Inorganica Chimica Acta 394 (2013) 472–480

Contents lists available at SciVerse ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

Synthesis, characterization and cytotoxicity studies of platinum(II) complexes with amino acid ligands in various coordination modes

Sangeeta Roy Chaudhuri [a,b], Goran N. Kaluđerović [a,b], Martin Bette [a], Jürgen Schmidt [c], Harry Schmidt [a], Reinhard Paschke [b], Dirk Steinborn [a,*]

[a] Institut für Chemie – Anorganische Chemie, Martin-Luther-Universität Halle-Wittenberg, Kurt-Mothes-Straße 2, D-06120 Halle, Germany
[b] Biozentrum, Martin-Luther-Universität Halle-Wittenberg, Weinbergweg 22, D-06120 Halle, Germany
[c] Abteilung für Natur- und Wirkstoffchemie, Leibniz-Institut für Pflanzenbiochemie, Weinberg 3, D-06120 Halle, Germany

ARTICLE INFO

Article history:
Received 18 June 2012
Received in revised form 28 August 2012
Accepted 31 August 2012
Available online 13 September 2012

Keywords:
Platinum carbonato complexes
Platinum amino acid complexes
Cytotoxicity
DNA laddering
Cell cycle

ABSTRACT

Reactions of $[Pt(CO_3)(PPh_3)_2]\cdot CH_2Cl_2$ (**1**) with non-substituted and alkyl substituted amino acids, NH(R)CH(R′)CO_2H (R/R′ = H/Me, **L1**; H/^{i}Pr, **L2**; H/CH_2CHMe_2, **L3**; Me/H, **L4**; Et/H, **L5**), in the presence of Tl[PF_6] in methanol afforded with liberation of CO_2 the formation of platinum(II) complexes of the type $[Pt(PPh_3)_2\{NHR–CHR'–C(O)O-\kappa N,\kappa O\}][PF_6]$ (R/R′ = H/Me, **2**; H/^{i}Pr, **3**; H/CH_2CHMe_2, **4**; Me/H, **5**; Et/H, **6**). Single-crystal X-ray diffraction analysis of complex **4** exhibited a square-planar coordination of the platinum atom having coordinated two triphenylphosphane ligands and a deprotonated κ*N*,κ*O*-coordinated leucine ligand (**L3**$_{-H}$). On varying the pK_a value of the amino group, platinum(II) complexes with different coordination modes of amino acid ligands were obtained. Thus, treatment of complex **1** with *N*-acetyl L-alanine (**L6**), possessing a comparatively highly acidic NH proton, in 1:1 ratio in methanol resulted in the formation of $[Pt(PPh_3)_2\{N(COMe)–CHMe–C(O)O-\kappa N,\kappa O\}]$ (**7**), while reacting *N*-phenyl glycine (**L7**) having a moderately acidic NH proton with complex **1** afforded a mixture of complexes $[Pt(PPh_3)_2\{NPh–CH_2–C(O)O-\kappa N,\kappa O\}]$ (**8**) and $[Pt(PPh_3)_2\{NHPh–CH_2–C(O)O-\kappa O\}_2]$ (**10**). Treatment of complex **1** with two equivalents of **L6**/**L7** in dichloromethane resulted in the formation of $[Pt(PPh_3)_2\{NHR–CHR'–C(O)O-\kappa O\}_2]$ (R/R′ = COMe/Me, **9**; Ph/H, **10**). An analogous reactivity was observed for L-lactic acid on treating with complex **1** in 1:1 and 2:1 ratio resulting in $[Pt(PPh_3)_2\{O–CHMe–C(O)O-\kappa O,\kappa O'\}]$ (**11**) and $[Pt(PPh_3)_2\{HO–CHMe–C(O)O-\kappa O\}_2]$ (**12**). The identities of all complexes have been proven by NMR (^{1}H, ^{13}C, ^{31}P) spectroscopic and high-resolution ESI mass-spectrometric investigations. *In vitro* cytotoxicity studies against human tumor cell lines (8505C, A2780, HeLa, SW480, and MCF-7) showed the highest activities for the neutral complex **7**. Furthermore, complexes **7** and **9** against the A2780 cell line induced an apoptotic mode of cell death, which was further supported by morphological investigation and DNA laddering. Cell cycle perturbation studies showed that both complexes induced faster cell death than cisplatin.

1. Introduction

Platinum complexes with bioligands attract attention both from the viewpoint of bioactivity, mainly due to their carcinostatic properties, and from the coordination chemistry perspective, because in many cases bioligands have various donor sites of similar properties at their disposal [1,2]. This may result into many different coordination modes and a detailed knowledge on them is a requirement for a deeper understanding of the role of metals in biochemistry. Furthermore, to get control over the coordination mode of bioligands to metals may be a prerequisite for a targeted development of metal-containing drugs.

Amino acids, like other biomolecules, can have a multitude of coordination modes. They can be monocoordinated (κ*O* or κ*N*) or dicoordinated in a chelating (κ*O*, κ*N* or $\kappa^2 O,O'$) or bridging (κ*O*:κ*N* or κ*O*:κ*O*′) fashion. A further versatility results from the coordination of the amino acid as anionic, dianionic or neutral (zwitterionic) ligand [3–7].

Although there is a multitude of ways to synthesize these complexes, but to acquire control over the coordination mode may be a challenge. Carbonato platinum(II) complexes which can react with acidic ligands under release of carbon dioxide may open a way to a targeted deprotonation of the amino acid. In this respect, they have been used so far to react with various acidic substrates. Thus, bis(phosphane)carbonatoplatinum(II) complexes $[Pt(CO_3)L_2]$ (2 L = 2 PR_3, dppe) were found to react with polyols containing at least one vicinal diol moiety (such as ethane-1,2-diol, L-ascorbic acid, D-mannitol) with formation of diolato complexes of the type

* Corresponding author. Tel.: +49 345 5525620; fax: +49 345 5527028.
E-mail address: steinborn@chemie.uni-halle.de (D. Steinborn).

0020-1693/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ica.2012.08.034

[Pt(O⌒O)L_2] [8–10]. Analogously, aromatic 1,2-diols (such as BINOL, 1,8,9,16-tetrahydroxytetraphenylene) [11–16] and silasesquioxane [17] reacted with deprotonation of phenolic OH groups and silanol groups, respectively. Reactions of [Pt(CO_3)L_2] complexes with dicarboxylic acids having a rigid backbone (e.g., $C_6H_4(CO_2H)_2$-1,4; *trans*,*trans*-HO_2CCH=CHCH=CHCO_2H) gave rise to the formation of dinuclear complexes with bridging dicarboxylato ligands, whereas in the presence of NH_4^+, additionally, under its deprotonation ammine complexes were formed [18]. Reactions of triketones having an enolizable β-diketone unit (heptane-2,4,6-trione, 1,5-diphenylpentane-1,3,5-trione) led to the deprotonation of either the enolic OH proton or the methylene group, thus forming platinum(II) complexes with β-diketonato type ligands or platinacyclobutan-3-one complexes, respectively [19–21]. Furthermore, reactions with trifluoromethyl sulfonamides (CF_3SO_2NH–CHPhCHPh–NHSO_2CF_3, CF_3SO_2NH–CHPhCHPhOH) under deprotonation of the relatively highly acidic NH groups [15,22,23] and, very recently, with 1,3-dithiols (HSCH_2–CH(OH)–CH_2SH, HSCH_2–CH(OSiMe_2CMe_3)–CH_2SH) under deprotonation of SH groups have been reported [24].

Herein, we report reactions of the bis(triphenylphosphane)carbonatoplatinum(II) complex [Pt(CO_3)(PPh_3)$_2$]·CH_2Cl_2 (**1**) with unsubstituted and substituted amino acids which proceed with deprotonation of the carboxylic group and, in dependence on the NH acidity and the solvent, of the amino group, too. In analogous reactions with lactic acid, a pronounced solvent dependence on the deprotonation of the alcoholic OH group was found. Finally, the *in vitro* cytotoxicity of the amino acid complexes and the corresponding ligand precursors against five human tumor cell lines has been investigated.

2. Results and discussion

2.1. Synthesis

Treatment of the complex [Pt(CO_3)(PPh_3)$_2$]·CH_2Cl_2 (**1**) with unsubstituted α-amino acids (L-alanine, **L1**; DL-valine, **L2**; l-leucine, **L3**) and alkyl substituted amino acids (*N*-methyl glycine, **L4**; *N*-ethyl glycine, **L5**) in 1:1 ratio in methanol, afforded the mononuclear cationic platinum(II) complexes **2′**–**6′** (Scheme 1, route **a**). Addition of 1.2 equivalents of Tl[PF_6] to the reaction solutions resulted in the formation of Tl_2CO_3 and cationic platinum(II) complexes **2**–**6** with anionic κ*N*,κ*O*-coordinated amino acid ligands (Scheme 1, route **b**). The complexes obtained are found to be air-stable white powders, which were isolated in yields between 73% and 86%, and they were characterized by ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy, HRMS-ESI measurements, and a single crystal X-ray diffraction analysis (**4**).

