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Synthesis, characterization, structural features and cytotoxicity of innovative zinc(II) complex derived from ONS-donor thio-Schiff base of acyl pyrazolone

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RESEARCH ARTICLE

ABSTRACT



doi: 10.5155/eurjchem.10.2.131-138.1858

 Received: 29 March 2019
 Received in revised form: 09 May 2019
 Accepted: 10 May 2019
 Published online: 30 June 2019
 Printed: 30 June 2019

KEYWORDS

 ONS donors
 Cytotoxicity
 Zn(II) complex
 Thio-Schiff base
 Single crystal structure
 Acyl pyrazolone derivatives

Novel acyl pyrazolone and thio-Schiff base of acyl pyrazolone ligand HL (2-((4-chlorophenyl)(1-(3-chlorophenyl)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)methylene)hydrazine-1-carbothioamide) has been synthesized and characterized by FT-IR and ¹H NMR techniques. This unusual coordinated complex [Zn(L)(CH₃COO)(H₂O)]CH₃CH₂OH has been synthesized and characterized by FT-IR, thermogravimetric analysis (TGA), UV-Vis spectroscopy and single crystal X-ray diffraction. ¹H NMR confirms the structure of the thio-Schiff base used in the study. The structural data reveal that mononuclear Zn(II) complex has distorted square pyramidal geometry. S-S interaction, Inter molecular and intra molecular H-Bond found in crystal packing of the complex. The complex has been screened for MTT assay against A549 cell lines. The Zn(II) complex is cytotoxic against the screened cell line at low concentration.

 Cite this: *Eur. J. Chem.* 2019, 10(2), 131-138

 Journal website: www.eurjchem.com

1. Introduction

The Schiff based ligands of acyl pyrazolones paying attention in recent time due to its coordination ability and decent biological activities [1-6]. Reaction between ketones (or aldehydes) and a thiosemicarbazide that gives thiosemicarbazones and which are called thio-Schiff and owing to their good binding capacity to metal they are very suitable in coordination chemistry [7]. Tautomer thione (-C=S) and thiol (-C-SH) forms (Figure 1) of thiosemicarbazone act as neutral or anionic bidentate ligands from the donor sites of azomethine N and S(thione/thiol) [8,9].

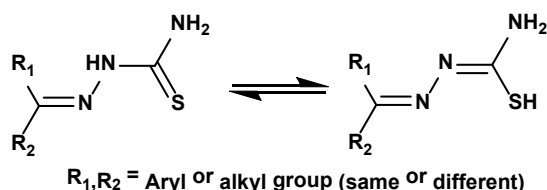


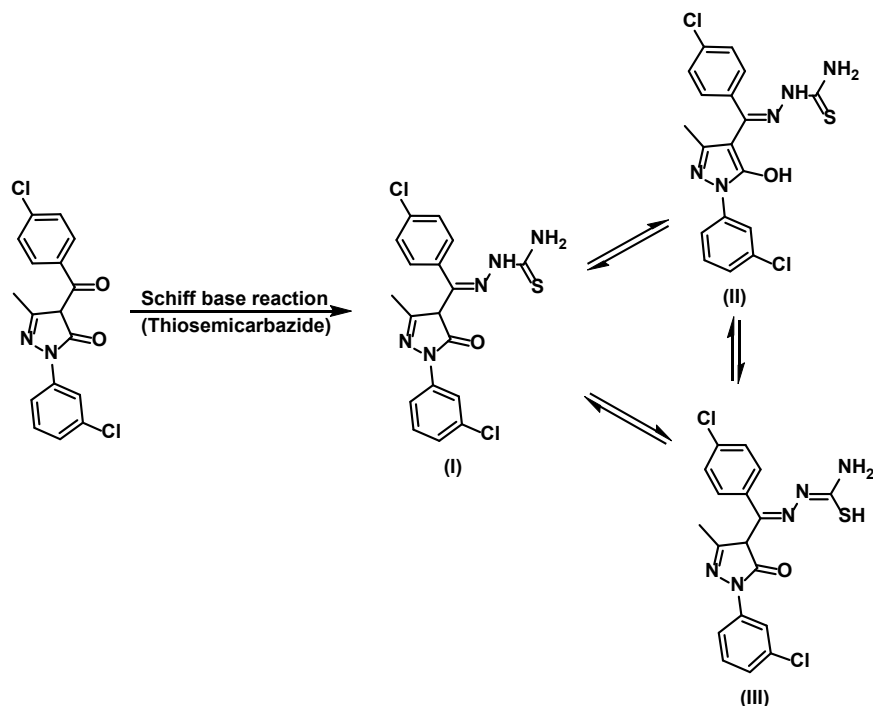
Figure 1. Thione, thiol tautomer of thiosemicarbazone.

