

## ORIGINAL ARTICLE

# Chemical and bioactive natural products from *Microthyriaceae* sp., an endophytic fungus from a tropical grass

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**Significance and Impact of the Study:** The present study attributes new antiparasitic and psychoactive biological activities to sterigmatocystin (**2**), and describes the structure elucidation of the new natural product integrasone B (**1**), which possesses a rare epoxyquinone with a lactone ring moiety. This is also the first report of sterigmatocystin (**2**) isolation in a fungal strain from this family, broadening the taxonomic range of sterigmatocystin-producing fungi. The study also presents taxonomic analyses indicating that strain F2611 is strongly supported as a member of the *Microthyriaceae* (Ascomycota), but is not a member of any previously known or sequenced genus.

## Keywords

antiparasitic activity, endophytic fungi, integrasone B, sterigmatocystin.

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## Abstract

In screening for natural products with antiparasitic activity, an endophytic fungus, strain F2611, isolated from above-ground tissue of the tropical grass *Paspalum conjugatum* (*Poaceae*) in Panama, was chosen for bioactive principle elucidation. Cultivation on malt extract agar (MEA) followed by bioassay-guided chromatographic fractionation of the extract led to the isolation of the new polyketide integrasone B (**1**) and two known mycotoxins, sterigmatocystin (**2**) and secosterigmatocystin (**3**). Sterigmatocystin (**2**) was found to be the main antiparasitic compound in the fermentation extract of this fungus, possessing potent and selective antiparasitic activity against *Trypanosoma cruzi*, the cause of Chagas disease, with an  $IC_{50}$  value of  $0.13 \mu\text{mol l}^{-1}$ . Compounds **2** and **3** showed high cytotoxicity against Vero cells ( $IC_{50}$  of  $0.06$  and  $0.97 \mu\text{mol l}^{-1}$ , respectively). The new natural product integrasone B (**1**), which was co-purified from the active fractions, constitutes the second report of a natural product possessing an epoxyquinone with a lactone ring and exhibited no significant biological activity. Strain F2611 represents a previously undescribed taxon within the *Microthyriaceae* (Dothideomycetes, Ascomycota).

## Introduction

Endophytic fungi are an important, diverse, and underexplored resource of natural bioactive products with potential applications in agriculture, medicine, and industry (Strobel *et al.* 2004; Gunatilaka 2006; Verma *et al.* 2009; Higginbotham *et al.* 2013). Especially in the past two decades, valuable bioactive compounds with antimicrobial, insecticidal, cytotoxic and anticancer activities have been discovered from endophytes including alkaloids, terpenoids, steroids, quinones, phenols and lactones (Zhang *et al.* 2006; Xu *et al.* 2008).

In 2004, Herath and co-workers isolated and determined the structure of the polyketide and epoxyquinone-based natural product integrasone from an unidentified sterile mycelium (Herath *et al.* 2004). In that report, integrasone was shown to inhibit HIV-1 integrase, the viral enzyme responsible for integrating the HIV provirus into the host genome (Craigie 2001). Interest in the epoxyquinone moiety led to the enantioselective total synthesis of integrasone (Metha and Roy 2005).

Sterigmatocystin (ST) is a carcinogenic polyketide (Versilovskis and De Saeger 2010) with antibiotic properties (Nekam and Polgar 1948) produced by species in several fungal genera (Rank *et al.* 2011). Among the mycotoxins, STs and the related aflatoxins produced by *Aspergillus flavus* and *A. parasiticus* have been extensively studied at the molecular level and are models for the study of fungal gene clusters (Sidhu 2011). Fungi capable of producing STs or aflatoxins are common contaminants of food, feed and indoor environments, and may also be plant and mammalian pathogens. Because of their large economic impact in the biotechnological, agricultural and food industries, it is important for human safety to understand the diversity of taxa that can produce these compounds (Nyikal *et al.* 2004; Wu 2004; Wagacha and Muthomi 2008; Versilovskis and De Saeger 2010; Rank *et al.* 2011).

