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Synthesis, characterization and cytotoxicity of a palladium(II) complex of 3-[(2-hydroxybenzylidene)amino]-2-thioxo-imidazolidin-4-one

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Abstract: The polydentate ligand 3-[(2-hydroxybenzylidene)amino]-2-thioxo-imidazolidin-4-one was synthesized in the intermolecular cyclocondensation reaction of 2-hydroxybenzaldehyde thiosemicarbazone and ethyl chloroacetate. A novel palladium(II) complex was obtained from *cis*-[$Pd(DMSO)_2Cl_2$] by nucleophilic substitution of both DMSO ligands with the iminic nitrogen and the thiolactamic sulfur from the ligand. The structures of the compounds were characterized based on their spectral data. The cytotoxic activities of the ligand and the palladium(II) complex were studied on the tumor cell lines: human colon carcinoma HCT-116 and SW-480 cells using the MTT viability test. The results showed that the investigated palladium(II) complex had a significantly greater cytotoxic effect compared to that of the ligand.

Keywords: thiohydantoins; palladium(II) complexes; cytotoxic activity.

INTRODUCTION

Thiohydantoins are sulfur analogs of hydantoins with one or both carbonyl group(s) replaced by a thiocarbonyl group.[1–5] Among the known thiohydantoins, 2-thiohydantoins (2-thioxoimidazolidin-4-ones) are the most well known due to their wide applications as intermediates and reagents as well as therapeutics, herbicides and fungicides. They are traditionally considered as useful intermediates in peptide synthesis and structure determination.[6] 2-Thiohydantoins are also used for other purposes, including textile printing,[7] as catalysts for polymerizations,[8] in the production of resins and plastics[9] and as qualitative reagents for the detec-

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tion of some metal ions upon complexation.[10] Thiohydantoins and their derivatives exist in many natural products.[11–13] In the past decades, great efforts have been devoted to introducing thiohydantoins and their derivatives into desirable substrates, such as pharmacophores and synthetic intermediates.[14–17] Compounds containing the thiohydantoin structural motif have been identified to display a wide range of biological activities. For example, many of them exhibit anticonvulsant,[18] anti-epileptic,[19] antimicrobial,[20] antiviral,[21] antineoplastic,[22] hypolipidemic,[23] antithrombotic,[24] and potential antitumor activities,[25,26] *etc*. Several derivatives are employed either as established drugs in clinical practice[2] or as fungicides and herbicides in agriculture.[27]

It is known[28] that coordination of these compounds with transition metal ions sometimes enhances their antiviral and antitumor activities. 2-Thiohydantoin molecules contain a thioamide fragment and can undergo thione–thiol tautomerism,[29] due to which they can be coordinated to metal ions through the lone electron pairs of the nitrogen or sulfur atoms, or both. Coordination with some transition metals was previously studied.[30–35]. The resulting complexes can contain 2-thiohydantoins as either neutral ligands or monoanions, which are formed upon deprotonation of the N–H group.[36–40] Most of the resulting complexes contain four-membered metalla-cycles.

Thiohydantoin molecules containing substituents with an additional donor atom can form chelate[40–43] or supramolecular[44] complexes in which the metal atom is coordinated by one or two thiohydantoin ligands in the neutral or deprotonated form.

The synthesis of coordination compounds that incorporate the thiohydantoin nucleus could have significant impact in the field of drug design and drug delivery.

In this paper, the synthesis and spectroscopic characterization of a palladium(II) complex of 3-[(2-hydroxybenzylidene)amino]-2-thioxoimidazolidin-4--one is described. The cytotoxic activities of the ligand and its palladium(II) complex were studied on two tumor cell lines of human colon carcinoma using the MTT viability test.

EXPERIMENTAL

Starting materials

All chemicals were purchased from commercial sources (Sigma-Aldrich, Fluka or Centrohem) and were used without further purification. Solvents were purified and dried by standard methods. Dimethyl sulfoxide (DMSO) and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were obtained from Serva, Germany. Dublecco's modified Eagle medium (DMEM) was obtained from Gibco Invitrogen. Fetal bovine serum (FBS) and trypsin–EDTA were obtained from PAA-The cell culture company, Austria.

