Asian Journal of Chemistry; Vol. 28, No. 11 (2016), 2446-2452

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2016.20013

Ni(II), Cu(II) and Pd(II) Complexes of Anisaldehyde-4-phenyl-thiosemicarbazone: Synthesis, Spectral Characterization and Biological Study

JAYANTAJIT BARUAH[1], GEETIKA BORAH[1,*] and DEVID KARDONG[2]

[1]Department of Chemistry, Dibrugarh University, Dibrugarh-786 004, India
[2]Department of Life Sciences, Dibrugarh University, Dibrugarh-786 004, India

*Corresponding author: Tel: +91 373 2370210; E-mail: geetikachem@yahoo.co.in

Received: 4 April 2016; *Accepted*: 27 June 2016; *Published online*: 10 August 2016; AJC-18015

Anisaldehyde-4-phenyl-thiosemicarbazone (HL) complexes of Ni(II), Cu(II) and Pd(II) have been synthesized. Conductivity measurements, spectroscopic (FTIR, UV-visible, ESI(+) mass, ^{13}C and ^{1}H NMR, ESR), cyclic voltammetry study and thermal analyses were used to propose their molecular formulations. Ni(II), Cu(II) and Pd(II) complexes show room temperature magnetic moments of 3.07, 1.59 and 0.00 BM, respectively. The ligand and the metal complexes have been screened *in vitro* for antibacterial activity against the bacterial strains *viz. P. vulgaris, S. aureus, E. coli, S. aeruginosa, B. subtillis* and *S. epidemidis* and for antifungal activity against the fungal strains *viz. A. niger, F. Oxosporum ciceri, F. oxosporum* NCIM1281 and *R. solani*. The ligand, Cu(II) and Pd(II) complexes have been tested for antitubercular activity against *Mycobacterium tuberculosis* H37RV strain and for anticancer activity against three human cancer lines. The Cu(II) complex was found to be most potent against *M. tuberculosis* H37RV strain and showed its MIC as 10 μM. The ligand and Cu(II) complex exhibited quite good growth inhibitory activity against most of the tested bacterial and fungal strains.

Keywords: Anisaldehyde-4-phenyl-thiosemicarbazone, Antibacterial, Antifungal, Antitubercular, Anticancer activity.

INTRODUCTION

Thiosemicarbazones constitute an important and versatile type of N,S-donor ligands because of their mixed hard-soft character and the variety of donor atoms they may possess [1]. They exhibit varied binding modes in different tautomeric forms and can stabilize various oxidation states of transition metal ions. Possibilities of varied binding modes of thiosemicarbazone ligands open up scopes for interesting studies with transition metal complex formation [2]. Many complexes have been found to possess excellent catalytic potentiality [3-6]. Moreover, these complexes are also known to possess high thermal stability, both in solid and solution, making them good candidates for high temperature homogenous catalysis [7]. However, there has been a growing interest in the last few years in these ligands and their metal complexes due to the broad spectrum of biological properties they possess, such as antifungal, antibacterial, anti-inflammatory, antiviral, anti-convulsant, antidepressant, anticancer along with good anti-HIV activity and structural similarities with natural biological substances [8-12]. The biological activity is mainly due to the presence of the imine (–N=CH–) and the thiazo (–N–C=S) groups near one another [13,14]. Biological properties of thiosemicarbazones have been studied since 1956 when Brockman *et al.* reported the antitumour activities of thiosemicarbazones derived from 2-formylpyridine and recently it has been developed as an antitumour drug and has received clinical phase II on several cancer types [15,16]. The nature of the substituent attached at N^4 influences the biological potentiality and it was reported that electron-rich substituents such as an aromatic ring with a halogen, methyl or a methoxy group resulted greater activity than electron withdrawing groups [17,18]. There are reports of significant antimalarial and cytotoxic activity in N^4-phenyl substituted thiosemicarbazones and such compounds were reported to demonstrate significantly enhanced anti-proliferative activity [19]. Transition metal complexes of thiosemicarbazones are also potential anticancer and chemotherapeutic agents. This key biological activity is often related with their capability to inhibit a crucial enzyme, ribonucleotide reductase for DNA biosynthesis and cell division [20]. It is well known that the biological potentialities of thiosemicarbazones can be enhanced and modified to their ability to form chelates with specific metal ions, nature of parent aldehyde or ketone group and terminal amino substituents [21]. Extensive research has been done on the preparation, characterization and biological activity of metal complexes containing heterocyclic thiosemicarbazones [9]. However, little attention has been paid to the systems

bearing non-heterocyclic aromatic ring. These systems have a unique flat electron rich aromatic surface, which may facilitate their interaction with nucleic acid, thereby acting as potent biologically active molecule. So, in continuation of our research work on transition metal complexes with non-heterocyclic thiosemicarbazones as well as this intriguing feature motivated us to carry out the present study [22]. We report herein, the synthesis and characterization of anisaldehyde-4-phenyl-thiosemicarbazone ligand and its Ni(II), Cu(II) and Pd(II) complexes . The antimicrobial activities of the ligand and complexes have been tested against standard bacterial and fungal strains. The ligand, Cu(II) and Pd(II) complexes have also been screened for antitubercular activity against *Mycobacterium tuberculosis* H37RV strain and for anticancer activity against three human cancer cell lines. It is noteworthy to mention that although, synthesis and structural features of similar complexes of anisaldehyde-4-phenyl-thiosemicarbazone with Co(II), Ni(II), Cu(II), Cd(II), Ru(III) and Rh(III) had been studied [23-26]. To the best our knowledge, the biological activity of Ni(II), Cu(II) complexes of this ligand are yet to be reported. Hence, there is resurgence of interest on thiosemicarbazone complexes in the present time, especially on their biological activity.

