Platinum(II/IV) complexes containing ethylenediamine-*N,N'*-di-2/3-propionate ester ligands induced caspase dependent apoptosis in cisplatin resistant colon cancer cells

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Materials and methods

$K_2[PtCl_4]$ and $Na_2[PtCl_6]$ was purchased from Merck and used without further purification. Infrared spectra were recorded on a Perkin-Elmer FTIR 31725-X spectrophotometer using the KBr pellet technique (4000-400 cm^{-1}). ^1H and ^{13}C NMR spectra were recorded on Bruker Avance 500 (500 MHz) spectrometer in DMSO-d_6 and $CDCl_3$. Elemental analyses for C, H and N were done on a Vario III CHNOS Elemental Analyzer, Elementar Analysensysteme GmbH.

Synthesis of esters L4·2HCl and L5·2HCl

These esters were prepared by using the esterification reaction previously described.[1,2] Thionyl chloride (4.0 ml, 55 mmol) was introduced into a flask containing 50 ml of respective alcohol (anhydrous conditions) over 1 h. After that 2.0 g (8.31 mmol) of corresponding H_2edda-type acid hydrochloride, $[H_4$edda-type$]Cl_2$, were added to the flask and the suspension was refluxed 16 h. The mixture was filtered and the filtrate was left for a few days at 4 ºC, yielding the crude product that was filtered off and washed with $CHCl_3$ (3 x 2 ml).

L4·2HCl: $[(S,S)$-$H_2(i$-Pr$)_2$eddp$]Cl_2$: yield: 1.76 g, 68%. Anal. calcd. for $C_{14}H_{30}Cl_2N_2O_4$ (%): C, 46.54; H, 8.37; N, 7.73. Found (%): C, 46.26; H, 8.14; N, 7.82. ^1H NMR [200 MHz, DMSO-d_6]: δ 1.22 (d, 12H, $^3J_{H,H}$ = 6.20 Hz, CH_3-*i*-Pr), 2.79 and 3.19 ('t' (AA'BB'), 8H, CH_2-β-ala), 3.33 (s, 4H, CH_2-(en)), 4.94 (m, 2H, CH-*i*-Pr), 9.72 (s, 4H, NH_2^+). ^{13}C NMR [50 MHz, DMSO-d_6]: δ 21.7 (CH_3-*i*-Pr), 30.6 and 42.3 (CH_2-β-ala), 42.7 (CH_2-(en)), 68.3 (CH-*i*-Pr), 169.6 (COO-*i*-Pr). IR [cm^{-1}]: ν 3435, 2976, 2727, 2691, 1738, 1404, 1179, 953, 791.

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L5·2HCl: $[(S,S)$-$H_2(i$-Bu$)_2$eddp$]Cl_2$: yield 1.58 g, 56%. Anal. calcd. for $C_{16}H_{34}Cl_2N_2O_4$ (%): C, 49.36; H, 8.80; N, 7.19. Found (%): C, 48.98; H, 8.52; N, 7.16. ^1H NMR [200 MHz, DMSO-d_6]: δ 0.90 (d, 12H, $^3J_{H,H}$ = 6,60 Hz, CH_3-*i*-Bu), 1.90 (m, 2H, CH-*i*-Bu), 2.85 and 3.21 ('t' (AA'BB'), 8H, CH_2-β-ala), 3.42 (s, 4H, CH_2-(en)), 3.87 (d, 4H, $^3J_{H,H}$ = 6.80 Hz, CH_2-*i*-Bu), 9.76 (s, 4H, NH_2^+). ^{13}C NMR [50 MHz, DMSO-d_6]: δ 19.1 (CH_3-*i*-Bu), 27.3 (CH-*i*-Bu), 30.3 and 42.3 (CH_2-β-ala), 42.7 (CH_2-(en)), 70.5 (CH-*i*-Bu), 170.1 (COO-*i*-Bu). IR [cm^{-1}]: ν 3445, 2966, 2712, 2438, 2406, 1743, 1377, 1202, 1156, 978, 860.

Synthesis of platinum(IV) and platinum(II) complexes, 3a–5a

Complexes were obtained by mixing 10 ml aqueous solution of $K_2[PtCl_4]$ (0.158 g, 0.512 mmol) or $Na_2[PtCl_6]$ (0.232 g, 0.512 mmol) and the respective ligand (**L3**·2HCl: 0.225 g, 0.512 mmol; **L4**·2HCl: 0.185 g, 0.512 mmol; **L5**·2HCl·H_2O: 0.200 g, 0.512 mmol). During 2 h of stirring 10 cm^3 of 0.1 $mol \cdot dm^{-3}$ LiOH were added in small portions to the reaction solution. Within this period a yellow precipitate was observed, filtered off and dried on the air.

