

**Ph.D. thesis**

**Development of the prototype of SOD  
mimetic Mitochondrial Permeability  
Transition inhibitors**

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## Abbreviations

mPT .....	Mitochondrial Permeability Transition
ROS .....	Reactive Oxygen Species
AiF .....	Apoptosis inducing faktor
EndoG .....	Endonuclease G
$\Delta\Psi$ .....	Mitochondrial Membrane Potential
CsA .....	Cyclosporine A
HO-3538 .....	(2-Methyl-3-(3,5-diiodo-4-{2-[N-ethyl,N-(1-hydroxy-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-ylmethyl) ethyl]}oxybenzoyl)benzofurane 2HCl salt)
SCAV .....	1-hydroxy-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-ylmethy
SOD .....	Superoxide dismutase

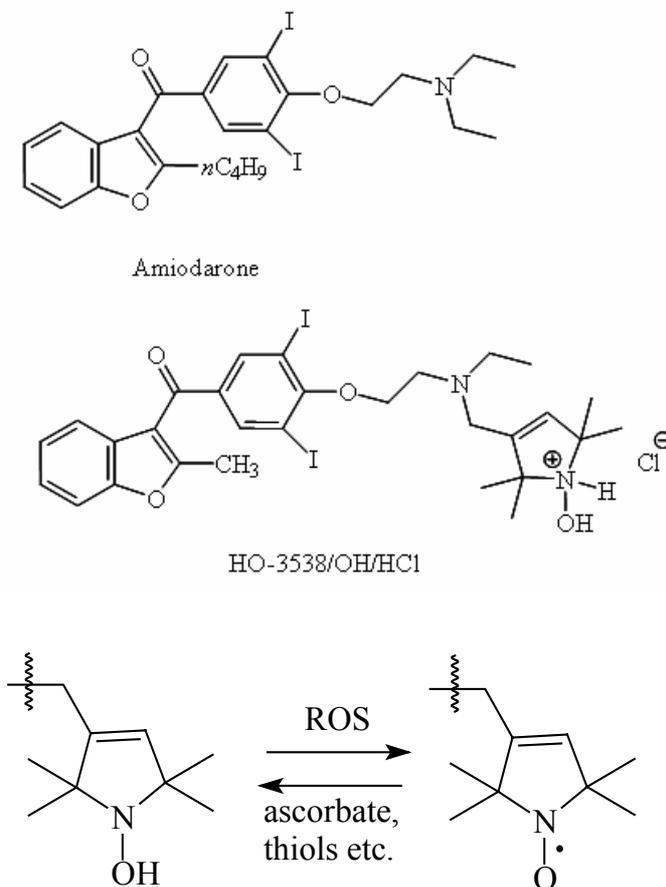
## Introduction

Amiodarone (2-butyl-3-benzofuranyl 4-[2-(diethylamino)-ethoxy]-3,5-diiodophenyl-ketone hydrochloride) is a class III antiarrhythmic agent used in the clinical practice for the treatment of various arrhythmias. We found that it presented a protective effect on the postischemic heart by enhancing the recovery of high-energy phosphate metabolites and inhibiting mPT at low concentrations. However, when administered at higher concentrations, it induced mitochondrial swelling, the collapse of the mitochondrial membrane potential ( $\Delta\psi$ ) and apoptosis.

Desethylamiodarone, the major metabolite of amiodarone, also has an antiarrhythmic activity, significantly increasing the action potential duration (class III antiarrhythmic effect) and decreasing the maximum rate of depolarization (class I antiarrhythmic effect) at clinically relevant concentrations. Desethylamiodarone rapidly accumulates in the lung after amiodarone treatment, sometimes in higher concentrations than amiodarone itself raising the possibility that this metabolite contributed to the effects of amiodarone. However, we found it to be more toxic than amiodarone in pulmonary cell types, suggesting that desethylamiodarone may play an important role in the development of the amiodarone treatment-induced pulmonary fibrosis.

