STUDIA UBB CHEMIA, LXI, 3, Tom I, 2016 (p. 109-116)
(RECOMMENDED CITATION)

Dedicated to Professor Luminița Silaghi-Dumitrescu on the occasion of her 65th anniversary

CYTOTOXIC ACTIVITY OF PALLADIUM (II) COMPLEXES OF (1E,6E)-1,7-*BIS*(4-(DIMETHYLAMINO)PHENYL)HEPTA-1,6-DIENE-3,5-DIONE AGAINST HUMAN COLON CARCINOMA

NATALIA MIKLÁŠOVÁ[a], ROMAN MIKLÁŠ[a], PIROSKA VIRAG[b], CORINA BIANCA TATOMIR[b], CRISTINA SZALONTAI[b], DIANA CENARIU[c], FERDINAND DEVÍNSKY[a], EVA FISCHER-FODOR[b]*

ABSTRACT. Two palladium(II) complexes of (1*E*,6*E*)-1,7-bis(4-(dimethilamino) phenyl)hepta-1,6-diene-3,5-dione were synthesized and structurally characterized with the aim of testing their cytotoxicity towards human colon tumor cells *in vitro*. Complexes **A** and **B** have the capacity to inhibit the cell growth in HT-29 and DLD-1 cell lines, the activity of **A** being superior as a result of a better accumulation inside the tumor cells.

Keywords: *palladium complexes, curcumin derivative, cytotoxicity, colon cancer, cellular uptake*

INTRODUCTION

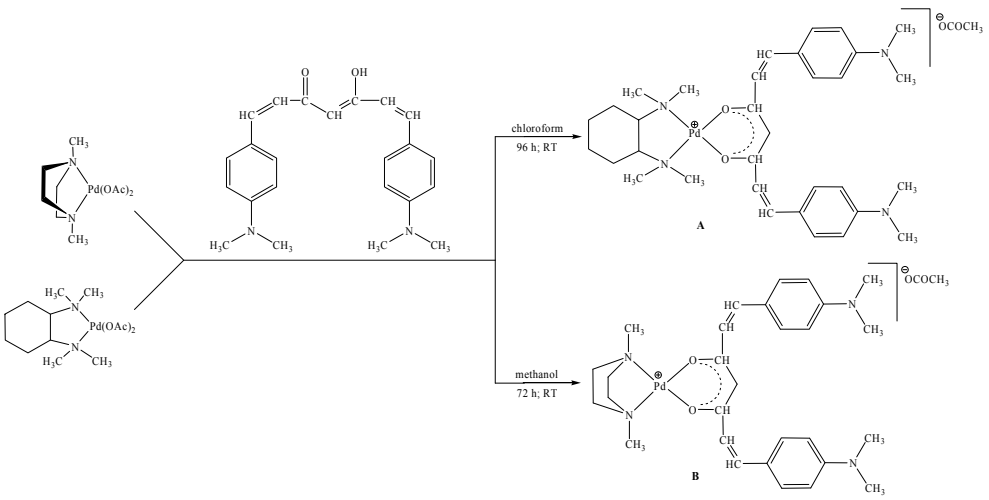
The natural extract curcumin exhibits *in vitro* antitumor effect against a variety of cancer cells [1], but this activity is not transposable to clinics due to the low bioavailability of the compound [2], which generates a minor effect on the cancer patients survival [3]. Seeking out for more active prodrugs,

