Some Amino Acid Derivatives Containing Selenium Part (II): Anticancer Activity and of Their Cu (II)-Complexes

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Abstract

Anticancer activity of two amino acid derivatives containing selenium [3,5 dinitrobenzoylamino- selenomethyl] Amino Acid; where amino acids; glycine; DNBASG (1) and alanine DNBASA (2) have been done. Anticancer properties against breast cancer (MCF-7) and carcinoma of the uterine cervix (HeLa) were accomplished for compounds (1) and (2) and show lower cytotoxic activity as compared to the antitumor agents 5-fluorouracil, cyclophosphamid and cisplatin. Cupric complexes of the two derivatives (I) and (2) were prepared but lack of solubility prevented the anticancer activity test to be done for them.

Key Words: Amino acids, Dinitrobenzoyl isocelenocyanate, Antibacterial activity.

1. Introduction

Some amino acid derivatives and their complexes have been investigated as having antitumor properties. The chloroacetyl derivatives of four para-substituted phenylalanine were prepared and tested for growth inhibitory activity on microbial antitumor prescreens (Otani.T. et al 1981). Boron analogues of the α-amino acids, ranged from simple glycine analogues such as H3NBH2COOH and Me2NHBH2 COOH to alanine analogues have expressed potent pharmacological activity including anticancer activity against in vitro cell cultures (Bernard et al.,1991). Nine amino acid derivatives of benzisoselenazole (BISA) as well as ebselen; [2-phenyl 1-1,2-benzisosenazol-3(2H)-one] exhibited apparent structure inhibition against human liver tumor cell (HCT-8) and human epidermal carcinoma cell (A431) (Xiao Yingxin Lin et al.,1996). Two new classes of elenazolidine-4®-carboxylic acids(2-oxo and 2-methyl-SCAs) were synthesized and may be clinically useful in cancer chemoprevention(Yang Xie et al 2001). Antitumor activity, in vitro, against leukemia HL-60 and liver cancer BEL-7402 of a series of amino acid ester derivatives containing S-fluorouracil were reported(Jing Xiong et al 2009) 4. Although platinum-based drugs such as cisplatin are powerful anticancer agents but has undesirable side effects, copper, gold and silver complexes are potential candidate to fullfil this need (Shiow Jin Tan et al 2010).Some selenoderivatives have activity against prostate cancer such as 1,4-phenylene bis(methylene)selenocyanate (p-XSC) (Sinha et al 2008), Ebselen (Sung 2006), Cupper-selone complex(Kiman et al 2010) and selenadiazole derivatives (Plano et al, 2010).

We reported her anticancer activity of some new amino acid derivatives containing selenium; DNBASG and DNBASA.

2. Experimental

2.1. Chemicals

All amino acid are from Fluka. All solvents are from BDH and used without further purification except DMSO which was consciously purified and dried and kept in dark container as in the literature method [(AlMudhaffar)]. 3,5-Dinitrobenzoyl Chloride is from Merck.

Potassium tetrachloroplatinate(II) is from Johnson Matty (Seabrook,N-H.). Argon gas (99.995%) is from JGC. Potassium selenocyanate was prepared as literature method[15]. Biological activity was accomplished by using LTF-Uni Jemp Autoclave for sterilizing and Escherichia (NCIC 5933) coli and Staphylococcus aureus (NCTC6571) for biological activity study.

Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco-Invitrogen (UK). Dimethyl sulfoxide (DMSO), neutral red and trypsin were obtained from AppliChem (Germany); thiazolyl blue tetrazolium bromide (MTT) was from Sigma-Aldrich Chemie GmbH (Germany). All other chemicals of the highest purity commercially available were purchased from local agents and distributors. All sterile plastic and syringe filters were from Orange Scientific (Belgium).

2.2. Preparation of Cu (II)-Complexes of (HL)

2.2.1. Preperation of 3,5 dinitro-N-[(benzoyl amino) selenoxomethly)-glycine (DNBASG)(1): and 3,5 dinitro-N-[(benzoyl amino) selenoxomethly)-alanine (2) ,**(HL)**, were carried out as in the literature method (Al-Mudhaffar M.H. 2012).

