Cytotoxic Activity of Platinum(II) and Palladium(II) Complexes of *N*-3-Pyridinylmethanesulfonamide: the Influence of Electroporation

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The series of complexes: *cis*-$[Pd(PMSA)_2X_2]$, *cis*-$[Pt(PMSA)_2X_2]$, *trans*-$[Pt(PMSA)_2I_2]$ and $[Pt(PMSA)_4]Cl_2$ (PMSA = *N*-3-pyridinylmethanesulfonamide; X = Cl, Br, I), previously synthesized and characterized by us, as well as the free ligand PMSA, were tested for their cytotoxic activity without electroporation – against murine leukemia F4N and human SKW-3 and MDA-MB-231 tumour cell lines – and with electroporation – against the latter two cell lines. The majority of the complexes exhibited cytotoxic effects ($IC_{50} < 100$ μmol/l) under the conditions of electroporation. Both *cis*- and *trans*-$[Pt(PMSA)_2I_2]$ had pronounced cytotoxic effects (29–61 μmol/l against MDA-MB-231 cells).

Key words: Platinum Complexes, Sulfonamides, Cytostatic Agents, Electroporation

Introduction

cis-Diamminedichloroplatinum (*cis*-$[Pt(NH_3)_2Cl_2]$, cisplatin) is the first and still the most important metal-based antineoplastic drug, which has been widely applied in cancer chemotherapy for the treatment of lung, head and neck, ovarian, bladder and testicular cancers (Boulikas and Vougiouka, 2003; Cohen and Lippard, 2001). The drug cisplatin has, however, numerous disadvantages, *e.g.* nephro- and neurotoxicity, as well as other severe side-effects and low aqueous solubility (Boulikas and Vougiouka, 2003; Kostova, 2006a). The search for metal cytostatics with improved therapeutic characteristics has led to the synthesis and screening of a vast number of new platinum and other metal complexes (Bose, 2002; Kostova, 2006b; Reedijk, 2003; Stordal *et al.*, 2007). A significant disadvantage of the "classical" (conventional) cisplatin analogues is their inefficacy against cisplatin-resistant tumours. For this reason, a topical trend is the design of "non-classical" platinum complexes which differ not only in their structure from cisplatin, but display distinct mechanisms of action and hence are quite promising for treating cisplatin-resistant tumours (Kostova, 2006a; van Zutphen *et al.*, 2006). After Farrell *et al.* (1989) and Van Beusichem and Farrell (1992) established the cytostatic activity of Pt(II) pyridine complexes with *trans* configuration, the interest in platinum coordination compounds with planar *N*-heterocyclic ligands considerably increased and now *trans*-platinum complexes with such ligands acquire the significance of a new class of non-conventional platinum cytostatic agents, that retain their activity against cisplatin-resistant cells (Jakupec *et al.*, 2003; Kostova, 2006a; Coluccia and Natile, 2007).

On the other hand, the antitumour activity of sulfonamide derivatives and its relation to the processes of enzyme inhibition have attracted much attention in the last years (Scozzafava *et al.*, 2003; Supuran *et al.*, 2004). Numerous metal complexes with sulfonamide ligands act as carbonic anhydrase inhibitors (Supuran *et al.*, 1994, 1996), and some of them exhibit cytotoxic activity (Chohan *et al.*, 2005). The molecule of *N*-3-pyridinylmethanesulfonamide (PMSA) combines both structural fragments, a *N*-heterocycle and a sulfonamide group. We have performed an *ab initio* quantum chemical and IR spectroscopic study on PMSA (Dodoff, 2000) and have firstly synthesized and structurally characterized Pd(II)

and Pt(II) complexes of this ligand (Dodoff, 2001; Dodoff *et al.*, 2004).

The application of high electric field pulses (rectangular or exponential) leads to a transient permeabilisation of the cellular membrane, and this phenomenon is known as electroporation (Gehl, 2003). Electroporation permits exogenous membrane proteins or DNA to be inserted into the cells (Neumann *et al.*, 1996; Tsoneva *et al.*, 2005). Electroporation, combined with the chemotherapeutic drugs bleomycin and cisplatin (electrochemotherapy), finds advanced application for the enhanced delivery of drugs into solid tumours (Serša *et al.*, 2000; Spugnini and Porrello, 2003; Fujimoto *et al.*, 2005; Spugnini *et al.*, 2008). A valuable advantage of this modern drug delivery method is the selective application of the drug onto the tumour (*e.g.* melanoma) while preserving the normal tissues (Serša *et al.*, 2000; Peycheva and Daskalov, 2004).

