# Multidrug resistance reversal effect of organosilicon, terpenoid and flavonoid derivatives on various tumor cell lines *in vitro*

*Ph.D. Thesis*

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# 1. INTRODUCTION

## 1.1. Drug resistance mechanisms

Cancer chemotherapy is limited by MDR (multidrug resistance) based on known biochemical mechanisms. MDR1 also called P-gp transporter is the most important efflux mechanism, which has role in development of cancer multidrug resistantce. Beside efflux mechanisms the intracellular drugresitance can be classified as follows:

1. **Alteration in drug target**
2. **Increasing in DNA repair mechanisms**
3. **Defective apoptotic pathways.**
4. **Reduced intracellular accumulation**: It is in association with overexpression of ABC transporters

## 1.2. ABC transporters

Over 200 proteins are involved in the transport of substrates accross biological membranes are members of the ABC (ATP-binding cassette) superfamily of proteins.

The most common member of ABC transporter is the 170 kDa **multidrug resitance protein 1** (**MDR1)** also called **P-glycoprotein (P-gp)**. The second distantly related ABC protein, the 190-kDa **multidrug resistance related protein 1 (MRP1)** facilitated the discovery of eight more genes within the same ABC subfamily. A third drug transporter, also distantly related to P-glycoprotein and the MRPs, is the **breast cancer resistance protein** (**BCRP)**. Although the **lung resistance protein (LRP)** is not an ABC transporter, it is frequently included in discussions of drug resistance, as it is expressed at high levels in drug-resistant cell lines and some tumours.

The **clinical relevance** of these proteins in multidrug resistance is not yet established except expression of MRP1 and P-glycoprotein which has been reported in a variety of hematological and solid tumors.

## 1.3. MDR modulators

The **first generation** of P-gp modulators transporter, including verapamil, cyclosporin (cyclosporin A), tamoxifen, and several calmodulin antagonists, were identified in the 1980s.

In vivo use of high doses from them resulting in unacceptable toxicity, moreover increased myelosupression, hyperbilirubinemia and altered disposition of the cytototoxins.

The **second generation P-gp** modulators include dexverapamil, dexniguldipine, valspodar (PSC 833), and biricodar (VX-710). These agents are more potent than their predecessors and also less toxic. Second-generation P-gp inhibitors have a better pharmacologic profile than the first generation compounds, but they also retain some characteristics that limit their clinical usefulness.

**The third generation** have high specifity for P-gp and not to other ABC transporters. The third generation P-gp inhibitors currently in clinical development include the tariquidar, zosuquidar and laniquidar.

## 1.4. MRP modulators

**Agosterol** A is a natural polyhydroxylated sterol. Few of its analogues were found to be powerful modulators.

**Flavonoids** have been largely explored as modulators of P-gp-mediated MDR. In the last few years, a growing number of reports have been published concerning the use of dietary flavonoids as MRP1 inhibitors.
The **nonsteroidal estrogen receptor mixed agonist/antagonist**, raloxifene was used as a pharmacophore model to conceive selective modulators of MRP1-mediated MDR.
Antiinflammatory drug, **indomethacin** chemosensitized MRP1-overexpressing cells.

Despite the fact that large numbers of chemicals were synthesized and evaluated, there is no resistance modifier to-date in the medical practice. Natural compounds of edible plants and vegetables are expected to be incomparably less toxic on normal cells, tissues of the host than synthetic compounds. Consequently, jatrophane diterpenes carotenoids and flavonoids were promising to study their MDR reversing effect on various tumor cell lines with multidrug resitance.

# 2. AIMS OF THE STUDY

The aim of our study was to examine new perspectives of chemotherapy in connection with modification of multidrug resistance of cancer by inhibiton of MDR1 and MRP. Our purpose was to find a less toxic multidrug resistant reversal agent than verapamil, valspodar or tariquidar among synthethic organosilicons and natural plant derivative carotenoids, flavonoids, tri- and diterpenoid compounds.
Main goals of the study in details:

