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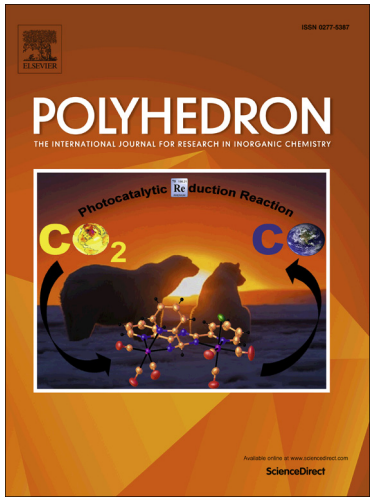

Cytotoxicity of palladium(II) complexes with some alkyl derivates of thiosalicylic acid. Crystal structure of the *bis*(S-butyl-thiosalicylate)palladium(II) complex, [Pd(S-bu-thiosal)$_2$]

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Cytotoxicity of palladium(II) complexes with some alkyl derivates of thiosalicylic acid. Crystal structure of the *bis*(S-butyl-thiosalicylate)palladium(II) complex, [Pd(S-bu-thiosal)$_2$]

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Abstract

The spectroscopically predicted structure of the obtained *bis*(S-butyl-thiosalicylate)palladium(II) complex, [Pd(S-bu-thiosal)$_2$], was confirmed by an X-ray structural study. The asymmetric unit of [Pd(S-bu-thiosal)$_2$] consists of neutral complex molecules, where the Pd(II) ion is placed in a *cis*-square-planar coordination environment formed by O and S atoms of two deprotonated S-butyl-thiosalicylic acid ligands. The cytotoxic effects of the S-alkyl (R = benzyl (**L1**), methyl (**L2**), ethyl (**L3**), propyl (**L4**) and butyl (**L5**)) derivatives of thiosalicylic acid and the corresponding palladium(II) complexes are reported here. The analysis of cancer cell viability showed that all the tested complexes are cytotoxic to human colon carcinoma cells (HCT-116 and CaCo-2) and human lung

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carcinoma epithelial cells (A549). The antitumor activities of the above mentioned Pd(II) complexes are higher in comparison to the corresponding ligands.

Keywords: palladium(II) complexes; crystal structure; cytotoxicity

1. Introduction

Since the seventies when *cisplatin,* with antitumor activity, was discovered by Rosenberg and co-workers [1,2], thousands of platinum complexes have been synthesized in order to obtain new platinum(IV) compounds with improved properties in comparison to the parent drug *cisplatin* [3-5]. It has been shown that platinum(II) complexes with antitumor activity are represented by the general formula $[Pt(am)_2X_2]$, having a *cis* geometry, where (am) is an inert amine ligand having at least one NH group and X is an easy leaving ligand. The leaving group should be an anion with a moderately strong coordination to the platinum(II) ion, as well as with a weak *trans* effect in order to avoid labilization of the amine ligand bond.

Those discoveries have increased the interest in obtaining efficient ligands and complexes of other metals. The palladium(II) complexes *cis*-diamminedichloridopalladium(II), *cis*-$[PdCl_2(NH_3)_2]$, and *cis*-1,2-diaminocyclohexanedichloridopalladium(II), *cis*-$[PdCl_2(DACH)]$, which are analogues to cisplatin, were among the first complexes used in clinical trials as anti-tumor agents [6].

Platinum(II) complexes are known to be thermodynamically and kinetically more stable than their palladium(II) analogues. The reactivity of the palladium(II) complexes for hydrolysis and exchange of the ligands is 10^5 times faster than the corresponding platinum(II) complexes. Therefore the palladium(II) complexes show lower antitumor activity, but higher toxicity [6-8].

Although the initial results showed no significant antitumor activity of the palladium(II) complexes, these complexes have been widely studied. Due to their high reactivity, palladium(II) complexes generally exhibit lower antitumor activity than *cisplatin* [6,7]. It was concluded that the lower activity of the palladium(II) complexes is the result of a very rapid exchange of the ligands and the inability of the complexes, with an unchanged structure, to reach biological targets, increasing the risk of adverse effects on biochemical processes in the cell. In order to overcome these problems some authors [9] suggested that the palladium(II) ion should be coordinated by chelating ligands, reducing the reactivity of the palladium center and increasing the stability of the complex.

A palladium(II) complex with a coumarine derivative was synthesized, showing approximately 7800 times higher activity than carboplatin for A549, K562 and HeLa cells [10,11]. This confirms the assumption that complexes with voluminous ligands have better antitumor activity. Also a large number of palladium(II) complexes with neutral ligands were tested, such as phosphate derivatives of quinoline [12,13], derivatives of pyridine [14,15] and pyrazole [16], and these complexes showed significant antitumor activity.

Thiosalicylic acid and its derivatives are used for metal determination [17,18], as modificators for graphite paste electrodes [19], as photoinitiators for free radical polymerization [20], in cosmetics [21], in disease treatments (in particular inflammatory, allergic and respiratory diseases) [22], as well as Ras-tumor growth inhibitors [23]. In our previously published papers we investigated the antimicrobial activity of some S-alkyl derivatives of thiosalicylic acids and the corresponding palladium(II) complexes. We concluded that all the tested compounds showed a low antimicrobial activity, but the palladium(II) complexes showed higher activity than the corresponding S-alkyl derivatives of thiosalicylic acid [24].

The aim of this paper is to research the *in vitro* cytotoxic effects of the earlier synthesized S-alkyl derivatives of thiosalicylic acid and the corresponding palladium(II) complexes, due to recent great interest in palladium(II) complexes as cytotoxic and anticancer drugs [25-27]. For the first time, we demonstrate that five newly synthesized palladium(II) complexes and their corresponding ligand precursors exhibit relevant cytotoxic properties to three different human cancer cell lines: CaCo-2, HCT116 and A549. The CaCo-2 cell line is a continuous line of heterogeneous human epithelial colorectal adenocarcinoma cells [28]. The HCT-116 cell line is an adherent epithelial cell line originating from human colorectal carcinoma [29], while the human lung carcinoma epithelial cell line A549 is the most usually used cancer cell line for *in vitro* research in the field of testing cytotoxicity and metabolism of new synthesized complexes towards human lung carcinoma epithelial cells [30].

2. Experimental

2.1. Materials and measurements

The reagents were obtained commercially and used without further purification. For the infrared spectra, a Perkin-Elmer Spectrum One FT-IR spectrometer was employed. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini-200 NMR spectrometer using TMS in DMSO-d_6 as an internal reference, at 22 ºC and with 10 mM solutions of the complexes. Elemental analyses were done on a Vario III CHNOS Elemental Analyzer, Elemental Analysensysteme GmbH.

2.2. Syntheses

2.2.1. General procedure for the synthesis of the S-alkyl thiosalicylic acids (L1)-(L5)

The thioacid ligands **L1**-**L5** were prepared by alkylation of thiosalicylic acid by means of the corresponding alkyl halogenides in alkaline water-ethanol solution. Thiosalicylic acid

(1 mmol) was added to a 100 mL round bottom flask containing 50 mL of a 30% solution of ethanol in water and stirred. A solution of NaOH (2 mmol in 5 mL of water) was added to the acid suspension, whereupon the solution became clear. The corresponding alkyl halogenide (2 mmol) was dissolved in 5 mL of ethanol and transferred to the stirred solution. The resulting mixture was kept overnight at 60 °C. The reaction mixture was transferred into a beaker and ethanol was evaporated off on a water bath. Diluted hydrochloric acid (2 mol/L) was added to the resulting water solution and S-alkyl thiosalicylic acid was precipitated as a white powder. The free acid was filtered off and washed with plenty of distilled water. The product was dried under vacuum overnight. Yield: 85-95%.

