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Preparation of *N*- and *O*-Palmitoyl Derivates of Fumonisin B1 Toxin and their Toxicity *in vitro* and *in vivo*

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1. Background of the Research and Aims

Background of the Research

The *O*- and *N*-acyl-FB1 toxin derivatives, which are the subject of my thesis, belong to the group of mycotoxins, which are produced by some field and warehouse mold species. Currently, hundreds of mycotoxins are known, but only a few of them can cause significant health problems. Mycotoxins can occur in many food industry raw materials, semi-finished and finished products (e.g. cereals, corn, grapes, raisins, beer, wine, coffee and cocoa beans, pistachios, peanuts, paprika, apple juice, milk).

Mycotoxins cause significant economic damage and food safety problems all over the world, they can contaminate grain seeds in almost every phase of cultivation, harvesting and storage, it is practically impossible to eliminate these natural toxic substances from the food chain. According to surveys, 25% and 20% of crops harvested in the world and in Europe are contaminated with mycotoxins (FAO, 1997). According to recent surveys using high-precision multi-mycotoxin analytical methods (HPLC/MSMS), the level of contamination can be as high as 80%, if different mycotoxin metabolites are also taken into account (Kovalsky et al., 2016).

The most dangerous mycotoxins are subject to strict food health limit values, and in the case of animal feed – with the exception of aflatoxin B1, for which a limit value already exists – recommendations apply. Pure reference materials (standards) are needed for the quantitative and qualitative determination of mycotoxins in various foods and feeds, and for the calibration of instruments. The reference materials are chemically synthesized and/or produced *in vitro* by various filamentous fungi under liquid or

solid fermentation conditions. After chemical synthesis or production, reference materials are extracted in sufficient purity using various extraction and chromatography methods.

Aims

1. During my research, I wanted to develop a suitable procedure for optimizing the solid-phase fermentation of the FB1 toxin using *Fusarium verticillioides* isolates.

2. My goal was the clean extraction of the FB1 toxin from the cultures using preparative flash and liquid chromatography methods.

3. My plan was to perform experiments on the synthetic acylation of the pure FB1 toxin with palmitoyl chloride, as well as on the purification of the acylated FB1 derivatives.

4. I wanted to determine the purity of the derivatives obtained from the synthetic acylation of the FB1 toxin by HPLC/MS methods.

5. In order to identify the synthetically produced components, I wanted to carry out a spiking experiment using the fungal extract produced during fermentation.

6. I planned to monitor the produced acylated FB1 toxin derivatives with high-resolution mass spectrometry (HRMS) and nuclear magnetic resonance spectroscopy (NMR).

7. During the animal experiment, which was one of the most important parts of my research, my goal was to investigate the toxicity of isolated/synthesized acylated fumonisins, using the FB1 toxin as a control.

2. Materials and Methods

The FB1 toxin was produced by a *Fusarium verticillioides* isolate on rice medium using a solid-phase fermentation method. The extract of the lyophilized rice cultures was filtered through a membrane filter. The filtrate was first purified on a strong anion exchange (SAX, then on a C18 column using flash chromatography. The FB1 toxin content of the fractions was determined off-line by an HPLC/MS device. Components eluting from the HPLC C18 column were detected with a mass spectrometer using a HPLC/ESI-MS device in positive ion mode.

The *N*-acylation of FB1 toxin was carried out in anhydrous tetrahydrofuran (THF) with a mixture of palmitoyl chloride and triethylamine (TEA) reagents. The *O*-acylation of FB1 toxin was performed with palmitoyl chloride and triethylamine reagents, but the concentration of the reagents was higher and the THF contained 2% water.

The samples taken from the reaction mixtures were analyzed by an high performance liquid chromatograph (Agilent 1100) connected to a mass spectrometer (Agilent 1946D) equipped with an electrospray ion source (ESI) using gradient elution, in positive ion mode.

In order to extract the acylated FB1 derivatives, the reaction products were purified by preparative HPLC (Hanbon NS421). The gradient separation was carried out on a C18 preparative column, at room temperature. The fractions were analyzed off-line by HPLC/MS.