2.2.2. Preperation of Cu(II) complex with the ligand DNBASG (1) were carried out by adding dropwise solution of $CuCl_2.3H_2O$ dissolved in H_2O to the ligand DNBASG(1) solution dissolved in methanol, in 1:2 molal ratio respectively. The orange brown solution darken and after 45 minutes and solid brown product formed, filtered and washed with water, methanol and diethyl ether. Then was kept in dissicater. m.p.› 300°C

2.2.3. Similarly Cu (II) complex with the second ligand DNBASA (2) was prepared giving brown solid product, worked up as in (ii), and was kept in dissicater. m.p.› 300° C

3. Physical Measurements

The IR-spectra were recorder on Shimadzu FTIR-8400 spectrophotometer using KBr pellets in the range 4000-400cm. Melting points were determined by a Galenkamp melting point apparatus without correction.

4. *Result and Discussion*

4.1. Infrared spectra: infrared spectra of the two copper complexes with the ligands DNBASG(1) and DNBASA(2) showed similar features to that of the ligands except the shift of carbonyl bands from about 1700 cm^{-1} to lower frequency, indicating the coordination of the Cu(II) via carboxylic group of the ligands (1) and (2).

4.2. Anticancer Activity Study

4.2.1. Compounds (DNBASG) 1 and DNBASA 2

The compounds 1 and 2 were initially dissolved in dimethylsulfoxide and then diluted in culture medium. The final concentration of DMSO in the stock solutions (where the concentration of the tested compound was 1 mg/mL) was 2%.

4.2.2. Antitumor Agents

Commercially available antitumor agents 5-Fluorouracil and Cisplatin were used as positive controls in our investigations.

4.2.3. Cell Cultures and Cultivation

Permanent cell lines established from breast cancer (MCF-7) and carcinoma of the uterine cervix (HeLa) were used as model systems in the present study. Both cell lines were obtained from Cell Culture Collection of the Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences.The cells were grown as monolayer cultures in D-MEM medium, supplemented with 5-10% fetal bovine serum, 100 U/mL penicillin and 100 □g/mL streptomycin. The cultures were maintained at 37 ºC in a humidified CO2 incubator (Thermo scientific, Hepa class 100). For routine passages adherent cells were detached using a mixture of 0.05% trypsin and 0.02% EDTA. The experiments were performed during the exponential phase of cell growth.

4.2.4. Cytotoxicity Assays

The cells were seeded in 96-well flat-bottomed microplates at a concentration of 1×104 cells/well. After the cells were grown for 24 h to a subconfluent state (~ 60-70%), the culture medium was removed and changed with media modified with different concentrations (20, 50, 100, 200 µg/ml) of the compounds tested.

Each solution was applied into 4 to 6 wells. Samples of cells grown in non-modified medium served as controls. After 24, 48 and 72 h of incubation, the effect of the compounds on cell viability and proliferation was examined by MTT (thiazolyl blue tetrazolium bromide) test (which reflects damage to mitochondria) and neutral red uptake cytotoxicity assay (NR) (indicates damage to lysosomes).

The MTT colorimetric assay of cell survival was performed as described by Mossman [1983]. The method consisted of three hours incubation with MTT solution (5 mg MTT in 10 mL D-MEM) at 37 ºC under 5% carbon dioxide and 95% air, followed by extraction with a mixture of absolute ethanol and DMSO (1:1, vol/vol) to dissolve the blue MTT formazan.

The NR assay was based on the method of Borenfreund and Puerner [1985]. To each well containing corresponding cells a medium consisting of NR (50 µg/mL, 0.1 mL) was added. The plate was placed in an incubator for 3 h for the uptake of vital dye. Thereafter, the medium with NR was removed and the cells were washed with phosphate buffered saline (PBS, pH 7.2) (0.2 mL/well), followed by the addition of 0.1 mL 1% acetic acid solution containing 50% ethanol to extract the dye from the cells.

Optical density was measured at 540 nm using an automatic microplate reader (TECAN, SunriseTM, Austria). Relative cell viability, expressed as a percentage of the untreated control (100% viability), was calculated for each concentration. "Concentration – response" curves were prepared and the effective concentrations of the compound - CC50 (causing a 50% reduction of cell viability) and/or CC90 (causing a 90% reduction of cell viability) were estimated (where possible) from these curves using Origin 6.1. All data points represent an average of three independent assays.

