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Synthesis, characterization and studies on antioxidant and molecular docking of metal complexes of 1-(benzo[d]thiazol-2-yl)thiourea

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Abstract. In the present study, a new thiourea derivative bearing benzothiazole ligand, 1-(benzo[d]thiazol-2-yl)thiourea (btt) and its ternary metal (Cu(II), Co(II) and Ni(II)) complexes were synthesized. The structural characterization was carried out by micro analysis, IR, ^{1}H-NMR, EPR, UV-Visible spectral analyses, molar conductance and thermal analysis studies. Spectral studies of complexes revealed that the metal complexes have distorted octahedral geometry. Molecular modelling study was performed to evaluate the recognition of target compounds at the 3MNG binding pocket. The docking results revealed that copper complex selectively binds to the crucial amino acid residues in the active site of 3MNG. The *in vitro* antioxidant activity of the ligand and its metal complexes was assayed by radical scavenging activity (DPPH, H_2O_2 and NO) and ferric reducing antioxidant power (FRAP) methods. The ligand showed moderate antioxidant activity whereas the metal complexes exhibited better antioxidant activity than that of the ligand. The results of the four methods proved that the copper complex is the most potent antioxidant among all the tested compounds.

Keywords. Benzo[d]thiazol; thiourea; metal complexes; antioxidant activity; molecular docking.

1. Introduction

Thioureas are important functional derivatives possessing good biological activities like antiviral, antibacterial, antifungal, antitubercular, anti-thyroidal, herbicidal and insecticidal.[1–4] These are also found as precursors or intermediates in the synthesis of a variety of heterocyclic systems such as imidazole-2-thiones, 2-imino-1,3-thiazolines, N-4-substituted-benzyl-*N*-tert-butylbenzyl thioureas as vanilloid receptors and antagonists in rat DRG neurons.[5] Further, nitrogen and sulfur donor atoms in thiourea provide a multitude of bonding possibilities, hence, its derivatives are versatile ligands and are able to coordinate to metal centers either as neutral ligands, mono anions or di-anions and form stable complexes.[6] They can behave as thiolate-type ligands, since a substituted thiourea bearing at least one hydrogen, $R^1R^2NC(=S)NHR^3$ can exist in a tautomeric thiolate form, i.e., $R^1R^2C(SH)=NR^3$. Metal complexes of thiourea derivatives are bio-active compounds and show wide range of biological activities.[7]

The thiazoles, containing N and S heterocycles, are found in many naturally occurring compounds.[8–11] Thiazoles are used as neuro protectors[12,13] and antioxidants.[14,15] They also form biologically potent metal complexes.[16–19] Metal complexes of thiazoles recently attracted attention of inorganic, metallo-organic as well as bio-inorganic chemists because of their extensive applications in wide ranging areas from material to biological sciences.[20]

Thioazoles attached with thiourea functional group are used as pharmaceutical agents.[21] The biological and synthetic significance of thiazole, and thiourea derivatives prompted us to synthesize new molecules: 1-(benzo[d]thiazol-2-yl)thiourea and its (Cu(II), Co(II) and Ni(II)) complexes. Transition metal ions such as Cu(II), Co(II) and Ni(II) were selected since they enhance biological activity.[22,23] The synthesized ligand and metal complexes were characterized by elemental analyses, IR, ^{1}H-NMR, ESR, UV-Visible spectra, molar conductance and thermal analysis studies. Further, the *in vitro* antioxidant activity and molecular modelling study against 3MNG binding pocket were performed.

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2. Experimental

2.1 *Materials and physical measurements*

Analytical grade (AR) chemicals with high purity were purchased from Sigma Aldrich, Spectrochem and S D Fine Chem. and used without further purification. The analysis of CHNS of ligand and metal complexes were carried out on Euro elemental analyzer. IR spectra were recorded on Bruker spectrometer. ^{1}H NMR was recorded on a Bruker Advanced Drx-200 MHz spectrometer with TMS as an internal standard. The melting points of the compounds were determined on Beijing XT-4-100x microscopic melting point apparatus and are uncorrected. TG/DTA curves for the complexes were recorded on Nietzsche thermo balance (model-STA 40 g) with PtVsP$_t$ 10% Rh thermocouple in dynamic air conditions between the room temperature (~20°C) and 1020°C (rate 10 K/min).