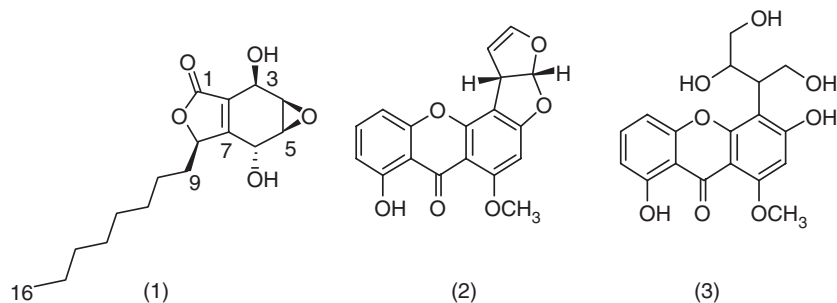
Here we report the isolation and structural elucidation of a new polyketide, integrasone B (1), together with the known mycotoxins sterigmatocystin (2) and secosterigmatocystin (3) (Fig. 1), from the culture of a previously undescribed fungal species, F2611. Sterigmatocystin (2)

was found to be the antiparasitic compound in the fermentation extract of this fungus, exhibiting selective antiparasitic bioactivity against the causal agent of Chagas disease, *Trypanosoma cruzi*, with an  $IC_{50}$  value of  $0.13 \mu\text{mol l}^{-1}$ . Sterigmatocystin (2) also showed antagonistic activity against the DAT receptor with a  $K_i$  value of  $2.23 \mu\text{mol l}^{-1}$ . Taxonomic assessment strongly supported placement of F2611 in the Microthyriaceae (Dothideomycetes, Ascomycota). The present study is the first report of sterigmatocystin production from this family.

## Results and discussion

The molecular formula of 1 was deduced by accurate mass measurement (HRMS–APCI-TOFMS) to be  $C_{16}H_{24}O_5$ , requiring five degrees of unsaturation. The IR spectrum indicated the presence of hydroxyl groups ( $3368 \text{ cm}^{-1}$ , br) and a hydrogen-bonded conjugated lactone ( $1703 \text{ cm}^{-1}$ ). The  $^{13}\text{C}$  NMR and DEPT135 spectra contained 16 carbon resonances, including one resulting from a methyl group and five resonances from  $\text{sp}^3$  methines with chemical shifts indicating to be connected to oxygen atoms, one of which was relatively deshielded ( $\delta_{\text{C}}$  84.4). Seven additional signals resulted from methylene groups. Three resonances were assigned to quaternary carbons, one of which was from a lactone carbonyl ( $\delta_{\text{C}}$  173.3) and the other two were from tetrasubstituted olefinic carbons at  $\delta_{\text{C}}$  126.0 and  $\delta_{\text{C}}$  162.0 (see Table 1 and Supporting Information). The  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum showed correlations from H-8 and H-3 with C1, evidencing the position of the carbonyl C1 in the lactone ring. The  $^1\text{H}$ - $^1\text{H}$  COSY spectrum suggested the presence of two spin systems, one of which consisted of four shielded oxymethines that were connected together in a sequence forming the continuous fragment H-3 through H-6. The second spin system corresponded to an aliphatic chain from the deshielded oxymethine H-8 to H<sub>3</sub>-16 through the correlations of seven methylenes in the aliphatic chain. Long range heteronuclear correlations from H-3 to C-2 and C-1, and from H-4 to C-2 and not with C-7 evidenced the C-1 to C-4 sub-structure (see Fig. 2). Both olefinic carbons C-2 and C-7 displayed correlations

**Figure 1** Structures of integrasone B (1), sterigmatocystin (2) and secosterigmatocystin (3), respectively.



**Table 1** 1D NMR spectroscopic analysis of integrasone B (400 MHz) in comparison with the reported 1D NMR data of integrasone (500 MHz) both in CD<sub>3</sub>OD. Complete 2D spectroscopic data for integrasone B are available in the Supporting Information