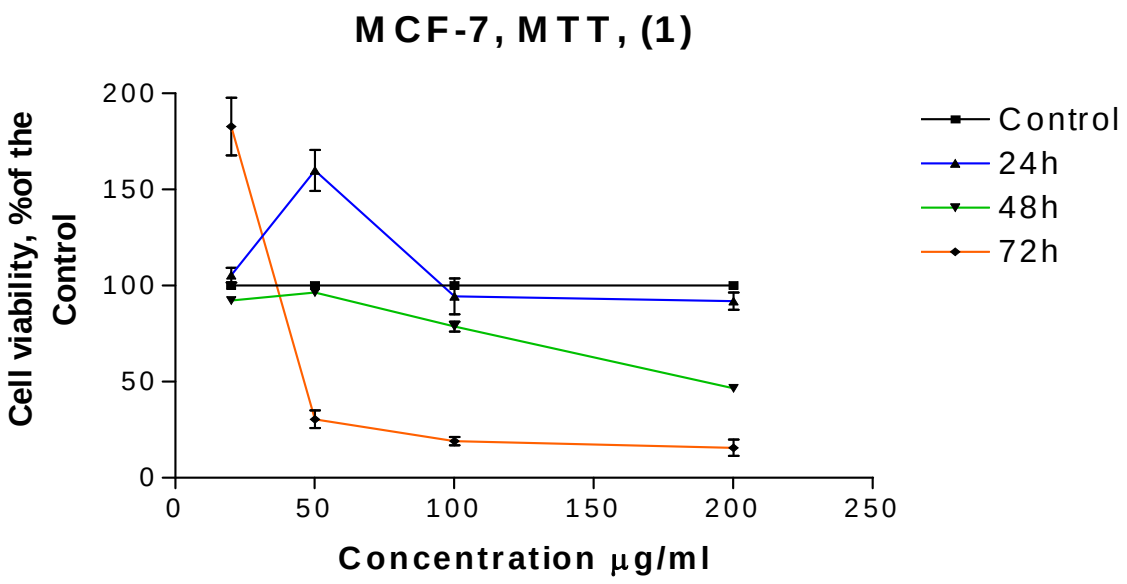
4.2.5. Statistical Analysis

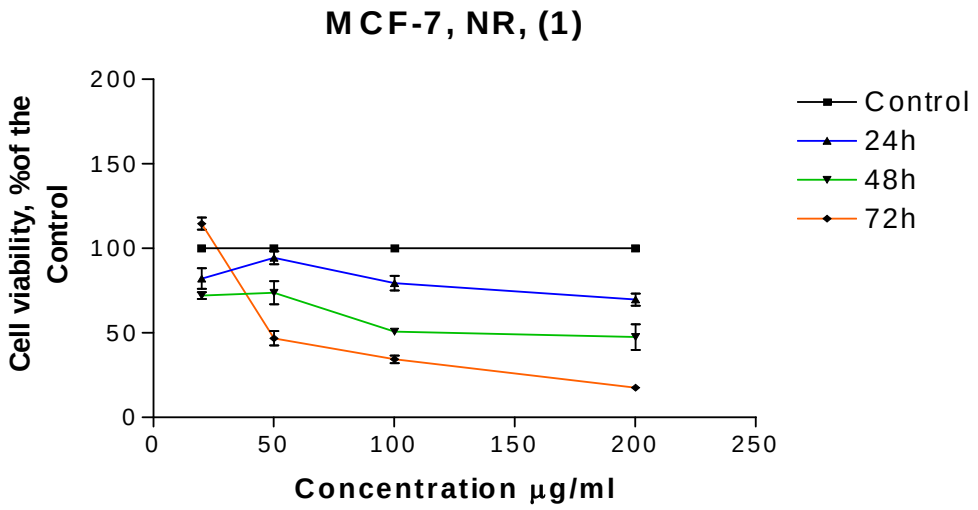
The data are presented as mean ± standard error of the mean. Statistical differences between control and treated groups were assessed using one-way analysis of variance (ANOVA) followed by Dunnett post-hoc test.

