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SYNTHESIS, CHARACTERIZATION AND UREASE INHIBITION STUDIES OF TRANSITION METAL COMPLEXES OF THIOUREAS BEARING IBUPROFEN MOIETY

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ABSTRACT

Starting from ibuprofen, a non-steroidal anti-inflammatory drug, *N,N'*-disubstituted thiourea derivatives were synthesized by refluxing the acid chloride of ibuprofen with potassium thiocyanate followed by substituted anilines to get *N*-2-(4-(2-methylpropyl)phenyl)propionoyl-*N´-(2´*-bromophenyl) thiourea **(1),** *N*-2-(4-(2-methylpropyl)phenyl)propionoyl-*N´*-(2´-chlorophenyl) thiourea **(2)** and *N*-2-(4-(2-methylpropyl)phenyl)propionoyl-N´-(3´,4´-dichlorophenyl) thiourea **(3).** Metal complexes **(4-15)** of **(1-3)** were synthesized by refluxing it with one equivalence salts of Co (II), Ni(II), Pb(II) and Cu(I). Structures of all the synthesized thiourea ligands and their metal complexes were determined by FTIR and ^{1}HNMR spectroscopy. Shift in stretching frequency in FTIR and resonance frequency in ^{1}HNMR spectroscopic data suggested that the ligands and metal are coordinated to afford respective metal complexes $M[L]_n$. The synthesized compounds were tested against urease enzyme and the results were compared with standard thiourea, as positive control. Most of the investigated compounds showed potential inhibitory activity against *Jack bean* urease. Compound **5** was found to be the most potent urease inhibitor with IC_{50} of 14.6 µM, whereas compounds 8**,** 11 and 12 possessed potent urease inhibition. The tested compounds can be taken as lead molecules for gastrointestinal ulcer therapy.

Key words: Amides, Thioureas, Metal complexes, Ibuprofen, Urease.

1. INTRODUCTION

Urease (urea aminohydrolase E.C. 3.5.1.5) is nickel containing metalloenzyme which belongs to the super family of amidohydrolases and phosphotriestreases, responsible for the hydrolytic decomposition of urea (Holm 1997). It was the first enzyme which crystallized from the plant source *Canavalia ensiformis* and was also the first protein containing nickel in the active site (Saeed et al. 2014; Saeed et al. 2013). Because of these characteristics urease is considered as a paradigm in the development of inorganic biochemistry. Urease catalyses the hydrolysis of urea in different plants, algae, fungi, and microorganisms to produce ammonia and carbamate (Mobley HL 1989). Enzyme catalysed hydrolysis-process occurred at a rate of approximately 10^{14} times the rate of the unanalyzed reaction and considered as the final step of nitrogen metabolism in all living organisms. Different types of urease enzymes can be isolated from bacteria, fungi, algae and plants but enzymatic activity is same due to the occarance of the same order of amino acid (Bacanamwo M 2002; Benini S 1999). Urease activity due to infectious microorganism is the major reason for many deceases in humans such as development of infection stones, pyelonephritis, stomach and peptic ulcer, urinary catheter encrustation, and hepatic com (Gripenberg-Lerche C 2000; Mobley et al. 1995; Mobley HL 1989). To overcome these diverse types of problems, urease inhibitors have received special attention over the past few years. These inhibitors generally divided into two classes, substrate structural analogs like hydroxamic acid (Kobashi K 1962,; Krajewska B 2009; Muri EMF 2003) and those which affect the mechanism of the reaction like phosphoramidat (Amtul Z 2002; Kot M 2001). Lansoprazole (Nagata K 1993), omeprazole (Kuehler TC 1995), thiol-compounds (Todd MJ 1989), quinines (Bundy LG 1973) and schiff base derivatives (Hanif M 2012) are reported as potent urease inhibitors.

On the other hand Ibuprofen, an analgesic drug, is famous for its anti-pyretic and anti-inflammatory properties and found promising to cut the tumor growth in colorectal cancer (KD 2003; McMillan DC 1995; Rao P 2008). The incorporation of ibuprofen moiety in metal complexes of thioureas has opened a new area of research in organometallics (Yuan YF 2001). Thioureas and their derivatives have gained the significance importance in the field of medicinal chemistry because of their biological activities such as: antituberculosis, analgesic, antimicrobial, antiarrhythmic, anti-HIV, anti-inflammatory, rodenticides, fungicide, herbicides and as inhibitors of phenoloxidase enzyme (Kumavat 2013). N, N'-Disubstituted thioureas are of great importance due to their ability to concentrate and separate the toxic metals. Therefore metal complexes of aroyl thiourea are excellent candidates to use as lixiviant, chromogenic ionophore towards fluoride ion detection, in opto-electric modulation, for developing polymer membrane ion-selective electrode and also exhibit great anti-bacterial, anti-fungal, anti-tumor, anti-viral, anti-mycobacterial and also posses urease inhibition activities (Arslan et al. 2006; D'Cruz OJ 2003; Del CR 2002; Dhumane NR 2006; Moro AC 2009; Sun MZ 2008; Tremblay L 1996). Thioureas and their derivative are considered as very effective inhibitory active agents against the urease enzyme (Aslam MA 2011). Considerable attention has been focused on transition metal complexes containing thioureas. Thioureas considered as very good chelating agents for transition metal complexes. These complexes play important role to inhibit the over activities of wide range of enzymes due to their specific molecular architectures (Carcu V 2000).

Literature reveals the efficiency of thioureas ligand toward complex formation so the aim of present study is the synthesis of some aroyl thiourea bearing ibuprofen moiety and their metal complexes with Ni(II), Pb(II), Co(II) and Cu(I) and their evaluation as urease inhibitor.

2. EXPERIMENTAL

2.1 Instruments

Melting points were determined using capillary tubes and an electrothermal melting point apparatus, model MEL-TEMP MP-D Mitamura Riken Kogyo, Japan, and were uncorrected.