2.2 *Preparation of the ligand: 1-(benzo[d]thiazol-2-yl) thiourea*

Aniline (0.1 mole, 9.10 mL) (**1**) and concentrated hydrochloric acid (15 mL) were mixed and the solution was warmed for 30 min. A saturated solution of ammonium thiocyanate in water (5 g in 10 mL) was added slowly to the above solution. The mixture was boiled until the solution attained the turbidity. This solution was poured in cold water. The precipitate was filtered and re-crystallized from aqueous ethanol to afford pure phenylthiourea (**2**). The phenylthiourea (20 mmol, 3.04 g) in chloroform (20 mL) was brominated by using bromine solution in chloroform (5%) till the orange-yellow colour appeared. The slurry was kept overnight at ambient temperature. The obtained precipitate was filtered and washed with chloroform until the color disappeared. The precipitate as hydrobromide was dissolved in rectified spirit (30 mL) and basified with ammonia solution (20 mL). The precipitate was filtered, washed with water, dried and recrystallized from ethanol:dichloromethane mixture (1:2) to get pure benzo[d]thiazole-2-amine (**3**). The compound (**3**) was treated with concentrated hydrochloric acid (20 mL) and warmed for 30 min. A saturated solution of ammonium thiocyanate in water (5 g in 10 mL) was added slowly to the above solution. The mixture was boiled until the solution got turbidity. The turbid solution was poured in cold water. The resulting precipitate was filtered and recrystallized from aqueous ethanol (80%) to obtain pure 1-(benzo[d]thiazol-2-yl)thiourea (**4**). Yellow solid, Yield: 80%, M.p.: 174°C, Anal. Calc. for $C_8H_7N_3S_2$: C, 45.91; H, 3.37; N, 20.08; S, 30.64%. Found: C, 45.86; H, 3.41; N, 20.05; S, 30.61%. IR (cm^{-1}); 3318 (w), 1565 (m), 842 (m), 710 (s). ^{1}H-NMR (DMSO-d$_6$): δ 2.54 (s, 1H, NH), 9.30 (2H, s, NH$_2$), 7.20-7.85 (4H, m, Aromatic). ^{13}C-NMR (DMSO-d6) in δ (ppm): 144.3, 169.01, 116.31, 123.3, 124.0, 127.2, 127.7, 128.3. Electronic spectra (DMF, cm^{-1}); 47619, 37735, 43516, 26667.

2.3 *Synthesis of Metal Complexes*

All the three metal complexes (**5-7**) were prepared by a general procedure. To the hot solution (60°C) of the appropriate metal chloride (1 mmol) in ethanol and water mixture (1:1, 25 mL), 1-(benzo[d]thiazol-2-yl) thiourea (**4**) (0.41 g, 2 mmol) was added to the same solvent mixture (20 mL). The resulting mixture was stirred under reflux for 1–2 h, the precipitate was filtered off, washed twice with a 1:1 ethanol: water mixture.

2.3a *[Cu(btt)$_2$Cl$_2$] (**5**)*: Green solid, Yield: 83%, M.p.: 246°C, Anal(%). Calcd(%). for $C_{16}H_{12}Cl_2CuN_6S_4$: C, 34.90, H, 2.23, N, 15.22, S, 23.26, Cu, 11.54, Cl, 12.89. Found: C, 34.88, H, 2.23, N, 15.22, S, 23.26, Cu, 11.54, Cl, 12.89%. IR data (KBr, cm^{-1}); 3329 (w), 1584 (m), 762 (m), 718 (s). ^{1}H-NMR (DMSO-d6; δ 10.23 (2H, s, NH$_2$), 7.25-7.91(4H, m, Aromatic). Electronic spectra (DMF, cm^{-1}); 15267, 16525, 17211, 21739.

2.3b *[Co(btt)$_2$Cl$_2$] (**6**)*: Reddish-brown solid, Yield: 79%, M.p.: 320°C, Anal(%). Calcd(%). For $C_{16}H_{12}Cl_2CoN_6S_4$: C, 35.20, H, 2.25, N, 15.36, S, 23.45, Co, 10.81, Cl 13.02. Found: C, 35.17, H, 2.21, N, 15.38, S, 23.47, Co, 10.79, Cl, 12.98. IR data (KBr, cm^{-1}): 3332 (w), 1585 (m), 786 (m), 724 (s). ^{1}H-NMR (DMSO-d$_6$; δ 9.07 (2H, s, NH$_2$), δ 7.40–7.82 (4H, m, Aromatic). Electronic spectra (DMF, cm^{-1}); 17856, 21734.

