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Original scientific paper

In vitro anticancer evaluation of novel triphenyltin(IV) compounds with some *N*-acetyl-*S*-naphthoquinonylcysteine derivatives

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Abstract: Triphenyltin(IV) compounds with naphthoquinone derivatives containing *N*-acetylcysteine, *N*-acetyl-*S*-(3,4-dihydro-3,4-dioxo-1-naphthyl)cysteine (1,2-NQC), **1**, and *N*-acetyl-*S*-(1,4-dihydro-1,4-dioxo-2-naphthyl)cysteine (1,4-NQC), **2**, were synthesized and characterized by elemental microanalysis, IR, multinuclear (^{1}H, ^{13}C, ^{119}Sn) NMR spectroscopy as well as HR-ESI mass spectrometry. With the aim of *in vitro* anticancer activity determination of ligand precursors and novel synthesized organotin(IV) compounds against human cervix adenocarcinoma (HeLa), human colon carcinoma (HT-29) and melanoma carcinoma cell line (B16F10), MTT colorimetric assay method was applied. The results indicate that synthesized compounds exhibited remarkable antiproliferative activity toward all tested cell lines with IC_{50} in the range from 0.17 to 0.87 μM. Complex **1** showed the greatest activity against HT-29 cells, with IC_{50} value of 0.21±0.01 μM, 119 times better than cisplatin, while complex **2** demonstrated the highest activity toward HeLa cells, IC_{50} = 0.17±0.01 μM, which is ~26 times better than cisplatin.

Keywords: organotin(IV) compounds; characterization; antitumor agents; cytotoxicity.

INTRODUCTION

Cancer is an illness that implies anomalous cell growth with the tendency of occupying and spreading to other parts of the body.[1-3] Cancer can start almost anywhere in the human body, which is made up of trillions of cells. The causes of cancer are not completely understood.[4] Global economic trends, associated

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with lifestyle, include inadequate nutrition, less physical activity and stress, significantly affect the occurrence of malignant and various chronic diseases.[5] The radiation, chemotherapy, and surgery are the main methods of treating tumors in humans. Cancer chemotherapeutic agents often can give patients provisional assuagement of symptoms, prolongation of life, and in some cases complete healing.

Last few decades metal-based complexes have been widely used in medicine as medicaments. Nowadays, cisplatin and the second generation of the platinum--based compounds (carboplatin and oxaliplatin) are still the most broadly used drugs in therapy for various types of cancer.[6] Nevertheless, these compounds do not exhibit selectivity between carcinogens and non-cancer cells, and therefore it causes the serious drawback such as nephro-, neuro-, ototoxicity, nausea and vomiting.[7–10] In order to overcome these issues numerous complexes containing gold, ruthenium, palladium, even tin and titanium have been synthesized and tested.[11–16] In recent years organotin(IV) complexes are of great interest due to their potential applications in biology, medicine, catalysis, and materials science.[17–20] Many *in vitro* studies have shown that some organotin(IV) compounds demonstrate better anticancer potential as drugs in comparison to cisplatin.[21,22] Additionally, a more recent study on SBA-15 mesoporous nanomaterial loaded with 6-(triphenylstannyl)hexan-1-ol exhibits a non-aggressive manner of action, greatly efficient toward tumor cells.[23]

Generally, biological activity is related with the nature of ligands which extenuate the movement of the complexes through the cell membrane.[24] The anionic ligands, such as carboxylates, are greatly efficient for tin(IV) compounds due to the higher stability of the obtained metal complexes. This could be explained by the steric and electronic factors of the organic part of the molecules on the tin and/or the carboxylate moiety which considerably contribute to the whole electronic structure of the molecule.[25] Although the mechanism of action of the tin(IV) complexes is still not completely clear, some studies showed that they may harm DNA in tumor cells which leads to prevention of cell proliferation and cell death.[26] One of the major problems of tin(IV) compounds is their poor solubility in water and therefore finding of novel organotin(IV) compounds that will overcome these shortcomings and exhibit better selectivity between normal and tumor cells is one of the goals of the researchers in this field.

