Bulgarian Chemical Communications, Volume 47, Special issue A (pp. 75 -79) 2015

Synthesis and bioactivity of new platinum and ruthenium complexes of 4-bromo-spiro-(fluorene-9,4'-imidazolidine)-2',5'-dithione

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Received July 24, 2014; Accepted February 19, 2015

The present study is focused on platinum and ruthenium complexes of 4-bromo-spiro-(fluorene-9,4'-imidazolidine)-2',5'-dithione (L), synthesized from $(NH_4)_2[PtCl_4]$ and $RuCl_3.H_2O$, and their potential cytotoxicity properties. The structure of the complexes obtained is researched by elemental analysis and means of spectroscopic UV-Vis, IR, FT-ATR methods. We have examined for the first time biological potential of new complexes on a retinoblastoma human cell line (WERI-Rb-1). The cytotoxic effect is evaluated by WST-assay (Roche Applied Science).

Key words: *4-bromo-spiro-(fluorene-9,4'-imidazolidine)-2',5'-dithione, metal complexes, cytotoxic effect*

INTRODUCTION

Hydantoin derivatives possess a wide array of important biochemical and pharmacological properties. Several of these (phenytoin, methetoin, mephenythoin, fosphenytoin, norantoin) are well-known anticonvulsive drugs [1], whereas others have been suggested to act as antiarrhythmics and antimicrobial agents, skeletal muscle relaxants and nonsteroidal antiandrogens [2]. Recently, antitumor effect of hydantoin derivatives has been described by several authors [3, 4].

Although hydantoin compounds are studied extensively, there is not much research on their anticancer effects. In a previous work of ours, we have reported a method for obtaining 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) (4-bromo-spiro-(fluorene-9,4'-imidazolidine)-2',5'-dithione) [5] and 3-amino-9'-fluorenespiro-5-hydantoin [6]. In the studies cited above, we have investigated cytotoxic activities of the two compounds on the retinoblastoma cell line WERI-Rb-1 and antibacterial effects towards Gram-positive, Gram-negative bacteria, as well as yeasts *C. albicans*. Recently, we studied the complexation properties of (9'-fluorene)-spiro-5-hydantoins and its 2-thio derivative [7]. The two platinum complexes show significant effects on cancer cell growth compared to their ligands.

In the current work we described the synthesis and reaction conditions to obtain of new Pt(II) and Ru(III) complexes of 4-bromo-spiro-(fluorene-9,4'-imidazolidine)-2',5'-dithione (L) with general formula given in Figure 1**,** as well as characterization of the obtained complexes and *in vitro* antiproliferative activity on human tumor cell line.

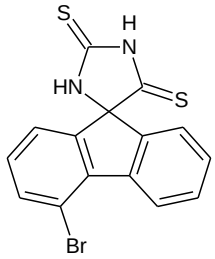

Fig. 1. General formula of (L).

EXPERIMENTAL

Metal salts ($(NH_4)_2[PtCl_4]$ and $RuCl_3.H_2O$ - Sigma-Aldrich or Merck) and solvents used for the synthesis of the complexes were with a p. a. qualification. UV/Vis spectra of L and its metal complexes were measured on a Lambda 9 Perkin-Elmer UV/Vis/NIR Spectrophotometer from 200 nm to 1000 nm. The IR spectra of L and its complexes were registered in KBr pellet on a Bruker FT-IR VERTEX 70 Spectrometer from 4000 cm^{-1} to 400 cm^{-1} at resolution 2 cm^{-1} with 25

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scans. Attenuated Total Reflection (ATR) spectra were registered on the same instrument by ATR accessory MIRacle™ with a one-reflection ZnSe element (Pike); the stirred crystals of L and its complexes were pressed by an anvil to the reflection element; the spectra were from 4500 cm^{-1} to 600 cm^{-1} at resolution 2 cm^{-1} with 16 scans.

1. Synthesis of Pt(II) and Ru(III) complexes of L

0.0004 mol (0.1445 g) of L in 10 cm^3 THF; 0.0002 mol (0.0746 g) $(NH_4)_2[PtCl_4]$ or 0.0002 mol (0.0450 g) $RuCl_3.H_2O$ in 10 cm^3 H_2O; 0.1 M solution of NaOH. 1 cm^3 of 0.1 M NaOH was added slowly to an L solution while stirring at pH = 9. The solution of the metal salts was added dropwise from a burette during stirring with electromagnetic stirrer. The precipitation of the formed complexes started after 72 h for Pt(II)L or 48 h for Ru(III)L, respectively. The complexes were formed as a brown or a black amorphous precipitates. The precipitates was filtered and washed with 10-20 cm^3 H_2O. These were dried over $CaCl_2$ for 2 weeks. It was found out that the complexes were soluble in THF and DMSO.

