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Inhibition of DNase I Enzyme with Nickel(II) Triphenylphosphine Complexes Incorporating Tridentate Schiff Base Ligands in Vitro

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Abstract: The nickel(II) complexes containing with ONS chelating 3-methoxy-salicylaldehyde-N^4-R thiosemicarbazones (R:-H_2,-propyl) and triphenylphosphine coligands have been synthesized. The structures of Ni(II)-centered metal complexes were approved by means of analytical and spectroscopic data. The solid-state structure of complex **2** bearing PPh3 as co-ligand was clarified by single crystal X-ray crystallography, which revealed square planar geometry around Ni(II) ion. The potential of these complexes to inhibit the DNase I enzyme, which uses DNA as a substrate, was investigated *in vitro*. The results revealed that the compounds inhibited the DNase enzyme directly and/or indirectly (by masking of DNA molecules) at ≥0.1 µg/mL concentrations *in vitro*.

Keywords: Nickel(II); thiosemicarbazone; X-ray; DNase I.

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INTRODUCTION

The reaction of thiosemicarbazide and an aldehyde or ketone results in thiosemicarbazone compounds. Thiosemicarbazones are an important class of chelating ligands which contain nitrogen and sulfur donor atoms and have extensive applications in various fields such as medicine, industry, analytical and organic processing (1-4). Mixed-thiosemicarbazones complexes bearing seconder ligand play essential roles in biological processes like activation of enzymes by metals (5-6). Particularly thiosemicarbazone-based nickel(II) complexes have shown significant antiviral (7), antibacterial (8) and anticancer activities (9). Investigations on interactions of DNA and thiosemicarbazone molecules have attracted significant attention over the last years (10). The square planar Ni(II)-thiosemicarbazone complexes were reported to have DNA interaction and topoisomerase II inhibition activity (11-13).

Deoxyribonuclease I, was the first enzyme to be recognized as specific for DNA, binds to the small groove of DNA and is often used as an enzymatic tool to study the interaction of DNA and proteins (14,15). DNases play a significant role in alimentary canal digestion and in pathogenesis of diverse diseases and apoptosis, while DNase inhibitors could modify or control those activities. Determining molecules that are able to cleaving/binding DNA has drawn great attention because of their important use in nanotechnology, therapeutic and biotechnology applications (16, 17).

Binding affinity in CT-DNA and protein and cytotoxicity activity against cancer cell lines HeLa, A549 and HepG2 of Ni(II) complexes consisting of 4-methoxysalicylaldehyde-N^4-R-thiosemicarbazone (R: H, Me, Et, Ph) and PPh_3 were investigated by Prabhakaran et al. The results showed that the complexes have important binding ability and cytotoxicity activity in contrast of their ligands. The binding affinity towards DNA and protein is decreased in order of C_2H_5>CH_3>H>C_6H_5 unlike in order of cytotoxic activity (11). Another paper of Prabhakaran is on DNA topoisomerase II inhibition activity of

nickel(II) complexes consisted of salicylaldehyde-N^4-R (R: Me, Ph)/2-hydroxynaphthaldehyde-N^4-R (R: Me)-thiosemicarbazone and PPh_3. The activity is decreased in order of [Ni(Nap-Me-tsc)(PPh_3)]>[Ni(Sal-Ph-tsc)(PPh_3)]>[Ni(Sal-Me-tsc)(PPh_3)].

In our previous study, two nickel complexes incorporating tridentate Schiff bases derived from 3-methoxy salicylaldehyde with triphenylphosphine were synthesized and characterized by various spectroscopic data (7). In this paper, the complexes **1**,**2** (Figure 1.) were firstly investigated for DNase I enzyme inhibition and the crystal structure of the complex **2** was performed by single-crystal diffraction.

