Toxicon xxx (2013) 1–8

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Cytotoxicity and phytotoxicity of trichothecene mycotoxins produced by *Fusarium* spp.☆

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ARTICLE INFO

Article history:
Received 27 February 2013
Received in revised form 25 July 2013
Accepted 30 July 2013
Available online xxxx

Keywords:
Bioherbicide
Phytotoxicity
Cytotoxicity
Trichothecenes
Vomitoxin
Fusarium spp.

ABSTRACT

Trichothecenes, a major class of mycotoxins produced by *Fusarium*, *Myrothecium*, and *Stachybotrys* species, are toxic to both plants and mammals. Simple trichothecenes, including type A (e.g., T-2 toxin) and type B (e.g., deoxynivalenol), are generally less toxic than macrocyclic trichothecenes. We sought to determine if simple trichothecenes are a potential source of candidates for development as bioherbicides, which require high phytotoxicity and low mammalian toxicity. We examined 28 simple trichothecenes *in vitro* for phytotoxicity using a small aquatic plant, *Lemna pausicostata*, and for mammalian toxicity using four cultured mammalian cell lines. Several structure–activity relationships were identified, including the following two, which may be relevant to bioherbicide development: peracetylation of type B trichothecenes and de-epoxidation of type A trichothecenes both substantially reduced mammalian toxicity with little effect on phytotoxicity. It was concluded that simple trichothecenes possessing strong phytotoxicity and minimal mammalian toxicity *in vitro* can be identified.

1. Introduction

Trichothecenes are a major class of mycotoxins produced by many species of *Fusarium*, *Myrothecium*, and *Stachybotrys* (Ueno, 1983; CAST, 2003). More than 120 trichothecenes are known. *Fusarium* mycotoxins are second only to aflatoxins in importance as causes of economic loss to agriculture, due to their toxicity to both plants and animals (Robens and Cardwell, 2003). Trichothecenes produced by *Myrothecium*, and *Stachybotrys* species are responsible for sick building syndrome and other problems with indoor air due to the mycotoxins present in spores released by the fungi (Kuhn and Ghannoum, 2003). In plants, tricothecenes produced by *Fusarium* spp. cause necrosis, chlorosis, and mortality enabling them to mediate a wide variety of plant diseases, including wilts, stalk rot, root rot and leaf rot in many important crop and ornamental plants (Ueno, 1983; Cutler, 1988; Cheeke, 1998; CAST, 2003; Harris et al., 1999; Wang et al., 2006). Examples include kudzu (Abbas et al., 2002), Orobanche (Zonno and Vurro, 2002), duckweed (Abbas et al., 1998a,b), and many grain crops (Morin et al., 2000; Tanaka et al., 1993). Trichothecenes are also powerful mammalian mycotoxins known to produce toxic response syndromes including rash, hemorrhage, diarrhea, vomiting and mortality (Ueno, 1983; Froquet et al., 2001). Trichothecenes produce their toxic effects by binding to 60s ribosomes and interrupting protein synthesis in eukaryotic cells (Bin-Umer et al., 2011; Cutler and Jarvis, 1985; Cutler, 1988; Feinberg and

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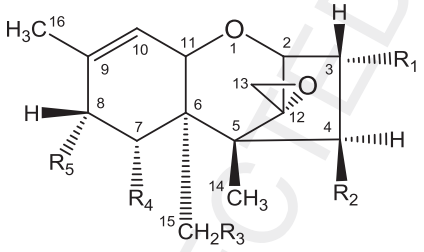
0041-0101/$ – see front matter © 2013 The Authors. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxicon.2013.07.026

McLaughlin, 1989; Masuda et al., 2007; McLaughlin et al., 1977; Nishiuchi et al., 2006; Yike et al., 1999). The genes responsible for the biosynthesis of trichothecenes have been cloned and the component enzymes extensively studied (Desjardins and Proctor, 2007).

The trichothecenes are a family of cyclic terpenoids, which are classified into simple and macrocyclic trichothecenes (Ueno, 1983). Simple trichothecenes are families of tetracyclic (Figs. 1 and 3) or tricyclic (Fig. 2) sesquiterpenoids with variations at five sites on the molecular framework. Type A simple trichothecenes have variations at four sites (C-3, C-4, C-7 and C-15) and are sub-divided into epoxy type (Fig. 1) and de-epoxy type (Fig. 2), which are structural analogs bearing an ethylene group, which has not been epoxidized, and they may be biosynthetic intermediates on the pathway to corresponding epoxidized derivatives. Type B simple trichothecenes (Fig. 3) have a carbonyl group at C-8 and variations at the same four sites (C-3, C-4, C-7 and C-15) as in Type A. Macrocyclic trichothecenes contain a fifth ring in the form of an 18-member triester macrolide produced by linking substituents on C-4 and C-15.