Cis-[Pd(DMSO)$_2$Cl$_2$][45] and 2-hydroxybenzaldehyde thiosemicarbazone[46] **1** were synthesized according to literature methods.

Instrumentations

The elemental analyses were performed by standard micro-methods using an Elemental Vario ELIII CHNSO analyzer.

The IR spectra were recorded on a Perkin-Elmer FTIR spectrometer, model Spectrum One.

The ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Gemini 2000 spectrometer at 200 and 50 MHz, respectively, in DMSO-d_6 solution using TMS as internal standard. D_2O exchange was applied to confirm the assignment of the nitrogen- and oxygen-bound protons.

The UV–Vis spectra were recorded on a Perkin-Elmer Lambda 35 double beam spectrophotometer in 1.00 cm quartz Suprasil cells.

The X-ray powder diffraction (XRPD) patterns were obtained on a Philips PW 1710 automated X-ray powder diffractometer using a Cu tube operated at 40 kV and 35 mA. The instrument was equipped with a diffracted beam curved graphite monochromator and a Xe-filled proportional counter. The diffractometer was calibrated with a silicon standard sample. For the Rietveld profile fitting method, the diffraction data were collected using the step-scanning mode in the 2θ range between 4 and 130° with 0.02° steps. The counting time was fixed to 5 s per step. The collected data were refined using FullProff software.

Ligand synthesis

The procedure for preparation of 3-[(2-hydroxybenzylidene)amino]-2-thioxoimidazolidin-4-one **2** was analogous to that described previously.[46] A mixture of 5.100 g **1** (26.15 mmol) and 3.204 g ethyl chloroacetate (26.15 mmol) in absolute methanol (150 cm^3) in the presence of 6.433 g fused sodium acetate (78.45 mmol) was heated under reflux for 6 h. The reaction mixture was cooled and poured into water. The precipitate was separated by filtration, washed with cold water and dried. The crude product was recrystallized from hot methanol. Yield: 4.548 g.

Complex synthesis

The synthesis of palladium(II) complex with 3-[(2-hydroxybenzylidene)amino]-2-thioxoimidazolidin-4-one **3** was achieved by dissolving 0.178 g **2** (0.76 mmol) in 10 cm^3 of absolute methanol under reflux until dissolution and then adding 10 cm^3 of a methanolic solution of $Pd(DMSO)_2Cl_2$ (0.252 g, 0.76 mmol) in three portions. Reaction mixture was heated under reflux for 12 h, cooled to 0 °C and the resulting orange–red precipitate was separated by filtration, washed with hot water, small amount of hot methanol and dried. The complex was recrystallized from hot methanol. Yield: 0.180 g.

Antiproliferative assay

Tumor cell lines were obtained from the American Type Culture Collection. The cells were maintained in DMEM supplemented with 10 % FBS, with 100 units mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. The cells were cultured in a humidified atmosphere with 5 % CO_2 at 37 °C. The cells were grown in 75 cm^2 culture bottles supplied with 15 mL DMEM, and after a few passages, the cells were plated in a 96-multiwell plate (10^4 cells $well^{-1}$). All studies were realized with cells at 70 to 80 % confluence. After 24 h of incubation of the cells, the medium was replaced with 100 μL medium containing various doses of **2** and **3** (0.1, 1, 10, 50, 100 and 500 μM) for 24 and 72 h. Untreated cells served as the control. After 24 and 72 h of treatment, the cell viability was determined by the MTT assay.[47] The proliferation test is based on the color reaction of mitochondrial dehydrogenase in living cells with MTT. At the end of the treatment period, MTT (final concentration 5 mg mL^{-1} PBS) was added to each well, which was then incubated at 37 °C in 5 % CO_2 atmosphere for 2–4 h. The colored crys-

tals of the produced formazan were dissolved in 150 μL DMSO. The absorbance was measured at 570 nm on Microplate Reader (Elisa 2100C). Cell proliferation was calculated as the ratio of the absorbance of the treated group divided by the absorbance of the control group, multiplied by 100 to give the percentage proliferation.