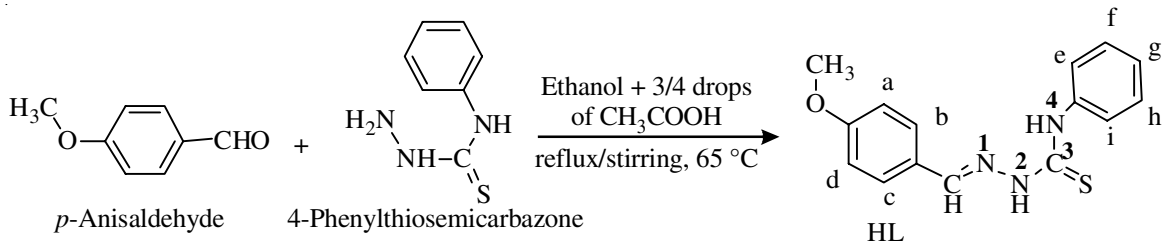
EXPERIMENTAL

All the chemicals used were of AnalaR grade and procured from Sigma-Aldrich and Fluka. Metal salts were purchased from E. Merck and used as received. Nutrient broth and Agar agar were purchased from Himedia. The standard bacterial and fungal strains were obtained from National Chemical Laboratory, Pune, India and North East Institute of Science and Technology, Jorhat, India respectively. The precursor complex $[Pd(Cod)Cl_2]$ was prepared according to literature method [27]. The melting points were determined using Buchi B450 melting point apparatus. Molar conductivity was measured in DMF (10^{-3} M) at 25 °C on an Elico CM180 digital conductivity bridge. The IR spectra were recorded on a Shimadzu Prestige 21 FTIR spectrophotometer using KBr disks in the range 4000-250 cm^{-1}. The UV-visible spectra were recorded in DMF solution using 1 cm^3 quartz cell in the range 800-200 nm on a Shimadzu-Graphicord UV-1700 spectrometer. Cyclic voltammetric measurements were performed in DMF solution with a CH instrument (model 600C) using platinum as working electrode, a platinum wire as auxiliary electrode and a Ag/AgCl as the reference electrode, with tetrabutylammonium perchlorate (TBAP) as the supporting electrolyte at a scan rate of 500 mV s^{-1}. TGA-DTA was recorded on a Perkin Elmer, Pyris Diamond TG/DTA analyzer. Elemental analyses were conducted on a Elementar Vario EL III Carlo Erba 1108 elemental analyzer. The room temperature magnetic moment of the complexes were measured on a Sherwood Scientific magnetic susceptibility balance, using $Hg[Co(NCS)_4]$ as the calibrating standard and also by Evans method [28]. The ESI(+) mass spectra were recorded on a Waters ZQ-4000 liquid chromatography-mass spectrometer. The ^1H (300 MHz) and ^{13}C (75.5 MHz) NMR spectra were recorded at room temperature on a Bruker Avance II 400 FT-NMR spectrometer at 27 °C, using DMSO-d_6 as solvent and tetramethylsilane (TMS) as standard. The X-band ESR spectra of the complexes were recorded at liquid nitrogen temperature in DMSO on JEOL Model JES FA200 using DPPH (g = 2.0036) as standard.

