A New Class of Antimetabolites: Pyridine Thioglycosides as Potential Anticancer Agents

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The present study was designed for highlighting and focusing on the cytotoxic activity of a new class of antimetabolites both on human cell lines, namely liver carcinoma cell line (Hepg2), lung carcinoma cell line (H460), breast carcinoma cell line (MCF7), brain carcinoma cell line (U251), and animal cell line EAC (Ehrlich ascites carcinoma cells). The results revealed that some of these modified deazapyrimidine thioglycosides have significant cytotoxic activity against EAC cells with growth inhibition percentage ranged between 80% to 90%. The possible inhibitory mechanism of the pyridine thioglycosides was explored by studying the cell cycle perturbation of thioglycosides against human cell lines *(in vitro)* as well as the most suitable time for maximum compound cytotoxic activity after 6, 18, and 24 h of incubation. To confirm the cytotoxic activity of these compounds, they have been tested for their apoptotic and antiproliferative activity *in vivo* against solid Ehrlich tumours using five groups of Swiss albino mice for 37 days from inoculation and three treatments, 250, 500 and 1,000 μg/kg body weight. There was significant reduction in Ehrlich tumour size in case of the 500 and 1,000 μg/kg body weight group but mild significant tumour reduction in the 250 μg/kg body weight group. Histograms of DNA per cell for each treatment group indicated that there was a dose-dependent increase in the preG$_1$ phase with a corresponding complete arrest of cells from entering the G$_2$/M phase compared to the untreated EAC group.

In conclusion, pyridine thioglycosides have proven good cytotoxic effects against EAC cells and also significant cytotoxic activity against the four tested human cell lines. Flow cytometric DNA ploidy analysis of pyridine thioglcyosides against the Hepg2 and U251 cell lines revealed that the postulated mechanism of action of pyridine thioglcyosides is cell cycle arrest in the S phase. This is similar to antimetabolites and cell cycle arrest in the G$_2$/M phase (M phase) in the same way as microtubule inhibitors like pyridine thioglycosides are cell-cycle-specific in the S phase and the M phase (in case of human cell lines) and have apoptotic effects (in case of animal cell line).

Key words: Anticancer Agents, Antimetabolites, Pyridine Thioglycosides

Introduction

Nucleosides of purines and pyrimidines are fundamental building blocks of biological systems that display a wide range of biological activities. Consequently, the search for nucleoside analogues that function as nontoxic, selective inhibitors for the control of viral diseases and cancer has been the subject of intense research, including the determination of their activity against the human immunodeficiency virus (HIV) (Bostrom and Erdmann, 1993; Johnston *et al.*, 1997; Dorr and Von Hott, 1994). In recent reports from our laboratory, we described the preparation of different novel functionalized pyrimidine and pyridine thioglycosides, which revealed antagonistic activity against human carcinoma cells and the HIV (Elgemeie and Attia, 2002, 2003, Elgemeie *et al.*, 1994, 1997, 1999, 2004). In an earlier brief communication we had reported the use of dihydropyridinethione glycosides as P-glycoprotein (Pgp) substrates or inhibitors in the protein glycosylation process (Scala *et al.*, 1997). These common features encouraged us to develop a new straightforward route for the synthesis of these compounds and to investigate their potential anticancer effect against many human cell lines (*in vitro* studies) as well as animal cell lines (Ehrlich ascites carcinoma, *in vivo* studies).

Material and Methods

Pyridine and dihydropyridine thioglycosides

The structure and molecular weight of all pyridine and dihydropyridine thioglycosides were confirmed by IR, MS, ^{1}H NMR and ^{13}C NMR spectra; they have been prepared following our reported procedures (Elgemeie and Attia, 2002, 2003; Elgemeie *et al.*, 1994, 1997, 1999, 2004).

Cell culture

Four human carcinoma cell lines were used in this study: U251 (brain carcinoma cell line), MCF7 (breast carcinoma cell line), Hepg2 (liver carcinoma cell line), and H460 (lung carcinoma cell line). They were obtained frozen in liquid nitrogen (−180 °C) from the American Type Culture Collection. The tumour cell lines were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing. Such cell lines are grown as "monolayer culture" in RPMI-1640 medium supplemented with 10% FBS, 100 units/ml penicillin and 2 mg/ml streptomycin. The cell lines were incubated at 37 °C in 5% CO_2/95% air in a humide atmosphere in a water-jacketed incubator (Revco, GS Laboratory Equipment, RCO 3000 TVBB, Asheville, NC, USA).

Antitumour activity of different thioglycosides against Ehrlich ascites carcinoma cells (mouse tumour)

A line of Ehrlich ascites carcinoma (EAC) cells was supplied through the courtesty of Dr. G. Klien, Amsterdam, Holland. The tumour line was maintained in female Swiss albino mice by intraperitoneal transplantation of $2.5 \cdot 10^6$ cells from one mouse to another in the Laboratory of Pharmacology and Clinical Pharmacy Department, National Cancer Institute, Cairo, Egypt. Antitumour activity against EAC cells for any drug occured in the following way:

The drug for which the cytotoxicity had to be investigated was prepared by adding 1 mg of solid thioglycosides to 0.1 ml dimethyl sulfoxide (DMSO) solvent. The mixture was then shaked for complete solvation of the drug, and the volume was completed to 1 ml by phosphate buffered saline (PBS, pH 7.4), then diluted by additional 4 ml of PBS to a concentration of 2 µg drug/10 µl of solvent.

Ascites fluid was withdrawn under aseptic conditions (ultraviolet laminar flow system) from tumour-bearing mice by needle aspiration from the peritoneal cavity, 7–10 d after EAC cells inoculation.

Each ml of EAC tumour cells obtained was diluted with 4 ml PBS, then determination and counting of EAC viable cells by trypan blue exclusion test were done as follows: (i) 50 µl of 0.05% trypan blue solution were added to 50 µl of the single cell suspension. (ii) The cells were examined under an inverted microscope (Olympus 1 × 70, Tokyo, Japan) using the haemocytometer, where dead cells were stained and viable cells not so that each ml contained $2.5 \cdot 10^6$ EAC cells (Lazarus *et al.*, 1966).

