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Synthesis, Characterization And Biological Activity of N- Thioureidomaleimic Acid and its Complexes with M(III) ions

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ABSTRACT

The N-thioureidomaleimic acid and its complexes with Cr (III) and La(III) ions have been prepared and characterized by using elemental analysis, thermal analysis, spectroscopy (I.R., u.v-vis., ^{1}H NMR, mass) and magnetic susceptibility measurements. The I.R. spectral data suggest that the thiosemicarbazide derivative coordinates to the metal ions as a tridentate ligand through sulfur, nitrogen and oxygen atoms. The thermal behavior of the complexes indicates thermal stabilities up to about 200^{0}C. The free ligand and its metal complexes have been tested in vitro against a number of microorganisms, to assess their antimicrobial properties.

INTRODUCTION

Thiosemicarbazides are interesting for their abilities in forming stable and intensely colored complexes, which are, then, useful for spectroscopic determination of some metal ions (Sorourddin et al., 2005), as well as their catalytic activities (Chawla et al., 2006). They are also potentially beneficial as antibacterial, antifungal and anticancer agents (Simonov et al., 2002). Aromatic thiosemicarbazide derivatives such as phenyl thiosmicarbazide, quinoline thiosemicarbazide and their complexes with some metal ions were reported (Henderson, 2003). Triphenyl thiosemicarbazide and its Pd(II), Pt(II) and Au(III) complexes were reported, too (Du and Liu, 2008). Many substituted maleamic acids and their substituted derivatives are used as antimicrobial agents and in polymer synthesis (Huanga et al., 2005; Garcina-Martinez et al., 2000; Sortino et al., 2008). Studies concerning the interaction of maleamic acids with metal ions are available (Lazarou et al., 2009). However, most of the studied topics concentrate on ligands with oxygen and nitrogen donors (Shen et al., 1997).

Thioureidmaleimic acid or maleylthiourea (N-thiocarbamoyl maleamic acid) is an important maleic acid monoureide. It can be obtained by the reaction of thiosemicarbazide and maleic acid anhydride. The compound can act as a tridentate ligand through S, N and O of the thioureido derivative. The present work aims to prepare a tridentate thiosemicarbazide derivative thioureidomaleimic acid and its complexes with Cr(III) and La(III). The biological activities of the ligand and its resulting complexes will also be investigated.

MATERIALS AND METHODS

$LaCl_3.7H_2O$ and $CrCl_3.6H_2O$ are of Analar grade and used without further purification. Thiosemicarbzide 98% and maleic acid anhydride 98% (Pareac) were used as received. Organic solvents used include; absolute ethyl alcohol, diethylether and acetonitrile HPLC grade, (Scharlau).

Synthesis of the ligand N-thioureidomaleimic acid (H_3L)

1.96 g (0.02 mol) of maleic acid anhydride was dissolved in 40 ml of warm acetonitrile and left to stand at room temperature. Meanwhile 1.82 g (0.02 mol) of thiosemicarbazide was refluxed in

50 ml of acetonitrile and after the dissolution was complete the solution was left to cool at room temperature. To the warm solution of the thiosemicarbazide in acetonitrile, maleic acid anhydride solution was added stepwise while stirring vigorously.

The mixture was then put in a refrigerator at about 5°C overnight. The yellowish precipitate formed was filtered, washed with water and ether and left to dry in air. The yield was 40%.

Synthesis of complexes

Synthesis of $[Cr(C_5H_5N_3O_3S)Cl(NH_3)(H_2O)].EtOH$

To a solution containing 0.3 g (0.00175mol) of N-thioureidomaleimic acid dissolved in 40 ml of absolute ethanol, was added 0.421 g (0.0016mol) of $CrCl_3.6H_2O$ dissolved in 40 ml of absolute ethanol while stirring. The mixture was heated for few minutes, followed by addition of drops of ethanolic solution of ammonia, and left to stand for five days. The dark brown solid was collected by filtration, washed with ethanol and left to dry in air.

