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Research Article

SYNTHESIS, CHARACTERIZATION, EGFR DOCKING, AND ANTICANCER EVALUATION OF ISATIN DIMETHYL MORPHOLINYL THIOSEMICARBAZONE AND ITS COPPER(II) COMPLEX

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ABSTRACT

Isatin-4-(2,6-dimethyl morpholinyl)-3-thiosemicarbazone (*Istdmpln*), and its copper(II) thiosemicarbazone (*Cu-istdmpln*) were prepared and analysed using CHNS analysis, mass spectrometry, NMR, UV-Vis, FTIR, EPR spectroscopy, and thermogravimetric analysis (TGA). Copper(II) thiosemicarbazone exhibited mononuclear square planar geometry with ONS linkage of thiosemicarbazone and a chloride ion. The absence of chemical shift (δ) at 4 ppm confirmed the formation of ligand in thione form. The shift of IR band corresponding to N–N toward higher energy and that of C=O and C=N toward lower energy confirmed the chelation of thiosemicarbazone to copper(II) ion. The Cu(II) complex exhibited significantly higher cell growth inhibition property than its ligand against both cancer cell lines ($IC_{50;}$ 13.01 µM, HCT116 and $IC_{50;}$ 31.19 µM, MDA-MB-231). The free ligand in aforementioned cell lines exhibited anticancer activity with the higher micromolar concentration (IC_{50}; 34.64 µM, HCT116 and IC_{50}; 43.99 µM, MDA-MB-231). The IC_{50} bar diagram showed that the Cu(II) complex is about 3 and 1.5 times more potent than the free ligand toward HCT116 and MDA-MB-231 cancer cells, respectively. Additionally, both ligand and copper(II) complex showed low toxicity toward normal embryonic kidney cell (HEK293) with IC_{50} 15.39-16.72 µM. Molecular docking analysis showed that both *Istdmpln* and *Cu-istdmpln* are effective in inhibiting the EGFR protein with the significant binding energy (kcal/mol) of -8.1 and -8.9, respectively. The remarkable result of anticancer potency on coordination of ligand to copper(II) ion encourages for mechanistic and *in vivo* studies of the synthesized compounds.

Keywords: Breast cancer, Colorectal cancer, Cu(II) complex, Isatin, Thiosemicarbazone

INTRODUCTION

Cancer is a significant global health challenge and the second most significant reason of death per year (Ferlay et al., 2015). Most of the current anticancer medications have serious side effects. So, synthesis of safe anticancer drugs is essential to reduce toxicity and multidrug resistance (Emami & Dadashpour, 2015; Wan et al., 2021). Heterocyclic rings, being important parts of biomolecules like RNA, DNA, haemoglobin, chlorophyll, enzymes, vitamins, etc., play a vital role in medicinal chemistry. Thus, the heterocyclic compounds are widely used as antibacterial, antioxidant, antifungal, anti-inflammatory, anticonvulsant, antiallergic, anticancer, herbicidal, enzyme inhibitors, anti-HIV, antidiabetic, insecticidal agents (Al-Mulla, 2017; Thakur et al., 2023). Isatin and its derivatives can interact with telomerase, tubulin, phosphatases, protein kinases, deoxyribose nucleic acid (DNA), or P-glycoprotein and exhibit anticancer activity (Vine et al., 2013). Isatin has tendency to enter the brain to inhibit enzymes, affect neurotransmitters, enhances pentobarbitone-induced effects, influences nervous system and shows antidepressant activity (Bhrigu et al., 2010). 5-Haloisatin and 5-nitroisatin thiosemicarbazones work as histone deacetylase (HDAC) inhibitors and carbonic anhydrase isoform IX (CA IX) inhibitors and increase the antiproliferative activity against cervical tumors (Eldehna et al., 2017; Singh et al., 2017; Singh et al., 2021). Thiosemicarbazones have ability to inhibit

ribonucleoside diphosphate reductase (RDR) that play a key role in the synthesis of DNA precursors (Singh et al., 2020, Hernandez et al., 2023). The 5-fluoroisatin thiosemicarbazone N(4) substitution by phenyl, methyl and ethyl showed strong HCT 116 cell growth inhibition at IC_{50} of 31.4 µM, 59.5 µM, and 66.6 µM respectively (Ali et al., 2014).

Copper has been used by humans for over 10,000 years and recently gaining renewed scientific attention due to its antimicrobial properties and potential use in healthcare environments (O'Gorman & Humphreys, 2012). It is necessary for keeping our nerve cells and our immune system healthy. It has significant importance in tumor patients because of its nature of accumulation in the tumor cells due to their selective permeability and its key role in DNA synthesis, enzymatic functions, intracellular redox potential regulation (Singh et al., 2020; Singh & Yadav, 2021). Copper is an essential nutrient for the human diet, though the body requires only a small quantity for good health (Osredkar et al., 2011). Copper ions (Cu I/II) can display significant biological activity due to their involvement in DNA cleavage, p53 protein activation, and cell death by generating reactive oxygen species (ROS) or impeding the progression of cell cycles (Singh et al., 2020). Copper complexes, known for their high oxidative potential, strong affinity for nucleobases, and effective binding to DNA as well as proteins are key players in redox reactions within cancer cells that cause ROS production. This copper complex tends to have less impact on healthy cells compared to cancer cells by selective inducing apoptosis (Banerjee et al., 2025). The distinct metabolic pathways and differential responses of cancer cells have contributed to the advancement of copper-based antineoplastic agents (Singh & Yadav, 2021). The presence of coordination sites in the complexes can enhance their biological activities due to increased lipophilicity of complexes (Lobana et al., 2009). N(4) substituted copper(II) complexes have been found to exhibit anticancer activity higher than their free ligands (Singh et al., 2021, 2022).

Considering the biological importance of heterocyclic compounds, thiosemicarbazones and copper, new N(4) 2,6-dimethyl morpholinyl isatin thiosemicarbazone (*Istdmpln*) and its copper(II) complex (*Cu-istdmpln*) were prepared and tested for anticancer activity against HCT116 and MDA-MB-231 and non- toxicity against normal cell; HEK293.

MATERIALS AND METHODS

Materials

N-methyl aniline (98%) was purchased from Alfa Aeser., Isatin (98%), and sodium chloroacetate (98%) were bought from Chemical center. 2,6-dimethyl morpholine was purchased from Sigma Aldrich. Acetonitrile (99.8%), diethyl ether (99%) and hydrochloric acid were bought from Merck. The hydrazine hydrate (99-100%), glacial acetic acid (99.8%) and methyl alcohol (99.8%) were taken from Fisher Scientific. CS_2 (99%) and absolute alcohol (99.9%) were purchased from Qualigens and Changshu Hongsheng, respectively and used as received. Melting point of the new compounds were noted using Optics Technology, Ordinary Model, melting point apparatus. The electronic transition bands were measured using the SHIMADZU UV-2600 series spectrophotometer at Pulchowk Engineering Campus, Tribhuvan University, Lalitpur using Barium sulphate as a coating on the integrating sphere of the spectrophotometer, in the range of 800–200 nm. Perkin-Elmer CHN-2400 was used for CHNS analysis of the compounds in Sophisticated Test and Instrumentation Centre **(**STIC), Cochin University of Science & Technology Campus, Kerala, India. FTIR spectra were recorded in the range of 4000–500 cm^{-1} at the Department of Chemistry, Amrit Campus, using a Fourier Transform Infrared (FTIR) spectrometer (Perkin Elmer, USA). Bruker NMR spectrometer having 400 MHz, 9.4 Tesla of Advance III HD model was used to measure the value of chemical shift (δ ppm). The data of ESI-HRMS (LC-QTOF-HRMS instrument having ACQUITY H-CLASS PLUS UPLC system coupled with XevoG₂ XS QTOF spectrometer, PDA detector, StepWave ion optics, QuanT of technology and XS Collision Cell) and EPR (JEOL Model JES FA200 instrument, 8.75 - 9.65 GHz X-Band frequency, 7×10^9 spins/0.1mT sensitivity, 2.35 µT resolution, -153 to +25 °C variable temperature frequency) were collected in Indian Institute of Technology Madras, Chennai. Thermal behavior of complex of copper(II) was studied by using DSC/TGA system of Simultaneous Thermal Analyzer-SDT 650 at Savitribai Phule Pune University, Pune, India into nitrogen atmosphere at 20–800 ºC with 10 ºC per minute heating rate.