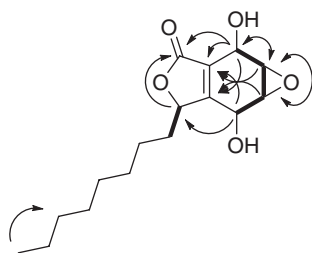
Position	Integrasone* $\delta_C$ , mult.	Integrasone B $\delta_C$ , mult.†‡	Integrasone* $\delta_H$ , (mult, J in Hz)	Integrasone B $\delta_H$ (mult, J in Hz)§
1	173.3, C	173.3, C		
2	126.0, C	125.9, C		
3	62.2, CH	62.2, CH	4.80, dt (3.0, 1.0)	4.79, m
4	55.7, CH	55.7, CH	3.60, dt (1.0, 3.5)	3.55, m
5	57.1, CH	57.1, CH	3.48, dd (3.5, 1.5)	3.47, dd, (3.6, 1.6)
6	62.1, CH	62.0, CH	4.68, s, br	4.67, s, br
7	162.0, C	162.0, C		
8	84.4, CH	84.4, CH	5.0, ddd (8.5, 3.0, 1.0)	4.98, ddd, (8.0, 3.5, 1.6)
9	34.2, CH <sub>2</sub>	34.1, CH <sub>2</sub>	2.11, dddd (14.0, 6.5, 5.0, 3.5)	2.11, m
10	25.9, CH <sub>2</sub>	25.9, CH <sub>2</sub>	1.60, dddd (15.0, 10.0, 8.5, 4.5)	1.59, dddd, (14.3, 10.2, 8.0, 4.5)
11	30.1, CH <sub>2</sub>	30.5, CH <sub>2</sub>	1.40, m	1.44, m
12	30.1, CH <sub>2</sub>	30.5, CH <sub>2</sub>	1.48, m	1.30, m
13	23.6, CH <sub>2</sub> ¶	30.4, CH <sub>2</sub>	1.36, m	1.30, m
14	32.8, CH <sub>2</sub> ¶	30.3, CH <sub>2</sub>	1.32, m	1.30, m
15	14.4, CH <sub>3</sub>	33.0, CH <sub>2</sub>	1.30, m	1.30, m
16		23.7, CH <sub>2</sub>	0.91, t (7.0)	1.28, m
		14.4, CH <sub>3</sub>		0.89, t, (7.0)

\*Data from [Herath *et al.* 2004].†Methanol-d<sub>4</sub> 100 MHz.

‡Determined by DEPT.

§Methanol-d<sub>4</sub>, 400 MHz.

¶Chemical shifts to be interchanged, see text.

**Figure 2** Key <sup>1</sup>H-<sup>13</sup>C HMBC, <sup>1</sup>H-<sup>1</sup>H NOE and <sup>1</sup>H-<sup>1</sup>H COSY correlations from integrasone B (1). (↷) HMBC; (→) COSY; (↪) NOE.

with H-8 from the lactone ring and the oxymethines H-3 and H-6 in the <sup>1</sup>H-<sup>13</sup>C HMBC spectrum, thus confirming the fusion of the six membered dihydroxy epoxy ring with the five membered lactone ring. Through <sup>1</sup>H-<sup>1</sup>H COSY, <sup>1</sup>H-<sup>13</sup>C HSQC correlations and <sup>13</sup>C NMR of the sp<sup>3</sup> methines C-4 and C-5 ( $\delta_C$  55.7 and 57.1, respectively), we deduced an epoxy structure to be present due to the correlations and resonances of these carbons (see Fig. 2).

For compound **1** the trivial name integrasone B is suggested by the presence of two additional methylene groups in the aliphatic chain, in comparison with the previously reported integrasone (Herath *et al.* 2004). As a note on the reported <sup>13</sup>C NMR chemical shifts of the

manuscript reporting the spectroscopic data of integrasone, we believe that the C-12 and C-13 chemical shift values are likely interchanged, i.e. the alkyl chain should be more upfield (see Table 1 and Herath *et al.* 2004). The relative configuration of **1** was established by inspecting the <sup>1</sup>H-<sup>1</sup>H NOESY spectrum, where strong correlations between H-3, H-4 and H-5, and a weak correlation between H-5 and H-6 were observed, indicating the same relative configuration as in integrasone. Furthermore, similar coupling constant values were determined for H-9 as in integrasone, supporting the same relative configuration for both (see Table 1 and Supporting Information). The optical rotation observed of integrasone B,  $[\alpha]_D^{25} = +10.7$  (c 1.5, MeOH), was similar to the value reported for integrasone,  $[\alpha]_D^{25} +16.7^\circ$  (c 1.5, MeOH), suggesting that they have the same absolute configuration.

