Calf Thymus DNA Intercalation by Anionic Ru(III) Complexes Containing Tridentate Schiff Bases Derived from 5-X-Substituted Salicylaldehyde and 2-Aminophenol

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Abstract: Sodium bis[N-2-oxyphenyl-5-substituted-salicylideneiminato-ONO]ruthenate(III) hemitriethylamine compounds of general formula $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$, where, $\text{R} = \text{C}_6\text{H}_4\text{O}$, $\text{X} = \text{Cl}, \text{Br}, \text{NO}_2$, salim = salicylideneiminato, Et_3N triethylamine, were synthesized and characterized on the basis of elemental analysis, MALDI-TOF (matrix-assisted laser desorption/ionization/time-of-flight) mass spectra, ^1H NMR (nuclear magnetic resonance), electronic spectra and cyclic voltammograms. Ru(III) is chelated with two O_2N anionic tridentate Schiff bases derived from 5-X-salicylaldehyde and 2-aminophenol. Spectroscopic titrations of the Ru(III) compounds with CT DNA (calf thymus DNA) in the LMCT (ligand metal charge transfer) region have shown moderate intercalation properties of compounds with binding constants $K_b = 2.06\text{--}3.85 \times 10^4 \text{ M}^{-1}$. Electrochemical evidence of intercalative mode of binding is based on the decrease of current and shift of anodic and cathodic peaks towards higher values of potential with μL -increment of CT DNA in Ru(III) compound solution.

Key words: Ruthenium, Schiff bases, salicylideneimine, CT DNA, intercalation.

1. Introduction

Ruthenium complexes are the subject of increasing interest in chemistry and interdisciplinary fields of science due to many significant properties, especially anticancer and antimetastatic properties [1-8] and the ability to catalyze a number of organic reactions [9-12] and the aging.

A number of ruthenium complexes showed electron transfer capability as a key feature in the development of new sensors [13-14], and appropriate thermal and electrical properties essential for applications in solar cells [15]. In the development of new compounds, as potential antitumor agents, DNA is still considered as the primary target. Metal compounds are known to

bind DNA by covalent or non-covalent mode. For covalent, either inter- or intra-strand mode of binding, easily leaving groups, e.g., chlorides, are generally required. Non-covalent binding mainly assumes groove binding or intercalation which means insertion of fused and planar aromatic moiety between base pairs of DNA. Many metal complex species, in particular neutral or positively charged, with some typical intercalating organic molecules containing extended π -electronic system, e.g., 2,2-bipyridine (bipy) or 1,10-phenanthroline (phen) have shown ability to intercalate DNA [16-21]. Some Ru(III) complexes with such ligands have also shown the property to bind DNA by intercalation [22-24].

Schiff bases derived from salicylaldehyde, either as neutral or anionic species, are considered to be very functional ligands, therefore many metal complexes

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with this type of ligands were reasonably synthesized for different purposes. Some of ruthenium complexes which contain ON (oxygen, nitrogen) or ONO (oxygen, nitrogen, oxygen) anionic salicylideneimine as ligands showed antifungal and antibacterial activity [25-27]. Recently, we reported the DNA binding properties of two anionic Ru(III) compounds containing anionic bidentate salicylideneimine ligands and two chlorides [28].

In continuation of our study on synthesis and characterization of Ru(III) compounds with Schiff bases and interaction with DNA, we report herein on Ru(III) compounds containing two anionic O₂N Schiff bases derived from 5-substituted-salicylaldehyde and 2-aminophenol and study of interaction with CT DNA (calf thymus DNA).

2. Experiments

All chemicals of analytical grade were purchased from Sigma Aldrich and Merck and were used without further purification. Calf thymus DNA type I-fibrous ($A_{260}/A_{280} = 1.3$) was purified with phenol-chloroform-isoamyl alcohol procedure until ratio $A_{260}/A_{280} = 1.8$ was obtained and then DNA was precipitated as sodium salt by adding 0.1 M acetate buffer (pH 4.60) and ethanol. The precipitate was dried at 50 °C and kept at 4 °C for further use. Solid nucleic acid of sufficient purity was suspended in 0.1 M Tris-HCl buffer, pH 7.40, and left one day for hydration at 4 °C. The stock solution of CT DNA was prepared in Tris-HCl buffer at pH 7.40 and stored at 4 °C for a maximum of 1-4 days. The concentration of CT DNA solution was calculated on the basis of DNA extinction coefficient $\epsilon = 6,600 \text{ M}^{-1} \cdot \text{cm}^{-1}$.