Synthesis of $[La(C_5H_5N_3O_3S)Cl(H_2O)_2].EtOH.H_2O$

To a solution containing 0.3 g (0.00175mol) of N-thioureidomaleimic acid dissolved in 40 ml of absolute ethanol, was added, with constant stirring, 0.50 g (0.00135mol) of $LaCl_3.7H_2O$ dissolved in 40 ml hot absolute ethanol. The mixture was heated on a hot plate for ten minutes, followed by addition of few drops of ethanol solution of ammonia. The mixture was left to stand at room temperature for 5 days. The white colored precipitate was collected by filtration, washed with ethanol and left to dry in air.

methods of analysis

Magnetic susceptibility measurements on powder sample were carried out on a Johnson Matthey magnetic susceptibility balance. Elemental analyses were carried in the micro analytical unit, Cairo University, using chemical analyzer Carlo – Erba model 1106. Uv-Vis. spectra were recorded using Shimadzu model 3101 PC spectrophotometer. Infrared spectra of solid sample were recorded on a Perkin-Elmer and Shimadzu model FT-IR 8400 S, spectrophotometers. Thermal analysis were recorded by Shimadzu Thermal analyzer model TGA-50H and DTA-50H, using nitrogen atmosphere at a flow rate of 20 ml min^{-1}. The heating rate was 10°C min^{-1} in the temperature range 30-1000 °C, using platinum crucibles. Mass spectra were recorded by Shimadzu model Q-P-2010 plus, spectrometer. ^1H NMR spectra were recorded on an NMR spectrometer model VX-300. The spectra were run at 300 MHz in deuterated dimethylsulfoxide (DMSO-d_6) at Cairo University, Faculty of Science, Department of Chemistry, NMR Lab.

Antimicrobial activity

The ligand and its complexes were tested as antifungal and antibacterial agents at Micro Analytical Centre, Faculty of Science, Cairo University.

Antifungal activity of the synthesized ligand H_3L and its complexes in term of their inhibition to the linear growth of Aspergillus *flavus* (fungus) and Candida *albicans* (fungus) was investigated, and their values were compared with that of the commercial drug Amphotericin B. However, the antibacterial activity was tested against Esherichia *coli* as gram negative bacteria and Staphylococcus *aureus* as gram positive bacteria. The investigated values were compared with that of the commercial antibacterial agent Tetracycline. The experiment control was DMSO.

RESULTS AND DISCUSSION

Characterization

The analytical data of the ligand and its complexes summarized in Table1 coincide with the proposed empirical formulae. The electronic impact mass spectrum of the ligand, Figure 1, shows molecular ion (M^+) peak at m/z = 189 a.m.u., corresponding to the species ($C_5H_7N_3O_3S^+$), conforming the molecular formula of the ligand. The spectrum also shows a series of peaks corresponding to the various fragments of the compound.

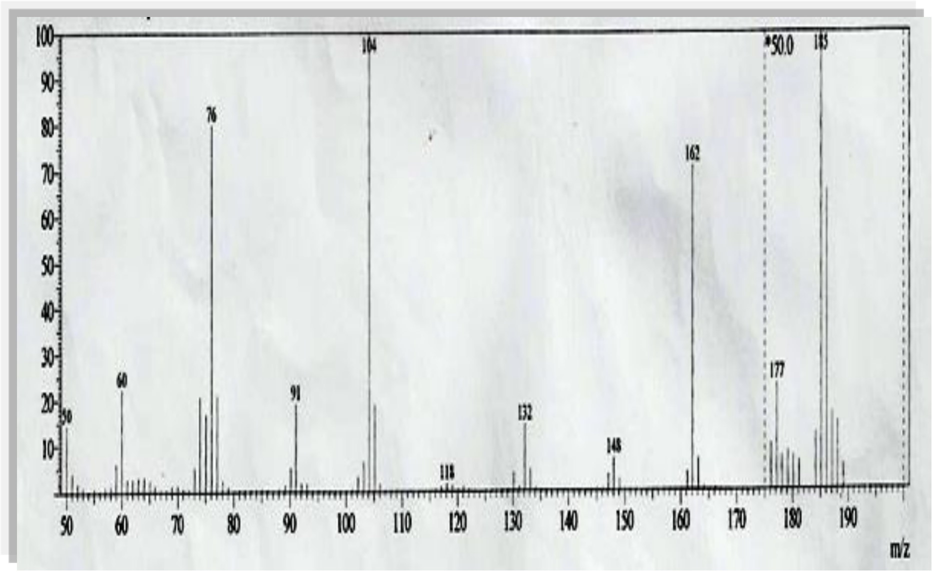


Figure (1): mass spectrum of the ligand (H_3L)