1. Examined cell lines were characterized to multidrug resistant properties by immuncytochemistry
2. Antiproliferative effects of potential resistant modifiers were studied
3. MDR reversal effect of organosilicon, terpenoid (jatrophane diterpens and carotenoids) and flavonoid compounds was systematically studied by human mdr1 gene transfected mouse lymphoma cell line
4. Reversal of MDR was also studied on drug resistant human breast cancer cell line KCR by carotenoid compounds
5. Reversal of MRP was investigated on human breast cancer cell line MDA-MB-231 (HTB-26) by terpenoid (jatrophane diterpens and carotenoids) and flavonoid compounds.
6. Reversal of multidrug resistance by organosilicon compounds on human larynx cancer Hep2C cell line
7. Combination of possible resistance modifiers and anticancer drugs in vitro was examined by checkerboard microplate method.
8. Comparison of the structure – activity relationship by physico-chemical parameters of carotenoids was analyzed by Computer model ADME/Boxes (computer program Adsorption, Distribution, Metabolism, Excretion).
9. Apoptosis induction as additional contributing factor of induced cancer cell death.

# 3. MATERIALS AND METHODS

## 3.1. Chemicals

**Synthetic compounds**

Compounds **alis-409** (1,3-dimethyl-1,3-p-fluorophenyl-1,3(3-morfolinopropyl)-1,3 disiloxan dihydrochlorid) and **alis-421** (1,3-dimethyl-1,3-(4-fluorophenyl)-1,3-[3(4-buthyl)-(1piperazinyl)-propyl]-1,3-disiloxan-tetrahydrochlorid). The silicion substituted alis

compounds were dissolved in DMSO by preparing stock solutions at a concentration 1.0 mg/ml.

**Natural compounds**:

**Macrocyclic diterpenes** of the jatrophane type and rearranged polycyclic derivatives were involved in the study as follows:

(**1**) pubescene A (3β,9α,15β-triacetoxy-7β-benzoyloxy-14-oxojatropha-5*E*,12*E*-diene); (**2**) pubescene B (3β,9α,15β-triacetoxy-7β-butanoyloxy-14-oxojatropha-5*E*,12*E*-diene); (**3**) pubescene C (3β,9α-diacetoxy-7β-benzoyloxy-15β-hydroxy-14-oxojatropha-5*E*,12*E*-diene); (**4**) pubescene D (3β,9α-diacetoxy-7β-benzoyloxy-15β-hydroxy-14-oxo-2βH-jatropha-5*E*,12*E*-diene); (**5**) 1b,5a,14a,17a-tetraacetoxy-3b-benzoyloxy-15b-hydroxy-9-oxo-paraliane; (**6**) portlandicine (2a,5a,14a,17a-tetraacetoxy-3b-benzoyloxy-15b-hydroxy-9-oxo-paraliane; (**7**) segetalol-1,5,14-triacetate-3-benzoate; (**8**) euphopubescenol [5α,8α,15β-triacetoxy-3α-benzoyloxy-4α-hydroxy-9,14-dioxo-13βH-jatropha-6(17),11*E*-diene]; (**9**) euphopubescene (3β,7β,8β,9α,14α,15β-hexaacetoxy-2βH-jatropha-5*E*,11*E*-diene); (**10**) pubescenol [5α, 8β,15β-triacetoxy-3β-benzoyloxy-4α-hydroxy-9,14-dioxo-13βH-jatropha-6(17),11*E*-diene]. All compounds were dissolved in DMSO.

**Carotenoids**: Lycopene, capsanthin, capsorubin, antheraxanthin, violaxanthin, α-carotene, β-carotene, α-cryptoxanthin, β-cryptoxanthin, zeaxanthin, lutein, neoxanthin, violeoxanthin, apple peel phytoxanthins and lycophill were isolated and identified earlier by our group and were taken from our store. In case of the newly isolated and identified carotenoids mutatochrome, aurochrome, flavoxantin**,** chrisanthemaxanthin, monoepoxy-β-carotene, diepoxy-β-carotene, 15,15`-dehydro-diepoxy-β-carotene, monoepoxy-α-carotene, capsochrome "upper" epimer, from natural (anti)-capsanthin-5,6-epoxide, capsochrome "lower" epimer, from natural (anti)-capsanthin-5,6-epoxide, capsochrome "upper" epimer,from semisynthetic (syn) capsanthin-5,6-epoxid, capsochrome "lower"epimer from semisynthetic (syn) capsanthin-5,6-epoxid, lutechrome, (8'R)-luteoxanthin, (8'S)-luteoxanthin, (13Z)-lutein (13'Z)-lutein, (13Z)-zeaxanthin, (9Z)-violaxanthin and (9'Z)-neoxanthin. Stock solutions were prepared in DMSO.