Their Zn (II) complexes show prospective fluorescence properties [10,11]. Several metal complexes showed respectable biological activities with significant thiosemicarbazone of acyl pyrazolone [12]. Selected metal complexes of thiosemicarbazone are octahedral, square planer, square pyramidal and trigonal bipyramidal are reported in recent time [12,13]. Very unique binding from three different molecules, penta coordinated square pyramidal geometry of thio-Schiff Zn(II) complex is reported in this article.

Metal complexes of thio-Schiff base ligands appreciated because of their antimicrobial, antifungal, antioxidant and catalytic activities [14-17]. Metal complexes of tridentate acyl pyrazolone based thiosemicarbazone showed some decent results of several bioactivities [12]. Herein, zinc(II) complex of thio-Schiff base of biological active acyl pyrazolone synthesized and reported sound result of cytotoxicity against A549 cells by MTT assay.

2. Experimental

2.1. Materials



Scheme 1. Synthesis route of ligand (HL).

The compound 1-(3-chlorophenyl)-3-methyl-1H-pyrazol-5(4H)-one (MCPMP) was obtained from Nutan Dye Chem., Sachin, Surat, India as free gift sample. 1,4-Dioxane and methanol were obtained from SD Fine Chem. Limited, Chennai, India. Calcium hydroxide, sodium acetate and zinc acetate dehydrate were obtained from LOBA Chem. Pvt. Ltd., Mumbai (India). Thiosemicarbazide obtained from Sisco Research Lab. Pvt. Ltd., Mumbai (India). Absolute ethanol was obtained from Baroda Chem. Industry Ltd. and was used after distillation.

2.2. Characterization techniques

The synthesized compounds were characterized using FT-IR and UV-visible spectroscopy, molar conductance and X-ray crystallography. Infrared spectra (4000-400 cm^{-1} , KBr pellets) of the samples were recorded on a model Bruker alpha FT-IR spectrophotometer with 4 scan number. The ^1H NMR spectrum of the ligand was recorded with a Bruker AV 400 MHz spectrometer using CDCl_3 as the solvent and TMS as an internal reference. Electronic spectra (in DMSO at room temperature) in the range 400-800 nm were recorded on a model Perkin Elmer Lambda 35 UV-VIS spectrophotometer. TGA analysis has been done on SII TG/DTA A6300 instrument. The molar conductivity of a 1×10^{-3} M solution of the complex in DMSO was measured at room temperature with a model Elico CM 180 digital direct reading deluxe digital conductivity meter. The zinc content was determined by using EDTA in volumetric analysis after decomposing the complex with HNO_3 .

2.3. Synthesis

2.3.1. Synthesis of 4-(4-chlorobenzoyl)-2-(3-chlorophenyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one [PCBMCPMP]

The acylation reaction of pyrazolone (MCPMP) was carried out by reported method [18]. Color: Yellow. Yield: 58%. M.p.: 105-107 $^\circ\text{C}$. ^1H NMR (400 MHz, CDCl_3 , δ , ppm): 2.14 (s, 3H, pyrazolone C-CH₃), 7.28-7.30 (m, 1H, Ar-H), 7.39-7.43 (m, 1H,

Ar-H), 7.51-7.63 (m, 4H, Ar-H), 7.83-7.85 (m, 1H, Ar-H), 7.963-7.968 (m, 1H, Ar-H), 11.65 (s, 1H, OH). FT-IR (KBr, ν , cm^{-1}): 1625 (s) (C=O, amide of pyrazolone), 1590 (m) (C=O, p-chloro ph), 966 (s) (N-N), 1486 (m) (Ar-C-C). Anal. calcd. for $\text{C}_{17}\text{H}_{12}\text{Cl}_2\text{N}_2\text{O}_2$: C, 58.81; H, 3.48; N, 8.07 Found: C, 58.70; H, 3.37; N, 8.22%. Λ_m ($\text{S.cm}^2.\text{mol}^{-1}$): 7.0.