*2.2.2. Preparation of the bis(S-benzyl-thiosalicylate)–palladium(II) complex, [Pd(S-bz-thiosal)$_2$] (**C1**)*

The palladium(II) complexes with S-alkyl derivatives of thiosalicylic acid were obtained by the previously described procedure [24]. $K_2[PdCl_4]$ (0.100 g, 0.3065 mmol) was dissolved in 10 mL of water on a steam bath and (S-benzyl)-2-thiosalicylic acid (**L1**) (0.1497 g, 0.613 mmol) was added into the solution. The resulting mixture was stirred for 2 h and during this time an aqueous solution of LiOH (0.0256 g, 0.613 mmol in 10 mL of water) was introduced. The complex [Pd(S-bz-thiosal)$_2$] (**C1**), as a yellow precipitate, was filtered, washed with water and air-dried. Yield: 0.11 g (58.70%). *Anal.* Calc. for $C_{28}H_{22}O_4S_2Pd$ (M_r = 592.98): C, 56.71; H, 3.74; S, 10.82. Found: C, 56.43; H, 3.85; S, 10.75%. IR (KBr, cm^{-1}): 3420, 3057, 1634, 1616, 1562, 1327, 1146, 753, 708, 698. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 4.05 (s, 4H, CH_2), 7.08-8.10 (m, 9H, Ar and bz). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 25.9 (CH_2), 124.1, 125.6, 125.7, 126.2, 126.3, 126.8, 127.3, 127.8, 129.5, 133.2, 136.2, 139.7 (Ar and bz), 171.5 (COO^-).

2.2.3. *Preparation of the bis(S-methyl-thiosalicylate)–palladium(II) complex, [Pd(S-met-thiosal)$_2$] (**C2**)*

The complex [Pd(S-met-thiosal)$_2$] (**C2**) was prepared as described in Section 2.2.2., using (S-methyl)-2-thiosalicylic acid (**L2**) (0.103 g, 0.613 mmol) instead of (S-benzyl)-2--thiosalicylic acid. Yield: 0.08 g (59.80%). *Anal.* Calc. for $C_{16}H_{14}O_4S_2Pd$ (M_r = 440.672): C, 43.61; H, 3.20; S, 14.52. Found: C, 43.41; H, 3.39; S, 14.21%. IR (KBr, cm^{-1}): 3419, 1619, 1597, 1399, 1385, 1332, 1306, 1142, 960, 865, 741, 693, 654. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 2.35 (s, 6H, CH_3), 7.19-8.08 (m, 8H, Ar). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 14.6 (CH_3), 123.6, 125.1, 125.2, 129.0, 132.7, 135.7, (Ar), 171.8 (COO^-).

*2.2.4. Preparation of the bis(S-ethyl-2-thiosalicylate)–palladium(II) complex, [Pd(S-et-thiosal)$_2$] (**C3**)*

The complex [Pd(S-et-thiosal)$_2$] (**C3**) was prepared as described in Section 2.2.2., using (S-ethyl)-2-thiosalicylic acid (**L3**) (0.1117 g, 0.613 mmol) instead of (S-benzyl)-2--thiosalicylic acid. Yield: 0.0832 g (57.90%). *Anal.* Calc. for $C_{18}H_{18}O_4S_2Pd$ (M_r = 468.856): C, 46.11; H, 3.87; S, 13.68. Found: C, 45.97; H, 3.93; S, 13.54%. IR (KBr, cm^{-1}): 1436, 1587, 1518, 1393, 752. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 1.27 (t, 6H, CH_3), 2.83 (q, 4H, CH_2), 7.11-8.08 (m, 8H, Ar). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 14.4 (CH_3), 13.8 (CH_2), 124.8, 125.3, 126.1, 128.7, 133.2, 135.9 (Ar), 172.0 (COO^-).

*2.2.5. Preparation of the bis(S-propyl-2-thiosalicylate)–palladium(II) complex, [Pd(S-pro-thiosal)$_2$] (**C4**)*

The complex [Pd(S-pro-thiosal)$_2$] (**C4**) was prepared as described in Section 2.2.2., using (S-propyl)-2-thiosalicylic acid (**L4**) (0.1203 g, 0.613 mmol) instead of (S-benzyl)-2-thiosalicylic acid. Yield: 0.0889 g (58.40%). *Anal.* Calc. for $C_{20}H_{22}O_4S_2Pd$ (M_r = 496.908):

C, 48.34; H, 4.46; S, 12.91. Found: C, 48.52; H, 4.11; S, 12.73%. IR (KBr, cm^{-1}): 1421, 1589, 1541, 1520, 1397, 752. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 0.98 (t, 6H, CH_3), 1.76 (m, 4H, CH_2), 2.84 (t, 4H, CH_2), 7.20-8.25 (m, 8H, Ar). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 13.2 (CH_3), 22.0 (CH_2), 27.6 (CH_2), 125.1, 126.6, 126.7, 130.5, 134.2, 137.2 (Ar), 172.5 (COO^-).

2.2.6. *Preparation of the bis(S-butyl-2-thiosalicylate)–palladium(II) complex, [Pd(S-bu-thiosal)$_2$] (**C5**)*

The complex [Pd(S-bu-thiosal)$_2$] (**C5**) was prepared as described in Section 2.2.2., using (S-butyl)-2-thiosalicylic acid (**L5**) (0.1289 g, 0.613 mmol) instead of (S-benzyl)-2-thiosalicylic acid. Yield: 0.0941 g (58.43%). *Anal.* Calc. for $C_{22}H_{26}O_4S_2Pd$ (M_r = 524.960): C, 50.33; H, 4.99; S, 12.22. Found: C, 50.52; H, 4.51; S, 12.56%. IR (KBr, cm^{-1}): 3419, 1619, 1597, 1399, 1385, 1332, 1306, 1142, 960, 865, 741, 693, 654. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 2.35 (s, 6H, CH_3), 7.19-8.08 (m, 8H, Ar). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 14.6 (CH_3), 123.6, 125.1, 125.2, 129.0, 132.7, 135.7, (Ar), 171.8 (COO^-).

2.3. X-ray experiment

The diffraction data from a selected single crystal of [Pd(S-bu-thiosal)$_2$] were collected at room temperature with an Oxford Diffraction Xcalibur Gemini S diffractometer equipped with MoKα radiation (λ = 0.71073 Å). Data integration and scaling of the reflections were performed with the CRYSALIS software [31]. Empirical absorption corrections of the diffracted intensities were performed with the SCALE3 ABSPACK [32] scaling algorithm implemented in the CRYSALIS suite.

The crystal structure was solved by direct methods using SIR2002 [33] and refined using the SHELXL program [34]. Hydrogen atoms attached to carbon atoms were placed at

geometrically idealized positions with C–H distances fixed to 0.96, 0.97 and 0.96 Å from methyl, methylene and phenyl C atoms, respectively. The isotropic displacement parameters of the hydrogen atoms were equal to 1.2 U_{eq} of the parent methylene and phenyl C atoms and 1.5 U_{eq} of the methyl C atoms. The crystallographic data are listed in Table 1. The PARST [35] program was used to perform geometrical calculations and the program ORTEP [36] was employed for molecular graphics.

