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Antiproliferative activity of Pt(IV) complexes with Lonidamine and Bexarotene ligands attached via succinate-ethylenediamine linker.

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We present the synthesis and cytotoxic potencies of new Pt(IV) complexes with lonidamine and bexarotene ligand tethered to Pt-center via a succinate-ethylenediamine linker. The *in vitro* results for a series of complexes with cisplatin, dichloride(ethane-1,2-diamine)platinum(II), or oxaliplatin core indicate that the addition to the structure lonidamine or bexarotene moiety can confer activity or selectivity of Pt(IV) complexes.

Introduction

Cisplatin, oxaliplatin and carboplatin are currently applied in > 50% of chemotherapeutic regimens. However, side effects such as general toxicity, low specificity, and the emergence of resistance often limit their application [1-4]. Utilizing Pt(IV) moiety became a successful synthetic strategy for the design of new Pt containing compounds capable of overcoming problems associated with classical Pt drugs, largely due to low general toxicity and higher stability [5, 6]. Several Pt(IV) compounds, namely tetraplatin, iproplatin, and satraplatin, have entered clinical trials but none has been approved as a drug. In Phase I tetraplatin evoked high nephrotoxicity due to a rapid reduction in the blood [4]. Iproplatin did not reach Phase III as it was insufficiently active due to a slow reduction [7]. Satraplatin was evaluated for the treatment of hormone-refractory prostate cancer but lack of convincing benefit for overall survival did not promote the drug to the clinic although clinical trials in combination with various organic drugs are currently in the active phase [8].

One of the strategies in the modern medicinal chemistry of anticancer drugs the preparation of dual-action drugs capable to bind to more than one molecular target [6, 9]. Combination of the platinum group and an organic moiety can generate dual activity compounds that, upon reduction in the cell environment, produce two or more active components with definitive molecular targets [10].

A variety of promising Pt(IV) complexes containing targeting axial ligands such as dichloroacetate [11], short peptides [12], inhibitors of glutathione-S-transferase [13-15], cyclooxygenase[16-18], p53 activators [19] or albumin-binding part [20] have been recently prepared. The approach of coupling the pharmacophore to a metal center through the linker is widely used. Several studies have shown advantages of different linkers in Pt(IV) carboxylate complexes: formylbenzoate platinum derivatives ligated with amines [21], PEG-containing linker for folic acid [11], amide linker for estradiol [22].

Recently we have shown that the introduction of Lonidamine, a selective inhibitor of aerobic glycolysis by targeting the mitochondria-bound hexokinase; active against lung, prostate, breast, and non-small cell lung cancer [23-25]), as well as Bexarotene, a selective agonist of retinoid X receptors used in cutaneous T-cell lymphoma to induce differentiation and apoptosis [26, 27]) significantly increased the cytotoxicity of Pt (IV) complexes [28, 29]. Lonidamine and Bexarotene served as axial ligands.

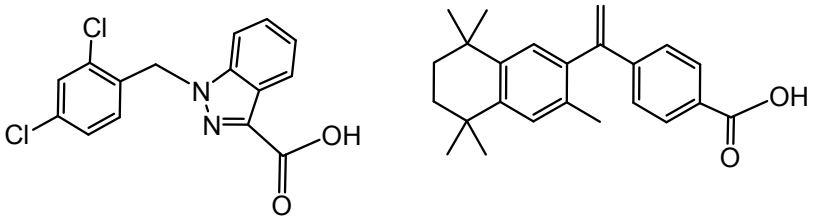


Figure 1. Lonidamine (left) and bexarotene (right).

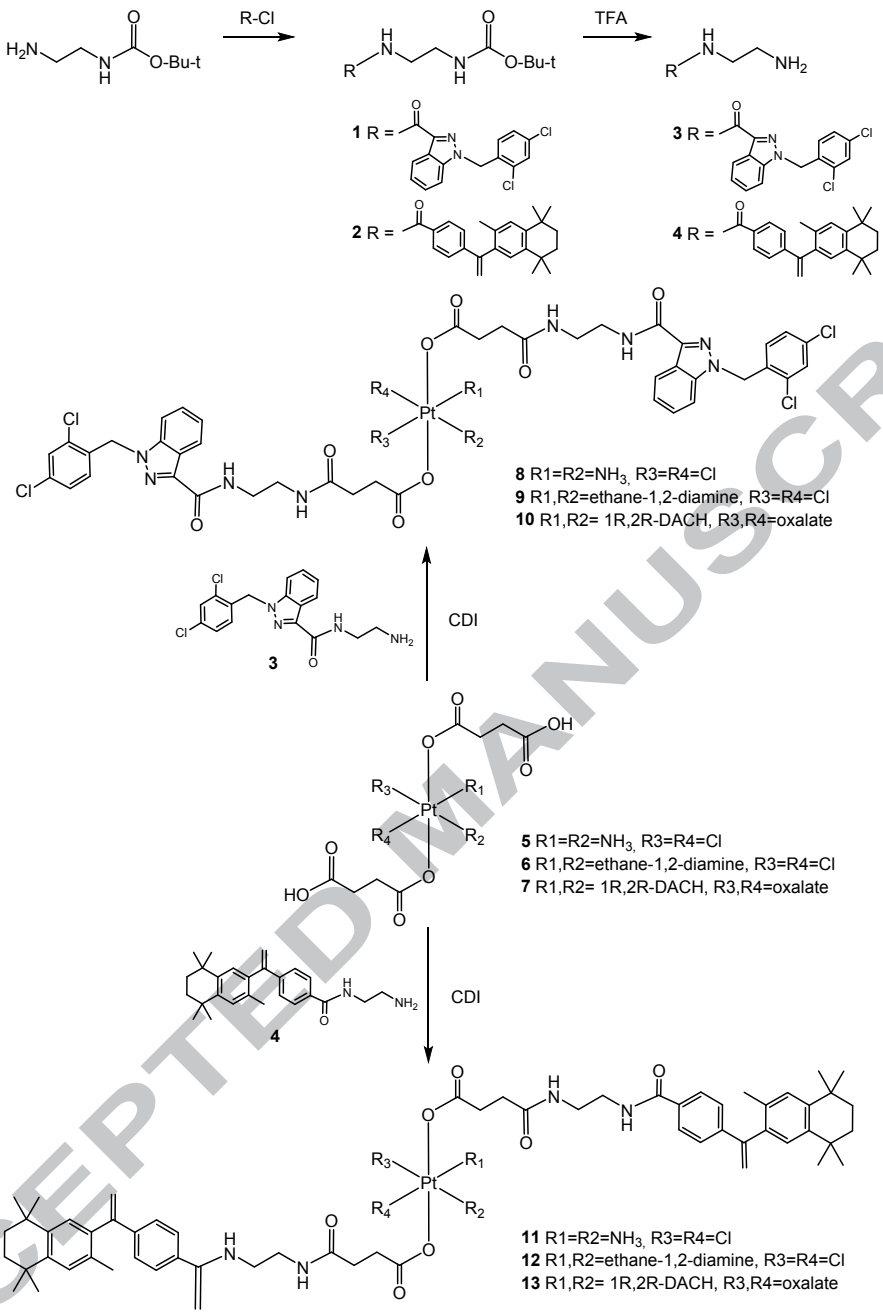
In this study, we report the synthesis and characterization, including antiproliferative activity, of new Pt(IV) complexes in which lonidamine and bexarotene were tethered to Pt via a succinate-ethylenediamine linker.

