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In silico analysis sheds light on the structural basis underlying the ribotoxicity of trichothecenes—A tool for supporting the hazard identification process

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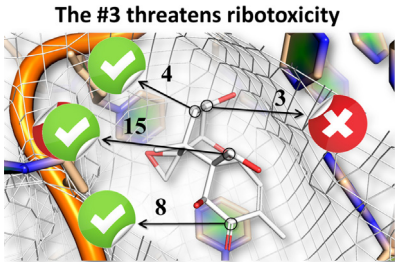
HIGHLIGHTS

- An *in silico* study on the structure-activity relationship of trichothecenes has been proposed.
- The structural basis underlying the ribotoxic activity of trichothecenes has been investigated.
- Modified forms never tested before have been analyzed to identify additional toxic metabolites.
- Steric hindrances in the position 3 of the sesquiterpenoid core may cause the loss of ribotoxicity.
- Positions 4,8 and 15 were found more influenced by hydrophobic/hydrophilic mismatch.

GRAPHICAL ABSTRACT



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ABSTRACT

Deoxynivalenol is a food borne mycotoxin belonging to the trichothecenes family that may cause severe injuries in human and animals. The inhibition of protein synthesis *via* the interaction with the ribosome has been identified as a crucial mechanism underlying toxic action. However, it is not still fully understood how and to what extent compounds belonging to trichothecenes family affect human and animal health. In turn, this scenario causes delay in managing the related health risk. Aimed at supporting the hazard identification process, the *in silico* analysis may be a straightforward tool to investigate the structure-activity relationship of trichothecenes, finding out molecules of possible concern to carry forth in the risk assessment process. In this framework, this work investigated through a molecular modeling approach the structural basis underlying the interaction with the ribosome under a structure-activity relationship perspective. To identify further forms possibly involved in the total trichothecenes-dependent ribotoxic load, the model was challenged with a set of 16 trichothecene modified forms found in plants, fungi and animals, including also compounds never tested before for the capability to bind and inhibit the ribosome. Among them, only the regiospecific glycosylation in the position 3 of the sesquiterpenoid scaffold (i.e. T-2 toxin-3-glucuronide, α and β isomers of T-2 toxin-3-glucoside and deoxynivalenol-3-glucuronide) was found impairing the interaction with the ribosome, while the other compounds tested (i.e. neosolaniol, nivalenol, fusarenon-X, diacetoxyscirpenol, NT-1 toxin, HT-2 toxin, 19- and 20-hydroxy-T-2 toxin, T-2 toxin triol and tetraol, and 15-deacetyl-T-2 toxin), were found

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potentially able to inhibit the ribosome. Accordingly, they should be included with high priority in further risk assessment studies in order to better characterize the trichothecenes-related hazard.

1. Introduction

Mycotoxins are low-molecular weight secondary metabolites produced by various species of filamentous fungi that can contaminate food and feed (Pitt et al., 2013), posing threat for health of humans and animals (Wu et al., 2014a). The most relevant food borne mycotoxins in terms of impact on public health and agro-economic losses are aflatoxins, zearalenone, trichothecenes, fumonisins, ochratoxins and patulin, which are mainly produced by the fungi species *Aspergillus, Fusarium* and *Penicillium* (Milićević et al., 2016). The broad class of trichothecenes is extremely prevalent worldwide and includes over 200 molecules (Grove, 2007). Among them, deoxynivalenol (DON) deserves particular attention as it is counted among the most common foodborne mycotoxins, found mainly in cereals and derived products (Pestka, 2010), and may cause severe injuries in human and animals (Payros et al., 2016) with a marked low-tolerability especially in monogastric animals (Hassan et al., 2015).

In vivo, DON and congeners elicit a wide number of outcomes including the damage at the level of gastro-intestinal apparatus, alteration in the normal food and feed intake, and nutrients absorption, immunosuppression and susceptibility to infection (Payros et al., 2016). Such generalized effects are elicited after the trichothecenes have interacted with a series of biological targets. Among them, the 60S large subunit of ribosome is regarded among the major ones, being involved in the inhibition of protein synthesis (McCormick et al., 2011). The structural architecture of ribosome-trichothecenes interaction has been recently disclosed by crystallographic studies, wherein DON and the two congeners T-2 toxin and verrucarin A were found to bind the A-site within the peptidyl transferase catalytic center (Garreau de Loubresse et al., 2014). Such a molecular event causes the inhibition of peptide elongation and induces the so defined ribotoxic stress (Pestka, 2008).