[a] *Research Department, Institute of Oncology "Prof.Dr.I.Chiricuta", 34-36 Republicii str., RO-400015, Cluj-Napoca, Romania*
[b] *Department of Chemical Theory of Drugs, Faculty of Pharmacy, Comenius University in Bratislava, Kalinčiakova 8, Bratislava 83104, Slovakia*
[c] *Faculty of Medicine, Iuliu Hatieganu University of Medicine and Pharmacy Cluj Napoca, 8, Babes str, RO-400012, Cluj Napoca, Romania*
** Corresponding author: fischer.eva@iocn.ro*

synthetic strategies were elaborated to obtain metal complexes of curcumin with enhanced selectivity *in vivo* [4]. We reported previously novel palladium complexes with curcumin derivative ligands having antiproliferative [5] and immunomodulatory effects [6]. The curcumin derivative used in synthesis of palladium complexes tested in this work, 1,7-bis(4-(dimethylamino)phenyl) hepta-1,6-diene-3,5-dione, has been reported and tested for its biological activity against activation of AP-1 family of transcription factors [7]. It was tested for its cytotoxic ability, displaying a comparable or better activity than curcumin in a series of different curcuminoids. Pabon [8] observed a comparable activity of the curcumin derivative, containing dimethylamino moieties, with the pure curcumin when the anti-oxidant activity was investigated by FRAP assay. The aim of the present study was to obtain new metal complexes with enhanced capacity to target the tumor cells; the selected biologic substrates were the human colon cancer cells, a tumor type where curcumin alone was proven to be efficient more in prevention than in cancer cure [3].

RESULTS AND DISCUSSION

The synthesis of curcumin derivative with dimethylamino groups on the aromatic rings follows the pathway described previously in the literature [9]. Palladium complexes with the curcumin like ligand and the precursor palladium complexes used in syntheses (**Scheme 1**) were prepared based on a procedure reported formerly in our papers [5, 6].



Scheme 1. Synthetic pathway for palladium(II) complexes **A** and **B**

Cytotoxicity

The biological tests showed that the (1*E*,6*E*)-1,7-bis(4-(dimethilamino) phenyl)hepta-1,6-diene-3,5-dione ligand has no toxic effect against colon cancer cell lines in the studied concentration range; the attempts to calculate IC_{50} values resulted ambiguous and unconverged data in a 95% confidence interval. The half inhibitory concentrations (IC_{50}) corresponding to complexes **A** and **B** were calculated using the sigmoidal dose-response curves (**Table 1**);

Table 1. Half inhibitory concentrations IC_{50} of curcuminoid like ligand and palladium(II) complexes **A** and **B**.

Compound	Cell line	IC50 value [μM] ± Standard deviation
Complex **A**	DLD-1	19.17 ± 4.35
	HT-29	5.54 ± 0.37
Complex **B**	DLD-1	49.65 ± 6.13
	HT-29	68.51 ± 6.56

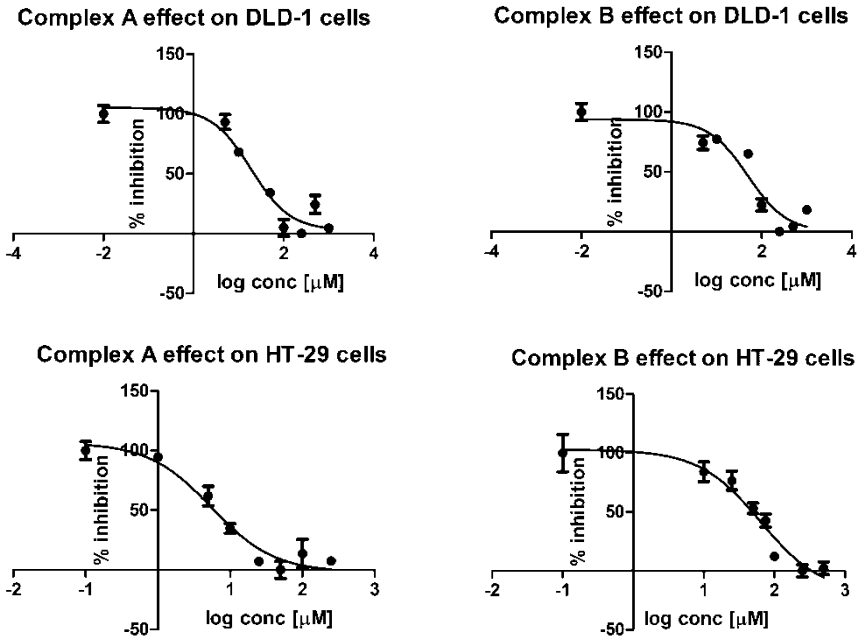


Figure 1. Sigmoidal dose-response curve corresponding to inhibitory activity of complexes **A** and **B** against DLD-1 and HT-29 cells growth.

