Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society

SYNTHESIS, CHARACTERIZATION, DENSITY FUNCTIONAL THEORY AND CYTOTOXICITY OF ISATIN THIOCARBOHYDRAZIDE AND ITS COPPER(II) AND ZINC(II) COMPLEXES

(Sintesis, Pencirian, Teori Fungsi Ketumpatan, dan Sitotoksikiti Isatin Tiokarboidrazida serta Kompleks Kuprum(II) dan Zink(II))

Ummi Liyana Mohamad Rodzi[1], Karimah Kassim[1,2*], Nageswara Rao Meroshine[3], and Nur Azzalia Kamaruzaman[3]

[1]*Faculty of Applied Sciences,*
Universiti Teknologi MARA (UiTM) 40450 Shah Alam, Selangor, Malaysia
[2]*Institute of Science,*
Universiti Teknologi MARA (UiTM) 40450 Shah Alam, Selangor, Malaysia
[3]*National Poison Centre,*
Universiti Sains Malaysia (USM), 11800 Pulau Pinang, Malaysia

[*]*Corresponding author: karimah@uitm.edu.my*

Received: 22 July 2023; Accepted: 3 October 2023; Published: 29 December 2023

Abstract

Isatin and thiocarbohydrazide exhibit a broad spectrum of biological functions including antibacterial, antiviral, antioxidant and antimalarial activities. In this study, copper(II) and zinc(II) complexes derived from isatin thiocarbohydrazide were synthesized and assessed for their potential as antileukemia agents. ^{1}H and ^{13}C NMR, infrared spectroscopy, UV-Vis spectroscopy, thermal and elemental analysis were used to elucidate the structures of the synthesized compounds. The complexation was observed from the shifting frequencies of the *v*(C=N) and the appearance of new peaks assigned as *v*(M-N) and *v*(M-O) at 499 and 583-594 cm^{-1}. The formation of the complexes is also supported by the d-d transition in UV-visible spectra. Using proper DFT functionals and basis sets, the optimized geometries and electronic energies of the complexes were ascertained. Theoretical simulations provide important insights into the stability and electrical structure of the complexes, assisting in understanding their potential biological actions. Cytotoxicity screening was conducted against the human leukemia K562 cell line and low activity was reported, thus indicating minimal toxic profile of the complexes.

Keywords: cytotoxicity, characterization, density functional theory, isatin, thiocarbohydrazide

Abstrak

Isatin dan tiokarboidrazida mempunyai fungsi biologi yang luas seperti aktiviti antibakteria, antivirus, antioksidan dan antimalaria. Dalam kajian ini, kompleks kuprum(II) dan zink(II) telah disintesis daripada isatin tiokarboidrazida dan dinilai potensinya sebagai agen antileukemia. Kaedah spektroskopi iaitu ^{1}H dan ^{13}C NMR, inframerah, UV-Vis, analisis haba dan unsur digunakan untuk menjelaskan struktur sebatian tersintesis. Pengkompleksan diperhatikan daripada frekuensi peralihan *v*(C=N) dan puncak penampilan baharu yang boleh ditetapkan kepada *v*(M-N) dan *v*(M-O) di 499dan 583-594 cm^{-1}. Pembentukan kompleks juga disokong oleh peralihan d-d dalam spektrum UV-Cahaya Nampak. Menggunakan fungsi dan set asas DFT yang sesuai, geometri

molekul dioptimumkan dan tenaga elektronik kompleks telah dikenalpasti. Simulasi teori memberikan gambaran penting tentang kestabilan dan struktur elektrik kompleks, membantu dalam pemahaman potensi tindakan biologi mereka. Saringan sitotoksisiti telah dijalankan terhadap garisan sel leukemia manusia K562 dan sitotoksisiti yang rendah dilaporkan, dengan itu menunjukkan profil toksik kompleks.

Kata kunci: sitotoksisiti, pencirian, teori fungsi ketumpatan, isatin, tiokarboidrazida

Introduction

In contemporary medicinal chemistry, isatin is a privileged scaffold with a wide range of biological activity. Using nitric and chromic acids, Erdmann and Laurent originally isolated it as an indigo oxidation byproduct [1]. Isatin consists of two cyclic rings, which are a six-membered ring with an aromatic character and a five-membered ring possessing an anti-aromatic character [1]. A literature survey reported that isatin is known to have anticancer [2, 3], antiviral [4, 5], antioxidant [6], analgesic [7] and antimicrobial [8, 9] properties. Another important part of the production of the ligand is the higher homologue of thiosemicarbazide, known as thiocarbohydrazide. Thiocarbohydrazide has an extra nitrogen atom that can serve as a metal-coordinating center [10]. Compared to thiosemicarbazide, there are limited studies on the theoretical study and cytotoxicity effects of the thiocarbohydrazide. Therefore, the present work was done to screen for their respective cytotoxicity activity against human leukemia K562 cancer cell lines.

Copper and zinc are chosen as the metal ions to be bound with the isatin-derived thiocarbohydrazide ligand in this work. Formerly, the addition of copper was reported to have increased the antiproliferative activity of salicylaldehyde monothiocarbohydrazide against breast cancer and human prostate adenocarcinoma cell lines [11]. On top of that, zinc(II) complexes also appear to be extremely appealing because zinc is substantially less toxic in larger dosages than other metals [12]. The computational analysis using the B3LYP method with 6-31G+ (d,p) and LANL2DZ basis set has been done on all the compounds to explore their electronic properties. Subsequently, all parameters like the relationships between the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energies were used to explain the anticancer activity.

