PhD Thesis

Investigation of molecular interactions of Ochratoxin A applying *in vitro* models

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1. Introduction

Ochratoxins are secondary metabolic products of different *Aspergillus* and *Penicillium* species. Based on their chemical structure we can distinguish Ochratoxin A, B and C toxins; Ochratoxin A (OTA) is highly the most toxic derivative. OTA is built up from a dihydroisocoumarin moiety linked with L-phenylalanine. OTA occurs mainly in the Balkan region, however, it is present world-wide in detectable amounts in cereals, fruits, bakery-, meat- and dairy products, wine, beer, coffee and even in eggs. Because of its wide occurrence and high thermal stability, its eradication from the food chain with the currently known methods seems to be impossible.

Since OTA is a food contaminant, it enters orally to the human organism; it has a high oral bioavailability (about 93% in humans). After the toxin reaches the bloodstream more than 99% of OTA binds to serum proteins (mainly to albumin), resulting in its very long plasma half-life (about 1 month). OTA binds to human serum albumin (HSA) in dianionic form; its main binding site is located at subdomain IIA. Some animal experiments suggest that displacement of OTA from albumin speeds up its elimination and prevents the accumulation of the toxin in target tissues. OTA is excreted mainly by urine.

OTA is primarily a nephrotoxic mycotoxin, however, several other adverse effects of OTA are also suggested. In recent years, numerous *in vitro* and *in vivo* studies have been performed in order to explore its exact mechanism of action. Based on these results many theories are known, on the other hand we still lack strong evidences. The suspected mechanisms are inhibition of protein synthesis and cellular energy production, DNA adduct formation, induction of oxidative/nitrosative stress, disruption of calcium homeostasis, provocation of apoptotic cell death and cell cycle arrest. At present there are no proven effective antidote or prevention strategies against OTA toxicity.

In the human organism OTA is able to interact with several molecules, e.g. ions, different peptides and proteins. One of the most important interactions is the complex formation with HSA because it plays a key role in the toxicokinetics of OTA. Further investigation of molecular interactions of OTA is reasonable and necessary for the deeper understanding of its mechanism of action and for developing potential prevention strategies.

2. Aims

Although, many theories have been published, the exact mechanism of action of OTA is currently unclear. We do not know which effect plays a key role in the mechanism of action of OTA during the long term human exposure of the toxin. In order to alleviate OTA-mediated negative impacts, the deeper understanding of its biochemical behavior is necessary. For this purpose our main goals were the followings:

- Investigation the physico-chemical behavior of OTA: Study of its interactions with ions, proteins and other endogenous molecules.
- Quantitative analysis of OTA-albumin interaction, identification of potential competitor molecules with low toxicity.
- Identification of agents which are suitable to decrease the absorption or to enhance the elimination of OTA.
- Investigation of *in vitro* toxic effects of OTA in cell cultures.
- Identification of agents which counteract with the toxicodynamic effects of OTA.

3. Methods

Hitachi F4500 fluorimeter and Fluorolog τ3 spectrofluorimetric system (Jobin-Yvon/SPEX) were used for fluorescence spectroscopic and fluorescence polarization measurements, experiments were performed at +25 °C (with the exception of thermodynamic studies) in the presence of air.

For quantitative determination of OTA and OTA^{2-}-Mg^{2+} complex with chromatography, integrated high performance liquid chromatography (HPLC) system (Agilent 1100) was applied, it was equipped with a quaternary pump, a degasser, an autosampler, an injector with a 100-µl sample loop, a column oven and a fluorescent detector. Data were recorded and evaluated using Agilent ChemStation (Rev.B.03.02-SR2) software. Separation was performed on a LiChroCART® 4 mm × 125 mm, 5-µm particle size, Merck Purospher STAR® RP-18e (endcapped) column with a guard cartridge (TR-C-160-K1; ABLE&E-Jasco) using binary gradient mobile phase.

