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## Metabolism and Resistance of *Fusarium* spp. to the Manzamine Alkaloids via a Putative Retro Pictet-Spengler Reaction and Utility of the Rational Design of Antimalarial and Antifungal Agents

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### Abstract

As a part of our continuing investigation of the manzamine alkaloids we studied the in vitro activity of the  $\beta$ -carboline containing manzamine alkaloids against *Fusarium solani*, *Fusarium oxysporium*, and *Fusarium proliferatum* by employing several bioassay techniques including one-dimensional direct bioautography, dilution, and plate susceptibility, and microtiter broth assays. In addition, we also studied the metabolism of the manzamine alkaloids by *Fusarium* spp. in order to facilitate the redesign of the compounds to prevent resistance of *Fusarium* spp. through metabolism. The present research reveals that the manzamine alkaloids are inactive against *Fusarium* spp. and the fungi transform manzamines via hydrolysis, reduction, and a retro Pictet-Spengler reaction. This is the first report to demonstrate an enzymatically retro Pictet-Spengler reaction. The results of this study reveal the utility of the rational design of metabolically stable antifungal agents from this class and the development of manzamine alkaloids as antimalarial drugs through the utilization of *Fusarium*'s metabolic products to reconstruct the molecule.

## Keywords

Manzamine; *Fusarium*spp; Biotransformation; Metabolism; Resistance

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## Introduction

The manzamines are a group of alkaloids with complex heterocyclic ring system bearing a  $\beta$ -carboline moiety. The compounds are a product of sponges and unique host-microbial community. Since the first report by Higa et al. in 1986, more than 60 additional manzamine-type alkaloids have been isolated from sponges collected from different geographical regions. To date, there are 16 species belonging to eight families of sponges that have been confirmed to yield the  $\beta$ -carboline containing manzamine and manzamine-related alkaloids. These sponges have been collected from Okinawa, the Philippines, Indonesia, the Red Sea, Italy, South Africa, and Papua New Guinea (Hu et al. 2003). The manzamine alkaloids are reported to possess a diverse range of bioactivities (Ichiba et al. 1988; Kobayashi et al. 1994; Kondo et al. 1992; Mayer et al. 2000; Edrada et al. 1996; El Sayed et al. 1997; Rao et al. 2003, 2004; Yousaf et al. 2004). Manzamine A has been shown to inhibit GSK-3 and CDK-5 and is effective in decreasing *tau* phosphorylation after treatment in a human neuroblastoma cell line. The underlying mechanism for these activities appears to be kinase inhibition (Hamann et al. 2007). Manzamine A is a promising drug lead for the treatment of malaria. In vivo studies showed that a single intraperitoneal and oral administration of manzamine A or 8-hydroxymanzamine A prolonged the survival of *Plasmodium berghei*-infected mice more than 10 days (Ang et al. 2000).

The fungicidal activity of manzamine A, *ent*-8-hydroxymanzamine A, and *ent*-manzamine F against phyto-pathogenic fungi was reported for *Stagonospora nodorum*, *Phytophthora infestans*, and *Pyricularia grisei* (Peng et al. 2003). However, the compounds showed no activity against *Puccinia recondita* and *Fusarium culmorum* with the exception of manzamine F which inhibited 31 % of the growth of *F. culmorum* at a concentration of 1.7  $\mu$ M. *Fusarium* spp. are common soil saprophytes and plant pathogens (Agrios 1988). The genus is also reported to be the cause of opportunistic infections in human immunocompromised patients (Guarro and Gene 1995). *Fusarium* spp. and its mycotoxins constitute major agricultural and health problems significantly impacting the economy in addition to human and animal health (Bryden et al. 2001). Opportunistic mycoses which are associated with the use of immunosuppressive drugs and AIDS combined with increasing numbers of fungi resistant to available antifungal drugs have driven the need for new antifungal drug leads (Kasanah and Hamann 2005). *Fusarium* spp. can cause a potentially severe opportunistic fungal infection that is primarily encountered in patients with leukemia, immunocompromised patients such as those with allogenic bone marrow or solid organ transplant recipients. There are five species of *Fusarium* that are most infective to humans: *Fusarium solani*, *Fusarium oxysporium*, *Fusarium moniliforme*, *Fusarium verticilloides* and *Fusarium proliferatum*. There are limited numbers of antifungal drugs effective against *Fusarium* spp. (Lionakis et al. 2003). Recent reports demonstrated that amphotericin B and voriconazole were active against strains of *F. solani* and *F. oxysporium*, while itraconazole showed minimal activity (Lewis et al. 2005). Another study showed in vitro synergy of

caspofungin and amphotericin B against clinical isolates of *Fusarium* spp. (Arikan et al. 2002). The discovery of novel antifungal leads that are defined, safe, and have a specific mechanism of action is essential for both the pharmaceutical as well as agrochemical industries (Wedge and Nagle 2000).

We have studied the biotransformation of manzamine alkaloids extensively in order to generate analogs that are more active, less toxic, and that could be utilized in the construction of semisynthetic libraries. 12,34-Oxamanzamine F was first isolated from a sponge and was also shown to be a product of biotransformation of *ent*-8-hydroxymanzamine A by *F. oxysporium* ATCC 7601 and *Nocardia* ATCC 21145 (Yousaf et al. 2002). *F. solani* and *Streptomyces seokies* were reported to transform 8-hydroxymanzamine A to manzamine A. Further studies showed that *F. solani* could transform manzamine F to manzamine E and manzamine X to 6-deoxymanzamine X (Kasanah et al. 2003, 2004). To date, no new manzamine analogs have been reported from microbial transformation studies suggesting that the products isolated from the sponge are likely generated through biotransformation and biocatalysis of manzamine A.

