Research paper

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PII: S0020-1693(16)30242-0
DOI: http://dx.doi.org/10.1016/j.ica.2016.05.013
Reference: ICA 17049

To appear in: *Inorganica Chimica Acta*

Received Date: 25 February 2016
Revised Date: 6 May 2016
Accepted Date: 6 May 2016

Please cite this article as: A. Khan, J.P. Jasinski, V.A. Smoleaski, K. Paul, G. Singh, R. Sharma, Synthesis, structure and cytotoxicity evaluation of complexes of N^1-substituted-isatin-3-thiosemicarbazone with copper(I) halides, *Inorganica Chimica Acta* (2016), doi: http://dx.doi.org/10.1016/j.ica.2016.05.013

Synthesis, structure and cytotoxicity evaluation of complexes of N^1-substituted-isatin-3-thiosemicarbazone with copper(I) halides

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Abstract

Synthesis of Isatin-N^1-methyl-thiosemicarbazone (H_2itsc-N^1-Me, H_2L^1) and isatin-N^1-ethyl-thiosemicarbazone (H_2itsc-N^1-Et, H_2L^2) has been carried out and the effect of substituents (at N^1 atom of isatin-3-thiosemicarbazones) on nuclearity of copper(I) halide complexes has been investigated. Reactions of copper(I) halides (X = I, Br, Cl) with H_2L^1 and H_2L^2 using Ph_3P as co-ligand in 1 : 1 : 1 (M : L : PPh_3) molar ratio in acetonitrile yielded complexes of stiochiometry [$CuX(H_2L^1)(Ph_3P)$] (X = I, **C1**; Br, **C2**; Cl, **C3**) and [$CuX(H_2L^2)(Ph_3P)$] (X = I, **C4**; Br, **C5**; Cl, **C6**) respectively. All these complexes have been characterized using analytical and spectroscopic data (IR, ^{1}H NMR and ESI mass). The single crystal structure has been solved for H_2L^1 and **C2**. The complex **C2** has distorted tetrahedral geometry around copper(I) and isatin-

N^1-methyl-thiosemicarbazone coordinated to metal center as neutral, bidentate, N^3, S- chelating ligand. Elemental analysis suggested the presence of one acetonitrile molecule in complexes **C3** and **C6** and half CH_3CN in complexes **C2** and **C4** as solvent of crystallization. MTT assay, supported by docking studies have revealed the cytotoxic nature of the compounds **C1**-**C6**.

Keywords: *isatin-N^1-methyl-thiosemicarbazone; isatin-N^1-ethyl-thiosemicarbazone; copper(I) halide; X-ray crystallography; cytotoxicity; docking.*

1. Introduction

Isatin and schiff bases of isatin derivatives possess a wide range of biological properties, like antibacterial[1-3], antifungal[4-6], antiviral[7–10], anti-HIV[11–13], antiprotozoal[14,15] and anti-helminthic[16,17] activities. Isatin on condensation with thiosemicarbazide forms three types of thiosemicarbazones: isatin-3-thiosemicarbazone (I), isatin-2-thiosemicarbazone (II) and isatin-2,3-bis(thiosemicarbazone) (III) (Chart 1).

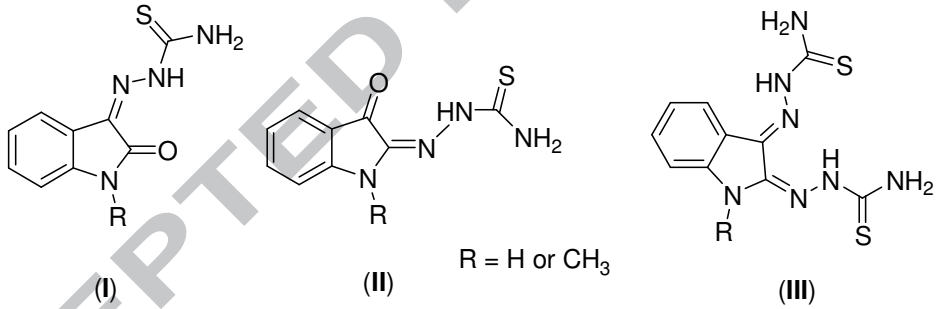
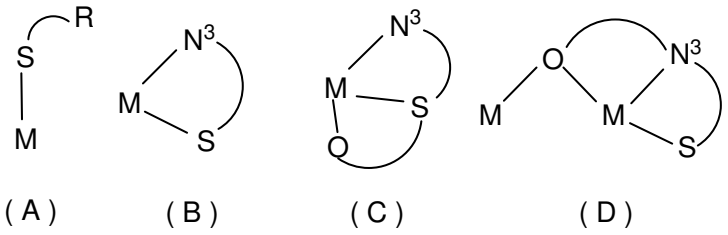


Chart 1

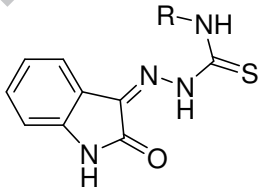
Out of these three, isatin-3-thiosemicarbazone has been of interest as 1-methylisatin-3-thiosemicarbazone was found to be active in the treatment of smallpox.[18-20] Moreover, a number of substituted isatin-3-thiosemicarbazones are structurally characterized.[21-31] Isatin-3-thiosemicarbazones are good O, N, S- donors and form complexes with metals. [32-42] They show variable bonding modes like, η^1-S, terminal (A)[36], N^3, S- chelation (B)[37], N^3, S, O- chelation (C)[37], N^3, S, O-chelation-cum-O-bridging (D)[38] (Chart 2).



(A) (B) (C) (D)

Chart 2

Recently, the chemistry of copper(I) halides with thiophene-2-carbaldehyde thiosemicarbazones and furan-2-carbaldehyde thiosemicarbazones have been reported and it was observed that substituents at C^2 and N^1 atoms of thiosemicarbazone influenced the bonding and nuclearity of copper(I) halide complexes.[42-45] Thus, we were interested in finding the similar effect in case of isatin-3-thiosemicarbazone. Un-substituted isatin-3-thiosemicarbazone reacted with copper(I) halides and Ph_3P in 1: 1: 2 molar ratio to form tetrahedral monomers.[36, 46] In present work, isatin-3-thiosemicarbazone with methyl (H_2itsc-N^1-Me) or ethyl group (H_2itsc-N^1-Et) group at N^1 atom have been synthesized (Chart 3) and the effect of substituent at N^1 atom on the nuclearity of their copper(I) complexes has been investigated. The synthesized complexes have been characterized using elemental analysis, spectroscopic studies (IR, ESI mass, ^{1}H NMR) and single crystal x-ray crystallography (Complex **C2**). These compounds were further investigated for their cytotoxic evaluations.