However, no other mode of coordination of the amino acid ligands was observed on treating complex **1** with excess or in deficiency of **L1**–**L5** (0.5–4 equivalents) in methanol. In all cases, formation of complexes **2′**–**6′** was observed. ^{1}H and ^{31}P NMR spectroscopy studies showed the signals of these complexes along with those of the unreacted amino acids and the unreacted complex **1**, respectively.

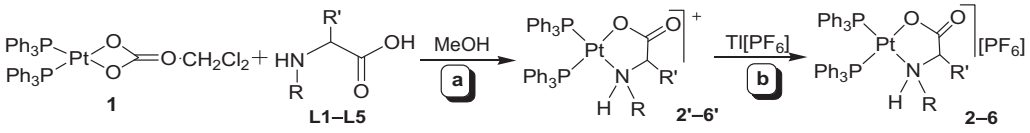
In contrast, on treating complex **1** with *N*-acetyl alanine (**L6**) which possesses a comparatively highly acidic NH proton in 1:1 ratio in methanol afforded the neutral platinum(II) complex **7** containing κ*N*,κ*O*-chelating dianionic amino acid ligand (Scheme 2, route **a**). On the other hand, on treating complex **1** with *N*-phenyl glycine (**L7**), an amino acid having a moderately acidic NH proton, in 1:1 ratio in methanol resulted in the formation of a mixture of complexes **8** and **10** (Scheme 2, routes **a**/**b**), bearing one dianionic κ*N*,κ*O*-chelating amino acid ligand and two anionic monodentately κ*O*-coordinated ligands, respectively. However, due to similar solubilities, complex **8** could not be prepared on a pure state.

Unlike the case described for **L1**–**L5**, changing the molar ratios of the platinum complex **1** and the amino acids with more acidic NH protons (**L6**, **L7**) resulted in a different course of reaction. Thus, treatment of complex **1** with **L6**/**L7** in 1:2 ratio in a protic solvent like methanol gave the complexes **9** and **10** with two anionic monodentately κ*O*-coordinated amino acid ligands along with unreacted complex **1** in case of **L7**. However, these latter complexes could be obtained as solely reaction product when working in an aprotic solvent like dichloromethane (Scheme 2, route **b**). The complexes **7**, **9**, and **10** were isolated in yields between 60% and 70% as air-stable white solids. They were characterized by ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy and by HRMS-ESI measurements.

Quite analogously, L-lactic acid (**L8**), an α-hydroxy acid with a moderately acidic OH group, was found to react with complex **1**. Using a 1:1 and 1:2 ratio in methanol, also afforded a mixture of two neutral platinum(II) complexes, one bears a chelating κ^2O,O'-coordinated dianionic lactato ligand (**11**) and the other one is a bis(lactato) complex with monodentately κ*O*-coordinated ligands (**12**). Due to its sparing solubility in methanol, complex **11** could be isolated in a pure state as white solid in 52% yield (Scheme 3, route **a**). On the other hand, reactions of complex **1** with **L8** in 1:1 and 1:2 ratio in an aprotic solvent (dichloromethane) proceed differently. Using a 1:1 ratio resulted in formation of complex **12** along with an unidentified platinum complex having non-equivalent phosphane ligands, whereas using a 1:2 ratio resulted in the formation of complex **12** exclusively (isolated yield 65%) (Scheme 3, route **b**). The identities of the two complexes **11** and **12** have been proven by NMR spectroscopy (^{1}H, ^{13}C, ^{31}P) and HRMS-ESI measurements.

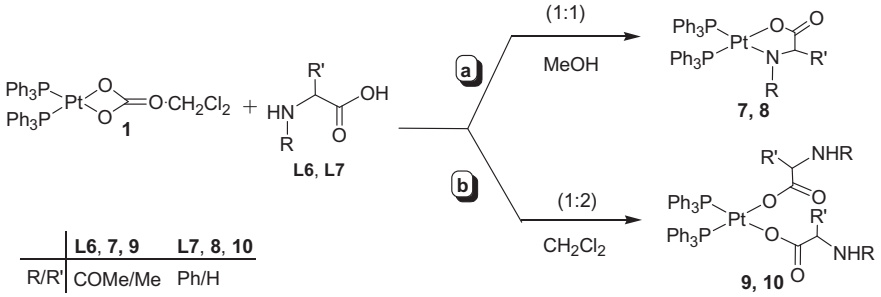
2.2. Spectroscopic investigations

Selected NMR spectroscopic parameters of complexes **2**–**12** are given in Table 1. All spectra show the expected signals in the expected shift range. The ^{1}H NMR spectra give unequivocally proof of the 1:1 and 1:2 stoichiometry, respectively, as given in Table 1. The ^{31}P NMR spectra of the complexes (**2**–**7**, **11**) exhibit the presence of two chemically non-equivalent P nuclei. They show AB spin patterns with the corresponding ABX satellite spectra (A, B = ^{31}P; X = ^{195}Pt). The signal assignment is based on the large differences in the two $^1J_{Pt,P}$ coupling constants. The much higher *trans* influence of the amide and alcoholate groups over that of the carboxyl-

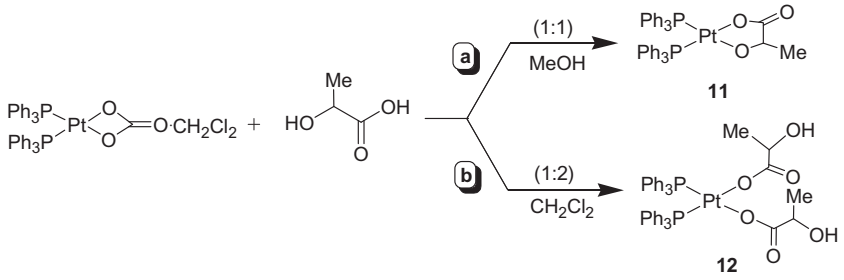


	L1, 2′, 2	L2, 3′, 3	L3, 4′, 4	L4, 5′, 5	L5, 6′, 6
R/R′	H/Me	H/^{i}Pr	H/CH_2CHMe_2	Me/H	Et/H

Scheme 1. Reactivity of [Pt(CO_3)(PPh_3)$_2$]·CH_2Cl_2 toward amino acids.



Scheme 2. Reactivity of $[Pt(CO_3)(PPh_3)_2]\cdot CH_2Cl_2$ toward *N*-substituted amino acids.



Scheme 3. Reactions of $[Pt(CO_3)(PPh_3)_2]\cdot CH_2Cl_2$ with L-lactic acid.

Table 1
Selected NMR spectroscopic data (δ in ppm, J in Hz) of complexes $[Pt(PPh_3)_2\{NHR–CHR'–C(O)O-\kappa N,\kappa O\}][PF_6]$ (**2**–**6**), $[Pt(PPh_3)_2\{NHCOMe–CHMe–C(O)O-\kappa N,\kappa O\}]$ (**7**), $[Pt(PPh_3)_2\{NHR–CHR'–C(O)O-\kappa O\}_2]$ (**9**, **10**), $[Pt(PPh_3)_2\{O'–CHMe–C(O)O-\kappa^2 O,O'\}]$ (**11**), and $[Pt(PPh_3)_2\{HO–CHMe–C(O)O-\kappa O\}_2]$ (**12**).

Complexes	$\delta_{P'}$ ($^1J_{Pt,P'}$)[a]	$\delta_{P''}$ ($^1J_{Pt,P''}$)[a]	$\delta_{\alpha\text{-}H}$ ($^2J_{H,H}$)	δ_C (COO)
2 (R/R′ = H/Me)	5.6 (3663)	7.7 (3508)	3.86	186.0
3 (R/R′ = H/^{i}Pr)	6.4 (3643)	7.3 (3471)	3.65	184.6
4 (R/R′ = H/CH_2CHMe_2)	7.2 (3570)	7.6 (3486)	3.69	187.0
5 (R/R′ = Me/H)	7.4 (3703)	8.2 (3381)	3.31 (16.7), 4.38 (16.7)	181.0
6 (R/R′ = Et/H)	6.5 (3703)	6.4 (3353)	3.46 (16.8), 4.29 (16.8)	183.0
7	6.4 (4091)	9.3 (3068)	4.41	190.0
9 (R/R′ = COMe/Me)	6.8 (3865)	–	3.64	175.6[b]
10 (R/R′ = Ph/H)	6.8 (3826)	–	3.03	174.1
11	12.3 (3911)	10.6 (3241)	4.66	194.1
12	5.7 (3856)	–	3.4	178.8

[a] P′ and P″ are *trans* to a carboxylate oxygen atom and nitrogen atom, respectively. In case of **11** P″ is *trans* to the alcoholate oxygen atom.
[b] Assignment of the signal is uncertain.

ate groups gives rise to smaller (3068–3508 Hz) and higher (3570–4091 Hz) $^1J_{Pt,P}$ coupling constants, respectively. The ^{31}P NMR spectra of the bis(amino acid) complexes **9**, **10**, and **12** show, as expected, a singlet phosphorus resonance with ^{195}Pt satellites. The magnitude of the $^1J_{Pt,P}$ coupling constants (3826–3865 Hz) and the comparison with requisite data of carboxylato complexes (both in κO and $\kappa O,\kappa O'$ coordination) having a PPh_3 ligand in *trans* position [25] (cf. also with the carbonato complex **1**: $^1J_{Pt,P}$ = 3702 - Hz [26]) show that the anionic amino acid ligands are coordinated through the carboxylate groups.