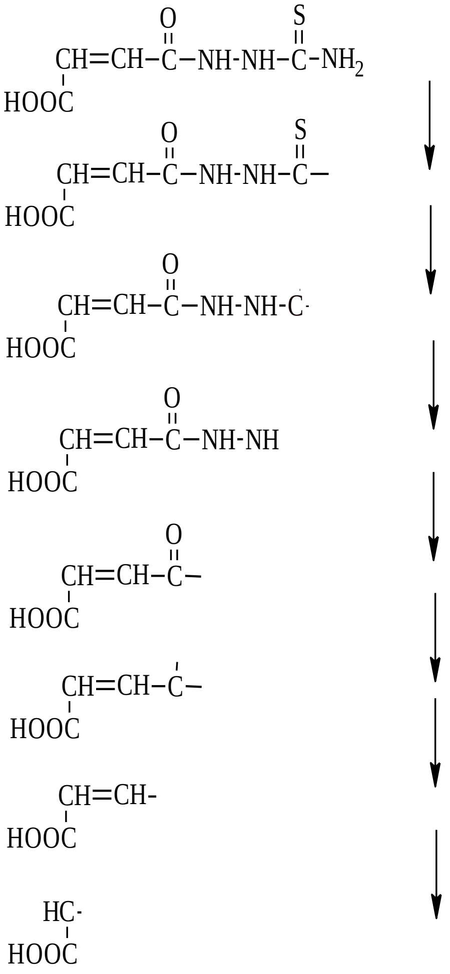
The intensities of the peaks give an indication of the relative stability of the fragments. The melting point of the synthesized ligand is 216°C with decomposition. Elemental chemical analysis data are shown in Table (1).

Table 1 : Analytical data of H_3L and its metal complexes.

Formulae	m.p °C	color	Elemental analysis found (calculated)%				Molecular weight found (calculated)
			C	N	S	H	
$C_5H_7N_3O_3S$ (H_3L)	216	Pale yellow	30.76 (31.75)	21.77 (22.22)	18.59 (16.93)	3.54 (3.70)	189(189)
$[Cr(C_5H_5N_3O_3S)Cl(NH_3)(H_2O)].EtOH$		Dark brown	24.61 (23.66)	13.97 (15.77)	- -	4.14 (4.50)	
$[La(C_5H_5N_3O_3S)Cl(H_2O)_2].EtOH.H_2O$		white	18.59 (18.22)	8.08 (9.11)	- -	3.13 (3.68)	

The results obtained indicate the formation of one type namely the 1:1 metal: ligand species.

The degradation pattern of the compound N- thioureidomaleimic acid is shown on Fig.(2)



Cal = 189	F = 189
Cal = 173	F = 173
Cal = 141	F = 141
Cal = 129	F = 129
Cal = 99	F = 99
Cal = 83	F = 79
Cal = 71	F = 71
Cal =58	F = 60

Figure (2) Degradation pattern of the compound N- thioureidomaleimic acid

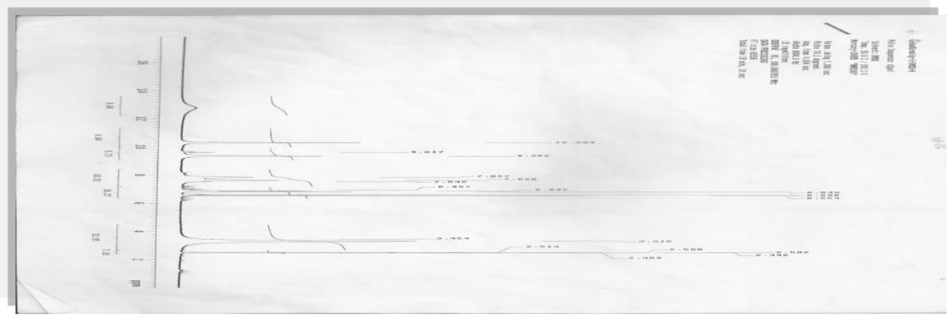


Figure (3) ^{1}H NMR spectrum of the ligand (H_3L)

The ^{1}H NMR spectrum of the ligand (H_3L), (Figure 3), shows two singlet signals at δ10.304 ppm and δ9.362 ppm, assigned to the protons of NH (COHN) and NH (HNCS), respectively. The doublet signal at δ7.857 ppm and δ7.628 ppm is due to NH_2 ($CSNH_2$) protons. The signal at δ 6.606 ppm is due to H(2H, HC=CH). The broad signal at δ 12.8 ppm is attributed to OH (COOH).