R= CH_3 (H_2L^1); C_2H_5 (H_2L^2)

Chart 3

Cancer figures among the leading cause of morbidity and mortality worldwide, with approximately 14 million new cases and 8.2 million cancer related deaths in 2012.[47] It is expected that annual cancer cases will rise from 14 million in 2012 to 22 within the next 2 decades.[47] These rising and alarming figures have prompted the discovery of some important anticancer drugs, currently used in the therapy and tremendous efforts are being taken towards identifying new efficient drug molecules.[48] Towards this end, we evaluated the cytotoxicity

potency of copper(I) complexes and further investigated the mechanism of action by molecular modelling studies.

2. Experimental

2.1. Materials and Methods

Potassium chloride, potassium bromide, potassium iodide and triphenylphosphine were purchased from Loba Pvt. Ltd, whereas isatin, 3-methylthiosemicarbazide and 3-ethyl thiosemicarbazides were procured from Aldrich Chemicals Ltd. Copper(I) iodide, bromide and chloride were prepared by the reduction of $CuSO_4 \cdot 5H_2O$ using SO_2 in the presence of KI, KBr or KCl in water.[49] C, H and N analyses were obtained with a Thermoelectron FLASHEA1112 CHNS analyzer. Infrared spectra were recorded from KBr pellets in the range 4000-200 cm^{-1} on a SHIMADZU FTIR 8400S spectrophotometer. Melting points were determined with an electrically heated Gallenkamp apparatus. ^1H NMR were recorded on an AV500 FT spectrometer operating at a frequency of 500 MHz using d^6-DMSO/$CDCl_3$ as the solvent with TMS as the internal standard. Crystal snaps were taken from OLYMPUS Magnus MLX Camera (40x and 100x). Mass spectra, ESI-methods, were recorded on Bruker Daltonics Esquire 300 mass spectrometer.

2.2. Synthesis of N^1-substituted-isatin-3-thiosemicarbazones

Isatin-N^1-methyl thiosemicarbazone (H_2L^1) and Isatin-N^1-ethyl thiosemicarbazone (H_2L^2) were prepared by refluxing isatin with their respective thiosemicarbazides in methanol for 8-10 h.

2.3. Procedure for Synthesis of Copper(I) Complexes C1-C6

[CuI(η^2-N^3, S-H_2itsc-N^1-Me)(Ph_3P)] C1

To a solution of CuI (0.050 g, 0.26 mmol) in 15 ml of acetonitrile was added, solid Ph_3P (0.068 g, 0.26 mmol) and stirred for 2-3 hrs. The white ppt. of [CuI(Ph_3P)] were formed. The acetonitrile was decanted off from the reaction and precipitates were dissolved in 15 ml chloroform. To it was added, solid H_2itsc-N^1-Me (0.061 g, 0.26 mmol) and the reaction mixture was stirred for 3-4 h. Yellow color clear solution thus obtained was filtered and kept for crystallization on slow evaporation at room temperature. The product obtained was recrystallized from acetonitrile. Yield: 0.061 g, 68%; m.p. 218-220 ºC. Elemental analysis: Found: C, 48.5; H,

3.7; N, 8.04; S, 4.36. $C_{28}H_{26}N_4PSOCuI$ requires, C, 48.9; H, 3.8; N, 8.16; S, 4.66%. Main IR peaks (KBr, cm^{-1}), υ(N–H), 3468s, 3402s; υ(–NH–) 3217m; (NH, isatin) 3142s; $\upsilon(C–H_{Ph})$, 3051s; $\upsilon(C–H_{Me})$, 2814; υ(C=O) 1687s; $\delta(NH_2)$ + υ(C=N) + υ(C–C), 1597, 1554s; υ(C=S) 826s (thioamide moiety), $\upsilon(P–C_{Ph})$, 1095s. ^{1}H NMR (DMSO, δ ppm): 12.64 s (1H NH_{isatin}), 11.23s (1H, N^2H), 7.76 s (1H N^1H), 7.67-6.93 m (19H, $C^{5,7,8,6}H$ + Ph_3P), 2.50 m (3H, CH_3). m/z (ESI) 707.5 (M^++ Na), 684.9 (M^+).

Complexes **C2-C6** were prepared by a similar method albeit with their respective metal salts.

[CuBr(η^2-N^3, S-H$_2$itsc-N^1-Me)(Ph$_3$P)] C2.

Yield: 0.080 g, 76%; m.p. 182-185 ºC. Elemental analysis, Found: C, 53.5; H, 4.0; N, 9.76; S, 4.39. $C_{28}H_{26}N_4SOPCuBr{\cdot}0.5CH_3CN$ requires: C, 53.0; H, 4.2; N, 9.54; S, 4.84. Main IR peaks (KBr, cm^{-1}), υ(N–H), 3306s, 3207s, 3051s, 3026s; (NH, isatin) 2983m; $\upsilon(C–H_{Me})$, 2935s; $\upsilon(C–H_{Ph})$, 3026; υ(C=O) 1707s; $\delta(NH_2)$ + υ(C=N) + υ(C–C), 1614s, 1556m, 1479m, 1464m; υ(C=S) 826s (thioamide moiety), $\upsilon(P–C_{Ph})$, 1095s. ^{1}H NMR (DMSO, δ ppm): 11.25 s (1H, NH_{isatin}), 9.50 s (1H, N^2H), 7.80 s (1H N^1H), 7.39-6.89 m (19H $C^{5,7,8,6}H$+Ph_3P), 3.06 m (3H, CH_3). m/z (ESI) 660 (M^++ Na), 637 (M^+).

[CuCl(η^2-N^3, S-H$_2$itsc-N^1-Me)(Ph$_3$P)] C3.