Infrared spectra (250-4000 cm^{-1}) were recorded on a Perkin-Elmer system 2000 FT-IR spectrometer and on Perkin-Elmer system 2000 FTIR spectrometer using nujol mull. The ^{1}HNMR were recorded on a Varian Associates Inova spectrometer (400 and 300 MHz) using deuterated solvents and tetramethyl silane (TMS) as a reference. ^{1}HNMR Spectra were also recorded on Bruker AM-400 NMR spectrophotometer using $CDCl_3$ as an internal reference. Chemical shifts are given in δ scale (ppm). However the splitting of proton resonances in the ^{1}HNMR spectra is defined as s = singlet, d = doublet, t = triplet and m = multiplet pattern.

2.2 Synthesis of the *N, N'*-Disubstituted thioureas (1-3)

To a 100 ml of two necked round bottom flask, freshly prepared solution of ibuprofen [2-(4-(2 methylpropyl)phenyl)propanoic acid] (0.0048 mol) was added to thionyl chloride(1.5 eq) and heated under reflux to give benzoyl chloride. The resulting reaction mixture was added dropwise to a suspension of potassium thiocyanate (0.0048 mol) and reaction mixture was refluxed for 30 min, then cooled to room temperature. A solution of substituted aniline (0.0048 mol) in dry acetone was added and the resulting mixture was stirred for 2 h. Completion of reaction was checked by TLC. On cooling, the reaction mixture was slowly poured into acidified (pH 4–5) chilled water and stirred well with a glass rod. The solid products **(1-3)** obtained were purified by slow evaporation technique (see Scheme 1) (Rasmussen CR 1988; Rauf MK 2006) .

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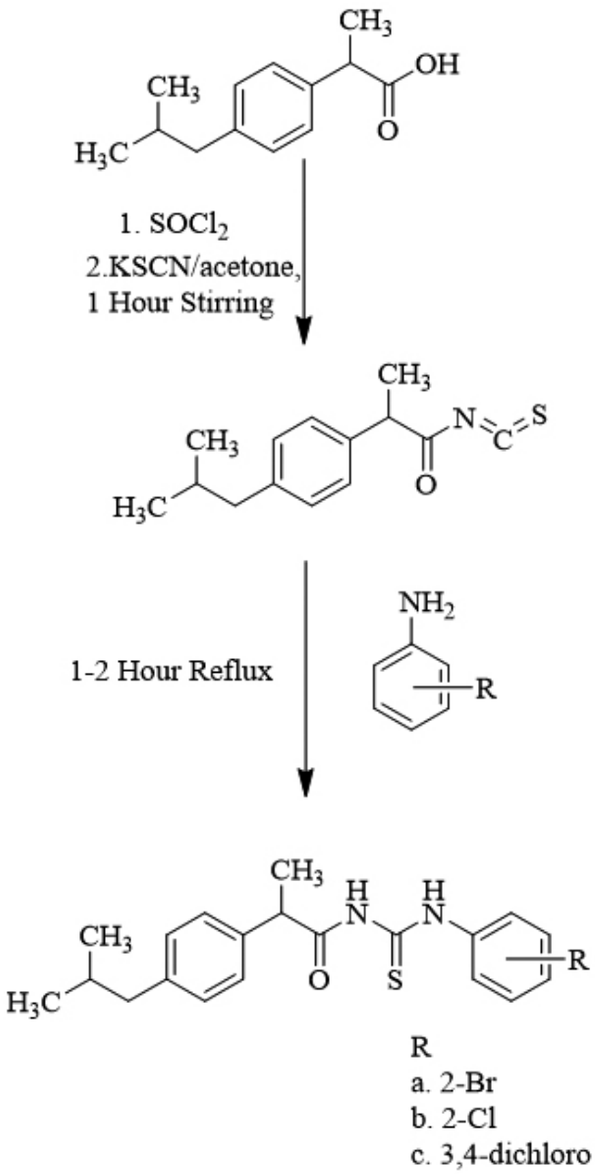


Scheme 1

Table 1. Physical data of the N,N'-disubstituted thioureas **(1-3)**.

No.	R	M.P(°C)	Yield (%)
1	2-Br	68-69	75
2	2-Cl	130-131	85
3	3,4-Dichloro	84-85	78

***N*-2-(4-(2-methylpropyl)phenyl)propionoyl-*N*´-**(2´-bromophenyl) thiourea (1)

Yellow solid, Yield: 75%, R_f(*n*-hexane : ethyl acetate, 9: 1) = 0.2. Melting point.: 68-69 °C. IR (cm^{-1}): ν (N-H) 3212 (w), ν (C=O) 1692 (s), ν (arom.C=C) 1589 (m), ν (C=S) 1236 (s), ν (C-N) 1158 (m). ^{1}HMR ($CDCl_3$): 12.43 (s, 1H, CSNH), 8.47 (s, 1H, CONH), 7.91 (brdd, 1H, ArH-5' J = 7.8, J = 1.9), 7.59 (dd, 1H, ArH-2', J = 8.1, J = 0.9), 7.41 (dt, 1H, ArH-4´, J = 8.1, J = 0.9) 7.11-7.30 (m, 5H, ArH-2,3,5,6,3') 3.70 (q, 1H, CH-CH_3, J = 14.1, J = 6.9), 2.49 (d, 2H, Ar-CH_2-H, J = 7.2), 1.84-1.90 (m, 1H, CH $(CH_3)_2$), 1.58 (d, 3H, CH_3-CH-Ar, J = 7.2), 0.93 (d 6H, $(CH_3)_2$-CH), J = 6.6).

***N*-2-(4-(2-methylpropyl)phenyl)propionoyl-*N*´-**(2´-chlorophenyl) thiourea (2)

Off white solid, Yield: 85%, R_f(*n*-hexane : ethyl acetate, 9:1) = 0.4, Melting point: 130-131°C, IR (cm^{-1}): ν (N-H) 3281 (w), ν (C=O) 1659 (s), ν (arom. C=C) 1588 (m), ν (C=S) 1268 (s), ν (C-N) 1176 (m). ^{1}HMR ($CDCl_3$): 12.39 (s, 1H, CSNH), 8.54 (s, 1H, CONH), 7.09-7.83 (m, 8H, ArH-2,3,5,6,2´,3´,5´,6´) 3.73 (q, 1H, CH-CH_3, J = 14.1, J = 6.9), 2.51 (d, 2H, Ar-CH_2-H, J = 7.2), 1.87-1.95 (m, 1H, CH-$(CH_3)_2$), 1.62 (d, 3H, CH_3-CH-Ar, J = 7.2), 0.97 (d, 6H, $(CH_3)_2$-CH), J = 6.6).

