EurJIC
European Journal of Inorganic Chemistry

A Journal of ChemPubSoc Europe

Accepted Article

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To be cited as: Eur. J. Inorg. Chem. 10.1002/ejic.201600857

Link to VoR: http://dx.doi.org/10.1002/ejic.201600857

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Enhancing the cytotoxic activity of anticancer Pt(IV) complexes by introduction of lonidamine as an axial ligand.

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Abstract: The synthesis and *in vitro* cytotoxicity of a series of Pt(IV) complexes with lonidamine as a ligand coordinated in axial position are described. The lonidamine was found to affect strongly the *in vitro* cytotoxic activity of these novel complexes, lowering the IC_{50} values down to the nanomolar range. Lipophilicity assessed in terms of log *P* showed no direct correlation with cytotoxicity.

Introduction

Nowadays platinum(II)-based chemotherapeutic agents are in the first-line for treatment of different types of cancers.[1] Much effort was devoted to understanding the mechanism of action of platinum drugs[2] and overcoming side effects and resistant phenomena.[3] Pt(IV) complexes have received considerable interest due to their kinetical inertness and specific reactivity in the reducing milieu of the cancer cell (activation by reduction), lower general toxicity, and possibility of oral administration.[4]

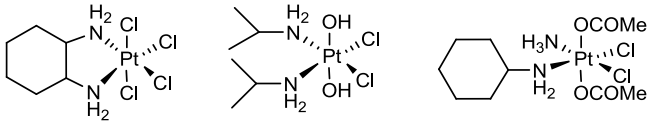

Figure 1. Examples of Pt(IV) complexes evaluated in clinical trials.

Several Pt(IV) compounds (see e.g. Figure 1) were tested in clinical trials, however none of them so far reached the approval stage. Tetraplatin was rejected due to a high toxicity that probably stemmed from its fast reduction in the blood,[5] whereas iproplatin demonstrated a low activity associated with slow reduction.[6] For satraplatin, evaluated in the treatment of hormone-refractory prostate cancer, lack of convincing benefit for overall survival did not let the drug to enter the clinic, though clinical trials in combination with various organic drugs are still undergoing.[7]

One of the synthetic advantages of Pt(IV) complexes is the possibility to covalently link biologically active ligands at the respective axial positions.[4c] In cancer chemotherapy, drug combinations are widely applied, and therefore incorporation of a bioactive ligand can, by the rule of thumb, increase the activity or selectivity profile of new complexes and tune the controlled release of an active organic moiety.[8] There is a proven record that such ligands exhibit their own activity and act as carriers in drug targeting (e.g., folic acid,[9] estradiol,[10] aspirin,[11]and short peptides[12]), inhibit a key enzyme (ethacrynic acid[13] as an inhibitor of glutathione-S-transferase, dichloroacetic acid[14] for pyruvate dehydrogenase kinase, or valproic acid[15] – histone deacetylase inhibitor), or inhibit immune checkpoint.[16]

As a selective aerobic glycolysis inhibitor, lonidamine was evaluated in clinical trials, being administrated as a single agent or in combination with other drugs against lung, prostate, breast, and in non-small cell lung cancer.[17] Phase II/III clinical trials for several chemotherapeutic programs that include lonidamine are still in progress. Selectivity of lonidamine due to inhibiting aerobic glycolysis in cancer cells while simultaneously enhancing aerobic glycolysis in normal cells might be attributed to the inhibition of mitochondria bound hexokinase, which is highly specific to malignant cells.[18] To our expectations, integration of a platinum moiety and a releasable organic inhibitor in one compound can provide a stronger tumor-inhibiting effect than that observed when anticancer platinum complexes were co-administrated with lonidamine.[19] Here, we report the synthesis, chemical characterization and evaluation of drug-like properties, such as lipophilicity and in-vitro antiproliferative activity, of the first anticancer complexes with the aerobic glycolysis inhibitor, lonidamine, covalently linked to the platinum(IV) center.

Results and Discussion

We combined the known pharmacophore lonidamine with Pt(IV) moiety by introduction of organic part as an axial ligand. Complexes **5–7** (Scheme 1) were prepared by reaction of dihydroxidoplatinum(IV) derivatives based on diamminedichloridoplatinum(II) (cisplatin), dichlorido(ethane-1,2-diamine)platinum(II), and [(1R,2R)-cyclohexane-1,2-diamine](oxalato)platinum(II) (oxaliplatin) with an excess of 1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxylic acid chloride obtained *in situ* from lonidamine and oxalyl chloride. Complex **8** was made up in a similar way, starting from acetato(1R,2R-cyclohexanediamine)hydroxido(oxalato)platinum(IV). In all reactions, pyridine was used as an acceptor of HCl. Complex **6** precipitated during the reaction because of its low solubility in acetone, while **5**, **7**, and **8** precipitated after evaporating the reaction mixture. Finally, pyridine hydrochloride was removed by washing the precipitates with water, hence yielding pure complexes.