Materials and Methods

Materials

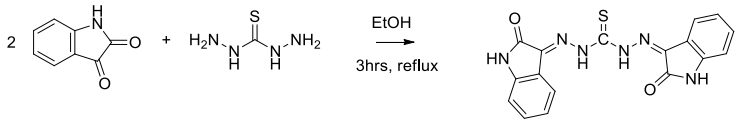
All chemicals used in this study were purchased from Sigma Aldrich Company, St Louis, USA and were used without further purification.

Instrumentation

Elemental analysis (C, H, N and S) for the ligand and complexes were determined using a Thermo Scientific Flash Smart analyzer. The magnetic susceptibility of the complexes was determined using Sherwood Auto Magnetic Susceptibility at room temperature. Molar conductivities were measured using a conductivity standard of 1413 µS/cm. Perkin Elmer 1600 Spectrometer was employed in obtaining the FTIR spectra for ligand and complexes as KBr disc in the region of 400-4000 cm^{-1}. Proton (^{1}H) and carbon (^{13}C) NMR (400 MHz) spectra were recorded using Jeol ECZS spectrometer in DMSO-d_6. The UV-Vis spectra were obtained on Perkin Lambda 365 in acetonitrile at $1x10^{-4}$ M solution using a quartz cuvette in the range of 200-800 nm. Thermal analyses were performed in nitrogen atmosphere using TA Instruments Q50 TGA analyzer with a heating rate of 10 °C/min in a temperature range between 25 and 500 °C. Powder XRD patterns were collected using PANalytical X'pert PRO in Bragg-Brentano reflecting geometry with CuKα (k= 1.5418740 Å). The X-ray tube was utilized for a copper tube operating at 45 kV and 40 mA. Data were collected in 2θ range of 5-90° with 0.0167° and 10 s exposure per step.

Synthesis of ligand

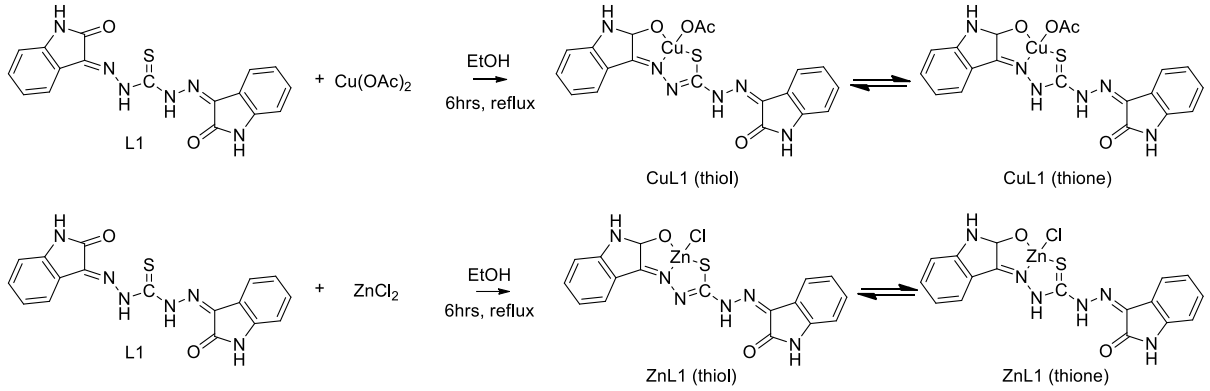
The ligand was prepared with a modification from previous studies [13, 14] by dissolving isatin and thiocarbohydrazide in hot absolute ethanol (30 mL) and the reaction mixture was refluxed for three hours. A yellow precipitate was filtered off, washed with cold ethanol and air-dried overnight. The remaining filtrate was left for recrystallization.



Scheme 1. Synthesis of L1

Synthesis of complexes

Cu(II) and Zn(II) complexes were synthesized by refluxing the ligand with metal salt (copper(II) acetate monohydrate and zinc(II) chloride) with the ratio of 1:1 in a solution of absolute ethanol for six hours. The resulting precipitates were filtered off, washed with cold ethanol and air-dried.



Scheme 2. Synthesis of CuL1 and ZnL1

Computational methods

The structures of the ligand and the complexes were built using Gaussian view 16.0. DFT calculations were then carried out using Gaussian 09 program and B3LYP method [15, 16]. The geometrical optimizations were done against the ligand using the 6-31G+ (d,p) [17] basis set and the complexes using LANL2DZ [18].

Cytotoxicity

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay described by Mossmann [19] was used to assess the cytotoxicity activity of all the synthesized compounds against human leukemia K562 cell line with slight modification. In a 96-well culture plate, the cells ($5x10^4$ cells/mL) were seeded and incubated for 24 hours. Subsequently, the cells were treated with the compounds at 100, 50, 25, 12.5, 6.25 and 3.125 µM for 48 hours. Each well received 20 µL of MTT solution, which was then incubated for an additional four hours to observe for cell viability. DMSO was used to dilute the purple formazan in the well and the absorbance was measured at 570 and 690 nm. The cytotoxicity activity of the compounds was expressed as IC_{50}. Doxorubicin was used as a positive control against the cancer cell line.

Results and Discussion

The compounds were colored, stable in air and insoluble in most organic solvents except for DMSO and DMA. The elemental analyses for the complexes displayed that they formed in a ratio of 1:1. The analytical findings and the theoretical formula are in good agreement. CuL1 possessed a magnetic moment of 1.71 B.M. which is relatively lower than the regular spin-only value of 1.73 B.M. and showed a square planar geometry, while ZnL1 is diamagnetic affording a tetrahedral geometry [20]. The physical and elemental analysis data are summarized in Table 1.