The resonances of the carboxylate C atoms of the complexes **2**–**7** and **11** having anionic/dianionic $\kappa O,\kappa N$ coordinated amino acid ligands fall in the range of δ 181.0–194.1 ppm, which is typical for such complexes [27,28]. The carboxylate C atoms of the complexes **9**, **10**, **12** gave signals in the range δ 174.1–178.8 ppm. Thus, they are significantly more shielded than in the chelate complexes, which is typical for complexes with monocoordinated (κO) amino acid ligands [27,29]. Noteworthy, in complexes **3** and **11** couplings of carboxylate C atoms to phosphorus ($^{3/4}J_{P,C}$) through the heteroatoms have been observed, although any coupling to ^{195}Pt is not discernible. Analogous couplings were observed in some other amino acid complexes of platinum [30].

Anionic chelating amino acid ligands produce invariably a downfield shift of the proton resonances, relative to their corresponding amino acid anions [31,32]. Furthermore, the resonances of the α protons were found to be more shielded in complexes with monodentately coordinated (κO) amino acid ligands than with bidentately coordinated ($\kappa O,\kappa N$; $\kappa^2 O,O'$) amino acid ligands (cf. complexes **7**/**9** and **11**/**12**). Thus, the downfield shift of α protons in complexes **2**–**7** and **11** gives an additional proof for a $\kappa O,\kappa N$ coordination.

Furthermore, as expected, the α-methylene protons of the *N*-substituted glycine complexes **5** and **6** are not equivalent and two separate doublets of doublet resonances are observed due to coupling to each other ($^2J_{H,H}$ = 16.7/16,8 Hz) and to NH protons ($^3J_{H,H}$ = 3.6–5.2 Hz) [27].

2.3. Molecular structure of the leucinato complex 4

Crystals of $[Pt(PPh_3)_2\{NH_2–CH(CH_2CHMe_2)–C(O)O\text{-}\kappa N,\kappa O\}][BF_4]$ (**4**; obtained as described before but using $Ag[BF_4]$ instead of $Tl[PF_6]$) suitable for X-ray diffraction analysis were obtained from dichloromethane solutions with a layer of diethyl ether at room temperature. In the asymmetric unit, two symmetry-independent cations and anions of very similar structure are found. The structure of one of the two cations is shown in Fig. 1 and selected structural parameters are given in the figure caption. The platinum atom is square-planar coordinated by two PPh_3 ligands and the $\kappa N,\kappa O$-chelating leucinato ligand (sum of angles around Pt1: 360.0/360.2°[1]). Deviations originate from the restricted bite of the chelating ligand (O1–Pt1–N1 80.3(3)/80.0(2)°). One of the *trans* angles (P2–Pt1–O1) is nearly linear (178.4(2)/176.3(2) Å), whereas the other one (P1–Pt1–N1) is severely bent (162.2(2)/163.0(2) Å).

The Pt1–N1 distances (2.102(6)/2.080(7) Å) and the Pt1–O1 distances (2.067(5)/2.024(6) Å) are approximately as long as those in structurally similar amino acid platinum(II) complexes (Pt–N: median: 2.044 Å, lower/higher quartile: 2.060/2.094 Å; Pt–O: median: 2.074 Å, lower/higher quartile: 2.033/2.073 Å; $n = 11$, n = number of observations) [33]. In accordance with the higher *trans* influence of the amino group over the carboxylate group [34] the Pt1–P1 bond (2.264(2)/2.272(2) Å) is much longer than the Pt1–P2 bond (2.243(2)/2.249(2) Å). The five-membered $PtNOC_2$ ring is twisted on C1–C2 and Pt1–N1, respectively.

Crystals of **4** are threaded by N1–H···F hydrogen bonds which connect the cationic platinum complexes with the $[BF_4]^-$ anions. The N1···F distances are between 2.94(1) and 3.15(1) Å.

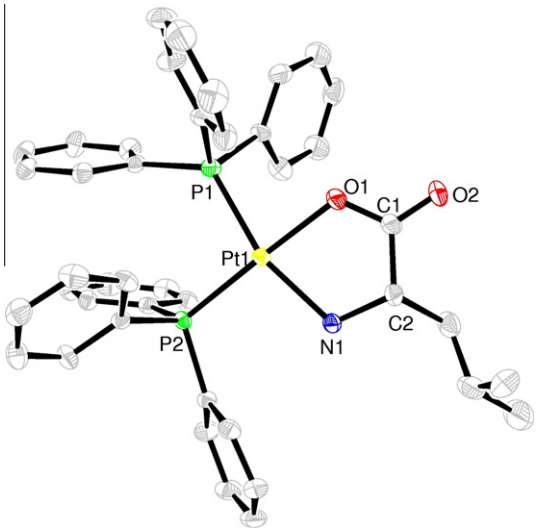


Fig. 1. Structure of one of the two symmetry-independent cations in crystals of $[Pt(PPh_3)_2\{NH_2–CH(CH_2CHMe_2)–C(O)O'\text{-}\kappa N,\kappa O'\}][BF_4]$ (**4**). The ellipsoids are drawn with a probability of 30%. Hydrogen atoms were omitted for clarity. Selected structural parameters (distances in Å, angles in °); the values for the two symmetry-independent molecules are separated by a slash: Pt1–P1 2.264(2)/2.272(2), Pt1–P2 2.243(2)/2.249(2), Pt1–N 2.102(6)/2.080(7), Pt1–O1 2.067(5)/2.024(6), C1–O1 1.30(1)/1.317(9), C1–O2 1.21(1)/1.217(9), Pl–Pt1–P2 98.02(8)/98.74(7), P1–Pt1–O1 83.6(2)/84.1(2), O1–Pt1–N1 80.3(3)/80.0(2), N1–Pt1–P2 98.1(2)/97.4(2), P1–Pt1–N1 162.2(2)/163.0(2), O1–Pt1–P2 178.4(2)/176.3(2).

2.4. In vitro studies

2.4.1. Cytotoxicity studies

In order to find possible structure–activity relationships, *in vitro* cytotoxicities of complexes **2**–**12** against the human tumor cell lines 8505C anaplastic thyroid cancer, A2780 ovarian cancer, HeLa epithelial cancer, SW480 colon cancer, and MCF-7 breast cancer were determined by using the sulforhodamine-B microculture colorimetric assay. The cytotoxicities of the amino acids/lactic acid, starting platinum(II) carbonato complex (**1**) and cisplatin [35,36] are included for comparison.

Treatment with **L1**–**L6** and **L8** did not show any toxicity in the dose range used (0.1–150 μM) except **L7** ($IC_{50} \geqslant 78$ μM). On the other hand, their corresponding complexes showed much higher activity (Table 2). The platinum(II) complexes **1**–**12** showed dose–dependent antiproliferative effect toward investigated cancer cell lines, see Fig. 2 as example. The highest and lowest activity of these complexes was observed in A2780 and HeLa cell lines, respectively. A medium activity was observed against 8505C, SW480, and MCF-7 cell lines except complexes with *N*-alkylated amino acid ligands (**5** and **6**) which showed maximum activity against MCF-7 cell line. Thus, alkylation of the amino group leads to a change in the selectivity of the complexes. Cytotoxicities of the cationic complexes **2**–**6** were found to be comparable with those of the starting platinum(II) precursor complex **1.** Only complexes **5** and **6** against MCF-7 cell line and complex **3** against A2780 cell line show cytotoxicities which are increased and reduced, respectively, roughly by factor two compared to complex **1** (see Table 2). Of all the studied complexes, complex **7** seems to be the most active against selected cell lines (IC_{50}: 3.4–9.9 μM). Explicitly, the neutral complex **7** having coordinated a deprotonated *N*-acetyl derivative of L-alanine was found to be 3–7 times more active than the cationic complex **2** having coordianted L-alanine. In comparison to cisplatin, complexes **1**–**12** are less active against investigated cell lines, except complex **7** which showed a comparable cytotoxicity against 8505C cell line.

2.4.2. Mode of cell death

The mode of cell death was investigated for the two most active platinum complexes (**7**, **9**) against ovarian A2780 cancer cell line. Discrimination of the living cells from dead ones can be done on the basis of membrane integrity using acridine orange/ethidium bromide (AO/EB) staining. Microphotographs of AO/EB double stained A2780 cells, which were pretreated for 24 h with complexes **7** and **9** and cisplatin (for comparison) are presented in Fig. 3. Control cells (non treated) have green nuclei and uniform chromatin with an intact cell membrane. In contrast, complexes **7** and **9**, as well as cisplatin induced apoptosis as seen by condensation and/or fragmentation of the nuclei. In addition, rounding of the cells was also observed when treated with complexes **7**, **9**, and cisplatin.

