European Journal of Medicinal Chemistry 44 (2009) 1623–1629

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and characterization of new Pt(II) and Pd(II) complexes with 2-quinolinecarboxaldehyde selenosemicarbazone: Cytotoxic activity evaluation of Cd(II), Zn(II), Ni(II), Pt(II) and Pd(II) complexes with heteroaromatic selenosemicarbazones

Nevenka Gligorijević [a], Tamara Todorović [b], Siniša Radulović [a], Dušan Sladić [b], Nenad Filipović [c], Dejan Gođevac [d], Dejan Jeremić [b], Katarina Anđelković [b,*]

[a] Laboratory for Experimental Pharmacology, Institute for Oncology and Radiology of Serbia, 11000 Belgrade, Serbia
[b] Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia
[c] Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11081 Zemun, Serbia
[d] Institute of Chemistry, Technology and Metallurgy, 11001 Belgrade, Serbia

ARTICLE INFO

Article history:
Received 12 June 2008
Received in revised form 25 July 2008
Accepted 28 July 2008
Available online 8 August 2008

Keywords:
Antitumor activity
Complexes
Heterocyclic compounds
Selenosemicarbazone
NMR spectroscopy

ABSTRACT

New complexes of Pt(II) and Pd(II) with 2-quinolinecarboxaldehyde selenosemicarbazone were synthesized and characterized by elemental analysis, NMR and IR spectroscopy and molar conductivity measurements. The assumed geometry of Pt(II) and Pd(II) complexes was square planar where the ligand was tridentately coordinated via the quinoline and imine nitrogen atoms and the selenium atom. The cytotoxic activity of the new Pt(II) and Pd(II) compounds, as well as of some previously synthesized Cd(II), Zn(II) and Ni(II) complexes with the same or analogous ligand, was tested against a panel of three human cancer cell lines: human cervix carcinoma cells (HeLa), human melanoma cells (FemX) and breast cancer cells (MDA-361). All investigated compounds, except Pt(II) complex, possess a strong dose-dependent cytotoxic activity of the same order of magnitude as cisplatin (CDDP). The investigation of potential of these compounds to induce HeLa cell cycle perturbations was also evaluated.

1. Introduction

Thiosemicarbazones and their complexes have been extensively studied for antibacterial, antiviral, antifungal and antitumor activities [1–6]. In contrast, there are only few studies on selenosemicarbazones which showed a greater biological activity in comparison to sulphur analogues [7–13]. Surprisingly, to the best of our knowledge there is just one study on antiproliferative activity of a selenosemicarbazone complex [13]. The study on the human tumor cell lines 41 M (ovarian carcinoma) and SK-BR-3 (mammary carcinoma) showed that gallium(III) complex with 2-acetylpyridine *N*,*N*-dimethylselenosemicarbazone has the most pronounced cytotoxic activity among gallium(III) complexes with oxygen and sulphur analogues. Moreover, the free selenosemicarbazone ligand showed a 2–3 times lower activity than its corresponding gallium(III) complex.

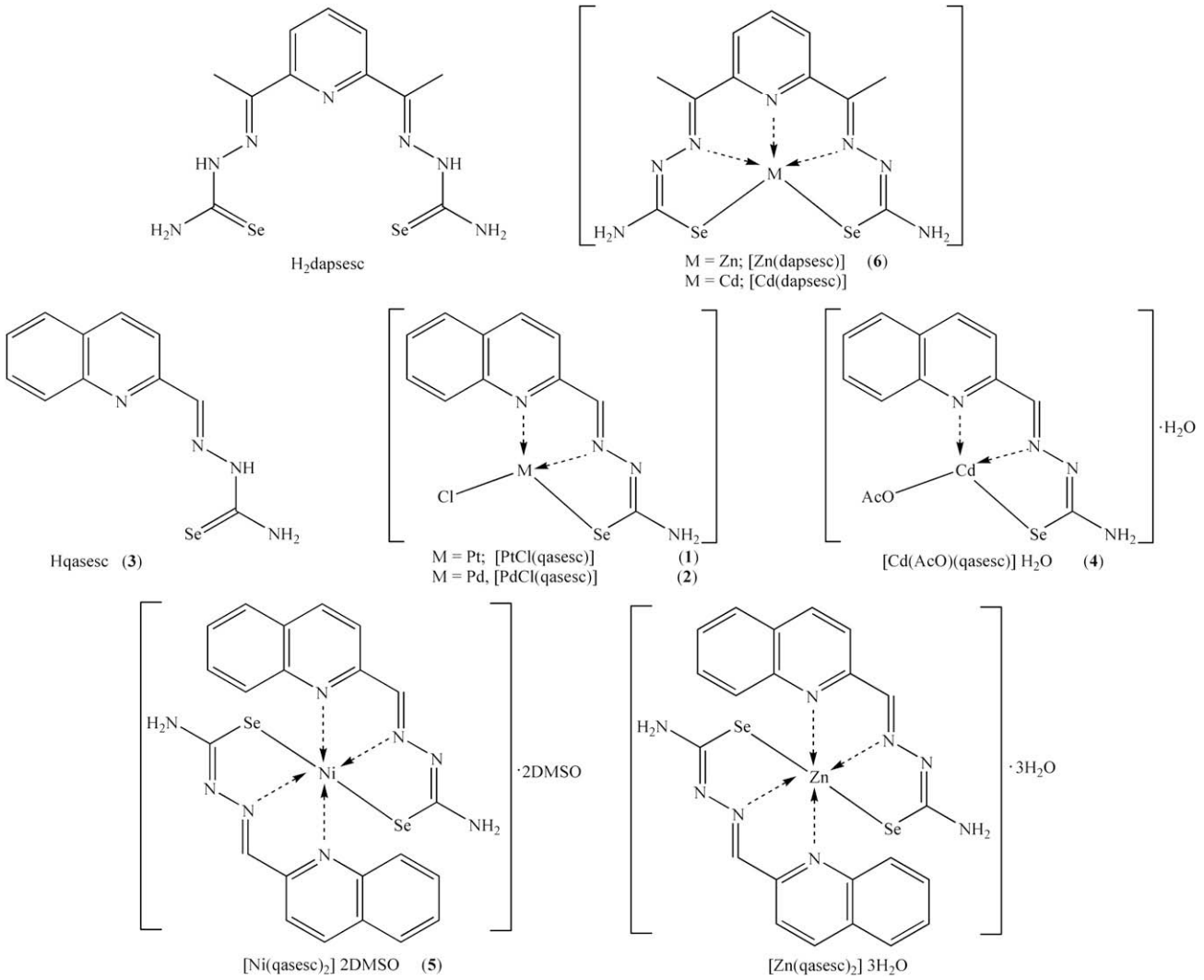
Selenosemicarbazones exhibit various binding modes with d-metals. Similar to sulphur analogues, they can act as mono- or bidentate ligands, and the coordination capacity can be extended if aldehyde or ketone part of molecule contains additional donor atom(s) suitable for chelation.