Table 1. Analytical data of the ligand and its metal complexes

Compounds	Molecular Formula	Color	Yield %	$\mu_{eff.}$ (B.M.)	Λm $\Omega^{-1}mol^{-1}cm^2$	% Found (Calc.)			
						C	H	N	S
L1	$C_{17}H_{12}N_6O_2S$	Yellow	91.46	-	-	56.38 (56.04)	3.58 (3.32)	22.46 (23.06)	8.03 (8.80)
CuL1	$C_{19}H_{20}CuN_6O_6S$	Black	85.78	1.71	0.010	43.73 (43.55)	3.80 (3.85)	16.28 (16.04)	6.10 (6.12)
ZnL1	$C_{17}H_{15}ClN_6O_3SZn$	Red	64.77	Diamagnetic	0.006	42.25 (42.17)	2.92 (3.12)	17.39 (17.36)	6.64 (5.90)

Infrared spectroscopy

The emergence of *v*(C=N) at 1697 cm^{-1} confirmed the formation of Schiff base ligand L1 according to Figure 1, while bands at 499 cm^{-1} and 583-594 cm^{-1} correspond to *v*(M-N) and *v*(M-O) respectively, showing the formation of the proposed complexes, CuL1 and ZnL1 [21, 22]. The strong *v*(C=O) band appeared at 1750 cm^{-1} in the free ligand and shifted in CuL1 and ZnL1. The shifting of the *v*(C=O) band indicates that the carbonyl oxygen participated in coordination with the metal ions [23]. The broad band recorded at 3254 cm^{-1} in the ligand spectrum was attributed to the stretching vibration for *v*(N-H). This band was later shifted to lower frequencies in CuL1 at 3160 cm^{-1} and ZnL1 at 3164 cm^{-1}. The O-H stretching of the water molecules, which are connected by a dense web of hydrogen bonds, results in a strong and broad absorption that is centered at 3474 cm^{-1} in ligand, 3460 cm^{-1} in CuL1 and 3429 cm^{-1} in ZnL1 [24]. The tetrahedral and square planar geometry of ZnL1 and CuL1 was stabilized through coordination with water molecules.

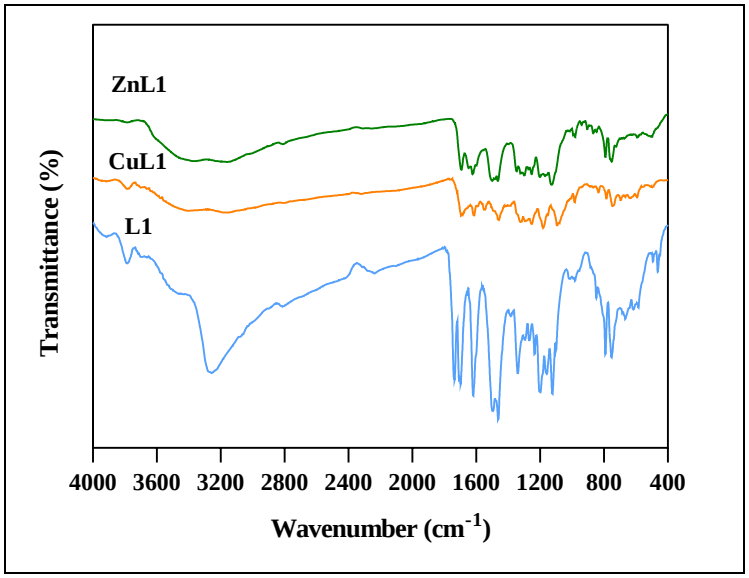


Figure 1. Infrared spectra of the ligand and metal complexes

^{1}H-NMR spectroscopy

The ^{1}H NMR spectra of both ligand and zinc(II) complex were examined to determine each distinct type of hydrogen nuclei. On the contrary, the NMR signal of the copper(II) complex was unable to be examined due to its paramagnetic property. Singlet peaks were found between 12.94 and 11.34 ppm in both spectra, which were attributed to isatin NH and NH thiocarbohydrazide, respectively. The multiplets of the aromatic protons of the isatin ring were recorded at 7.60, 7.42, 7.14, and 6.98 ppm. ZnL1 showed similar aromatic proton peak.

^{13}C-NMR spectroscopy

Based on the ^{13}C NMR spectrum, L1 displays its aromatic carbons between 112.16-138.35 ppm. The signal for thione (C=S) was detected at 175.30 ppm while those for azomethine (C=N) and carbonyl carbon (C=O) were detected at 162.55 and 138.50 ppm, respectively. Thione signal in ZnL1 appeared at a higher range of 178.50 ppm compared to its parent ligand. The peak was seen for azomethine and carbonyl carbon in the range of 164.0 ppm and 144.0 ppm. As a result, it can be inferred that azomethine nitrogen, carbonyl oxygen, and thione sulphur participated in the coordination of complexes, which was further supported by the results from the infrared spectroscopy [25].

UV-visible spectroscopy

The ligand's UV-visible spectrum in Figure 2 showed two absorption peaks that were identified as $\pi \rightarrow \pi^*$ for benzene and $n \rightarrow \pi^*$ transitions for the azomethine group (C=N) at 243 nm and 373 nm, respectively [26]. The two peaks appeared to have bathochromic or red shifting in both copper(II) and zinc(II) complexes. A redshift stipulates that the energy of the π-π^* transition decreases due to metal chelating. The copper(II) and zinc(II) complexes spectra displayed ligand-to-metal transfer (LCMT) peaks 509 and 484 nm [27].