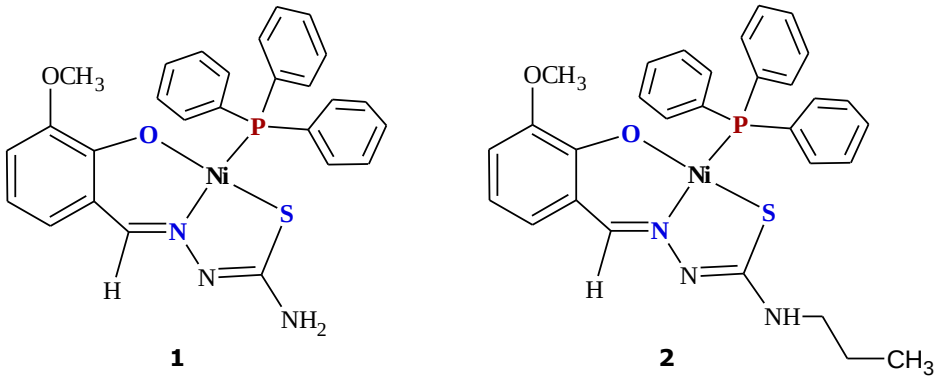

Figure 1. The chemical diagrams of complexes (**1, 2**).

EXPERIMENTAL SECTION

Synthesis

The nickel complexes were prepared by the literature method as follows (7, 12). Reactions of the solution of thiosemicarbazone ligands (1 mmol) in dichloromethane (10 mL) with the solution of dichlorobistriphenylphosphine nickel(II) (1 mmol) in 10 mL absolute ethanol in equivalent amounts were resulted by giving the tetra-coordinated Ni(II) complexes (Figure 1). The structure of Ni(II) complexes were characterized by means of analytical and spectroscopic data (7). The complex **2**, was in the form of fine crystals, soluble in alcohols and chlorinated hydrocarbons. Recrystallization of complex **2** was resulted in the composition of [Ni(L)(PPh_3)].

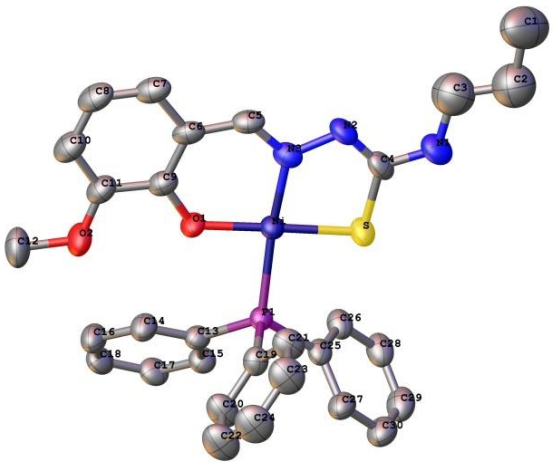

Figure 2. Crystal structure of the complex **2**.

X-ray crystallography

The data of intensity of the complex was recorded on a Bruker D8 VENTURE diffractometer equipped with PHOTON100 detector at 304 K temperature using graphite- monochromated Mo Ka radiation (k = 0.71073 Å) by applying the multi-scan method. SHELXS program of the SHELXTL-1997 (18) software is used to solve of the structure which is refined by full-matrix least-squares methods with SHELXL-2014/7 (19, 20). Absorption corrections were performed using SADABS (21). The intensity data were integrated by SAINT software package using a wide-frame algorithm (22). All H atoms were placed in calculated positions and treated using a riding model, fixing the bond lengths at 0.82, 0.93, 0.97, 0.97 and 0.96 Å for NH, aromatic CH, methine, methylene, methyl atoms, respectively. The details of the data collection and structure solution are collected in Table 1.

Deoxyribonuclease I (DNase I) inhibition activity

DNase I enzyme (Sigma-D4227), isolated from bovine pancreas, was used to determine the effects of complexes on DNase enzyme activity *in vitro*. The DNase enzyme was dissolved in a buffer containing 10 mM Tris (pH:7.5), 10 mM $CaCl_2$, and 50% (v/v) glycerin with ~10U/µl and stored at -20 °C. The pHKP-Luc plasmid, purified by the plasmid DNA isolation kit from *Escherichia coli* DH5α cells, was used as the DNA molecule. Plasmid DNA was obtained from K.Turan (23). The reaction was carried out in 15 µL of DNase I buffer (10 mM tris, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, pH 7.5) containing 2 m units of DNase, 1 µg of plasmid DNA and 0.1, 0.01 or 0.001 µg of complex by allowing to stand for 20 minutes at 37 °C. At the end of the period, DNase I enzyme was inactivated by heating the reaction mixtures at 75 °C for 10 min. To clarify the enzymatic digestion of plasmid DNAs, the reaction mixtures were analysed with agarose gel electrophoresis. For this purpose, the samples were mixed with x 6 concentrated gel loading buffer at the 1:5 ratio and applied to 1% agarose gel. Electrophoresis was completed in 25 minutes under a constant voltage of 100 V in TAE buffer. DNA was visualized with a UV transilluminator and photographed.