2.3c *[Ni(btt)$_2$Cl$_2$] (**7**)*: Pale green solid, Yield: 82%, M.p.: 284°C, Anal(%). Calcd(%). For $C_{16}H_{12}Cl_2NiN_6S_4$: C, 35.22, H, 2.25, N, 15.36, S, 23.45, Ni, 10.81, Cl 13.02. Found: C, 35.19, H, 2.21, N, 15.39, S, 23.48, Ni, 10.75, Cl, 12.98. IR data (KBr, cm^{-1}); 3326 (w), 1585 (m), 774 (m), 719 (s). ^{1}H-NMR (DMSO-d$_6$; δ 9.71 (2H, s, NH$_2$), δ 7.25-7.91 (4H, m, Aromatic). Electronic spectra (DMF, cm^{-1}); 24271, 15360, 9576.

2.4 *Molecular docking*

Molecular modeling studies were performed using Molecular Operating Environment (MOE) version

2008.10 for the synthesized compounds, which exhibited promising and lower antioxidant activity *in vitro* to find the preferred binding conformations in the receptor.[24] Three dimensional structures of the ligand and metal complexes were outlined by Hyper Chem software which is a refined molecular modeling environment that uniting with quantum chemical calculations, molecular mechanics and dynamics.[25] The starting coordinates of the human antioxidant enzyme in complex[26,27] with the competitive inhibitor DTT (PDB: 3MNG) were taken from the Protein Data Bank (http://www.rcsb.org/pdb). The docking study was carried out by the protein loaded in MOE and the errors of the protein were corrected by the *structure preparation processes* in MOE. After the corrections, the ligand molecules were removed and the polar hydrogen atoms were added to the isolated target with their standard geometry. Energy minimization (MMFF94x, gradient: 0.05) was performed and the lowest energy conformers were selected as the global minimal for modeling study. The active site was identified from PDB Sum as well as correlated with 'Site Finder' module of MOE to define the docking site for the ligands. The standard protocol implemented in MOE 2008.10 was followed for docking procedure. The selected ligands were docked against the lead competitive inhibitor ligand DTT at the crystal enzyme structure of the target protein and the best energy conformations of receptor-ligand were studied, and the energy of binding was calculated as the difference between the energy of the complex and the individual energies of enzyme and ligand.

2.5 *Antioxidant activity*

2.5a *1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay*: DPPH has been widely used to evaluate the free radical scavenging capacity of different antioxidants. The radical in the DPPH has a strong absorption maximum at 517 nm and the absorbance of DPPH decreases, when the radical reacts with the antioxidant. All the tested samples in various concentrations (50, 75 and 100 μg/mL) were prepared in MeOH and the homogeneous solutions were achieved by stirring. Aliquot of test sample (1 mL) was added to 4 mL of 0.004% (w/v) methanol solution of DPPH and the reaction mixture was vortexed for 1 min and kept at room temperature for 30 min in the dark to complete the reaction. The absorbance was read against blank at 517 nm. The synthetic antioxidant BHT was used as positive control. The ability of the tested samples at tested concentration to scavenge DPPH radicals was calculated using equation 1. The experiment was carried out in triplicate and the average values calculated.

$$\%\ \text{of scavenging} = \left[1 - \left(A_{sample} - A_{sample-blank}\right) / A_{control}\right] \times 100 \quad (1)$$

Here, $A_{control}$ is the absorbance of the control (DPPH solution without the test compound) and A_{sample} is the absorbance of the test sample (DPPH solution with the test compound) and $A_{sample-blank}$ is the absorbance of the sample solution (the test compound solution without DPPH).

2.5b *Hydrogen peroxide (H_2O_2) scavenging activity*: The hydroxyl radical ($OH^{\bullet}$) in aqueous media was generated through the Fenton reaction. The solutions of the test compounds were prepared in DMF. The reaction mixture containing 2.5 mL of 0.15 M phosphate buffer (pH = 7.4), 0.5 mL of 114 μM safranin, 1 mL of 945 μM EDTA-Fe(II), 1 mL of 3% H_2O_2 and 30 μL of the test compound solution were prepared. The reaction mixture without the test compound was used as the control. The reaction mixtures were incubated at 37°C for 60 min on a water bath. Absorbances (A_i, A_0, A_c) at 520 nm were measured. The scavenging ratio for $OH^{\bullet}$ was calculated from equation 2.

