Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2014

43

5-10

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: _____

Original Scientific Paper

Spectrophotometric determination of binding constants of Ru(III) salicylideneimine complexes with CT DNA

Ljubijankić S., Zahirović A., Memišević M., Ljubijankić N., Kahrović E.*

Faculty of Science, Department of Chemistry, Zmaja od Bosne 33-35, Sarajevo, Bosnia-Herzegovina

Article info
Received: 19/11/2014
Accepted: 07/12/2014

Keywords:
ruthenium(III),
Schiff bases,
CT DNA,
spectroscopic titration,
salicylideneimine

***Corresponding author**:
Emira Kahrović
E-mail: emira_kahrovic@yahoo.com
Phone: 033 279 910

Abstract: The interactions of Ru(III) complexes with Schiff bases derived from salicylaldehyde and aminophenol, butylamine and naphthylamine with general formula Na[Ru(N-R-5-X-salim)$_2$] (R = C_6H_4O, X = H, Cl, Br, NO_2), Na[$RuCl_2$(N-R-5-X-salim)$_2$] (R = C_4H_9, X = H, Cl, Br, NO_2) and [Ru(N-R-5-X-salim)$_3$] (R = $C_{10}H_7$, X = H, Cl, Br) with CT DNA, were investigated by spectroscopic titration. Experimental data show that Ru(III) complexes with salicylideneimine bind CT DNA with constants of 10^4 M^{-1}. The results indicate the influence of 5-X-substituents on K_b values.

INTRODUCTION

Ruthenium as a coordination center is interesting due to a number of reasons, especially because its complexes can act as catalysts, electron-transfer mediators or anticancer agents (Bruijnincx and Sadler, 2008; Keppler *et all* 1989) depending on a coordination environment (Bratsos *et all*, 2007). Schiff bases are organic ligands with many suitable properties that qualify them as excellent candidates for the design and development of novel metal complexes. The properties of Schiff bases, such as antibacterial, antiviral and anticancer ones are much more extensive when in metal complexes (Drozdzak *et all*, 2005; Muray *et all*, 1978). In addition, Schiff bases are able to tune the redox potentials of metal center in complex compounds. Some ruthenium complexes with salicylaldimine showed significant biological activity against some bacteria, in contrast to the less active free ligands[6] (Chittilappilly and Yusuff, 2008). For design and development of metal-based drugs (Kahrović, 2011), the activation mode and transport in biological environment is essential. There are many proofs that biologically active ruthenium compounds with anticancer properties can be activated either by hydrolysis or by reduction "*in situ*". It is generally thought that DNA is main target molecule for metal-based chemotherapeutic agents (Piggot *et all*, 2004).

The aim of this study was investigation of interaction of Ru(III) salicylaldimine complexes with CT DNA. The compounds are formulated by general formula Na[Ru(N-R-5-X-salim)$_2$] (R = C_6H_4O, X = H, Cl, Br, NO_2), Na[$RuCl_2$(N-R-5-X-salim)$_2$] (R = C_4H_9, X = H, Cl, Br, NO_2) and [Ru(N-R-5-X-salim)$_3$] (R = $C_{10}H_7$, X = H, Cl, Br).

EXPERIMENTAL

Methods and materials

All chemicals were purchased from commercial sources and used without further purification. Calf thymus DNA (CT DNA) was obtained from Merck (Type I, highly polymerized) and was purified using phenol-chloroform-isoamyl alcohol extraction until satisfactory A_{260}/A_{280}=1.8 ratio was achieved. The stock solution of CT DNA (c = $2.21 \cdot 10^{-3}$ molL^{-1}) was prepared in Tris-HCl buffer at pH 7.4 and stored at 4 °C for 1−4 days. The concentration of