***N*-2-(4-(2-methylpropyl)phenyl)propionoyl-*N*´-**(3´,4´-dichloroPhenyl) thiourea (3)

Off white solid. Yield: 78%. R_f (*n*-Hexane : Ethyl acetate, 9:1) = 0.3. Melting point: 84-85°C, IR (cm^{-1}): ν (N-H) 3299 (w), ν (C=O) 1688 (s), ν (arom. C=C) 1575 (m), ν (C=S) 1200 (s), ν (C-N) 1151 (m). ^{1}HMR ($CDCl_3$): 12.27 (s, 1H, CSNH), 8.69 (s, 1H, CONH), 7.17-7.94 (m, 8H, ArH-2,3,5,6,2´,3´,5´,6´) 4.49 (q, 1H, CH-CH_3, J = 13.8, J = 6.9), 2.45 (d, 2H, Ar-CH_2-H, J = 7.2), 1.89-1.97 (m, 1H, CH $(CH_3)_2$), 1.67 (d, 3H, CH_3-CH-Ar, J = 7.2), 0.94 (d, 6H, $(CH_3)_2$-CH), J = 6.6).

2.3. Synthesis of the Metal Complexes (4-15)

2.3.1. Co(II) complex of N,N´-disubstituted thiourea (4-6)

The Co (II) complexes **(4-6)** were prepared according to a prescribed method [9, 15]. The freshly prepared solution of metal salt $CoCl_2.6H_2O$

(0.00021 moles) in ethanol (EtOH) was added dropwise to the ligand in a 1:1 molar ratio with a small excess of ligand in dichloromethane (DCM) and stirred for 6 hours. The green color precipitates were filtered and recrystallized from an ethanole–dichloromethane mixture (1 : 1) (Scheme 2) (Douglass 1934; Nadeem S 2009).

Co(II) complex of *N*-2-(4-(2-methylpropyl)phenyl)propionoyl-*N′*-(2′-bromophenyl) thiourea (4)

Green solid. Yield: 73%. Melting point: 98-99 °C. IR (cm^{-1}): ν (C=O) 1696 (s), ν (arom.C=C) 1589 (m), ν (C=S) 1238 (s), ν (C-N) 1162 (m). ^{1}HMR ($CDCl_3$) 8.36 (s, 1H, CSNH), 7.01-7.52 (m, 8H, ArH-2,3,5,6,2′,3′,5′,6′) 3.76 (q, 1H, CH-CH_3, J = 13.8, J = 6.9), 2.38 (d, 2H, Ar-CH_2-H, J = 7.2), 1.84-1.99 (m, 1H, CH-$(CH_3)_2$), 1.44 (d, 3H, CH_3-CH-Ar, J = 7.2), 0.86 (d, 6H, $(CH_3)_2$-CH), J = 6.6), *Anal, Calc.* for $C_{43}H_{55}Br_2CoN_4O_2S_2$: C, 54.78, H, 5.88, N, 5.94. Found: C, 55.18, H, 5.48, N, 5.54.

Co(II) complex of *N*-2-(4-(2-methylpropyl)phenyl)propionoyl-*N′*-(2′-chlorophenyl) thiourea (5)

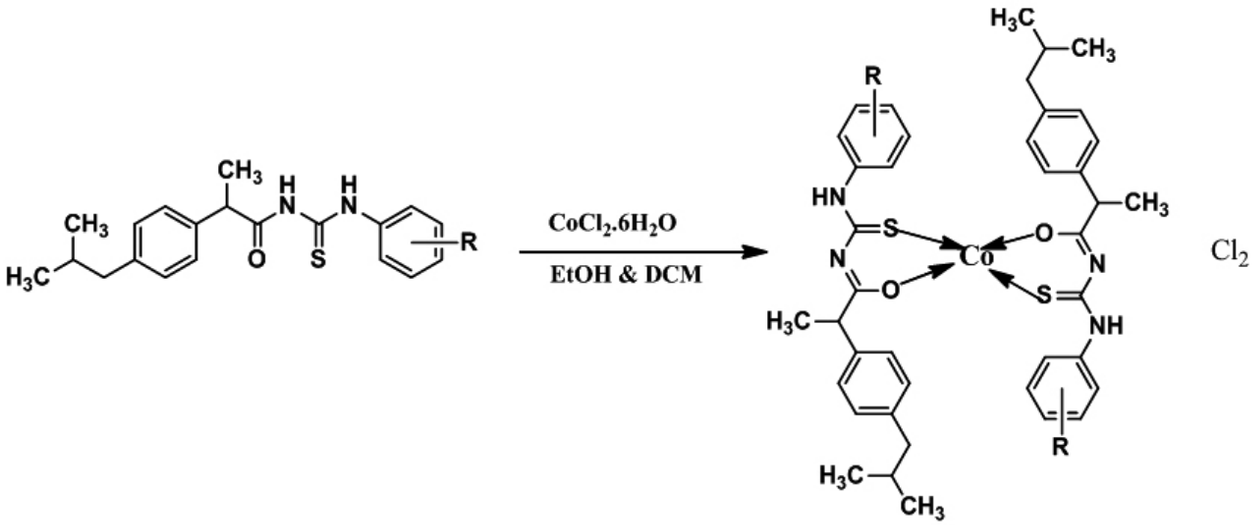
Green solid. Yield: 79%. Melting point: 95-96 °C. IR (cm^{-1}): ν (C=O) 1686 (s), ν (arom.C=C) 1591 (m), ν (C=S) 1231 (s), ν (C-N) 1192 (m).^{1}HMR ($CDCl_3$) 8.32 (s, 1H, CSNH), 6.41-6.97 (m, 8H, ArH-2,3,5,6,2′,3′,5′,6′) 3.01 (q, 1H, CH-CH_3, J = 13.8, J = 6.9), 1.73 (d, 2H, Ar-CH_2-H, J = 7.2), 1.10-1.26 (m, 1H, CH-$(CH_3)_2$), 0.83 (d, 3H, CH_3-CH-Ar, J = 7.2), 0.15 (d, 6H, $(CH_3)_2$-CH), J = 6.6), *Anal, Calc.* for $C_{43}H_{55}Cl_2CoN_4O_2S_2$: C, 60.48, H, 6.49, N, 6.56. Found: C, 60.88, H, 6.08, N, 6.15.