For each drug three sterile test tubes were prepared: One contained 25 µg/ml drug + 100 µl EAC + 875 µl PBS; one contained 50 µg/ml drug + 100 µl EAC + 850 µl PBS; one contained 100 µg/ml drug + 100 µl EAC + 800 µl PBS.

Another two extra sterile test tubes were prepared: A DMSO tube containing 800 µl PBS + 100 µl EAC + 100 µl DMSO (in which 0.4 ml saline was added to 0.1 ml DMSO, then 0.1 ml of this solution was rediluted with another 0.4 ml PBS, 100 µl of this previous solution were taken and added to the DMSO tube) and a control tube containing 900 µl PBS + 100 µl EAC. The sterile tubes were maintained under a constant overlay of 5% CO_2 and kept in a water bath at 37 °C for 2 h.

The EAC viable cells were determined and counted by the trypan blue exclusion test as follows: (i) 50 µl of 0.05% trypan blue solution were added to 50 µl of the single cell suspension. (ii) The cells were examined under an inverted microscope using a haemocytometer, where dead cells were stained and viable cells not.

Percent survival of cells = $(T/C) \cdot 100$ and percent growth inhibition = $(C − T/C) \cdot 100$ for each specimen were calculated, where T and C represent the number of viable cells in a unit volume and the number of total (viable + dead) cells in the same unit volume, respectively.

Sulforhodamine B (SRB) assay

The assay was carried out according to Skehan *et al.* (1990). Viability was determined by trypan blue exclusion using an inverted microscope.

Cells were seeded in 96-well microtiter plates at a concentration of $5 \cdot 10^4 – 10^5$ cells/well in

fresh medium and left to attach to the plates for 24 h. After 24 h, cells were incubated with the appropriate concentration of drugs and completed to a total of 200 μl/well using fresh medium. Incubation was continued for 48 h. Control cells were treated with vehicle alone. For each drug concentration (0, 1, 2.5, 5, and 10 μg/ml) three wells were used. Following 48 h of treatment, the cells were fixed with 50 μl cold 50% trichloroacetic acid for 1 h at 4 °C. Wells were washed 5 times with water and stained for 30 min at room temperature with 50 μl 0.4% SRB (sulforhodamine B) dissolved in 1% acetic acid. The wells were then washed 4 times with 1% acetic acid. The plates were air-dried, and the dye was solubilized with 100 μl/well of 10 mM Tris base (pH 10.5) for 5 min on a shaker (Orbital shaker OS 20, Boeco, Hamburg, Germany) at 1600 rpm. The optical density (OD) of each well was measured spectrophotometrically at 564 nm with an Elisa microplate reader (Meter tech Σ 960, Fort Lauderdale, FL, USA). The mean background absorbance was subtracted automatically, and mean values for each drug concentration were calculated. The percentage of cell survival was calculated as follows: Survival fraction = OD (treated cells)/OD (control cells).

The IC$_{50}$ values (the concentrations of thioglycoside required to produce 50% inhibition of cell growth) were calculated using sigmoidal dose response curve fitting models (Graph Pad, Prizm software).

Tumour transplantation

Solid tumour was produced by intramuscular (i.m.) inoculation in the right thigh of a female mouse with 0.2 ml (2.5 · 10^6 cells) of EAC cells after dilution. The control mice received an equal volume (0.2 ml) of saline only. The animals were used, when their tumour had grown to about 10 mm in diameter (9 d after tumour inoculation; such period was believed to permit the development of the tumour without causing death of inoculated animals).

Compound **2A** was dissolved in a small volume of DMSO, completed to the appropriate volume with saline, and injected intraperitoneally in different treatments (250, 500 and 1,000 μg/kg body weight) 9 d after tumour inoculation, 3 times a week up to 37 d after tumour inoculation.

Tumour volume measurement

The size of a solid tumour was measured using a vernir caliper, starting from the ninth day post tumour inoculation. The tumour volume was calculated by the following formula, according to reported literature (Papadopoulos *et al.*, 1989): tumour volume (mm^3) = 4/3 ϖ $(A/2)^2$ $(B/2)$ = 0.52 A^2B, where A and B are the minor and major axes of the tumour, respectively.

Flowcytometric cell cycle analysis

The method was carried out according to Shanky *et al.* (1993) and Dressler (1990).

In vitro experiment: Cells were seeded in 96-well microtiter plates at 5 · 10^4 – 10^5 cells/well in fresh medium and left to attach to the plates for 24 h. Thereafter, cells were incubated with the appropriate concentration of thioglycosides, completed to a total of 200 μl/well using fresh medium; incubation was continued for 48 h. Control cells were treated with vehicle alone. For each drug concentration, three wells were used. Plates were removed from the incubator and the culture medium gently discarded. 1 ml of propidium iodide solution was added into each DNAcon3 test tube containing dehydrated buffer mixture. 50 μl of cell suspension containing a minimum of 200,000 cells (nuclei) were added to each DNAcon3 test tube. All sample preparations were stored at 2–8 °C for 60 min. Flow cytometric DNA ploidy analysis was performed by acquiring a minimum of 200,000 nuclei. Cell cycle analysis was performed with the Epics XL coulter software package (Beckman Coulter Corp., Brea, CA, USA).

