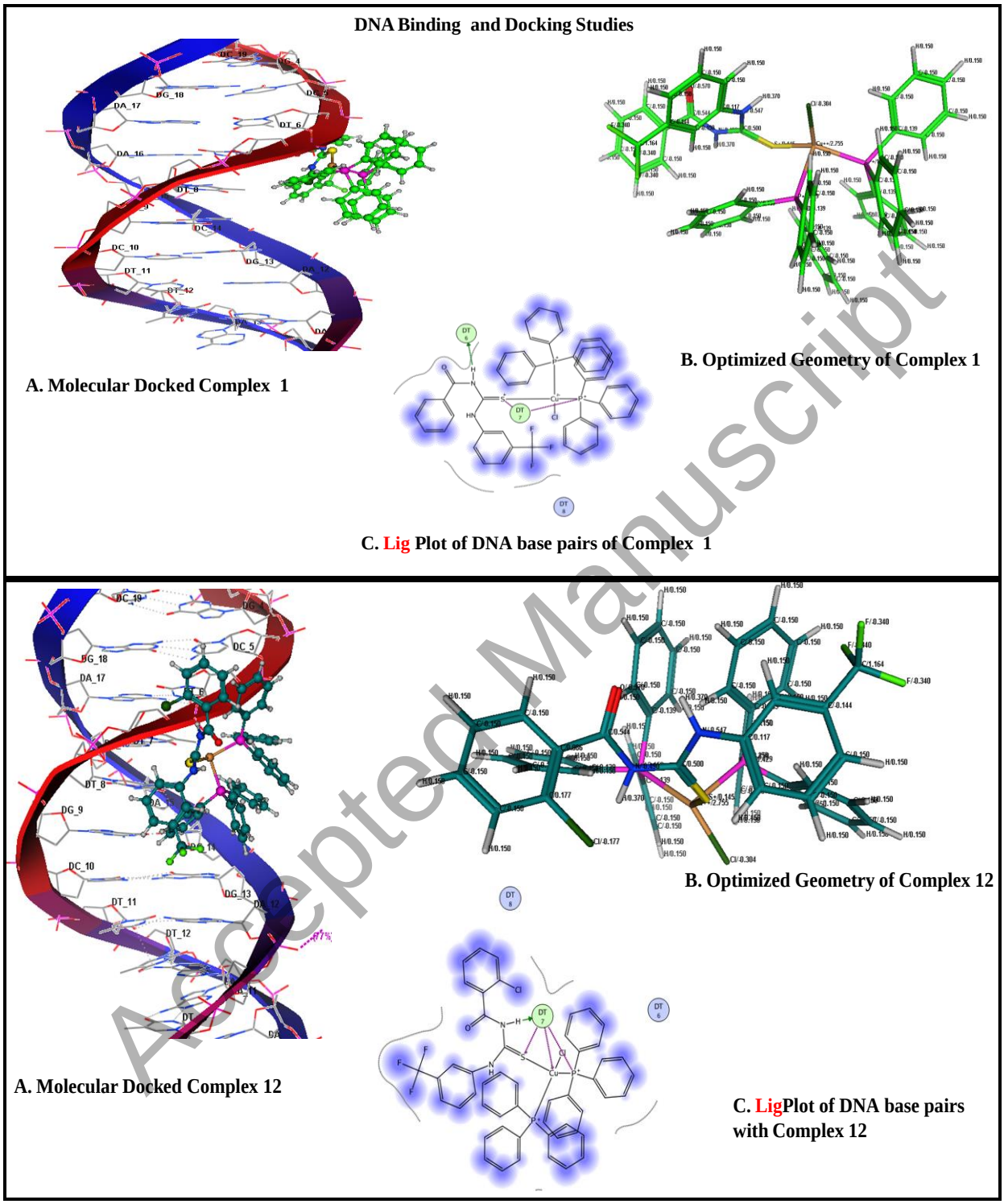

Figure 7. DNA binding and docking studies of complexes **1** and **12**.

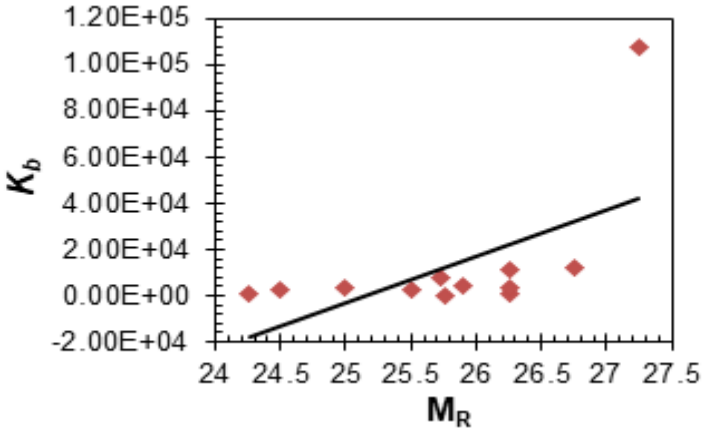

Figure 8. Plot of formation constant K_b *vs.* molar refractivity (M_R).