Furthermore, to confirm findings from AO/EB double staining assay, floating A2780 cells after treatment with IC_{90} concentrations were collected and analyzed by laddering technique (Fig. 3). For A2780 cells treated with platinum(II) compounds **7** and **9**, DNA appeared as characteristic ladder-like fragments which is the biochemical hallmark of apoptosis. The results are compared to the negative control (untreated cells) where no laddering pattern or smear (indicating necrosis) was seen and to the positive control cisplatin (induces apoptosis).

2.4.3. Cell cycle investigation

To investigate the effects of platinum(II) complexes **7** and **9** (cisplatin used as a positive control) on cell cycle progression, A2780 cells were treated for 24 h and 48 h with IC_{50} and IC_{90} concentrations and analyzed by flow cytometry. The obtained results (see Fig. 4 for example and Supplemental material) indicated that when A2780 cells were exposed for 24 h to IC_{50} and IC_{90} concentrations of **7** and **9** drastically decreased the cell population in all phases

[1] Here and in the following the values for the two symmetry independent molecules are given separated by a slash.

Table 2
Cytotoxicity of investigated compounds against 8505C (anaplastic thyroid carcinoma), A2780 (ovarian carcinoma), HeLa (human epithelial carcinoma), SW480 (human colon carcinoma), and MCF7 (breast carcinoma) cell lines represented by the IC_{50} values [μM].[a]

Compound	IC_{50} ± SD				
	8505C	A2780	HeLa	SW480	MCF-7
L1–**L6**, **L8**			>150.0		
L7	135.2 ± 1.7	104.7 ± 3.7	>150.0	121.2 ± 1.7	77.7 ± 4.6
1	36.6 ± 0.5	17.3 ± 1.3	66.9 ± 3.5	29.4 ± 2.7	23.0 ± 1.9
2	26.0 ± 1.9	17.3 ± 0.3	50.4 ± 2.4	27.3 ± 1.3	22.0 ± 2.1
3	39.9 ± 0.5	31.1 ± 1.4	57.8 ± 2.3	46.3 ± 2.8	29.1 ± 1.4
4	27.7 ± 1.1	12.9 ± 0.5	39.9 ± 2.7	25.8 ± 63.8	21.9 ± 1.7
5	32.6 ± 1.4	23.4 ± 2.1	42.7 ± 4.7	29.4 ± 2.4	10.7 ± 1.4
6	31.3 ± 1.6	23.8 ± 2.2	41.0 ± 3.1	27.9 ± 1.6	13.4 ± 1.2
7	7.2 ± 0.5	3.4 ± 0.6	9.9 ± 1.5	7.4 ± 0.6	8.7 ± 4.3
9	18.0 ± 0.2	10.0 ± 0.1	54.5 ± 1.9	27.6 ± 2.1	28.9 ± 1.2
10	30.9 ± 1.3	28.2 ± 1.5	28.4 ± 3.1	28.2 ± 3.5	16.0 ± 1.4
11	15.0 ± 0.4	15.4 ± 0.3	50.8 ± 6.5	17.1 ± 0.1	18.5 ± 1.5
12	24.2 ± 0.8	15.7 ± 0.4	64.1 ± 1.8	25.4 ± 0.3	27.6 ± 1.3
Cisplatin	5.02 ± 0.23	0.55 ± 0.03	4.6 ± 0.34	3.2	2.03 ± 0.11

[a] Mean values ± SD (standard deviation) from three experiments.

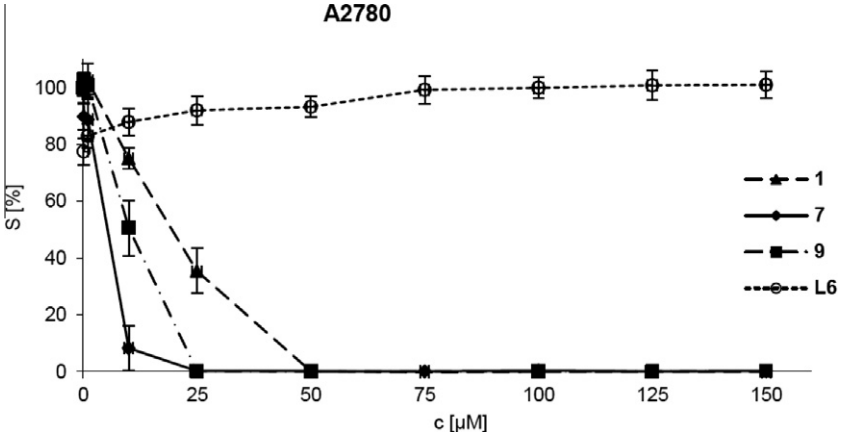


Fig. 2. Representative graph showing survival after 96 h of the A2780 cell line treated with **L6** and platinum(II) complexes **1**, **7**, and **9**.

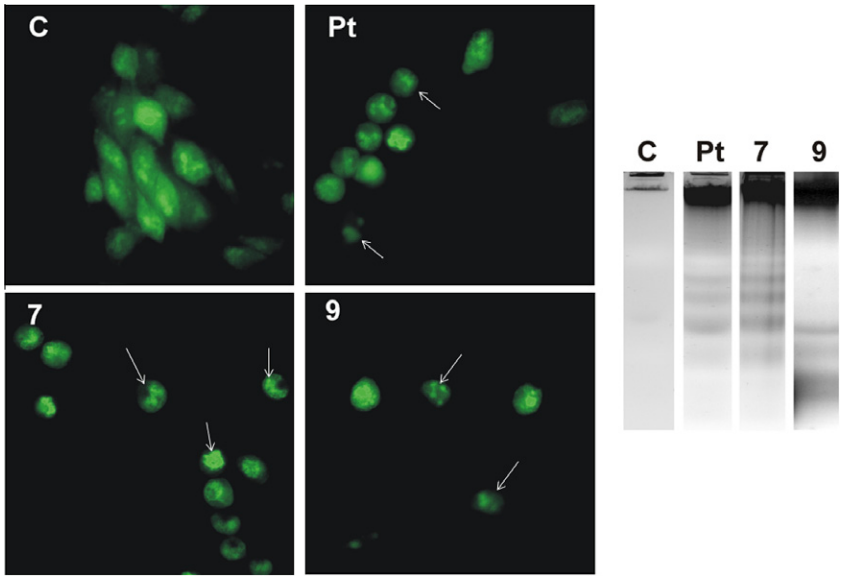


Fig. 3. Left: AO/EB double stained A2780 cells: untreated (C – control) and treated with cisplatin (Pt) and complexes **7** and **9**. Right: DNA laddering of A2780 cells untreated (C – control) and treated with IC_{90} concentrations of cisplatin (Pt), complexes **7** and **9**. Arrows indicate shrinkage of nuclei and chromatin condensation.

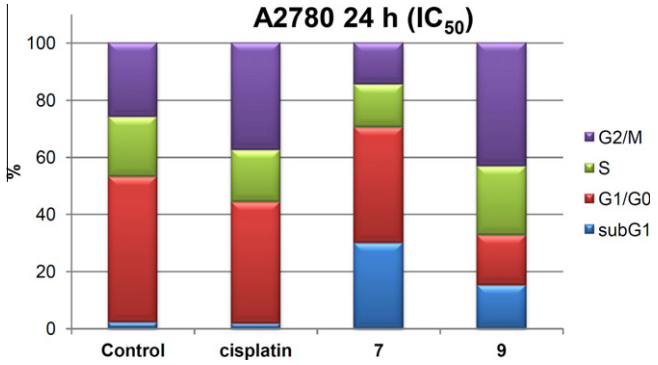


Fig. 4. Cell cycle analysis of A2780 cell line treated for 24 h to IC_{50} concentrations of cisplatin and complexes **7** and **9**; control – untreated cells.

of the cell cycle. Consequently, an increasement in the number of cells in sub-G1 phase was observed. Cisplatin slightly increased the relative number of A2780 cells in the G2/M phases when treated for 24 h at IC_{50} and IC_{90} concentrations. On treatment of A2780 cells with IC_{50} and IC_{90} concentration of complexes **7** and **9** for 48 h also show a decrease in the cell population in G1, S, and G2/M phases and relative increasement in the cells in sub-G1 phase. Unlike cisplatin, platinum complexes **7** and **9** did not induce a significant cell cycle arrest in any cell cycle phase but rather directly provoked an increase in the number of apoptotic cells with concomitant decline of all other cell cycle phases in A2780 cells. Thus, the cell cycle perturbation studies indicate that complex **7** induced apoptosis (see Section 2.4.2) of the A2780 cells faster than complex **9** and both complexes faster than cisplatin.

