



New *trans* dichloro (triphenylphosphine)platinum(II) complexes containing *N*-(butyl),*N*-(arylmethyl)amino ligands: Synthesis, cytotoxicity and mechanism of action



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ABSTRACT

Some new platinum(II) complexes have been prepared, of general formula *trans*-[PtCl₂(PPh₃)(NH(Bu)CH₂Ar)], where the dimension of the Ar residue in the secondary amines has been varied from small phenyl to large pyrenyl group. The obtained complexes, tested *in vitro* towards a panel of human tumor cell lines showed an interesting antiproliferative effect on both cisplatin-sensitive and -resistant cells. For the most cytotoxic derivative **2a** the investigation on the mechanism of action highlighted the ability to induce apoptosis on resistant cells and interestingly, to inhibit the catalytic activity of topoisomerase II.

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1. Introduction

Since the discover of *cis*-[PtCl₂(NH₃)₂] (cisplatin) anticancer properties,¹ hundreds of new platinum(II) complexes have been tested for their antiproliferative activity.² Among them, *trans* complexes also containing phosphorous based ligands,³ are promising, and in some cases they have shown a good activity towards cisplatin resistant cell lines.^{3a,h} We have recently prepared a series of [PtCl₂(PPh₃)(L)] complexes,⁴ where L was a neutral ligand. Most of them were tested *in vitro*^{4c,d} against HeLa, H460 and A549 human tumor cell lines and our results showed that a higher antiproliferative activity could be observed in the case of *trans* compounds and L = R₂NH.^{4d} In particular, the highest antiproliferative activity was observed for L = NH(CH₂CH₂OH)₂ and the investigation about its mechanism of action highlighted the capacity to affect the mitochondrial functions,^{4c} with a mode of action different from cisplatin. Moreover, its interaction with a phospholipid bilayer, used as a model of the cellular membrane, proved to be different from that of cisplatin,⁵ suggesting that even the mode of crossing the cellular membrane could be different.

In this contest and with the aim of expanding knowledge about structure–activity relationship, we prepared four new platinum(II)

complexes, of general structure *trans*-[PtCl₂(PPh₃)(NH(Bu)CH₂Ar)], where the dimension of the aromatic residue on the secondary amines was varied (Ar = Phenyl, 1-Naphthyl, 9-Anthracenyl, 2-Pyrenyl).

In addition, *trans*-[PtCl₂(PPh₃)(*p*-Tolu)] (*p*-Tolu = 4-methylaniline) was prepared, as a model devoid of the CH₂ spacer between the aromatic moiety and the coordinated nitrogen.

In the present work, we report the synthesis and characterization of the secondary amines ArCH₂NHBu and of the corresponding *trans*-platinum(II) complexes, together with the antiproliferative activity towards a panel of tumor cell lines, both sensitive and resistant to cisplatin. For the most interesting complex, the study on the mechanism of action is reported. In particular, the death pathway on resistant cells was analyzed by flow cytometry and moreover, the binding to salmon testes DNA and the effect on cell cycle were determined in comparison with cisplatin. Finally, the ability to interfere with the catalytic activity of the nuclear enzymes topoisomerase I and II was investigated.

2. Materials and methods

2.1. General experimental conditions

All manipulations were performed under a dinitrogen atmosphere, if not otherwise stated. Solvents and liquid reagents were

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dried according to reported procedures. ^1H , ^{13}C , ^{31}P and ^{195}Pt NMR spectra were recorded with a Bruker 'Avance DRX400' spectrometer, in CDCl_3 solution if not otherwise stated. Chemical shifts were measured in ppm (δ) from TMS by residual solvent peaks for ^1H and ^{13}C , from aqueous (D_2O) H_3PO_4 (85%) for ^{31}P and from aqueous (D_2O) hexachloroplatinic acid for ^{195}Pt NMR. A sealed capillary containing C_6D_6 was introduced in the NMR tube to lock the spectrometer to the deuterium signal when non-deuterated solvents were used. FTIR spectra in solid phase were recorded with a Perkin-Elmer 'Spectrum One' spectrometer, equipped with an ATR accessory. IR data are expressed in cm^{-1} . Elemental analyses (C, H, N) were performed at Dipartimento di Scienze e Tecnologie Chimiche, Università di Udine. *cis*-[PtCl₂(NCMe)(PPh₃)]^{4a} was prepared according to a reported procedure.

2.2. Synthesis of ArCH₂NHBu

In a round-bottomed flask equipped with magnetic stirrer, condenser and dropping funnel, a solution of ArCHO in ethanol ([ArCHO] \approx 0.37 mol/L) was treated with butylamine ([BuNH₂]/[ArCHO] = 2.1 mol/mol). The mixture was refluxed until the complete conversion of ArCHO into ArCH=NHBu was reached [IR spectroscopy, $\tilde{\nu}_{\text{CHO}}$ (\approx 1680 cm^{-1}) versus $\tilde{\nu}_{\text{CH=N}}$ (\approx 1640 cm^{-1})]. The solution was cooled (0 °C) and an excess of NaBH₄ ([NaBH₄]/[ArCHO] = 2.5/1 mol/mol) was added in small portions. When the vigorous evolution of dihydrogen ceased, the mixture was warmed (25 °C) and stirring was continued (10 h). Ethanol and excess BuNH₂ were evaporated under reduced pressure, water was added and the aqueous solution was extracted in Et₂O (3 \times 100 mL). Organic extracts were washed with water (3 \times 30 mL) and dried over anhydrous Na₂SO₄, then solvents were evaporated under reduced pressure. For each amine prepared the purification method, the percentage isolated yield and the spectroscopic characterization are reported.

2.2.1. *N,N*-Benzylbutylamine (PhCH₂NHBu, 1a)⁷

Ar = Ph; filtration over a short package of anhydrous Al₂O₃; colorless liquid; 73%. *Anal.* Calcd for C₁₁H₁₇N: C, 80.9; H, 10.5; N, 8.6. Found: C, 80.8; H, 10.3; N, 8.6. IR (ATR): 3340 (b), 3087, 3064, 3028, 2957, 2928, 2872, 2815, 1495, 1454, 1120, 1076, 1029, 730, 696; ^1H NMR: 7.27–7.18 (m, 5H, H_{arom}), 3.73 (s, 2H, PhCH₂), 2.58 (t, 2H, J = 7.3 Hz, NHCH₂), 2.50 (br s, 1H, NH), 1.46 (quint, 2H, J = 7.3 Hz, NHCH₂CH₂), 1.28 (sest, 2H, J = 7.5 Hz, CH₂CH₃), 0.87 (t, 3H, J = 7.5 Hz, CH₃); ^{13}C NMR: 139.8, 128.4, 128.3, 127.0, 53.8, 48.9, 31.9, 20.4, 13.9.

2.2.2. *N,N*-(1-Naphtylmethyl)butylamine (1-NpCH₂NHBu, 1b)⁷

Ar = 1-Np; distillation under reduced pressure (130 °C/0.5 Torr); colorless oil; 98%. *Anal.* Calcd for C₁₅H₁₉N: C, 84.5; H, 9.0; N, 6.6. Found: C, 85.0; H, 9.2; N, 7.0. IR (ATR): 3350 (b), 3048, 2956, 2928, 2870, 2828, 1590, 1576, 1509, 1457, 1396, 1377, 1333, 1236, 1225; ^1H NMR: 8.11–7.41 (m, 7H, H_{ar}), 4.22 (s, 2H, ArCH₂), 2.73 (t, 2H, J = 7.0 Hz, NHCH₂CH₂), 1.73 (br s, 1H, NH), 1.53 (m, 2H, NHCH₂CH₂), 1.35 (m, 2H, CH₂CH₂CH₃), 0.91 (t, 3H, J = 7.0 Hz, CH₃); ^{13}C NMR: 136.0, 133.9, 131.8, 128.8, 127.7, 126.1, 126.0, 125.6, 125.4, 123.6, 51.6, 49.8, 32.2, 20.6, 14.1.