Results and Discussion

Synthesis and characterization

Reactions were carried out in argon atmosphere and DMF as a solvent due to the low solubility of all reagents. Complexes **8-13** (Scheme 1) were synthesized starting from succinate platinum(IV) analogues of cisplatin **5**, oxaliplatin **7** and the ethylenediamine analogue. The ethylenediamine moiety was prepared in the reaction of acid chlorides obtained *in situ* from lonidamine or bexarotene and an excess of oxalyl chloride with Boc-protected ethylenediamine and subsequent removal of the Boc-group. The resulting amines were introduced into the peptide synthesis reaction with the carboxyl group of the platinum part using carbonyl diimidazole (CDI) as a coupling reagent. Pure complexes were isolated using column chromatography on Silica Gel.

Complexes **8–13** were characterized by ^{1}H, $^{13}C\{^{1}H\}$, ^{15}N, ^{195}Pt 1D and 2D NMR spectroscopy, ESI mass spectrometry and elemental analysis. The coordination sphere of the Pt(IV) centre was confirmed by ^{195}Pt NMR spectroscopy: for **10**-**13** the resonance at 3231 ppm and 3232 ppm, respectively, indicate a $Pt(IV)N_2O_4$ coordination sphere while resonance at 2843, 2654, 2843 and 2655 indicates the formation of $Pt(IV)Cl_2N_2O_2$ configured complexes.



Scheme 1. Synthesis of amines and Pt(IV) complexes **8-13**

Cytotoxicity

Cytotoxicity of complexes **8–13**, as well as of lonidamine, bexarotene and cisplatin was determined against human cancer cell lines (colon carcinoma SW480, non-small cell lung

carcinoma A549, MCF7 breast and its doxorubicin/cisplatin resistant analogue variant MCF7D, and on immortalized human keratinocytes HaCat) by an MTT colourimetric assay.

Table 1. Cytotoxicity of complexes **8–13**, lonidamine, bexarotene and cisplatin (IC_{50} value after 72 h of incubation, μM.)

	SW480	**A549**	**MCF7**	**MCF7D**	**HaCat**
Lonidamine	>90	>90	30±10	60±5	25±10
8	48±6	>100	27±4	53±7	>100
9	43±1	>100	3.4±0.5	5.9±0.1	42±8
10	>100	>100	56±12	>100	>100
Bexarotene	80±10	85±9	67±13	71±21	>90
11	24±0,8	>100	50±6	>100	48±10
12	0.8±0.1	1±0.01	0.75±0.1	0.85±01	0.7±0.01
13	35±0,6	52±5	23±5	24±3,2	23±2.5
Cisplatin	17.6±5	20.1±1.9	15.2±5	70±12	4.8±1.2

Complexes showed a marginal activity in the micromolar range of concentrations. The most cytotoxic was **12** with the bexarotene ligand active against a number of cancer cell lines/ However, no preferential potency for malignant vs non-malignant cells was found. Interestingly, complex **9** with lonidamine showed some activity against breast cancer cells and almost no activity against HaCat cells. It is worth to mention that in the experiment with mechanical mixture of ligand **3** and cis-dichlorideethylenediamineplatinum(II) in the ratio 2:1 the IC50 value on the MC7 cell line was found close to the value of cis-dichlorideethylenediamineplatinum(II) (18.3 and 16.5 μM correspondingly) confirming the synergistic effect of two active part in the complex **9**.

Conclusions

In this study, several Pt(IV) complexes with bexarotene and lonidamine moiety linked to the platinum centre were synthesized and characterized. The *in vitro* results for a series of complexes with cisplatin, dichloride(ethane-1,2-diamine)platinum(II), or oxaliplatin core indicate that the biologically active moiety can confer activity or selectivity of Pt(IV) complexes. The complex **12** displays the most promising cytotoxic profile, while **9** showed higher activity against breast cancer cell lines with some preference towards cancer cells.

Experimental

Materials

(OC-6-33)-(diamine)bis(3-carboxypropanoato)(dichlorido)platinum(IV) [30], (OC-6-33)- bis(3-carboxypropanoato)dichlorido(ethane-1,2-diamine)platinum(IV) [31] and (OC-6-33)-bis(3-carboxypropanoato)(trans-1R,2R-diaminocyclohexane)oxalatoplatinum(IV) [30] prepared using published procedure. The purity of all starting compounds was 95% or higher.

Physical measurements

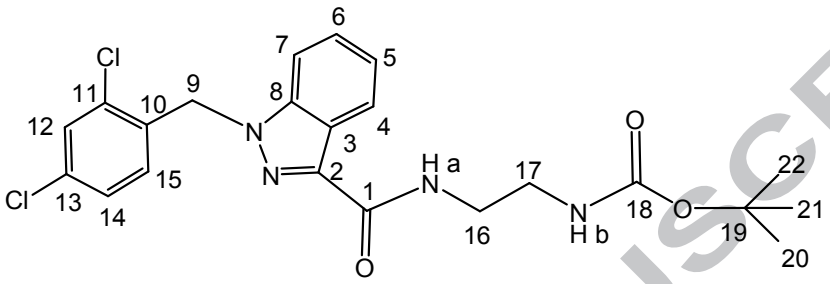
NMR spectra were recorded on a Bruker FT-NMR Avance III 500 MHz instrument at 500.32 (^{1}H), 125.81 (^{13}C), 50.70 (^{15}N) and 107.57 (^{195}Pt) MHz. 2D NMR measurements were carried out using standard pulse programs. Chemical shifts were referenced relative to the solvent signal for ^{1}H and ^{13}C spectra. For ^{15}N and ^{195}Pt spectra, the external standards NH_4Cl and $K_2[PtCl_4]$ were used. ESI mass spectra were recorded on LC/MSn ion trap mass spectrometer amaZon SL (Bruker, Bremen, Germany) with MeOH as a solvent. Elemental analysis was performed at Moscow State University with MicroCube Elementar analyzer.