Elemental analysis for $PtL_2.2H_2O$; $C_{30}H_{20}N_4S_4Br_2PtO_2$; Mw = 951.65 g/mol; calc./(exp.) C% - 37.8 / (37.2); H% - 2.1 / (2.0); N% - 5.9 / (6.2); $Ru_2L_2L_{-4H}Cl_2.2H_2O$; $C_{90}H_{54}N_{12}S_{12}Br_6Ru_2O_2Cl_2$; Mw = 2472.72 g/mol; calc./(exp.) C% - 43.7 / (43.1); H% - 2.2 / (2.1); N% - 6.8 / (7.0)

IR (ν_{max}, cm^{-1}) L: 3374, 3150, 3066, 2919, 1605, 1575, 1515, 1465, 1448, 1407, 1232, 1216, 1194, 1102, 1064, 773, 754

IR (ν_{max}, cm^{-1}) Pt(II)L: 3500, 3392, 3250, 3065, 2922, 1607, 1513, 1465, 1448, 1405, 1233, 1215, 1191, 1103, 1064, 773, 757

IR (ν_{max}, cm^{-1}) Ru(III)L: 3400, 3100, 3065, 2973, 2872, 1606, 1508, 1465, 1448, 1406, 1234, 1215, 1194, 1106, 1064, 774, 757.

The ATR spectral data obtained of the free ligand L and its new complexes are on situation in University of Plovdiv, Department of the corresponding author.

2. WST-1 cell proliferation assay

The cytotoxic effect of Pt(II)L and Ru(III)L complexes were assessed on a retinoblastoma cell line (WERI-Rb1, ATCC-HTB-169) using WST-1 assay (Cat. No11 644 807 001, Roche). Cells were seeded in 96-well flat-bottom plates at a density of $6{,}5x10^4$ cells/well. After a cultivation period of 24h, the compounds were added at a concentration of 50 μM and incubated for 24 and 48h respectively. WST-1 was added to the cells at these time points and incubated for 4h. WST-1 is a colorimetric assay for the nonradioactive quantification of cell proliferation, viability and cytotoxicity. Absorbance was measured on ELISA SUNRISE Reader. The wavelength for measuring the absorbance of the formazan product is 450 nm and the reference filter was set at 620 nm in accordance with the WST-1 manual. Cells grown in culture media alone and in appropriate concentrations of DMSO were used as controls. The percentage of viable cells was calculated as a ratio of the OD value of the sample to the OD value of the control. Descriptive statistics was done in Excel to determine mean and SD values.

RESULTS AND DISSCUSION

Complexation with Pt(II) and Ru(III) were conducted under alkaline conditions using metal salts namely $(NH_4)_2[PtCl_4]$ and $RuCl_3.H_2O$ at molar ratio $M:L:OH^- = 2:4:1$. Neutral complexes were synthesized and isolated as brown and black precipitates, respectively. All complexes were investigated by means of elemental analysis, UV-Vis FT-ATR and IR spectroscopy. The elemental analysis data show metal-to-ligand ratio 1:2 and presence of two water molecules for Pt(II)L complex. All UV/Vis spectra were registered in THF. Maxima in the UV/Vis spectra of the free ligand L were observed at λ_{max} = 234 nm, 241 nm, 268 nm, 279 nm, 294 nm. Maxima in the UV/Vis spectra of Pt(II)L and Ru(III)L complexes were observed at 238 nm, 243 nm, 271 nm, 290 nm, 380 nm and 239 nm, 269 nm, 294 nm, 380 nm, respectively. In the UV/Vis spectra of the two metal complexes, one new maximum appeared at 380 nm.

The presence of two thioamide groups renders four donor atoms for coordination to the metal center: two S-atoms from C=S groups and two N-atoms from NH groups. The fact that both thioamide groups are part of a common heterocycle, where p-conjugation occurs, makes almost impossible the unambiguous assignment of the observed IR frequencies to individual vibrational modes. Selected vibrational frequencies observed in the IR spectra of the complexes and the free ligand L are given in Experimental part. The bands at 3374 cm^{-1} and 3150 cm^{-1} of the free ligand L may refer to the stretching vibrations of two N-H groups of the hydantoin ring. In the IR spectrum of the PtL complex, the same bands have been observed at 3392 and 3250 cm^{-1}. The two bands shift to higher frequency by 18 and 100 cm^{-1} as compared to the corresponding free ligand bands. In the IR spectrum of the RuL complex one of the N-H band has disappeared and the second band