Methods

Ligand structure preparation

The structures of the synthesized compounds were depicted in 2D form using Chemdraw software and then converted into 3D form by using Avogadro software (https://avogadro.cc/) in BIOVIA Discovery

studio software. Later, the structures were saved in PDBQT (PDB (Protein Data Bank)+Q(Charges)+T(Atom Types)) format after minimizing the energy in AutoDock Tools software (https://vina.scripps.edu/).

Receptor preparation

The crystal structure of the EGFR with pyrrolo[3,2-d]pyrimidine (PDB ID: 3W2S) was downloaded from the Protein Data Bank, RCSB (https://www.rcsb.org/). The binding sites of the protein was predicted as binding pocket of co-crystallized ligand which were verified by a comprehensive review of the literature, prior to this research (Sogabe et al., 2013). The EGFR protein was obtained by extracting all the chain structures apart from the target protein structure and saving them in the PDB format using the BIOVIA Discovery Studio software https://www.3ds.com/products/biovia/discovery-studio. The structures were saved in the PDBQT format after being optimized with Kollmann charges and adding polar hydrogen in the AutoDock tools (Morris et al., 2009). The Grid box with dimensions of 24×22×20 Å was defined to enclose the key binding site residues, ensuring covering the ligand-binding pocket.

Molecular docking experiment

Molecular docking was carried out to evaluate the binding mechanisms and affinity of the ligands towards the known 3D structure of EGFR protein. Using AutoDock 1.5.7, the ligand and its complex were prepared and docked into the active site *via* AutoDock Vina under default parameters. In each case, nine different binding conformations were analysed depending on their calculated binding free energy (ΔG, kcal/mol).

Intermolecular interactions between the components were analyzed post-docking using BIOVIA Discovery Studio Visualizer. The stability of complex was identified by studying its binding energies with specific hydrogen bond interactions involving key catalytic residues. For validating the docking procedure, the ligand in its co-crystalline state was re-docked into the receptor and the binding pocket was deemed acceptable if the RMSD value between the two was below 2 Å.

Preparation of 2,6-dimethyl morpholinyl thiosemicarbazide

N(4) 2, 6- dimethyl morpholinyl thiosemicarbazide was prepared *via* three steps of reactions (Fig. 1)

Step-I

Synthesis of carboxymethyl N-methyl-N-phenyldithiocarbamate (I)

A mixture of 21.6 mL (200 mmol, 21.2 g) N-methyl aniline, 12 mL (200 mmol, 15.2 g) carbon disulphide, and 8.4 g (210 mmol) of NaOH was stirred for 4 h to get a straw-colored solution. 23.2 g (200 mmol) of sodium chloroacetate was added to the straw-colored solution and then kept in dark for 17 h. 25 mL of conc. HCl was added to it to get a creamy yellowish-white solid (compound I) which was dried in oven at 40 ºC to get a white solid of melting point of 197–198 ºC (Scovil, 1991; Pokharel et al., 2025).

Step-II

Synthesis of N-methyl N-phenyl hydrazine carbothioamide (II)

A mixture of 17.7 g of compound (I), 20 mL of 98% hydrazine hydrate ($H_2NNH_2.H_2O$) and 10 mL of distilled water was heated on a steam bath at 85 ºC for 30 min. The white residue (compound II) was dried in an oven at 40 ºC. The compound was recrystallized by a mixture of 50 mL absolute ethanol (99.8% EtOH) and 25 mL distilled water and then dried at 40 ºC again. After cooling for 20 min, the white crystalline product was collected by filtration, washed successively with 2:1 mixture of ethanol and distilled water, then with pure ethanol, and kept in oven for drying at 40 °C for 12 h. The final product was obtained as white crystals with a melting point of 122–124 ºC (Scovil 1991; Pokharel et al., 2025).

Step-III

Synthesis of N(4) 2,6-dimethyl morpholinyl thiosemicarbazide (III)

A mixture of 1.00 g (5.52 mmol) of compound (**II**), 0.6357 g (5.52 mmol) of 2,6-dimethyl morpholine and 10 mL acetonitrile was condensed at 85 °C for 45 min. The crystal (compound III) obtained after filtration was dried in the oven at 40 °C and recrystallized in methanol (Scovil 1991; Pokharel et al., 2025). Yield: 80%. mp:112 °C.

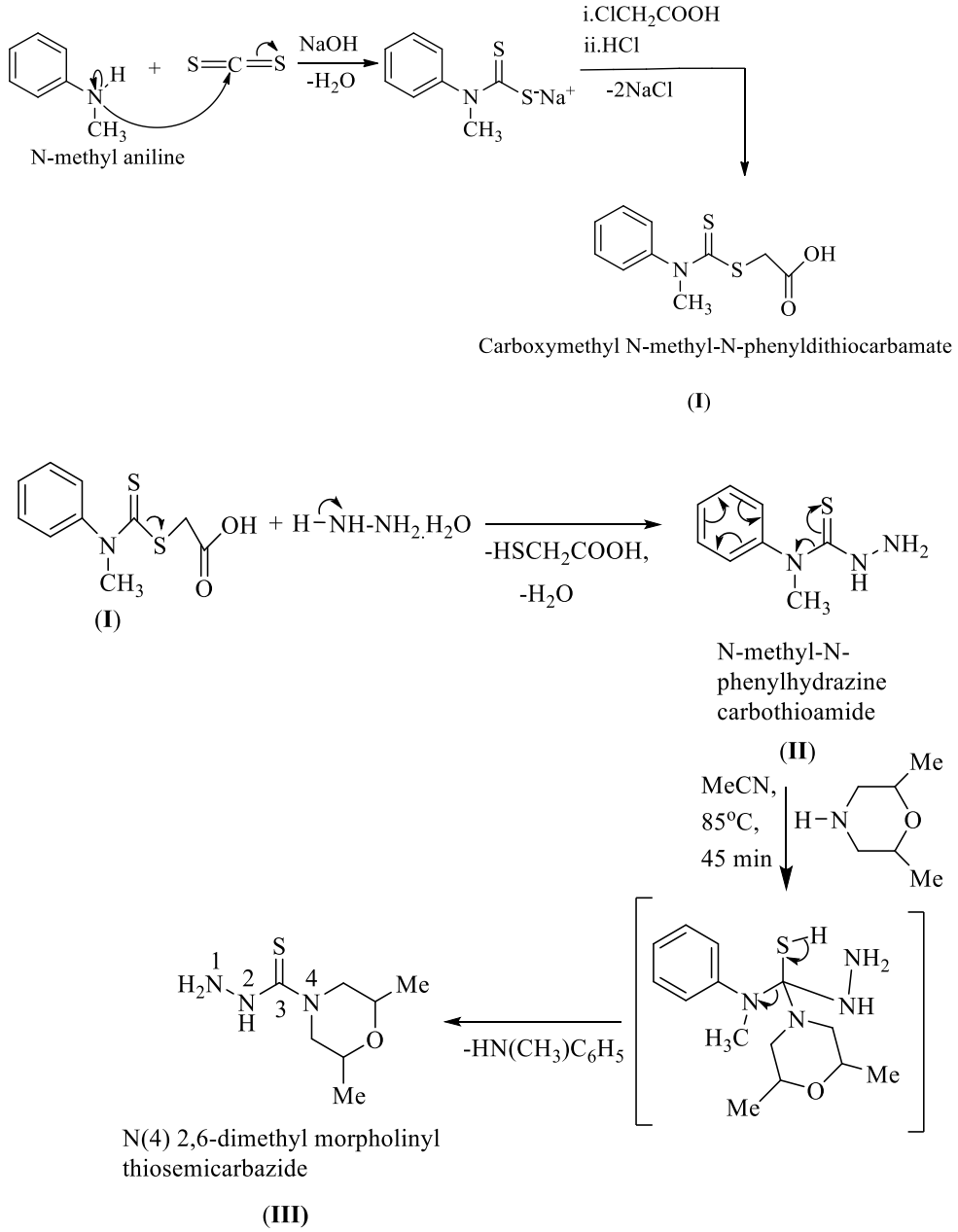

Figure 1. Synthesis of 2,6-dimethylmorpholine-4-carbothiohydrazide (III)

Synthesis of isatin-4-(2,6-dimethyl morpholinyl)-3-thiosemicarbazone

Isatin-4-(2,6-dimethyl morpholinyl)-3-thiosemicarbazone was prepared by refluxing a mixed solution of 0.189 g (1 mmol) thiosemicarbazide (compound III), 0.1471 g (1 mmol) isatin, 15 mL ethanol and 2–3 drops of glacial acetic acid at 80 ºC for 6 h (Fig. 2). The product was filtered, washed with EtOH and dried in oven at 50 ºC (Ali et al., 2014; Muralisankar et al., 2016).