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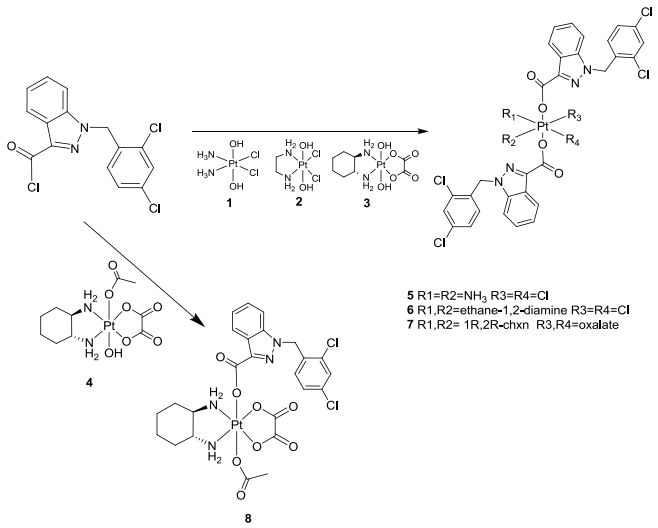


Scheme 1. Synthesis of Pt(IV) complexes **5-8**.

Complexes **5**–**8** were characterized by ^{1}H, $^{13}C\{^{1}H\}$, ^{15}N, ^{195}Pt 1D and 2D NMR spectroscopy, ESI mass spectrometry and elemental analysis. In the $^{13}C\{^{1}H\}$ spectra, we observed a shift of the carboxylic group that confirms the attachment of lonidamine to the platinum center. In addition, in the proton and carbon spectra for complexes **5**–**7**, only one set of signals was recorded confirming formation of the bis-substituted complexes. In ESI mass spectra in methanol, the most abundant peaks were assigned to the $[M+H^+]^+$ ion in the positive ion mode or to $[M-H^+]^-$ ion in the negative ion mode, respectively, containing the characteristic isotope pattern for such type of platinum complexes. For all mass spectra, experimental isotopic patterns are in good agreement with calculated ones.

Monitoring of the coordination sphere around the platinum(IV) center using ^{195}Pt NMR spectroscopy is well described in the literature.[20] For complexes **5** and **6**, the respective resonance at 2842 and 2633 ppm indicates the formation of $Pt(IV)Cl_2N_2O_2$ configured complexes. Likewise, for complexes **7** and **8** resonances at 3235 and 3240 ppm indicate a $Pt(IV)N_2O_4$ coordination sphere. As shown earlier, the nature of carboxylates in the axial position has no influence on the resonance in ^{195}Pt spectra.[21]

The molecular structure of **7** was determined by X-ray diffraction analysis at 200 K (Figure 2). Re-crystallization of the complex from acetone and diethyl ether gave crystals suitable for crystallographic analysis. Selected bond distances and angles for complex **7** are reported in Table 1. Crystal data and the structure refinement details are given in Table 3.

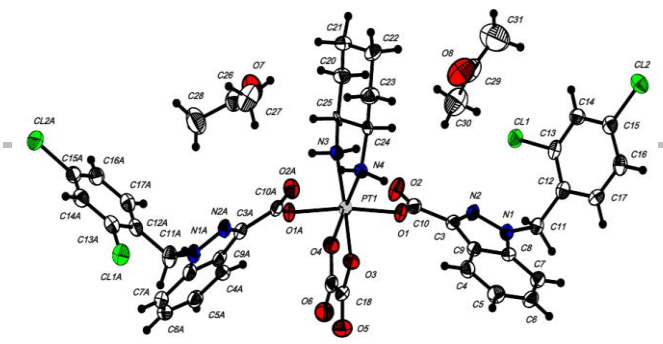


Figure 2. Molecular structure of complex **7** with the atom numbering scheme. Thermal displacement ellipsoids are given at the 50% probability level.

Complex **7** was found to be a monomer in solid state, with distorted octahedron geometry around the Pt center (Figure 2). The bond distances Pt–O and Pt–N vary between 2.007(3) and 2.055(4) Å. The bond angles around Pt(IV) vary between 83.29(16) and 98.35(15) and 171.75(14) and 177.96(17)°. The crystal structure of **7** contains two acetone molecules without any interactions with the complex; this finding explains low stability of the crystals at room temperature.