A Berthold Lumat LB9507 luminometer was applied to quantify ATP concentrations. Flow cytometry measurements were performed on a Beckman-Coulter FC500 flow cytometer, data analysis was done using CXP software. Promega GloMax®-Multi Microplate Multimode Reader was used for ROS-measurements.
MDCK (tubular kidney, immortalized, ATCC: CCL 34) adhering cells were cultured in DMEM (high glucose, 4.5 g/l) with 10% FBS plus penicillin (100 U/ml) and streptomycin (100 μg/ml) in 25 cm^2 sterile plastic flasks (VWR) and in 96-well sterile plastic plates (VWR), furthermore, 6-well sterile plastic plates (VWR) were applied for flow cytometric analyses. Cells were kept at 37 °C in the presence of 5% CO_2 in humidified atmosphere. All sterile work was carried out in an Aireguard-126300 (Nuaire) vertical laminar box.
Total protein, ATP and ROS concentrations were determined using fluorescamine, luciferin-luciferase and C400 reagents, respectively. Viability of control and treated cells was evaluated applying calcein and propidium iodide – FITC-AnnexinV viability assays. For cell cycle analysis, after permeabilization cells were labeled with propidium iodide.
All experiments were repeated at least three times. Statistical analyses were performed with One-Way ANOVA using IBM SPSS Statistics software (Version 20). $P < 0.05$ was set as the level of significance.

4. Results and discussion

4.1. Interaction of Ochratoxin A with serum albumin. Fluorescence characteristics of OTA are highly influenced by the microenvironment. Depending on the pH, OTA could be present in nonionic, monoanionic (OTA^-) and dianionic (OTA^{2-}) forms. Under physiological conditions dianionic OTA predominates because pH 7.4 is higher than pKa values of OTA, furthermore, the high concentration of alkali earth ions also shift the equilibrium toward the formation of OTA^{2-}.
Nonionic and monoanionic OTA show the same spectra (λ_{exc} = 332 nm, λ_{em} = 451 nm). On the other hand, fluorescence excitation and emission wavelength maxima of OTA^{2-} are different from the two other forms (λ_{exc} = 380 nm, λ_{em} = 443 nm) and it represents a much higher fluorescence signal. Interaction of OTA with albumin results in spectral changes in its fluorescence: the excitation and emission wavelength maxima of OTA^{2-}-HSA complex are 393 and 446 nm, respectively. Furthermore, the

fluorescence intensity of OTA^{2-}-HSA complex is considerably higher than that of OTA^{2-} in the same concentration.

Since the complex formation of OTA with albumin plays a key role in the toxicokinetics of OTA, it is one of the main subjects of our interest. Complex stability of OTA-HSA was determined applying three different experimentations (fluorescence intensity, polarization and quenching) and in all cases association constants (K) were higher than 10^7 dm^3/mol. This value is very high compared to other albumin-ligand complexes and it explains the extremely long plasma half-life of OTA.

The binding affinities of OTA with different albumin species show major differences. Association constants of OTA-albumin complexes were quantified with human (HSA), bovine (BSA) and rat serum albumin (RSA) in PBS buffer (pH 7.4) at 25 °C. The determined values are the following: $\log K_{OTA\text{-}HSA} = 7.65 \pm 0.36$, $\log K_{OTA\text{-}BSA} = 6.48 \pm 0.22$ and $\log K_{OTA\text{-}RSA} = 6.17 \pm 0.12$. Our results demonstrate that 15-fold and 30-fold higher complex stabilities were observed in the case of OTA-HSA complex compared to OTA-BSA and OTA-RSA complexes, respectively.

Thereafter, a previously published fluorescence polarization-based method was compared to two intensity-based approaches to verify its suitability for quantitative investigation of OTA-HSA interaction. The results of the three applications show very similar data, indicating that we are able to follow precisely and quantitatively the complex formation of OTA with HSA. To investigate competitive interactions of OTA with different drugs and flavonoids for binding to HSA, the fluorescence polarization model was applied subsequently.

4.2. Competitive interaction of drugs with OTA for binding to HSA. In this part of the studies our aim was to identify further drug molecules which are able to displace OTA from the surface of HSA. 13 different drugs were examined using 1.0 μM OTA, 1.7 μM HSA and 40, 70 and 100 μM drug concentration. Experiments were performed in PBS buffer (pH = 7.4) at 25 °C. Acetylsalicylic acid, nifedipine, phenobarbital, phenytoin, theophylline and verapamil did not show any interaction with the OTA-HSA complex. In contrast, ibuprofen, indometacin, furosemide, glipizide, phenylbutazone, simvastatin and warfarin proved to be as effective competitors. The most conspicuous effect was represented by indometacin and phenylbutazone. In competing efficiency these two drugs were followed by warfarin, furosemide and ibuprofen (in this order). Finally, glipizide and simvastatin can be mentioned with a lower but measurable

effect. The competitive interaction of some NSAIDs (piroxicam, indometacin) and warfarin with OTA had already been reported in previous studies. On the other hand, the identification of furosemide, glipizide and simvastatin as effective competitors, furthermore, the absence of displacement in the case of acetylsalicylic acid and phenobarbital (in contrast with previous assumptions) are new observations. We suggest that this explorative investigation will be useful in the future to examine the effect of these drugs on OTA toxicity in humans using epidemiologic approaches.