Istdmpln: Yield: 79%; colour: yellow; mp: 260 ºC. Anal. Calcd (found)% for $C_{15}H_{18}N_4O_2S$ (318.40): C, 56.59(56.47); H, 5.70 (5.65); N, 17.60 (17.55); S, 11.03 (10.01). FTIR (KBr pellet) ν cm^{-1}: 3192 m (H–N; indole & azomethine), 3073 m (Ar-C-H), 2972 m, 2975 s (C-H), 1689 s (C=O), 1588 s (C=N), 1124 s (N–N), 1352 s, 787 s (C=S). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 13.14 (s, 1H, N3-H), 11.24 (s, 1H, N1-H), 12.69 (s, 1H, O-H of CONH enolization) 7.67 (d, 1H, C4-H), 7.40 (dd, 1H, C6-H), 7.11 (dd, 1H, C5-H), 6.95 (d, 1H, C7-H), 3.57 (t, 2H, C12-H; C13-H), 3.36 (d, 4H, C11-H; C14-H), 1.06 (s, 6H, C15-H; C16-H). ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 182.77(C10), 163.32 (C2), 143.79(C5), 142.58(C9), 131.56(C3), 128.19(C6), 126.96(C4), 119.96(C8), 111.47(C7), 56.55(C12, C13), 43.48(C11, C14) 18.75(C15, C16). UV–Vis (solid state) λ nm: 340, 228. ESI-HRMS (Positive): *m/z* (calcd (found)) 351.1491 (351.1156) $[M+H]^+$.CH_3OH.

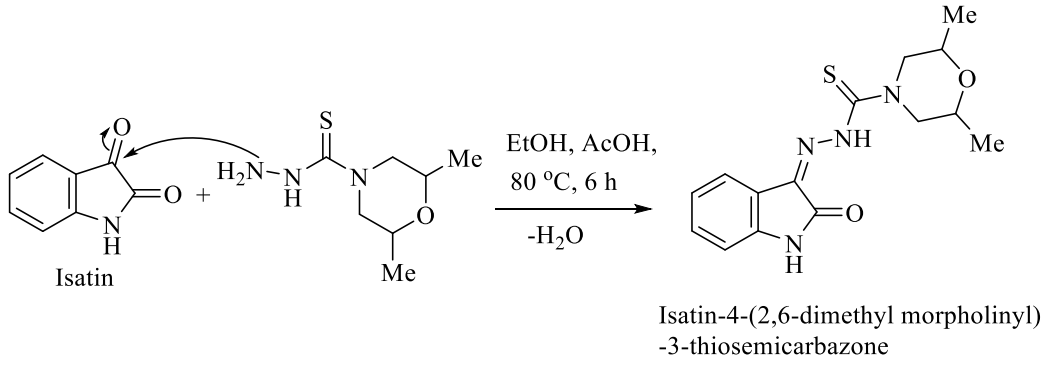

Figure 2. Synthesis of isatin-4-(2,6-dimethyl morpholinyl)-3-thiosemicarbazone

Synthesis of copper (II) complex of isatin-4-(2,6-dimethyl morpholinyl)-3-thiosemicarbazone

An equimolar mixture of thiosemicarbazone (1 mmol, 0.318 g) and $CuCl_2.2H_2O$ (1 mmol, 0.170 g) in ethanol (15 mL) was refluxed at 80 ºC for 6 h to get copper(II) thiosemicarbazone (Fig. 3). The complex was filtered, washed with EtOH and then with Et_2O and dried in oven at 50 ºC overnight and then 80 ºC for 2 h (West et al., 1995, Singh et al., 2021).

Cu-istdmpln: Yield: 80%; colour: deep brown; mp: 264 ºC. Anal. Calcd (found)% for $C_{15}H_{17}ClCuN_4O_2S$ (416.38): C, 43.27 (43.10); H, 4.12 (3.97); N, 13.46 (13.40); S, 7.70 (7.62). FTIR (KBr pellet) ν cm^{-1}: 3306 m (H–N; indole), 3041 m (Ar-C-H), 2975 s, (C-H), 1662 s (C=O), 1573 s (C=N), 1177 s (N=N), 1327 s, 724 s (C=S). UV–Vis (solid state) λ nm: 652, 440, 350, 227. ESI-HRMS (Positive): *m/z* (calcd (found)) 453.9694 (454.0588) $[M+K]^+$. EPR (9165.176 MHz, 298K): $g_{\parallel}$(2.2977), $g_{\perp}$ (2.0723), g_{av} or g (2.1474), G (4.2200). TGA: Endothermic pattern, anhydrous, stable to air.

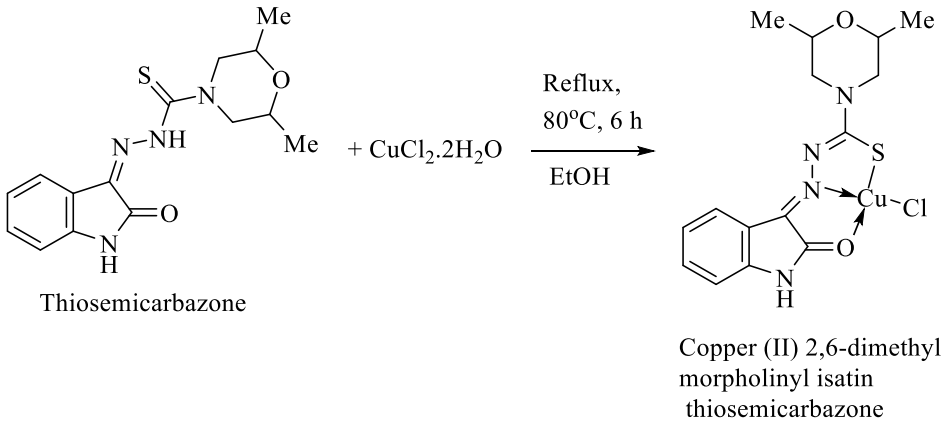

Figure 3. Synthesis of copper(II) isatin-4-(2,6-dimethyl morpholinyl)-3-thiosemicarbazone

Cells and culture medium

Cell lines; HCT116, MDA-MB-231, and HEK293 were taken for *in vitro* anticancer and non-toxicity screening of the compounds. Cultures of cells were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 5% fetal bovine serum (FBS) and supplemented with antibiotic -antimycotic (100X) Gibco catalogue number 15240062 solutions prepared with 10,000 units/mL penicillin G sodium, 10,000 µg/mL streptomycin sulfate, and 25µg/mL amphotericin B in 0.85% saline in a tissue culture incubator maintained at 37 ºC with 5% CO_2. The inhibition percentage was calculated using the percentage viability data.

MTT assay

MTT Assay was done to check the viability of the cells. Cells were seeded at a density of 1×10^4

cells/well in a 96 well plate and incubated overnight at 37 ºC until they reached 90% confluence. Each well was then filled with 50 µg of MTT (HiMedia) and incubated at 37 ºC for four hours. A solubilizing buffer (10% w/v SDS in 0.01 N HCl) was used to dissolve the generated formazan crystals overnight. At 570 nm, absorbance was measured on the multiplate reader. In order to calculate the cell vitality of treated cells, the cell viability of untreated cells was set at 100%. MTT assay of all the synthesized compounds was carried out using three different concentrations (1 µM, 5 µM, and 10 µM). The cells showed no cell inhibition (100% cell viability) in control.