Here we report the cytotoxic effect of PMSA and its Pd(II) and Pt(II) complexes **1**–**6** (Fig. 1) under usual conditions, *i. e.* without applying an electric field and under the conditions of electroporation.

Material and Methods

Compounds

The ligand PMSA was prepared by a known procedure (Jones and Katritzki, 1961) and purified as described by us (Dodoff, 2000). The synthesis of the complexes tested, **1**–**6**, was described in detail elsewhere (Dodoff, 2001). Cisplatin of the quality *pro analysi* and *N,N*-dimethylformamide (DMF), >99.8%, were purchased from Fluka. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and (3*Z*)-5-amino-3-[[4-[4-[(2*Z*)-2-(8-amino-1-oxo-3,6-disulfonaphthalen-2-ylidene)hydrazinyl]-3-methylphenyl]-2-methylphenyl]hydrazinylidene]-4-oxonaphthalene-2,7-disulfonic acid (trypan blue) were from Sigma.

Cell lines

Murine erythroleukemia cells, clone F4N (Dube *et al.*, 1975), were cultured in Dulbecco's modified Eagle medium (Gibco, Grand Island, NY, USA) supplemented with 10% calf serum, under 5% CO_2 atmosphere at 37 °C, and passaged every day at a concentration of $5 \cdot 10^5$ cells/ml.

The human T-cell leukemia cell line SKW-3 was established from the peripheral blood of a man with T-cell chronic lymphocytic leukemia (Hirono *et al.*, 1979). The human breast cancer cell line MDA-MB-231 was originally derived from a pleural effusion and grown adherently (Cailleau *et al.*, 1974). Both cell lines were grown in RPMI-1640 medium (Lonza, Walkersville, USA), supplemented with 10% fetal calf serum (FCS) and 1% L-glutamine, at 37 °C in an incubator with humid atmosphere and 5% CO_2. The cells were passaged two or three times per week to keep them in the log phase.

Cytotoxicity assays and electroporation protocol

The compounds studied were dissolved immediately before use in DMF (cisplatin, PMSA and complexes **1**–**5**) or sterile distilled water (complex **6**) to obtain stock solutions, which were then diluted to obtain the desired final concentrations. Control samples (except those for **6**) were incubated in the presence of 1% DMF.

F4N cells

Exponentially growing cells were incubated in culture medium with varying concentrations (100, 200 and 400 μmol/l) of the test compounds at 37 °C in an atmosphere containing 5% CO_2. After 24, 48 and 72 h of drug exposure the cells were counted hemocytometrically. The number of dead cells was determined by staining with trypan blue. The cell growth inhibition was expressed as the percentage of living cells in the treated samples with respect to that of the control. The mean of three determinations was taken.

SKW-3 and MDA-MB-231 cells

The cells were harvested, centrifuged and resuspended in electroporation medium (0.3 M mannitol solution in sterile distilled water, with 0.1 mM Mg^{2+} and 0.1 mM Ca^{2+} added) to give a final concentration of $5 \cdot 10^6$ cells/ml ($5 \cdot 10^5$ cells in 100 μl of sample). To those 100-μl samples, the corresponding amounts of the drugs were added. The samples were subjected or not to electroporation (electroporated or non-electroporated samples). Immediately after the treatment all samples were additionally diluted with RPMI-1640 complete medium to give a total volume of 1 ml ($5 \cdot 10^5$ cells/ml), and then 100-μl fractions ($5 \cdot 10^4$ cells) from each sample were distributed

into an 8-well column of a sterile 96-well plate and left for 72 h for incubation (37 °C, 5% CO_2). The final concentrations of the compounds were 1, 10 and 100 μmol/l.

