Synthesis, Characterization and Biological activity of 2-bis (1H imidazole-2-yl) methylene) thiosemicarbazone and its divalent transition metal complexes

By

A.Gobara

University of dalanj, faculty of education, Sudan

Musa A.Ahmed

University of Gezira Medani, Sudan

Doi: 10.33850/ejev.2020.73466

استلام البحث : ١٨ / ٢ /٢٠٢٠ قبول النشر: ٢ / ٣ / ٢٠٢٠

Abstract

2-bis(imidazole-2-yl)methylene) thiosemicarbazone and its complexes with Pd (II), Cu (II), Hg (II), and Cd (II),ions have been prepared and characterized by elemental analysis, molar conductivity, spectroscopy (IR, uv. VIS, 1H NMR, mass spectrometry) and magnetic moment. The thiosemicarbazone coordinates to the metal ions via sulfur and azomethine nitrogen atom. The free ligand and its metal complexes have been tested in vitro against a number of microorganisms, to assess their antimicrobial properties. Antibacterial screening of the ligand and its complexes was conducted against *Streptococcus pneumonia* (G+), *Bacillis subtilis* (G+), *Pseudomonas aeruginosa* (G-), Escherichia coli (G-), also antifungal activity was examined against *Aspergillus fumigates* and *Candida albicans*. The experimental results show that nearly some complexes exhibit antibacterial activity highest than the free ligand but less than the standard.

Keywords: Antimicrobial, diffusion method, Potato dextrose agar.

INTRODUCTION:

Thiosemicarbazones are compounds that have been studied for a considerable period of time for their biological properties. Traces of interest date back to the beginning of the 20th century but the first reports on their medical applications began to appear in the fifties as drugs against tuberculosis (Bavin et al, 1950), and leprosy (Koch and stuttom ,1950). In the sixties the antiviral properties were discovered (Kune .1964) and a huge amount of research was carried out that eventually led to the commercialization of thiosemisazone. Recently Triapine 3-aminopyridine-2-carboxaldehyde thiosemicarbazone has been developed as an anticancer drug (Nutting, et al, 2009 and Goh et al, 2008). Presently, the areas in which thiosemicarbazones are receiving more attention can be classified according to their antitumor (Blanz ; 1968*)*, antifungal (Mitral et al, 1981), antibacterial (Dobek et al, 1980) and antiviral activities (Pirrung et al, 2005), and in all cases their action has been shown to involve interaction with metal ions (Finch et al., 1999).

Transition metal complexes of thiosemicarbazones are widely studied, especially because of their chemical and biological properties. According to the transition metal and the parent aldehyde or ketone associated to the thiosemicarbazide moiety, the thiosemicarbazone complexes are developed for analytical chemistry (Sarma et al., 2005), can be engaged in catalysis or pre-catalysis,(Pandiarajan et al., 2013) in addition to their biological properties. All these peculiar properties prompt many authors to study transition metal complexes of thiosemicarbazone in order to understand the relationship between these activities and their molecular structures. New imidazole derived thiosemicarbazones were prepared by condensation of 4(5)-imidazole carboxaldehyde, 4-(1H-imidazole-1-yl) benzaldehyde and 4-(1H-imidazole-1-yl) acetophenone with a thiosemicarbazide. All compounds were characterized by quantitative elemental analysis, IR and NMR techniques. The structures were determined by single crystal X-ray diffraction. The

antifungal activities of the compounds were evaluated. None of the compounds exhibited significant activity against Aspergillus flavus and Candida albicans, while 4(5)-imidazolecarboxaldehyde thiosemicarbazone) and4-(1H-imidazole-1-yl) benzaldehyde thiosemicabazone were highly and selectively active against Cladosporium cladosporioides. In many cases, activity was superior to that of the reference compound nystatin (Débora C. Reis et al. 2013).

Material and Methods

Unless otherwise stated all metal chlorides are of analytical (AR) grade and used without further purification. All chemicals, reagents and solvents were of the analytical grade (AR). Thiosemicarbzide 98%(Pareac, EU), 1H imdazole-2-carboaldahyde (Sigma, USA). $PdCl_2$ provided by Johnson and Matthey chemicals limited; $HgCl_2$ provided by CDH, extra pure. Magnetic susceptibility measurements on powder samples were carried out on a Johnson Mathey magnetic moment balance. Elemental analysis were carried out in the micro analytical unit at Cairo University, using chemical analyzer Carlo – Erba model 1106. Uv-Vis. spectra were carried out using Shimadzu models 3101 PC and 1800 spectrophotometers. Infrared spectra of solid samples were recorded on a Perkin-Elmer and Shimadzu model FT-IR 8400 S, spectrophotometers.