3a: $[PtCl_4\{(c$-Pe$)_2$eddip$\}]\cdot 2H_2O$: yield 98 mg, 18%. Anal. calcd. for $C_{18}H_{32}Cl_4N_2O_4Pt$ (%): C, 30.31; H, 5.09; N, 3.93. Found (%): C, 30.50; H, 5.15; N, 3.63. ^1H NMR [500 MHz, DMSO-d_6]: δ 1.30-1.95 (m, 16H, CH_2-*c*-Pe), 1.48 (d, 6H, $^3J_{H,H}$ = 6.80 Hz, CH_3), 2.87-3.12 (m, 4H, CH_2-(en)), 4.16 (m, 2H, CH), 5.15 (m, 2H, CH-*c*-Pe), 7.19 (m, 2H, NH). ^{13}C NMR [125 MHz, DMSO-d_6]: δ 16.6 (CH_3), 23.4 (CH_2-*c*-Pe), 32.2 (CH_2-*c*-Pe), 51.6 (CH_2-(en)), 59.8 (CH), 78.9 (CH-*c*-Pe), 170.6 (COO-*c*-Pe). IR [cm^{-1}]: ν 3114, 2961, 2873, 1723, 1449, 1254, 1204, 1108, 943, 858.

3b: $[PtCl_2\{(c$-Pe$)_2$eddip$\}]\cdot H_2O$: yield 235 mg, 81 %. Anal. calcd. for $C_{18}H_{32}Cl_2N_2O_4Pt\cdot H_2O$ (%): C, 34.62; H, 5.49; N, 4.49. Found (%): C, 34.81; H, 5.62; N, 4.34. ^1H NMR [500 MHz, DMSO-d_6]: isomer A: δ 1.50-1.85 (m, 16H, CH_2-*c*-Pe), 1.53 (d, 6H, $^3J_{H,H}$ = 7,05 Hz, CH_3), 2.46- 2.83 (m, 4H, CH_2-(en)), 4.26 (m, 2H, CH), 5.09 (m, 2H, CH-*c*-Pe) 6.25-6.95 (m, 2H, NH); isomer B: δ 1.50-1.85 (m, 16H, CH_2-*c*-Pe), 1.38 (d, 6H, $^3J_{H,H}$ = 7,26 Hz, CH_3), 2.74-2.99 (m, 4H, CH_2-(en)), 3.89 (m, 2H, CH), 5.09 (m, 2H, CH-*c*-Pe), 7.30-7.70 (m, 2H, NH). ^{13}C NMR [125 MHz, DMSO-d_6]: isomer A: δ 14.5 (CH_3), 23.9 (CH_2-*c*-Pe), 32.7 (CH_2-*c*-Pe), 48.8

(CH_2-(en)), 56.3 (CH), 78.4 (CH-*c*-Pe), 170.1 (COO-*c*-Pe); isomer B: δ 15.4 (CH_3), 23.9 (CH_2-*c*-Pe), 32.7 (CH_2-*c*-Pe), 50.7 (CH_2-(en)), 57.7 (CH), 77.8 (CH-*c*-Pe), 170.6 (COO-*c*-Pe); isomer ratio A/B = 3/2. IR [cm^{-1}]: ν 3095, 2959, 2872, 1730, 1449, 1210, 1119, 957, 839.

4a: $[PtCl_4\{(i\text{-}Pr)_2eddp\}]\cdot H_2O$: yield 176 mg, 61%. Anal. calcd. for $C_{14}H_{28}Cl_4N_2O_4Pt\cdot H_2O$ (%): C, 26.14; H, 4.70; N, 4.35. Found (%): C, 25.72; H, 4.85; N, 4.31. ^{1}H NMR [200 MHz, DMSO-d_6]: δ 1.20 (d, 12H, $^3J_{H,H}$ = 6,20 Hz, CH_3-*i*-Pr), 2.83 (m, 8H, CH_2-β-ala), 3.23-3.79 (m, 4H, CH_2-(en)), 4.92 (m, 2H, CH-*i*-Pr), 7.67 (m, 2H, NH). ^{13}C NMR [50 MHz, DMSO-d_6]: δ 21.8 (CH_3-*i*-Pr), 30.7 (CH_2-β-ala), 48.2 and 54.4 (CH_2-(en)), 68.0 (CH-*i*-Pr), 170.6 (COO-*i*-Pr). IR [cm^{-1}]: ν 3194, 2981, 1717, 1375, 1205, 1180, 1103, 976, 824.