**Flavonoids:** Rotenone, catechin, neohesperidin, naringin, chrysin, robinin, floretin, floridzin, robinetin, dihydrorobinetin, kaempferol, dihydrofisetin, dihydroquercetin, sakuranin and sakuratenin were provided by Sándor Antus, Department of Organic Chemistry, University of Debrecen, Hungary. Formononetin, amorphigenin, afrormosin, 6a,12a-dehydroamorphigenin and (+)-12-hydroxyamorphigenin were provided by Judit Hohmann, Department of Pharmacognosy, Faculty of Pharmacy, Albert Szent-Györgyi Medical Center, University of Szeged, Hungary. Epigallocatechin was purchased from Sigma (St Louis, MO, USA). All of the tested compounds were dissolved in dimethylsulfoxide (SERVA, Feinbiochemica, Heidelberg, Germany).

### 3.2. Cell cultures

- **3.2.1.** **L5178** mouse T-cell lymphoma cells
- **3.2.2.** Drug-resistant subline of breast cancer **MCF7/KCR**cells and its parental cell line
- **3.2.3.** Breast cancer **MDA-MB-231** (ATCC: HTB-26) cells
- **3.2.4.** **Hep2** (ATCC: CCL-23) pharyngeal carcinoma cell line

### 3.3. Immunocytochemistry

Cells were harvested and resuspended in serum-free media; the density of the cell suspension was $5\times10^5$ /ml. One hundred μl was cytocentrifugated for 5 minutes on 1000 rpm. The samples were fixed in 4°C acetone for 10 minutes and were washed in TBS buffer (pH 7, 6) for 5 minutes. Endogenous peroxidases were quenched in 0.3% $H_2O_2$ for

15 minutes. The samples were incubated with primary antibodies (MRP: MRPm6, monoclonal (ICN), P-gp: NCL-PGLYm, monoclonal (Novocastra) in suitable dilutions (1:5, 1:10, 1:20) for one hour at room temperature.
The samples were washed in TBS buffer for 3×5 minutes and were incubated with the secondary antibody (DAKO EnVision™ System, CA, USA) for 30 minutes. The samples were washed in TBS for 3×5 minutes. Diaminobenzidin (DAKO, CA, USA) was used as the chromogen. Sections were counterstained with haematoxylin and mounted.

### 3.4. Assay for antiproliferative and cytotoxic effect

The effects of increasing concentrations of the drugs alone on cell growth were tested in 96-well flat-bottomed microtiter plates. The compounds were diluted in a volume of 50 L medium. Then, 1×104 cells in 0.1 mL of medium were added to each well, with the exception of the medium control wells. The culture plates were further incubated at 37 °C for 72 h. (In case of cytotoxicity test the cell number were 3×104 and the incubatuion time was 24 h.) At the end of the incubation period, 20 μL of MTT (thiazolyl blue, Sigma, St Louis, MO, USA) solution (from a 5 mg/mL stock) was added to each well. After incubation at 37 °C for 4 h, 100 μL of Sodium dodecyl sulfate (SDS) (Sigma, St Louis, MO, USA) solution (10%) was measured into each well and the plates were further incubated at 37 °C overnight. The cell growth was determined by measuring the optical density (OD) at 550 nm (ref. 630 nm) with a Dynatech MRX vertical beam ELISA reader. Inhibition of cell growth (as a percentage) was determined according to the formula:

$$100 - \left[ \frac{OD\,sample - OD\,medium\ control}{OD\,cell\ control - OD\,medium\,control} \right] \times 100$$