3. Summary and conclusions

The carbonato platinum complex $[Pt(CO_3)(PPh_3)_2]\cdot CH_2Cl_2$ (**1**) has been found to react with amino acids and lactic acid, without adding an external base, with liberation of CO_2 yielding platinum complexes with monodeprotonated amino acid/lactic acid ligands of type **I** ($[Pt(PPh_3)_2\{HX\frown C(O)O\text{-}\kappa O\}_2]$; HX = NHR, OH) or type **II** ($[Pt(PPh_3)_2\{HRN\frown C(O)O\text{-}\kappa N,\kappa O\}][PF_6]$) and with doubly deprotonated amino acid/lactic acid ligands of type **III** ($[Pt(PPh_3)_2(X\frown C(O)O\text{-}\kappa X,\kappa O\}]$), respectively. The type of complex formed does not depend primarily on the ratio of starting carbonato platinum complex (**1**) and amino acid/lactic acid used in the synthesis, but on the solvent and the acidity of the NHR group tuned via the substituent R. A deprotonation of the carboxylic group takes place both in aprotic (dichloromethane) and protic solvents (methanol), whereupon the formation of type **I** complexes is favored in dichloromethane and that of type **II** complexes in methanol. An additional deprotonation of the OH group in lactic acid and of the NHR group in amino acids leading to type **III** complexes was only found in methanol and for amino acids with more acidic amino groups (R = COMe, Ph). Furthermore, the *in vitro* cytotoxicities of these complexes against five human cancer cell lines indicated that the neutral type **III** complex **7** is the most active one. It proved to be only slightly less active than cisplatin and, moreover, to induce apoptosis (shown for the cell line A2780), but faster than cisplatin.

4. Experimental section

4.1. General remarks

All reactions were carried out in an atmosphere of dry argon using standard Schlenk and vacuum line techniques, but the work-up procedures and the spectral measurements could be conducted under aerobic conditions. The solvents used in these reactions were dried by standard methods and freshly distilled under argon prior to use. NMR spectra (^1H, ^{13}C, ^{31}P; ^{13}C and ^{31}P NMR spectra are generally broadband proton decoupled) were recorded at 27 °C on Varian Gemini 200, VXR 400, and Unity 500 spectrometers. Chemical shifts are relative to solvent signals ($CDCl_3$; δ_H 7.24, δ_C 77.0) as internal references. H_3PO_4 (85%) was used as external reference for ^{31}P NMR spectra. High-resolution ESI mass spectra were obtained from a Bruker Apex III Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker Daltonics) equipped with an Infinity cell, a 7.0 T superconducting magnet (Bruker), an rf-only hexapole ion guide, and an external APOLLO electrospray ion source (Agilent, off-axis spray). The sample solutions were introduced continuously via a syringe pump with a flow rate of 120 $\mu L \cdot h^{-1}$. *cis*-$[PtCl_2(PPh_3)_2]$ was prepared as reported in literature [37]. $[Pt(CO_3)(PPh_3)_2]\cdot CH_2Cl_2$ (**1**) was prepared according to the procedure given in literature [26], but using a 7-fold excess of Ag_2CO_3 and a much longer (5 days) reaction time (yield 75%). All other chemicals were commercially available and used as supplied without any further purification.

4.2. Synthesis of the platinum(II) complexes

4.2.1. Synthesis of $[Pt(PPh_3)_2\{NHR–CHR'–C(O)O\text{-}\kappa N,\kappa O\}][PF_6]$ (**2**–**6**)

Amino acids (**L1**–**L5**, 0.22 mmol) were added to a suspension of $[Pt(CO_3)(PPh_3)_2]\cdot CH_2Cl_2$ (**1**; 160.0 mg, 0.19 mmol) in methanol (5 mL). The reaction mixture was stirred overnight at room temperature resulting in a colorless solution. Then, $Tl[PF_6]$ (76.9 mg, 0.22 mmol) was added and the reaction mixture was further stirred for 2 h. A white precipitate started appearing within 10 min. Dichloromethane (5 mL) was added and the reaction mixture was stirred for about 30 min resulting in a dissolution of the majority of the precipitate. The reaction mixture was then passed through Celite and the Celite pad was washed with CH_2Cl_2 (6 × 5 mL). The combined organic phases were concentrated under reduced pressure almost to dryness. Addition of diethyl ether (10 mL) resulted in a white precipitate, which was filtered off, washed with diethyl ether (3 × 2 mL) and dried under vacuum.

R/R′ = H/Me (**2**). Yield: 146.0 mg (83%). HRMS (ESI): *m*/*z* Calc. for $[C_{39}H_{36}O_2NP_2Pt]^+$: 807.1868; found for $[M]^+$: 807.1864. ^1H NMR (400 MHz, $CDCl_3$): δ 1.12 (d, $^3J_{H,H}$ = 6.8 Hz, 3H, C*H*$_3$), 3.22 (s (br), 2H, N*H*$_2$), 3.86 (m, 1H, C*H*), 7.16–7.55 (m, 30H, Ph). ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.0 (s, *C*H$_3$), 53.5 (s, *C*H), 125.0 (d, $^1J_{P,C}$ = 66.5 - Hz, *i*-*C*, Ph), 126.1 (d, $^1J_{P,C}$ = 63.2 Hz, *i*-*C*, Ph), 129.2 (d, $^3J_{P,C}$ = 11.5 - Hz, *m*-*C*, Ph), 129.4 (d, $^3J_{P,C}$ = 11.7 Hz, *m*-*C*, Ph), 133.8 (d, $^4J_{P,C}$ = 2.7 Hz, *p*-*C*, Ph), 134.2 (d, $^4J_{P,C}$ = 2.7 Hz, *p*-*C*, Ph), 134.0 (d, $^2J_{P,C}$ = 10.9 Hz, *o*-*C*, Ph), 134.6 (d, $^2J_{P,C}$ = 10.9 Hz, *o*-*C*, Ph), 186.0 (s, *C*OO). ^{31}P NMR (162 MHz, $CDCl_3$): δ −142.7 (sept, $^1J_{P,F}$ = 713.1 Hz, *P*F_6^-), 5.6 (d + d, $^2J_{P',P''}$ = 24.2 Hz, $^1J_{Pt,P'}$ = 3663 Hz, P′), 7.7 (d + d, $^2J_{P',P''}$ = 24.2 Hz, $^1J_{Pt,P''}$ = 3508 Hz, P″).

R/R′ = H/^{i}Pr (**3**). Yield: 156.0 mg (86%). HRMS (ESI): *m*/*z* Calc. for $[C_{41}H_{40}O_2NP_2Pt]^+$: 835.2180; found for $[M]^+$: 835.2177. ^1H NMR (400 MHz, $CDCl_3$): δ 0.71 (d, $^3J_{H,H}$ = 7.0 Hz, 3H, CH(C*H*$_3$)$_2$), 0.84 (d, $^3J_{H,H}$ = 6.9 Hz, 3H, CH(C*H*$_3$)$_2$), 2.13 (m, 1H, C*H*(CH$_3$)$_2$), 2.37 (m, 1H, N*H*$_2$), 3.65 (m, 1H, C*H*NH$_2$), 3.94 (m, 1H, N*H*$_2$), 7.23–7.62 (m, 30H, Ph). ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.9 (s, CH(CH$_3$)*C*H$_3$), 18.8 (s, CH(*C*H$_3$)CH$_3$), 31.0 (s, *C*H(CH$_3$)$_2$), 61.9 (s, *C*HCOO), 124.8 (d, $^1J_{P,C}$ = 64.8 Hz, *i*-*C*, Ph), 125.8 (d, $^1J_{P,C}$ = 63.7 Hz, *i*-*C*, Ph), 129.1 (d, $^3J_{P,C}$ = 11.5 Hz, *m*-*C*, Ph), 129.5 (d, $^3J_{P,C}$ = 11.6 Hz, *m*-*C*, Ph), 132.3 {br (s), *p*-*C*, Ph}, 132.6 {br (s), *p*-*C*, Ph}, 133.9 (d, $^2J_{P,C}$ = 10.1 - Hz, *o*-*C*, Ph), 134.5 (d, $^2J_{P,C}$ = 10.7 Hz, *o*-*C*, Ph), 184.6 (d, $^{3/4}J_{P,C}$ = 5.3 - Hz, *C*OO). ^{31}P NMR (162 MHz, $CDCl_3$): δ −142.7 (sept, $^1J_{P,F}$ = 712.1 Hz, *P*F_6^-), 6.4 (d + d, $^2J_{P',P''}$ = 24.1 Hz, $^1J_{Pt,P'}$ = 3643 Hz, P′), 7.3 (d + d, $^2J_{P',P''}$ = 24.1 Hz, $^1J_{Pt,P''}$ = 3471 Hz, P″).

