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Role of some styryl-heterocycles in the control of ochratoxin A biosynthesis

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Ochratoxin A (OTA) is a mycotoxin whose dangers have been sufficient for many countries to regulate its presence in various foods. In different countries, the black Aspergilli group, in particular *Aspergillus carbonarius*, causes the highest OTA contamination in fruit. Here we describe the effects of different styryl-heterocyclic compounds on the prevention of OTA biosynthesis by *A. carbonarius* cultured in a conducive liquid medium. The most effective and long-lasting control of OTA biosynthesis was achieved with (*E*)-3,5-dimethoxy-(2-thienyl) styrene (**10b**) and (*E*)-3,4,5-trimethoxy-(3-thienyl) styrene (**11d**). In fungal cultures treated with these compounds at 50 ppm, OTA biosynthesis decreased by 65% and 90%, respectively, after 8 days of incubation. A lower reactivity enhances the inhibition of OTA biosynthesis, in particular long-term. This study underlines the greater effectiveness of the phenyl ring substitution model as compared with that of the thiophene substitution model. Natural compounds present in edible plants having a styryl-heterocyclic scaffold may be effective inhibitors of OTA biosynthesis.

Keywords: Ochratoxin A, Thienyl-styrenes, Control effect.

INTRODUCTION

Ochratoxin A (OTA) is a mycotoxin well known for causing severe health hazards for humans and animals [1]. In particular, OTA interferes with tRNA and inhibits protein synthesis, resulting mainly in kidney pathologies [2]. This mycotoxin is synthesized by several different widespread fungal species, some of which (e.g., *Penicillium viridicatum*) are particularly adapted for cold environments, whereas others (e.g., *Aspergillus carbonarius*) are adapted for temperate or warm environments. Despite the huge diversity among OTA-producing fungi, it has been shown that oxidative stress in the cellular environment is commonly associated with this mycotoxin's biosynthesis [3]. Taking this correlation into account, different studies have been conducted for testing molecules whose antioxidant activity is used to control the biosynthesis of some mycotoxins [4, 5]. Furthermore, the antioxidant activity of the *cis* and *trans* isomers of several analogues of resveratrol and pterostilbene was investigated [6].

Considering these studies, we investigated the biological evaluation of various synthetic *trans*-styryl-heterocycle derivatives in order to preliminarily correlate their structure–activity relationships, and find a molecule with a significant and long-lasting inhibitive effect on OTA biosynthesis that could also serve as a new lead for chemical optimization**.** Monoatomic five-membered heteroaromatic compounds such as furan, thiophene or pyrrole display higher reactivity in oxidative conditions compared to the phenyl ring. The replacement of the phenyl ring with a heteroaromatic one with a stilbene pattern generates styryl-heterocycles with potentially strong biological activity [7]. The styryl-heterocycles can inhibit the production of nitric oxide [8], HIV-1 integrase [9], cell viability [10], cyclooxygenase and lipoxygenase activity [11-13], the growth of *Mycobacterium tuberculosis* [14] and *Botrytis cinerea* [15], and ochratoxin A biosynthesis by *Aspergillus carbonarius* [7, 16]. The type and efficacy of biological effects of styryl-heterocycles depend on several factors such as the number and nature of heteroatoms in the heterocycle (oxygen, nitrogen, or sulfur), the heterocycle ring's dimensions (5- or 6-membered), and the functions present on the phenyl ring.

In this study, *Aspergillus carbonarius* was used as OTA-producing fungus because this fungal species is widely diffused (particularly in temperate/warm environments) and most *A. carbonarius* strains can produce large quantities of OTA. Our aim was to investigate the effects of some styryl-heterocyclic compounds on the biosynthesis of OTA.