DNA was calculated based on extinction coefficient 6600 M^{-1} cm^{-1} at 260 nm wavelength (Meadows *et all*, 1993). The complexes of general formula Na[Ru(N-R-5-X-salim)$_2$] (R = C_6H_4O, X = H, Cl, Br, NO_2), Na[$RuCl_2$(N-R-5-X-salim)$_2$] (R =C_4H_9, X = H, Cl, Br, NO_2) and [Ru(N-R-5-X-salim)$_3$] (R = $C_{10}H_7$, X = H, Cl, Br) were prepared according to published procedures (Ljubijankić *et all*, 2013; Kahrović, 2014). The purity of Ru(III) compounds was checked by infrared spectroscopy. K_b values were calculated on the basis of spectroscopic titrations of Ru(III) complexes with fixed concentrations, while increasing concentration of CT DNA. The titrations were performed in Tris-HCl buffer pH 7.42, c = 0.1 $molL^{-1}$ in presence of NaCl, c = 0.15 $molL^{-1}$. The complexes were initially dissolved in small volume of DMSO (420-1745 μL) due to insolubility in water and solutions were diluted with Tris-HCl buffer, to 2 mL giving the concentrations about 2 · 10^{-5} $molL^{-1}$. The titrations were performed by addition of 5μL-volumes of CT DNA solution, compensated in the blank, to the metal complexes solutions. The molar ratio of Ru(III) compounds and DNA were in the range of 0 - 3.85. Each titration was repeated three times.

RESULTS AND DISCUSSION

Interaction of metal complex compounds with DNA is significant initial signal about potential biological activity of a tested compound. Activity of ruthenium compounds toward DNA, as a key target for anticancer drugs, may originate from either covalent binding to DNA or non-covalent interaction. In the case of covalent interaction, the complex compounds are able to bind nucleobases, while non-covalent binding comprises electrostatic interaction of positively charged species with phosphate backbone, intercalation or groove binding. The mode of interaction depends on metal center and ligands. Schiff bases derived from 5-X-salicylaldehyde and different amines are chelating ligands, that are able to stabilize Ru(III) in solutions. Spectroscopic study of interaction of Na[Ru(N-R-5-X-salim)$_2$] (R = C_6H_4O, X = H, Cl, Br, NO_2), [Ru(N-R-5-X-salim)$_3$] (R = $C_{10}H_7$, X = H, Cl, Br) and Na[$RuCl_2$(N-R-5-X-salim)$_2$] (R = C_4H_9, X = H, Cl, Br, NO_2) with CT DNA has been performed by titration of fixed concentration of complex compounds with increasing concentrations of calf thymus DNA (CT DNA) in the [DNA] / [complex] ratio range of 0−3.85. The binding constant, K_b were calculated using following equation (Bratsos *et all*, 2007) :

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

ε_a, ε_f and ε_b represent apparent extinction coefficients for particular measurements (A_{obs} / [DNA]), free complex and completely bound form, respectively. By plotting [DNA] / ($\varepsilon_a - \varepsilon_f$) vs [DNA], K_b is obtained as the ratio of the slope and intercept.

Figures 1-3 show the titrations of the selected complexes from three series of compounds, Na[Ru(N-C_6H_4O-5-X-salim)$_2$], [Ru(N- $C_{10}H_7$-5-X-salim)$_3$] and Na[$RuCl_2$(N-C_4H_9-5-X-salim)$_2$] with CT DNA.

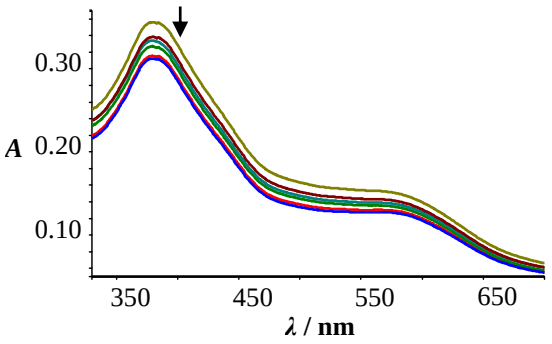


Figure 1. Spectrophotometric titration of Na[Ru(N-C_6H_4O-5-H-salim)$_2$], c = 2.21·10^{-5} $molL^{-1}$, with increasing concentration of CT DNA (stock solution, c = 2.21·10^{-3} $molL^{-1}$) in Tris-HCl buffer pH 7.42, c = 0.1 $molL^{-1}$ in the presence of NaCl, c = 0.15 $molL^{-1}$