Biological activity was the result of one individual experiment, performed in triplicate for each dose. The magnitude of correlation between the variables was determined using an SPSS (Chicago, IL) statistical software package (SPSS for Windows, ver. 17, 2008). The effect of each extract are expressed by IC_{50} (inhibitory dose which inhibits 50 % of cell growth) and by the magnitude of maximal effect in exposed cells. The IC_{50} values were calculated from the dose curves using the CalcuSyn computer program.

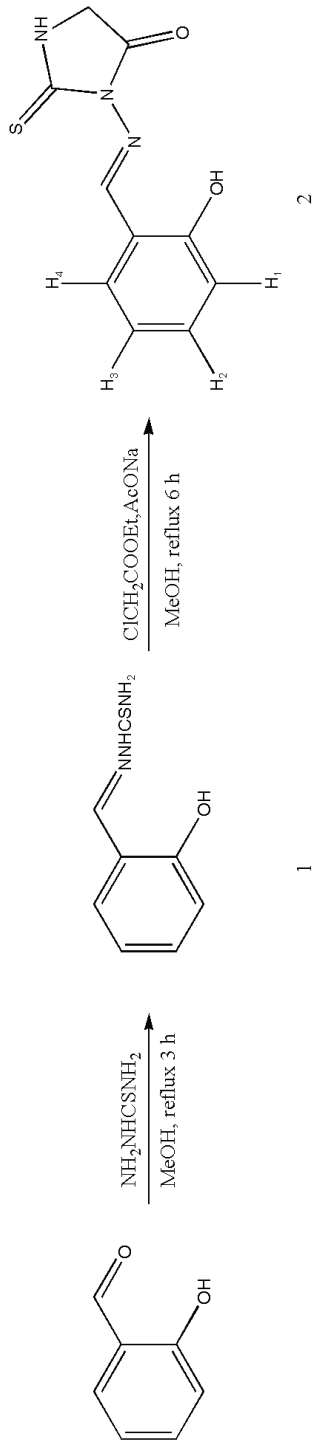
RESULTS AND DISCUSSION

The polydentate ligand, 3-[(2-hydroxybenzylidene)amino]-2-thioxoimidazolidin-4-one **2**, was obtained by intermolecular cyclocondesation reaction of 2-hydroxybenzaldehyde thiosemicarbazone and ethyl chloroacetate (Scheme 1).[46]

Yield: 74.0 %. Anal. Calcd. for $C_{10}H_9N_3O_2S$: C, 51.05; H, 3.86; N, 17.86 %. Found: C, 51.08; H, 3.88; N, 17.89 %; IR (KBr, cm^{-1}): 3443 (O–H), 3318 (NH), 3173, 3031 (CH Ar), 2987, 2808 (CH_2), 1721 (C=O), 1616 (C=N), 1604, 1538, 1490 (C=C Ar), 1464, 1368, 1282, 1266 (C=S), 1202 (C–O), 1112, 1062, 949, 830, 753 (δ CH Ar), 627; ^{1}H-NMR (DMSO-d_6, δ / ppm): 3.97 (2H, *s*, CH_2), 6.94 (2H, *m*, H1–, H2–Ar), 7.30 (1H, *dt*, *J* = 7.8 and 1.8 Hz, H3–Ar), 7.75 (1H, *dd*, *J* = 8.0 and 1.9 Hz, H4–Ar), 8.64 (1H, *s* CH=N), 10.88 (1H, *s* OH), 12.06 (1H, *bs* NH); ^{13}C-NMR (50 MHz, DMSO-d_6, δ / ppm = 33.53 (CH_2), 116.51, 118.63, 119.69, 130.76, 132.34, 156.60 (Ar), 158.19 (C=N), 174.04 (C=O), 177.94 (C=S); UV–Vis (DMSO, λ_{max} / nm): 305.91, 338.97, 351.14.