2.3.2. (Z)-2-((4-chlorophenyl)(1-(3-chlorophenyl)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)methylene)hydrazine-1-carbothioamide [HL]

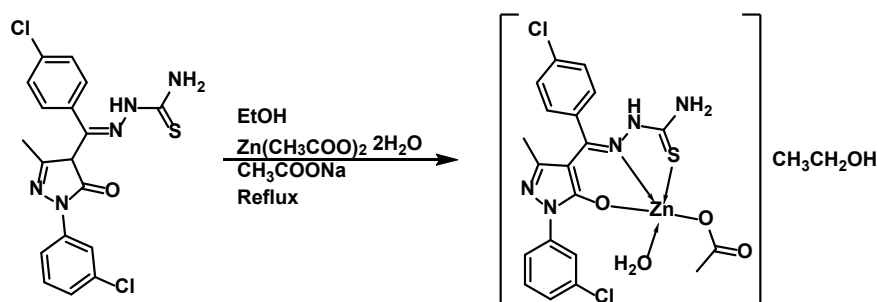
PCBMCPMP (0.347 g, 1 mmol) and thiosemicarbazide (0.091 g, 1 mmol) in ethanol (50 mL) were refluxed for 6 h in round bottom flask. During the reflux a microcrystalline yellow compound [HL] separated out, which was isolated by filtration, dried in air and finally crystallized in the suitable solvent (Scheme 1). Color: Yellow. Yield: 82.34 %. M.p.: 188-190 $^\circ\text{C}$. ^1H NMR (400 MHz, CDCl_3 , δ , ppm): 2.14 (s, 3H, pyrazolone C-CH₃), 2.24 (s, 2H, NH₂-tsc), 3.50 (s, 1H, NH-tsc), 7.28-7.31 (m, 1H, Ar-H), 7.39-7.43 (m, 1H, Ar-H), 7.52-7.63 (m, 4H, Ar-H), 7.96-7.97 (m, 1H, Ar-H), 7.84-7.85 (m, 1H, Ar-H), 10.19 (s, 1H, OH). FT-IR (KBr, ν , cm^{-1}): 3331 (b) (NH₂), 3168 (b) (NH-tsc), 1662 (C=O, pyrazolone), 1014 (m) (C=S), 1602 (m) (C=N, cyclic), 958 (s) (N-N), 1481 (m) (Ph-C-C), 2960 (b) (Ph-C-H). Anal. calcd. for $\text{C}_{18}\text{H}_{15}\text{Cl}_2\text{N}_5\text{OS}$: C, 51.44; H, 3.60; N, 16.66. Found: C, 51.25; H, 3.56; N, 16.57%. UV/Vis (DMSO, λ_{max} , nm, ϵ): 271 (1.83). Λ_m ($\text{S.cm}^2.\text{mol}^{-1}$): 8.8.

2.4. Synthesis of complex $[\text{Zn}(\text{L})(\text{CH}_3\text{COO})(\text{OH}_2)]\text{CH}_3\text{CH}_2\text{OH}$

To a solution of $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ (0.2195 g, 1 mmol) in methanol (5 mL), a solution of HL (0.420 g, 1 mmol) in methanol (10 mL) was added while stirring. After half an hour add a methanolic solution of sodium acetate. The reaction mixture was refluxed for 4 h. After the reflux, the clear reaction mixture was placed for a week at 4 $^\circ\text{C}$ temperature. After a week light yellow crystalline product was obtained. The synthesis of the complex can be summarized by Scheme 2. Color: Yellowish white. Yield: 65 %. M.p.: 200-202 $^\circ\text{C}$.

Table 1. Crystal data and structure refinement for $[\text{Zn}(\text{L})(\text{H}_2\text{O})(\text{CH}_3\text{COO})]\text{CH}_3\text{CH}_2\text{OH}$.