Herein, the synthesis, characterization, and antiproliferative activity of novel triphenyltin(IV) compounds with two *N*-acetyl-*S*-naphthoquinonylcysteine, [Ph_3Sn(1,2-NQC)], **1**, and [Ph_3Sn(1,4-NQC)], **2** (1,2-NQC = *N*-acetyl-*S*-(3,4--dihydro-3,4-dioxo-1-naphthyl)cysteine); 1,4-NQC = *N*-acetyl-*S*-(1,4-dihydro--1,4-dioxo-2-naphthyl)cysteine) has been reported. The compounds were characterized by IR, ^{1}H-, ^{13}C- and ^{119}Sn-NMR spectroscopy, HR-ESI-MS and elemental microanalysis. Additionally, *in vitro* cytotoxic activity of ligand precursors and

synthesized complexes was determined versus human cervix adenocarcinoma (HeLa), human colon carcinoma (HT-29), and melanoma carcinoma cell line (B16-F10) by the MTT colorimetric assay method.

EXPERIMENTAL

Chemicals and methods

The ligand precursors *N*-acetyl-*S*-(3,4-dihydro-3,4-dioxo-1-naphthyl)cysteine (1,2-NQC), and *N*-acetyl-*S*-(1,4-dihydro-1,4-dioxo-2-naphthyl)cysteine (1,4-NQC), were custom-synthesised by KAdem Custom Chem, Gottingen (Germany). Elemental analyses were performed on an Elemental Vario EL III microanalyzer. IR spectra were recorded on a Nicolet 5700 FT--IR (Thermo, Madison, WI, USA) spectrometer in the range 4000–400 cm^{-1}. NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer; ^{1}H-NMR (400.13 MHz): internal standard solvent, external standard $(CH_3)_4Si$; $^{13}C\{1H\}$-NMR (100.6 MHz): internal standard solvent, external standard $(CH3)_4Si$; $^{119}Sn\{1H\}$-NMR (149.2 MHz): internal standard solvent, external standard $(CH_3)_4Sn$. Reagents and solvents were of commercial reagent grade quality and used without further purification. The positive ion HR-ESI-MS were obtained with an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Germany) equipped with an HESI electrospray ion source (positive spray voltage 4.5 kV, negative spray voltage 3.5 kV, capillary temperature 275 °C, source heater temperature 250 °C, FTMS resolution 30000). Analytical data are given as Supplementary material to this paper.

Synthesis of complexes

A suspension of the 1,2-NQC (50.1 mg; 0.157 mmol) and 1,4-NQC (50.1 mg; 0.157 mmol), respectively, in distilled water (1.57 mL) was treated with 157 μL 1 M KOH and stirred for 15 min at ambient temperature while a clear solution was formed. Then 1.57 mL methanolic solution of Ph_3SnCl (60.35 mg; 0.157 mmol) was added dropwise in the reaction mixture, the reaction was stirred for 1 h and white precipitate formed. The precipitate was filtered off, washed twice with 7 mL of cold distilled water and then dried under vacuum. The numbering of carbon atoms for ^{13}C-NMR is presented in Scheme 1.

Biological experiments

Preparation of drug solutions. DMSO (Sigma–Aldrich, St. Louis, MO, USA) was used as solvent for stock solution preparation of investigated compounds at the concentrations of 20 mM. Working solutions were prepared diluting appropriate stock solutions by completed medium. RPMI-1640 (Sigma–Aldrich, St. Louis, MO, USA) containing 10 % fetal bovine serum (FBS; Biochrom AG, Berlin, Germany) and penicillin/streptomycin (Sigma–Aldrich, St. Louis, MO, USA) was used as completed medium.

Cell lines. HeLa cervix adenocarcinoma cell line, HT-29 human colon carcinoma cells and B16-F10 melanoma carcinoma cell line were grown in completed RPMI-1640 medium.