RESULTS AND DISCUSSION

FTIR spectroscopy

In the thiosemicarbazone **(***Istdmpln***)** the broad band present in the region of 3192 cm^{-1} was ascribed to the stretching vibration of both indole ν(N-H) and azomethine ν(N-H). The polar ν(C=O) group exhibited strong stretching band at 1689 cm^{-1} (Ali et al., 2014). The strong absorption band, observed at 1588 cm^{-1} attributed to the stretching vibrations of ν(C=N) thereby confirming the formation of thiosemicarbazone (Chaudhary et al., 2023). The ν(N-N) group of thiosemicarbazone moiety showed strong absorption band at 1124 cm^{-1} (Singh et al., 2021; Chaudhary et al., 2023). The absence of absorption band in the region 2700-2500 cm^{-1} specific to thiol group ruled out the existence of thione-thiol tautomerism in the solid thiosemicarbazone (Turkkan et al., 2017). The presence of strong absorption bands at 1352 cm^{-1} and 787 cm^{-1} attributed to the ν(C=S) of ligand thereby confirming its existence in thione form (Pokharel et al., 2025). The stretching vibrations attributed to Ar-C-H, and aliphatic C-H were seen at 3073 cm^{-1} and 2972 cm^{-1} respectively (Shin et al., 2015).

Cu(II) thiosemicarbazone complex was formed by the coordination of thiol thiosemicarbazone after deprotonation of azomethane ν (N-H) group. ν(N-H) of indole moiety exhibited the band in FTIR spectra at 3306 cm^{-1} (Aneesrahman et al., 2019). Strong bands related to ν(C=O) and ν(C=N) groups of thiosemicarbazone moiety shifted toward the lower frequency demonstrating their coordination to copper(II) ion and were detected at 1662 cm^{-1} and 1573 cm^{-1}, respectively (Pawar et al., 2021; Banerjee et al., 2025). Ar–C–H, and aliphatic C-H stretching bands due to the coordination of ligand to copper(II) ion were seen at 3041 cm^{-1} and 2975 cm^{-1} respectively (Shin et al., 2015).

Coordination of copper(II) ion by enolate form of thiosemicarbazone resulted to increase electron density through the formation new −N=C−S⁻ skeleton of thiosemicarbazone moiety. As a result, the spectral band, characteristics of ν(N–N) shifted to higher frequency in copper(II) complex of thiosemicarbazone and hence observed at 1177 cm^{-1} (Joseph et al., 2006; Almeida et al., 2020; Singh et al., 2021) (Supplementary Materials: S1-S2). In the complex, the strong bands characteristics of ν(C=S) of thioamide group of thiosemicarbazone showed negative shift and hence found toward slightly lower frequencies at 1327 cm^{-1} and 724 cm^{-1} (Singh et al., 2021).

NMR spectroscopy

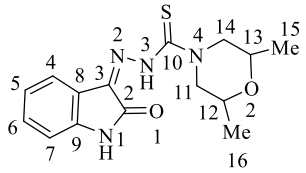

Figure 4. Structure of the ligand

In the thiosemicarbazone (Fig. 4), absence of a sharp signal at δ 4 ppm, characteristic of thiol (–SH) proton indicates that the thiosemicarbazone exist as neutral thione tautomeric form in solid state as it is confirmed by IR spectrum (Jouad et al., 2001). The spectrum exhibited two separate singlets in the downfield region at δ 13.14 ppm and δ 11.24 ppm attributed to the proton of =N–NH of thiosemicarbazone and indole N–H moieties, respectively (Singh et al., 2021). The chemical shift present in the downfield region (δ 13.14–11.24 ppm) appeared due to high magnetic field experienced by proton bonded to electronegative atoms. The chemical shift appeared at δ 12.69 ppm attributed to the –OH group of resonating –CONH of the isatin ring. The peaks of aromatic protons were observed in the form of singlet and multiplet in the region of δ 7.67–6.95 ppm (Rodriguez-Arguelles et al., 1999; Pokharel et al., 2025).

The aliphatic protons of 2,6-dimethylmorpholinyl showed the broad multiplet signals in the up field at δ 3.36 ppm (d, H11 & H14), and δ 3.57 ppm (t, H12 & H13) and doublet peak at δ 1.06 ppm (d, H15 & H16) (Labisbal et al., 2000; Singh et al., 2022). In

^{13}C NMR three distinct peaks were observed at downfield region δ 182.77 ppm, δ 163.32 ppm and δ 131.56 ppm attributed to sp^2 hybridized carbon atoms of C=S, C=O and C=N groups, respectively (Ali et al., 2014). The compound showed the chemical shift values δ (ppm): 143.79(C5), 142.58(C9), 131.56(C3), 128.19(C6), 126.96(C4), 119.96(C8), 111.47(C7) for aromatic carbon atoms (Bain et al., 1997, Rodriguez-Arguelles et al., 1999, Pokharel et al., 2025). The spectra of aliphatic 2,6-dimethylmorpholinyl carbon (sp^3-C) atoms were seen at up field at 43.48 ppm (C11, C14, neighbour of less electronegative N-atom) and δ 56.55 ppm (C12, C13, neighbour of high electronegative O-atom), and δ 18.75 ppm of two methyl groups (C15, C16) (Labisbal et al., 2000; Haribabu et al., 2016; Singh et al., 2022) (Supplementary Materials: S3-S4).

UV-Visible spectroscopy

The prominent bands were observed in the range of 228–227 nm due to π→π* electronic transition of benzene ring of ligand and their copper(II) complexes (West et al., 1997; Akinchan, 2004; El-Saied et al., 2019). The ligand and its copper(II) compound exhibited broad absorption bands at 340 nm and 350 nm, respectively as a result of n→π* intramolecular electronic transition viz. the bands due to the electronic transition of azomethine (–C=N), carbonyl (–C=O) and thioamide (–HN–C=S) groups (Da Silva et al., 2013; Haribabu et al., 2016; Muralisankar et al., 2016). The observed data showed that there is no change of π→π* electronic transition in ligand and copper(II) complex. The n→π* electronic transition from –C=O, –C=N, and –HN–C=S appeared at higher wavelength due to their involvement in coordination to copper(II) ion.

On coordination to Cu(II) ion by tridentate thiosemicarbazone anion, the absorption bands of uncomplexed ligand shifted to higher wavelength region of 440 nm due to the transitions of ligand to metal charge transfer (LMCT) (Singh et al., 2021; Singh et al., 2022). Such type of coordination also confirms the participation of –C=N, –C=O and –HN–C=S groups in the complex formation (Akinchan 2004; Da Silva et al., 2013).

Electronic spectra of the complex in solid form displayed absorption peaks of a broad low-intensity band in the regions 652 nm due to the d-d transitions of the copper(II) ions by $d_{x^2-y^2} \rightarrow d_{z^2}$ in square planar geometry (Pokharel et al., 2025) (supplementary Materials: S5).

Mass spectrometry

The study of ESI-HRMS in positive mode exhibited methanol adduct peak, $[M+H]^+.CH_3OH$ for ligand due to the use of methanol solvent during its crystallization or sample preparation (Duraisamy et al., 2011). In the same way, the copper(II) complex showed potassium adduct ion peak, $[M+K]^+$ which is commonly observed in electrospray ionization mass spectra of coordination compound due to trace alkali metal ions present in solvents or glassware (Singh et al., 2022; Shahi et al., 2023). These peaks are in accordance with the molecular masses of proposed molecules (supplementary Materials: S6, S7).

EPR spectroscopy

The EPR spectral data: $g_{\parallel}$ (2.2977) > $g_{\perp}$ (2.0723) > 2.0023 and G (4.2200) > 4 of Cu-istdmpln confirmed the formation of mononuclear complex with square planar or distorted tetragonal geometry having an unpaired electron in $d_{x^2-y^2}$ ground state (Muralisankar et al, 2016, Pokharel et al, 2025). According to Hathaway & Billing (1970) there is no exchange interaction between nuclear centres when the Cu(II) complex has G > 4. In this condition neighbouring Cu centers are far apart, orbital overlapping becomes negligible and hence electron spin exchange cannot occur effectively (supplementary Materials: S8).