Identification code	$[\text{Zn}(\text{L})(\text{H}_2\text{O})(\text{CH}_3\text{COO})]\text{CH}_3\text{CH}_2\text{OH}$
Empirical formula	$\text{C}_{22}\text{H}_{25}\text{Cl}_2\text{N}_5\text{O}_5\text{SZn}$
Formula weight	607.80
Temperature (K)	273.0
Crystal system	Triclinic
Space group	P-1
a (Å)	8.1030(7)
b (Å)	12.1220(10)
c (Å)	14.9628(12)
α (°)	72.777(7)
β (°)	75.541(7)
γ (°)	88.486(7)
Volume (Å ³)	1357.6(2)
Z	2
ρ_{calc} (g/cm ³)	1.487
μ (mm ⁻¹)	1.219
F(000)	624.0
Crystal size (mm ³)	0.3 × 0.2 × 0.2
Radiation	MoK α ($\lambda = 0.71073$)
2 θ range for data collection (°)	6.128 to 57.92
Index ranges	-10 ≤ h ≤ 10, -14 ≤ k ≤ 15, -11 ≤ l ≤ 20
Reflections collected	6488
Independent reflections	5415 [R _{int} = 0.0196, R _{sigma} = 0.0554]
Data/restraints/parameters	5415/0/330
Goodness-of-fit on F ²	1.026
Final R indexes [$I \geq 2\sigma(I)$]	R ₁ = 0.0484, wR ₂ = 0.1086
Final R indexes [all data]	R ₁ = 0.0698, wR ₂ = 0.1231
Largest diff. peak/hole (e Å ⁻³)	0.94/-0.84

**Scheme 2.** Synthesis route of zinc (II) complex.

FT-IR (KBr, ν , cm^{-1}): 3408 (b) (NH-), 1160 (C=N, azomethine), 862 (s) (N-N), 1474 (m) (Ph-C-C), 1612 (s) (C=N, cyclic), 1139 (m) (N-N), 3159 (b) (NH₂), 1589 (m) (C=N, cyclic), 2927 (b) (Ph-C-H), 1015 (m) (C=S), 511 (s) (Zn-N), 480 (s) (Zn-O), 445 (s) (S-O). Anal. calcd. for $\text{C}_{22}\text{H}_{25}\text{Cl}_2\text{N}_5\text{O}_5\text{SZn}$: C, 43.47; H, 4.15; N, 11.52. Found: C, 43.36; H, 3.95; N, 11.75%. UV/Vis (DMSO, λ_{max} , nm, ϵ): 288 (1.40). Λ_m (S.cm².mol⁻¹): 3.3.

2.5. X-ray structure determination

Single-crystal X-ray diffraction measurement for one of the Zn(II) complex was carried out. X-ray intensity data of Reflections collected 6488 (Independent reflections 5415) were collected on Bruker CCD area-detector diffractometer equipped with graphite monochromated MoK α radiation ($\lambda = 0.71073$). The crystal used for data collection was of dimensions 0.30 mm × 0.20 mm × 0.20 mm. The structure was solved by methods using SHELXS97 [19]. All non-hydrogen atoms of the molecule were located in the best E-map. Full-matrix least-squares refinement was carried out using SHELXL97 [19]. The final refinement cycles converged to an R1 = 0.0698 and wR2 = 0.1231 for the observed data. Residual electron densities ranged from 0.94/-0.84 e.Å⁻³. The crystallographic data are summarized in Table 1. An ORTEP [20] view of the complex with 50% possibility is shown in Figure 2 [21]. The geometry of the molecule has been calculated using the software PLATON [22] and PARST [23].