In vivo experiment: All fat and connective tissue was removed from solid EAC tissue samples. The tissue was kept wet using RPMI medium and carefully minced using a scalpel until complete disaggregation. The cell suspension, consisting of recovered cells with PBS, was filtered using a 50-μm pore size mesh and centrifuged below 1,000 rpm. 50 μl of 0.05% trypan blue solution were added to 50 μl of the cell pellet suspension. The cells were examined under an inverted microscope using a homocytometer. Nonstained (viable) cells were counted, and the cells were then diluted to give the concentration of single cell suspension required for proper flow cytometric DNA ploidy analysis which was 50 μg PI per 1 to 5 · 10^6 cells (nuclei) per ml. 1 ml of propidium iodide solution was added into each DNAcon3
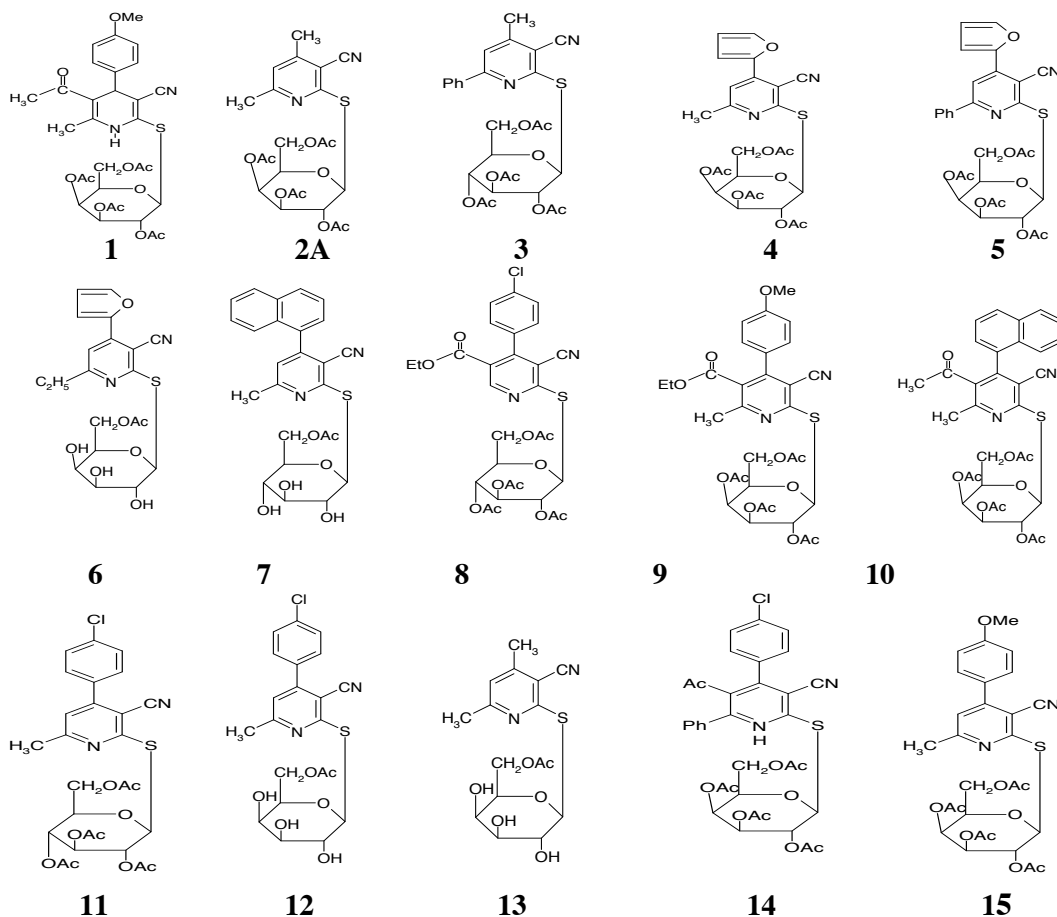
Fig. 1. Chemical structures of compounds **1–28**, **3B**, and **6B**.

test tube containing dehydrated buffer mixture. 50 μl of cell suspension containing a minimum of 200,000 cells (nuclei) were added to each DNA-con3 test tube. All sample preparations were stored at 2–8 °C for 60 min. Flow cytometric DNA ploidy analysis was performed by acquiring a minimum of 200,000 nuclei. Cell cycle analysis was performed with the Epics XL coulter software package (Beckman Coulter Corp.).

Results

In vitro study

Flow cytometric DNA ploidy analysis was performed in the H460 cell line treated for 48 h with the six compounds (**6**, **1**, **8**, **3B**, **6B**, and **10**, Fig. 1) in a concentration of 10 μg/ml which have shown

sufficient cytotoxic activity against this line. A histogram of DNA per cell was obtained for each specimen as presented in Table I.

Table I. Cell cycle modifications (in %) induced by six different thioglycosides at 10 μg/ml in H460 cells after 48 h of incubation.

Drug	H460		
	G_0/G_1	S	G_2/M
Control	87.9	3.3	7.5
6	82.3	6.05	6.95
1	84	5.57	8.88
8	79.3	4.64	10.2
3B	87.2	2.48	8.53
6B	85.5	3.37	8.64
10	85.2	2.58	10.4

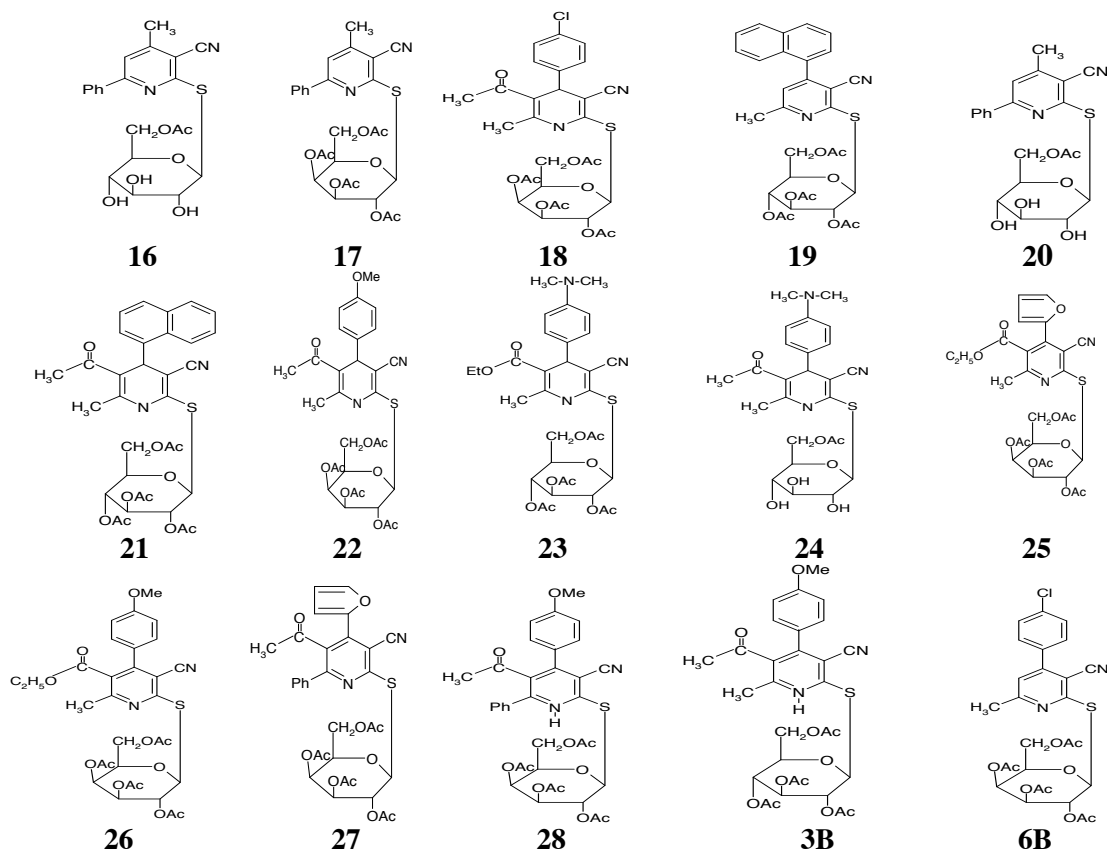Values are presented as percentages of cells at the indicated cell cycle phases.