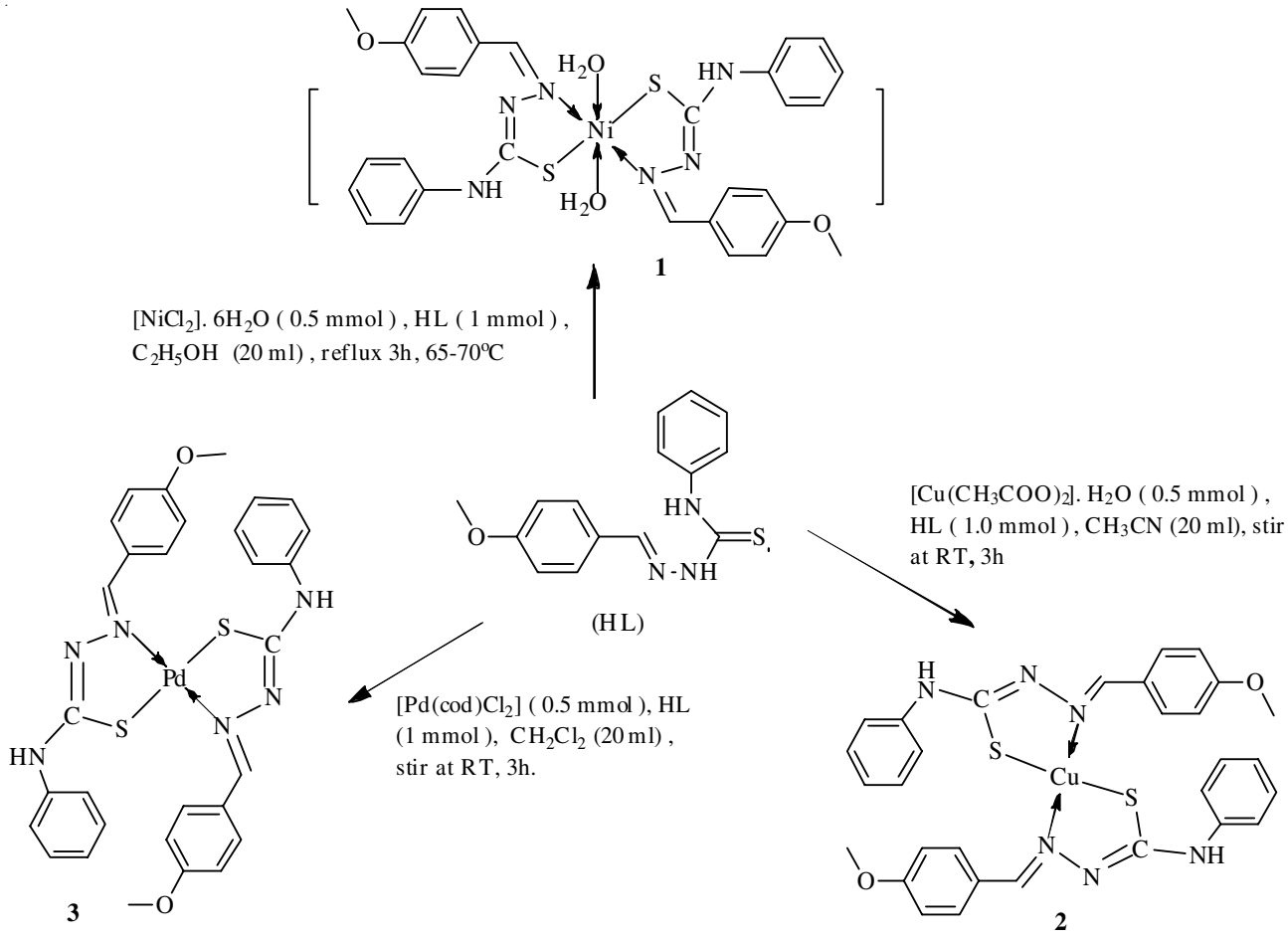
Synthesis of anisaldehyde-4-phenylthiosemicarbazone ligand (HL): The ligand has been synthesized by reacting 1.36 g (10 mmol) of anisaldehyde and 1.65 g (10 mmol) of 4-phenylthiosemicarbazide (1:1 molar ratio) in 25 mL ethanol with few drops of acetic acid (**Scheme-I**) [24]. White crystalline solid; Yield: 85 %. FW: 285 g mol^{-1}; m.p.: 194 °C; UV–visible [DMSO, cm^{-1} (log ε)]: 29940 (2.83); Selected IR bands (KBr, cm^{-1}): (N^4-H) 3327, (N^2-H) 3152, (C=N) 1603, (N-N) 1171, (C=S) 827; ^1H NMR [300 MHz, DMSO-d_6, δ ppm]: 11.69 (s, 1H, N^2-H), 10.04 (s, 1H, N^4-H), 8.10 (s, 1H, CH=N), 7.84 (d, 2H, H^b, H^c), 7.54 (d, 2H, H^e, H^i), 7.35 (dd, 2H, H^f, H^h), 7.19 (t, 1H, H_g), 6.96 (d, 2H, H^a, H^d), 3.78 (s, 3H, OCH_3); ^{13}C NMR [75.5 MHz, DMSO-d_6, δ ppm]: 175.5 (C=S), 160.8 (Ph-C), 142.9 (CH), 138.9 (Ph-C), 114.1-129.3 (Ph-C), 55.2 (CH_3); ESI(+) MS *m/z* $[M]^+$: 285, $[M+H]^+$: 286; Anal. calcd. for $C_{15}H_{15}N_3OS$ (%): C, 63.16; H, 5.26; N, 14.74; S, 11.23. Found: C, 63.58; H, 4.98; N, 14.19; S, 10.83.

$[Ni(L)_2(H_2O)_2]$ (1): 0.285 g of HL (1 mmol) and 0.119 g of $NiCl_2{\cdot}6H_2O$ (0.5 mmol) was mixed in 20 mL ethanol and refluxed at 65-70 °C for 3 h. The purple coloured solution was evaporated under reduced pressure, recrystallized with methanol and dried over fused $CaCl_2$ in a desiccator (**Scheme-II**). Dark purple powdered solid; Yield: 68 %. FW: 662.71 g mol^{-1}; λ_m (1 mmol L^{-1}, DMF): 07 Ω^{-1} cm^2 mol^{-1}; UV–visible [DMSO, cm^{-1} (log ε)]: 21598 (1.69), 17730 (1.29); Selected IR bands (KBr, ν_{max}, cm^{-1}): (O-H) 3520, (N^4-H) 3281, (C=N) 1597, (N-N) 1175, (C-S) 596, (Co-N) 432, 413, (Co-S) 365; ESI(+)MS *m/z* $[M+1]^+$: 664, $[M-2H_2O+1]^+$: 628, $[M-L]^+$: 379, $[M-L-2H_2O]$: 343; Anal. calcd. for $C_{30}H_{32}N_6NiO_4S_2$ (%): C, 52.32; H, 4.83; N, 12.68; S, 9.66. Found: C, 52.86; H, 4.97; N, 12.26; S, 9.19.