EXPERIMENTAL

Chemicals

The solvents (HPLC grade) and the reagents used were purchased from Sigma-Aldrich S.r.l. (Milan, Italy). Flash column chromatography was conducted on silica gel 230-400 mesh (Merck S.p.A., Milan). Reactions were monitored by TLC using Merck silica gel 60F-254 plates with UV indicator and/or visualized with phosphomolybdic acid (10% sol in EtOH). HPLC analyses were performed by using an

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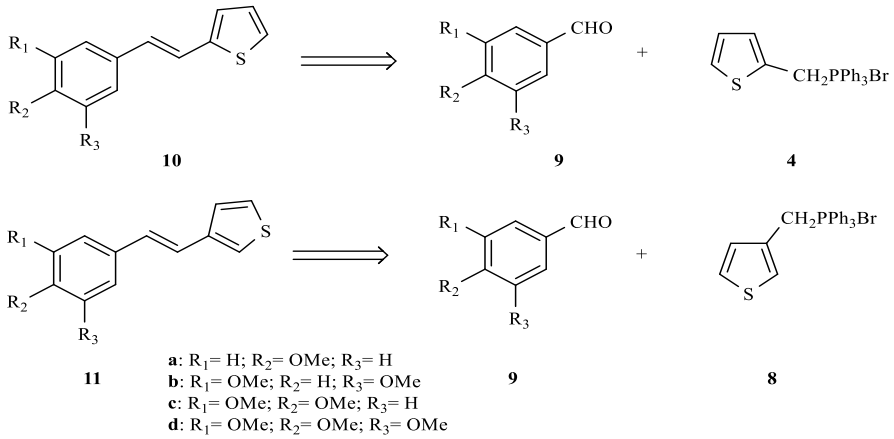
Agilent 1260 Liquid Chromatograph equipped with a Diode Array Detector (DAD). The ^{1}H and ^{13}C spectra were recorded with a Varian Mercury 3000 spectrometer at 300Mhz and 75MHz, in $CDCl_3$ as solvent. ^{1}H-NMR chemical shifts (δ) are expressed in parts per million (ppm), coupling constants (*J*) were measured in Hertz (Hz), and coupling patterns are indicated as s (singlet), d (doublet), t (triplet), q (quartet), bs (broad singlet), bd (broad doublet), m (multiplet). ^{13}C-NMR results (in ppm) were measured in correlation to $CDCl_3$ (δ=77.0 ppm for centerline).

All the experiments were performed using an *Aspergillus carbonarius* strain able to synthesize high concentrations of OTA isolated from wine grapes grown in Manduria (TA, Italy) in 2010. The fungus was maintained in Czapek-Dox (CD) Agar slants at 25°C, and 10-day cultures were used for conidia inoculum in liquid medium. The experiments to assess the effect of the synthesized molecules were performed using Czapek-Dox Yeast (CDY) 0.5%. Fungal growth was evaluated by weighing the mycelial part of the *A. carbonarius* culture after drying at 80°C for 48 h.

*Retrosynthetic analysis of 2-thienyl-styrenes **10** and 3-thienyl-styrenes **11** (Scheme 1).*

The stilbenoid structure was achieved by means of the Wittig reaction between the appropriate aromatic aldehydes **9** and the suitable thienyl-methylene ylides, which were generated *in situ* from the corresponding phosphonium salts **4** and **8**.



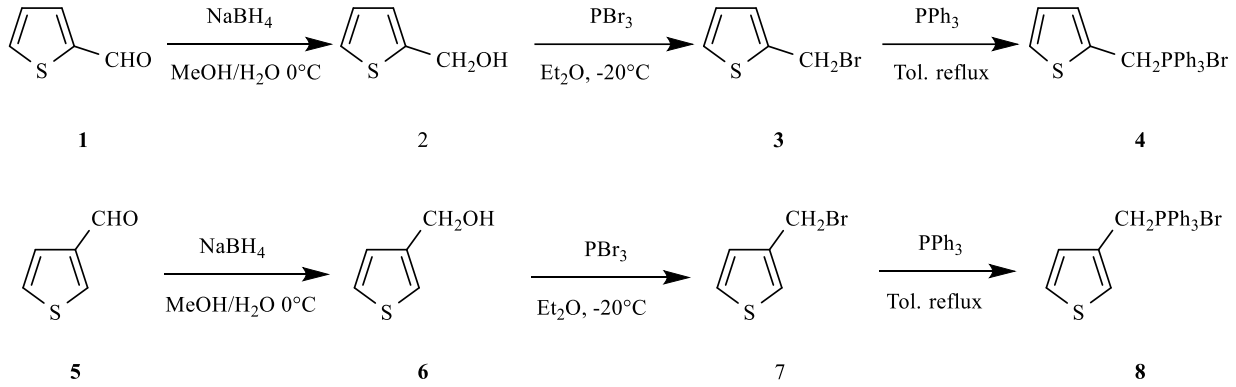
Scheme 1. Retrosynthetic analyses of the 2- and 3-thienyl-styrenes **10** and **11**.