The electroporation of the samples was performed in chambers equipped with flat parallel electrodes (BioRad Laboratories, Richmond, CA, USA)and an electrode distance of 0.4 cm. Each electroporated sample was treated with one rectangular pulse of 1000 V/cm for 5 ms. A detailed description of the electroporation equipment used can be found in the literature (Haenze *et al.*, 1998). The controls of the electroporated set of samples were subjected to an electrical pulse in the absence of any drug. The controls of the non-electroporated set of samples were subjected neither to electrical nor to drug treatments.

The treated samples and the controls were seeded in 96-well plates (flat tissue bottom; Becton Dickinson, Heidelberg, Germany) at a density of $5 \cdot 10^4$ cells/100 μl well. The plates were placed for 72 h at 37 °C in an incubator with humidified atmosphere and 5% CO_2. For each treatment, at least 8 wells were used. The cell viability fraction was determined by the Mosmann MTT dye-reduction assay (Mosmann, 1983) with some modifications (Konstantinov *et al.*, 1998). In brief, after the incubation period of the samples, MTT solution (10 mg/ml) in phosphate buffer saline (PBS) was added (10 μl/well). The plates were further incubated for 4 h at 37 °C and the formazan crystals formed were dissolved by adding 110 μl acidified (0.04 M HCl) 2-propanol per well. Absorption was measured by an automated microtiter plate spectrophotometer (Anthos) at 540 nm, reference filter at 690 nm. A mixture of complete medium (100 μl), MTT stock (10 μl) and 110 μl acidified (0.04 M HCl) 2-propanol was used as blank solution. The cell growth inhibition was expressed as the percentage of the absorption of the treated sample with respect to that of the control. The mean of at least three determinations was taken.

The 50%-inhibitory concentration (IC_{50}) is defined as the drug concentration that reduces twice the number of living cells (trypan blue assay) or the absorption (MTT assay) as compared to the control. The IC_{50} values were obtained by linear regression using Origin 6.0 software.

Flow cytometry (FACS) analysis

The MDA-MB-231 cell line was used for these experiments. The samples were subjected to cisplatin or PMSA and its complexes at two different concentrations (10 and 100 μmol/l) in the presence or absence of electrical pulses and then further incubated for 72 h (37 ºC, 5% CO_2). After that, FACS analysis of each sample was performed according to Watson and Erta (1992). Briefly, controls or treated samples were pelleted, washed with cold PBS and re-suspended in 100 μl PBS and 300 μl of 96% ethanol. The cells were kept at –20 °C. On the day of FACS measurements, the samples were centrifuged and re-suspended in 500 μl PBS containing 20 μg/ml RNAse and 20 μg/ml propidium iodide at room temperature. $1–2 \cdot 10^4$ Cells/sample were analyzed with a FACS Calibur cell sorter (Becton Dickinson), equipped with a 488 nm air-cooled argon laser, using CellQuest software.

Results and Discussion

Cytotoxic effect

F4N cells

The compounds were at first tested against this cell line without electroporation. PMSA and its complexes did not exhibit important cytotoxic effects: the most active was complex **1** [IC_{50} = (248 ± 8) μmol/l (72 h of drug exposure)], whereas the IC_{50} values for the free ligand and the other complexes were above 400 μmol/l.

SKW-3 and MDA-MB-231 cells

The cytotoxic effect of the free ligand and its complexes (Fig. 1) was examined with and without electroporation. The results of cell growth inhibition assays are presented in Figs. 2 and 3, and the IC_{50} values are collected in Table I.

If we accept an IC_{50} value below 100 μmol/l as criterion for interesting activity, only the *cis*-diiodo complex **4** exhibited this degree of cytotoxic activity against SKW-3 cells without electroporation [IC_{50} = (69 ± 13) μmol/l], whereas its *trans*-analogue **5** showed marginal activity [IC_{50} = (102 ± 8) μmol/l]. The electroporation, however, considerably increased the cytotoxicity of all compounds tested against SKW-3 cells. Complexes **3**–**6** exhibited IC_{50} values lower than 100 μmol/l. The most active was again the *cis*-diiodo complex **4**, showing a significant effect [IC_{50} = (20 ± 2)

Table I. 50% Inhibitory concentrations (IC_{50}) of cisplatin, PMSA and its complexes against SKW-3 and MDA-MB-231 cells without and with electroporation. Values are means of at least three independent experiments ± standard deviations (SD).