The structural identification experiments of the purified components were performed on a 700 MHz NMR spectrometer (Bruker) equipped with a Prodigy TCI cryoprobe measuring head.

The HPLC/HRMS measurements were performed using a UHPLC system connected to a Q Exactive Plus hybrid quadrupole-orbitrap mass spectrometer (Thermo Fisher Scientific) equipped with a heated electrospray ion source (HESI). The separation of the purified components was carried out on a C18 HPLC column using a binary gradient. An isocratic and then a linear gradient was used to separate the components. The samples were measured in both positive and negative ion modes.

A spiking experiment was performed in order to identify the acylated FB1 derivatives produced by *F. verticillioides* during the fermentation. During the experiment, the fungal extract was spiked individually with the purified and NMR-identified acylated FB1-derivatives (3-*O*-, 5-*O*- and *N*-palmitoyl-FB1) using the autosampler of the HPLC device. After each spiking, the samples were analyzed in positive ion mode using an HPLC device connected to a mass spectrometer equipped with an electrospray ion source (ESI).

During the spectroscopic experiment, the spectra were collected using an F-4500 fluorimeter and UV-Vis photometer at 25 °C. The effects of the examined FB1-derivatives and palmitic acid on the emission spectrum of human serum albumin (HSA) were tested in phosphate buffered saline (PBS, pH 7.4). The changes in the emission signals of the albumin was evaluated at 340 nm. The internal filter effects of the ligand molecules were corrected based on the ultraviolet-visible spectrum (UV/Vis).

The HSA-bound fractions of albumin and the tested fumonisins were sedimented by ultracentrifugation, then the free fraction of the ligand molecules was quantitatified using an HPLC/MS instrument. Gradient elution was used to separate the components. During the analysis, an internal standard mixture (1 ng FB1-^{13}C34 and 1 ng *N*-C17:0-FB1 in ACN-water (1:1 v/v%)) was added to each sample by the autosampler. The MS data were collected in positive ion selective ion monitoring (SIM) mode.

In order to examine the binding sites and to test the effect of fumonisins on the binding site-HSA interaction, ultrafiltration experiments were performed. The effects of FB1, *N*-pal-FB1, 5-*O*-pal-FB1 and FB4 toxins were investigated with Site I (warfarin), Site II (naproxen) and FA1 (S-camptothecin) ligands using ultrafiltration methods. The concentrations of warfarin, naproxen and S-camptothecin in the supernatants were determined by HPLC. Statistical differences were evaluated with one-way ANOVA and Tukey post-hoc tests, using SPSS Statistics software.

The structures of *N*-pal-FB1 and 5-*O*-pal-FB1 were built in Maestro program. The energy minimization of the ligands was performed using OpenBabel, followed by steepest descent and conjugate gradient minimization. The obtained structures were energy-minimized using the PM7 parameter set and the quantum chemistry program package (MOPAC). Gasteiger–Marsili partial charges were assigned to the ligand atoms using the AutoDock Tools program. The AutoDock 4.2.6 program was used to dock the ligands to human serum albumin.

To study the *in vivo* toxicity of fumonisin derivatives, we used a laboratory-reared AB zebrafish (*Danio rerio*) line. The Zebrafish

Embryo Toxicity Test (ZETA) was performed to test the toxicity. Embryos aged 96 hpf ("hours post fertilization") were exposed to specific concentrations of each compound for 24 hours. Mortality and developmental abnormalities were evaluated using a stereomicroscope. For the statistical analysis of lethal and non-lethal effects, two-way ANOVA and Tukey's post hoc tests were used. The analysis was performed using GraphPad Prism 9 software.