Fig. 1 continued

In the control specimen of the H460 cell line, cells were distributed as follows: Nearly 88% were in the G_0/G_1 phase, 3.3% were in the S phase, and 7.5% were in the G_2/M phase. Treatment of the cells with the compounds didn't give any significant change in the percentage of cells in the G_0/G_1, S, and G_2/M phases compared to the control specimen.

In addition, flow cytometric DNA analysis was performed in the H460 cell line treated with **10** (Fig. 1) at 20 μg/ml. The percentage of cells in the G_0/G_1, S, and G_2/M phases and is shown in Table II.

In the control specimen of the H460 cell line, cells were distributed as follows: More than 94% were in the G_0/G_1 phase, 1.19% were in the S phase, and 3.81% were in the G_2/M phase. The cell cycle distribution analysis revealed that treatment of cells with higher concentrations of **10** didn't give a significant change in the percentage of cells in the G_0/G_1, S, and G_2/M phase compared to the control, although there was mild depletion of the S phase (from 1.19% to 1.07%).

Flow cytometric DNA ploidy analysis was performed in the Hepg2 cell line treated with **21** (Fig. 1), which is the most active compound against this line, for 6 h, 18 h, and 24 h in a concentration of 20 μg/ml. Histograms of DNA per cell were obtained for each individual incubation

Table II. Cell cycle modifications (in %) induced by compound **10** at 20 μg/ml in H460 cells after 48 h of treatment.

Drug	H460		
	G_0/G_1	S	G_2/M
Control	94.2	1.19	3.81
10	94.2	1.07	4.25

Values are presented as percentages of cells at the indicated cell cycle phases.
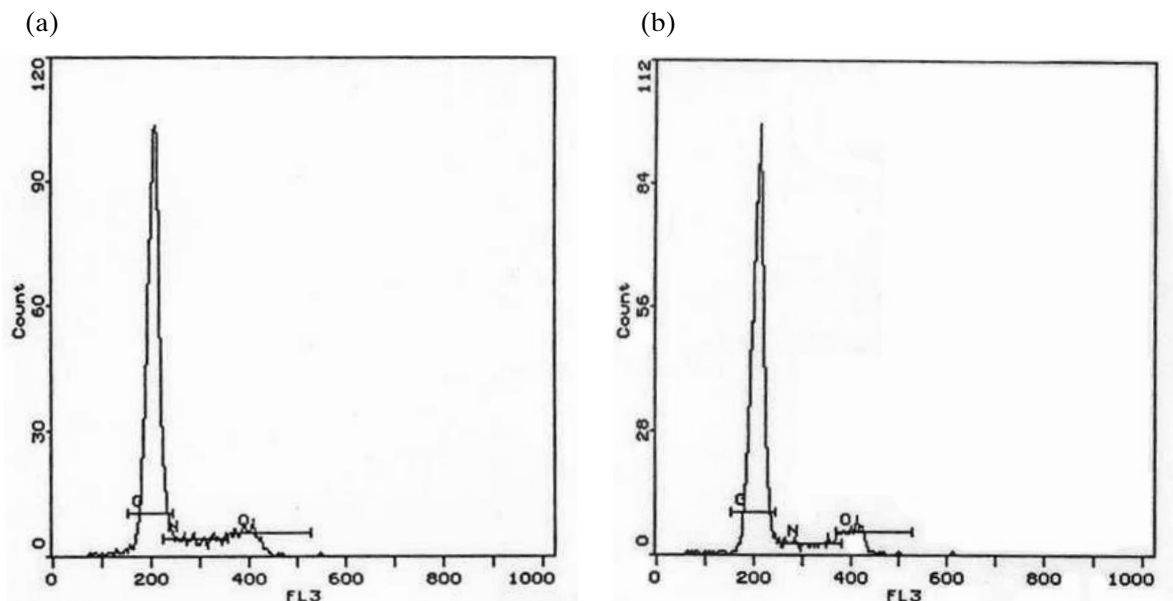
(a)

(b)