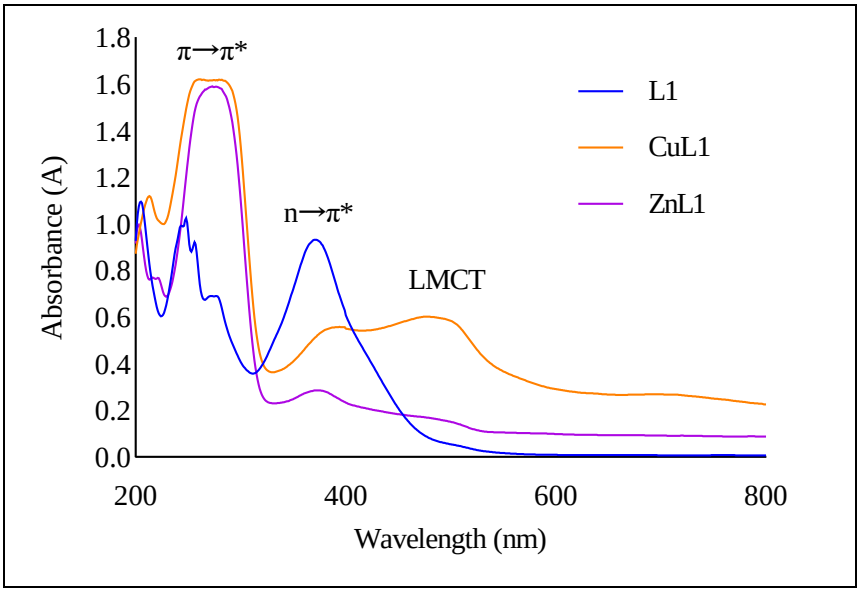


Figure 2. UV-visible spectra of the synthesized compounds

TGA studies

The thermal stability of the copper(II) and zinc(II) complexes was determined using the thermogravimetric (TGA) analysis. TGA aids in separating the coordinated water molecule present in the compound from the lattice water molecule [28]. Based on the curves in Figure 3 and data in Table 2, CuL1 breaks down into two phases, according to the thermogram. Dehydration of the initial lattice water molecule, with a weight loss of 2.30 percent, occurs in the first stage at temperatures between 50 and 125 °C. The second step showed a weight loss of 2.57 % indicating the elimination of the second lattice water molecule. Adversely, ZnL1 revealed a decomposition of water molecules through one stage with a weight loss of 3.10 %.

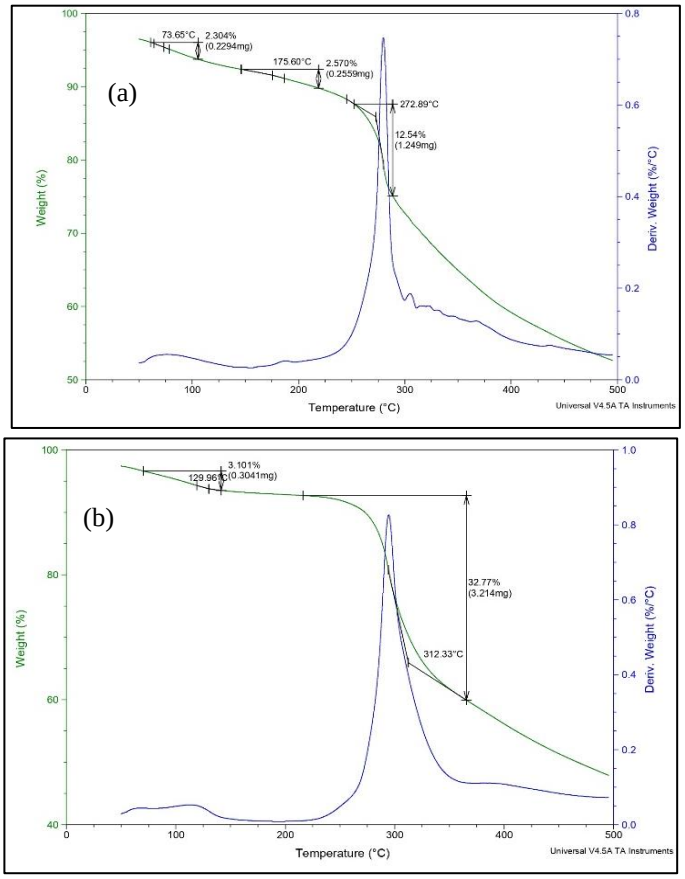


Figure 3. Thermogravimetric curves for (a) CuL1 and (b) ZnL1

Table 2. Decomposition temperature and the percentage weight loss of complexes

Compound	Decomposition Temperature (°C)	Weight Loss (%)		Lost Species
		Found	Calc.	
CuL1	50-125	2.30	3.43	One lattice water molecule
	150-200	2.57	3.43	One lattice water molecule
ZnL1	100-130	3.10	3.72	One lattice water molecule

Powder XRD studies

All compounds were then examined using powder diffraction XRD patterns due to unsuccessful attempts in generating ligand and complexes as single crystals. The 2θ = 5-90 range lattice constants were used to record the powder diffraction patterns. The compounds were found to be crystalline (L1) and semi-crystalline (CuL1 and ZnL1). The X-ray diffractometry patterns for complexes displayed in Figure 4 are entirely distinct from those of the ligands, indicating the discovery of new compounds [29].