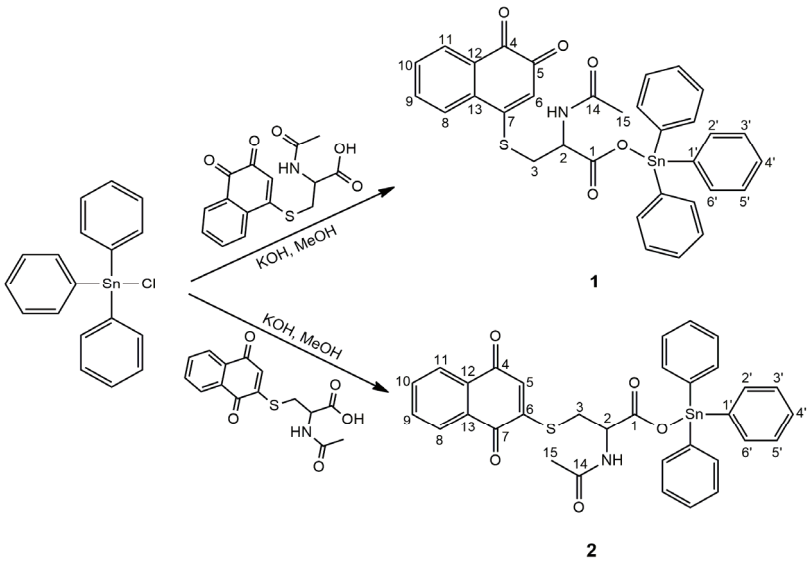
Determination of cell survival. Target cells HeLa (2000 cells/well), HT-29 (5000 cells/ /well), and B16-F10 (5000 cells/well), were seeded in appropriate cell density into the 96-well flat-bottomed microtitre plates. Approximately 24 h after seeding, various working solutions with a different concentration (**1** and **2**: 0.10, 0.25, 0.50, 0.75, 1.00 and 2.50 μM; 1,2-NQC and 1,4-NQC: 1, 10, 25, 50, 75 and 100 μM) were added to the wells. In the control wells only completed medium was added. The concentration of DMSO in wells was always less than 0.5 %, concentration non-toxic for the cells. Upon treatment for 72 h, cell survival rates were determined by the MTT assay, according to the literature.[27,28] Briefly, after washing with 50 μL of PBS the cells were treated with 50 μL of MTT solution (5 mg in 1 mL of PBS) and samples

were incubated (ca. 45 min) at 37 °C. The cells were examined microscopically for formazan (black precipitate) development. The supernatant was discarded from each well and the formazan dissolved in DMSO. The number of viable cells was determined using Spectramax plate reader (Molecular Devices, San Jose, CA, USA) at 570 nm with a background wavelength of 670 nm. For the calculation of the IC_{50} value, a four-parameter logistic function was used and the results presented as a mean of three independent trials. Evaluations were performed in three technical and biological replicates.

RESULTS AND DISCUSSION

Synthesis and characterization

In the reaction of Ph_3SnCl and equimolar quantity of ligand precursors, *N*-acetyl-*S*-(3,4-dihydro-3,4-dioxo-1-naphthyl)cysteine (1,2-NQC) and *N*-acetyl--*S*-(1,4-dihydro-1,4-dioxo-2-naphthyl)cysteine (1,4-NQC), previously deprotonated with KOH, desired compounds were obtained as yellow products in good yields (Scheme 1). The synthesized complexes were characterized by IR spectroscopy, multinuclear NMR spectroscopy, mass spectrometry as well as elemental microanalysis. Applied techniques show that complexes, isolated as yellow powders, are of high purity.



Scheme 1. Synthesis of thiphenyltin(IV) compounds.

IR spectra of novel compounds exhibited strong ν(C=O) stretching band around 1700 cm^{-1} similar to the ligand precursor, demonstrating that coordination of carbonyl oxygen atoms to the tin(IV) center did not occur. Additionally, two strong bands belonging to the asymmetric and symmetric absorptions of the COO moiety were found in regions 1645–1640 and 1372–1368 cm^{-1}. Moreover,

the divergence between these two vibrations (>200 cm^{-1}) suggests monodentate coordination of the carboxylate ligand.[29,30] Furthermore, the medium band which corresponds to the Sn–O vibration occurs at 450 cm^{-1}.