3. Results

Acylation of FB1 Toxin with Palmitoyl Chloride/Triethylamine Reagents

The formation of *N*-palmitoyl-FB1 occurred rapidly (within 30 min) at room temperature, with a conversion over 90%. The *O*-acylation of the FB1 toxin took place much more slowly. 3-*O*-palmitoyl-FB1 formed first, then 5-*O*-palmitoyl-FB1 slowly appeared. The concentration of 3-*O*-palmitoyl-FB1 reached its maximum value in 48 hours. At that time, based on the area percentage evaluation, 38.9% 3-*O*-palmitoyl-FB1, 33.7% 5-*O*-palmitoyl-FB1 and a total of 27.4% intermediates were present in the reaction mixture. As time progressed, the composition shifted towards the formation of 5-*O*-palmitoyl-FB1. Five days after starting the reaction, 80% 5-*O*-palmitoyl-FB1 toxin was observed in the reaction mixture containing 2% water, along with 20% 3-*O*-palmitoyl-FB1. In this case, 3-*O*-palmitoyl-FB1 was considered as an intermediate. 3-*O*-palmitoyl-FB1 was not stable even after purification by preparative HPLC. In the fractions eluting from the preparative HPLC column, *O*→*N*-acyl migration was observed during evaporation and lyophilization, which resulted in the formation of approximately 10% *N*-palmitoyl-FB1 from 3-*O*-palmitoyl-FB1.

NMR Investigation of the *O*- and *N*-acylated FB1 Compounds

The structures of the 3-*O*-, 5-*O*- and *N*-palmitoyl-FB1 compounds were confirmed by high-resolution ^{1}H and ^{13}C NMR spectroscopy with a 700 MHz NMR spectrometer. Previously, only ^{1}H spectral data were reported on the *N*-palmitoyl-FB1 toxin using a

lower magnetic field (400 MHz) NMR device (Harrer et al., 2013). The resonances were assigned based on one (1D) and two-dimensional (2D) NMR experiments. The 2D ^{1}H-^{13}C HSQC-CLIP-COSY spectrum edited with ^{13}C-multiplicity differentiates between CH/CH_3 and CH_2 resonances, so the identification of connectivities within different spin systems is feasible and simple.

All main chain resonances were assigned, however most of the palmitoyl CH_2 and tricarballyl acyl (TCA) CH, CH_2 chemical shifts remained ambiguous due to significant spectral overlaps. However, the position of the palmitoyl substitutions could be verified based on the three-bond heteronuclear correlations between the palmitoyl carbonyl and H3, H5, NH protons of the FB1 main chain. As expected, the TCA groups were located at the C14 and C15 positions in all three compounds, as evidenced by the HMBC linkages between the H14-H15 protons and the TCA carbonyls. Furthermore, the ^{1}H and ^{13}C main chainchemical shift values in positions 2-5 show the different palmitoyl substitution patterns of the components. Protons near palmitoyl carbonyls were significantly downshifted, and this perturbation was also observed in neighboring resonances. The stability of the 3-*O*-palmitoyl-FB1 sample showed a solvent dependence: a mixture of 3-*O*- and *N*-palmitoyl-FB1 was detected in DMSO-d6 by NMR. Unlike the other compounds, this sample was dissolved and analyzed in CD_3CN/D_2O to prevent rapid *O*→*N*-acyl migration. As a result, the spectral traces of the *N*-derivative disappeared from all 3-*O*-palmitoyl-FB1 measurements.

Characterisation of the Reaction Products by HPLC-ESI-HRMS

In the positive ion full scan ESI-HRMS spectra for 3-*O*- and 5-*O*-palmitoyl-FB1 toxins, only the protonated molecule $[M+H]^+$ was observed at 960.623 *m/z*, while in the case of *N*-palmitoyl-FB1, Na-adduction $[M+Na]^+$ also appeared with significant intensity in the spectrum at 982.60 *m/z*. In the negative ion ESI-HRMS spectrum, the deprotonated molecule (at 958.611 *m/z*) was accompanied by the $[M-2H+Na]^-$ ion at 980.592 *m/z*.