In our previous paper, synthesis of the first heteroaromatic bis(selenosemicarbazone), 2,6-diacetylpyridine bis(selenosemicarbazone) (H_2dapsesc) and the corresponding Cd(II) and Zn(II) complexes, [Cd(dapsesc)] and [Zn(dapsesc)] (Scheme 1), was reported [14]. In these complexes the ligand is coordinated as a pentadentate, via both selenium atoms, pyridine and both imine nitrogen atoms. A series of complexes, namely $[Zn(qasesc)_2]\cdot 3H_2O$, $[Cd(qasesc)(AcO)]\cdot H_2O$ and $[Ni(qasesc)_2]\cdot 2DMSO$ was synthesized with another selenosemicarbazone ligand 2-quinolinecarboxaldehyde selenosemicarbazone (Hqasesc) (Scheme 1) [15]. In these complexes the ligand is coordinated as a tridentate in the monoanionic form, via the imine and quinoline nitrogen atoms and the selenium atom. The biological activity of the ligands and the complexes was tested by the brine shrimp test (toxicity to *Artemia salina*) [15], which is in good correlation with cytotoxic activity [16]. All the compounds showed moderate activity, the complexes being more active than the ligands.

As a continuation of our work in this field, in the present paper synthesis and spectroscopic characterization of new Pt(II) and

* Corresponding author. Tel.: +381 11 3336 739; fax: +381 11 3282 750.
E-mail address: kka@chem.bg.ac.yu (K. Anđelković).

0223-5234/$ – see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.07.033



Scheme 1.

Pd(II) selenosemicarbazone complexes (Scheme 1) are reported. In order to study the influence of the nature of the central metal ion in selenosemicarbazone complexes on cytotoxicity, the cytotoxic activity of new compounds as well as of some previously synthesized ones was tested against a panel of three human cancer cell lines. The investigation of potential of these compounds to induce HeLa cell cycle perturbations is also part of the present work.

2. Chemistry

Selenosemicarbazide, 2-quinolinecarboxaldehyde and 2,6-diacetylpyridine were obtained from Acros Organics (BVBA, Geel, Belgium). Potassium tetrachloroplatinate(II) (98%), potassium tetrachloropalladate(II) (98%), cadmium acetate dihydrate (reagent grade 98%), nickel acetate tetrahydrate (reagent grade 98%), zinc acetate dihydrate (reagent grade 98%) and cisplatin (CDDP) were obtained from Aldrich (Sigma–Aldrich Chemie GmbH, Steinheim, Germany). All solvents (reagent grade) were obtained from commercial suppliers and used without further purification. The ligand H_2dapsesc was synthesized as described previously by adding ethanolic solution of 2,6-diacetylpyridine into warm ethanolic suspension of selenosemicarbazide in the molar ratio 1:2 [14]. The pH value of the reaction mixture was adjusted to ~4 by adding glacial acetic acid. The ligand Hqasesc was obtained by condensation of 2-quinolinecarboxaldehyde and selenosemicarbazide in the molar ratio of 1:1 in ethanol (pH ~ 4) [15].

3. Pharmacology

The activity of the new complexes and some related previously synthesized ones was tested against three tumor cell lines: human cervix carcinoma cells (HeLa), human melanoma cells (FemX) and breast cancer cells (MDA-361). The complexes differed in the nature of the central metal ion, while the ligands were of selenosemicarbazone type, principally quinoline derivative Hqasesc, and in one case pyridine derivative H_2dapsesc. IC_{50} values were determined for the periods of 48 and 72 h (only complexes **1** and **2**). Since most complexes showed a strong dose-dependant cytotoxic activity, quantitative analysis of cell cycle phase distribution and potential of inducing apoptosis was performed by flow-cytometric analysis of the DNA content in fixed HeLa cells.

4. Results and discussion

4.1. Chemistry

By reaction of potassium tetrachloroplatinate(II) or potassium tetrachloropalladate(II) with ligand Hqasesc in equimolar ratio

(1:1) the corresponding complexes were obtained. The mole ratio of reacting species did not influence the composition of the products. All the synthesized complexes are non-electrolytes, as determined by molar conductivity measurements. Elemental microanalysis showed that in both Pt(II) and Pd(II) complexes there is a deprotonated ligand molecule and a chloride ion. The following formulae of new compounds can be derived: [PtCl(qasesc)] (**1**) and [PdCl(qasesc)] (**2**).