R/R′ = H/$CH_2CH(CH_3)_2$ (**4**). Yield: 140 mg (76%). HRMS (ESI): *m*/*z* Calc. for $[C_{42}H_{42}O_2NP_2Pt]^+$: 849.2340; found for $[M]^+$: 849.2333. ^1H

NMR (400 MHz, $CDCl_3$): δ 0.60 (d, $^3J_{H,H}$ = 6.4 Hz, 3H, CH(CH_3)CH_3), 0.71 (d, $^3J_{H,H}$ = 6.8 Hz, 3H, CH(CH_3)CH_3), 0.98 (m, 1H, $CH(CH_3)_2$), 1.63 (m, 2H, CH_2), 2.9 (br, 1H, NH_2), 3.69 (br, 2H, $NH + CHCH_2$), 7.16–7.63 (m, 30H, Ph). ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.5 (s, CH(CH_3)CH_3), 23.3 (s, CH(CH_3)CH_3), 25.7 (s, $CH(CH_3)_2$), 43.7 (s, CH_2), 56.2 (s, $CHCH_2$), 125.3 (d, $^1J_{P,C}$ = 66.7 Hz, *i*-*C*, Ph), 127.4 (d, $^1J_{P,C}$ = 64.3 Hz, *i*-*C*, Ph), 129.9 (d, $^3J_{P,C}$ = 11.6 Hz, *m*-*C*, Ph), 130.5 (d, $^3J_{P,C}$ = 11.6 Hz, *m*-*C*, Ph), 133.3 (d, $^4J_{P,C}$ = 2.4 Hz, *p*-*C*, Ph), 133.8 (d, $^4J_{P,C}$ = 2.9 Hz, *p*-*C*, Ph), 135.4 (d, $^2J_{P,C}$ = 11.1 Hz, *o*-*C*, Ph), 135.8 (d, $^2J_{P,C}$ = 10.6 Hz, *o*-*C*, Ph), 187.0 (s, *C*OO). ^{31}P NMR (162 MHz, $CDCl_3$): δ −142.7 (sept, $^1J_{P,F}$ = 712.1 Hz, PF_6^-), 7.2 (d + d, $^2J_{P',P''}$ = 24.7 Hz, $^1J_{Pt,P'}$ = 3570 Hz, P′), 7.6 (d + d, $^2J_{P',P''}$ = 24.6 Hz, $^1J_{Pt,P''}$ = 3486 Hz, P″).

R/R′ = Me/H (**5**). Yield: 140 mg (79%). HRMS (ESI): *m*/*z* Calc. for $[C_{39}H_{36}O_2NP_2Pt]^+$: 807.1865; found for $[M]^+$: 807.1864. ^1H NMR (200 MHz, $CDCl_3$): δ 2.33 (br, 3H, CH_3), 3.1 (br, 1H, N*H*), 3.31 (dd, $^2J_{H,H}$ = 16.7 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H, CH_2), 4.38 (dd, $^2J_{H,H}$ = 16.7 Hz, $^3J_{H,H}$ = 5.2 Hz, 1H, CH_2), 7.24–7.65 (m, 30H, Ph). ^{13}C NMR (125 MHz, $CDCl_3$): 41.4 (s, CH_3), 57.3 (s, CH_2), 125.4 (d, $^1J_{P,C}$ = 65.0 Hz, *i*-*C*, Ph), 126.0 (d, $^1J_{P,C}$ = 64.2 Hz, *i*-*C*, Ph), 128.9 (d, $^3J_{P,C}$ = 11.6 Hz, *m*-*C*, Ph), 129.7 (d, $^3J_{P,C}$ = 11.5 Hz, *m*-*C*, Ph), 132.1 {br (s), *p*-*C*, Ph}, 132.7 {br (s), *p*-*C*, Ph}, 133.9 (d, $^2J_{P,C}$ = 10.8 Hz, *o*-*C*, Ph), 134.5 (d, $^2J_{P,C}$ = 10.5 Hz, *o*-*C*, Ph), 181.0 (s, *C*OO). ^{31}P NMR (81 MHz, $CDCl_3$): δ −142.7 (sept, $^1J_{P,F}$ = 712.1 Hz, PF_6^-), 7.4 (d + d, $^2J_{P',P''}$ = 23.7 Hz, $^1J_{Pt,P'}$ = 3703 Hz, P′), 8.2 (d + d, $^2J_{P',P''}$ = 23.7 Hz, $^1J_{Pt,P''}$ = 3381 Hz, P″).

R/R′ = Et/H (**6**). Yield: 131 mg (73%) HRMS (ESI): *m*/*z* Calc. for $[C_{40}H_{38}O_2NP_2Pt]^+$: 821.2011; found for $[M]^+$: 821.2020. ^1H NMR (400 MHz, $CDCl_3$): δ 0.60 (t, $^3J_{H,H}$ = 6.2 Hz, 3H, CH_3), 2.76 (br, 1H, N*H*), 2.86 (br, 2H, CH_2CH_3), 3.46 (dd, $^2J_{H,H}$ = 16.8 Hz, $^3J_{H,H}$ = 3.6 Hz, 1H, CH_2), 4.29 (dd, $^2J_{H,H}$ = 16.8 Hz, $^3J_{H,H}$ = 4.5 Hz, 1H, CH_2), 7.24–7.68 (m, 30H, Ph). ^{13}C NMR (125 MHz, $CDCl_3$): 12.0 (s, CH_3), 48.8 (s, CH_2CH_3), 52.5 (s, CH_2), 124.9, 125.4, 125.6, 126.1, 129.2 (d, $^3J_{P,C}$ = 10.7 Hz, *m*-*C*, Ph), 129.8 (d, $^3J_{P,C}$ = 10.9 Hz, *m*-*C*, Ph), 132.3 (d, $^4J_{P,C}$ = 1.6 Hz, *p*-*C*, Ph), 132.7 (d, $^4J_{P,C}$ = 1.8 Hz, *p*-*C*, Ph), 133.9 (d, $^2J_{P,C}$ = 9.9 Hz, *o*-*C*, Ph), 134.5 (d, $^2J_{P,C}$ = 9.6 Hz, *o*-*C*, Ph), 183.0 (s, *C*OO). ^{31}P NMR (81 MHz, $CDCl_3$): δ −142.7 (sept, $^1J_{P,F}$ = 712.1 Hz, PF_6^-), 6.4 (d + d, $^2J_{P',P''}$ = 24.0 Hz, $^1J_{Pt,P''}$ = 3353 Hz), 6.5 (d + d, $^2J_{P',P''}$ = 23.1 Hz, $^1J_{Pt,P'}$ = 3703 Hz).

4.2.2. Synthesis of [Pt(PPh$_3$)$_2${N(COMe)–CHMe–C(O)O-κN,κO}] (**7**)

N-Acetyl L-alanine (**L6**) (27.2 mg, 0.21 mmol) was added to a suspension of $[Pt(CO_3)(PPh_3)_2]\cdot CH_2Cl_2$ (**1**; 150.0 mg, 0.17 mmol) in MeOH (5 mL). The reaction mixture was stirred overnight at room temperature. Then the solvent was completely removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (0.5 mL) and addition of diethyl ether (10 mL) resulted in precipitation of a white solid which was filtered off, washed with diethyl ether (3 × 2 mL) and dried under vacuum.

Yield: 99 mg (63%). HRMS (ESI): *m*/*z* Calc. for $[C_{41}H_{37}NaO_3P_2$-NPt]: 871.1798; found for $[M+Na]^+$: 871.1789. ^1H NMR (400 MHz, CD_3OD): δ 1.42 (s, 3H, C(O)CH_3), 1.71 (d, $^3J_{H,H}$ = 7.0 Hz, 3H, CH_3), 4.41 (m, 1H, *CH*), 7.11–7.84 (m, 30H, Ph). ^{13}C NMR (125 MHz, CD_3-OD) δ 19.4/21.8 (s/s, C(O)CH_3, $CHCH_3$), 62.9 (s, *C*H), 126.3–135.8 (aromatic *C*), 173.3 (s, *C*O), 190.0 (*C*OO) ^{31}P NMR (81 MHz, CD_3OD): δ 6.4 (d+d, $^2J_{P',P''}$ = 24.7 Hz, $^1J_{Pt,P'}$ = 4091 Hz, P′), 9.3 (d+d, $^2J_{P'',P'}$ = 24.7 Hz, $^1J_{Pt,P''}$ = 3068 Hz, P″).

4.2.3. Synthesis of [Pt(PPh$_3$)$_2${NHR–CHR′–C(O)O-κO}$_2$] (**9**, **10**) and [Pt(PPh$_3$)$_2${HO–CHMe–C(O)O-κO}$_2$] (**12**)

The requisite amino acid **L6**/**L7** and lactic acid **L8**, respectively, (0.41 mmol) were added to a solution of $[Pt(CO_3)(PPh_3)_2]\cdot CH_2Cl_2$ (**1**; 160.0 mg, 0.19 mmol) in CH_2Cl_2 (5 mL) and the reaction mixture was stirred overnight at room temperature. The solution was concentrated under reduced pressure almost to dryness. Addition of diethyl ether (10 mL) resulted in precipitation of a white solid which was filtered off, washed with diethyl ether (3 × 2 mL) and dried under vacuum.