*Synthesis of the phosphonium salts: 2-thienyl-methylen-phosphonium bromide **4** and 3-thienyl-methylene-phosphonium bromide **8** (Scheme 2)*

To perform this synthesis, $NaBH_4$ (10 mmol) was added portionwise to a solution of 2-formyl-thiophene **1** (10 mmol) in methanol (10 mL) at 0°C. After 30 min, the aldehyde **1** was consumed and the reaction mixtures were concentrated under reduced pressure. The material was dissolved in ethyl ether and washed with brine. The organic solution was dried over anhydrous Na_2SO_4 and, after filtration, the solvent was evaporated *in vacuum* to obtain alcohol 2-(hydroxymethyl) thiophene **2** (9.5 mmol) as pale yellow liquid. The alcohol **2** was dissolved in anhydrous diethyl ether (15 mL) at 0°C, and PBr_3 (19.2 mmol) was slowly added to the well-stirred solution. After 1 h the alcohol completely reacted (TLC: hexane/diethyl ether 95:5 v/v) and cold water was slowly added. The extraction process was accomplished by using diethyl ether (3×30mL). The organic layer was washed with water, a solution of $NaHCO_3$ 2M, and brine; then it was dried over anhydrous Na_2SO_4. Filtration and evaporation of diethyl ether *in vacuum* at room temperature resulted in 2-(bromomethyl) thiophene **3** (7.6 mmol) as brownish oil. The bromide **3** was dissolved into 40 mL of toluene, and triphenylphosphine (10.1 mmol) was added portionwise at room temperature. The reaction was conducted at reflux and the total consumption of the bromide **3** was monitored by TLC (hexane/diethyl ether 98:2 v/v). The suspension was filtered through a Büchner funnel, and the solid part was washed with cold toluene and dried *in vacuum*. This resulted in solid 2-thienyl-methylen-triphenyl-phosphonium bromide **4** (6.94 mmol). The overall yield from **1** is 69%. ^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm): 7.80-7.60 (m, 15H), 7.38 (bd, 1H *J* = 4.9 Hz), 7.12 (d, 1H, *J* = 5.1 Hz), 6.71 (m, 1H), 5.57 (d, 2H, *J* = 13.7 Hz). Anal. Calcd for $C_{23}H_{20}BrPS$: C, 62.88; H, 4.59; S, 7.30. Found: C, 62.96; H, 4.67; S, 7.33.

The 3-thienyl-triphenyl-phosphonium bromide **8** was synthesized from 3-formyl-thiophene **5** in the same way, the overall yield was 68%. ^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm): 7.80-7.60 (m, 15H), 7.35

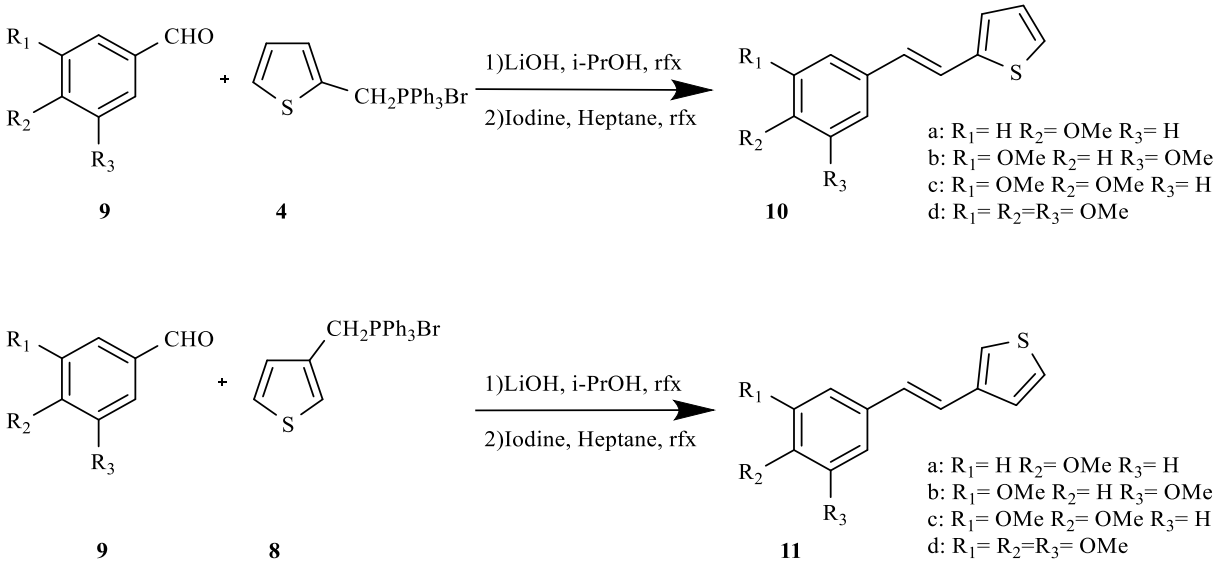
(bs, 1H), 7.12 (d, 1H, *J* = 4.9 Hz), 6.69 (d, 1H, *J* = 5.0 Hz), 5.61 (d, 2H, *J* = 13.9 Hz). Anal. Calcd for $C_{23}H_{20}BrPS$: C, 62.88; H, 4.59; S, 7.30. Found: C, 62.98; H, 4.64; S, 7.35.



