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-salicylideneiminate-ONO]ruthenate(III) hemi-triethylamine compounds of general formula Na[Ru(N-R-5-X-salim)]₂·0.5Et₃N, where, R = C₆H₄O, X = Cl, Br, NO₂, salim = salicylideneiminate, Et₃N 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 voltamograms. Ru(III) is chelated with two O₂N 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 \( K_b = 2.06-3.85 \times 10^4 \text{ M}^{-1} \). 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 interdisciplin ary fields of science due to many significant properties, especially anticancer and antimitomastic 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 \( \pi \)-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...
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 salicylidenimine 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 salicylidenimine 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 $\varepsilon = 6,600$ M$^{-1}$·cm$^{-1}$.

2.1 Synthesis

2.1.1 General Procedure for Synthesis of Na[Ru(N-R-5-X-salim)$_2$]·0.5Et$_3$N

The solution of 0.76 mmol of appropriate N-2-hydroxyphenyl-5-X-salicylideneimine ($X = \text{Cl, Br, NO}_2$) in 50 mL absolute ethanol was mixed with 0.76 mmol triethylamine, afterwards 0.38 mmol RuCl$_3$·3H$_2$O 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/dichloromethane (1/1, v/v). The compounds were dried at 80 °C. Yield: 60%-71%.

2.1.2 Na[Ru(N-R-5-Cl-salim)$_2$]·0.5Et$_3$N

Anal. Calc. for NaRuC$_{26}$H$_{16}$Cl$_2$N$_2$O$_4$·0.5C$_6$H$_{15}$N (%): C 52.30, H 3.56, N 5.26. Found (%): C 52.32, H 4.15, N 5.55. MALDI-TOF/TOF MS $m/z$: 595.9354 ([C$_{26}$H$_{16}$N$_2$O$_4$Cl$_2$Ru]$^-$, 100%). IR (KBr) $\nu_{\text{max}}$/cm$^{-1}$: 1,598 vs (C=N), 1,287 m (C-O phen), 1,033 w (C$_2$N). IR (CsI) $\nu_{\text{max}}$/cm$^{-1}$: 460 w (Ru-N), 387 w (Ru-O). UV/Vis [CH$_2$Cl$_2$, $\lambda_{\text{max}}$/nm]: 344, 395, 580 ($\varepsilon$/M$^{-1}$·cm$^{-1}$): 12,394, 14,457, 5,320. $^1$H NMR (nuclear magnetic resonance) Na[Ru(N-R-5-Cl-salim)$_2$]·0.5C$_6$H$_{15}$N (600 MHz, acetone-d6) $\delta$ 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$_d$, $J = 7.9$ 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)$_2$]·0.5Et$_3$N

Anal. Calc. for NaRuC$_{26}$H$_{16}$Br$_2$N$_2$O$_4$·0.5C$_6$H$_{15}$N (%): 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 ([C$_{26}$H$_{16}$N$_2$O$_4$Br$_2$Ru]$^-$, 100%). IR (KBr) $\nu_{\text{max}}$/cm$^{-1}$: 1,598 vs. (C=N), 1,286 m (C-O phen), 1,032 w (C$_2$N). IR (CsI) $\nu_{\text{max}}$/cm$^{-1}$: 469 w (Ru-N), 393 w (Ru-O). UV/Vis [CH$_2$Cl$_2$, $\lambda_{\text{max}}$/nm]: 345, 391, 588 ($\varepsilon$/M$^{-1}$·cm$^{-1}$): 13,060, 14,461, 5,150. $^1$H NMR Na[Ru(N-R-5-Br-salim)$_2$]·0.5C$_6$H$_{15}$N (600 MHz, acetone-d6) $\delta$ 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$_e$, $J = 2.5$ Hz, 0.8 Hz), 7.65 dd (4 H$_d$, $J = 8.8$ Hz, 2.6 Hz), 7.21 dd (4 H$_h$, $J = 9.0$ Hz, 2.5 Hz), 7.03 td (4 H$_f$,
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3.1 Synthesis

N-2-hydroxyphenyl-5-substituted-salicylideneamines, 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), Na[Ru(N-R-5-X-salim)₂]·0.5Et₃N, where, X = Cl, 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 NO₂ substituted salicyldenedimine was prepared as an analogue. The starting compounds RuCl₃·3H₂O 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, dichlormethane.

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 Na[Ru(N-R-5-X-salim)₂]·0.5Et₃N. Mass spectra showed molecular ions (M⁺) at m/z (100%) at 591.9534, 681.8532, 614.0018 for [RuC₂₆H₁₆Cl₂N₂O₄]⁻, [RuC₂₆H₁₆Br₂N₂O₄]⁻ and [RuC₂₆H₁₆N₂O₆]⁻, respectively.

Ru(III) in octahedral coordination is chelated with two O₂N tridentate Schiff bases trough 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 Na[Ru(N-R-5-X-salim)₂]·0.5Et₃N showed absence of singlet in region 10 ppm to 12 ppm 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 metha-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 [Ru(N-R-5-X-salim)₂]⁻·0.5Et₃N are shown in Fig. 2.