In the positive ion ESI-HRMS/MS spectrum of molecular ions, the same fragment ions were observed as previously in the case of the 3D ion trap mass analyzer (Bartók et al., 2010; Bartók et al., 2013). The most abundant fragment ion at 157 *m/z* value in the negative ion HRMS/MS spectra formed from tricarballylic acid (TCA) by water loss ($[TCA-H_2O-H]^-$). These fragment ions appeared as TCA anhydride (TCAD) and/or ketene (TCAK). These ions (157 *m/z*) did not appear in the spectra recorded earlier with the 3D ion trap mass analyzer, because their *m/z* value was below 1/3 of the *m/z* value of the molecular ion to be fragmented (the 1/3 rule of 3D ion trap mass analyzers). However, the splitting of TCAD/TCAK from the molecule was visible in several fragment ions in the mass spectrum obtained by a 3D ion trap mass analyzer (Bartók et al., 2010; Bartók et al., 2013).

Spiking of Fungal Extract with Synthesised Acylated FB1 Derivatives

Spiking 5-*O*-palmitoyl-FB1 or *N*-palmitoyl-FB1 to the original extract increased the area of the previously hypothesized peaks (Bartók et al., 2013). However, spiking of the original fungal

extract with the 3-*O*-palmitoyl-FB1 toxin did not result in an increase of the peak previously thought to be 3-*O*-palmitoyl-FB1, but appeared at a lower retention time, where the fungal extract contained no measurable component. Consequently, it can be stated that the fungus did not produce 3-*O*-palmitoyl-FB1 toxin. It was surprising that supplementation with the toxin 3-*O*-palmitoyl-FB1 also resulted in an increase in the peak height of *N*-palmitoyl-FB1, which could be explained by the instability of 3-*O*-palmitoyl-FB1. During the centrifugal evaporation and lyophilization of the pure 3-*O*-palmitoyl-FB1 fractions, the *O*→*N* acyl migration resulted in the formation of about 10% *N*-palmitoyl-FB1 toxin in the crystalline final product. The component previously thought to be a 3-*O*-palmitoyl-FB1-derivative in the fungal extract is presumably a 10-*O*-palmitoyl-FB1-derivative, as its mass spectra clearly indicate the palmitoylation of FB1 (Bartók et al., 2013). The only free group where acylation can still take place is the OH group on the C10 atom.

Effect of Fumonisins and Palmitic Acid on the Emission Spectrum of HSA

FB1 and FB4 toxins did not significantly change the emission spectrum of albumin. Only the highest concentration of palmitic acid (10 μM) increased HSA fluorescence. However, both *N*-pal-FB1 and 5-*O*-pal-FB1 gradually increased the fluorescence intensity of HSA accompanied by blueshifts (*N*-pal-FB1: 340 nm → 330 nm; 5-*O*-pal-FB1: 340 nm → 329 nm) in the emission wavelength maximum of the protein. The fumonisins and palmitic acid showed no fluorescence in the absence of albumin and no absorption in the wavelength range used.

The Interaction of Fumonisins with HSA Based on Ultracentrifugation Studies

Albumin reduced the amounts of *N*-pal-FB1 and 5-*O*-pal-FB1 below LOD (limit of detection), and FB1 and FB4 levels were reduced by approximately 50% and 75%, respectively. Based on these data, the binding constants of FB1–HSA (K = 1.6 × 103 L/mol) and FB4–HSA (K = 6.6 × 103 L/mol) were determined assuming 1:1 stoichiometry of fumonisin-albumin complexes (Fliszár-Nyúl et al., 2022). Considering the strong removal of palmitoyl-FB1 derivatives by HSA, the experiments were repeated with HSA concentrations of 2, 5 and 10 μM. Interestingly, even 2 μM HSA significantly decreased the concentration of *N*-pal-FB1 and 5-*O*-pal-FB1 in the supernatant.

