Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 81 (8) 897–905 (2016)
JSCS–4895
UDC 546.924+546.962+544.421.032.4:
536.5.004.12:615.277–188
Original scientific paper

Cytotoxic Pt(IV) and Ru(II) complexes containing a biologically relevant edda-type ligand: A comparative study of their thermal properties

LJILJANA E. MIHAJLOVIĆ-LALIĆ[1]*, LJILJANA DAMJANOVIĆ[2], MAJA ŠUMAR-RISTOVIĆ[2], ALEKSANDAR SAVIĆ[3], TIBOR J. SABO[3], VERA DONDUR[1#] and SANJA GRGURIĆ-ŠIPKA[3]

[1]*Innovation Centre of the Faculty of Chemistry, Studentski trg 12–16, Belgrade, Serbia,* [2]*Faculty of Physical Chemistry, University of Belgrade, Studentski trg 12–16, Belgrade, Serbia and* [3]*Faculty of Chemistry, University of Belgrade, Studentski trg 12–16, Belgrade, Serbia*

(Received 20 March, revised 12 May, accepted 20 May 2016)

Abstract. The thermal behaviour of a Pt(IV) and a Ru(II) complex coordinated to dibutyl (*S*,*S*)-α,α'-(1,2-ethanediyldiimino)biscyclohexanepropanoate was investigated using thermogravimetry (TG) and differential scanning calorimetry (DSC). The study included an investigation of the thermal decomposition of these complexes in the temperature range of 30 to 590 °C and an evaluation of the activation energy for the first decomposition steps. For both metal complexes, broad DSC peaks indicated complex thermal transformation processes. The two-step decomposition of the Pt(IV) complex started at 175 and ended at about 418 °C, leaving elemental platinum as the final residue. On the other hand, the Ru(II) analogue decomposed in three stages. Thermal degradation was evident beginning at 144 °C and suggested the decomposition of a coordinated ligand as the dominant process. For this complex, the proposed final residue was RuO_2. Kinetic parameters for the first decomposition step were obtained by means of the multi-heating rates method, in this case the Kissinger–Akahira–Sunose (KAS) method. The mean activation energy calculated for $0.2 < \alpha < 0.8$ were 122.0 kJ mol^{-1} for the Pt(IV) and 118.9 kJ mol^{-1} for the Ru(II) complex and decreased constantly, a characteristic of a multi-step process.

Keywords: platinum (IV) complex; ruthenium (II) complex; anti-tumor activity; activation energy; TG–DSC.

* Corresponding author. E-mail: ljiljanam@chem.bg.ac.rs
# Serbian Chemical Society member.
doi: 10.2298/JSC160320059M

INTRODUCTION

The field of metal-containing drugs was established by Rosenberg's revolutionary discovery of cisplatin in the late 1960s.[1,2] Despite its benefits in clinical treatment, cisplatin displayed significant side effects[3,4] that could not be neglected. Numerous platinum as well as ruthenium, palladium, gold and even osmium complexes have been synthesized in the past decades[5–9] but therapeutic use is still limited to only a few of them (carboplatin, oxaliplatin, NAMI-A, KP1019).[10–12] In this sense, platinum- and ruthenium-based drugs are dominant compounds in the field of bioinorganic chemistry considering their cytotoxicity and determined mechanism of action.[13] Modern studies commonly include synthetic procedures and characterization of novel compounds followed by examination of their redox properties and interaction with biomolecules under physiological conditions.[14,15] Hitherto, only a few studies have included detailed examination of the thermal behaviour of the complexes.[16–18] Primarily, investigation of the thermal behaviour of various metal complexes was performed in order to link metal–ligand bonding and their structural properties.[19] In this way, it was possible to confirm the coordination mode of ligands and to test the stability of synthesized compounds at higher temperatures. Recently a few studies have suggested that the thermal characterization of potential antitumor agents could be used as a potential method for the determination of the fate compounds in the human body.[20–22]

The synthesis, characterization and antitumor activity of Pt(IV) and Ru(II) complexes bearing cyclohexyl functionalized ethylendiamine-*N*,*N*′-diacetate-type ligands was reported.[23,24] The main advantage of complexes containing edda-(ethylenediamine)-type ligands is their strong anticancer activity. Most of these complexes exhibit strong antitumor potential toward various cancer cell lines (U251, C6, L929, B16, A375, U251, B16 and PC3).[23,24] Among all reported Pt(IV) and Ru(II) complexes with this type of ligand, the compounds with *n*-butyl esters of (*S*,*S*)-α,α'-(1,2-ethanediyldiimino)biscyclohexanepropanoic acid ((*S*,*S*)-*n*Bu_2eddchxp) were the most active ones and for this reason they were selected for investigation of their thermal properties in this study.