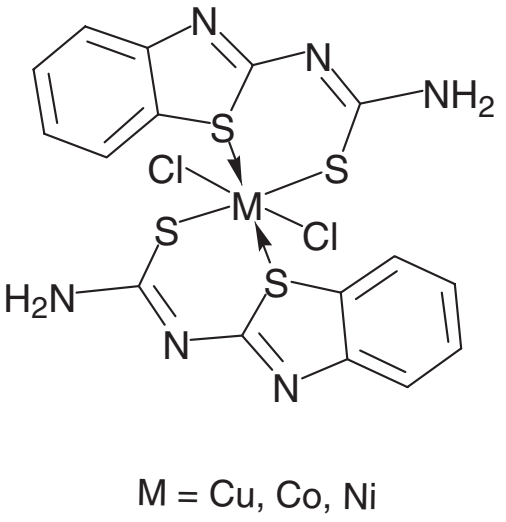
$$Scavenging\ ratio\ (\%) = \frac{A_i - A_0}{A_c - A_0} \times 100 \quad (2)$$

Where, A_i is the absorbance in the presence of the tested compound, A_0 is the absorbance in the absence of the tested compound and A_c is the absorbance in the absence of the tested compound, EDTA-Fe(II) and H_2O_2.

2.5c *Nitric oxide (NO) scavenging activity*: Nitric oxide scavenging activity of the tested samples was measured using a slightly modified protocol of Green *et al*. and Marcocci *et al*.[28,29] All the tested samples in various concentrations (50, 75 and 100 μg/mL) were prepared in DMF and homogeneous solutions were achieved by stirring. Nitric oxide radicals (NO) were generated from 1 mL of sodium nitroprusside (10 mM) and 1.5 mL of phosphate buffer saline (0.2 M, pH 7.4) were added to the test compounds and incubated for 150 min at 25°C. The reaction mixture of the above samples (1 mL each) was treated with 1 mL of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthylene diamine dihydrochloride). The absorbance was measured at 546 nm. Butylated hydroxy toluene (BHT) was used as a positive control. The ability to scavenge the NO radicals was calculated by equation 3.

$$\%\ of\ Scavenging\ = \frac{A_{control} - A_{sample}}{A_{\text{control}}} \times 100 \quad (3)$$

2.5d *Ferric reducing antioxidant power (FRAP)*: The reducing power of synthesized compounds was determined according to the method of Oyaizu.[30] Various concentrations of the tested compounds, 50, 75 and 100 mM were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferric cyanide and incubated at 50°C for 20 min. 2.5 mL of 10% trichloro acetic acid was added to this mixture and centrifuged at 3000 rpm for 20 min. The upper layer (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride and the absorbance was measured at 700 nm using a spectrophotometer (Shimadzu 160A). Increase in the absorbance of the reaction mixture indicates higher reducing power. The experiment was repeated in triplicate and mean reducing power values are summarized in Figure S11 (in Supplementary Information, SI). BHT was used as standard and compared with the reducing power of the synthesized complexes.



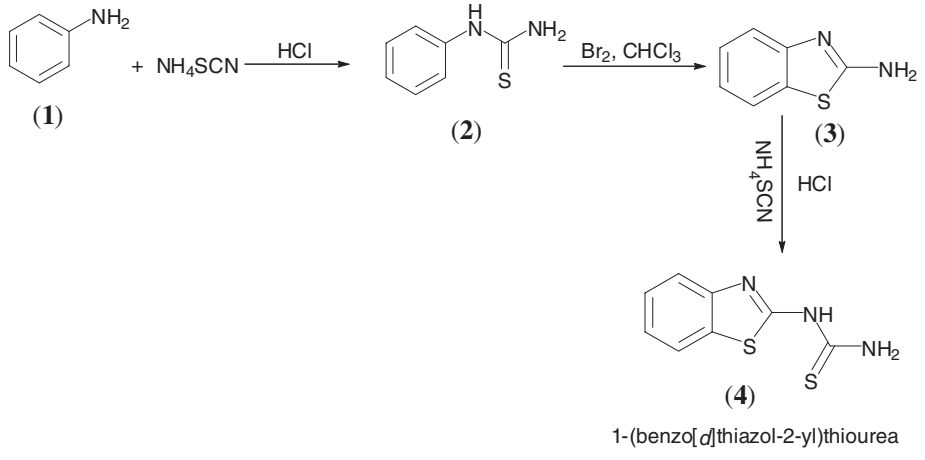
Scheme 2. Proposed structure for metal complexes.

