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Research Article

Antibacterial, Cytotoxic Studies and Characterization of Some Newly Synthesized Symmetrical N^3,$N^{3\prime}$-Bis(disubstituted)isophthalyl-bis(thioureas) and Their Cu(II) and Ni(II) Complexes

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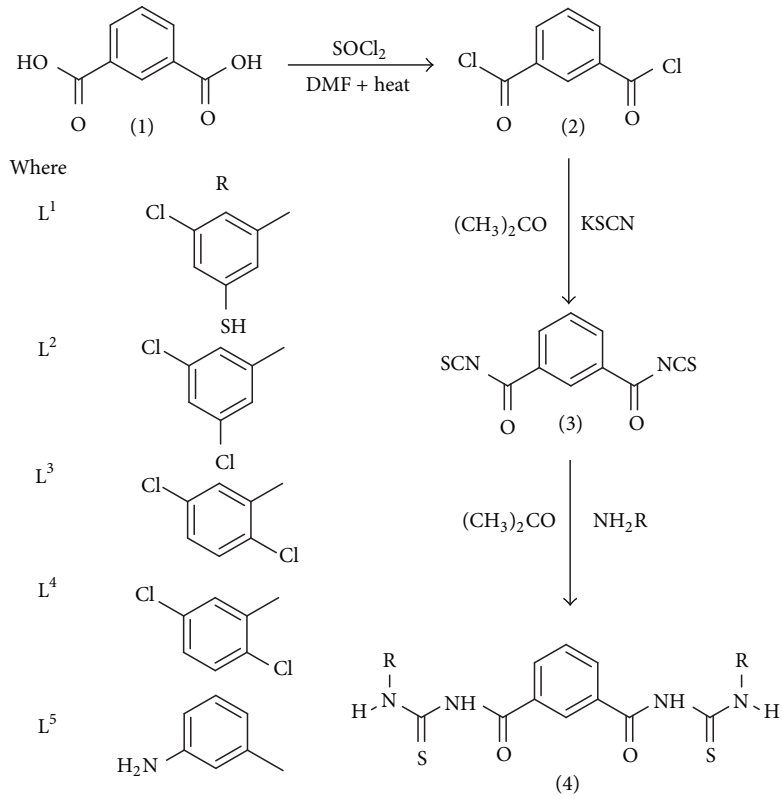
A series of some novel N^3,$N^{3\prime}$-bis(disubstituted)isophthalyl-bis(thioureas) compounds with general formula $[C_6H_4 \cdot \{CONHCSNHR\}_2]$, where R = 2-$ClC_6H_4S$ (L^1), 3,5-$(Cl)_2C_6H_3$ (L^2), 2,4-$(Cl)_2C_6H_3$ (L^3), 2,5-$(Cl)_2C_6H_3$ (L^4), and 2-$NH_2C_6H_4$ (L^5), and their Cu(II) and Ni(II) complexes (C^1–C^{10}) have been synthesized. These compounds (L^1–L^5) and their metal(II) complexes (C^1–C^{10}) have been characterized by elemental analysis, infrared spectroscopy, ^{1}H NMR and ^{13}C NMR spectroscopy, magnetic moments, and electronic spectral measurements. The ligands are coordinated to metal atom in a bidentate pattern producing a neutral complex of the type $[ML]_2$. These compounds (L^1–L^5) and their metal(II) complexes (C^1–C^{10}) were also screened for their antibacterial and cytotoxic activities.

1. Introduction

Thioureas, an emerging class of compounds, were first synthesized by Neucki [1]. Thiourea derivatives hold broad range of applications in the field of medicine, agriculture, and analytical chemistry. These compounds show a comprehensive range of biological activities such as antiviral [2, 3], antibacterial [4], fungicidal [5–7], analgesic, herbicidal [8, 9], plant growth regulating [10], antiaggregating [11], antiarrhythmic [12], local anesthetic [13], and antihyperlipidemic activities [14]. Some thioureas have been recently described as effective antitumor and nonnucleoside inhibitors of HIV reverse transcriptase [15]. Recently reported [16] some dithiourea derivatives exhibited cytotoxicity against various cancer cells, and one of these indicated best inhibition activities against KB and CNE2 with IC_{50} values of 10.72 and 9.91 micrometer, respectively. In view of these results, our interest increased in the synthesis of some new bis(thiourea) derivatives which were characterized by spectroscopic techniques such as FTIR, ^{1}H NMR, and ^{13}C NMR. These derivatives are stable and contain at least two potential donor atoms as O and S. These have been found to display surprisingly rich coordination chemistry at their active sites especially with transition metals. Metalloorganic chemistry is becoming an emerging area of research due to the demand for new metal-based antibacterial and antifungal compounds [17, 18]. Many investigations have proved that binding of a drug to a metalloelement enhances its activity, and in some cases, the complex possesses even more healing properties than the parent drug [19]. Recently, a number of attempts have been made to obtain Cu(II) and Ni(II) complexes with thioureas [20–22]. In view of these observations, we became interested



Scheme 1: Synthesis of disubstituted bisthiourea derivatives (ligands L^1–L^5).

in the synthesis of some new bis(thiourea) derivatives and their Cu(II) and Ni(II) complexes. In present work, these compounds were synthesized, isolated, and characterized by elemental analyses, infrared spectroscopy, ^{1}H NMR and ^{13}C NMR spectroscopy, magnetic moments, and electronic spectral measurements. These compounds were also screened for their antibacterial and cytotoxic studies.