Co(II) complex of *N*-2-(4-(2-methylpropyl)phenyl)propionoyl-*N′*-(3′,4′-dichlorophenyl) thiourea (6)

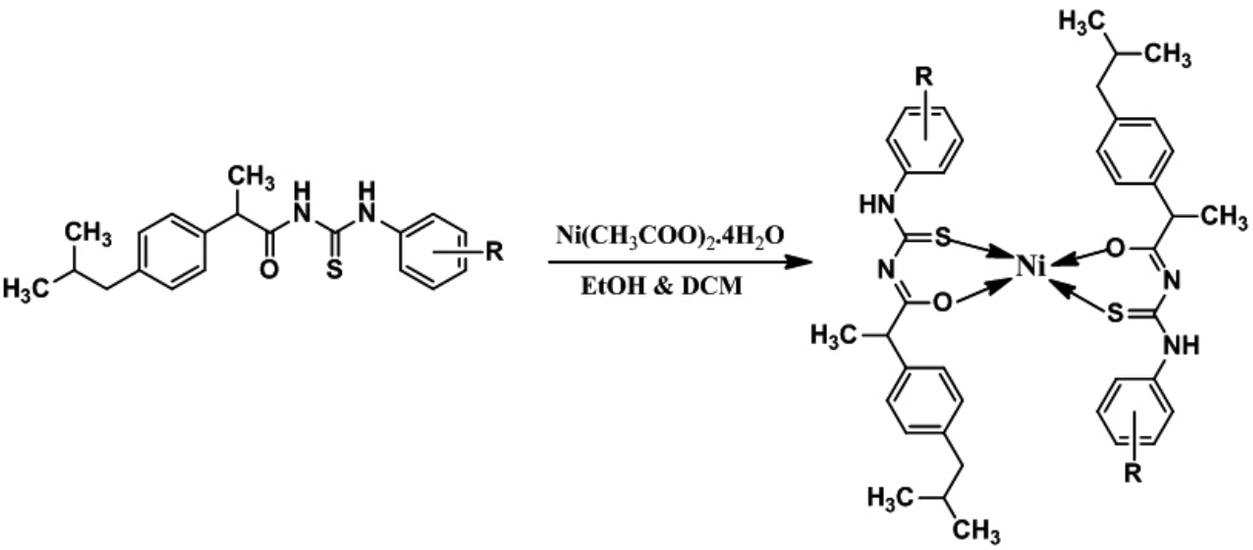
Brown solid. Yield: 84%. Melting point: 102-103 °C. IR (cm^{-1}): ν (C=O) 1695 (s), ν (arom.C=C) 1576 (m), ν (C=S) 1236 (s), ν (C-N) 1149 (m). ^{1}HMR ($CDCl_3$): 7.97 (s, 1H, CSNH), 7.06-7.81 (m, 8H, ArH-2,3,5,6,2′,3′,5′,6′) 4.27 (q, 1H, CH-CH_3, J = 13.8, J = 6.9), 2.39 (d, 2H, Ar-CH_2-H, J = 7.2), 1.74-1.86 (m, 1H, CH-$(CH_3)_2$), 1.52 (d, 3H, CH_3-CH-Ar, J = 7.2), 0.79 (d, 6H, $(CH_3)_2$-CH), J = 6.6), *Anal, Calc.* for $C_{43}H_{53}Cl_4CoN_4O_2S_2$: C, 55.97, H, 5.79, N, 6.07. Found: C, 55.57, H, 6.19, N, 6.03.

2.3.2. Ni(II) complex of *N,N′*-disubstituted thiourea (7-9)

A solution of the $Ni(CH_3COO)_2$ (0.00021 mol) in EtOH (4 ml) was added dropwise to a solution of the ligand (0.00042 mol) in DCM (13 ml) at room temperature and the resulting reaction mixture was stirred for 4 hours. The solid products obtained were filtered and recrystallized from dichloromethane-ethanol mixture (1:1) (Scheme 3) (Beyer L 1975; Douglass 1934).



Scheme 2



Scheme 3

Ni(II) complex of *N*-2-(4-(2-methylpropyl)phenyl)propionoyl-*N*′-(2′-bromophenyl) thiourea (7)

Yellow solid. Yield: 59%. Melting point: 91-92 °C. IR (cm^{-1}): ν (C=O) 1659 (s), ν (arom.C=C) 1587 (m), ν (C=S) 1241 (s), ν (C-N) 1148 (m). ^{1}HMR ($CDCl_3$) 8.29 (s, 1H, CSNH), 7.06-7.59 (m, 8H, ArH-2,3,5,6,2′,3′,5′,6′) 3.81 (q, 1H, CH-CH_3, J = 13.8, J = 6.9), 2.40 (d, 2H, Ar-CH_2-H, J = 7.2), 1.72-1.87 (m, 1H, CH-$(CH_3)_2$), 1.38 (d, 3H, CH_3-CH-Ar, J = 7.2), 0.85 (d, 6H, $(CH_3)_2$-CH), J = 6.6), *Anal, Calc.* for $C_{43}H_{53}Cl_4NiN_4O_2S_2$: C, 55.97, H, 5.79, N, 6.07. Found: C, 55.57, H, 6.19, N, 6.03.