4.2. NMR spectroscopy

The ligand coordination mode in new complexes **1** and **2** was determined by NMR spectroscopy results (^{1}H, ^{13}C, ^{77}Se), 2D NOESY (Nuclear Overhauser Effect Spectroscopy) spectra and 2D heteronuclear correlation spectra (^{1}H–^{13}C, ^{1}H–^{15}N, ^{1}H–^{77}Se). Due to low sensitivity of 1D ^{15}N NMR, ^{15}N chemical shifts are derived from 2D HSQC (Heteronuclear Single Quantum Coherence) and 2D HMBC (Heteronuclear Multiple Bond Coherence). Coordination via the quinoline nitrogen could be evidenced by strong upfield ^{1}H NMR shift of C3H signal, and strong downfield shifts of C4H and especially C8H signals with respect to the ligand [15], as well as by downfield shifts of signals of C3 and C4 in ^{13}C NMR spectra. Furthermore, there is a strong upfield ^{15}N NMR shift of N1 signal from 311 ppm in the ligand to 227 ppm in the Pt(II) complex and 238 ppm in the Pd(II) complex (Fig. 1). Coordination via the azomethine nitrogen can be shown by the change in chemical shift of the hydrogen C9H, bound directly to it, upon complexation. There is also an upfield shift of C9 signal in ^{13}C NMR spectrum, and an upfield shift of N2 signal from 330 ppm in ^{15}N NMR spectrum of the ligand to 281 and 294 ppm in the NMR spectra of Pt(II) and Pd(II) complexes, respectively. Coordination via selenium can be proven using ^{77}Se NMR spectra in which there is a strong downfield shift of the signal by 228 ppm upon complexation with Pt(II) (satisfactory ^{77}Se spectrum could not be obtained for Pd(II) complex due to its low solubility). In ^{1}H NMR spectra of both complexes N3H proton signal (at 12.08 ppm in the ligand) is missing, indicating coordination of the ligand in deprotonated form. Also, in the ^{15}N NMR spectra the signal of N3 is shifted strongly downfield from 178 ppm in the ligand, to 280 and 296 ppm in Pt(II) and Pd(II) complexes, respectively. Finally, in both complexes there is only a weak ^{15}N shift of N4 from 121 ppm in the ligand to 101 ppm in Pt(II) complex and 100 ppm in Pd(II) complex, indicating no coordination via this atom. The evidence presented shows that in both complexes the ligand is coordinated in monodeprotonated form via the quinoline and azometine nitrogen atoms and the selenium atom, the fourth coordination site being occupied by chloride.

4.3. Pharmacology

4.3.1. Cytotoxic activity

The cytotoxic activity of the selenosemicarbazone complexes **1**, **2**, **4**–**6** and the ligand **3** (Scheme 1), as well as corresponding metal salts, was tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide assay (MTT assay) against a panel of three tumor cell lines: HeLa, FemX and MDA-361 cells. Cells were treated for 48 h with the desired range of concentrations of investigated compounds. Other selenosemicarbazone complexes, namely [Zn(qasesc)$_2$], [Cd(dapsesc)] as well as the ligand H_2dapsesc (Scheme 1) could not be tested due to low solubility.

The results indicate that, as shown by IC_{50} values (determined from cell survival diagrams), all investigated compounds, except complex **1**, possess a strong dose-dependent cytotoxic activity (Fig. 2, Table 1), of the same order of magnitude as CDDP. The complexes were significantly more active than the corresponding metal salts. Also, the complexes with Hqasesc were more active than the ligand itself. The most sensitive cell line was FemX (Fig. 2B).

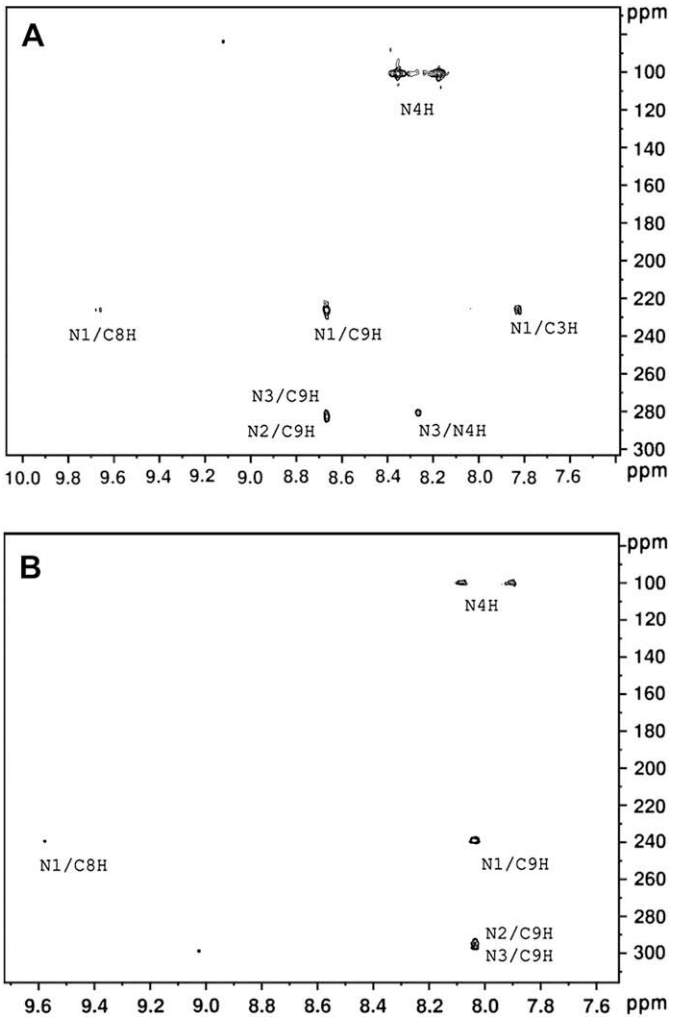


Fig. 1. N, H HMBC spectrum of [PtCl(qasesc)] (A) and [PdCl(qasesc)] (B).