Cell lines and culture conditions

The MCF7, MCF7D (gift of N.I.Moiseeva), HaCat, A549, and SW480 cell lines were cultured in Dulbecco modified Eagle's medium (DMEM; PanEco, Russia) with 10% fetal bovine serum (HyClone, USA) and antibiotics (PanEco, Russia) in 5% CO_2, 37ºC. The compounds were pre-dissolved at 20 mM in dimethyl sulfoxide (DMSO) and added to the cell culture at the required concentration with maximum DMSO content of 0.5 v/v%. Cells in 96-well plates (7×10^3 cells/well) were treated with various concentrations of **3**, **4**, cisplatin or bexarotene at 37ºC for 72 h. Cell viability was determined using the MTT assay as follows: cells were incubated at 37ºC for 4 h with 20 µl of 5 mg/ml solution of 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (Sigma-Aldrich, St. Louis, USA). The supernatant was discarded and formazan was

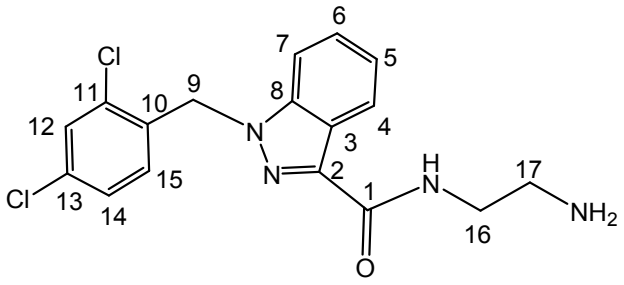
dissolved in 150 µl of DMSO. The optical density of the solution was measured at 550 nm on a multiwell plate reader (Multiskan FC, Thermo Fisher Scientifics, USA). The percentage of viable (i.e., MTT converting) cells was calculated from the absorbance of untreated cells (100%). Each experiment was repeated three times, each concentration was tested in three replicates.

Tert-butyl-2-(1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxamido)ethylcarbamate *1*



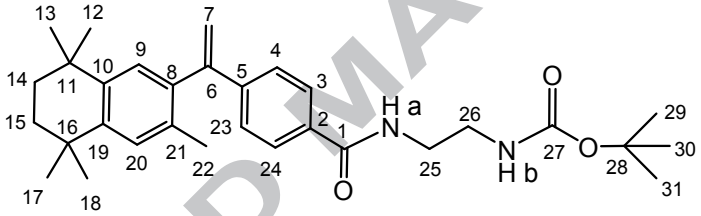
Oxalyl chloride (2.0 mL, 23.3 mmol) and one-two drops of DMF was added to a stirred suspension of 1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxylic acid (500 mg, 1.55 mmol) in CH_2Cl_2 (25 mL). The reaction mixture was refluxed for 1 h until a clear solution was formed. Solvent and unreacted oxalyl chloride were removed under reduced pressure to yield the acid chloride as a pale yellow solid that was used without purification. A solution of tert-butyl N-(2-aminoethyl)carbamate (248 mg, 1.54 mmol) in CH_2Cl_2 (5 mL) was added to a mixture of 1-(2,4-dichlorobenzyl)-1H-indazole-3-carbonyl chloride and triethylamine (433 µL, 3.12 mmol) in CH_2Cl_2 (40 mL). The reaction mixture was stirred 12 h at room temperature and washed with a saturated solution of $NaHCO_3$ (2x20 mL). The organic phase was dried with Na_2SO_4 and the solvent was removed under reduced pressure. Hexane (20 mL) and diethyl ether (5 mL) were added to a yellow oil and precipitated white product was filtered off and dried under reduced pressure. Yield: 415 mg (57%), elem. anal. calc (%) for ($C_{22}H_{24}Cl_2N_4O_3$): C 57.13, H 5.23, N 12.12, found: C 56.68, H 4.92, N 11.79. ^{1}H NMR ($CDCl_3$) δ: 8.42 (dt, 1H, J=8.1, 1.0 Hz, H4), 7.49-7.29 (m, 5H, H5, H6, H7, H12, NH*a*), 7.13 (dd, 1H, J=8.4, 2.1 Hz, H14), 6.70 (d, 1H, J=8.1 Hz, H15), 5.68 (s, 2H, H9), 5.02 (brs, 1H, NH*b*), 3.66-3.58 (m, 2H, H16), 3.48-3.38 (m, 2H, H17), 1.42 (s, 9H, H20, H21, H22) ppm. ^{13}C{^{1}H} NMR ($CDCl_3$) δ: 163.1 (C1), 156.4 (C18), 141.1 (C8), 138.2 (C2), 134.5 (C10), 133.2 (C11, C13), 132.3 (C3), 129.5 (C12, C15), 127.6 (C14), 127.4 (C6),123.0 (C4, S5), 109.3 (C7), 79.9 (C19), 50.0 (C9), 40.7 (C17), 39.7 (C16), 28.3 (C20, C21, C22) ppm. **^{15}N NMR** ($CDCl_3$) δ: 81.7 (NH*a*), 57.4 (NH*b*) ppm.

N-(2-aminoethyl)-1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxamide *3*



Trifluoroacetic acid (7 mL) was added to tert-butyl 2-(1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxamido)ethylcarbamate **1** (380 mg, 0.82 mmol) and stirred for 20 min. Trifluoroacetic acid was removed under reduced pressure, oil residue was dissolved in CH_2Cl_2 (30 mL) and washed with a saturated solution of $NaHCO_3$ (25 mL). The organic phase was dried over Na_2SO_4, the solvent was removed under reduced pressure and dried under reduced pressure. Amine was used without further purification.

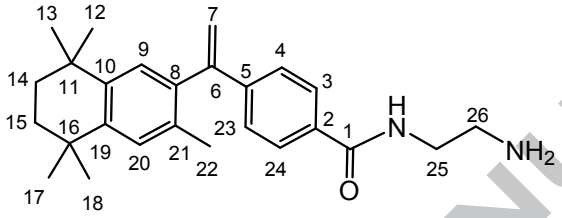
Tert-butyl-2-(4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphtyl)vinyl) benzamide)ethylcarbamate *2*



Oxalyl chloride (4.0 mL, 45.6 mmol) and one-two drops of DMF was added to a stirred suspension of 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphtyl)-vinyl]benzoic acid (1.0 g, 2.87 mmol) in CH_2Cl_2 (40 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 tert-butyl N-(2-aminoethyl)carbamate (448 mg, 2.8 mmol) in CH_2Cl_2 (5 mL) was added to a mixture of 1-(2,4-dichlorobenzyl)-1H-indazole-3-carbonyl chloride and triethylamine (776 μL, 5.6 mmol) in CH_2Cl_2 (40 mL). The reaction mixture was stirred 12 h at room temperature and washed with saturated solution of $NaHCO_3$ (2x20 mL). The organic phase was dried with Na_2SO_4 and the solvent was removed under reduced pressure, hexane (20 mL) and diethyl ether (5 mL) were added to yellow oil and precipitated white product was filtered off and dried under reduced pressure. Yield: 890 mg (63%), elem. anal. calc (%) for ($C_{31}H_{42}N_2O_3$): C 75.88, H 8.63, N 5.71, found: C 75.58, H 9.03, N 5.27. ^{1}H NMR ($CDCl_3$) δ: 7.75 (d, 2H, J=8.1 Hz, H3, H24), 7.34 (d, 2H, J=8.1 Hz, H4, H23), 7.14 (s, 2H, H20, NHa), 7,09 (s, 1H, H9), 5.80 (s,1H, H7), 5.31 (s,1H, H7), 5.02 (t, 1H, J=5.0 Hz, NHb), 3.59 (m, 2H, H25), 3.41 (m, 2H, H26), 1.95 (s, 3H, C22), 1.72