2.2.3. *N,N*-(9-Anthracenylmethyl)butylamine (9-AnthCH₂NHBu, 1c)⁸

Ar = 9-Anth; the sample was used in the following synthesis without further purification; yellow, low melting solid; 88%. *Anal.* Calcd for C₁₉H₂₁N: C, 86.6; H, 8.0; N, 5.3. Found: C, 86.3; H, 8.0; N, 5.2. IR (ATR): 3330 (b), 3052, 2954, 2926, 2858, 1623, 1524, 1445, 1376, 1337, 1320, 1158, 1107, 952, 882, 839, 788, 727; ^1H NMR: 8.37–8.31 (m, 3H, H_{ar}), 7.96 (d, 2H, H_{ar}), 7.53–7.41 (m, 4H, H_{ar}), 4.71 (s, 2H, ArCH₂), 2.84 (t, 2H, J = 7.3 Hz, NHCH₂CH₂), 2.37

(br s, 1H, NH), 1.56 (m, 2H, NHCH₂CH₂), 1.33 (m, 2H, CH₂CH₂CH₃), 0.88 (t, 3H, J = 7.3 Hz, CH₃); ^{13}C NMR: 131.6, 131.1, 130.4, 129.2, 127.4, 126.2, 125.0, 124.1, 50.1, 45.6, 32.0, 20.5, 14.0.

2.2.4. *N,N*-(2-Pyrenylmethyl)butylamine (2-PyrCH₂NHBu, 1d)⁹

Ar = 2-Pyr; the sample was used in the following synthesis without further purification; colorless solid; 98%. *Anal.* Calcd for C₂₁H₂₁N: C, 87.4; H, 7.4; N, 4.8. Found: C, 87.7; H, 7.3; N, 4.8. IR (ATR): 3380 (b), 3039, 2952, 2927, 2894, 2859, 2814, 1601, 1586, 1475, 1443, 1415, 1393, 1369, 1331, 1299, 1183, 1117, 1094, 839, 824, 801, 743, 710, 679; ^1H NMR: 8.34–7.96 (m, 9H, H_{ar}), 4.45 (s, 2H, ArCH₂), 2.76 (t, 2H, J = 7.0 Hz, NHCH₂CH₂), 1.54 (m, 3H, NHCH₂CH₂), 1.35 (m, 2H, CH₂CH₂CH₃), 0.89 (t, 3H, J = 7.0 Hz, CH₃); ^{13}C NMR: 134.2, 131.4, 130.9, 130.6, 129.1, 127.6, 127.5 (2C), 127.0, 126.9, 125.9, 125.1, 125.0 (2C), 124.7, 123.2, 51.9, 49.8, 32.3, 20.6, 14.1.

2.3. Synthesis of *trans*-[PtCl₂(PPh₃){NH(Bu)CH₂Ar}] (2a–d)

In a round-bottomed flask equipped with magnetic stirrer, condenser and dropping funnel, a suspension of *cis*-[PtCl₂(CH₃CN)(PPh₃)] in 10 mL of 1,2-DCE was refluxed (84 °C) and treated with a solution containing the secondary amine (ArCH₂)BuNH in 2.0 mL of the same solvent (*cis*-[PtCl₂(CH₃CN)(PPh₃)]/[R₂NH] molar ratio = 1:1.2). The progress of the reaction was monitored by ^{31}P NMR spectroscopy until the complete conversion of the precursor into the desired product was observed (3 h). Solvent was eliminated under vacuum (0.01 mmHg), the residue was dissolved in CH₂Cl₂ (5 mL) and treated with heptane (10 mL). Solid products were recovered by filtration and dried under vacuum (0.01 mmHg). The same procedure was followed for the preparation of *trans*-[PtCl₂(PPh₃)(*p*-Tolu)] (3). For each of the *trans*-[PtCl₂(PPh₃)(amine)] complexes the amine used, the isolated % yield and the spectroscopic characterization are indicated.

2.3.1. *trans*-[PtCl₂(PPh₃){NH(Bu)(CH₂Ph)}] (2a)

PhCH₂NHBu; 50%; *Anal.* Calcd for C₂₉H₃₂Cl₂NPPt: C, 50.4; H, 4.7; N, 2.0. Found: C, 50.3; H, 4.4; N, 2.0. IR (ATR): 3195 m, 2964, 2929, 2871, 2792, 2358, 1811, 1588, 1496, 1480, 1384, 1312, 1097 s, 1010, 977, 911, 866, 707 m, 691 s; ^1H NMR: 7.66–7.32 (m, 20H, H_{arom}), 4.50 (dd, [$^2J_{\text{H-H}} = 12.4$ Hz, $^3J_{\text{H-H}} = 6.7$ Hz], 1H, PhCHH), 4.03–3.81 (br s [$^2J_{\text{H-Pt}} = 73.0$ Hz]+m, 2H, NH + PhCHH), 3.25 (m, 1H, NHCHH), 2.66 (m, 1H, NHCHH), 2.24 (m, 1H, NHCH₂CHH), 1.86 (m, 1H, NHCH₂CHH), 1.38 (m, 2H, CH₂CH₃), 0.92 (t, 3H, CH₃); ^{13}C NMR: 148.0, 134.7 (d, [$J_{\text{C-P}} = 10.5$ Hz]), 130.7 (d, [$J_{\text{C-P}} = 2.0$ Hz]), 130.0, 129.8, 127.9 (d, [$J_{\text{C-P}} = 56.5$ Hz]), 127.9 (d, [$J_{\text{C-P}} = 11.2$ Hz]), 56.3, 50.7, 31.0, 20.4, 13.9; ^{31}P NMR: 5.25 ($^1J_{\text{P-Pt}} = 3576$ Hz); ^{195}Pt NMR: –3669 ($^1J_{\text{Pt-P}} = 3576$ Hz).

2.3.2. *trans*-[PtCl₂(PPh₃){NH(Bu)(CH₂Np)}] (2b)

1-NpCH₂NHBu; 86%; *Anal.* Calcd for C₃₃H₃₄Cl₂NPPt: 0.5(CH₂Cl₂): C, 51.3; H, 4.5; N, 1.8. Found: C, 52.3; H, 4.5; N, 1.9. IR (ATR): 3250, 1481, 1433, 1265, 1098, 1014, 800, 726, 690; ^1H NMR: 8.24 (d, 1H, H_{Np}), 7.87 (m, 2H, 2H_{Np}), 7.64–7.32 (m, 19H, 4H_{Np} + Ph), 5.05 (d, [$^2J_{\text{H-H}} = 12.0$ Hz], 1H, NpCHH), 4.37–4.17 (br s [$^2J_{\text{H-Pt}} = 81.0$ Hz]+d, [$^2J_{\text{H-H}} = 12.0$ Hz], 2H, NH + NpCHH), 3.29 (m, 1H, NHCHH), 2.51 (m, 1H, NHCHH), 2.20 (m, 1H, NHCH₂CHH), 1.77 (m, 1H, NHCH₂CHH), 1.31 (m, 1H, CHHCH₃), 1.22 (m, 1H, CHHCH₃), 0.82 (t, 3H, CH₃); ^{13}C NMR: 134.8 (d, [$^2J_{\text{C-P}} = 10.2$ Hz]), 134.0, 131.7, 131.4, 130.8, 129.4, 129.0, 128.9 (d, [$^1J_{\text{C-P}} = 62$ Hz]), 127.9 (d, [$^2J_{\text{C-P}} = 11.1$ Hz]), 127.9, 127.1, 126.1, 125.2, 123.3, 53.7, 49.9, 31.1, 20.0, 13.8; ^{31}P NMR: 4.09 ($^1J_{\text{P-Pt}} = 3580$ Hz); ^{195}Pt NMR: –3620 ($^1J_{\text{Pt-P}} = 3580$ Hz).