2.1 Synthesis

2.1.1 General Procedure for Synthesis of Na[Ru(N-R-5-X-salim)₂].0.5 Et₃N

The solution of 0.76 mmol of appropriate N-2-hydroxyphenyl-5-X-salicylideneimine (X = Cl, Br, NO₂) in 50 mL absolute ethanol was mixed with

0.76 mmol triethylamine, afterwards 0.38 mmol RuCl₃·3H₂O in 5 mL absolute ethanol was added. The solution was heated in a rotary evaporator at 60-70 °C during 4 h and then was cooled to room temperature. Small volume of water solution (0.5 mL) containing 0.38 mmol NaCl was added and resulting solution was left over night at ambient temperature. The precipitate was filtered off, washed with cold water, ethanol and petrol ether. Recrystallization was performed from absolute ethanol/dichlorometan (1/1, v/v). The compounds were dried at 80 °C. Yield: 60%-71%.

2.1.2 Na[Ru(N-R-5-Cl-salim)₂].0.5Et₃N

Anal. Calc. for NaRuC₂₆H₁₆Cl₂N₂O₄·0.5C₆H₁₅N (%): C 52.30, H 3.56, N 5.26. Found (%): C 52.32, H 4.15, N 5.55. MALDI-TOF/TOF MS m/z : 591.9534 ([C₂₆H₁₆N₂O₄Cl₂Ru]⁺, 100%). IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$: 1,598 vs (C=N), 1,287 m (C-O_{phen}), 1,033 w (C₂N). IR (CsI) $\nu_{\text{max}}/\text{cm}^{-1}$: 460 w (Ru-N), 387 w (Ru-O). UV/Vis [CH₂Cl₂, $\lambda_{\text{max}}/\text{nm}$]: 344, 395, 580 ($\epsilon/\text{M}^{-1} \cdot \text{cm}^{-1}$): 12,394, 14,457, 5,320. ¹H NMR (nuclear magnetic resonance) Na [Ru(N-R-5-Cl-salim)₂].0.5 C₆H₁₅N (600 MHz, acetone-d₆) δ 10.23 s (4 H_d), 7.94 dd (4 H_c, $J = 8.0$ Hz, 0.5 Hz), 7.82 td (4 H_g, $J = 6.9$ Hz, 1.3 Hz), 7.54 dd (4 H_e, $J = 8.8$ Hz, 2.8 Hz), 7.34 dd (4 H_a, $J = 7.9$ Hz, 0.8 Hz), 7.12 dd (4 H_h, $J = 9.0$ Hz, 2.7 Hz), 6.88 td (4 H_f, $J = 7.5$ Hz, 4.1 Hz), 3.10 q (6 H_j, $J = 4.7$ Hz), 1.18 t (9 H_i, $J = 7.3$ Hz).

2.1.3 Na[Ru(N-R-5-Br-salim)₂].0.5Et₃N

Anal. Calc. for NaRuC₂₆H₁₆Br₂N₂O₄·0.5 C₆H₁₅N (%): C 46.14, H 3.14, N 4.64. Found (%): C 47.46, H 3.84, N 5.04. MALDI-TOF/TOF MS m/z : 681.8532 ([C₂₆H₁₆N₂O₄ Br₂Ru]⁺, (100%). IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$: 1,598 vs. (C=N), 1,286 m (C-O_{phen}), 1,032 w (C₂N). IR (CsI) $\nu_{\text{max}}/\text{cm}^{-1}$: 469 w (Ru-N), 393 w (Ru-O). UV/Vis [CH₂Cl₂, $\lambda_{\text{max}}/\text{nm}$]: 345, 391, 588 ($\epsilon/\text{M}^{-1} \cdot \text{cm}^{-1}$): 13,060, 14,461, 5,150. ¹H NMR Na[Ru(N-R-5-Br-salim)₂].0.5 C₆H₁₅N (600 MHz, acetone-d₆) δ 10.21 s (4 H_d), 7.93 dd (4 H_c, $J = 8.0$ Hz, 0.6 Hz), 7.82 td (4 H_g, $J = 8.2$ Hz, 1.1 Hz), 7.77 dd (4 H_a, $J = 2.5$ Hz, 0.8 Hz), 7.65 dd (4 H_e, $J = 8.8$ Hz, 2.6 Hz), 7.21 dd (4 H_h, $J = 9.0$ Hz, 2.5 Hz), 7.03 td (4 H_f,

$J = 7.9$ Hz, 0.9 Hz), 3.09 q ($6H_j$, $J = 4.4$ Hz), 1.18 t ($9H_i$, $J = 7.3$ Hz).