Table 1. Selected bond lengths and angles for complex **7**.

Bond lengths (Å)		Angles (°)	
Pt1–O1	2.007(3)	O1–Pt1–O3	86.06(15)
Pt1–O3	2.012(3)	O1–Pt1–O4	92.33(14)
Pt1–O4	2.013(3)	O3–Pt1–O4	84.40(14)
Pt1–O1A	2.024(3)	O1–Pt1–O1A	171.75(14)
Pt1–N4	2.037(4)	O3–Pt1–O1A	86.36(15)
Pt1–N3	2.055(4)	O4–Pt1–O1A	83.69(14)
		O1–Pt1–N4	85.64(15)
		O3–Pt1–N4	95.68(15)
		O4–Pt1–N4	177.96(17)
		O1A–Pt1–N4	98.35(15)
		O1–Pt1–N3	96.44(16)
		O3–Pt1–N3	177.21(16)
		O4–Pt1–N3	96.72(16)
		O1A–Pt1–N3	91.21(15)
		N4–Pt1–N3	83.29(16)

Cytotoxicity of complexes **5–8**, as well as of lonidamine and cisplatin, was determined in human MCF7 (breast cancer) cell line and its doxorubicin/cisplatin resistant analogue, MCF7D, in colon carcinoma SW480 cells, in the non-small cell lung carcinoma cell line A549, and in the immortalized human keratinocyte cell line HaCat (nonmalignant) by means of standard MTT colorimetric assay. For comparison, cytotoxicity data taken from literature for the precursor complexes. Cytotoxic activity of the compounds was evaluated as the IC_{50} value after 72 h of incubation (Table 2).

In MCF7, MCF7D, SW480, and A549 cell lines, complexes **5–8** showed considerably higher toxicity than lonidamine or cisplatin. In immortalized keratinocytes HaCat, compound **5** exhibited the same cytotoxic activity as lonidamine showing low degree of specificity towards the cancer cells. A similar effect was observed for complex **7**, while complexes 6 and 8 displayed stronger cytotoxicity (0.04±0.015 and 0.21±0.06 μM, respectively). The highest activity of all the complexes was found against breast cancer cells, with IC_{50} values in the MCF7 cell line falling into the nanomolar range. In spite of the fact that IC_{50} values for cisplatin and lonidamine were **3–4** times higher against doxorubicin/cisplatin-resistant MCF7D in comparison with MCF7 measurements, no difference between cytotoxicity of the tested compounds against both MCF7 and MCF7D was observed.

In summary, compound **6** showed the highest cytotoxic activity against MCF7, MCF7D, HaCat, and SW480 cell lines, with nanomolar IC_{50} values. Consequently, this compound can be

recommended for a more detailed investigation directed toward the assessment of its cytotoxic profile, interactions with important biomolecules, and *in vivo* evaluation.

Table 2. Cytotoxicity of complexes **5–8**, lonidamine and precursor complexes in different cell lines and log *P* values

compounds	IC_{50} (μM)[a]					log *P*	
	SW480	A549	MCF7	MCF7D	HaCat	Shake-flask	MEKC
5	1±0.5	2.1±0.7	0.45±0.3	0.6±0.15	2.5±1.1	1.76	1.69
6	0.13±0.06	1.5±0.9	0.03±0.008	0.07±0.024	0.04±0.015	1.31	1.60
7	0.7±0.2	1.2±0.4	0.5±0.25	0.33±0.01	1.2±0.5	1.79	1.77
8	2.1±1.0	3.9±2.4	0.5±0.2	0.5±0.1	0.21±0.06	1.84	1.84
lonidamine	>30	>40	8.5±2.1	27±14	2.5±0.9	1.30[22]	1.30
cisplatin	10±6	21.0±1.7	16±5	64±7	56±15	-	-
Oxaliplatin	0.9±0.3[23]	11.5±3.9[23]	43.8[25]				
$Pt(en)Cl_2$		20.6±4.1[24]					

[a] Errors represent the standard error of the mean.

Liphophilicity, expressed in the terms of log *P*, is one of important parameters in drug discovery. This characteristic is included in the Lipinsky rule of 5 and can be used as an indication for bioavailability of drug-like molecules and the possibility of oral administration.[26]

Two different methods (shake-flask and micellar electrokinetic chromatography, MEKC), applied according to previously developed experimental protocols, were used for log *P* determination of new complexes. The log *P* values of complexes **5–8** and lonidamine are collected in Table 2. (Note that because of a fairly high hydrophobicity of newly developed complexes, an adjustment of MEKC conditions toward lower surfactant concentration was necessary. As can be seen from Table 2, the data of both methods used to determine log *P* are in good agreement. However, for such a limited number of compounds, it was not possible to observe any strict relationship between log *P* and the structure of equatorial ligands (see Scheme 1).