R/R′ = C(O)Me/Me, (**9**). Yield: 109 mg (60%). HRMS (ESI): *m*/*z* Calc. for $[C_{41}H_{38}O_3P_2NPt]^+$: 849.1971; found for $[M-\mathbf{L6}_{-H}]^+$: 849.1969. ^1H NMR (400 MHz, $CDCl_3$): δ 0.79 (d, $^3J_{H,H}$ = 6.8 Hz, 6H, $CHCH_3$), 1.75 (s, 6H, C(O)CH_3), 3.64 (dq, $^3J_{H,H}$ = 5.8 Hz, $^3J_{H,H}$ = 6.8 Hz, 2H, *CH*), 6.12 (d, $^3J_{H,H}$ = 5.8 Hz, 2H, N*H*), 7.15–7.54 (m, 30H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.8 (s, $CHCH_3$), 23.4 (s, $COCH_3$), 50.5 (s, *C*H), 127.5 (m*,[2] *i*-C_{Ph}), 128.2 ('t'*,[3] *m*-C_{Ph}), 131.1 {br (s), *p*-C_{Ph}}, 134.5 ('t'*, *o*-C_{Ph}), 168.6/175.6 (s/s, $COCH_3$, *C*OO). ^{31}P NMR (162 MHz, CD_2-Cl_2): δ 6.8 (s + d, $^1J_{Pt,\ P}$ = 3865 Hz).

R/R′ = Ph/H (**10**). Yield: 132 mg (70%). HRMS (ESI): *m*/*z* Calc. for $[C_{44}H_{38}O_2P_2NPt]^+$: 869.2016; found for $[M-\mathbf{L7}_{-H}]^+$: 869.2020. ^1H NMR (200 MHz, CD_2Cl_2): δ 3.03 (s, 4H, CH_2), 4.1 (br, 2H, N*H*), 6.25–7.60 (m, 40H, Ph). ^{13}C NMR (125 MHz, $CDCl_3$): 47.6 (s, CH_2), 112.6–147.9 (aromatic *C*), 174.1 (s, *C*OO). ^{31}P NMR (81 MHz, CD_2-Cl_2): δ 6.8 (s + d, $^1J_{Pt,P}$ = 3826 Hz).

12 Yield: 108 mg (65%). HRMS (ESI): *m*/*z* Calc. for $[C_{39}H_{35}P_2O_3$-Pt]$^+$: 808.1701; found for $[M-\mathbf{L8}_{-H}]^+$: 808.1704. ^1H NMR (400 MHz, $CDCl_3$): δ 0.8 (br, 6H, CH_3), 3.4 (br, 2H, *CH*), 4.2 (br, O*H*), 7.18–7.60 (m, 30H, Ph). ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.5 (s, CH_3), 68.3 (m, *C*H), 128.1 ('t'*, *m*-C_{Ph}), 131.1 (br, *p*-C_{Ph}), 134.5 ('d'*, *o*-C_{Ph}), 178.8 (s, *C*OO); *i*-C resonance not found. ^{31}P NMR (162 MHz, $CDCl_3$): δ 5.7 (s + d, $^1J_{Pt,P}$ = 3856 Hz).

4.2.4. Synthesis of [Pt(PPh$_3$)$_2${O–CHMe–C(O)O-κO,κO′}] (**11**)

Anhydrous L-lactic acid (**L8**) (17.5 mg, 0.19 mmol) was added to a suspension of $[Pt(CO_3)(PPh_3)_2]\cdot CH_2Cl_2$ (**1**; 140.0 mg, 0.16 mmol) in MeOH (5 mL). The reaction mixture was stirred overnight at room temperature. The white solid precipitated during the reaction was filtered off, washed with diethyl ether (3 × 2 mL) and dried under vacuum.

Yield: 78 mg (52%). HRMS (ESI): *m*/*z* Calc. for $[C_{39}H_{34}NaP_2O_3$-Pt]$^+$: 830.1524; found for $[M]^+$: 830.1523. ^1H NMR (200 MHz, $CDCl_3$): δ 1.35 (d, $^3J_{H,H}$ = 6.7 Hz, 3H, CH_3), 4.66 (m, 1H, *CH*), 7.11–7.52 (m, 30H, Ph). ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.9 (s, CH_3), 75.7 (s, *C*H), 127.9 (d, $^3J_{P,C}$ = 11.3 Hz, *m*-*C*, Ph), 128.0 (d, $^1J_{P,C}$ = 63.7 Hz, *i*-*C*, Ph), 128.1 (d, $^3J_{P,C}$ = 11.0 Hz, *m*-*C*, Ph), 129.1 (d, $^1J_{P,C}$ = 59.7 Hz, *i*-*C*, Ph), 130.7 (d, $^4J_{P,C}$ = 2.2 Hz, *p*-*C*, Ph), 130.8 (d, $^4J_{P,C}$ = 2.4 Hz, *p*-*C*, Ph), 134.4 (d, $^2J_{P,C}$ = 10.9 Hz, *o*-*C*, Ph), 134.6 (d, $^2J_{P,C}$ = 10.8 Hz, *o*-*C*, Ph), 194.1 (m, *C*OO). ^{31}P NMR (81 MHz, $CDCl_3$): δ 10.6 (d + d, $^2J_{P',P''}$ = 24.8 Hz, $^1J_{Pt,P''}$ = 3241 Hz, P″), 12.3 (d + d, $^2J_{P'',P'}$ = 24.8 Hz, $^1J_{Pt,P'}$ = 3911 Hz, P′).

4.3. X-ray crystallography

Single crystals suitable for X-ray diffraction measurements of **4** were obtained from CH_2Cl_2 solutions with a layer of diethyl ether. Data were collected with a STOE IPDS diffractometer at 200(2) K using Mo Kα radiation (λ = 0.71073 A, graphite monochromator). A summary of the crystallographic data, the data collection parameters, and the refinement parameters is given in Table 3. Absorption correction was applied empirically with the PLATON program package (T_{min}/T_{max} 0.13/0.16) [38]. The structure was solved with direct methods using SHELXS-97 [39] and refined using full-matrix least-square routines against F^2 with SHELXL-97 [40]. All non-hydrogen atoms were refined with anisotropic displacement parameters and hydrogen atoms with isotropic ones. Hydrogen atoms were placed in calculated positions according to the riding model. ORTEP III was used for graphical presentation [41].

[2] Here and in the following a star (*) represents the X part of a ABX spin system.

[3] Here and in the following 't' and 'd' represent the pseudo triplet and pseudo doublet, respectively.

Table 3
Crystallographic data, data collection parameters, and refinement parameters for complex **4**.

Empirical formula	$C_{42}H_{42}BF_4NO_2P_2Pt$
M_r	936.61
Crystal system	monoclinic
Space group	$P2_1$
a (Å)	12.7036(9)
b (Å)	23.773(2)
c (Å)	13.5624(9)
β (°)	106.322(7)
V (Å^3)	3930.8(5)
Z	4
D_{calc} (g cm^{-1})	1.583
μ(Mo K$_\alpha$) (mm^{-1})	3.707
F(000)	1864
θ range (°)	1.88–26.08
Reflection collected	35449
Reflection observed [$I > 2\sigma(I)$]	13222
Reflection independent (R_{int})	15350 (0.0451)
Data/restraints/parameter	15350/1/953
Goodness-of-fit (GOF) on F^2	0.954
$R1$, wR_2 [$I > 2\sigma(I)$]	0.0324, 0.0649
$R1$, wR_2 (all data)	0.0425, 0.0676
Largest difference in peak and hole (e Å^{-3})	1.097 and −0.849

4.4. In vitro studies

4.4.1. Cell cultures and cytotoxicity assay

The human tumor cell lines: human anaplastic thyroid cancer (8505C), ovarian cancer (A2780), human colon cancer (SW480), human epithelial cancer (HeLa), and human breast cancer (MCF-7) were maintained as monolayers in nutrient medium at 37 °C in a humidified atmosphere with 5% CO_2. Nutrient medium was RPMI-1640 (PAA Laboratories) supplemented with 10% fetal bovine serum (Biochrom AG) and penicillin/streptomycin (PAA Laboratories).

The cytotoxic activity of the investigated compounds was measured by the sulforhodamine-B (SRB, Sigma Aldrich) microculture colorimetric assay after 96 h [42]. Stock solutions of investigated amino acids, platinum complexes, and of the reference compound (cisplatin) were made in dimethyl sulfoxide (DMSO) at concentration of 20 mM and diluted by nutrient medium to the various working concentrations. Final concentrations achieved in treated wells were in the range from 0.1 to 150 μM and tested in quadruplicate. All experiments were done in triplicate. The final concentrations (<0.1%) of DMSO were non-toxic to the cells. Absorbance was measured at 570 nm using a 96-well plate reader (SpectraFluor Plus Tecan, Germany). The IC_{50} and IC_{90} values, defined as the concentrations of the compound at which 50% or 90% cell inhibition was observed, were estimated from the dose response curves.