2.3.3. *trans*-[PtCl₂(PPh₃){NH(Bu)(CH₂Anthr)}] (2c)

9-AnthrCH₂NHBu; 57%; *Anal.* Calcd for C₃₇H₃₆Cl₂NPt: C, 56.1; H, 4.6; N, 1.8. Found: C, 55.7; H, 4.5; N, 1.8. IR (ATR): 3250, 3047, 2921, 1622, 1525, 1481, 1433, 1310, 1186, 1156, 1099, ¹H NMR: 8.49 (m, 2H, 2H_{Anth}), 8.02 (m, 2H, 2H_{Anth}), 7.72–7.32 (m, 20H, 5H_{Anth} + Ph), 5.42 (d, [²J_{H-H} = 13.5 Hz]), 1H, AnthCHH), 5.27 (d, [²J_{H-H} = 13.5 Hz]), 1H, AnthCHH), 4.50 (br s, [²J_{H-Pt} = 80.0 Hz]), 1H, NH), 3.46 (m, 1H, NHCHH), 2.30 (m, 1H, NHCHH), 2.16 (m, 1H, NHCH₂CHH), 1.73 (m, 1H, NHCH₂CHH), 1.21 (m, 1H, CHHCH₃), 1.08 (m, 1H, CHHCH₃), 0.71 (t, 3H, CH₃); ¹³C NMR: 134.8 (d, [²J_{C-P} = 10.2 Hz]), 131.5, 131.1, 130.8, 129.4, 128.9 (d, [¹J_{C-P} = 62 Hz]), 128.6, 127.9 (d, [²J_{C-P} = 11.0 Hz]), 127.0, 126.7, 125.3, 124.0, 49.5, 48.0, 31.3, 19.8, 13.7; ³¹P NMR: 4.16 (¹J_{P-Pt} = 3573 Hz); ¹⁹⁵Pt NMR: –3618 (¹J_{Pt-P} = 3573 Hz).

2.3.4. *trans*-[PtCl₂(PPh₃){NH(Bu)(CH₂Pyren)}] (2d)

2-PyrenCH₂NHBu; 63%; Calcd for C₃₉H₃₆Cl₂NPt·CH₂Cl₂: C, 53.3; H, 4.2; N, 1.6. Found: C, 54.1; H, 4.1; N, 1.6. IR (ATR): 3229, 3042, 2957, 2929, 1590, 1482, 1433, 1185, 1100, 1078, 999, 850; ¹H NMR: 8.49 (d, 1H, H_{Pyren}), 8.20–8.00 (m, 7H, 7H_{Pyren}), 7.98 (t, 1H, H_{Pyren}) 7.59–7.13 (m, 15H, Ph), 5.28 (br s, 1H, PyrenCHH), 4.63 (br s, 1H, PyrenCHH), 4.39 (br s, [²J_{H-Pt} = 79.0 Hz]), 1H, NH), 3.43 (m, 1H, NHCHH), 2.60 (m, 1H, NHCHH), 2.22 (m, 1H, NHCH₂CHH), 1.81 (m, 1H, NHCH₂CHH), 1.33 (m, 1H, CHHCH₃), 1.23 (m, 1H, CHHCH₃), 0.82 (t, 3H, CH₃); ¹³C NMR: 134.8 (d, [²J_{C-P} = 10.2 Hz]), 131.7, 131.4, 130.9, 130.7, 129.7, 128.9 (d, [¹J_{C-P} = 61 Hz]), 127.9 (d, [²J_{C-P} = 10.7 Hz]), 127.5 (2C), 126.2 (2C), 125.5 (2C), 125.2, 124.9, 124.8, 123.0 (2C), 53.9, 50.4, 31.2, 20.1, 13.9; ³¹P NMR: 4.05 (¹J_{P-Pt} = 3590 Hz); ¹⁹⁵Pt NMR: –3618 (¹J_{Pt-P} = 3590 Hz).

2.3.5. *trans*-[PtCl₂(PPh₃)(*p*-Tolu)] (3)

4-Methylaniline (*p*-toluidine); 95%; mp: 251–254 °C (lit. 247–249 °C);¹⁰ *Anal.* Calcd for C₂₅H₂₄Cl₂NPt: C, 47.3; H, 3.8; N, 2.2. Found: C, 47.0; H, 3.7; N, 2.3. IR (ATR): 3212, 3128, 3052, 1559, 1509, 1480, 1433, 1097, 814, 754, 745, 691; ¹H NMR: 7.72–7.67 (m, 6H, H_{arom} Phosphine), 7.46–7.38 (m, 9H, H_{arom} Phosphine), 7.32 (d, 2H, *J* = 8.2 Hz, H_{arom} Toluidine), 7.14 (d, 2H, *J* = 8.2 Hz, H_{arom} Toluidine), 5.31 (br s, 2H, [²J_{H-Pt} = 46.0 Hz, NH₂]), 2.35 (s, 3H, CH₃); ¹³C NMR: 136.8, 135.2, 134.8 (d, [²J_{C-P} = 10.5 Hz]), 130.9, 129.9, 128.3 (d, [¹J_{C-P} = 64.0 Hz]), 128.0 (d, [²J_{C-P} = 11.3 Hz]), 121.5, 20.9; ³¹P NMR: 3.63 (¹J_{P-Pt} = 3773 Hz); ¹⁹⁵Pt NMR: –3614 (¹J_{Pt-P} = 3773 Hz).

2.4. Inhibition growth assay

HeLa (human cervix adenocarcinoma) were grown in Nutrient Mixture F-12 [HAM] (Sigma Chemical Co.); H460 (large cell lung carcinoma) and MSTO-211H (human biphasic mesothelioma) were grown in RPMI 1640 (Sigma Chemical Co.) supplemented with 2.38 g/L HEPES, 0.11 g/L pyruvate sodium and 2.5 g/L glucose; A2780 (human ovarian carcinoma) and A2780cis (human ovarian carcinoma cisplatin-resistant) were grown in RPMI 1640 (Sigma Chemical Co.). 1.5 g/L NaHCO₃, 10% heat-inactivated fetal calf serum (Invitrogen), 100 U/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL amphotericin B (Sigma Chemical Co.) were added to the media. The cells were cultured at 37 °C in a moist atmosphere of 5% carbon dioxide in air. Cells (2.5–3 × 10⁴) were seeded into each well of a 24-well cell culture plate. After incubation for 24 h, various concentrations (from 0.25 to 20 µM) of the test agents were added to the complete medium and incubated for a further 72 h. Stock solutions of new complexes were made in dimethylsulfoxide at 5 mM concentration and then diluted with complete medium in such a way that the final amount of solvent in each well did not exceed 0.5%. Cisplatin was dissolved in 0.9% NaCl. A Trypan blue assay was performed to determine cell viability. Cytotoxicity data were expressed as

GI₅₀ values, i.e., the concentration of the test agent inducing 50% reduction in cell number compared with control cultures.

2.5. Evaluation of apoptotic cell death by Annexin V-FITC and propidium iodide staining

To detect phosphatidylserine translocation from the inner face to the outer surface of plasma membrane, a FITC Annexin V Apoptosis Detection Kit I (BD Pharmingen) was used.

A2780cis cells (2.0 × 10⁵) were seeded into each cell culture plate in complete growth medium. After incubation for 24 h the test agents were added at the indicated concentrations and cells were incubated for a further 18 h. After treatment, cells were centrifuged and resuspended at 10⁶ cells/mL in binding buffer. Cell suspensions (100 µL) were added with Annexin V-FITC and propidium iodide (PI) as indicated by the supplier's instructions, and incubated for 15 min at room temperature in the dark. The populations of Annexin V-negative/PI-negative cells (viable), Annexin V-positive/PI-negative cells (early apoptosis), Annexin V-positive/PI-positive cells (late apoptosis) and Annexin V-negative/PI-positive cells (necrosis) were evaluated by FACSCanto II flow cytometer (Becton–Dickinson, Mountain View, CA).¹¹

2.6. Cell cycle analysis

A2780 cells (2.0 × 10⁵) were seeded into each cell culture plate in complete growth medium. After incubation for 24 h the test agents were added to the complete medium at the indicated concentrations and cells were incubated for a further 18 h. After treatment, 300–500 × 10³ cells were fixed twice with phosphate buffered saline. Cells were incubated with 0.1 mg/mL RNase and 36 µg/mL PI at room temperature for 20 min and the analysis was performed using a FACSCanto II flow cytometer (Becton–Dickinson, Mountain View, CA).

2.7. Nucleic acids

Salmon testes DNA was purchased from Sigma Chemical Company. The DNA concentration was determined using extinction coefficient 6600 M⁻¹ cm⁻¹ at 260 nm. pBR322 DNA was purchased from Fermentas Life Sciences.

2.8. Quantitative platinum and phosphorus analysis

The quantitative platinum and phosphorus analyses were performed according to a previous established method.¹² Briefly, an aqueous solution of salmon testes DNA (9 × 10⁻⁴ M) was incubated at 37 °C with test compound (stock solution 5 mM in DMSO) or cisplatin (stock solution 2 mM in 0.9% NaCl) to reach a [DNA]/[drug] = 10. At fixed incubation time, aliquots of exact volume were collected and DNA was precipitated with Na-acetate (up to 0.3 M concentration) and cold ethanol (2 vol). The precipitated DNA was washed with 70% ethanol, dried and then dissolved in a measured volume of milliQ water. The samples were then mineralized by treating with HNO₃ (65%) at 90 °C for 20 min. Finally the samples were re-suspended in diluted HCl to determine the P and Pt content.