It should be noted that for metal-based drugs with low to moderate lipophilicity, the order of log *P* is equivalent to the order of increased drug accumulation inside the cell and hence cytotoxicity. However, when the cytotoxicity of all compounds was compared to log *P*, no correlation was evident. Furthermore, the least lipophilic complex **6** showed the highest activity. This most plausible explanation behind this observation is that neither lipophilicity nor accumulation corresponds to the activation of bioreductive Pt(IV) prodrugs in a reducing environment of cancer cells, which is likely dependent on the rate of reduction.[27]

Conclusions

In this study, a unique approach to increasing the anticancer activity of platinum(IV) complexes was proposed, taking advantage of their axial ligation by lonidamine. The *in vitro* results for a series of complexes with a cisplatin, dichlorido(ethane-1,2-diamine)platinum(II), or oxaliplatin core indicate that lonidamine exhibits remarkable influences on their activity, which is superior to that of the majority of known platinum(IV) prodrugs, as well as to the positive control cisplatin. Of the tested compounds, complex **6** displays the most attractive biological performance, thus being worth of further investigations the mechanism of action and *in vivo* study.

Experimental Section

Materials

All solvents were purified and degassed prior to use.[28] Methanol (HPLC grade, Fisher) and Millipore water (18.2 MΩ; Millipore, Molsheim, France) were used for ESI-MS and MEKC studies.(OC-6-33)-Dichlorido(ethane-1,2-diamine)bis[(4-propylamino)-4-oxobutanato]platinum(IV) (**9**, log *P* – 0.43[29]) and (OC-6-33)-dichlorido(ethane-1,2-diamine)bis[(4-propyloxy)-4-oxobutanato]platinum(IV) (**10**, log *P* 0.70[29]) used as reference points in MEKC measurements were synthesized in the University of Vienna. SDS (Sigma-Aldrich, St. Louis, USA), Sudan III and oxalyl chloride (Fluka, St. Louis, USA) were used as purchased.

Physical Measurements

NMR spectra were recorded on a Bruker FT-NMR Avance III 500 MHz instrument at 500.32 (^{1}H), 125.81 (^{13}C), 50.70 (^{15}N) and 107.57 (^{195}Pt) MHz. 2D NMR measurements were carried out using standard pulse programs. Chemical shifts were referenced relative to the solvent signal for ^{1}H and ^{13}C spectra. For ^{15}N and ^{195}Pt spectra, the external standards, NH_4Cl an $K_2[PtCl_4]$, were used, respectively. Elemental analysis was performed by the Microanalytical Laboratory of the University of Vienna using a 2400 CHN elemental analyzer (Perkin–Elmer) and at Moscow State University with MicroCube Elementar analyzer. ESI mass spectra were recorded using a LC/MSn ion trap mass spectrometer amaZon SL (Bruker, Bremen, Germany) with MeOH as solvent. ICP-MS measurements were performed on a X-7 quadrupole mass-spectrometer (Thermo Elemental, USA) equipped with a standard low-volume glass spray chamber (Peltier, cooled at 3 °C) and a concentric glass nebulizer at a sample up-take rate of 0.8 mL min^{-1}. Before analysis, the instrument was tuned to achieve maximum Pt count rate and ^{115}In served as internal standard. All MEKC separations were carried out on a CAPEL 105M (Lumex Instruments, St. Petersburg, Russia) equipped with an on-column UV-vis detector and controlled by an Elphoran software. Fused-silica capillaries of 60 cm total length (50 cm effective length), 75 μm inner diameter, were provided by Polymicro Technologies (Phoenix, USA). Further details concerning MEKC system operational parameters and protocols for capillary initialization and pre- and between-run conditioning can be found elsewhere.[29]

log *P*

Using the shake-flask method log *P* values of complexes **5–8** were obtained similarly to other sparingly soluble metal complexes, as described elsewhere.[30] Briefly, the saturated solution of each compound in water (pre-saturated with n-octanol) was prepared by agitating in the ultrasonic bath. The supernatant was filtrated through a 0.45 μm membrane filter and shaken with an equal volume of water-saturated n-octanol for 2 h using a mechanical shaker. Platinum concentrations in the aqueous phase after phase separation (C_{aq}) and in the initial saturated solution (C_0) were determined by ICP-MS. Partition coefficients were calculated from the equation:

$$\log P = \log [(C_0 - C_{aq}) / C_{aq}] \quad (1)$$

In addition, log *P* values were determined with a MEKC method. A 6×10^{-4} M solution of each compound in acetone, as well as of two previously studied Pt(IV) complexes (**9** and **10**), was introduced hydrodynamically into the capillary filled with the micellar background electrolyte (30 mM SDS in 20 mM sodium tetraborate solution) and run separately by applying a high voltage. Acetone and Sudan III were used as markers for the electroosmotic flow (EOF) and micelles, respectively. Migration times of each analyte and marker were used to calculate the retention factor, *k*, according to the following equation

$$k = \frac{t - t_0}{t_0\left(1 - \frac{t_R}{t_{mc}}\right)} \quad (2)$$

(t_0, t_{mc} and t_R are migration times of EOF marker, micelles, and the complex, respectively). The corresponding log *P* value was assessed similarly to the previously established approach to ascertain the relationship between log P and log *k* .[29]

Cell lines and culture conditions

MCF7, MCF7D (a kind gift of N.I. Moiseeva), HaCat, A549, and SW480 cell lines were cultured in DMEM (PanEco, Russia) with 10% FBS (HyClone, USA) and antibiotics (PanEco, Russia) in 5% CO_2 at 37ºC. The compounds were pre-dissolved at 20 mM in DMSO and then added to the cell culture medium at the required concentration with a maximum DMSO content of 0.5 v/v%. At these concentrations, DMSO has no effect on cell viability. Cells were cultured in 96-well plates (7×103 cells/well) and treated with various concentrations of complexes **5–8** (from 0.01μM to 50μM) as well as with lonidamine and cisplatin at 37ºC for 72 h. Cell viability was determined using the MTT assay, which quantifies the mitochondrial activity Then, the cells were incubated at 37ºC for 4 h with 20 μl of 5 mg/ml solution of MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich, St. Louis, USA). The supernatant was discarded and cells were dissolved in 150 μl of dimethyl sulfoxide. The optical density of the solution was measured at 550 nm using a multiwell plate reader (Multiskan FC, Thermo Fisher Scientifics, USA) and the percentage of surviving cells was calculated from the absorbance of untreated cells. Each experiment was repeated at least three times and each concentration was tested in at least three replicates.

Crystallographic data collection and structure determination

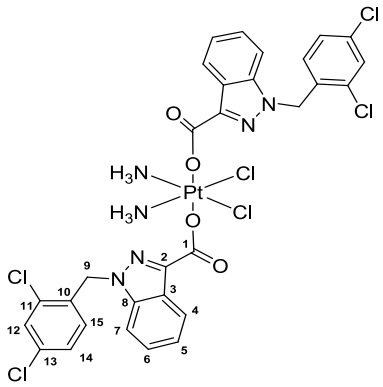
The data of **7** were collected by using STOE diffractometer Pilatus100K detector, focusing mirror collimation CuKα (1.54086Å) radiation, rotation method mode at 200 K. STOE X-AREA software was used for cells refinement and data reduction. Data collection and image processing was performed with X-Area 1.67 (STOE & Cie GmbH, Darmstadt, Germany, 2013). Intensity data were scaled with LANA (part of X-Area) in order to minimize differences of intensities of symmetry-equivalent reflections (multi-scan method). The structures were solved with direct methods and refined by full-matrix least-squares procedures on F^2 with SHELXL97.[31] The non-hydrogen atoms were refined by using the anisotropic full matrix least-square procedure; hydrogen atoms were located at calculated positions and refined *via* the "riding model". Refinement was made against 8251 reflections. 604 parameters were refined using 20 restraints. CCDC 1492004 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data via www.ccdc.cam.ac.uk/data_request/cif. The structures of complexes were drawn using the MERCURY CSD 3.1 program.[32]

Table 3. Selected bond lengths and angles for complex **7**.