$[Cu(L)_2]$ (2): 0.285 g of HL (1 mmol) and 0.099 g of $Cu(CH_3COO)_2.H_2O$ (0.5 mmol) was mixed in 20 mL acetonitrile and stirred at room temperature for 3 h. The solution turned dirty green and on standing for 1 h brown precipitate was formed. It was washed with acetonitrile and dried over fused $CaCl_2$ in a desiccator (**Scheme-II**). Brown



Scheme-I: Synthesis of ligand (HL)



Scheme-II: Synthesis of complexes

powdered solid; Yield: 65 %. FW: 631.5 g mol^{-1}; λ_m (1 mmol L^{-1}, DMF): 16 Ω^{-1} cm^2 mol^{-1}; UV–visible [DMSO, cm^{-1} (log ε)]: 23041 (2.35), 14374 (1.42); Selected IR bands (KBr, ν_{max}, cm^{-1}): (N^4-H) 3306, (C=N) 1600, (N-N) 1173, (C-S) 624, (Co-N) 403, (Co-S) 366; ESI(+)MS *m/z* $[M]^+$: 631, $[M+1]^+$: 632; Anal. calcd. for $C_{30}CuH_{28}N_6OS_2$ (%): C, 57.01; H, 4.43; N, 13.30; S, 10.13. Found: C, 56.38; H, 4.27; N, 13.64; S, 9.87.

$[Pd(L)_2]$ (3): 0.285 g of HL (1 mmol) and 0.143 g of $[Pd(Cod)Cl_2]$ (0.5 mmol) was dissolved in 20 mL dichloromethane and stirred at room temperature for 3 h. The red precipitate formed was washed with dichloromethane and finally dried over fused $CaCl_2$ in a desiccator (**Scheme-II**). Dark red microcrystalline solid; Yield: 72 %. FW: 674.4 g mol^{-1}; λ_m (1 mmol L^{-1}, DMF): 15 Ω^{-1} cm^2 mol^{-1}; UV–visible [DMSO, cm^{-1} (log ε)]: 20747 (1.09); Selected IR bands (KBr, ν_{max}, cm^{-1}): (N^3-H) 3240, (N^2-H) 3125, (C=N) 1574, (N-N) 1175, (C-S) 620, (Co-N) 434, (Co-S) 388; ^{1}H NMR [300 MHz, DMSO-d_6, δ ppm]: 9.79 (s, 1H, N^4-H), 8.30 (s, 1H, CH=N), 8.14 (d, 2H, H^a, H^d) 7.52 (d, 2H, H^b, H^c), 7.50 (d, 2H, H^e, H^i), 7.36 (dd, 2H, H^f, H^h), 7.07 (t, 1H, H^g), 3.85 (s, 3H, OCH_3). ^{13}C NMR [75 MHz, DMSO-d_6, δ ppm]: 167.9 (C=S), 162.1 (Ph-C), 155.1 (CH), 140.6 (Ph-C), 114.1-134.6 (Ph-C), 55.5 (CH_3); ESI(+)MS *m/z* $[M+1]^+$: 675.22; Anal. calcd. for $C_{30}H_{28}N_6O_2PdS_2$ (%): C, 53.38; H, 4.15; N, 9.08; S, 6.92. Found: C, 52.32; H, 3.87; N, 9.40; S, 7.25.

Test for antimicrobial activity: The antibacterial activity of the synthesized compounds were tested *in vitro* by the agar well method against standard bacterial strains, *viz.*, *Escherichia coli* (ATCC 25922) [Gram-negative], *Pseudomonas aeruginosa* (ATCC 10662) [Gram-negative], *Staphylococcus aureus* (ATCC 25923) [Gram-positive], *Proteus vulgaris* (NCTC 8313) [Gram-positive] and *Bacillus subtilis* (TCC 6633) [Gram-positive], maintained at 4 °C in nutrient agar media. The antifungal activity was tested against the standard strains *viz*. *Aspergillus niger*, *Fusarium oxysporum ciceri*, *Fusarium oxysporum* NCIM 1281 and *Rhisoctonia solani*. These were also maintained at 4 °C in potato dextrose agar (PDA) media. The subculture of the strains was done in regular interval of 2 months. Streptomycin disc (30 µg) and flucanazole disc (25 µg) were used as standards, respectively. The MIC of each test sample is expressed in terms of zone of inhibition (mm) after incubation at 37 °C for 24 h.

Test for antitubercular activity: The ligand, complexes **2** and **3** were tested for *in vitro* antitubercular activity against the standard *Mycobacterium tuberculosis* H37RV strain. The zone of inhibition assay was performed as follows: 10 µL of 10 mM drug was loaded over Whatmann filter paper No. 1 discs and dried. Discs were placed in a dish plated with four weeks old bacterial culture on 7H11 agar media supplemented with OADC (growth supplement) and incubated at 37 °C for four weeks for the bacterial growth. Zone of inhibition was measured using digital Vernier caliper (Mitutoyo, Japan) [29]. Minimum inhibitory concentration assay was performed as follows. 2,700 µL of 7H9 broth supplemented with ADC

(growth supplement) in glass tubes was inoculated with 10 µL of bacterial culture from four weeks old culture. 300 µL of each drug at 5 different concentrations as 100, 10, 1, 0.1 and 0.01 µM in duplicate were added to each tube and incubated at 37 °C for six weeks. Growth was monitored under light. Inhibition was confirmed in tubes showing clear media. No inhibition was confirmed in tubes showing bacterial growth as precipitate at the bottom of the tube.

Anticancer study: The ligand, the complexes **2** and **3** were tested *in vitro* for anticancer activity on three tumor cell lines *viz*. human breast cancer cell line MCF7, human hepatoma cell line HEPG2, human lung cancer cell line A549 using the sulforhodamine B (SRB) assay method following standard protocol [30,31]. The cell lines were obtained from NCI, USA and NCCS, Pune. Cell density was maintained at 5×10^3 cells/well and DMSO was used as the carrier. The growth control of the cells in percentage was recorded against different concentrations of the test compounds *viz.* 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M and LC_{50}, TGI and GI_{50} were determined as average of three independent experiments. Adriamycin was used as the positive control.