4.3. Results

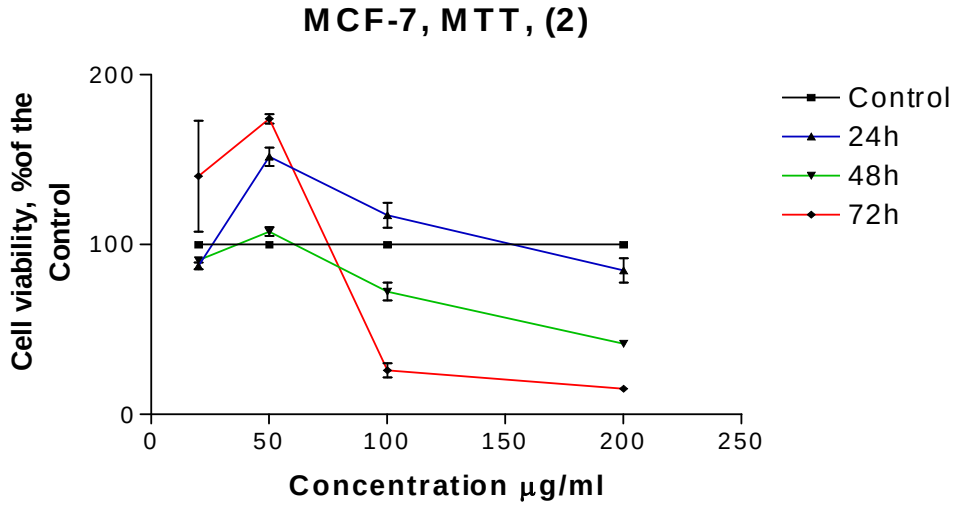
4.3.1. Concentration-response curves (The data about viability of cells treated with compounds 1 and 2 are also presented in Table 1)4.3.2. MCF-7 – MTT and NR

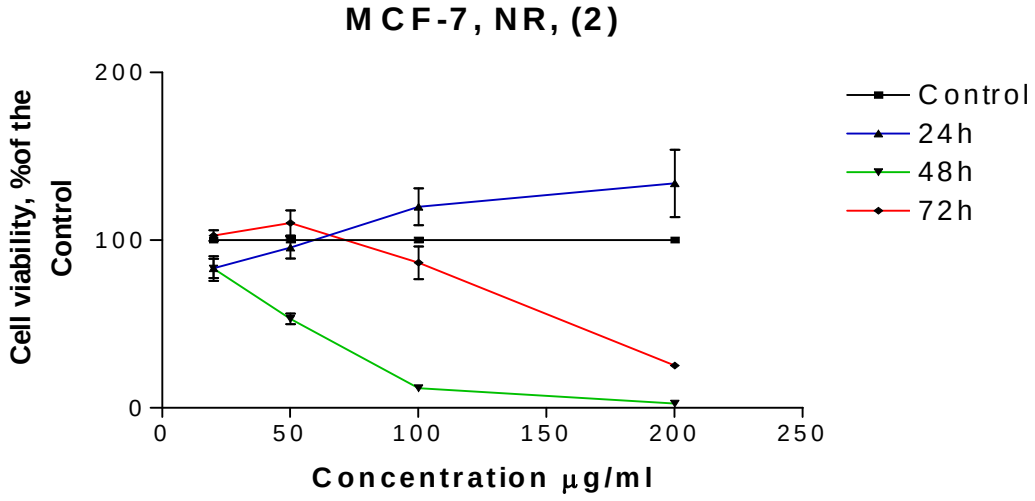
A - Compound (1)




MCF-7, NR, (1)
Cell viability, %of the Control
200
150
100
50
0
0
50
100
150
200
250
Concentration µg/ml
Control
24h
48h
72h

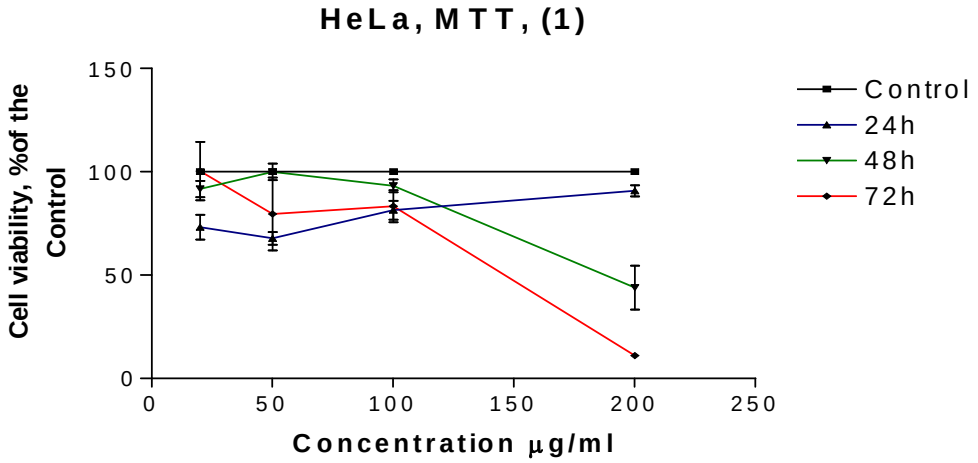
B- Compound (2)