$2.1.4$ Na[Ru(N-R-5-NO₂-salim)₂] $\cdot 0.5Et_3N$

Anal. Calc. for NaRuC₂₆H₁₆N₄O₈ $\cdot 0.5$ C₆H₁₅N (%): C 50.69, H 3.45, N 9.17. Found (%): C 49.58, H 4.05, N 8.63. MALDI-TOF/TOF MS m/z : 614.0018 ([C₂₆H₁₆N₄O₈Ru]⁺, 100%). IR (KBr) ν_{max}/cm^{-1} : 1,595 vs. (C=N), 1,300 m (C-O_{phen}), 1,033 w (C₂N). IR (CsI) ν_{max}/cm^{-1} : 471 w (Ru-N), 384 w (Ru-O). UV/Vis [CH₂Cl₂, λ_{max}/nm]: 335, 371, 589 ($\epsilon/M^{-1}cm^{-1}$): 17,273, 18,941, 5,137. ¹H NMR Na[Ru(N-R-5-NO₂-salim)₂] $\cdot 0.5$ C₆H₁₅N (600 MHz, acetone-d₆) δ 10.28 s (4 H_d), 9.68 dd (4 H_c, $J = 7.7$ Hz, 0.24 Hz), 8.16 dd (4 H_a, $J = 2.3$ Hz, 0.5 Hz), 8.33 dd (4 H_b, $J = 9.1$ Hz, 2.8 Hz), 8.03 dd (4 H_e, $J = 8.3$ Hz, 0.84 Hz), 7.92 td (4 H_g, $J = 9.3$ Hz, 2.7 Hz), 7.38 dd (4 H_h, $J = 8.52$ Hz, 0.82 Hz), 7.06 td (4 H_f, $J = 8.4$ Hz, 1.3 Hz), 3.10 q ($6H_j$, $J = 7.2$ Hz), 1.18 t ($9H_i$, $J = 7.3$ Hz).

2.2 Physical Measurements

Mass spectra were recorded on a complex 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) equipped with Nd: YAG laser, wavelength of 355 nm. The sample was resuspended in 10 μ L THAP/DAC MALDI matrix (1.9 mg 2,4,6-Trihydroxyacetophenone and 2.3 mg ammonium hydrogen citrate in 1 mL mixed solvent acetonitrile/H₂O, 1/1, v/v) and 1 μ L was set on the MALDI plate. Mass spectra were recorded at 200 Hz in a negative mode, in the range m/z values 10-1,000 Da with focus on the mass 500 Da. As an internal standard in the negative mode, riboflavin and 3-aminosalicylic acid were used.

¹H NMR spectra were recorded on 600 MHz Bruker BioSpin GmbH instrument in acetone-d₆ using TMS (tetramethylsilane) as internal standard.

Infrared spectra were collected on Perkin Elmer BX FT-IR (fourier transform infrared) spectrophotometer as KBr pellets in the region 4,000-400 cm^{-1} and for

detection Ru-N and Ru-O absorptions CsJ pellets were used in the region 600-380 cm^{-1} .

Electronic spectra were recorded on Perkin Elemer UV/visible lambda 35 spectrophotometer in CH₂Cl₂ solution over the range 200-700 nm.

Cyclic voltammograms of Na[Ru(N-R-5-X-salim)₂] $\cdot 0.5Et_3N$ were recorded on an electrochemical workstation Autolab potentiostat/galvanostat (PGSTAT 12) using three electrode system: glassy carbon electrode as working, Ag/AgCl as reference electrode and Pt wire. We used acetonitrile as solvent and tetraethylammonium perchlorate as supporting electrolyte. Electrochemical titrations of Na[Ru(N-R-5-X-salim)₂] $\cdot 0.5Et_3N$ with CT DNA were performed on the same instrument with the same three-electrode system in Tris-HCl buffered water solutions in potential range -1.0 V to -0.1 V. The compounds were initially dissolved in DMSO (dimethyl sulfoxide), afterwards diluted with Tris-HCl buffer, pH = 7.40, in the presence of 150 mM NaCl, up to a total volume of 2 mL. DNA is added in 25 μ L portions and cyclic voltammograms were recorded after each addition of DNA.

Spectroscopic titration of Ru(III) compounds with CT DNA was performed by the successive addition of DNA (six portions of 10 μ L) in 2 mL of the compound solutions at pH 7.40 in the presence of 150 mM NaCl. Spectra were recorded 2 min after addition of the DNA in the range 200-700 nm. Each addition of DNA was compensated in the blank.