Compound	IC_{50} ± SD [μmol/l]			
	SKW-3 cells		MDA-MB-231 cells	
	without electroporation	with electroporation	without electroporation	with electroporation
Cisplatin	36 ± 2	6 ± 1	46 ± 4	2 ± 2
PMSA	[a]	[c]	100 ± 12	80 ± 20
1	[b]	117 ± 8	61 ± 6	58 ± 5
2	[a]	134 ± 10	90 ± 11	32 ± 8
3	[a]	84 ± 6	71 ± 8	38 ± 7
4	69 ± 13	20 ± 2	56 ± 5	29 ± 6
5	102 ± 8	63 ± 5	61 ± 5	43 ± 9
6	[a]	94 ± 24	[b]	60 ± 14

[a] No inhibition of cell growth at concentrations up to 100 μmol/l.
[b] Not more than 20% inhibition of cell growth at concentrations up to 100 μmol/l.
[c] Not more than 30% inhibition of cell growth at concentrations up to 100 μmol/l.

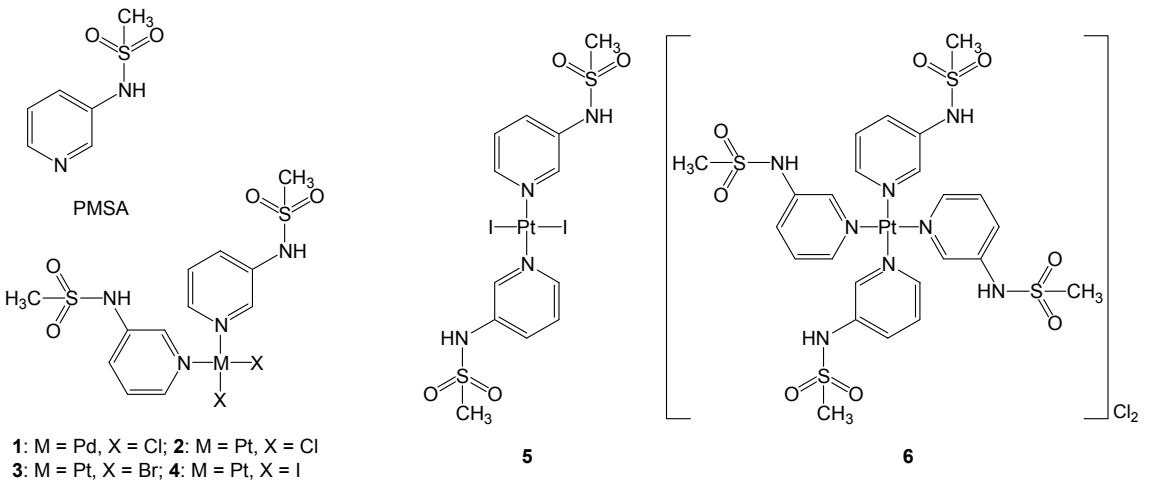

Fig. 1. Chemical structures of PMSA and its complexes **1**–**6**.

μmol/l], followed by its *trans*-isomer, which was three times less active. In this cell line the use of electroporation caused a 3.5-fold increase in the cytotoxicity of **4**, whereas for cisplatin the effect was six-fold, in comparison with the result without electroporation.

With the exception of the tetra-amine cationic complex **6**, all the tested compounds exhibited interesting cytotoxic activity (marginal value for PMSA) against MDA-MB-231 cells, even without electroporation. The activity of **1**, **4** and **5** was practically the same ($IC_{50} \approx 60$ μmol/l), and it was only 1.3-fold smaller than that of cisplatin. This result is especially important taking into account that MDA-MB-231 is a cisplatin-resistant tumour cell line (Chu *et al.*, 2005). Under the conditions of electroporation all the compounds, including the free ligand, showed increased cytotoxic activity against this cell line. As before, the *cis*-diiodo Pt(II) complex **4** was the most active [IC_{50} = (29 ± 6) μmol/l]. Practically the same activity exhibited its dichloro analogue **2**. The *trans*-diiodo complex **5** also showed a considerable cytotoxicity [IC_{50} = (43 ± 9) μmol/l]. In the case of MDA-MB-231 cells, the electroporation caused a more than 20-fold increase in the cisplatin cytotoxicity [IC_{50} =