Scheme 2. Synthesis of the phosphonium salts (**4**) and (**8**) used in the Wittig reaction.

Synthesis of 2-thienyl-methoxy-styrenes and 3-thienyl-methoxy-styrenes (Scheme 3).

An amount of $LiOH \cdot H_2O$ (4 mmol) was added to a stirred solution of phosphonium salt **4** or **8** (3 mmol) in isopropyl alcohol (10 mL). After 15 min the benzaldehyde **9** (3 mmol) was added to the mixture, the reaction was refluxed until complete consumption of **9** (TLC n-hexane/ethyl acetate 9:1 v/v), and the solution was brought to 25°C. The solvent was evaporated *in vacuum* and the crude product was purified by flash chromatography by eluting with a mixture of hexane/diethyl ether 9:1 (v/v). The residue was dissolved in 20 mL of CH_2Cl_2; the solution was washed with brine (3×10 mL) and dried over $MgSO_4$. The solvent was removed *in vacuum* to yield the desired 2-thienyl-methoxy-styrenes **10** and 3-thienyl-methoxy-styrenes **11** (ca. 2.7–2.8 mmol) as a mixture of *E*-and *Z*-isomers (*Z*/*E* ratio from 1:1 to 5:1). The *cis/trans* isomers were separated by silica gel column chromatography (hexane/ether), or the *Z*/*E* mixtures were converted to the *E*-isomers by heating with catalytic amounts of iodine in refluxing heptane. The *Z*-isomers or mixtures of two stereoisomers *E* and *Z* (1 mmol) were dissolved in heptane (10 mL). A catalytic amount of iodine (ca. 1-3 mg) was added to this solution and heated at reflux for 12 h. The reaction mixture was diluted with 20 mL of diethyl ether and washed with saturated aqueous $Na_2S_2O_3$ (10 mL) and brine (2×10 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuum* to provide the desired *E*-isomers. Although the synthesized compounds are already known, the synthetic procedure conducted in this study presents elements of originality with respect to those reported in the literature [8, 16, 17]. The isolated compounds were characterized by ^{1}H-NMR and ^{13}C-NMR spectroscopy.



Scheme 3. Synthesis of 2-thienyl-styrenes (**10)** and 3-thienyl-styrenes (**11**) by Wittig reaction

OTA extraction and analysis

To extract OTA, 25 mL of $CHCl_3$ were added to the same volume of the fungal cultural filtrate acidified by 1% of 0.1M H_3PO_4 and mixed for 1 min; after 5 min the organic phase was collected and the extraction was repeated three times. The collected extracts were concentrated in a rotary evaporator, redissolved in the mobile phase used for HPLC, and analyzed. An isocratic mixture of CH_3CN:H_2O:formic acid 99:99:2 v/v/v was used as mobile phase, and an Agilent YMC column (150 × 2 mm; 2.1 nm) equipped with a pre-column filter as stationary phase. The spectra were collected at 333 nm (maximum of OTA absorbance). The quantification of OTA was performed using the external standard method by comparing the peak values obtained by the pure standard with those having the same retention time obtained by analyzing the samples. The amount of OTA is expressed in ppm., with 1 ppm corresponding to 1 μg of OTA per mL of culture medium (CDY 0.5%). The evaluation of peak purity by DAD was also performed. A stock solution of OTA was prepared by dissolving 5 mg of OTA in 2 mL of toluene acidified by 1% formic acid and stored at -20°C. Working solutions were prepared by serial dilutions of the stock solution and stored at 4°C for 7 days.

