The CellScan technology for *in vitro* studies on novel platinum complexes with organoarsenic ligands†

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The present paper examines one of the multiple uses of the CellScan technique, its utilization in the biological evaluation of novel compounds, in order to improve the methods concerning the prediction of their anti-proliferative effects and application as anti-cancer drugs. The CellScan is a laser scanning static cytometer, enabling repetitive spectroscopic measurements in intact living cells. The detection of cell viability and apoptosis is possible, based on the transformations that occur in the cytoplasm matrix of tumour cells influenced by cytotoxic compounds. The measurement of fluorescence changes, due to this phenomenon, is possible with the CellScan system. The potential of this technology to detect the *in vitro* effects of the inhibitory molecules on tumour cells was demonstrated, making this method a valuable tool for chemosensitivity tests. We synthesized and fully characterised three novel platinum complexes of tertiary arsine ligands: *trans*-[PtI_2(2-^{i}PrOC_6H_4AsPh_2)$_2$] (**1**), *trans*-[$PtCl_2$(2-MeOC_6H_4AsPh_2)$_2$] (**2**) and *cis*-[$PtCl_2$(2-HOC_6H_4AsPh_2)$_2$] (**3**). The three compounds are biologically active against tumour cells and their cytotoxicity is comparable with standard drugs. Measurements using the CellScan technology correlate well with the results provided by other bioassay methods.

Introduction

Metal-based chemotherapeutic agents, above all platinum complexes, are an important class of anti-tumour compounds that have had a significant impact on the treatment of a variety of tumours for nearly 30 years.

The unique efficiency observed with standard platinum-based drugs, such as cisplatin, *cis*-diamminecyclobutanedicarboxylatoplatinum(II) (carboplatin) and 1,2-diaminocyclohexaneoxalatoplatinum(II) (oxaliplatin) in cancer treatment, fuelled the development of new platinum analogues.[1] In the last decade, it was demonstrated that several newly synthesized platinum compounds exhibit biological activity against tumour cells, and their antimitotic properties were proven by several procedures.[2–8]

The CellScan technology is based on a spectrometric method which allows repetitive multi-parameter measurements to be performed on intact living cells exposed to different external stimuli. The equipment is designed to perform fluorescence-based cytometric studies.[9] Using this straightforward and rapid method, it was possible to establish the cytotoxicity and apoptotic capacity of chemotherapeutic agents.[10,11] Our aim is to establish a procedure to study cytotoxicity, genotoxicity, and anti-proliferative effects of novel compounds.

We synthesized a new group of platinum complexes with the general formula [PtX_2L_2] (X = I, Cl; L = diphenylarsine ligand) in order to determine their potential biological activity. These three platinum complexes have organometallic ligands with structures, which lead us to presume that they exhibit biological effects: *trans*-[PtI_2(2-^{i}PrOC_6H_4AsPh_2)$_2$] (**1**), *trans*-[$PtCl_2$(2-MeOC_6H_4AsPh_2)$_2$] (**2**) and *cis*-[$PtCl_2$(2-HOC_6H_4AsPh_2)$_2$] (**3**). Each structure contains two identical tertiary arsine groups bonded to platin, and the only difference between these groups being one moiety, which is isopropyl for compound (**1**), methyl for (**2**) and hydroxyl for compound (**3**). The molecular structures of (**1**), (**2**) and (**3**) are analogous.

For this purpose, we used the CellScan system, which permits the detection of fluorescence intensity (FI) and polarization (FP) of different cell types. There is a strong correlation between the inhibition of cell growth induced by different chemotherapeutic agents and the resultant changes in FI and FP. Thus, changes can be revealed by staining treated cells with fluorescent dyes such Fluorescein diacetate (FDA) and Rhodamine 123 (Rh123). Hyperpolarization of the fluorescence intensity detected by the CellScan system can identify apoptotic cells within a complex cell population; it is a marker of the early apoptotic processes.[12]

To verify the accuracy of the new method, we correlated the results obtained in this way with those determined with customary procedures, such as the MTT (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) viability test[13] and one of the standard methods for assessing DNA damage, namely, the single-cell gel 'comet' assay.[14,15]

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† Electronic supplementary information (ESI) available: POV-Ray representations, atomic coordinates and displacement parameters, anisotropic displacement parameters, bond lengths and angles for **1**, **2** and **3**. CCDC reference numbers 693566–693568. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b802364f

Tertiary arsine complexes of platinum and other metals have been recently reported,[16–18] but there is no information regarding their biological activity. Among phenylarsines, the information on the biological activity was reported for phenylarsine oxide and dichlorophenylarsine,[19–23] and the phenylarsine compounds attached to biomolecules like peptides and enzymes were studied.[24,25] Phenylarsine oxide derivates are well known as anti-trypanosome agents and they are used as drugs.[26–28]

