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Anti-growth effect of a novel *trans*-dichloridobis[2-(2-hydroxyethyl)pyridine]platinum (II) complex via induction of apoptosis on breast cancer cell lines

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ABSTRACT

Breast cancer still continues to be the leading cause of cancer-related mortality in women worldwide. Although advances have been made in the treatment of this disease during the past decade, new approaches and novel compounds are urgently needed. The aim of this study was to evaluate the cytotoxic activity of *trans*-[$PtCl_2$(2-hepy)$_2$] [2-hepy=2-(2-hydroxyethyl) pyridine] on breast cancer cell lines, MCF-7 and MDA-MB-231. The platinum (II) complex was synthesized and characterized by our laboratory working group. Anti-growth effect was assayed by the MTT and ATP viability assays and also monitored real-time using xCELLigence system. The mode of cell death was evaluated by using the fluorescence microscopy (Hoechst 33342 + Calcein-AM + Propidium iodide staining), Western blotting (cleaved PARP and caspase 3, total caspase 8), flow cytometry (quantitative analysis of live, early/late apoptotic, dead cells and caspase 3/7 activity) and the RT-PCR (the genes analyzed were BCL-2L10, BIK, BAX, BCL-2, FASLG, HRK, TNFRSF10B, and TNFRSF10A). The platinum (II) complex had anti-growth effect in a dose dependent manner in vitro. Cells were killed by apoptosis as evidenced by the pyknotic nuclei, cleavage of poly-(ADP-ribose) polymerase (PARP) and induction of active caspase-3. These results suggest that the complex might represent a potentially active novel drug for the breast cancer treatment and warrants further studies due to its promising cytotoxic activity.

1. Introduction

Chemotherapy is currently the most widely used treatment option for cancer disease which continues to increase largely globally.[1] Among currently used chemotherapeutics cisplatin, carboplatin and oxaliplatin are platinum-based chemotherapeutics and regularly used in the treatment of solid tumors.[2] Platinum therapy associated inadequacies such as acquired resistance[3] and toxic side effects[4] emphasizes the need for new anticancer drugs with new mechanisms of action. Several research groups showed that platinum (II) complexes have significant anti-tumor activities against different types of cancers in vitro and in vivo.[5–11]

Although transplatin, the *trans* isomer of cisplatin, is not clinically used, some *trans*-derivatives showed cytotoxic activity profiles comparable to cisplatin.[12] *Trans*-configured platinum (II) complexes undergo several undesired reactions, which result in the lack of anticancer activity.[13,14] The effects of leaving groups

Abbreviations: 7-AAD, 7-amino-actinomycin D; Abs, absorbance; ATP, adenosine-5′-triphosphate; BCL-2, B-cell lymphoma 2; BCL2L10, BCL2-like 10; BIK, BCL2-interacting killer; BAX, BCL2-associated X protein; Calcein AM, the acetomethoxy derivate of Calcein; cDNA, complementary DNA; CI, cell index; DNA, deoxyribonucleic acid; DMSO, dimethyl sulfoxide; FASLG, FAS ligand; HCl, hydrochloric acid; HRK, Harakiri; HRP, horseradish peroxidase; IC_{50}, 50% inhibitory concentration of drug; IC_{90}, 90% inhibitory concentration of drug; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide thiazolyl blue; PARP, poly (ADP-ribose) polymerase; PBS, phosphate buffered saline; PI, Propidium iodide; RNA, ribonucleic acid; RPMI, Roswell Park Memorial Institute medium; RIPA, radioimmunoprecipitation assay; RTCA, real-time cell analyzer; RT-PCR, real time polymerase chain reaction; SD, standard deviation; SDS–PAGE, Sodium dodecyl sulfate polyacrylamide gel electrophoresis; TNFRSF10A, tumor necrosis factor receptor superfamily member 10a; TNFRSF10B, tumor necrosis factor receptor superfamily member 10b.

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and the aromatic heterocyclic planar systems on efficiency, kinetics, and DNA reactivity mode have been investigated and may partly help to explain the cytotoxic properties of transplatinum derivatives.[15,16]

In this context, our research group has recently reported the synthesis, characterization and DNA binding affinity of *trans*-configured palladium (II) and platinum (II) complexes, in which both NH_3 ligands in cisplatin were exchanged by two hydroxyalkylpyridines.[17] Among the complexes, *trans*-[$PtCl_2$(2-hepy)$_2$], where 2-hepy is 2-(2-hydroxyethyl)pyridine (Fig. 1), showed high DNA binding affinity as well as high anticancer activity against human colon cancer cells (Colo 320 and Colo 741), human lung cancer cells (H1299) and rat transformed fibroblasts (5RP7, compared to transplatin and clinically used platinum drugs such as cisplatin, carboplatin and oxaliplatin.[17]

The present study describes in vitro cytotoxic activity of *trans*-[$PtCl_2$(2-hepy)$_2$] against breast cancer cells. The platinum (II) complex has been found to exhibit anti-growth effects against breast cancer cell lines (MCF-7 and MDA-MB-231) in vitro. The action mechanism of the complex, such as caspase 3/7 activation, cleavage of PARP and quantitative analysis of early/late apoptotic cells and apoptotic cell death related genes expression were also studied.

2. Materials and methods

2.1. Chemicals and cell culture

The platinum (II) complex was synthesized by Icsel et al.[17] This complex was dissolved in DMSO/ethanol (1:1) at a concentration of 50 mM as a stock solution and further dilutions were made in culture medium. Different concentrations of platinum (II) complex ranging from 1.56 to 50 μM were used. Breast cancer cell lines, MCF-7 and MDA-MB-231, were cultured in RPMI 1640 medium supplemented with penicillin G (100U/ml), streptomycin (100 μg/ml), L-glutamine, and 5% fetal bovine serum at 37 °C in a humidified atmosphere containing 5% CO_2.