Fig. 2. (a) Cell cycle analysis of the untreated Hepg2 cell line 6 h after the exponential phase. (b) Cell cycle analysis of the Hepg2 cell line treated with compound **21** for 6 h.
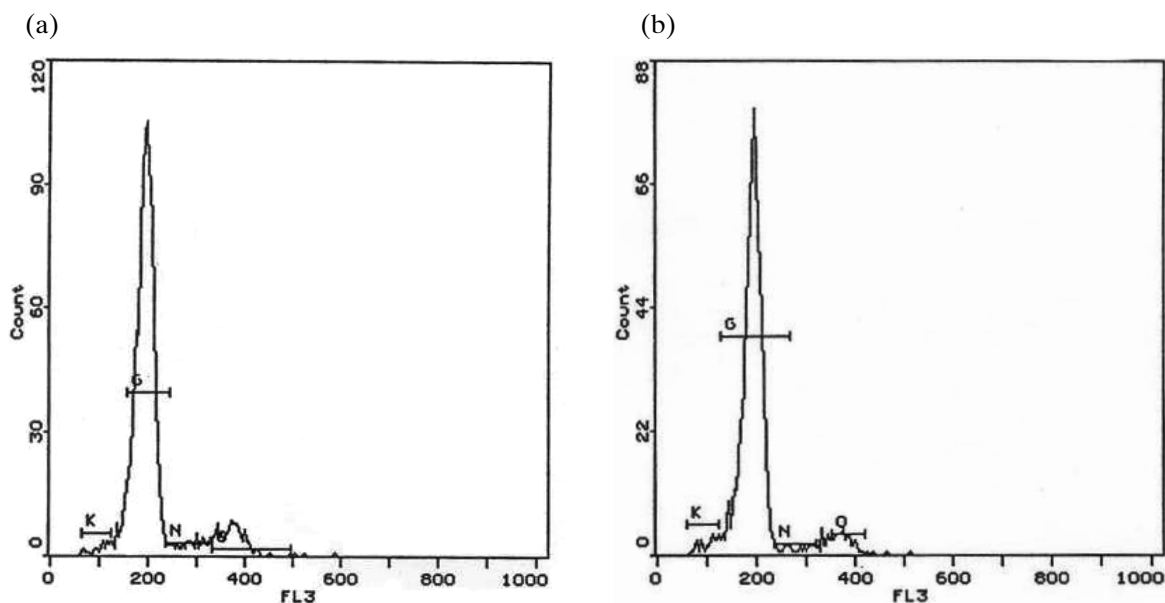
(a)

(b)

Fig. 3. (a) Cell cycle analysis of the untreated Hepg2 cell line 18 h after the exponential phase. (b) Cell cycle analysis of the Hepg2 cell line treated with compound **21** for 18 h.

time as presented in Figs. 2, 3, and 4 and the percentage of cells in the G_0/G_1, S, and G_2/M phases are shown in Table III.

Cell cycle distribution analysis in the control specimen of the Hepg2 cell line after 6 h of incubation showed 76.1% in the G_0/G_1 phase, 16.4%

in the S phase, and 10.4% in the G_2/M phase, *i.e.* treatment of the Hepg2 cell line with **21** for 6 h led to moderate depletion of the S phase (from 16.4% to 10.5%) with no significant change in the percentage of cells in the G_0/G_1 and G_2/M phases. While the cell cycle distribution analysis
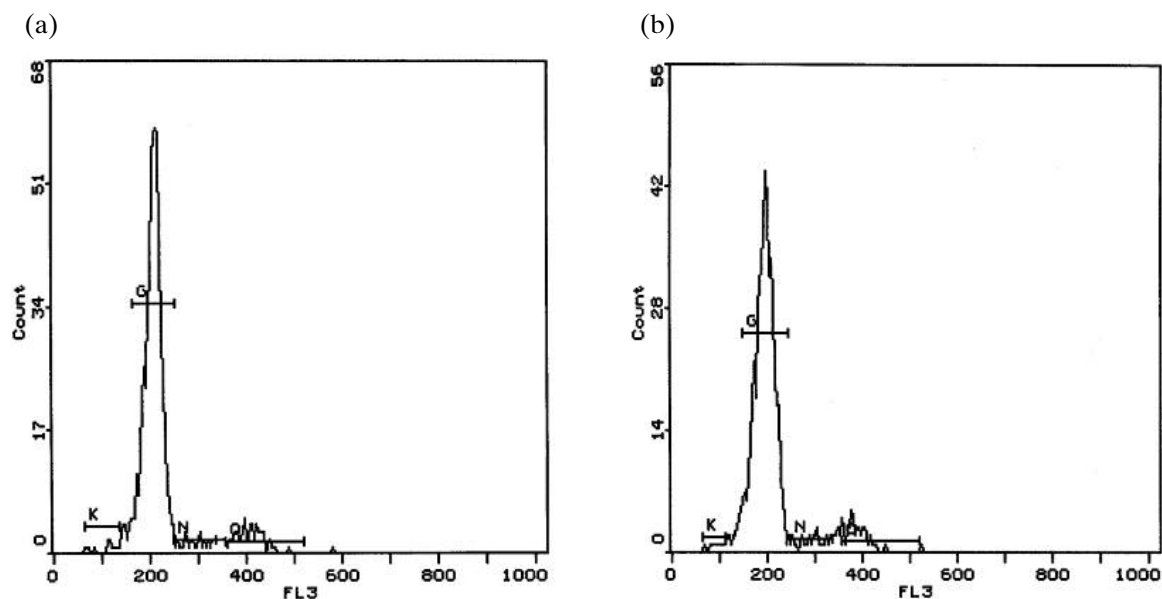
(a) (b)

Fig. 4. (a) Cell cycle analysis of the untreated Hepg2 cell line 24 h after the exponential phase. (b) Cell cycle analysis of the Hepg2 cell line treated with compound **21** for 24 h.

in the control group of the Hepg2 cell line after 18 h of incubation revealed 84.9% in the G_0/G_1 phase, 3.79% in the S phase, and 11.9% in the G_2/M phase, treatment of the Hepg2 cell line with **21** for 18 h revealed that there was a quiet significant decrease in the S phase compared to the control (from 3.79% in the control to 3.5% in the test specimen) and also there was a significant decrease in the G_2/M phase by about 50% (from 11.9% in control to 5.8% in test specimen) with no significant change in the percentage of cells in the G_0/G_1 phase. On the other hand the cell cycle distribution analysis in the control group of the Hepg2 cell line after 24 h of incubation revealed 84.4% in the G_0/G_1 phase, 5.03% in the S phase, and 8.52% in the G_2/M phase. Treatment of the Hepg2 cell line with **21** for 24 h have shown that there was a significant decrease in the S phase compared to the control (from 5.03% in the control to 4.39% in the test specimen) and also there was a moderate decrease in the G_2/M phase compared to the control (from 8.52% in the control to 7.27% in the test specimen) with no significant change in the percentage of cells in the G_0/G_1 phase.