Empirical formula	$C_{38}H_{32}Cl_4N_6O_8Pt$, $2(C_3H_6O)$
Fw	1153.74
Space group	$P\bar{1}$
Syngony	Triclinic
a (Å)	12.7080(10)
b (Å)	13.5260(10)
c (Å)	14.2440(10)
α (°)	101.883(6)
β (°)	93.235(6)
γ (°)	106.228(7)
V (Å^3)	2283.5(3)
$_{calc.}$, g/cm^3	1.67
Z	2
$_{max/min}$ (e/Å^3)	2.109/-1.561
	CuKα
μ (mm^{-1})	8.445
R_1/wR_2 ($I \geq 2\sigma(I)$)	0.0397/0.0875
GOOF	0.999

Synthesis

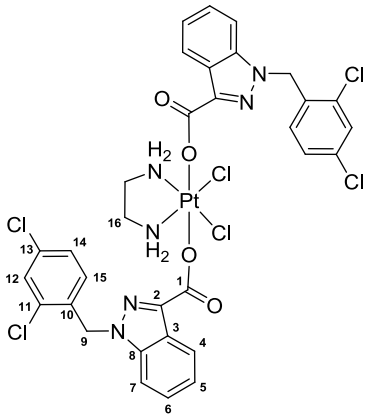
(OC-6-33)-(diammine)dichloridobis(1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxylate)platinum(IV) 5



Oxalyl chloride (1.2 mL, 14 mmol), one-two drops of DMF was added to a stirred suspension of 1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxylic acid [33] (321 mg, 1 mmol) in CH_2Cl_2 (25 mL). The reaction mixture was refluxed for 1 h until a clear solution was formed. Solvent and unreacted oxalyl chloride were removed under reduced pressure to yield the acid chloride as a pale yellow solid that was used without purification. A solution of 1-(2,4-dichlorobenzyl)-1H-indazole-3-carbonyl chloride in acetone (10 mL) was added to a stirred suspension of (OC-6-33)-(diammine)dichloridodihydroxidoplatinum(IV)[34] (65 mg, 0.2 mmol) and pyridine (120 μl, 1.5 mmol) in acetone (10 mL). The reaction mixture was stirred for 12h at room temperature, concentrated to ~3 mL and a formed white precipitate was filtered off, washed with diethyl ether (2 x 5 mL), water (3 x 3mL) and dried under reduced pressure. Yield: 78 mg, (42%), m.p. . 210–212 °C (decomp), elem. anal. calc (%) for ($C_{30}H_{24}Cl_6N_6O_4Pt$): C 38.32, H 2.57, N 8.94, found: C 38.21, H 2.91, N 9.03. 1H NMR ([d6]-DMSO) δ: 8.38 (d, 2H, J = 8.2 Hz, H4), 7.75 (d, 2H, J = 8.6 Hz, H7), 7.71 (d, 2H, J = 2.1 Hz, H12), 7.47 (t, 2H, J = 7.3 Hz, H6), 7.40 (dd, 2H, J = 2.1, 8.4 Hz, H14), 7.31 (t, 2H, J = 7.6 Hz, H5), 6.90 (brs, 6H, NH_3), 6.86 (d, 2H, J = 8.4 Hz, H15), 5.83 (s, 4H, H9) ppm. 13C{1H} ([d6]-DMSO) δ: 169.5 (C1), 141.2 (C8), 137.3 (C2), 134.1 (C10), 133.7 (C11/13), 133.6 (C11/13), 131.1 (C15), 129.6 (C12), 128.2 (C14), 127.0 (C6), 124.1 (C4), 123.6 (C3), 122.8 (C5), 110.5 (C7), 50.0 (C9)

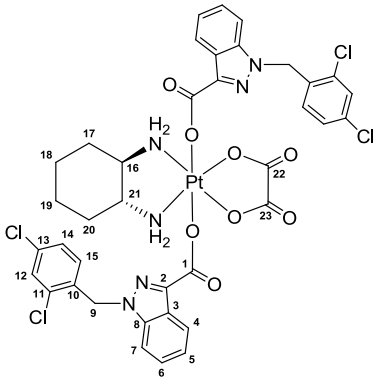
ppm. 195Pt NMR ([d6]-DMSO): δ = 2842 ppm. ^{15}N NMR ([d6]-DMSO): δ = -40.6 (NH_2) ppm. ESI-MS: m/z: 963 $[M + H^+]^+$.

(OC-6-33)-dichloridobis(1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxylato)(ethane-1,2-diamine)platinum(IV) 6