Preparation of the ligand

0.0911g (0.001mol) of thiosemicarbazide was dissolved in 20 ml of absolute ethanol, then heated until the dissolution was complete, to this solution was added 0.192 g (0.002 mol) of ^{1}H imdazole -2-carboxaldehyde dissolved in 20 ml hot absolute ethanol, followed by addition of a few drops of concentrated HCl. The mixture was refluxed on a water bath for two hours, and left to stand at room temperature overnight. The yellow powder precipitate was filtered, washed with ethanol and left to dry in air. The product was

recrystallized from ethanol. The yield obtained was 89.13%, melting point is 209 ^{0}C.

Preparation of the metals complexes

The metal complexes were all prepared by the same procedure. To a stirred solution containing 0.494g (0.002mol) of 2-bis (^{1}H imidazole-2-yl)methylene) thiosemicarbazone (dissolved in 20 ml of hot absolute ethanol), a solution containing the appropriate weight of the metal chloride equivalent to (0.001mol) in 20 ml hot absolute ethanol was added while stirring. The mixture was refluxed for 1 hour followed by addition of few drops of NaOH (1M). The solid precipitate was collected by filtration, washed with ethanol and left to dry under shadow.

Biological Activity

Antifungal activity of the synthesized ligand (HL) and its complexes in term of their inhibition to the linear growth of *Aspergillus flavus*, and *Candida albicans* was investigated. Potato dextrose agar (PDA) was used to evaluate the effect of the compounds under investigation on the mycelia linear growth of the two tested fungi. Fifty milliliters of the medium were poured into 150 ml conical flasks and autoclaved at 121° C for 20 minutes. Three drops of 25% lactic acid were added to prevent bacterial contamination. Dilutions of each of the tested compounds were carried out (v/v) by dissolving appropriate amounts of each compound in 10 ml DMSO. Equal volumes of DMSO containing diluted compounds were added to sterile molten (40C) PDA to get a series of different concentrations for each compound in PDA. A zero concentration treatment was prepared for each fungus which contains equivalent volume of DMSO only and used as control. Compounds amended PDA was dispensed aseptically into 9 centimeter Petri dishes. Plugs of mycelium were cut from the margins of actively grow thing cultures of the fungi and placed in the center of compound-amended and amended PDA plates with four replicate plates for each fungus. All plates were incubated at 25C. Colony

diameter in (mm) was measured after three days and the inhibition zone was calculated for each compound. The growth inhibition percentage diameter of the fungal colony using the equation (C-T)x 100/C, where C is the diameter of the fungus colony in the control plate after three days and T is the diameter of the fungus colony in the tested plates after the same period of time. The antibacterial activity of the ligand and its complexes were tested using diffusion method against *Staphylococcus aureus* as gram positive bacteria and *Esherichia coli* as gram negative bacteria. Nutrient agar (NA) medium was used. The test compounds were dissolved in DMSO. 25 ml of nutrient agar (NA) were placed in Petri plates. After solidification, the test bacteria was spread over the medium using a spreader. Discs of What-mann no. 1 filter paper saturated with the test compounds were placed at four equidistant places from the center in the incubated Petri plates. Filter paper discs treated with DMSO served as control and Tetracycline was used as standard drug. The Petri dishes were kept in a refrigerator for 24 hours for pre-diffusion and then incubated for 72 hours at 38 °C and the inhibition zone around each disc was measured. The zone of inhibition was carefully calculated in millimeters.

Results and discussion

The analytical data of the ligand and complexes are in agreement with the empirical formulae shown in Table1. The results obtained indicate the formation of one type namely the 1:2 metal: ligand species. The molar conductivities (in micro semen) in carbon tetrachloride (Table1) at room temperature showed them to be non-electrolytes. Figure 1 shows the postulated structure of the thiosemicarbazone ligand.