The Effect of Fumonisins and Palmitic Acid on the Interaction of Site Markers and Human Serum Albumin

The filtered fractions of the studied site markers were not affected by the FB1 toxin. Furthermore, the albumin binding of warfarin and naproxen was not affected by FB4, but it moderately increased the level of S-camptothecin even at lower concentrations (5 and 10 μM) in the filtrate. Palmitic acid reduced the concentration of warfarin in the filtrate. However, *N*-pal-FB1 and 5-*O*-pal-FB1 showed the opposite effect: their higher concentration (20 μM) resulted in significantly increased warfarin levels in the filtrates. Palmitic acid and *N*-pal-FB1 caused moderate and high naproxen concentrations in the filtrate. In contrast, 5-*O*-pal-FB1 did not affect the naproxen-HSA interaction. In addition, palmitic acid and 5-*O*-pal-FB1 did not change the albumin binding of S-camptothecin, while *N*-pal-FB1 induced a concentration-dependent increase of the FA1 site marker in the filtered fraction.

Molecular Modeling Studies

Based on the individual 100-line docking runs, our results indicate that their docking locations are located at the entrance of Site II: in the first rank for 5-*O*-pal-FB1 and in the third rank for *N*-pal-FB1. Furthermore, *N*-pal-FB1 has two top-ranked binding modes near to Site I.

At the entrance of Site II, *N*-pal-FB1 interacts with amino acids R410, K413, K414, E492, K541, and K545. In blind docking studies, the first ranked binding mode of 5-*O*-pal-FB1 was found 6.2 Å away from the experimental binding mode of diazepam (the known ligand of Site II), where the complex is stabilized by hydrophilic (R410, E542), hydrophobic (L387, L394) and ionic (K541, K545) interactions.

Toxic Impacts of Fumonisins on Zebrafish Embryos

During the 24-hour treatment of zebrafish embryos (between 96 and 120 hpf), no death was observed either in the E3 medium or in the solvent controls. The FB1 and FB4 toxin did not cause death even at a concentration of 200 μM, in contrast, treatment with the 5-*O*-pal-FB1 toxin at a concentration of 100 and 200 μM resulted in 10 and 30% mortality, respectively. Furthermore, the *N*-pal-FB1 toxin caused 100% mortality even at a concentration of 6.25 μM.

The sublethal effects of FB1, FB4 and 5-*O*-pal-FB1 were demonstrated at concentrations of 3.12 μM and 200 μM. Since sublethal effects can only be tested in live zebrafish embryos, and *N*-pal-FB1 caused 100% mortality even at 6.25 μM, we evaluated *N*-pal-FB1-induced malformations only at 3.12 μM. Uninflated swimbladder was the only sublethal effect induced by the tested

fumonisins observed at the concentration of 3.12 μM. This effect was most often caused by *N*-pal-FB1 (80%), followed by 5-*O*-pal-FB1 (50%), FB4 (30%) and FB1 (25%) toxins.

At 200 μM, FB1, FB4 and 5-*O*-pal-FB1 induced an uninflated swim bladder in the majority of embryos, with 5-*O*-pal-FB1 inducing (100%) the highest abundance of this phenotype. Abnormal yolk coloration was observed following treatment with 200 μM FB1 (15%; statistically not significant), FB4 (40%) and 5-*O*-pal-FB1 (100%). FB1 did not cause head malformations (deformation of the lower jaw and olfactory region), whereas these deformities occurred in the embryos exposed to 200 μM FB4 (40%) and 5-*O*-pal-FB1 (100%). Body axis curvature (71%) and edemas (15%, statistically not significant) were induced only by 5-*O*-pal-FB1 (200 μM) treatment.