2.2. Determination of cytotoxic activity

2.2.1. The MTT assay

MCF-7 and MDA-MB-231 cells were seeded at a density of 5×10^3 cells per well of 96-well plate in 100 μl medium. After 24 h 100 μl of platinum (II) complex was added (used at the range of 1.56–50 μM) and cells were subsequently incubated for 48 h. The untreated cells received only the medium without using any drugs for control (maximum viability). Each experiment was

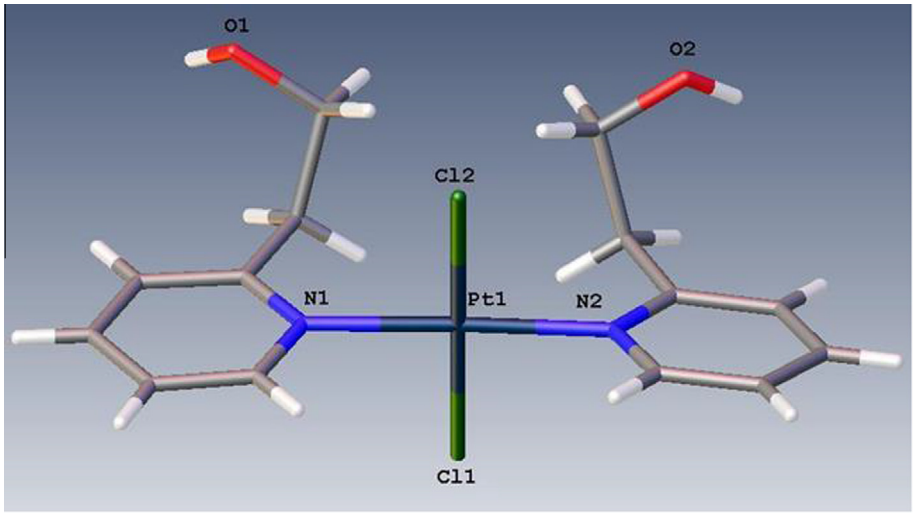

Figure 1. The structure of *trans*-[$PtCl_2$(2-hepy)$_2$].

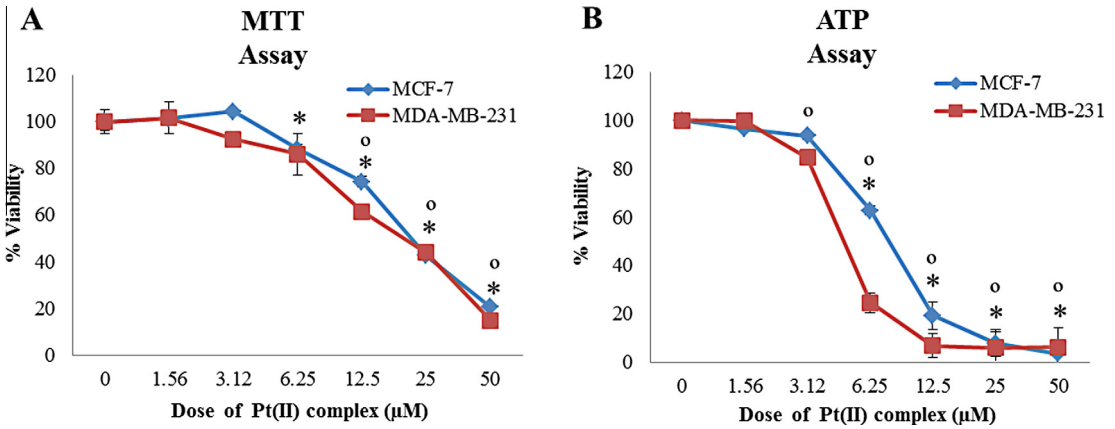

Figure 2. Anti growth effect of the platinum (II) complex. Viability of MCF-7 and MDA-MB-231 cells after the treatment with varying doses of platinum (II) complex for 48 h was measured by (A) MTT and (B) ATP viability assays. The results are mean of three independent experiments and the error bars indicate SD. *(for MCF-7) and °(for MDA-MB-231) denotes statistically significant differences in comparison with control ($p < 0.005$).

carried out twice in triplicates. MTT was first prepared as a stock solution of 5 mg/ml in phosphate buffer (PBS, pH of 7.2) and then filtered. At the end of the treatment period (48 h), with six different doses in triplicates, 20 μl of MTT solution was added to each well. After incubation for 4 h at 37 °C, 100 μl of solubilizing buffer (10% sodium dodecyl sulfate dissolved in 0.01 N HCl) was added to each well. After overnight incubation, the 96-well plate was read by a spectrophotometer at 570 nm for absorbance density values to determine the cell viability. Cell viability of treated cells was calculated in reference to the untreated control cells using the formula as viability (%) = [100 × (Sample Abs)/(Control Abs)], where Abs is the absorbance value at 570 nm. The assay was performed two times and the results were given as mean ± SD of independent experiments.

2.2.2. The ATP assay

The cells were seeded and treated as done in the MTT assay. The ATP assay uses the highly sensitive 'firefly' reaction to determine the level of cellular ATP as an indirect measure to assess the number of viable cells.[18] The seeding and treatment conditions as well as the calculation of viability were all performed as for the MTT assay (see above). At the end of the treatment period (48 h) with six different doses in triplicates, the ATP assay was used for luminometric measurement of cell growth (viability) according to the standard protocol of the manufacturer with a little modification in which 150 μl of medium was removed from each well before the addition of 50 μl of somatic cell ATP releasing agent (ATP Bioluminescent Somatic Cell Assay Kit, Sigma, Steinheim, Germany). After mixing thoroughly, the microplates were allowed to stand on the bench for 20–30 min at room temperature before 50 μl medium from each well was transferred to a white non-translucent plate. After that, 50 μl of luciferin–luciferase counting reagent was added. The microplates were measured using a count integration time of 1 s at luminometer (Bio-Tek, Vermont, USA). The assay was performed three times and the results were given as mean ± SD of independent experiments.