RESULTS AND DISCUSSION

The stoichiometries of anisaldehyde-4-phenylthiosemicarbazone and the metal complexes $[Ni(L)_2(H_2O)_2]$ (**1**), $[Cu(L)_2]$ (**2**) and $[Pd(L)_2]$ (**3**) were suggested on the basis of micro-analyses, ESI-mass, infrared, UV-visible, NMR, ESR, magnetic moment measurement and molar conductivity. All the complexes are microcrystalline solid and found stable at room temperature. The complexes are insoluble in most of the common polar and non-polar solvents. They are, however, soluble in DMF, in which conductivity measurements were made, showing all complexes to be non-electrolytes.

ESI(+)Mass: The ESI-mass spectrum of the ligand exhibits peaks at *m/z* 285 and 286, corresponding to $[M]^+$ and $[M+1]^+$ respectively. Complex1 displays peaks at *m/z* 664, 628, 379 and 343, attributed to $[M+1]^+$, $[M-2H_2O+1]^+$, $[M-L]^+$ and $[M-L-2H_2O]^+$ ions, respectively. Complex **2** shows peaks at *m/z* 631 and 632, could be assigned to $[M]^+$ and $[M+1]^+$, respectively, while for complex 3 peak at *m/z* 675.22 was suggested to $[M+1]^+$.

Infrared spectra: The ν(C=N) vibration at 1603 cm^{-1} in the infrared spectrum of HL confirms the formation of the thiosemicarbazone. Absorptions observed at 3152 and 3327 cm^{-1} were attributed to $\nu(N^2\text{-H})$ and $\nu(N^4\text{-H})$, respectively. The ν(N-N) vibration was found at 1171 cm^{-1}. A strong absorption at 827 cm^{-1} could be assigned to ν(C=S). The ν(C=N) absorption underwent considerable shift ($\Delta\nu$ =3-29 cm^{-1}) in the spectra of the complexes, suggesting coordination of the imine nitrogen. The band due to ν(C=S) disappeared with simultaneous appearance of a new band at 624-594 cm^{-1} for all the complexes, assignable to ν(C-S), suggesting coordination *via* C=S in the thiol form. This was further supported by the appearance of a new band at 434-403cm^{-1} and at 388-365cm^{-1} assigned to ν(M-N) and ν(M-S) [29,32], respectively in all the complexes which support the proposed mode of coordination.

UV-visible spectrum: The electronic spectra of the ligand and the complexes were recorded in 10^{-3}M DMF solution. Complex **1** shows two broad bands centered at 17,730 and 21,598 cm^{-1} attributed to $^3A_{2g(F)} \rightarrow {}^3T_{1g(F)}$ and $^3A_{2g(F)} \rightarrow {}^3T_{1g(P)}$ transitions respectively, suggested an octahedral geometry for the Ni(II) complex [33]. The absence of any peak below 10,000 cm^{-1} eliminates the possibility of a tetrahedral environment in this complex [34]. Complex **2** demonstrates one broad peak at 14,374 cm^{-1} may be suggested to d→d transition in a square-planar geometry for the complex, while the strong peak at 23041 cm^{-1} is assignable to ligand→Cu(II) charge transfer transition [32]. Complex **3** displays one broad peak at 20,747 cm^{-1} assigned to $^1A_{1g} \rightarrow {}^1A_{2g}$ in a square-planar geometry.

NMR spectra: The ^{13}C NMR spectral peaks are in good agreement with the suggested structures. The HL showed signals at 175.5, 142.9, 55.2 ppm attributed to thioamide (C=S), azomethine (-HC=N) and $-CH_3$ carbon atoms, respectively. The signals for the phenyl ring carbon were found in the range 114-161 ppm. In ^1H NMR spectrum of HL singlet peaks at 11.69, 10.04 and 8.10 ppm could be assigned to N^2H, N^4H and C^8H respectively, while the singlet at 3.78 ppm is corresponds to $-CH_3$ protons. The *para* methoxy phenyl protons show two doublets, one of which upon coordination with palladium shows a strong downfield shift ($\Delta\delta$ = 1.18ppm). The NHPh protons resonances were observed as one doublet, one double of doublet and one triplet, which upon metallation with palladium exhibit upfield shifts. Considerable shift in the ^{13}C signals were also observed for the complex **3**. The complex **3** does not show any peak attributable to a N^2H proton, indicating thioenolization of N^2H-C=S group to N^2=C-SH on complexation. The NMR spectra of complexes **1** and **2** could not be resolved due to paramagnetic nature of the complexes.

DTA-TGA analysis: The thermogravimetric analysis of HL shows endothermic weight loss (37.5 %) of the fragment $[CH_3O\text{-Ph}]$ in the temperature range 170-214 °C. This is followed by loss of 47.7 % up to 319 °C and 14.7 % up to 600 °C, attributed to the loss of [CS-NH-Ph] and the fragment [CH=N-NH], respectively. The TGA curve of complex **1** exhibits an endothermic weight loss of 5.4 % in the temperature range 110-172 °C attributed to the loss of 2 mol of coordinated H_2O [24]. This is followed by steady loss of the remaining mass. The complex **2** exhibits a continuous exothermic weight loss (58.3 %) at 128-520 °C, which may be assigned to the loss of $[(CH_3O\text{-Ph-CH})+Ph]$ fragment. With the increase of temperature gradual loss of the remaining mass was observed. Complex **3** initially loses weight (16.1 %) in the temperature range 100-248 °C, which conforms to the loss of $[CH_3\text{-O-Ph}]$ fragment. This is followed by a sharp exothermic loss (36.7 %) in the temperature range 208-290 °C, attributed to the loss of $[CS\text{-}NH_2]$ fragment. The remaining fragment along with Pd is steadily lost up to 690 °C. The TGA data also support assigned molecular formulations of the complexes.