2.4. In vitro cytotoxicity studies

2.4.1. Preparation of drug solutions

Stock solutions of the tested palladium(II) complexes and ligand precursors were made in dimethyl sulfoxide (DMSO) at a concentration of 20 mM, filtered through a 0.22 mm Millipore filter before use and diluted by a nutrient medium to various working concentrations. The concentration of DMSO in most concentrated working solutions was 1% (v/v). The nutrient medium used was HEPES (4-(2-hydroxyethyl)-1--piperazineethanesulfonic acid) buffered RPMI-1640 supplemented with streptomycin (100 mg/mL), penicillin (100 IU/mL) and 10% fetal bovine serum (FBS). MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide was dissolved (5 mg/mL) in a phosphate buffer saline (PBS) having a pH of 7.2, and filtered through a 0.22 mm Millipore filter before use. All reagents were purchased from Sigma Chemicals (Sigma Aldrich, Munich, Germany).

2.4.2. Cell lines

The human cancer colon cell line CaCo-2 and the human lung carcinoma epithelial cell line A549 were purchased from the American Type Culture Collection (ATCC, Manassas, USA). Human cancer colon cell line HCT-116 was kindly provided by Dr Danijela

Vignjević (Institute Curie, Paris, France). The fibroblasts, isolated as previously described [37], were kindly provided by Dr Biljana Ljujić (Centre for Molecular Medicine and Stem Cell Research, Kragujevac, Serbia) and, as non-cancer cells, were used for studying any unwanted cytotoxicity of the tested complexes.

CaCo-2 and HCT-116 cells were maintained in RPMI 1640 (Sigma Aldrich, Munich, Germany) supplemented with 10% fetal bovine serum, FBS (Sigma), penicillin G (100 IU/mL), streptomycin (100 μg/mL), and in a humidified atmosphere of 95% air/5% CO_2 at 37 °C. The A549 cells and fibroblasts were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS, 100 IU/mL penicillin G and 100 μg/mL streptomycin (Sigma). The cell number and viability were determined by trypan blue staining. CaCo-2, HCT-116, A549 cells and fibroblasts in passage 3 were used throughout these experiments.

2.4.3. Assessment of cytotoxicity

The effects of selected complexes on the viability of CaCo-2, HCT-116 and A549 cancer cells and fibroblasts were determined using an MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test. The CaCo-2, HCT-116 and A549 cells and fibroblasts were diluted with medium to 5 x 10^4 cells/mL, and aliquots (5 x 10^3 cells/100 μL) were placed in individual wells in 96-multiplates. After 24 hours, the cells were treated with selected concentrations of the complexes for 3 days: the medium was exchanged with 100 μL of different compounds, which had been serially diluted 2-fold in the medium to concentrations ranging from 250 μM to 3.9 μM. Control wells were prepared by the addition of a culture medium. The wells containing a culture medium without cells were used as blanks. After incubation, the drug containing medium was discarded and replaced with serum free medium containing 15% of MTT (5 mg/mL) dye. After an additional 4 h of incubation at 37°C in a 5% CO_2 incubator, the medium with MTT was removed and DMSO (150 μL) with glycine buffer

(20 μL) was added to dissolve the blue formazan crystals. The plates were shaken for 10 min. The optical density of each well was determined at 595 nm. The percentage cytotoxicity was calculated using the formula:

% cytotoxicity = 100-((TS-BG0)-E/(TS-BG0) x 100)

where BG0 is the background of the medium alone, TS is the total viability/spontaneous death of untreated target cells and E is the experimental well.

3. Results and discussion

3.1. Chemistry

Palladium(II) complexes with S-alkyl derivatives of thiosalicylic acid, [Pd(S-alkyl-thiosal)$_2$], were obtained by the direct reaction of $K_2[PdCl_4]$ with the S-alkyl derivatives of thiosalicylic acid (in a molar ratio of 1:2) in water solution, as described earlier [24]. On the basis of the IR and NMR spectra of the ligands and the corresponding Pd(II) complexes, we concluded that the ligands are bidentately coordinated to the palladium(II) ion, but claim nothing about the geometry of complexes. Based on S,O-coordination of all the ligands and the crystal structure of the [Pd(S-bz-thiosal)$_2$] [24] and [Pd(S-bu-thiosal)$_2$] complexes, it can be assumed that the other complexes occur in the form of a *cis*-S-*cis*-O geometric isomer.

*3.2. Description of the crystal structure of bis(S-butyl-2-thiosalicylate)-palladium(II) (**C5**)*

The crystal structure of [Pd(S-bu-thiosal)$_2$], together with the atom-labelling scheme, is presented in Fig. 1. Selected structural parameters are listed in Table 2. The asymmetric unit of [Pd(S-bu-thiosal)$_2$] consists of neutral complex molecules, where Pd(II) is placed in a *cis*-square-planar coordination environment formed by O and S atoms of two deprotonated

S-butyl-thiosalicylic acid ligands (Fig. 1). The coordination about the Pd atom is essentially planar, with a maximum deviation for the O1a donor atom of -0.013(1) Å.

A CSD search [38] reveals that the recently reported crystal structure of the Pd(II) complex with S-benzyl-thiosalicylate, [Pd(S-bz-thiosal)$_2$], represents the only example of a Pd(II) complex containing two bidentately coordinated thiosalicylate ligands [24]. The geometrical properties of the novel [Pd(S-bu-thiosal)$_2$] and [Pd(S-bz-thiosal)$_2$] complexes could be closely compared. Although both of the complexes coordinate thiosalicylate ligands in a *cis*-square-planar fashion, they differ significantly in the spatial orientation of the thiosalicylate rings and corresponding S-substituents. Namely, in the title compound the same type of residues take the same orientation with respect to the PdO_2S_2 plane, while in the former compound the same residues occupy opposite sides of the coordination plane. Apart from this major structural difference, the geometrical features of the two coordination spheres comprising the S-butyl and S-benzyl [24] derivatives are similar. The Pd–S distances of the complexes are in good agreement, while the Pd–O bonds are approximately 0.01 Å shorter in the present compound. The distribution of coordination angles in both complexes is within 4° from the ideal values, with the largest angle being between the voluminous S atoms (*cis*-S–Pd–S). The difference between the *trans*-O–Pd–S angles is more evident, as in [Pd(S-bu-thiosal)$_2$] they are somewhat closer to linearity [175.59(8) and 177.65(9)°] than in the [Pd(S-bz-thiosal)$_2$] complex [172.52(15) and 172.77(14)°]. Accordingly, the previously reported complex [24] displays higher deviations of the constituent atoms from the PdO_2S_2 coordination plane (root-mean-square deviation 0.13 Å) in comparison to the present one (r.m.s. 0.01 Å).

The different spatial distribution of the ligands residues and the less distorted coordination geometry in [Pd(S-bu-thiosal)$_2$] in comparison to [Pd(S-bz-thiosal)$_2$] suggests a dissimilar steric influence of the corresponding S-substituents. A comparison of the sets of

angles involving the S atom in two complexes reveals fair differences. In [Pd(S-bu-thiosal)$_2$], similarly to [Pd(S-bz-thiosal)$_2$] and complexes comprising rigid thiosalate chelates [39-44], there is a relatively acute angle at S [the mean value of the Pd–S1–C3 angle is 100.7(3)°], in contrast to the angle at the O donor [the mean value of the Pd–O1–C1 angle is 129(1)°]. The C3–S1–C8 angle, which relates the S-substituent to the aromatic part of the ligand, is slightly smaller in the S-butyl derivative [mean value 103(1)°] than in the S-benzyl derivative [mean value 106(1)°], suggesting less steric hindrance of residues in the first complex. A major difference could be observed if one compares the Pd–S1–C8–C9 torsion angle, which describes the further directionality of the S-butyl and S-benzyl substituents. The mean values of the Pd–S1–C8–C9 torsion angles are 167(1) and 60(1)° in [Pd(S-bu-thiosal)$_2$] and [Pd(S-bz-thiosal)$_2$] respectively.