The fermentation extract of the fungus F2611 exhibited higher activity against *T. cruzi* than cytotoxic activity, indicating a possible therapeutic window. Antiparasitic (*T. cruzi*) bioassay-guided fractionation of the extract of F2611 led to the isolation of the known mycotoxins sterigmatocystin (**2**) and secosterigmatocystin (**3**). Sterigmatocystin (**2**) was found to possess potent and selective antiparasitic activity against *T. cruzi*, the cause of Chagas disease, with an IC<sub>50</sub> value of 0.13  $\mu\text{mol l}^{-1}$ , while revealing no activity against *Plasmodium falciparum* at the

30  $\mu\text{mol l}^{-1}$  level. Secosterigmatocystin (**3**) revealed no activity in either antiparasitic assay at the 30  $\mu\text{mol l}^{-1}$  level. Compound **1** exhibited no cytotoxic activity at the 30  $\mu\text{mol l}^{-1}$  level, whilst pure compounds **2** and **3** revealed high cytotoxicity against Vero cells ( $\text{IC}_{50}$  of 0.06 and 0.97  $\mu\text{mol l}^{-1}$ , respectively). The cytotoxic activity of these mycotoxins has previously been described in the literature (Pachter and Steyn 1985). Sterigmatocystin (**2**) was also subjected to several psychoactive receptor assays and revealed antagonistic activity against the DAT receptor with a  $K_i$  value of 2.23  $\mu\text{mol l}^{-1}$ .

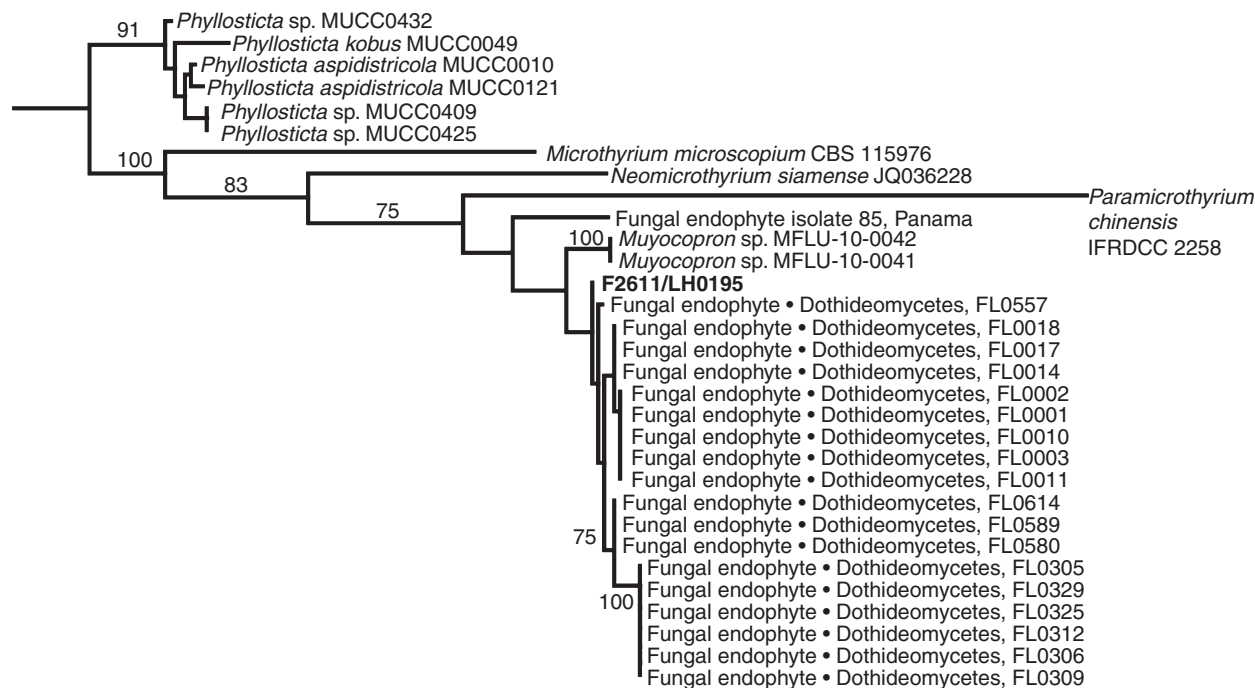
This is the first report of sterigmatocystin production from a fungus belonging to this family. Sterigmatocystin (ST) is a carcinogenic polyketide produced by species in several fungal genera (Versilovskis and De Saeger 2010; Rank *et al.* 2011). A large gene cluster codes for production of this highly toxic, mutagenic and carcinogenic secondary metabolite, which is also a biogenetic precursor to aflatoxins and a genetic study model for fungal horizontal gene transfer (Brown *et al.* 1996; Bennett and Klich 2003; Sidhu 2011). ST and related aflatoxins have been reported from many species in phylogenetically and ecologically disparate genera, as recently reviewed (Rank *et al.* 2011). Although the fungus from the present study could not be described to the species level, phylogenetic analysis reveals its placement in the Dothideomycetes (Ascomycota; see below). The production of a complicated natural product