The analyses of P and Pt were performed by inductively coupled plasma atomic emission spectrometry (ICP-AES) at emission lines λ(P) = 178.290 nm and λ(Pt) = 214.423 nm. A Spectroflame Modula sequential and simultaneous ICP-spectrometer (ICP SPECTRO Arcos with EndOnPlasma torch) equipped with a capillary cross-flow Meinhard nebulizer was used (Spectro Analytical). Analytical determinations were performed using a plasma power of 1.2 kW, a radiofrequency generator of 27.12 MHz and an argon gas flow

with nebulizer, auxiliary, and coolant set at 1, 0.5 and 14 L min⁻¹, respectively.

2.9. DNA topoisomerase relaxation assay

Supercoiled pBR322 plasmid DNA (0.25 µg) was incubated for 30 min at 37 °C in 20 µL reaction buffer containing test compound dissolved in DMSO (0.5 µL) at indicated concentrations, then 1 U topoisomerase II (USB Corporation) or 2 U topoisomerase I (Topogen) were added and reactions performed for further 60 min at 37 °C.

Reactions were stopped by adding 4 µL stop buffer (5% SDS, 0.125% bromophenol blue and 25% glycerol), 50 µg/mL proteinase K (Sigma) and incubating for a further 30 min at 37 °C. The samples were separated by electrophoresis on 1% agarose gel at room temperature. The gels were stained with ethidium bromide 1 µg/mL in TAE buffer, transilluminated by UV light, and fluorescence emission was visualized by a CCD camera coupled to a Bio-Rad Gel Doc XR apparatus.

2.10. Topoisomerase II-mediated DNA cleavage

Reaction mixtures (20 µL) containing 10 mM Tris–HCl (pH = 7.9), 50 mM NaCl, 50 mM KCl, 5 mM MgCl₂, 0.1 mM EDTA, 15 µg/mL bovine serum albumin (BSA), 1 mM ATP, 0.25 µg pBR322 plasmid DNA, 10 U topoisomerase II (human recombinant topoisomerase II, USB), and test compounds dissolved in DMSO (0.5 µL) were incubated for 60 min at 37 °C. Reactions were stopped by adding 4 µL of stop buffer (5% SDS, 0.125% bromophenol blue and 25% glycerol) and 50 µg/mL proteinase K (Sigma) and incubating for a further 30 min at 37 °C. The samples were separated by electrophoresis on 1% agarose gel containing ethidium bromide 0.5 µg/mL at room temperature in TBE buffer (0.09 M Tris–borate and 0.002 M EDTA).

3. Results and discussion

3.1. Chemistry

The preparation of secondary amines ArCH₂NH(CH₂)₃CH₃ was carried out starting from the corresponding aromatic aldehydes ArCHO and according to a well known procedure¹³ reported in Scheme 1.

Briefly, aromatic aldehydes were reacted with *n*-butylamine in refluxing ethanol, affording the corresponding imines, which were not isolated, but easily identified by IR spectroscopy, where C=O stretching disappeared and C=N stretching was observed. The following, smooth reduction of the intermediates by sodium borohydride was carried out in situ and, after the usual work-up and

Table 1
Some significant spectroscopic data for complexes **2**

Compound	$\bar{\nu}_{\text{N-H}}^{\text{a}}$ (cm ⁻¹)	³¹ P NMR ^b (ppm)	¹⁹⁵ Pt NMR ^{b,c} (ppm)	¹ J _{P-Pt} (Hz)
2a	3195	5.25	-3669	3576
2b	3205	4.09	-3620	3580
2c	3250	4.16	-3618	3573
2d	3229	4.05	-3618	3590
<i>trans</i> -[PtCl ₂ (PPh ₃)(NHET ₂)] ^d	3219	4.30	-3612	3560

^a FTIR (ATR).

^b Solvent = CDCl₃.

^c Doublet.

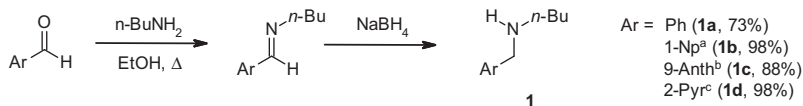
^d See Ref. 4b.

purification procedures, amines ArCH₂NH(CH₂)₃CH₃ **1a–d** were obtained in very good yields (Scheme 1). Their spectroscopic characterization (IR, ¹H and ¹³C NMR) is reported in the experimental part and is in agreement with the expected structures.

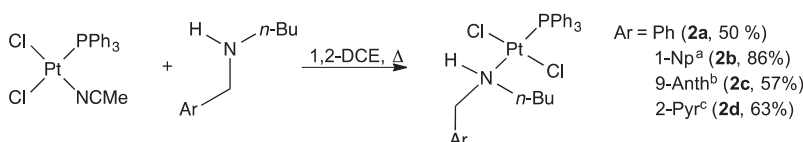
As for the synthesis of platinum complexes, they were prepared by reacting the described secondary amines with *cis*-[PtCl₂(PPh₃)(NCMe)],^{4a} under experimental conditions allowing the precursor isomerization and the following substitution of coordinated nitrile,^{4b} to afford *trans*-[PtCl₂(PPh₃)(NH(Bu)(CH₂Ar))] (Scheme 2). The reactions, monitored by ³¹P NMR spectroscopy to detect the disappearing of the precursor signal, were completed in 2–3 h and afforded, after the usual work-up procedures, crystalline samples of the desired complexes **2a–d** in good yields. The same procedure was followed to prepare *trans*-[PtCl₂(PPh₃)(*p*-Tolu)] (**3**) in 95% isolated yield.

New prepared complexes **2** and **3** were insoluble in water and ethanol, but soluble ([complex] > 5 mM) in dimethylsulfoxide.

The new platinum complexes were characterized by elemental analysis, IR and NMR (¹H, ¹³C, ³¹P and ¹⁹⁵Pt) spectroscopies. The most significant IR, as well as ³¹P and ¹⁹⁵Pt NMR data are reported in Table 1. In the IR spectrum of each complex a narrow, medium-weak band was observed around 3200 cm⁻¹, attributable to coordinated amine N–H stretching, while both aromatic and saturated C–H stretching vibrations were observed between 3050 and 2900 cm⁻¹. In ³¹P NMR spectra, a single signal with satellites was observed, thus confirming the chemio-^{4b} and stereoselectivity of the process; this datum was also confirmed by the presence, in the ¹⁹⁵Pt NMR spectra, of a single signal with a doublet multiplicity. Moreover, both the observed chemical shifts and ¹J_{P-Pt} coupling constants were in agreement with data previously collected for analogous complexes^{4b–d} where the *trans* stereochemistry had been structurally determined.^{4b} For ease of comparison, data for *trans*-[PtCl₂(PPh₃)(NHET₂)] are included in Table 1.

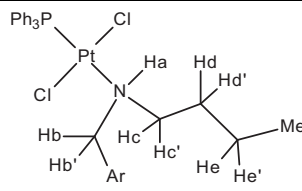


Scheme 1. Synthesis of ArCH₂NH(CH₂)₃CH₃. Reagents and conditions: (a) Np = Naphthyl; (b) Anth = Anthracenyl; (c) Pyr = Pyrenyl.



Scheme 2. Synthesis of *trans*-[PtCl₂(PPh₃)(NH(Bu)(CH₂Ar))]. Reagents and conditions: (a) Np = Naphthyl; (b) Anth = Anthracenyl; (c) Pyr = Pyrenyl.