For comparison and investigation on influence of nature of central metal ion in new selenosemicarbazone complexes on cytotoxicity it is important to notice that palladium(II) complex has higher cytotoxicity than platinum(II) complex with the same ligand system. This may be explained by higher reactivity and/or lower stability of palladium complexes [17,18].

Because platinum(II) complex was inactive on all cell lines in the range of investigated concentrations, the time of incubation was prolonged to 72 h. However, cytotoxic activity of platinum(II) complex was still low. This complex showed a moderate cytotoxic activity only on HeLa cells. In the case of palladium(II) complex after incubation of 72 h there was no important difference in activity, except in the case of MDA-361 cells where activity was two times higher than activity after incubation period of 48 h (Table 1).

The higher activity of the complexes is probably a consequence of the non-ionic nature of all complexes which might facilitate their diffusion through biological membranes [19].

4.3.2. Cell cycle alterations

In order to investigate the mechanism of action of investigated compounds the potential of inducing cell cycle perturbations of HeLa cells was analyzed. Flow-cytometric quantitative analysis of cell cycle phase distribution after treatment with investigated compounds was determined after staining fixed HeLa cells with propidium iodide (PI). Compared to control cells it was determined that, after 24 h of continual incubation, complexes **4**–**6** and ligand **3**

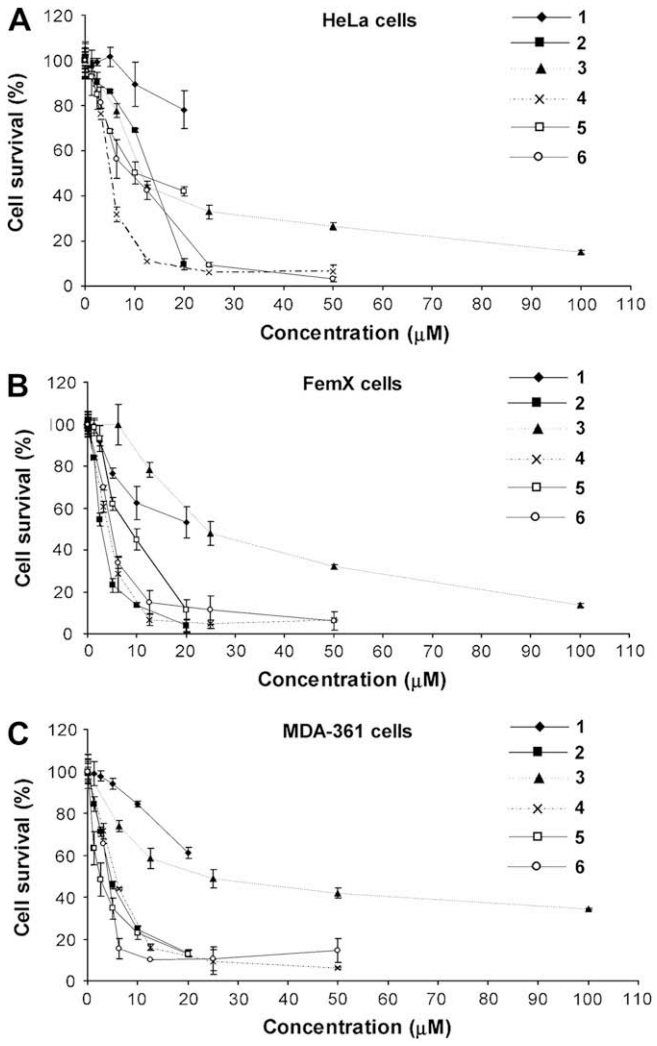


Fig. 2. Diagrams of HeLa (A), FemX (B) and MDA-361 (C) cell survival (%) after 48 h of continual investigated compound action. Data are representative for one out of three separate experiments with standard deviation.

induce an increase in the percentage of sub-G1 cells (apoptotic), especially complex **5** (over 20%), and decrease the percentage of cells in G1 phase, in a concentration dependent manner (Fig. 3, Table 2). The percentage of cells in G2 phase was also lower after treatment with complexes **5** and **6** and ligand **3**, while complex **4** induced increase of the fraction cells in G2 phase. Increase of the percentage of cells in S phase was determined only after treatment with complexes **4** and **6**.

Platinum(II) and palladium(II) complexes with the same ligand system induced higher perturbations of cell cycle after treatment with lower concentrations (Fig. 3, Table 2). An increase of the percent of cells in sub-G1 phase (apoptotic), and decrease of the percentage of cells in G1 phase were observed after treatment with lower concentrations. After treatment with higher concentrations perturbations of cell cycle were minor. With prolonged time of incubation (48 h) with complexes **1** and **2** perturbations of cell cycle were higher, especially induced by complex **2**, which induced an increase of the fraction of apoptotic cells, decrease of the fraction of cells in G1 and G2 phases and increase of the fraction of cells in S phase (not shown).

Cell cycle analysis of HeLa cells after treatment with investigated complexes revealed their potential to induce cell cycle perturbations, and to increase the number of hypodiploid cells, with fragmented DNA, suggesting their ability to induce tumor cells apoptosis. The complexes that induced the biggest perturbations of cell cycle were palladium(II) and nickel(II) complexes. It is important to mention that ligand **3** alone had an important potential to induce cell cycle perturbations. The reference compound CDDP, after 48 h continual action on HeLa cells, induced increase of the percent of apoptotic (sub-G1) cells in a concentration dependant manner (Table 2). The percent of cells in G1 and G2 phase decrease, while the percent of cells in S phase was higher than in the control. The apoptotic activity of the complexes **2** and **5** was in the range of the apoptotic activity of reference compound CDDP.