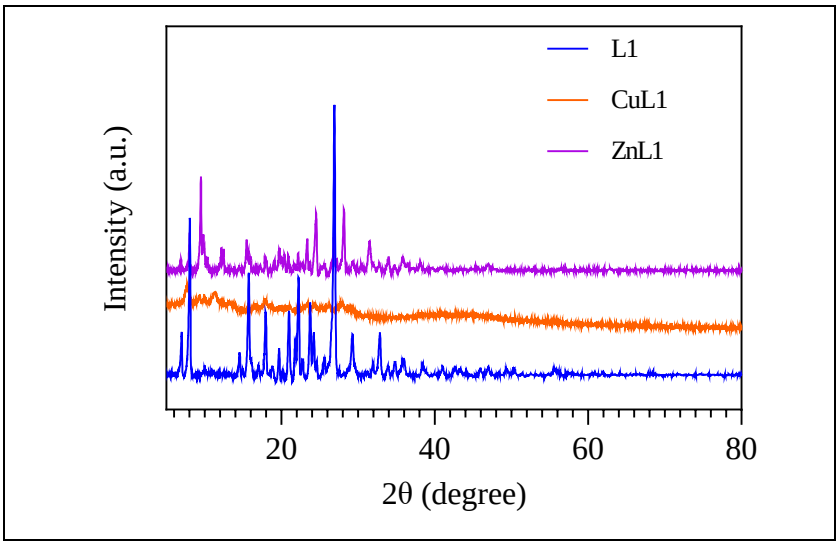


Figure 4. Powder XRD patterns for all synthesized compounds

Computational analysis: Frontier molecular orbitals

The frontier molecular orbitals are used to explore the chemical reactivity of the molecules. The highest occupied molecular orbitals (HOMO) and the lowest unoccupied molecular orbitals (LUMO) are split into molecular orbitals alpha (spin ↑) and beta (spin ↓) because of the doublet multiplicity in the complexes' molecular geometry. The B3LYP/6-31G+ (d,p) and LANDL2DZ level theory's calculations of the molecular orbital energies (E_{HOMO} and E_{LUMO}) reveal that they are all negative, indicating that the ligand and both copper(II) and zinc(II) complexes are stable. The HOMO region of the ligand and complexes are mainly localized around the electronegative sulphur and nitrogen atoms. Both atoms are bonded to the metal ions and are able to attract electrons away from their aromatic rings, thus reducing the HOMO electron density around the rings. This explains why the rings contributed significantly to the LUMO as according to Figure 5. The value of the energy gap between the HOMO and LUMO in Table 3 conveyed the stability and reactivity of each compound. The energy gap of L1 is 3.143 eV, which is considered to be similar to that of a previously reported Schiff base [30]. In comparison, the energy gap of the zinc complex for the alpha spin is the lowest among the ligand and copper complex, indicating that the zinc complex has a greater affinity for receiving electrons [31].

Table 3. The HOMO, LUMO and gap energies of the synthesized compounds

Compounds		**E_{HOMO} (eV)**	**E_{LUMO} (eV)**	**ΔEgap (eV)**
L1		-6.044	-2.901	3.143
CuL1	Alpha	-6.006	-3.085	2.921
	Beta	-6.007	-3.500	2.507
ZnL1	Alpha	-4.987	-3.187	1.800
	Beta	-6.193	-3.434	2.759

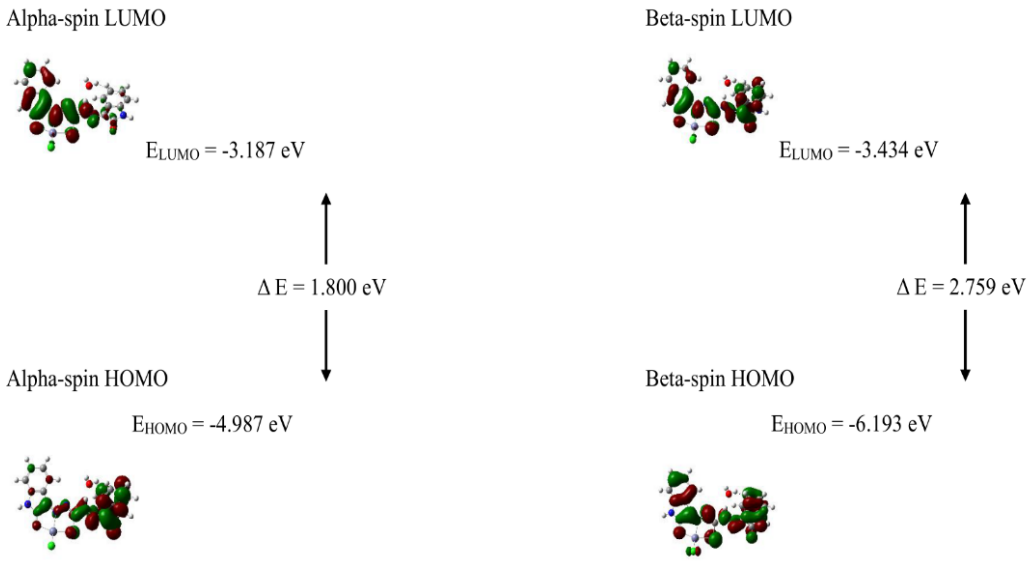


Figure 5. The energy gaps for ZnL1

Global chemical reactivity descriptors (GCRD)

Soft molecules are made of molecular systems with low gap energies and low chemical hardness (η), which in this case is ZnL1 as shown in Table 4. The small energy of ZnL1 indicates that the charge may be transferred easily, and therefore provides an impact on the compounds biological activity [32]. The negative value of chemical potential (μ) indicated that the molecule is capable of accepting electrons from the environment [33] and ZnL1 is considered to have the lowest. Thus, it is suggested that ZnL1 will have the highest chemical reactivity. The dipole moment is one of the parameters responsible for the potential biological activity. The compound with the highest polarity, ZnL1 is expected to display high bio-efficiency [34].