Similarly to [Pd(S-bz-thiosal)$_2$, the six membered chelate rings of [Pd(S-bu-thiosal)$_2$] are in skew chair conformations. In [Pd(S-bu-thiosal)$_2$] alone, the chelate rings of the A and B molecular halves show a significant difference in the C1–C2–C3–S1 torsion angle, which is equal to 1.2(6) and -9.3(6)°, respectively. In addition the Pd–O1–C1 angle has the value of 132.1(3) and 126.5(3)° in the A and B halves, respectively. The two halves further differ in the level of inclination of their phenyl rings with respect to coordination plane, forming the different dihedral angles of 50.38(1) and 63.9(1)°.

In the crystal packing, molecules of [Pd(S-bu-thiosal)$_2$] associate by means of weak C–H…O and C–H…π interactions (Table 3). Two equivalent C4–H4…O2 interactions connect the complex molecules into a two-dimensional sheet parallel to the *bc* plane, Fig. 2. The arrangement of the molecules along the *a* axis is mostly supported by interactions of two S-butyl groups as C–H donors. The engagement of these fragments in the intermolecular interactions is dissimilar. Placed approximately in between the two phenyl rings of the neighbouring molecule, the S-butyl group of fragment A engages in three C–H…π

interactions. At the same time the S-butyl group of fragment B is placed approximately at the same level as the oxygen donors of the same neighboring molecule, forming the strongest C8b−H8b1...O1b interaction (H…O1b = 2.47 Å). The terminal C atoms of the S-butyl group of fragment B are not engaged in any interactions and, in contrast to those in fragment A, display noticeable disorder.

3.3. In vitro cytotoxic activity

Cytotoxicity assay experiments were conducted to determine the effects of the newly synthesized palladium(II) complexes on the cell viability of human colon and lung carcinoma cells. The analysis of the cancer cell viability showed that all the tested complexes are cytotoxic to human colon carcinoma cells (HCT-116 and CaCo-2, Figures 3 and 4) and human lung carcinoma epithelial cells (A549, Fig. 5). The cytotoxic effect is dose dependent: a concentration decrease of the tested complexes is followed by a considerable increase of tumor cell viability.

The tested palladium(II) complexes showed moderate and high cytotoxcities against human colon and lung carcinoma cells (Figs. 3-5), and low cytotoxicities against fibroblasts (Fig. 6). In comparison with the other tested palladium(II) complexes and cisplatin, **C3** showed the highest cytotoxicity at all the tested concentrations and against all tested cancer cell lines (Figs. 3-5). The IC_{50} value of palladium(II) complex **C3** was 3-4 times lower than the IC_{50} value of palladium(II) complex **C4** and more than 10-15 times lower compared to the same value of the other tested palladium(II) complexes (Table 4). In addition, the IC_{50} values of palladium(II) complex **C3** were lower than the IC_{50} values for cisplatin for all the cancer cell lines (Table 4). Importantly, the cytotoxicity of **C3** complex was above 98% for all cancer cell lines at the highest tested concentration (250 μL), while at the same concentration the cytotoxicity of cisplatin was above 98% only against CaCo-2 cells. Additionally, the

percentage of dead HCT-116, CaCo-2 and A549 cells were above 20% after 72h treatment with the lowest tested concentration (3.9 μL) of **C3**, suggesting that this complex has potent anti-tumor activity. The lowest activity against all the tested cancer cell lines was observed for the complex **C5**. The order of the cytotoxic activity of the palladium(II) complexes and cisplatin, **C3** > cisplatin > **C4** > **C2** > **C1** > **C5**, was the same against all the tested cancer cells.

Further, we also demonstrated that the cytotoxicities of the palladium(II) complexes against cancer cell lines were significantly higher than those of their ligand precursors. The ligand precursors showed moderate to low cytotoxicities against HCT-116, CaCo-2 and A549 cancer cell lines (Figs. 3-5), and very low cytotoxicities against fibroblasts (Fig. 6). Cisplatin showed a significantly higher cytotoxicity than the ligand precursors at all tested concentrations and against all cancer cell lines (Figs. 3-5). The cytotoxic activity of the ligand precursors against all the cell lines correlated with cytotoxicities of the palladium(II) complexes (cisplatin> **L3** > **L4** > **L2** > **L1** > **L5**).

Fig. 1 shows that the palladium(II) ion is "blocked" by coordination of two ligands, but the molecules are more opened on the Pd-S side than the Pd-O side. This might be a reason for an easier interaction with a DNA molecule, breaking of the Pd-S bonds and finally binding to DNA, knowing that Pd-S is kinetically labile [45].

It is important to note that all the tested palladium(II) complexes and their ligand precursors as well as cisplatin showed low cytotoxicity against fibroblasts. At the highest tested concentration (250 μL) the percentage of dead fibroblasts was below 50% for all the tested palladium(II) complexes and their ligand precursors, and at the lowest tested concentration (3.9 μL) none of the tested compounds were cytotoxic against fibroblasts.

Due to the obtained results, the complex **C3** seems to be a good candidate (among the tested palladium(II) complexes) for future pharmacological investigation in the field of colon and lung cancer research.

Both palladium(II)complexes characterized by single crystal X-ray analysis (**C1** and **C5**) generally exhibit the lowest cytotoxic activity. Since these two compounds display significant conformational differences it is not possible to find any connection between their structural properties and level of activity (different conformational properties versus low activity in both cases). However, since **C1** and **C5** possess the most voluminous S-substituents, it seems that the size of the substituent has some influence on the cytotoxic activity (where less voluminous S-substituents are favorable).

4. Conclusion

The cytotoxicities of the reported palladium(II) complexes were significantly higher than those of their ligand precursors. The tested palladium(II) complexes showed moderate and high cytotoxicities against human colon and lung carcinoma cells and low cytotoxicities against fibroblasts. In comparison with other tested palladium(II) complexes and cisplatin, palladium(II) complex **C3** showed the highest cytotoxicity at all tested concentrations and against all tested cancer cell lines.

The molecular structure of the mononuclear complex [Pd(S-bu-thiosal)$_2$] was determined by the single-crystal X-ray diffraction method. Although two deprotonated S-butyl-thiosalicylic acid ligands form a symmetric *cis*-square-planar coordination around the Pd(II) metal atom, they show a significant difference in their conformation. The major differences between the two molecular halves are: (i) the value of the C1–C2–C3–S1 torsion angles in the chelate rings [1.2(6) and -9.3(6)°], (ii) the spatial orientation of the S-substituents and (iii) the inclination angle of the two phenyl rings with respect to the

coordination plane (dihedral angles of 50.38(1) and 63.9(1)°). The engagement of the two S-bu-thiosal ligands in intermolecular interactions is also dissimilar.

Acknowledgment

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Appendix A. Supplementary data

CCDC 1027150 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk.