In the ^{1}H-NMR spectra, a set of two dissimilar multiplets, at *ca*. 7.8 and 7.4 ppm, belonging to the *m*-protons and to the *o*- and *p*-protons of $SnPh_3$ moiety, respectively, have been detected. The protons belonging to the aromatic ring of naphthoquinone showed chemical shifts between 7.68 and 8.03 ppm. Furthermore, the hydrogen atom of the secondary amide appeared in ^{1}H-NMR spectra at 8.1 ppm. Expectedly, protons of *N*-acetyl moiety resonated as singlet at 1.80 ppm, while the methylene protons of cysteine moiety were observed as doublet at approximately 3.3 ppm. Coupling with tin nucleus can be observed as satellite nearby resonances of *o*-H atoms from the Ph_3Sn moiety. $^{13}C\{^{1}H\}$-NMR spectra of the complexes showed the expected signals for the phenyl groups, as well as the signals corresponding to the 1,2- and 1,4-naphthoquinone. Furthermore, a characteristic signal at *ca*. 170 ppm has been remarked in the spectra and designated to the carbon atom of the COO group, while the $^{119}Sn\{^{1}H\}$-NMR spectrum of complexes **1** and **2** have shown one signal at –83.1 and –84.5 ppm, respectively, suggesting the tetrahedral geometry of triphenyltin(IV) carboxylato complexes.[31] HR-ESI-MS gave evidence for molecular composition of the organotin(IV) compounds. In both cases, $[M+H]^+$ species are found.

In vitro *cytotoxicity*

In Fig. 1, action of different concentrations of novel complexes toward HeLa, HT-29 and B16-F10 cell survival using MTT assay, after 72 h of continual exposure, are given. Additionally, in Table I are provided the results of *in vitro* anticancer activity presented as IC_{50} value which is defined as the concentration of complex that reduces a proliferation rate of the cancer cells by 50 % in comparison with control, untreated cells. As can be seen, investigated compounds obstructed the growth in all examined cells in a dose-dependent mode, with the

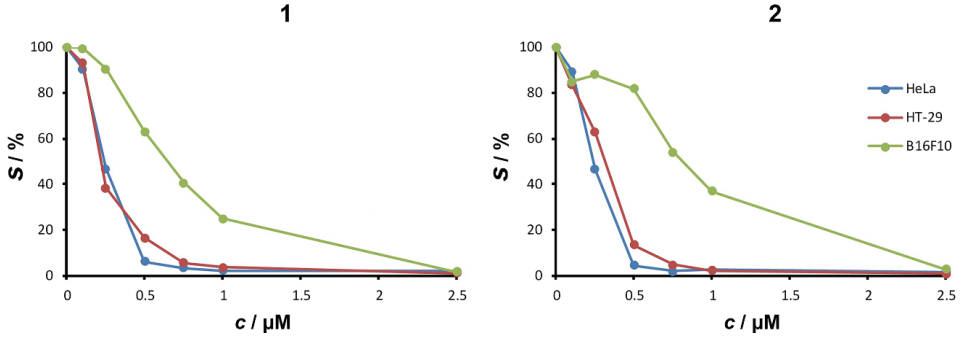


Fig. 1. Survival of tumor cells as a function of different concentrations of synthesized compounds **1** and **2** determined by MTT test, after 72 h incubation.

range of IC_{50} values from 0.17 to 26.99 μM. The results have shown that ligand precursors exhibited medium to good antiproliferative activity toward cancerogenic cells. Ligand precursor 1,4-NQC showed better activity than 1,2-NQC, and expressed the highest action against B16-F10 cells (IC_{50} = 6.30±1.35 μM), comparable to that of cisplatin. On the other hand, the synthesized complexes demonstrated remarkable activity toward all investigated tumor cell lines with IC_{50} values of 0.17 to 0.87 μM.