In this paper, we report the metabolism of manzamine as one possible mechanism of inherent resistance of *Fusarium* spp. to this class of alkaloids. In addition, we discuss the impact of resistance and modification of structure of this lead class of compounds of antimalarial and antifungal agents. The manzamine alkaloids isolated from the sponge *Acanthostrongylophora* sp. were evaluated for in vitro anti-fungal activity against the phytopathogenic strains of *F. solani*, *F. oxysporum*, and *F. proliferatum*. We employed several bioassays including one-dimensional direct-bioautography, dilution, and plate susceptibility and micro-titer broth assays in order to establish in vitro activity of the manzamine alkaloids against *Fusarium* spp. and to carefully characterize the products.

## Material and Methods

### General Experimental Section

Liquid chromatography–mass spectrometry (LC-MS) was measured on a Bruker Daltonik GmbH, Germany, equipped with diode array detector (Agilent Technologies), Agilent 1100 binary pump and Autosampler (Agilent). The electrospray ionization positive mass spectra were acquired using a micrOTOF series mass spectrometer. Conditions, optimized using flow injection of standard and sample solutions, were as follows: electrospray ionization capillary voltage, 4.5 kV; end plate offset voltage, –500 V; nebulizer pressure, 2 bar; dry gas flow, 6 L/min; dry gas temperature 180 °C; source detector voltage 1,600 V; and TOF detector voltage 2,190 V. The electrospray ionization gas was nitrogen. All TOF measurements were performed at high resolution and the TOF analyzer was scanned at  $m/z$  50–1,000 with a 1-s integration time. A flow rate of 0.4 mL/min was used for the analytical column C-8 (2), Phenomenex, Luna 5u, 150×4.6 mm. HPLC grade water and acetonitrile both containing 0.1 % formic acid were used as the mobile phase with a 15-min elution time.

## Chromatographic Conditions

Thin layer chromatography (TLC) analysis for monitoring the metabolite was carried out on precoated silica gel UV<sub>254</sub> plates (E. Merck, Darmstadt, Germany); mobile phase, hexanes/acetone (7:3) and chloroform/methanol (9:1). Detection of metabolites was performed under UV light at a wavelength of 254 nm. The crude extracts were purified using silica gel gravitational column and eluted with hexanes/acetone (from 100 to 70 % hexanes) and chloroform/methanol (from 100 to 70 % chloroform). Purification of metabolites was carried out by RP-HPLC, Phenomenex, Luna 5u, C-8, 100 Å, 250×10 mm column, a gradient of water/acetonitrile (0.1 % TFA) from 30 to 100 % acetonitrile over 60 min at a flow rate of 5 mL/min. The metabolites were detected at a wavelength of 254 nm.

## Fusarium Isolates

*F. solani* (F-0007), *F. oxysporum* (F-0001), and *F. proliferatum* (F0029-1) were obtained from USDA-ARS Laboratory, Natural Products Utilization Research Unit, and National Center for Natural Products Research, the University of Mississippi. The fungi were isolated from an orchid species by Dr. D. E. Wedge and identified by Dr. W. H. Elmer of the Connecticut Agricultural Experiment Station. The cultures were stored on silica gel in the USDA-NPURL repository. The isolates were found to be pathogenic to orchid species.

## Manzamine Alkaloids

Manzamine alkaloids were isolated from a marine sponge of the genus *Acanthostrongylophora* sp. collected from Manado, Indonesia. The structures of manzamine and its analogs used in this study are shown in Fig. 1. The isolation and characterization of compounds used as standards was described previously in Rao et al. 2004.

## Conidia Preparation

*Fusarium* spp. cultures were initiated on potato dextrose agar (PDA, Difco, Detroit, MI) from spores. Conidia were subsequently harvested from 7-day-old culture by flooding plates with 10-mL sterile distilled water and dislodging using an L-shaped glass rod. Conidial suspensions were filtered through sterile Miracloth (Calbiochem-Novabiochem Corp., La Jolla, CA) to remove mycelia. The concentration of conidia was determined photometrically at 625 nm from a standard curve. The conidia suspension ( $3 \times 10^5$  conidia/mL) was used for all experiments.

## One-Dimensional Direct Bioautography

Each compound was dissolved in chloroform at a concentration of 100 µg/mL (175–180 µM) and 1 mg/mL (1.75–1.8 mM). One hundred microliters of solution was spotted on a glass TLC plate (silica gel GF, 250 µm, 10×20 cm, Uniplates, Analtech, Inc., Newark, DE) and allowed to dry. The initial concentration of each compound tested was 100 µg/mL (175–180 µM). After drying, the plates were sprayed with conidial solution of *Fusarium* spp. under sterile conditions. The conidial solution was prepared with potato dextrose broth 12 g/500 mL, 0.1 % bacto agar, 0.1 % Tween-80 and contained  $3 \times 10^5$  conidia/mL of each *Fusarium* spp. Plates were placed in a moisture chamber (Pioneer Plastic, Inc., Dixon, KY) and incubated for 3 days at 26 °C. After incubation plates were removed from the moisture

chamber, dried at room temperature, and exhaustively sprayed with 0.25 % MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide, Sigma, St. Louis, MO) prepared in phosphate buffer pH 7 (Sigma, St Louis, MO). The plates were placed back into a moisture chamber and incubated further at 26 °C for another day. The active compound was visualized showing clear zones of fungal growth inhibition on TLC plates with purple background. Amphotericin B, captan, benomyl, and azoxystrobin were used as positive controls to evaluate the activity of manzamine and its analogs. If the compound showed no antifungal activity, the concentration was increased to 1 mg/mL (1.75–1.8 mM) to investigate resistance of *Fusarium* spp. to the manzamine alkaloids.