EXPERIMENTAL

Measurements were performed using a thermobalance coupled with differential scanning calorimeter (TG-DSC 111 from Setaram, Caluire-et-Cuire, France) consisting of a quartz micro-reactor, heated in a vertical furnace. The experiments were performed under a dynamic helium atmosphere as the carrier gas (30 cm^3 min^{-1}) in the temperature range from 30 to 590 °C at heating rates of 5, 8 and 12 °C min^{-1}. The investigated complexes, **C1**, [$PtCl_4${(*S*,*S*)-*n*Bu_2eddchxp}] and **C2**, [(η^6-*p*-cymene)RuCl{(*S*,*S*)-*n*Bu_2eddchxp}]PF_6 (Fig. 1), were synthesized according to published procedures[23,24] and characterized by infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy. The IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer using the ATR technique. The ^{1}H spectra were obtained using Varian Gemini 200 and Bruker Avance III 500 spectrometers. The samples were dissolved in deuter-

ated dimethyl sulfoxide (DMSO-d_6) with tetramethylsilane (TMS) as the reference. The obtained results for both compounds (Supplementary material to this paper) were in agreement with previously reported data.[23,24]

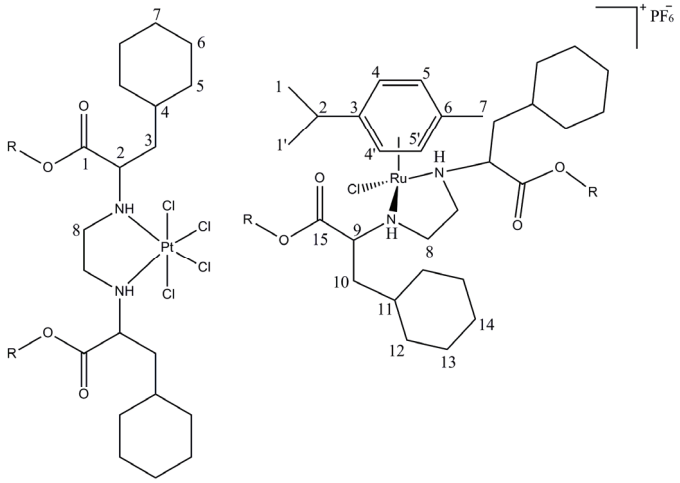

Fig. 1. Structures of the studied compounds **C1**, [$PtCl_4${(*S*,*S*)-*n*Bu_2eddchxp}], and **C2**, ([[(η^6-*p*-cymene)RuCl{(*S*,*S*)-*n*Bu_2eddchxp}]PF_6).

For a preparation of complex **C1**, dibutyl (*S*,*S*)-α,α'-(1,2-ethanediyldiimino)biscyclohexanepropanoate dihydrochloride (0.18 g, 0.41 mmol) was added to 15 mL of an aqueous solution of $K_2[PtCl_6]$ (0.20 g, 0.41 mmol) at 80 °C. The mixture was stirred for 8 h while a solution of LiOH (16.5 mL, 0.19 mol dm^{-3}) was added portion-wise. The formed precipitate was collected by filtration, washed with water and dried. Compound **C1** was obtained as yellow powder.[23] Yield: 52 %.

Compound **C2** was synthesized in the reaction of [(η^6-*p*-cymene)$RuCl_2]_2$ (0.12 g, 0.20 mmol), previously dissolved in methanol (11 mL) with a suspension of ligand dibutyl (*S*,*S*)-α,α'-(1,2-ethanediyldiimino)biscyclohexanepropanoate dihydrochloride (0.22 g, 0.40 mmol) in methanol (10 mL) previously neutralized with $LiOH{\cdot}H_2O$ (0.03 g, 0.80 mmol). The reaction mixture was stirred at 40 °C for 2.5 h. After the addition of NH_4PF_6 (0.11 g, 0.68 mmol), a fine yellow–orange solid was isolated.[24] Yield: 49 %.