Table 2
Chemical shifts of diastereotopic hydrogens in ^1H NMR spectra for *trans*-[PtCl₂(PPh₃)₂](NH(Bu)CH₂Ar)]

	Hb; Hb' ($\Delta\delta$, ppm)	Hc; Hc' ($\Delta\delta$, ppm)	Hd; Hd' ($\Delta\delta$, ppm)	He; He' ($\Delta\delta$, ppm)
	2a (Ar = Ph) 2b (Ar = 1-Np) 2c (Ar = 9-Anth) 2d (Ar = 2-Pyr)	4.50; 3.81 (0.69) 5.05; 4.17 (0.88) 5.42; 5.27 (0.15) 5.28; 4.63 (0.65)	3.25; 2.66 (0.59) 3.29; 2.51 (0.78) 3.46; 2.30 (1.16) 3.43; 2.60 (0.83)	2.24; 1.86 (0.38) 2.20; 1.77 (0.43) 2.16; 1.73 (0.43) 2.22; 1.81 (0.41)

The coordination of amines to platinum was also evident in ^1H NMR spectra, recorded in CDCl₃ solution. In each sample, NH originated a broad singlet signal around 4.1–4.4 ppm, where a further broadening near the baseline allowed to measure a $^2J_{\text{H-Pt}}$ coupling constant of ~ 80 Hz. Moreover, due to the presence of the chiral nitrogen (Table 2), the hydrogen nuclei on the amine moiety are diastereotopic and, hence, non equivalent. Thus, Hb and Hb' (Table 2) originate two, well separated doublet signals, with a $^2J_{\text{H-H}}$ coupling constant of ~ 13 Hz, typically observed for coupling between geminal hydrogen nuclei.¹⁴ Two distinct signals of multiplet were observed, as expected, also for Hc and Hc' (Table 2). The non equivalence of geminal hydrogen atoms was observed along the whole butyl chain, with Hd,d' and He,e' (Table 2) generating well separated sets of multiplets, despite their growing distance from the chiral center.

3.2. Antiproliferative activity

The antiproliferative activity of the new complexes **2a–d** and **3** was evaluated on a panel of human tumor cell lines: HeLa (human cervix adenocarcinoma), H460 (large cell lung carcinoma), MSTO-211H (human biphasic mesothelioma), A2780 (human ovarian carcinoma) and A2780cis (human ovarian carcinoma cisplatin-resistant). The cisplatin was taken as reference drug.

The results have been expressed as GI₅₀ values, i.e., the concentration of compound able to induce the death of 50% of the cells with respect to a control culture, and have been reported in Table 3.

The obtained results indicate for all complexes belonging to the **2** series the ability to exert a significant antiproliferative effect towards all cell lines taken into consideration. In particular, **2a–d** show in all cases GI₅₀ values lower than 20 μM and interestingly, **2a**, characterized by the less bulky Ar residue, appears the most active. Otherwise, complex **3**, which differs from **2a** for the absence of the *n*-butyl residue and of the methylene spacer at the level of the secondary amine, shows a low cytotoxicity profile with GI₅₀ values detectable only on A2780 and A2780cis cell lines.

Table 3
Cell growth inhibition values in the presence of examined complexes and cisplatin

Complex	Cell line GI ₅₀ ^a (μM)				
	HeLa	H460	MSTO-211H	A2780	A2780cis
2a	5.31 \pm 0.65	7.33 \pm 0.86	8.93 \pm 0.27	3.55 \pm 0.26	3.63 \pm 0.21
2b	9.35 \pm 1.28	13.3 \pm 0.7	14.9 \pm 1.3	6.41 \pm 0.65	5.91 \pm 1.14
2c	16.3 \pm 1.4	17.7 \pm 1.2	18.5 \pm 1.4	7.97 \pm 0.45	12.6 \pm 1.1
2d	19.0 \pm 1.4	9.03 \pm 0.98	17.5 \pm 1.2	4.90 \pm 0.75	6.95 \pm 1.45
3	>20	>20	>20	17.7 \pm 0.4	18.7 \pm 1.4
Cisplatin ^b	1.60 \pm 0.37	0.83 \pm 0.11	1.3 \pm 0.22	0.80 \pm 0.06	7.23 \pm 0.26

^a Values are the mean \pm SD of at least three independent experiments.

^b Taken as reference drug.

Taking into consideration the chemical structures of the novel **2a–c** complexes, it is possible to observe an opposite relationship between cytotoxicity and the dimension of Ar residue in the secondary amines although the differences in GI₅₀ values are quite small. Indeed, the benzyl-substituted **2a** exerts the highest cell effect, which decreases of about 1.7 and 2.6 times for the naphthyl (**2b**) and the anthracenyl derivative (**2c**), respectively. Otherwise, the pyrenyl derivative **2d** shows an antiproliferative effect somewhat dependent on cell line.

The well-known drug cisplatin, taken as reference compound, is more cytotoxic than the new *trans*-platinum(II) complexes in all sensitive cell lines, showing GI₅₀ values low micromolar or submicromolar. Moreover, as expected, in A2780cis cells a decrease in cytotoxicity of about one order of magnitude with respect to the sensitive A2780 cells, is attained. In this connection, it is interesting to note that unlike cisplatin, **2a–d** induce a comparable effect on both sensitive and resistant A2780cis cells. In particular, **2a** exerts on resistant cells an antiproliferative effect significantly higher with respect to that induced by the drug, thus suggesting the involvement of cell pathways different from those of cisplatin.

3.3. Determination of apoptosis

To investigate the mechanism of cytotoxicity induced by **2a** on A2780cis cells, flow cytometry analysis was performed in the presence of Annexin V-FITC and DNA-specific dye propidium iodide. The results are shown in Figure 1A as dot plots and the percentage data are reported as histograms in Figure 1B. By increasing the concentration of **2a** a notable decrease in viable cells is obtained, accompanied by a concurrent increase in apoptotic (both early and late apoptosis) cells. Actually, the percentage of viable cells is reduced from 88.2% in the absence of complex (control) to 43.2% after incubation for 18 h in the presence of **2a** at 25 μM . In the same experimental conditions, the percentage of apoptotic cells increases from 9.3% to 47.9%. Otherwise, the percentage of necrotic cells varies only scarcely, reaching a percentage of 8.9% at the maximum concentration of **2a** taken into account. These

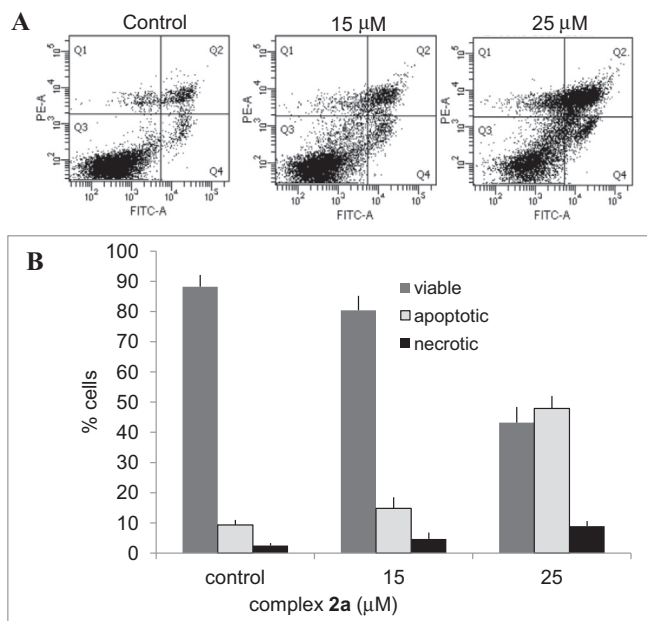


Figure 1. Flow cytometry analysis of cell death induced by **2a**. (A) Dot plots of the flow cytometry analysis performed on A2780cis cells labeled with Annexin V-FITC and propidium iodide after 18 h of treatment with different concentrations of **2a**. (B) The histograms show the percentage of viable (Q3), early and late apoptotic (Q4 + Q2) and necrotic (Q1) cells. Values are the mean \pm SD of four independent experiments.

results confirm the capacity of **2a** to overcome the resistance of A2780cis, cisplatin-resistant ovarian cancer cells, and highlight its ability to induce the apoptotic pathway in a dose-dependent manner pointing to a different mechanism of action or to an additional molecular target with respect to cisplatin. On the basis of the above results, we investigated more in depth the biological effects of **2a** and in particular, its ability to interact with some biomacromolecules.

