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Synthesis, Caracterization and Interaction with CT DNA of Novel Cationic Complex Ru(III) with Indazole and Schiff Base Derived from 5-Chlorosalicylaldehyde

Begić-Hairlahović S.*, Kahrović E., Turkušić E.

Department of Chemistry, Faculty of Science, University of Sarajevo, Sarajevo 71 000, Bosnia and Herzegovina

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***Corresponding author:**
E-mail: sabinab2009@gmail.com
Phone: 00-387-33-279950
Fax: 00-387-33-649359

Abstract: Novel cationic complex compound of Ru(III) with indazole and Schiff base derived from 5-chlorosalicylaldehyde and aniline has been synthesized. Formulation and characterization of the complex was performed using CHN analysis, MALDI-TOF/TOF mass spectrometry, FT-IR spectroscopy and UV/Visible spectrophotometry. In the octahedral environment of Ru(III), coordination of bidentate Schiff bases occurs through azomethine nitrogen and deprotonated phenolic oxygen while in indazole via nitrogen atom. The interaction of the complex with CT DNA (calf thymus DNA) was carried out under physiological conditions using spectrophotometric titration.

INTRODUCTION

In the last decades, ruthenium complexes have being in the focus of the interest, primarily because of their anticancer (Keppler *et al*, 1989), antibacterial (Kahrovic, Bektas, *et al*, 2014), catalytic (Kahrovic, 2011) or electron transfer mediated activity (Turkusic *et al*, 2012). The properties of the complex compounds depend on coordination environment. Some Ru(III) complexes with Schiff bases derived from salicylaldehyde and substituted salicylaldehyde (Kahrovic *et al*, 2010; Kahrovic, 2014) are described as moderate intercalators (Ljubijankić *et al*, 2013; Kahrović, *et al*, 2014) and electrochemical mediators for the low potential amperometric determination of ascorbic acid (Kahrovic and Turkusic, 2012) or L-cysteine (Turkusic and Kahrovic, 2012). A relatively small number of complexes containing Schiff base derived from 5-chlorosalicylaldehyde are described in literature (Blagus *et al*, 2010).

N-heterocyclic compounds, as a component of some vitamins and drugs, play a key role in many biological systems (Bayari *et al*, 2003). Two ruthenium complexes with *N*-heterocyclic ligands, imidazolium trans-imidazoledimethyl sulfoxide-tetrachlororuthenat (NAMI-A) and trans-[tetrachlorobis(1H-indazole)ruthenate(III)] (KP1019) have been reported as anticancer agents, against metastatic and colon cancers, respectively (Lakhai *et al*, 2004). Because of structural similarity to the nucleic bases, adenine and guanine, indazole is very interesting ligand.

In this study, we aimed to synthesise novel Ru(III) cationic complex with indazole and Schiff base derived from 5-chlorosalicylaldehyde and investigate interaction with CT DNA (calf thymus DNA).

EXPERIMENTAL

Materials and Physical Measurements

All chemicals were commercially available with analytical grade of purity and were used without further purification. CT DNA type 1-fibrous was purified using phenol-chloroform-isoamyl alcohol extraction method until ratio A_{260}/A_{280} = 1.8 was obtained.

Elemental analysis was obtained on a Perkin Elmer 2400 Series CHNSO/O Analyzer.

Mass spectrum was recorded by matrix-assisted laser desorption/ionization–time-of-flight MALDI-TOF/TOF mass spectrometer (4800 Plus MALDI TOF/TOF analyzer, Applied Biosystems Inc., Foster City, CA, USA) in the positive ion reflector mode. A small amount of sample was mixed with 10 μL of MALDI matrix (DHAP (2,6-dihydroxyacetophenone); 5 mg/mL) and 1 μL was spotted on MALDI plate. The number of 1600 shots per spectrum were acquired in m/z range of 100-1000 Da (focus mass 500 Da, delay time 300 ns). As an internal standard in the positive mode,thiamine mononitrate, azithromycin 591 and 794 and B 12 were used.

The infrared spectra were recorded as KBr pellets on a Perkin Elmer spectrum BX FTIR System in the region 4000-400 cm^{-1}.