The IR spectral data (Table 2) of the ligand (H_3L) shows bands at 3423 cm^{-1} (s) 3282 cm^{-1} (s) and 3172 cm^{-1} (s) assigned to ν(OH), ν(CONH) and ν (NH_2) vibrations, respectively. The band at 1685 cm^{-1} (s) is assigned to ν (COOH), the carbonyl oxygen group. The band observed at 1274 cm^{-1} is assignable to ν(CN).

The band assigned to ν(CN), in the free ligand is shifted from 1274 cm^{-1} to 1208 cm^{-1} and 1192 cm^{-1} in Cr(III) and La(III) complexes, respectively. This indicates that the ligand is coordinated to the metal ions through the nitrogen atom of the (CONH) group. Disappearance of the band at 1685cm^{-1}

characteristic of the ν(COOH), indicates the involvement of the carboxylate group in complexation. Coordination of the ligand through thiolate sufur is

evidenced by the disappearance of the band at 827 cm^{-1} assignable to ν (CS), due to embolization of the group (NHC=S) with a subsequent deportation of the (N=C-S-H). This is evidenced by the appearance of new bands at 1547 cm^{-1} and 1542 cm^{-1}in both complexes, due to ν(C=N).

Table (2): IR spectral data for the ligand H_3L and its complexes

Compound	ν(OH)	ν(NH_2)	ν(NH)	ν(COOH)	ν(CN)	ν(CS)	ν(N=C)
$C_5H_7N_3O_3S$	3423(s)	3282(s)	3172(b)	1685(s)	1274	827	-
$[Cr(C_5H_5N_3O_3S)Cl(NH3)H_2O].EtOH$	3418	3301(s)	-	-	1208	-	1547
$[La(C_5H_5N_3O_3S)Cl\ (HO)_2]EtOH.H_2O$	-		-	-	1192	-	1542

Solid state Uv, electronic absorption data of the two complexes and their the moments are shown in Table (3).

Table (3): Electronic spectra and magnetic moments of the complexes **of** the ligand H_3L

NO.	Complex	Electronic spectra(nm)	μ_{eff} (B.M)
1	$[Cr(C_5H_5N_3O_3S)Cl(NH_3)(OH_2)]$ .EtOH	232, 271.5, 310, 502, 767	4.43

2	[La($C_5H_5N_3O_3S$)Cl(H_2O)$_2$].EtOH.H_2O	212,311, 359, 516,610, 661, 777	0.00

Thermal analysis of the complexes of the ligand (H_3L)

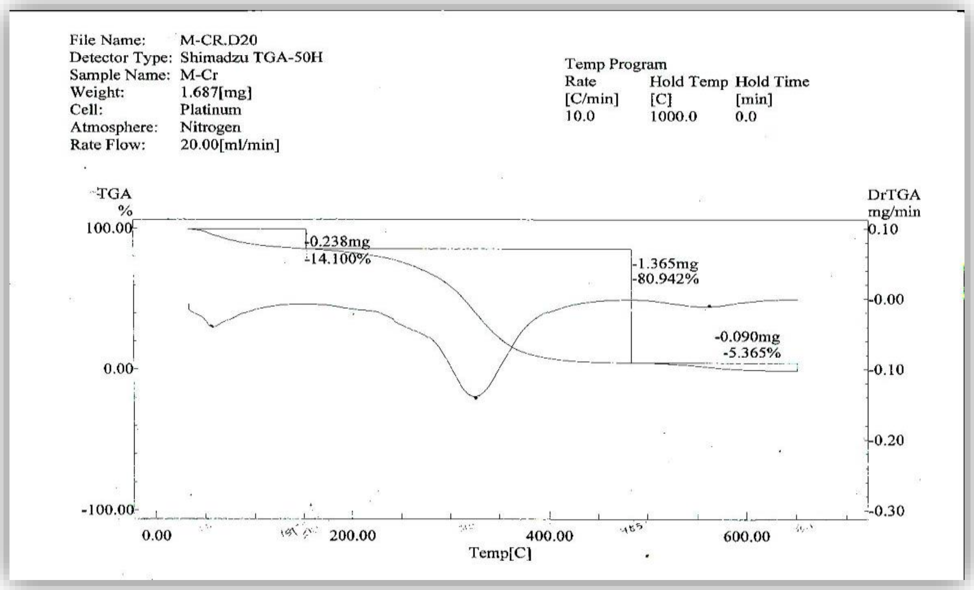
The thermogravimetric analysis of the complexes of the ligand N-thioueidomaleimic acid (H_3L) with Cr(III) and La(III) along with the % weight losses at different temperatures are recorded in Table 4. The Cr(III) complex decomposes in three stages, while the La(III) complex shows four decomposition stages. The complexes show both endothermic and exothermic peaks within the temperature ranges of decomposition.