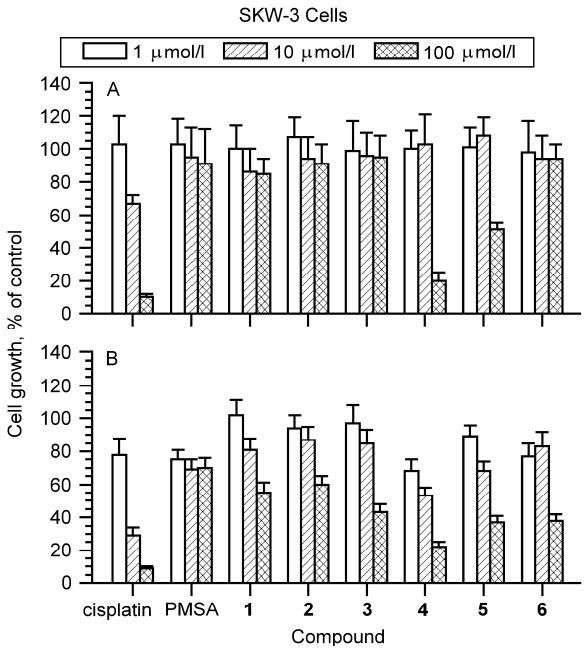

Fig. 2. Cytotoxic activity of different concentrations of cisplatin, PMSA and its complexes **1**–**6** on SKW-3 cells (A) without and (B) with electroporation. Values are means of at least three independent experiments ± standard deviations.

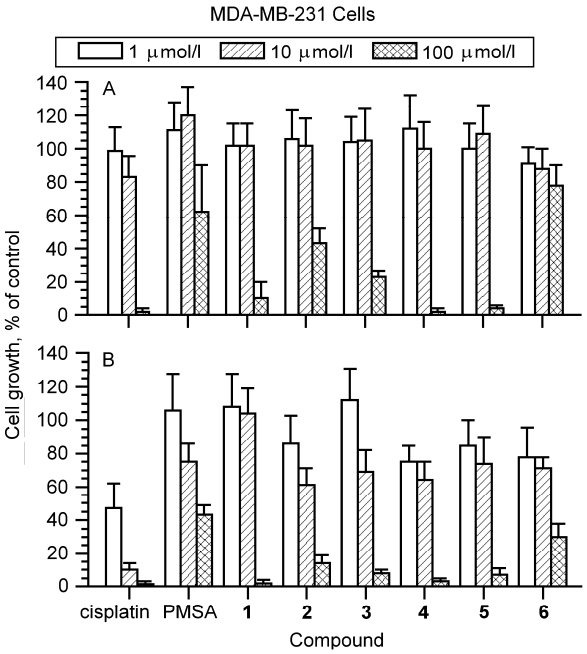

Fig. 3. Cytotoxic activity of different concentrations of cisplatin, PMSA and its complexes **1**–**6** on MDA-MB-231 cells (A) without and (B) with electroporation. Values are means of at least three independent experiments ± standard deviations.

(2 ± 2) μmol/l]. For the dichloro Pt(II) complex **2**, the activity-enhancing effect of electroporation was three-fold, whereas there was no effect in the case of its Pd(II) analogue.

Cell cycle FACS analysis

The cell cycle FACS measurements were used to determine whether the new compounds provoke an apoptotic response among the treated cell population and to compare this effect with that of cisplatin. The red fluorescence intensity of propidium iodide (with emission maximum at *ca.* 620 nm) was used for the quantitative determination of the distribution among the cell cycle phases in each sample. A representative set of results including the control, cisplatin and complex **3** are illustrated in Fig. 4. The ligand and the remaining complexes studied gave quantitatively the same pattern. The FACS analysis results showed that electroporation alone did not influence dramatically the apoptosis level of the sample, but treatment with 10 μmol/l cisplatin and electroporation increased the amount of apoptotic cells after 72 h of incubation. The induction of apoptosis by cisplatin is well known and was described previously (Henkels and Turchi, 1999; Park *et al.*, 2002; Schwerdt *et al.*, 2005). Unlike cisplatin, however, the treatment with PMSA and its complexes did not provoke a pronounced apoptotic response within the sample sets subjected to electroporation or not (Fig. 4), even at a concentration of 100 μmol/l. This finding implies that the mechanism of cytotoxic action of the compounds studied differs from that of cisplatin and, probably, does not involve apoptosis triggering. It should be pointed out, however, that some authors suggest a different mechanism of cell death induction for cisplatin as well (Gonzalez *et al.*, 2001).