Electronic spectra, hydrolysis experiment and CT DNA binding were measured on a Perkin Elmer lambda 35 spectrophotometer. Electronic spectra were recorded in CH_2Cl_2 solution over the range 190-700 nm. Hydrolysis of Ru(III) complex was performed by adding the concentrated solution of complex in DMSO to Tris-HCl buffer solution (pH 7.4). Spectra were recorded every 10 minutes within two hours. The stock solution of CT DNA was prepared in Tris-HCl buffer pH 7.4 and stored at 4 °C up to 4 days. The concentration of DNA was determined spectrophotometrically at 260 nm using a molar extinction coefficient of 6600 M^{-1} cm^{-1} (Meadows *et al.*, 1993). Concentrated stock solution of complex was prepared by initial dissolution of complex in a small amount of DMSO and diluting with Tris-HCl buffer to the final concentration. Spectrophotometric titration of Ru(III) solution of fixed concentration with CT DNA was performed by the successive addition of DNA (in portion of 10 μL) in 2 mL of the compound solutions at pH 7.4. Each addition of DMSO, DNA or complex was compensated in the blank. The determination of the binding constant, K_b, has performed on the basis of spectrophotometric titration of complex with CT DNA by recording spectra in the range of 350 – 600 nm. The binding constant is calculated on the basis of changes in absorptions at 583 nm.

Cyclovoltammograms were recorded on an electrochemical workstation Autolab potentiostat/galvanostat (PGSTAT 12) using three electrode system: glassy carbon as working electrode, Ag/AgCl as reference and Pt-wire as counter electrode. Cyclovoltammograms were recorded in acetonitrile (MeCN) with sodium perchlorate($NaClO_4$) as supporting electrolyte in the potential range of -1.0 to 0.0 V, with scan rate 0.2 V/s and in *N*, *N*-dimethylformamide (DMF) with tetraethylammonium bromide (Et_4NBr) as suppoting electrolyte in the potential range of -1.1 to -0.5 V with scan rate 0.2 V/s .

Synthesis of the complex, [Ru(*N*-5-Cl-salim)$_2$(Ind)$_2$]Cl, Diindazole–bis-[*N*-phenyl-5-chlorosalicylideneiminato-*O,N*]ruthenium(III) chloride

Schiff base, *N*-phenyl-5-chlorosalicylideneimine, hereinafter *N*-Ph-5-Cl-salimH and starting compound, sodium dichloro-bis-[*N*-phenyl-salicylideneiminato-*N,O*]ruthenate(III), hereinafter Na[$RuCl_2$(*N*-Ph-5-Cl-salim)$_2$] were synthesized according to the published procedures (Kahrović *et al*, 2010; Ljubijankić *et al*, 2013). Starting compound was used for the synthesis of novel cationic complex without any purification. The purity of starting compound was checked by using IR spectroscopy.

Cationic complex, [Ru(*N*-5-Cl-salim)$_2$(Ind)$_2$]Cl was prepared in reaction of starting compound with indazole in molar ratio 1 : 2 in absolute ethanol. The amount of 23.6 mg (0.2 mmol) indazole in absolute ethanol (2 mL) was added to the solution of 6.55 mg (0.1 mmol) of pulverized Na[$RuCl_2$(*N*-5-Cl-salim)$_2$] in 60 mL hot absolute ethanol. Reaction mixture was refluxed at temperature 75 °C for five hours whereby the solution has changed color from dark green to dark blue. The volume of the solution was reduced under vacuum distillation to about one-quarter of initial volume. The resulting solution was kept in an ice-salt bath for five days. The green solid was filtered off and washed with water until the negative reaction for chloride ions.

Yield: 73 %.

Diindazole–bis-[*N*-phenyl-5-chlorosalicylideneiminato-*O,N*]ruthenium(III) chloride: Anal. calcd for $C_{40}H_{30}Cl_3N_6O_2Ru$: C 57.60, H 3.63, N 10.78. Found: C 55.53, H 4.51, N 9.80; MALDI TOF/TOF MS (*m*/*z*) calcd for $[C_{40}H_{30}Cl_2N_6O_2Ru]^+$, 798.0850; found, 798.0870; IR (KBr, cm^{-1}) 3444 [ν(N–H)], 1695 [ν(C=N)], 1356 m [ν(C–N)], 1076 [ν(N–N)], 669 w [ν(Ru–N), 421 w [ν(Ru–O)] UV-Vis (CH_2Cl_2) I_{max} (log e) 230 (4.63), 282 nm (4.32).