### 3.5. Assay for reversal of MDR in mouse lymphoma cells

The L5178 MDR and L5178Y parent cell lines were grown in McCoy's 5A medium containing 10% heat-inactivated horse serum, L-glutamine and antibiotics. The cells were adjusted to a density of 2×106/mL, resuspended in serum-free McCoy's 5A medium and distributed in 0.5-mL aliquots into Eppendorf centrifuge tubes. The tested compounds were added at various concentrations in different volumes (2.0-20.0 μL) of the 1.0-10.0 mg/mL stock solutions and the samples were incubated for 10 min at room temperature. Next, 10 μL (5.2 μM final concentration) of the indicator rhodamine 123 (Sigma, St Louis, MO, USA) was added to the samples and the cells were incubated for a further 20 min at 37 °C, washed twice and resuspended in 0.5 mL PBS for analysis. The fluorescence of the cell population was measured with a Beckton Dickinson FACScan flow cytometer. Verapamil (EGIS, Hungarian Pharmaceutical Company, Budapest, Hungary) was used as a positive control in the rhodamine 123 exclusion experiments. The percentage mean fluorescence intensity was calculated for the treated MDR and parental cell lines as compared with the untreated cells. An activity ratio R was calculated via the following equation on the basis of the measured fluorescence values:

$$R = \frac{MDR\,treated \,/\, MDR\,control}{parental\,treated \,/\, parental\,control}$$

### 3.6. Assay for reversal of MDR drug-resistant subline of breast cancer MCF7 cells

See at assay for reversal of MDR in mouse lymphoma cells.

### 3.7. Reversal of MRP in the MDA-MB-231 (HTB-26) human brest cancer cell line

The cells were distributed onto a 6-well plate, each well containing $2.5\times10^5$ cells in 5 mL of culture medium. These cells were grown for 72 h for the experiment. The culture medium was then changed to 1 mL of serum-free medium per well and the tested compounds were added in various amounts (4-40 □L) from a 1.0 mg/mL stock solution, and the cells were incubated for 10 min at room temperature. Next 10 □L of 2',7'-bis(2-carboxyethyl)-5(6)-carboxy-fluorescein acetoxymethyl ester (BCECF-AM) (Sigma, St Louis, MO, USA) was added to the samples, to give a final concentration of 2.6 □M, and the cells were then incubated for a further 20 min at 37 °C, next washed in phosphate-bufferd saline (PBS) and detached with trypsin-versen solution {0.25%(w/v) trypsin – 0.03%(w/v) EDTA} to harvest the cells from the 6-well plate. The cells from each well were transferred to Eppendorf centrifuge tubes and washed once in culture medium and PBS. The samples were resuspended in 0.5 mL of PBS for measurement. The fluorescence of the cell population was analyzed by flow cytometry using a Beckton Dickinson FACScan instrument. Indomethacin (Sigma, St Louis, MO, USA) in a final concentration of 20 □M was used as a positive control in the experiments. The fluorescence activity was determined as the ratio of the fluorescence activities of treated and untreated samples.

### 3.8. Checkerboard microplate method as model for combination therapy

It was applied to study the effects of drug interactions between resistance modifiers and cytotoxic compound on cancer cells.

The effects of the anticancer drug epirubicin and the resistance modifiers in combination were studied on various cancer cell lines. The dilutions of epirubicin (A) were made in a horizontal direction, and the dilutions of resistance modifiers (B) vertically in the microtiter plate in 100□L volume. The cell suspension in the tissue culture medium was distributed into each well in 100 μL containing $5\times10^4$ cells. The plates were incubated for 72 h at 37 °C in a $CO_2$ incubator. The cell growth rate was determined after MTT staining and the intensity of the blue color was measured on a micro ELISA reader. Drug interactions were evaluated according to the following system:

$FIC_A = ID_{50A \text{ in combination}} / ID_{50A \text{ alone}}$
$FIC_B = ID_{50B \text{ in combination}} / ID_{50B \text{ alone}}$
ID=inhibitory dose
FIC=fractional inhibitory concentration
FIX=fractional inhibitory index

$FIX = FIC_A + FIC_B$
FIX = 0.51-1Additive effect
FIX < 0.5 Synergism
FIX=1 - 2 Indifferent effect
FIX > 2 Antagonism

### 3.9. The prediction of P-glycoprotein substrate specificity for clinical studies

For tested compounds values of Hydrogen donors, Hydrogen acceptors, Abraham's alpha, Abraham's beta, total polar surface area (TPSA), logP and McGowan volume were calculated by ADME/Boxes (computer program) available from Pharma Algorithms Inc., 2004 Canada; www-ap-algorithms.com.