Yield: 0.115 g,72 %; m.p. 182-185 ºC. Elemental analysis: Found: C, 56.2; H, 4.4; N, 11.00, S, 4.99. $C_{28}H_{25}N4SOPCuCl{\cdot}CH_3CN$ requires, C, 56.6; H, 4.4; N, 11.26; S, 5.03. Main IR peaks (KBr, cm^{-1}), υ(N–H), 3470s, 3402s, 3215m; υ(–NH–) 3132m; (NH, isatin) 2982m; $\upsilon(C–H_{Me})$, 2893, 2823; υ(C=O) 1687s; $\delta(NH_2)$ + υ(C=N) + υ(C–C), 1653, 1620s, 1543s, 1467s; υ(C=S) 827s (thioamide moiety), $\upsilon(P–C_{Ph})$, 1103s. ^{1}H NMR (DMSO, $CDCl_3$, δ ppm): 11.23 s (1H, NH_{isatin}) 9.42 s (1H, N^2H), 7.78 s (1H, N^1H), 7.39-6.91 m (19H, $C^{5,7,8,6}H$ + Ph_3P), 3.10 m (3H, CH_3). m/z (ESI) 616 (M^++ Na), 593 (M^+).

[CuI(η^2-N^3, S-H_2itsc-N^1-Et)(Ph_3P)] C4.

Yield: 0.065 g, 71%; m.p. 197-200 ºC. Elemental analysis, Found: C, 49.5; H, 3.8; N, 7.96; S, 4.53. $C_{29}H_{28}N_4SOPCuI$ requires, C, 49.6; H, 3.9; N, 7.98; S, 4.56. Main IR peaks (KBr, cm^{-1}), υ(N–H), 3306s, 3273s; υ(–NH–) 3180m; (NH, isatin) 3053s; υ(C–H_{Ph}), 2982w, 2929w; υ(C–H_{Et}), 2829; υ(C=O) 1685s; δ(NH_2) + υ(C=N) + υ(C–C), 1651s, 1591m, 1543s; υ(C=S) 827s (thioamide moiety), υ(P–C_{Ph}), 1094s. ^{1}H NMR (DMSO, δ ppm): 11.24 s (1H, NH_{isatin}), 9.5 s(1H, N^2H), 7.8 s (1H, N^1H), 7.46-6.9 m (19H, $C^{5,7,8,6}H$+Ph_3P), 2.5 t (3H, CH_3), 1.206 m (2H, CH_2). m/z (ESI) 722 (M^+ + Na), 699 (M^+).

[CuBr(η^2-N^3, S-H_2itsc-N^1-Et)(Ph_3P)] C5.

Yield: 0.087 g, 74%; m.p. 182-185 ºC. Elemental analysis, Found: C, 53.4; H, 4.0; N, 9.17; S, 4.81. $C_{29}H_{28}N_4PSOCuBr{\cdot}0.5CH_3CN$ requires, C, 53.5; H, 4.4; N, 9.36; S, 4.75. Main IR peaks (KBr, cm^{-1}), υ(N–H), 3308s; υ(–NH–) 3176s; (NH, isatin) 3049s, 3012s 2980m; υ(C–H_{Ph}), 2928s; υ(C–H_{Et}), 2889; υ(C=O) 1705s; δ(NH_2) + υ(C=N) + υ(C–C), 1614s, 1570m, 1543s, 1523m, 1458s; υ(C=S) 828s (thioamide moiety), υ(P–C_{Ph}), 1094s. ^{1}H NMR (DMSO, δ ppm): 11.23 s (1H, NH_{isatin}), 9.56 s (1H, N^2H), 7.9 s (1H, N^1H), 7.34-6.90 m (19H, $C^{5,7,8,6}H$ + Ph_3P), 2.5 t (3H, CH_3), 1.179 m (2H, -CH_2-). m/z (ESI) 674 (M^+ + Na), 651 (M^+).

[CuCl(η^2-N^3, S-H_2itsc-N^1-Et)(Ph_3P)] C6.

Yield: 0.018 g, 72%; m.p. 182-184 ºC. Elemental analysis, Found: C, 57.2; H, 4.3; N, 10.07; S, 4.62. $C_{29}H_{28}N_4PSOCuCl{\cdot}CH_3CN$ requires, C, 57.3; H, 4.8; N, 10.78; S, 4.93. Main IR peaks (KBr, cm^{-1}), υ(N–H), 3306m; υ(–NH–) 3161m; (NH, isatin) 2982m; υ(C–H_{Et}), 2929; υ(C=O) 1687s; δ(NH_2) + υ(C=N) + υ(C–C), 1653, 1620s, 1543s, 1467s; υ(C=S) 827s (thioamide moiety), υ(P–C_{Ph}), 1093s. ^{1}H NMR (DMSO,$CDCl_3$ δ ppm): 11.23 s (1H, NH_{isatin}), 9.46 s (1H, N^2H), 7.80 s (1H, N^1H),7.38-6.91 m (19H, $C^{5,7,8,6}H$+Ph_3P), 2.53 t (3H, CH_3), 1.18 m (2H, CH_2). m/z (ESI) 630.1 (M^+ + Na), 607.1 (M^+).

2.4. X-ray Crystallography

A single crystal was used for data collection with Agilent, Eos, Gemini (**C2**) and *X'calibur* CCD (**H_2L^1**), equipped with graphite monochromated Mo-Kα radiation (λ = 0.71073 Å). Crystal data was collected at 173 (2) (**C2**) and 293(2) (**H_2L^1**) K. Data was processed with CrysAlisPro, Agilent Technologies (**C2**), CrysAlisPro, Oxford Diffraction Ltd (**H_2L^1**) (data collection) and CrysAlisPro RED (cell refinement, data reduction). [50] For structure solution program SHELXS-97 (**H_2L^1**) [51] was used, whereas structure refinement was done by SHELXL (**C2**)[52] and SHELXL-97 (**H_2L^1**). Atomic scattering factors taken from "International Tables for Crystallography".[53]

2.5. Cytotoxicity studies

2.5.1. Cell culture

L123 (human lung cells) and HepG2 (Human hepatocellular carcinoma cells) was cultured in Dulbecco's modified Eagle's medium with 10% fetal bovine serum (heat inactivated), 100 units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, and 2.5 μg mL^{-1} amphotericin B, at 37 ^oC in a saturated humidity atmosphere containing 95% air/5% CO_2.[54] The cell lines were harvested when they reached 80% confluence to maintain exponential growth.