2.6. MTT cell viability assay

A549 (human lung carcinoma) cells were procured from National Centre of Cell Sciences, Pune, India. Cells were maintained at 37 °C and 5% CO₂. Cells were seeded (1×10^5 cells) in a T25 flask and cultured in DMEM containing 10% FBS and 1% antibiotic-antimycotic solution with trypsinization at every third day and sub-culturing with a TPVG solution. 1 mg test complex was dissolved in 5 μL DMSO solvent and later, 1, 5, 10, 25, 50, 100, 200, 500 and 1000 $\mu\text{g}/\text{mL}$ doses were tested against A549 cells for cytotoxicity using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assay. A549 cells (7×10^3 cells/well) were seeded in 96-well culture plates (Tarson India Pvt. Ltd.) for 24 h in absence or presence of complex. Later, 10 μL MTT (5 mg/mL) was added to the wells and plates were incubated at 37 °C for 4 h to form the formazan crystals. The culture media was discarded and wells were washed with PBS. The resultant formazan was dissolved in 150 μL of DMSO and absorbance was read at 540 nm in Synergy HTX-Multimode Reader (Biotek, USA).

2.7. Statistical analysis

The data was analysed for statistical significance using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test and the results were expressed as

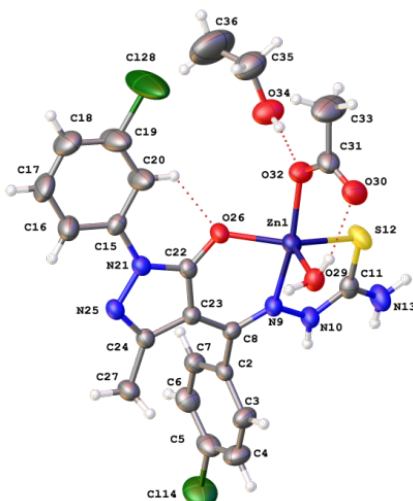


Figure 2. Crystal structure of Zn(II) complex.

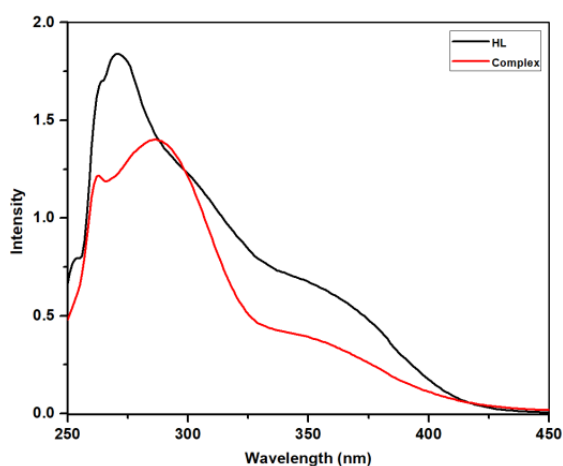


Figure 3. UV-Vis spectroscopy of ligand and complex.

mean \pm SEM using Graph Pad Prism version 5.0 for Windows, Graph Pad Software, San Diego, California, USA.

3. Result and discussion

3.1. IR spectroscopy

IR spectroscopy study and comparison between ligand complex indicating that ligand HL is reacted as monoionic tridentate ligand in zinc(II) complex with thionesulphur, azomethine nitrogen and amidic -C=O to -C-O- in pyrazolone moiety. The IR spectra of the ligand display sharp band at 3331 cm^{-1} as indicated NH_2 group remain unchanged and just slightly shifted to 3159 cm^{-1} in complex IR-spectrum [24]. Medium intensity bands in the region 2960-2926 cm^{-1} due to -N-H vibration changed in complex 2968-2927 cm^{-1} and IR stretching of C=S is 1014 cm^{-1} in the ligand (HL) that showed in complex at 1015 cm^{-1} . These bands of IR spectra clearly indicating -N-H and -C=S remain unchanged in complex [12,24]. In comparison between IR spectra of ligand and complex indicating unchanged thione-sulphur and azomethine nitrogen in coordination zinc(II) complex. Carbonyl bond (C=O) band observed at 1625 cm^{-1} of ligand disappear in complex and new bond (-C-O-) a sharp intense band appeared at 1160 cm^{-1} the complex, where shift to higher frequency region is seen, due to de-protonation of enol (-C-OH) form

(Scheme 1) of ligand and the appearance of new bond of (-C-O-) in complex indicating the ONS monoionic tridentate behaviour of the ligand (HL). Bonds between metal and ligand showed bands at 511, 480 and 445 cm^{-1} recognized to (Zn-N), (Zn-O) and (Zn-S), respectively.