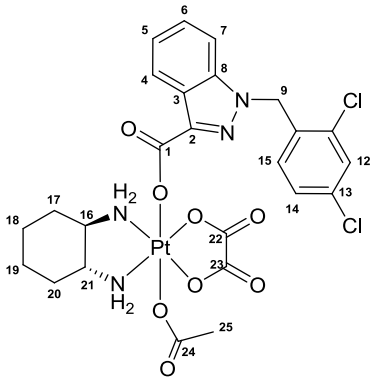
Complex **6** was prepared similar to complex **5** starting from 1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxylic acid (635 mg, 2 mmol), oxalyl chloride (2.5 mL, 29 mmol), (OC-6-33)-dichlorido(ethane-1,2-diamine)dihydroxidoplatinum(IV)[35] (144 mg, 0.4 mmol) and pyridine (254 µl, 3.2 mmol). The reaction mixture was stirred overnight and formed white solid was filtered off, washed with cold acetone (2 x 5 mL), diethyl ether (2 x 5 mL) and dried under reduced pressure. Yield: 147 mg, (38%), m.p. 215–217 °C (decomp), elem. anal. calc (%) for ($C_{32}H_{26}Cl_6N_6O_4Pt$): C 39.77, H 2.71, N 8.70, found: C 39.80, H 3.03, N 8.31. ^{1}H NMR ([d7]-DMF) δ: 9.17 (brs, 1H, NH_2), 8.55 (d, 2H, *J* = 8.1 Hz, H4), 7.85 (d, 2H, *J* = 8.6 Hz, H7), 7.71 (d, 2H, *J* = 2.1 Hz, H12), 7.52 (t, 2H, *J* = 7.6 Hz, H6), 7.45 (dd, 2H, *J* = 2.1, 8.5 Hz, H14), 7.36 (t, 2H, *J* = 7.54 Hz, H5), 7. 09 (d, 2H, *J* = 8.3 Hz, H15), 5.90 (s, 4H, H9), 3.23 (s, 4H, H16) ppm. ^{13}C{^{1}H} ([d7]-DMF) δ: 170.7 (C1), 141.3 (C8), 137.3 (C2), 133.9 (C10), 133.8 (C11/13), 133.7 (C11/13), 131.2 (C15), 129.3 (C12), 127.9 (C14), 126.9 (C6), 124.0 (C4), 123.7 (C3), 122.5 (C5), 110.1 (C7), 50.0 (C16), 49.9 (C9) ppm. ^{195}Pt NMR ([d7]-DMF): δ = 2633 ppm. ^{15}N NMR ([d7]-DMF): δ = -3.7 (NH_2) ppm. ESI-MS: m/z: 967 $[M + H^+]^+$.

(OC-6-33)-(trans-1R,2R-diaminocyclohexane)bis(1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxylate)oxalatoplatinum(IV) 7



Complex **7** was prepared similar to complex **5** starting from 1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxylic acid (789 mg, 2.46 mmol), oxalyl chloride (3.5 mL, 40.8 mmol), (OC-6-33)-(trans-1R,2R-diaminocyclohexane)dihydroxido(oxalato)platinum(IV)[36] (240 mg, 0.56 mmol) and pyridine (50 µl, 0,62 mmol). The reaction mixture was stirred for 12h at room temperature, concentrated to ~3 mL and a formed white precipitate was filtered off, washed with diethyl ether (2 x 5 mL), water (3 x 3 mL) and dried under reduced pressure. Yield: 410 mg, (71%), m.p. 170–172 °C (decomp), elem. anal. calc (%) for ($C_{38}H_{32}Cl_4N_6O_8Pt$): C 43.99, H 3.11, N 8.10, found: C 43.86, H 3.28, N 7.92. ^{1}H NMR ([d6]-DMSO) δ: 8.74 (d, 2H, J = 7.5 Hz, NH_2), 8.60 (t, 2H, J = 10.0, NH_2), 8.07 (d, 2H, 8.2, H4), 7.82 (d, 2H J = 8.6 Hz, H7), 7.71 (d, 2H, J = 2.1 Hz, H12), 7.51-7.47 (m, 2H, H6), 7.41 (dd, 2H, J = 2.2, 8.4 Hz, H14), 7.33-7.28 (m, 2H, H5), 7.02 (d, 2H, J = 8.4 Hz, H15), 5.82 (s, 4H, H9), 2.89 (brs, 2H, H16, H21), 2.18 (d, 2H, J = 10.5 Hz, H17, H20), 1.53 (d, 4H, J = 6.2 Hz, H17, H18, H19, H20), 1.16 (t, 2H, J = 11.7 Hz, H18, H19) ppm. ^{13}C{^{1}H} ([d6]-DMSO) δ: 169.6 (C1), 163.9 (C22, C23), 141.2 (C8), 136.6 (C2), 133.9 (C10), 133.9 (C11/13), 133.8 (C11/13), 131.7 (C15), 129.6 (C12), 128.2 (C14), 127.4 (C6), 123.2 (C3), 123.2 (C5), 122.8 (C4), 110.9 (C7), 61.8 (C16, C21), 50.1 (C9), 31.4 (C17, C20), 24.1 (C18, C19) ppm. ^{195}Pt NMR ([d6]-DMSO): δ = 3235 ppm. ^{15}N NMR ([d6]-DMSO): δ = -5.9 (NH_2), -6.1 (NH_2) ppm. ESIMS: m/z: 1037 $[M + H^+]^+$.