Each experiment was repeated twice, with three replicates of each measure. The data were statistically analysed using Statistica software (7.0 StatSoft). Mean values were evaluated using ANOVA and compared using Fisher's Protected LSD Test (P =0.05).

RESULTS AND DISCUSSION

The described compounds were synthesized by the Wittig reaction using the commercially available benzaldehydes **9** carrying methoxy groups in different positions, and the 2-phosphonium salt **4** or the 3-phosphonium salt **8** (Scheme 1).

The synthesis of phosphonium salts **4** and **8** starts from 2-formyl-thiophene **1** and 3-formyl-thiophene **5** through 3 steps: 1) reduction, 2) bromination, and 3) phosphorylation, as shown in Scheme 2.

The Wittig reaction was accomplished by using lithium hydroxide because this compound is an efficient base in this reaction [17] due to its ability to extract, in *iso*-propyl alcohol, the proton from the phosphonium bromides **4** and **8**, thereby yielding, *in situ*, the corresponding ylides. The reaction of these ylides with the methoxy-benzaldehydes **9a-d** provides thienyl-styrene derivatives **10** and **11** in high yields (Scheme 3).

The final olefin products **10a-d** and **11a-d** were obtained as *cis*/*trans* isomers. Their structures were determined from the characteristic ^{1}H-NMR spectra and the geometries of the double bonds were established by comparing the ^{1}H-NMR spectra of the isomeric hydrogen pairs.

The olefin protons of the *Z*-isomers were at 0.3-0.4 ppm higher field than those of the olefin protons of *E*-isomers. The coupling constant of the vinyl protons of the *E*-isomers was about 16 Hz, whereas the *Z*-isomers coupling constant was 12 Hz. The *Z*/*E* mixtures were converted to the *E*-isomers by heating with catalytic amounts of iodine in refluxing heptane [18] when the separation of the *Z*/*E*-isomers was not possible by chromatography.

Among the 2-thienyl-styrenes assayed (compounds **10a-d**), compound **10b** (*E*-3,5-dimethoxy-(2-thienyl) styrene) was the most effective at inhibiting OTA biosynthesis (Fig. 1 a,b).

It is worth noting that Caruso *et al.* [15], testing 2-furyl styrenes with aromatic substitutions models equal to those of the molecules **10a**, **10b** and **10d**, showed that the *E*-3,5-dimethoxy-(2-furyl) styrene is the most effective at inhibiting the mycelial growth of *Botrytis cinerea*, while still taking into account the differences in the reactivity between furyl and thienyl moiety and in the biological activity considered.

Moreover, the *E*-3,4,5-trimethoxy-(2-thienyl) styrene **10d** also showed a significant ability to control OTA biosynthesis.

Among the 3-thienyl-styrenes assayed (compounds **11a-d**), compounds **11b** (*E*-3,5-dimethoxy-(3-thienyl) styrene) and **11d** (*E*-3,4,5-trimethoxy-(3-thienyl) styrene) displayed the strongest biological activity.

Compounds **10a** and **10b,** as well as **11a** and **11b,** have been selected for their similarity to resveratrol's structure (3,5,4'-trihydroxy stilbene) concerning the substitution pattern of the phenyl ring. Compounds **10c** and **10d,** as well as **11c** and **11d,** have been selected because they present at least two substituents in cathecolic position. Several studies report that such a substitution pattern leads to greater biological activity compared to that fostered by other substitution patterns [4, 19, 20].

In our experiments, the 3,5-dimethoxy and the 3,4,5-trimethoxy derivatives yielded the best results in controlling OTA biosynthesis, while 3,4-dimethoxy compounds resulted in significantly lower inhibition.