Thermogravimetric analysis

The copper(II) complex showed endothermic TGA pattern (Antony et al., 2013; Chandraleka & Chandramohan, 2014). The multistep decomposition patterns indicate sequential degradation of specific structural moieties. The compound showed the mass loss of 3.86% and 5.57% at the temperature range 38.41–82.61 ºC and 82.61– 234.51 ºC due to the evaporation of moisture in the matrix (Sharma et al., 2005; Konstantinovic et al., 2007; Pokharel et al., 2025).

The endothermic graph also showed the decomposition of 2,6-dimethyl thiomorpholine moiety ($=C–N(CH_2–CH(CH_3))_2O$) of thiosemicarbazide part and remaining part of thisemicarbazide with amide group of isatin ring (–NH–C–C=N–N–S–) by 32.18% (30.30%) and 25.31% (23.80%) [exp (calc)] in the region of 234.51–458.58 ºC and 458.58–708.19 ºC,

respectively (Singh et al., 2021; Pokharel et al., 2025). The complex was found as CuO residue at the temperature of 708.19 ºC and above (Huseynova et al., 2019; Singh et al., 2022) (Supplementary Materials: S9).

Overall, the thermogravimetric results corroborate the proposed compositions and coordination modes of the complexes, in agreement with elemental analysis and spectroscopic characterization data.

Anticancer activity

Isatin-based thiosemicarbazone and its Cu(II) complex showed inhibition of cancer cell viability, and induction of apoptosis, which together can explain selective cytotoxicity toward MDA-MB-231, and HCT116 while exhibiting relative non-toxicity toward HEK293 cells.

The IC_{50} values of the compounds in HCT116, MDA-MB-231 and HEK293 cell lines are given in Table 1. The compounds exhibited potent anticancer activity toward both cell lines; the free ligand with $IC_{50,}$ 34.64 and 43.99 µM in and HCT116, and MDA-MB-231 cells, respectively. While in Cu(II) complex, IC_{50} in aforementioned cells was observed as 13.01 and 31.19 µM, respectively. Additionally, the ligand and complex showed low toxicity toward normal cell, HEK293 with IC_{50} 16.72-15.39 µM.

Table 1. *In vitro* anticancer screening data (IC_{50} µM) of the synthesized compounds

Compounds → **Cell line ↓**	***Istdmpln*** **(µM)**	***Cu-istdmpln*** **(µM)**
HCT116	34.64	13.01
MDA-MB-231	43.99	31.19
HEK293	16.72	15.39

Bar diagram in Figure 5 showed that the copper(II) complex experienced much higher cell growth inhibition (IC_{50} 31.19–13.01 µM) than its ligand (IC_{50} 43.99–34.64 µM) toward both cancer cell lines. Thus, anticancer potency of ligand gets increased on coordination to the Cu(II) ion.

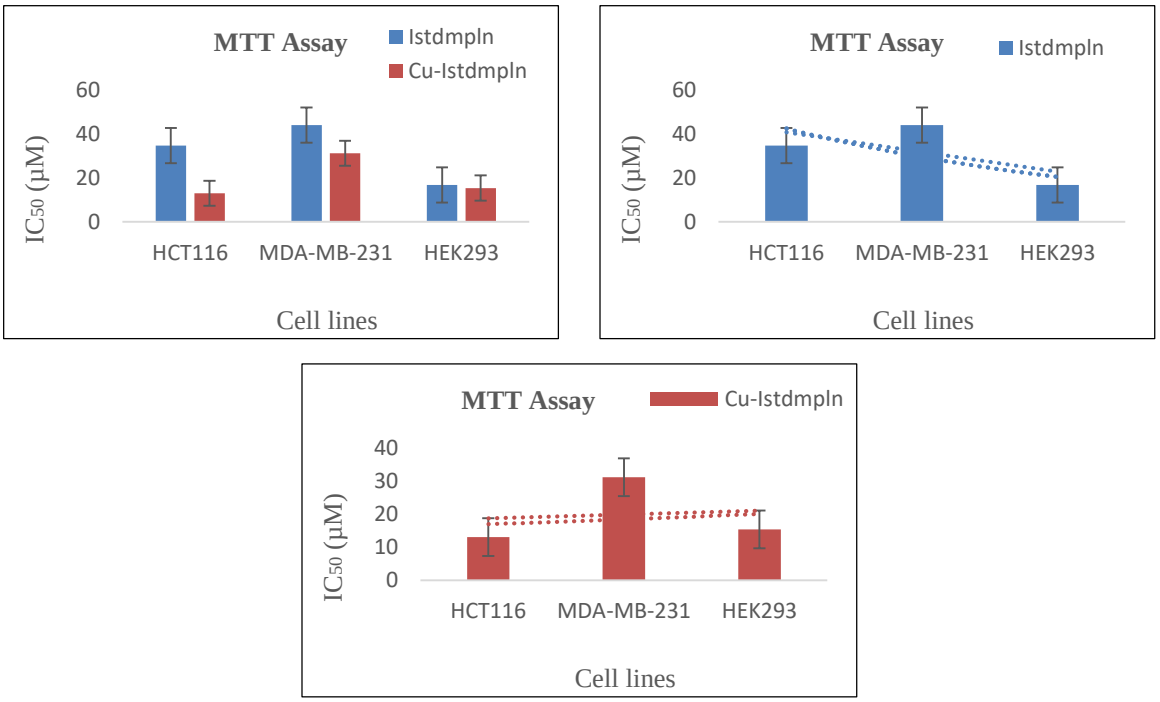

Figure 5. Cell viability assay of thiosemicarbazone (*Istdmpln*) and its Cu(II) complex (*Cu-istdmpln*) with their trend line to show the trend of cell growth inhibition

***In silico* study**

The binding energy, interacting amino acids and the quantity of hydrogen bonds are the main metrics used to assess the result of molecular docking. *Istdmpln* and its copper complex *(Cu-istdmpln*) were docked with the EGFR protein. The molecular docking research showed that ligand attach to EGFR active pockets more strongly than the reference ligand, Pyrrolo[3,2-d]pyrimidine-based Epidermal Growth Factor Receptor, EGFRT790M/L858R mutant inhibitors. Hydrogen bonds are important in the recognition of protein-ligand interactions and play vital role in selecting appropriate binding affinity and stabilization of protein-ligand complexes (Bitencourt-Ferreira et al., 2019). The low binding energy of *Cu-istdmpln* toward EFRG protein indicates that the complex binds to the active site of the proteins more strongly and stably.

They showed binding interaction with catalytic sites of the receptor as indicated in Table 2 below. From Figures 6a, 6b, and 6c, it can be observed that both compounds had a good alignment in the binding site of EGFR, which is close to that of the reference compound.

Table 2. Molecular docking of synthesized ligand and its copper complex and reference drug

Results	***Istdmpln***	***Cu-istdmpln***	**Pyrrolo[3,2-d]pyrimidine (Sogabe et al., 2013)**
Binding Energy (kcal/mol)	-8.1	-8.9	-8.6
Interacted residues	Cys 797 & Met 793 (H-bond), Leu 844 & Leu 718 (π-σ), Ala 743 & Val 726 (Alkyl)	Cys 797 (H- bond), Leu 844 (π-σ), Leu 718, Val 726 & Ala 743 (π-Alkyl)	Cys 797 & Leu 718 (H-bond), Val 726 (π-σ), Ala 722, Leu 844 & Ala 743 (π -alkyl)

The molecular docking results indicate that the ligand *Istdmpln* exhibits favorable binding interactions with the EGFR active site. The ligand forms hydrogen bonds with the catalytic residues Cys797 and Met793, with a binding energy of −8.1 kcal/mol, which is comparable to that of the reference drug (−8.6 kcal/mol). In addition, the ligand interacts with several other important amino acid residues through hydrophobic interactions within the binding pocket. With a binding energy of −8.9 kcal/mol, the metal complex *Cu-istdmpln* exhibits an even greater binding affinity and establishes a hydrogen bond with the catalytic residue Cys797. Overall, molecular docking experiments indicated that *Istdmpln* as well as its Cu(II) complex can interact strongly with EGFR binding site *via* hydrogen bond and hydrophobic interactions; Cu-istdmpln showed the highest binding affinity (−8.9 kcal/mol), higher than that of the free ligand and the reference EGFR inhibitor.