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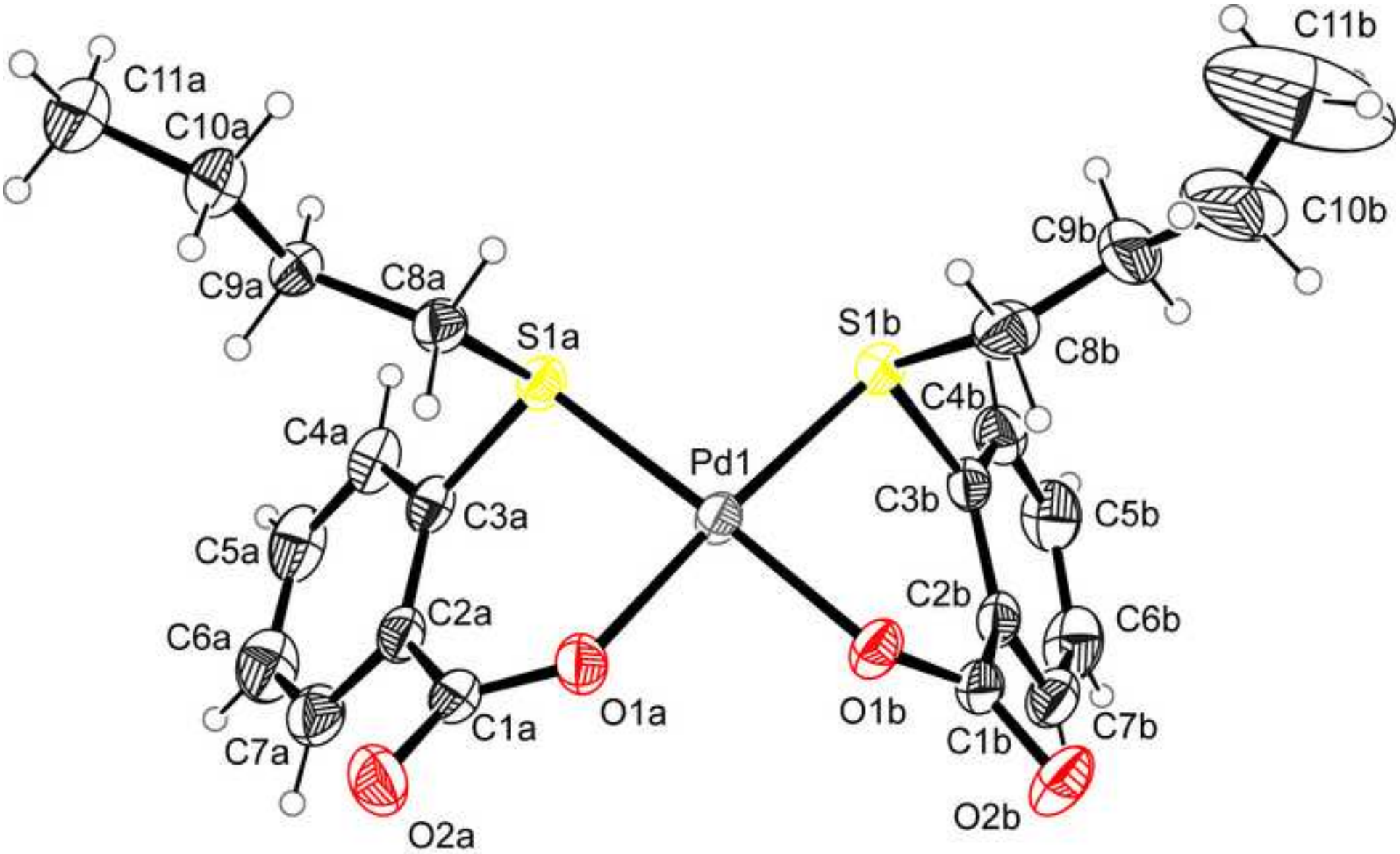
Table 1. Crystallographic data for [Pd(S-bu-thiosal)$_2$].

Empirical formula	$C_{22}H_{26}O_4PdS_2$
Formula weight	524.95
Color, crystal shape	yellow, prism
Crystal size (mm^3)	0.18 x 0.15 x 0.14
Temperature (K)	293(2)
Wavelength (Å)	0.71073
Crystal system	Orthorhombic
Space group	*Pbca*
Unit cell dimensions	
a (Å)	10.3458(3)
b (Å)	20.5915(6)
c (Å)	20.7746(6)
V (Å^3)	4425.7(2)
Z	8
D_{calc} (Mg/m^3)	1.576
μ (mm^{-1})	1.053
θ range for data collection (°)	2.95 to 29.10
Reflections collected	16607
Independent reflections, R_{int}	5257, 0.0405
Completeness (%) to θ = 26°	99.9
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5257 / 0 / 264
Goodness-of-fit on F^2	1.095
Final R_1/wR_2 indices [$I > 2\sigma(I)$]	0.0552/ 0.1014
Largest diff. peak and hole (e Å^{-3})	0.682 /−0.560

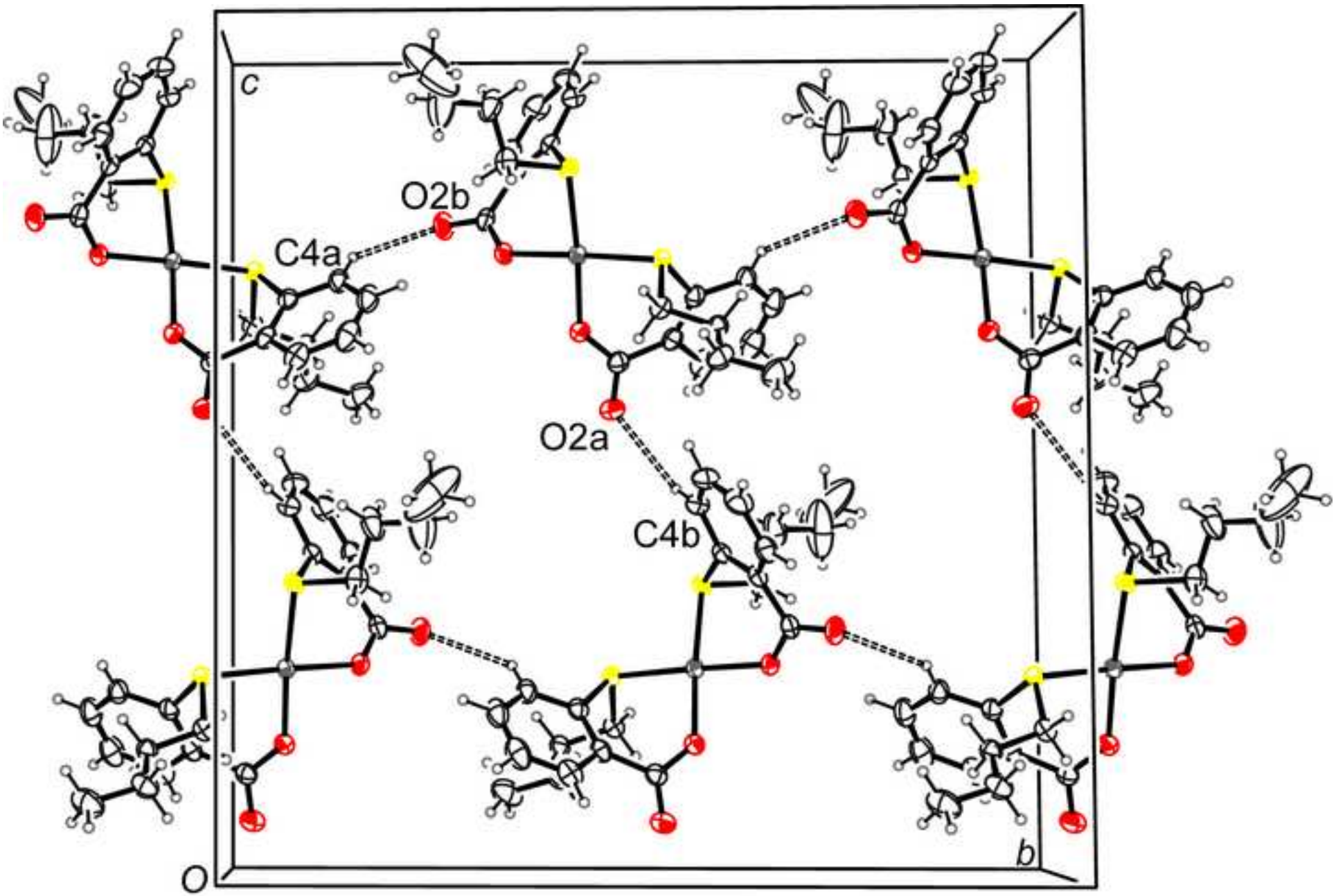
Table 2. Selected bond lengths (Å) and angles (º) for [Pd(S-bu-thiosal)$_2$].

