Supporting Information

(Carboxydiamine)Pt(II) complexes of a combretastatin A-4 analogous chalcone: influence of the diamine ligand on DNA binding and anticancer effects

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General synthetic information

Reagents and solvents (p.a.) were purchased from Acros, Aldrich, Alfa Aesar, or Merck. (D,L)-2,3-Di-t-butoxycarbonylaminopropionic acid (4) was prepared according to a literature procedure. For column chromatography Merck silica gel 60 (230-400 mesh) and distilled solvents were used. The following instruments were used: melting points (uncorrected), Electrothermal 9100; IR spectra, Perkin-Elmer Spectrum One FT-IR spectrophotometer with ATR sampling unit; nuclear magnetic resonance spectra, Bruker Avance 300 spectrometer; chemical shifts are given in parts per million (δ) downfield from tetramethylsilane as internal standard; mass spectra, Varian MAT 311A (EI); microanalyses, Perkin-Elmer 2400 CHN elemental analyzer.


Microscopy with Giemsa staining

A 6-well plate was prepared by attaching round optical-glass slides (Ø 1.5 cm) to the bottom of the wells. A confluent cell layer in a Petri dish was trypsinised and diluted with medium to a cell concentration of 0.75×10^5 cells/mL. Each well was filled with 2 mL of this suspension so that the round optical-glass slides were completely covered. For attachment of the cells the plate was incubated at 37°C and 5% CO₂ for about 24 h. The X-cross was performed with 10 μL tips and IC₁₀ concentrations of the test substances were added to the wells (one compound per well). As controls one well was filled with a 1:1000 dilution of DMF, another one with the plain cell culture. The plate was incubated (5% CO₂ and 95% humidity, 37°C) for various periods, the medium was discarded and the adherent cells were washed with PBS and a mixture of PBS and methanol (1:1.5), and covered with methanol for 15 min for fixation on the surface of the glass plates. The solvent was discarded and the cells were washed with fresh methanol. In order to stain the melanoma cells the glass plates were completely covered for 5 min with a 1:15 dilution of Giemsa dye, prepared with methanol/water (7:3). Tap water was added to the wells (about twice the volume of the Giemsa dilution), incubation was continued for 2 min, and the aqueous Giemsa solution was discarded. After rinsing with water the glass plates with the cells were fixed and dried on microscopy slides. Pictures of the cell samples were taken with an optical microscope and the software AxioVs 40 V 4.5.0.0 (Carl Zeiss Imaging Solutions GmbH) and analysed with the AxioVision program.
NMR-kinetics of reaction of complex 3 with N-acetyl-L-cysteine

**Figure.** $^1$H NMR monitoring of the reaction of complex 3 (5 mM) with N-acetyl-L-cysteine (50 mM) in DMF-d$_7$ at room temperature and under exclusion of moisture.
Figure: $^1$H NMR spectrum of the reaction of complex 3 (3.1 mM) with 10 equiv. $N$-acetyl-$L$-cysteine (31 mM) in D$_2$O/DMF-$d_7$ (1:1, v/v) at room temperature after 18 h.

The reaction proceeded faster than in the corresponding experiment run under water free conditions. 2/3 of starting complex 3 were converted into the mono-adduct after 18 h. The resonances of 2H ($\delta = 9.81$ ppm) and 4H ($\delta = 8.55$ ppm) protons shifted towards higher field [$\delta = 9.28$ ppm (2H-C); $\delta = 8.36$ ppm (4H-C)] in the cysteine adduct.