Ni(II) complex of *N*-2-(4-(2-methylpropyl)phenyl)propionoyl-*N*′-(2′-chlorophenyl) thiourea (8)

Yellowish orange. Yield: 67%. Melting point: 100-101°C. IR (cm^{-1}): ν (C=O) 1691 (s), ν (arom.C=C) 1589 (m), ν (C=S) 1250 (s), ν (C-N) 1158 (m). ^{1}HMR ($CDCl_3$) 8.18 (s, 1H, CSNH), 6.87-7.39 (m, 8H, ArH-2,3,5,6,2′,3′,5′,6′) 3.51 (q, 1H, CH-CH_3, J = 13.8, J = 6.9), 2.42 (d, 2H, Ar-CH_2-H, J = 7.2), 1.56-1.69 (m, 1H, CH-$(CH_3)_2$), 1.36 (d, 3H, CH_3-CH-Ar, J = 7.2), 0.62 (d, 6H, $(CH_3)_2$-CH), J = 6.6), *Anal, Calc.* for $C_{43}H_{55}Br_2N_4NiO_2S_2$: C, 54.79, H, 5.88, N, 5.94. Found: C, 54.39, H, 5.48, N, 6.34 %.

Ni(II) complex of *N*-2-(4-(2-methylpropyl)phenyl)propionoyl-*N*′-(3′,4′-dichlorophenyl) thiourea (9)

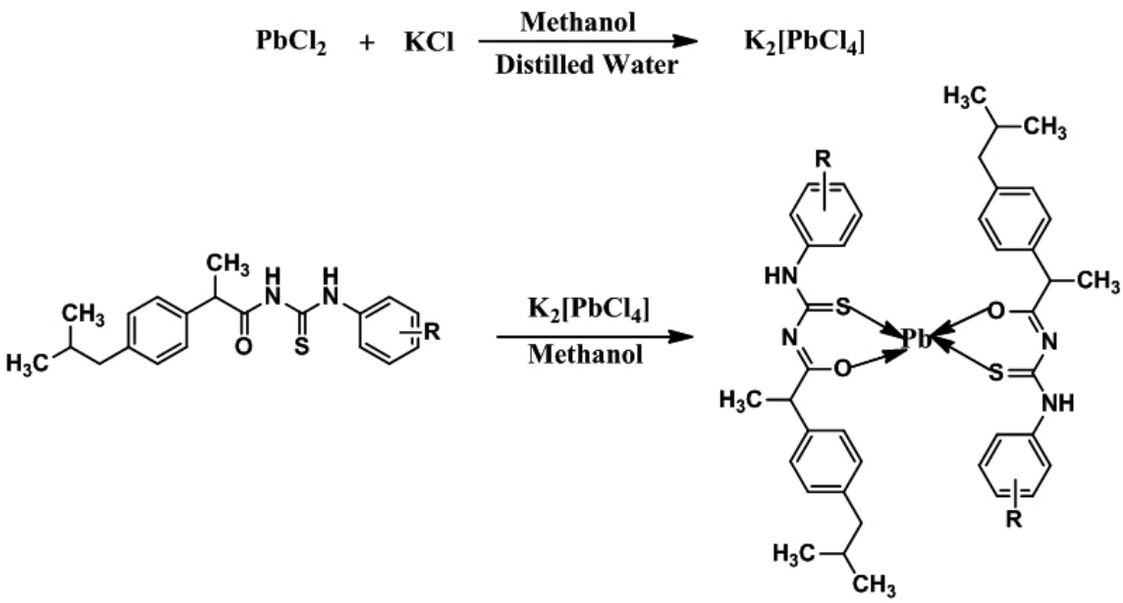
Color: brown. Yield: 84%. m.p.: 102-103 °C dec. IR (cm^{-1}): ν (N-H) 3387 (w), ν (C=O) 1692 (s), ν (arom.C=C) 1576 (m), ν (C=S) 1236 (s), ν (C-N) 1149 (m), NMR data; ^{1}HMR ($CDCl_3$) 7.97 (s, 1H, CSNH), 7.06-7.81 (m, 8H, ArH-2,3,5,6,2′,3′,5′,6′) 4.27 (q, 1H, CH-CH_3, J = 13.8, J = 6.9), 2.39 (d, 2H, Ar-CH_2-H, J = 7.2), 1.74-1.86 (m, 1H, CH-$(CH_3)_2$), 1.52 (d, 3H, CH_3-CH-Ar, J = 7.2), 0.79 (d, 6H, $(CH_3)_2$-CH), J = 6.6). *Anal, Calc.* for $C_{43}H_{55}Cl_4N_4NiO_2S_2$: C, 55.98, H, 5.79, N, 6.07. Found: C, 56.38, H, 5.39, N, 6.03.

2.3.3. Pb(II) complex of N,N′-disubstituted thiourea (10-12)

Synthesis of $K_2[PbCl_4]$

$K_2[PbCl_4]$ was synthesized by reacting excess of KCl with $PbCl_2$. In this regard $PbCl_2$ (0.000179 mol) was suspended in methanol and KCl solution was added drop-wise to above solution and reaction mixture was stirred for 3 hours. At the end, the off white suspension obtained was cooled in ice bath for 1 hour to afford off white precipitates of $K_2[PbCl_4]$.

For the synthesis of Pb(II) complexes (12-15) the ligand solution (0.000358 moles) in 10 mL of methanol was added drop wise to above solution of $K_2[PbCl_4]$ and the reaction mixture was stirred for 6 hours. The yellowish precipitates were filtered off and allowed to stand at room temperature for 2 days. Purification of the crude was carried out by using methanol as recrystalising solvent (Scheme 4) (Rauf MK 2010)



Scheme 4

Pb(II) complex of N-2-(4-(2-methylpropyl)phenyl)propionoyl-N′-(2′-bromophenyl) thiourea (10)

Yellow solid. Yield: 78%. Melting point. 90-91°C. IR (cm^{-1}): ν (C=O) 1667 (s), ν (arom.C=C) 1589 (m), ν (C=S) 1239 (s), ν (C-N) 1159 (m). ^{1}HMR ($CDCl_3$) 8.19 (s, 1H, CSNH) 7.47 (dd, 1H, ArH-5' J = 7.8, J = 1.5), 7.37 (dd, 1H, ArH-2', J = 8.1, J = 1.5), 7.30 (d, 1H, ArH-4′, J = 7.8) 7.19-7.27 (m, 4H, ArH-2,3,5,6) 7.14 (d, 1H, ArH-3′, J = 8.1) 3.88 (q, 1H, CH-CH_3, J = 13.8, J = 6.9), 2.46 (d, 2H, Ar-CH_2-H, J = 7.2), 1.78-1.91 (m, 1H, CH-$(CH_3)_2$), 1.50 (d, 3H, CH_3-CH-Ar, J = 7.2), 0.89 (d 6H, $(CH_3)_2$-CH), J = 6.6), *Anal, Calc.* for $C_{43}H_{55}Br_2N_4O_2PdS_2$: C, 52.15, H, 5.60, N, 5.66. Found: C, 51.85, H, 5.20, N, 6.06 %.