DNase I inhibition studies

The potential inhibition activity of the complexes on DNase I was investigated by gel electrophoresis (Figure 4). It is observed that almost entire DNase I activity were inhibited by the complexes at the concentration of 0.1 µg/mL. In the case of the diluted complex concentration of 0.01 and 0.001 µg/mL. It is revealed that the activity of the enzyme decreased. It is suggested that the inhibition efficiency of the complexes on DNase I enzyme protects the DNA structure. The observed inhibition activities against DNase I could be associated with the coordinatively unsaturated square planar geometry of the metal center which causes binding the DNA to the available vacant sites. Another possibility is that the complexes can directly and/or indirectly inhibit the DNase I enzyme. Regarding, Prabhakaran et al. has investigated DNA topoisomerase II inhibition activity of nickel(II)-PPh_3 complexes containing the thiosemicarbazones coordinated in ONS fashion. This study showed increase in the electron deficiency on metal centre and the formation of coordinative unsaturated square planar geometry was attributed to the binding of topoisomerase enzyme to the metal centre (13).

RESULTS AND DISCUSSION

Crystal structure studies

The reaction of $[Ni(PPh_3)_2Cl_2]$ and the thiosemicarbazones in the same ratio gave yields, the diamagnetic Ni(II)-complexes 1, 2 containing PPh_3 co-ligand. The complexes were coordinated to Ni(II) by giving two protons from thiosemicarbazone via phenolic –OH and thiol group. In to approve the definite structure of the complex **2**, crystallographic analysis has been carried out. ORTEP-3 (19) drawing of the complex is illustrated in Figure 2. whilst the bond angles/lengths are presented in Table 2. Complex **2** includes the dibasic form of the ligand which acts as tridentate ligand by the nitrogen, sulfur and oxygen atoms resulting in the formation of six and five-membered chelate ring with O–Ni–N and S–Ni–N bite angles of 95(3)°and 86.6(2)°, respectively. The triphenylphosphine group forms the fourth coordination of Ni(II). The C–S bond-distance is 1.745(9) Å, revealing that thiolate form of thiosemicarbazone bound to metal. The P(1)–Ni–N(3) and S(1)–Ni–O(1) bond-angles deviate remarkably from 180° which shows that is significant distortion in NiSNOP core around nickel atom. Packing diagram of the complex **2** is shown in Figure 3. According to the figure, no hydrogen-bonds or important intermolecular-interactions in the structure were observed.

Table 1 Crystal data and structure refinement parameters for complex **2**.

Parameter	**2**
CCDC depository	1873557
Color/shape	Brown / Rod
Chemical formula	$C_{30}H_{30}N_3NiO_2PS$
Formula weight	586.31
Temperature (K)	304
Wavelength (Å)	0.71073 (Mo Kα)
Crystal system	Monoclinic
Space group	*P 21*
Unit cell parameters	
a, b, c (Å)	11.546(4), 8.050(2), 15.287(6)
α, β, γ (°)	90, 97.490 (11), 90
Volume (Å^3)	1408.7(9)
Z	2
D_{calc} (g/cm^3)	1.382
μ (mm^{-1})	0.852
Absorption correction	Multi-scan
T_{min}, T_{max}	0.931, 0.983
F_{000}	612
Crystal size (mm^3)	0.020 × 0.070 × 0.200
Diffractometer	Bruker D8 VENTURE
Measurement method	multi scan
Index ranges	-13 ≤ h ≤ 13, -8≤ k ≤ 9, -18 ≤ l ≤ 18
θ range for data collection (°)	2.37 to 25.00
Reflections collected	10369
Independent reflections	4699
Observed reflections	2684
R_{int}	0.0658
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	4699/398/349
Goodness-of-fit on F^2	1.033
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0542, wR_2 = 0.1050
R indices (all data)	R_1 = 0.0956, wR_2 = 0.1186
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e/Å^3)	0.777, -0.440

Table 2 Important geometric parameters for complexes **2**.