Table 1
Anti-growth parameters for platinum (II) complex determined by the ATP assay following 48 h treatment against MCF-7 and MDA-MB-231 cell lines

Cells	*IC_{50} (μM)	**IC_{90} (μM)
MCF-7	8.08 ± 0.21	22.59 ± 0.46
MDA-MB-231	4.92 ± 0.17	11.32 ± 0.25

* IC_{50} is defined as the dose inhibiting 50% of viability after the treatment with the complex.
** IC_{90} is defined as the dose inhibiting 90% of viability after the treatment with the complex.

2.2.3. RTCA system

Following the background impedance measurements as explained in the booklet of the manufacturer, MCF-7 and MDA-MB-231 cells (20,000 cells in 100 μl) were seeded in each well of 96-well E-plate (xCELLigence RTCA, Roche, Germany). Following 30 min incubation at 37 °C in the cell culture incubator, E-plate 96 was placed into the system. After 24 h 100 μl medium removed from wells without damaging the cells. Subsequently, the cells were exposed to 100 μl of medium containing different doses of the platinum (II) complex (1.56–50 μM). Adhesion, growth and proliferation of the cells was monitored every 1 h for a period of up to 48 h via the incorporated sensor electrode arrays of the cell-loaded 96-well E-Plate. All experiments were run for 48 h

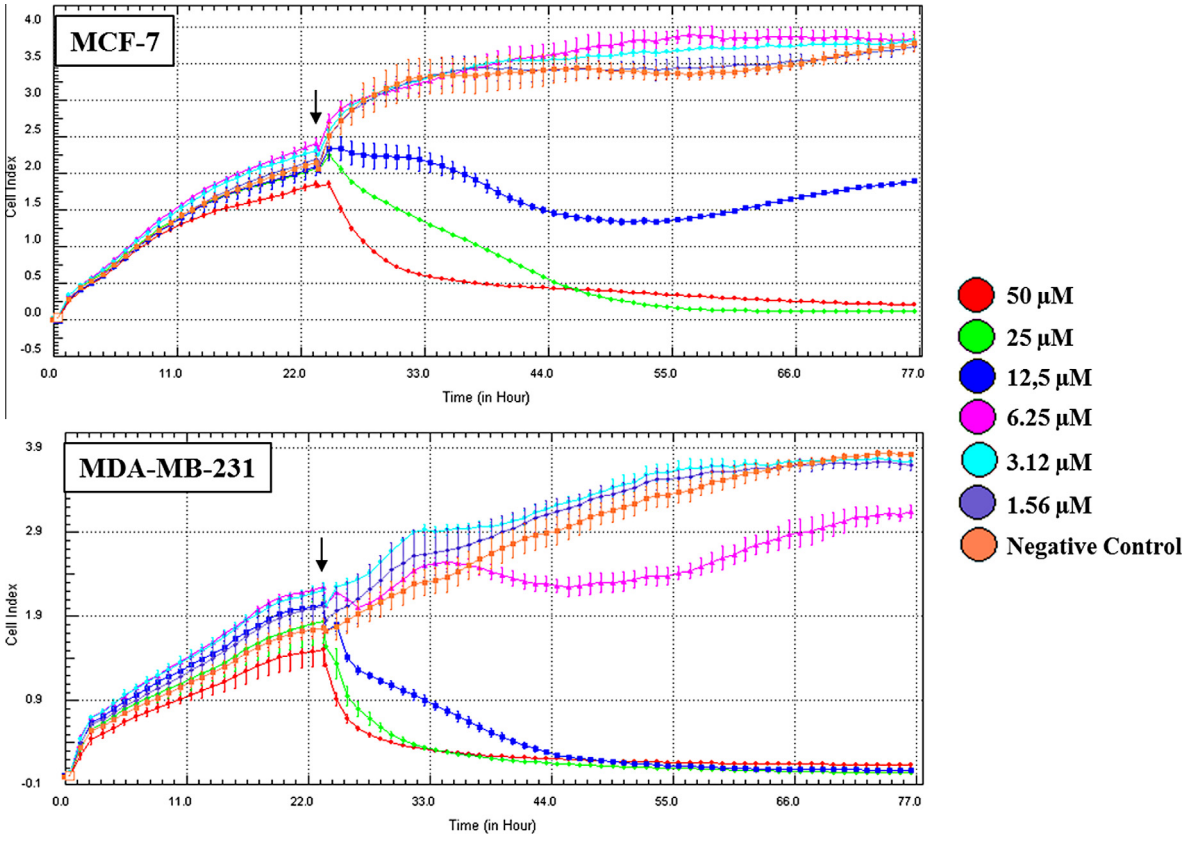

Figure 3. Real time analyses of anti growth effect. The CI values for 1.56–50 μM of the platinum (II) complex for 48 h on MCF-7 and MDA-MB-231 cells are shown. The concentrations represented by different colors. Assay was performed as described in materials and methods. Cytotoxic, cytostatic and antiproliferative doses are clearly observed.

and time dependent CI graph was produced by the device using the RTCA software program of the manufacturer.

2.3. Determination of cell death mode

2.3.1. Fluorescence microscopy

The nuclear and membrane morphology of treated and untreated cells were examined after staining them with fluorescent dyes, Hoechst 33342, PI and Calcein-AM. Hoechst 33342 is a dye that can bind DNA and stains all of the alive or dead (apoptotic or necrotic) cells. In this study, nuclei of the platinum (II) complex treated cells, which were stained with Hoechst 33342 dye, was observed smaller and brighter compared to control. Calcein-AM can pass through cell membrane, but itself is not a fluorescent molecule. In living cells esterases (a group of enzymes) interacts with non-fluorescent Calcein-AM leading to the production of fluorescent Calcein, giving out green fluorescent. Therefore, Calcein-AM is utilized to label only living cells. On the other hand, PI is a fluorescent dye which can only pass through cells that have membrane damage, thereby staining specifically the late apoptotic/secondary necrotic and primary necrotic cells.