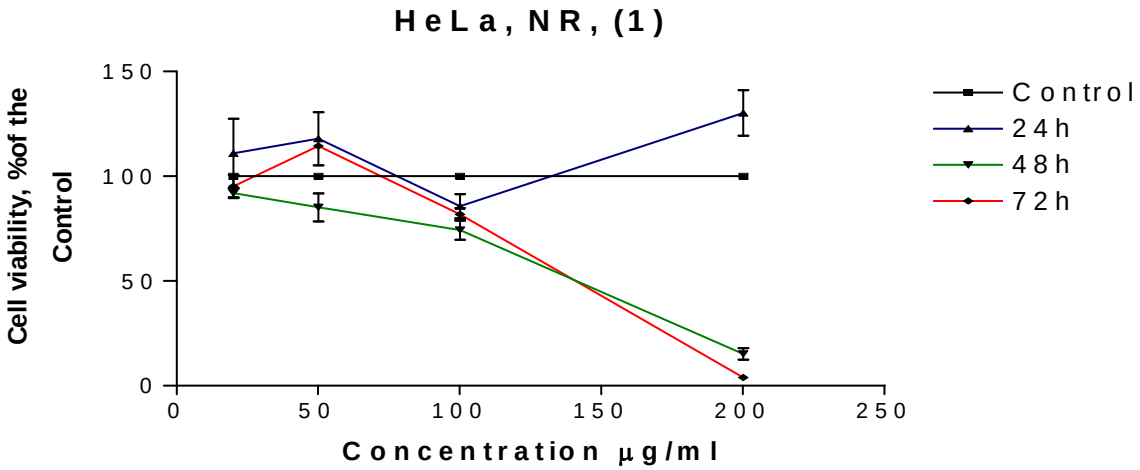

MCF-7, MTT, (2)
Cell viability, %of the Control
200
100
0
0
50
100
150
200
250
Concentration µg/ml
Control
24h
48h
72h


MCF-7, NR, (2)
Cell viability, %of the Control
200
100
0
0
50
100
150
200
250
Concentration µg/ml
Control
24h
48h
72h

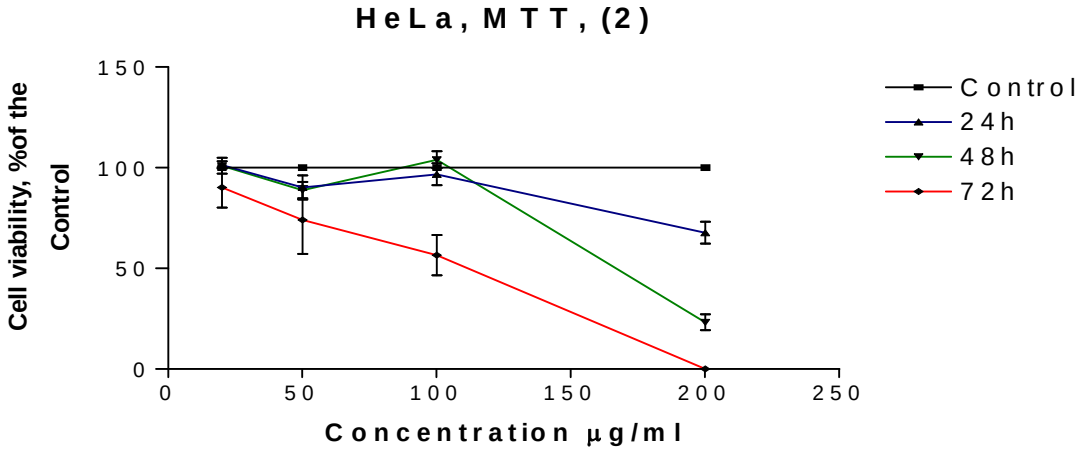
4.3.3. HeLa - MTT and NR

A-Compound (1)