4.3. Competitive interaction of flavonoids with OTA for binding to HSA. Recent studies highlighted that dietary flavonoids bind to serum albumin with high affinity, occupying the same binding site (Sudlow's site I) than OTA. Furthermore, toxicity of flavonoids is low thus we can apply them in higher amounts without known side effects. Therefore, this molecular group seems to be suitable for the displacement of OTA from HSA and using this principle the elimination of OTA from the human organism can be enhanced. In our experiment, 12 different flavonoid aglycones were tested using the same conditions described in the previous section (4.2.) with the exception of the applied flavonoid concentrations (10, 25 and 50 μM, respectively). Comparing the effectiveness of flavonoids belonging to various subclasses, we found that the tested flavones and flavonols possess the ability to remove OTA from its albumin complex, while flavanones and isoflavonoids showed negligible or no displacing properties. These findings suggest that the presence of a C2-C3 double bond in ring C in conjugation with 4-carbonyl group (as in flavones and flavonols) might be critical for the binding affinity. Although, daidzen possesses these important structural features, it was an ineffective competitor. This could be due to the fact that the phenyl group in the isoflavonoid structure is substituted at the C3 position of the dihydropyrane ring instead of regular C2 position. Interpreting the data for all three flavonoid concentrations in the competition experiments, galangin showed far the highest effect followed by quercetin. They both belong to flavonols possessing C3 hydroxyl group which also seems to have an important role. The comparison of obtained results for all tested flavonols showed that the presence of hydroxyl groups in the ring B obviously reduces their competing abilities. Moreover, their properties also depend on the number and position of the substituents. For example quercetin with the C2',C3'-dihydroxy structures in the ring B was a better competitor than morin with

C1',C3'-hydroxyl groups as well as myricetin which has three hydroxyl groups (C2',C3',C4') in the B ring. While the C3-hydroxyl group in ring C causes higher binding strength, it is possible that the ortho-hydroxyl group (position C1') of morin and the two meta-hydroxyl groups (positions C2' and C4') of myricetin can reduce the binding affinity because of steric reasons. Although, a few animal studies suggest the positive effect of displacement of OTA from its albumin-bound form, we still lack strong evidences on its beneficial action. Therefore, it is feasible to postulate that the increased concentration of free OTA results in its increased metabolism but besides the detoxification in the body (e.g. formation of 4-hydroxy-OTA or 10-hydroxy-OTA metabolites) the formation of potential toxic or reactive metabolites cannot be excluded. However, at present we do not know any research that identifies the negative impact of this type of interaction.

4.4. Interaction of OTA with alkali and alkaline earth ions. In this part of the studies 0.1 M TRIS-HCl buffer (pH 7.4) was applied. Experiments were performed using the chloride salts of Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+} and Ba^{2+} ions. In the presence of alkali earth ions (Li^+, Na^+ and K^+) the fluorescence wavelength maxima of OTA did not change but alkali earth ions shifted the chemical equilibrium towards the formation of its dianionic form; the order of its magnitude was $Li^+ > Na^+ \sim K^+$. In contrast, the presence of alkaline earth metal ions caused the shifting of both the excitation and emission wavelength maxima of OTA (Mg^{2+}: λ_{exc} = 375 nm, λ_{em} = 427 nm; Ca^{2+}: λ_{exc} = 375 nm, λ_{em} = 433 nm; Ba^{2+}: λ_{exc} = 377 nm, λ_{em} = 439 nm) and a significant increase of its fluorescence intensities was also observed. Our investigations highlighted that OTA^{2-} is highly the more preferred form for complex formation.