5. Conclusion

Two new Pt(II) and Pd(II) complexes with 2-quinolinecarboxaldehyde selenosemicarbazone were synthesized. The ligand was coordinated as a tridentate via the quinoline and azomethine nitrogen atoms and the selenium atom, as shown by NMR spectroscopy. The fourth coordination site was occupied by chloride ion. The overall geometry of both complexes was square planar, based on composition, coordination number, mode of coordination and diamagnetic nature of the complexes. The new complexes, as well as three previously synthesized complexes with the same ligand or a structurally similar one, were tested for antitumor activity against a panel of three cell lines. All the complexes, except Pt(II) complex, showed a strong dose-dependent cytotoxic activity, probably due to their non-electrolyte nature. Cell cycle analysis of HeLa cells after treatment with investigated complexes, displayed that the

Table 1
Cytotoxicity results expressed as IC_{50} values which are determined from cell survival diagrams after 48 and 72 h treatment

Compounds		IC_{50} (μM)					
		HeLa		FemX		MDA-361	
		48 h	72 h	48 h	72 h	48 h	72 h
[PtCl(qasesc)]	(**1**)	>20	19.35 ± 0.12	>20	>20	>20	>20
[PdCl(qasesc)]	(**2**)	11.35 ± 2.49	9.30 ± 2.36	2.94 ± 0.84	2.30 ± 0.77	4.67 ± 0.65	2.41 ± 0.55
Hqasesc	(**3**)	15.31 ± 5.36	–	20.89 ± 4.67	–	26.08 ± 3.70	–
$[Cd(AcO)(qasesc)]\cdot H_2O$	(**4**)	5.88 ± 1.29	–	4.79 ± 0.89	–	6.36 ± 1.51	–
$[Ni(qasesc)_2]\cdot 2DMSO$	(**5**)	9.76 ± 0.98	–	2.96 ± 1.28	–	2.79 ± 0.79	–
[Zn(dapsesc)]	(**6**)	12.17 ± 5.25	–	4.52 ± 1.7	–	4.94 ± 1.51	–
$Zn(AcO)_2\cdot 2H_2O$		>100	–	>100	–	>100	–
$Cd(AcO)_2\cdot 2H_2O$		47.86 ± 3.68	–	38.75 ± 3.89	–	81.29 ± 0.32	–
$Ni(AcO)_2\cdot 4H_2O$		82.65 ± 4.84	–	91.91 ± 7.94	–	>100	–
$K_2[PtCl_4]$		>100	–	>100	–	>100	–
$K_2[PdCl_4]$		>100	–	>100	–	>100	–
CDDP		6.95 ± 1.69	–	2.0 ± 0.3	–	14.7 ± 1.2	–

Values of IC_{50} represent the average of three experiments, each experiment performed in three replicates. The sign (>) indicates that IC_{50} value is not reached in the examined range of concentrations (the sign is in front of the maximum value of the concentration in the examined range of concentrations).

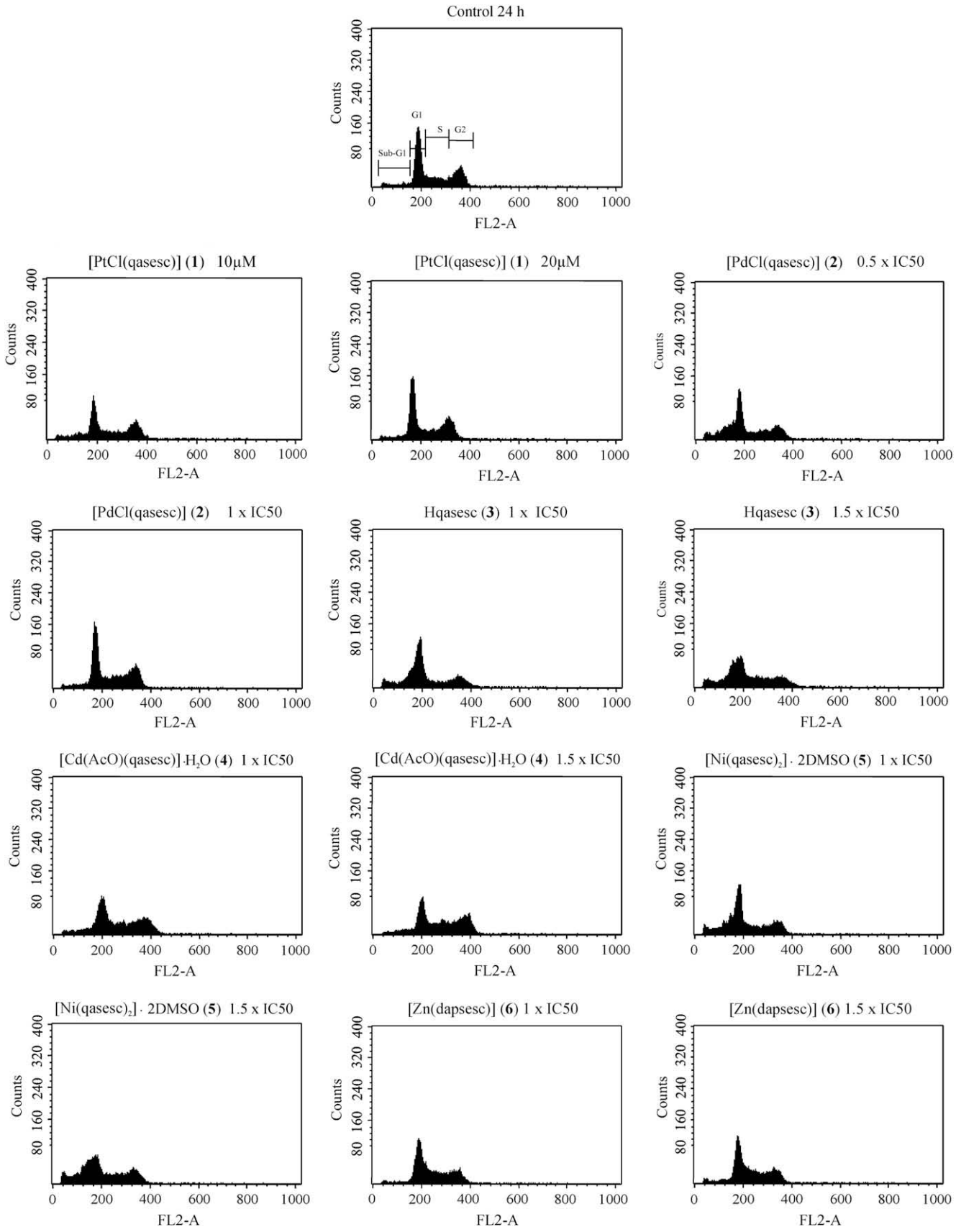


Fig. 3. The histograms from a representative experiment for HeLa cell cycle analysis after 24 h continual incubation with medium (control), and investigated compounds.