B-Compound (2)



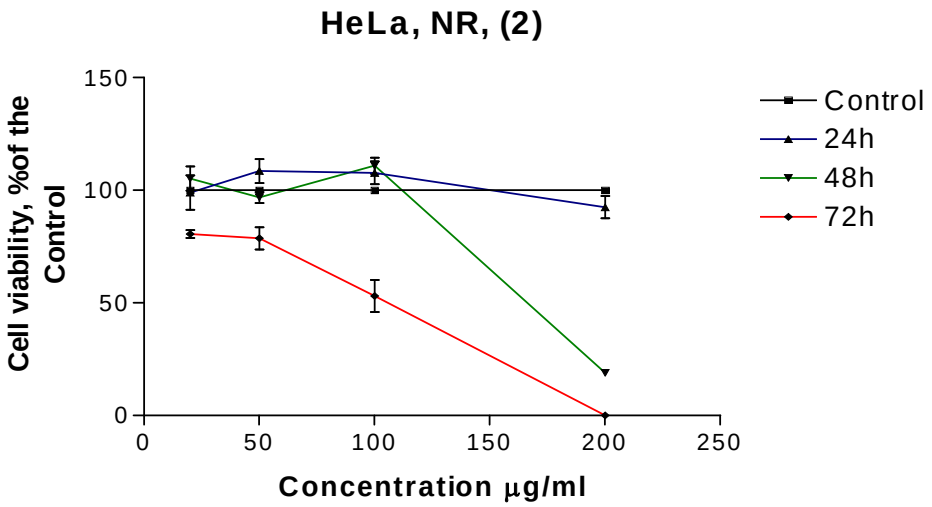


Table 1. Cell viability (% of the control)

		48h			
		MTT Test		NRTest	
Compound	Concentration	HeLa	MCF-7	HeLa	MCF-7
	20	91.66 ±3.95	92.27 ±0.65	91.98 +/-2.05	72.09+/-2.02**
	50	99.92 ±4.03	96.35 ±0.49	85.14 +/-6.71	73.77+/-6.89**
	100	93.19 ±3.16	78.70 ±2.61	74.36 +/-4.66**	50.81+/- 1.10**
1	200	43.94 ±10.59**	46.55 ±0.56**	15.22 +/-2.77**	47.54+/- 7.57**
	20	100.97 +/-3.91	90.74+/-1.31	105.20 +/-5.38	83.12+/-7.35
	50	88.83 +/-4.06	107.65+/-2.59	96.76 +/-2.43	53.05+/-3.20**
	100	103.95 +/-4.24	72.29+/-5.27*	110.91 +/-3.55	11.71+/-0.92**
2	200	23.24 +/-3.89**	41.52+/-0.84**	18.88 +/-0.92**	2.51+/-0.02**

		48h			
		MTT Test		NRTest	
Compound	Concentration	HeLa	MCF-7	HeLa	MCF-7
	20	91.66 ±3.95	92.27 ±0.65	91.98 +/-2.05	72.09+/-2.02**
	50	99.92 ±4.03	96.35 ±0.49	85.14 +/-6.71	73.77+/-6.89**
	100	93.19 ±3.16	78.70 ±2.61	74.36 +/-4.66**	50.81+/- 1.10**
1	200	43.94 ±10.59**	46.55 ±0.56**	15.22 +/-2.77**	47.54+/- 7.57**
	20	100.97 +/-3.91	90.74+/-1.31	105.20 +/-5.38	83.12+/-7.35
	50	88.83 +/-4.06	107.65+/-2.59	96.76 +/-2.43	53.05+/-3.20**
	100	103.95 +/-4.24	72.29+/-5.27*	110.91 +/-3.55	11.71+/-0.92**
2	200	23.24 +/-3.89**	41.52+/-0.84**	18.88 +/-0.92**	2.51+/-0.02**