Conclusion

Most representatives of the Pt(II) and Pd(II) complexes with the sulfonamide substituted pyridine ligand PMSA exhibited cytotoxic activity against SKW-3 and MDA-MB-231 tumour cells

under the conditions of electroporation. Against the cisplatin-resistant cells MDA-MB-231, the non-ionic complexes were also active without electroporation. The most active complex was *cis*-[Pt(PMSA)$_2$I$_2$]; its *trans*-isomer showed also significant activity. Platinum complexes of PMSA with *cis*- and *trans*-configuration deserve further attention as potential cytostatic agents.

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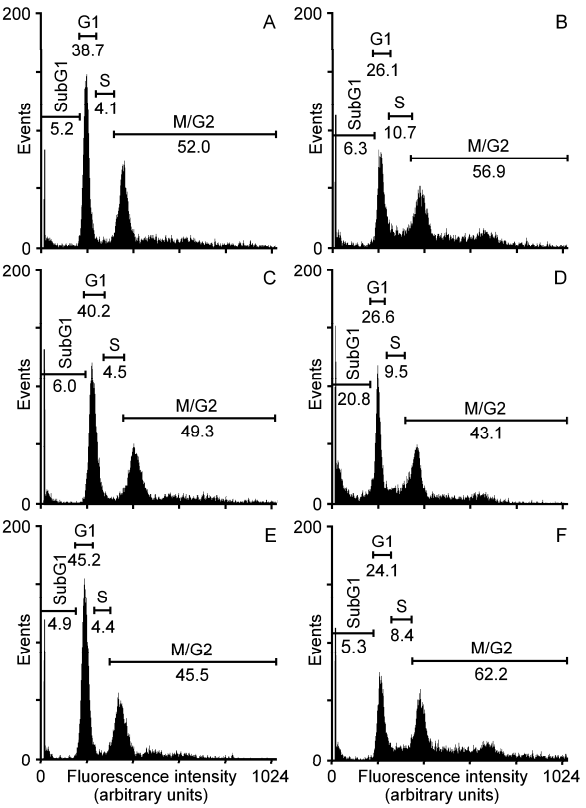

Fig. 4. MDA-MB-231 cell cycle FACS analysis of: (A, B) control samples without and with electroporation; (C, D) samples treated with 10 μmol/l of cisplatin without and with electroporation; (E, F) samples treated with 10 μmol/l of **3** without and with electroporation. The different cell cycle phases are indicated by the markers: SubG1 phase (apoptotic cells), G1 phase (2n cells), S phase (synthetic), and M/G2 phase (mitotic). The exact values of the four markers are given below each marker as percentage of the total amount of cells in the sample.

Bose R. N. (2002), Biomolecular targets for platinum antitumor drugs. Mini-Rev. Med. Chem. **2**, 103–111.

Boulikas T. and Vougiouka M. (2003), Cisplatin and platinum drugs at the molecular level (Review). Oncol. Rep. **10**, 1663–1682.

Cailleau R., Young R., Olivé M., and Reeves W. J. Jr. (1974), Breast tumor cell lines from pleural effusions. J. Natl. Cancer Inst. **53**, 661–674.

Chohan Z. H., Hassan M., Khan K. M., and Supuran C. T. (2005), *In vitro* antibacterial, antifungal and cytotoxic properties of sulfonamide-derived Schiff's bases and their metal complexes. J. Enzyme Inhib. Med. Chem. **20**, 183–188.

Chu F., Barkinge J., Hawkins S., Gudi R., Salgia R., and Kanteti P. V. S. (2005), Expression of Siva-1 protein or its putative amphipathic helical region enhances cisplatin-induced apoptosis in breast cancer cells: effect of elevated levels of BCL-2. Cancer Res. **65**, 5301–5309.