The cardioprotective effect of amiodarone is due, at least in part, to the inhibition of mitochondrial permeability transition (mPT) at lower concentrations. However, when administered in higher concentrations, it induced mitochondrial swelling as well as the collapse of the mitochondrial membrane potential ( $\Delta\psi$ ). Desethylamiodarone, the major metabolite of amiodarone — reported by some authors to be the major cause of the amiodarone administration induced toxicity — does not inhibit mPT at any concentration, and induce swelling and the collapse of the membrane potential at higher concentrations. The difference between the effect of amiodarone and desethylamiodarone is due to the absence of an ethyl side chain from the amino group of desethylamiodarone. This led us to the conclusion that the structural modification of amiodarone can improve its inhibitory effect on mPT as well as its beneficial effect in ischemia-reperfusion injuries. Considering its advantages, a lot of effort has been made in the past decade to improve pharmacokinetic

properties of amiodarone, mainly by chemical alterations of the original molecule such as synthesis of monoiodo derivatives, introduction of carboxymethoxy side chain instead of tertiary amine or substitute the original n-butyl group for an isobutyl ester.



**Fig. 1. The chemical structures of amiodarone HO-3538 and SCAV**

The chemical structures of amiodarone (2-butyl-3-benzofuranyl 4-[2-(diethylamino)-ethoxy]-3,5-diiodophenyl-ketone hydrochloride), HO-3538 (2-Methyl-3-(3,5-diiodo-4-{2-[N-ethyl,N-(1-hydroxy-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-ylmethyl) ethyl]}oxybenzoyl)benzofurane 2HCl salt). The 1-hydroxy-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-ylmethyl component (SCAV) of HO-3538 possesses ROS scavenging activity. The oxidized form can be reduced by thiols, ascorbate, GSSH, etc.

Prof. Hideg and his co-workers provided numerous paramagnetic and diamagnetic amiodarone derivatives synthesized by them for screening their effect on the mitochondrial permeability transition. In this study, we analyzed the effect of HO-3538 (Fig.1), the most effective novel amiodarone analogue, in which an ethyl side chain is substituted with 1-

hydroxy-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-ylmethyl (SCAV) that has been described to possess free-radical scavenging activity on the permeability transition *in vitro* and in cultured cells as well as *in situ* on ischemia-reperfusion in Langendorff-perfused rat hearts. This amiodarone analogue can form the basis of developing novel amiodarone analogues that have the same or enhanced efficiency as amiodarone but with fewer side effects.

## Materials and Methods

**Chemicals.** Cyclosporin A (CsA) was from BIOMOL Research Laboratories (Plymouth Meeting, PA). Rhodamine 123 (Rh123), carboxy-H2DCFDA and dihydrorhodamine123 (DRh123) were from Molecular Probes (Eugene, OR), and desethylamiodarone (Dea) was a gift from Professor Varro (Department of Pharmacology and Pharmacotherapy, University of Szeged, Szeged, Hungary), anti-cytochrome c monoclonal antibody was from Pharmingen (San Diego, CA), anti-AIF polyclonal antibody was from Oncogene (San Diego, CA), HO-3538 (2-Methyl-3-(3,5-diiodo-4-{2-[N-ethyl,N-(1-hydroxy-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-ylmethyl) ethyl]}oxybenzoyl)benzofurane.2HCl salt), and other amiodarone analogues were produced as described previously (Kallai et al., 2005). All other compounds were from Sigma-Aldrich (St. Louis, MO) unless otherwise stated.

**Animals.** Male Wistar rats weighing 300-350 g were used for this study. Rats were handled in accordance with the Guide for the Care and Use of Laboratory Animals.

**Heart Perfusion.** Hearts were perfused via the aorta as described before (Szabados et al., 1999) in the absence or presence of different concentrations of amiodarone, desethylamiodarone, HO-3538 and SCAV. The perfusion medium was a modified phosphate-free Krebs-Henseleit buffer. The perfusate was adjusted to pH 7.4 and bubbled with 95% O<sub>2</sub>, 5% CO<sub>2</sub> through a glass oxygenator. After washing (non-recirculating period of 15 min), hearts were perfused under normoxic conditions for 10 minutes; the flow was subsequently discontinued for 30 minutes by inflating a balloon (ischemia), which was followed by 15 minutes of reperfusion. Drugs at the indicated concentration were added at the beginning of the normoxic perfusion phase.

**NMR spectroscopy.** NMR spectra were recorded with a Varian <sup>UNITY</sup>INOVA 400 WB instrument. <sup>31</sup>P measurements (161.90 MHz) of perfused hearts were run at 37°C in a Z•SPEC® 20-mm broadband probe (Nalorac Co., Martinez, CA, USA) applying WALTZ proton decoupling ( $\gamma$ B2= 1.2 kHz) during acquisition. Field homogeneity was adjusted by following the <sup>1</sup>H signal ( $w_{1/2}$  = 10-15 Hz). Spectra were collected with a time resolution of 3 min by accumulating 120 transients in each FID.