Cell viability, %		72h			
		MTT Test		NR Test	
Compound	Concentration	HeLa	MCF-7	HeLa	MCF-7
1	20	100.30 ±14.14	182.75 ±15.00**	95.16 +/-5.55	114.65 +/-3.61
	50	79.53 ±17.61	30.49 ±4.62**	114.44 +/-1.10	46.82+/-4.22**
	100	83.33 ±7.74	19.04 ±2.20**	81.80 +/-2.99	34.40 +/- 2.26**
	200	11.09 ±1.21**	15.65 ±4.20**	3.99 +/-0.19**	17.56 +/- 1.75**
2	20	90.17 +/-10.01	140.25 +/-32.71	80.56+/-1.78	102.71 +/-3.19
	50	74.01 +/-16.78	174.02 +/-2.72**	78.62+/-4.89*	110.18 +/-7.51
	100	56.56 +/-10.02**	25.93 +/-4.10**	53.10+/-7.14**	86.51 +/-9.70
	200	0.00 +/-0.00**	15.04 +/-0.18**	0.00 +/-0.00**	25.27 +/-1.21**

** $p < 0.05$; **$p < 0.01$*

2.2. CC50 and CC90 of the compounds 1 and 2 (Table 2-5)

Table 2. Cytotoxic activity (CC_{50} and CC_{90}) of Compound 1 - MCF 7

Cell line	MCF-7					
Cytotoxicity Assay	MTT			NR		
Treatment Period	24 h	48 h	72 h	24 h	48 h	72 h
CC_{50}	n.d.	188.7	46.4	n.d.	140.6	48.6
CC_{90}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 3. Cytotoxic activity (CC50 and CC90) of Compound 1 - HeLa

Cell line	HeLa					
Cytotoxicity Assay	MTT			NR		
Treatment Period	24 h	48 h	72 h	24 h	48 h	72 h
CC_{50}	n.d.	186.5	146.2	n.d.	141.1	141.1
CC_{90}	n.d.	n.d.	n.d.	n.d.	n.d.	192.2

Table 4. Cytotoxic activity (CC50 and CC90) of Compound 2 – MCF-7

Cell line	MCF-7					
Cytotoxicity Assay	MTT			NR		
Treatment Period	24 h	48 h	72 h	24 h	48 h	72 h
CC_{50}	n.d.	171.8	92.3	n.d.	53.1	160
CC_{90}	n.d.	n.d.	n.d	n.d.	n.d.	n.d

Table 5. Cytotoxic activity (CC50 and CC90) - Compund (2) - HeLa

Cell line	HeLa					
Cytotoxicity Assay	MTT			NR		
Treatment Period	24 h	48 h	72 h	24 h	48 h	72 h
CC_{50}	n.d.	167.2	111.6	n.d.	166.7	105.4
CC_{90}	n.d.	n.d.	181.4	n.d.	n.d.	180.3

CC50 (CC90) – concentrations (µg/ml), that reduce cell viability by 50% (90%) n.d. - CC50 and CC90 were not determined because at all concentrations administered the cell viability was > 50% or > 10%, respectively

MTT – MTT test

NR – neutral red uptake cytotoxicity assay

2.3. CC50 and CC90 of the antitumor agents (Table 6)

Table 6. Cytotoxic activity (CC50 and CC90) of the antitumor agents

Antitumor agents	Treatment Period	MCF-7	HeLa
5-Fluorouracil	48h	-.	0,83
	72h	-	-
Cisplatin	24h	2.7	-
	48h	0.9	26.5
	72h	-	5.0

5. *Conclusions*

i- Both compounds (1 and 2) express similar cytotoxicity and/or antiproliferative properties

ii - Both cell lines (HeLa and MCF-7) exhibit similar chemosensitivity to the compounds tested.

iii - The compounds 1 and 2 show lower cytotoxic activity as compared to the antitumor agents 5-fluorouracil, cyclophosphamid and cisplatin.

4. Ongoing Experiments

In order to clarify better the biological activity (cytotoxic, antiproliferative) of the compounds tested (1 and 2) some other experiments are performed such as:

4.1. Evaluation of the putative cytotoxic/antiproliferative activity of the compounds in nontumor human cells (permanent cell line Lep 3) for comparative analysis with the tumor cells HeLa and MCF-7.