Table 2
Data of HeLa cell cycle analysis after 24 h of continual treatment with investigated compounds

Compound			% Sub-G1	% G1	% S	% G2
Control			5.30	47.94	20.14	26.68
[PtCl(qasesc)]	(**1**)	10 μM	12.07	37.84	23.43	26.42
		20 μM	5.49	44.88	20.64	29.08
[PdCl(qasesc)]	(**2**)	$0.5 \times IC_{50}$	24.95	38.60	18.72	19.61
		$1 \times IC_{50}$	5.86	40.31	28.82	25.29
Hqasesc	(**3**)	$1 \times IC_{50}$	18.81	41.28	13.82	15.33
		$1.5 \times IC_{50}$	19.78	40.03	23.56	15.07
$[Cd(AcO)(qasesc)]\cdot H_2O$	(**4**)	$1 \times IC_{50}$	8.51	38.48	23.69	27.40
		$1.5 \times IC_{50}$	7.56	32.63	29.72	30.35
$[Ni(qasesc)_2]\cdot 2DMSO$	(**5**)	$1 \times IC_{50}$	22.18	44.69	19.34	15.04
		$1.5 \times IC_{50}$	24.44	37.10	19.29	17.08
[Zn(dapsesc)]	(**6**)	$1 \times IC_{50}$	3.70	46.05	33.32	17.13
		$1.5 \times IC_{50}$	7.35	46.95	29.98	16.02
CDDP		$1 \times IC_{50}$ (48 h)	23.46	22.83	38.71	10.71

complexes, particularly Pd(II) and Ni(II) ones, induced cell cycle perturbations, and increased the number of hypodiploid cells, with fragmented DNA, indicating their ability to induce tumor cell apoptosis, comparable to reference compound CDDP.

These results might contribute to the design and research of new metal-based antitumor agents and to introduce selenosemicarbazones as new interesting ligands with potential for future research in this field.

6. Experimental protocols

6.1. Chemistry

6.1.1. Physical measurements

Elemental analysis (C, H, N) was performed by the standard micromethods using the ELEMENTAR Vario ELIII C.H.N.S=O analyser. IR spectra were recorded on Perkin–Elmer FT-IR 1725X spectrophotometer by the KBr technique in the region 4000–400 cm^{-1}. Abbreviations used for IR spectra: vs, very strong; s, strong; m, medium; w, weak. Molar conductivities were measured at room temperature (298 K) on the digital conductometer JENWAY-4009. NMR spectral assignments and structural parameters were obtained by combined use of ^1H homonuclear spectroscopy (2D NOESY) and multinuclear proton-detected spectroscopy (2D HSQC, 2D HMBC). The NMR spectra were performed on Bruker Avance 500 equipped with broad-band direct probe. All spectra were measured at 298 K. 2D NOESY spectra were collected with 4 scans per t1-increment and mixing time of 1 s, while ^1H–^{13}C heteronuclear 2D HSQC and 2D HMBC experiments were obtained with 6 scans per t1-increment. ^1H–^{77}Se heteronuclear 2D HSQC spectra were obtained with 16 scans per 128 t1-increments. Directly detected 1D ^{77}Se spectra at 95 MHz were obtained with 10 000 scans. Chemical shifts are given on δ scale relative to tetramethylsilane (TMS) as internal standard for ^1H and ^{13}C, relative to urea (77.0 ppm) as external standard for ^{15}N, or indirect referencing to TMS as ^1H standard for ^{77}Se. Abbreviations used for NMR spectra: s, singlet; d, doublet; t, triplet; br, broad; ovlp, overlapping.

6.1.2. Synthesis of the complexes [PtCl(qasesc)] (**1**) and [PdCl(qasesc)] (**2**)

Title complexes were synthesized according to a general procedure. To a solution of 2-quinolinecarboxaldehyde selenosemicarbazone (**3**) (0.05 g, 0.18 mmol) in dimethyl sulfoxide (2.5 mL), a solution of potassium tetrachloroplatinate(II) (0.07 g, 0.18 mmol) or potassium tetrachloropalladate(II) (0.06 g, 0.18 mmol) in dimethyl sulfoxide (2.5 mL) was added. The reaction mixture was heated at 55 °C with stirring for 1 h followed by cooling to room temperature. Crystals of the complexes **1** and **2**, which were not suitable for X-ray analysis, were obtained by vapor diffusion of ethanol into the DMSO mother liquor. Crystals were filtered off, washed with cold ethanol and ether and dried in vacuo.