Electrochemical study: The redox behaviour of the complexes was examined by means of cyclic voltammetry (potential range -4.0 to + 4.0 V with scan rate 500 mV s^{-1}) and the data is demonstrated in Table-1. The cyclic voltammogram of HL exhibits only a cathodic peak at -1.18 V, which could be assigned to $[HL]/[HL]^{n-}$. The absence of anodic peak in the reverse scan indicates that the electrochemical process was coupled with a chemical reaction [26,35]. The ligand also

shows one irreversible oxidative response at +0.35 V [ΔEp = 200 mV]. For the complexes **1** and **2**, the cathodic peaks are at -1.25 and -1.44 V, respectively, could be attributed to ligand-based reduction [26]. Compared with the HL, these peaks are all shifted to the negative side, indicating that the complexes are much harder to be reduced. It was observed that the reduction peak value of the complex **2** is much more negative than the complex **1**. This indicates that the property of the metal ion has a significant influence on the redox behaviour of the relevant complex. Moreover, the complexes **1** and **2** exhibit an oxidative couple at +0.20 V [ΔEp = 110 mV] and +0.29 V [ΔEp = 210 mV], respectively. These are believed to be the ligand-based oxidations, but the presence of Ni(II) and Cu(II) ions have caused the electronic transition mode of the ligand L, little different from that of the free ligand HL. The complex **3** in its cyclic voltammogram demonstrated one irreversible reductive response at -0.89 V [ΔEp = 440 mV]. This feature could be assigned to the Pd(II)/Pd(0) redox couple [36], since palladium complexes of π-acceptor ligands undergo two-electron reduction with the stabilization of Pd(0) [37]. Another couple at +0.85V could be tentatively ascribed for irreversible Pd(II)/Pd(IV) couple [ΔEp = 770 mV]. Extremely high peak to peak separation values for the complex indicated that the redox processes are quite non-Nernstian [36]. As the electron transfer processes are irreversible, the species that are initially formed in the electrode process may either get decomposed or react further to give products that are electro-inactive.

TABLE-1
ELECTROCHEMICAL DATA OF THE LIGAND AND THE METAL COMPLEXES

	First electrode couple			Second electrode couple		
	Ep_a (V)	Ep_c (V)	ΔEp (mV)	Ep_a (V)	Ep_c (V)	ΔEp (mV)
HL	–	-1.18	–	0.35	0.15	200
Complex **1**	–	-1.25	–	0.20	0.09	110
Complex **2**	–	-1.44	–	0.29	0.08	210
Complex **3**	-0.45	-0.89	440	0.85	0.08	770

*$E_{1/2} = (E_{pc} + E_{pa})/2$

Magnetic susceptibility measurements: The magnetic moment value of Ni(II) complex is found to be 3.07 BM which suggests an octahedral geometry of the complex [38]. The measured moment is slightly greater than the μ spin-only value (2.83 B.M.), although octahedral Ni(II) complex with $^3A_{2g}$ ($T_{2g}^6e_g^2$) ground state has no orbital contribution. The excited state $^3T_{2g}$ ($T_{2g}^5e_g^3$) carry orbital contribution. In a system having such an excited state, the spin-orbit coupling brings about some mixing of the ground state with the excited state, thus forcing some orbital contribution [39]. The value of Cu(II) complex is 1.59 BM indicating the presence of one unpaired electron on the metal ion, while the magnetic moment value (0.00 BM) of Pd(II) complex is indicative of square-planar arrangement of the ligands around the metal ion.

ESR study: The X-band ESR spectra of the polycrystalline samples of the nickel(II) and copper(II) complexes were recorded at liquid nitrogen temperature. The spectrum of the Ni(II) complex (Fig. 1a) shows one single broad peak with isotropic g value, 2.013. The observed single line EPR is indicative of negligible zero-field splitting [40]. Most of the earlier works on Ni(II) ion at 77 K or 4 K have reported isotropic g value and close to 2.2 [41,42]. The Cu(II) complex exhibits two g values at 2.022 and 2.174 (Fig. 1b). Absence of super hyperfine coupling between Cu(II) and ligated N-atoms indicates that N-atoms are not in the same plane with the Cu(II) ion. This excludes the possibility of perfect square-planar geometry for the complex.

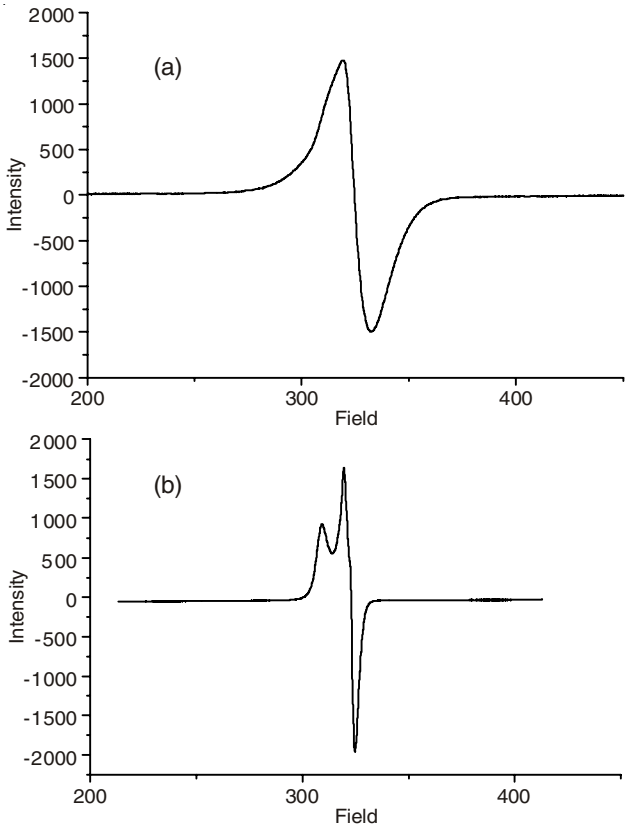
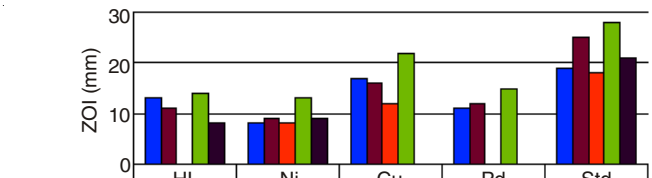