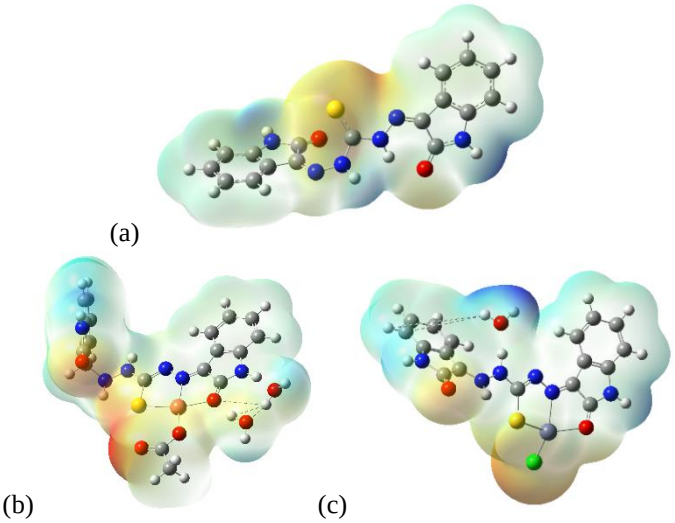
Table 4. The electronic parameters of the ligand and metal complexes

Property	Ligand and Metal Complexes				
	L1	CuL1		ZnL1	
		α	β	α	β
EA (eV)	2.901	3.085	3.500	3.187	3.434
IP (eV)	6.044	6.006	6.007	4.987	6.193
X (eV)	4.473	4.546	4.754	4.087	4.814
η (eV)	1.572	1.461	1.254	0.900	1.380
S (eV^{-1})	0.318	0.342	0.399	0.556	0.362
μ (eV)	-4.473	-4.546	-4.754	-4.087	-4.814
ω	6.363	7.073	9.011	9.280	8.397
Total Energy (Hartree)	-1532.21	-1722.05		-1300.98	
Dipole moment (Debye)	1.885	9.452		14.394	

Molecular electrostatic potential (MEP)

The binding properties of the compounds are presented using the molecular electrostatic potential (MEP) map in Figure 6. The electron density is displayed by the molecular electrostatic potential, which helps to identify the sites of electrophilic and nucleophilic reactions as well as hydrogen bonding interactions [35]. The electronegative potential which is represented by red color is concentrated around the oxygen atoms in the isatin moiety of all the compounds and the acetate ion of the copper complex. The blue color portrays the

electropositive potential, which is centered around the nitrogen atoms and water molecules.



(a) (b) (c)

Figure 6. MEP diagrams of (a) L1, (b) CuL1 and (c) ZnL1

Cytotoxicity

The cytotoxicity of all synthesized compounds was evaluated against human leukemia K562 cell line, whereby three independent studies were conducted and data for each test were reported in triplicates. The anticancer drug, Doxorubicin was used as the positive control. Based on the results shown in Table 5, the ligand and its complexes gave IC_{50} values of more than 100 µM. This indicated that the compounds are poor inhibitors of the human cancer cell line, thus proving to have a low toxicity effect. L1, CuL1 and ZnL1 show low toxicity to human cells due to the presence of the imine group in the structure that may interfere with the cytotoxicity of the compounds [36]. According to Noruzi and co-workers, the toxicity of copper derivate increased as the concentration increased to 200 µg/mL [37].

Table 5. IC_{50} values of the compounds against human leukemia K562 cancer cell line

	IC_{50} values in cancer cells (µM)
Compound	K562
L1	>100
CuL1	>100
ZnL1	>100
Doxorubicin[a]	0.26 ± 0.06

[a]= *reference drug*

Conclusion

Copper(II) and zinc(II) complexes were synthesized through a condensation process with a Schiff base ligand, N,2-bis(2-oxoindolin-3-ylidene)hydrazine carbothioamide resulting in excellent yields. The characterization using spectroscopic techniques confirmed the formation of the desired complexes and ensured their chemical structure. The molar conductivity values for the complexes gave an insight into their non-electrolytic properties. The infrared spectra highlight the shifting of *ν*(C=N) and the appearance of new bands which are *ν*(M-N) and *ν*(M-O) for complexes. Apart from that, ^{1}H and ^{13}C NMR spectra displayed peaks that located the protons and carbon present in each compound. UV-visible spectra confirmed the complexation of metal ions with ligand using the d-d transition. The zinc(II) complex has a better tendency to accept electrons since it has the lowest

energy gap for the alpha spin compared to the ligand and copper(II) complex. From the chemical reactivity descriptors, ZnL1 was predicted to have better cytotoxicity activity compared to the ligand and CuL1. However, low cytotoxicity was reported for all compounds against human leukemia K562 cancer cell line. This indicated a potential development for all compounds due to their minimal toxic profile. In addition, the contradiction between experimental and theoretical results may be due to the solubility of the compounds. Colored compounds may have interfered with the reading of the OD values.

Acknowledgement

The experiments were carried out using the facilities in Universiti Teknologi MARA and Universiti Sains Malaysia. This research was funded by Universiti Teknologi MARA under the grant number 600-RMC/GIP 5/3 (040/2022).