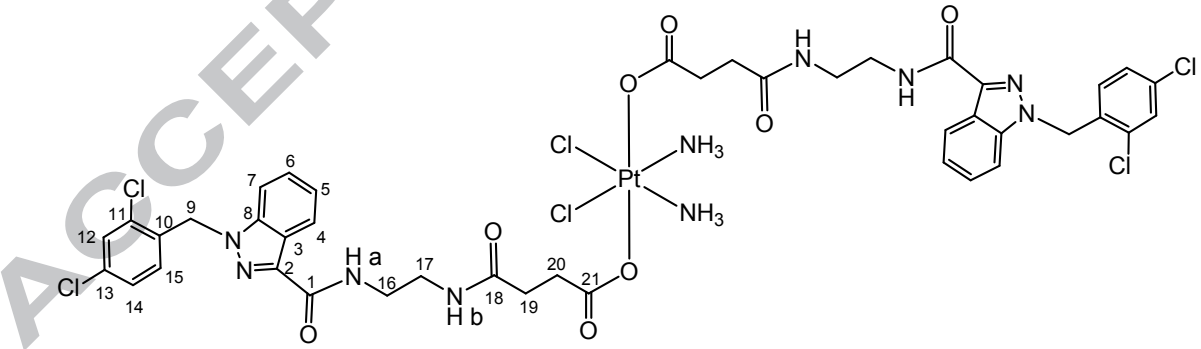
(s, 4H, H14, H15), 1.43 (s, 9H, H29, H30, H31), 1.32 (s, 6H, H12, H13, H17, H18), 1.29 (s, 6H, H12, H13, H17, H18) ppm. $^{13}C\{^{1}H\}$ NMR ($CDCl_3$) δ: 167.6 (C1), 157.5 (C27), 149.1 (C6), 144.3 (C19), 144.2 (C5), 142.3 (C10), 138.1 (C8), 132.9 (C2), 132.8 (C21), 128.0 (C9, S20), 127.0 (C3, C24), 126.7 (C4, C23), 116.3 (C7), 79.9 (C28), 42.0 (C25), 40.1 (C26), 35.2 (C14, C15), 34.0 (C11/C16), 34.0 (C11/ C16), 31.9 (C12, C13/C17, C18), 31.8 (C12, C13, C17, C18), 28.3 (C29, C30, C31), 19.9 (C22) ppm. **^{15}N NMR** ($CDCl_3$) δ: 85.6 (NH*a*), 59.6 (NH*b*) ppm.

N-(2-aminoethyl)-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphtalene-2-yl)vinyl)benzamide *4*



Trifluoroacetic acid (7 mL) was added to tert-butyl-2-(4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphtyl)vinyl)benz-amide)ethylcarbamate **2** (370 mg, 0.755 mmol) and stirred for 20 min. Trifluoroacetic acid was removed under reduced pressure, oil residue was dissolved in CH_2Cl_2 (30 mL) and washed with saturated solution of $NaHCO_3$ (25 ml). Organic phase was dried over Na_2SO_4, the solvent was removed under reduced pressure and product was dried under reduced pressure. Amine was used without further purification.

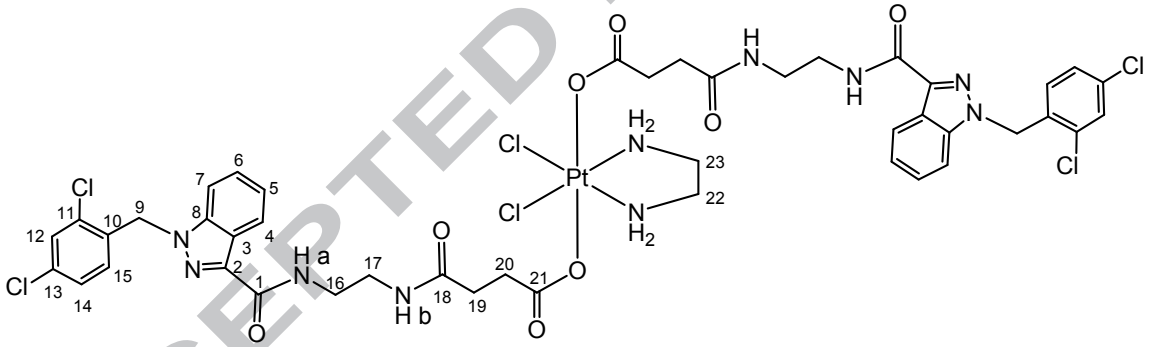
(OC-6-33)-(Diamine)dichlorido(bis(4-(2-(1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxamido)ethylamino)-4-oxobutanoate))platinum(IV) *8*



Carbonyldiimidazole (CDI) (89 mg, 0.55 mmol) in dry DMF (10 mL) was added to a suspension of (OC-6-33)-(diamine)bis(3-carboxypropanoato)(dichlorido)platinum(IV) (173 mg, 0.31 mmol) in dry DMF (10 mL) and the mixture heated to 60°C. After 20 min of stirring the solution was cooled down to room temperature and CO_2 was removed by flushing with argon for 5 min. N-(2-

aminoethyl)-1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxamide **3** (218 mg, 0.60 mmol) in dry DMF (10 mL) was added to the solution and the reaction mixture was stirred for 12 h at room temperature. The solvent was removed under reduced pressure and the product was purified by column chromatography on SilicaGel (CH_2Cl_2/EtOH, 6:1). Yield: 74 mg (22%), m.p. 92–94 °C (decomp), elem. anal. calc (%) for ($C_{42}H_{44}Cl_6N_{10}O_8Pt \cdot 2H_2O$): C 40.01, H 3.84, N 11.11, found: C 39.90, H 3.78, N 11.17. **^{1}H NMR** ([d6]-DMSO) δ: 8.38 (t, 2H, J=5.4 Hz, NH*a*), 8.23 (d, 2H, J=7.9 Hz, H4),7.98 (t, 2H, J=5.6 Hz, NH*b*), 7.74 (d, 2H, J=8.4 Hz, H7), 7.71 (s, 2H, H12), 7.48 (t, 2H, J=7.3 Hz, H6), 7.37 (d, 2H, J=7.8 Hz, H14), 7.31 (t, 2H, J=7.2 Hz, H5), 6.82 (d, 2H, J=8.2 Hz, H15), 6.51 (brs, 6H, NH3), 5.83 (s, 4H, H9), 3.40 (m, 4H, H16), 3.29 (m, 4H, H17), 2.46 (t, 4H, J=6.5 Hz, H19/H20), 2.28 (t, 4H, J=6.7 Hz, H19/H20) ppm. **^{13}C{^{1}H} NMR** ([d6]-DMSO) δ: 180.5 (C21), 172.1 (C18), 162.3 (C1), 141.5 (C8), 138.7 (C2), 133.9 (C10), 133.7 (C11/C13), 133.4 (C11/C13), 130.8 (C15), 129.5 (C12), 128.3 (C14), 127.6 (C6), 123.1 (C5), 122.7 (C3), 122.6 (C4), 110.8 (C7), 50.0 (C9), 39.0 (C16/C17), 38.8 (C16/C17), 31.9 (C19/C20), 31.8 (C19/C20) ppm. **^{15}N NMR** ([d6]-DMSO) δ: -40.2 (NH3). 86.0 (NH*a*), 92.2 (NH*b*) ppm. **^{195}Pt** ([d6]-DMSO) δ: 2843 ppm. **ESI-MS**: m/z = 1223 $[M - H^+]^-$.