4. Conclusions and Recommendations

It was already mentioned that *Fusarium verticillioides* strains are capable of biosynthesis of *O*-acyl-FB1 derivatives. Bartók et al. (2010) identified these components as esterified FB1 toxins (EFB1, iso- EFB1), indicating the fatty acid involved in the esterification (linoleic-, oleic- or palmitic acid) in the last two characters of the name (EFB1LA, iso-FB1LA, EFB1OA, iso-EFB1OA, EFB1PA, iso-EFB1PA). A few years later, it was noticed that certain strains are also capable of the biosynthesis of *N*-acyl-FB1 derivatives (Bartók et al., 2013). A total of six *O*-acyl- and three *N*-acyl-FB1 components were detected, and their MS2 spectrum was recorded with a 3D ion trap mass spectrometer, and their exact masses were determined with a time-of-flight mass spectrometer. Three different acyl groups (palmitoyl, linoleoyl and oleoyl) were observed. Norred et al. (2001) described the *N*→*O*-acyl migration of the *N*-acetyl group of FA1 toxin (which is actually *N*-acetyl-FB1) to the OH group of the C3 and C5 atoms of the carbon chain under acidic conditions. Based on this, Bartók et al. believed that their chromatograms also showed the 3-*O*-, 5-*O*- and *N*-acyl-FB1 derivatives in this elution order. In addition, with the production of the *N*-palmitoyl-FB1 toxin, it was previously shown that the *N*-acyl-FB1 derivatives eluted after the *O*-acyl-FB1 derivatives, when spinking the reaction mixture to the *F. verticillioides* fungal extract. By HPLC/ESI-MS analysis of the samples in both positive and negative ion mode, it is easy to determine whether the separated acylated FB1 derivatives are *O*- or *N*-acylated compounds, because *O*-acyl derivatives give a higher signal/noise ratio in positive ion mode (the primary amino groups are easily protonated under acidic conditions), while *N*-acyl derivatives give a

higher signal/noise ratio in negative ion mode (when the primary amino group can no longer be protonated due to acylation).

During the spiking experiment, we confirmed that the *F. verticillioides* strain produced both 5-*O*-pal-FB1 and *N*-pal-FB1 toxins, since the addition of the synthetically produced palmitoyl derivatives to the original fungal extract increased the area of the previously assumed peaks (Bartók et al., 2013). In contrast, supplementing the extract with 3-*O*-palmitoyl-FB1 did not result in an increase in the peak previously attributed to 3-*O*-palmitoyl-FB1, but appeared within a shorter retention time, where the fungal extract contained no measurable component, i.e. the strain did not produce 3-*O*-palmitoyl-FB1 toxin. In addition, during the spiking experiment of 3-*O*-palmitoyl-FB1, the peak height of *N*-palmitoyl-FB1 increased. This was due to the fact that 3-*O*-palmitoyl-FB1 is extremely unstable. It was mentioned earlier that during the centrifugal evaporation and lyophilization of pure 3-*O*-palmitoyl-FB1 fractions, approximately 10% of *N*-palmitoyl-FB1 toxin was formed in the crystalline final product as a result of *O*→*N* acyl migration. Since it was proved that the fungus did not produce 3-*O*-palmitoyl-FB1, and the only group where acylation could still take place was the OH group at the C10 atom, the derivative previously thought to be a 3-*O*-pal-FB1 component is presumably 10-*O*-pal-FB1. Bartók et al. (2010; 2013) described that the *F. verticillioides* strain they studied produces not only palmitoyl, but also linoleyl and oleoyl-FB1 derivatives. Due to the similarity in the acylation of the fatty acids in these products, it is expected that the derivatives previously thought to be 3-*O*-linoleoyl-FB1 and 3-*O*-oleoyl-FB1 are actually also 10-*O*-acyl derivatives. In order to identify these compounds, they should be isolated from solid-phase fermentation samples and then their

structure should be verified by NMR and mass spectroscopy, since the OH group located on the C10 atom is not preferred during synthetic acylation.