Table 1: Analytical data of HL^2 and its metal Complexes.

compound	Color	Elemental analysis (found) and calculated (%) (to compare the results)			
		C	N	H	S
HL^2	Yellow	43.7 (43.2)	39.65 (38.3)	3.67 (4.12)	12.9 (11.8)
$[Cu(C_9H_9N_7S)_2]Cl_2, 2H_2O$	Green	34.37 (34.7)	31.18 (30.12)	2.88 (2.22)	10.19 (9.43)
$[Pd (C_9H_8N_7S)2]$	Reddish yellow	23.02 (23.4)	23.2 (22.6)	2.7 (2.14)	12.29 (12.3)
$[Cd(C_9H_9N_7S)_2Cl_2]$	Gray	31.83 (32.2)	28.93 (26.02)	2.68 (3.75)	9.46 (7.06)
$[Hg(C_9H_9N_7S)_2Cl_2]$	White	28.22 (28.81)	25.6 (25.09)	2.37 (2.12)	8.37 (8.03)

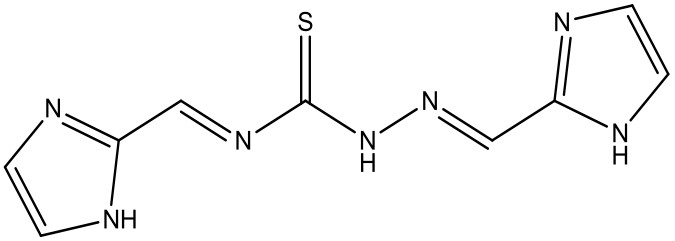

Fig.1 the postulated structure of the thiosemicarbazone ligand

1H NMR spectra

For free ligand 2-bis ^1H imidazole-2-yl methylene thiosemicarbazone the absorption band at 1527 cm^{-1} is assigned to ν (C=N), the band at 848 cm^{-1} is assigned to ν (C=S), and the band at 3163 is assigned to ν (NH).

Table (2): 1HNMR spectral data (ppm) of the ligand (HL)

ligand	ν(NH)	ν (C=N)	ν (C=S)
HL^2	3163	1527	848

Vibration Spectra

The I.R spectral data of the ligand HL2 and its complexes are shown in Table (3).

Table (3): IR spectral data (4000-400cm^{-1}) of the ligand HL^2 and its complexes.

compound	ν (C=N)	ν (NH)	ν (C=S)
HL^2	1527	3163	798
$[Cu(C_9H_9N_7S)_2Cl_2.2H_2O$	1531	3151	821
$[Pd\ (C_9H_8N_7S)_2]$	1535	--	829
$[Cd\ (C_9H_9N_7S)_2Cl_2]$	1523	3155	817
$[Hg(C_9H_9N_7S)_2]Cl_2$	1512	3154	829

The Infra-red spectral data of Co (II) with the ligand 2-bis (1H imidazole-2-yl) methylene) thiosemicarbazone HL^2, the band at 1527 cm^{-1} assigned to ν (C=N) is shifted to 1531 cm^{-1}, indicating coordination of the ligand through the azomethine nitrogen. The band assigned to ν (C=S) is shifted from 848 cm^{-1} to 821 cm^{-1} indicating the involvement of sulfur in complexation. The band at 1527 cm^{-1} assigned to ν(C=N) in the free ligand 2-bis (1H imidazole-2-yl) methylene) thiosemicarbazone (HL^2), is shifted to a higher frequency. in the complex $[Pd\ (C_9H_8N_7S)_2]$ namely to 1535 cm^{-1}. This indicates coordination of the ligand to the metal ion through azomethine nitrogen. The absorption band at 3163 cm^{-1} assigned to ν(NH) is disappeared in the complex spectrum. This may be due to deprotonation of the thiol sulfur atom. The band at 848 cm^{-1} assigned to ν (C=S) in the free ligand is shifted to 829 cm^{-1} this illustrates coordination of Pd (II) ion with sulfur (Manimaran a, 2012). In the complex $[Cd(C_9H_9N_7S)_2Cl_2)]$, the band at 1527 cm^{-1} assigned to ν (C=N) is shifted to 1523 cm^{-1}, indicating coordination of the ligand through the azomethine nitrogen. The band assigned to ν (C=S) is shifted from 848 cm^{-1} to 817 cm^{-1} indicating the involvement of sulfur atom in complexation. In the complex of Hg(II) with the ligand HL^2, $[Hg\ (C_9H_9N_7S)_2\ Cl_2]$, the band at 1527 cm^{-1} assigned to ν (C=N) is shifted to 1512 cm^{-1}, indicating coordination of the ligand through the azomethine nitrogen. The band assigned to ν (C=S) is shifted from 848 cm^{-1} to 829 cm^{-1} indicating the involvement of sulfur atom in complexation.