3. Results and Discussion

Keeping in mind the biological importance of the thiourea derivatives bearing heterocyclic scaffold and metal complexes, the ligand, 1-(benzo[d]thiazol-2-yl)thiourea **(4)** was synthesized from the aniline **(1)** as depicted in scheme 1. All the synthesized intermediates and final product were recrystallized from methanol and used for further study. Later, the desired metal complexes, copper(II), cobalt(II) and nickel(II) were prepared (scheme 2). Analytical data indicated the formation of 1:2 metal complexes of ligands with Co(II), Cu(II) and Ni(II) metal ions. The molar conductance values of the complexes (measured in 10^{-3} M DMF) are in the range of 15–18 Ω^{-1} cm^2 mol^{-1} indicating the non-electrolytic nature of the complexs. Solubility of metal complexes is low in water, ethanol, chloroform, acetone and most organic solvents, but have solubility in DMSO, DMF and decompose at higher temperature.

3.1 *IR Spectral Analysis*

The FT-IR spectra of ligand and its metal complexes are shown in figure S1. The bands at 1517 and 751 cm^{-1} due to thioamide stretching and bending vibrations, respectively, of the free ligand are shifted to lower values indicating coordination of thiolate sulphur to the metal ions (table S1). This negative shift of ν (C=S) in the complexes has already been indicated by Campbell.[31] In the spectra of all the complexes, a new strong band is found in the range of 1612–1649 cm^{-1}, which may be due to the newly formed ν(C=N) bond as a result of enolization. A strong band observed at 751 cm^{-1} is attributed to ν(C–S–C) of thiazole ring.



Scheme 1. Synthetic route for ligand.

Complexation of the ligand with metals leads to shift in the band to lower frequency. This is probably due to the coordination properties of the ligand and suggesting the involvement of sulfur atoms in the bonding with the metal ions.[32] The bands near 435–450 cm^{-1} are ascribed to ν (M–S).[33] This presents a good evidence for the participation of thiazole and thiourea sulfur atoms in complex formation.

3.2 *Nuclear magnetic resonance spectroscopy*

The formation of ligand was supported by the ^1H NMR spectrum, by the appearance of a sharp singlet at 2.54 ppm, corresponding to the N(2)H proton figure S2). The ^1H-NMR spectra of the complexes when compared with the parent ligand did not show the signal for N(2)H at 2.54 ppm indicating that the ligand is coordinated to the metal ions in all the complexes in the thiolate form. The multisignals within the range of 7.20–7.85 ppm are assigned to the aromatic protons of benzothiazole ring. These signals are shifted towards low field in the metal complexes. A singlet for two protons at δ 9.30 is assigned to NH_2 protons and shifted towards up field in the complexes. This information suggests the adjustment of electronic current upon coordination of >C=S group to the metal ion.

3.3 *Electron paramagnetic resonance spectrum of the Cu(II) complex*

The X-band EPR spectrum of Cu(II) complex was recorded at room temperature (figure S3). The g_{iso} values and the geometric parameter G, i.e. the measurement of exchange interaction between the copper centers were evaluated by using equations 4 and 5:

$$g_{iso} = \frac{g_{||} + g_{\perp}}{3} \tag{4}$$

$$G = \frac{g_{||} - 2.0023}{g_{\perp} - 2.0023} \tag{5}$$

The evaluated value of g tensor parameters shows the order as $g_{||} > g_{\perp} > 2.0023$ which reveals that $d_{x^2-y^2}$ is the ground state[34] and also indicates that the unpaired electron is localized in $d_{x^2-y^2}$ orbital of the Cu(II) ion. The tetragonal structure is thus confirmed for the complexes. The complexes in the present study showed the value of G<4 (G = 2.1383) which indicated the effective interaction between the copper centers and the ligand.[35]

3.4 *Electronic spectrum of the 1-(benzo[d]thiazol-2-yl)thiourea and its complexes*

Electronic spectral data of the ligand and its metal complexes are shown in table S2. The electronic spectrum of the ligand exhibited absorption bands at 47,619 cm^{-1} and 37,735 cm^{-1} corresponding to π-π* transitions of the benzenoid system of benzothiazole moiety.[36] The absorption band at 43,516 cm^{-1} is attributed to the π-π* transitions of the thiazole moiety.[37] The broad band at 26,667 cm^{-1} is due to the transition within the molecule, essentially an intra molecular charge transfer interaction. The electronic absorption spectrum of Cu(II) complex exhibited three broad, low intensity bands centered at 15,267 cm^{-1}, 16525 cm^{-1} and 17,211 cm^{-1}. These bands might reasonably be assigned to Cu(II) d–d transitions, $^2B_{1g} \rightarrow {}^2A_{1g}$ ($d_{x^2-y^2}$–d_{z^2}), $^2B_{1g} \rightarrow {}^2B_{2g}$ ($d_{x^2-y^2}$–d_{xy}) and $^2B_{1g} \rightarrow {}^2Eg$ ($d_{x^2-y^2}$–d_{xz}, d_{yz}) respectively. These d–d transitions are normally close in energy. These transitions are due to the ^2Eg and $^2T_{2g}$ states of the octahedral Cu(II) ion (d9) split under the influence of the asymmetric filling causing the tetragonal distortion. A shoulder band observed at 21,739 cm^{-1} is due to ligand–metal charge transfer (LMCT). The electronic spectrum of Ni(II) complex displayed three broad bands at 24,271 cm^{-1} (ν1), 15,360 cm^{-1} (ν2) and 9576 cm^{-1} (ν3) which are assigned to the spin-allowed transitions from the ground state $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ states, respectively. The positions of bands indicated that the Ni(II) complex has six coordinated tetragonal geometry. The electronic spectrum of Co(II) complex showed two spin-allowed transitions at 17856 and 21734 cm^{-1} which are assignable to $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transitions respectively. The positions of bands indicated that the Co(II) complex has six coordinated tetragonal geometry.