by phylogenetically diverse fungi informs our understanding of the evolution of secondary metabolism and the complex gene clusters coding for STs and aflatoxins (Rank *et al.* 2011; Slot and Rokas 2011).

Epoxyquinones with a lactone ring are very rarely found in nature. To our knowledge this is the second report of such structures, after integrasone. A database search for similar substructures yielded only 1,4-dihydroxy aromatic lactone analogs. Although integrasone B was co-purified from the active fractions but not the active antiparasitic compound, we tested integrasone B against HIV integrase, based on the plausible activity stated in the literature. Surprisingly it was not active even at the 333  $\mu\text{mol l}^{-1}$  level. Integrasone B (**1**) was tested against a panel of 42 psychoactive receptors, the MCF-7 human breast cancer cells and the parasites *T. cruzi* and *P. falciparum* (tested at the 10  $\mu\text{g ml}^{-1}$  level), but showed no activity.

The VLC fractions of the F2611 extract were also tested in antibacterial assays against *Bacillus subtilis* and *Pseudomonas aeruginosa* (at the 50  $\mu\text{g ml}^{-1}$  level) but showed no activity.

Taxonomic analysis indicates that F2611 represents a potentially undescribed fungal taxon. The resulting analysis (Fig. 3 and Supporting Information) provided strong support for placement of F2611 within the Microthyriaceae, but did not place it within any currently known or previously sequenced genus.



**Figure 3** Result of maximum likelihood analysis placing F2611 within the Microthyriaceae, where it is related to a clade that includes an undescribed species of endophytic fungi first published by U'Ren *et al.* (2012). Values at internodes indicate maximum likelihood bootstrap support; overall likelihood, -6369.896171.

## Material and methods

### General experimental procedures

Optical rotations were measured with a Rudolf Research Analytical Autopol III 6971 automatic polarimeter. UV and IR spectra were obtained with a WATERS 996, Photodiode Array Detector and a Shimadzu, IRAFFINITY-1, Fourier transform infrared spectrophotometer, respectively. NMR spectra were recorded on a JEOL spectrometer with standard pulse sequences operating at 400 MHz and on a BRUKER spectrometer with standard pulse sequences operating at 300 MHz spectra. Spectra were referenced to residual solvent signals for CD<sub>3</sub>OD and CDCl<sub>3</sub>. LC-MS and HR-MS were recorded on a JMS-LC mate (APCI+ ionization) JEOL mass spectrometer. The HPLC was an Agilent 1100 series. Reverse phase HPLC was performed on a Waters 600 model system with a photodiode array UV detector 2996. HPLC columns were RP-HPLC XTerra<sup>®</sup>Prep (RP18, 10 μm, 10 × 250 mm, Part No. 186001028), used with a 2 ml min<sup>-1</sup> flow and a semi preparative RP-HPLC column (Column SymmetryPrep<sup>™</sup> Waters C<sub>18</sub> 7 μm, 19–300 mm column), used with a 4 ml min<sup>-1</sup> flow. Merck silica gel 60 (0.040–0.063 mm, 70–230 mesh) was used for vacuum liquid chromatography (VLC). Columns were wet-packed under vacuum with petroleum ether (PE). Before applying the sample solution, the columns were equilibrated with the first designated eluent. Standard columns for crude extract fractionation had dimensions of 13 × 4 cm.