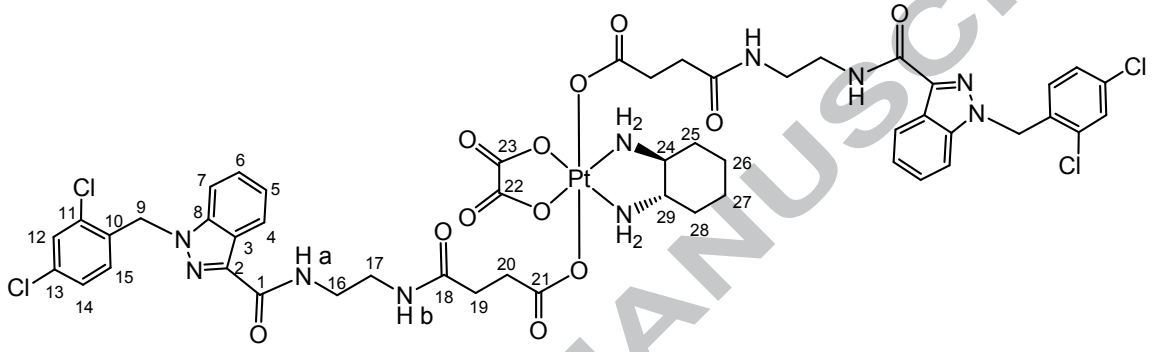
(OC-6-33)-Dichlorido(bis(4-(2-(1-(2,4-dichloridobenzyl)-1H-indazole-3-carboxamido)ethylamine)-4-oxobutanoato))(ethane-1,2-diamine)platinum(IV) *9*



Compound was prepared similar to complex **8** from CDI (105 mg, 0.65 mmol), (OC-6-33)- bis(3-carboxypropanoato)dichlorido(ethane-1,2-diamine)platinum(IV) (173 mg, 0.31 mmol) and N-(2-aminoethyl)-1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxamide **3** (260 mg, 0.72 mmol). Yield: 140 mg (36%), m.p. 175-177 °C (decomp), elem. anal. calc (%) for ($C_{44}H_{46}Cl_6N_{10}O_8Pt$): C 42.25, H 3.71, N 11.20, found C 41.86, H 3.62, N 10.96. **^{1}H NMR** ([d6]-DMSO) δ: 8.43 (brs, 4H, NH2), 8.36 (t, 2H, J=5.90 Hz, NH*a*), 8.23 (d, 2H, J=8.2 Hz, H4), 7.95 (t, 2H, J=5.6 Hz, NH*b*), 7.74 (d, 2H, J=8.6 Hz, H7), 7.70 (d, 2H, J=2.1 Hz, H12), 7.50 (m, 2H, H6), 7.37 (dd, 2H, J=8.4, 2.2 Hz, H14), 7.33-7.28 (m, 2H, H5), 6.82 (d, 2H, J=8.4 Hz, H15), 5.82 (s, 4H, H9), 3.38-3.32 (m, 4H, H16), 3.26-3.19 (m, 4H, H17), 2.68 (brs., 4H, H22, H23), 2,47 (t, 4H, J=7.4 Hz, H19/H20), 2.29

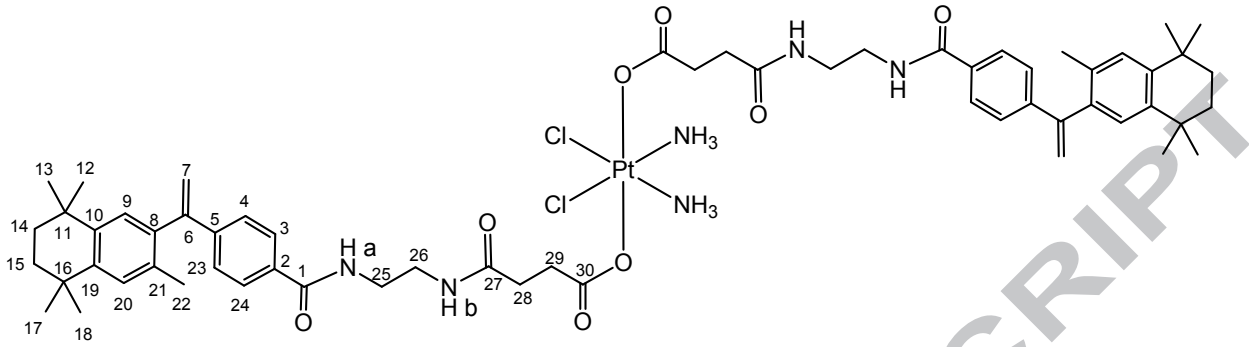
(t, 4H, J=7.3 Hz, H19/H20) ppm. **^{13}C{^{1}H} NMR** ([d6]-DMSO) δ: 181.7 (C21), 171.9 (C18), 162.3 (C1), 141.5 (C8), 138.7 (C2), 133.9 (C10), 133.7 (C11/C13), 133.4 (C11/C13), 130.8 (C15), 129.5 (C12), 128.3 (C14), 127.6 (C6), 123.1 (C5), 122.7 (C3), 122.6 (C5), 110.8 (C7), 50.0 (C9), 49.1 (C22/C23), 39.1 (C16/C17), 38.8 (C16/C17), 32.2 (C19/C20), 31.8 (C19/C20) ppm. **^{15}N NMR** ([d6]-DMSO) δ: -5.1 (NH_2), 85.1 NH*a*), 91.0 (NH*b*) ppm. ^{195}Pt NMR ([d6]-DMSO) δ: 2654 ppm. **ESI-MS**: m/z: 1249 [M - H^+]$^-$.