Also flow cytometric DNA ploidy analysis was performed in U251 cells treated with **10** (Fig. 1), which is the most active compound against this

Table III. Cell cycle modifications (in %) induced by compound **21** at 20 µg/ml in Hepg2 cells after 6, 18, and 24 h of incubation.

Drug	Hepg2		
	G_0/G_1	S	G_2/M
Control, 6 h	76.1	16.4	10.4
Test, 6 h	77.4	10.5	10.7
Control, 18 h	84.9	3.79	11.9
Test, 18 h	84.9	3.5	5.8
Control, 24 h	84.4	5.03	8.52
Test, 24 h	84.9	4.39	7.27

Values are presented as percentages of cells at the indicated cell cycle phases.

line, for 6 h, 18 h, and 24 h in a concentration of 20 µg/ml. Histograms of DNA per cell were obtained for each individual incubation time, and the percentage of cells in the G_0/G_1, S, and G_2/M phases is shown in Table IV.

Cell cycle distribution analysis in control specimen of the U251 cell line after 6 h of incubation revealed 63.2% in the G_0/G_1 phase, 10% in the S phase, and 20.7% in the G_2/M phase. Treatment of the U251 cell line with **10** for 6 h led to significant accumulation of cells in the S and G_2/M phase compared to control specimen. While the cell cycle distribution analysis in the control group of

the U251 cell line after 18 h of incubation showed 76.3% in the G_0/G_1 phase, 8.7% in the S phase, and 9.42% in the G_2/M phase, treatment of the U251 cell line with **10** for 18 h revealed that there was a significant decrease in the S phase compared to the control (from 8.7% in the control to 7.8% in the test specimen) and also there was a significant decrease in the G_2/M phase (from 9.42% in the control to 6.6% in the test specimen) with no significant change in the percentage of cells in the G_0/G_1 phase. On the other hand the cell cycle distribution analysis in the control group of the U251 cell line after 24 h of incubation revealed 79.4% in the G_0/G_1 phase, 7.7% in the S phase, and 10% in the G_2/M phase. Treatment of the U251 cell line with **10** for 24 h have shown that there was a moderate decrease in the S phase compared to the control (from 7.7% in the control to 7.15% in the test specimen) and also there was a moderate decrease in the G_2/M phase compared to the control (from 10% in the control to 8.5% in the test specimen) with no significant change in the percentage of cells in the G_0/G_1 phase.

Table IV. Cell cycle modifications (in %) induced by compound **10** at 20 μg/ml in U251 cells after 6, 18, and 24 h of incubation.

Drug	U251		
	G_0/G_1	S	G_2/M
Control, 6 h	63.2	10	20.7
Test, 6 h	63	12.4	27.5
Control, 18 h	76.3	8.7	9.42
Test, 18 h	81.1	7.8	6.6
Control, 24 h	79.4	7.7	10
Test, 24 h	78.1	7.15	8.5

Values are presented as percentages of cells at the indicated cell cycle phases.

In vivo study

Table V illustrates the flow cytometric DNA ploidy analysis of EAC cells 37 days after solid tumour inoculation without any treatment or injected with **2A** (Fig. 1) at doses of 250, 500, and 1,000 μg/kg body weight, three times weekly starting at the ninth day post inoculation till day 37 of tumour inoculation. Histograms of DNA per cell were obtained for each treatment group as presented in Fig. 5, and the percentage of cells in the G_0/G_1, S, and G_2/M phases are shown in Table V.

In the control group, cells were distributed as follows: 67.8% in the G_0/G_1 phase, 12.6% in the S phase, and 12.3% in the G_2/M phase. The cell cycle distribution analysis in the group of mice inoculated with EAC and treated with 250 μg/kg body weight of **2A** have shown that the percentage of cells in the preG1 phase increased which wasn't found in the control group indicating that the number of apoptotic cells (22.2% in the preG1 phase) increased compared to the control group and there was a complete decrease in cells entering the G_2/M phase. Also in the animals inoculated with EAC and treated with 500 μg/kg body weight of **2A** cells were distributed so that the percentage of cells in the preG1 phase increased more than in the 250 μg/kg body weight group due to increasing the concentration of the compound indicating that the percentage of apoptotic cells may be proportional to the increasing compound concentration (33% in the preG1 phase). Also there was a complete decrease in cells entering the G_2/M phase. Similarly in the 1,000 μg/kg body weight group, the percentage of preG1 phase cells increased more than in the 250 μg/kg body weight group and 500 μg/kg body weight group due to increasing compound concentration and increasing percentage of apoptotic cells (51% in the preG1

Table V. Cell cycle modifications (in %) induced by 250, 500, and 1,000 μg/kg body weight (b.w.) treatment with **2A** of EAC tumour cells for 37 d, three times weekly.

Drug	EAC			
	preG$_1$	G_0/G_1	S	G_2/M
Control EAC	–	67.8 ± 1.8	12.6 ± 2.1	12.3 ± 3.7
EAC + 250 μg/kg b.w.	22.2 ± 0.77	52 ± 1.05	16.1 ± 1.53	–
EAC + 500 μg/kg b.w.	33 ± 2.9	36.13 ± 3.04	17.63 ± 1.3	–
EAC + 1,000 μg/kg b.w.	51 ± 2.445	26.05 ± 2.55	10.135 ± 0.465	–