Bond lengths (Å)		**Bond angles (°)**	
Ni1—P1	2.216(2)	P1—Ni1—S1	90.28(9)
Ni1—S1	2.137(2)	P1—Ni1—O1	88.32(17)
Ni1—O1	1.865(5)	P1—Ni1—N3	171.0(2)
Ni1—N3	1.882(6)	S1—Ni1—O1	178.08(19)
S1—C4	1.745(9)	S1—Ni1—N3	86.6(2)
O1—C9	1.313(9)	O1—Ni1—N3	95.0(3)
N2—N3	1.402(9)	S1—C4—N1	117.9(7)
N1—C3	1.473(14)	S1—C4—N2	123.6(7)
N1—C4	1.345(12)	N1—N2—C9	113.1(2)
N2—C4	1.286(11)	N2—C9—N3	119.8(3)
N3—C5	1.305(10)	N2—N3—C5	113.8(7)
		N3—C5—C6	127.5(8)
		C4—N3—C3	118.9(10)

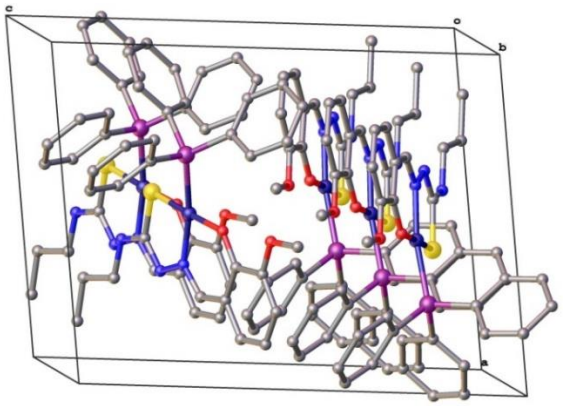

Figure 3. Molecular packing of the complex **2.**

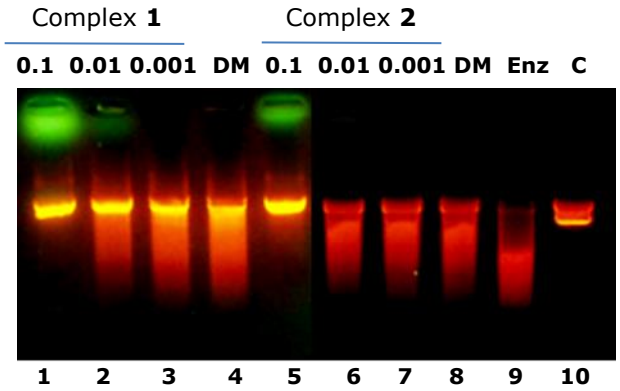

Figure 4. The diagram of the agarose gel electrophoresis shows the inhibition of DNase I enzyme by Ni(II) complexes **1, 2**: lanes 1-3 (for complex **1**): 0.1 µg/mL; 0.01 µg/mL; 0.001 µg/mL, respectively; lane 4 (DM): DNase I enzyme with DMSO control; lanes 5-7 (for complex **2**): 0.1 µg/mL; 0.01 µg/mL; 0.001 µg/mL, respectively; lane 8 (DM): DNase I enzyme with DMSO control; lane 9 (Enz): plasmid DNA incubated with DNase I; lane 10 (C): pHKP-Luc plasmid DNA alone.

CONCLUSION

The nickel-PPh_3 complex **2** of 3-methoxy-salicylaldehyde-N^4-propyl-thiosemicarbazone was identified by X-ray crystallographic techniques, which confirmed the dibasic forms (L^{2-}) of propyl-substituted in the nickel centered chelates. The thiosemicarbazone complexes containing N4-long chain alkyl substituent are few in literatures. The synthesized compounds have a slightly distorted square planar geometry involving the thiosemicarbazone, coordinated via ONS mode. The DNase I enzyme inhibition of Ni(II)-thiosemicarbazone complexes, which uses DNA as a substrate, was observed in electrophoresis *in vitro*. studies for the first time.

The asset of electron withdrawing groups in the coordinated thiosemicarbazones causes the increment in the electron lack on the metal. Thus, it is thought that the coordinative unsaturated square-planar geometry maybe in charge of the binding of DNA to nickel(II).

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