6.1.2.1. Compound ***1***. Yield: 0.07 g (84.2%). Anal. Calcd. for $C_{11}H_9ClPtN_4Se$ (506.7): C, 26.07; H, 1.79; N, 11.06. Found: C, 26.35; H, 1.93; N, 11.04%. IR spectrum in KBr, cm^{-1}: 3383 (m), 3291 (m), 3104 (m), 1639 (s), 1577 (m), 1476 (vs), 1450 (vs), 1390 (m), 1316 (m), 1144 (s), 747 (w). ^1H NMR (500.26 MHz, DMSO-d_6): $\delta = 7.67$ (t, 1H, C6H, $^3J_{H,H} = 7.5$ Hz), 7.82 (d, ovlp, 1H, C3H, $^3J_{H,H} = 8.5$ Hz), 7.84 (br t, ovlp, 1H, C7H, $^3J_{H,H} = 7.5$ Hz), 8.03 (d, 1H, C5H, $^3J_{H,H} = 8.0$ Hz), 8.26 (br s, 2H, N4H), 8.66 (s, 1H, C9H), 8.70 (d, 1H, C4H, $^3J_{H,H} = 8.5$ Hz), 9.66 (d, 1H, C8H, $^3J_{H,H} = 9.0$ Hz). ^{13}C NMR (125.79 MHz, DMSO-d_6): $\delta = 121.7$ (C3), 126.6 (C8), 128.8 (C6), 129.1 (C5), 130.3 (C4a), 132.9 (C7), 141.9 (C4), 148.5 (C8a), 151.1 (C9), 161.0 (C2), 174.6 (C10). Λ_M (1×10^{-3} M, DMSO): 14.8 Ω^{-1} cm^2 mol^{-1}.

6.1.2.2. Compound ***2***. Yield: 0.05 g (68.7%). Anal. Calcd. for $C_{11}H_9ClPdN_4Se$ (418.0): C, 31.60; H, 2.17; N, 13.40. Found: C, 31.48; H, 2.21; N, 13.23%. IR spectrum in KBr, cm^{-1}: 3374 (m), 3289 (m), 3070 (m), 1637 (s), 1581 (m), 1473 (vs), 1448 (vs), 1386 (m), 1141 (s), 749 (w). ^1H NMR (500.26 MHz, DMSO-d_6): $\delta = 7.66$ (t, 1H, C6H, $^3J_{H,H} = 7.4$ Hz), 7.76 (d, 1H, C3H, $^3J_{H,H} = 8.4$ Hz), 7.81 (t, 1H, C7H, $^3J_{H,H} = 7.5$ Hz), 7.94 (br s, ovlp, 2H, N4H), 7.96 (d, ovlp, 1H, C5H, $^3J_{H,H} = 7.4$ Hz), 8.02 (s, 1H, C9H), 8.54 (d, 1H, C4H, $^3J_{H,H} = 8.4$ Hz), 9.61 (d, 1H, C8H, $^3J_{H,H} = 9.0$ Hz). ^{13}C NMR (125.79 MHz, DMSO-d_6): $\delta = 120.8$ (C3), 127.1 (C6), 127.7 (C5), 128.6 (C8), 131.7 (C4a), 132.4 (C7), 140.3 (C4), 146.8 (C8a), 148.7 (C9), 157.8 (C2), 172.2 (C10). Λ_M (1×10^{-3} M, DMSO): 12.6 Ω^{-1} cm^2 mol^{-1}.

6.1.3. Synthesis of the complexes $[Cd(AcO)(qasesc)]\cdot H_2O$ (**4**), $[Ni(qasesc)_2]\cdot 2DMSO$ (**5**) and [Zn(dapsesc)] (**6**)

The Cd(II) and Ni(II) complexes with Hqasesc ligand were prepared as described previously [15]. In short, the complexes were synthesized by adding aqueous solution of $Ni(AcO)_2\cdot 4H_2O$, or ethanolic solution of $Cd(AcO)_2\cdot 2H_2O$ into solution of Hqasesc in ethanol. Nickel(II) complex was purified by vapor diffusion using the DMSO solution as an inner solution and ethanol as an outer solvent. The Zn(II) complex with H_2dapsesc ligand was synthesized by adding aqueous solution of $Zn(CH_3COO)_2\cdot 2H_2O$ into suspension of the ligand in ethanol [14].

6.2. Pharmacology

6.2.1. Cell culture

HeLa, FemX and MDA-361 cells were maintained as monolayer culture in the Roswell Park Memorial Institute (RPMI) 1640 nutrient medium (Sigma Chemicals Co, USA). RPMI 1640 nutrient

medium was prepared in sterile ionized water, supplemented with penicillin (192 U/mL), streptomycin (200 μg/mL), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) (25 mM), L-glutamine (3 mM) and 10% of heat-inactivated fetal calf serum (FCS) (pH 7.2). The cells were grown at 37 °C in 5% CO_2 and humidified air atmosphere, by twice weekly subculture.

6.2.2. Cytotoxicity assay

Cytotoxicity of the investigated compounds was determined using MTT assay (Sigma) [20]. Cells were seeded in 96-well cell culture plates (NUNC) HeLa (2000 c/w), FemX (1500 c/w) and MDA-361 (7000 c/w) in culture medium and grown for 24 h. Stock solutions of investigated compounds were made in DMSO at concentrations of 2 mM for **1**, **2**, **3** and **5**, and 5 mM for **4** and **6**, and afterwards diluted with nutrient medium to desired final concentrations. Stock solutions of acetate salts of zinc(II), cadmium(II) and nickel(II) were made in distilled water at concentration of 10 mM and afterwards diluted with nutrient medium. Stock solutions of $K_2[PtCl_4]$ and $K_2[PdCl_4]$ were made in DMSO at concentration of 10 mM and afterwards diluted with nutrient medium to desired final concentrations. CDDP stock solution was made in 0.9% NaCl at concentration of 10 mM and afterwards diluted with nutrient medium to desired final concentrations (in range up to 100 μM). The final concentration of DMSO per well did not exceed 1%. Solutions of various concentrations of examined compounds were added to the wells, except the control wells where only nutrient medium was added. All samples were done in triplicate. Nutrient medium with corresponding agent concentrations but without target cells, was used as a blank, also in triplicate. Cells with investigated compounds were incubated for 48 and 72 h (only complexes **1** and **2**) at 37 °C, with 5% CO_2 in humidified atmosphere. After incubation period, 20 μL of MTT solution, 5 mg/mL in phosphate buffer solution (PBS), pH 7.2 were added to each well. Samples were incubated for 4 h at 37 °C, with 5% CO_2 in humidified atmosphere. Formazan crystals were dissolved in 100 μL 10% sodium dodecyl sulphate (SDS) in 0.01 M HCl. Absorbance was recorded on an enzyme-linked immunosorbent assay (ELISA) reader after 24 h at a wavelength of 570 nm. Concentration of IC_{50} (μM) was defined as the concentration of drug producing 50% inhibition of cell survival. It is determined from cell survival diagrams.