Fig. 1. (a) ESR spectrum of complex **1**, (b) ESR spectrum of complex **2**

Biological study

Antibacterial property: The results of *in vitro* test for antibacterial properties of the compounds against five test organisms have been shown as zone of inhibition (ZOI) and their minimal inhibitory concentrations (MIC) in Fig. 2 and Table-2, respectively. The ligand and complexes showed good activity against *P. vulgaris* and minimum to no activity against *B. subtilis* and *S. aureus*. It was observed that the activity was



	HL	Ni	Cu	Pd	Std.
E. coli	13	8	17	11	19
P. aeruginosa	11	9	16	12	25
B. subtilis	0	8	12	0	18
P. vulgaris	14	13	22	15	28
S. aureus	8	9	0	0	21

Fig. 2. Chart showing antibacterial activity

TABLE-2
MIC VALUES OF TEST COMPOUNDS AGAINST BACTERIAL AND FUNGAL STRAINS (μg/mL)

Microbial strain	*E. coli*	*P. aeruginosa*	*B. subtillis*	*P. vulgaris*	*S. aureus*	*F. oxo.* NCIM1281	*F. oxo. ciceri*	*R. solani*	*A. niger*
HL	62.5	125	–	62.5	250	125	62.5	250	–
Complex **1**	500	250	500	125	500	–	–	–	–
Complex **2**	62.5	125	62.5	31.5	–	125	62.5	250	–
Complex **3**	125	125	–	62.5	–	–	–	–	–

significantly increased with the copper complex and slightly with the palladium complex. Maximum activity was recorded for complex **2** against *P. vulgaris* (21 mm) and *E. coli* (17 mm) with MIC of 31.25 and 62.5 μg/mL, respectively.

Antitubercular activity: The results of antitubercular activity tested on *Mycobacterium tuberculosis* H37RV strain is shown in Fig. 3. The ligand demonstrated moderate activity with a ZOI of 12 mm and MIC of 10 μM, while complex **2** exhibited quite good activity with ZOI and MIC of 20 mm and 10 μM, respectively. No significant activity was recorded for complex **3**. Oh *et. al.* have synthesized a series of 1,2,4-triazole derivatives and evaluated their antitubercular activity against *M. tuberculosis* H37RV. The most active compounds showed their MIC against the strain as 12.5 μM [43]. So our results indicate that the Cu(II) complex of anisaldehyde-4-phenyl-thiosemicarbazone is more potent against *M. tuberculosis* H37RV and can be considered as lead molecule for further development of new candidates of this type with high anti-tubercular activity.

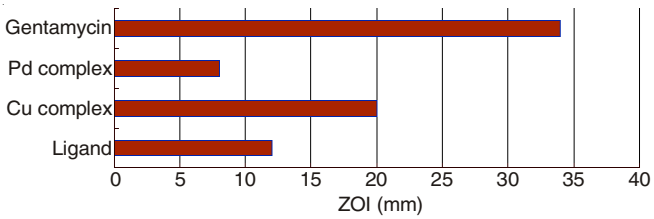


Fig. 3. Chart showing antitubercular activity

Antifungal property: Fig. 4 and Table-2 depict the results of ZOI and MIC values obtained in the *in vitro* testing of antifungal property of the ligand and the complexes. The ligand was found to be active against the strains *F. oxysporum* NCIM1281, *F. oxysporum ciceri* and *R. solani* while activity of the complex **2** was found to be slightly lower than that of the ligand. The complexes **1** and **3** were found to be inactive against all of the fungal strains.

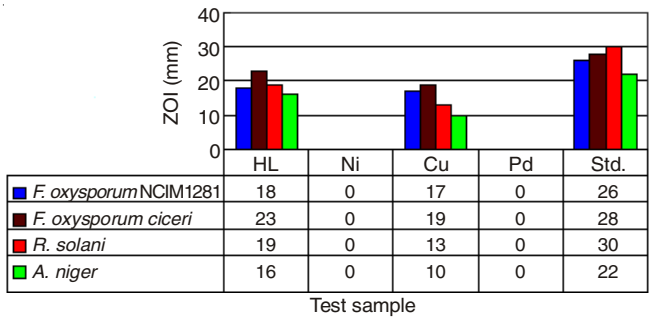


Fig. 4. Chart showing antifungal activity

Anticancer property: The LC_{50}, TGI and GI_{50} values (in μM) for the ligand, complexes **2** and **3** against the tested human tumor cell lines are given in Table-3. Fig. 5 depicts the mean graph plot of concentration of the test compounds against controlled growth percentage of the cell lines at various concentrations. The tested compounds are found almost inactive, as indicated by GI_{50} values. However, the complex **2** exhibited antiproliferative activity against all the three cell lines at concentrations above 10 μM (GI_{50} in range 46.5-12.3). The ligand showed potential activity against human breast cancer line MCF7 (GI_{50} = 75.7). The complex **2** showed better anti-proliferative effect against A549 at 10 μM and against MCF7 at 100 μM on comparison with the standard drug.