2. Experimental

2.1. Materials and Methods. All chemicals used were of analytical reagent grade (AR) and of the highest purity available. These include isophthalic acid, thionyl chloride, potassium thiocyanate, 3-amino-5-chlorobenzenethiol, 3,5-dichloroaniline, 2,4-dichloroaniline, 2,5-dichloroaniline, and 3-aminoaniline. The organic solvents used include acetone, absolute ethyl alcohol, and dimethylformamide (DMF). These solvents were either spectroscopically pure or purified by the recommended methods and tested for their spectral purity. Deionized water collected from all-glass equipment was used wherever required.

2.2. General Procedure for Synthesis of Compounds (L^1–L^5). A solution of isophthalyl chloride (2) (0.1 mol), obtained by the reaction of isophthalic acid with thionyl chloride, was prepared in dry acetone solvent. Potassium thiocyanate (0.2 mol), previously dried at 80°C for two hours, was added to above solution and stirred for one hour at room temperature to obtain the isophthalyl isothiocyanate (3). This solution was mixed with a solution of primary amines (0.2 mol) and stirred for 24 hours at room temperature to get the target disubstituted bisthiourea derivatives (L^1–L^5) in good to excellent yields (Scheme 1). The mixture was then poured into sufficient quantity of ice cold water, and the product was settled as white to yellow precipitate which was filtered, washed with cold water, and dried in vacuum desiccator. For further purification, the products were recrystallized from DMF.

N^3, $N^{3'}$-Bis(3-chloro-5-mercaptophenyl)isophthalyl-bis(thiourea) (L^1). Yellow solid; Yield: 68%; m.p. 204°C; FTIR (KBr, cm^{-1}): 3345 ν(N–H), 756 ν(C–Cl), 1664 ν(C=O), 1600, 1530, 1459 ν(C=C), 1135 ν(C=S). ^{1}H NMR (DMSO-d_6, δ, ppm): 12.47 (s, 2H, 2CSNH), 8.56 (s, 2H, 2CONH), 8.20–7.71 (m, 4H, isophthalyl Ar-H), 7.13–6.62 (m, 6H, amine Ar-H), 3.46 (s, 2H, 2SH). ^{13}C NMR (DMSO-d_6, δ, ppm): 125.3 (C_1), 133.9 (C_2), 132.2 (C_3), 128.5 (C_4), 165.1 (C_5), 182.4 (C_6), 138.1 (C_7), 127.2 (C_8), 129.4 (C_9), 126.7 (C_{10}), 128.2 (C_{11}), 129.8 (C_{12}). Anal. Calcd. (%) for $C_{22}H_{16}Cl_2N_4O_2S_4$ (567.55): C, 46.56; H, 2.84; Cl, 12.49; N, 9.87; O, 5.64; S, 22.60. Found (%): C, 46.59; H, 2.78; Cl, 12.54; N, 9.89; O, 5.61; S, 22.71.

N^3, $N^{3'}$-Bis(3,5-dichlorophenyl)isophthalyl-bis(thiourea) (L^2). Yellowish solid; Yield: 72%; m.p. 205°C; FTIR (KBr, cm^{-1}): 3334 ν(N–H), 759 ν(C–Cl), 1654 ν(C=O), 1607, 1545, 1490

ν(C=C) 1155 ν(C=S). ^{1}H NMR (DMSO-d_6, δ, ppm): 12.32 (m, 2H, CSNH), 8.65 (s, 2H, 2CONH), 8.26–7.714 (m, 4H, isophthalyl Ar-H) 7.43–7.08 (s, 6H, amine Ar-H). ^{13}C NMR (DMSO-d_6, δ, ppm): 123.9 (C_1), 134.2 (C_2), 131.4 (C_3), 128.5 (C_4), 165.9 (C_5), 180.1 (C_6), 138.1 (C_7), 127.2 (C_8), 129.3 (C_9), 125.6 (C_{10}), 128.9 (C_{11}), 129.1 (C_{12}). Anal. Calcd. (%) for $C_{22}H_{14}Cl_4N_4O_2S_2$ (576.92): C, 46.17; H, 2.47; Cl, 24.78; N, 9.79; O, 5.59; S, 11.21. Found (%): C, 46.14; H, 2.41; Cl, 24.76; N, 9.67; O, 5.62; S, 11.33.

N^3, $N^{3'}$-Bis(2,4-chlorophenyl)isophthalyl-bis(thiourea) (L^3). Yellow solid; Yield: 70%; m.p. 196°C. FTIR (KBr, cm^{-1}): 3338 ν(N–H), 752 ν(C–Cl), 1658 ν(C=O), 1592, 1480, 1526 ν(C=C), 1161 ν(C=S). ^{1}H NMR (DMSO-d_6, δ, ppm): 12.31 (m, 2H, CSNH), 8.51 (s, 2H, 2CONH), 8.25–7.74 (m, 4H, isophthalyl Ar-H) 7.37–7.06 (s, 6H, amine Ar-H). ^{13}C NMR (DMSO-d_6, δ, ppm): 125.1 (C_1), 134.2 (C_2), 132.4 (C_3), 126.5 (C_4), 164.5 (C_5), 182.3 (C_6), 138.3 (C_7), 126.7 (C_8), 129.7 (C_9), 125.9 (C_{10}). Anal. Calcd. (%) for $C_{22}H_{14}Cl_4N_4O_2S_2$ (576.92): C, 46.17; H, 2.47; Cl, 24.78; N, 9.79; O, 5.59; S, 11.21. Found (%): C, 46.11; H, 2.48; Cl, 24.16; N, 9.25; O, 5.54; S, 11.31.