a lower IC_{50} value indicates more significant toxicity. The inhibitory effect of complex **A** against tumor cells growth is superior to the effect of complex **B** in both DLD-1 and HT-29 cell lines *in vitro* (**Figure 1**). The IC_{50} value for compound **A** is markedly decreased in HT-29 cells. The complexes show a superior toxicity when compared with the nearly inactive ligand.

Cellular accumulation

The (1*E*,6*E*)-1,7-bis(4-(dimethilamino)phenyl)hepta-1,6-diene-3,5-dione and its complexes (**A** and **B**) are fluorescent molecules, and their accumulation in the cancer cells can be tracked using fluorescence intensity measurements on treated cells.

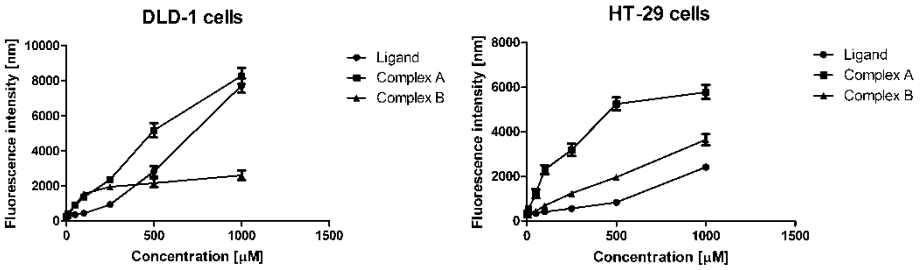


Figure 2. The compounds cellular uptake into DLD-1 cells following 24 hours treatment with the compounds, measured using fluorescence at 620nm emission, 540nm excitation; median values obtained from 6 independent experiments

Table 2. Linear regression data proving the dose-response relationship between the compounds concentration and their cellular accumulation rate within 24 hours treatment of DLD-1 and HT-29 cells; $p<0.0001$ (the deviation from zero was significant for all compounds)

Best-fit values	Ligand	Complex **A**	Complex **B**
DLD-1 cells			
Slope	7.20 ± 0.28	8.163 ± 0.26	2.27 ± 0.24
r^2	0.94	0.96	0.65
F	675.4	998.4	86.32
Deviation from zero	Significant	Significant	Significant
HT-29 cells			
Slope	1.99 ± 0.08	5.89 ± 0.43	3.37 ± 0.11
r^2	0.93	0.81	0.95
F	624.9	186.5	881.0
Deviation from zero	Significant	Significant	Significant

The compounds accumulate in HT-29 and even in the *K-ras* mutant DLD-1 cells after 24 hours of exposure (**Figure 2**) and the cellular uptake in all compounds was dose-related (**Table 2**). Complex **A** displays the biggest accumulation rate in both DLD-1 and HT-29 cells (one-way analysis of variance, Bonferroni post-test, $p<0.05$). The curcumin like ligand is selective, with good affinity for the DLD-1 line but with lower uptake in the HT-29 cells. The accumulation of complex **B** was better in HT-29 cells at 1mM concentration, and the decrease was very rapid. In DLD-1 cells a plateau was observed above 250 μM, where, despite the dose augmentation, the cells do not take up more complex. Although complex **A** is the most cytotoxic, there is no statistically significant correlation between the intracellular fluorescence and the IC_{50} values of studied compounds (nonparametric Spearman correlation, p value 0.917).