Results and discussion

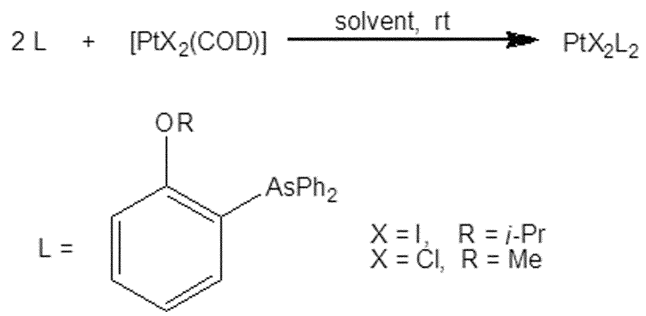
Synthesis and characterization of Pt complexes with organoarsine ligands

Complexes of the general formula $[PtX_2L_2]$, (X = I, L = (2-isopropoxyphenyl)diphenylarsine, X = Cl, L = (2-methoxyphenyl)diphenylarsine, (2-hydroxyphenyl)diphenylarsine) were prepared by the reaction of the arsine ligands with $[PtX_2(COD)]$ (COD = 1,5-cyclooctadiene) in a molar ratio of 2 : 1 (Scheme 1).

In the case of the complexes with diphenylarsinophenol ethers (**1** and **2**) both reagents were dissolved in dichloromethane with stirring at room temperature, and the final products were recrystallized from chloroform or from dichloromethane to obtain crystals suitable for X-ray diffraction.

The diphenylarsinophenol containing platinum complex (**3**) was prepared by slow diffusion of solvents; the arsine ligand was dissolved in toluene and added very slowly as a layer to a solution of $[PtCl_2(COD)]$ in dichloromethane. The final product was isolated as crystals suitable for X-ray structure determination.

In all complexes, the geometry around the metal center is square-planar and the organoarsenic ligands are coordinated to the transition metal through the arsenic atom. Similar complexes of platinum and palladium with O and P donor ligands have been reported in the literature.[29]



Scheme 1 General procedure for the synthesis of platinum complexes with the tertiary arsine ligands.

Fig. 1 and Fig. 2 present the molecular structure of the new platinum compounds.

In *cis*-$[PtCl_2(2\text{-}HOC_6H_4AsPh_2)_2]$, two independent molecules are present in the asymmetric unit (Fig. 2) and they have similar structural properties.

Biological effects

In the order to study the possible anti-proliferative and anti-mitotic effects of the compounds, we established their chemosensitivity on tumour and normal cell lines. We chose the MLS human ovarian cancer cell line, the M1–15 highly metastatic melanoma cell line, and the Hfl-1 normal human lung fibroblast cell line. The MLS cells were cultivated in a DMEM cell growth media, supplemented with glutamine, antibiotics and fetal bovine serum. M1–15 requires a RPMI-1640 medium, while Hfl-1 cells are cultivated in the nutrient-rich medium F-12. The cell cultures were maintained at 37 °C under a CO_2-enriched sterile atmosphere in an incubator for cell cultures.

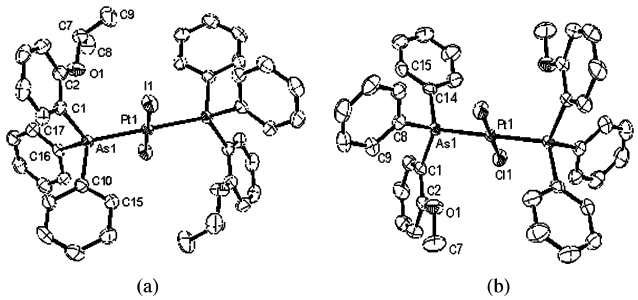


Fig. 1 ORTEP representations of the molecular structure of *trans*-$[PtI_2(2\text{-}^{i}PrOC_6H_4AsPh_2)_2]$ (**1**) (left) and *trans*-$[PtCl_2(2\text{-}MeOC_6H_4AsPh_2)_2]$ (**2**) (right); 50% ellipsoids are shown (hydrogen atoms are omitted for clarity).

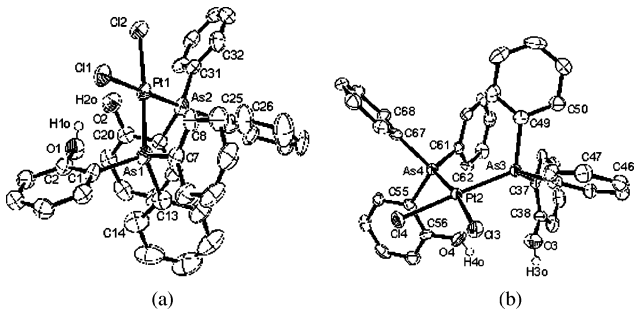


Fig. 2 ORTEP representation of the molecular structure of *cis*-$[PtCl_2(2\text{-}HOC_6H_4AsPh_2)_2]$ (**3**), with both independent molecules revealed; 50% ellipsoids are shown (hydrogen atoms are omitted for clarity).