3.2. Electronic spectroscopy

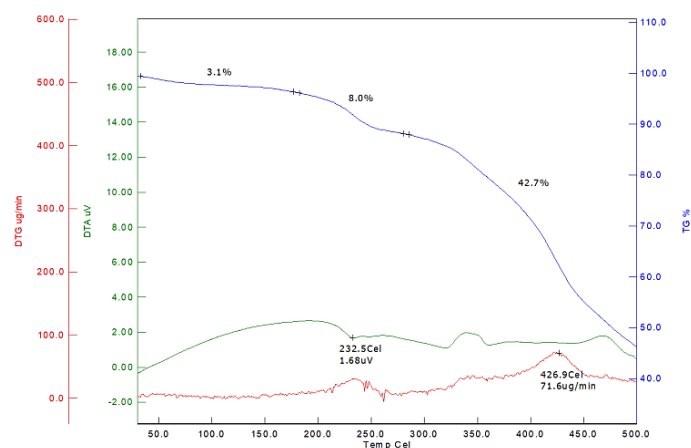
The transfer of an electron from one atom to another called charge transfer (C.T.). The UV-Vis bands are related with C.T. of metal to ligand and ligand to metal in metal complex. The visible spectrum for the complex under examination was measured in DMSO (Figure 3). Two bands were observed around wavelength 280 and 350 nm indicated charge transfer in complex [18]. As expected, there was no any d-d band for Zinc complex due to the completely filled 3d orbital in d^{10} metal complex [18].

3.3. Thermogravimetric analysis (TGA)

The thermogravimetric analysis (TGA) is an analytical method used to define thermal stability of a compound and its division of volatile decomposition products by monitoring the mass loss that occurs as sample is heated. TGA of complex has been completed under nitrogen atmosphere from 27 to 500 $^{\circ}\text{C}$.

Table 2. Important bond lengths and bond angles.

Atom-Atom	Length (Å)	Atom- Atom-Atom	Angle (°)
Zn1-S12	2.3293(10)	O26-Zn1-S12	145.25(8)
Zn1-O26	1.977(2)	O26-Zn1-O29	107.39(10)
Zn1-O29	2.010(2)	O26-Zn1-O32	87.18(10)
Zn1-O32	2.090(2)	O26-Zn1-N9	86.48(9)
Zn1-N9	2.198(3)	O29-Zn1-S12	107.01(7)
S12-C11	1.695(3)	O29-Zn1-O32	95.93(10)
C114-C5	1.747(4)	O29-Zn1-N9	100.82(10)
C128-C19	1.740(5)	O32-Zn1-S12	94.09(8)
O26-C22	1.287(4)	O32-Zn1-N9	163.19(10)
O32-C31	1.271(4)	N9-Zn1-S12	82.43(7)
O30-C31	1.232(5)	C11-S12-Zn1	99.24(12)
N9-N10	1.384(4)	C22-O26-Zn1	122.7(2)
N9-C8	1.305(4)	C31-O32-Zn1	123.8(2)
N10-C11	1.347(4)	N10-N9-Zn1	114.31(19)
N21-N25	1.393(3)	C8-N9-Zn1	127.6(2)
N21-C22	1.367(4)	C8-N9-N10	117.9(3)
N21-C15	1.415(4)	C11-N10-N9	120.3(3)
N25-C24	1.317(4)	N25-N21-C15	119.6(3)
O34-C35	1.411(5)	C22-N21-N25	110.8(3)
N13-C11	1.318(4)	C22-N21-C15	129.2(3)
		C24-N25-N21	105.7(3)
		N10-C11-S12	123.2(3)
		N13-C11-S12	119.7(3)
		N13-C11-N10	117.0(3)
		C24-C23-C8	130.2(3)
		C22-C23-C24	104.6(3)
		C22-C23-C8	124.8(3)
		N25-C24-C23	112.1(3)
		N25-C24-C27	118.2(3)
		C23-C24-C27	129.6(3)

**Figure 4.** TG-DTA of complex.

The first mass loss was observed up to 150 °C probably is due to loss of coordinated one molecule of water. Second mass loss observed between 160-260 °C that indicating the coordinated acetate group [25]. Thus the thermogravimetric curve of the Figure 4 indicate that thermal properties of Zn(II) complex is completely approving its thermal stability. After two mass losses there is one continuous mass loss displayed in complex TGA study. That above 300 °C to more than 500 °C, the complexes decompose slowly due to fragmentation and thermal degradation of the organic moiety of thio-Schiff based thiosemicarbazone of acyl pyrazolone.