The (TG) curve of [Cr($C_5H_5N_3O_3S$)Cl(NH_3)(OH_2)].EtOH are shown in figure 4. The thermogram exhibits three main decomposition steps. The first step of decomposition within the temperature range (30-150°C) corresponds to the loss of ethanol molecule at 70°C with mass loss of 14.00% (calculated 13.00%). The second and third steps within the temperature range (150-600°C) corresponds to mass loss of 86.00% (calculated 86.76°C). The overall weight loss amounts to 100.00% (calculated 99.71%).

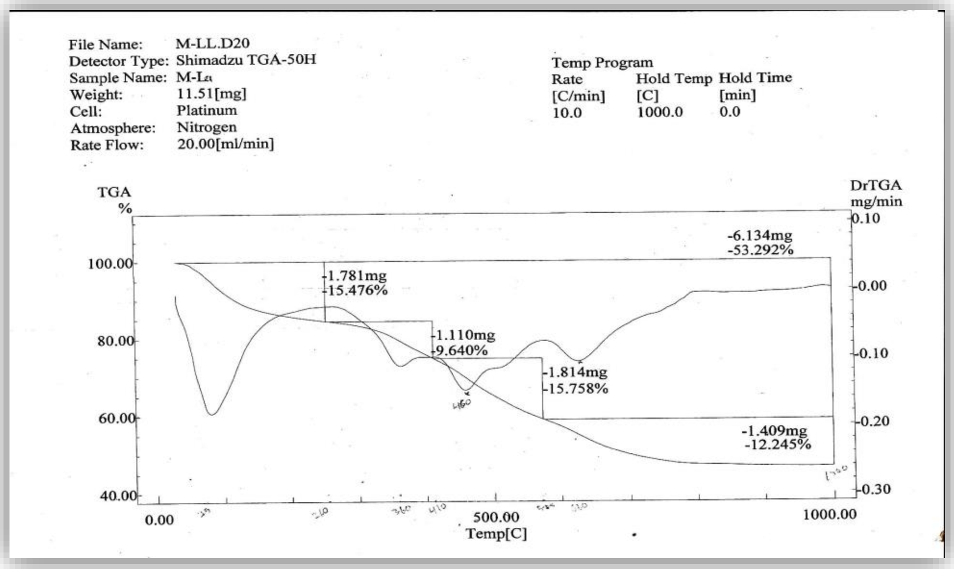
The thermogram of [La($C_5H_5N_3O_3S$)Cl(H_2O)$_2$].EtOH.H_2O is given in figure 5. It exhibits three decomposition steps. The first step of decomposition within the temperature range (30-260°C) corresponds to the loss of one EtOH molecule and water molecule of hydration with mass loss of 13.94% (calculated 15.5%). The second step within the temperature range (260-410°C) corresponds to the loss of 5H atoms, two coordinated H_2O with mass loss of 9.6% (calculated 8.9%). The third decomposition step within the temperature range (410-575 °C) corresponds to the loss of CSCl with mass loss of 15.8% (calculated 17.1%). The fourth decomposition step within the temperature range (575-1000°C) with mass loss of 12.2% (calculated 11.71%). The overall weight loss amounts to 53.119% (calculated 51.64%), that is about 48% of the compound remains undecomposed.