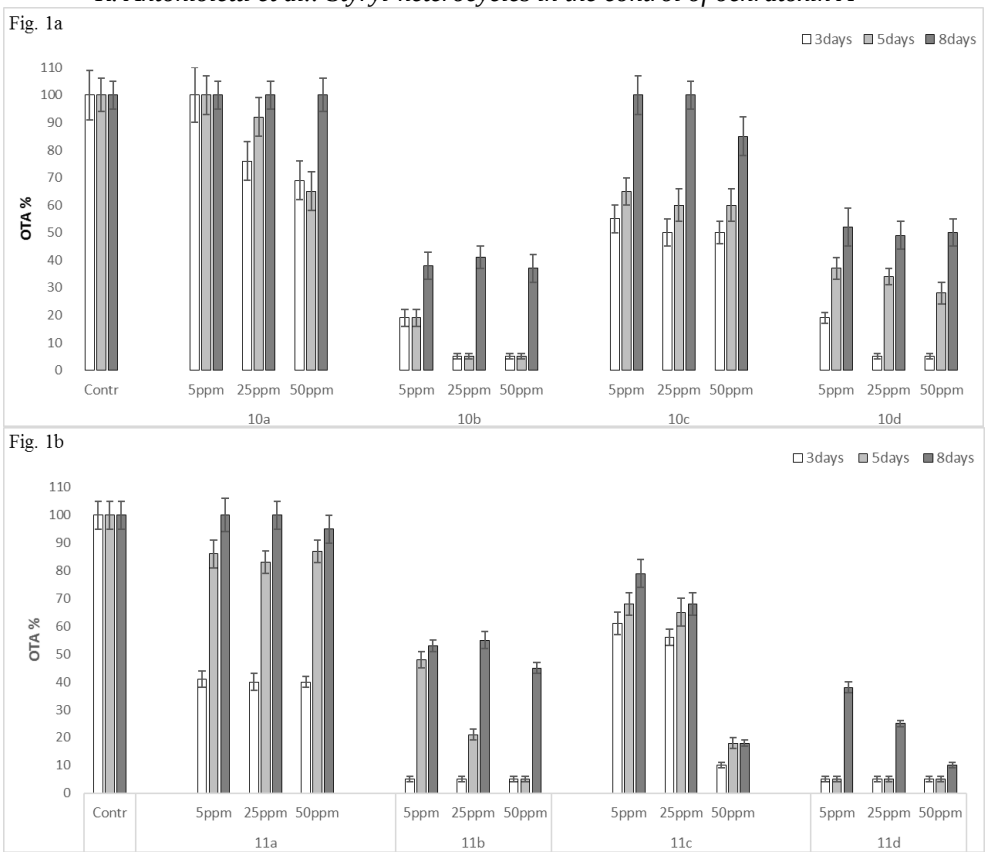


Fig. 1 a,b. Effect of the assayed 2-thienyl (a) and 3-thienyl (b) compounds on OTA biosynthesis. Means marked by the same letter are not significantly different (Fisher's Protected LSD Test, P=0.05).

Note that when the *para* position is occupied, the 3-thienyl-styrenes **11a**, **11c**, and **11d** yielded better results than those of the 2-thienyl-styrenes **10a**, **10c**, and **10d**, whereas, when the *para* position is unsubstituted, the 2-thienyl-styrene **10b** was better at inhibiting OTA production than was the 3-thienyl-styrene **11b (**Fig. 1 a,b**).** In particular, in the case of molecules having unsubstituted *para* position (**10b** and **11b**), due to the presence of 2 free α positions in the heterocycle, the 3-thienyl derivative (**11b**) displays a greater reactivity than the corresponding 2-thienyl derivative (**10b**). This may explain the shorter duration of the **11b** compound, which was able to significantly inhibit OTA production through the 5th day of the experiment but was not effective on the 8th day of incubation. On the other hand, molecule **10b** is able to maintain the ability to inhibit OTA biosynthesis over time (Fig. 1). These results are consistent with those obtained by Lee [8], who tested the inhibiting activity on NO production by LPS-activated RAW264.7 macrophage cells, although that biological system responds more readily.

CONCLUSION

This study highlights the importance of the phenyl ring's substitution pattern in determining the ability of the molecule to control OTA biosynthesis by *Aspergillus carbonarius*. However, the substitution pattern of the heterocyclic ring also was influential, albeit to a lesser extent. It is also important to highlight that the molecules **10a** and **11a**, both of which are mono substituted in *para* position, showed the lowest control effect on OTA biosynthesis.