N^3, $N^{3'}$-Bis(2,5-dichlorophenyl)isophthalyl-bis(thiourea) (L^4). Yellowish solid; Yield: 59%; m.p. 194°C. FTIR (KBr, cm^{-1}): 3362, ν(N–H), 1655 ν(C=O), 1600, 1516, 1474 ν(C=C), 1197 ν(C=S). ^{1}H NMR (DMSO-d_6, δ, ppm): 12.29 (m, 2H, 2CSNH), 9.12 (s, 2H, 2CONH), 8.56–7.81 (m, 4H, isophthalyl Ar-H), 7.43–6.73 (m, 6H, amine Ar-H). ^{13}C NMR (DMSO-d_6, δ, ppm): 124.1 (C_1), 133.4 (C_2), 133.4 (C_3), 127.6 (C_4), 166.4 (C_5), 182.2 (C_6), 137.3 (C_7), 127.8 (C_8), 129.1 (C_9), 128.6 (C_{10}), 128.8 (C_{11}), 129.1 (C_{12}). Anal. Calcd. (%) for $C_{22}H_{14}Cl_4N_4O_2S_2$ (576.92): C, 46.17; H, 2.47; Cl, 24.78; N, 9.79; O, 5.59; S, 11.21. Found (%): C, 46.14; H, 2.43; Cl, 24.18; N, 9.23; O, 5.55; S, 11.34.

N^3, $N^{3'}$-Bis-(3-aminophenyl)isophthalyl-bis(thiourea) (L^5). Grey solid; Yield: 72%; m.p. 201°C; IR (KBr, νmax, cm^{-1}): 3330, 3211 ν(N–H), 1685 ν(C=O), 1640, 1535, 1458 ν(C=C), 1270, 1148 ν(C=S). ^{1}H NMR (300 MHz, DMSO-d_6, Me4Si): δ (ppm): 12.33 (s, 2H, 2CSNH), 9.81 (s, 2H, 2CONH), 8.25–7.64 (m, 12H, isophthalyl Ar-H), 7.14–6.47 (m, 12H, amine Ar-H), 5.22 (s, 4H, 2NH2). ^{13}C NMR (DMSO-d_6, δ, ppm): 123.4 (C_1), 133.6 (C_2), 132.9 (C_3), 125.9 (C_4), 167.6 (C_5), 184.1 (C_6), 135.2 (C_7), 126.9 (C_8), 128.3 (C_9), 122.6 (C_{10}), 130.2 (C_{11}), 129.5 (C_{12}). Anal. Calcd. (%) for $C_{22}H_{20}N_6O_2S_2$ (464.56): C, 56.88; H, 4.34; O, 18.09; S, 13.80. Found (%): C, 56.93; H, 4.33; O, 18.16; S, 13.79.

2.3. General Procedure for Synthesis of Metal(II) Complexes. A solution of the bisthiourea (0.05 mol) in DMF (15 mL) was added to a solution of MCl_2 (0.05 mol) in DMF (15 mL), while M = Cu for (C^1–C^5) and M = Ni for (C^6–C^{10}). The mixture was refluxed for 6 hours at room temperature and then concentrated to one-third volume and kept at room temperature for 2 hours. The solid product formed was filtered, washed with DMF, and dried.

N^3, $N^{3'}$-Bis(3-chloro-5-mercaptophenyl)isophthalyl-bis(thiourea) Copper(II) Complex (C^1). Orange red solid; Yield: 69%; m.p. 287°C; IR (KBr, νmax, cm^{-1}): 3345 ν(N–H), 756 ν(C–Cl), 1600, 1531, 1459 ν(C=C), 1514 ν(C–O), 366 ν(M–S), 463 ν(M–O). Anal. Calcd. (%) for $Cu_2C_{44}H_{28}Cl_4N_8O_4S_8$ (1258.17): C, 42.00; H, 2.24; N, 8.91; S, 20.39; Cl, 11.27; Cu, 10.10. Found (%): C, 42.07; H, 2.28; N, 8.86; S, 20.41; Cl, 11.24; Cu, 1011.

N^3, $N^{3'}$-Bis(3,5-dichlorophenyl)isophthalyl-bis(thiourea) Copper(II) Complex (C^2). Orange red solid; Yield: 71%; m.p. 287°C; IR (KBr, νmax, cm^{-1}): 3333 ν(N–H), 750 ν(C–Cl), 1600, 1545, 1490 ν(C=C) 1604 ν(C–O), 365 ν(M–S), 445 ν(M–O). Anal. Calcd. (%) for $Cu_2C_{44}H_{24}Cl_8N_8O_4S_4$ (1257.98): C, 41.69; H, 1.91; N, 8.84; S, 10.12; Cl, 22.37; Cu, 10.03. Found (%): C, 41.64; H, 1.90; N, 8.86; S, 10.12; Cl, 22.31; Cu, 10.12.