RESULTS AND DISCUSSION

Synthesis and Spectroscopic Studies

The starting complex, Na[$RuCl_2$(*N*-Ph-5-Cl-salim)$_2$] was synthesized from $RuCl_3$ and freshly prepared ligand, 5-chlorosalicilydeneimine, in absolute ethanol solution in molar ratio 1:2. The freshly prepared Na[$RuCl_2$(*N*-Ph-5-Cl-salim)$_2$] compound was used for the synthesis of novel cationic complex.

[Ru(*N*-5-Cl-salim)$_2$(Ind)$_2$]Cl was prepared from absolute ethanol solutions containing starting compound and indazole in molar ratio 1 : 2. The synthesis of the cationic complex was carried out in relative mild conditions by replacement of two easily outgoing chloride ions in starting compound with indazole. The scheme of preparation is showed in Figure 1. The green solid is stable in air, insoluble in water, soluble in acetonitrile (MeCN), dimethyl sulphoxyde (DMSO), dimethylformamide (DMF).

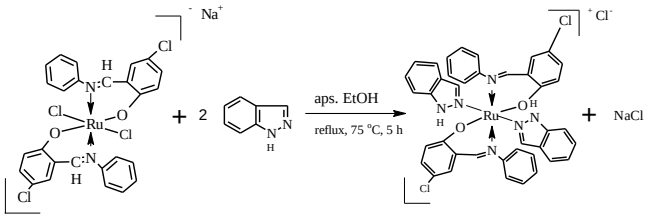

Figure 1: Synthesis of [Ru(*N*-5-Cl-salim)$_2$(Ind)$_2$]Cl

On the basis on CHN elemental analysis, mass spectra, infrared and UV/Visible spectroscopic measurements the compound formula was formulated as [Ru(*N*-5-Cl-salim)$_2$(Ind)$_2$]Cl. Mass spectra showed molecular ion (M^+) at *m/z* (100%) = 798.0870 which corresponds to $[C_{40}H_{30}Cl_2N_6O_2Ru]^+$.

The characteristic IR frequencies of starting Ru(III) compound, free indazole and [Ru(*N*-5-Cl-salim)$_2$(Ind)$_2$]Cl are given in Table 1. Coordination of indazole to Ru(III) through electronic pair on the atom nitrogen undoubtedly affects the C=N and C–N stretching vibrations which were shifted for 6 and 10 cm^{-1}, respectively, towards higher wavenumbers.

Stretching vibration of N–H bond in free indazole appears at 3410 cm^{-1}. In [Ru(*N*-5-Cl-salim)$_2$(Ind)$_2$]Cl this vibration is coupled with vibration in the starting Ru(III) compound and appears at 3444 cm^{-1}. The weak bands at 669 and 421 cm^{-1} could be attributed to Ru – N and Ru – O, respectively. Characteristic IR vibrations of starting Na[RuCl$_2$(*N*-Ph-5-Cl-salim)$_2$] are azomethine C=N, C-O phenolic , Ru-N and Ru-O which are not affected after coordination of indazole.

Infrared spectra of [Ru(*N*-Ph-5-Cl-salim)$_2$(Ind)$_2$]Cl showed that in octahedral environment of Ru(III), coordination of bidentate Schiff base occurs through azomethine nitrogen and deprotonated phenolic oxygen while in indazole via nitrogen atom (Table1).