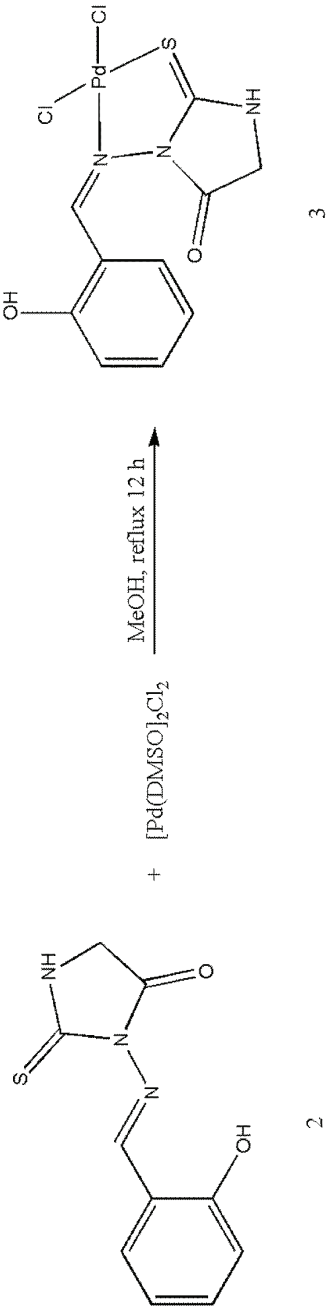
The novel palladium(II) complex **3** was obtained by nucleophilic substitution of both DMSO ligands from *cis*-[Pd(DMSO)$_2$Cl$_2$] with the iminic nitrogen and thiolactamic sulfur from ligand **2** (Scheme 2). A non-charged complex was isolated as an orange–red amorphous precipitate. The metal complex was non-hygroscopic, soluble in DMSO, sparingly soluble in methanol and ethanol and insoluble in water. The analytical data revealed 1:1 (metal:ligand) stoichiometry. The structures of the ligand and complex were established by its elemental composition and IR, NMR and electronic spectra.

Yield: 57.7 %. Anal. Calcd. for $C_{10}H_9Cl_2N_3O_2PdS$: C, 29.11; H, 2.22; N, 10.18 %. Found: C, 29.08; H, 2.18; N, 10.16 %; IR (KBr, cm^{-1}) 3436 (O–H), 3153 (NH), 2925 (CH_2), 2853, 1719 (C=O), 1602 (C=N), 1533, 1400, 1385, 1332 (C=S), 1254, 1201 (C–O), 1154, 755 (δ CH Ar); ^{1}H-NMR (DMSO-d_6, δ / ppm): 3.16 (2H, *s* CH_2), 6.64 (1H, *t*, *J* = 7.0 Hz, H2–Ar), 6.93 (1H, *d*, *J* = 8.0 Hz, H1–Ar), 7.29 (1H, *dt*, *J* = 8.6 and 1.9 Hz, H3–Ar), 7.48 (1H, *dd*, *J* = 7.6 and 1.9 Hz, H4–Ar), 8.10 (1H, *s* CH=N), 9.78 (1H, *s* OH), 10.89 (1H, *bs* NH). ^{13}C--NMR (50 MHz, DMSO-d_6, δ / ppm) 48.77 (CH_2), 115.32, 118.12, 119.67, 133.47, 134.26, 148.71 (Ar), 161.31 (C=N), 169.91 (C=O), 193.26 (C=S). UV––Vis (DMSO, λ_{max} / nm): 259.25, 304.72, 340.39, 403.33.



1 2

Scheme 1. Synthesis of 3-[(2-hydroxybenzylidene)amino]-2-thioxoimidazolidin-4-one **2**.