For this purpose, MCF-7 and MDA-MB-231 cells were seeded in a 96-well plate at the density of 1×10^4 cells per well in 100 μl culture medium. Then, the cells were treated with platinum (II) complex for 48 h. Following the treatment, the cells were incubated for 20 min in the dark at 37 °C with the staining solution (Hoechst 33342, PI and Calcein-AM) of Cell Viability Imaging Kit (Roche, Mannheim, Germany) as instructed by the manufacturer.

2.3.2. Evaluation of Annexin-V staining and caspase 3/7 activity by Muse cytofluorimetric analyzer

The Annexin V/Dead Cell kit utilizes a fluorescent dye conjugated to Annexin-V to detect phosphatidylserine on the external membrane of apoptotic cells and a cell death dye 7-AAD that provides information on the membrane integrity or cell death. 7-AAD is excluded from living healthy cells, as well as early apoptotic cells. The caspase-3/7 Kit determines the percentage of cells in various stages of apoptosis based on activity of caspase-3/7. This kit also utilizes the same dead marker, 7-AAD. By observing the events of graphical plots of both Annexin V and caspase-3/7 assays, populations of cells were classified into four distinguished groups:

Viable cells: Annexin V (−) or caspase 3/7 (−) and 7-AAD (−), lower-left quadrant (LL).

Early apoptotic cells: Annexin V (+) or caspase 3/7 (+) and 7-AAD (−), lower-right quadrant (LR).

Late apoptotic cells: Annexin V (+) or caspase 3/7 (+) and 7-AAD (+), upper-right quadrant (UR).

Cells that have died through non-apoptotic pathway: Annexin V (−) or caspase 3/7 (−) and 7-AAD (+), upper-left quadrant (UL).

Cells were seeded in a 6-well culture plate at a density of 2×10^5 cells/well were cultured overnight in RPMI supplemented with 5% fetal bovine serum. The next day cells were exposed to different doses of the platinum (II) complex (6.25, 12.5 and 25 μM) for 6 and 12 h. MCF-7 and MDA-MB-231 cells were harvested by trypsinisation and analyzed for the detection of early/late apoptosis and cell death mode using Annexin V/Dead Cell (kit MCH100105, Millipore, Darmstadt, Germany) and caspase 3/7

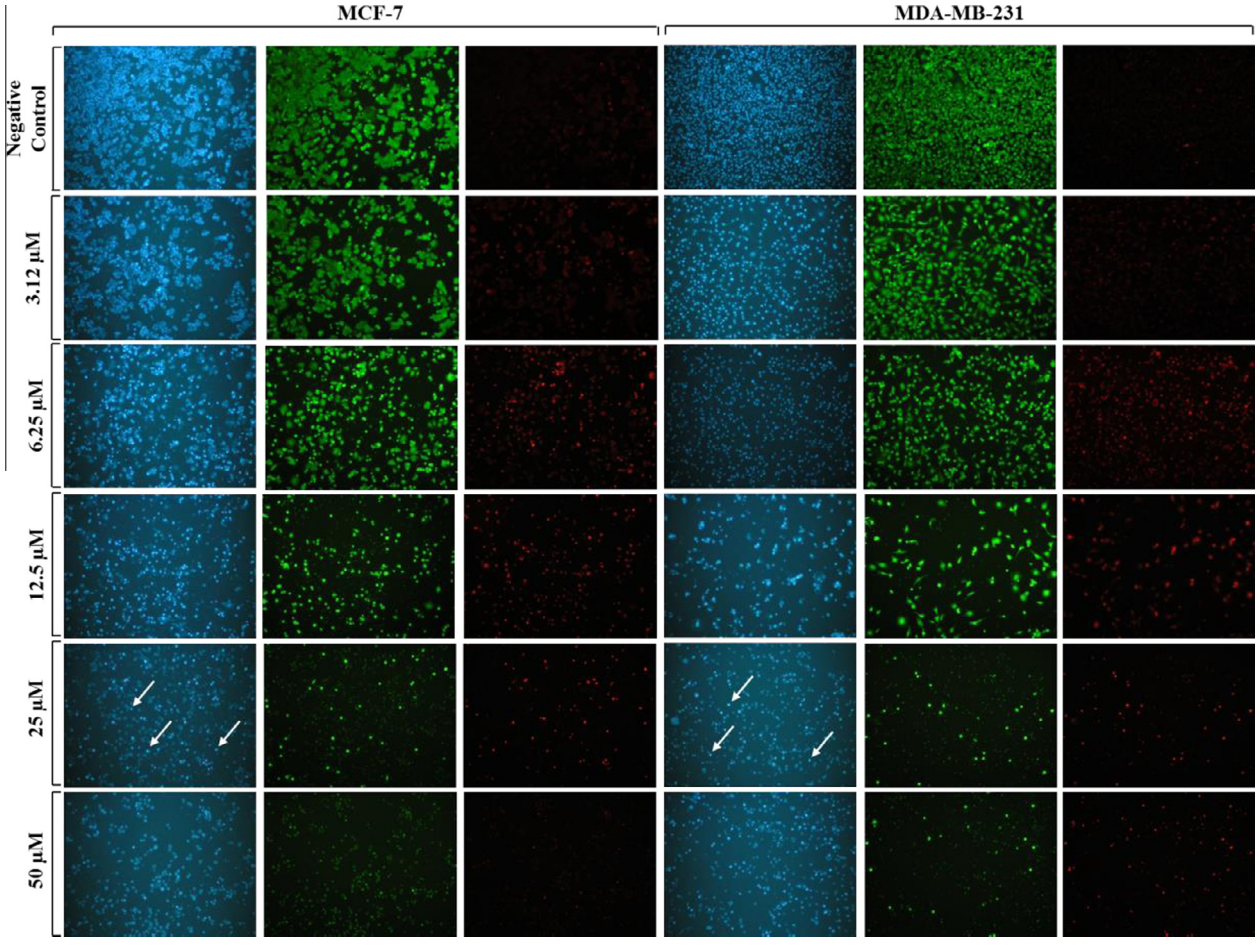

Figure 4. Visualization of cell death by fluorescent microscopy. Images of MCF-7 and MDA-MB-231 cells stained with Hoechst 33342 (blue), Calcein-AM (green) or PI (red) after being treated with six different concentrations ranging between 3.12 and 50 μM of platinum (II) complex for 48 h. Arrows indicate pyknotic nuclei which is a well known feature of apoptosis.