IC50 values. The effectiveness of the platinum complexes against cell proliferation was measured using a quantitative colorimetric assay. As described previously,[14] 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was used to assess cytotoxicity. This pale yellow salt is reduced only by the mitochondria of living cells to its blue formazan derivative. Using a multiwell scanning spectrophotometer (ELISA reader) we measured the colour intensity, which is proportional to the number of living cells.

The test was performed on MLS, M1–15 and Hfl-1 cells. In 96-welled flat-bottomed microculture plates, cells were suspended in a concentration of 12 500 cells per well, in 200 µl of cell culture media. After 24 h incubation in the incubator, each compound was screened for *in vitro* anticancer activity by treating the cell lines with a wide range of concentrations of the platinum complex solutions. For **1**, **2** and **3**, we used the concentration range 0.01–90 µM mL^{-1}, for carboplatin and oxaliplatin the range was between 0.01–50 µM. The solvent containing the upper limit 1 : 100 ethanol–PBS (phosphate buffered salt solution) was tested separately and displayed no cytotoxic effect, which could affect our results. Three different experiments were performed for each complex.

Measurements were made after 48 h of incubation with the complexes, by removing the cell culture media, staining the cells with the MTT dye, and then by dissolving the formazan crystals with dimethylsulfoxyde (DMSO). The chemosensitivity was expressed as an IC50 value, that is, the concentration of the compound causing 50% cell death. IC50 values were obtained from dose-response curves after fitting the data to a linear quadratic equation using the Graph Pad Prism biostatistics program (95% confidence intervals, $r^2 = 0.94$, $P < 0.05$).

The IC50 values obtained are higher against normal Hfl-1 cells in all cases (Fig. 3), that is, platinum complexes **1**, **2** and **3** are more toxic against MLS ovarian tumour cells. The less toxic is **1** and the most toxic is **3**. IC50 values of carboplatin and oxaliplatin against MLS ovarian tumour cells are represented for comparison with novel synthesized compounds.

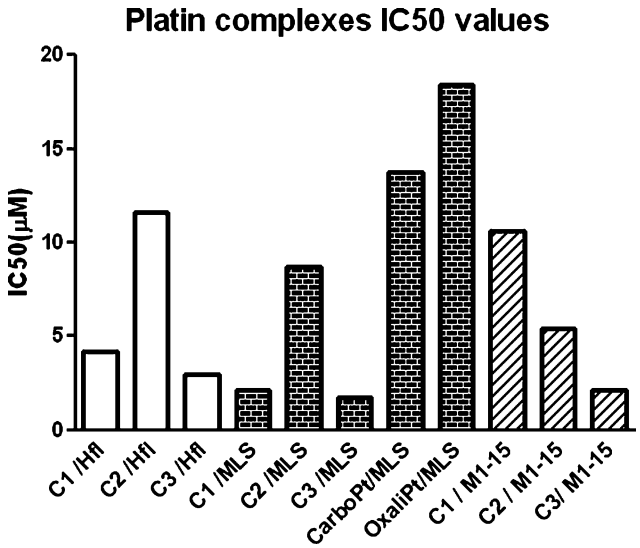


Fig. 3 The influence of complexes **1**, **2** and **3** on normal (Hfl-1) and tumour (MLS and M1–15) cell lines, expressed as IC50 values. C1 represents the platinum complex **1**, C2 complex **2** and C3 complex **3**, CarboPt is the abbreviation for carboplatin and OxaliPt is the abbreviation for oxaliplatin.

The cytotoxicity of the platinum complexes studied is comparable with the anti-tumour activity of carboplatin, a widely used drug in ovarian cancer treatment. The studied compounds are all more active than oxaliplatin against ovarian tumor cells.

The highly metastatic M1–15 melanoma cell line is more resistant to the complexes and the IC50 values show a lower cytotoxicity in comparison with the MLS cells; however, the compounds are still more active against the melanoma cell line *versus* normal Hfl-1 cells, except **3**, which shows a lower toxicity against the highly metastatic M1–15 cells.

These values helped us to choose the concentrations used for the CellScan determinations.

CellScan spectrometry. In this experiment, we utilized the MLS, Hfl-1 and M1–15 cell lines. The cells were suspended in growth media in 6-well microplates, and were incubated for 24 h, to allow them to attach to the plate surface. The plates were treated with **1**, **2** and **3** solutions, and also with carboplatin and oxaliplatin. As a control, we used untreated cells. In each case, we used a concentration of 5 mM. After 48 h of incubation with the cytotoxic agents, the cells were stained with the fluorescent dyes Fluorescein diacetate and Rhodamine 123, and measurements were made with the CellScan system.