2.5.2. MTT assay

The MTT assay is a standard colorimetric assay, in which mitochondrial activity is measured by splitting tetrazolium salts with mitochondrial dehydrogenases in viable cells only.[55] For viability testing, MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide, M2128 from Sigma) cell proliferation assay was carried out. The cell monolayers in exponential growth were harvested using 0.25% trypsin and single-cell suspensions were obtained by repeated pipetting. Only viable cells were used in the assay. Exponentially growing cells were plated at 1.2×10^4 cells per well into 96-well plates (Costar, Corning, NY, USA) and incubated for 48 h before the addition of drugs to achieve the maximum confluency of the cells. Stock solutions were prepared by dissolving the compounds in 10% (v/v) DMSO and further diluted with fresh complete medium to achieve 1M concentration. Cells were incubated with different concentrations of

cisplatin (reference drug) and test compounds for 48 h at 37 ^{o}C in 5% CO_2 humidified incubator together with untreated control sample. At appropriate time points, cells were washed in PBS, treated with 50 µL MTT solution (5 mgmL^{-1}, tetrazolium salt) and incubated for 4 h at 37^{o}C. At the end of the incubation period, the medium was removed and pure DMSO 150 µL was added to each well. The metabolized MTT product dissolved in DMSO was quantified by measuring the absorbance at 570 nm on an Microplate reader (iMark, BIORAD) with a reference wavelength of 655 nm. All assays were performed in triplicate and repeated thrice.

The percentage inhibition was calculated, from the data, using the formula given below, and IC_{50} values were calculated using nonlinear regression analysis.

$$\frac{\text{Mean OD of untreated cells (control) - Mean OD of treated cells} \times 100}{\text{Mean OD of untreated cells (control)}}$$

The IC_{50} concentration was determined as the dose that needs to be required to kill 50% of the cells.

2.6. Molecular docking study

Docking studies

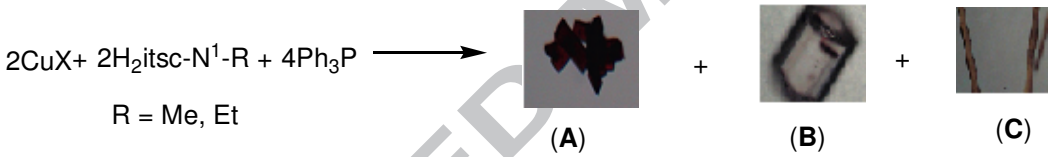
Compounds were built using the builder tool kit of the software package Argus Lab 4.0.1.23 and energy minimized with semi-empirical quantum mechanical method PM3. Crystal coordinates of Hodgkin lymphoma enzyme and DNA were downloaded from protein data bank and in the molecule tree view of the software, the monomeric structures of the crystal co-ordinate was selected and the active site was defined as 15 Å around the ligand. Validation of the docking programme was checked by docking the known inhibitors of the respective enzymes in their binding sites. The molecule to be docked in the active site of the enzyme was pasted in the work space carrying the structure of the enzyme. The docking programme implements an efficient grid based docking algorithm which approximates an exhaustive search within the free volume of the S19 binding site cavity. The conformational space was explored by the geometry optimization of the flexible ligand (rings were treated as rigid) in combination with the incremental construction of the ligand torsions. Thus, docking occurs between the flexible ligand parts of the compound and enzyme. The docking was repeated several times (approx. 10000 iterations) until no change in the position of the ligand and a constant value of the binding energy was observed. The ligand orientation was determined by a shape scoring function based on Ascore and the final positions

were ranked by lowest interaction energy values. H-bond between the respective compound and enzyme were explored.

3. Results and Discussion

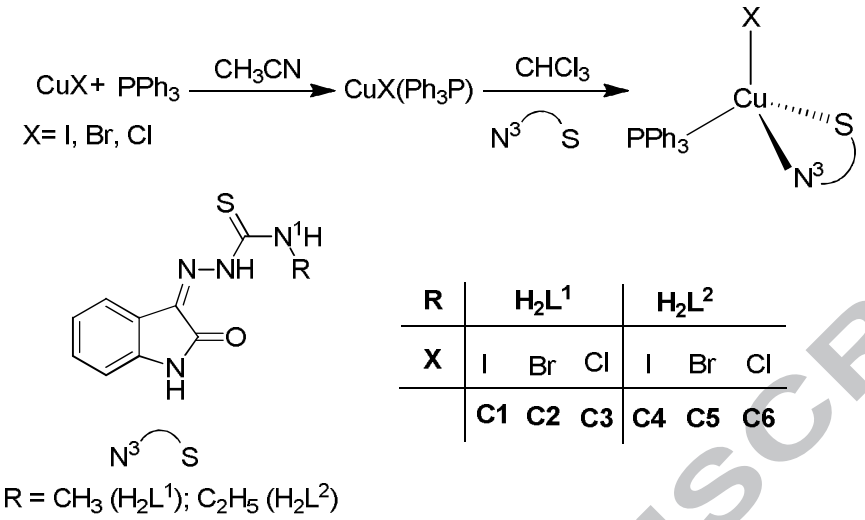
3.1. Chemistry

Reaction of un-substituted isatin-3-thiosemicarbazone (H_2itsc) with copper(I) halides and Ph_3P in 1 : 1 : 1 (M : L : PPh_3) molar ratio formed insoluble orange colored compounds. To solubilize them and to get crystalline product, one mole of Ph_3P had been further added, which formed tetrahedral monomeric complexes, [CuX(η^1-S-H_2itsc)($Ph_3P)_2$] (X = I, Cl, Br).[36, 46] However, with substituted isatin-3-thiosemicarbazone (H_2itsc-N^1-R) (R = Me, Et), the similar reaction in 1 : 1 : 2 molar ratio formed three types of crystals (i) Red crystals of stoichiometry, [CuX(H_2itscR)(Ph_3P)] (**A**), (ii) white crystals of formula, [CuX($Ph_3P)_3$] (**B**) and (iii) yellow needles of free ligand (**C**). The whole reaction sequence is given in Scheme 1, the characterization data of B and C is provided in supplementary information.