(OC-6-33)-(Trans-1R,2R-diamincyclohexane)bis(4-(1-(2,4-dichloridobenzyl)-1H-indazole-3-carboxamido)ethylamine)-4-oxobutanoato)oxalatoplatinum(IV) *10*



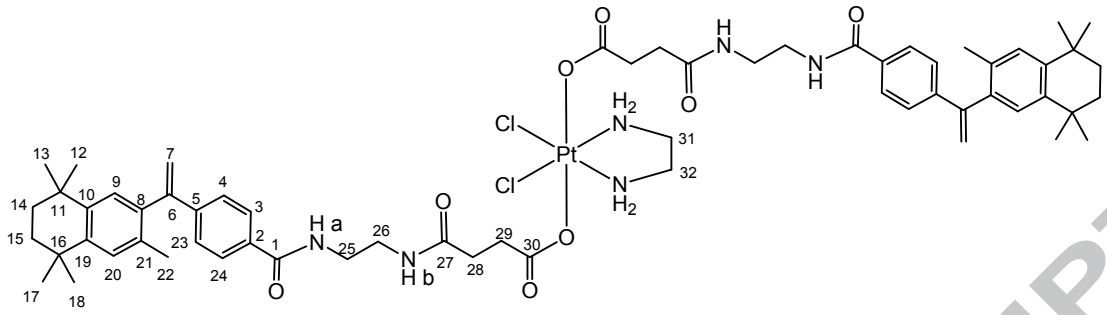
Complex was prepared similar to comlex **8** from CDI (88 mg, 0.54 mmol), (OC-6-33)-bis(3-carboxypropanoato)(trans-1R,2R-diaminocyclohexane)oxalatoplatinum(IV) (170 mg, 0.27 mmol) and N-(2-aminoethyl)-1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxamide **3** (210 mg, 0.58 mmol). Yield: 80 mg (22%), m.p. 109-110 °C (decomp), elem. anal. calc (%) for ($C_{50}H_{52}Cl_4N_{10}O_{12}Pt$.): C 45.43, H 3.96, N 10.60, found: C 44.95, H 3.99, N 10.85. **^{1}H NMR** ([d6]-DMSO) δ: 8.38 (t, 2H, J=5.9 Hz, NH2), 8.34 (brs, 2H, NH*a*), 8.24 (m, 2H, H4), 8.14 (t, 2H, J=9.0 Hz, NH*b*), 7.99 (t, 2H, J=5.6 Hz, NH2), 7.75 (m, 2H, H7), 7.71 (d, 2H, J=2.2 Hz, H12), 7.48 (m, 2H, H6), 7.37 (dd, 2H, J=8.4, 2.2 Hz,H14), 7.30 (m, 2H, H5), 6.81 (d, 2H, J=8.4 Hz, H15), 5.83 (s, 4H, H9), 3.35 (m, 4H, H16), 3.25-3.18 (m, 4H, H17), 2.68 (brs, 2H, H24, H29), 2.51-2.47 (m, 4H, H19/H20), 2.39-2.21 (m, 4H, H19/H20), 2.09 (d, 2H, J=11.9 Hz, H25, H28), 1.50-1.33 (m, 4H, H25, H26, H27, H28), 1.16 (t, 2H, J=9.6 Hz, H26, H27) ppm. **^{13}C{^{1}H} NMR** ([d6]-DMSO) δ: 180.7 (C21), 171.9 (C18), 163.8 (C22, C23), 162.3 (C1), 141.5 (C8), 138.7 (C2), 133.9 (C10), 133.7 (C11/C13), 133.4 (C11/C13), 130.8 (C15), 129.5 (C12), 128.3 (C14), 127.6 (C6), 123.1 (C5), 122.7 (C3), 122.5 (C4), 110.8 (C7), 61.2 (C24, C29), 50.0 (C9), 39.1 (C16/C17), 38.8 (C16/C17), 31.6 (C19/C20), 31.5 (C19/C20), 31.4 (C25, S28), 23.9 (C26, C27) ppm. **^{15}N NMR** ([d6]-DMSO) δ: -6.7 (NH_2), -6.6 (NH_2), 85.8 (NH*a*), 91.1 (NH*b*) ppm. **^{195}Pt NMR**([d6]-DMSO) δ: 3231 ppm. **ESI-MS**: m/z = 1345 [M + Na^+]$^+$.

(OC-6-33)-(Diamine)dichlorido(bis(4-(4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphtyl)vinyl) benzamide)ethylamine)-4-oxobutanoato))platinum(IV) ***11***



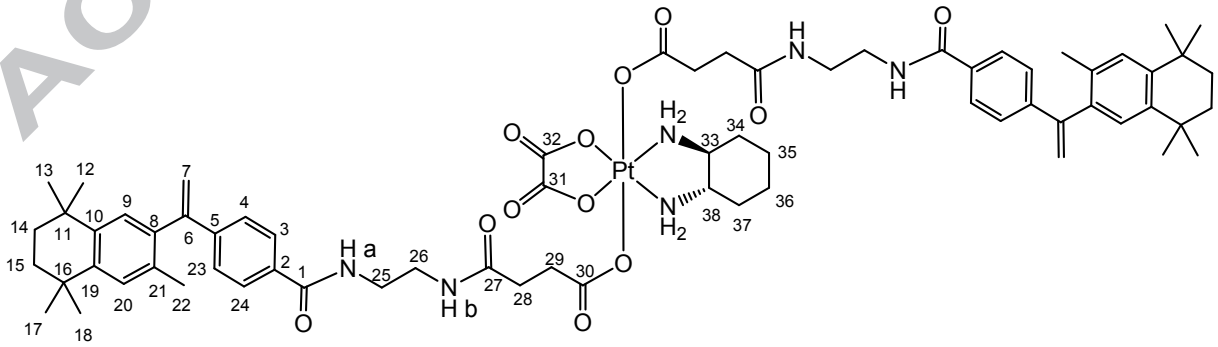
CDI (94 mg, 0.58 mmol) in dry DMF (10 mL) was added to a suspension of (OC-6-33)-(diamine)bis(3-carboxypropanoato)(dichlorido)platinum(IV) (150 mg, 0.28 mmol) in dry DMF (10 mL) and the mixture heated to 60°C. After 20 min of stirring the solution was cooled down to room temperature and CO_2 was removed by flushing with argon for 5 min. N-(2-aminoethyl)-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphtalene-2-yl)vinyl)benzamide **4** (226 mg, 0.58 mmol) in dry DMF (10 mL) was added to the solution and the reaction mixture was stirred for 12 h at room temperature. The solvent was removed under reduced pressure and the product was purified by column chromatography on SilicaGel (CH_2Cl_2/EtOH, 10:1). Yield: 91 mg (26%), m.p. 151-153 °C (decomp), elem. anal. calc (%) for ($C_{60}H_{80}Cl_2N_6O_8Pt$·H2O):C 55.55, H 6.37, N 6.48, found: C 55.52, H 6.51, N 6.84. **^{1}H NMR** ([d6]-DMSO) δ: 8.47 (t, 2H, J=5.5 Hz, NH*a*), 7.97 (c, 2H, J=5.6 Hz, NH*b*), 7.79 (d, 4H, J=8.57 Hz, H3, H24), 7.30 (d, 4H, J=8.4 Hz, H4, H23), 7.15 (s, 2H, H20), 7.08 (s, 2H, H9), 6.50 (brs, 6H, NH3), 5.89 (s, 2H, H7), 5.23 (s, 2H, H7), 3.30 (m, 4H, H25), 3.24-3.16 (m, 4H, H26), 2.46 (t, 4H, J=7.5 Hz, H28, H29), 2.29 (t, 4H, J=7.5 Hz, H28, H29), 1.90 (s, 6H, H22), 1.65 (s, 8H, H14, H15), 1.26 (c, 12H, H12, H13, H17, H18), 1.23 (c, 12H, H12, H13, H17, H18) ppm. **^{13}C{^{1}H} NMR** ([d6]-DMSO) δ: 180.4 (C30), 172.1 (C27), 166.5 (C1), 148.7 (C6), 144.2 (C19), 143.2 (C5), 142.3 (C10), 138.4 (C8), 134.0 (C2), 132.6 (C21), 128.3 (C20), 127.9 (C9), 127.7 (C3, C24), 126.4 (C4, C23), 117.0 (C7), 39.5 (C25), 38.8 (C26), 35.1 (C14, C15), 34.1 (C11/ C16), 34.0 (C11/ C16), 32.1 (C12, C13/C17, C18), 32.1 (C12, C13/C17, C18), 32.0 (C28/C29), 31.8 (C28/C29), 20.0 (C22) ppm. **^{15}N NMR** ([d6]-DMSO) δ: -41.4 (NH3), 87.3 (NH*a*), 91.6 (NH*b*) ppm. **^{195}Pt** ([d6]-DMSO) δ: 2843 ppm. **ESI-MS**: m/z = 1277 $[M - H^+]^-$.