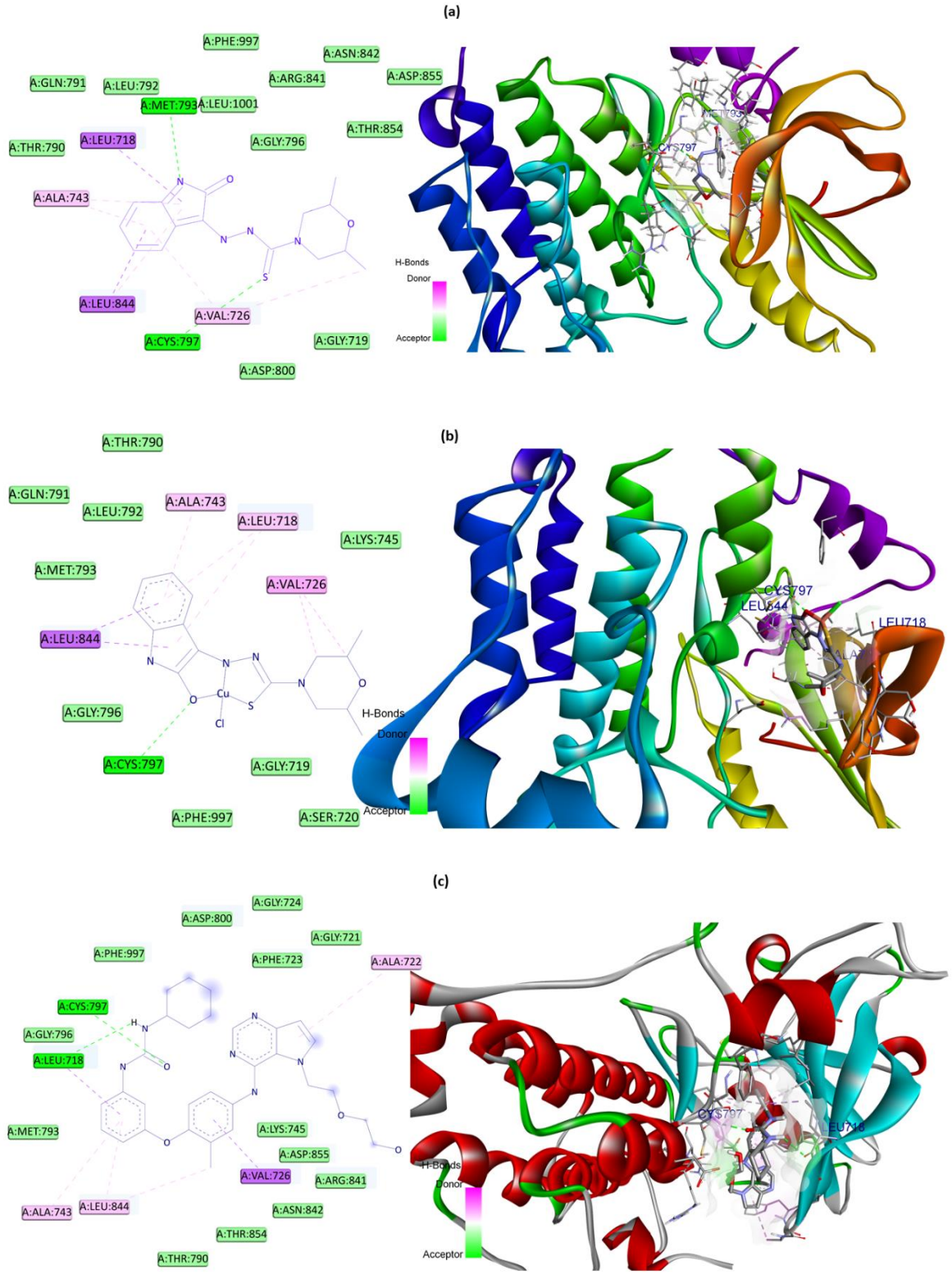

Figure 6. 2D and 3D interaction diagram of (a) *Istdmpln* (b) *Cu-istdmpln* (c) reference drug with EGFR

CONCLUSION

The synthesized thiosemicarbazone and its Cu(II) complex showed cell growth inhibition in micromolar concentrations (HCT116, IC_{50} 34.64–13.01 µM; MDA-MB-231, IC_{50} 43.99–31.19 µM). These compounds showed low toxicity toward HEK293 (IC_{50} 16.72–15.39 µM). It has been found that the anticancer potency gets increased on coordination of ligand to copper(II) ion which is also

supported by molecular docking study. *Cu-istdmpln* exhibited excellent cell inhibition property (IC_{50} 13.01 µM) toward HCT116. This research has explored the need for further study of synthesized compounds for *in vivo* and mechanism of action against cancer cell lines.

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AUTHORS CONTRIBUTION

Conceptualization: NKS; Methodology: NKS; Validation: PNY, NKS; Investigation: DK, NT, AS; Data analysis: NKS, PNY, PPM, SPM, AAK; Writing original draft: NKS; Writing-review & editing: NKS, PNY; Supervision: PNY; Funding acquisition: NKS

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

ETHICAL STATEMENT

Our original unpublished and unsubmitted work used cancer and normal cells for *in vitro* anticancer screening by getting the ethical approval certificate from the Ethical Review Board of Tribhuvan University (ERB-082-18).

DATA AVAILABILITY STATEMENT

On request, the corresponding authors of the article can provide the data (S1-S9).

SUPPLEMENTARY INFORMATION

Figure S1: FTIR spectrum of ligand (*Istdmpln*)
Figure S2: FTIR spectrum of copper (II) complex (*Cu-istdmpln*).
Figure S3: ^{1}HNMR spectrum (400 MHz, DMSO-d_6) of ligand (*Istdmpln)*
Figure S4: ^{13}CNMR spectrum (400 MHz, DMSO-d_6) of ligand (*Istdmpln)*
Figure S5: UV-Visible electronic transitions of the compounds (*Istdmpln* and *Cu-istdmpln*)
Figure S6: ESI-HRMS spectra of the compound (*Istdmpln*)
Figure S7: ESI-HRMS spectra of the compound (*Cu-istdmpln*)
Figure S8: EPR spectra of copper (II) thiosemicarbazone (*Cu-istdmpln*)
Figure S9: TGA pattern of copper (II) thiosemicarbazone (*Cu-istdmpln*)

REFERENCES

Akinchan, N. T. (2004). Spectroscopic studies on isatin-3-semicarbazone and isatin-3-thiosemicarbazone. *Global Journal of Pure and Applied Sciences*, *10*(4), 545–553. https://doi.org/10.4314/gjpas.v10i4.16437

Ali, A. Q., Teoh, S. G., Salhin, A., Eltayeb, N. E., Ahamed, M. B. K., & Majid, A. M. S. A. (2014). Synthesis of isatin thiosemicarbazones derivatives: In vitro anti-cancer, DNA binding and cleavage activities. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *125*, 440–448. https://doi.org/10.1016/j.saa.2014.01.118

Almeida, C. M., de Carvalho, J. G. M., Fujimori, M., França, E. L., Honorio-França, A. C., Parreira, R. L. T., Orenha, R. P., & Gatto, C. C. (2020). Structural investigation of group 10 metal complexes with thiosemicarbazone: Crystal structure, mass spectrometry, Hirshfeld surface and in vitro antitumor activity. *Structural Chemistry*, *31*, 2093–2103. https://doi.org/10.1007/s11224-020-01564-2

Al-Mulla, A. (2017). A review: Biological importance of heterocyclic compounds. *Der Pharma Chemica*, *9*(13), 141–147.