3.4. Conductance measurement

The conductivities of zinc(II) metal complex and ligand in DMSO were measured with a calibrated conductivity cell at room temperature. The low conductivity values in DMSO reveal Complexes are non-electrolytic in nature. The conductivity of each solution (1×10^{-3} M) was measured and used for further calculations where possible, specific conductivities and molar conductivities. Ligand (HL) and its

zinc(II) complex molar conductance values are 8.8 and 3.3 $\text{ohm}^{-1}\text{cm}^2\text{mol}^{-1}$, respectively.

$$\text{Sp. conductivity} = \text{cell constant} \times \text{conductivity} \quad (1)$$

$$\text{Molar conductivity} = \frac{\text{Sp. conductivity} \times 1000}{\text{Molarity of solution}} \quad (2)$$

3.5. Crystallographic data

The molecular structure of zinc complex and the atom labelling scheme is shown in Figure 2. The main bond distances and angles are listed in Table 2. As shown in Figure 1, the Zn(II) ion is penta-coordinated by three oxygen atoms of one acyl pyrazolone ligand, one oxygen atom of water molecule and one oxygen atom of acetate group. The one oxygen, one thione-sulphur and one azomethine nitrogen of ligand (L) and one oxygen of acetate molecules occupy the basal sites of the square pyramidal structure, whereas the water molecule binds the zinc at the apical site.

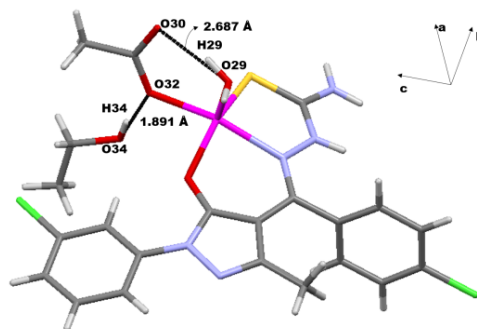


Figure 5. Intra molecule H-Bond in complex.

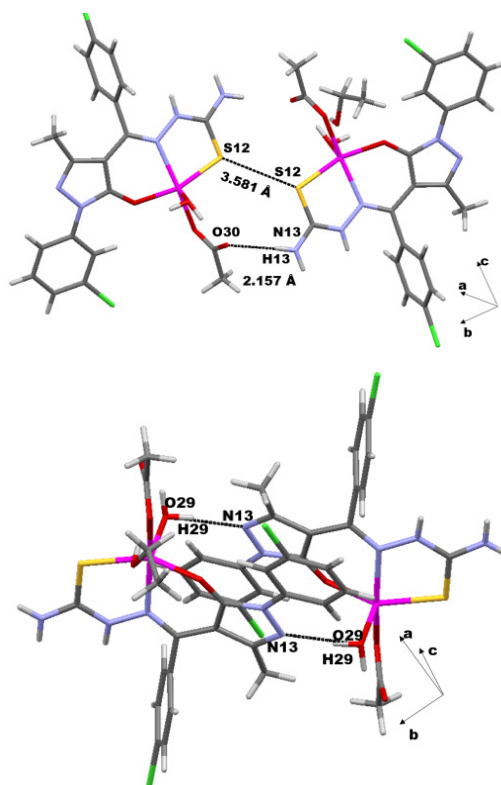


Figure 6. Intermolecule H-bond, S-S interactions.

In this complex, the combination of the solvent EtOH into the outer-sphere of the complex without coordination bond. The geometry around the metal is now distorted square pyramidal.