TABLE I. Concentrations of ligand precursors 1,2-NQC and 1,4-NQC, synthesized compounds **1** and **2**, and cisplatin that were able to induce a 50 % decrease in cell survival (IC_{50} in μM), after 72 h of incubation (mean ± *SD*)

Compound	Cell line		
	HeLa	HT-29	B16F10
1,2-NQC	26.99±4.33	12.54±0.30	12.10±0.60
1,4-NQC	8.62±1.09	8.25±1.76	6.30±1.35
1	0.24±0.01	0.21±0.01	0.60±0.09
2	0.17±0.01	0.30±0.02	0.87±0.05
Cisplatin	4.40±0.30	25.0±0.30	4.20±0.35

Complex **1** have shown the greatest activity versus HT-29 cells, (IC_{50} = = 0.21±0.01 μM), which is 119 times higher activity than cisplatin, while complex **2** expressed the highest antiproliferative activity toward HeLa cells, IC_{50} = = 0.17±0.01 μM, which is *ca*. 26 times better than cisplatin. Furthermore, the synthesized compounds have shown similar antiproliferative activity in comparison to other triphenyltin(IV) carboxylate complexes toward the same cell lines,[32] and comparable or even better activity to those which were incubated for a longer time.[33,34] Based on the obtained results, complexes **1** and **2** will be further examined as potential antitumor agents.

CONCLUSION

Two novel triphenyltin(IV) compounds bearing carboxylato ligands were synthesized and characterized by standard techniques. The ligand precursors and their corresponding tin(IV) complexes have been examined for their *in vitro* cytotoxic potential toward human cervix adenocarcinoma (HeLa), human colon carcinoma (HT-29) and melanoma (B16-F10) cell lines. Carboxylic acids showed moderate to good activity with the IC_{50} value ranging from 6.30 to 26.99 μM, in some cases comparable to cisplatin. On the other hand tin(IV) complexes demonstrated an extremely high anticancer potential toward the evaluated tumor cells, showing much lower IC_{50} values in comparison to those of cisplatin. Complex **2** presented the best activity, against HeLa cells, with IC_{50} values of 0.17±0.01 μM, approximately 26 times lower than cisplatin, while complex **1** showed 119 times better activity than cisplatin against HT-29 cells with IC_{50} value of 0.21±0.01 μM. Therefore, synthesized complexes **1** and **2** could be con-

sidered excellent candidates for some further *in vitro* examinations against normal and other cancerogenic cell lines and depending on the outcome obtained, for possible *in vivo* investigations.

SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: http:// //www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

In vitro АНТИТУМОРСКА ПРОЦЕНА НОВИХ ЈЕДИЊЕЊА ТРИФЕНИЛКАЛАЈА(IV) СА ДЕРИВАТИМА *N*-АЦЕТИЛ-*S*-НАФТАХИНОНИЛ-ЦИСТЕИНА

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Једињења трифенилкалаја(IV) са дериватима нафтахинона који садрже *N*-ацетилцистеин, *N*-ацетил-*S*-(3,4-дихидро-3,4-диоксо-1-нафтил)цистеин, (1,2-NQC), **1**, и *N*-ацетил-*S*-(1,4-дихидро-1,4-диоксо-2-нафтил)цистеин, (1,4-NQC), **2**, су синтетисана и окарактерисана уз помоћ елементалне микроанализе, IR, мултинуклеарне (^{1}H, ^{13}C, ^{119}Sn) NMR спектроскопије као и HR-ESI масене спектрометрије. *In vitro* антитуморска активност лиганада и новосинтетисаних комплекса је испитина на хуманим ћелијским линијама аденокарцинома грлића материце (HeLa), карцинома дебелог црева (HT-29) и меланома (B16-F10), уз помоћ MTT теста. Резултати су показали да синтетисана једињења имају значајну антипролиферативну активност према свим испитаним ћелијским линијама са IC_{50} вредностима у интервалу од 0,17 до 0,87 μM. Комплекс **1** показује највећу активност према HT-29 ћелијама са IC_{50} вредношћу 0,21±0,01 μM, што је 119 пута боља активност од цисплатине, док комплекс **2** показује највећу активност према HeLa ћелијској линији са IC_{50} вредношћу 0,17±0,01 μM што је приближно 26 пута већа активност од цисплатине.