**Cell Culture.** PANC-1 human pancreatic epithelioid carcinoma cells, BRL 3A rat liver cells, H9C2 mouse cardiomyocytes, Jurkat cells, and WRL-68 human liver cells were from American Type Culture Collection. The cell lines were grown in humidified 5% CO<sub>2</sub> atmosphere at 37°C. The cells were maintained as monolayer adherent culture in Dulbecco's modified Eagle's medium containing 1% antibiotic-antimycotic solution (Sigma Chemical, Poole, Dorset, UK) and 10% fetal calf serum. Cells were passaged at intervals of 3 days.

**Western Blot Analysis.** Frozen heart muscle samples were mechanically homogenized in liquid nitrogen and the nuclear fraction was prepared as described previously (Schmitt et al., 2002). Equal amounts of nuclear extracts were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12% gel) and then transferred to a nitrocellulose membrane. Membranes were blocked using 5% dry milk. The blots probed with antibodies against AIF, Endo G and cyt-c were detected by immunoblotting from each fraction as described under the "Detection of mitochondrial protein release" section. The antigen-antibody complex was visualized on an X-ray film using secondary antibodies linked to horseradish peroxidase (1:1000; Sigma-Aldrich). The experiments were repeated three times for each group, and the results are demonstrated by photomicrograph of a representative blot.

**Isolation of mitochondria.** Rats were sacrificed by decapitation and the mitochondria were isolated from the liver and the heart by differential centrifugation as described by a standard protocol (Schneider et al., 1950). The only difference among the organs was in the primary homogenization protocol; liver was squeezed through a liver press, while pooled heart tissue from 5 rats was minced with a blender. All isolated mitochondria were purified by Percoll gradient centrifuging (Sims et al., 1990), and the mitochondrial protein concentrations were determined by the biuret method with bovine serum albumine as the standard.

**Mitochondrial permeability transition.** mPT was monitored by following the accompanying large amplitude swelling via the decrease in absorbance at 540 nm (Varbiro et al., 2001) measured at room temperature by a Perkin-Elmer fluorimeter (London, UK) in reflectance mode. Briefly, mitochondria at the concentration of 1 mg protein/ml were pre-incubated in the assay buffer for 60 seconds.

**Mitochondrial membrane potential.** The membrane potential was monitored by fluorescence of Rh123, released from the mitochondria following the induction of permeability transition at room temperature by using a Perkin-Elmer fluorimeter (London,

UK) at an excitation wavelength of 495 and an emission wavelength of 535 nm. Briefly, mitochondria at the concentration of 1 mg protein/ml were pre-incubated in the assay buffer containing 1  $\mu$ M Rh123 and the studied substances for 60 seconds.

**The Determination of ROS Formation.** ROS formation was detected as described previously (Varbiro et al., 2001) by the fluorescence of Rh123 formed by ROS-induced oxidation of the nonfluorescent DRh123 in situ at an excitation wavelength of 495 nm and an emission wavelength of 535 nm by a fluorimeter (PerkinElmer Life Sciences). The ROS-induced oxidation of N-acetyl-8-dodecyl-3,7-dihydroxyphenoxazine forms N-acetyl-8-dodecyl-resorufin (resorufin), which exhibits strong red fluorescence. This product is well retained in living cells and organelles by virtue of its lipophilic tail, making it possible to detect ROS production in the lipid phase. The method is the same as described above except for changing the excitation wavelength to 578 nm and the emission wavelength to 597 nm. ROS formation was calculated from the slope of the registration curves.

**Quantitative mass spectrometry (MS) with electron spray ionisation and HPLC-MS.** The samples and the calibration standards of HO-3538 were dissolved in acetonitrile. The sample solutions were introduced into the ion source at the flow rate of 150  $\mu$ L per hour via a PEEK capillary (Upchurch Scientific Inc., Oak Harbor, WA, USA). A Bruker Esquire HCT ion trap mass spectrometer equipped with an atmospheric electrospray ionization source (Bruker Daltonics, Bremen, Germany) was employed for mass detection. The ion source was operated in positive and negative mode. Nitrogen was used as drying gas at 300  $^{\circ}$ C, with a flow rate of 12 liter per minute, the pressure of the nebulizer was set at 20 psi. We used the Smart Parameter Setting (SPS) with target mass of 729 m/z. The scanning mass to charge range was 50 to 1200 m/z with a scanning speed of 26000 m/z per second. Maximum accumulation time was 200 ms.

