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Synthesis, characterization, and antimicrobial activity of 4-imidazolecarboxaldehyde thiosemicarbazone and its Pt(II) and Pd(II) complexes

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ABSTRACT

Schiff bases are versatile ligands, synthesized via condensation of primary amines with carbonyl compounds. In this study, equimolar amounts of 4-imidazolecarboxaldehyde and thiosemicarbazide were combined and the Schiff base 4-imidazolecarboxaldehyde thiosemicarbazone was prepared as a new bidentate complexing agent. The synthesized ligand was reacted with palladium (II) and platinum (II) ions yielding air-stable complexes. For characterization purpose, infrared spectra, mass spectra, electronic spectra, thermal analysis, proton nuclear magnetic resonance and ¹³C-carbon nuclear magnetic resonance spectra studies were carried out on the obtained complexes and ligand. The characterization data showed that the ligand acts as a bidentate coordinate to the metal ions through azomethine nitrogen and sulfur atoms. An *in vitro* antimicrobial investigation was also carried out for the free ligand and its metal complexes against four bacteria; *Bacillus cereus*, *Staphylococcus aureus* (Gram-positive), *Escherichia coli* and *Salmonella typhimurium* (Gram-negative) and one Fungi; *Candida albicans*, to assess their antimicrobial properties by disc diffusion technique. Antimicrobial activity of the prepared complexes showed higher activity than the free ligand.

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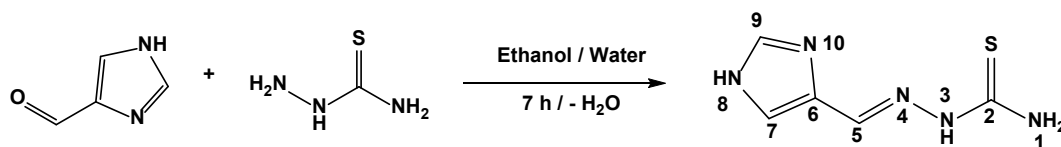
1. Introduction

Thiosemicarbazones, an important class of synthetic compounds, have a variety of applications due to their wide spectrum of biological activities which include antifungal, antibacterial, anti-inflammatory, antimalarial antiviral, antitumoral, anticancer and among others as well as parasitocidal activity [1-12]. Thiosemicarbazones are very active ligands, in particular, that contain an imidazole moiety which is known to play an important role in biological systems as a part of the histidine residue in peptides and proteins and it has been shown that their biological activities are related to their ability to coordinate to metal centers in enzymes [13-15]. There are some research groups work about imidazole thiosemicarbazone derivatives and their metal complexes such as West *et al.*, Casas *et al.* and Rodriguez-Arguelles *et al.* [16-18]. West *et al.* have reported the synthesis of the substituted imidazole-2-carbaldehydethiosemicarbazone derivatives and some of their

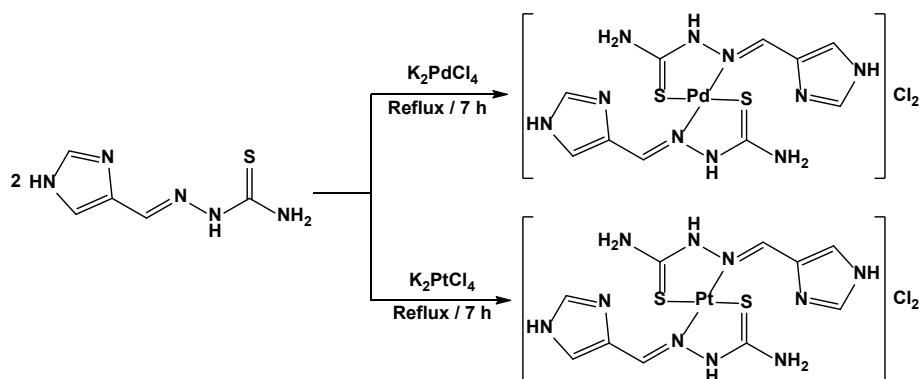
copper(II) complexes [16]. Casas *et al.* synthesized an imidazole-2-carbaldehydethiosemicarbazone ligand and some of its diorganotin (IV) complexes [17]. Rodriguez-Arguelles *et al.* also prepared cobalt (II) and nickel (II) complexes from 2-carbaldehyde thiosemicarbazone ligands and compared their coordinative behavior besides their antimicrobial activities [18]. In this paper, the preparation and characterization of 4-imidazolecarboxaldehyde thiosemicarbazone ligand and its metal complexes were described. In addition, *in vitro* antimicrobial investigation was also carried out for the ligand and its metal complexes against four bacteria; *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium* and one Fungi; *Candida albicans*.