RESULTS AND DISCUSSION

The main objective of this study was to analyze the thermal behaviour of the biologically active complexes **C1** and **C2**. The recorded TG and DSC curves for complexes **C1** and **C2** are shown in Fig. 2. The TG and DSC parameters for the investigated complexes (stages of decomposition, their temperatures ranges, corresponding DSC_{max} values, transferred heat values, mass loss percentages and evolved moieties for each stage) are presented in Table I.

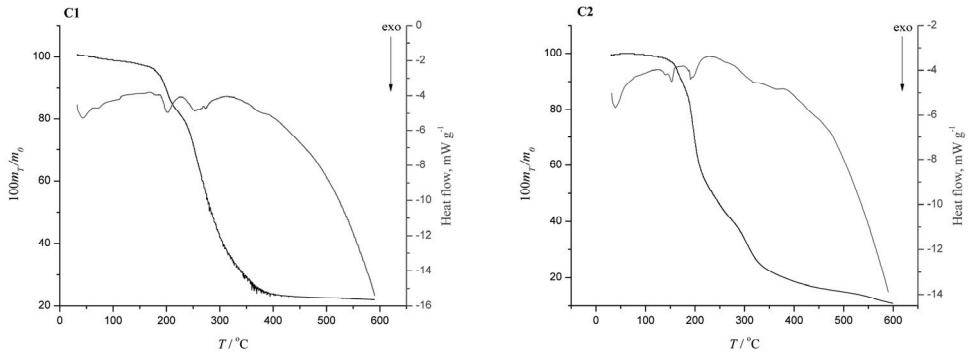

Fig. 2. Thermoanalytical curves of complexes **C1** and **C2** obtained at a heating rate β = 5 °C min^{-1} under a helium flow.

TABLE I. TG and DSC decomposition parameters for **C1** and **C2** at β = 5 °C min^{-1}

Complex	Stage	Temperature range, °C	DSC_{max} °C	ΔH kJ kg^{-1}	Mass loss, %		Evolved moiety
					Found	Theor.	
C1	I	175–225	202	− 847.1	17.59	17.34	2HCl, 2Cl
	II	230–396	254	−1644.6	58.55	58.80	$C_{28}H_{50}N_2O_4$
	Residue	418–590	–	–	23.26	23.85	Pt
C2	I	114–167	153	−575.5	16.38	16.17	PF_6^-
	II	179–228	193	−775.4	23.98	22.64	$C_{10}H_{14}Cl$
	III	271–381	317	−1353.5	38.47	44.92	$C_{28}H_{52}N_2O_2$
	Residue	406–590	–	–	14.39	14.84	RuO_2

*Thermal analysis of complex **C1***

The TG curve obtained for complex **C1** (Fig. 2) indicates its thermal stability up to 175 °C when decomposition begins (Table I). The first step occurs in the temperature range from 175 to 225 °C and represents a fast mass loss (Δm = = 19.18 %) originating from all four chlorido ligands in the axial position. This assumption is primarily made based on satisfying agreement with theoretical mass loss value (Δm = 17.34 %) and probably originates from elimination of HCl and chlorido ligands. In the same temperature region, a dominant peak in DSC curve is observed (Fig. 2) at T_{max}= 202 °C. Above 230 °C, a significant mass loss due to decomposition of the organic moiety occurs.[25] The elimination of the organic ligand appears over a large temperature range (230–396 °C). Based on the TG curve, this process occurs in two steps. The first step appears in the temperature range from 230 to 313 °C (Δm = 37.37 %), while the second occurs from 315 to 396 °C (Δm = 17.27 %). The sum of corresponding mass losses for these two steps equals mass of the whole ligand (Δm = 54.64 %) and is clearly assigned to the $C_{28}H_{52}N_2O_4$ moiety.[25] The complexity of this degradation step is also obvious in the DSC curve. The broad, rather complex DSC peak reflects the multiplicity of the degradation process caused by the bulkiness of the ligands.