3.4. Binding to DNA

It was already widely demonstrated that DNA constitutes the main target responsible for the antiproliferative activity of cisplatin.¹⁵ Moreover, for some *trans*-diiodophosphine Pt(II) complexes the interaction with DNA was demonstrated and a correlation of such ability with cytotoxicity was also supposed.¹⁶ In this connection, the capacity of the most active complex **2a** to coordinate DNA was investigated by ICP-AES technique, in comparison with cisplatin (Fig. 2). In detail, salmon testes DNA platination was determined for both **2a** and the drug, at incubation time from 0 to 48 h. Furthermore, in each sample also the total amount of phosphorus was estimated, as internal standard for DNA content.¹²

The performed quantitative evaluation indicates for complex **2a** an ability to bind to DNA clearly lower with respect to that obtained for cisplatin. In particular, after 48 h of incubation in the presence of the reference drug, about 700 ppb of Pt is bound to DNA, while for **2a**, an amount of about 200 ppb is detected in the same experimental conditions. These results appear in agreement with the data reported in Table 3, showing for the novel complex, GI₅₀ values higher than those of the drug in all sensitive cells taken into consideration, i.e., a lower antiproliferative activity. Then, for both **2a** and cisplatin a similar mechanism of action, involving the platination of DNA, could be assumed. Nevertheless, due to the ability of **2a** to exert a comparable cytotoxic effect on both A2780 and A2780cis cells, the involvement of further intracellular targets was hypothesized and investigated.

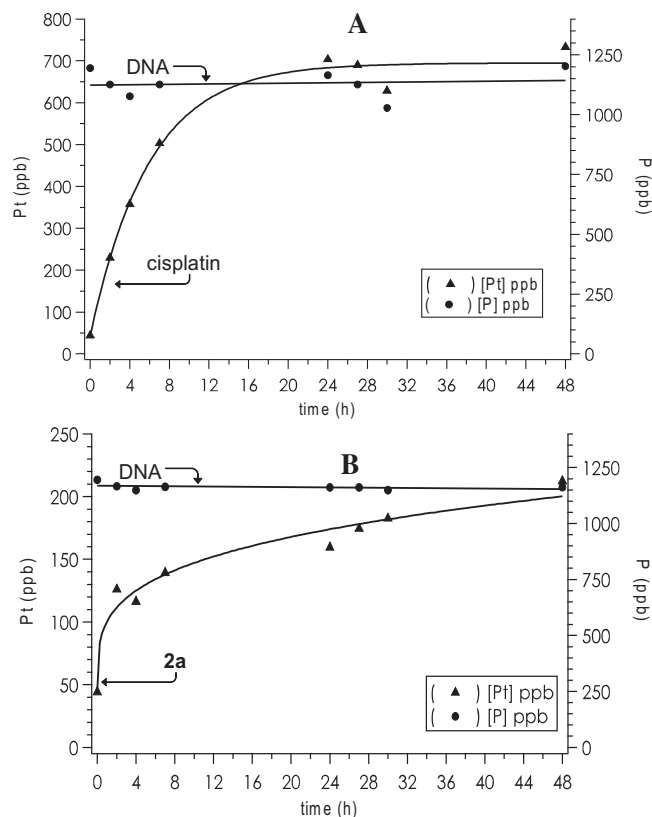


Figure 2. Binding of cisplatin (A) and **2a** (B) to salmon testes DNA (9×10^{-4} M) as a function of incubation time. [DNA]/[drug] = 10.

3.5. Effect on cell cycle distribution

Most anticancer drugs block the cell cycle in either the S or G₂/M phase and also cisplatin inhibits cancer cell growth by arresting the cell cycle progression through these phases.^{17,18}

In order to compare the effect of **2a** with that of cisplatin, flow cytometric analysis was performed on A2780 cells, stained with PI and analyzed for DNA/cell content. The cells were incubated in the absence (control) or in the presence of different concentrations (from 1 to 15 μM) of cisplatin or **2a**, and the results are shown in Figure 3A and B, respectively.

In detail, for the reference drug a dose-dependent arrest in S phase is highlighted (Fig. 3A), as previously reported by some authors.¹⁸ Otherwise, complex **2a** induces a notable increase in subG₀ population (Fig. 3B). Interestingly, the occurrence of a hypodiploid DNA peak can be retained indicative of apoptosis,¹⁹ and it appears in accordance with the ability of **2a** to induce apoptosis on cells.

3.6. Effect on topoisomerases

Topoisomerases are nuclear enzymes able to solve topological problems that arise during replication, transcription and chromosome segregation, as a consequence of the double-stranded helix nature of DNA.²⁰ Moreover, due to their catalytic activity, which introduces into DNA single (topoisomerase I) or double (topoisomerase II) strand breaks, a role of these enzymes in the resolution of intermediates that are found in recombinational repair, was also postulated.²¹ Furthermore, it is to underline that a significant decrease in the rate of repair of cisplatin-induced DNA interstrand cross-links was demonstrated in human glioblastoma cells after exposure to novobiocin, a known topoisomerase II inhibitor.²²

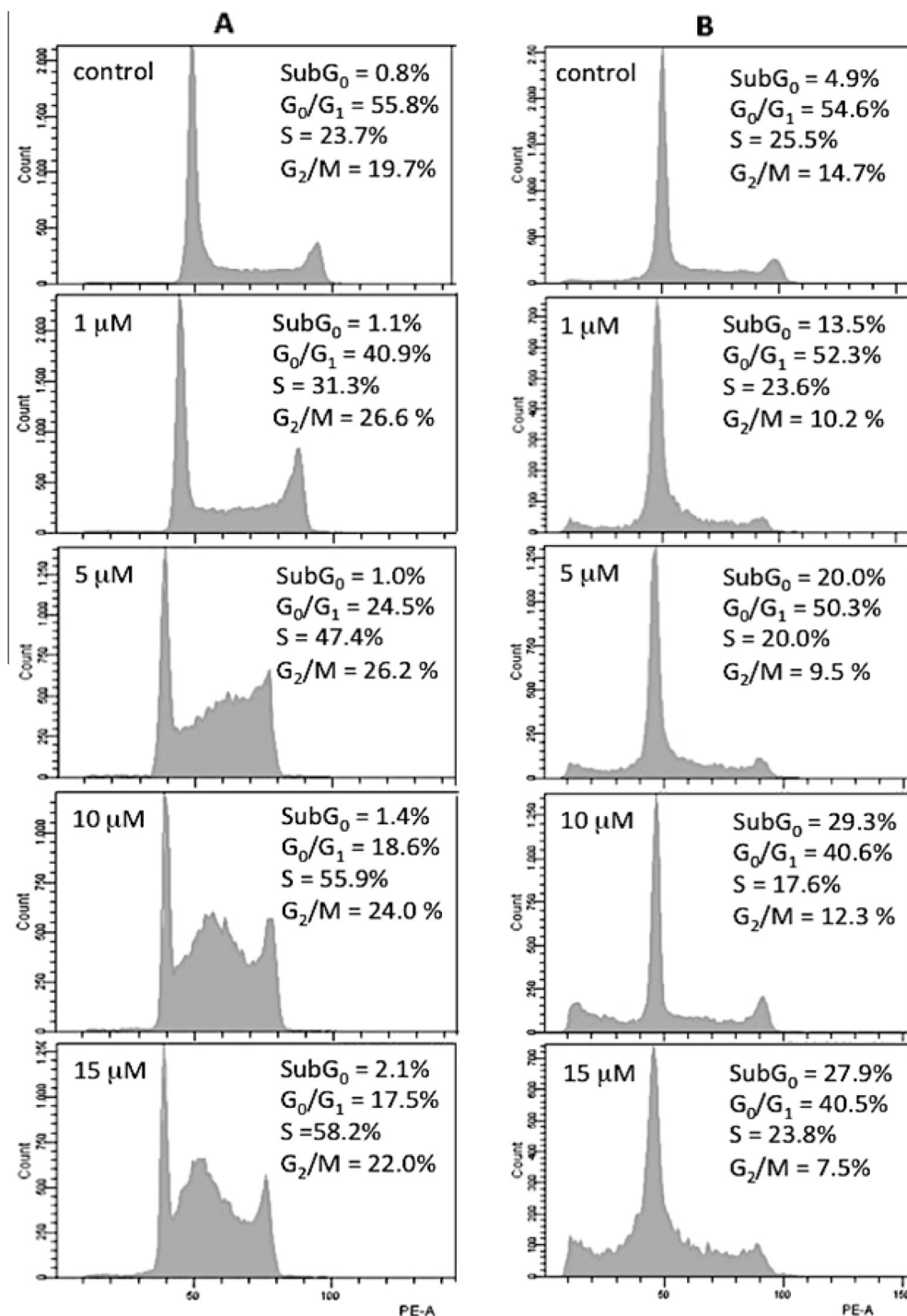


Figure 3. Cell cycle distribution of A2780 cells cultured in the presence of cisplatin (A) or complex **2a** (B) for 18 h at indicated concentrations.