Cohen S. M. and Lippard J. S. (2001), Cisplatin: from DNA damage to cancer chemotherapy. Prog. Nucl. Acid Res. Mol. Biol. **67**, 93–130.

Coluccia M. and Natile G. (2007), *trans*-Platinum complexes in cancer therapy. Anti-cancer Agents Med. Chem. **7**, 111–123.

Dodoff N. I. (2000), Conformational and vibrational analysis of *N*-3-pyridinylmethanesulfonamide. Internet J. Vibr. Spectrosc. **4**, **3**, **5** (http://www.ijvs.com/volume4/edition3/section3.html).

Dodoff N. I. (2001), Palladium(II) and platinum(II) complexes of *N*-3-pyridinylmethanesulfonamide. Z. Naturforsch. **56b**, 1217–1226.

Dodoff N. I., Varga R. A., and Kovala-Demertzi D. (2004), Crystal structure of *N*-3-pyridinylmethanesulfonamide and *trans*-diiodobis(*N*-3-pyridinylmethanesulfonamide)-platinum(II). Z. Naturforsch. **59b**, 1070–1076.

Dube S. K., Pragnell I. B., Kluge N., Gaedicke G., Steinheider G., and Ostertag W. (1975), Induction of endogenous and of spleen focus-forming viruses during dimethylsulfoxide-induced differentiation of mouse erythroleukemia cell transformed by spleen focus-forming virus. Proc. Natl. Acad. Sci. USA **72**, 1863–1867.

Farrell N., Ha T. T. B., Souchard J.-P., Wimmer F. L., Cross S., and Johnson N. P. (1989), Cytostatic *trans*-platinum(II) complexes. J. Med. Chem. **32**, 2240–1241.

Fujimoto T., Maeda H., Kubo K., Sugita Y., Nakashima T., Sato E., Tanaka Y., Madachi M., Aiba M., and Kameyama Y. (2005), Enhanced antitumor effect of cisplatin with low-voltage electrochemotherapy in hamster oral fibrosarcoma. J. Int. Med. Res. **33**, 507–512.

Gehl J. (2003), Electroporation: theory and methods, perspectives for drug delivery, gene therapy and research. Acta Physiol. Scand. **177**, 437–447.

Gonzalez V. M., Fuertes M. A., Alonso C., and Perez J. M. (2001), Is cisplatin-induced cell death always produced by apoptosis? Mol. Pharmacol. **59**, 657–663.

Haenze J., Fisher L., Koenen M., Worgall S., and Rascher W. (1998), Electroporation of nucleic acids into prokaryotic and eukaryotic cells by square wave pulses. Biotechnol. Tech. **12**, 159–162.

Henkels K. M. and Turchi J. J. (1999), Cisplatin-induced apoptosis proceeds by caspase-3-dependent and -independent pathways in cisplatin-resistant and -sensitive human ovarian cancer cell lines. Cancer Res. **59**, 3077–3083.

Hirono T., Kishimoto T., Kuritani T., Muraguchi A., Yamamura Y., Ralph P., and Good R. A. (1979), *In vitro* immune response of human peripheral lymphocytes. IV. Specific induction of human suppressor T cells by an antiserum to the T leukemia cell line HSB. J. Immunol. **123**, 1133–1140.

Jakupec M. A., Galanski M., and Keppler B. K. (2003), Tumor-inhibiting platinum complexes – state of the art and future perspectives. Rev. Physiol. Biochem. Pharmacol. **146**, 1–53.

Jones R. A. and Katritzki A. R. (1961), Potentially tautomeric pyridines. Part III. 2-, 3-, and 4-methanesulfonylpyridines. J. Chem. Soc., 378–384.

Konstantinov S. M., Eibl H., and Berger M. R. (1998), Alkylphosphocholines induce apoptosis in HL-60 and U-937 leukemic cells. Cancer Chemother. Pharmacol. **41**, 210–216.

Kostova I. (2006a), Platinum complexes as anticancer agents. Rec. Pat. Anti-Cancer Drug Discov. **1**, 1–22.

Kostova I. (2006b), Gold coordination complexes as anticancer agents. Anti-Cancer Agents Med. Chem. **6**, 19–32.

Mosmann R. (1983), Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods **139**, 55–63.