2 3

Scheme 2. Synthesis of the palladium(II) complex with 3-[(2-hydroxybenzylidene)amino]-2-thioxoimidazolidin-4-one **3**.

Spectroscopic characterization

In order to confirm complex formation and gain insight into the structure of obtained compounds, the IR spectra of the free ligand and its complex were recorded. The absence of the strong band at ≈2500 cm^{-1}, assignable to the ν(S–H) mode, indicate that both compounds exist in the thiono (C=S) form in the solid state. Comparison of vibrational frequencies of the free ligand with those of the complex showed a shift in the C=S band to a higher frequency by 66 cm^{-1}. This is evidence that the thionic sulfur atom was involved in the coordination.[32,48] In addition, the C=N absorption band was shifted to a lower frequency by 14 cm^{-1}. Consequently, the iminic nitrogen atom is also presumably involved in the coordination.[49] The non-coordination of O is shown by the C=O band shifting only very slightly upon complexation. The broad band observed in region 3153 cm^{-1} due to N–H stretch was shifted in complex, probably because of an adjustment in the current arising due to coordination of the thionic sulfur.

Further evidence for the coordinating mode of the thiohydantoin **2** was obtained from the NMR spectra in DMSO-d_6. In the ^{1}H-NMR spectrum of the ligand, there is no resonance at *ca*. 4.0 ppm, attributed to the resonance of the –SH proton, while the appearance of a broad peak at 12.06 ppm due to the –NH proton indicates that even in a polar solvent such as DMSO, it remains in the thione form. Such a large chemical shift for an –NH proton was already reported for similar systems.[50] Various types of primary and secondary hydrogen bond interactions are possible in the studied system. The phenolic H-atom could form an intramolecular hydrogen bond with the iminic nitrogen and additionally, as donating bifurcated, with the carbonyl group (Fig. 1). This proposal was confirmed experimentally by recording the ^{1}H-NMR spectrum of ligand **2** in the presence of NaOH. Upon transformation of the ligand into the phenolate form, the OH proton at 10.88 ppm disappeared while the other protons shift upfield (the –NH proton shifted from 12.06 to 10.98 ppm, Fig. 2.) This fact also could explain the upfield shifts of the corresponding signals in complex **3**. It seems that coordination prevented the formation of an intramolecular hydrogen bond because after complexation, iminic nitrogen has no lone electron pair. The –NH proton signal of the thiohydantoin shifted upfield and appeared at 10.89 ppm in complex **3**. The up-

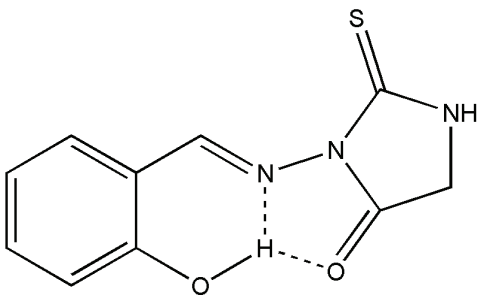


Fig. 1. Intramolecular hydrogen bond interaction in ligand **2**.

field shift of the iminic proton signal by 0.54 ppm is clear evidence for coordination by the iminic nitrogen.