Moreover, an increase in topoisomerase I and II activity was detected in cisplatin-resistant ovarian cancer and murine leukemia cell line, respectively^{23,24} and interestingly, a number of topoisomerase inhibitors showed notably cytotoxicity on cisplatin-resistant cells.^{25–27} Overall, these results suggest a role of topoisomerases in the occurrence of cisplatin resistance and, in this connection, the possible effect of **2a** on catalytic activity of both topoisomerase I and II was evaluated (Fig. 4A and B, respectively).

Figure 4A shows the effect of test complex on the relaxation of supercoiled plasmid DNA mediated by topoisomerase I. The

supercoiled DNA (lane DNA) is relaxed by the enzyme into a series of topoisomers (lane topo I), characterized by lower electrophoretic mobility with respect to the supercoiled form. The complex **2a**, tested at 5, 10 and 20 μM concentration, appears unable to affect the catalytic activity of topoisomerase I also at the higher concentration taken into consideration, as demonstrated by the occurrence of the same pattern of topoisomers as in the control (lane topo I). Similar experiments were also performed, by incubating topoisomerase II (lane topo II) in the presence of the same concentrations of **2a** (Fig. 4B). The obtained results show that **2a** prevents the relaxation of DNA catalyzed by

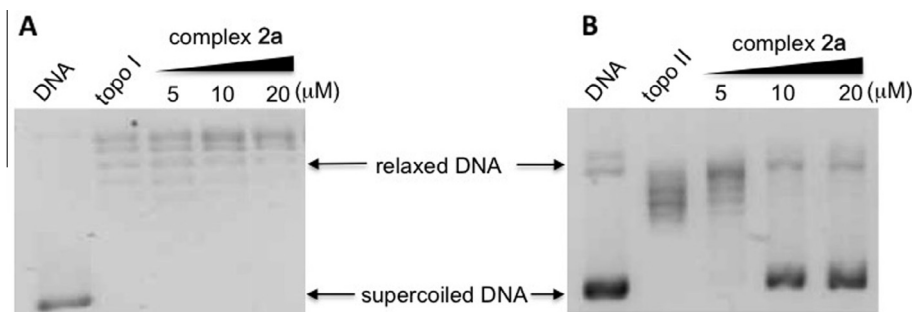


Figure 4. Effect of **2a** on relaxation of supercoiled pBR322 DNA by human recombinant topoisomerase I (A) and II (B).

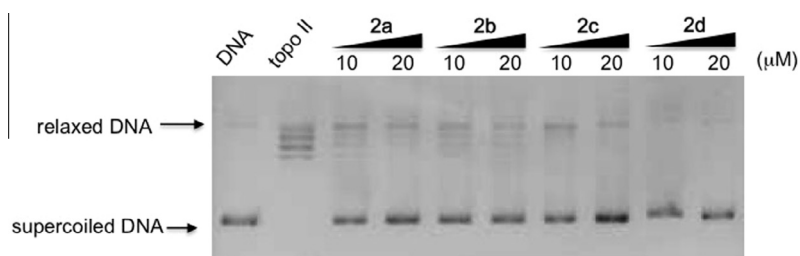


Figure 5. Effect of **2a–d** on relaxation of supercoiled pBR322 DNA by human recombinant topoisomerase II.

the enzyme and notably, the inhibition is complete already at 10 μM concentration, pointing out this complex as an effective inhibitor of topoisomerase II.

The structurally related **2b–d** were also evaluated, in comparison with **2a**, for the capacity to inhibit the relaxation activity of topoisomerase II and all test complexes showed a significant effect (Fig. 5).

Some important clinically used antitumor anti-topoisomerase II agents, interfere with the enzyme activity by stabilizing a covalent intermediate, the cleavable complex, into a lethal damage for the cell.^{28–30} These drugs are called topoisomerase II poisons and their mechanism of action and role in the anticancer activity has been widely discussed.³¹ The occurrence of the cleavable complex can be highlighted experimentally by the enzyme-dependent formation of linear from supercoiled DNA. The ability of **2a** to inhibit significantly the relaxation activity mediated by topoisomerase II prompted us to investigate if a poisoning effect could be demonstrated for this complex. Figure 6 shows the results of the cleavable complex assay performed with topoisomerase II in the presence of 50 and 100 μM concentrations of **2a**. The *m*-AMSA, a well known topoisomerase II poison,³² was taken as reference at 10 μM concentration. The supercoiled pBR322 DNA (lane DNA) was relaxed

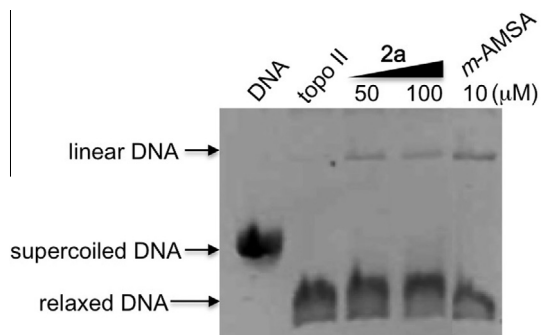


Figure 6. Effect of **2a** on the stabilization of the covalent DNA-topoisomerase II complex.

by the enzyme (lane topo II) and the addition of *m*-AMSA induces, as expected, the formation of linear DNA. Also complex **2a** appears able to stabilize the cleavable complex, even if the amount corresponding to the linear DNA appears lower and detectable at higher concentrations with respect to that induced by the reference drug.

4. Conclusions

Platinum(II) complexes containing triphenylphosphine and *N* (butyl),*N*(arylmethyl)amino ligands were synthesized and studied. The evaluation of the cytotoxicity on a panel of human tumor cell lines showed the ability of the complexes to induce an interesting antiproliferative activity with GI_{50} values in most cases in the low micromolar range. In particular, it is noteworthy the ability of the new complexes to exert a comparable cytotoxicity on both sensitive and resistant cell lines, with complex **2a** as the most effective. The ability to overcome resistance prompted an investigation on the mechanism of action that revealed a different effect on cell cycle and, in particular, an interesting capacity to interfere with the catalytic activity of topoisomerase II. Based on the role played by this enzyme on the occurrence of resistance, it could be assumed that the inhibitory effect exerted by **2a–d** could be responsible for overcoming the resistance.

Considering the results obtained in this study, it may be concluded that the new *trans* platinum(II) complexes could represent a model for the development of novel *trans*-Pt(II) based drugs able to exert an antiproliferative effect on cisplatin resistant cells.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmc.2016.04.067>.