### Fungal material

Living, fresh, and apparently healthy individuals of the tropical grass *Paspalum conjugatum* (Poaceae; buffalo grass, carabao grass, or sour paspalum) were collected in Colon, Panama (plant sample ID 276) and were identified by Dr. Alicia Ibáñez. Fungal strain F2611 was isolated on malt extract agar (2%) from a surface-sterilized, healthy, mature leaf following Arnold and Lutzoni (2007). The fungal strain was deposited in the Gilbertson Herbarium at the University of Arizona and at the Smithsonian Tropical Research Institute in Panama (strain number F2611).

### Fermentation and extraction

The fungus F2611 was pre-cultured in 400 ml malt extract broth (MEB, Sigma-Aldrich) for 10 days to generate a large mycelial mass. A thin layer of liquid pre-culture (5–7 ml) was used to inoculate the surface of (MEA) medium in 145 mm Petri dishes. The liquid evaporated after 4–6 days and thus saturated the agar surface with fungal biomass. F2611 was cultured for 24 days in 150

MEA petri dishes (10 l MEA) with constant light and at room temperature (25–30°C). An extraction with 5 l EtOAc yielded 2.2 g of extract.

### Isolation and structure elucidation of compounds

2.2 g of extract were subjected to a VLC fractionation using silica as solid phase and a gradient solvent system with petroleum ether : acetone of (50 : 1), (5 : 1), (2 : 1), (1 : 1), 100% acetone, 100% acetonitrile and 100% MeOH, yielding seven fractions. Fraction 2 (petroleum ether : acetone of 5 : 1) was subjected to RP-HPLC fractionation using a solvent system of acetonitrile : water (65 : 35) to yield compound 2, sterigmatocystin (subfraction 6, *t<sub>R</sub>* 43 min, 25.6 mg). Fraction 3 (petroleum ether/acetone of 2 : 1) was subjected to RP-HPLC fractionation using a solvent system of acetonitrile : water (70 : 30) to yield compound 1 (subfraction 4, *t<sub>R</sub>* 29 min, 92.3 mg). Fraction 6 (100% acetonitrile) was subjected to RP-HPLC fractionation using a solvent system of acetonitrile : water (40/60) to yield compound 3, secosterigmatocystin (subfraction 3, *t<sub>R</sub>* 15 min., 3.3 mg). Due to the high yield of the compounds in several fractions, all compounds were isolated only partially in regard to the total amount.

### Fungal identification

Because the isolate did not produce diagnostic fruiting structures in culture, phylogenetic analyses of molecular sequence data were used to determine its taxonomic placement. After total genomic DNA extraction (Arnold and Lutzoni 2007), the nuclear ribosomal internal transcribed spacers and 5.8S gene (ITS rDNA; approx. 600 base pairs [bp]) and an adjacent portion of the nuclear ribosomal large subunit (LSU rDNA; approx. 500 bp) were amplified as a single fragment by PCR. The edited consensus sequence (Supporting Information) was submitted to GenBank under accession KJ469652.

Phylogenetic analyses (described in full in Supporting Information) provided strong support for placement of F2611 within the Microthyriaceae, but did not place it within any currently known or sequenced genus. Therefore, we designate the strain Microthyriaceae sp. F2611. This placement was strongly supported and could not have been inferred using BLAST results alone (Supporting Information).

### Bioassays

Binding assays against a panel of 44 psychoactive receptors (activity considered with at least 50% inhibition at the 10 μmol l<sup>-1</sup> level against 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1D</sub>, 5-HT<sub>1E</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>3</sub>, 5-HT<sub>5A</sub>, 5-HT<sub>6</sub>,