Pb(II) complex of N-2-(4-(2-methylpropyl)phenyl)propionoyl-N′-(2′-chlorophenyl) thiourea (11)

Yellow solid. Yield: 91%. Melting point. 118-119°C. IR (cm^{-1}): ν (C=O) 1681 (s), ν (arom.C=C) 1589 (m), ν (C=S) 1250 (s), ν (C-N) 1158 (m). ^{1}HMR ($CDCl_3$) 8.26 (s, 1H, CSNH), 6.91-7.46 (m, 8H, ArH-2,3,5,6,2′,3′,5′,6′) 3.29 (q, 1H, CH-CH_3, J = 14.1, J = 6.9), 2.31 (d, 2H, Ar-CH_2-H, J = 7.2), 1.63-1.79 (m, 1H, CH-$(CH_3)_2$), 1.41 (d, 3H, CH_3-CH-Ar, J = 7.2), 0.71 (d, 6H, $(CH_3)_2$-CH), J = 6.6), *Anal, Calc.* for $C_{43}H_{55}Cl_2N_4O_2PdS_2$: C, 57.30, H, 6.15, N, 6.22. Found: C, 57.70, H, 6.55, N, 6.44 %.

Pb(II) complex of N-2-(4-(2-methylpropyl)phenyl)propionoyl-N′-(3′,4′-dichlorophenyl) thiourea (12)

Yellow solid. Yield: 71%. Melting point. 105-106°C. IR (cm^{-1}): ν (C=O) 1692 (s), ν (arom.C=C) 1576 (m), ν (C=S) 1265 (s), ν (C-N) 1150 (m). ^{1}HMR ($CDCl_3$) 7.72 (s, 1H, CSNH), 7.16-7.64 (m, 8H, ArH-2,3,5,6,2′,3′,5′,6′) 4.28 (q, 1H, CH-CH_3, J = 14.1, J = 6.9), 2.44 (d, 2H, Ar-CH_2-H, J = 7.2), 1.82-1.98 (m, 1H, CH-$(CH_3)_2$), 1.59 (d, 3H, CH_3-CH-Ar, J = 7.2), 0.91 (d, 6H, $(CH_3)_2$-CH), J = 6.6), *Anal, Calc.* for $C_{43}H_{53}Cl_4N_4O_2PdS_2$: C, 53.23, H, 5.51, N, 5.77. Found: C, 543.63, H, 5.11, N, 6.17 %.

2.3.4. Cu(I) complex of N,N′-Disubstituted thiourea (13-15)

For the synthesis of Cu(I) complexes **(13-15)** thiourea ligand (0.000625 mol) was dissolved in 35 ml of acidified methanol (3.5 ml, 0.5% HCl) on stirring. Powdered Cu(I)Cl (0.00025 moles) was dissolved in the above solution and resulting suspension was allowed to stir for 4 hours. The yellowish precipitates of Cu(I) complexes were filtered and washed well with cold methanol.The solid products were purified by slow evaporation technique (Scheme 5) (Rauf MK 2009).

Cu(I) complex of N-2-(4-(2-methylpropyl)phenyl)propionoyl-N′-(2′-bromophenyl) thiourea (13)

Yellow solid. Yield: 74%. Melting point. 82-83 °C. IR (cm^{-1}): ν (C=O) 1666 (s), ν (arom.C=C) 1585 (m), ν (C=S) 1238 (s), ν (C-N) 1168 (m). ^{1}HMR ($CDCl_3$) 8.45 (s, 1H, CSNH) 7.02-7.93 (m, 8H, ArH-2,3,5,6,2′,3',4′,5′) 3.82 (q, 1H, CH-CH_3, J = 13.8, J = 6.9), 2.41 (d, 2H, ArCH_2-H, J = 7.2), 1.81-1.94 (m, 1H, CH-$(CH_3)_2$), 1.64 (d, 3H, CH_3-CH-Ar, J = 7.2), 0.84 (d, 6H, $(CH_3)_2$-CH), J = 6.6), *Anal, Calc.* for $C_{43}H_{55}Br_2CuN_4O_2S_2$: C, 54.15, H, 5.85, N, 5.91. Found: C, 54.55, H, 5.45, N, 6.31 %.

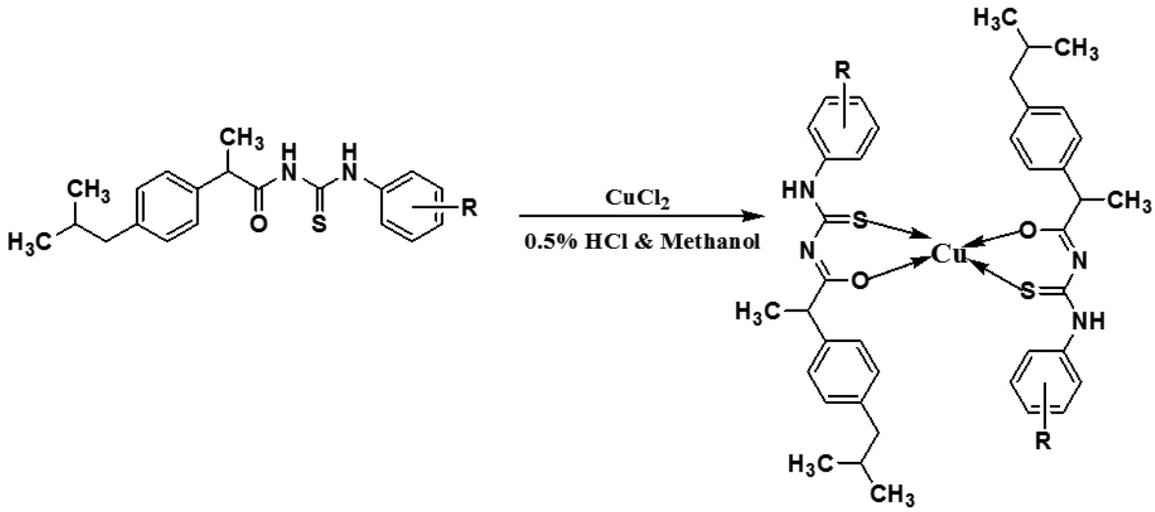
Cu(I) complex of N-2-(4-(2-methylpropyl)phenyl)propionoyl-N′-(2′-chlorophenyl) thiourea (14)