It is known that the DLD-1 and HT-29 tumor cells are sensitive to platinum-based-based drugs [10]. The accumulation of the platinum-based compounds in the colorectal tumor cells is related to the drug transporter molecules belonging to ABC family such ABCB1 and ABCG2 drug efflux pumps [11]. The alteration of ABCB1 and ABCG2 in colon carcinoma cells lead to a decrease of the cytotoxic drugs efficacy [12], including the metal-based drugs such as oxaliplatin.

The most toxic compound, complex **A** was proven to be one with the best accumulation in the cell, in agreement with previous studies which demonstrated a relation between the cellular uptake of Pd(II) complexes and their cytotoxic activity [13].

On the assumption that the favourable *in vitro* effect of complex **A** could be translated to chemotherapy, the compound might be functionalized [14] or encapsulated [15], to obtain a satisfactory *in vivo* activity.

CONCLUSIONS

Despite its capacity to accumulate in tumor cells, the curcuminoid ligand showed no toxicity against colon adenocarcinoma cell lines, IC_{50} values being relatively high, above the highest concentration used in biological tests. Once incorporated inside the cells, the fluorescence of the three compounds was detected at 620 nm emission, with 540 nm excitation. The cellular uptake of the two palladium complexes **A** and **B** exhibit different patterns: complex **A** incorporation was superior to **B** in both cell lines. Complex **A** displays also the most important *in vitro* inhibitory growth capacity against colon carcinoma HT-29 and *K-ras* mutant DLD-1 cells.

EXPERIMENTAL SECTION

All chemicals necessary in the syntheses were of reagent grade and were used as they received.

All synthesized products were structurally characterized by NMR spectra, measured on a Varian Gemini 2000 spectrometer at working frequencies 300 Mz (for ^{1}H-NMR) and 75 Mz (for ^{13}C-NMR). Spectra were measured in $CDCl_3$, using as internal standard TMS. The infrared spectra were recorded on a Nicolet 6700 FT-IR spectrophotometer, scanning between 400 and 4000 cm^{-1}. UV-Vis spectra were measured with a Genesys 10S UV-Vis spectrophotometer in ethanol at a concentration of 10^{-5} mol/L. Purification of curcumin derivative and complexes **A** and **B** was done by column chromatography performed on silica gel (0.035-0.070 mm 60 Å, Acros). Melting points were determined with a Koffler apparatus without correction.

Syntheses: Complex **A:** 0.38 mmol (0.14 g) of (1*E*,6*E*)-1,7-bis(4-(dimethylamino)phenyl)hepta-1,6-diene-3,5-dione were dissolved in 5 mL of chloroform and to this solution were added dropwise 0.38 mmol (0.15 g) of palladium precursor complex containing *N,N,N',N'*-tetramethylcyclohexane-1,2-diamine dissolved in 3 mL of chloroform. Reaction was left on stirring for 96 h at room temperature, then the mixture was filtered and the solvent evaporated under vacuum. The final product was obtained, after purification *via* silica gel chromatography ($CHCl_3$:MeOH, 9:1) as a reddish powder (0.07 g, 26 %). M.p.: 170 °C (decomposed without melting).

^{1}H-NMR (300 Mz, $CDCl_3$) δ (ppm): 1.24-1.36 (m, 2H); 1.42-1.54 (m, 2H); 1.81 (d, 2H); 1.89 (s, 3H); 2.18 (d, 2H); 2.82 (s, 6H); 2.84 (s, 6H); 3.01 (s, 12H); 3.18 (m, 2H); 5.72 (s, 1H); 6.57 (d, 2H); 6.73 (d, 4H); 7.40 (d, 2H); 7.45 (d, 4H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 25.02 (1C); 25.28 (2C); 25.56 (2C); 40.32 (4C); 43.56 (2C); 73.18 (4C); 105.34 (1C); 113.17 (4C); 120.79 (2C); 124.35 (2C); 130.90 (4C); 142.04 (2C); 153.43 (2C); 179.61 (2C). IR ν_{max} (cm^{-1}): 3359; 2931; 1727; 1660; 1597; 1508; 1431; 1407; 1342; 1298; 1272; 1165; 1070; 995; 971; 862; 847; 823; 692; 632. UV-Vis λ_{max} (nm): 272; 504.