The final step occurs around 418 °C and shows mass loss of Δm = 23.26 %. This result clearly indicates that final residue consists of elemental platinum as the evaluated experimental value of the mass loss fully corresponds to the theoretical one (Δm = 23.85 %). The obtained results are in agreement with literature since platinum complexes generally decompose either to platinum carbides or elemental platinum.[26,27] Taking all the given data into account, the multi-step decomposition of **C1** starts at 175 and ends around 418 °C leaving elemental platinum as the final residue.

*Thermal analysis of complex **C2***

The TG and DSC curves obtained for complex **C2** (Fig. 2) suggest that decomposition occurs in at least three major steps (Table I). The Ru(II) complex starts to decompose at 114 °C. The first step noted in the temperature range from 114 to 167 °C shows a mass loss of 16.38 %, which indicates the loss of counter ion species, PF_6^-. This stage is also manifested in corresponding DSC curve as a complex peak, obviously involving two overlapping processes. The following two steps (II and III), corresponding to the loss of the organic part, cover the wide temperature range from 179 to 381 °C.[28] In this part of the TG plot, total mass loss equals 62.45 % clearly indicating the organic ligand release. DSC curve is in an agreement with proposed thermal decomposition mechanism. The second peak is asymmetric and broad with T_{max} at 193 °C. The third peak in DSC curve (271–381 °C) corresponds to the noticeable change in the slope of the plot. The largest mass loss (≈38.47 %) was detected in this temperature range since it probably involves decomposition of the ligand, dibutyl (*S*,*S*)-α,α'-(1,2-ethanediyldiimino)biscyclohexanepropanoate. Finally, the investigated Ru(II) complex decomposed to RuO_2 (Δm = 14.39 %). Literature data showed that complex degradation of Ru compounds eventually leads to RuO_2 as the final residue.[28,29]

Solid-state kinetics and isoconversional method for the evaluation of activation energy

Kinetic analyses of solid-state transformations are based on a single-step kinetic equation:

$$\frac{\mathrm{d}\alpha}{\mathrm{d}t} = k(T)f(\alpha) \tag{1}$$

where $k(T)$ is the rate constant, t is the time, T is the temperature, α is the fractional extent of reaction, and $f(\alpha)$ is a conversion function which depends on the particular reaction model.

The temperature dependence of the rate conversion is introduced by replacing $k(T)$ with the Arrhenius equation, which gives the relation:

$$\frac{\mathrm{d}\alpha}{\mathrm{d}t} = A\exp\left(-\frac{E_\mathrm{a}}{RT}\right)f(\alpha) \tag{2}$$

where A (the pre-exponential factor) and E_a (the activation energy) are the Arrhenius parameters and R is the gas constant.

For non-isothermal measurements at constant heating rate, β, Eq. (2) transforms to:

$$\beta\frac{\mathrm{d}\alpha}{\mathrm{d}T} = A\exp\left(-\frac{E_\mathrm{a}}{RT}\right)f(\alpha) \tag{3}$$

where $\mathrm{d}\alpha/\mathrm{d}t \equiv \beta(\mathrm{d}\alpha/\mathrm{d}T)$.

The great majority of detailed kinetic studies start with an evaluation of the activation energy values of thermal-induced processes.[30–32] Generally, isoconversional methods can be linear (when the activation energy values are determined from the slope of a straight line) or non-linear (when the activation energy values are determined from a specific minimum condition).[33] Within this work, isoconversional or "model-free" kinetic procedures were used, such as the Kissinger–Akahira–Sunose (KAS) integral linear method, which is based on the following equation:[34,35]

$$\ln\left(\frac{\beta}{T_\alpha^2}\right) = \ln\left(-\frac{A_\mathrm{a}R}{E_\mathrm{a}}f(\alpha)\right) - \frac{E_\mathrm{a}}{RT\alpha} \tag{4}$$

where α is the degree of conversion and $f(\alpha)$ is the integral conversion function.

Therefore, the calculations of the activation energy included determination of the slopes for the graph dependences $\ln(\beta/T^2)$ *vs.* $(1/T)$ and $\ln(\beta)$ *vs.* $(1/T)$ for α = const. Plots of α *vs.* T (Fig. 3) were obtained from the experimental DSC curves recorded at three constant heating rates and they all refer to the first degradation stage defined in Table I (Fig. S-1 of the Supplementary material to this paper). As expected, the obtained $\alpha = f(T)$ plots are sigmoid-shaped[36] for both complexes and shifted to higher temperatures with increasing heating rate, indicating that thermal activation steps are involved in the degradation (Fig. 3).