The CellScan apparatus is a laser scanning, static, multi-parameter cytometer[10,30] measuring both fluorescence intensity (FI) and polarization fluorescence (FP) of individually fluorescent-labelled cells. The simplicity of this system provides a new perspective in clinical studies and in the basic research area. The CellScan system enables a repetitive spectroscopic measurements on intact living cells within a population under certain physiological conditions. It incorporates a unique 'cell carrier', which allows for repeated high-precision measurements by fixing the location of the cell in the cell carrier. We can perform repetitive measurements on the same populations and on the same cells after various stimulations and different set-ups of the scanner.[31]

The 'core' of the carrier is a 2 mm square grid of precisely dimensioned conical apertures, 5–8 μm in diameter, spaced at 20 μm, which can hold 10 000 cells, each loaded and maintained in a separate trap (Fig. 4). Each aperture is dimensioned to hold one single cell; lymphocytes and human tumour cell dimensions are compatible with the aperture diameter. We used carriers with 8 μm sized apertures. The cells were loaded in the grid using a gradient of pressure. A negative pressure of 1–5 mm H_2O, maintained between the upper and the lower surfaces of the carrier, caused a rapid fixing of the cells into the orifices. The loaded cells, once trapped into the conical orifice, can not leave this and remain fixed during various procedures and manipulations: staining, washing, microscopic and spectrometric measurements.

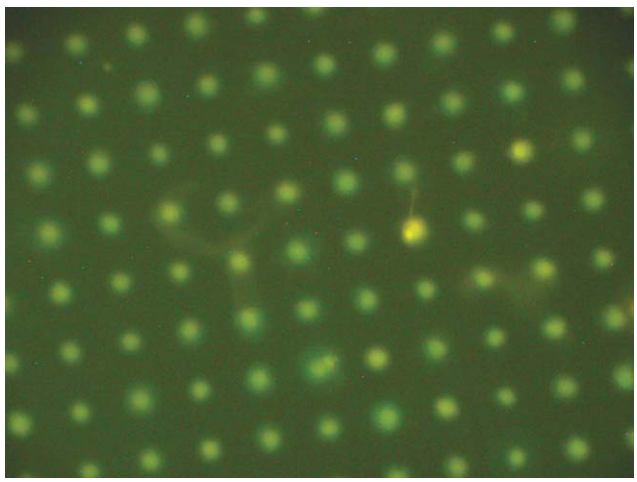


Fig. 4 Fluorescent inverse phase microscope image of FDA stained MLS tumour cells trapped in the orifices of the cell carrier (20-fold magnitude).

The static He–Cd laser cytometer scans each hole and displays the spread of intensity (FI) on the *x* axis and polarization (FP) on the *y* axis on the screen (Fig. 5). The illumination intensity of the laser beam is 5 μW, the intensity and polarization values measured at two wavelengths (527 and 510 nm) characterize each living cell. The measurements are made using a preset photon-counting technique, in which the same number of photons is counted for each cell, regardless of emissions intensity, so that the measurement has the same precision both for weakly and strongly emitting cells.

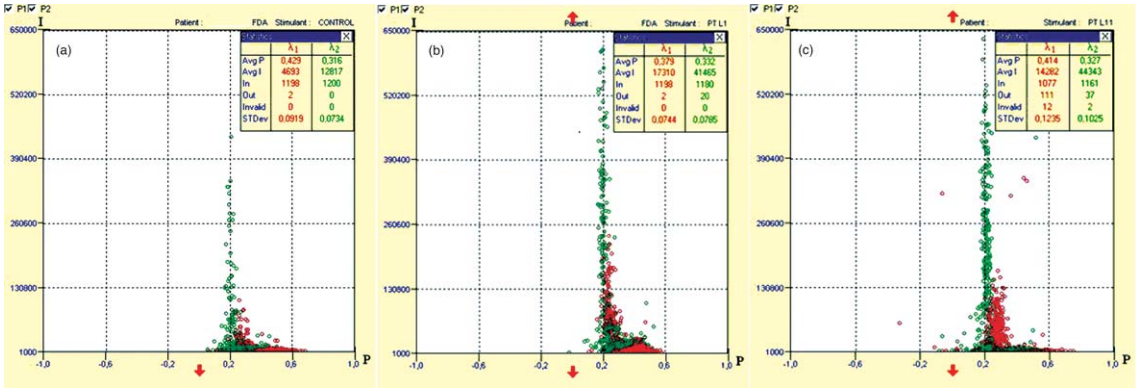


Fig. 5 Histogram provided by the CellScan: the polarization and the intensity for each cell, measured at two wavelengths are represented by red and green dots. In Fig. 5a the untreated (control) MLS cells are shown, (b) and (c) represents FI and FP of MLS cells treated with complex **1**, respectively.