Conclusion

From the spectroscopic and analytical investigations the molecular formulae for the complexes **1-3** have been proposed

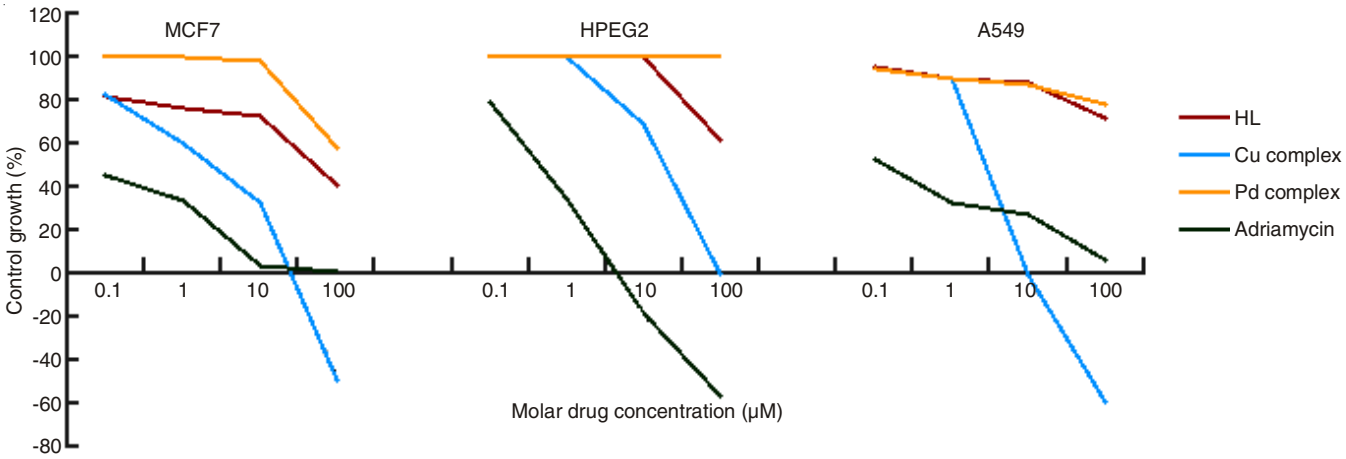


Fig. 5. Anticancer activity-growth curve

TABLE-3
LC_{50}, TGI AND GI_{50} DATA OF HL, COMPLEX **2** AND **3** AGAINST HUMAN CANCER CELLS

Cell line	Breast cancer-MCF7			Hepatoma-HEPG2			Lung cancer-A549		
	LC_{50}	TGI	GI_{50}	LC_{50}	TGI	GI_{50}	LC_{50}	TGI	GI_{50}
HL	> 100	> 100	75.7	> 100	> 100	> 100	> 100	> 100	> 100
Complex **2**	> 100	56.0	**12.3**	> 100	98.8	**46.5**	89.7	51.9	**14.2**
Complex **3**	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
Adriamycin	> 100	96.1	< 0.1	89.6	38.4	< 0.1	> 100	> 100	< 0.1

as $[Ni(L)_2(H_2O)_2]$, $[Cu(L)_2]$ and $[Pd(L)_2]$, respectively. The magnetic moment value of $[Ni(L)_2(H_2O)_2]$ (**1**) is found to be 3.07 BM indicating octahedral geometry of the complex. The ESR and visible spectra also support the geometry. The magnetic moment value of Cu(II) complex is 1.59 BM indicating presence of one unpaired electron on the metal ion, while the magnetic moment value (0.00 BM) of Pd(II) complex is indicative of square-planar arrangement of the ligands around the metal ion . From the nature of ESR as well as visible spectra of $[Cu(L)_2]$ (**2**), distorted square-planar geometry can be tentatively proposed for the complex. The ligand coordinates through its azomethine nitrogen and thiolate sulphur atoms to the metal ion in all the complexes and acts as a mono anionic bidentate ligand. The biological study reveals that the ligand anisaldehyde-4-phenyl-thiosemicarbazone has potent antibacterial and antifungal property. Its bacterial activity varies on coordination with various metals and remarkably increases in the Cu(II) complex. The antifungal activity of the Cu(II) complex was slightly lower than the ligand and was found to be nullified in the Ni(II) and Pd(II) complex. The Cu(II) complex exhibited quite good antitubercular activity against *M. tuberculosis* H37RV with ZOI and MIC of 20 mm and 1 μM, respectively. It has higher antiproliferative activity than that of the ligand. These biological activities of the complexes may not have any clinical significance, but the synthesis and study of similar and modified ligands and complexes can be an interesting field of research for obtaining potent biologically active compounds which may constitute futuristic drug candidate prototype.

ACKNOWLEDGEMENTS

The authors thank SAIF, STIC, Cochin, India for elemental analysis, SAIF, IIT, Madras for magnetic measurements and SAIF, NEHU, Shillong, India for ESI-mass, ^1H and ^{13}C NMR facilities. The services of ACTREC, Tata Memorial Centre, Mumbai, India for anticancer study and Deshpande Laboratories Pvt. Ltd., Bhopal, India for antitubercular study are gratefully acknowledged.

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