### Agar Susceptibility Assay

This test was performed on a Petri dish containing 10-mL PDA (Difco). A manzamine solution and antifungal standard (1 mg/mL) were spread on agar and allowed to dry. The conidia suspension *Fusarium* spp. (100 µL) was spread equally over the plate. The negative control consisted of a conidia suspension, solvent (chloroform), and no fungicidal compound. The positive control consisted of conidia suspension and antifungal standards (benomyl, captan, azoxystrobin, amphotericin B). All plates were incubated at 28 °C for 5 days. Each experiment was replicated three times. The compound was considered active if it inhibited the growth of *Fusarium* spp. on plates at concentration tested.

### Microdilution Broth Assay

Manzamine and its analogs were evaluated in a dose response format for antifungal activity using a 96-well microdilution broth assay at concentrations of 0.3, 3, and 30 µM against *F. proliferatum*, *F. solani*, and *F. oxysporium* according to procedure published by Wedge et al. in 1998. Microtiter plates (Nunc, Micro Well, Roskilde, Denmark) were covered with plastic lids and incubated in a growth chamber at 24±1 °C for a 12-h photoperiod under 60±5 µmol/m<sup>2</sup> s. Fungal growth was monitored photometrically by measuring absorbance at 620 nm for 24, 48, and 72 h. Mean absorbance values and standard error were used to evaluate fungal growth.

### Biotransformation Experiments

Cultures of *Fusarium* spp. were prepared as follows: 25 mL of potato dextrose broth in 125-mL flask was inoculated with 3×10<sup>5</sup> conidia/mL and fed manzamine A (**1**), 8-hydroxymanzamine A (**2**), ircinal A (**3**), and manzamine F (**5**) at a concentration of 100 µg/mL. The metabolites were monitored by TLC after 5 and 7 days. Cultures that contained metabolites were harvested after 7 days, while other cultures which did not contain any metabolites were allowed to grow for 2 weeks. The metabolites were analyzed as follows: the cultures were extracted with chloroform and ethyl acetate and evaporated under vacuum. The crude extracts were evaluated by TLC and subjected to separation using column chromatography to yield fractions. Further purification of fractions was done using SPE C-8 (Alltech) and evaluated by LC-MS to validate metabolite production.

## Quantitative Analysis of Metabolites

Separate standard calibration curves were prepared over a concentration range of 0.2–20 µg/mL for 8-hydroxymanzamine A (**2**), ircinal A (**3**), and manzamine F (**5**). For each curve, six different concentrations (0.2, 0.5, 1.0, 5.0, 10, and 20 µg/mL) were used and the calculated peak area plotted against the concentration of each metabolite. The data showed a linear relationship with correlation coefficients ( $R^2$ ) of 0.994, 0.969, and 0.999 for alkaloids **2**, **3**, and **5**, respectively. The quantitation of metabolites was accomplished by comparing the relative peak area of the extracted ion chromatogram of  $m/z$  565±0.5, 411±0.5, and 581±0.5, in accordance to each metabolite present in the mixture for **2**, **3**, and **5**, respectively.

## Results

### The in vitro bioactivity of manzamine alkaloids against *Fusarium* spp

Three bioassays were employed to examine the in vitro activity of manzamine alkaloids against *Fusarium* spp. In summary, the data collected from the microdilution broth assay exhibited that *F. oxysporum*, *F. proliferatum*, and *F. solani* were not susceptible to the manzamine alkaloids. The fungi were sensitive to the antifungal standards, and the response depends both upon the fungus and metabolites. *F. oxysporum* and *F. solani* are more susceptible to amphotericin B while *F. proliferatum* is more susceptible to captan (Figs. 2, 3, and 4).

### Metabolisms of Manzamine Alkaloids by *Fusarium* spp

The metabolism study of the manzamine alkaloids by *Fusarium* spp. was designed to help improve our understanding of how *Fusarium* spp. are inherently resistant to these types of compound and to provide an opportunity to redesign this class of kinase inhibitors for optimized activity and reduced toxicity. The metabolism studies were conducted for manzamines **1**, **2**, and **5**. Herein, we discuss biotransformation of **5** to **4** in detail. Other biotransformation products are tabulated in Table 1.

Data from LC-MS spectra (Fig. 5) revealed a single signal at retention time 6.2 min that corresponds to the authentic standard of **6** at  $m/z$  581.3313. No traces of signal were observed that correspond to other standard manzamine alkaloids at the LC-MS detection limit (10 pg).

Purification of metabolites by HPLC yielded four major fractions which were analyzed further by LC-MS and compared with authentic manzamine standards. Chromatograms of four fractions after HPLC are shown in Fig. 6.

The chromatograms show that fractions 1, 2, and 3 contain a mixture of metabolites while fraction 4 possesses a single compound. Mass spectra analysis of each signal (Fig. 7) revealed that the signal with a retention time of 3.9 min was **4** ( $m/z$  413.3359), while the signal eluting at 6.2 min represented **5** ( $m/z$  581.3313). The signal at the retention time of 9.3 min corresponded to **2** ( $m/z$  565.3781) while **3** ( $m/z$  411.3198) was detected as a peak with retention time at 11.0 min.

LC-MS analysis revealed that **2**, **4**, and starting material **5**, were obtained from fraction 1 while fraction 2 contained **2** as a major metabolite along with **5** and **3**. Fraction 3 contained **2** and **3**, while fraction 4 showed **3** as a major metabolite. In this study, we showed that metabolite **5** was converted to **2**, **3**, and **4**. The yield of **2** recovered from the culture was  $0.98\% \pm 0.02$ . Ircinal A **3** was determined as a major metabolite with a yield of  $4.49\% \pm 0.96$ . The starting material **5** was recovered from the culture in a yield of  $1.27\% \pm 0.035$ . The transformation of **5** by *F. solani* may involve several pathways. Optimization of the biotransformation conditions improves the yield of desired metabolites from certain biotransformation pathways. This is the first report of a retro Pictet-Spengler reaction catalyzed by fungi. The capability of *Fusarium* spp. to transform antifungal compounds is also reported in a number of papers (Turbek et al. 1992; Yue et al. 1998; Zhang and Smith 1983).