Bond	*A*	*B*
Pd1−O1	2.013(3)	2.013(3)
Pd1−S1	2.2526(11)	2.2572(11)
S1−C3	1.784(4)	1.777(4)
S1−C8	1.818(4)	1.822(5)
O1−C1	1.284(5)	1.292(5)
O2−C1	1.216(5)	1.216(5)
C1−C2	1.517(6)	1.503(6)
C2−C3	1.392(6)	1.401(6)
O1−Pd1−S1	90.75(8)	88.76(9)
Pd1−O1−C1	132.1(3)	126.5(3)
Pd1−S1−C3	102.62(14)	98.88(14)
O1−C1−C2	119.7(4)	118.4(4)
O1−C1−O2	121.9(4)	121.3(4)
C1−C2−C3	125.4(4)	124.7(4)
C2−C3−S1	123.3(3)	123.5(3)
O1a−Pd1−O1b	86.92(11)	
S1a−Pd1−S1b	93.58(4)	
O1a−Pd1−S1b	175.59(8)	
O1b−Pd1−S1a	177.65(9)	

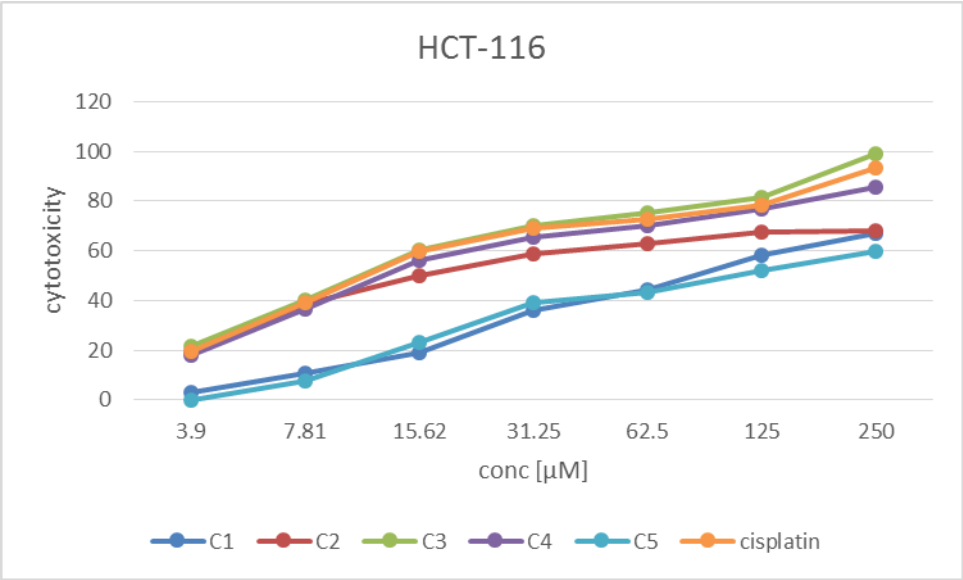
Figures



Figures



Figures



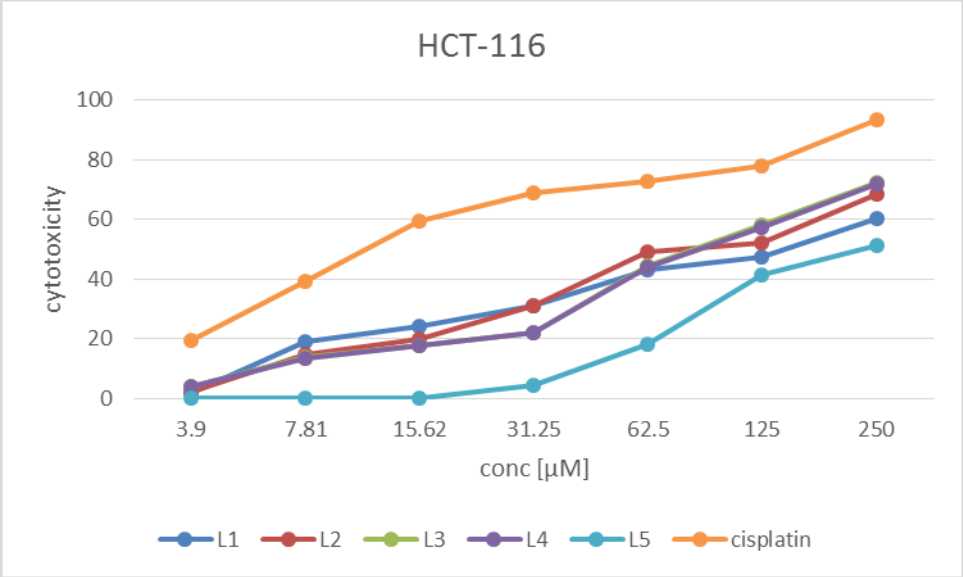


Fig. 3. Representative graphs of HCT-116 cell survival after 72 h cell growth in the presence of palladium(II)-complexes, cisplatin and ligand precursors.