(OC-6-33)-Dichloridobis(4-(2-(4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphtyl)vinyl)benzamide)ethylamine)-4-oxobutanoato))(ethane-1,2-diamin)-platinum(IV) ***12***



Complex was prepared similar to **11** from CDI (75 mg, 0.46 mmol), (OC-6-33)- bis(3-carboxypropanoato)dichlorido(ethane-1,2-diamine)platinum(IV) (128 mg, 0.23 mmol) and N-(2-aminoethyl)-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphtalene-2-yl)vinyl)benzamide **4** (188 mg, 0.48 mmol). Yield: 130 mg (46%), m.p. 153-155 °C (decomp), elem. anal. calc (%) for ($C_{62}H_{82}Cl_2N_6O_8Pt \cdot H_2O$.): C 56.27, H 6.40, N 6.34, found: C 55.95, H 6.54, N 6.41. **^{1}H NMR** [d6]-DMSO) δ: 8.56-8.35 (m, 6H, NH*a*, NH_2), 7.95 (t, 2H, J=5.7 Hz, NH*b*), 7.79 (d, 4H, J=8.5 Hz, H3, H24), 7.30 (d, 4H, J=8.5 Hz, H4, H23), 7.15 (s, 2H, H20), 7.07 (s, 2H, H9), 5.89 (s, 2H, H7), 5.23 (s, 2H, H7), 3.32-3.24 (m, 4H, H25), 3.24-3.16 (m, 4H, H26), 2.67 (brs, 4H, H31, H32), 2.47 (t, 4H, J=7,4 Hz, H28, H29), 2.29 (t, 4H, J=7,3 Hz, H28, H29), 1.91 (s, 6H, H22), 1.66 (s, 8H, H14, H15), 1.27 (c, 12H, H12, H13, H17, H18), 1.23 (c, 12H, H12, H13, H17, H18) ppm. **^{13}C{^{1}H} NMR** ([d6]-DMSO) δ: 181.7 (C30), 172.0 (C27), 166.5 (C1), 148.7 (C6), 144.2 (C19), 143.3 (C5), 142.3 (C10), 138.4 (C8), 134.0 (C2), 132.6 (C21), 128.3 (C20), 127.9 (C9), 127.7 (C3, C24), 126.4 (C4, C23), 117.0 (C7), 49.2 (C31, S32), 39.5 (C25), 38.9 (C26), 35.1 (C14, C15), 34.1 (C11/S16), 34.0 (S11/C16), 32.2 (C28/C29), 32.1 (C12, C13/C17, C18), 32.1 (C12, C13/C17, C18), 31.8 (C28/C29), 20.0 (C22) ppm. **^{15}N NMR** ([d6]-DMSO) δ: -6.6 (NH_2), 87.9 (NH*a*), 92.8 (NH*b*) ppm. **^{195}Pt NMR** ([d6]-DMSO) δ: 2655 ppm. **ESI-MS**: m/z = 1303.5 [M - H^+]$^-$.

(OC-6-33)-(Trans-1R,2R-diaminecyclohexane)bis(4-(4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphtyl)vinyl) benzamide)ethylaminne)-4-oxobutanoato))oxalato-platinum(IV) *13*



Complex was prepared similar to **11** from CDI (67 mg, 0.41 mmol), (OC-6-33)-bis(3-carboxypropanoato)(trans-1R,2R-diaminocyclohexane)oxalatoplatinum(IV) (130 mg, 0.21 mmol) and N-(2-aminoethyl)-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphtalene-2-yl)vinyl)benzamide **4** (270 mg, 0.69 mmol). Yield: 130 mg (46%), m.p. 153-155 °C (decomp), elem. anal. calc (%) for ($C_{68}H_{88}N_6O_{12}Pt$): C 59.33, H 6.44, N 6.11, found: C 59.08, H 6.74, N 6.39. **^{1}H NMR** ([d6]-DMSO) δ: 8.74 (t, 2H, J=5.6 Hz, NH*a*), 8.35 (d, 2H, J=6.2 Hz, NH_2), 8.13 (t, 2H, J=9.9 Hz, NH_2), 7.99 (t, 2H, J=5.7 Hz, NH*b*), 7.79 (d, 4H, J=8.6 Hz, H3, H24), 7.30 (d, 4H, J=8.5 Hz, H4, H23), 7.15 (s, 2H, H20), 7.07 (s, 2H, H9), 5.89 (s, 2H, H7), 5.23 (s, 2H, H7), 3.30 (m, 4H, H25), 3.23-3.17 (m, 4H, H26), 2.71-2.60 (m, 2H, H33, H38), 2.54-2.46 (m, 4H, H28, H29), 2.34-2.25 (m, 4H, H28, H29), 2.09 (d, 2H, J=11,2 Hz, H34, H37), 1.90 (s, 6H, H22), 1.65 (s, 8H, H14, H15), 1.49-1.35 (m, 4H, H34, H35, H36, H37), 1.26 (c, 12H, H12, H13, H17, H18), 1.23 (c, 12H, H12, H13, H17, H18), 1.19-1.11 (m, 2H, H35, H36) ppm. **^{13}C{^{1}H} NMR** ([d6]-DMSO) δ: 180.6 (C30), 172.0 (C27), 166.5 (C1), 163.9 (C31, C32), 148.7 (C6), 144.2 (C19), 143.2 (C5), 142.3 (C10), 138.4 (C8), 134.0 (C2), 132.6 (C21), 128.3 (C20), 127.9 (C9), 127.7 (C3, C24), 126.4 (C4, C23), 117.0 (C7), 61.2 (C33, C38), 39.5 (C25), 38.8 (C26), 35.1 (C14, C15), 34.1 (C11/ C16), 34.0 (C11/ C16), 32.1 (C12, C13/C17, C18), 32.1 (C12, C13/C17, C18), 31.6 (C28/C29), 31.5 (C28/C29), 31.4 (C34, C37), 23.9 (C35, C36), 20.0 (C22) ppm. **^{15}N NMR** ([d6]-DMSO) δ: -6.5 (NH_2), -6.4 (NH_2), 87.0 (NH*a*), 91.0 (NH*b*) ppm. **^{195}Pt NMR** ([d6]-DMSO) δ: 3232 ppm. **ESI-MC**: m/z: 1400 $[M + Na^+]^+$.