## Discussion

The cumulative data generated from these bioassays demonstrated that *F. oxysporum*, *F. solani*, and *F. proliferatum* are resistant to the manzamine alkaloids. Antifungal resistance is a broad concept that can be described as failure of a fungus to respond to the antifungal agent. The in vitro antifungal resistance has been classified as primary (intrinsic) and secondary (acquired). Primary resistance is present prior to exposure to antifungals while secondary resistance develops after exposure to antifungal agents due to genotypic alterations (Kontoyiannis and Lewis 2002). The resistance of *Fusarium* spp. to the manzamine alkaloids is clearly an example of intrinsic resistance due to the fact that *Fusarium* spp. has never been exposed to the natural products. Intrinsic resistance was reported for zygomycetes such as *Fusarium* spp., *Acremonium* spp., *Scedosporium* spp., and dematiaceous fungi (White et al. 1998; Perea and Patterson 2002). Based on metabolites detected from the culture, we propose that **5** was transformed by *F. solani* to **4** through the formation of **2** and **3**. The proposed route of biotransformation is shown in Fig. 8.

We propose here a possible mechanism of microbial transformation from metabolite **5** to **2** through a series of reasonable transformations. The first step is the reduction of the azacyclooctanone ring to a secondary alcohol moiety which is carried out in the presence of NADPH. The second step is the dehydration of the alcohol to afford a cyclooctene species which in turn participates in a [1,3]-sigmatropic rearrangement to yield **2**.

In addition, the microbial metabolism of **2** to **3** (Fig. 9) is proposed to be either a reduction followed by a retro Pictet-Spengler (mechanism A) or hydrolysis-reductive cleavage (mechanism B) pathway. Mechanism A consists of enzymatic reduction of the heteroaromatic ring to the tetrahydro moiety which in turn undergoes a retro Pictet-Spengler reaction to yield **3** and hydroxytryptamine. A retro Pictet-Spengler reaction could be involved either in an intermediate with direct bond-cleavage route (path a) or pass through a bond-rearrangement to a spiro skeleton which is converted to the iminium moiety (path b). Mechanism B involves the hydrolysis of the imine group on the  $\beta$ -carboline ring, followed by stabilization of the hemiaminal species to form the corresponding amino and ketone moieties. Reductive cleavage of the ketone with NADPH would result in the formation of the target compound **3** and 3-(2-amino-vinyl)-1*H*-indol-7-ol as a by-product. During the

study of the capability of *Fusarium* spp. to modify manzamine alkaloids, we observed the metabolism products presented in Table 1.

The metabolism studies of the manzamine alkaloids revealed some highly promising results. Several mechanisms were involved in the transformation of the manzamine alkaloids including oxidation, reduction, hydrolysis, dehydration, and the retro Pictet-Spengler reaction. *F. solani* metabolized **5** to **2** followed by the formation of **3** and further reduction to **4**. Reduction of **3** to **4** was validated when *F. solani* was fed metabolite **3**. No metabolite was detected when *F. oxysporum* was fed metabolites **2** and **3**; however, the fungus transformed **5** to **2** as well as **1** to **2**. *F. proliferatum* showed the capability to transform the manzamine alkaloids to non-related manzamine metabolites. Reduction is the most dominant reaction catalyzed by *Fusarium* spp. in this study whereas a retro Pictet-Spengler reaction is the most important and useful mechanism for optimizing this class of drug leads. The biotransformation products are significantly different from those previously reported due to the variety of media used in the biotransformation studies. However, all the products can be found in the sponge (Kasanah et al. 2003, 2004). Fungal pathogens have strategies to counter the effects of antifungal agents in many ways including the biosynthesis of degradative enzymes, overexpression of target sites, altering the target sites, and activating efflux systems (White et al. 1998; Loeffler and Stevens 2003). Biotransformation and degradation of antifungal compounds derived from plants by phytopathogenic fungi including *Fusarium* spp. are common mechanisms of resistance (Morrissey and Osbourn 1999, Van Etten et al. 1989) and explain how these prolific phytopathogenic fungi may be resistant to many antifungal agents active against other fungi.

### The Implication for Antifungal Drug Discovery

The morbidity and mortality associated with resistance and opportunistic fungal infections continue to increase. Seated mycosis caused by resistant filamentous fungi such as *Fusarium* spp., *Scedosporium prolificans*, and *Aspergillus terreus* requires new classes of antifungal agents with new specific targets, better activity and safety than existing antifungal drugs (Canuto and Rodero 2002). Numerous marine natural products have been reported to possess antifungal activity (Li et al. 1998; Molinski 2004). There are limited reports regarding the activity of these compounds against *Fusarium* spp. We learned from the results presented here that the  $\beta$ -carboline moiety is not a viable pharmacophore against *Fusarium* spp. due to the fungus' ability to metabolize this functional group. There are many marine natural products which were discovered as antifungal leads but are cytotoxic as well as many cytotoxic leads which are good antifungal agents. Cytotoxic compounds will indeed exhibit antifungal activity in vitro. Manzamine A was discovered as a cytotoxic compound with a  $\beta$ -carboline ring system which is essential for activity.  $\beta$ -Carboline alkaloids are widely distributed in nature including plants, marine organisms, insects, as well as mammals. This pharmacophore is interesting due to the diverse biological activity such as inhibiting CDK, topoisomerase I, and monoamine oxidase, and interacting with benzodiazepine and 5-hydroxyserotonin receptors and intercalating DNA (Cao et al. 2007). Our results revealed that all manzamine alkaloids with or without modification to the  $\beta$ -carboline ring are inactive against *Fusarium* spp.