Scheme **1**

Compounds of stoichiometry, [CuX(H_2itscR)(Ph_3P)] (R=Me, H_2L^1; Et, H_2L^2) were of our interest and were obtained by another route. Copper(I) halides (X = I, Br, Cl) were reacted with Ph_3P in 1 : 1 molar ratio to form white solid of composition, CuX(Ph_3P) in acetinitrile. Acetonitrile was then removed and precipitates were dissovled in chlorofrom and one mole of H_2L^1 and H_2L^2 was added to get complexes of stiochiometry, [Cu(η^2-N^3, S-H_2itsc-N^1-Me)(Ph_3P)] (X = I, **C1**; Br, **C2**; Cl, **C3**) and [CuX(η^2-N^3, S-H_2itsc-N^1-Et)(Ph_3P)] (X = I, **C4**; Br, **C5**; Cl, **C6**) respectively (Scheme 2).



Scheme **2**

The ν(N-H) bands in IR spectra of complexes can be divided into three categories: (i) bands between 3490–3200 cm^{-1} due to (symmetric and asymmetric stretching mode) amino group, (ii) band in the ranges, 3051–3081 cm^{-1}, due to NH of isatin ring, (iii) band in the ranges, 3132-3149 cm^{-1} due to amide (-NH-) group. The presence of all these bands in the complexes **C1**-**C6**, suggest that no deprotonation occurs at any nitrogen atom and ligand is coordinating in neutral form. The characteristic ν(C=S) band in H_2L^1 and H_2L^2 appeared at 835 and 837 cm^{-1} respectively and showed shift to lower region (826-828 cm^{-1} **C1**-**C6**) in their complexes. This shift indicates the binding of metal ion with thione sulfur. The presence of ν(P-C_{Ph}) in the range, 1093-1103 cm^{-1} ensures the coordination of triphenylphosphine to the copper center.[36, 46]

In ^{1}H NMR spectra of ligands, –N^2H- proton appeared at δ11.22 (H_2L^1) and δ11.21 ppm (H_2L^2), which showed an upfield shift in complexes (**C2**-**C6**) (δ9.33 - 9.56 ppm). Similarly, the NH proton of isatin ring and N^1H proton appeared at δ12.60 (H_2L^1) and at δ12.55 (H_2L^2). Both these signals also showed an upfield shift in complexes **C2-** C**6** vis-à-vis free ligand and no change was observed in complex **C1**. The presence of all these protons indicate that no deprotonation take place during complexation and isatin thiosemicarbazone ligands coordinate to the copper center in neutral form. The shift in the position of these protons also ensure complexation. The C^5H, C^6H, C^7H and C^8H ring protons of thiosemicarbazone ligands appeared in range, δ7.67-6.89 ppm in the complexes. The CH_3 group at N^1H gives a doublet (in range δ

3.17-2.50 ppm, **C1**-**C3**) and due to ethyl group a triplet (at δ2.5 ppm) and a multiplet (in the range δ1.18-1.20 ppm, **C4**-**C6**).

3.2. Structure of N^1-methyl-isatin-3-thiosemicarbazone (H_2L^1) and Complex C2

The molecular structure of ligand N^1-methyl-isatin-3-thiosemicarbazone (**H_2L^1**) and Complex **C2** is given in Figure 1 and Figure 2 respectively. The crystallographic data and important bond parameters are given in Tables **1** and **2** respectively.

Structure of H_2L^1

The ligand N^1-methyl-isatin-3-thiosemicarbazone crystallizes in monoclinic crystal system with space group $P2_1/c$. The two rings of isatin moiety (five and six membered) are nearly planer and the dihedral angle between mean plane of both rings is 2.08(5)°, which is close to that of N^1-ethyl-isatin-3-thiosemicarbazone, (H_2itsc-N^1-Et, 2.1°) and N^1-hexamethyleniminyl isatin-3-thiosemicarbazone, (H_2itsc-N-Hm, 0.5°).[33] The angle describing plane of the thiosemicarbazone moiety, N(1)–N(2)–C(3)–S(1) and N(1)–N(2)–C(3)–N(3) is 1.8 and 1.6° respectively.

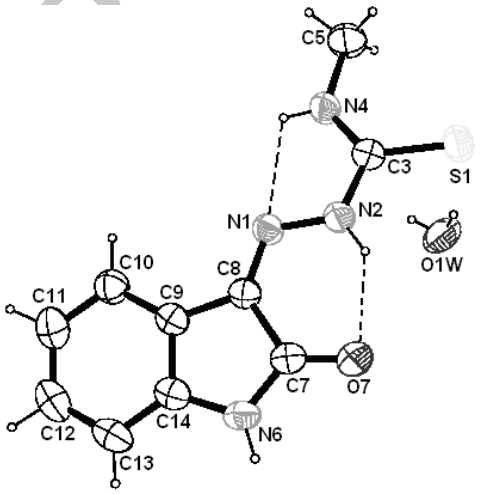


Figure 1. ORTEP view of H_2itsc-N^1-Me (**H_2L^1**), showing the atom-labeling scheme

H_2itsc-N^1-Me, show Z – configuration about C(8)=N(1) double bond due to intramolecular H-bonding between N(2)H of thiosemicarbazone and O(7) of isatin similar to unsubstituted and substituted isatin-3-thiosemicarbazones (Table 2).[34] The formation of five

membered ring due to H-bonding between N(4)H and N(1) leads to E-configuration about N(2)-C(3) similar to H_2itsc and H_2itsc-N^1-Et, but opposite to Z-configuration in case of H_2itsc-N^1-Hm, where no such H-bonding is possible.[33]

Structure of [CuBr(η^2-N^3, S-H_2itsc-N^1-Me)(Ph_3P)] C2:

Complex **C2** crystallizes in triclinic crystal system with space group P-1. In this complex, phosphorous atom from triphenylphosphine ligand, one bromide atom, imino nitrogen (N^3) and thione sulfur of thio-ligand formed corners of tetrahedron with copper(I) in center. N^1-methyl-isatin-3-thiosemicarbazone coordinate as neutral ligand binding to copper(I) in N^3, S- chelation mode. The Cu–S bond distance, 2.3274(10) Å is close to that of 2.3566(10) Å in [CuBr(η^1-S-H_2itsc)($Ph_3P)_2$][46], but shorter than, 2.4156(6) Å in [Cu(η^2-N^3, S-Hitsc)$Ph_3P)_2$]·NO_3.[56] Cu–Br bond length in **C2**, 2.4219(6) Å is less than sum of ionic radii of Cu and Br (Cu^+, Br^-, 2.73 Å). [57] This Cu–Br bond length is also shorter than that of 2.5459(6) Å in [CuBr(η^1-S-H_2itsc)($Ph_3P)_2$][46]. Cu-N bond distance, 2.108(3) Å is close to that found in literature. [56, 58-59] The C–S bond length, 1.689(3) Å is close to 1.6880(15) Å in free ligand (H_2L^1) as well in literature[56, 58-59], which indicates binding of ligand to metal center in thione (C=S) form. The bond angles around copper in range, 84.64(8)-121.35(3)° reveal distorted tetrahedral geometry. The maximum distortion of 121.35(3)° is found to be in P–Cu–Br angle. The Cu-S-C angle, 95.61(11)° in **C2** is close to 96.91(12)° in [(Ph_3P)Cu(μ-Br)(μ_3-S, N^3–Hcptsc)CuBr(PPh_3)], where cyclohexanone thiosemicarbazone (Hcptsc) binds to metal in N^3,S-chelation-cum S-bridging mode.[60] This angle is much shorter than 113.30(12)° in [CuBr(η^1-S-H_2itsc)($Ph_3P)_2$], where the thiosemicarbazone was acting as terminal monodentate ligand.[46]

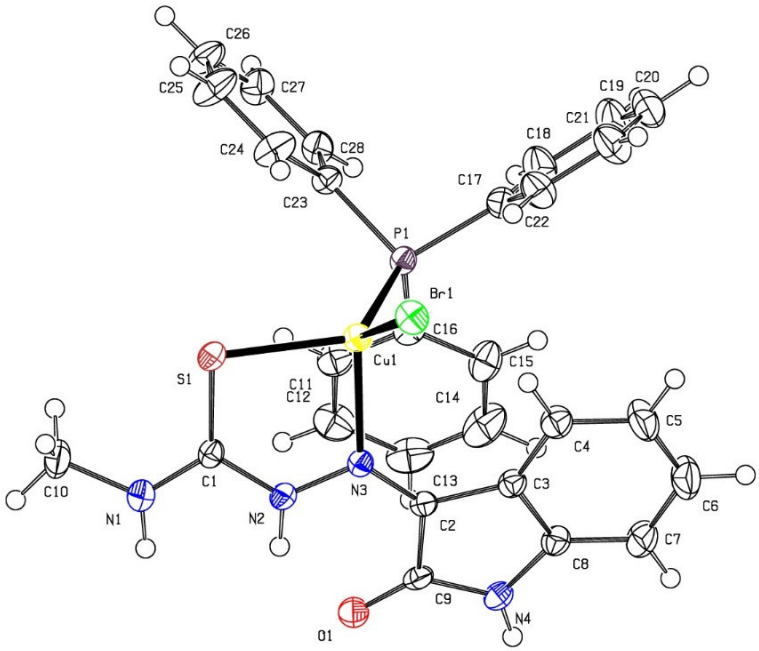


Figure 2. ORTEP view of **C2** showing the atom-labeling scheme

In **C2**, inter-molecular H-bonding beween pyrrole hydrogen (N^4H) of one molecule and bromide atom of other molecule, $N^4H\cdots Br$, 2.778Å leads to formation of H-bonded dimer. An additional CH···HC, 2.322 Å interaction between phenyl ring of triphenylphosphine of two molecules is also present. These interactions are repeated to form 1D chain. Inter-molecular H-bonding between prrrole nitrogen of one chain and Phenyl hydrogen of triphenylphosphine molecule of second chain, $HN^4\cdots HC_{Ph,}$ 2.733 Å lead to formation of 2D sheet (Figure 3).

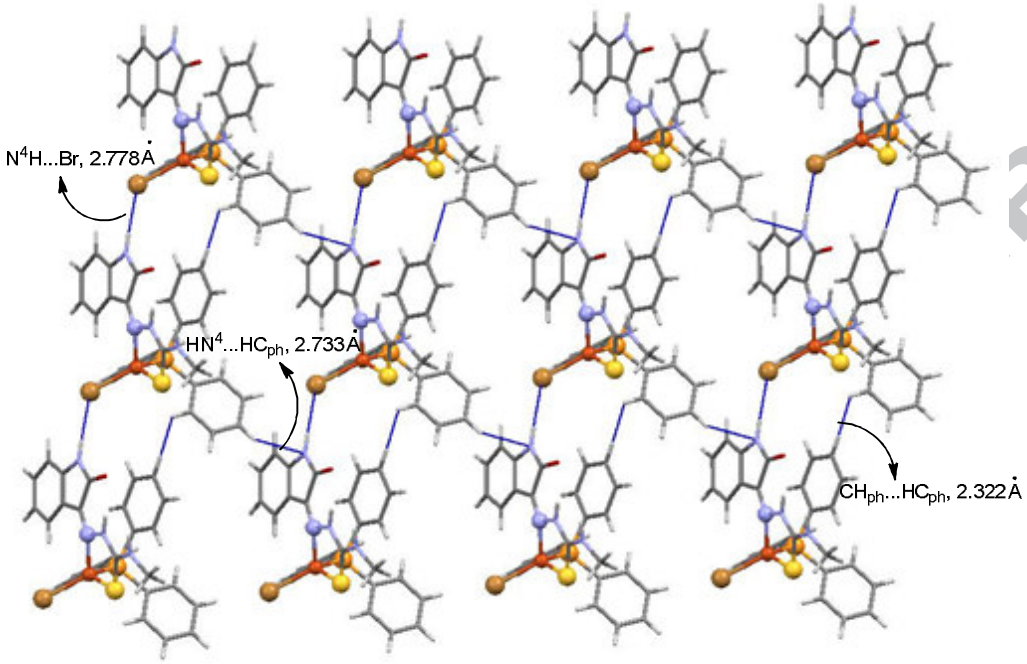


Figure 3. Packing diagram of **C2** showing H-bonding and various interactions

Table 1: Crystallographic data for H_2itsc-N^1-Me (**H_2L^1**)