Table (4) UV-vis spectral (nm) of band, Solubility, molar Conductivity and magnetic moment of complexes of the ligands HL^2

Complex	Electronic spectra (nm)	Molar Conductivity $Ohm^{-1}cm^2mol^{-1}$	Mag.Momet μ (B.M)
$[Cu(C_9H_9N_7S)Cl_2]$ $2H_2O$	217, 255, 458, 543	2.77	1.86
$[Pd\ (C_9H_8N_7S)_2)]$	269, 341	0.00	diamagnetic
$[Cd(C_9H_9N_7S)_2]$	276, 341	0.00	diamagnetic
$[Hg(C_9H_9N_7S)_2\ Cl_2]$	271, 316	0.00	diamagnetic

The observed magnetic moment value of the complex is 1.80 B.M. The $[Cu\ (C_9H_9N_7S)_2]Cl_2$ complex shows bands at 46082 cm^{-1} (217 nm),28169 cm^{-1} (255 nm) and 13850 cm^{-1} (534 nm). The observed magnetic moment is 1.86 BM, this suggest distorted square planar geometry of the copper center.(Amna Qasem Ali et al ; 2014). the complex Pd $(C_9H_8N_7S)_2$ exhibits bands at 3717 cm^{-1} (269 nm), and 29325 cm^{-1} (341nm) this bands 3717 cm^{-1} (269 nm), and 29325 cm^{-1} (341nm) may be due to LMCT from ligand to Pd (HL^2) ion. for Cd $(C_9H_9N_7S)_2Cl_2)$ are attributable to the $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ respectively (Mohammad et al, 2003). On the basis of analytical, conductance and spectral data octahedral geometry is suggested (Mohammad et al, 2003). The Complex $[Hg\ (C_9H_9N_7S)_2\ Cl_2]$ show bands 37174 cm^{-1} (276 nm), and 29325 cm^{-1} (316 nm).

Results of the microbial studies

The antimicrobial screening data for the ligand and its complexes are shown in Tables (5) and (6).The ligand and its complexes exhibit higher activity against bacteria than fungi. The experimental results show that nearly all complexes exhibit antibacterial activity higher than the free ligand, but less than the standard. This fact can be understood in terms of the chelation theory which states that upon complexation the polarity of the metal ion is

reduced which increase the lipophilicity of the metal complex enabling them to cross the cell membrane easily (Mohamed G, 2009). The free ligand was active against *Aspergillus fumigates*, but less than the standard drug, but its complexes activity showed very least antifungal activity, especially those of Hg(II), Cu(II),Cd(II). The complexation with Pd (II) showed highest activity nears to that the standard drug.

Table (5). Antiacterial activity of the ligand (HL) and its complexes and the standard antibacterial on the tested G^- (*Gentamicin)* and G^+ (*Ampicillin)* bacteria.

	Zone of inhibition in diameter using (1mg/ml of sample)			
Sample	Gram positive bacteria		Gram negative bacteria	
	S.Pneumoniae	*B. subtilis*	*P.aeruginosa*	*E.Coli*
(mg/ml)	Mean	Mean	Mean	Mean
Ampicillin (Positive)	23.8	32.4	----	----
Gentamicin (negative)	----	----	22.6	27.3
HL^2	15.9	20.3	17.5	21.8
$[Cu(C_9H_9N_7S)_2Cl_2]\ 2H_2O$	20.6	27.2	18.7	22.5
$[Pd\ (C_9H_8N_7S)_2]$	22.6	19.4	14.5	16.7
$[Cd(C_9H_9N_7S)_2]$	21.9	12.8	17.3	22.6
$[Hg(C_9H_9N_7S)_2]$	13.6	21.2	19.4	25.8

Table (6): Antifungal screening data of the ligand (HL) and its complexes.

	Fungal inhibition zone % (conc. In mg/ml)	
Sample	*Aspergillus fumigatus*	*Candida albicans*
Control DMSO	0.0	0.0
Amphotericin B	23.7	25.4
HL^2	19.5	15.2
$[Cu(C_9H_9N_7S)Cl_2]2H_2O$	12.0	17.7
$[Pd\ (C_9H_8N_7S)Cl_2)]$	16.3	22.8

$[Cd(C_9H_9N_7S)_2]$	14.6	18.2
$[Hg(C_9H_9N_7S)_2]$	21.8	19.3

REFERENCES

Amna Qasem Ali , Siang Guan Teoh ,Naser Eltaher Eltayeb , Mohamed B. Khadeer Ahamed , A.M.S. Abdul Majid,(2014). Synthesis of copper(II) complexes of isatin thiosemicarbazone derivatives: In vitro anti-cancer, DNA binding, and cleavage activities, Polyhedron 74 - 6–15

Bavin EM, Rees RJW, Robson JM, Seiler M, Seymour DE, Suddaby D.(1950), The tuberculostatic activity of some thiosemicarbazones. J Pharm Pharmacol 2: 764-72.