References and notes

- Rosenberg, B.; Van Camp, L.; Krigas, T. *Nature* **1965**, *205*, 698.
- For some recent review articles see: (a) Han, X.; Sun, J.; Wang, Y.; He, Z. *Med. Res. Rev.* **2015**, *35*, 1268; (b) Wilson, J. J.; Lippard, S. J. *Chem. Rev.* **2014**, *114*, 4470; (c) Cutillas, N.; Yellol, G. S.; de Haro, C.; Vicente, C.; Rodríguez, V.; Ruiz, J. *Coord. Chem. Rev.* **2013**, *257*, 2784; (d) Johnstone, T. C.; Wilson, J. J.; Lippard, S. J. *Inorg. Chem.* **2013**, *52*, 12234; (e) Quiroga, A. G. *J. Inorg. Biochem.* **2012**, *114*, 106; (f) Tang, C.-H.; Parham, C.; Shocron, E.; McMahon, G.; Patel, N. *Cancer Chemother. Pharmacol.* **2011**, *67*, 1389; (g) Margiotta, N.; Denora, N.; Ostuni, R.; Laquintana, V.; Anderson, A.; Johnson, S. W.; Trapani, G.; Natile, G. *J. Med. Chem.* **2010**, *53*, 5144; (h) Olszewski, U.; Ach, F.; Ulsperger, E.; Baumgartner, G.; Zeillinger, R.; Bednarski, P.; Hamilton, G.; Coluccia, M. *Met. Based Drugs* **2009**. <http://dx.doi.org/10.1155/2009/348916>. Article ID 348916; (i) Lovejoy, K. S.; Lippard, S. J. *Dalton Trans.* **2009**, *48*, 10651; (j) Kalinowska-Lis, U.; Ochocki, J.; Matlawska-Wasowska, K. *Coord. Chem. Rev.* **2008**, *252*, 1328.
- (a) Pérez, C.; Díaz-García, V.; Agudo-López, A.; del Solar, V.; Cabrera, S.; Agulló-Ortuño, M. T.; Navarro-Ranninger, C.; Alemán, J.; López-Martín, J. A. *Eur. J. Med. Chem.* **2014**, *76*, 360; (b) del Solar, V.; Quiñones-Lombraña, A.; Cabrera, S.; Padrón, J. M.; Ríos-Luci, C.; Alvarez-Valdés, A.; Navarro-Ranninger, C.; Alemán, J. *J. Inorg. Biochem.* **2013**, *127*, 128; (c) Řezníček, T.; Dostala, L.; Růžička, A.; Vinklerek, J.; Řežáčova, M.; Jambor, R. *Appl. Organomet. Chem.* **2012**, *26*, 237; (d) Chellan, P.; Land, K. M.; Shokar, A.; Au, A.; An, S. H.; Clavel, C. M.; Dyson, P. J.; de Kock, C.; Smith, P. J.; Chibale, K.; Smith, G. S. *Organometallics* **2012**, *31*, 5791; (e) Quiroga, A. G.; Ramos-Lima, F. J.; Alvarez-Valdés, A.; Font-Bardía, M.; Bergamo, A.; Sava, G.; Navarro-Ranninger, C. *Polyhedron* **2011**, *30*, 1646; (f) Alemán, J.; del Solar, V.; Alvarez-Valdés, A.; Ríos-Luci, C.; Padrón, J. M.; Navarro-Ranninger, C. *Med. Chem. Commun.* **2011**, *2*, 789; (g) Bergamini, P.; Marchesi, E.; Bertolasi, V.; Fogagnolo, M.; Scarpantonio, L.; Manfredini, S.; Vertuani, S.; Canella, A. *Eur. J. Inorg. Chem.* **2008**, *2008*, 529; (h) Ramos-Lima, F. J.; Quiroga, A. G.; García-Serrelede, B.; Blanco, F.; Carnero, A.; Navarro-Ranninger, C. *J. Med. Chem.* **2007**, *50*, 2194; (i) Messere, A.; Fabbri, E.; Borgatti, M.; Gambari, R.; Di Blasio, B.; Pedone, C.; Romanelli, A. *J. Inorg. Biochem.* **2007**, *101*, 254; (j) Bergamini, P.; Bertolasi, V.; Marvelli, L.; Canella, A.; Gavioli, R.; Mantovani, N.; Manas, S.; Romero, A. *Inorg. Chem.* **2007**, *46*, 4267.
- (a) Belli Dell'Amico, D.; Labella, L.; Marchetti, F.; Samaritani, S. *Dalton Trans.* **2012**, *41*, 1389; (b) Belli Dell'Amico, D.; Broglia, C.; Labella, L.; Marchetti, F.; Mendola, D.; Samaritani, S. *Inorg. Chim. Acta* **2013**, *395*, 181; (c) Dalla Via, L.; García-Argáez, A. N.; Adami, A.; Grancara, S.; Martinis, P.; Toninello, A.; Belli Dell'Amico, D.; Labella, L.; Samaritani, S. *Bioorg. Med. Chem.* **2013**, *21*, 6965; (d) Belli Dell'Amico, D.; Dalla Via, L.; García-Argáez, A. N.; Labella, L.; Marchetti, F.; Samaritani, S. *Polyhedron* **2015**, *85*, 685.
- Nierzwicki, L.; Wieczor, M.; Censi, V.; Baginski, M.; Calucci, L.; Samaritani, S.; Czub, J.; Forte, C. *Phys. Chem. Chem. Phys.* **2015**, *17*, 1458.
- Armarego, W. L. F.; Chai, C. L. L. *Purification of Laboratory Chemicals*, 6th ed.; Butterworth-Heinemann, 2009.
- Shankaraiah, N.; Markandeya, N.; Srinivasulu, V.; Sreekanth, K.; Reddy, C. S.; Santos, L. S.; Kamal, A. *J. Org. Chem.* **2012**, *76*, 7017.
- Wang, C.; Delcros, J.-G.; Biggerstaff, J.; Phanstiel, O. *J. Med. Chem.* **2003**, *46*, 2663.
- Marcus, E.; Fitzpatrick, J. T. *J. Org. Chem.* **1959**, *24*, 1031.
- (a) Hartley, F. R.; Searle, G. W. *Inorg. Chem.* **1973**, *12*, 1949; (b) Crocker, C.; Goggin, P. L. *J. Chem. Res. Syn.* **1978**, 93.
- van Engeland, M.; Nieland, L. J.; Ramaekers, F. C.; Schutte, B.; Reutlingsperger, C. P. *Cytometry* **1998**, *31*, 1.
- Dalla Via, L.; Santi, S.; Di Noto, V.; Venzo, A.; Agostinelli, E.; Calcabrini, A.; Condello, M.; Toninello, A. *J. Biol. Inorg. Chem.* **2011**, *16*, 695.
- Emerson, W. S. *Org. React.* **1948**, *4*, 174.
- Silverstein, R. M.; Webster, F. X.; Kiemle, D. J. *Spectrometric Identification of Organic Compounds*, 7th ed.; John Wiley and Sons, 2005.
- Jamieson, E. R.; Lippard, S. J. *Chem. Rev.* **1999**, *99*, 2467.
- Medrano, A.; Dennis, S. M.; Alvarez-Valdés, A.; Perles, J.; McGregor Mason, T.; Quiroga, A. G. *Dalton Trans.* **2015**, *44*, 3557.
- Gümüş, F.; Eren, G.; Açıık, L.; Çelebi, A.; Öztürk, F.; Yılmaz, S.; Sagkan, R. I.; Gür, S.; Özkul, A.; Elmali, A.; Elerman, Y. *J. Med. Chem.* **2009**, *52*, 1345.
- Zhao, J.; Gou, S.; Sun, Y.; Yin, R.; Wang, Z. *Chem. Eur. J.* **2012**, *18*, 14276.
- Ormerod, M. G. *Leukemia* **1998**, *12*, 1013.
- Nitiss, J. L. *Nat. Rev. Cancer* **2009**, *9*, 327.
- Wang, J. C. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 430.
- Ali-Osman, F.; Berger, M. S.; Rajagopal, S.; Spence, A.; Livingston, R. B. *Cancer Res.* **1993**, *53*, 5663.
- Minagawa, Y.; Kigawa, J.; Itamochi, H.; Terakawa, N. *Hum. Cell* **2001**, *14*, 237.
- Barret, J.-M.; Calsou, P.; Larsen, A. K.; Salles, B. *Mol. Pharmacol.* **1994**, *46*, 431.
- Raspaglio, G.; Ferlini, C.; Mozzetti, S.; Prislei, S.; Gallo, D.; Das, N.; Scambia, G. *Biochem. Pharmacol.* **2005**, *69*, 113.
- Ferlin, M. G.; Marzano, C.; Gandin, V.; Dall'Acqua, S.; Dalla Via, L. *ChemMedChem* **2009**, *4*, 363.
- Thibault, B.; Clement, E.; Zorza, G.; Meignan, S.; Delord, J.-P.; Couderc, B.; Bailly, C.; Narducci, F.; Vandenberghe, I.; Kruczynski, A.; Guilbaud, N.; Ferré, P.; Annereau, J.-P. *Cancer Lett.* **2016**, *370*, 10.
- Liu, L. F. *Annu. Rev. Biochem.* **1989**, *58*, 351.
- Chen, A. Y.; Liu, L. F. *Annu. Rev. Pharmacol. Toxicol.* **1994**, *34*, 191.
- Watt, P. M.; Hickson, I. D. *Biochem. J.* **1994**, *303*, 681.
- Pommier, Y.; Leo, E.; Zhang, H. L.; Marchand, C. *Chem. Biol.* **2010**, *17*, 421.
- Nelson, E. M.; Tewey, K. M.; Liu, L. F. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1361.