Yellow solid. Yield: 54%. Melting point. 110-111 °C. IR (cm^{-1}): IR (cm^{-1}):

ν (C=O) 1692 (s), ν (arom.C=C) 1588 (m), ν (C=S) 1261 (s), ν (C-N) 1175 (m). ^{1}HMR ($CDCl_3$) 8.39 (s, 1H, CSNH) 6.97-7.59 (m, 8H, ArH-2,3,5,6,2´,3',4´,5´) 3.44 (q, 1H, CH-CH_3, J = 13.8, J = 6.9), 2.26 (d, 2H, Ar-CH_2-H, J = 7.2), 1.55-1.72 (m, 1H, CH-$(CH_3)_2$), 1.29 (d, 3H, CH_3-CH-Ar, J = 7.2), 0.68 (d, 6H, $(CH_3)_2$-CH), J = 6.6), *Anal, Calc.* for $C_{43}H_{55}Cl_2CuN_4O_2S_2$: C, 60.16, H, 6.46, N, 6.53. Found: C, 60.46, H, 6.06, N, 6.93 %.

Cu(I) complex of N-2-(4-(2-methylpropyl)phenyl)propionoyl-N´-(3´,4´-dichlorophenyl) thiourea (15)

Yellow solid. Yield: 81%. Melting point. 149-150°C. IR (cm^{-1}): ν (C=O) 1693 (s), ν (arom.C=C) 1505 (m), ν (C=S) 1268 (s), ν (C-N) 1151 (m). ^{1}HMR ($CDCl_3$) 7.89 (s, 1H, CSNH), 7.11-7.77 (m, 8H, ArH-2,3,5,6,2´,3´,5´,6´) 4.31 (q, 1H, CH-CH_3, J = 13.8, J = 6.9), 2.53 (d, 2H, Ar-CH_2-H, J = 7.2), 1.81-1.92 (m, 1H, CH-$(CH_3)_2$), 1.58 (d, 3H, CH_3-CH-Ar, J = 7.2), 0.96 (d, 6H, $(CH_3)_2$-CH), J = 6.6), *Anal, Calc.* for $C_{43}H_{53}Br_2CuN_4O_2S_2$: C, 55.69, H, 5.76, N, 6.04. Found: C, 55.29, H, 5.39, N, 6.44 %.



Scheme 5

Table 2. Physical Data of the metal complexes of N,N'-disubstituted thioureas **(4-15)**.

No.	R	MX	M.P(ºC)	Yield (%)
4	2-Br	Co	98-99	73
5	2-Cl	Co	95-96	79
6	3,4-Dichloro	Co	102-103	84
7	2-Br	Ni	91-92	59
8	2-Cl	Ni	100-101	67
9	3,4-Dichloro	Ni	102-103	84
10	2-Br	Pb	90-91	98
11	2-Cl	Pb	118-119	71
12	3,4-Dichloro	Pb	105-106	91
13	2-Br	Cu	82-83	74
14	2-Cl	Cu	110-111	54
15	3,4-Dichloro	Cu	149-150	81

2.4. Urease inhibition Assay

The amount of ammonia produced was measured by indophenol method and its absorbance provided the enzyme activity (Weatherburn 1967). The assay mixture containing 40 µL buffer (100 mM urea, 1 mM EDTA, 0.01 M K_2HPO_4, 0.01 M $LiCl_2$, pH 8.2), 10 µL of test compound and 10 µL of enzyme (5 U/mL) were incubated in a 96 well plate for 30 minutes at 37°C. In addition, solutions of 40 µL of phenol reagent (1%, w/v phenol, 0.005%, w/v sodium nitroprusside) and 40 µL of alkali reagent (0.5%, w/v NaOH, 0.1% active chloride NaOCl) were added to each well. Experiments were performed in triplicate and thiourea was used as reference drug. Microplate reader (Bio-TekELx 800, Instruments, Inc., USA) was used to read the absorbance at 625 nm (Kumavat PP 2013).

3. RESULTS AND DISCUSSIONS

3.1 Infrared spectra

The FTIR spectral data of N,N´-disubstituted thioureas **(1-3)** show absorption bands at υ_{max}/cm^{-1} 3281-3381, 3442–3462 (NH), 1659-1692 (C=O), 1527–1595(CN), 1575-1589 (C=C), 1200-1236 (C=S),1148–1162(C–S), 1151-1171 (CN). The addition of the amine to the C=N double bond results in the formation of the desired compounds. As a consequence, the strong band at around 2000 cm^{-1} (N=C=S) in the isocyanate disappears. Instead of the having normal carbonyl absorption around υ_{max}/cm^{-1} 1710 and a medium (C=O) absorption band around 1659–1692 indicated a possible hydrogen bond formation between the H-atom of the CSNH and the O-atom of the carbonyl group (Scheme 1). A comparative absorption pattern study of the complexes **(4-15)** with the values of the free ligand suggested a significant effect in stretching frequencies of (CN) and (CS) groups due to coordination of the ligand to the Co, Ni, Pb and Cu atom. In the FTIR spectra of the N,N´-disubstituted thiourea ligands **(1-3)** the N-H stretching vibration is observed in the range 3212-3299 cm^{-1} as a broad band. This band disappears upon metal complex formation of related ligands. This behavior suggests that the ligand is coordinated to the metal atom and lost amine proton. Moreover, the slight shift in stretching frequency of C=O in coordinated compound as compared to ligand also accounted the involvement of the carbonyl group in coordination which confirmed the formation of desire compound(Hakan A 2003; Losada J 2000). A shift in frequency was also be expected for the C=S stretching vibration but this stretching vibration could not be assigned unambiguously, because it is located in the fingerprint zone of the IR spectra (Costa 1984).