5-HT<sub>7</sub>,  $\alpha_{1A}$ ,  $\alpha_{1B}$ ,  $\alpha_{1D}$ ,  $\alpha_{2A}$ ,  $\alpha_{2B}$ ,  $\alpha_{2C}$ ,  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$ , BZP Rat Brain Site, D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>, D<sub>5</sub>, DAT,  $\delta$ ,  $\kappa$ ,  $\mu$ , GABA<sub>A</sub>, H<sub>1</sub>, H<sub>2</sub>, H<sub>3</sub>, H<sub>4</sub>, M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub>, M<sub>4</sub>, M<sub>5</sub>, NET, SERT,  $\sigma_1$ ,  $\sigma_2$ ) are fully described in the literature (University of North Carolina at Chapel Hill, National Institute of Mental Health, Psychoactive Drug Screening Program, Assay Protocol Book). Antiparasitic assays against *Trypanosoma cruzi* and *P. falciparum*, anticancer assays against cell line MCF-7, and mammalian cell cytotoxicity assays were performed as described by Moreno *et al.* (2011). HIV Integrase inhibition assays were performed following Zhao *et al.* (2012). Extracts and VLC fractions were tested for antimicrobial activity against *B. subtilis* and *Escherichia coli* using the Kirby-Bauer disk diffusion method (Bauer *et al.* 1966) with some changes (see Supporting Information).

#### Integrasone B (1)

White yellowish solid (9.2 mg l<sup>-1</sup>, 4–20%);  $[\alpha]_D^{25} = +10.7$  (c 1.5, MeOH); UV (MeOH)  $\lambda_{max}$ , nm (log  $\epsilon$ ): 213 (2.08); IR (KBr)  $\nu_{max}$ : 3368, 2924, 1703, 1366, 1231, 1097 cm<sup>-1</sup>; <sup>1</sup>H NMR and <sup>13</sup>C NMR (see Table 1 and 2D NMR in Supporting Information); LRMS–APCI (*m/z*): 297.0 [M+H]<sup>+</sup>; HR-APCI-TOFMS = 297.1522 [M+H]<sup>+</sup> (calcd for C<sub>16</sub>H<sub>25</sub>O<sub>5</sub> 297.1702), 329.1831 [M+CH<sub>3</sub>OH+H]<sup>+</sup>.

#### Sterigmatocystin and secosterigmatocystin

1D NMR data (see Supporting Information) and MS data [APCI+ mode, *m/z* 325 for sterigmatocystin (M+H<sup>+</sup>) and *m/z* 363 for secosterigmatocystin (M+H<sup>+</sup>)] were compared to the data reported in the literature for a direct structural assignment (Metha and Roy 2005).

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#### Conflict of Interest

The authors declare no conflict of interest.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1**  $^1\text{H}$  NMR spectra (400 MHz, methanol- $d_4$ ) of compound **1**.

**Figure S2**  $^{13}\text{C}$  NMR spectra (100 MHz, methanol- $d_4$ ) of compound **1**.

**Figure S3**  $^1\text{H}$ - $^1\text{H}$  COSY NMR data of compound **1**.

**Figure S4**  $^1\text{H}$ - $^{13}\text{C}$  HMBC NMR data of compound **1**.

**Figure S5**  $^1\text{H}$ - $^1\text{H}$  NOESY NMR data for compound **1**.

**Figure S6** Separation chromatogram for compound **1**.

**Figure S7** Ultra-violet spectra for compound **1**.

**Figure S8** Infra-red spectra for compound **1**.

**Figure S9** 1D and 2D NMR spectroscopic data for compound **1**.

**Figure S10** MS data for compound **1**.

**Figure S11**  $^1\text{H}$  NMR spectra (300 MHz,  $\text{CDCl}_3$ ) of compound **2**.

**Figure S12**  $^{13}\text{C}$  NMR spectra (75 MHz,  $\text{CDCl}_3$ ) of compound **2**.

**Figure S13**  $^1\text{H}$  NMR spectra (300 MHz, MeOD) of compound **3**.

**Figure S14** MS data for compound **3** ( $m/z$  363, APCI+ mode,  $\text{M}+\text{H}^+$ ).

**Figure S15**  $\text{K}_i$  determination of the antagonistic activity of sterigmatocystin (compound **2**) against the DAT receptor.

**Figure S16** Bioactivity results of compounds **2** and **3** against *T. cruzi*, *P. falciparum* and the Vero cells line.

**Figure S17** Fungal identification & respective references.

**Figure S18** Antimicrobial bioassays description.