The zinc atom of complex occupies a distorted square-pyramidal environment (SP), formed by three oxygen atoms [O(26), O(29) and O(32)] supplied by one pyrazolone based ligand, water molecule and acetate molecule, respectively. The fourth and fifth coordination site is occupied by one nitrogen atom and sulphur atom of ligand molecule. The Zn-O distances are Zn(1)-O(26) = 1.977(2) Å and Zn(1)-O(29) = 2.010(2) Å and Zn(1)-O(32) is 2.090(2) Å. Where the distance between Zn(1)-S(12) = 2.3293(10) Å [26] and Zn(1)-N(9) = 2.198(3) Å.

In the complex, displayed intra-molecular hydrogen bond (O34-H34...O32) between H34-O34 of EtOH solvent and O32 of acetate, and additional one is H29-O29 of water molecule and O30 of acetate (Figure 5).

Furthermore in inter molecule hydrogen bond observed between H13-N13...O30 where H13-N13 of thio-Schiff base of acyl pyrazolone. Another one is H29-O29...N25 H-bond where

H29-O29 of coordinated water molecule and N25 of pyrazolone ring.

In the Figure 6, very interesting illustrated that the structure of zinc(II) complex exhibiting a sulphur-sulphur (S...S) interaction of 3.581 Å in identical complex molecules found within the crystallographic cell. The C=S bond is formally a double bond (1.695(3) Å) and the adjacent C-N(H) bond (C11-N10 = 1.347(4) Å) was consistent with a thioamide tautomer (Table 2, Figure 2) [27].

3.6. Cell culture and cell viability assay

Cytotoxicity of the synthesized metal complex was assessed against A549 cells by MTT assay. Doses 1, 5 and 10 µg/mL accounted for a weak cytotoxicity response with more than 70% cells being viable at the end of 24 h. But the subsequent doses of 325-1000 µg/mL accounted for 11% viability thus indicating at significant toxicity induced by the metal complex. These results are in agreement with previous report on anticancer potential of pyrazolone based complexes [28-30].

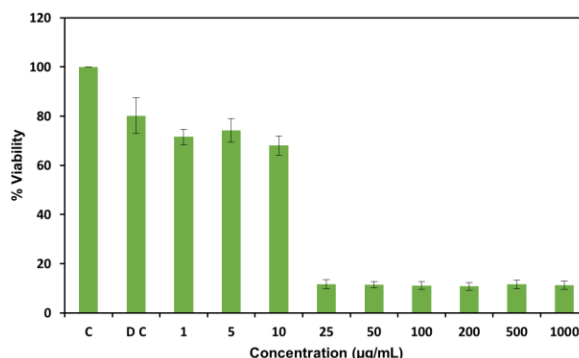


Figure 7. Cytotoxicity of complex [Zn(H₂O)(CH₃COO)]₂C₂H₅OH (DC=DMSO control).

It is hypothesized in the present study that Zn(II) chelation by pyrazolone based ligand accounts for cytotoxicity observed herein. MTT assay is focused at reporting the viability of a cell as a result of mitochondrial function and loss of viability is implicated on mitochondrial dysfunction [31,32]. Results obtained herein suggest mitochondrial dysfunction induced by metal complexes results in loss of cell viability of A549 cells (Figure 7).

4. Conclusion

The acyl pyrazolone and its Schiff based with thiosemicarbazide has been synthesised and characterized by FT-IR, ¹H NMR and UV-Vis spectroscopy. The ligand typically behaved as monoionic tridentate in nature coordinated through thione sulphur, azomethine nitrogen and amidic -C=O to -C-O- in pyrazolone moiety. Zinc(II) complex of thio-Schiff ligand synthesised and characterised by FT-IR, single crystal X-ray diffraction, UV-vis spectroscopy and conductance measurement. The Zn(II) enjoys square pyramidal environment. The complex was also screened for the cytotoxicity against the A549 lung cancer cell lines and found active.

Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.

Author contributions: All authors contributed equally to this work.

Ethical approval: All ethical guidelines have been adhered.

Sample availability: Samples of the compounds are available from the author.

Acknowledgment

The authors are thankful to Head, Department of Chemistry for providing necessary facilities required to carry out this work.

Supporting information

CCDC-1906438 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via <https://www.ccdc.cam.ac.uk/structures/>, or by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033.

Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.

Author contributions: All authors contributed equally to this work.

Ethical approval: All ethical guidelines have been adhered.

Sample availability: Samples of the compounds are available from the author.

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