N^3, $N^{3'}$-Bis(2,4-dichlorophenyl)isophthalyl-bis(thiourea) Copper(II) Complex (C^3). Orange red solid; Yield: 72%; m.p. 288°C; IR (KBr, νmax, cm^{-1}): 3338 ν(N–H), 752 ν(C–Cl), 1592, 1483, 1524 ν(C=C), 1508 ν(C–O), 363 ν(M–S), 432 ν(M–O). Anal. Calcd. (%) for $Cu_2C_{44}H_{24}Cl_8N_8O_4S_4$ (1257.98): C, 41.69; H, 1.91; N, 8.84; S, 10.12; Cl, 22.37; Cu, 10.03. Found (%): C, 41.65; H, 1.95; N, 8.87; S, 10.11; Cl, 22.36; Cu, 10.04.

N^3, $N^{3'}$-Bis(2,5-dichlorophenyl)isophthalyl-bis(thiourea) Copper(II) Complex (C^4). Orange red solid; Yield: 73%; m.p. 286°C; IR (KBr, νmax, cm^{-1}): 3359, ν(N–H), 1600, 1516, 1474 ν(C=C), 1459 ν(C–O), 357 ν(M–S), 429 ν(M–O). Anal. Calcd. (%) for $Cu_2C_{44}H_{24}Cl_8N_8O_4S_4$ (1257.98): C, 41.69; H, 1.91; N, 8.84; S, 10.12; Cl, 22.37; Cu, 10.03. Found (%): C, 41.62; H, 1.93; N, 8.82; S, 10.17; Cl, 22.35; Cu, 10.07.

N^3, $N^{3'}$-Bis-(3-aminophenyl)isophthalyl-bis(thiourea) Copper (II) Complex (C^5). Orange red solid; Yield: 69%; m.p. 285°C; IR (KBr, νmax, cm^{-1}): 3343 ν(N–H), 1682 ν(C=N), 1589, 1523, 1481 ν(C=C), 1591 ν(C–O), 352 ν(M–S), 435 ν(M–O). Anal. Calcd. (%) for $Cu_2C_{44}H_{36}N_{12}O_4S_4$ (1052.19): C, 50.23; H, 3.45; N, 15.97; S, 12.19; Cu, 12.08. Found (%): C, 50.22; H, 3.49; N, 15.89; S, 12.17; Cu, 12.11.

N^3, $N^{3'}$-Bis(3-chloro-5-mercaptophenyl)isophthalyl-bis(thiourea) Nickel(II) Complex (C^6). Red solid; Yield: 68%; m.p. 292°C; IR (KBr, νmax, cm^{-1}): 3346 ν(N–H), 755 ν(C–Cl), 1612, 1530, 1459 ν(C=C), 1511 ν(C–O), 355 ν(M–S), 461 ν(M–O). Anal. Calcd. (%) for $Ni_2C_{44}H_{28}Cl_4N_8O_4S_8$ (1248.46): C, 42.33; H, 2.26; N, 8.98; S, 20.55; Cl, 11.36; Ni, 9.40. Found (%): C, 42.19; H, 2.29; N, 8.97; S, 20.49; Cl, 11.24; Ni, 9.29.

N^3, $N^{3'}$-Bis(3,5-dichlorophenyl)isophthalyl-bis(thiourea) Nickel(II) Complex (C^7). Red solid; Yield: 73%; m.p. 292°C; IR (KBr, νmax, cm^{-1}): 3334 ν(N–H), 752 ν(C–Cl), 1607, 1545, 1491 ν(C=C) 1609 ν(C–O), 367 ν(M–S), 456 ν(M–O). Anal. Calcd. (%) for $Ni_2C_{44}H_{24}Cl_8N_8O_4S_4$ (1190.72): C, 42.01; H, 1.92; N, 8.91; S, 10.20; Cl, 22.55; Ni, 9.33. Found (%): C, 42.02; H, 1.96; N, 8.89; S, 10.32; Cl, 22.41; Ni, 9.34.

N^3, $N^{3'}$-Bis(2,4-dichlorophenyl)isophthalyl-bis(thiourea) Nickel(II) Complex (C^8). Red solid; Yield: 74%; m.p. 294°C; IR (KBr, νmax, cm^{-1}): 3336 ν(N–H), 751 ν(C–Cl), 1591, 1482, 1526 ν(C=C), 1504 ν(C–O), 354 ν(M–S), 437 ν(M–O). Anal. Calcd. (%) for $Ni_2C_{44}H_{24}Cl_8N_8O_4S_4$ (1190.72): C, 42.01; H, 1.92; N, 8.91; S, 10.20; Cl, 22.55; Ni, 9.33. Found (%): C, 42.04; H, 1.91; N, 8.95; S, 10.31; Cl, 22.41; Ni, 9.32.

N^3, $N^{3'}$-Bis(2,5-dichlorophenyl)isophthalyl-bis(thiourea) Nickel(II) Complex (C^9). Red solid; Yield: 72%; m.p. 293°C; IR (KBr, νmax, cm^{-1}): 3362, ν(N–H), 1609, 1526, 1471 ν(C=C), 1464 ν(C–O), 360 ν(M–S), 442 ν(M–O). Anal. Calcd. (%) for $Ni_2C_{44}H_{24}Cl_8N_8O_4S_4$ (1190.72): C, 42.01; H, 1.92; N, 8.91; S, 10.20; Cl, 22.55; Ni, 9.33. Found (%): C, 42.09; H, 1.90; N, 8.93; S, 10.29; Cl, 22.47; Ni, 9.31.