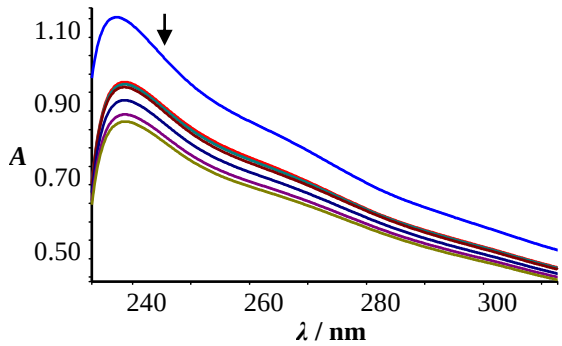


Figure 2. Spectrophotometric titration of [Ru(N-$C_{10}H_7$-5-H-salim)$_3$], c = 2.20·10^{-5} $molL^{-1}$, with increasing concentration of CT DNA (stock solution, c = 2.21·10^{-3} $molL^{-1}$) in Tris-HCl buffer pH 7.42, c = 0.1 $molL^{-1}$ in the presence of NaCl, c = 0.15 $molL^{-1}$

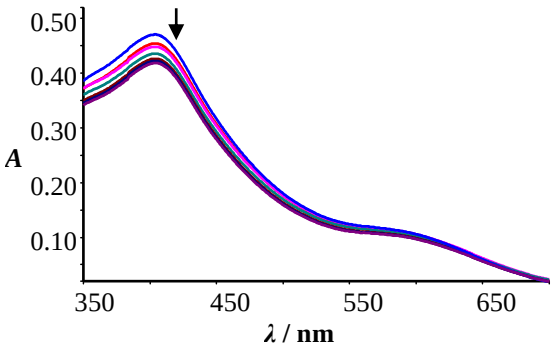
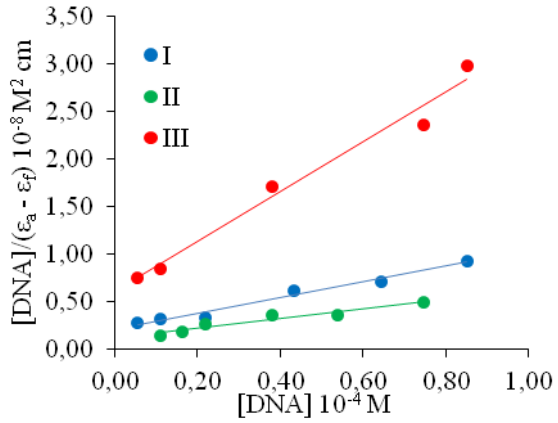


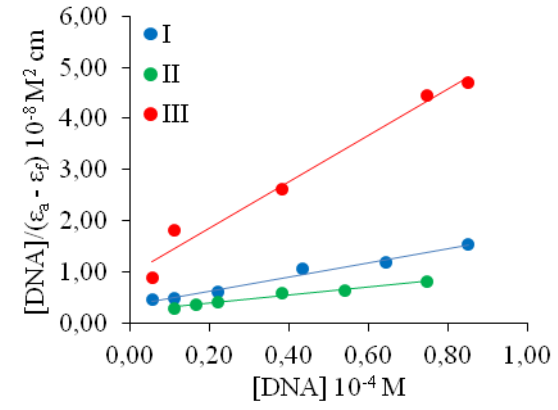
Figure 3. Spectrophotometric titration of Na[$RuCl_2$(N-C_4H_9-5-H-salim)$_2$], c = 2.19·10^{-5} $molL^{-1}$, with increasing concentration of CT DNA (stock solution, c = 2.21·10^{-3} $molL^{-1}$) in Tris-HCl buffer pH 7.42, c = 0.1 $molL^{-1}$ in the presence of NaCl, c = 0.15 $molL^{-1}$