3.2.2 Infrared Spectra

Infrared spectra of free Schiff bases and Na[Ru(N-R-5-X-salim)₂]·0.5Et₃N are in accordance with coordination trough azomethine nitrogen and deprotonated phenolic oxygen atoms, respectively.
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 Na[Ru(N-R-5-X-salim)\(_2\)]\(\cdot\)0.5Et\(_3\)N compounds at 1,598-1,595 cm\(^{-1}\). Coordination through deprotoned 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 Na[Ru(N-R-5-X-salim)\(_2\)]\(\cdot\)0.5Et\(_3\)N were found at 1,287-1,300 cm\(^{-1}\). The presence of solvate triethylamine molecule is in accordance with weak frequencies corresponding to \(\nu_2\) 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 Et\(_3\)N…H (azomethine) secondary interaction. Weak Ru-N and Ru-O frequencies in Na[Ru(N-R-5-X-salim)\(_2\)]\(\cdot\)0.5Et\(_3\)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 Na[Ru(N-R-5-X-salim)\(_2\)]\(\cdot\)0.5Et\(_3\)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 \(\nu(O)\rightarrow\pi^*\) and n(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 \(t_2g^5\rightarrow\pi^*_g\) spin-allowed electron transition of low-spin Ru(III).

3.3 Cyclic Voltammetry

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

The ratio \(i_s/i_a\) 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 [RuO\(_2\)N\(_2\)] skeleton compared to [RuO\(_2\)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 Na[Ru(N-R-5-X-salim)\(_2\)]\(\cdot\)0.5Et\(_3\)N and N-RH-5-X-salimH. |
|----------|-------------------|-------------------|-------------------|-------------------|
| X        | \(\nu(CH=N)\) (cm\(^{-1}\)) | \(\nu(C-O)\) (cm\(^{-1}\)) | \(\nu(Ru-N)\) (cm\(^{-1}\)) | \(\nu(Ru-O)\) (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 Na[Ru(N-R-5-X-salim)2]·0.5Et3N and N-RH-5-X-salimH.

| X    | λ[π→π*] (nm) | λ[n(O)→π*] (nm) | λ[n(N)→π*] (nm) | λ[LMCT] (nm) |
|------|--------------|-----------------|-----------------|--------------|
| Cl   | 230 (230)    | 296 (269)       | 344 (366)       | 395 (-)      |
| Br   | 230 (230)    | 297 (269)       | 345 (366)       | 391 (-)      |
| NO2  | 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 Na[Ru(N-R-5-X-salim)2]·0.5Et3N (X = Cl, Br, NO2) in acetonitrile/tetraethylammonium perchlorate; working electrode GCE vs. Ag/AgCl; scan rate 300 mV·s⁻¹.

Table 3: The potentials of Na[Ru(N-R-5-X-salim)2]·0.5Et3N.

| X    | $E_a$ (mV) | $E_f$ (mV) | $E_{1/2}$ (mV) | $AE_{pr}$ (mV) |
|------|------------|------------|---------------|---------------|
| Cl   | -895       | -712       | -804          | 183           |
| Br   | -883       | -742       | -812          | 141           |
| NO2  | -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 Na[Ru(N-R-5-X-salim)2]·0.5Et3N with CT DNA was carried out by titrating a fixed amount of the complex with increasing amount of calf thymus CT DNA. The [DNA]/[complex] ratio was between 0-2.28 for chloro, 0-1.54 for bromo- and 0-2.30 for NO2 derivative. The binding constants $K_b$ were calculated on the basis of Eq. (1) [30]:

$$\frac{[\text{DNA}]}{(e_a - e_f)} = \frac{[\text{DNA}]}{(e_b - e_f)} + \frac{1}{K_b(e_a - e_f)}$$

where, $e_a$, $e_f$ and $e_b$ represent extinction coefficients for particular measurements ($\lambda_{obs}/[\text{DNA}]$), free complex and completely bound form, respectively. The binding constant $K_b$ is obtained as the ratio of the slope and intercept in graph $[\text{DNA}]/(e_a - e_f)$ vs. [DNA]. Binding constants are shown in Figs. 4 and 5. Apparent hypochromism in the region of LMCT absorptions and values of $K_b$ (2.06-3.85 × 10⁴ M⁻¹) designate Na[Ru(N-R-5-X-salim)2]·0.5Et3N as moderate DNA-intercalators. The binding constants are given in Table 4. The impact of 5-X-substituent on intercalative
Table 4  The binding constants of Na[Ru(N-R-5-X-salim)₂]·0.5Et₃N to CT DNA.

| X   | 10⁴ K_b (M⁻¹) |
|-----|---------------|
| Cl  | 2.06          |
| Br  | 2.18          |
| NO₂ | 3.85          |

Fig. 6  Electrochemical evidence for intercalation 29 μM Na[Ru(N-R-5-Cl-salim)₂]·0.5Et₃N in the presence of increasing concentration of CT DNA (0-50 μM) under physiological condition: Tris-HCl, pH 7.40, 150 mM NaCl; working electrode GCE vs. Ag/AgCl; scan rate 800 mV·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 Na[Ru(N-R-5-X-salim)₂]·0.5Et₃N. 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 [DNA]/[complex] was within the range 0-1.8 for X = Cl, and 0-1.5 for X= Br, NO₂.

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 Na[Ru(N-R-5-X-salim)₂]·0.5Et₃N were synthesized and characterized by elemental analysis, different spectroscopic techniques and cyclic voltammetry. Ruthenium is chelated by two tridentate Schiff base in RuO₄N₂ 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 = 2.06-3.85 × 10⁴ 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 Na[Ru(N-R-5-X-salim)₂]·0.5Et₃N affects an increase of binding constants with increasing electron withdrawing properties of substituent in order Cl ~ Br < NO₂.

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