## The Significance of a Proposed Retro Pictet-Spengler Reaction in the Optimization of Antimalarial and Antifungal Activity

The Pictet-Spengler reaction is an important reaction for the generation of the tetrahydro- $\beta$ -carboline and tetrahydroisoquinoline ring systems (Nielsen et al. 2003). This type of reaction is widely involved in the biosynthesis of alkaloids derived from tryptophan including the manzamine alkaloids. The reverse reaction known as the retro Pictet-Spengler reaction is rarely reported and involves the reverse catalytic ring opening and hydrolysis of an iminium group (Zhang et al. 1989). Microbial transformations offer a high degree of stereospecificity and eliminate the need to protect and deprotect functional groups (Abraham and Spassov 2002; Rathbone and Bruce 2002). A biocatalytic approach to performing a retro Pictet-Spengler reaction would be highly valuable in the production of **3** which is an important starting material for the development of manzamine-related analogs as antimalarial and antifungal agents. The production of **3**, either directly from bacterial biosynthesis or transformation, can have a big impact on how this class of compounds will be developed in the future. The biotransformation of **5** to **3** is particularly significant due to the reasonable yield and the absence of biological activity associated with **5**.

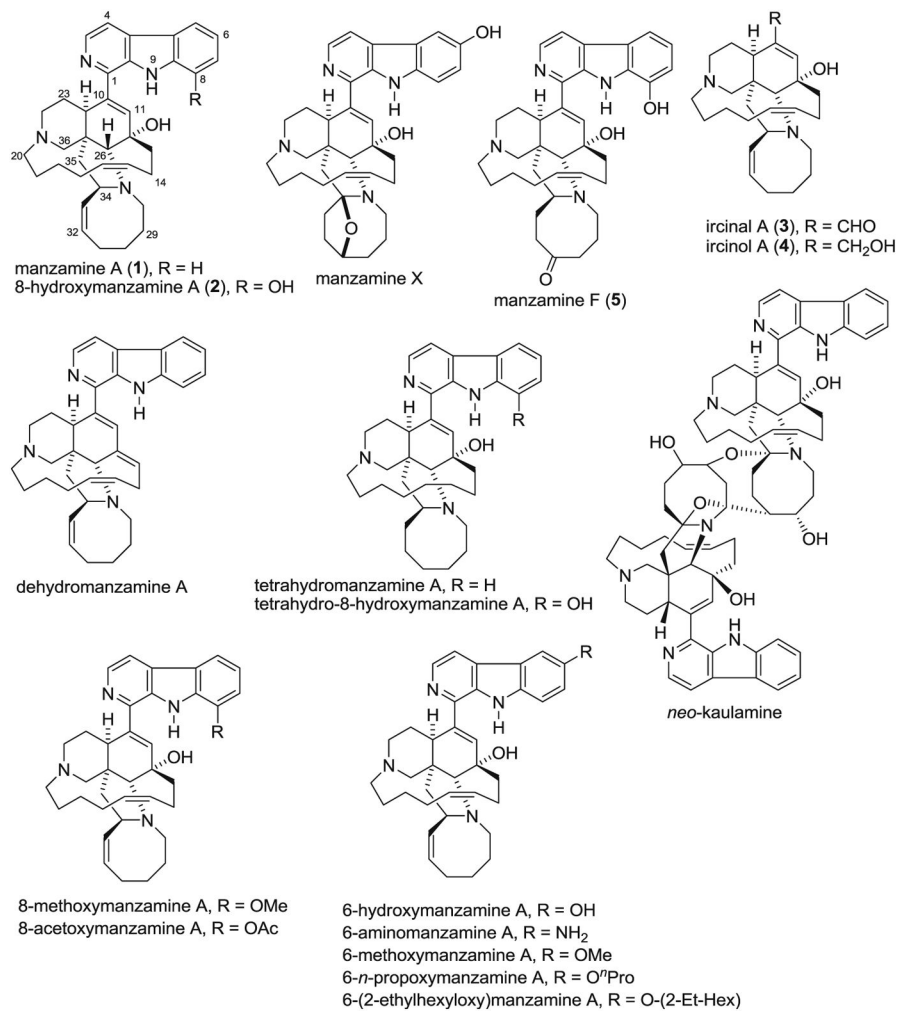
We have shown that there is a loss of activity that may be attributed in part due to metabolism through a unique mechanism although other contributing factors or mechanisms cannot be dismissed. We report here the metabolism of **6** to **2**, **3**, and **4** by *F. solani* through a putative retro Pictet-Spengler mechanism. This transformation is highly significant due to the fact that **3** is an essential starting material in the derivatization of manzamine analogs and **5** is inactive and isolated in high yield from the sponge. Although the classification of this transformation as a retro Pictet-Spengler reaction needs further validation with stable isotopes, no other reasonable explanation can currently be provided. In addition, the results suggest that replacing the  $\beta$ -carboline moiety of these natural products with the heterocyclic moiety may yield antifungal kinase inhibitors that are metabolically stable.