The spectroscopic results of titrations in the regions known as LMCT (ligand → metal charge transfer) or in the region of aromatic π → π* electronic transitions showed hypochromic effect and bathochromic shifts (0-2 nm), suggesting non-covalent interaction of Ru(III) salicylideneimine complexes with DNA. Since the red shift might be an indication of the mode of binding to DNA, the lack or negligible red shift in presented measurements suggests very weak intercalative mode or rather major groove binding. Table 1. Gives all data, significant for the procedure of K_b determination, for selected $Na[Ru(N\text{-}C_6H_4O\text{-}5\text{-}H\text{-}salim)_2]$.

Table 1: Determination of binding constant of $Na[Ru(N\text{-}C_6H_4O\text{-}5\text{-}H\text{-}salim)_2]$ with CT DNA at two wavelengths with repeated measurements

λ / nm	#	V_{DNA} μL	[complex] 10^{-5} M	[DNA] / [complex]	[DNA]/($\varepsilon_a - \varepsilon_f$) 10^{-8} M^2 cm	Graphical determination of binding constant
380	I	0	2.21	0.00	-	
		5	2.20	0.25	2.74	
		10	2.20	0.50	3.10	
		20	2.19	0.99	3.35	
		40	2.17	1.96	6.15	
		60	2.15	2.91	7.13	
		80	2.13	3.85	9.31	
	II	0	2.21	0.00	-	
		10	2.20	0.50	1.32	
		15	2.20	0.74	1.84	
		20	2.19	0.99	2.58	
		35	2.17	1.72	3.53	
		50	2.15	2.44	3.58	
		70	2.13	3.38	4.91	
	III	0	2.21	0.00	-	
		5	2.20	0.25	7.55	
		10	2.20	0.50	8.48	
		35	2.17	1.72	17.2	
		70	2.14	3.38	23.6	
		80	2.13	3.85	29.9	

#	Slope 10^{-4} M cm	Intercept 10^{-8} M^2 cm	r^2	K_b / 10^4 M^{-1}
I	8.35	2.08	0.980	4.01
II	5.16	1.10	0.935	4.68
III	2.64	6.04	0.979	4.37
$K_b \pm \sigma$ / 10^4 M^{-1}				**4.35 ± 0.34**

λ / nm	#	V_{DNA} μL	[complex] 10^{-5} M	[DNA] / [complex]	[DNA]/($\varepsilon_a - \varepsilon_f$) 10^{-8} M^2 cm	Graphical determination of binding constant
581	I	0	2.21	0.00	-	
		5	2.20	0.25	4.62	
		10	2.20	0.50	4.80	
		20	2.19	0.99	6.08	
		40	2.17	1.96	10.7	
		60	2.15	2.91	12.0	
		80	2.13	3.85	15.5	
	II	0	2.21	0.00	-	
		10	2.20	0.50	2.89	
		15	2.20	0.74	3.66	
		20	2.19	0.99	4.11	
		35	2.17	1.72	5.82	
		50	2.15	2.44	6.38	
		70	2.13	3.38	8.27	
	III	0	2.21	0.00	-	
		5	2.20	0.25	8.94	
		10	2.20	0.50	18.2	
		35	2.17	1.72	26.1	
		70	2.14	3.38	44.5	
		80	2.13	3.85	47.1	

#	Slope 10^{-4} M cm	Intercept 10^{-8} M^2 cm	r^2	K_b / 10^4 M^{-1}
I	13.9	3.57	0.980	3.91
II	8.06	2.29	0.982	3.53
III	4.52	9.59	0.977	4.71
$K_b \pm \sigma$ / 10^4 M^{-1}				**4.05 ± 0.61**

The titration experiments were carried out under the same experimental conditions and obtained data were processed on the same way for all compounds. The binding constants are summarized in Table 2.