The fluorescence polarization (FP) indicates the entropy of the fluorescent molecules, that is, the relative rotational freedom. The FP of a fluorochrome increases with increasing viscosity or restriction of molecular rotation. Cell activation and apoptosis involve conformational changes of cytosolic enzymes and proteins, that is, intracellular matrix reorganization. The more the molecule's rotational movement is restricted, the higher the FP value, and *vice versa*. The fluorescence intensity (FI) is expressed as the number of photon counts per unit of time and the FI of the fluorochrome reflects intracellular content.

The dyes used for the measurements are FDA and Rh123. FDA is a non-fluorescent dye, which is converted to its fluorescent form by enzymatic hydrolysis only in living cells and it is an indicator of cell viability. Rh 123 is a lipophilic cationic fluorochrome that selectively accumulates in the mitochondria of living cells and whose specific uptake or release depends upon the mitochondrial membrane potential and it therefore reflects mitochondrial activity. A drop in the FI and an increase in the FP of Rh123 was attributed to early apoptosis.[11,13]

A FP value obtained as a result of the measurements using FDA-stained cells gives us information about the viability of the cells, and the inhibitory effect of the treatment. The cell growth inhibition is demonstrated if the treated cell population displays a high FP value and a high FI value than the untreated control and no hyperpolarization of FDA or Rh123 was observed in the resistant cells.[9,10] In our experiment, an increase of FP was observed for the platinum complex **1**, **2** and **3** relative to the control for both the MLS and M1–15 cells (Fig. 5).

Measurements show the cytotoxic activity of the studied platinum complexes and the fact that against MLS cell lines they display a cytotoxic effect as high as carboplatin and oxaliplatin. The increase of the FP values ratio is not so prominent in the experiment with the highly metastatic M1–15 cells.

The Rh123 fluorescent marker gives us information about the early apoptotic processes, which affect cytoplasmic viscosity and alterations in cell permeability. The values from Table 1 show us that **2** displays the most significant capacity to induce apoptosis, in this case, the FP increase is the biggest, both for MLS and M1–15 cells, although against M1–15 cells we observed a smaller increase in FP values. Despite its IC50 value, **3** does not induce apoptosis.

Platinum tertiary arsine cytotoxicity (cell growth inhibition) lies in a range that is comparable with other compounds that have less toxic ligands. The complexes have poor solubility and are stable in ethanol and phosphate buffered saline solutions, without the release of the ligand.

Comet assay. We also performed the alkaline single-cell gel electrophoresis or 'comet' assay, which detects DNA strand breaks, alkali-labile sites and cross-links in eukaryotic cells. It is based on electrophoretic mobility and reveals changes in the tertiary DNA structure of the exposed cells. The procedure is widely used in testing novel chemicals for genotoxicity, including platinum complexes and organometallic compounds.[32–35]

MLS tumour cells were treated with complexes **1**, **2** and **3**, carboplatin and oxaliplatin at the same concentrations used for the polarization measurements. The cells were placed in six-well plates, and they were incubated in growth media for 24 h, to allow them to attach to the plate surface. Treatment was made with **1**, **2** and **3** solutions, and also with carboplatin and oxaliplatin. As a control, we used untreated cells. We used the same concentrations

Table 1 Fluorescence polarization values

	FP values obtained with FDA staining/a.u.			FP values obtained with Rh123 staining/a.u.	
Stimulant	Hfl-1	MLS	M1–15	MLS	M1–15
Control	0.409	0.316	0.263	0.362	0.415
Oxaliplatin	0.453	0.275	0.241	0.358	0.439
Carboplatin	0.461	0.330	0.274	0.398	0.404
Complex **1**	0.420	0.332	0.275	0.371	0.419
Complex **2**	0.412	0.321	0.297	0.391	0.426
Complex **3**	0.439	0.327	0.285	0.352	0.404

Table 2 Lesion scores (LS) and tail factors (TF) for platinum complexes and platin-based drugs

Stage	Control	Oxaliplatin	Carboplatin	Complex **1**	Complex **2**	Complex **3**
0	72	2	13	20	13	19
1	15	58	49	47	23	17
2	8	29	18	9	17	7
3	2	3	0	2	11	1
4	3	8	20	22	24	56
Sum	100	100	100	100	100	100
LS	35	157	165	159	186	258
TF	7.95	25.83	31.35	34.98	41.00	59.98

as for the static cytometry measurements and 48 h effects were considered.

The method used by us was described previously[36] and it was performed according to the alkaline version of the comet assay test.[10,11] Cells incorporated in agarose gel on a microscope slide are lysed with an alkaline lysis solution (pH > 10) to denude nucleotides containing supercoiled loops of DNA. After this operation, gels are placed in a pH > 13 buffer solution for electrophoretic migration, during which damaged DNA loops containing breaks lose their supercoils and migrate to the anode.