## 4. RESULTS

### 4.1. Immuncytochemistry of cell lines

The presence of MRP on MDA-MB 231 was detected by immunocytochemistry with MRPm6, monoclonal antibody. High level of P-gp expression was also detected on the membrane of L5178Y human *mdr1* transfected mouse lymphoma cells and on the membrane surface of the drug-resistant subline of MCF7 (KCR) by NCL-PGLYm mouse monoclonal antibody. High expression of both MDR1 and MRP proteins were detected on cell surface of human larynx carcinoma Hep2C cell line by the above mentioned antibodies.

### 4.2. Antiproliferative and cytotoxic effect of potential resistant modifiers

It is suggested to decrease the toxicity of possible resistance modifiers that is why it is important to know the antiproliferative and toxic property of different compounds before in vitro combination. The antiproliferative effect and cytotoxic effect are very different, in case of organosilicon, terpenoid and flavonoid derivatives, generally the human *mdr1* gene transfected mouse lymphoma is more sensitive than human cancer cell lines.

### 4.3. Reversal of multidrug resistance on mouse lymphoma

Pubescences A-D were shown to enhance drug retention in the cells by inhibiting the efflux-pump activity mediated by P-glycoprotein. The results showed concentration dependence for all the compounds. Pubescene A and D exhibited the highest effect in reversing MDR activity and manifold activity, when compared to that of the positive control verapamil**.**

Alpha- and beta-carotene exhibited no effect. The most effective compounds were capsanthin, lycophyll and capsorubin. Moderately effective carotenoids were violaxanthin, lycopene, lutein, zeaxanthin and antheraxanthin.

Hydroxylation on the left ring in case of capsanthin and capsorubin a hydroxylation on the right five membered ring possibly responsible for the very high resistance reversal effect on the human MDR1 gene transfected mouse lymphoma cells.

Treatment of the cancer cells with flavoxanthin, + chrysanthemaxanthin mixture and capochromes resulted in an extremely high fluorescence activity ratio. Different isomers of luteoxanthin and (Z)-carotenoids (cis-carotenoids) exhibited a high fluorescence activity ration on mouse lymphoma cells, however structurally related luteochrome was hardly effective as a reversing agent.

When flavonoids were tested on the mouse lymphoma cells, rotenone, chrysin, floretin and epigallocatechin displayed a dose-dependent increase in the fluorescence activity ratio (FAR). Dihydroquercetin, dihydrofisetin, dihydrorobinetin and floridzin reduced the rhodamine 123 accumulation in the cells.

Of the isoflavonoids, amorphigenin and formononetin proved to have the strongest MDR-reversal effects.

### 4.4. Reversal of multidrug resistance on doxorubicine selected drugresistant subline of MCF7 (KCR)

When the carotenoid isomers were tested on KCR cell line, much less inhibition of MDR efflux pumps was found than for the human *mdr*1 gene transfected mouse lymphoma cells in the presence of the carotenoids.

### 4.5. Reversal of MRP on breast cancer cell line MDA-MB-231 (HTB-26)

R123 accumulation was very low in case of **1** and **4** it was only 20%. However the presence of MDR1 was detected by specific monoclonal antibody but it seems that this protein remained ineffective, because neither verapamil nor pubescences could enhance the fluorescence activity ratio.

The same compounds were tested on MRP, the carboxyfluorescein (BCECF-AM) accumulation of the MDA-MB-231 breast cancer cells was measured. Increased fluorescence activity was shown by some compounds such as **1, 8, 9**.

When the carotenoid compounds were tested on MRP, the carboxyfluorescein accumulation of the MDA-MB-231 breast cancer cells was measured. Several compounds like mutatochorme, 15-15'dehidroβcarotene, monoepoxy-α-carotene, (8'S)-luteoxantin and (13Z)-lutein (13'Z)-lutein caused higher accumulation than positive control indometacin.

Chrysin, robinin, kaempferol, dihydroquercetin and epigallocatechin were as effective as

the indometacin control, elevating the BCECF-AM accumulation by 20-60%. Rotenone, catechin and sakuranin reduced the drug accumulation in the cells, because of their toxical effect.
Among isoflavonoids formononetin increased the drug accumulation in both cell lines as compared with the control. Afrormosin was only moderately effective on the MRP reversal of the tumor cells. 6a12a-Dehydroamorphigenin and (+)-12a-hydroxyamorphigenin had only marginal effects as compared with indomethacin-treated MRP cells.