Table 1: Characteristic vibrations (cm^{-1}) in FT-IR spectra of starting compound, cationic complex and free indazole

	Starting compound	Cationic complex	Free Indazole
ν(N–H) $_{Ind}$	-	3444	3410
ν(C=C) $_{Ind}$	-	1622	1621
ν(C=N)$_{SB}$	1607	1607	-
ν(C=N)$_{Ind}$	-	1695	1689
ν(C–N)$_{Ind}$	-	1356	1356
ν(C=O$_{Ph}$)$_{SE}$	1298	1298	-
ν(N–N) $_{Ind}$	-	1086	1076
ν(Ru–N)	668	669	-
ν(Ru–O)	419	421	-

$_{SB}$ – assigned vibrations in Schiff base; $_{Ind}$ – assigned vibrations in Indazole

Electronic spectra of Na[RuCl$_2$(*N*-Ph-5-Cl-salim)$_2$], [Ru(*N*-5-Cl-salim)$_2$(Ind)$_2$]Cl and free indazole were recorded in CH_2Cl_2. Electronic spectra of indazole has two absorption bands which could be attributed to $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transitions.

Weak broad absorption in the electronic spectra of starting copound centered at 609 nm in the region of d-d spin alowed transition of low spin t_{2g}^5 Ru(III) can be assigned to ($^2T_{2g}\rightarrow{}^2A_{2g}$). In the spectra of [Ru(*N*-5-Cl-salim)$_2$(Ind)$_2$]Cl this transition moves towards lower value of wavelength (574 nm). Replacement of two chloride in the starting compound with *N*-donor ligands, that split stronger crystal field than chlorides results in higher separation energies of d-atomic orbitals and moves d-d transition to higher energies. The shift of $n\rightarrow\pi^*$ transition in free indazole from 285 nm to 282 nm in [Ru(*N*-5-Cl-salim)$_2$(Ind)$_2$]Cl is an evidence of coordination of indazole through nitrogen atom.

Table 2: Characteristic absorptions in electronic spectra of Na[RuCl$_2$(*N*-Ph-5-Cl-salim)$_2$], [Ru(*N*-Ph-5-Cl-salim)$_2$(Ind)$_2$]Cl and indazole in dichlormethane

Compound	$\pi\rightarrow\pi^*$	$n\rightarrow\pi^*$	IL (SB);	d-d
Na[RuCl$_2$(*N*-Ph-5-Cl-salim)$_2$	244	sh	348	609
Indazole	250	285	-	-
[Ru(*N*-Ph-5-Cl-salim)$_2$(Ind)$_2$]Cl	230	282	sh	574

$\pi\rightarrow\pi^*$ - electronic transition of delocalized electrons of the aromatic system; $n\rightarrow\pi^*$ - electronic transitions of the atoms of azomethine group or free electron pair on the N atom of indazole with aromatic π electrons; IL (SB) – intraligand transition of whole molecule of Schiff base; d-d – transition of low spin complex; sh-shoulder.

Cyclovoltammetry

Redox potential of Ru(III) complexes is an important characteristic which determines its behavior in biological environment. It is considered that the activity of some Ru(III) complexes which are known as antitumor agents is achieved by reduction *in situ*.

Starting compound and cationic complex are insoluble in water and therefore cyclovoltammograms were recorded in non-aqueous solvents, MeCN and DMF in the presence of Et_4NBr and $NaClO_4$ as supporting electrolytes using glassy carbon electrode as working electrode. All data are given *vs* Ag/AgCl reference electrode (Table 3).

Cyclovoltammograms of [Ru(*N*-Ph-5-Cl-salim)$_2$(Ind)$_2$]Cl and Na[RuCl$_2$(*N*-Ph-5-Cl-salim)$_2$] in MeCN and DMF show defined E_{pa} anodic peaks and E_{pc} chatodic peaks. The half-wave potentials, assigned to $Ru_{(III)}/Ru_{(II)}$ couple, are quite negative and for starting compound in MeCN/Et_4NBr system is -0.832 V and for final product is -0.844 V. Formal potential, $E_{1/2}$ in DMF/$NaClO_4$ system for starting compound is -0.626 and for final product is -0.563 V. This values indicated that Ru(III) is stabilized through ON_2 ligands. The separation peaks (E_k-E_a) and ratio i_k/i_a in cyclovoltammograms in both systems indicate quasi-reversible one-electron processes.

Cyclovoltammograms recorded in MeCN/Et_4NBr showed higher peak separations and reversibility according to the DMF/$NaClO_4$ system. This is in agreement with coordination ability of solvents.