4.4.2. AO/EB double staining assay

Morphological features of A2780 cell death induced by the studied compounds were analyzed after staining of the treated cells with a mixture of acridine orange (AO) and ethidium bromide (EB) [43]. When AO enters into the cells it fluoresces green. EB can enter only when the cell membrane is disrupted and is used to detect necrotic morphological changes, especially nuclear morphology. 1×10^4 of A2780 cells was seeded on the eight-chamber slide. Following 24 h-incubation, the IC_{90} concentration of the studied compounds (**7**, **9**, and cisplatin) was added to the cells. After 24 h, cells were washed with PBS and stained with a 10 μL mixture of AO (0.01 μg/mL) and EB (0.02 μg/mL). Ten minutes later, cells were examined by fluorescent microscopy and photographed (400×).

4.4.3. DNA fragmentation assay

Determination of apoptotic cell death was performed by DNA gel electrophoresis. Briefly, 1×10^6 A2780 cells were treated with the respective IC_{90} doses of complexes **7** and **9**, respectively, for 48 h. Floating cells induced by drug exposure were collected, washed with PBS, and lysed with lysis buffer (100 mM Tris–HCl pH 8.0; 20 mM EDTA; 0.8% SDS; all from Sigma Aldrich). Then they were treated with RNAase A at 37 °C for 2 h and proteinase K at 50 °C (both from Roche Diagnostics, Mannheim, Germany). Control sample was obtained by harvesting untreated A2780 cells and isolating DNA according to the same procedure described above. DNA laddering was observed by running the samples on 2% agarose gel followed by ethidium bromide (Sigma Aldrich) staining.

4.4.4. Cell cycle analysis

For this assay, 1×10^5 A2780 cells were seeded in a six-well plate with 5 mL of medium. After 24 h-incubation, cisplatin, **7** and **9** were added at their respective IC_{50} and IC_{90} concentrations. Following a 24- and 48 h-incubation, cells were harvested by mild trypsinization, collected by centrifugation, and washed with PBS. Adherent and floating cells were both resuspended in 100 μL of PBS and fixed with 2 mL of 70% ethanol at 4 °C for at least 1 h. The fixed samples were then centrifuged, and the cell pellet was washed with 2 mL of staining buffer (PBS + 2% FCS + 0.01% NaN_3) and again centrifuged. The cell pellet was then resuspended in 100 μL of RNase A (1 mg/mL) and incubated for 30 min at 37 °C. At the end of incubation, the samples are treated with propidium iodide (20 μg/mL of staining buffer) and allowed to stand for at least 30 min before analysis. The fluorescence intensity was determined by an Attune® Acoustic Focusing Cytometer equipped with Attune® Cytometric Software. Each analysis was carried out using about 1×10^4 events.

Acknowledgments

The authors would like to acknowledge Mrs. Renate Herzog for technical support and Mrs. Bianka Siewert for the help in cell culture laboratory as well as BioSolutions Halle GmbH for providing cell culture facilities.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ica.2012.08.034.

References

[1] A.-M. Florea, D. Büsselberg, Cancers 3 (2011) 1351.
[2] B. Lippert (Ed.), Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug, Verlag Helv. Chim. Acta Zürich (1999).
[3] L.F. Krylova, Khim. Interesakh Ustoich. Razvit. 7 (1999) 465.
[4] T.G. Appleton, in [2], pp. 363–376.
[5] T.G. Appleton, Coord. Chem. Rev. 166 (1997) 313.
[6] A. Iakovidis, N. Hadjiliadis, Coord. Chem. Rev. 135 (136) (1994) 17.
[7] L.M. Volshtein, Koord. Khim. 1 (1975) 595.
[8] M.A. Andrews, E.J. Voss, G.L. Gould, W.T. Klooster, T.F. Koetzle, J. Am. Chem. Soc. 116 (1994) 5730.
[9] W.T. Klooster, E.J. Voss, Inorg. Chim. Acta 285 (1999) 10.
[10] P. Bergamini, E. Marchesi, A. Marchi, V. Bertolasi, M. Fogagnolo, A. Canella, Eur. J. Inorg. Chem. (2008) 3056.
[11] N.M. Brunkan, P.S. White, M.R. Gagné, Angew. Chem., Int. Ed. 37 (1998) 1579.
[12] J.J. Becker, P.S. White, M.R. Gagné, Organometallics 22 (2003) 3245.
[13] J.-F. Wen, W. Hong, K. Yuan, T.C.W. Mak, H.N.C. Wong, J. Org. Chem. 68 (2003) 8918.
[14] H.-Y. Peng, C.-K. Lam, T.C.W. Mak, Z. Cai, W.-T. Ma, Y.-X. Li, H.N.C. Wong, J. Am. Chem. Soc. 127 (2005) 9603.
[15] M.D. Tudor, J.J. Becker, P.S. White, M.R. Gagné, Organometallics 19 (2000) 4376.
[16] A.O. Larsen, R.A. Taylor, P.S. White, M.R. Gagné, Organometallics 18 (1999) 5157.

[17] H.C.L. Abbenhuis, R.A. van Santen, A.D. Burrows, M.T. Palmer, H. Kooijman, M. Lutz, A.L. Spek, Chem. Commun. (1998) 2627.
[18] A.D. Burrows, D.M.P. Mingos, S.E. Lawrence, A.J.P. White, D.J. Williams, J. Chem. Soc., Dalton Trans. (1997) 1295.
[19] A. Imran, R.D.W. Kemmitt, A.J.W. Markwick, P. McKenna, D.R. Russell, L.J.S. Sherry, J. Chem. Soc., Dalton Trans. (1985) 549.
[20] D.A. Clarke, R.D.W. Kemmitt, M.A. Mazid, P. McKenna, D.R. Russell, M.D. Schilling, L.J.S. Sherry, J. Chem. Soc., Dalton Trans. (1984) 1993.
[21] S. Okeya, Y. Nakamura, S. Kawaguchi, T. Hinomoto, Bull. Chem. Soc. Jpn. 55 (1982) 477.
[22] J.J. Becker, P.S. White, M.R. Gagné, Inorg. Chem. 38 (1999) 798.
[23] K. Mikami, H. Kakuno, K. Aikawa, Angew. Chem., Int. Ed. 44 (2005) 7257.
[24] C. Mügge, C. Rothenburger, A. Beyer, H. Görls, C. Gabbiani, A. Casini, E. Michelucci, I. Landini, S. Nobili, E. Mini, L. Messori, W. Weigand, Dalton Trans. 40 (2011) 2006.
[25] A.L. Tan, P.M.N. Low, Z.-Y. Zhou, W. Zheng, B.-M. Wu, T.C.W. Mak, T.S.A. Hor, J. Chem. Soc., Dalton Trans. (1996) 2207.
[26] M.A. Andrews, G.L. Gould, W.T. Klooster, K.S. Koenig, E.J. Voss, Inorg. Chem. 35 (1996) 5478.
[27] T.G. Appleton, J.R. Hall, S.F. Ralph, Inorg. Chem. 24 (1985) 673.
[28] O.W. Howarth, P. Moore, N. Winterton, J. Chem. Soc., Dalton Trans. (1974) 2271.
[29] T.G. Appleton, J.R. Hall, J. Chem. Soc., Chem. Commun. (1983) 911.
[30] R.D.W. Kemmitt, S. Mason, J. Fawcett, D.R. Russell, J. Chem. Soc., Dalton Trans. (1992) 1165.
[31] A. Iakovidis, N. Hadjiliadis, H. Schöllhorn, U. Thewalt, G. Trötscher, Inorg. Chim. Acta 164 (1989) 221.
[32] G.C.K. Roberts, O. Jardetzky, Adv. Protein Chem. 24 (1970) 447.
[33] Cambridge Structural Database, Version 5.31, The Cambridge Crystallographic Data Centre, Cambridge, UK, 2009.
[34] T.G. Appleton, H.C. Clark, L.E. Manzer, Coord. Chem. Rev. 10 (1973) 335.
[35] G.N. Kaluđerović, H. Kommera, S. Schwieger, A. Paethanom, M. Kunze, H. Schmidt, R. Paschke, D. Steinborn, Dalton Trans. (2009) 10720.
[36] M. Scholz, G.N. Kaluđerović, H. Kommera, R. Paschke, J. Will, W.S. Sheldrick, E. Hey-Hawkins, Eur. J. Med. Chem. 46 (2011) 1131.
[37] F.R. Hartley, Organomet. Chem. Rev. A 6 (1970) 119.
[38] a) MULscanABS, PLATON for Windows, University of Glasgow, 2008.;
b) A.L. Spek, J. Appl. Crystallogr. 36 (2003) 7.
[39] G.M. Sheldrick, SHELXS-97, Program for Crystal Structure Solution, University of Göttingen, Göttingen, 1998.
[40] G.M. Sheldrick, SHELXL-97, Program for the Refinement of Crystal Structures, University of Göttingen, Göttingen, 1997.
[41] L.J. Farrugia, J. Appl. Crystallogr. 30 (1997) 565.
[42] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, J. Natl. Cancer Inst. 82 (1990) 1107.
[43] N.K. Banda, W.C. Satterfield, A. Dunlap, K.S. Steimer, R. Kurrle, T.H. Finkel, Apoptosis 1 (1996) 49.