Thereafter, stability constants of OTA-cation complexes were quantified using Hyperquard2006 program package (Protonic Software). The determined log*K* values were the followings: OTA^{2-}-Mg^{2+} = 3.40 ± 0.01, OTA^{2-}-Ca^{2+} = 2.78 ± 0.01, OTA^{2-}-Ba^{2+} = 1.92 ± 0.02. These data demonstrate that the stability of OTA^{2-}-Mg^{2+} complex is 4-fold higher than OTA^{2-}-Ca^{2+} and 30-fold higher than OTA^{2-}-Ba^{2+}. Thermodynamic studies (performed by using the van't Hoff theory) suggest that complex formation of OTA with cations is an entropy-driven process because the desolvation of alkaline earth ions results in entropy production. The investigation of OTA^{2-}-Mg^{2+} interaction in PBS buffer (pH 7.4) showed that 15,000-fold higher Mg^{2+} concentration is needed than OTA to achieve the complete

saturation of OTA with magnesium ion. Based on this observation, we suggest that the protein-free fraction of OTA is partially presented as magnesium complex in body fluids.

4.5. Application of OTA^{2-}-Mg^{2+} complex formation for HPLC-FLD system. Since the OTA^{2-}-Mg^{2+} complex is relatively stable and because of the complex formation of OTA^{2-} with Mg^{2+} results in the significant elevation of its fluorescence, we hypothesized that the interaction maybe suitable for developing more sensitive fluorescence analytical methods, e.g. in the case of chromatographic analyses. We tested this idea on HPLC-FLD instrument. The applied alkaline mobile phase [Triethylamine buffer (triethylamine hydrochloride/triethylamine 5 mM, pH 9.8) – acetonitrile; the gradient elution was as follows: from 0 to 10 min, acetonitrile was increased from 17.5% (v/v) to 25%, while triethylamine buffer was decreased from 82.5% to 75%. From 10 to 12 min, acetonitrile and buffer returned to the initial values.] was supplemented with Mg^{2+} (its buffer component contained 5 mM $MgCl_2$). Because of this slight modification of the eluent, the sensitivity of the chromatographic method doubled. So we can significantly increase the sensitivity without the considerable elevation of costs. In addition, the sample preparation is not affected by the application of Mg^{2+} in the mobile phase.

4.6. Interaction of OTA with zinc ions. Besides alkali and alkaline earth ions other cations were also investigated. Our results showed that Zn^{2+} ions are able to form complex with OTA. Similarly to OTA^{2-}-Mg^{2+} interaction, a shift of excitation and emission wavelength maxima (λ_{exc} = 370 nm, λ_{em} = 431 nm), an elevation of fluorescence signal and a preference towards dianionic OTA were observed. log*K* = 3.78 (± 0.02) was determined for OTA^{2-}-Zn^{2+} complex as stability constant in 0.1 M TRIS-HCl buffer (pH 7.4) at 25 °C. This log*K* value suggests about 2.5-fold higher stability than that was observed for OTA^{2-}-Mg^{2+} complex. Since recent *in vitro* studies showed that adverse effects of OTA can be alleviated by zinc supplementation, it is possible that OTA^{2-}-Zn^{2+} complex formation has a biological role.

4.7. Interaction of OTA with cyclodextrins. The interactions of OTA with β- and γ-cyclodextrins were reported in recent publications. On the other hand, the stability of OTA-cyclodextrin complexes is low and several times complex formation with cyclodextrins does not result in fluorescence changes on the spectra of OTA. We tested different chemically modified β-cyclodextrins (β-CD) to find a derivative which is able to form more stable

complex with OTA than previously published OTA-CD complexes. In addition, the higher affinity of the CD toward dianionic OTA was a further objective. Both requirements were fulfilled by QABCD ([2-hydroxy-3-N,N,N-trimethylamino]-propyl-β-cyclodextrin-chloride). Unfortunately, the inclusion of OTA by QABCD did not result in the changes of its fluorescence. On the other hand, cyclodextrins are large enough to decrease significantly the rotational freedom of OTA, therefore, the interaction can be followed by fluorescence polarization technique. Based on our calculations, the stability of OTA^{2-}-QABCD complex is 200-fold higher than determined for OTA^{2-}-β-CD complex. The increase of complex stability originates from the ionic interaction which stabilizes the OTA^{2-}-QABCD complex. Furthermore, QABCD forms 130-fold more stable complex with dianionic than monoanionic OTA. In addition, the log*K* value was determined for OTA^{2-}-QABCD complex (4.46 ± 0.09) suggests 10-fold higher complex stability compared to the most stable OTA-CD complex described in the literature. The appropriate chemical modification of cyclodextrins most probably will allow us to decrease the absorption of OTA from the intestinal tract or even to increase its elimination from the human organism.