**Statistical Analysis.** Statistical analysis was performed by analysis of variance and all of the data were expressed as the mean  $\pm$  S.E.M. Significant differences were evaluated by use of unpaired Student's *t* test and *P* values below 0.05 were considered to be significant (\*).

## Objectives

- Comparing the effect of amiodarone and its major metabolite desethylamiodarone on the mitochondrial permeability transition
- Analyzing the concentration dependent effect of amiodarone and desethylamiodarone on cardiac energy metabolism
- Screening newly synthesised amiodarone analogues based on their effect on the mitochondrial permeability transition and cardiac energy metabolism.
- Functionally evaluating the potential drug candidate amiodarone analogue HO-3538 in various experimental settings.
- Verifying that HO-3538 accumulates in the mitochondria and exert ROS scavenging effect in situ.

## Conclusions

- We report that amiodarone exerts a biphasic effect on the mitochondria with protective effects in lower concentrations and toxic properties manifesting when present at higher concentrations. On the other hand desethylamiodarone had toxic effects only. In higher concentrations amiodarone as well as desethylamiodarone can induce a CsA-independent mitochondrial swelling, thus contributing to the toxic property of the drug. Although both amiodarone and desethylamiodarone have similar antiarrhythmic properties, only amiodarone possesses a cardioprotective effect, and the frequently manifesting side effects during long-term amiodarone therapy could be related, at least in part, to the accumulation of desethylamiodarone.
- We present clear evidence that amiodarone at low concentrations, unlike desethylamiodarone, protects the mitochondrial energy metabolism of the perfused heart during ischemia and reperfusion as detected by real-time *in situ*  $^{31}\text{P}$  NMR measurements. We also demonstrated that cardiac and noncardiac cells are more susceptible to desethylamiodarone than amiodarone.
- We report that out of the newly synthesised amiodarone analogues, the compound labelled as HO-3538 seemed to be a good starting point for further toxicology and pharmacokinetic studies in rats. In this analogue, an ethyl side chain is substituted with 1-hydroxy-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-ylmethyl (SCAV) that has been described to possess free-radical scavenging activity. We tested HO-3538 on the permeability transition *in vitro* and in cultured cells as well as on ischemia-reperfusion in Langendorff-perfused rat hearts.
- The best derivate HO-3538 had much lower toxicity and higher cardioprotective effect than amiodarone. Simultaneously, its efficacy in inhibiting mPT *in vitro* and *in vivo*

was found similar to amiodarone, whereas its antioxidant effect was superior to that of amiodarone.

- HO-3538 was found to be taken up preferably into the mitochondria by quantitative mass spectrometry, therefore it could exert mitochondrial protection. In conclusion, HO-3538, the first representative of mPT inhibitors with free-radical scavenging properties demonstrated significant cytoprotection and cardioprotection with potential therapeutic relevance.

## References

- Cassarino, D. S.; Parks, J. K.; Parker, W. D. Jr.; Bennett, J. P. Jr. : The parkinsonian neurotoxin MPP 1 opens the mitochondrial permeability transition pore and releases cytochrome c in isolated mitochondria via an oxidative mechanism. *Biochim. Biophys.Acta* **1453**:49–62, 1999
- Kalai, T.; Varbiro, G.; Bogнар, Z.; Palfi, A.; Hanto, K.; Bogнар, B.; Osz, E.; Sumegi, B.; Hideg, K. Synthesis and evaluation of the permeability transition inhibitory characteristic of paramagnetic and diamagnetic amiodarone derivatives. *Bioorg Med Chem* **13**:2629-2636; 2005.
- Kannan, R.; Miller, S.; Perez, V and Singh, BN Sensitive method for the measurement of amiodarone and desethylamiodarone in serum and tissue and its application to disposition studies. *J Chromatogr* **385**:225–232; 1987.
- Schneider, W.C.; Hageboom, G.H. Cytochemical studies of mammalian tissues. III. Isocitric dehydrogenase and triphosphopyridine nucleotide-cytochrome c reductase of mouse liver. *J Biol Chem* **186**:417-427; 1950.
- Schmitt, J. P.; Schunkert, H.; Birnbaum, D. E.; Aebert, H. Kinetics of heat shock protein 70 synthesis in the human heart after cold cardioplegic arrest. *Eurn J Cardio-Thor Surg* **22**:415-420; 2002.
- Shimizu, S.; Narita, M.; Tsujimoto, Y.: Bcl-2 family proteins regulate the release of apoptogenic cytochrome c by the mitochondrial channel VDAC. *Nature* **399**:483–487, 1999
- Sims, N.R. Rapid isolation of metabolically active mitochondria from rat brain and subregions using Percoll density gradient centrifugation. *J Neurochem* **55**:698-707; 1990.
- Susin, S. A.; Lorenzo, H. K.; Zamzami, N.; Marzo, I.; Brenner, C.; Larochette, N.; Pre'vost, M.-C.; Alzari, P. M.; Kroemer, G.: Mitochondrial release of caspase-2 and -9 during the apoptotic process. *J. Exp. Med.* **189**:381–393, 1999
- Szabados, E.; Fisher, M. G.; Gallyas, F. Jr.; Kispal, Gy.; Sumegi, B. Enhanced ADP-ribosylation and its diminution by lipoamide following ischemia-reperfusion in perfused rat heart. *Free Rad Biol Med* **27**:1103-1113; 1999.
- Varbiro, G.; Veres, B.; Gallyas, F. Jr.; Sumegi, B. Direct effect of Taxol on free radical formation and mitochondrial permeability transition. *Free Rad Biol Med* **31**:548-558; 2001