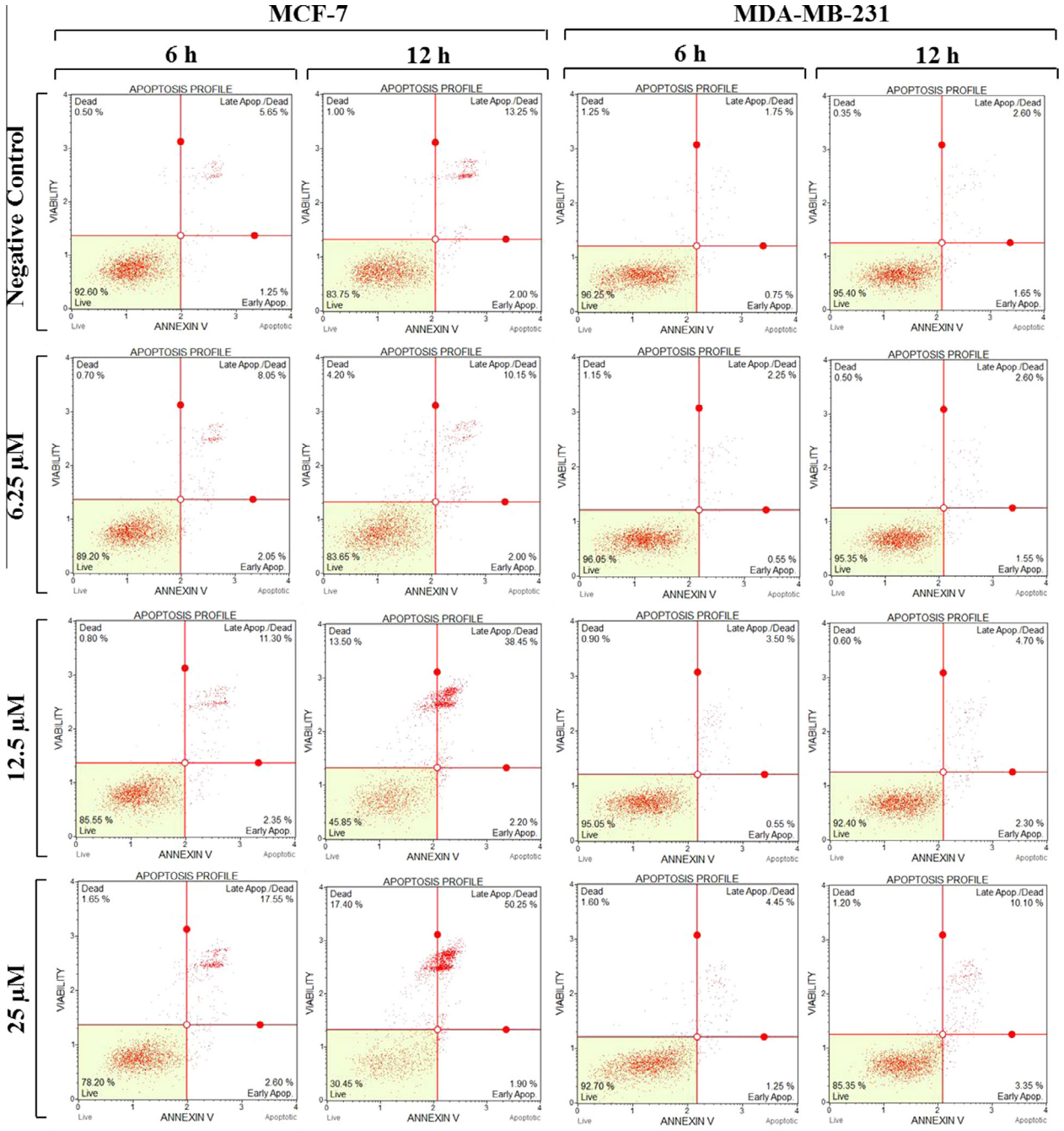

Figure 5. Flow cytometric analysis of Annexin-V Staining. MCF-7 and MDA-MB-231 cells were treated with platinum (II) complex (6.25, 12.5 and 25 µM) for 6 and 12 h. Based on positivity for phosphatidylserine exposure (positivity for Annexin V) in apoptotic cells and simultaneous detection of dead cells (7-AAD dye positivity), four population detected in each sample by fluorimetric separation: non-apoptotic live (lower left (LL)), non-apoptotic dead (upper left (UL)), early apoptotic (lower right (LR)) and late apoptotic (upper right (UR)).

(kit MCH100108, Millipore, Darmstadt, Germany), respectively, according to the manufacturer's instructions.

2.3.3. RT-PCR

One million cells per well were cultured overnight in 6-well cell culture plates in RPMI supplemented with 5% fetal bovine serum. The next day cells were exposed to 25 µM of platinum (II) complex for 6 h. At the end of the incubation period MCF-7 and MDA-MB-231 cells were harvested for RNA isolation. Cellular RNA was isolated using the High Pure RNA Isolation Kit (Roche, Mannheim, Germany). 1 µg of each total RNA sample was used for cDNA synthesis with the Transcriptor First Strand cDNA Synthesis Kit (Roche, Mannheim, Germany). A single cDNA synthesis reaction using 5 µg total RNA was used as template for each RealTime ready Human Apoptosis Custom Panel (Roche, Mannheim, Germany). Total PCR reaction volume per well was 20 µl with the LightCycler480 Probes Master (Roche, Mannheim, Germany). Sample setup and analysis were accomplished using the macro file for each gene panel with the LightCycler 480 Software 1.5.