Acknowledgements

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References

[1] A.-M. Florea, D. Büsselberg, Cancers, 3 (2011) 1351-1371.
[2] T. Lazarević, A. Rilak, Ž.D. Bugarčić, Eur. J. Med. Chem., 142 (2017) 8-31.
[3] R. Oun, Y.E. Moussa, N.J. Wheate, Dalton Trans., 47 (2018) 6645-6653.
[4] N.J. Wheate, S. Walker, G.E. Craig, R. Oun, Dalton Trans., 39 (2010) 8113-8127.
[5] J.J. Wilson, S.J. Lippard, Chem. Rev. (Washington, DC, U. S.), 114 (2014) 4470-4495.
[6] R.G. Kenny, C.J. Marmion, Chem. Rev. (Washington, DC, U. S.), 119 (2019) 1058-1137.
[7] H. Anderson, J. Wagstaff, D. Crowther, R. Swindell, M.J. Lind, J. McGregor, M.S. Timms, D. Brown, P. Palmer, Eur. J. Cancer Clin. Oncol., 24 (1988) 1471-1479.
[8] M. Hanif, C.G. Hartinger, Future Med. Chem., 10 (2018) 615-617.

[9] S. Luciana, J.B.M.J. Francisco, M.I. Hamilton, F.R. Frederico, K.S. Rajeev, M.B.F. Jose, S.D.S. Marcelo, T.S. Marcus, Curr. Drug Targets, 18 (2017) 592-604.

[10] D. Gibson, Dalton Trans., 45 (2016) 12983-12991.

[11] S. Dhar, Z. Liu, J. Thomale, H. Dai, S.J. Lippard, J. Am. Chem. Soc., 130 (2008) 11467-11476.

[12] S. Mukhopadhyay, C.M. Barnes, A. Haskel, S.M. Short, K.R. Barnes, S.J. Lippard, Bioconjug. Chem., 19 (2008) 39-49.

[13] W.H. Ang, A. De Luca, C. Chapuis-Bernasconi, L. Juillerat-Jeanneret, M. Lo Bello, P.J. Dyson, ChemMedChem, 2 (2007) 1799-1806.

[14] W.H. Ang, I. Khalaila, C.S. Allardyce, L. Juillerat-Jeanneret, P.J. Dyson, J. Am. Chem. Soc., 127 (2005) 1382-1383.

[15] W.H. Ang, L.J. Parker, A. De Luca, L. Juillerat-Jeanneret, C.J. Morton, M. Lo Bello, M.W. Parker, P.J. Dyson, Angew. Chem., Int. Ed. Engl., 48 (2009) 3854-3857.

[16] Q. Cheng, H. Shi, H. Wang, Y. Min, J. Wang, Y. Liu, Chem. Commun., 50 (2014) 7427-7430.

[17] W. Neumann, B.C. Crews, L.J. Marnett, E. Hey-Hawkins, ChemMedChem, 9 (2014) 1150-1153.

[18] W. Neumann, B.C. Crews, M.B. Sarosi, C.M. Daniel, K. Ghebreselasie, M.S. Scholz, L.J. Marnett, E. Hey-Hawkins, ChemMedChem, 10 (2015) 183-192.

[19] L. Ma, R. Ma, Y. Wang, X. Zhu, J. Zhang, H.C. Chan, X. Chen, W. Zhang, S.-K. Chiu, G. Zhu, Chem. Commun., 51 (2015) 6301-6304.

[20] Y.-R. Zheng, K. Suntharalingam, T.C. Johnstone, H. Yoo, W. Lin, J.G. Brooks, S.J. Lippard, J. Am. Chem. Soc., 136 (2014) 8790-8798.

[21] D.Y.Q. Wong, J.Y. Lau, W.H. Ang, Dalton Trans., 41 (2012) 6104-6111.

[22] K.R. Barnes, A. Kutikov, S.J. Lippard, Chem. Biol. (Oxford, U. K.), 11 (2004) 557-564.

[23] A. Floridi, M.G. Paggi, S. D'Atri, C. De Martino, M.L. Marcante, B. Silvestrini, A. Caputo, Cancer Res., 41 (1981) 4661-4666.

[24] U. Gatzemeier, F. Cavalli, K. Häussinger, E. Kaukel, G. Koschel, G. Martinelli, R. Neuhauss, J. von Pawel, Semin. Oncol., 18 (1991) 42-48.

[25] A. Nista, C. De Martino, W. Malorni, M.L. Marcante, B. Silvestrini, A. Floridi, Exp. Mol. Pathol., 42 (1985) 194-205.

[26] M. Duvic, A.G. Martin, Y. Kim, E. Olsen, G.S. Wood, C.A. Crowley, R.C. Yocum, Arch. Dermatol., 137 (2001) 581-593.

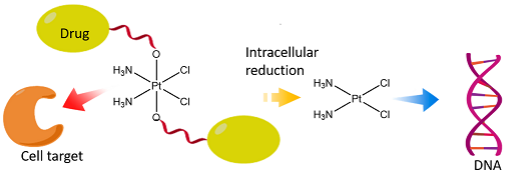
[27] W. Kempf, N. Kettelhack, M. Duvic, G. Burg, Hematol. Oncol. Clin. North Am., 17 (2003) 1405-1419.

[28] Y.N. Nosova, L.S. Foteeva, I.V. Zenin, T.I. Fetisov, K.I. Kirsanov, M.G. Yakubovskaya, T.A. Antonenko, V.A. Tafeenko, L.A. Aslanov, A.A. Lobas, M.V. Gorshkov, M. Galanski, B.K. Keppler, A.R. Timerbaev, E.R. Milaeva, A.A. Nazarov, Eur. J. Inorg. Chem., 2017 (2017) 1785-1791.

[29] Y.N. Nosova, I.V. Zenin, V.P. Maximova, E.M. Zhidkova, K.I. Kirsanov, E.A. Lesovaya, A.A. Lobas, M.V. Gorshkov, O.N. Kovaleva, E.R. Milaeva, M. Galanski, B.K. Keppler, A.A. Nazarov, Bioinorg. Chem. Appl., 2017 (2017) 1-6.

[30] M.R. Reithofer, S.M. Valiahdi, M.A. Jakupec, V.B. Arion, A. Egger, M. Galanski, B.K. Keppler, J. Med. Chem., 50 (2007) 6692-6699.

[31] M. Reithofer, M. Galanski, A. Roller, B.K. Keppler, Eur. J. Inorg. Chem., 2006 (2006) 2612-2617.



Highlights:

The new Pt(IV) complexes with lonidamine and bexarotene ligand tethered to Pt-center via a succinate-ethylenediamine linker were prepared.

The *in vitro* results indicate that incorporating the lonidamine or bexarotene moiety can confer activity and selectivity of Pt(IV) complexes.