Table 3: Characteristic potentials of $Na[RuCl_2(N\text{-}Ph\text{-}5\text{-}Cl\text{-}salim)_2]$ and $[Ru(N\text{-}5\text{-}Cl\text{-}salim)_2(Ind)_2]Cl$ from cyclovoltammetric measurements in different systems

	$Na[RuCl_2(N\text{-}Ph\text{-}5\text{-}Cl\text{-}salim)_2]$		$[Ru(N\text{-}5\text{-}Cl\text{-}salim)_2(Ind)_2]Cl$	
	MeCN/Et_4NBr	DMF/$NaClO_4$	MeCN/Et_4NBr	DMF/$NaClO_4$
E_{pc} / V	-0.924	-0.858	-0.945	-0.869
E_{pa} / V	-0.740	-0.394	-0.742	-0.257
$E_{1/2}$/ V	-0.832	-0.626	-0.844	-0.563
ΔE / V	0.184	0.464	0.203	0.590

All data are given *vs* Ag/AgCl reference electrode

Interaction of cationic complex with CT DNA

Behavior of complex compounds in solution is of great importance for biological activity. The hydrolysis of $[Ru(N\text{-}5\text{-}Cl\text{-}salim)_2(Ind)_2]Cl$ was monitored in Tris-HCl buffer (pH 7.4). Hydrolytic profile showed stability in the solution (Figure 2).

Spectrophotometric titration is a method of choice to study the interactions between metal complex and DNA. In this research, the titration was performed by adding increasing amount of CT DNA to the Ru(III) solution of fixed concentration keeping the [DNA] / [complex] ratio in range from 0 to 2.48. The binding constant was calculated on the basis of equation (1) (Pyle *et al*, 1989):

$$\frac{[DNA]}{[\varepsilon_a - \varepsilon_b]} = \frac{[DNA]}{[\varepsilon_b - \varepsilon_f]} + \frac{1}{Kb(\varepsilon_b - \varepsilon_f)} \quad (1)$$

where ε_a, ε_f, ε_b represent exinction coefficients for particular measurements (A_{obs} / [DNA]), free complex and completely bound form, respectively. In plots of [DNA] / ($\varepsilon_a - \varepsilon_f$) *versus* [DNA], the binding constant, K_b is given by the ratio of slope to the intercept. Experimental data for spectrophotometric titration of $[Ru(N\text{-}5\text{-}Cl\text{-}salim)_2(Ind)_2]Cl$ with CT DNA are given in Table 4 and Figures 3 and 4.

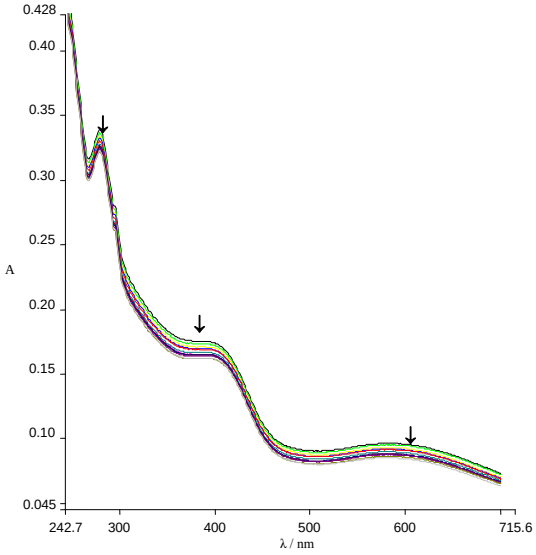

Figure 2: Hydrolysis of $[Ru(N\text{-}5\text{-}Cl\text{-}salim)_2(Ind)_2]Cl$ in 0.1 M Tris-HCl buffer (pH 7.4); c = 3.36 x 10^{-5} M

