SUPPLEMENTARY MATERIAL

Bioactive Compounds from the Endophytic Fungus *Fusarium proliferatum*

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The crude extract of an endophytic fungus isolated from *Syzygium cordatum* and identified as *Fusarium proliferatum* showed 100% cytotoxicity against the brine shrimp *Artemia salina* at 100 µg/ml. Seven coloured biologically active metabolites, including ergosta-5,7,22-trien-3β-ol, nectriafurone-8-methyl ether, 9-*O*-methyl fusarubin, bostrycoidin, bostrycoidin-9-methyl ether, and 8-hydroxy-5,6-dimethoxy-2-methyl-3-(2-oxo-propyl)-1,4-naphthoquinone, were isolated from the extract.

Keywords: endophytic fungus; *Syzygium cordatum*; *Fusarium proliferatum*; naphthoquinone

Experimental

1 General experimental procedures

NMR spectra were recorded on Varian Unity 300 (300.145 MHz) and Varian Inova 500 (499.876 MHz) spectrometers. ESI-MS was recorded on a Finnigan LCQ with quaternary pump Rheos 4000 (Flux Instrument). ESI-HR mass spectra were measured on a Micromass LCT spectrometer coupled with a HP 1100 HPLC with a Diode Array Detector. UV/vis spectra were measured on a Perkin-Elmer Lambda 15 UV/vis spectrometer. Column chromatography was done on silica gel 230-400 mesh; preparative thin layer chromatography (PTLC) was performed on silica gel P/UV254, *R_f* values were obtained on Polygram SIL G/UV254 cards with CH₂Cl₂/MeOH (all Macherey-Nagel & Co., Düren, Germany). Size exclusion chromatography was done on Sephadex LH-20 (Lipophilic Sephadex; Amersham Biosciences, Ltd., Purchased from Sigma-Aldrich Chemie, Steinheim, Germany). For microbiological methods and conditions of culture preparation see ref. (Maskey et al. 2002, Zhang et al. 2008).

*2 Isolation and identification of the fungal *Fusarium proliferatum* strain 749A7b*

Endophytic isolations were done from *Syzygium cordatum* trees in the northern KwaZulu/Natal region of South Africa, according to (Marsberg et al. 2014). During preliminary screens from the isolate collections, strain 749A7b showed promise to contain quinonoid substances. Culturally the isolate resembled a *Fusarium* species. The identity was refined by sequencing the Internal Transcribed Spacer (ITS) region of the ribosomal operon (White et al. 1990) following protocols of Marsberg et al. (2014). The resultant DNA sequences from the forward and reverse primers were merged to form a contig with the online program MEGA 6 (Tamura et al. 2013) and searched against the online DNA database Genbank and a *Fusarium* specific database (Geiser et al. 2004). These yielded the likely identity of *F. proliferatum*.

The stock cultures were maintained at 4°C on Malt Extract Agar (2%, Biolab, Johannesburg) agar medium without antibiotics.

3 *Fermentation, activity tests, and isolation of the bioactive compounds from the strain*

The fungal strain 749A7b was pre-cultivated on M₂ agar (consisting of malt extract (10 g), yeast extract (4 g), glucose (4 g), and bacto agar (20 g) dissolved in 1000 mL) plates at 28°C for six days. Well-grown plates were used to inoculate 12 P-flasks each containing 1L of potato dextrose agar (potato (200 g), dextrose (2 g), agar (20 g), and 1000 mL water) broth. The flasks were incubated as resting cultures for three weeks at room temperature. To the well-grown cultures, 5 ml chloroform was added to destroy the spores formed. The cultures were harvested and extracted with ethyl acetate exhaustively and evaporated under vacuum to dryness. The obtained crude extract was tested for cytotoxicity against *A. salina* and for antimicrobial activity using a disk diffusion assay. The red crude extract (5 g) was chromatographed on silica gel using a stepwise CH₂Cl₂/MeOH gradient to yield at first four fractions (Figure S1). Work-up of the first fraction yielded two sub-fractions consisting of fat and ergosta-5,7,22-trien-3 β -ol **1** (Figure S2), which were separated through crystallization. The second fraction was purified by PTLC and Sephadex LH 20 to afford bostrycoidin (**5**). Fraction 3 was also purified in the same way as Fraction 2 and produced 9-*O*-methyl-fusarubin **3** (Figures S3 and S4) and bostrycoidin-9-methyl ether **4** (Figures S5 and S6). Fraction 4 was separated using PTLC and further purified to afford nectriafurone-7-methyl ether (**2**) and 8-hydroxy-5,6-dimethoxy-2-methyl-3-(2-oxo-propyl)-1,4-naphthoquinone **6** (Figures S7 and S8). A fifth fraction produced Compound **7**, which had similar spectral characteristics (Figures S9-S13) to Compound **2**, but could not be identified unambiguously.