Regulations and recommendations have been enforced in many countries to reduce the potential exposure to the food borne mycotoxins, thereby preserving the health and wellbeing of humans and animals. Nonetheless, only DON is regulated worldwide among the trichothecenes, in spite of the wide numbers of modified forms potentially found in food and feed (Nathanail et al., 2015), basically due to the paucity of data for the most of such compounds. This scenario ultimately makes disputable the consistency of the current regulations in respect to the real risk for health. Indeed, risk assessors are currently moving toward the setting of group health-based guidance values for foodborne mycotoxins taking into account all those forms manifesting toxicity, as sustained by the European Food Safety Authority (Alexander et al., 2016). Ultimately, this is the foreground perspective to better understand the toxicological role of trichothecenes and to manage more effectively the mycotoxins-related risk. Moreover, the identification of non- or less-toxic forms may serve to design focused strategies to chemically modify mycotoxins in the framework of mitigating the abundance of toxicologically active forms in contaminated food and feedstuff.

In this light, the investigation of toxicodynamic of DON and congeners, with emphasis on which modifications preserve the capability to interact with the A-site, are needed to: i) provide a solid foothold in the hazard identification process at the early stage; and ii) allow the straightforward and evidence-based hierarchizing of compounds for supporting experimental trials. However, the lack of commercial availability of the most part of modified mycotoxins, including those of DON and congeners, makes extremely costly and time consuming a wide-scale assessment (Dellafiora and Dall'Asta, 2016). In the recent years, *in silico* analysis has turned out to be an effective analytical tool for assessing the interaction between mycotoxins and biological targets (Cozzini and Dellafiora, 2012), including the ribosome-trichothecenes interaction (Pierron et al., 2016), and to extrapolate the structural features underlying the toxicity of some trichothecenes as well (Steinmetz et al., 2009). Hence, the computational approach can be reliably used contextually to gain insights on the structure-activity relationship of DON and congeners.

Therefore, the present work investigated the structural basis underlying the interaction between a set of trichothecenes with

Table 1
Effects of chemical modifications on ribotoxicity experimentally observed and computational categorization of compounds.

Compound	Effects on ribosome inhibition	Computed Score	Assigned category [a]	Main formation route
DON	Ribotoxic[b] [c]	−103.4	A	Fungal metabolite
T-2 toxin (T2)	Preserved [b]	−127.9	A	Fungal metabolite
NX-3	Preserved [c]	−103.7	A	Fungal metabolite
Verrucarin A	Preserved [b]	−139.9	A	Fungal metabolite
15-acetyl-DON	Preserved [d]	−106.6	A	Fungal metabolite
3-acetyl-DON	Detoxified [d]	−96.9	B	Fungal metabolite
DON-15-sulfate	Detoxified [e]	−101.4	B	Plant/Animal metabolite
DON-3-sulfate	Detoxified [e]	−63.2	B	Plant/Animal metabolite
EN139544	Detoxified [f]	−51.6	B	Chemical synthesis
DON-3-glucoside	Detoxified [g]	−95.8	B	Plant metabolite
DOM-1	Detoxified [h]	−94.1	B	Bacterial metabolite
3-*epi*-DON	Detoxified [h]	−99.1	B	Bacterial metabolite
NX-2	Detoxified [c]	−82.5	B	Fungal metabolite

[a] A indicates compounds with a score comparable to or higher than DON; B indicates compounds whit a score worse than DON.
[b] Garreau de Loubresse et al. (2014).
[c] Varga et al. (2015).
[d] Payros et al. (2016).
[e] Warth et al. (2016).
[f] Wu et al. (2014b).
[g] Poppenberger et al. (2003).
[h] Pierron et al. (2016).