3. Results and Discussion

3.1 Synthesis

N-2-hydroxyphenyl-5-substituted-salicylideneimines, N-RH-5-X-salimH, were prepared from absolute ethanol solutions containing equimolar quantities of 2-aminophenol and 5-X-salicylaldehyde where X = Cl, Br, NO₂. Reactions were performed at 50 °C during 20 min. The orange solids were purified by recrystallization from warm absolute ethanol with good yields of 80%-90%.

Sodium bis[N-2-oxyphenyl-5-substituted-salicylideneiminato-ONO]ruthenate(III), $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$, where, $\text{X} = \text{Cl}, \text{Br}$, have been synthesized according to published procedure for complex anions [29], but for the purpose of interaction with DNA, the complex anions were precipitated as sodium salts. The compound containing NO_2 substituted salicylideneimine was prepared as an analogue. The starting compounds $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$ and N-RH-5-X-salimH were used in molar ratio 1:2. Deprotonation of phenolic oxygen atoms from ligands was performed by adding triethylamine in molar ratio 1:2, respectively. Reactions were performed at 60-70 °C for 4 h in rotary evaporator. The precipitation was performed with stoichiometric amounts of NaCl in water solution. The scheme of synthesis is shown in Fig. 1. The solids are stable in air, insoluble in water, soluble in acetonitrile, N,N-DMF (dimethylformamide), acetone, ethyl alcohol, DMSO, dichloromethane.

On the basis on CHN (carbon nitrogen hydrogen) elemental analysis, mass spectra, ^1H NMR, infrared and UV/visible spectroscopic measurements the compounds were formulated as $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$. Mass spectra showed molecular ions (M^-) at m/z (100%) at 591.9534, 681.8532, 614.0018 for $[\text{RuC}_{26}\text{H}_{16}\text{Cl}_2\text{N}_2\text{O}_4]^-$, $[\text{RuC}_{26}\text{H}_{16}\text{Br}_2\text{N}_2\text{O}_4]^-$ and $[\text{RuC}_{26}\text{H}_{16}\text{N}_4\text{O}_8]^-$, respectively.

Ru(III) in octahedral coordination is chelated with two O_2N tridentate Schiff bases through azomethine nitrogen and deprotonated phenolic oxygen atoms. Elemental analysis, IR and ^1H NMR spectra are in accordance with presence of one solvate molecule of triethylamine per two complex anions.

3.2 Spectroscopic Measurements

3.2.1 ^1H NMR Spectra

^1H NMR spectra of $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$ showed absence of singlet in region 10 ppm to 12 ppm

Fig. 1 Synthesis of $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2]$, ($\text{X} = \text{Cl}, \text{Br}, \text{NO}_2$).

corresponding to phenolic hydrogen, consequently confirming that N-2-oxyphenyl-5-X-salicylideneimines bind Ru(III) via deprotonated phenolic oxygen atoms. Singlets, positioned in this region (10.21 ppm to 10.28 ppm), with small variations in the chemical shift (0.09 ppm), correspond to azomethine hydrogen which is slightly sensitive to 5-X substituent on salicylaldehyde. The positions of azomethine singlet correspond to hydrogen bonding of azomethine group with triethylamine. Four doublets of doublets and two triplets of doublets in region 7 ppm to 9 ppm correspond to six aromatic hydrogen atoms with ortho-coupling constants J_{HH} between 7.9 Hz and 9.3 Hz and meta-coupling constants J_{HH} 0.8 Hz to 2.6 Hz. Quartet at 3.1 ppm and triplet at 1.18 ppm confirm methylene and methyl hydrogen respectively in ratio 1:1.5. ^1H NMR data strongly support proposed structures of complexes. Different hydrogen atoms in the structure of $[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$ are shown in Fig. 2.

3.2.2 Infrared Spectra

Infrared spectra of free Schiff bases and $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$ are in accordance with coordination through azomethine nitrogen and

Fig. 2 The structure of $[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$ with different hydrogen atoms which correspond to ^1H NMR spectra.