Complex **B**: (1*E*,6*E*)-1,7-bis(4-(dimethylamino)phenyl)hepta-1,6-diene-3,5-dione (0.67 mmol, 0.24 g) was dissolved in 10 mL of methanol. To this solution was added dropwise the palladium precursor complex containing *N,N'*-dimethylpiperazine (0.67 mmol, 0.23 g) dissolved in 5 mL of dry methanol. Reaction mixture was stirred at room temperature for 72 h. Final product was purified by silica gel chromatography ($CHCl_3$:MeOH, 9:1), being isolated as a reddish powder (0.06 g, 14 %). M.p.: 195 °C (decomposed without melting).

^{1}H-NMR (300 Mz, $CDCl_3$) δ (ppm): 1.89 (s, 3H); 2.59 (s, 6H); 2.72 (d, 4H); 3.00 (s, 12H); 3.84 (d, 4H); 5.74 (s, 1H); 6.57 (d, 2H); 6.71 (d, 4H); 7.44 (dd, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 24.14 (1C); 40.33 (4C); 46.57 (2C); 59.50 (4C); 105.76 (1C); 113.15 (4C); 120.61 (2C); 124.42 (2C); 130.89 (4C); 142.37 (2C); 153.33 (2C); 179.42 (2C).

IR ν_{max} (cm^{-1}): 3414; 1592; 1497; 1429; 1409; 1364; 1298; 1232; 1158; 1125; 994; 969; 946; 858; 818; 794; 688; 643. UV-Vis λ_{max} (nm): 272; 503.

The absorption of complexes **A** and **B** was monitored by UV-vis spectra in an interval of 96 hours, each spectrum being recorded once in 24 hours. No significant changes of characteristic wavelengths were observed, thus the two palladium complexes **A** and **B** are considered stable in ethanolic solution and atmospheric conditions.

Biologic activity: To evaluate the curcumin like ligand and its palladium complexes **A** and **B**, a pair of *in vitro* cultivated human colon adenocarcinoma cell lines from the European Collection of Cell Cultures (Salisbury, UK) was used as biologic system. The cell lines were the human colon adenocarcinoma HT-29 and DLD-1 cells, respectively, epithelial, adherent cells with high proliferation rate. They were cultivated as described earlier [10] using media acquired from Sigma Aldrich (St Louis, USA); at 37 °C in a sterile Unitherm incubator (from Uniequip, Planegg, Germany) with humidified atmosphere and 5% CO_2 content. Cells passage was made at 85-90% confluence, using Trypsin-EDTA solution.

For cytotoxicity test the cells were plated on 96-well microplates (Nunclon delta surface plates from Thermo Fischer Scientific, Waltham, MA, USA) at a concentration of $13x10^3$ cell/well in 190 µl media and they were incubated for 48 hours. At subconfluency they were treated with 10 µl of ligand or complex. The curcumin derivative and the complexes **A** and **B**, were dissolved in absolute ethanol, to obtain a stock solution of 2 mM. From these stock solutions, it was prepared working solutions through a serial dilution in PBS, from 1000 to 10 µM for each compound. Dissolved compounds were intensively colored, therefore for every concentration color controls were required, by applying the solution in wells without cells, containing cell culture media only. As reference values, untreated cells were used.

The cytotoxicity of the compounds was measured in triplicate, using the MTT dye (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, from Sigma Aldrich), as described earlier [16] the 96-well plates were measured in colorimetry using a Synergy 2.0 microplate reader (from BioTek Company, Winooski, USA) at 570 nm wavelength. Two independent measurements were performed. The absorbance of each well reflected the number of viable cells present.