The results presented in Fig. 3 were obtained using the KAS method. All the calculations refer to the first degradation stage. The temperature range for the first degradation steps for the investigated complexes are given in Table I. The mean values of the activation energy corresponding to the first thermal decomposition step and determined for the conversion degree range $0.2 < \alpha < 0.8$ (step of a 0.05) are: $\bar{E}_\mathrm{a}$ (**C1**) = 122.0±0.5 kJ mol^{-1} and $\bar{E}_\mathrm{a}$ (**C2**) = 118.9±0.5 kJ mol^{-1}.

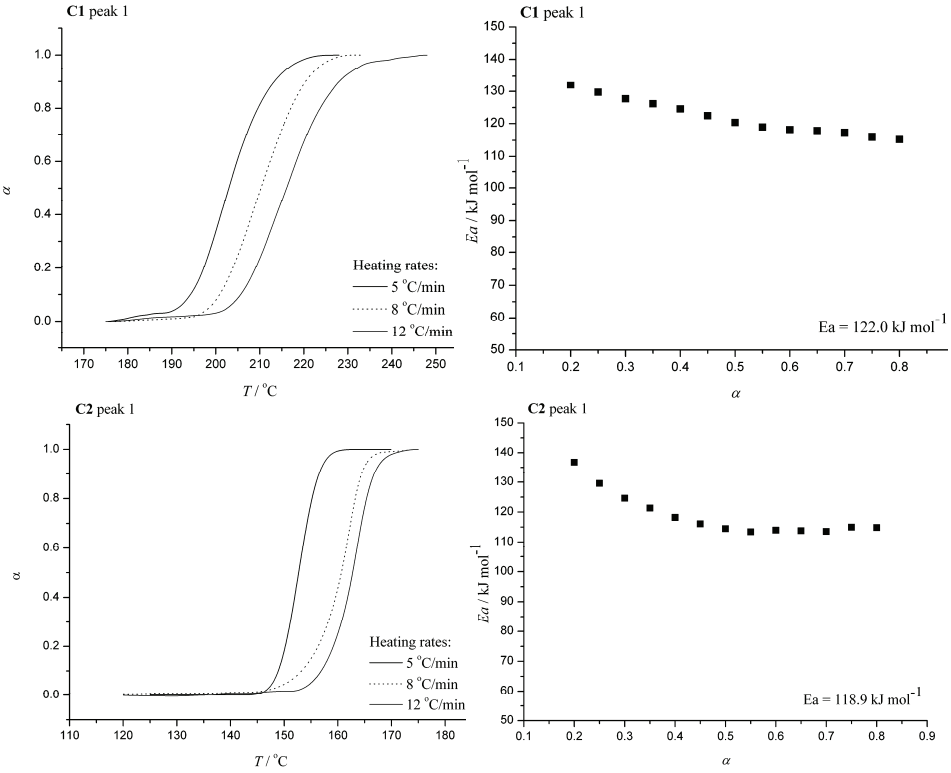

Fig. 3. Fractional conversion (α) as a function of temperature (T) and apparent activation energies as a function of fractional conversion (α) in the range $0.2 < \alpha < 0.8$ for the KAS method.

CONCLUSIONS

The thermal behaviour of two metal complexes (Pt(IV) and Ru(II)) bearing the dibutyl (*S,S*)-α,α'-(1,2-ethanediyldiimino)biscyclohexanepropanoate ligand were examined over the temperature range from 30 to 590 °C. Both TG and DSC data indicated that the thermal decomposition of these compounds is a multi-step degradation process. The decomposition of the Pt(IV) complex starts at 175 °C and includes two steps. The first reflects the loss of HCl and chlorido ligands, and second corresponds to a major loss originating from the loss of the organic ligand. The final residue of the decomposition according to the mass loss value was elemental platinum. In this case, all the thermal transformations were completed below 418 °C. The Ru(II) analogue was significantly less stable than the Pt(IV) compound since its degradation started at 114 °C. In the first stage, the observed mass loss corresponds to the mass of the counter ion (PF_6^-). The following two steps involve the loss of two organic moieties, first the cymene part, and then the cyclohexyl part. Eventually, the Ru(II) complex fully decomposed

to leave RuO_2 as the final residue at 406 °C. Evaluation of the isoconversional activation energies suggests that the decomposition of both compounds is a complex process, probably involving more than one step. Overall, the degradation is a stepwise process as might be expected for the complex structures of the Pt(IV) and Ru(II) complexes. The investigated compounds are representatives of the edda-metallo-drug-family based on their strong antitumor activity. Thermal stability up to 100 °C was demonstrated. Therefore, the thermal stability for this type of compound does not represent any kind of drawback for further clinical studies.