	H_2L^1	**C2**
Crystal description	Block shaped	Prism
Crystal colour	Orange	Red
Crystal size	0.30 x 0.20 x 0.20 mm	0.34 x 0.24 x 0.12 mm
Empirical formula	$C_{10}H_{10}N_4OS.H_2O$	$C_{28}H_{25}BrCuN_4OPS$
Formula weight	252.30	640.00
Radiation, Wavelength	Mo *K*α, 0.71073 Å	Mo *K*α, 0.71073 Å
Unit cell dimensions	a = 8.9306(2), b = 13.0538(3), c = 10.2347(2) Å, β = 93.105(2)^o	a = 9.8769(7), b = 11.2821(8), c = 14.1915(11) Å, α = 93.964(6), β = 102.013(6), γ = 105.636(6)^o
Crystal system	Monoclinic	Triclinic

Space group	$P2_1/c$	P-1
Unit cell volume	1191.39(4) $Å^3$	1476.3(2) $Å^3$
No. of molecules per unit cell, Z	4	2
Temperature	293(2) K	173(2) K
Absorption coefficient	0.268 mm^{-1}	2.245 mm^{-1}
F(000)	528	648
Scan mode	ω scan	ω scan
θ range for entire data collection	3.48 <θ< 24.99 °	3.000<θ< 32.743º
Range of indices	h= -10 to 10, k= -15 to 15, l= -12 to 12	h= -14 to 14, k= -16 to 15, l= -21 to 21
Reflections collected / unique	24272 / 2083	18759 / 9734
Reflections observed (I > 2σ(I))	1798	6849
R_{int}	0.0404	0.0998
R_{sigma}	0.0171	0.0671
Final R	0.0320	0.0998
$wR(F^2)$	0.0835	0.1677

Table 2: Selected bond distance (Å) and bond angles (°) for H_2itsc-N^1-Me (**H_2L^1**)

H_2L^1			
S(1) – C(3)	1.6880(15)	N(1) – N(2) – C(3)	120.41(13)
N(1) – C(8)	1.2906(19)	N(4) – C(3) – N(2)	116.97(14)
N(1) – N(2)	1.3561(18)	N(4) – C(3) – S(1)	125.32(12)
N(2) – C(3)	1.364(2)	N(2) – C(3) – S(1)	117.71(11)
C(3) – N(4)	1.312(2)	C(3) – N(4) – C(5)	123.80(16)
N(4) – C(5)	1.455(2)	O(7) – C(7) – N(6)	127.44(15)

C(7) – O(7)	1.236(2)	O(7) – C(7) – C(8)	126.07(14)
C(7) – C(8)	1.507(2)	N(6) – C(7) – C(8)	106.48(14)
C(8) – N(1) – N(2)	116.55(13)	N(1) – C(8) – C(9)	126.13(14)
[CuBr(η^2-N^3, S-H_2itsc-N^1-Me)(Ph_3P)] C2:			
Cu(1) – Br(1)	2.4219(6)	P(1) – Cu(1) – Br(1)	121.35(3)
Cu(1) – S(1)	2.3274(10)	P(1) – Cu(1) – S(1)	113.66(4)
Cu(1) – P(1)	2.2300(10)	P(1) – Cu(1) – N(3)	105.57(8)
Cu(1) – N(3)	2.108(3)	S(1) – Cu(1) – Br(1)	111.37(3)
N(3) – N(2)	1.361(4)	Br(1) – Cu(1) – N(3)	114.24(8)
C(1) – N(1)	1.330(5)	N(3) – Cu(1) – S(1)	84.64(8)
C(1) – S(1)	1.678(4)	Cu(1) – S(1) – C(1)	96.91(12)

3.3. Cytotoxicity and docking studies

MTT assay was used to assess the % cell viability of the newly synthesized compounds against L123 (human lung cells) and HepG2 (Hepatocellular carcinoma cells) cell lines to check their cytotoxicity profile. Cisplatin was used as a reference drug. A sub-confluent population of L123and HepG2 cells was treated with increasing concentration of these compounds and the number of viable cells was measured after 48 h by MTT cell viability assay. The concentration range of all the compounds was 1.55-50 μM. The cytotoxicity of complexes **C1**-**C6** was found to be concentration-dependent. The data reported in Table **3** suggest that all the complexes showed different levels of cytotoxicity. IC_{50} value for Complexes **C1**-**C6** fall in range, 8.31-25.50 μM (L123) and 18.65-22.50 μM (HepG2) (Table **3**).

Table 3: Cytotoxicity profile of synthesized compounds and reference drug Cisplatin*.

Compounds→ ($IC_{50}\mu M$)[a]							
Cell lines↓	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**Cisplatin**
L123	18.31	25.86	22.50	18.01	24.54	22.45	8.5
HepG2	22.56	25.50	18.65	20.54	22.65	18.65	9.65

[a]IC_{50} = the concentration of compound that inhibits 50% of cell growth.

*Each cytotoxic activity was performed in triplicate and average result is given with an error range of ±0.05

3.4 Molecular modeling studies

Based on the *in vitro* anticancer activity to the Hodgkin lymphoma cell-line L1236, one of the active complex **2** was selected to evaluate the putative binding mode with amino acids in the active site of the enzyme using molecular docking technique. To confirm the binding affinity or probable binding site, docking study of cisplatin was first carried out with Hodgkin lymphoma cell line (PDB ID 1QOK)[61] using builder tool kit of software package ArgusLab 4.0.1 (www.arguslab.com).[62] It was observed that NH_2 groups of cisplatin binds with the cysteine 184 (d = 2.51 Å) and 248 (d = 2.76 Å) amino acid residues through hydrogen bonding. Moreover, it also showed binding interactions with histidine 194 (d = 1.03 Å) and glutamine 249 (d = 2.62 Å) amino acid residue. Encouraging from the results of cisplatin with Hodgkin lymphoma cell line and finding out the probable binding sites from these interactions, docking was then performed by placing complex **2** into the binding site of target-specific region (where cisplatin showed its interactions) of the Hodgkin lymphoma cell-line (PDB ID 1QOK). The docked model of cisplatin and complex **2** with Hodgkin lymphoma enzyme was shown in Figure **4**. Complex **2** is surrounded by side chains of various amino acids such as F196, H194, Y192 and Y231, indicating that the molecule is well fitted in the active pocket. The docking results revealed that the interaction of complex **2** with Hodgkin lymphoma is dominated by hydrogen bonding interactions with tyrosine (Y247), glutamine (Q249) and cysteine (C248 and C184) amino acid residues. As shown in Figure 4, NH of complex **2** showed hydrogen bonding interactions with S of cysteine (C248 and C184) amino acid residues and N of tryptophan (W195) of 1QOK (bond

distance: NH…S = 2.96 Å in case of C248, NH…S = 2.73 Å in case of C184 and NH…N = 2.81 Å of W195). NH group of indole ring of complex **2** also showed hydrogen bonding interactions with oxygen of glutamine (Q249) and tyrosine (Y247) amino acid residue (bond distance: NH…O = 2.41 Å in case of Q249 and NH…O = 1.98 Å in case of Y247). Therefore, docking of complex **2** in the active site of enzyme indicated the probable mode of action for anticancer activity.