3.5 *Thermal analysis of the metal complexes*

In the present investigation, the thermo gravimetry analysis (TG-DTA) of the complexes was carried out in the temperature range of 20–1020°C with a sample heating rate 10°C/min in a static nitrogen atmosphere. During the heating, the complexes have undergone a series of thermal changes associated with a weight loss in the samples. The TGA curves of the complexes (figures S4–S6) show three stages of decomposition within the temperature range of 160–770°C. The first stage of decomposition is observed at 170°C to 320°C which corresponds to the loss of HCl. This process is accomplished by exothermic peaks observed

at 270–280°C. Second stage occurred within the temperature range of 280–620°C. This stage involves the loss of thiourea moiety. This degradation is accomplished by exo-effects with maxima 370–390°C. Third stage of decomposition is observed in the temperature range of 400–770°C. For cobalt complex, fourth stage is observed in between 760 to 870°C. This process is accompanied by an endo-effect observed at 870°C. Above this temperature, no weight change is observed, leaving the metal oxide as the final residue.

3.6 *Molecular docking studies*

The antioxidant assay of the synthesized ligand and its metal complexes has revealed that the copper complex exhibited more antioxidant activity, almost equal to the standard, BHT. In order to interpret the binding mode of the compounds in comparison to the competitive antioxidant inhibitor, 1,2-dithiane-4,5-diol (DTT),[26] the molecular modeling study was conducted for these compounds at the human antioxidant enzyme (3MNG) pocket. The starting coordinate of the human antioxidant enzyme in complex[27] with the competitive inhibitor DTT (PDB: 3MNG) was obtained from the Protein Data Bank (http://www.rcsb.org/pdb). The 3MNG pocket was loaded into Molecular Operating Environment MOE version 2008.10[24] with high resolution of 2.70 Å and a library was constructed for these selected molecules with receptor. The active site was identified from PDB Sum and recognized the residues, Gly 46, Gln 133, Asn 24, Val 94, Glu 16, Arg 86 to be interacting with DNA as well as correlated with 'Site Finder' module of MOE to define the docking site for the ligands. Hydrophobic and hydrophilic spheres are used to identify the interactive positions which will be the potential ligand binding sites in each possible position.

The binding recognition profile of these synthesized molecules was attained by performing the docking simulation at 3MNG pocket. The most common H-interactions in the docked structures, bond lengths, bond angles and binding energies are shown in table 1. In the docked conformers, all the compounds were showing best bonding energies in the order of **5** (−97.4802 kcal/mol) > **4** (−30.3237) > **7** (−25.7574) > **6** (−20.5211) > DTT (−12.3869). Interestingly, it was observed that the active molecules, even the least active molecule **6**, showed better score than antioxidant competitive inhibitor, DTT.

The competitive antioxidant inhibitor, DTT (Figure S7a) formed two bifurcated H-bonds with the key amino acid residues, Gly 47 and Cys 47; the latter is an essential residue for binding and biological activity. Besides, one more H-bond was formed with the Thr 44.[38] The docked conformer of ligand (figure S7b) showed no hydrogen bonds among active site residues of 3MNG protein, but it has hydrophobic and electrostatic interactions among ligand and active site residues of 3MNG. The molecular docking of the copper complex (figure S7c) indicated that it forms one stable hydrogen bonding with a bond length 2.76 Å from CN of copper complex with the CN group of the key pocket residue of Gly 46. Nickel complex was found to be forming two hydrogen bondings (figure S7d) with bond of lengths 2.89 and 2.57 Å from –CN of the complex with the –CO and –CN group of the key pocket residue of Asn24. Cobalt complex showed two hydrogen bonds with bond lengths of 2.73 and 2.41 Å from –NC of the complex with –CO groups of Glu16 and Val94 (figure 1). Finally, the molecular docking studies for the selected compounds revealed that the synthesized compounds are antioxidant competitive inhibitors in comparison to antioxidant inhibitor, DTT at 3MNG binding pocket.