SUPPLEMENTARY MATERIAL

The spectral data and DSC curves for **C1** and **C2**, are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

Acknowledgements. The authors acknowledge the support from the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project Nos. 172035, 172055 and 172018).

ИЗВОД

ЦИТОТОКСИЧНИ Pt(IV) И Ru(II) КОМПЛЕКСИ СА БИОЛОШКИ РЕЛЕВАНТНИМ ЕДДА-ТИПОМ ЛИГАНДА: УПОРЕДНА СТУДИЈА ЊИХОВИХ ТЕРМИЧКИХ СВОЈСТАВА

ЉИЉАНА Е. МИХАЈЛОВИЋ-ЛАЛИЋ[1], ЉИЉАНА ДАМЈАНОВИЋ[2], МАЈА ШУМАР-РИСТОВИЋ[3], АЛЕКСАНДАР САВИЋ[3], ВЕРА ДОНДУР[2], ТИБОР Ј. САБО[3] и САЊА ГРГУРИЋ-ШИПКА[3]

[1]Иновациони центар Хемијског факултета, Студентски трг 12–16, Београд, [2]Факултет за физичку хемију, Универзитет у Београду, Студентски трг 12–16, Београд и [3]Хемијски факултет, Универзитет у Београду, Студентски трг 12–16, Београд

Термичко понашање Pt(IV) и Ru(II) комплекса координованих за дибутил-(*S*,*S*)-$-\alpha,\alpha$-(1,2-етандиилдиимино)бисциклохексанпропаноат је испитивано коришћењем термогравиметрије (ТГ) и дифенцијалне сканирајуће калориметрије (DSC). Студија је обухватила испитивање термичког разлагања наведених комплекса у температурском опсегу од 30 до 590 °C и процену енергије активације првог корака разлагања. За оба метална комплекса, широки DSC максимум је указао на сложени термички процес. Двостепено разлагање Pt(IV) комплекса почиње на 175 и завршава се на око 418 °C остављајући елементалну платину као крајњи производ. С друге стране, разлагање његовог Ru(II) аналога се одвија у три ступња. Термодинамички ефекти су примећени на 144 °C и указали су на разлагање координованог лиганда као доминатни процес. За овај комплекс, предложени крајњи остатак је RuO_2. У погледу основних кинетичких израчунавања, добијени су кинетички параметри првог ступња разлагања на основу Kissinger–Akahira–Sunose (KAC) методе. Средње енергије активације израчунате за $0,2 < \alpha < 0,8$ износе 122,0 kJ mol^{-1} за Pt(IV) и 118,9 kJ mol^{-1} за Ru(II) комплекс и константно опадају описујући вишестепени процес.

(Примљено 20. марта, ревидирано 12. маја, прихваћено 20. маја 2016)

REFERENCES

1. B. Rosenberg, L. Van Camp, T. Krigas, *Nature* **205** (1965) 698
2. B. Rosenberg, L. Van Camp, J. E. Trosko, V. H. Mansour, *Nature* **222** (1969) 385
3. T. Boulikas, M. Vougiouka, *Oncol. Rep.* **10** (2003) 1663