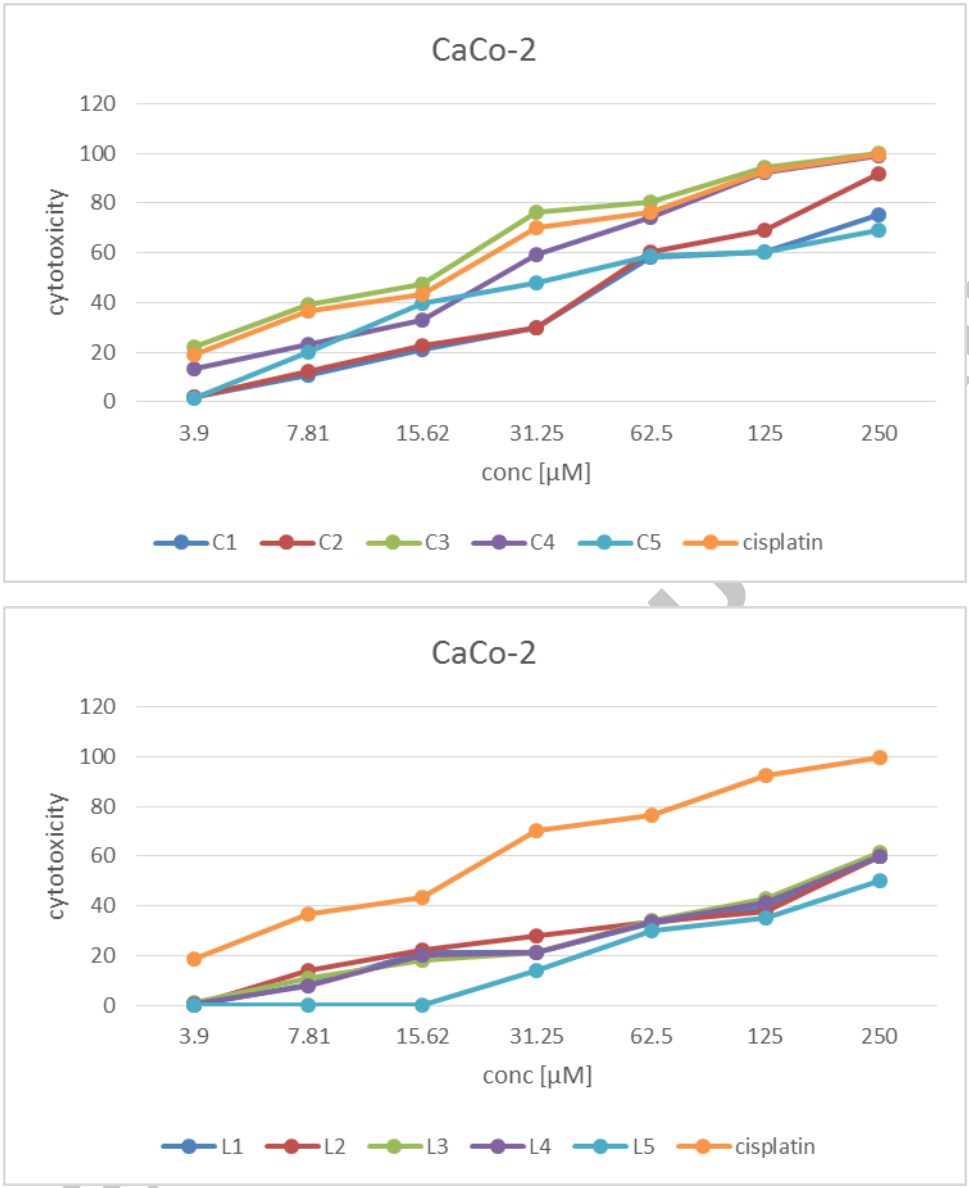


Fig. 4. Representative graphs of CaCo-2 cell survival after 72 h cell growth in the presence of palladium(II)-complexes, cisplatin and ligand precursors.

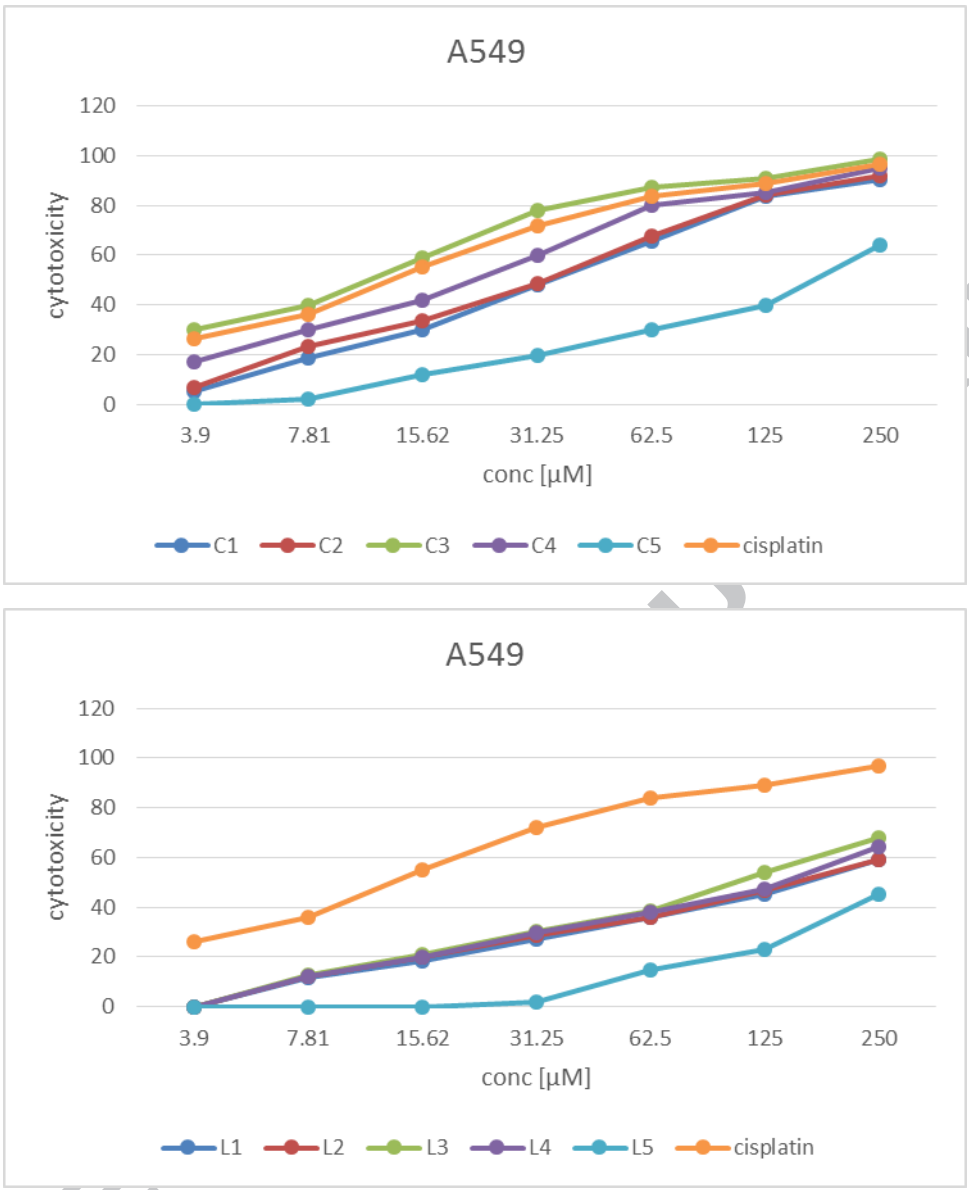


Fig. 5. Representative graphs of A549 survival after 72 h cell growth in the presence of palladium(II)-complexes, cisplatin and ligand precursors.