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REFERENCES

1. J. Jadidi-Niaragh, G. Ghalamfarsa, M. Yousefi, M. N. Tabrizi, F. Shokri, *Tumor Biol*. **34** (2013) 2031 https://doi.org/10.1007/s13277-013-0832-x
2. G. Ghalamfarsa, A. Hadina, M. Yousefi, F. Jadidi-Niaragh, *Tumor Biol*. **34** (2013) 1349 https://doi.org/10.1007/s13277-013-0743-x
3. M. U. Haque, N. Ferdiousi, S. R. Sajon, *Int. J. Pharmacogn.* (*Panchkula, India*) (2016) 55 https://doi.org/10.13040/IJPSR.0975-8232.IJP.3(2).55-66
4. P. Anand, A. B. Kunnumakara, C. Sundaram, K. B. Harikumar, S. T. Tharakan, O. S. Lai, B. Sung, B. B. Aggarwal, *Pharm. Res*. **25** (2008) 2097 https://doi.org/10.1007/s11095-008-9661-9
5. T. H. Elizabeth, E. T. H. Fontham, *Ca - Cancer J. Clin.* **59** (2009) 5 https://doi.org/10.3322/caac.20000

6. *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*, B. Lippert, Ed., John Wiley & Sons, Inc., New York. 1999
7. B. Rosenberg, *Adv. Exp. Med. Biol.* **91** (1977) 129
8. C. A. Rabic, M. E. Dolan, *Cancer Treat. Rev*. **33** (2007) 9 https://doi.org/10.1016/j.ctrv.2006.09.006
9. Y. W. Jung, S. J. Lippard, *Chem. Rev*. **107** (2007) 1387 https://doi.org/10.1021/cr068207j
10. G. N. Kaluđerović, R. Paschke, *Curr. Med. Chem*. **18** (2011) 4738 https://doi.org/10.2174/092986711797535308
11. A. Molter, S. Kathrein, B. Kircher, F. Mohr, *Dalton Trans*. **47** (2018) 5055 https://doi.org/10.1039/C7DT04180B
12. L. Perdisatt, S. Moqadasi, L. O'Neill, G. Hessman, A. Ghion, M. Q. M. Warraich, A. Casey, C. O'Connor, *J. Inorg. Chem*. **182** (2018) 71 https://doi.org/10.1016/j.jinorgbio.2018.01.018
13. N. Pantelić, B. B. Zmejkovski, B. Kolundžija, M. Đorđić Crnogorac, J. M. Vujić, B. Dojčinović, S. R. Trifunović, T. P. Stanojković, T. J. Sabo, G. N. Kaluđerović, *J. Inorg. Biochem*. **172** (2017) 55 http://dx.doi.org/10.1016/j.jinorgbio.2017.04.001
14. N. Pantelić, B. B. Zmejkovski, T. P. Stanojković, T. J. Sabo, G. N. Kaluđerović, *Eur. J. Med. Chem*. **90** (2015) 766 http://dx.doi.org/10.1016/j.ejmech.2014.12.019
15. N. Pantelić, B. B. Zmejkovski, J. Trifunović-Macedoljan, A. Savić, D. Stanković, A. Damjanović, Z. Juranić, G. N. Kaluđerović, T. J. Sabo, *J. Inorg. Biochem*. **128** (2013) 146 http://dx.doi.org/10.1016/j.jinorgbio.2013.08.002
16. S. Gómez-Ruiz, T. P. Stanojkovic, G. N. Kaluđerović, *Appl. Organomet. Chem*. **26** (2012) 383 https://doi.org/10.1002/aoc.2878
17. N. Muhammad, Z. U. Rehman, S. Shujah, S. Ali, A. Shah, A. Meetsma, *J. Coord. Chem*. **67** (2014) 1110 https://doi.org/10.1080/00958972.2014.898755
18. H. M. Wahba, M. J. Stevenson, A. Mansour, J. Sygusch, D. E. Wilcox, J. G. Omichinski, *J. Am. Chem. Soc.* **139** (2017) 910 https://doi.org/10.1021/jacs.6b11327
19. T. S. B. Baul, P. Kehie, A. Duthie, N. Guchhait, N. Raviprakash, R. B. Mokhamatam, S. K. Manna, N. Armata, M. Scopelliti, R. Wang, U. Englert, *J. Inorg. Biochem*. **168** (2017) 76 https://doi.org/10.1016/j.jinorgbio.2016.12.001
20. X. Han, M. Tian, X. Xiao, J. Liang, D. Zhu, *J. Iran. Chem. Soc*. **15** (2018) 513 https://doi.org/10.1007/s13738-017-1251-5
21. M. Yousefi, M. Safari, M. B. Torbati, V. M. Kazemiha, H. Sanati, *Appl. Organomet. Chem*. **26** (2012) 438 https://doi.org/10.1002/aoc.2885
22. G. Gasser, I. Otto, N. Metzler-Nolte, *J. Med. Chem*. **54** (2011) 3 https://doi.org/10.1021/jm100020w
23. M. Z. Bulatović, D. Maksimović-Ivanić, C. Bensing, S. Gomez-Ruiz, D. Steinborn, H. Schmidt. M. Mojić, A. Korać, I. Golić, D. Perez-Quintanilla, M. Momčilović, S. Mijatović, G. N. Kaluđerović, *Angew. Chem. Int. Ed*. **53** (2014) 5982 https://doi.org/10.1002/anie.201400763
24. M. Sirajuddin, S. Ali, V. McKee, M. Sohail, H. Pasha, *Eur. J. Med. Chem*. **84** (2014) 343 https://doi.org/10.1016/j.ejmech.2014.07.028
25. F. Javed, S. Ali, S. Shahzadi, S. K. Sharma, K. Qanungo, M. N. Tahir, N. A. Shah, M. R. Khan, N. Khalid, *J. Inorg. Organomet. Polym*. **26** (2016) 48 https://doi.org/10.1007/s10904-015-0303-5
26. Y. G. Yang, M. Hong, L. D. Xu, J. C. Cui, G. L. Chang, D. C. Li, C. Z. Li, *J. Organomet. Chem*. **804** (2016) 48 https://doi.org/10.1016/j.jorganchem.2015.12.041