### 4.6. Reversal of multidrug resistance by organosilicon compounds on human larynx cancer Hep2C cell line

Apparently the rhodamine and carboxyfluorescein accumulation was increased in the presence of low concentration of Alis 409 remarkably while the rhodamine accumulation was increased moderately in the presence of 0.4 µg/ml Alis 409. In case of Alis 421 also the lower concentrations have higher activity, probably due to some toxical effect at higher concentration.

### 4.7. In vitro combinations (checkerboard method)

However in the short term accumulation assays the Alis compounds seemed to be ineffective in the longer combination. Both of them could enhanced the effect of epirubicin on human larynx cancer Hep2C cell line.
Pubescene A showed the highest activity against efflux pump MRP however it had only moderate effect in longer in vitro combination assay on MDA-MB-231 cell line. Pubescenes A and D enhanced the effect of epirubicin on human mdr1 gene transfected mouse lymphoma cell line.
Of the possible resistance modifiers (13*Z*)-zeaxanthin was able to enhance the antiproliferative effect of epirubicin synergistically on both cell lines. Luteoxanthin and (*Z*)-carotenoid treatment was also effective. Monoepoxy-β-carotene remained almost ineffective in combination treatment.
Despite the relatively low degree of inhibition on the P-gp efflux pump of KCR human breastcancer cells, the combinations of epirubicin and some carotenoids resulted in synergism in the antiproliferative action of epirubicin.
Carotenoids mutatochrome, 15-15'dehidro-β-carotene, monoepoxy-α-carotene and (8'S) Luteoxanthin which had higher activity of MRP inhibition were only moderately effective in vitro combination on MDA-MB-231 cell line.
Of the resistance modifiers chrysin and amorphigenin were able to enhance the antiproliferative activity of epirubicin on mouse lymphoma cells. Rotenone significantly had only a marginal additive antiproliferative effect in combination with epirubicin. On the MDA-MB-231 breast cancer cell line, formononetin and kaempferol exhibited synergism.

### 4.8. Comparison of the structure – activity relationship by physico-chemical parameters of carotenoids was analyzed by Computer model ADME/Boxes (computer program Adsorption, Distribution, Metabolism, Excretion)

Based on the fluorescence activity ratio values and the chemical structures of the studied compounds some correlation was found between the resistance modifier action and physicochemical parameters of the tested compounds.
In further analysis we decided which physico-chemical property is the most significant in describing MDR inhibitor capacity of tested carotenoids. Our finding that TPSA is one of the most important factors (however itself is not enough)in describing the MDR inhibitor capacity.

# 5. DISCUSSION

In general P-gp can be inhibited on direct and indirect ways. Direct: a) blocking drug binding site either competitively, non competitively or allosterically, b) interfering ATP hydrolysis. Indirect: altering integrity of cell membrane lipids.

Four groups of compounds were studied: **organosilicon**, **jatrophane-type diterpenes**, **carotenoids**, **flavonoid derivatives**.

Some **organosilicon** compounds are well known cytostatic drugs: 2.6-cis-diphenylcyclotetrasiloxane (Cisobitan) was found to be partially effective in the in vivo treatment of patients with prostate carcinoma.

The high toxicity and side-effects of organosilicon compounds limited their application in the therapy of cancer. The possible application of organosilicon compounds is a new approach for the reversal of MDR. Alis 409 and Alis 421 display marked effectivity for reversal of the MDR of mdr-1 cells. Both P-gp and MRP inhibitory effects were low on human cancer cell lines in drug accumulation assay nevertheless significant synergism was observed in vitro combination of Alis compounds and epirubicin on human larynx carcinoma Hep2C cell line.

Several derivatives of polyfunctional **diterpenes** isolated from Euphorbiaceae have been considered as potent inhibitors of multidrug resistance. The high anti-MDR potency of jatrophanes pubescene A and D which were also very effective in combination, indicated that this type of diterpenes are considered as a new chemotype of P-gp inhibitors. Moreover Pubescene D has significant affinity both MRP and MDR inhibition.

Recently, G. Corea's group also reported jatrophane diterpenes from Mediterranean *Euphorbia* species as powerful MDR-modifier. The biological activities of the diterpenes were assayed through their ability to inhibit P-gp-mediated daunomycin efflux of cancer cells, related to cyclosporine A.