Aneesrahman, K. N., Ramaiah, K., Rohini, G., Stefy, G. P., Bhuvanesh, N. S. P., & Sreekanth, A. (2019). Synthesis and characterisations of copper(II) complexes of 5-methoxyisatin thiosemicarbazones: Effect of N-terminal substitution on DNA/protein binding and biological activities. *Inorganica Chimica Acta*, *492*, 131–141. https://doi.org/10.1016/j.ica.2019.04.025

Antony, R., Manickam, S. T. D., Saravanan, K., Karuppasamy, K., & Balakumar, S. (2013). Synthesis, spectroscopic and catalytic studies of Cu(II), Co(II) and Ni(II) complexes immobilized on Schiff base modified chitosan. *Journal of Molecular Structure*, *1050*, 53–60. https://doi.org/10.1016/j.molstruc.2013.07.006

Bain, G. A., West, D. X., Krejci, J., Valdes-Martinez, J., Hernández-Ortega, S., & Toscano, R. A. (1997). Synthetic and spectroscopic investigations of N(4)-substituted isatin thiosemicarbazones and their copper complexes. *Polyhedron*, *16*(5), 855–862. https://doi.org/10.1016/S0277-5387(96)00323-3

Banerjee, I., Kumar, P., Singh, N. K., Yadav, P. N., & Pokharel, Y. R. (2025). Evaluation of anticancer potency of Cu-isatin-1-(2-pyridyl)piperazine thiosemicarbazone (Cu-Istpypz) against epidermoid carcinoma cell line (A431). *Results in Chemistry*, *13*, 101993. https://doi.org/10.1016/j.rechem.2024.101993

Bhrigu, B., Pathak, D., Siddiqui, N., Alam, M. S., & Ahsan, W. (2010). Search for biologically active isatins: A short review. *International Journal of Pharmaceutical Sciences and Drug Research*, *2*, 229–235.

Bitencourt-Ferreira, G., Veit-Acosta, M., & De Azevedo, W. F. (2019). Hydrogen bonds in protein–ligand complexes. In W. F. De Azevedo (Ed.), *Docking screens for drug discovery* (pp. 93–107). Springer. https://doi.org/10.1007/978-1-4939-9752-7_7

Chandraleka, S., & Chandramohan, G. (2014). Synthesis, characterization and thermal analysis of the copper(II) complexes with 2,2-bipyridyl and 1,10-phenanthroline. *African Journal of Pure and Applied Chemistry*, *8*(10), 162–175. https://doi.org/10.5897/AJPAC2014.0592

Chaudhary, U., Dawa, D., Banerjee, I., Sharma, S., Mahiya, K., Rauf, A., Pokharel, Y. R., & Yadav, P. N. (2023). Anticancer potency of N(4)-ring incorporated-5-methoxyisatin thiosemicarbazones. *Journal of Molecular Structure*, *1274*, 134549. https://doi.org/10.1016/j.molstruc.2022.134549

Da Silva, J. G., Recio Despaigne, A. A., Louro, S. R. W., Bandeira, C. C., Souza-Fagundes, E. M., & Beraldo, H. (2013). Cytotoxic activity, albumin and DNA binding of new copper(II) complexes with chalcone-derived thiosemicarbazones. *European Journal of Medicinal Chemistry*, *65*, 415–426. https://doi.org/10.1016/j.ejmech.2013.04.036

Duraisamy Senthil Raja, D., Bhuvanesh, N. S. P., & Natarajan, K. (2011). Biological evaluation of a novel water soluble sulphur bridged binuclear copper(II) thiosemicarbazone complex. *European Journal of Medicinal Chemistry*, *46*(9), 4584–4594. https://doi.org/10.1016/j.ejmech.2011.07.038

Eldehna, W. M., Almahli, H., Al-Ansary, G. H., Ghabbour, H. A., Aly, M. H., Ismael, O. E., Al-Dhfyan, A., & Abdel-Aziz, H. A. (2017). Synthesis and in vitro anti-proliferative activity of some novel isatins conjugated with quinazoline/phthalazine hydrazines against triple-negative breast cancer MDA-MB-231 cells as apoptosis-inducing agents. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *32*(1), 600–613.https://doi.org/10.1080/14756366.2017.1279155

El-Saied, F., El-Aarag, B., Salem, T., Said, G., Khalifa, S. A. M., & El-Seedi, H. R. (2019). Synthesis, characterization, and in vivo anti-cancer activity of new metal complexes derived from isatin-N(4)antipyrine thiosemicarbazone ligand against Ehrlich ascites carcinoma cells. *Molecules*, 24(18), 3313. https://doi.org/10.3390/molecules24183313

Emami, S., & Dadashpour, S. (2015). Current developments of coumarin-based anti-cancer agents in medicinal chemistry. *European Journal of Medicinal Chemistry*, *102*, 611–630. https://doi.org/10.1016/j.ejmech.2015.08.033

Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D. M., Forman, D., & Bray, F. (2015). Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *International Journal of Cancer*, *136*(5), E359–E386. https://doi.org/10.1002/ijc.29210

Haribabu, J., Subhashree, G. R., Saranya, S., Gomathi, K., Karvembu, R., & Gayathri, D. (2016). Isatin based thiosemicarbazone derivatives as potential bioactive agents: Anti-oxidant and molecular docking studies. *Journal of Molecular Structure*, *1110*, 185–195. https://doi.org/10.1016/j.molstruc.2016.01.044

Hathaway, B. J., & Billing, D. E. (1970). The electronic properties and stereochemistry of mono-nuclear complexes of the copper(II) ion. *Coordination Chemistry Reviews*, *5*(2), 143–207. https://doi.org/10.1016/S0010-8545(00)80135-6

Hernandez, W., Carrasco, F., Vaisberg, A., Spodine, E., Icker, M., Krautscheid, H., Beyer, L., Tamariz-Angeles, C., & Olivera-Gonzales, P. (2023). Novel thiosemicarbazone derivatives from furan-2-carbaldehyde: Synthesis, characterization, crystal structures, and antibacterial, antifungal, antioxidant, and antitumor activities. *Journal of Chemistry, 2023(1)*, 5413236. https://doi.org/10.1155/2023/5413236

Huseynova, M. T., Aliyeva, M. N., Medjidov, A. A., Sahin, O., & Yalcin, B. (2019). Cu(II) complex with thiosemicarbazone of glyoxylic acid as an anion ligand in a polymeric structure. *Journal of Molecular Structure, 1176*, 895–900 https://doi.org/10.1016/j.molstruc.2018.08.090

Joseph, M., Kuriakose, M., Kurup, M. R. P., Suresh, E., Kishore, A., & Bhat, S. G. (2006). Structural, antimicrobial and spectral studies of copper(II) complexes of 2-benzoylpyridine N(4)-phenyl thiosemicarbazone. *Polyhedron, 25*(1), 61–70. https://doi.org/10.1016/j.poly.2005.07.006

Jouad, E. M., Larcher, G., Allain, M., Riou, A., Bouet, G. M., Khan, M. A., & Do Thanh, X. (2001). Synthesis, structure and biological activity of nickel(II) complexes of 5-methyl-2-furfural thiosemicarbazone. *Journal of Inorganic Biochemistry, 86*(2–3), 565–571. https://doi.org/10.1016/S0162-0134(01)00220-3

Konstantinovic, S. S., Radovanovic, B. C., & Krkljes, A. (2007). Thermal behaviour of Co(II), Ni(II), Cu(II), Zn(II), Hg(II) and Pd(II) complexes with isatin-β-thiosemicarbazone. *Journal of Thermal Analysis and Calorimetry, 90*(2), 525–531. https://doi.org/10.1007/s10973-006-7794-9

Labisbal, E., Sousa, A., Castiñeiras, A., Garcia-Vazquez, J. A., Romero, J., & West, D. X. (2000). Spectral and structural studies of metal complexes of isatin 3-hexamethyleneiminylthiosemicarbazone prepared electrochemically. *Polyhedron, 19*(10), 1255–1262. https://doi.org/10.1016/S0277-5387(00)00383-1

Lobana, T. S., Sharma, R., Bawa, G., & Khanna, S. (2009). Bonding and structure trends of thiosemicarbazone derivatives of metals: An overview. *Coordination Chemistry Reviews, 253*(7–8), 977–1055. https://doi.org/10.1016/j.ccr.2008.07.004

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry, 30*(16), 2785–2791. https://doi.org/10.1002/jcc.21256

Muralisankar, M., Bhuvanesh, N. S. P., & Sreekanth, A. (2016). Synthesis, X-ray crystal structure, DNA/protein binding and DNA cleavage studies of novel copper(II) complexes of N-substituted isatin thiosemicarbazone ligands. *New Journal of Chemistry, 40*, 2661–2679. https://doi.org/10.1039/C5NJ02806J

Muralisankar, M., Sujith, S., Bhuvanesh, N. S. P., & Sreekanth, A. (2016). Synthesis and crystal structure of new monometallic and bimetallic copper(II) complexes with N-substituted isatin thiosemicarbazone ligands: Effects on DNA/protein binding, DNA cleavage, and anticancer activity. *Polyhedron, 118*, 103–117. https://doi.org/10.1016/j.poly.2016.08.038