2.3.4. SDS–PAGE and Western blotting

Cells were seeded in 25 cm^2 flasks and treated with platinum (II) complex (6.25, 12.5 and 25 µM) when the cells reached 70% confluency. The cells were scraped and washed with ice-cold PBS after 24 h treatment. The cells were then lysed in RIPA lysis buffer (Santa Cruz Biotechnology Inc., CA, USA) containing protease

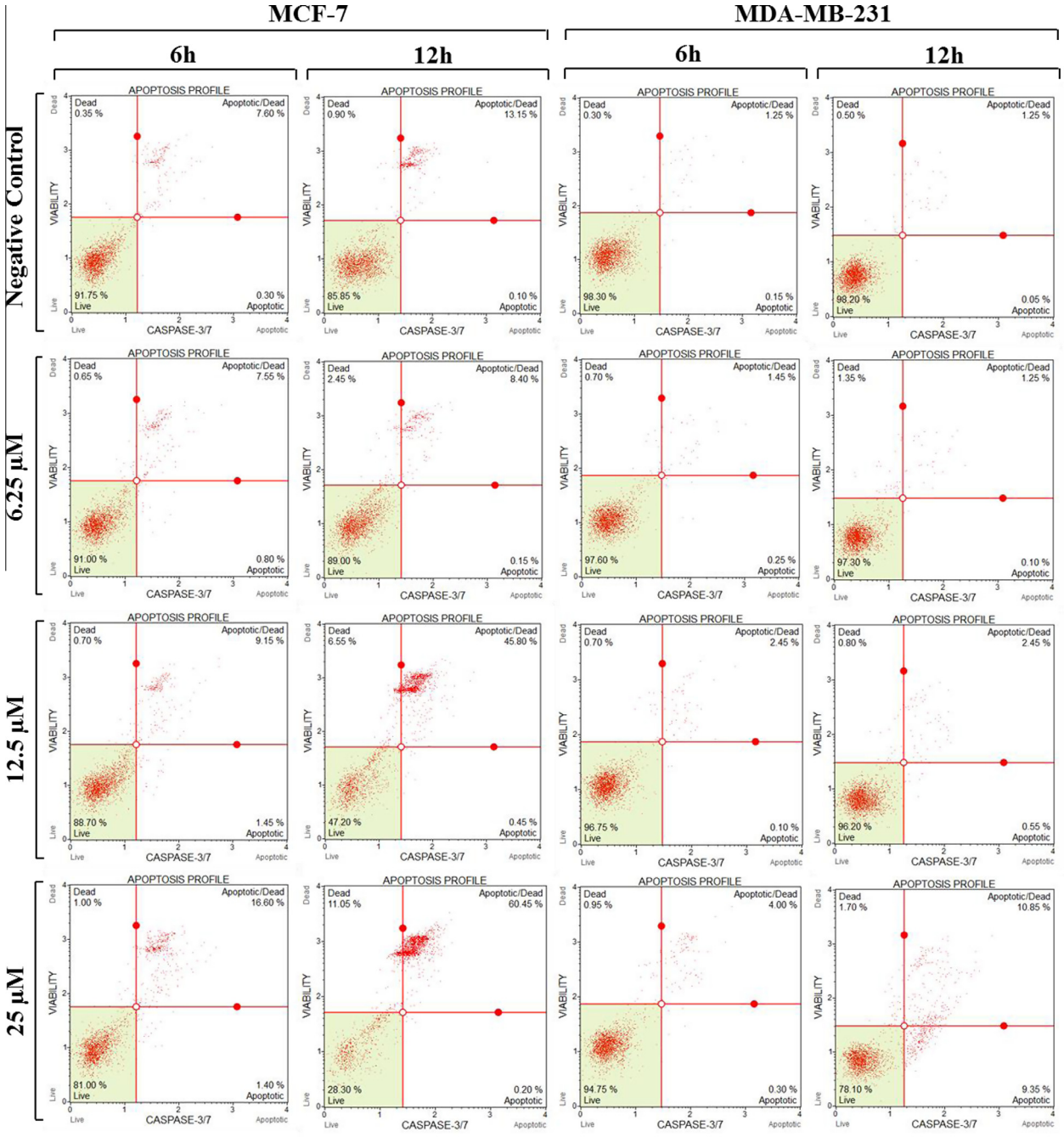

Figure 6. Flow cytometric activity of caspase 3/7. MCF-7 and MDA-MB-231 cells were treated with platinum (II) complex (6.25, 12.5 and 25 µM) for 6 and 12 h. Based on positivity for caspase 3/7 activity in apoptotic cells and simultaneous detection of dead cells (7-AAD dye positivity), four population detected in each sample by fluorimetric separation: non-apoptotic live (lower left (LL)), non-apoptotic dead (upper left (UL)), early apoptotic (lower right (LR)) and late apoptotic (upper right (UR)).

inhibitors at 4 °C for 30 min. The solutions were centrifuged at 4 °C for 10 min at 10,000*g* to separate proteins from other cellular particles. Equal amounts of protein (40 µg protein/lane) were subjected to 12% SDS–PAGE and then transferred to a nitrocellulose membrane. Western blotting was performed using rabbit anti-PARP monoclonal antibody (1:1000 dilution; Cell Signaling, MA, USA), rabbit anti-cleaved caspase-3 antibody (1:1000 dilution; Cell Signaling, MA, USA), rabbit anti-procaspase 8 antibody (1:1000 dilution; Cell Signaling, MA, USA) and rabbit anti-β-actin monoclonal antibody (1:1000 dilution; Cell Signaling, MA, USA). HRP-linked anti-rabbit IgG antibodies (1:2000 dilution; Cell Signaling, MA, USA) and LumiGLO reagent and horse radish peroxide (Cell Signaling, MA, USA) were used to detect primary antibodies according to the manufacturer's instructions. The membrane was stripped for subsequent detections with different antibodies. Bound antibodies were visualized on Fusion FX-7 imaging device (Vilber Lourmat, Torcy, France).