The large number of plant diseases caused by *Fusarium* species and the large number of trichothecene toxins produced by them suggests that trichothecenes may be a potential source of bioherbicides (Abbas et al., 1998a,b; Amusa, 2006). To be an effective bioherbicide for use in controlling weeds and other applications such as defoliants, a natural product must exhibit strong phytotoxicity and low mammalian toxicity. In the present study we have selected two *in vitro* toxicity tests for use as a prescreen for a bioherbicide, bleaching and growth inhibition of *Lemna pausicostata* (duckweed) in axenic cultures as an indicator of phytotoxicity, and inhibition of cell proliferation in a bank of various mammalian permanent cell lines as an indicator of mammalian toxicity. We have used these bioassays to pre-screen a collection of 28 simple trichothecenes with a wide range of structural variations. The study was restricted to simple trichothecenes because macrocyclic trichothecenes (e.g., verrucarins, roridins) usually exhibit stronger mammalian toxicity than all but the most toxic of the simple trichothecenes (i.e., T-2) (Abbas et al., 2002; Ge et al., 2009). Structure–activity relationships among simple trichothecenes have been reported previously by Madhyastha et al. (1994), who examined a panel of 16 analogs for toxicity to a yeast, *Kluyveromyces marxianus*, and by Desjardins et al. (2007), who examined a panel of 24 biosynthetic precursors, intermediates and completed toxins for phytotoxicity in an *Arabidopsis thaliana* detached leaf assay.



Toxin	R_1	R_2	R_3	R_4	R_5	P/C[a]
T-2 toxin type						
HT-2 toxin	OH	OH	$OCOCH_3$	H	$OCOCH_2CH(CH_3)_2$	0.03
3'-Hydroxy-HT-2 toxin	OH	OH	$OCOCH_3$	H	$OCOCH_2C(OH)(CH_3)_2$	0.57
T-2 toxin	OH	$OCOCH_3$	$OCOCH_3$	H	$OCOCH_2CH(CH_3)_2$	0.04
3'-Hydroxy-T-2 toxin	OH	$OCOCH_3$	$OCOCH_3$	H	$OCOCH_2C(OH)(CH_3)_2$	0.02
3-Acetyl-T-2 toxin	$OCOCH_3$	$OCOCH_3$	$OCOCH_3$	H	$OCOCH_2CH(CH_3)_2$	0.08
3'-Hydroxy-3-Acetyl-T-2 toxin	$OCOCH_3$	$OCOCH_3$	$OCOCH_3$	H	$OCOCH_2C(OH)(CH_3)_2$	0.00
T-2 tetraol	OH	OH	OH	H	OH	0.13
Neosolaniol	OH	$OCOCH_3$	$OCOCH_3$	H	OH	0.18
3-Acetyl neosolaniol	$OCOCH_3$	$OCOCH_3$	$OCOCH_3$	H	OH	0.93
8-Acetyl neosolaniol	OH	$OCOCH_3$	$OCOCH_3$	H	$OCOCH_3$	0.02
3,7,8,15-Tetraacetyl-T-2 tetraol	$OCOCH_3$	$OCOCH_3$	$OCOCH_3$	H	$OCOCH_3$	0.04
Diacetoxyscirpenol type:						
Scirpentriol	OH	OH	OH	H	H	2.43
4,15-Diacetoxyscirpenol	OH	$OCOCH_3$	$OCOCH_3$	H	H	0.05
3,4,15-Triacetoxyscirpenol	$OCOCH_3$	$OCOCH_3$	$OCOCH_3$	H	H	0.02

[a] P/C = Ratio of phytotoxicity of the test substance, measured as % growth reduction of duckweed, to its mammalian cytotoxicity expressed as the ratio of the IC_{50} value for a non-toxic trichothecene (3,7,15-triacetyldeoxynivalenol, 10μM) to the mean IC_{50} value measured with the four cell lines studied.

Fig. 1. Structures and phytotoxicity-to-cytotoxicity ratios of type A trichothecene toxins (epoxy-type) included in this study.