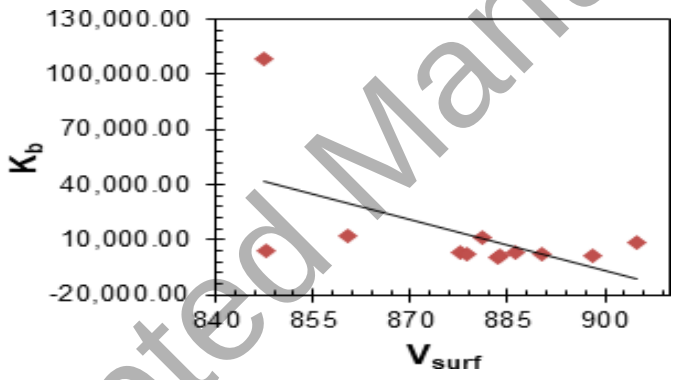

Figure 9. Plot of formation constant K_b *vs.* hydrophobic surface area (V_{surf}).

Table 1. Crystal structures data and refinement parameters.

Crystal parameters	Complex **1**	Complex **2**	Complex **12**
CCDC No.	1834952	1834953	1834958
Chemical formula	$C_{51}H_{41}ClCuF_3N_2OP_2S$	$C_{51}H_{44}ClCuN_2O_2P_2S$	$C_{50}H_{40}Cl_2CuN_2OP_2S,CF_3$
Crystal color	Yellow	Light yellow	Light yellow
Fw; *F* (000)	947.85; 976.00	909.87; 1888	982.29; 2016
T (K)	100	100	100
Radiation Cu Kα wavelength (Å)	1.54178	1.54178	1.54178
Crystal system	Triclinic	Monoclinic	Monoclinic
Space group	P-1	$P2_1/c$	$P2_1/c$
a (Å)	11.1549(2)	10.4078(4)	18.1452(4)
b (Å)	12.6450(2)	22.2400(8)	13.2566(3)
c (Å)	17.5217(2)	18.7709(7)	19.0281(4)
α (deg)	80.8140(10)	90	90
β (deg)	79.8950(10)	90.1750(10)	94.1390(10)
γ (deg)	67.6180(10)	90	90
Z	2	4	4
V ($Å^3$)	2238.13(6)	4344.9(3)	4565.15(17)
ρ calcd ($g \cdot cm^{-3}$)	1.406	1.391	1.429
μ (mm^{-1})	2.788	2.763	3.280
Crystal size/mm^3	0.24 × 0.20 × 0.16	0.28 × 0.2 × 0.18	0.4 × 0.28 × 0.16
θ range (deg); completeness	2.575 − 72.120; 0.976	3.081 − 72.112; 0.999	2.441 − 72.145; 0.998
2θ range for data collection/°	5.15 to 144.24	6.162 to 144.224	4.882 to 144.29
Collected reflections; Rσ	93515	119651	121155
Unique reflections; Rint	93515; 0.0225	0.0322; 0.0885	121155; 0.0331
Independent reflections	8550 [Rint = 0.0225, Rsigma = 0.0094]	8548 [Rint = 0.0350, Rsigma = 0.0140]	8980 [Rint = 0.0331, Rsigma = 0.0118]
Data / restraints / parameters	550 / 0 / 567	8548 / 0 / 550	8980 / 117 / 604
Index ranges	$-13 \le h \le 13$, $-15 \le k \le 15$, $-21 \le l \le 21$	$-12 \le h \le 12$, $-27 \le k \le 26$, $-23 \le l \le 23$	$-22 \le h \le 22$, $-16 \le k \le 16$, $-23 \le l \le 23$
Final R indexes [I>=2σ (I)]	R1 = 0.0271, wR2 = 0.0723	R1 = 0.0315, wR2 = 0.0878	R1 = 0.0439, wR2 = 0.1131
Final R indexes [all data]	R1 = 0.0273 wR2 = 0.0725	R1 = 0.0322, wR2 = 0.0885	R1 = 0.0439, wR2 = 0.1131
Goodness of fit on F^2	1.078	1.041	1.072
Largest diff peak and hole/ e $Å^{-3}$	0.466 and -0.312	0.50 and -0.31	0.92 and -1.23

Graphical abstract