Based on the high stability of palmitic acid-albumin complexes (Ashbrook et al., 1975; Rose et al., 1994), we assumed that the palmitoyl derivatives of FB1 toxin will also interact with the protein. Thus, the interactions of the toxins FB1, *N*-pal-FB1, 5-*O*-pal-FB1 and FB4 with HSA were investigated using fluorescence spectroscopy, ultrafiltration, ultracentrifugation and modeling studies, and it was assumed that *N*-pal-FB1 and 5-*O*-pal-FB1 causes higher acute toxicity than FB1 and FB4 toxins. In addition, in order to investigate the toxic effect of FB1, *N*-pal-FB1, 5-*O*-pal-FB1 and FB4, we tested the mortality and sublethal toxic effect caused by mycotoxins on zebrafish embryos. During our research, we came to the conclusion that the binding constants of the FB1–HSA and FB4–HSA complexes are low, whereas palmitoyl-FB1 derivatives bind to the protein with high affinity. Furthermore, *N*-pal-FB1 and 5-*O*-pal-FB1 likely occupy multiple high-affinity binding sites on albumin. *N*-pal-FB1 at a concentration of 6.25 μM caused 100% mortality, while very high levels (100 μM and 200 μM) of the other mycotoxins tested caused lower (5-*O*-pal-FB1) or zero mortality (FB1 and FB4). Considering the sublethal effects of these mycotoxins on zebrafish embryos, the following order of toxicity was established: *N*-pal-FB1 > 5-*O*-pal-FB1 > FB4 > FB1. Our results support that *N*-pal-FB1 and 5-*O*-pal-FB1 bind to serum albumin with a much higher affinity than FB1, therefore the toxicokinetic properties of palmitoyl derivatives may show large differences compared to FB1 toxin. Our research includes the first *in vivo* toxicity data for *N*-pal-FB1, 5-*O*-pal-FB1 and

FB4. Our results highlight the toxicological significance of the acylated derivatives of FB1.

Considering that currently in the European Union the limit value for food and the recommendation for animal feed is only set for the sum of FB1 and FB2 toxins, it would be important to examine the occurrence of the acylated forms of fumonisins in various raw materials, semi-finished products and finished products in the food industry and if necessary, limit values for acylated FB1 derivatives should be established.

5. New Scientific Results

Based on my investigations carried out in line with my objectives, we reached the following results:

1. The pure FB1 toxin was successfully acylated with palmitoyl chloride/triethylamine reagent, resulting in the production of *N*-pal-FB1, 3-*O*-pal-FB1 and 5-*O*-pal-FB1 toxins. We proved that *N*-acylation took place in the presence of anhydrous THF, while *O*-acylation took place when 2% (v/v) water was added to THF.
2. The acylated FB1 derivates in the fungal extract were successfully identified using a spiking experiment.
3. The produced acylated FB1 derivatives were identified by high-resolution mass spectrometry (HRMS) and nuclear magnetic resonance spectroscopy (NMR).
4. Based on the results of our fluorescence spectroscopy, ultracentrifugation, ultrafiltration and modeling studies, we proved that the *N*-pal-FB1 and 5-*O*-pal-FB1 toxins bind to human serum albumin with a higher affinity than the FB1 toxin itself.
5. In order to investigate the toxicity of the produced and identified acylated FB1 derivatives, we tested the mortality and sublethal toxic effects caused by these fumonsin derivates on zebrafish embryos. In our experiment, we proved that the examined acylated FB1 derivatives (*N*-pal-FB1 and 5-*O*-pal-FB1) were more toxic than FB1 and FB4.

6. Publications on the Topic of the Dissertation

- Csenki, Z., Bartók, T., Bock, I., Horváth, L., Lemli, B., Zsidó, B.Z., Angeli, C., Hetényi, C., Szabó, I., Urbányi, B., Kovács, M., Poór, M. (2023): Interaction of Fumonisin B1, *N*-Palmitoyl-Fumonisin B1, 5-*O*-Palmitoyl-Fumonisin B1, and Fumonisin B4 Mycotoxins with Human Serum Albumin and Their Toxic Impacts on Zebrafish Embryos. *Biomolecules* 13, 755.
- Iqbal, N., Czékus, Z., Angeli, C., Bartók, T., Poór, P., Ördögh, A. (2023): Fumonisin B1-Induced Oxidative Burst Perturbed Photosynthetic Activity and Affected Antioxidant Enzymatic Response in Tomato Plants in Ethylene-Dependent Manner. *Journal of Plant Growth Regulation* 42, 1865-1878.
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- Miklós, G., Angeli, C., Ambrus, Á., Nagy, A., Kardos, V., Zentai, A., Kerekes, K., Farkas, Z., Jóźwiak, Á., Bartók, T. (2020): Detection of Aflatoxins in Different Matrices and Food-Chain Positions. *Frontiers in Microbiology* 11, 21.