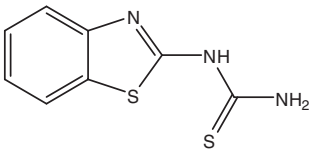
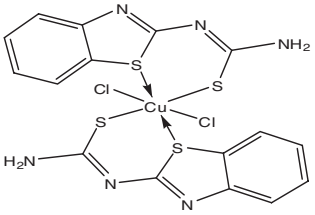
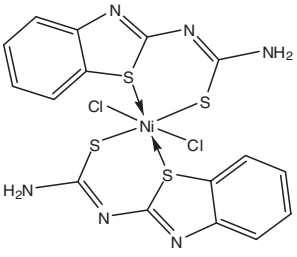
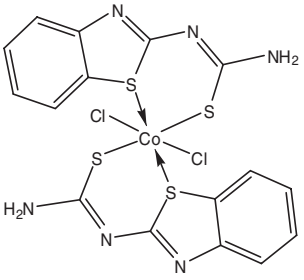
3.7 *Antioxidant activity of ligand and complexes*

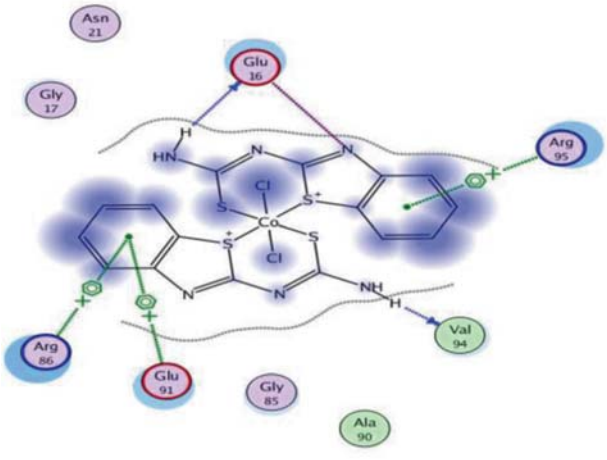
In vitro antioxidant activity of 1-(benzo[d]thiazol-2-yl)thiourea and its metal (Cu(II), Ni(II) and Co(II)) complexes was evaluated by using radical scavenging methods, DPPH, H_2O_2, NO and reducing power method (FRAP). The antioxidant property of the tested samples was evaluated at different concentrations (50, 75 and 100 μg/mL) and butylated hydroxyl toluene (BHT) was used as a standard for the comparison of the activity.

3.7a *DPPH radical scavenging activity*: DPPH radical scavenging activity (RSA) evaluation is a standard assay in antioxidant activity studies. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517 nm due to DPPH free radical. This purple colour generally fades/disappears when an antioxidant is present in the medium. More potent antioxidants are able to reduce the absorbance rapidly.

DPPH radical scavenging activity data (figure S8) of the synthesized ligand and metal complexes states that all the compounds showed potency, high to moderate activity. The metal complexes exhibited more radical scavenging activity than that of the ligand. Cu(II) metal complex exhibited potent antioxidant activity almost close to the standard, BHT and Co(II) complex showed lower antioxidant activity, and Ni(II) complex and the ligand showed moderate activity.

Table 1. Molecular docking parameter of ligand and its metal complexes.

S.No.	Product	Interactions Protein—Ligand	Binding energies (kcal/mol)	Bond angle(°)	Bond length (Å)
1		Lys 32, Lys 33, Gly 133, Asp 134	−30.3237	–	–
2		Gly 46CN—NC	−97.4802	107.6	2.76
3		Asn 24CO—NC Asn 24CN—NC	−20.5211	119.0 136.5	2.89 2.57
4		Glu 16CO—NC Val 94CO—NC	−25.7574	120.6 136.4	2.73 2.41