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## List of Publications

### Publications supporting the dissertation:

**Zita Bognar**, Tamas Kalai, Anita Palfi, Katalin Hanto, Balazs Bognar, Laszlo Mark, Zoltan Szabo, Antal Tapodi, Balazs Radnai, Zsolt Sarszegi, Arpad Szanto, Ferenc Gallyas Jr., Kalman Hideg, Balazs Sumegi, Gabor Varbiro.: A novel SOD-mimetic permeability transition inhibitor agent protects ischemic heart by inhibiting both apoptotic and necrotic cell death. **Free Radical Biology & Medicine** (2006) 41:835–848 I.F.: 4.971

Kalai T., Varbiro G., **Bognar Z.**, Palfi A., Hanto K., Bognar B., Osz E., Sumegi B., Hideg K.: Synthesis and evaluation of the permeability transition inhibitory characteristics of paramagnetic and diamagnetic amiodarone derivatives. **Bioorg. and Med. Chem.** (2005) 13:2629-2636. I.F.: 2.286

Varbiro G., Toth A., Tapodi A., **Bognar Z.**, Veres B., Sumegi B., Gallyas F.: Protective effect of amiodarone but not N-desethylamiodarone on postischemic hearts by the inhibition of mitochondrial permeability transition. **J. Pharm. Exp. Ther.** (2003) 307:615-25.  
I.F.: 4.337

### Other publications:

Tapodi A, Debreceni B, Hanto K, **Bognar Z**, Wittmann I, Gallyas F Jr, Varbiro G, Sumegi B.: Pivotal role of Akt activation in mitochondrial protection and cell survival by poly(ADP-ribose)polymerase-1 inhibition in oxidative stress. **J. Biol. Chem.** 2005 Oct 21;280 (42):35767-75. I.F.: 6.355

Sumegi B., Kovacs K., Veres B., Radnai B., Varbiro G., **Bognar Z.**, Toth A., Gallyas F. Jr.: Oxidative Stress and the Endoplasmic Reticulum. In: Benedetti A., Banhegyi G., Burchell A. (Eds). **Endoplasmic reticulum: A metabolic compartment**. IOS Press, (2005) pp.121-130.

### **Abstracts, posters and presentations supporting the dissertation:**

Varbiro G., **Bognar Z.**, Palfi A., Hanto K., Kalai T., Hideg K., Sumegi B.: The effect of amiodarone analogues on the mitochondrial apoptotic process. Evry, France. 3<sup>rd</sup> European Conference and Course – Advanced Methods for Industrial production Purification and Characterization of Gene Vectors. 2004 June 14-26

**Bognar Z.**, Varbiro G., Palfi A., Hanto K., Kalai T., Hideg K., Sumegi B.: The protection of post-ischemic hearts by HO3538, a potent amiodarone analogue through inhibition of the mitochondrial apoptotic pathway, XXXIV. Membrán Transzport Konferencia, Sümeg, 2004. június 1-4.