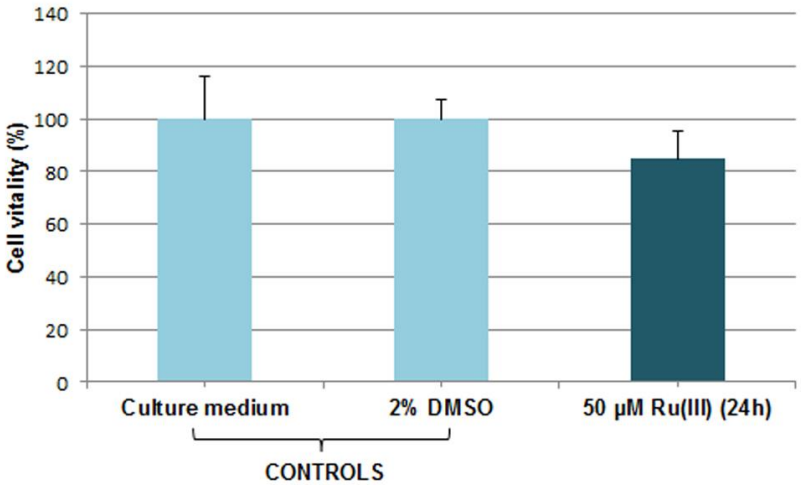

Fig. 2. Effect of the Ru(III)L complex on the proliferation of WERI-Rb 1 cells measured by WST-1 assay at 24h of treatment. The results represent mean values of three measurements. Error bars are SD values.

shifts to a lower frequency by 50 cm^{-1} as compared to the corresponding free ligand. The bands of free ligand L at 1605 cm^{-1} and 1575 cm^{-1} can be attributed to the stretching vibration of the two C=S groups of the hydantoin ring. In the IR spectrum of PtL and RuL complexes the band at 1607 cm^{-1} and 1606 cm^{-1} can be attributed to the stretching vibration of C=S group, respectively. In the IR spectrum of PtL and RuL complexes the second band of C=S group has disappeared. This fact shows that one of the two thiocarbonyl groups of the ligand hydantoin ring participates in the coordination with a metal ion. In the IR spectrum of the PtL and RuL complexes one new band has been observed at 3500 cm^{-1} and 3400 cm^{-1}, respectively. This band may refer to the stretching vibrations of OH^- group. In the IR spectra of the free ligand and its PtL and RuL complexes the band at 3066 cm^{-1} and 3065 cm^{-1}, 3065 cm^{-1} have been for stretching vibrations of CH in fluorene moiety, respectively.

Our results show that the Ru(III)L complex has a moderate cytotoxic effect on the human retinoblastoma cell line WERI-Rb1 (Figure 2). We have found that prolonged incubation periods do not influence cell viability. In contrast, the complex Pt(II)L does not perturb cell proliferation at all. This could be explained by the fact that this compound has a low solubility and the highest achievable concentration at which we were able to test it was only 50 µM. We have previously shown that the ligand 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) significantly reduces the number of cells in a time-dependent manner [5].

CONCLUSIONS

The synthesis of two new Pt(II) and Ru(III) complexes with 4-bromo-spiro-(fluorene-9,4'-imidazolidine)-2',5'-dithione have been described. The structure of the obtained complexes was verified by elemental analysis, UV-Vis, FT-ATR and IR spectroscopy. Our results imply that two newly synthesized compounds could not act as potential anticancer agents since they do not significantly inhibit the growth of retinoblastoma cells at the tested concentration.

Acknowledgements. *Financial support by the National Science Fund of Bulgaria, (Contract DFNI BO1/0014) is gratefully acknowledged.*

We are grateful also to Assoc. Prof. P. Penchev from University of Plovdiv for spectra measurements.

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СИНТЕЗ И БИОЛОГИЧНА АКТИВНОСТ НА НОВИ Pt(II) И Ru(III) КОМПЛЕКСИ НА 4-БРОМО-СПИРО-(ФЛУОРЕН-9,4'-ИМИДАЗОЛИДИН)-2',5'-ДИТИОН

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Постъпила на 24 юли, 2014 г.; приета на 19 февруари, 2015 г.

(Резюме)

Целта на настоящата разработка е получаването на нови Pt(II) и Ru(III) комплекси на 4-бромо-спиро-(флуорен-9,4'-имидазолидин)-2',5'-дитион и изследване на техните потенциални цитотоксични свойства. Структурата на получените метални комплекси е изследвана с елементен анализ и UV-Vis, ИЧ, FT-ATR спектроскопия. За пръв път е проучен потенциалният цитотоксичен ефект на новополучените комплекси върху човешка ретинобластомна клетъчна линия (WERI-Rb-1, ATCC-HTB-169). За оценка на клетъчната пролиферация и жизненост е използван комерсиалния Cell Proliferation Reagent - WST-1 тест (Cat. No11 644 807 001, Roche Applied Science).