both phenolic oxygen atoms in Schiff base. The characteristic frequencies of compounds and free ligands are given in Table 1. Azomethine group appears in spectra of free N-RH-5-X-salimH at 1,630-1,629 cm^{-1} while after coordination the frequency of azomethine is significantly shifted toward lower values for 32-29 cm^{-1} and appears in corresponding $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$ compounds at 1,598-1,595 cm^{-1} . Coordination through deprotonated phenolic oxygen caused the shift of C-O absorption towards higher values of wave numbers for 15-19 cm^{-1} as a result of the formation of C-O(Ru) instead of C-O(H) group. Phenolic C-O(H) frequencies appear at 1,272-1,281 cm^{-1} in free ligands, while in $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$ were found at 1,287-1,300 cm^{-1} . The presence of solvate triethylamine molecule is in accordance with weak frequencies corresponding to C_2N asymmetric stretching at 1,033-1,032 cm^{-1} and also with broad absorptions centred on 3,430 cm^{-1} which can be attributed to $\text{Et}_3\text{N} \cdots \text{H}$ (azomethine) secondary interaction. Weak Ru-N and Ru-O frequencies in $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$ were found at 460-471 cm^{-1} and 387-384 cm^{-1} , respectively.

3.2.3 Electronic Spectra

Electronic spectra of free N-RH-5-X-salimH and $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$, recorded in dichloromethane, are in good agreement with the mode of coordination of the ligands. Electronic spectra of free Schiff bases have three or four in the case of X = NO_2 compound, well-defined absorption bands (Table 2). Coordination of Schiff bases significantly changes the position of the $\text{n}(\text{O}) \rightarrow \pi^*$ and $\text{n}(\text{N}) \rightarrow \pi^*$ bands by moving them toward higher and lower wavelength respectively due to bonding trough deprotonated phenolic oxygen and lone pair on

azomethine nitrogen. Two new bands were found in the spectra of complex compounds, LMCT bands in the region 371-395 nm and very weak, broad and poorly defined absorptions in the region 580-589 nm corresponding to $\text{t}_{2g}^5 \rightarrow \text{t}_{2g}^4 \text{e}_g^1$ spin-allowed electron transition of low-spin Ru(III).

3.3 Cyclic Voltammetry

Cyclic voltammograms of $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$ were recorded in acetonitrile in the presence of tetraethylammonium perchlorate as supporting electrolyte using glassy carbon working electrode (Fig. 3).

The ratio i_k/i_a and E_k-E_a separations in cyclic voltammograms indicate quasi-reversible one-electron processes. Cathodic peaks appear in the potential range -883 mV to -915 mV, anodic peaks at -712 mV to -742 mV with peaks separations 141-183 mV. Formal potentials $E_{1/2}$, assigned to Ru(III)/Ru(II) couple, are quite negative varying from -804 mV to -824 mV. The characteristic potentials are given in Table 3. The values are consistent with increased stabilization of Ru(III) through $[\text{RuO}_4\text{N}_2]$ skeleton compared to $[\text{RuO}_2\text{N}_2]$ in the compounds with similar type of ligand [28].

3.4 Calf Thymus DNA Binding

3.4.1 Spectroscopic Evidence for Intercalation

Study of the interaction of metal compounds with DNA is an important step in the evaluation of the biological properties of the compounds. Many metal complexes with fused and planar aromatic ligands, or aromatic heterocyclic rings, are described as DNA intercalators. In addition, external minor or major groove binding or external binding to the phosphate backbone is possible. Spectrophotometric titration of

Table 1 IR frequencies for $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$ and N-RH-5-X-salimH.

X	$\nu(\text{CH}=\text{N}) (\text{cm}^{-1})$	$\nu(\text{C-O}) (\text{cm}^{-1})$	$\nu(\text{Ru-N}) (\text{cm}^{-1})$	$\nu(\text{Ru-O}) (\text{cm}^{-1})$
Cl	1,598 (1,630)	1,287 (1,272)	460 (-)	387 (-)
Br	1,598 (1,629)	1,286 (1,270)	469 (-)	387 (-)
NO_2	1,595 (1,629)	1,300 (1,281)	471 (-)	384 (-)

Values in parentheses refer to the free ligand N-RH-5-X-salimH.

Table 2 Electronic spectra of $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$ and N-RH-5-X-salimH .

X	$\lambda[\pi \rightarrow \pi^*]$ (nm)	$\lambda[n(\text{O}) \rightarrow \pi^*]$ (nm)	$\lambda[n(\text{N}) \rightarrow \pi^*]$ (nm)	$\lambda[\text{LMCT}]$ (nm)
Cl	230 (230)	296 (269)	344 (366)	395 (-)
Br	230 (230)	297 (269)	345 (366)	391 (-)
NO_2	233 (229)	295 (266)	335 (347)	371 (-)

Values in parentheses refer to the free ligand N-RH-5-X-salimH .