4.8. Effects of OTA on MDCK cells. After the 24-hour treatment of MDCK cells with 0-15 μM OTA, different cellular parameters were determined. Cellular total protein and ATP concentrations and the fluorescence of calcein significantly decreased in OTA-exposed samples. Fluorescence intensity of calcein showed linear correlation with the number of living cells (counted with a Bürker-type counting chamber). Based on our experiments, the changes in total protein levels show very similar characteristics to the decrease in the number on living cells. These observations suggest that in our experimental model the inhibition of protein synthesis by OTA is not dominant. In contrast, significant decrease of ATP/protein ratio and elevation of ROS/protein level were determined. Propidium iodide – FITC-AnnexinV viability assay highlights that the treatment with OTA results in the elevated number of apoptotic and/or necrotic cells, however, only extremely high OTA concentrations cause considerable effect. On the other hand, even 5 μM OTA has influence on the cell cycle: concentration dependent G2/M phase cell cycle arrest was observed, while the number of G0/G1 phase cells decreased and number of S phase cells did not change.

4.9. Effects of diosmetin on OTA-exposed cells. Numerous substances were tested during our experiments to develop an agent which is suitable to alleviate the toxic effects of OTA on MDCK cells. When the flavonoid aglycone diosmetin (DIOS) was tested, an unexpected phenomenon was observed. 6-12-hour treatment with 5-15 μM DIOS resulted in the significant elevation of ATP concentrations in MDCK cells and after 24 hour ATP levels returned to the control level. Calcein viability assay and total protein levels did not show any negative effect of DIOS on living cells compared to that of control. To understand the mechanism of action of DIOS different enzyme inhibitors were applied: sodium fluoride (glycolysis inhibitor), 3-bromopyruvate (glycolysis and pyruvate dehydrogenase inhibitor) and oligomycin A (ATP synthase inhibitor). Interestingly, sodium fluoride and 3-bromopyruvate did not decrease the impact of DIOS on ATP levels. In contrast, oligomycin A completely abolished it. Further investigations are needed to identify the exact mechanism of action of DIOS.

Thereafter, the effect of DIOS on OTA-exposed cells was tested. Based on our previous observations, low OTA concentrations (5-15 μM) cause major changes on ATP levels at least after 24-hour treatment. On the other hand, DIOS seems to be effective under shorter incubation times (6-12 hours). Therefore MDCK cells were treated with 15 μM OTA and after 12-hour incubation the system was supplemented/co-treated with 0-15 μM DIOS (without the replacement of the previous medium) and cells were incubated for further 12 hours. Each applied DIOS concentrations (5, 10 and 15 μM) resulted in the significant elevation of ATP/protein ratio in OTA-co-treated cells. The impact is so effective that 15 μM DIOS restores completely the intracellular ATP levels. ROS/protein levels were also quantified, DIOS caused significant but not dramatic decrease of ROS/protein ratios in co-treated (both OTA and DIOS) cells compared to the simply OTA-exposed cells. Unfortunately, the negative effect of OTA on cell cycle cannot be alleviated with DIOS or with antioxidants.

5. Conclusions, new findings

- We verified the suitability of the previously described fluorescence polarization-based method for quantification of OTA-HSA interaction as well.
- We determined the complex stabilities of OTA-albumin complexes applying human, bovine and rat serum albumin under the same conditions.
- We established that furosemide, glipizide and simvastatin were able while phenobarbital and acetylsalicylic acid (contrary to the earlier assumptions) were unable to displace OTA from HSA.
- We described and chemically and quantitatively characterized the competitive interaction between flavonoid aglycones and OTA for binding to HSA.
- We identified and characterized the interactions of OTA with alkali earth ions, alkaline earth ions and zinc ions. We developed a novel HPLC-FLD method based on the principle of the higher fluorescence of OTA^{2-}-Mg^{2+} complex.
- We described at the first time the OTA^{2-}-QABCD interaction which complex is more stable than the previously examined OTA-CD complexes. Furthermore, QABCD shows strong preference towards the dianionic form of OTA.
- We demonstrated that the main toxic mechanism of OTA in MDCK cells is the G2 phase cell cycle arrest. Antioxidants cannot alleviate this effect.
- We demonstrated the positive effect of diosmetin on cellular ATP levels and that diosmetin is able to restore ATP levels in OTA-exposed MDCK cells.