(E)-4-methoxy-(2-thienyl) styrene 10a:

Yield: 87%. ^{1}H-NMR, δ: 3.81 (s, 3H); 6.88 (d, 1H, *J* = 16.1 Hz); 6.90 (d, 2H, *J* = 8.8 Hz); 6.96-7.06 (m, 2H); 7.10 (d, 1H, *J* = 16.1 Hz); 7.15 (d, 1H, *J* = 5.4 Hz); 7.40 (d, 2H, *J* = 8.8 Hz). ^{13}C-NMR, δ: 159.2, 143.2, 129.7, 128.0, 127.5, 127.4, 125.3, 123.7, 119.7, 114.1, 55.2. Anal. Calcd for $C_{13}H_{12}OS$: C, 72.19; H, 5.59; S, 14.82. Found: C, 72.26; H, 5.65; S, 14.80.

(E)-3,5-dimethoxy-(2-thienyl) styrene 10b:

Yield: 95%. **^{1}**H NMR, δ: 3.75 (s, 6H); 6.31 (t, *J* = 4 Hz, 1H); 6.55 (m, 2H); 6.78 (d, *J* = 16.2 Hz, 1H); 6.93 (dd, *J* = 3.3, 5.1 Hz, 1H); 7.00 (bd, *J* = 3.0 Hz, 1H); 7.13 (d, *J* = 16 Hz, 1H); 7.21 (d, *J* = 5.0 Hz, 1H). ^{13}C NMR, δ: 160.9, 142.6, 138.9, 128.2, 127.6, 126.3, 124.5, 122.3, 104.3, 100.0, 55.3. Anal. Calcd for $C_{14}H_{14}O_2S$: C, 68.26; H, 5.73; S, 13.02. Found: C, 68.32; H, 5.81; S, 13.06.

(E)-3,4-dimethoxy-(2-thienyl) styrene 10c:

Yield: 91%. ^{1}H-NMR, δ: 3.90 (s, 3H); 3.94 (s, 3H); 6.85 (d, 1H, *J*= 8.8 Hz); 6.88 (d, 1H, *J* = 16.0

Hz); 6.98–7.04 (m, 4H); 7.11 (d, 1H, *J* = 16.0 Hz); 7.17 (d, 1H, *J* = 4.6 Hz). ^{13}C-NMR, δ:149.3, 149.1, 138.2, 130.2, 128.3, 127.7, 125.6, 124.0, 120.2, 119.8, 111.4, 108.7, 56.1, 56.0. Anal. Calcd for $C_{14}H_{14}O_2S$: C, 68.26; H, 5.73; S, 13.02. Foud: C, 68.31; H, 5.79; S, 12.98.

(E)-3,4,5-trimethoxy-(2-thienyl) styrene 10d:

Yield: 93%. **^{1}**H NMR, δ: 3.87 (3H, s), 3.88 (s, 6H), 6.68 (bd, *J* = 4 Hz, 2H); 6.72 (d, *J* = 16 Hz, 1H); 7.00 (dd, *J* = 3.1, 5.0 Hz, 1H); 7.06 (bd, *J* = 5.1 Hz, 1H); 7.14 (d, *J* = 15.98 Hz, 1H); 7.17 (d, *J* = 4.75 Hz, 1H). ^{13}C NMR, δ: 153.6, 142.9, 135.8, 132.9, 128.6, 128.5, 127.8, 126.2, 124.5, 124.4, 121.5, 103.6, 61.1, 56.3. Anal. Calcd for $C_{15}H_{16}O_3S$: C, 65.19; H, 5.84; S, 11.60. Found: C, 65.25; H, 5.92; S, 11.64.

(E)-4-methoxy-(3-thienyl) styrene 11a:

Yield: 83%. ^{1}H-NMR, δ: 3.81 (s, 3H); 6.92 (d, *J* = 16.2 Hz, 1H); 6.95 (m, 1H); 7.00 (d, *J* = 8.6 Hz, 2H); 6.99 (d, *J* = 16.3 Hz, 1H); 7.20 (dd, *J* = 2.8, 1.3 Hz, 1H); 7.33-7.28 (m, 1H); 7.40 (d, *J* = 8.6 Hz, 2H). ^{13}C-NMR, δ: 159.6, 140.8, 130.6, 128.7, 127.9, 126.4, 125.3, 121.9, 121.4, 114.6, 55.7. Anal. Calcd for $C_{13}H_{12}OS$: C, 72.19; H, 5.59; S, 14.82. Found: C, 72.29; H, 5.64; S, 14.85.