Fig. 3 Cyclic voltammograms of $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$ ($\text{X} = \text{Cl}, \text{Br}, \text{NO}_2$) in acetonitrile/tetraethylammonium perchlorate; working electrode GCE vs. Ag/AgCl ; scan rate $300 \text{ mV} \cdot \text{s}^{-1}$.**Table 3** The potentials of $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$.

X	E_k (mV)	E_a (mV)	$E_{1/2}$ (mV)	ΔE_p (mV)
Cl	-895	-712	-804	183
Br	-883	-742	-812	141
NO_2	-915	-734	-824	181

metal complex with DNA is a useful method to estimate binding mode and calculate binding constant K_b . In this work, spectroscopic study of interaction of $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$ with CT DNA was carried out by titrating a fixed amount of the complex with increasing amount of calf thymus CT DNA. The $[\text{DNA}]/[\text{complex}]$ ratio was between 0-2.28 for chloro, 0-1.54 for bromo- and 0-2.30 for NO_2 derivative. The binding constants K_b were calculated on the basis of Eq. (1) [30]:

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

where, ε_a , ε_f and ε_b represent extinction coefficients for particular measurements ($A_{\text{obs}}/[\text{DNA}]$), free complex and completely bound form, respectively. The binding constant K_b is obtained as the ratio of the slope and Spectroscopic titration and graphical calculation of

intercept in graph $[\text{DNA}]/(\varepsilon_a - \varepsilon_f)$ vs. $[\text{DNA}]$. binding constant are shown in Figs. 4 and 5. Apparent hypochromism in the region of LMCT absorptions and values of K_b ($2.06\text{-}3.85 \times 10^4 \text{ M}^{-1}$) designate $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$ as moderate DNA-intercalators. The binding constants are given in Table 4. The impact of 5-X-substituent on intercalative

Fig. 4 The spectroscopic titration of $31.5 \mu\text{M}$ $\text{Na}[\text{Ru}(\text{N-R-5-Br-salim})_2] \cdot 0.5\text{Et}_3\text{N}$ with increasing concentration of CT DNA ($0\text{-}49 \mu\text{M}$) in Tris-HCl buffer, pH 7.40 and 150 mM NaCl. **Fig. 5** Plot of $(\varepsilon_f - \varepsilon_a)$ vs. $[\text{DNA}]/[\text{DNA}]$.

Table 4 The binding constants of $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$ to CT DNA.

X	$10^4 K_b (\text{M}^{-1})$
Cl	2.06
Br	2.18
NO_2	3.85

Fig. 6 Electrochemical evidence for intercalation $29 \mu\text{M}$ $\text{Na}[\text{Ru}(\text{N-R-5-Cl-salim})_2] \cdot 0.5\text{Et}_3\text{N}$ in the presence of increasing concentration of CT DNA ($0\text{--}50 \mu\text{M}$) under physiological condition: Tris-HCl, pH 7.40, 150 mM NaCl; working electrode GCE vs. Ag/AgCl; scan rate $800 \text{ mV} \cdot \text{s}^{-1}$; step potential 50 mV.

activities of the compounds correlates with electron withdrawing properties and ability of substituent to decrease electron density from π -system making it less nucleophilic.

3.4.2 Electrochemical Evidence for Intercalation

Cyclic voltammetry is also a useful technique available for studying the interaction of metal complexes with DNA. Procedure is carried out by successive adding of μL -volumes of DNA to the solution containing fixed amount of $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$. Intercalation is followed by the current decrease and shift of cathodic and anodic peaks towards more positive values of potentials, though the peaks are poorly visible in aqueous solution due to insolubility of compounds in water (Fig. 6). The molar ratio r $[\text{DNA}]/[\text{complex}]$ was within the range 0–1.8 for $\text{X} = \text{Cl}$, and 0–1.5 for $\text{X} = \text{Br}, \text{NO}_2$.

4. Conclusions

We reported here on synthesis of Ru(III)

compounds with 5-X substituted salicylideneimine ligands derived from salicylaldehyde and 2-aminophenol. The compounds of the general formula $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$ were synthesized and characterized by elemental analysis, different spectroscopic techniques and cyclic voltammetry. Ruthenium is chelated by two tridentate Schiff base in RuO_4N_2 octahedral skeleton. The interaction with CT DNA was studied by spectrophotometric titration and cyclic voltammetry indicating capability of compounds to intercalate CT DNA. The quantification of affinity of each compound toward DNA was done by binding constants, $K_b = 2.06\text{--}3.85 \times 10^4 \text{ M}^{-1}$ which correspond to intercalative mode of binding. The absorption spectral bands at LMCT region showed hypochromism in the presence of increasing amount of DNA. The lack of measurable evidence for redshift is in accordance with values of binding constants and moderate intercalating properties of the compounds. Substituent X in $\text{Na}[\text{Ru}(\text{N-R-5-X-salim})_2] \cdot 0.5\text{Et}_3\text{N}$ affects an increase of binding constants with increasing electron withdrawing properties of substituent in order $\text{Cl} \sim \text{Br} < \text{NO}_2$.