(OC-6-44)-Acetato(trans-1R,2R-diaminocyclohexane)(1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxylato)(oxalato)platinum(IV) 8



Complex **8** was prepared similar to complex **5** starting from of 1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxylic acid (220 mg, 0.69 mmol), oxalyl chloride (1.0 mL, 11.7 mmol), (OC-6-44)-acetato(trans-1R, 2R--diaminocyclohexane)hydroxido(oxalato)platinum(IV)[37] (156 mg, 0.33 mmol) and with pyridine (57 µl, 3.2 mmol). The reaction mixture was stirred for 12h at room temperature, concentrated to ~3 mL and a formed white precipitate was filtered off, washed with diethyl ether (2 x 5 mL), water (3 x 3 mL) and dried under reduced pressure. Yield: 120 mg, (47%), m.p. 195-200 °C (decomp), elem. anal. calc (%) for ($C_{25}H_{26}Cl_2N_4O_8Pt$): C 38.67, H 3.38, N 7.22, found: C 38.32, H 3.71, N 7.15. ^{1}H NMR ([d6]-DMSO) δ: 8.53 (brs, 3H, NH_2), 8.35 (brs, 1H, NH_2), 8.02 (d, 1H, *J* = 8.2 Hz, H4), 7.78 (d, 1H, *J* = 8.6 Hz, H7), 7.68 (d, 1H, *J* = 2.0 Hz, H12), 7.47 (t, 1H, *J* = 7.8 Hz, H6), 7.39(dd, 1H, *J* = 2.0, 8.3 Hz, H14), 7.28 (t, 1H, *J* = 7.6 Hz, H5), 6. 99 (d, 1H, *J* = 8.4 Hz, H15), 5.80 (s, 2H, H9), 2.83-2.74 (m, 1H, H16/21), 2.69-2.61 (m, 1H, H16/21), 2.19-2.11 (m, 2H, H17, H20), 2.00 (s, 1H, H25), 1.54-1.46 (m, 4H, H17, H18, H19, H20), 1.25-1.15 (m, 2H, H18, H19) ppm. ^{13}C{^{1}H} ([d6]-DMSO) δ: 178.6 (C24), 169.9 (C1), 163.9 (C22/23), 163.9 (C22/23), 141.2 (C8), 136.8 (C2), 133.9 (C10), 133.8 (C11/13), 133.7 (C11/13), 131.65 (C15), 129.53 (C12), 128.20 (C14), 127.35 (C6), 123.3 (C3), 123.09 (C5), 122.8 (C4), 110.9 (C7), 61.9 (C16/21), 61.5 (C16/21), 50.1 (C9), 31.4 (C17/20), 31.3 (C17/20), 24.0 (C18, C19), 23.4 (C25) ppm. ^{195}Pt NMR ([d6]-DMSO): δ = 3240 ppm. ^{15}N NMR ([d6]-DMSO): δ = -7.4 (NH_2), 7.6 (NH_2) ppm. ESI-MS: m/z: 775 $[M - H^+]^-$.

Acknowledgements

We thank the Russian Foundation for Basic Research (grant No. 16-33-00373, L.F.), (grant No. 16-03-00743 Y.N.N., I.V.Z., A.A.N.), Russian science foundation (grant No. 14-03-00483 Y.N.N, K.I.K, A.A.N, E.R.M (biological study)) for financial support. The authors also acknowledge support from Moscow State University Program of Development (NMR measurements).

Keywords: Platinum complexes • Ionidamine • log *P* • cytotoxicity

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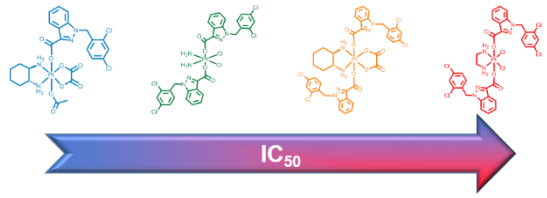
Layout 2:

FULL PAPER



A series of Pt(IV) complexes with lonidamine as a ligand coordinated in axial position is reported. The lonidamine was found to affect strongly the *in vitro* cytotoxic activity lowering the IC_{50} values down to the nanomolar range.

Key Topic: metals in medicine

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Enhancing the cytotoxic activity of anticancer Pt(IV) complexes by introduction of lonidamine as an axial ligand.