5a: $[PtCl_4\{(i\text{-}Bu)_2eddp\}]\cdot H_2O$: yield 182 mg, 61%. Anal. calcd. for $C_{14}H_{28}Cl_4N_2O_4Pt\cdot H_2O$ (%): C, 29.41; H, 4.94; N, 4.29. Found (%): C, 29.67; H, 4.79; N, 4.46. ^{1}H NMR [200 MHz, DMSO-d_6]: δ 0.90 (d, 12H, $^3J_{H,H}$ = 6,60 Hz, CH_3-*i*-Bu), 1.89 (m, 2H, CH-*i*-Bu), 2.89 (m, 8H, CH_2-β-ala), 3.25-3,50 (m, 4H, CH_2-(en)), 3.85 (d, 4H, $^3J_{H,H}$ = 6.20 Hz, CH_2-*i*-Bu), 7.70 (m, 2H, NH). ^{13}C NMR [50 MHz, DMSO-d_6]: δ 19.0 (CH_3-*i*-Bu), 27.3 (CH-*i*-Bu), 30.4 (CH_2-β-ala), 48.1 and 54.4 (CH_2-(en)), 70.3 (CH-*i*-Bu), 171.1 (COO-*i*-Bu). IR [cm^{-1}]: ν 3172, 2961, 2874, 1725, 1452, 1382, 1205, 1181, 988, 853.

X-ray structure determination for 3a

The crystals were obtained mixing diluted aqueous solutions of $Na_2[PtCl_6]$ and **L3**·2HCl. After few days on room temperature yellow crystals were obtained. The data of complex **3a** were collected with a CCD Oxford Xcalibur S difractometer ($\lambda(Mo_{K\alpha})$ = 0.71073 Å) using multiscan mode (Table S1). Semi-empirical from equivalents absorption corrections were carried out with SCALE3 ABSPACK software.[3] The structure was solved with direct method.[4] Structure refinement was carried out with SHELXL-97.[5] All non-hydrogen atoms were refined anisotropically, and H atoms were placed at calculated positions and refined isotropically using the riding model. ORTEP-3 program has been used for representation of the structures.[6] The Flack parameter was used to determine the absolute configuration of a structural model determined by single-crystal structure.[7] Details regarding crystallographic data and selected bond lengths and angles are included in Table S2.. Crystallographic data for the structural analyse of **3a** have been deposited with the Cambridge Crystallographic Data Centre, CCDC-855121. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-1223-336033; E-mail: deposit@ccdc.cam.ac.uk or http//www.ccdc.cam.ac.uk).

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Table S1. Crystallographic data for the structural analyse of **3a**.

Empirical formula	$C_{18}H_{32}Cl_4N_2O_4Pt$
Formula weight	677.35
Temperature	130(2) K
Wavelength	71.073 pm
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 1089.35(2) pm
	b = 1449.89(2) pm
	c = 1518.23(3) pm
Volume	2.39795(7) nm^3
Z	4
Density (calculated)	1.876 Mg/m^3
Absorption coefficient	6.323 mm^{-1}
F(000)	1328
Crystal size	0.12 × 0.08 × 0.08 mm^3
Theta range for data collection	2.68 to 30.51°.
Index ranges	–15<=h<=15, –20<=k<=20, –21<=l<=21
Reflections collected	84425
Independent reflections	7306 [R(int) = 0.0779]
Completeness to θ = 30.51°	99.9 %
Max. and min. transmission	1 and 0.79542
Data / restraints / parameters	7306 / 12 / 212
Goodness-of-fit on F^2	0.965
Final R indices [I>2σ(I)]	R_1 = 0.0397, wR_2 = 0.0801
R indices (all data)	R_1 = 0.0521, wR_2 = 0.0824
Absolute structure parameter	–0.002(7)
Largest diff. peak and hole	3.762 and –1.587 $e.Å^{-3}$

Table S2. Selected bond lengths (pm) and angles (º) for **3a**.

Pt1 –N1	209.7(5)	N1–Pt1–N2	84.6(2)
Pt1–N2	209.8(5)	N1–Pt1–Cl3	177.6(2)
Pt1–Cl3	230.6(2)	N2–Pt1–Cl3	93.0(1)
Pt1–Cl1	230.6(2)	N1–Pt1–Cl1	92.9(2)
Pt1–Cl2	230.8(1)	N2–Pt1–Cl1	177.5(1)
Pt1–Cl4	231.3(1)	Cl3–Pt1–Cl1	89.52(6)
O1–C5	120.0(7)	N1–Pt1–Cl2	88.1(1)
O2–C5	132.0(8)	N2–Pt1–Cl2	90.1(1)
O2–C6	148.2(8)	Cl3–Pt1–Cl2	92.01(6)
O3–C13	119.0(7)	Cl1–Pt1–Cl2	90.14(6)
O4–C13	132.4(8)	N1–Pt1–Cl4	88.6(1)
O4–C14	147.8(9)	N2–Pt1–Cl4	87.6(1)
		Cl3–Pt1–Cl4	91.21(6)
		Cl1–Pt1–Cl4	92.07(6)
		Cl2–Pt1–Cl4	176.11(7)