N^3, $N^{3'}$-Bis-(3-aminophenyl)isophthalyl-bis(thiourea) Nickel (II) Complex (C^{10}). Red solid; Yield: 75%; m.p. 297°C; IR (KBr, νmax, cm^{-1}): 3341 ν(N–H), 1678 ν(C=N), 1588, 1513, 1482 ν(C=C), 1592 ν(C–O), 351 ν(M–S), 443 ν(M–O). Anal. Calcd. (%) for $Ni_2C_{44}H_{36}N_{12}O_4S_4$ (1042.48): C, 50.69; H, 3.48; N, 16.12; S, 12.30; Ni, 11.26. Found (%): C, 50.63; H, 3.51; N, 16.09; S, 12.27; Ni, 11.30.

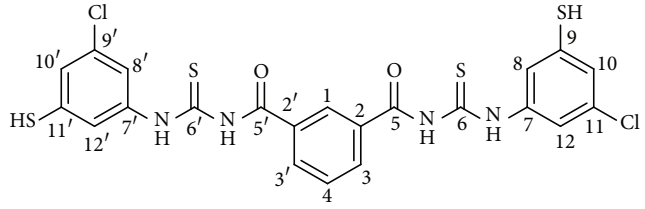
2.4. Characterization. Elemental microanalyses of the separated solids for C, H, N, and S and metal were performed on a PE-2400 CHNS analyzer. The analyses were repeated twice to check the accuracy of data. Infrared spectra were recorded on an Alpha Centauri FT-IR spectrophotometer in wave number region 250–4000 cm^{-1}. The spectra were recorded with the help of KBr pallets. The ^1H NMR and ^{13}C NMR were recorded using FT-80 instrument, and DMSO-d_6 was used as solvent and Me_4Si as internal standard. UV visible spectra were obtained in DMF on a Hitachi U-2000 double-beam spectrophotometer. Room temperature magnetic susceptibility measurements were carried out using a Sherwood-Scientific Gouy magnetic balance (Calibrant: $Hg[Co(SCN)_4]$).

2.5. Pharmacology. Antibacterial activity of synthesized ligands (L^1–L^5) and their metal(II) complexes (C^1–C^{10}) was determined by using the disc diffusion method [18] against various gram-negative and gram-positive bacteria at a concentration of 200 μg/100 μL in DMSO solution. Ampicillin (100 μL/disc) and ciprofloxacin (100 μL/disc) were used as standard drugs. Twenty-four-hour-old cultures, containing approximately 1.5×10^6 (CFU/mL), were spread on the surface of Nutrient Agar (NA) plates. The discs (6 mm diameter) were impregnated with (100 μL/disc) test samples and then placed aseptically on the inoculated agar media. Experimental plates were incubated at 37°C for 24 hours. Antibacterial activity was determined by measuring the diameter of the inhibition zone (IZ) and compared with standard drugs. The IZ values from 25 to 35 mm were taken as potent and from 20 to 25 mm as strong, and values greater than 10 mm were considered as moderate activity. *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella sonnei*, *Salmonella typhi*, and *Pseudomonas aeruginosa* were used as the bacterial tested organisms. In vitro cytotoxic activity of all the synthesized ligands (L^1–L^5) and their metal(II) complexes (C^1–C^{10}) were studied using the protocol of Meyer et al. [23]. Brine shrimp (*Artemia salina* Leach) eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm), filled with artificial sea water, which was prepared with commercial salt mixture and double-distilled water. Data were analyzed by Finney computer program to determine the LD_{50} values [24].

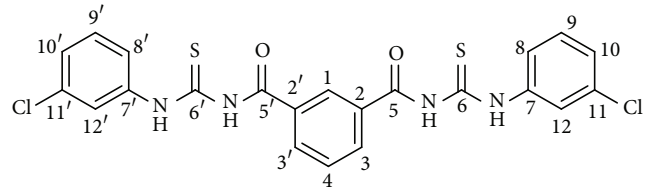
3. Results and Discussion

3.1. Chemistry. The synthetic route for the newly synthesized compounds, N^3, $N^{3'}$-bis(disubstituted)isophthalyl-bis(thioureas) (L^1–L^5), is illustrated and outlined in Scheme 1. The proposed structure for metal(II) complexes is presented in Figure 2. Isophthalyl chloride 2 was treated with anhydrous KSCN using dry acetone as solvent to give isophthalyl isothiocyanate 3 in quantitative yield. The isophthalyl isothiocyanate 3 are useful synthetic building blocks which may be efficiently used for the synthesis of N,N′-disubstituted thioureas and benzenedicarbonyl bisthioureas [15]. In the present work, the isothiocyanate 3 was not isolated and treated directly with some primary amines to give the corresponding dithiourea derivatives (L^1–L^5) in good to excellent yields. Since the addition to –N=C=S system and nucleophilic substitution at carbonyl-carbon atom may compete with one another, it has been noticed that isothiocyanate 3 reacted additively with amines to give corresponding bisthiourea derivatives (L^1–L^5). The target compounds (L^1–L^5) were purified by recrystallization from DMF and characterized by IR and ^1H NMR data. Metal complexation was carried out in DMF solvent. A solution of the thiourea (0.05 mol) in DMF (15 cm^3) was added to a solution of MCl_2 (0.05 mol) in DMF (15 cm^3), while M = Cu for (C^1–C^5) and M = Ni for (C^6–C^{10}) (Figure 2). The mixture was refluxed for 6 hours then concentrated to one-third volume and kept at room temperature for 2 hours. The solid product formed was filtered, washed with DMF, and dried. All the synthesized metal(II) complexes were characterized by IR, magnetic moments, and electronic spectral measurements.