Ergosta-5,7,22-trien-3 β -ol (1): White needles (30 mg), blue on TLC with anisaldehyde/sulphuric acid, R_f = 0.38 (CH₂Cl₂/3% MeOH). - ¹H NMR (CDCl₃, 300 MHz): δ 0.69 (3H, s, 19-*H*), 0.80 (3H, d, 28-*H*), 0.86 (3H, d, 26-*H*), 1.06 (1H, m, 14-*H*), 1.10 (3H, s, 18-*H*), 1.11 (3H, d, 27-*H*), 1.15 (1H, m, 17-*H*), 1.39 (2H, m, 12-*H*), 1.50 (2H, m, 15-*H*), 1.53 (1H, m, 24-*H*), 1.54 (1H, m, 9-*H*), 1.67 (3H, d, 21-*H*), 1.72 (2H, m, 7-*H*), 1.82 (2H, m, 5-*H*), 1.83 (2H, m, 1-*H*), 1.86 (2H, m, 2-*H*), 2.01 (2H, m, 20-*H*), 2.04 (2H, m, 6-*H*), 2.23 (2H, m, 4-*H*), 3.58 (1H, m, 3-*H*), 5.17 (2H, m, 22-*H*, 23-*H*), 5.45 (1H, m, 7-*H*), 5.54 (1H, dd, J = 5.2, 2.4, 6-*H*).

Nectriafurone-8-methyl ether (2): Yellow solid (3 mg). - ¹H NMR (300 MHz, CDCl₃): δ 1.60 (3H, d, J = 7, 2'-CH₃), 4.00 (3H, s, OCH₃), 4.01 (3H, s, OCH₃), 5.19 (1H, m, 1'-*H*), 6.80 (1H, s,

3-*H*), 7.98 (1H, s, 6-*H*), 13.30 (2H, s, *OH*). – (+)-**ESIMS** m/z (%) 659 ($[2\text{ M} + \text{Na}]^+$, 75), 341 ($[\text{M} + \text{Na}]^+$, 25). – (-)-**ESIMS** m/z (%) 317 ($[\text{M} - \text{H}]^-$, 75), – (+)-**HRESIMS** m/z 341.0629 $[\text{M} + \text{Na}]^+$, (calcd. 343.0629 for $\text{C}_{16}\text{H}_{14}\text{NaO}_7$).

9-*O*-Methylfusarubin (3): Red solid (2.5 mg). – ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.42 (3H, s, 15- CH_3), 2.40 (1H, d, $J = 18$ Hz, 4-*H*_{ax}), 2.45 (1H, d, $J = 18$ Hz, 4-*H*_{eq}), 3.98 (3H, s, 6- OCH_3), 3.99 (3H, s, 8- OCH_3), 4.50 (2H, dd, $J = 18, 2.8$ Hz, 1- CH_2), 7.00 (1H, s, 7-*H*), 12.90 (1H, s, 5-*OH*); ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$): δ 28.2 (C-15), 31.7 (C-4), 56.4 (OMe-15), 56.6 (OMe-6), 57.6 (C-1), 93.1 (C-3), 104.0 (C-7), 108.6 (C-12), 113.4 (C-11), 136.8 (C-14), 144.6 (C-13), 147.5 (C-5), 155.1 (C-6), 155.8 (C-8), 178.7 (C-9), 189.0 (C-10). – (+)-**ESIMS** m/z (%) 663 ($[2\text{ M} + \text{Na}]^+$, 100), 343 ($[\text{M} + \text{Na}]^+$, 25). – (-)-**ESIMS** m/z (%) 319 ($[\text{M} - \text{H}]^-$, 100), – (+)-**HRESIMS** m/z 343.0789 $[\text{M} + \text{Na}]^+$, (calcd. 343.0789 for $\text{C}_{16}\text{H}_{16}\text{NaO}_7$).

Bostrycoidin-9-methyl ether (4): Red powder (15 mg). – ^1H NMR (CDCl_3 , 300 MHz): δ 2.78 (3H, s, 3- CH_3), 4.02 (6H, s, 2x OCH_3), 6.82 (1H, s, 7-*H*), 7.82 (1H, s, 4-*H*), 9.41 (1H, s, 1-*H*), 13.24 (1H, s, *OH*); – ^{13}C NMR (CDCl_3): δ 25.12 (3- CH_3), 56.50 (9- OCH_3), 57.15 (7- OCH_3), 104.38 (C-8), 110.3 (C-9a), 110.5 (C-5a), 117.07 (C-4), 126.6 (C-10a), 138.00 (C-4a), 143.3 (C-1), 150.00 (C-6), 154.9 (C-7), 161.3 (C-9), 164.12 (C-3), 183.9 (C-10), 188.5 (C-5). – (+)-**ESIMS**: $m/z = 322$ $[\text{M} + \text{Na}]^+$, – (-)-**ESIMS**: m/z 298 $[\text{M} - \text{H}]^-$.