References

1. Kakkar, R. (2019). Isatin and its derivatives: a survey of recent syntheses, reactions, and applications. *Medicinal Chemistry Communication*, 10(3): 351-368.
2. Raju, R., Chidambaram, K., Chandrasekaran, B., Bayan, M. F., Maity, T. K., Alkahtani, A. M. and Chandramoorthy, H. C. (2023). Synthesis, pharmacological evaluation, and molecular modeling studies of novel isatin hybrids as potential anticancer agents. *Journal of Saudi Chemical Society*, 27(2): 101598.
3. Singh, N. K., Shrestha, S., Shahi, N., Choudhary, R., Kumbhar, A., Pokharel, Y. and Yadav, P. (2021). Anticancer potential of N(4) substituted 5-nitroisatin thiosemicarbazones and their copper(II) complexes. *Rasayan Journal Chemistry*, 14: 31788.
4. Elsaman, T., Mohamed, M. S., Eltayib, E. M., Abdel-Aziz, H. A., Abdalla, A. E., Munir, M. U. and Mohamed, M. A. (2022). Isatin derivatives as broad-spectrum antiviral agents: The current landscape. *Medicinal Chemistry Research*, 2022: 1-30.
5. Meleddu, R., Distinto, S., Corona, A., Tramontano, E., Bianco, G., Melis, C., ... and Maccioni, E. (2017). Isatin thiazoline hybrids as dual inhibitors of HIV-1 reverse transcriptase. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 32(1): 130-136.
6. Muğlu, H., Sönmez, F., Çavuş, M. S., Kurt, B. Z. and Yakan, H. (2023). New Schiff bases based on isatin and (thio)/carbohydrazone: preparation, experimental–theoretical spectroscopic characterization, and DFT approach to antioxidant characteristics. *Research on Chemical Intermediates*, 49(4): 1463-1484.
7. Lahari, K. and Sundararajan, R. (2020). Design and synthesis of novel isatin derivatives as potent analgesic, anti-inflammatory and antimicrobial agents. *Journal of Chemical Sciences*, 132: 1-15.
8. Jarrahpour, A., Jowkar, Z., Haghighijoo, Z., Heiran, R., Rad, J. A., Sinou, V., ... and Özdemir, N. (2022). Synthesis, in-vitro biological evaluation, and molecular docking study of novel spiro-β-lactam-isatin hybrids. *Medicinal Chemistry Research*, 31(6): 1026-1034.
9. Hassan, A. S. (2022). Antimicrobial evaluation, in silico ADMET prediction, molecular docking, and molecular electrostatic potential of pyrazole-isatin and pyrazole-indole hybrid molecules. *Journal of the Iranian Chemical Society*, 19(8): 3577-3589.
10. Bonaccorso, C., Marzo, T. and La Mendola, D. (2019). Biological applications of thiocarbohydrazones and their metal complexes: A perspective review. *Pharmaceuticals*, 13(1): 4.
11. Bonaccorso, C., Grasso, G., Musso, N., Barresi, V., Condorelli, D. F., La Mendola, D. and Rizzarelli, E. (2018). Water soluble glucose derivative of thiocarbohydrazone acts as ionophore with cytotoxic effects on tumor cells. *Journal of Inorganic Biochemistry*, 182: 92-102.
12. Pellei, M., Del Bello, F., Porchia, M. and Santini, C. (2021). Zinc coordination complexes as anticancer agents. *Coordination Chemistry Reviews*, 445: 214088.
13. Gangarapu, K., Manda, S., Jallapally, A., Thota, S., Karki, S. S., Balzarini, J., ... and Tokuda, H. (2014). Synthesis of thiocarbohydrazide and carbohydrazide derivatives as possible biologically active agents. *Medicinal Chemistry Research*, 23: 1046-1056.