2. Experimental

2.1. Materials and measurements



Scheme 1. Preparation of 4-imidazolecarboxaldehyde thiosemicarbazone (4-ITSC).



Scheme 2. Preparation of Pd(II) and Pt(II) complexes of 4-ITSC.

All chemicals and solvents of the highest analytical grade were used as received from Sigma-Aldrich and Alfa-Aesar. The melting points of the synthesized compounds were determined by a capillary method in a Thomas Hoover apparatus. Mass spectrum of the ligand was carried out on Esquire LC-00084 electronic spray ionization (ESI) Mass spectrometer. Infrared spectra of the ligand and its metal complexes were recorded on Vertex-183387000 FT-IR spectrometer by using KBr disk in the range 4000-400 cm^{-1} . ^1H and ^{13}C NMR spectra of the ligand and its metal complexes were recorded on a Bruker AV-III 600 operating at 600 MHz for ^1H and 150 MHz for ^{13}C by using $\text{DMSO}-d_6$ as a solvent. UV-Vis spectra in solid state were recorded on a Cary-EL05123055 4000 UV-Vis spectrophotometer in the range 200-800 nm. Thermal analysis was carried out using DTA/TG HIGH RG 2/S thermal analyser. All measurements were carried out at technical University of Dresden, Germany.

2.2. Synthesis of 4-imidazolecarboxaldehyde thiosemicarbazone (4-ITSC)

Equimolar amounts of thiosemicarbazide (910 mg, 0.001 mol) and 4-imidazole carboxaldehyde (960 mg, 0.001 mol) were dissolved in 20 mL of ethanol:water (60:40, v:v) and refluxed for 7 h in an oil bath at 78 °C [18-21]. The solution was allowed to cool at room temperature and left for slow solvent evaporation. After several days pale yellow crystals were obtained. The crystals were separated, washed with cold ethanol, and dried under vacuum (Scheme 1).

4-Imidazolecarboxaldehyde thiosemicarbazone: Color: Pale yellow. Yield: 72%. M.p.: 205-207 °C. FT-IR (KBr, ν , cm^{-1}): 3397, 3202 (NH_2), 3011 (NH), 1606 (C=N), 1103 (N-N), 851 (C=S). ^1H NMR (600 MHz, $\text{DMSO}-d_6$, δ , ppm): 12.4 (s, 1H, HN8), 8.2 (s, 1H, HC5), 7.3 (s, 2H, HN1), 8.3 (s, 1H, HN3), 8.0 (s, 1H, HC7), 7.7 (s, 1H, HC9). ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$, ppm): 177.88 (C2), 135.54 (C6), 136.60 (C5), 132.13 (C9), 121.60 (C7). Ms (ESI, m/z): 170.0 [M, $\text{C}_5\text{H}_7\text{N}_5\text{S}$] $^+$. UV-Vis (λ_{max} , nm): 313.

2.3. Synthesis of complexes

2.3.1. Synthesis of $[\text{Pd}(4\text{-ITSC})\text{Cl}_2]$

K_2PdCl_4 (16.32 mg, 5.0×10^{-5} mol) was dissolved in hot ethanol and then mixed with a hot ethanolic solution containing 16.9 mg (1.0×10^{-4} mol) of 4-ITSC. The mixture was refluxed for 7 h at 78 °C in an oil bath and then allowed to cool at room temperature and left for slow solvent evaporation for several days. The colored precipitate was filtered off, washed with cold ethanol, and dried in air and vacuum oven (Scheme 2) [18,21]. Color: Orange. Yield: 77%. M.p: 220-222 °C. FT-IR (KBr, ν , cm^{-1}): 3400, 3298 (NH_2), 3115 (NH), 1613 (C=N), 816 (C=S), 1116 (N-N). ^1H NMR (600 MHz, $\text{DMSO}-d_6$, δ , ppm): 11.8 (s, 2H, HN8), 8.1 (s, 2H, HC5), 7.4 (s, 4H, HN1), 8.5 (s, 2H, HN3), 8.4 (s, 2H, HC7), 7.8 (s, 2H, HC9). ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$, δ , ppm): 179.21 (C2), 135.97 (C6), 141.13 (C5), 129.21 (C9), 120.58 (C7). UV-Vis (λ_{max} , nm): 226, 313, 376.