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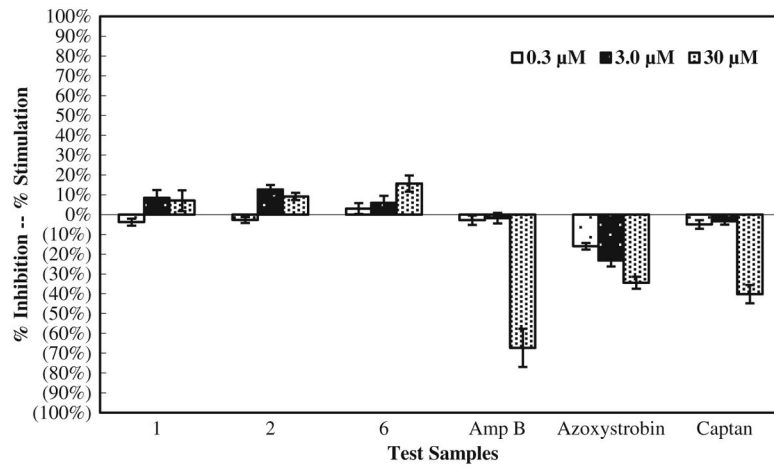
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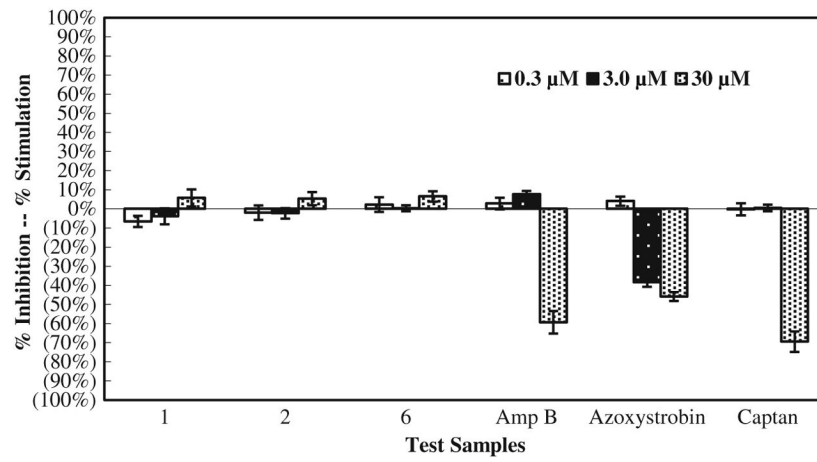
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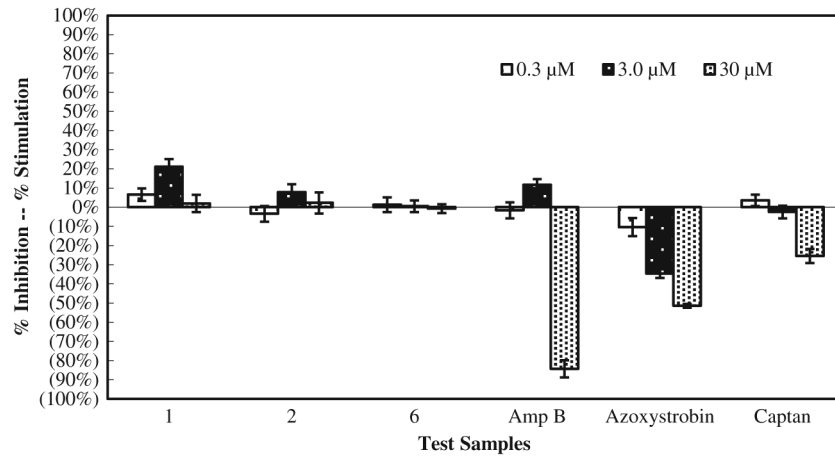
**Fig. 1.**  
Structures of manzamine alkaloids and its analogs used in this study



**Fig. 2.**  
The growth of *F. oxysporum* in a dose-dependent response to manzamine and its analogs in a microdilution broth assay



**Fig. 3.** The growth of *F. proliferatum* in a dose-dependent response to manzamine and its analogs in a microdilution broth assay



**Fig. 4.** The growth of *F. solani* in a dose-dependent response to manzamine and its analogs in a microdilution broth assay