The damaged cells display an increased electrophoretic migration of the DNA fragments by forming outlines resembling comets, in contrast to the round appearance of undamaged DNA. The length and form of the comet 'tail' reveal the levels of damage. We considered five possible stages based on the distance of DNA migration 'tail length'. The migration of the DNA fragments from the nucleus is visualized by manual microscopic analysis, which is equal in sensitivity to computerized analysis.[37]

We evaluated the DNA damage by means of two parameters: lesion score (LS) and tail factor (TF), both expressed in arbitrary units. The lesion score represents the product of the cells ratio and stage. The tail factor quantifies the global damage in the cells by associating the average proportion of DNA migrated in the comet tail and it represents the percentage of DNA in the tail relative to the amount of DNA in the entire comet. We considered the values obtained for untreated cells as a 'control' reference for each case.

In every case, we obtained a positive result, that is, a significant increase in LS and TF *versus* the control. The comet assay results show that DNA damage induced by the commonly used platinum-based drugs and the complexes **1** and **2** are comparable, while compound **3** induces the most strand lesions among the studied compounds (Table 2). Massive amounts of stage 1 and stage 2 lesions support the genotoxicity of platinum-based drugs described in the literature. In the case of complex **3**, the huge number of stage 4 lesions is confirmation of the cytotoxic effect. Complexes **1** and **2** show genotoxicity and an increase in stage 2 lesions is also observed. Complex **2** also exhibits many stage 3 lesions, which confirm its cytotoxic effect.

Conclusions

The CellScan system has already proved its utility in a number of important biochemical, immunological, kinetic, pharmacological and physicochemical applications and is an appropriate tool for analysing newly synthesized compounds. It is a simple and rapid method to assess cytotoxicity and genotoxicity. The results obtained using this technique are confirmed by literature data and by convergence with the data acquired by other widely used methods. It requires only a small number of cells to perform the *in vitro* studies, and the measurements can be repeated on the same cell population because the cell carrier used by the system has some unique characteristics that permit the positions of living cells during the experiment to be maintained and revealed.

The new platinum complexes with tertiary arsine ligands exhibit biological activity. Their activity against normal and tumour cells is differentiated as all are more active against tumour cell lines. Genotoxicity is evidenced for complexes **1** and **2**. Complex **1** is less toxic, while **2** has the greatest apoptosis-induction capacity and the largest gradient, in effect, against normal/malignant cells. We suppose that this is a consequence of the presence of the methyl moiety, which leads to methylation of the nucleic acids. The cytotoxicity of the compounds is analogous to that of standard anticancer chemotherapeutic platinum drugs and it is comparable with that of other new platinum complexes that exhibit biological activity.

The CellScan technology was useful in the analysis of the three compounds as it has a high sensitivity and selectivity. This procedure can replace other more difficult and lengthy methods for assessing biological activity.

The studied compounds are also suitable for testing *in vivo*. Similar compounds with improved solubility in biologically compatible solvents should be synthesized in order to develop anticancer drugs.

Experimental

Materials

Complex synthesis. All reactions were carried out under an atmosphere of dry nitrogen using standard Schlenk or vacuum line techniques. The solvents were purified (toluene refluxed over Na–benzophenone; CH_2Cl_2 and *n*-hexane refluxed over powdered CaH_2) and distilled under nitrogen. [PtX_2(COD)] (X = Cl, I) was prepared according to the literature procedure.[38] All chemicals were of reagent grade and were used as received: 1,5-cyclooctadiene was purchased from Merck and $H_2[PtCl_6]\cdot 6H_2O$ was generously donated by Umicore AG & Co KG, Hanau-Wolfgang, Germany.

Biological effects. For this purpose we performed cell cultures on both tumour and normal cell lines.

The Hfl-1 cell line was acquired from Sigma Aldrich Corporation from the European Collection of Cell Cultures; The M1–15 melanoma cell line was a generous gift from Prof. Falus Andras,

Semmelweiss University, Budapest, Hungary, whereas the MLS ovarian cell line was a donation from Dr Yael S. Schiffenbauer from MedisEl Company, Lod, Israel.

The cells were defrosted carefully, cultivated in special culture flasks with adequate cell growth media, under sterile conditions. Growth conditions were assured using incubators with a stable temperature (37 °C), 5% CO_2 atmosphere and constant humidity.

Several cell passages were performed using enzymatic procedures. Experiments were made when confluence of the cells achieved 80% on the flask surface. Cell lines were adherent, and they were seeded on 96-well cell culture plates for the IC50 measurements, and on 6-well plates for the cytometric and comet assay tests.

The cells were incubated for 24 h before adding the platinum complexes. After the treatment with sterile solutions with differing concentrations of complexes **1**, **2** and **3** and platinum drugs, respectively, the culture plates were kept in incubator for another 24 and 48 h before the measurements were taken.