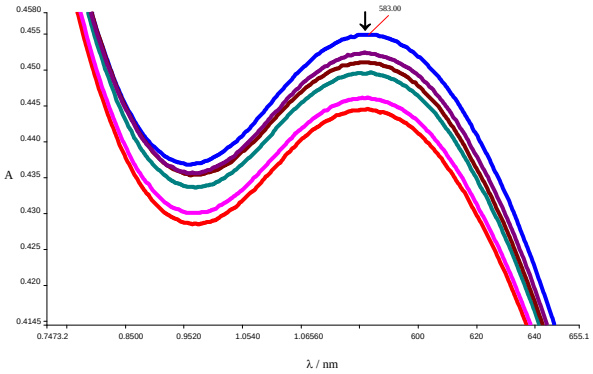

Figure 3: The spectrophotometric titration of 5.17 x 10^{-5} M $[Ru(N\text{-}5\text{-}Cl\text{-}salim)_2(Ind)_2]Cl$ with increasing concentration of CT DNA (0 – 1.24 x 10^{-4} M) in 0.1 M Tris-HCl buffer (pH 7.4)

Table 4: Spectrophotometric titration of $[Ru(N\text{-}5\text{-}Cl\text{-}salim)_2(Ind)_2]Cl$ with CT DNA

[complex] / 10^{-6} M	V_{DNA} / μL	[DNA] 10^{-6} M	[DNA]/ [complex]	10^{-7} [DNA]/ (ε_f-ε_a) M^2 cm
51.7	0	0	0	-
51.5	10	18.2	0.35	0.74
51.2	20	36.3	0.71	1.39
50.5	50	89.3	1.77	3.15
50.2	60	107	2.12	2.99
50.0	70	124	2.48	3.16

The binding constant (K_b) was calculated on the basis of decrease of absorptions at 583 nm. Spectral feature gives hypochromic effect, but bathochromic shift, which is characteristic for intercalation, is not evident. The calculated binding constant of 4.9 x 10^4 M^{-1} indicates non-covalent binding of complex to CT DNA which is described as major groove binding.

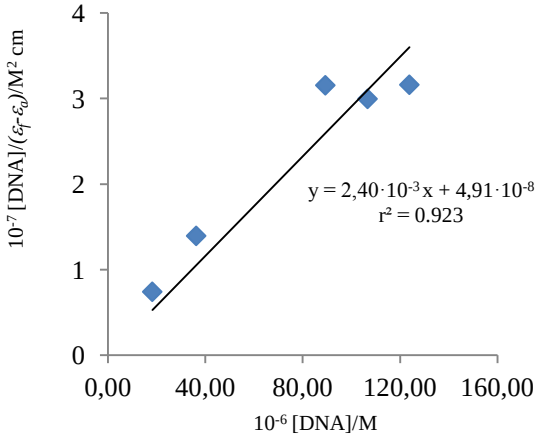

Figure 4: Graphical calculation of K_b(4.9 x 10^4 M^{-1}) on the basis of spectrophotometric titration of $[Ru(N\text{-}5\text{-}Cl\text{-}salim)_2(Ind)_2]Cl$ by CT DNA

CONCLUSION

Novel cationic complex of Ru(III) with indazole and Schiff base derived from 5-chlorosalicyladehyde and aniline was synthesized. Based on CHN elemental analysis, mass spectra, infrared and UV/visible spectroscopic measurements, the complex was formulated as $[Ru(N\text{-}5\text{-}Cl\text{-}salim)_2(Ind)_2]Cl$. In the octahedral environment of Ru(III), coordination of bidentate Schiff bases occurs through azomethine nitrogen and deprotonated phenolic oxygen while in indazole via nitrogen atom. DNA binding studie was investigated by spectrophotometric titration. The binding constant (K_b) of cationic complex was determined as 4.9×10^4 M^{-1}. According to this result novel cationic complex act as major groove binder.

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Summary / Sažetak

Sintetiziran je novi katjonski kompleksni spoj sa indazolom i Šifovom bazom izvedenom iz 5-hlorsalicilaldehida. Formulacija i karakterizacija kompleksa napravljena je na bazi CHN analize, MALDI-TOF/TOF masene spektrometrije, FT-IR spektroskopije i UV/Vidljive spektrofotometrije. U oktaedarskom okruženju Ru(III), koordinacija bidentatne Schiffove baze se ostvaruje preko azometinskog azota i deprotoniranog fenolnog kisika, dok se kod heterociklusa ostvaruje preko atoma azota. Interakcija kompleksa sa CT DNA (DNA iz timusa govečeta) provedena je u fiziološkim uslovima korištenjem spektrofotometrijske titracije.