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References

- [1] Hartmann, M.; Keppler, B. K. Inorganic Anticancer Agents: Their Chemistry and Antitumor Properties. *Comments Inorg. Chem.* **1995**, *16*(6), 339–372.
- [2] Keppler, B. K. Metal Complexes as Anticancer Agents—The Future Role of Inorganic Chemistry in Cancer Therapy. *New J. Chem.* **1990**, *14*(6–7), 389–403.
- [3] Keppler, B. K.; Berger, M. R.; Klenner, T.; Heim, M. E. Metal Complexes as Antitumor Agents. *Adv. Drug Res.* **1990**, *19*, 243–310.
- [4] Alessio, E.; Mestroni, G.; Bergamo, A.; Sava, G. Ruthenium Anticancer Drugs. *Met. Ions Biol. Syst.* **2004**, *42*, 323–352.
- [5] Sava, G.; Pacor, S.; Mestroni, G.; Alessio, E. $\text{Na}[\text{trans-RuCl}_4(\text{DMSO})\text{Im}]$, a Metal Complex of

- Ruthenium with Antimetastatic Properties. *Clin. Exp. Metastasis* **1992**, *10*(4), 273-280.
- [6] Alessio, E.; Mestroni, G.; Bergamo, A.; Sava, G. Ruthenium Antimetastatic Agents. *Curr. Top. Med. Chem.* **2004**, *4*(15), 1525-1535.
- [7] Van, R. S. H.; Sadler, P. J. Current Applications and Future Potential for Bioinorganic Chemistry in the Development of Anticancer Drugs. *Drug Discovery Today* **2009**, *14*(23), 1089-1097.
- [8] Bratsos, I.; Jedner, S.; Gianferrara, T.; Alessio, E. Ruthenium Anticancer Compounds: Challenges and Expectations. *Chimia Int. J. Chem.* **2007**, *61*(11), 692-697.
- [9] Tominaga, K. I.; Sasaki, Y. Ruthenium Complex-Catalyzed Hydroformylation of Alkenes with Carbon Dioxide. *Catal. Commun.* **2000**, *1*(1), 1-3.
- [10] Karvembu, R.; Hemalatha, S.; Prabhakaran, R.; Natarajan, K. Synthesis, Characterization and Catalytic Activities of Ruthenium Complexes Containing Triphenylphosphine/Triphenylarsine and Tetradentate Schiff Bases. *Inorg. Chem. Commun.* **2003**, *6*(5), 486-490.
- [11] Cozzi, P. G. Metal-Salen Schiff Base Complexes in Catalysis: Practical Aspects. *Chem. Soc. Rev.* **2004**, *33*(7), 410-421.
- [12] Cheung, K. C.; Wong, W. L.; Ma, D. L.; Lai, T. S.; Wong, K. Y. Transition Metal Complexes as Electrocatalysts—Development and Applications in Electro-Oxidation Reactions. *Coord. Chem. Rev.* **2007**, *251*(17), 2367-2385.
- [13] Turkušić, E.; Kahrović, E. Development of New Low Potential Amperometric Sensor for L-cysteine Based on Carbon Ink Modification by Tetraethylammonium Dichloro-bis[N-phenyl-5-bromosalicylideniminato-N,O]ruthenate(III). *TTEM* **2012**, *7*(3), 1300-1303.
- [14] Kahrović, E.; Turkušić, E. New Ruthenium Complexes with Schiff Bases as Mediators for the Low Potential Amperometric Determination of Ascorbic Acid, Part II: Voltametric and Amperometric Evidence of Mediation with Bromo-derivative of Tetraethylammonium Dichloro-bis[N-phenyl-5-halogeno-salicylideniminato-N,O] Ruthenate(III). *HealthMED* **2012**, *6*(3), 1046-1049.
- [15] Guimaraes, R. R.; Parussulo, A. L.; Toma, H. E.; Araki, K. New Tunable Ruthenium Complex Dyes for TiO₂ Solar Cells. *Inorg. Chim. Acta* **2013**, *404*, 23-28.
- [16] Messori, L.; Marcon, G.; Innocenti, A.; Gallori, E.; Franchi, M.; Orioli, P. Molecular Recognition of Metal Complexes by DNA: A Comparative Study of the Interactions of the Parent Complexes [PtCl(TERPY)]Cl and [AuCl(TERPY)]Cl₂ with Double Stranded DNA. *Bioinorg. Chem. Appl.* **2005**, *3*(3-4), 239-253.