Table (4): Thermoanalytical results (TGA and DTA) of the complexes of the ligand H_3L

Compound	TG range (°C)	No. of steps	Found (Calculated) Mass loss (%)	Total mass loss (%)	Assignments
[Cr($C_5H_5N_3O_3S$)Cl(NH_3)(H_2O)].EtOH	30-150		14(12.95)	99.71	Loss of EtOH
	150-600		23(24)	(100)	loss of ligand+metal
[La($C_5H_5N_3O_3S$)Cl(OH)$_2$] EtOH.H_2O	30-260	1	15.5(13.98)	53.12	
	260-410	2	9.6(8.9)	(51.64)	loss of H_2O+EtOH
	410-575	3	15.8(17.13)		loss of ligand
	575-1000	4	12.2(11.71)		



Figure(4): TGA curve of the complex Cr($C_5H_5N_3O_3S$)Cl(NH_3)(OH_2)].EtOH



Figure(5): TGA curve of the complex [La($C_5H_5N_3O_3S$)Cl(H_2O)$_2$].EtOH.H_2O

Antifungal studies of the Ligand (H_3L) and its complexes

The complexes of Cr(III), and La(III) with the ligand N- thioureidomaleimic acid (H_3L) were tested for in vitro antifungal activity against Aspergillus *flavus* and Candida *albicans* fungi. Amphotericin B was used as standard fungicide. The results of the antifungal activity of the free ligand and the complexes are summarized in Table (5).It is observed that the free ligand possesses antifungal activity against the test fungi but less than that of the activity of the standard antifungal agent, amphotericin B.

The complex $[La(C_5H_5N_3O_3S)Cl(OH_2)_2].EtOH.H_2O$ did not show any activity against Aspergillus *flavus* and Candida *albican* fungi, while $[Cr(C_5H_5N_3O_3S)Cl(NH_3)(OH_2)]EtOH$ was active, but its activity was less than that of the free ligand. In conclusion, all the compounds of the present study displayed non to weak activity against Aspergillus *flavus* and Candida *albicans* fungi.

Antibacterial studies of the ligand (H_3L) and its complexes

The two complexes and the ligand were tested for in vitro antibacterial activity against Esherichia *coli* (G^-) and Staphylococcus *aureus* (G^+) bacteria. The results of the antibacterial activity of the free ligand and the complexes are summarized in Table 6. The antibacterial data recorded, indicate that the activity of the free ligand was zero on complexation with La(III), and decreased on complexation with Cr(III).

Table (5): Antifungal screening data of the ligand (H_3L) and its complexes

NO.	(mm/mg s)	Inhibition zone diameter	
	Sample	Aspergillus *flavus* (Fungus)	Candida *albicans* (Fungus)
1.	Control DMSO	0.0	0.0
2.	Standard Amphotericin B (Antifungal agent)	18	19
3.	Ligand(**H_3L**)	12	14
4.	$[Cr(C_5H_5N_3O_3S)Cl(NH_3)OH_2].EtOH$	13	12
5.	$[La(C_5H_5N_3O_3S)Cl(OH_2)_2].EtOH.H_2O$	0.0	0.0

Table (6): Bacterial activity of the ligand (H_3L) and its complexes and the standard antibacterial (Tetracycline) on the tested G^- and G^+ bacteria

NO.	(mm/mg s)	Inhibition zone diameter	
	Sample	Escherichia *coli* (G^-)	Staphylococcus *aureus* (G^+)
1.	Control ; DMSO	0.0	0.0
2.	Standard; Tetracycline	32	30
3.	Ligand(**H_3L**)	15	16
4.	$[Cr(C_5H_5N_3O_3S)Cl(NH_3)(OH_2)].EtOH$	10	10
5.	$[La(C_5H_5N_3O_3S)Cl(OH_2)].EtOH.H_2O$	0.0	0.0

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مخلص الدراسة

تم تحضير المركب N– ثيويوريدو حمض الماليك ومعقداته مع كلٍ من الكروم (III) واللانثانيوم (III) وأجريت تجارب معملية لتحقق من التركيب عن طريق التحليل العنصري لتحديد نسبة العناصر (C, N, H, S) في كل مركب ، وباستخدام طيف الأشعة تحت الحمراء والرنين النووي المغنطيسي وطيف الكتلة وقياسات الحساسية المغنطيسية. أثبت التحليل بالأشعة تحت الحمراء أن مشتق الثوسمي كاريازايد يتناسق مع الأيونات الفلزية كلاقط ثلاث السن عبر ذرات النتروجين والكبريت والأوكسجين ، وأوضحت نتائج دراسة الثبات الحراري بأن المعقدات تحت الدراسة ثابتة حرارياً حتي والي 200^{0} م ، وأن أول خطوة في التحليل الوزني الحراري هي فقدان جزيئات ماء وكحول ثم تفكك بقية المركب لاحقاً. تم اختبار فعالية اللاقط الحر ومركباته ضد عدد من الأحياء الدقيقة.