O'Gorman, J., & Humphreys, H. (2012). Application of copper to prevent and control infection: Where are we now? *Journal of Hospital Infection, 81*(4), 217–223. https://doi.org/10.1016/j.jhin.2012.05.009

Osredkar, J., & Šuštar, N. (2011). Copper and zinc: Biological role and significance of copper/zinc imbalance. *Journal of Clinical Toxicology, 3*, 495. https://doi.org/10.4172/2161-0495.S3-001

Pawar, S., Amate, A., Chakravarty, D., Butcher, R. J., & Kumbhar, A. A. (2021). Cu(II) complexes of 2-indole thiocarbohydrazones: Synthesis, characterization and DNA cleavage studies. *Journal of Chemical Sciences, 133*, 107. https://doi.org/10.1007/s12039-021-01962-x

Pokharel, Y. R., Singh, N. K., Sharma, S., Wangchuk, T., Shahi, N., Mahiya, K., Choudhary, R. K., Kumbhar, A. A., & Yadav, P. N. (2025). Synthesis, characterization, and in vitro anticancer activity of a series of N(4) dimethyl isatin thiosemicarbazones and their copper(II) complexes. *Journal of Molecular Structure, 1327*, 141157.https://doi.org/10.1016/j.molstruc.2024.141157

Rodriguez-Arguelles, M. C., Sanchez, A., Ferrari, M. B., Fava, G. G., Pelizzi, C., Pelosi, G., Albertini, R., Lunghi, P., & Pinelli, S. (1999). Transition-metal complexes of isatin-β-thiosemicarbazone: X-ray crystal structure of two nickel complexes. *Journal of Inorganic Biochemistry, 73*, 7–15. https://doi.org/10.1016/S0162-0134(98)10085-5

Scovill, J. P. (1991). A facile synthesis of thiosemicarbazides and thiosemicarbazones by transamination. *Phosphorus, Sulfur, and Silicon and the Related Elements, 60*(1–2), 15–19. https://doi.org/10.1080/10426509108233920

Shahi, N., Yadav, P. N., Chaudhary, U., Saad, M., Mahiya, K., Khan, A., Shafi, S., & Pokharel, Y. R. (2023). 5-Methoxyisatin N(4)-pyrrolidinyl thiosemicarbazone (MeOIstPyrd) restores

mutant p53 and inhibits the growth of skin cancer cells, in vitro. *ACS Omega*, *8*(35), 31998–32016. https://doi.org/10.1021/acsomega.3c03824

Sharma, S., Athar, F., Maurya, M. R., Naqvi, F., & Azam, A. (2005). Novel bidentate complexes of Cu(II) derived from 5-nitrofuran-2-carboxaldehyde thiosemicarbazones with antiamoebic activity against *E. histolytica*. *European Journal of Medicinal Chemistry*, *40*(6), 557–562. https://doi.org/10.1016/j.ejmech.2005.01.003

Shin, S. M., Park, J. K., & Jung, S. M. (2015). Changes of aromatic CH and aliphatic CH in in-situ FT-IR spectra of bituminous coals in the thermoplastic range. *ISIJ International*, *55*(8), 1591–1598. https://doi.org/10.2355/isijinternational.ISIJINT-2014-625

Singh, A., Raghuwanshi, K., Patel, V. K., Jain, D. K., Veerasamy, R., Dixit, A., & Rajak, H. (2017). Assessment of 5-substituted isatin as surface recognition group: Synthesis and antiproliferative evaluation of hydroxamates as histone deacetylase inhibitors. *Pharmaceutical Chemistry Journal*, *51*(5), 366–374. https://doi.org/10.1007/s11094-017-1616-1

Singh, N. K., & Yadav, P. N. (2021). Anticancer potency of copper(I) complexes against cancer cell lines: A review. *Journal of Institute of Science and Technology*, *26*(1), 128–140. https://doi.org/10.3126/jist.v26i1.37840

Singh, N. K., Kumbhar, A. A., Pokharel, Y. R., & Yadav, P. N. (2020). Anticancer potency of copper(II) complexes of thiosemicarbazones. *Journal of Inorganic Biochemistry*, *210*, 111134. https://doi.org/10.1016/j.jinorgbio.2020.111134

Singh, N. K., Sharma, S., Krishnakumar, A., Choudhary, R. K., Kumbhar, A. A., Butcher, R. J., Pokharel, Y. R., & Yadav, P. N. (2022). Exploration of anticancer potency of N(4)-thiomorpholinyl isatin/5-haloisatin thiosemicarbazones on coordination to Cu^{2+} ion. *Inorganic Chemistry Communications*, *143*, 109767. https://doi.org/10.1016/j.inoche.2022.109767

Singh, N. K., Shrestha, S., Shahi, N., Choudhary, R. K., Kumbhar, A. A., Pokharel, Y. R., & Yadav, P. N. (2021). Enhancement of anticancer activity of N(4)-1-(2-pyridyl)piperazinyl 5-nitroisatin thiosemicarbazone on chelation with copper(II). *Asian Journal of Chemistry*, *33*(3), 557–564. https://doi.org/10.14233/ajchem.2021.23004

Singh, N. K., Shrestha, S., Shahi, N., Choudhary, R. K., Kumbhar, A. A., Pokharel, Y. R., & Yadav, P. N. (2021). Anticancer potential of N(4)-substituted 5-nitroisatin thiosemicarbazones and their copper(II) complexes. *Rasayan Journal of Chemistry*, *14*(3), 1600–1610. https://doi.org/10.31788/RJC.2021.1436422

Sogabe, S., Kawakita, Y., Igaki, S., Iwata, H., Miki, H., Cary, D. R., Takagi, T., Takagi, S., Ohta, Y., & Ishikawa, T. (2013). Structure-based approach for the discovery of pyrrolo[3,2-d]pyrimidine-based EGFR T790M/L858R mutant inhibitors. *ACS Medicinal Chemistry Letters*, *4*(2), 201–205. https://doi.org/10.1021/ml300327z

Thakur, D., Kumar, V., Tyagi, S. K., & Parmar, R. S. (2023). Review on the medicinal significance of heterocyclic compounds. *International Journal of Science, and Engineering Technology*, *11*(VII), 351–357. *https://doi.org/10.22214/ijraset.2023.54569*

Turkkan, E., Sayin, U., Erbilen, N., Pehlivanoglu, S., Gokce, E., Tasdemir, H. U., Saf, A. O., Leyla, G., & Akgemci, E. G. (2017). Anticancer, antimicrobial, spectral, voltammetric and DFT studies with Cu(II) complexes of 2-hydroxy-5-methoxyacetophenone thiosemicarbazone and its N(4)-substituted derivatives. *Journal of Organometallic Chemistry*, *831*, 23–35. https://doi.org/10.1016/j.jorganchem.2016.12.020

Vine, K. L., Matesic, L., Locke, J. M., & Skropeta, D. (2013). Recent highlights in the development of isatin-based anticancer agents. In M. Prudhomme (Ed.), *Advances in anticancer agents in medicinal chemistry* (pp. 254–312). Bentham Science Publishers.

Wan, Y., Fang, G., Chen, H., Deng, X., & Tang, Z. (2021). Sulfonamide derivatives as potential anticancer agents and their SAR elucidation. *European Journal of Medicinal Chemistry*, *226*, 113837. https://doi.org/10.1016/j.ejmech.2021.113837

West, D. X., Ives, J. S., Bain, G. A., Liberta, A. E., Valdés-Martínez, J., Ebert, K. H., & Hernández-Ortega, S. (1997). Copper(II) and nickel(II) complexes of 2,3-butanedione bis(N(3)-substituted thiosemicarbazones). *Polyhedron*, *16*(11), 1895–1905. https://doi.org/10.1016/S0277-5387(96)00468-8

West, D. X., Thientanavanich, I., & Liberta, A. E. (1995). Copper(II) complexes of 6-methyl-2-acetylpyridine-N(4)-substituted thiosemicarbazones. *Transition Metal Chemistry*, *20*(3), 303–308. https://doi.org/10.1007/BF00143498