Blanz Jr., F. French,(***1968)*** *Cancer Res.*

Dobek, D. 1. Klavrnan, E.J. Dickson Jr., J. P. Scovill, E. C. Trarnont,

Finch RA, Liu MC, Cory AH, Cory JG, Sartorelli AC (1999), Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone; 3-AP): an inhibitor of ribonucleotide reductase with antineoplastic activity. Adv Enzyme Regul 39: 3-12

Koch O, Stuttgen G.(1950),Clinical and experimental studies on the effects of thiosemicarbazones. Naunyn Schmiedebergs Arch Exp Pathol Pharmakol Vol 210: **409-23.**

Kune GA. (1964).To-day's drugs: methisazone. Br Med J 2: 621

Mitral, Sharma, Singh, J. P. Tandon,(1981). *Curr.* Sci, 50, and the references therein

Mohamed G., Ibrahim N.A. and Attia H.A. (2009). Synthesis an antifungicidal activity of some transition metal complexes with benzimidazole dithiocarbamate ligand. Spectrochimica Actra part A 72:610-615.

Mohammad Akbar Ali a, Aminul Huq Mirza a, Mohammad Nazimuddin b, Raju Ahmed b, Lawrence R. Gahan c, Paul V. Bernhardt (2003). Synthesis and characterization of mono-

and bis-ligand zinc(II) and cadmium(II) complexes of the di-2-pyridylketone Schiff base of Sbenzyl dithiocarbazate (Hdpksbz) and the X-ray crystal structures of the[Zn(dpksbz)2] and [Cd(dpksbz)NCS]2 complexes. Polyhedron 22 pp 1471-1479

Nutting CM, van Herpen CML, Miah AB, (2009). Phase II study of 3-AP Triapine in patients with recurrent or metastatic head and neck squamous cell carcinoma. Ann Oncol 20: 1275-9

Pandiarajan, Ramesh, Liu, R. Suresh, (2013), Inorg. Chem. Commun. pp:33 -33

Pirrung, M.C.;Pansare, S.V.; Sarma, K.D.; Keith, K.A.; Kern, E.R (2005). Combinatorial optimization of isatin-*β*-thiosemicarbazones as anti-poxvirus agents. J. Med. Chem, 48, 3045-3050

Sarma, Kumar, Reddy, Reddy, (2005) , J. Agric. Food Chem. 53 - 5492

Suni V, M.R. Prathapachandra Kurup, Munirathinam Nethaji, (2007). Studies on Co(II) and Co(III) complexes of di-2-pyridyl ketone N(4)-cyclohexyl and N(4)-phenyl thiosemicarbazones. Polyhedron 26 - 5203–5209

Goh BC, Tan EH, *et al*. A (2008),multicenter phase II trial of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, Triapine) and gemcitabine in advanced non-small-cell lung cancer with pharmacokinetic evaluation using peripheral blood mononuclear cells. Invest New Drugs 26: 169-73

Gehad G. .; A. Ibrahimb.; A.E. Attia .; (2009) .Synthesis and anti-fungicidal activity of some transition metal complexes with benzimidazole dithiocarbamate ligand, Spectrochimica Acta Part A **72** -610–615..

Musa A.ELrahman,(2012). Synthesis, characterization and biological activity of some N, O and S donor ligands and their metal ion complexes. Unpublished Ph,D Thesis. University of Gezira

Mihail Revenco , Petru Bulmaga , Elena Jora , Oleg Palamarciuc , Victor Kravtsov ,Paulina Bourosh,(2014). Specificity of salicylaldehyde S-alkylisothiosemicarbazones coordination in palladium(II) complexes Polyhedron 80 -250–255

El-Asmy A.A. and G.A.A .Al-Hazmi,(2008). Synthesis and spectral feature of benzophenone-substituted thiosemicarbazones and their Ni(II) and Cu(II) complexes. Spectrochimica Acta Part A: Mol.Biomol.spectros 1-6

Débora C. Reis, Angel A. Recio Despaigne, Jeferson G. Da Silva, Nayane F. Silva, Camila F. Vilela,Isolda C. Mendes, Jacqueline A. Takahashi and Heloisa Beraldo,(2013). Structural Studies and Investigation on the Activity of Imidazole-Derived Thiosemicarbazones and Hydrazones against Crop-Related Fungi, *Molecules*, *18*, 12645-12662; doi:10.3390/molecules181012645