Table 2. Binding constants of complexes Na[Ru(N-R-5-X-salim)$_2$] (R = C_6H_4O, X = H, Cl, Br, NO_2), Na[$RuCl_2$(N-R-5-X-salim)$_2$] (R = C_4H_9, X = H, Cl, Br, NO_2) and [Ru(N-R-5-X-salim)$_3$] (R = $C_{10}H_7$, X = H, Cl, Br), with CT DNA

R	X	$K_b \pm \sigma$ / M^{-1} [λ_1 / nm]	$K_b \pm \sigma$ / M^{-1} [λ_2 / nm]
C_6H_4O	H	$(4.35 \pm 0.34)\cdot10^4$ [380]	$(4.05 \pm 0.61)\cdot10^4$ [581]
	Cl	$(2.13 \pm 0.15)\cdot10^4$ [387]	$(1.70 \pm 0.24)\cdot10^4$ [560]
	Br	$(2.79 \pm 0.18)\cdot10^4$ [388]	$(2.14 \pm 0.68)\cdot10^4$ [558]
	NO_2	$(3.59 \pm 0.30)\cdot10^4$ [380]	$(2.78 \pm 0.69)\cdot10^4$ [591]
C_4H_9	H	-	$(2.20 \pm 0.28)\cdot10^4$ [403]
	Cl	$(8.52 \pm 3.30)\cdot10^3$ [349]	$(6.66 \pm 1.38)\cdot10^3$ [403]
	Br	$(4.35 \pm 1.18)\cdot10^2$ [354]	$(7.11 \pm 1.64)\cdot10^2$ [408]
	NO_2	$(8.69 \pm 0.90)\cdot10^3$ [384]	-
$C_{10}H_7$	H	$(7.40 \pm 0.43)\cdot10^4$ [237]	-
	Cl	$(4.65 \pm 0.22)\cdot10^4$ [238]	-
	Br	$(2.54 \pm 0.27)\cdot10^4$ [238]	-

The binding constants of the complex Ru(III) derived from 5-substituted salicylaldehyde and aminophenol, Na [Ru (N-R-5-X-salim)$_2$], where R = C_6H_4O, and X = H, Cl, Br, NO_2 show decreases in the series H> NO_2> Br ≈ Cl($4.35 \pm 0.34\cdot10^4$ M^{-1}) > ($3.59 \pm 0.30\cdot10^4$ M^{-1})> ($2.79 \pm 0.18\cdot10^4$ M^{-1}) ≈ ($2.13 \pm 0.15\cdot10^4$ M^{-1})

The complex where X = H has the highest binding constant. The bromo- and chloro-derivatives have close K_b values due to similar size and similar electronic effects on the electron density of the π-system of the ligand. Although K_b values differ, the influence of 5-X-substituents on salicylideneimine ligands could not be considered as substantial one under described experimental conditions.

The binding constants of Ru(III) complexes derived from 5-X-substituted salicylaldehyde and naphthylamine, [Ru(N-R-5-X-salim)$_3$] with R = $C_{10}H_7$, and X is H, Cl, Br, were determined at the wavelengths around 240 nm where intense aromatic π→ π* electronic transitions occurred since there is no significant absorptions in visible region of the spectra. The noticeable decrease of K_b values in order H ($7.40 \pm 0.43\cdot10^4$ M^{-1}) >Cl ($4.65 \pm 0.22 \cdot10^4$ M^{-1})> Br ($2.54 \pm 0.27\cdot10^4$ M^{-1}) indicates definite influence of 5-X-substituents and correlates with ability of atoms to decrease electronic density or aromatic ring.

The complex compounds of Ru(III) derived from 5-substituted salicylaldehyde and butylamine, Na[$RuCl_2$(N-R-5-X-salim)$_2$], where R = C_4H_9, and X = H, Cl, Br, NO_2 show the significant difference in K_b values changing according to the following order H ($2.20 \pm 0.28\cdot10^4$ M^{-1}) > NO_2 ($8.69 \pm 0.90)\cdot10^3$ M^{-1}≈ Cl ($8.52 \pm 3.30\cdot10^3$ M^{-1}) > Br ($4.35 \pm 1.18\cdot10^2$ M^{-1}).