2.4. Statistical analyses

All statistical analyses were performed by using the SPSS 20.0 statistical software for Windows. The significance was calculated using one-way analysis of variance (ANOVA). A value of $p < 0.005$ was considered statistically significant. Results were expressed as mean ± SD.

3. Results and discussion

3.1. Anti-growth activity of platinum (II) complex

Anti-growth activity of platinum (II) complex was investigated by employing the MTT assay and then the results were confirmed by a more sensitive assay, the ATP assay (Fig. 2). The viability was assessed 48 h after treatment with platinum (II) complex at six different concentrations ranging between 1.56 and 50 μM. It was found that the treatments exhibited statistically significant anti-growth effects in a dose-dependent manner at most of the doses applied compared to the control (marked as * and ° in the relevant figure) ($p < 0.005$). According to the dose response curves, the complex had same growth-inhibitory effect on both cells.

IC_{50} and IC_{90} values were calculated on the basis of the results of the ATP assay and were shown in Table 1. IC_{50} values were 8.08 and 4.92 μM for MCF-7 and MDA-MB-231 cell lines, respectively. MDA-MB-231 cell line was slightly more sensitive to the complex, compared to MCF-7 cells. Cisplatin was used as a reference drug and IC_{50} values were determined as 18.02 μM for MCF-7 and 17.82 μM for MDA-MB-231 cell lines after 48 h treatment by the ATP assay (Supplemental data). Thus, resulting IC_{50} values from the treatment with platinum (II) complex for both of the cell lines are considerably lower compared to cisplatin.

The anti-growth effect of the platinum (II) complex was also investigated by the RTCA system (xCELLigence®) for further analyses (Fig. 3). This system enables to determine the individual doses of the complex causing the cytotoxic, cytostatic or anti-proliferative effects. The application of the complex carried out 24 h after seeding the cells (as shown by arrow). The relatively higher doses of 25 and 50 μM caused a cytotoxic effect on both cell lines. 12.5 μM was also cytotoxic to MDA-MB-231 cells whereas it seems to cause a cytostatic effect on MCF-7 cells. At 6.25 μM and lower doses, the complex resulted in antiproliferative effect on both cell lines. The results indicated that MDA-MB-231 cells was slightly more sensitive than MCF-7 cells which were in accordance with the results of MTT and ATP viability assays.

Platinum (II) complexes have potential antitumor effects for different cancer types.[19–21] Indeed, we found that the platinum (II) complex had a significant anti-growth effect against MCF-7 and MDA-MB-231 human breast cancer cell lines in a dose dependent manner. In a previous study in which a platinum (II) complex was used against MCF-7 and MDA-MB-231 breast cancer cells, it was reported that the IC_{50} values were 32 ± 2 μM and 20 ± 2 μM, respectively.[22] This difference may rely on the structure of the platinum (II) complexes.

3.2. Cell death mode induced by platinum (II) complex

3.2.1. Flourescence staining

The detection of cell death mode was first made on the basis of both the nuclear morphology and the cell membrane integrity using a fluorescent microscope. For this purpose MCF-7 and MDA-MB-231 cells were treated with all doses of platinum (II) complex for 48 h except the dose of 1.56 μM that is not toxic to both cells. Following treatments, the cells were stained with the triple staining solution. It was observed that doses of 6.25–50 μM of the complex resulted in cell shrinkage and chromatin condensation in both cell types which are hallmarks of apoptosis in a dose-dependent manner (Fig. 4). At higher doses of the complex (25 and 50 μM) these cells were secondary necrotic evidenced by pyknotic nuclei with PI staining positivity. In addition, cell density reduced in a dose dependent manner which also confirmed the results of the viability assays. The elucidation of the mechanism by which the agents induce apoptosis is important in terms of the translation of the knowledge into the clinics.[23] We, therefore, investigated the mechanism by which the platinum (II) complex induced apoptosis.

3.2.2. Quantitative analysis of live, early/late apoptotic, dead cells and caspase 3/7 activity

The present complex was shown to induce apoptotic cell death (mainly late apoptosis) in a time and dose-dependent manner in both cells (Fig. 5). This observation was further supported by flow cytometric analysis of caspase 3/7 activity, which revealed a significant increase in late apoptotic cells in both cell lines (Fig. 6). Increases of Annexin-V (+) and caspase 3/7 (+) cells in MCF-7 cells was more profound compared to MDA-MB-231 cells in both assays. The percentage of Annexin-V (+) cells in MDA-MB-231 slightly increased in a time and dose dependant manner although as not apparent as in MCF-7 cells. Moreover, changes in the nuclear morphology and the presence of caspase 3/7 activity provided an evidence that MDA-MB-231 cells underwent apoptosis.

Numerous studies reported that several platinum (II)-derived complexes induced apoptosis in MCF-7 and MDA-MB-231 breast cancer cell lines.[24,25,9] Among these studies Agnieszka et al. reported that platinum (II) derivatives were more effective than cisplatin and led to the increase in the levels of active caspases 3, 8 and 9.[9] The results of the present work imply that platinum (II) complex may induce apoptotic cell death through the caspase dependent pathway in MCF-7 and MDA-MB-231 cell lines.

3.2.3. Determination of apoptosis-related gene expressions

To confirm the apoptosis at gene level, the expressions of some apoptosis-related genes (BCL2L10, BIK, BAX, BCL-2, FASLG, HRK, TNFRSF10A, TNFRSF10B) in both cell lines were measured after the treatment with 25 μM (a toxic dose) of platinum (II) complex for 6 h (a previously decided best time point for such experiment). Figure 7 shows that FASLG (a ligand for cell surface death receptor, FAS) and anti-apoptotic BCL2L10 was increased about 90-fold in MCF-7 cells compared to untreated control cells. The expression of HRK gene (pro-apoptotic gene) is also significantly increased in this cell line. Similarly, in one of our previous studies,[26] the combination treatment of fenretinide and indole-3-carbinol increased the expression of FASLG and HRK genes in MCF-7 cells, implying that these genes may play a primary role in apoptosis of this cell line. Interestingly, expressions of these two genes decreased about 8-fold (FASLG) and 5-fold (HRK) compared to untreated cells in MDA-MB-231 cells, implying no function of these genes in the apoptotic machinery of this cell line. Moreover, there was no increase in fold change in apoptosis-related gene expressions studied. In fact we expected apoptosis-suggesting expressions in this