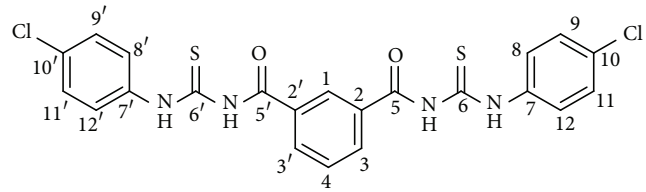
3.2. IR Spectra. In IR spectrum, the ν(C=S) peak appeared in the region 1135–1197 cm^{-1}, whereas the N–H peaks appeared from 3330 to 3362 cm^{-1}. Carbonyl absorption bands were observed in the region of 1654–1685 cm^{-1} for ligands. On comparison of the IR spectra of the ligands with their metal(II) complexes, different results were obtained. The most striking changes are that the N–H stretching frequency in the free ligands disappears completely in agreement with both ligand and complex structure and the complexation reaction. Another striking change is observed for the carbonyl stretching vibrations which shift to higher frequencies upon complexation of the thiourea ligands because the deprotonation induces delocalization of the carbonyl stretching vibration and confirming coordination through oxygen. Due to this deprotonation which induces delocalization and the ν(C=O) cm^{-1} stretching vibration frequency decreases by



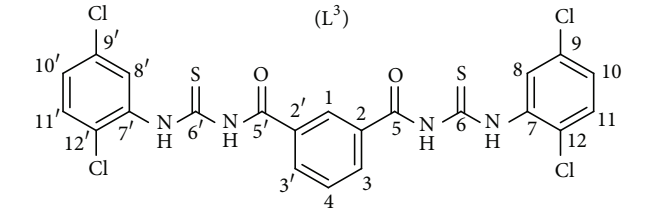
(L^1)



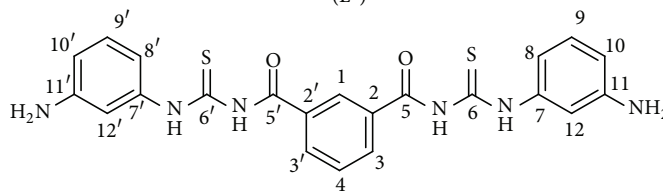
(L^2)



(L^3)



(L^4)



(L^5)

Figure 1: Bisthiourea derivatives (ligands L^1–L^5).

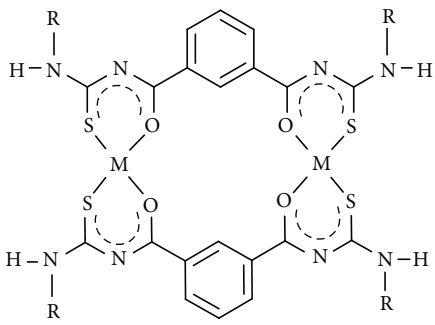


Figure 2: Proposed structure for metal(II) complexes (C^1–C^{10}) where M = Cu(II) for (C^1–C^5) and M = Ni(II) for (C^6–C^{10}).

about 150 cm^{-1}. The same trend is observed for the thiocarbonyl stretching vibration frequencies, which are observed at approximately 1135–1197 cm^{-1} in the free ligands and shift to higher frequency after complexation; unfortunately, this vibration could not be assigned unambiguously because it is located in the fingerprint zone of the IR spectra. Moreover, in the far infrared region the bands at 335–367 cm^{-1} and 429–463 cm^{-1} attributed to ν(M–S) and ν(M–O) were observed for all the metal(II) complexes.

3.3. ^{1}*H NMR Spectra.* In ^{1}H NMR spectra, the CSN^1-H protons appeared as singlet in the range δ 12.29–12.47 ppm whereas CON^3-H protons appeared at δ 8.51–9.81 ppm,

Table 1: Electronic spectra and magnetic moments of metal(II) complexes.