4. A. Florea, D. Büsselberg, *Cancers* **3** (2011) 1351
5. J. J. Wilson, S. J. Lippard, *Chem. Rev.* **114** (2014) 4470
6. A. Bergamo, C. Gaiddon, J. H. M. Schellens, J. H. Beijnen, G. Sava, *J. Inorg. Biochem.* **106** (2012) 90
7. A. R. Kapdi, I. J. S. Fairlamb, *Chem. Soc. Rev.* **43** (2014) 4751
8. S. Nobili, E. Mini, I. Landini, C. Gabbiani, A. Casini, L. Messori, *Med. Res. Rev.* **30** (2010) 550
9. M. Hanif, M. V. Babak, C. G. Hartinger, *Drug Discovery Today* **19** (2014) 1640
10. A. J. Di Pasqua, J. Goodisman, J. C. Dabrowiak, *Inorg. Chim. Acta* **389** (2012) 29
11. J. M. Pérez, M. A. Fuertes, C. Alonso, *Metal Compounds in Cancer Chemotherapy*, Research Signpost, Trivandrum, India, 2005, pp. 155–185
12. R. Trondl, P. Heffeter, C. R. Kowol, M. A. Jakupec, W. Berger, B. K. Keppler, *Chem. Sci.* **5** (2014) 2925
13. S. Spreckelmeyer, C. Orvig, A. Casini, *Molecules* **19** (2014) 15584
14. E. Wexselblatt, D. Gibson, *J. Inorg. Biochem.* **117** (2012) 220
15. B. J. Pages, D. L. Ang, E. P. Wright, J. R. Aldrich-Wright, *Dalton Trans.* **44** (2015) 3505
16. B. A. Howell, P. Chhetri, A. Dumitrascu, K. N. Stanton, *J. Therm. Anal. Calorim.* **102** (2010) 499
17. R. Olar, M. Badea, E. Cristurean, V. Lazar, R. Cernat, C. Balotescu, *J. Therm. Anal. Calorim.* **80** (2005) 451
18. V. Uivarosi, M. Badea, R. Olar, D. Marinescu, T. O. Nicolescu, G. M. Nitulescu, *J. Therm. Anal. Calorim.* **105** (2011) 645
19. V. Uivarosi, M. Badea, V. Aldea, L. Chirigiu, R. Olar, *J. Therm. Anal. Calorim.* **111** (2013) 1177
20. M. Juhász, S. Takahashi, T. Fujii, *J. Anal. Appl. Pyrolysis* **91** (2011) 114
21. S. M. A. Katib, *J. Therm. Anal. Calorim.* **103** (2011) 647
22. N. T. A. Ghani, A. M. Mansour, *Eur. J. Med. Chem.* **47** (2012) 399
23. J. M. Lazić, Lj. Vučićević, S. Grgurić-Šipka, K. Janjetović, G. N. Kaluđerović, M. Misirkić, M. Gruden-Pavlović, D. Popadić, R. Paschke, V. Trajković, T. J. Sabo, *ChemMedChem* **5** (2010) 881
24. A. Savić, M. Dulović, J. M. Poljarević, S. Misirlić-Denčić, M. Jovanović, A. Bogdanović, V. Trajković, T. J. Sabo, S. Grgurić-Šipka, I. Marković, *Chem. Med. Chem.* **6** (2011) 1884
25. Y. Baran, I. Kaya, M. Turkyılmaz, *J. Therm. Anal. Calorim.* **107** (2012) 869
26. A. Bakalova, *J. Therm. Anal. Calorim.* **96** (2009) 593
27. N. T. Abdel Ghani, A. M. Mansour, *Eur. J. Med. Chem.* **47** (2012) 399
28. M. Badea, R. Olar, D. Marinescu, V. Uivarosi, T. O. Nicolescu, D. Iacob, *J. Therm. Anal. Calorim.* **99** (2010) 829
29. M. Badea, R. Olar, D. Marinescu, V. Uivarosi, D. Iacob, *J. Therm. Anal. Calorim.* **97** (2009) 735
30. J. W. Huang, C. C. Chang, C. C. Kang, M. Y. Yeh, *Thermochim. Acta* **468** (2008) 66
31. R. Svoboda, J. Málek, *J. Therm. Anal. Calorim.* **115** (2014) 1961
32. D. M. Minić, B. Adnađević, *Thermochim. Acta* **474** (2008) 41
33. A. Rotaru, G. Brătulescu, P. Rotaru, *Thermochim. Acta* **489** (2009) 63
34. H. E. Kissinger, *Anal. Chem*. **29** (1957) 1702
35. T. Akahira, T. Sunose, *Res. Rep. Chiba Inst. Technol*. **16** (1971) 22
36. S. Vyazovkin, *J. Comput. Chem.* **18** (1997) 393.