(E)-3,5-dimethoxy-(3-thienyl) styrene 11b:

Yield: 87%. **^{1}**H NMR, δ: 3.82 (s, 6H); 6.38 (t, *J* = 2Hz, 1H); 6.61 (d, *J* = 2Hz, 2H); 6.65 (m, 1H); 6.74 (d, *J* = 16.2 Hz, 1H); 6.95 (d, *J* = 16.1Hz, 1H); 7.4 (m, 1H); 7.53 (s, 1H). ^{13}C-NMR, δ: 160.1, 136.5, 135.1, 131.5, 126.1, 125.8, 124.1, 120.1, 104.3, 56.9. Anal. Calcd for $C_{14}H_{14}O_2S$: C, 68.26; H, 5.73; S, 13.02. Found: C, 68.35; H, 5.79; S, 13.05.

(E)-3,4-dimethoxy-(3-thienyl) styrene 11c:

Yield: 95%. ^{1}H-NMR, δ: 3.90 (s, 3H); 3.94 (s, 3H); 6.85 (d, 1H, *J* = 7.9 Hz); 6.93 (d, 1H, *J* = 16.1 Hz); 7.03 (m, 2H); 7.09 (d, 1H, *J* = 16.1 Hz); 7.21-7.33 (m, 3H). ^{13}C-NMR, δ: 148.8, 148.5, 140.0, 130.2, 128.2, 125.9, 124.6, 121.5, 120.8, 119.4, 110.9, 108.2, 55.6, 55.5. Anal. Calcd for $C_{14}H_{14}O_2S$: C, 68.26; H, 5.73; S, 13.02. Found: C, 68.36; H, 5.82; S, 13.07.

(E)-3,4,5-trimethoxy-(3-thienyl) styrene 11d:

Yield: 88%. **^{1}**H NMR, δ: 3.87 (s, 3H), 3.94 (s, 6H); 6.70 (s, 2H); 6.88 (d, *J* = 16.2 Hz, 1H); 7.04 (d, *J*= 16.2 Hz, 1H); 7.21-7.33 (m, 3H). ^{13}C-NMR, δ: 152.7, 139.7, 136.1, 133.9, 131.2, 129.2, 128.5, 126.2, 120.3, 102.5, 59.8, 56.3, 56.1. Anal. Calcd for $C_{15}H_{16}O_3S$: C, 65.19; H, 5.84; S, 11.60. Found: C, 65.27; H, 5.90; S, 11.57.

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РОЛЯ НА НЯКОИ СТИРИЛОВИ ХЕТЕРОЦИКЛИ ЗА КОНТРОЛ НА БИОСИНТЕЗА НА ОКРАТОКСИН А

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(Резюме)

Ократоксин А (ОТА) е микотоксин, чието опасно действие е причина много страни да регулират присъствието му в различни храни. Черната Aspergilli група, и по-специално *Aspergillus carbonarius*, причинява най-голямо замърсяване с ОТА в плодовете. В настоящата работа е описано влиянието на различни стирил-хетероциклени съединения върху превенцията на биосинтеза на ОТА от *A. carbonarius,* отглеждана в проводима течна среда. Най-ефективен и продължителен контрол на биосинтеза на ОТА се постига с (*E*)-3,5-диметокси-(2-тиенил) стирен (**10b**) и (*E*)-3,4,5-триметокси-(3-тиенил) стирен (**11d**). В гъбични култури, третирани с тези съединения на ниво 50 ppm, синтезът на ОТА намалява съответно с 65% и 90% след инкубация от 8 дни. По-ниската реактивоспособност повишава инхибирането на биосинтеза на ОТА особено в дългосрочен план. Установено е, че моделът на заместване на фенолния пръстен е от голямо значение за заместителния модел на тиофена. Природните съединения, присъстващи в ядливите растения, които имат стирилов хетероциклен скелет, може да са ефективни инхибитори на биосинтеза на ОТА.