No.	λmax (cm^{-1})	Band assignment	B.M.	No.	λmax (cm^{-1})	Band assignment	B.M.
C^1	11868 18657	$^2B_1g \rightarrow {}^2A_1g$ $^2B_1g \rightarrow {}^2B_2g$	1.72	C^6	15987 18820 23765	$^1A_1g \rightarrow {}^1A_2g$ $^1A_1g \rightarrow {}^1B_2g$ $^1A_1g \rightarrow {}^1Eg$	dia
C^2	11734 18854	$^2B_1g \rightarrow {}^2A_1g$ $^2B_1g \rightarrow {}^2B_2g$	1.73	C^7	16560 18678 24340	$^1A_1g \rightarrow {}^1A_2g$ $^1A_1g \rightarrow {}^1B_2g$ $^1A_1g \rightarrow {}^1Eg$	dia
C^3	11470 18782	$^2B_1g \rightarrow {}^2A_1g$ $^2B_1g \rightarrow {}^2B_2g$	1.76	C^8	15443 18450 24300	$^1A_1g \rightarrow {}^1A_2g$ $^1A_1g \rightarrow {}^1B_2g$ $^1A_1g \rightarrow {}^1Eg$	dia
C^4	11765 18377	$^2B_1g \rightarrow {}^2A_1g$ $^2B_1g \rightarrow {}^2B_2g$	1.75	C^9	16970 19876 23345	$^1A_1g \rightarrow {}^1A_2g$ $^1A_1g \rightarrow {}^1B_2g$ $^1A_1g \rightarrow {}^1Eg$	dia
C^5	11561 18438	$^2B_1g \rightarrow {}^2A_1g$ $^2B_1g \rightarrow {}^2B_2g$	1.76	C^{10}	16821 19823 23347	$^1A_1g \rightarrow {}^1A_2g$ $^1A_1g \rightarrow {}^1B_2g$ $^1A_1g \rightarrow {}^1Eg$	dia

depending upon the nature of the group attached to N^3. The appearance of N^1-H proton at higher frequency may be attributed to the presence of carbonyl and thiocarbonyl groups which exert a strong deshielding effect. The ^{1}H NMR (DMSO) spectrum revealed signals at δ 12.29–12.47 ppm (2H, NH) in all the compounds (L^1–L^5) which indicates the NH group between (C=O) and (C=S) group remains unaffected regardless of attached to terminal N atom. The aromatic protons of the parent isophthalyl group appeared in the range of δ 7.33–8.56 ppm. While the aromatic protons of amine showed peaks in the range of δ 6.33–7.43.

3.4. ^{13}C NMR Spectra. The ^{13}C NMR spectra of the ligands (L^1–L^5) were taken in DMSO-d_6. The ^{13}C NMR spectral data are reported along with their possible assignments in the experimental section, and all the carbons were found in their expected region [25]. The conclusions drawn from these studies provided further support to the modes of bonding already explained in the IR and ^{1}H NMR spectral data. The ^{13}C NMR spectra of the ligands (L^1–L^5) showed the carbonyl carbon (C_5) at 164.5–167.6 ppm. The spectra of same ligands displayed thiocarbonyl carbons (C_6) in the region 180.1–184.1 ppm. Furthermore, all the ligands showed central benzene ring peaks in the region 123.4–134.2 ppm. The molecular structures of ligands are given in Figure 1.

3.5. Electronic Spectra and Magnetic Susceptibility Measurements of Complexes. The electronic spectra of Ni(II) complexes display bands in the regions of 15443–16970, 18450–19876, and 23345–24340 cm^{-1} assignable to $^1A_1g \rightarrow {}^1A_2g$ (ν_1), $^1A_1g \rightarrow {}^1B_2g$ (ν_2), and $^1A_1g \rightarrow {}^1Eg$ (ν_3) transitions, respectively, characteristic of square planar nickel(II) complexes. The first two bands are pure d-d transitions while the ν_3 band obviously was enveloped by a strong charge transfer transition. The assumed square planar geometry and diamagnetic d^8 configuration of Ni^{2+} complexes is confirmed from the value of its room temperature magnetic moment of zero. Cu(II) complexes displayed bands at 11470–11868 and 18377–18854 cm^{-1}, which may be assigned to the transitions $^2B_1g \rightarrow {}^2A_1g$ $_{(dx2-y2 \rightarrow dz2)}$ (ν_1), $^2B_1g \rightarrow {}^2B_2g$ $_{(dx2-y2 \rightarrow dz2)}$ (ν_2). The third band at around 28600 cm^{-1} may be due to charge transfer transitions. These observed transitions and magnetic moment values (1.7 to 1.76) B.M. suggest that Cu^{2+} complexes are square planar (Table 1).

3.6. Pharmacology

3.6.1. Antibacterial Assay. In vitro antibacterial activity of all the synthesized compounds was tested against six different bacterial strains [26]. The compounds (L^1–L^5) and their metal(II) complexes exhibited potential activity against all tested bacteria with highest inhibition zones (Table 2). L^1–L^4 showed strong activity against *B. subtilis, S. aureus, E. coli, S. sonnei, S. typhi,* and *P. aeruginosa*. L^5 showed no activity against all bacterial strains. However, the metal(II) complexes of L^5 showed activity against various bacterial strains. Ampicillin and Ciprofloxacin were used as standard antibiotics studied for comparing the results. All the compounds showed less activity as compared to standard drugs. The results of the present investigation demonstrated significant ($P < 0.05$) variations in the antibacterial activity of the compounds.

3.6.2. Cytotoxic Bioassay. Cytotoxicity (brine shrimp bioassay) was determined for all the ligands and their metal(II) complexes. The cytotoxicity is expressed as LD_{50}, that is, concentration, at which 50% of the viable cells were killed under the assay conditions. From the data recorded in (Table 3), it is evident that compound (C^4) displayed highest cytotoxic activity ($LD_{50} \geq 5.67 \times 10^{-4}$ moles/mL) against *Artemia salina*. Similarly compounds C^3, C^5 showed potent cytotoxic activity. All other synthesized compounds were almost inactive in this assay. It was interesting to note that complexation with copper increased cytotoxicity. These findings may help to serve as a basis for future direction

Table 2: In vitro antibacterial activity of ligands (L^1–L^5) and their metal(II) complexes.