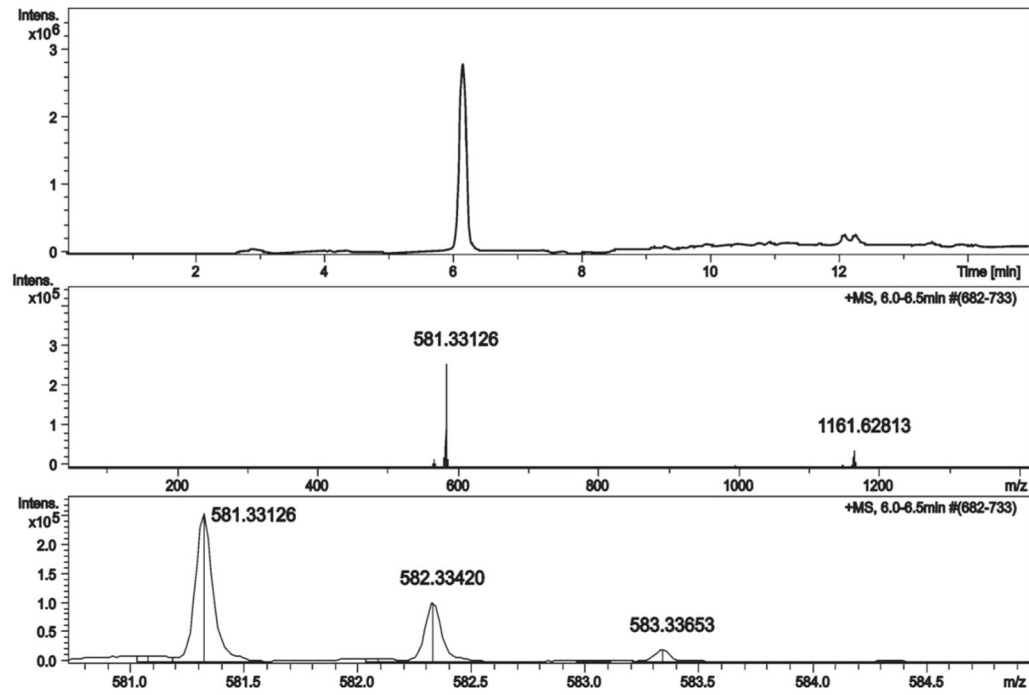
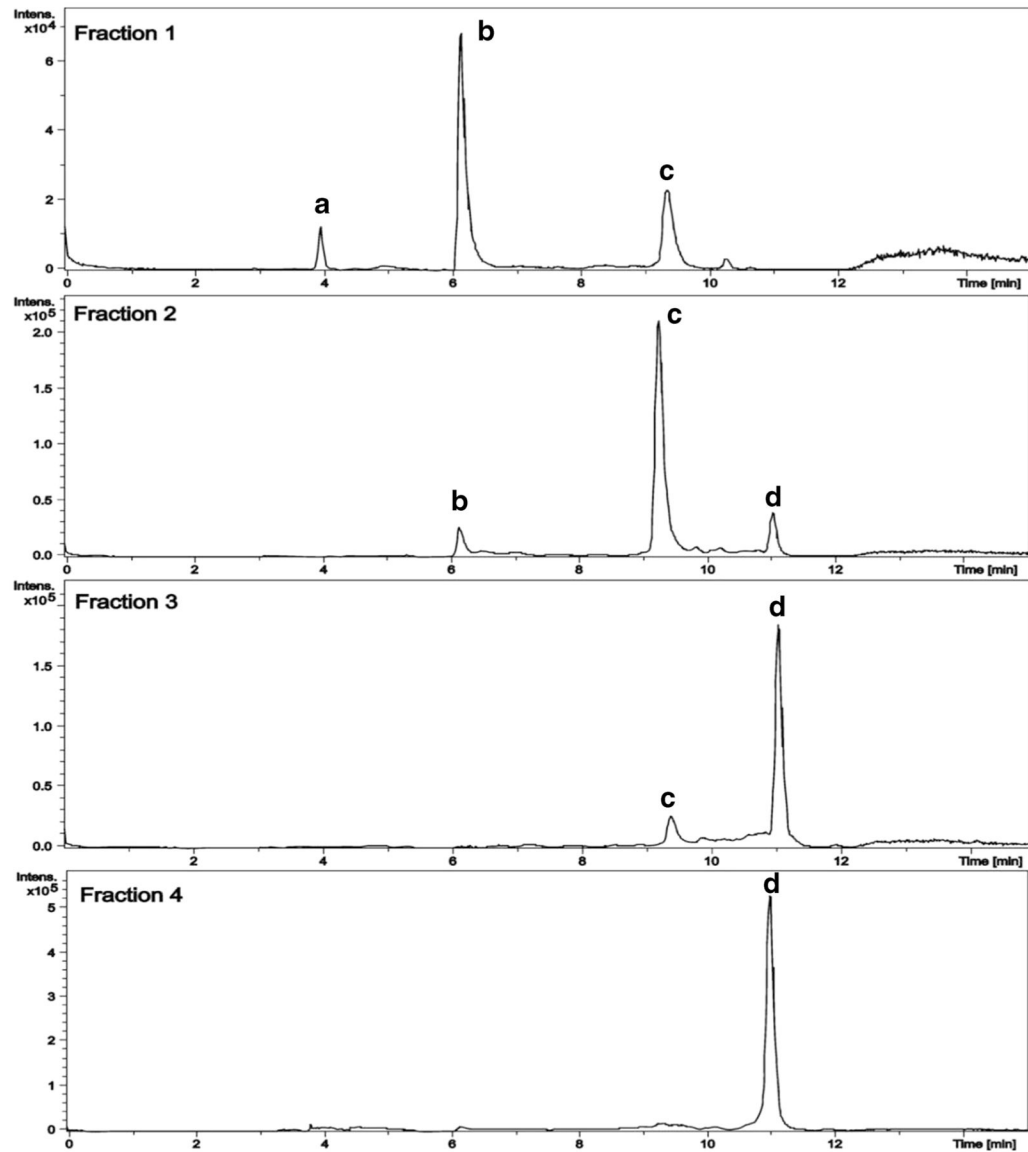
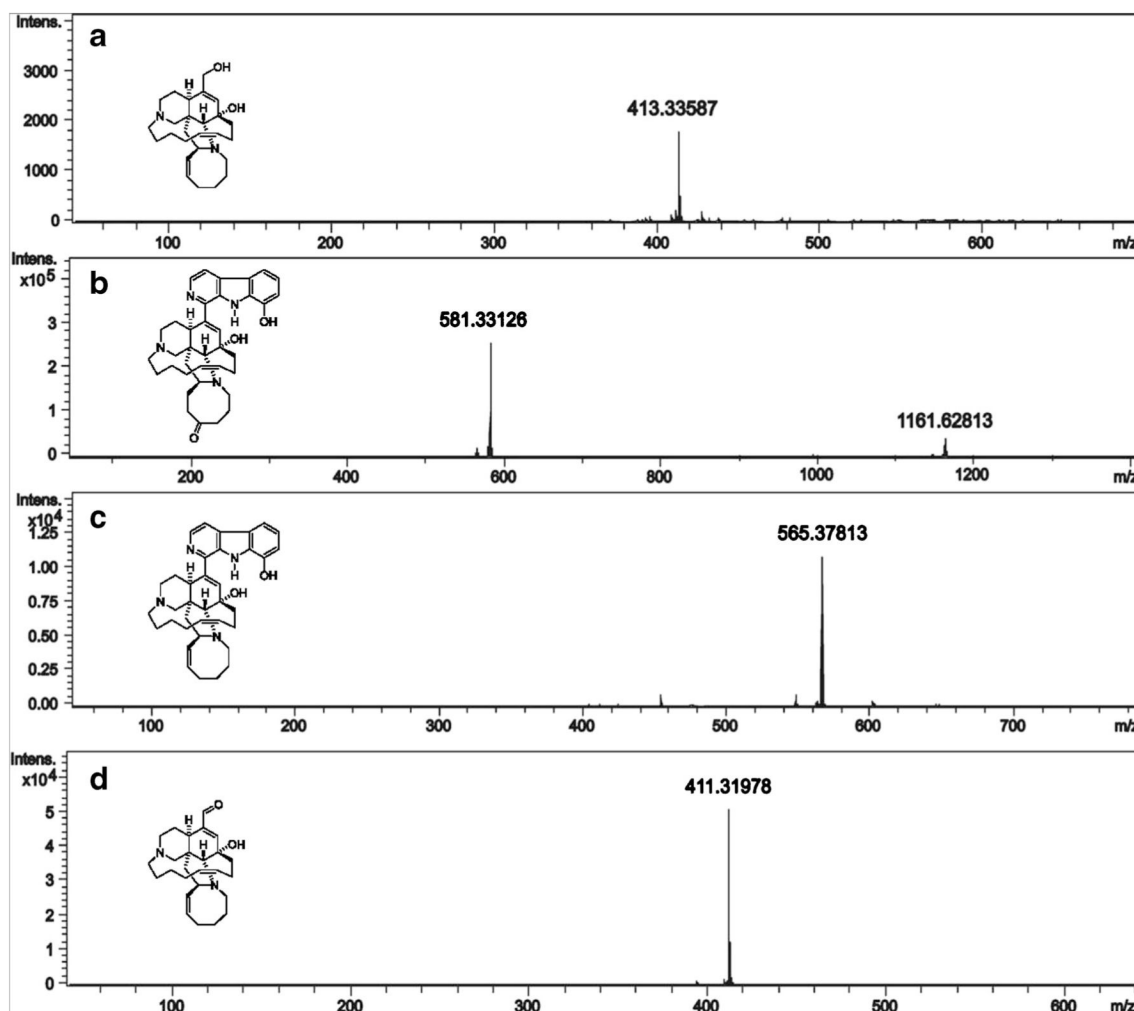