CONCLUSION

The experimental results showed that interactions of Ru(III) with Schiff bases derived from 5-substituted salicylaldehydes and aminophenol or naphthylamine with CT DNA are moderate, with K_b values of order 10^4 M^{-1}, suggesting the weak intercalative mode or stronger groove binding. The binding constants correlate with the lack or minor red shift. In the case of butyl derivative, the significant deference in K_b values, from 10^{-4} to 10^{-2} M^{-1}, showed essential effect of 5-X-substituents affecting the capability and mode of non-covalent binding of Ru(III) complexes to DNA. The ability of studied Ru(III) complexes with salicylideneimine to bind DNA stimulate further investigations of this class of compounds.

REFERENCES

Alessio, E., Mestroni, G., Nardin, G., Attia, W. M., Calligaris, M., Sava, G., and Zorzet, S. (1988). Cis- and trans-dihalotetrakis (dimethyl sulfoxide) ruthenium (II) complexes (RuX_2(DMSO) $_4$; X= Cl, Br): synthesis, structure, and antitumor activity. *Inorganic Chemistry*, 27(23), 4099-4106.

Bergamo, A., Gaiddon, C., Schellens, J. H. M., Beijnen, J. H., and Sava, G. (2012). Approaching tumour therapy beyond platinum drugs: status of the art and perspectives of ruthenium drug candidates. *Journal of Inorganic Biochemistry*, 106 (1), 90-99.

Bratsos, I., Jedner, S., Gianferrara, T., and Alessio, E. (2007). Ruthenium anticancer compounds: challenges and expectations. *CHIMIA International Journal for Chemistry*, 61(11), 692-697.

Bruijnincx, P. C., and Sadler, P. J. (2008). New trends for metal complexes with anticancer activity. Current opinion in chemical biology, 12 (2), 197-206.

Chittilappilly P. S. and Yusuff K. K. M. (2008). Synthesis, characterization and biological properties of ruthenium(III) Schiff base complexes derived from 3-hydroxyquinoxaline-2-carboxaldehyde and salicylaldehyde. *Indian J. Chem.* 47A 848−853.

Cory M., McKee D.D., Kagan J., Henry D.W and Miller J.A. (1985). Design, synthesis and DNA binding properties of bifunctionalintercalators. Comprasion of polymethylene and diphenylether chains connecting phenanthridines. *J. Am. Chem. Soc*. 107:2528-2536

Drozdzak R., Ledoux N., Allaert B., Dragutan I., Dragutan V., and Verpoort F. (**2005**). RationalDesign and Convenient Synthesis of a Novel Family of Ruthenium Complexes with O,N-Bidentate Ligands. *Central Eur. J. Chem*. 3, 404-416.

Kahrović. E. (2014). Ruthenium: Synthesis, Physicochemical Properties and Applications. Keeler, G. P. (Ed.), Ruthenium Compounds with Schiff Bases: Design and Promising Applications of Salicylideneimine Complexes. (p.p. 269-283). *NOVA Publishers*

Kahrović, E. (2011). Chemical feature of inorganic compounds as anticancer agents. *Healt Med*, 5/5, 1112 – 1116.

Kahrovic, E., Zahirovic, A., and Turkusic, E. (2014). Calf Thymus DNA Intercalation by Anionic Ru (III) Complexes Containing Tridentate Schiff Bases Derived from 5-X-Substituted Salicyladehyde and 2-Aminophenol. *Journal of Chemistry and Chemical Engeenering*, 8, 335-343.

Kelly, J. M., Tossi, A. B., McConnell, D. J., and OhUigin, C. (1985). A study of the interactions of some polypyridylruthenium (II) complexes with DNA using fluorescence spectroscopy, topoisomerisation and thermal denaturation. Nucleic Acids Research, 13(17), 6017-6034.

Keppler, B. K., Henn, M., Juhl, U. M., Berger, M. R., Niebl, R. and Wagner, F. E. (1989). New ruthenium complexes for the treatment of cancer. In Ruthenium and Other Non-Platinum Metal Complexes in Cancer Chemotherapy (pp. 41-69). *Springer Berlin Heidelberg*.