Each value is the mean ± SEM of six values (mice). Values are presented as percentages of cells at the indicated cell cycle phase.
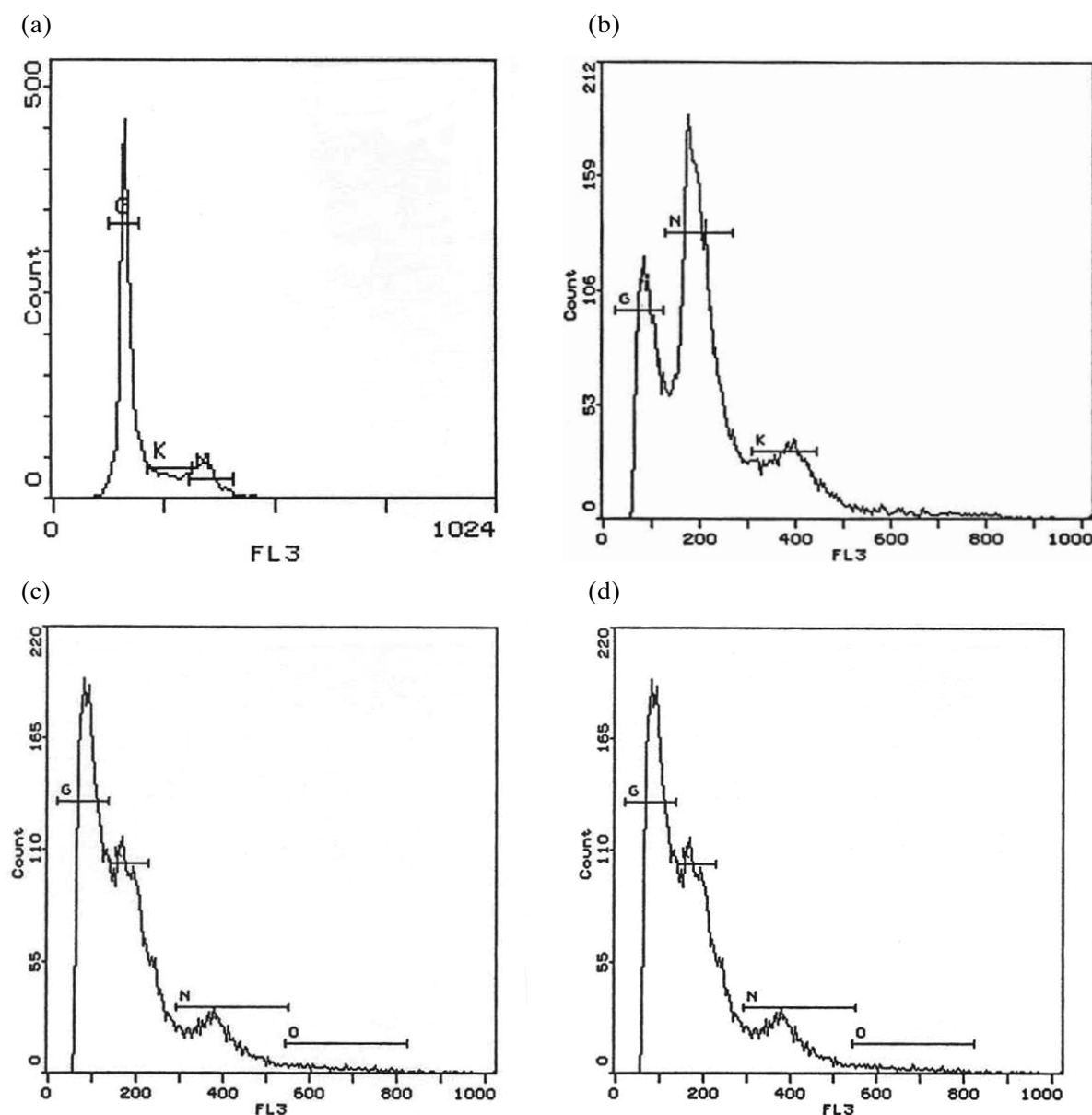
(a)

(b)

(c)

(d)

Fig. 5. (a) Cell cycle analysis of untreated EAC cells 37 d after inoculation. (b) Cell cycle analysis of EAC tumour cells treated with compound **2A** at 250 μg/kg body weight for 37 d, three times weekly. (c) Cell cycle analysis of EAC tumour cells treated with compound **2A** at 500 μg/kg body weight for 37 d, three times weekly. (d) Cell cycle analysis of EAC tumour cells treated with compound **2A** at 1,000 μg/kg body weight for 37 d, three times weekly.

phase). Similarly there was a complete decrease in cells entering the G_2/M phase.

As shown in Fig. 6 the tumour volume of the EAC group progressively increased in size reaching about four times of its initial volume at the end of the experimental period 37 days after inoculation.

Intraperitoneal administration of **2A** at 250 μg/kg body weight three times weekly resulted in mild reduction of the tumour size compared to the untreated EAC group, while administration of **2A** at 500 μg/kg body weight resulted in significant reduction of the tumour size, and at 1,000 μg/kg body weight it resulted in high reduction of
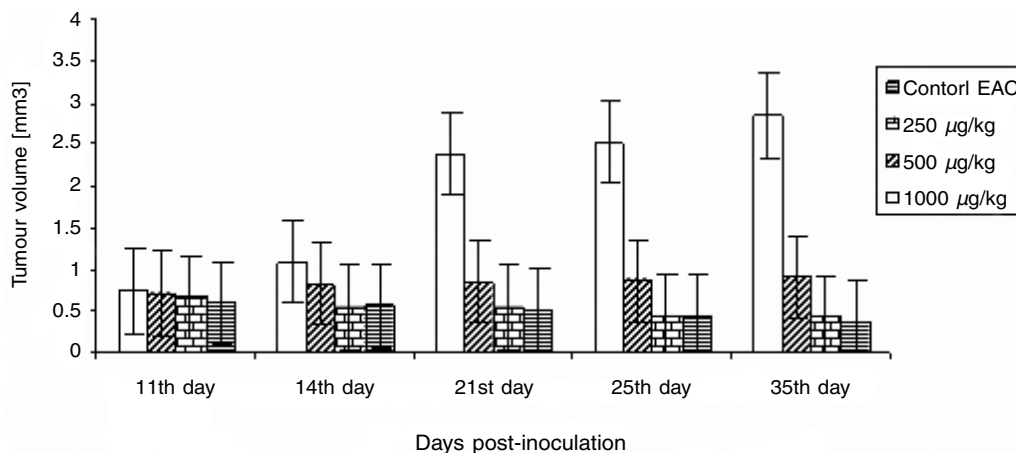
Fig. 6. Volume of solid EAC tumour cells in mice treated with 250, 500, and 1,000 μg/kg body weight of compound **2A** for 37 d.

the tumour size compared with the untreated EAC group.