Comparing certain pairs of the isolated compounds evidenced the importance of different part of the molecule, such as substitutions on ring A and at C-5, C-8, C-9, C-14 and C-15.

The MDR-reversing effects of the **carotenoids** tested in the present experiments exhibited a structure-activity relationship. It seems that the configuration of (*Z*) compounds has a stronger binding capacity toward the P-gp than the linear (all-E) forms.

Recent studies suggest that the membrane structure may play a critical role in the MDR activities. It has also been suggested that polar carotenoids (*e.g.* lutein or zeaxanthin) generally being incorporated better than non-polar β-carotene. This phenomenon could explain the higher activity of polar carotenoids in MDR reversal. Moreover, polar carotenoids are preferentially located in the region of unsaturated lipids of the membrane and it may be presumed that there are differences in the ratio of saturated and unsaturated fatty acids in different cell lines, which may be responsible for the variation in the MDR-modulating effect of carotenoids on the mouse lymphoma and resistant MCF7 cell lines in our experiments.

It is reported that the carotenoids partition into the hydrophobic core of the membrane and cause a decrease in lipid fluidity. The reduced membrane fluidity may sterically hinder diffusion of drugs and decrease the kinetics of drug excretion.

While (Z) stereoisomeric form of carotenoids, seemed to be more effective on P-gp inhibition; they were ineffective on MRP inhibition which can also been explain by functional and structural differences of two efflux pumps.

It seems that the most relevant factor among physico-chemical properties of carotenoids is TPSA total polar surface area, however is not enough in itself for prediction of P-gp inhibitory activity.

Other studies have demonstrated that some dietary **flavonoids** may modulate organic anion and GSH transport, ATPase and drug resistance. In our experiments, chrysin and

rotenoid derivatives, had P-gp modulating activity. The most effective cell proliferation inhibitor was the mitochondrial electron transport inhibitor rotenone which also could be responsible for caspase dependent and independent cell death. With hydroxyl(-OH) group at the position of 29th carbon moderated the toxicity of amorphigenin compare to rotenone, although it has remained significant.
The concentrations of the test compounds had great effects on the outcome of the experiments. A low concentration of quercetin, for an example, activated the activity of P-gp, whereas a high concentration inhibited that of P-gp.

## 6. NEW STATEMENTS

1. Detecting P-gp molecule by mABs is not always equal with increased efflux activity, P-gp resistant phenotype of cancer cells, because in our experiments immunocytochemistry signed high presence of P-gp on MDA-MB-231, however in vitro experiments these proteins remained non-functional (or with very low activity) in comparison to transfected cell line or other cells with acquired resistance.
2. Beside the human *mdr1* gene transfected mouse lymphoma the **organosilicon** compounds seem to be effective on human Hep2 cell line with chemo and radioresistance. These results are encouraging, *in vivo* investigations are in progress with organosilicon compounds on Hep2C transplanted SCID mice.
3. There is not enough data to draw for conclusion about the ideal structure of **jatrophane diterpenes** with the best P-gp inhibitory activity.The high anti-MDR potency of jatrophanes pubescene A and D were effective in combination with epirubicin in vitro, Pubescene D also had shown significant MRP inhibition. The results indicated that this type of diterpenes are considered as a new chemotype of MDR1 and MRP1 inhibitors, which can be regarded as promising lead compounds for drug development programs.
4. It seems that the effect of **carotenoids** on MDR reversal and apoptotic effect of tumor cells depend on the chemical structure, polarity as well as membrane incorporation ability of carotenoid derivatives.
5. The importance of computer analysis of structure-activity relationship (SAR) should be emphasized in connection with MDR reversal. In further P-gp reversal experiment also seems beneficial to use ADME computer program to predict the P-gp inhibitory probability to save money and time.
6. While effective carotenoids interact in an indirect way with P-gp through membrane – lipid and protein interactions, flavonoids (rotenone and amorphigenin) would rather affect directly the ATP binding domain of protein.
7. Membrane mediated mechanisms of MDR reversal through a putative modulation of membrane fluidity are likely to play a key role in reversal of resistance by carotenoids similarly to Tweens. Interaction of some carotenes change the membrane fluidity consequently the membrane mediated conformational changes of P-gp can be blocked.

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## JOURNAL ARTICLES RELATED TO THE THESIS

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# ABSTRACTS

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