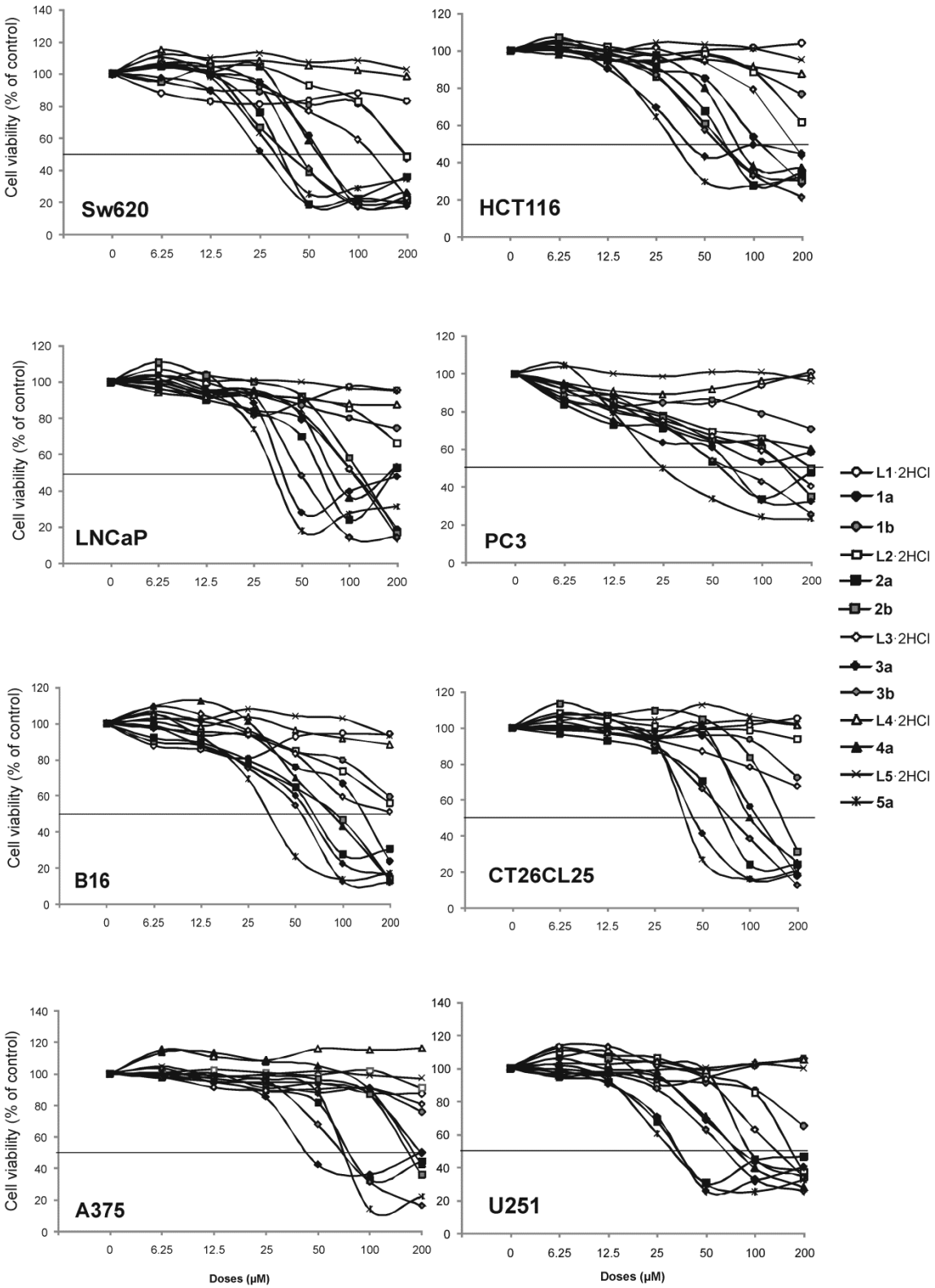

Fig. S1. The effect of ligand precursors and their platinum(II/IV) on viability of malignant cells. Cells (1×10^4 /well) were treated with a range of concentrations of tested compounds for 24 h, after which cell viability was determined by CV assay. The data are presented as mean from representative of three independent experiments while SD was less than 10 %.