We made 3 independent experiments for the MTT test and measurements were made directly using the 96-well plates.

For the cytometric and comet assay tests, a removal from the 6-well plates was made mechanically and not enzymatically to avoid accidental cell damage, which could be caused by the trypsine. For the cytometric assay we made 3 different experiments, and for the comet assay, for each compound, we performed 3 different electrophoretic migrations on 4 separate microscope slides.

Cell cultures were performed using Dulbecco's modified eagles medium, RPMI 1640 and F-12 cell growth media from Sigma Aldrich Corporation. Phosphate buffered salt solution, trypsine EDTA, MTT salt, ethidium bromide, low melting point agarose, type I agarose, FDA and Rh 123 fluorescent dyes were also provided by Sigma Aldrich. NaCl p.a., NaOH p.a. and EDTA·2HCl p.a. were obtained from Merck & Co. Inc., and TrizmaHCl from Fluka Analytics.

The supplier of all the disposables used for the biological experiments was Nalgene Nunc International.

The chemotherapeutical drugs, carboplatin (Carboplatin) and oxaliplatin (Eloxantine), were procured from Sindan Pharma.

Instrumentation

Characterization of new compounds. Infrared spectra were recorded on a Perkin-Elmer System 2000 FTIR spectrometer scanning between 400–4000 cm^{-1} using KBr disks and between 200–400 cm^{-1} using CsI plates, and also on a Bruker-Vector 22 spectrometer scanning between 200–4000 cm^{-1}.

The ^1H NMR spectra of the transition metal complexes were recorded on an AVANCE 300 spectrometer (Bruker). The chemical shifts for the ^1H NMR spectra are reported in parts per million (ppm) at 300 MHz with tetramethylsilane as the standard. The assignments were based on those for the unbound ligand and those for analogous complexes. The elemental analyses were recorded on a VARIO EL (Heraeus).

The crystallographic data for complexes **1** and **2** were collected on a Siemens CCD diffractometer (SMART) using MoKα radiation ($\lambda = 0.71073$ Å) and omega-scan rotation (Table 3). Data reduction was performed with SAINT (area-detector integration software, version 6.01 (1999), Siemens Industrial Automation, Inc., Madison, WI) including the program SADABS (G. M. Sheldrick, SADABS, Program for Scaling and Correction of Area-Detector data, Göttingen, 1997) for empirical absorption correction.

The crystallographic data for complex **3** were collected on a Stoe-IPDS imaging plate diffractometer (phi-scan rotation and MoKα radiation ($\lambda = 0.71073$ Å) and the numerical absorption correction was performed with X-RED (Version 1.22 (2001), STOE Data Reduction Program, STOE & Cie GmbH, Darmstadt).

Biological effects

The cell culture laboratory is equipped with Heto Holten and Laminair laminar hoods, with Hettich centrifuges, with humidified Heto Holten Cellhouse 154 and Uniequip CO_2 series incubators with CO_2 admission.

The fluorescence was tested using a Desktop CellScan apparatus [MedisEl Technologies, Lod, Israel]. The cell carriers were provided by MedisEl. The cytotoxicity was determined using a Tecan Sunrise ELISA reader with Magellan operating system. For the single-cell gel assay the electrophoresis was performed in a Consort electrophoresis chamber and the power source was Consort type EV231.

Image analysis of microscope slides for the comet assay test were made using a Nikon fluorescence microscope with CCD image capture and Lucia analyzer software. The image capture of the fluorescent-marked tumour cells on the cell carrier surface was made using a Zeiss Observer D1 optical microscope with an Axiovision Release 4.3.6/2007 software. The statistical analysis was performed using the Graph Pad Prism 5 biostatistics program.

Preparation of complexes 1 and 2

Synthesis of ligand solution. (2-isopropoxyphenyl)diphenylarsine (0.04 g, 0.11 mmol) for complex **1** and (2-methoxyphenyl)diphenylarsine (0.17 g, 0.5 mmol) for complex **2**, in dichloromethane was added to a dichloromethane solution of [PtI_2(COD)] (COD = 1, 5-cyclooctadiene) (0.03 g, 0.053 mmol) for **1** and [$PtCl_2$(COD)] (0.09 g, 0.025 mmol) for **2**. The reaction mixture was stirred at room temperature until a precipitate was formed. The precipitate was filtered and then recrystallized from dichloromethane and *n*-hexane to give the corresponding complexes.

1. Yield: 0.03 g, 46%, orange crystals, mp 271 °C. Found%: C 42.4, H 3.5, I 12.5. Calcd% $C_{42}H_{42}As_2I_2O_2Pt$: C 42.8, H 3.6, I 12.7. IR/cm^{-1}: 333 (Pt–I), 279 (Pt–As). δ_H/ppm (300 MHz, $CDCl_3$, Me_4Si) 1.09 (d, 12H, CH_3, $J = 6.04$ Hz), 4.50 (m, 2H, CH), 6.79–6.88 (m, 4H), 7.35–7.39 (m, 16H), 7.84–7.87 (m, 8H).