Compounds	Test microorganisms/diameter of inhibition zone (IZ in mm)					
	Gram-positive bacteria		Gram-negative bacteria			
	B. subtilis	*S. aureus*	*E. coli*	*S. sonnei*	*S. typhi*	*P. aeruginosa*
L^1	34 ± 0.83^b	33 ± 1.67^c	35 ± 1.35^b	33 ± 1.64^b	31 ± 1.12^b	25 ± 0.76^c
L^2	28 ± 0.81^c	$32 \pm 2.11b^c$	31 ± 0.75^c	32 ± 0.63^d	27 ± 1.63^b	28 ± 0.81^b
L^3	32 ± 1.67^b	29 ± 1.45^c	31 ± 1.23^b	30 ± 1.63^b	29 ± 0.0^b	22 ± 0.70^c
L^4	34 ± 0.83^b	33 ± 1.42^c	32 ± 1.63^b	32 ± 0.63^d	27 ± 1.63^b	35 ± 1.35^b
L^5	—	—	—	—	—	—
C^1	37 ± 1.48^b	36 ± 1.34^c	37 ± 0.78^b	35 ± 1.34^b	34 ± 1.7^b	29 ± 0.74^c
C^2	29 ± 0.86^c	30 ± 2.11^{bc}	24 ± 0.75^c	21 ± 0.68^d	30 ± 1.65^b	31 ± 0.84^b
C^3	33 ± 1.67^b	29 ± 1.45^c	32 ± 1.63^b	34 ± 1.63^b	29 ± 0.0^b	22 ± 0.70^c
C^4	35 ± 0.81^e	36 ± 1.45^c	32 ± 1.63^b	33 ± 0.66^d	35 ± 1.20^a	32 ± 0.70^c
C^5	34 ± 0.41^c	35 ± 1.25^b	31 ± 1.20^a	34 ± 0.45^a	36 ± 1.0^f	30 ± 0.50^b
C^6	35 ± 0.83^b	34 ± 1.67^c	36 ± 1.35^b	37 ± 1.64^b	31 ± 1.12^b	31 ± 0.76^c
C^7	30 ± 0.85^c	31 ± 2.37^{bc}	29 ± 0.87^c	27 ± 0.66^d	31 ± 1.69^b	32 ± 0.83^b
C^8	34 ± 0.45^f	28 ± 0.21^f	33 ± 0.91^b	31 ± 1.30^e	30 ± 1.21^c	26 ± 0.81^e
C^9	34 ± 2.11^{bc}	35 ± 0.85^c	33 ± 0.0^b	34 ± 1.45^c	30 ± 1.44^c	31 ± 1.69^b
C^{10}	33 ± 0.01^b	34 ± 0.25^c	31 ± 0.10^c	33 ± 1.05^a	30 ± 1.04^c	31 ± 1.09^b
S^1	38 ± 0.41^a	36 ± 1.82^a	38 ± 1.61^a	36 ± 1.63^a	35 ± 1.63^a	34 ± 1.47^a
S^2	38 ± 0.81^a	35 ± 1.41^a	38 ± 1.20^a	37 ± 0.0^a	34 ± 0.0^a	33 ± 0.0^a

S^1: Ampicillin, S^2: Ciprofloxacin, —: NIL.
Values are mean ± SD of three separate experiments.
Letters in superscript show the significance of the results against single strain.

Table 3: Brine shrimp bioassay of ligands (L^1–L^5) and their metal(II) complexes (C^1–C^{10}).

Compounds	LD_{50} (M)	Compounds	LD_{50} (M)	Compounds	LD_{50} (M)
L^1	$>2.05 \times 10^{-4}$	C^1	$>5.32 \times 10^{-3}$	C^6	$>4.05 \times 10^{-3}$
L^2	$>1.93 \times 10^{-3}$	C^2	$>5.43 \times 10^{-3}$	C^7	$>3.53 \times 10^{-3}$
L^3	$>1.87 \times 10^{-3}$	C^3	$>1.76 \times 10^{-4}$	C^8	$>1.65 \times 10^{-5}$
L^4	$>2.01 \times 10^{-3}$	C^4	$>5.67 \times 10^{-4}$	C^9	$>4.39 \times 10^{-4}$
L^5	$>3.05 \times 10^{-3}$	C^5	$>4.21 \times 10^{-4}$	C^{10}	$>4.12 \times 10^{-4}$

towards the development of bacteriostatic agents of lower cytotoxicity.

4. Conclusion

Some novel $N^3, N^{3'}$-bis(disubstituted)isophthalyl-bis(thioureas) and their metal(II) complexes have been synthesized and characterized by analytical and spectral (IR, ^{1}H NMR and ^{13}C NMR, electronic) techniques. Antibacterial activity of these compounds was studied against bacterial strains. Some compounds showed potential activity against some bacterial strains and others exhibited strong antibacterial activity. These compounds were also screened for their cytotoxic inhibition activities. The outcomes of these studies also show the transition metal(II) complexes to be more antibacterial against one or more species as compared to the uncomplexed ligands. It was concluded that these compounds may be the potential source of active antibacterial agents.

Conflict of Interests

All the authors of this paper have no conflict of interests in publishing this material. All the coauthors agreed to publish this work.

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