2.3.2. Synthesis of $[\text{Pt}(4\text{-ITSC})_2]\text{Cl}_2$

K_2PtCl_4 (20.75 mg, 5.0×10^{-5} mol) of was dissolved in hot ethanol and then mixed with a hot ethanolic solution containing 16.9 mg (1.0×10^{-4} mol) of 4-ITSC. The mixture was refluxed for 7 h at 78 °C in an oil bath and then allowed to cool at room temperature and left for slow solvent evaporation for several days. The colored precipitate was obtained, filtered off, washed with cold ethanol, and dried in air and vacuum oven (Scheme 2) [18,21]. Color: Orange. Yield: 82%. M.p: 230-232 °C. FT-IR (KBr, ν , cm^{-1}): 3390, 3303 (NH_2), 3098 (NH), 1618 (C=N), 853 (C=S), 1082 (N-N). ^1H NMR (600 MHz, $\text{DMSO}-d_6$, δ , ppm): 11.9 (s, 2H, HN8), 8.2 (s, 2H, HC5), 7.2 (s, 4H, HN1), 8.6 (s, 2H, HN3), 8.3 (s, 2H, HC7), 7.9 (s, 2H, HC9). ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$, δ , ppm): 178.21 (C2), 135.82 (C6), 142.68 (C5), 129.06 (C9), 120.70 (C7). UV-Vis (λ_{max} , nm): 210, 226, 265.

2.4. Antimicrobial activity

2.4.1. Antifungal screening

Preliminary antifungal screenings of the prepared compounds at different concentrations were performed. Potato dextrose agar medium was prepared by using potato, dextrose, agar-agar and distilled water. Appropriate amount of the compounds in DMSO was added to potato dextrose agar medium in order to get a concentration of 100 and 200 $\mu\text{g}/\text{mL}$ of compound in the medium.

Table 1. Thermal analysis of the synthesized Pd (II) and Pt (II) complexes.

Complex	TG (°C)	DTA (°C) *	Total mass loss (%) *	Metallic residue (%) *
Pd(4-ITSC) ₂ Cl ₂	30-1050	220 (-), 312 (-)	80.35 (79.37)	Pd 19.65 (20.63)
Pt(4-ITSC) ₂ Cl ₂	30-1050	220 (-), 609 (-)	70.27 (67.72)	Pt 29.73 (32.28)

* DTA: (-) Exothermic; Mass loss: Found (Calculated).

The medium was poured into a set of two Petri plates under aseptic conditions in a laminar flow hood. When the medium in the plates was solidified, a mycelial disc of 0.5 cm in diameter was cut from the periphery of the seven days old culture and it was aseptically inoculated upside down in the center of the Petri plates. These treated Petri plates were incubated at 26±1 °C until fungal growth in the control Petri plates was almost complete. The mycelial growth of fungi (mm) in each Petri plate was measured [22].

2.4.2. Antibacterial screening

The paper disc diffusion method was used to screen the antibacterial activity of the prepared compounds and performed by using Mueller Hinton agar (MHA). The ligand and its complexes were carried out according to the National Committee for Clinical Laboratory Standards Guidelines. Bacterial suspension was diluted with sterile physiological solution to 108 cfu/mL (Turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on the surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No. 1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µL of a solution of each sample. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured [23].

3. Results and discussion

3.1. Synthesis

The synthesis of ligand containing an imidazole ring is outlined in Scheme 1. The corresponding palladium (II) and platinum (II) complexes were obtained in two steps. In the first step, the ligand was synthesized by condensing equimolar quantities of thiosemicarbazide with 4-imidazole carboxaldehyde in ethanol: water mixture. In the second step, the prepared ligand was refluxed with metal salt in 1:2 M ratio, to obtain the desired complexes. The obtained compounds were characterized by UV-Vis, FTIR, ¹H NMR, ¹³C NMR, Mass spectrometry, and thermogravimetric analyses. Some physical characteristics of the ligand and its corresponding metal complexes are given in experimental section.

The infrared absorption bands become very useful for determining the mode of coordination of the ligands to metal. In the IR spectra, the absorption peaks at 3397 and 3202 cm⁻¹ are assigned to ν(NH₂). The absorption peak at 3011 cm⁻¹ is assigned to ν(N-H). In both complexes, the presence of a band in this region (3115 and 3098 cm⁻¹) corresponds to NH vibration, which indicates that the ligand is coordinated in the neutral form. The strong band observed at 1606 cm⁻¹ in the free ligand has been assigned to ν(C=N) stretching vibrations [18,24,25]. On complexation, this bands were observed to be shifted to higher frequencies (1613-1618 cm⁻¹) [18,24,25]. These results indicate that the imine nitrogen is coordinated to the metal ion. The ligand showed a medium band at 851 cm⁻¹ ascribed to ν(C=S) vibrations. These absorption bands shift to lower and higher frequencies on the coordination of the thiocarbonyl sulfur to palladium (II) or platinum (II) ions. These results agree with other thiosemicarbazone complexes. In addition, the vibrational frequencies of the NH₂ groups remain unchanged for the ligand and metal complexes. This evidence

indicates the noncoordination of the NH₂ group on the metal ion [10,18,26,27].