Experimental section

A solution of DMF-$d_7$/D$_2$O (1.2 mL, 1:1, v/v) containing complex 3 (2.8 mg, 0.0037 mmol, 3.1 mM) and a ten-fold excess of $N$-acetyl-$L$-cysteine (6.1 mg, 0.037 mmol, 31 mM) was filled into an NMR tube and incubated at room temperature for 18 h. $^1$H NMR spectra were recorded on a Bruker DRX-300 spectrometer (300 MHz) immediately and after 18 h. Chemical shifts ($\delta$) are given in parts per million (ppm) downfield from tetramethylsilane (TMS) as internal standard.
Cell culture conditions
Sources of cells: HL-60: German Collection of Biological Material (DSMZ), Braunschweig; melanoma 518A2: Department of Oncology, University Halle; HCT-116: University Hospital Erlangen (all Germany); BxPC-3 and MDA-MB-231: American Type Culture Collection (Manassas, VA). HL-60 and HCT-116 cells were grown in RPMI-1640 medium, 518A2 cells in Dulbecco’s modified Eagle medium (DMEM), both supplemented with 10% fetal calf serum (FCS), 100 IU/mL penicillin G, 100 µg/mL streptomycin sulfate, 0.25 µg/mL amphotericin B, and 250 µg/mL gentamycin (all from Gibco, Egenstein, Germany). BxPC-3 and MDA-MB-231 cells were grown in DMEM medium supplemented with 10% FBS, 100 units/mL of penicillin and 100 µg/mL of streptomycin. The cells were maintained (5% CO2, 95% humidity, 37 °C) and serially passaged after trypsinisation with 0.05% trypsin / 0.02% EDTA.

Growth inhibition (MTT) assay
MTT was used to identify viable cells that reduce it to a violet formazan.\(^2\) HL-60 cells (5×10^5 mL^{-1}), 518A2 and HCT-116 cells (5×10^4 mL^{-1}), BxPC-3 cells (3×10^3 cells/well), and MDA-MB-231 cells (2×10^3 cells/well) were seeded and cultured for 24 h on 96-well microplates. Incubation (5% CO2, 95% humidity, 37 °C) of cells following treatment with the test compounds (dilution series ranging from 0.0001 to 100 µM in DMSO) was continued for 48 or 72 h. Blanks and solvent controls were treated identically. In the case of the BxPC-3 and MDA-MB-231 cells, 25 µL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for further 2 h at 37 °C. Upon termination, the supernatant was aspirated and the formazan was dissolved in isopropanol (100 µL) by mixing for 30 min on a gyratory shaker. The absorbance was measured at 595 nm on an Ultra Multifunctional Microplate Reader (TECAN, Durham, NC). In the case of the other cells, a 5 mg mL^{-1} stock solution of MTT in phosphate buffered saline was added to a final MTT concentration of 0.05% (HL-60, 518A2). After 2 h the precipitated formazan was dissolved in 10% sodium dodecylsulfate in DMSO containing 0.6% acetic acid in the case of HL-60 cells. For the adherent cells the microplates were swiftly turned, flicked and blotted to discard the medium prior to adding the mixture. The microplates were shaken in the dark (30 min) and left in the incubator (8 h). Absorbance at 570 nm and 630 nm was measured with an ELISA microplate reader (MWG-BIOTECH). Experiments were carried out in triplicate, the percentage of viable cells quoted was calculated as the mean ± S.D. with respect to the controls set to 100%.

Apoptosis induction by complexes 3 and 6 (DNA–Histone ELISA assay)

The Cell Death Detection Kit (Roche, Palo Alto, CA) was used to detect apoptosis in BxPC-3 pancreatic and MDA-MB-231 breast cancer cells. Briefly, cells were treated with test compounds for 72 h. After treatment, the cytoplasmic histone/DNA fragments from these cells were extracted and incubated in microtiter plate modules coated with anti-histone antibodies. Subsequently, the peroxidase-conjugated anti-DNA antibody was used for the detection of immobilised histone/DNA fragments followed by colour development with ABTS substrate for peroxidase. The absorbance of the samples was determined by using an Ultra Multifunctional Microplate Reader (TECAN) at 405 nm.


Figure. Apoptosis rate of MDA-MB-231 breast carcinoma cells treated with compounds 3 and 6 for 72 h as to DNA-histone ELISA assays.