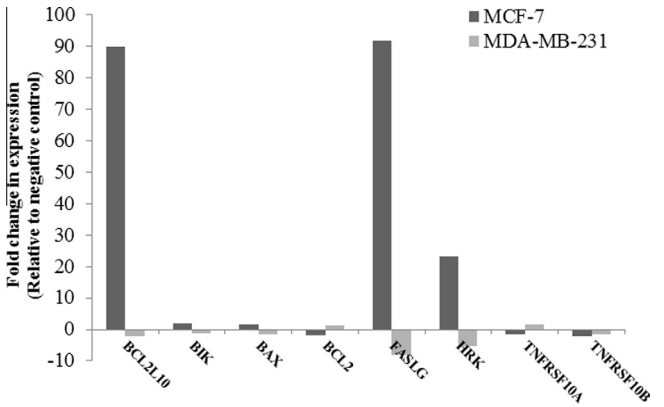

Figure 7. Real-time PCR analysis of apoptosis-related genes. MCF-7 and MDA-MB-231 cells were treated with 25 μM (a toxic dose) of platinum (II) complex for 6 h (a previously decided best time point) and then the analysis was performed as explained in the materials and methods.

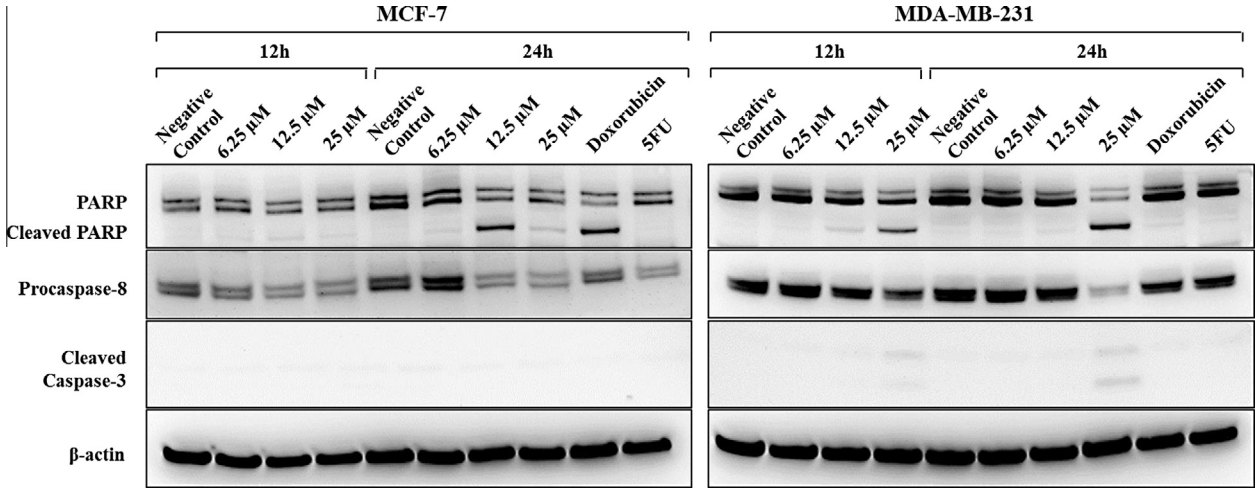

Figure 8. Western blot analysis of apoptosis-relevant protein levels. Cells were treated for 24 h with 6.25, 12.5 and 25 μM of platinum (II) complex for 24 h. Doxorubicin and 5-FU were used as positive control treatments. β-Actin was used as loading control.

cell line. This paradox led us to suggest the possibility that other pro-apoptotic genes (e.g., TNFR, p53, bid, bim) could have been involved in the cell death of this cell line.

3.2.4. Confirmation of apoptosis by Western blotting

Caspase activation (cleavage of procaspase to active caspase) is regarded as a hallmark of apoptosis[27] and caspase 3 is a central effector caspase in many types of cells and mediates the cleavage of itself, other downstream caspases, and other caspase substrates such as cytokeratin 18, and PARP.[28] To detect these apoptosis-relevant protein (cleaved PARP and caspase 3, total caspase 8) levels, we treated the MCF-7 and MDA-MB-231 cells for 24 h with platinum (II) complex of 6.25, 12.5 and 25 μM. As shown in Figure 8 activation of caspase-3 and PARP cleavage at 25 μM concentration of platinum (II) complex suggest apoptotic cell death in MDA-MB-231 cell line which is consistent with the flow cytometric detection of increased caspase 3/7 activity at this dose and time point in this cell line. In addition, depletion of procaspase-8 suggests the activation of extrinsic apoptotic pathway. The amount of procaspase-8 decreased in various doses at both time points in MCF-7 cells. Interestingly, PARP cleavage observed at 12.5 μM concentration, but not at 25 μM, hence suggesting the occurrence of possible cell necrosis at 25 μM dose. The expression of cleaved caspase-3 was not expected in MCF-7 cells because they do not express this protein. Therefore, we suggest that platinum (II) complex may induce apoptotic cell death through the caspase dependent pathway in MCF-7 and MDA-MB-231 cell lines. In one of our recent studies, a platinum (II) saccharinate complex of hydroxymethylpyridine also triggered apoptosis with increased caspase-3 activity and cleaved PARP levels in A-549 lung cancer cells.[29]

In conclusion, the present platinum (II) complex demonstrated anti-cancer activity by inducing apoptosis in MCF-7 and MDA-MB-231 breast cancer cells in a dose-dependent manner. These findings might advance future anti-cancer drug design strategy for breast cancer treatment and thus the synthesis of more *trans*-Pt (II) complexes may be required, which warrants for future in vitro and in vivo studies.

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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.2015.06.037.

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