Lakhai, J. M., van den Bongard, D., Pluim, D., Beijnen, J. H., and Schellens, J. H. (2004). A phase I and pharmacological study with imidazolium-trans-DMSO-imidazole-tetrachlororuthenate, a novel ruthenium anticancer agent. *Clinical Cancer Research*, 10(11), 3717-3727.

Ljubijankić, N., Zahirović, A., Turkušić, E., and Kahrović, E. (2013). DNA Binding Properties of Two Ruthenium (III) Complexes Containing Schiff Bases Derived from Salicylaldehyde: Spectroscopic and Electrochemical Evidence of CT DNA Intercalation. *Croatica Chemica Acta*, 86(2), 215-222.

Meadows K. A.,. Liu F., Sou J. , Hudson B. P. and McMillin D. R. (1993). Spectroscopic and photophysical studies of the binding interactions between copper phenanthroline complexes and RNA. *Inorganic Chemistry*, 32, 2919–2923.

Murray, K. S., van den Bergen, A. M., and West, B. O. (1978). Ruthenium complexes with tetradentate salicylaldimine Schiff base. *Aust. J. Chem.* 31, 203–207.

Pascoe, J. M., and Roberts, J. J. (1974). Interactions between mammalian cell DNA and inorganic platinum compounds—I: DNA interstrand cross-linking and cytotoxic properties of platinum (II) compounds. *Biochemical pharmacology*, 23(9), 1345-1357.

Piggot P. M. T., Hall L. A., White A. J. P., and Williams D. J(2004).Synthesis of ruthenium(II) monosubstituted squarates: 1. Procedural considerations. *Inorg. Chim. Acta* 357, 250−258.

Pyle, A. M., Rehmann, J. P., Meshoyrer, R., Kumar, C. V., Turro, N. J., and Barton, J. K. (1989). Mixed-ligand complexes of ruthenium (II): factors governing binding to DNA. *Journal of the American Chemical Society*, 111(8), 3051-3058.

Reedijk, J. (1987). The mechanism of action of platinum anti-tumor drugs. *Pure Appl Chem*, 59(2), 181-92.

Rixe, O., Ortuzar, W., Alvarez, M., Parker, R., Reed, E., Paull, K., and Fojo, T. (1996). Oxaliplatin, tetraplatin, cisplatin, and carboplatin: spectrum of activity in drug-resistant cell lines and in the cell lines of the National Cancer Institute's Anticancer Drug Screen panel. *Biochemical pharmacology*, 52(12), 1855-1865.

Rosenberg, B., and Vancamp, L. (1969). Platinum compounds: a new class of potent antitumour agents. *Nature* 222, 385-386.

Scholl, M., Ding, S., Lee, C. W., and Grubbs, R. H. (1999). Synthesis and Activity of a New Generation of Ruthenium-Based Olefin Metathesis Catalysts Coordinated with 1, 3-Dimesityl-4, 5-dihydroimidazol-2-ylidene Ligands. *Organic Letters*, 1(6), 953-956.

Summary / Sažetak

Interakcije Ru(III) kompleksa sa Schiff-ovim bazama izvedenim iz salicilaldehida i aminofenola, butilamina i naftilamina, opštih formula Na[Ru(NR-5-X-salim)$_2$] (R = C_6H_4O, X = H, Cl, Br, NO_2), Na[$RuCl_2$(NR-5-X-salim)$_2$] (R = C_4H_9, X = H, Cl, Br, NO_2) i [Ru(NR-5-X-salim)$_3$] (R = $C_{10}H_7$, X = H, Cl, Br) sa CT DNK, ispitivane su spektroskopskim titracijama. Eksperimentalni podaci pokazuju da se kompleksi Ru(III) sa salicilideniminima vezuju na CT DNK, sa konstantama vezivanja reda veličine 10^4 M^{-1}. Rezultati ukazuju na utjecaj 5-X-supstituenta na vrijednosti konstanti vezivanja.