27. T. Mosmann, *J. Immunol. Methods* **65** (1983) 55 https://doi.org/10.1016/0022-1759(83)90303-4
28. M. Ohno, T. Abe, *J. Immunol. Methods* **145** (1991) 199 https://doi.org/10.1016/0022-1759(91)90327-C
29. G. B. Deacon, R. J. Philips, *Coord. Chem. Rev*. **33** (1980) 227 https://doi.org/10.1016/S0010-8545(00)80455-5
30. G. N. Kaluđerović, H. Kommera, E. Hey-Hawkins, R. Paschke, S. Gómez-Ruiz, *Metallomics* **2** (2010) 419 https://doi.org/10.1039/C0MT00007H
31. S. Gómez-Ruiz, S. Prashar, T. Walther, M. Fajardo, D. Steinborn, R. Paschke, G. N. Kaluđerović, *Polyhedron* **29** (2010) 16 https://doi.org/10.1016/j.poly.2009.05.056
32. S. Gómez -Ruiz, G. N. Kaluđerović, S. Prashar, E. Hey-Hawkins, A. Erić, Ž. Žižak, Z. D. Juranić, *J. Inorg. Biochem*. **102** (2008) 2087 https://doi:10.1016/j.jinorgbio.2008.07.009
33. G. N. Kaluđerović, R. Paschke, S. Prashar, S. Gómez-Ruiz, *J. Organomet. Chem*. **695** (2010) 1883 https://doi:10.1016/j.jorganchem.2010.04.029
34. A. Molter, G. N. Kaluđerović, H. Kommera, R. Paschke, T. Langer, R. Pöttgen, F. Mohr, *J. Organomet. Chem*. **701** (2012) 80 https://doi:10.1016/j.jorganchem.2011.12.027.