Fig. 5.  
LC-MS spectra of starting material (manzamine F 5)

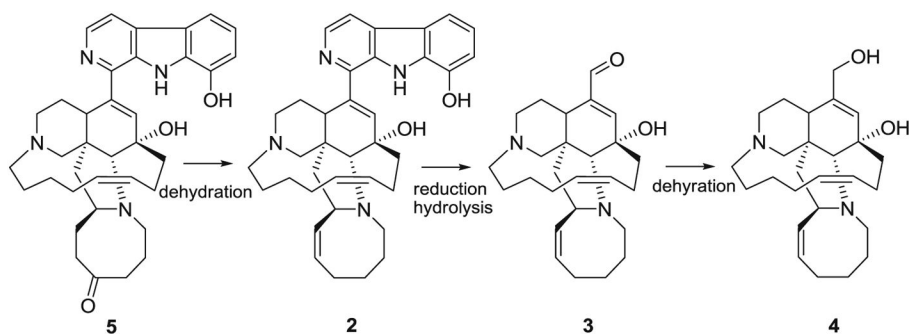




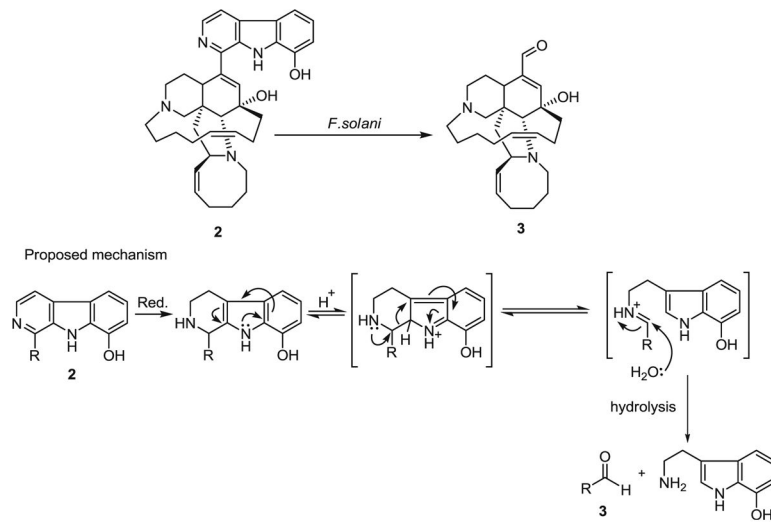
**Fig. 6.** LC-MS chromatograms of each fraction after purification by HPLC. **a**ircinol A (**4**); **b**manzamine F (**5**); **c**8-hydroxymanzamine A (**2**); **d**ircinal A (**3**) (refer to Fig. 7 for mass spectra analysis)



**Fig. 7.**  
Mass spectral analysis of each metabolite. **a** ircinol A (4); **b** manzamine F (5); **c** 8-hydroxymanzamine A (2); **d** ircinal A (3)



**Fig. 8.**  
Proposed route of biotransformation of manzamine F (5) by *F. solani*



**Fig. 9.**  
Proposed mechanism of biotransformation of **2** to **3**

**Table 1**Metabolites of manzamine alkaloids by *Fusarium* spp.

<i>Fusarium</i> spp.	Starting compounds	Metabolites
<i>F. solani</i> (F-0007)	Manzamine A (1)	8-Hydroxymanzamine A (2)
	Manzamine F (5)	8-Hydroxymanzamine A (2) Ircinal A (3), ircinol A (4).
	Ircinal a (3)	Ircinol a (4)
	8-Hydroxymanzamine a (2)	Ircinal a (3)
<i>F. oxysporium</i> (F-0001)	Manzamine a (1)	8-Hydroxymanzamine A (2)
	Manzamine F (5)	Ircinol A (4) 8-Hydroxymanzamine A (2)
	Ircinal A (3)	No metabolite
	8-Hydroxymanzamine A (2)	No metabolite
<i>F. proliferatum</i> (F-0029-1)	Manzamine A (1)	No metabolite
	Manzamine F (5)	No metabolite
	Ircinal A (3)	No metabolite
	8-Hydroxymanzamine A (2)	No metabolite