3.2 ^{1}HNMR spectra

In the ^{1}HNMR spectral data of ligands **(1-3)** show that the NH hydrogen resonates at a frequency significantly downfield from other resonances in the spectra. The proton chemical shifts of both CONH and CSNH are found around 12.27-12.43 and 8.47-8.69 ppm for free and hydrogen bonded NH, respectively, and aromatic protons are found in the resonating frequency range 7.09-7.91 ppm (Rauf MK 2008). A comparative ^{1}HNMR spectral study of the complexes **(4-15)** reveals that the N–H signal appears at 12.43, 12.39, and 12.27 ppm in the ^{1}H NMR spectrum of ligands **1, 2 and 3** respectively, and is not present in the corresponding complexes as a consequence of coordination, in agreement with IR results. Furthermore, all the other chemical shift values of ^{1}H NMR data agree with the literature and expected structure of the complex (Fig 1). Hence, all the characteristic resonating shifts were identified by their intensity and multiplicity patterns (Hakan A 2003; Losada J 2000).

3.3 Assay for urease inhibition

Transition metal complexes of thioureas were evaluated against *Jack bean* urease *in vitro.* The results are presented in Table 3. Initially the compounds were screened at a concentration of 1 mM. The compounds which exhibited

more than 50% inhibition were further selected for complete characterization. All of the transition metal complexes of thioureas were potent inhibitors of *Jack bean* urease. Thiourea was used as the reference compound for the assay, and its value is also included in Table 3. The screened compounds exhibited excellent inhibitory activity in micromolar range. Among the tested compounds, **5** was the most potent with IC_{50} being 14.1 µM. Other potent compounds were 8, 11 and 12 having 16.5, 18.0 and 16.9 µM, respectively. Upon further modification, moderate activity was exhibited by 4, 6, 9, 10, 13 and 14, their IC_{50} ranging from 24.6-58.1 µM. Among the synthesized analogues, compound **7** was least potent with IC_{50} values greater than 68.9 µM. Compound **5** has showed potent inhibition due to presence of chloro group at meta position and metal ligand is Co (II) in this case. The presence of chloro group at meta position enhances the urease inhibition activity as shown by results in case of compounds **5, 8** and **11**. Whereas, the substitution of bromo group at same position reduces the activity many folds irrespective of metal ligand attached i-e; compounds 4, 7, 10 and 13 (Table 3).

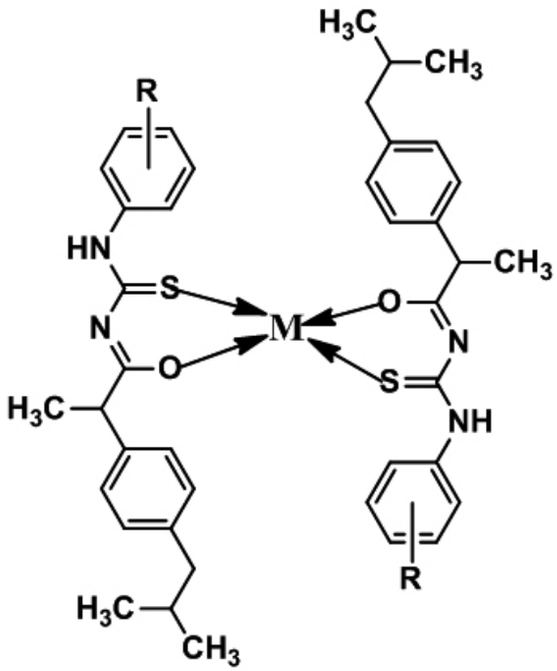


Fig 1: Most expected structure of the metal(Cu(II), Co(II), Ni(II), Pb(II)) complexes of ibuprofen substituted thioureas.

Table 3 Inhibitory activity of metal complexes of thioureas **(4-15)** against *Jack bean* urease.

Compounds	R	MX	IC_{50} (µM) ± SEM
4	2-Br	Co	32.9 ± 14.1
5	2-Cl	Co	14.6 ± 3.3
6	3,4-Dichloro	Co	24.6 ± 7.45
7	2-Br	Ni	68.9 ± 4.92
8	2-Cl	Ni	16.5 ± 10.4
9	3,4-Dichloro	Ni	34.9 ± 4.51
10	2-Br	Pb	58.1 ± 18.2
11	2-Cl	Pb	18.0 ± 3.94
12	3,4-Dichloro	Pb	16.9 ± 8.51
13	2-Br	Cu	37.3 ± 25.9
14	2-Cl	Cu	37.7 ± 0.76
15	3,4-Dichloro	Cu	-
Standard	Thiourea	-	21.1 ± 1.23

4. CONCLUSION

A series of thiourea derivatives bearing ibuprofen moiety *N*-2-(4-(2-methylpropyl)phenyl)propionoyl-*N′*-(2′-bromophenyl) thiourea (1), *N*-2-(4-(2-methylpropyl)phenyl)propionoyl-*N′*-(2′-chlorophenyl) thiourea (2), *N*-2-(4-(2-methylpropyl)phenyl)propionoyl-*N′*-(3′,4′-dichlorophenyl) thiourea (3) and their corresponding complexes of Co(II) (4-6), Ni(II) (7-9), Pb(II) (10-12) and Cu(I) (13-15) were synthesized and characterized by FTIR and ^{1}HNMR spectroscopy. The synthesized thiourea derivatives were evaluated for *Jack bean* urease inhibition activity. All of the compounds showed significant inhibition against *Jack bean* urease due to the similarity of their basic skeleton with urease substrate. The compound (**5**) showed excellent inhibitor activity having IC_{50} value 14.6 ± 3.3 M.

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