Discussion

Pyridine thioglycosides were recently reported by us as a new class of antimetabolites which exert inhibitory effects on both DNA and RNA containing viruses (Scala *et al.*, 1997). The structural requirements for Pgp-blocking activity within this particular group of pyridine thioglycosides are quite broad; effective blockers were found with the pyridine and dihydropyridine structure. For further highlighting the cytotoxic activity of pyridine thioglycosides, our current study was established aiming to investigate the cytotoxic, antiproliferative and apoptotic effect of thioglycosides on both human cell lines and mouse tumour.

The closest assumed mechanism for such thioglycosides as revealed from flow cytometric DNA ploidy analysis results indicated that pyridine thioglycosides may act in the same way as antimetabolites, by inhibiting key enzymes in the biosynthesis of purine and pyrimidine that are incorporated in the DNA molecule. Pyridine thioglycosides may also act like vincristine and vinblastine which bind to tubulin blocking the ability

of tubulin to polymerize. They form microtubules instead of paracrystalline aggregates consisting of tubulin dimers and the alkaloid drug formed. The resulting dysfunctional spindle apparatus frozen in the metaphase, preventing chromosomal segregation and cell proliferation, may act like taxanes (*e.g.* paclitaxel) which bind reversibly to tubulin. But unlike vinblastine and vincristine it promotes polymerization and stabilization of the polymer rather than disassembly, thus it shifts the depolymerization-polymerization to favour the formation of microtubules. Stable microtubules formed in the presence of paclitaxel are dysfunctional causing the death of the cell in the metaphase (Rowinsky and Donehower, 2001).

In conclusion, thioglycosides have been prove to have good cytotoxic effects against EAC cells as well as four human cell lines tested in the current study. The postulated mechanism of action of pyridine thioglcyosides is cell cycle arrest in the S phase similar to antimetabolites and cell cycle arrest in the G_2/M phase (M phase) in the same way like microtubules inhibitors. Thus pyridine thioglycosides are cell-cycle-specific in the S phase and M phase (in case of human cell lines) and show apoptotic effects (in case of animal cell line).

Bender R. A. and Hamel E. (1985), *Vinca* alkaloids. In: The Cancer Chemotherapy (Chabner B. A. and Pinedo H. M., eds.). Elsevier, Amsterdam, New York, Oxford, p. 100.

Bostrom B. and Erdmann G. (1993), Cellular pharmacology of 6-mercaptopurine in acute lymphoblastic leukemia. Am. J. Pediatr. Hematol. Oncol. **15**, 80–86.

Dorr R. T. and Von Hott D. H. (1994), Cancer Chemotherapy Handbook. Appleton and Lange, Norwalk, pp. 680–685 and pp. 893–898.

Dressler L. G. (1990), Controls, standard, and histogram interpretation in DNA flow cytometry. Methods Cell Biol. **33**, 157–171.

Elgemeie G. H. and Attia A. M. (2002), First gylcoside synthesis via piperidinium salts of heterocyclic nitrogen bases: the synthesis of a new class of dihydropyridine thioglycosides. J. Carbohydrate Chem. **21**, 325–340.

Elgemeie G. H. and Attia A. M. (2003), A new class of dihydropyridine thioglycosides via piperdinium salts. Synth. Commun. **33**, 2243–2260.

Elgemeie G. H., Attia A. M., Farag D. S., and Sherif S. M. (1994), Nucleotides and nucleosides: direct route to condensed pyridinethione carbocyclic nucleosides related to 3-deazauridine. J. Chem. Soc. Perkin Trans. 1, 1285–1289.

Elgemeie G. H., Attia A. M., and Shehada L. (1997), Synthesis of some novel condensed pyridine-2(1*H*)-thiones and related glycosides. Tetrahedron **53**, 17441–17448.

Elgemeie G. H., Attia A. M., and Hussain B. A. (1999), Synthesis of *N*-glycosylated pyridines as new antimetabolite agents. Nucleosides Nucleotides **18**, 2335–2350.

Elgemeie G. H., Hussein M. M., and Alkharasani S. A. (2004), A total synthesis of a new class of biazine thioglycosides. J. Carbohydrate Chem. **23**, 468–480.

Johnston P. G., Akimoto C. H., Grem J. L., Grossbare M. L., Chabner B. A., and Allegra C. J. (1997), Cancer chemotherapy and biological response modifiers. In: Antimetabolites (Pinedo H. M., Longo D. L., and Chabner B. A., eds.). Elsevier, Amsterdam, pp. 1–39.

Lazarus H., Tegeler M., Marzone H., Leroy J., Boone B., and Foley G. (1966), Determination of sensitivity of individual biopsy specimens to potential inhibitory agents. Evaluation of some explant cultures as assay systems. Cancer Chemother. Rep. **50**, 543–554.

Papadopoulos D., Kimller B. F., Estes N. C., and Durham F. J. (1989), Growth delay effect of combined interstitial hypothermia and brachytherapy in a rat solid tumour model. Anticancer Res. **9**, 45.

Rowinsky E. and Donehower R. (2001), Anti-microtubule agents. In: Cancer Chemotherapy and Biotherapy Principles and Practice, 2nd ed. (Chabner B. and Longo D., eds.). Lippincott-Raven Publishers, Philadelphia, pp. 263–297.

Scala S., Akhmed N., Rau U. S., Paull K., Lan L., and Elgemeie G. H. (1997), P-Glycoprotein substrates and antagonists cluster into two distinct groups. Mol. Pharmacol. **51**, 1024–1030.

Shankey T. V., Rabinovitch P. S., Bagwell B., Bauer K. D., Doque R. E., and Wheeless L. (1993), Guidelines for implementation of clinical DNA cytometry. Cytometry **14**, 472–477.

Skehan P., Storeng R., Scudiero D., Monks A., McMahon J., and Boyd M. R. (1990), New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. **82**, 1107–1112.