For cellular uptake, the cells were seeded on 96-well microplates and treated the same as for MTT testing. Six wells were used for every concentration. After 24-hours of incubation with studied compounds, the cell culture media was gently removed; wells were washed twice with PBS and filled with 100 µl PBS. The plates were measured in fluorescence, at 540 nm excitation and 620 nm emission, using the Synergy 2.0 microplate reader.

The results were analyzed with the Graph Pad Prism 5 software (from GraphPad Software, La Jolla, USA).

ACKNOWLEDGMENTS

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0516-12, by VEGA 1/0346/16, by the Romanian National Authority for Scientific Research and Innovation, CCCDI – UEFISCDI Grant for Exploratory Research Project PN-II-ID-PCE-2011-3-1057 and PNIII subprogram 3.1 Romanian-South African cooperation grant no. 9BM/2016 AFROMET.

REFERENCES

1. M. Fridlender, Y. Kapulnik, H. Koltai, *Frontiers in Plant Sciences*, **2015**, *6*, 799.
2. M. Kumaravel, P. Sankar, R. Rukkumani. *European Review for Medical and Pharmacological Sciences,* **2012**, *16*, 1900.
3. G. Bar-Sela, R. Epelbaum, M. Schaffer, *Current Medicinal Chemistry*, **2010**, *17*, 190.
4. S. Wanninger, V. Lorenz, A. Subhan, F.T. Edelmann. *Chemical Society Reviews*, **2015**, *44*, 4986.
5. N. Miklasova, E. Fischer-Fodor, R. Miklas,L. Kuckova, J. Kozisek,T. Liptaj, O. Soritau, J. Valentova, F. Devinsky, *Inorganic Chemical Communication*, **2014**, *46*, 229.
6. E. Fischer-Fodor, R. Mikláš, L.T. Krausz, P. Virag, D.C. Moldovan, M. Perde Schrepler, I. Berindan-Neagoe, F. Devínsky, N. Miklášová, *Studia UBB Chemia*, **2015**, LX, 93.
7. H. Hatcher, R. Planalp, J. Cho, F.M. Torti, S.V. Torti, *Cellular and Molecular Life Sciences*, **2008**, *65*, 1631.
8. H.J.J. Pabon, *Recueil des Travaux Chimiques des PaysBas*, **1964**, *83*, 379.
9. P.J. Roughley, D.A. Whiting, *Journal of the Chemical Society*, *Perkin Transactions 1*, **1973**, 2379.
10. S. Cetean, C. Căinap, O. Soriţău, C. Tatomir, P. Virag, A. Hangan, L. Oprean, R. Oprean, *Revista Romana de Medicina de Laborator*, **2015**, *23*, 439.
11. L. Ekblad ,J. Kjellström, A. Johnsson, *Anticancer Drugs*, **2010**, *21*, 523.
12. D. Theile, S. Grebhardt, W.E. Haefeli, J. Weiss, *Biochemical Pharmacology*, **2009**, *78*, 1366.
13. F.C. S. de Paula, W. Guerra, I.R. Silva, J.N. Silveira, F.V. Botelho, L.Q. Vieira, E.C. Pereira-Maia, *Chemistry & Biodiversity*, **2008**, *5*, 2124.
14. R. Kotcherlakota, A.K. Barui, S. Prashar, M. Fajardo, D. Briones, A. Rodríguez-Diéguez, C. R. Patra, S. Gómez-Ruiz, *Biomaterials Science*, **2016**, *4*, 448.
15. X. Yang, Z. Li, N. Wang, L. Li, L. Song, T. He, L. Sun, Z. Wang, Q. Wu, N. Luo, C. Yi, C. Gong, *Scientific Reports*, **2015**, *5*, 10322.
16. L. Timko, E. Fischer-Fodor, M. Garajová, M. Mrva, G. Chereches, F. Ondriska, M. Bukovský, M. Lukáč, J. Karlovská, J. Kubincová, F. Devínsky, *European Journal of Medicinal Chemistry*, **2015**, *93*, 263.