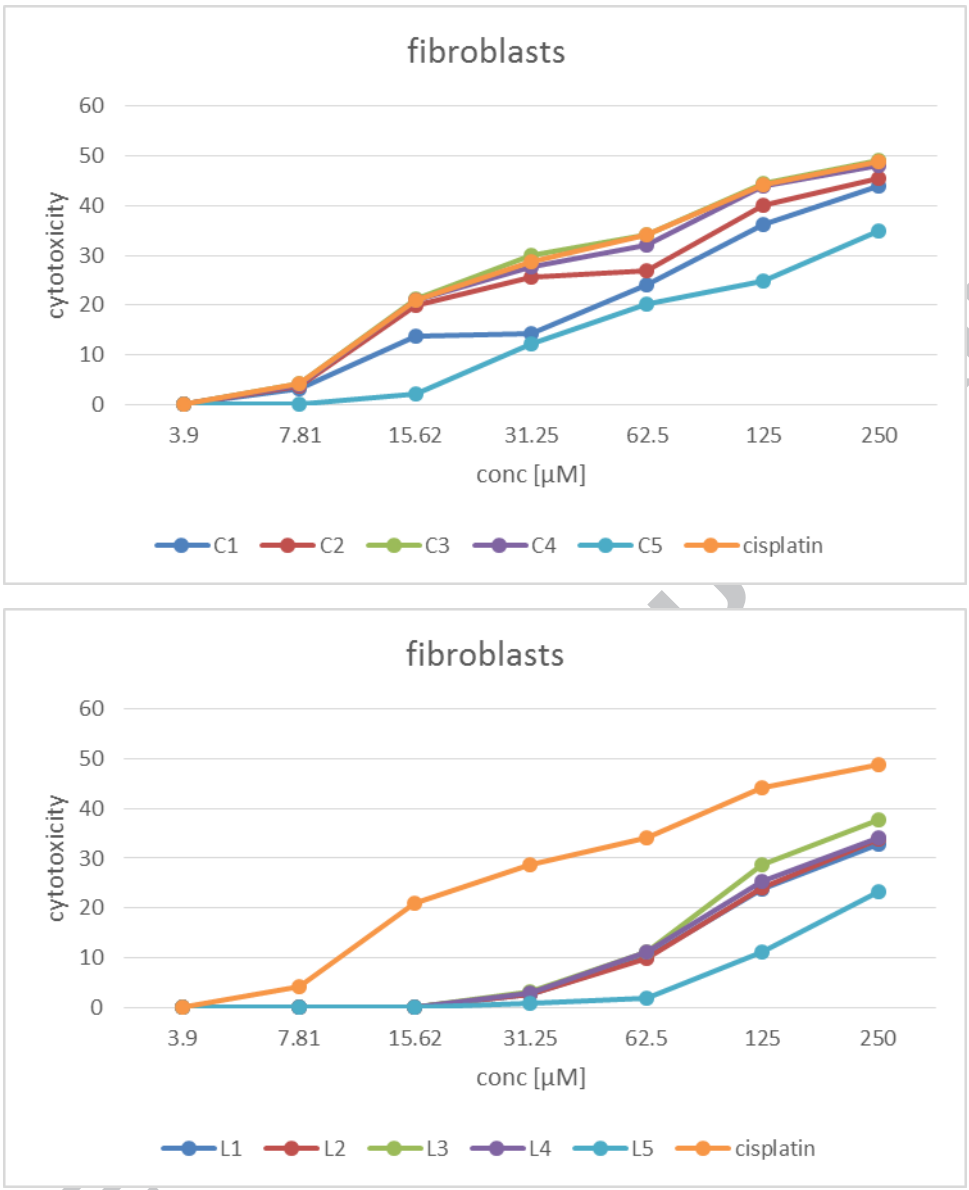


Fig. 6. Representative graphs of fibroblasts survival after 72 h cell growth in the presence of palladium(II)-complexes, cisplatin and ligand precursors.

Table 3. Geometrical parameters (Å, °) of the hydrogen bonds and C−H… π interactions.

D−H…A	D−H	D…A	H…A	D−H…A
C4a−H4a...O2b[i]	0.93	3.345(6)	2.59	139
C4b−H4b...O2a[ii]	0.93	3.324(6)	2.58	137
C8b −H8b1...O1b[iii]	0.97	3.422(6)	2.47	168
C−H… π	C−H	H…Cg	H-Perp	C−H…Cg
C8a−H8a1...Cg1 [iii]	0.97	2.82	2.78	124
C8a−H8a2...Cg1 [iii]	0.97	3.20	3.02	98
C9a−H9a2...Cg2 [iii]	0.97	3.21	2.68	137

Cg1 and Cg2 are the centroids of the phenyl rings in fragments A and B, respectively. Symmetry codes: (i) −x+1, y+1/2, −z+3/2; (ii) −x+1/2, −y, z+1/2; (iii) x−1/2, y, −z+3/2.

Table 4. IC_{50} (μM)[a] values after 72 h of action of the investigated complexes, cisplatin and ligand precursors on HCT116, CaCo2, A549 cells and fibroblasts, as determined by an MTT assay.

Compound	HCT-116 cells	CaCo-2 cells	A549 cells	fibroblasts
C1	105.71 +/-18.2	116.48 +/- 20.4	77.12 +/- 14.7	301.85 +/- 39.1
C2	30.31 +/-6.2	91.42 +/- 15.8	66.07 +/- 12.4	277.69+/- 28.7
C3	2.24 +/- 0.3	19.69 +/- 3.8	2.14 +/- 0.6	217.12 +/- 24.8
C4	9.65 +/- 1.4	38.47 +/- 10.1	43.07 +/- 11.9	230.12 +/- 31.5
C5	128.16 +/- 21.4	110.62 +/- 19.8	171.95 +/- 25.4	343.37 +/- 48.2
cisplatin	3.06 +/- 0.25	21.11+/- 3.28	3.12+/- 0.26	218.25+/-28.12
L1	184.97 +/- 28.4	182.68 +/- 27.7	204.58 +/- 29.9	415.75 +/- 50.7
L2	158.51 +/- 23.1	186.21 +/- 26.5	198.39 +/- 27.4	400.26 +/- 51.8
L3	144.31 +/- 20.4	180.61 +/- 28.4	157.34 +/- 22.1	324.19 +/- 45.2
L4	147.99 +/- 20.5	182.12 +/- 27.1	181.74 +/- 26.5	384.75 +/- 55.7
L5	224.81 +/- 32.3	236.6 +/- 30.3	288.94 +/- 41.1	579.94 +/- 65.9

[a] Mean values +/- SD (standard deviation) from three experiments.

Figure legends

Figure 1. Crystal structure and atom numbering scheme of [Pd(S-bu-thiosal)$_2$]. Displacement ellipsoids are drawn at the 30% probability level.

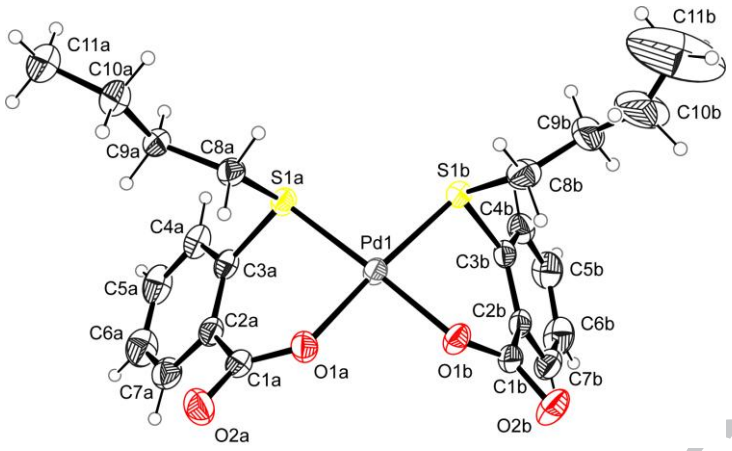
Figure 2. Two dimensional molecular sheet formed by equivalent C4-H…O2 interactions.

Figure 3. Representative graphs of HCT-116 cell survival after 72 h cell growth in the presence of the palladium(II) complexes, cisplatin and ligand precursors.

Figure 4. Representative graphs of CaCo-2 cell survival after 72 h cell growth in the presence of the palladium(II) complexes, cisplatin and ligand precursors.

Figure 5. Representative graphs of A549 survival after 72 h cell growth in the presence of the palladium(II) complexes, cisplatin and ligand precursors.

Figure 6. Representative graphs of fibroblasts survival after 72 h cell growth in the presence of the palladium(II) complexes, cisplatin and ligand precursors.



The molecular structure of the mononuclear complex [Pd(S-bu-thiosal)$_2$] was determined by the single-crystal X-ray diffraction method. Although the two deprotonated S-butyl-thiosalicylic acid ligands form a symmetric *cis*-square-planar coordination around the Pd(II) metal atom, they show significant differences in their conformation. The cytotoxicities of the palladium(II) complexes with S-alkyl derivatives of thiosalicylic acid were significantly higher than those of their ligand precursors.