Bostrycoidin (5): Red powder (4 mg). – ^1H NMR (CDCl_3): δ 2.80 (3H, s, 3- CH_3), 4.02 (3H, s, 7- OCH_3), 6.76 (1H, s, 8-*H*), 7.96 (1H, s, 4-*H*), 9.50 (1H, s, 1-*H*), 13.49 (1H, s, 6-*OH*), 13.20 (1H, s, 9-*OH*). ^{13}C NMR (CDCl_3): δ 25.3 (3- CH_3), 56.8 (7- OCH_3), 107.9 (C-8), 118.0 (C-4), 124.6 (C-10a), 138.7 (C-4a), 143.3 (C-1), 151.2 (C-6), 157.9 (C-7), 161.3 (C-9), 165.4 (C-3), 183.9 (C-10), 186.4 (C-5). (+)-**ESIMS**: $m/z = 308$ $[\text{M} + \text{Na}]^+$. – (-)-**ESIMS**: m/z 284 $[\text{M} - \text{H}]^-$.

8-Hydroxy-5,6-dimethoxy-2-methyl-3-(2-oxopropyl)-1,4-naphthoquinone (6): Red powder (1.5 mg). – ^1H NMR (CDCl_3 , 300.0 MHz): δ 2.00 (3H, s, 2- CH_3), 2.21 (3H, s, - COCH_3), 3.80 (2H, s, COCH_2), 3.85 (3H, s, OCH_3), 4.00 (3H, s, OCH_3), 7.12 (1H, s), 13.80 (1H, bs, *OH*). ^{13}C NMR (CDCl_3): δ 13.4 (2- CH_3), 29.8 (COCH_3), 40.7 (COCH_2), 56.4 (OCH_3), 56.7 (OCH_3), 104.4, 109.2, 113.4 (CH), 138.0, 147.0, 149.2, 154.8, 155.4, 180.2, 189.3, 203.8. – **EIMS** $m/z = 304$ $[\text{M}]^+$ ($\text{C}_{16}\text{H}_{16}\text{O}_6$).

Compound 7, $\text{C}_{17}\text{H}_{16}\text{O}_7$: Red solid (<1 mg). – ^1H NMR (CDCl_3 , 300.0 MHz): δ 1.42 (3H, t, CH_3), 2.01 (1H, s, *OH*), 3.96 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 4.02 (1H, m, *CH*), 5.53 (2H, m,

CH_2), 6.22 (1H, s, CH), 6.74 (1H, s, CH), 13.10 (1H, bs, OH). ^{13}C NMR (CDCl_3): δ 20.63 (CH_3), 56.35 (CH_3O), 56.92 (CH_3O), 63.30 (CH), 92.79 (CH_2), 95.56, 103.44 (CH), 110.65, 114.57, 124.30 (CH), 134.54, 148.71, 155.09, 155.33, 160.31, 179.51 ($\text{C}=\text{O}$), 188.76 ($\text{C}=\text{O}$). – (-)-**ESIMS** m/z (%) 331 ($[\text{M} - \text{H}]^-$, 72), (+)-**HRESIMS** m/z 687.2 $[\text{2M} + \text{Na}]^+$, 355.0782 $[\text{M} + \text{Na}]^+$ (calcd. 355.0788 for $\text{C}_{17}\text{H}_{16}\text{NaO}_7$).

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Figure S1: Work-up scheme for the fungus *Fusarium proliferatum* strain 749A7b

Figure S2: ^1H NMR spectrum (CDCl_3 , 300 MHz) of ergosta-5,7,22-trien-3 β -ol (**1**)

Figure S3: ^1H NMR spectrum ($\text{DMSO}-d_6$, 300 MHz) of 9-*O*-ethyl fusarubin (**3**)

Figure S4: ^{13}C NMR spectrum ($\text{DMSO}-d_6$, 125 MHz) of 9-*O*-methyl fusarubin (**3**)

Figure S5: ^1H NMR spectrum (CDCl_3 , 300 MHz) of bostrycoidin-9-methyl ether (**4**)

Figure S6: ^{13}C NMR spectrum (CDCl_3 , 125 MHz) of bostrycoidin-9-methyl ether (**4**)

Figure S7: ^1H NMR spectrum ($\text{DMSO}-d_6$, 300 MHz) of 8-hydroxy-5,6-dimethoxy-2-methyl-3-(2-oxopropyl)-1,4-naphthoquinone (**6**)

Figure S8: ^{13}C NMR spectrum ($\text{DMSO}-d_6$, 125 MHz) of 8-hydroxy-5,6-dimethoxy-2-methyl-3-(2-oxopropyl)-1,4-naphthoquinone (**6**).

Figure S9: ^1H NMR (CDCl_3 , 300 MHz) spectrum of Compound 7

Figure S10: ^{13}C NMR of (CDCl_3 , 125 MHz) spectrum of Compound 7

Figure S1: ESI-MS of Compound 7

Figure S2: (+)-HRESIMS of Compound 7

Figure S3: (-)-HRESIMS of Compound **7**