A **B**

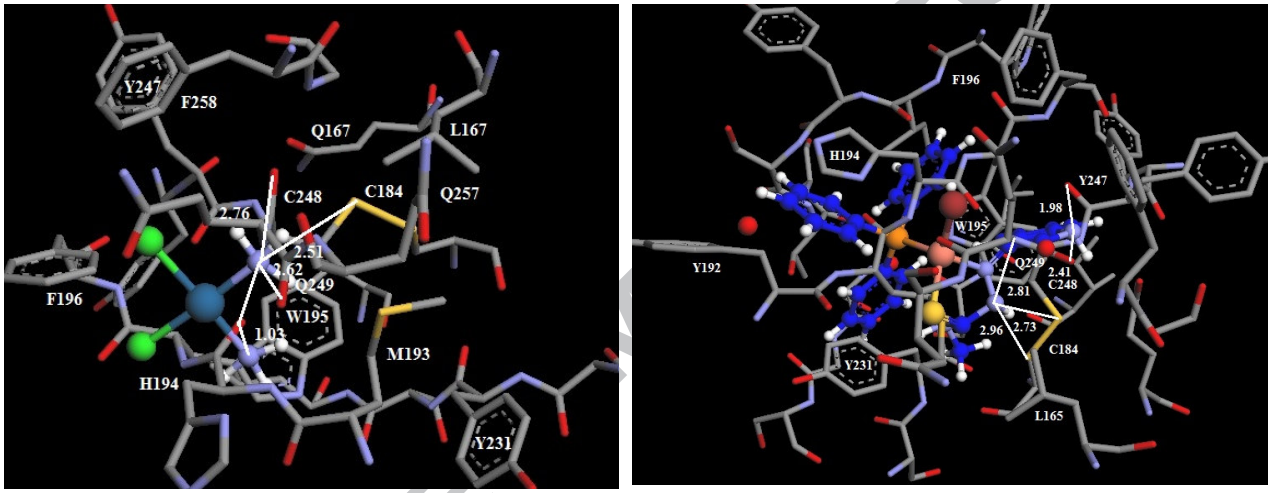


Figure 4 Docking of cisplatin (**A**) and complex **2** (**B**) in the active site of Hodgkin lymphoma (PDB ID 1QOK)

Molecular docking is an interesting tool to predict the possible drug–DNA interactions because the mode of action of cisplatin is directly associated with their binding to DNA. Thus, cisplatin showed interactions with DNA, crosslinked DNA in several different ways, interfering with cell division by mitosis and kill cells. To explore the most feasible binding site or binding affinity, docking studies have been performed on cisplatin and complex **2** with DNA (PDB ID: 1BNA). It has been demonstrated that both cisplatin and complex **2** showed interactions with DNA, the probable mode of action for anticancer activity (Supporting Information).

Conclusion

Previous reports have suggested that un-substituted isatin-3-thiosemicarbazone formed mononuclear tetrahedral complexes with copper(I) halides whereas the structure of complex **C2** revealed that substituent at N^1 (methyl) of isatin-3-thiosemicarbazone has changed the binding mode of ligand from terminal to N^3, S- chelation of its copper (I) bromide complex. The analytical data supports same result for other complexes also. The *in vitro* cytotoxicity evaluation showed moderate activity for these compounds. Docking study of **C2** reveal that the molecule is well fitted in the active pocket of Hodgkin lymphoma enzyme and the interaction of complex **C2** with Hodgkin lymphoma for the binding site is dominated by hydrogen bonding interactions with Y247, Q249, C248 and C184 amino acid residues.

Acknowledgements:

The authors thank Lovely Professional University for providing all the facilities to carry out the experimental work. JPJ acknowledges the NSF–MRI program (Grant No. CHE-1039027) for funds to purchase the X-ray diffractometer.

Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary data is available from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK. Fax: + 44-1223-336033 email: deposit@ccdc.cam.ac.uk) on request quoting the deposition number CCDC 842975 for structure of **H_2L^1 and** 1448643 for **C2.**

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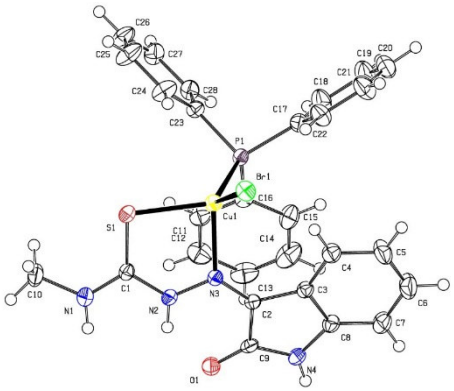
62	Compounds were constructed and docked with builder tool kit of software package ArgusLab 4.0.1 (www.arguslab.com).

Graphical Abstract (Pictorial):



C2

Synopsis

Unsubstituted isatin-3-thiosemicarbazone formed mononuclear tetrahedral complexes with copper(I) halides whereas the structure of complex **C2** revealed that substituent at N^1 (methyl) of isatin-3-thiosemicarbazone has changed the binding mode of ligand from terminal to N^3, S-chelation of its copper (I) bromide complex.

Highlights

1. Substituent at N^1 (methyl) of isatin-3-thiosemicarbazone has changed the binding mode of ligand from terminal to N^3, S- chelation of its copper (I) bromide complex.
2. The *in vitro* cytotoxicity evaluation of complexes showed moderate activity for these compounds.
3. Docking study of **C2** reveal that the molecule is well fitted in the active pocket of Hodgkin lymphoma enzyme.