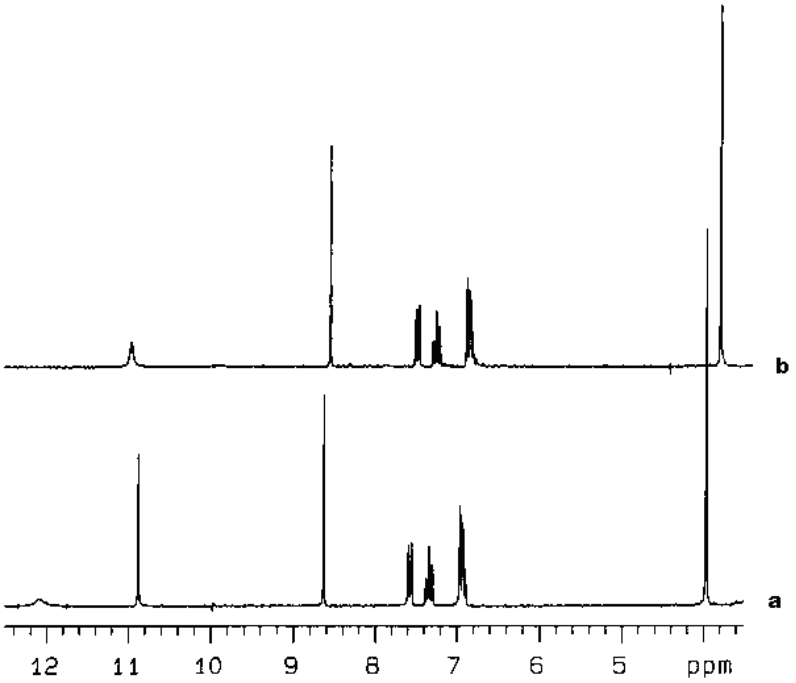


Fig. 2. ^{1}H-NMR spectra of ligand **2** (a) and ligand **2** after the addition of a small amount of solid NaOH (b).

The ^{13}C-NMR chemical shifts of the C=S and C=N resonances reflected the involvement of the sulfur from the thiohydantoin ring and the iminic nitrogen in an intermolecular interaction.

Based on foregoing discussion, the ligand behaves as a neutral NS-bidentate coordinative agent towards the palladium(II) ion.

In the electronic spectra, two intense absorption bands were exhibited at 305.91 and 351.14 nm for the ligand and at 304.72 and 403.33 nm for the complex. Three d–d spin allowed singlet–singlet and three spin forbidden singlet–triplet transitions are predicted for square-planar complexes of palladium(II). However, strong charge-transfer transitions may interfere and prevent the observation of some of the expected bands, especially for complexes containing sulfur donor atoms.[51] The prominent strong band at 403.33 nm was assigned to a combination of intraligand and ligand to metal charge transfer absorptions and d–d bands, which supports the idea of a square-planar environment for the metal ion.

In the XRPD pattern of the ligand **2**, low intensity and fairly broad peaks showed the poor crystallinity of the substance. The low intensities, poorly de-

fined peaks on the XRPD pattern of complex **3** demonstrated its low degree of crystallinity. The broad hump around 10° 2θ indicates the existence of an amorphous phase and might represent extremely poor or partial crystallinity of complex **3**. The existence of an amorphous hump between 6 and 15° 2θ in the pattern of complex **3** is obvious in comparison with the ligand **2**. In addition, there is no clear phase transition, but the metal–ligand complex disrupted the structure, causing the poor crystallinity demonstrated in the diffraction pattern.

Due to the poor solubility of the isolated complex in suitable solvents, no single crystal of sufficient quality could be obtained. Therefore, attention was focused on spectroscopic methods for the structural characterization of complex **3**, which suggested coordination of the ligand to palladium *via* the iminic nitrogen and the thionic sulfur (Scheme 2).

Antiproliferative activity

After seeding in standard DMEM medium, the cells were exposed to different concentrations of ligand **2** and complex **3** for 24 and 72 h at 37 °C. The results obtained with the antiproliferative assays are represented through IC_{50} values (Table I).

Comparing the cytotoxic effect of the complex and ligand, a significant difference was observed. Namely, while the complex exerted extreme cytotoxicity with IC_{50} values for 72-h treatment of 6.51 and 8.89 μM for HCT-116 and SW-480 cell lines respectively (Table I), the ligand did not show a considerable cytotoxic effect, even though decreased cell viability was generated with increasing ligand concentration.