14. Sathisha, M. P., Revankar, V. K. and Pai, K. S. R. (2008). Synthesis, structure, electrochemistry, and spectral characterization of bis-isatin thiocarbohydrazone metal complexes and their antitumor activity against Ehrlich ascites carcinoma in Swiss albino mice. *Metal-Based Drugs*, 2008: 362105.
15. Lee, C., Yang, W. and Parr, R. G. (1988). Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Physical Review B*, 37(2): 785.
16. Becke, A. D. (1993). A new mixing of Hartree–Fock and local density-functional theories. *The Journal of Chemical Physics*, 98(2): 1372-1377.
17. Casas, J. S., Casanova, N., García-Tasende, M. S., Sánchez, A., Sordo, J., Touceda, Á. and Vázquez, S. (2010). Back to the coordination modes of the thiosemicarbazonate chain: New insights from diorganolead (IV) and lead (II) derivatives of isatin-3-thiosemicarbazone. *European Journal of Inorganic Chemistry*, 31: 4992-5004.
18. Hashem, H. E., Mohamed, E. A., Farag, A. A., Negm, N. A. and Azmy, E. A. (2021). New heterocyclic Schiff base-metal complex: Synthesis, characterization, density functional theory study, and antimicrobial evaluation. *Applied Organometallic Chemistry*, 35(9): e6322.
19. Mossmann, P. B. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal Immunology Methods*, 65: 49-53.
20. Cristóvão, B. (2011). Spectral, thermal and magnetic properties of Cu (II) and Ni (II) complexes with Schiff base ligands. *Journal of the Serbian Chemical Society*, 76(12): 1639-1648.
21. Orif, M. I. and Abdel-Rhman, M. H. (2015). Synthesis, spectral and structural studies on some new isonicotinic thiosemicarbazide complexes and its biological activity. *Polyhedron*, 98: 162-179.
22. Mohamed, G. G., Omar, M. M., Moustafa, B. S., AbdEl-Halim, H. F. and Farag, N. A. (2022). Spectroscopic investigation, thermal, molecular structure, antimicrobial and anticancer activity with modelling studies of some metal complexes derived from isatin Schiff base ligand. *Inorganic Chemistry Communications*, 141: 109606.
23. Fernandes, T. S., Melo, W. D., Kalinke, L. H., Rabelo, R., Valdo, A. K., da Silva, C. C., ... and Cangussu, D. (2018). 2D and 3D mixed M II/Cu II metal–organic frameworks (M= Ca and Sr) with N, N′-2, 6-pyridinebis (oxamate) and oxalate: preparation and magneto-structural study. *Dalton Transactions*, 47(33): 11539-11553.
24. Nakamoto, K. (1986). Infrared spectra of inorganic and coordination compounds. 4th edition. New York: Wiley.
25. Kumar, R., Kumar, S. and Bala, M. (2020). Synthesis, characterisation and antibacterial activity of some indole derivatives and their inclusion complexes with β-cyclodextrin. *Oriental Journal of Chemistry*, 36(5): 923.
26. Haribabu, J., Balakrishnan, N., Swaminathan, S., Peter, J., Gayathri, D., Echeverria, C., ... and Karvembu, R. (2021). Synthesis, cytotoxicity and docking studies (with SARS-CoV-2) of water-soluble binuclear Ru-p-cymene complex holding indole thiosemicarbazone ligand. *Inorganic Chemistry Communications*, 134: 109029.
27. Ahmed, A. and Lal, R. A. (2017). Synthesis, characterization and electrochemical studies of copper (II) complexes derived from succinoyl-and adipoyldihydrazones. *Arabian Journal of Chemistry*, 10: S901-S908.
28. Jayakumar, K., Seena, E. B., Kurup, M. P., Kaya, S., Serdaroğlu, G., Suresh, E. and Marzouki, R. (2022). Spectral, thermal and DFT studies of novel nickel (II) complexes of 2-benzoylpyridine-N4-methyl-3-thiosemicarbazone: Crystal structure of a square planar azido-nickel (II) complex. *Journal of Molecular Structure*, 1253: 132257.
29. Sykuła, A., Nowak, A., Garribba, E., Dzeikala, A., Rowińska-Żyrek, M., Czerwińska, J., ... and Łodyga-Chruścińska, E. (2023). Spectroscopic characterization and biological activity of hesperetin Schiff bases and their Cu (II) complexes. *International Journal of Molecular Sciences*, 24(1): 761.
30. Yakan, H., Cavuş, M. S., Kurt, B. Z., Muğlu, H., Sönmez, F. and Güzel, E. (2021). A new series of asymmetric bis-isatin derivatives containing urea/thiourea moiety: Preparation, spectroscopic elucidation, antioxidant properties and theoretical

calculations. *Journal of Molecular Structure*, 1239: 130495.

31. Abdel-Rahman, L. H., Ismail, N. M., Ismael, M., Abu-Dief, A. M. and Ahmed, E. A. H. (2017). Synthesis, characterization, DFT calculations and biological studies of Mn(II), Fe(II), Co(II) and Cd(II) complexes based on a tetradentate ONNO donor Schiff base ligand. *Journal of Molecular Structure*, 1134: 851-862.
32. Fayed, T. A., Gaber, M., Abu El-Reash, G. M. and El-Gamil, M. M. (2020). Structural, DFT/B3LYP and molecular docking studies of binuclear thiosemicarbazide Copper (II) complexes and their biological investigations. *Applied Organometallic Chemistry*, 34(9): e5800.
33. Mahmoud, W. H., Mohamed, G. G. and El-Sayed, O. Y. (2018). Coordination compounds of some transition metal ions with new Schiff base ligand derived from dibenzoyl methane. Structural characterization, thermal behavior, molecular structure, antimicrobial, anticancer activity and molecular docking studies. *Applied Organometallic Chemistry*, 32(2): e4051.
34. El-Ghamry, M. A., Elzawawi, F. M., Aziz, A. A. A., Nassir, K. M. and Abu-El-Wafa, S. M. (2022). New Schiff base ligand and its novel Cr(III), Mn(II), Co(II), Ni(II), Cu(II), Zn(II) complexes: Spectral investigation, biological applications, and semiconducting properties. *Scientific Reports*, 12(1): 17942.
35. Jacob, J. M., Kurup, M. P., Nisha, K., Serdaroğlu, G. and Kaya, S. (2020). Mixed ligand copper(II) chelates derived from an O, N, S-donor tridentate thiosemicarbazone: Synthesis, spectral aspects, FMO, and NBO analysis. *Polyhedron*, 189:114736.
36. Kovala-Demertzi, D., Demertzis, M. A., Miller, J. R., Papadopoulou, C., Dodorou, C. and Filousis, G. (2001). Platinum(II) complexes with 2-acetyl pyridine thiosemicarbazone: synthesis, crystal structure, spectral properties, antimicrobial and antitumour activity. *Journal of Inorganic Biochemistry*, 86(2-3): 555-563.
37. Bahojb Noruzi, E., Shaabani, B., Geremia, S., Hickey, N., Nitti, P. and Kafil, H. S. (2020). Synthesis, crystal structure, and biological activity of a multidentate calix [4] arene ligand doubly functionalized by 2-hydroxybenzeledene-thiosemicarbazone. *Molecules*, 25(2): 370.