4.2. The effect of the compounds on colony-forming ability of tumor cells – This is a long-term study (16-day treatment) that will provide us information about the effect of the compounds on 3D colonies. It has to be mentioned here that MTT and NR which are the most widely used cytotoxicity assays in experimental oncology and oncopharmacology are short-term investigations (24-72h) with monolayer (2D) cell cultures.

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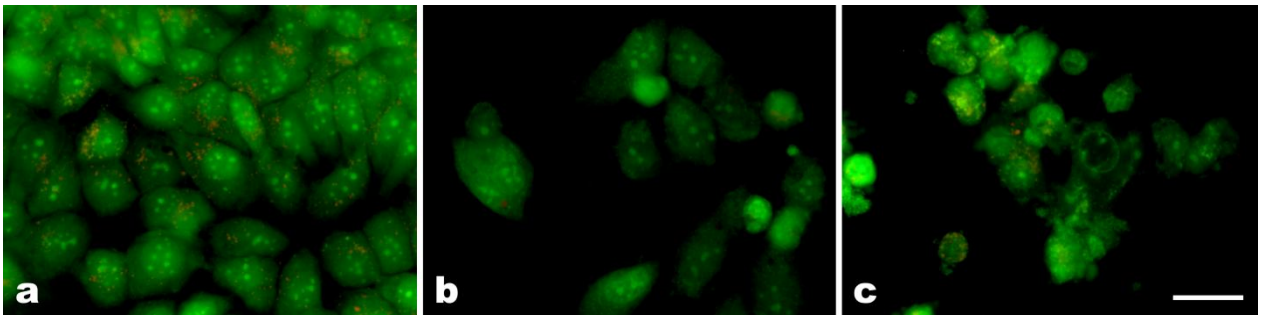
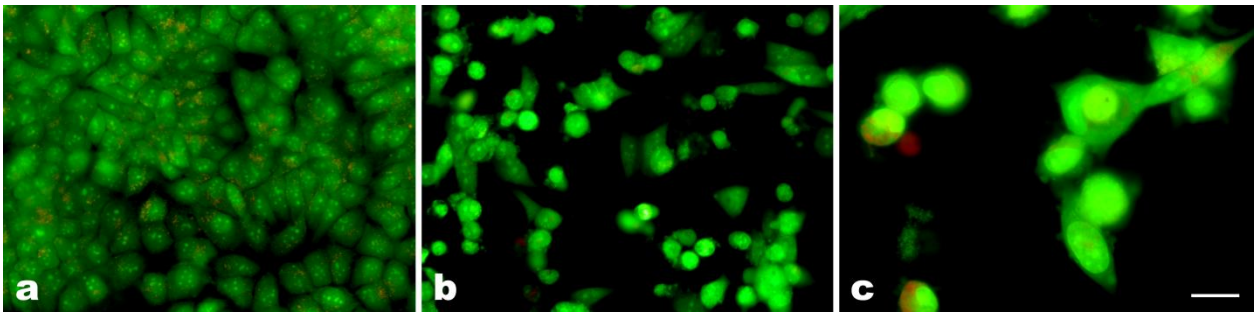


A complete monolayer of MCF7 cells with a pale green nuclear fluorescence, bright yellow-green nucleoli as well as considerably more dull green fluorescence of the cytoplasm. The cytoplasm include focal perinucler lysosomal accumulations with granular bright orange-red fluorescence (a). Considerable MCF7 cell lost as well as about 20% cell shrinkage and chromatin condensation at the 72th following the treatment with c 100 □g/ml DNBASG (b) or MCF7 cell lost and totally cell shrinkage and chromatin condensation at the 72th following the treatment with c 200 □g/ml D (c). Bar = 50 □m



A complete monolayer of MCF7 cells with a pale green nuclear fluorescence, bright yellow-green nucleoli as well as considerably more dull green fluorescence of the cytoplasm. The cytoplasm include focal perinucler lysosomal accumulations with granular bright orange-red fluorescence (a). Considerable MCF7 cell lost as well as cell shrinkage and chromatin condensation at the 72th following the treatment with c 100 □g/ml Dhafir-2 (b) or MCF7 cell lost with swelling of the remaining cells at the 72th following the treatment with c 200 □g/ml Dhafir-2 (c). Bar = 50 □m.

Fig.1. Cytopathological changes in MCF-7 human breast cells cultured in the presence of DNBASG(1) and DNBASA(2)