TABLE I. Growth inhibitory effects – IC_{50} values (μM) of the Pd(II) complex and its ligand on HCT-116 and SW-480 cell lines after 24 and 72 h of treatment

Compound	HCT-116		SW-480	
	24 h	72 h	24 h	72 h
Pd(II) complex	16.98±0.21	6.51±0.02	15.73±0.25	8.89±0.36
Ligand	336.75±2.20	>500	>500	366.00±2.55
Cisplatin	293.52±2.36	58.55±1.23	325.19±2.35	49.58±1.54

Complex **3** exerted very high cytotoxic activity, *i.e.*, with increasing complex concentration, the cytotoxicity increased. A statistically significant difference in cytotoxicity after 24 h of treatment was noticed compared to that after 72 h of treatment. Interestingly, studying the complex activity it was shown that sensitivity of investigated cell lines are of similar extent.

Considering the cytotoxicity of the ligand, it was noticed from the IC_{50} values (Table I) and from the measured absorbances that the cytotoxicity increased with increasing applied concentration of the ligand. It was shown that the SW-480 cells exhibited greater sensitivity to the ligand 72 h after treatment (IC_{50} =

366 μM) compared to after 24 h (> 500 μM). Interestingly, the HCT-116 cells show exactly the opposite trend, *i.e.*, a greater cytotoxic effect was observed 24 h (IC_{50} = 337 μM) after treatment compared to after 72 h (> 500 μM). This was probably due to the acute effect of the investigated ligand on the SW-480 cells. Although the cytotoxicity of the ligand was quantified and statistically processed, it is important to notice that this cytotoxic effect was certainly not significant, especially in comparison with the activity of the complex.

The results show that the investigated complex had a significantly greater cytotoxicity compared to that of cisplatin and further opened up the possibility for the synthesis of similar complexes of palladium(II).

CONCLUSIONS

A novel palladium(II) complex was synthesized by substitution reaction of *cis*-[$Pd(DMSO)_2Cl_2$] with a 2-thiohydantoin type ligand. Based on spectral data, it is proposed that 3-[(2-hydroxybenzylidene)amino]-2-thioxoimidazolidin-4-one acts as a bidentate ligand, making use of the thiolactamic sulfur and iminic nitrogen for coordination to the central metal ion. The cytotoxicity of this complex was investigated on two independent colon cancer cell lines (HCT-116 and SW-480) using the MTT viability test. The complex exhibited very high cytotoxic activity and showed a cytotoxic effect that was much better than that of the ligand. The observed cytotoxicity could be pursued to obtain a potential chemotherapeutic drug.

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ИЗВОД

СИНТЕЗА, КАРАКТЕРИЗАЦИЈА И ЦИТОТОКСИЧНОСТ ПАЛАДИЈУМ(II) КОМПЛЕКСА СА 3-[(2-ХИДРОКСИБЕНЗИЛИДЕН)АМИНО]-2-ТИОКСОИМИДАЗОЛИДИН-4-ОНОМ

БИЉАНА ШМИТ[1], РАДОСЛАВ З. ПАВЛОВИЋ[1], АНА РАДОСАВЉЕВИЋ-МИХАИЛОВИЋ[2], АЊА ДОШЕН[2], МИЛЕНА Г. ЂУРЧИЋ[1], ДРАГАНА С. ШЕКЛИЋ[1] и МАРКО Н. ЖИВАНОВИЋ[1]

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Полидентатни лиганд, 3-[(2-хидроксибензилиден)амино]-2-тиоксоимидазолидин-4-он синтетисан је интермолекулском циклокондензацијом 2-хидроксибензалдехидтиосемикарбазона и етил-хлороацетата. Нов паладијум(II) комплекс добијен је нуклеофилном супституцијом оба DMSO лиганда из *cis*-[$Pd(DMSO)_2Cl_2$] иминским азотом и тиолактамским сумпором из лиганда. Структуре једињења су окарактерисане на основу њихових спектралних података. Цитотоксична активност лиганда и паладијум(II) комплекса испитивана је на туморским ћелијским линијама: хуманог карцинома колона HCT-116 и SW-480 помоћу MTT теста вијабилности. Испитивани паладијум(II) комплекс испољава веома висок цитотоксични ефекат, много бољи од лиганда.

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