6.2.3. Flow-cytometric analysis of cell cycle phase distribution

Quantitative analysis of cell cycle phase distribution and potential of inducing apoptosis was achieved by flow-cytometric analysis of the DNA content in fixed HeLa cells, after staining with PI [21].

HeLa cells in exponential phase of growth, at the density of 2×10^5 cells/Petri dish (dimensions 60×5 mm, NUNC) were continually exposed to investigated compounds for 24 h at concentrations corresponding to their $1 \times IC_{50}$ and $1.5 \times IC_{50}$ values for **3**, **4**, **5**, and **6**, 10 and 20 μM for **1** and $0.5 \times IC_{50}$ and $1 \times IC_{50}$ for the complex **2**. In addition, for complexes **1** and **2** time of incubation was prolonged to 48 h with concentrations of 10 and 20 μM. After 24 and 48 h of continual treatment, HeLa cells were collected by trypsinization, washed twice with ice-cold PBS, and fixed for 30 min in 70% EtOH. Fixed cells were washed again with PBS, and incubated with RNase A (1 mg/mL) for 30 min at 37 °C. Just before flow-cytometric analysis cells were stained with PI at concentration of 400 μg/mL. Cell cycle phase distribution was analyzed using a fluorescence activated sorting cells (FACS), Calibur Becton Dickinson flow cytometer and Cell Quest computer software.

Acknowledgment

This work was supported by the Ministry of Science of the Republic of Serbia (Grants 142062 and 145035).

Appendix. Supplementary data

Supplementary data associated with this article contain ^{77}Se NMR spectra for the ligand **3** and the complex **1**, as well as ^{1}H and ^{13}C NMR chemical shifts for the ligand **3** and complexes **1** and **2**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2008.07.033.

References

[1] H. Beraldo, D. Gambino, Mini Rev. Med. Chem. 4 (2004) 31–39.
[2] H. Beraldo, Quim. Nova 27 (2004) 461–471.
[3] K.H. Reddy, J. Indian Chem. Soc. 80 (2003) 69–78.
[4] S.N. Pandeya, J.R. Dimmock, Pharmazie 48 (1993) 659–666.
[5] D.X. West, A.E. Liberta, S.B. Padhye, R.C. Chikate, P.B. Sonawane, A.S. Kumbhar, R.G. Yerande, Coord. Chem. Rev. 123 (1993) 49–71.
[6] D.X. West, S.B. Padhye, P.B. Sonawane, Struct. Bond. 76 (1991) 1–50.
[7] M. Liu, P.L. Xu, Z.J. Wang, Yaoxue Xuebao 27 (1992) 388–393.
[8] S.R. Turk, C. Shipman Jr., J.C. Drach, J. Gen. Virol. 67 (1986) 1625–1632.
[9] H.G. Mautner, W.D. Kumler, Y. Okano, P. Robertson, Antibiot. Khimoter. 6 (1956) 51–55.
[10] K. Bednarz, Diss. Pharm. 10 (1958) 93–98.
[11] K.C. Agrawal, B.A. Booth, R.L. Michaud, E.C. Moore, A.C. Sartorelli, Biochem. Pharmacol. 23 (1974) 2421–2429.
[12] W. Brucker, H.G. Rohde, Pharmazie 23 (1968) 310–315.
[13] C.R. Kowol, R. Eichinger, M.A. Jakupec, M. Galanski, V.B. Arion, B.K. Keppler, J. Inorg. Biochem. 101 (2007) 1946–1957.
[14] T.R. Todorović, A. Bacchi, G. Pelizzi, N.O. Juranić, D.M. Sladić, I.D. Brčeski, K.K. Anđelković, Inorg. Chem. Commun. 9 (2006) 862–865.
[15] T.R. Todorović, A. Bacchi, N.O. Juranić, D.M. Sladić, G. Pelizzi, T.T. Božić, N.R. Filipović, K.K. Anđelković, Polyhedron 26 (2007) 3428–3436.
[16] B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, J.L. McLaughlin, Planta Med. 45 (1982) 31–34.
[17] A.G. Quiroga, J.M. Perez, E.I. Montero, D.X. West, C. Alonso, C. Navaro-Ranninger, J. Inorg. Biochem. 75 (1999) 293–301.
[18] N. Farrel, in: R. Ugo, B.R. James (Eds.), Catalysis by Metal Complexes, Reidel-Kluwer, Dordrecht, 1989, pp. 183–207.
[19] R. Rosenberg Verl. Helvetica Chimica Acta, in: B. Lippert (Ed.), Cisplatin Chemistry and Biochemistry of a Leading Anticancer Drug, Wiley-VCH, Zürich, Weinheim, 1999, pp. 3–29.
[20] R. Supino, Methods in Molecular Biology, in: S. O'Hare, C.K. Atterwill (Eds.), In Vitro Toxicity Testing Protocols, Humana Press, New Jersey, 1995, pp. 137–149.
[21] M.G. Ormerod, Analysis of DNA – General Methods, in: M.G. Ormerod (Ed.), Flow Cytometry, a Practical Approach, Oxford University Press, New York, 1994, pp. 119–125.