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7. List of publications

7.1. Publications related to the present PhD thesis

Poór M, Kunsági-Máté S, Bencsik T, Petrik J, Vladimir-Knežević S, Kőszegi T, Flavonoid aglycones can compete with Ochratoxin A for human serum albumin: A new possible mode of action. *INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES* 51 (2012) 279-283. **[IF: 2.596]**

Poór M, Kunsági-Máté S, Czibulya Z, Li Y, Peles-Lemli B, Petrik J, Vladimir-Knežević S, Kőszegi T, Fluorescence spectroscopic investigation of competitive interactions between ochratoxin A and 13 drug molecules for binding to human serum albumin. *LUMINESCENCE* 28 (2013) 726-733. **[IF: 1.273]**

Poór M, Kunsági-Máté S, Matisz G, Li Y, Czibulya Z, Peles-Lemli B, Kőszegi T, Interaction of alkali and alkaline earth ions with Ochratoxin A. *JOURNAL OF LUMINESCENCE* 135 (2013) 276-280. **[IF: 2.144]**

Poór M, Li Y, Matisz G, Kiss L, Kunsági-Máté S, Kőszegi T, Quantitation of species differences in albumin-ligand interactions for bovine, human and rat serum albumins using fluorescence spectroscopy: A test case with some Sudlow's site I ligands. *JOURNAL OF LUMINESCENCE* 145 (2014) 767-773. **[IF: 2.144]**

Poór M, Veres B, Jakus PB, Antus C, Montskó G, Zrínyi Z, Vladimir-Knežević S, Petrik J, Kőszegi T, Flavonoid diosmetin increases ATP levels in kidney cells and relieves ATP depleting effect of ochratoxin A. *JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY B: BIOLOGY* 132 (2014) 1-9. **[IF: 3.110]**

Poór M, Kuzma M, Matisz G, Li Y, Perjési P, Kunsági-Máté S, Kőszegi T, Further aspects of ochratoxin A – cation interactions: complex formation with zinc ions and a novel analytical application of ochratoxin A – magnesium interaction in the HPLC-FLD system. *TOXINS (BASEL)* 6 (2014) 1295-1307. **[IF: 2.129]**

Li Y, Czibulya Z, **Poór M**, Lecomte S, Kiss L, Harte E, Kőszegi T, Kunsági-Máté S, Thermodynamic study of the effects of ethanol on the interaction of ochratoxin A with human serum albumin. *JOURNAL OF LUMINESCENCE* 148 (2014) 18-25. **[IF: 2.144]**

7.2. Presentations and posters related to the present PhD thesis

Poór M, Kőszegi T, Kunsági-Máté S, Competitive Interaction between Flavonoid Aglycons and Ochratoxin A During their Binding to Human Serum Albumin. ***The Third Asian Spectroscopy Conference*** (Xiamen, China, 28.11.2011-01.12.2012) *[poster]*

Poór M, Kőszegi T, Li Y, Czibulya Z, Kunsági-Máté S, Investigation of competitive interaction between Ochratoxin A and drug molecules for serum albumin. ***10th Conference of Colloid Chemistry*** (Budapest, Hungary, 09.08.2012-31.08.2012) *[poster]*

Poór M, Li Y, Matisz G, Kőszegi T, Kunsági-Máté S, Interaction of Ochratoxin A with alkaline earth ions. ***1st Symposium on Weak Molecular Interactions*** (Pécs, Hungary, 05.03.2013-06.03.2013) *[presentation]*

7.3. Other publications

Poór M, Li Y, Kunsági-Máté S, Petrik J, Vladimir-Knežević S, Kőszegi T, Molecular displacement of warfarin from human serum albumin by flavonoid aglycones. *JOURNAL OF LUMINESCENCE* 142 (2013) 122-127. **[IF: 2.144]**

Poór M, Li Y, Kunsági-Máté S, Varga Z, Hunyadi A, Dankó B, Chang FR, Wu YC, Kőszegi T, Protoapigenone derivatives: albumin binding properties and effects on HepG2 cells. *JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY B: BIOLOGY* 124 (2013) 20-26. **[IF: 3.110]**

7.4. Other presentations and posters

Poór M, Kunsági-Máté S, Li Y, Kőszegi T, A new possible mechanism of flavonoid-drug interaction: flavonoids are able to displace warfarin from human serum albumin. ***20th IFCC-EFLM European Congress of Clinical Chemistry and Laboratory Medicine*** (Milano, Italy, 19.05.2013-23.05.2013) *[poster]*