2. Yield: 0.15 g, 64%, pale yellow crystals, mp 274 °C. Found: C 48.4, H 3.4, Cl 7.1. Calcd% $C_{38}H_{34}As_2Cl_2O_2Pt$: C 48.6, H 3.6, Cl 7.5%, IR/cm^{-1}: 334 (Pt–Cl), 282 (Pt–As). δ_H/ppm (300 MHz, $CDCl_3$, Me_4Si): 3.84 (s, 6H, CH_3), 6.89–7.04 (m, 6H), 7.38–7.43 (m, 14H), 7.82 (m, 8H).

Preparation of complex 3

A solution of (2-hydroxyphenyl)diphenylarsine (0.03 g, 0.09 mmol) in toluene was added as a layer over a solution of [$PtCl_2$(COD)] (0.04 g, 0.044 mmol) in dichloromethane. As

Table 3 Crystallographic data for single-crystal X-ray structures of compounds **1**, **2** and **3**

Compound	**1**	**2**	**3**
Empirical formula	$C_{42}H_{42}As_2I_2O_2Pt$	$C_{42}H_{42}As_2Cl_{10}O_2Pt$	$C_{36}H_{30}As_2Cl_2O_2Pt$
Formula weight	1177.49	1278.19	910.43
Temperature/K	213(2)	210(2)	213(2)
λ/pm	71.073	71.073	71.073
Crystal system	Monoclinic	Triclinic	Triclinic
Space group	$P2_1/c$	$P\bar{1}$	$P\bar{1}$
a/pm	930.16(16)	919.1(3)	1124.10(7)
b/pm	1821.6(3)	1169.0(3)	1594.26(12)
c/pm	1215.6(2)	1344.4(4)	1861.95(12)
α/°	90	114.160(4)	83.834(8)
β/°	106.261(3)	92.344(4)	84.727(8)
γ/°	90	105.966(4)	85.694(9)
V/nm^3	1.9773(6)	1.2476(6)	3.2963(4)
Z	2	1	4
D_{calcd}/Mg m^{-3}	1.978	1.701	1.835
Absorption coefficient/mm^{-1}	6.805	4.697	6.443
F(000)	1120	624	1760
Crystal size/mm^3	0.20 × 0.10 × 0.10	0.30 × 0.20 × 0.20	0.20 × 0.10 × 0.10
Theta range for data collection (Θ_{min}–Θ_{max})/°	2.07–29.40	2.02–29.24	2.21–28.00
Index ranges	$-12 \le h \le 12$, $-21 \le k \le 23$, $-15 \le l \le 10$	$-12 \le h \le 12$, $-15 \le k \le 15$, $-17 \le l \le 18$	$-14 \le h \le 14$, $-18 \le k \le 21$, $-24 \le l \le 24$
Reflections collected	12 871	13 002	22 550
Independent reflections	4868 [$R_{int} = 0.0259$]	6044 [$R_{int} = 0.0230$]	14 616 [$R_{int} = 0.0410$]
Completeness to Θ_{max}	89.3%	89.2%	91.8%
Absorption correction	SADABS	SADABS	Numerical
Min./max. transmission	0.5494/0.3431	0.4535/0.3331	0.5651/0.3590
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Restraints/parameters	4868/0/225	6044/0/260	14 616/0/775
Goodness-of-fit on F^2	1.041	1.080	0.706
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0271$, w$R_2 = 0.0645$	$R_1 = 0.0332$, w$R_2 = 0.0789$	$R_1 = 0.0326$, w$R_2 = 0.0551$
R indices (all data)	$R_1 = 0.0395$, w$R_2 = 0.0685$	$R_1 = 0.0384$, w$R_2 = 0.0809$	$R_1 = 0.0763$, w$R_2 = 0.0605$
Largest diffraction peak and hole/e Å^{-3}	1.014 and –1.436	2.527 and –1.441	1.134 and –1.420

a result of the slow diffusion of the two solutions, complex **3** was obtained (0.02 g, 49%) as pale yellow crystals suitable for X-ray analysis, mp 239 °C. Found: C 47.9, H 3.2, Cl 8.0. Calcd% $C_{36}H_{30}As_2Cl_2O_2Pt$: C 47.5, H 3.3, Cl 7.8%, IR/cm^{-1}: 317, 325 (Pt–Cl), 277 (Pt–As). δ_H/ppm (300 MHz, $CDCl_3$, Me_4Si): 6.34 (dd, 2H, $J_{1,2} = 6.98$, $J_{1,3} = 1.13$), 6.87–7.21 (m, 6H), 7.39–7.45 (m, 12H), 7.74 (m, 8H). An OH resonance was not observed.

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