- [17] Patel, K. K.; Plummer, E. A.; Darwish, M.; Rodger, A.; Hannon, M. J. Aryl Substituted Ruthenium Bis-terpyridine Complexes: Intercalation and Groove Binding with DNA. *J. Inorg. Biochem.* **2002**, *91*(1), 220-229.
- [18] Önfelt, B.; Lincoln, P.; Nordén, B. Enantioselective DNA Threading Dynamics by Phenazine-Linked [Ru(phen)2dppz]²⁺ Dimers. *J. Am. Chem. Soc.* **2001**, *123*(16), 3630-3637.
- [19] Wang, X. L.; Chao, H.; Li, H.; Hong, X. L.; Liu, Y. J.; Tan, L. F.; et al. DNA Interactions of Cobalt(III) Mixed-Polypyridyl Complexes Containing Asymmetric Ligands. *J. Inorg. Biochem.* **2004**, *98*(6), 1143-1150.
- [20] Mahadevan, S.; Palaniandavar, M. Spectral and Electrochemical Behavior of Copper(II)-Phenanthrolines Bound to Calf Thymus DNA. [(5,6-dimethyl-OP)2Cu]²⁺ (5,6-dimethyl-OP = 5,6-dimethyl-1,10-phenanthroline) Induces a Conformational Transition from B to Z DNA. *Inorg. Chem.* **1998**, *37*(16), 3927-3934.
- [21] Maheswari, P. U.; Palaniandavar, M. DNA Binding and Cleavage Activity of [Ru(NH₃)₄(diimine)]Cl₂ Complexes. *Inorg. Chim. Acta* **2004**, *357*(4), 901-912.
- [22] Barton, J. K.; Danishefsky, A.; Goldberg, J. Tris(phenanthroline) Ruthenium(II): Stereoselectivity in Binding to DNA. *J. Am. Chem. Soc.* **1984**, *106*(7), 2172-2176.
- [23] Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B. Tris(phenanthroline) Ruthenium(II) Enantiomer Interactions with DNA: Mode and Specificity of Binding. *Biochem* **1993**, *32*(10), 2573-2584.
- [24] Morgan, R. J.; Chatterjee, S.; Baker, A. D.; Strekas, T. C. Effects of Ligand Planarity and Peripheral Charge on Intercalative Binding of Ru(2,2'-bipyridine)₂L₂⁺ to Calf Thymus DNA. *Inorg. Chem.* **1991**, *30*(12), 2687-2692.
- [25] Dharmaraj, N.; Viswanathamurthi, P.; Natarajan, K. Ruthenium(II) Complexes Containing Bidentate Schiff Bases and Their Antifungal Activity. *Trans. Metal Chem.* **2001**, *26*(1-2), 105-109.
- [26] Ramesh, R.; Maheswaran, S. Synthesis, Spectra, Dioxygen Affinity and Antifungal Activity of Ru(III) Schiff Base Complexes. *J. Inorg. Biochem.* **2003**, *96*(4), 457-462.
- [27] Jayabalakrishnan, C.; Natarajan, K. Ruthenium(II) Carbonyl Complexes with Tridentate Schiff Bases and Their Antibacterial Activity. *Trans. Metal Chem.* **2002**, *27*(1), 75-79.
- [28] Ljubijankić, N.; Zahirović, A.; Turkušić, E.; Kahrović, E. DNA Binding Properties of Two Ruthenium(III) Complexes Containing Schiff Bases Derived from Salicylaldehyde: Spectroscopic and Electrochemical Evidence of CT DNA Intercalation. *Croat. Chem. Acta* **2013**, *86*(2), 215-222.
- [29] Kahrović, E.; Dehari, S.; Dehari, D.; Reçi, H.; Begić, S.;

Ljubijankić, N. Synthesis and Characterization of New Ru(III) Complexes with Monobasic (NO) and Dibasic (ONO) Schiff Bases Derived from Salicylaldehydes. *TTEM* **2010**, 5(4), 799-803.

[30] Pyle, A. M.; Rehmann, J. P.; Meshoyrer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K. Mixed-ligand Complexes of Ruthenium(II): Factors Governing Binding to DNA. *J. Am. Chem. Soc.* **1989**, 111(8), 3051-3058.