The electronic spectra of 4-ITSC showed that a strong absorption band at 313 nm. This band assigned to the π → π* transition of the azomethine group [24,25]. The intra-ligand transitions for Pd (II) complex were observed 226, 313, 376 nm and for Pt (II) complex was observed in 210, 226, 265 nm and these bands are mainly due to π → π* and n → π* transitions [24,25].

The ¹H and ¹³C NMR spectra and chemical shift values of the ligand and corresponding metal complexes were recorded in DMSO-*d*₆ solvent. The ¹H NMR of 4-ITSC ligand and its metal complexes show signal at δ 8.3, 8.5, and 8.6 ppm have been assigned to δ(NHCS) protons and the signals at δ 7.3, 7.4 and 7.2 ppm have been assigned to δ(CSNH₂) protons. The signals at δ 8.2, 8.1, and 8.2 ppm assignable to azomethine protons (CH=N). The downfield chemical shift in the spectra of Pd (II) and Pt (II) complexes indicated the coordination through the azomethine nitrogen to the metal atom resulting in the formation of a coordinate N→M linkage and all imidazole ring protons were observed in the expected regions [18,24-28].

The ¹³C NMR spectra revealed the presence of an expected number of signals corresponding to different types of carbon atoms present in the compounds. The spectra of the Schiff base ligand exhibit a strong band at δ 177.88 ppm due to C=S group. In the complex formation, the position of this band undergoes an up-field shift [24-28] to δ 179.21 and 178.21 ppm. This indicates that sulphur is involved in coordination (S→M linkage). These results in agreement with a previously published paper [24-27].

Generally, not much is known about the thermal properties of transition metal complexes of imidazolecarboxaldehyde thiosemicarbazones. The thermal behavior of the palladium (II) and platinum (II) complexes of the synthesized imidazolecarboxaldehyde thiosemicarbazones were studied under argon atmosphere using thermal analyzer (DTA/TG) and the results shown in Table 1. The thermal decomposition of the complexes was recorded from ambient temperature to 1050 °C. The results showed that the complexes generally decomposed in several thermal events (Decomposition steps). The complexes lost moisture around 100 °C, and then started to decompose at a temperature above this limit. The total weight loss around 1050 °C is nearly 70-80% and this mass loss equals the loss of two moles of 4-ITSC and chloride ligands in agreement with the proposed metal:ligand ratio of 1:2 of the complex [24]. The remaining weights correspond to the metallic residue as shown in Table 1. The coordinative mode of compounds studied here becomes plain in palladium (II) and platinum (II) complexes of 4-imidazolecarboxaldehyde thiosemicarbazone in which the ligand behaves as NS donor giving square planar geometry.

3.2. Antimicrobial activity

The synthesized compounds were screened *in vitro* for their antibacterial activity against four pathogenic bacteria; *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* and one fungus; *Candida albicans* at a concentration of 100 and 200 µg/mL with DMSO as the solvent. The results showed that the tested compounds possess moderate antimicrobial activity against most of the tested organisms, as shown in Table 2.

Table 2. Antimicrobial activity of synthesized thiosemicarbazone and its complexes.

Compound	Concentration ($\mu\text{g/mL}$)	Diameter of inhibition zone (mm)				
		Gram+ve bacteria		Gram-ve bacteria		Fungi
		<i>B. cereus</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>C. Albicans</i>
4-ITSC	100	10	07	07	06	00
	200	10	11	12	10	09
Pd(4-ITSC) ₂ Cl ₂	100	11	09	16	11	09
	200	12	12	19	14	10
Pt(4-ITSC) ₂ Cl ₂	100	11	11	11	11	10
	200	12	12	11	13	11
Gentamicin	200	20	14	17	16	-

In similar previous studies, the evaluation of antibacterial activity of the synthesized compounds exhibit a moderate inhibitory effect on the microbial proliferation and only against some Gram-positive bacteria [18,20,21,23,25,28]. The palladium complex was more effective against *E. coli* than standard drug.

4. Conclusion

In this study, condensation reaction was adopted for preparing a new Schiff base ligand; 4-imidazolecarboxaldehyde thiosemicarbazone. The ligand and its metal complexes were fully characterized by several techniques and the antibacterial and antifungal activities of the ligand and its metal complexes were also evaluated.

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Disclosure statement

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

Author contributions: All authors are contributed in 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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