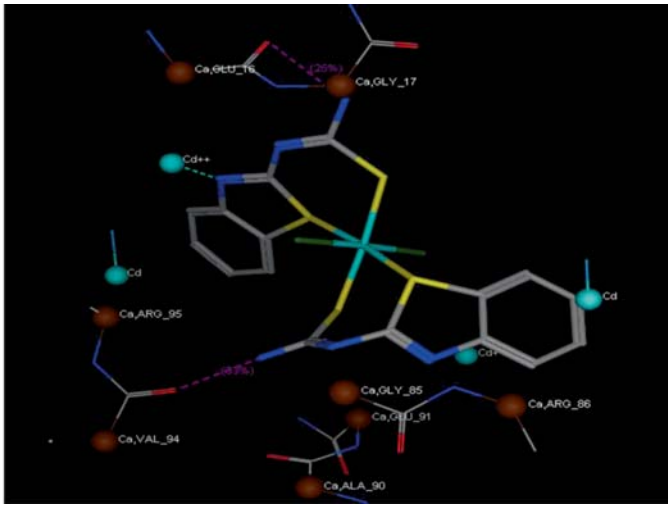


Figure 1. Interaction mode of the cobalt complex docked and minimized in the 3MNG binding pocket.

3.7b *Hydrogen peroxide scavenging activity*: Hydrogen peroxide is formed *in vivo* by many oxidizing enzymes such as superoxide dismutase in biological systems. It can cross membranes and may slowly oxidize a number of compounds.[39] It is used in the respiratory burst of activated phagocytes. It is known that H_2O_2 is toxic and induces cell death. Hydrogen peroxide can attack many cellular energy producing systems. The ability of 1-(benzo[d]thiazol-2-yl)thiourea (btt) and its metal complexes to scavenge hydrogen peroxide at different concentrations was studied and the results (figure S9) were compared with the standard. The results showed that the free ligand and its metal complexes exhibited an effective hydrogen peroxide scavenging activity.

3.7c *Nitric Oxide (NO) Scavenging Activity*: NO may divert $O_2^{\cdot -}$ mediated toxic reactions to other oxidative and less damaging, pathways, thus protecting $O_2^{\cdot -}$ sensitive target molecules. Nitric oxide undergoes a facile radical-radical reaction with $O_2^{\cdot -}$, to yield $ONOO^-$, a reaction that is three times faster than the SOD-catalyzed dismutation of $O_2^{\cdot -}$. This diversion of $O_2^{\cdot -}$ through $ONOO^-$ oxidation and decomposition pathways would also limit H_2O_2 accumulation and subsequent reactions of H_2O_2, by decreasing the amount of $O_2^{\cdot -}$ available for spontaneous or SOD-catalyzed dismutation. We find that the inhibitory effect of the compounds tested on $NO^{\bullet}$ is concentration-dependent and suppression ratio increases with increasing sample concentrations (figure S10). The copper(II) complex is the most effective among all the complexes.

3.7d *Ferric reducing antioxidant power (FRAP)*: Figure S11 shows the reducing power of 1-(benzo[d] thiazol-2-yl)thiourea (btt) and its metal complexes. In this assay, the yellow colour of the test solution changes to various shades of green and blue depending upon the reducing power of each compound. The presence of reducers (i.e., antioxidants) causes the reaction of Fe^{3+}/ferricyanide complex to the ferrous form giving, after addition of trichloro acetic acid and ferric chloride, the Perl's Prussion blue that can be monitored at 700 nm. The reducing power of the standard (BHT) at various concentrations showed higher absorbance value compared to that of the new compounds. The reducing power of the new complexes solutions increased with increase in concentration. Metal complexes showed much better activity than the ligand. All the three complexes showed moderate to high activity which are slightly less than that of the standard: BHT>Cu>Ni>Co> Ligand.

4. Conclusions

The ligand, 1-(benzo[*d*]thiazol-2-yl)thiourea (btt) and its metal(II) complexes (Cu, Co and Ni) have been synthesized and characterized by spectral and analytical data. The spectral data showed that the thiourea derivative exists as bidendate ligand by bonding to the metal ions through the deprotonated thiol group and thiazole sulphur. The analytical data showed the presence of one metal ion per two ligand molecules and suggest a mononuclear structure for the complexes. The correlation of the experimental data allows assigning a tetragonal geometry to all the three synthesized complexes. Biological activity studies revealed that the antioxidant activity of the ligand was found to be enhanced on complexation with metal ions. Among the studied complexes, copper complex exhibited high antioxidant activity. Finally, the molecular docking studies of the synthesized compounds were carried out. Molecular docking studies revealed that all the synthesized compounds have relatively less binding energy as compared to the standard drug and may be considered as a good antioxidant agents. Hence this study has widened the scope of developing this type of metal based drugs as promising antioxidant agents.

Supplementary Information

Figures S1 to S11, and tables S1 and S2 are provided in the supplementary information. Supplementary Information is available at www.ias.ac.in/chemsci.

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