**Bognar Z.**, Varbiro G., Palfi A., Veres B., Tapodi A., Radnai B., Sumegi B.: An amiodarone analogue, HO3538 protects ischemic hearts by inhibiting the mitochondrial apoptotic pathway. Istanbul, Turkey. 4<sup>th</sup> European Workshop on Cell Death. 2004. May 11-16.

**Bognár Z.**, Szántó Á., Szabó A., Hantó K., Ifj. Gallyas F., Sümegi B.: Egy módosított amiodarone analóg és az amiodarone szerepének összehasonlítása apoptotikus és nekrotikus sejthalálban. Biokémiai vándorgyűlés 2006 , Pécs, 2006. szept.

Arpad Szanto, **Zita Bognar**, Katalin Hanto,, Aliz Szabo, Viktoria Nemeth, Antal Tapodi, Kalman Hideg, Gabor Varbiro, Ferenc Gallyas Jr., Balazs Sumegi.: The protection of post-ischemic hearts by HO3538, a potent amiodarone analogue through inhibition of the mitochondrial apoptotic pathway, Cagliari, Italy, 14th Euroconferre on Apoptosis. 2006. Sept. 30 - Okt. 4.

### **Other abstracts, posters and presentations**

Dr. Nagy J., Pandur E., Szabó A., Montskó G., **Bognár Z.**, Sipos K.: A humán RNáz L Inhibitor szerepe a transzlációban, V. MagyarSejtanalitikai Konferencia, Budapest, 2006.05.04-06.

Radnai B., Veres B., Hantó K., Jakus P., Tapodi A., Várbíró G., **Bognár Z.**, Vető S. és Sümegi B.: Egy új PARP-gátló szer, a HO-3089 hatása az LPS stimulálta egér makrofág sejtvonalon, VI. Magyar Genetikai Kongresszus és XIII. Sejt- és Fejlődésbiológiai Napok Eger, 2005. április 10-12

Varbiro G., **Bognar Z.**, Veres B., Tapodi A., Radnai B., Sumegi B.: The effect of phosphatidic acid on the permeability transition and membrane potential in isolated rat liver mitochondria. (Abstract) **Gene Therapy** 11: S168-S168 72 Suppl. 1, (2004)

**Bognar Z.**, Varbiro G., Veress B., Kovacs K., Osz E., Sumegi B.: The direct induction of mitochondrial free radical formation by Taxol. (Abstract) **Free Radical Research** 37;Suppl. 1:110 (2003)

Varbiro G., **Bognar Z.**, Radnai B., Tapodi A., Hanto K., Jakus P., Gallyas F.Jr., Sumegi B.: Poly (ADP) ribose polymerase inhibitors influence the Taxol induced cell death in cultured cells. Miami, USA. Miami Nature Biotechnology Winter Symposium. 2005 February 5-9.

Radnai B., Veres B., Varbiro G., Tapodi A., **Bognar Z.**, Sumegi B.: The role of 4HQ – a PARP1 inhibitor – on LPS stimulated murine macrophage cells. Dubrovnik, Croatia. FEBS Lecture Course on cellular signaling & 4<sup>th</sup> Dubrovnik Signaling Conference 2004. May 21-27.

Varbiro G., **Bognar Z.**, Veres B., Tapodi A., Radnai A., Sumegi B.: Phosphatidic acid induces permeability transition and the collapse of the membrane potential in isolated rat liver mitochondria. Istanbul, Turkey. 4<sup>th</sup> European Workshop on Cell Death. 2004. May 11-16.

Varbiro G., **Bognar Z.**, Veres B., Tapodi A., Radnai A., Sumegi B.: The effect of phosphatidic acid on the permeability transition and membrane potential in isolated rat liver mitochondria. Bellaterra, Spain. 2<sup>nd</sup> European Conference and Course – Towards clinical gene therapy: pre-clinical gene transfer assessment. 2004. February 1-14.

Varbiro G., **Bognar Z.**, Veres B., Tapodi A., Radnai A., Sumegi B.: The effect of phosphatidic acid on the permeability transition and membrane potential in isolated rat liver mitochondria. Madrid, Spain. CNIO Cancer Conference: Apoptosis and Cancer. 2003. December 1-3.

**Bognar Z.**, Varbiro G., Veress B., Kovacs K., Osz E., Sumegi B.: The direct induction of mitochondrial free radical formation by Taxol. Ionannia, Greece, Free Radicals and Oxidative Stress: Chemistry, Biochemistry and Pathophysiological Implications. 2003. June 26-29.