Neumann E., Kakorin S., Tsoneva I., Nikolova B., and Tomov T. (1996), Calcium mediated DNA adsorption to yeast cells and kinetics of cell transformation by electroporation. Biophys. J. **71**, 868–877.

Park M. S., De Leon M., and Devarajan P. (2002), Cisplatin induces apoptosis in LLC-PK1 cells via activation of mitochondrial pathways. J. Am. Soc. Nephrol. **13**, 858–865.

Peycheva E. and Daskalov I. (2004), Electrochemotherapy of skin tumours: comparison of two electroporation protocols. J. BUOn **9**, 47–50.

Reedijk J. (2003), New clues for platinum antitumor chemistry: Kinetically controlled metal binding to DNA. Proc. Natl. Acad. Sci. USA **100**, 3611–3616.

Schwerdt G., Freudinger R., Schuster C., Weber F., Thews O., and Gekle M. (2005), Cisplatin-induced apoptosis is enhanced by hypoxia and by inhibition of mitochondria in renal collecting duct cells. Toxicol. Sci. **85**, 735–742.

Scozzafava A., Owa T., Mastrolorenzo A., and Supuran C. T. (2003), Anticancer and antiviral sulfonamides. Curr. Med. Chem. **10**, 925–953.

Serša G., Štabuc B., Čemažar M., Miklavčič D., and Rudolf Z. (2000), Electrochemotherapy with cisplatin: clinical experience in malignant melanoma patients. Clin. Cancer Res. **6**, 863–867.

Spugnini E. P. and Porrello A. (2003), Potentiation of chemotherapy in companion animals with spontaneous large neoplasms by application of biphasic electric pulses. J. Exp. Clin. Cancer Res. **22**, 421–430.

Spugnini E. P., Citro G., D'Avino A., and Baldi A. (2008), Potential role of electrochemotherapy for the treatment of soft tissue sarcoma: first insights from preclinical studies in animals. Int. J. Biochem. Cell Biol. **40**, 159–163.

Stordal B., Pavlakis N., and Davey R. (2007), Oxaliplatin for the treatment of cisplatin resistant cancer: A systematic review. Cancer Treat. Rev. **33**, 347–357.

Supuran C. T., Mironov V., Tudor V., and Banciu M. D. (1994), Carbonic anhydrase inhibitors. Part 21. Arylsulfonylhydrazides and their Cu(II) complexes are non-competitive isozyme II inhibitors. Rev. Roum. Chim. **39**, 587–592.

Supuran C. T., Mironov V., and Tudor V. (1996), Carbonic anhydrase inhibitors. Part 22. 4-Toluenesulfonylhydrazide complexes with Ni(II) and Pd(II) are dual isozyme inhibitors. Rev. Roum. Chim. **41**, 337–341.

Supuran C. T., Casini A., Mastrolorenzo A., and Scozzafava A. (2004), COX-2 selective inhibitors, carbonic anhydrase inhibition and anticancer properties of sulfonamides belonging to this class of pharmacological agents. Mini-Rev. Med. Chem. **4**, 625–632.

Tsoneva I., Nikolova B., Georgieva M., Genova M., Tomov T., Rols M. R., and Berger M. R. (2005), Induction of apoptosis by electrotransfer of positively charged proteins as cytochrome C and histone H1 into cells. Biochim. Biophys. Acta **1721**, 55–64.

Van Beusichem M. and Farrell N. (1992), Activation of the *trans* geometry in platinum antitumor complexes. Synthesis, characterization and biological activity of complexes with planar ligands pyridine, *N*-methylimidazole, thiazole, and quinoline. Crystal and molecular structure of *trans*-dichlorobis(thiazole)platinum(II). Inorg. Chem. **31**, 634–639.

van Zutphen S., Pantoja E., Soriano R., Soro C., Tooke D. M., Spek A. L., den Dulk H., Brouwer J., and Reedijk J. (2006), New antitumor active platinum compounds containing carboxylate ligands in *trans* geometry: synthesis, crystal structure and biological activity. Dalton Trans., 1020–1023.

Watson J. and Erta E. (1992), Flow cytometry. In: Animal Cell Culture (Freshley R., ed.). IRL Press, Oxford, pp. 165–213.