

Experimental and Supporting Information for:

**Fusarochromene, a novel tryptophan-derived metabolite from  
*Fusarium sacchari*.**

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## Instrumentation

NMR spectra of compounds were obtained using JEOL: Delta GX 270, lambda 300, Eclipse 300, Delta GX 400, Eclipse 400, or Varian: VNMR 600, iNOVA 600, VNMR 500 or VNMR 400 spectrometers. Unless otherwise stated NMR spectra were acquired in CDCl<sub>3</sub> with the residual CHCl<sub>3</sub> ( $\delta$ H 7.27/ $\delta$ C 77.0) signal as a reference.

ESIMS were obtained using a Waters Platform II ESI MS detector, a Waters ZQ MS or a Waters Quattro Micro triple quad ESI MS. HRESIMS data were obtained using a Bruker daltonics FT-ICR mass spectrometer. HRMS were obtained using a VG Autospec.

IR spectra were determined on a Perkin Elmer Spectrum 400 FT-IR equipped with a diamond cell. UV absorption was recorded on a Waters 996 Diode Array Detector.

Melting points were determined using a Gallenkamp electrothermal apparatus and are uncorrected.

HPLC and LC-MS analysis was carried out on: a HP 1050 HPLC system; A Dionex summit HPLC system equipped with a Polymer labs ELS2000 evaporative light scattering detector; a Waters 600 HPLC system equipped with a PDA 998 diode array detector and a micromass platform II MS detector, or a waters ZQ MS detector with an ESCi probe, or a Waters system comprising a 2767 sample manager, a 2545 Binary gradient module, a 2998 Photodiode array detector, a waters 2424 Evaporative light scattering detector and a waters Quattro Micro triple quad ESI MS with a Z spray ESCi MS probe.

Preparative HPLC was carried out using a Dionex Summit HPLC system equipped with an ISCO foxy jr fraction collector and a Polymer labs ELS2000 evaporative light scattering detector, or on a Waters system comprising a 2767 sample manager, a 2545 Binary gradient module, a 515 make up pump, a system fluidics organizer, a 2998 Photodiode array detector, a waters 2424 Evaporative light scattering detector and a Waters Quattro Micro triple quad ESI MS with a Z spray ESCi MS probe.

## Strains

*Fusarium sacchari*, originally isolated from field-grown sugar cane plants, was from the First Culture Bank, University of the Punjab (Lahore, Pakistan) supplied by Dr. Asifa Munawar, School of Biological Sciences, University of Bristol. It was propagated on Czapek Dox (CD) Agar (15 gm L<sup>-1</sup>) at 28 °C for 22 days.

## Isolation of Fusarochromene

*F. sacchari* was cultured in Czapek dox broth (100 mL per 250 mL flask) at 28 °C, shaking at 200 rpm on an orbital gyratory shaker for a typical incubation period of 10 days. All broth cultures were inoculated

with 300  $\mu\text{L}$  of spore suspension and all agar cultures were inoculated with 80  $\mu\text{L}$  of spore suspension containing  $10^{10}$  spores  $\text{mL}^{-1}$  per 100 mL of medium. The whole broth culture of *F. sacchari* (100 mL) was homogenised then filtered. The filtrate was acidified (pH 4) by addition of c.HCl (aq), then extracted into EtOAc ( $2 \times 100$  mL) and the organic solvent was evaporated under vacuum to afford a crude extract ( $400$  mg  $\text{L}^{-1}$ ). The extract was re-dissolved in acetone (at  $100$  mg  $\text{mL}^{-1}$ ) for HPLC analysis. Crude extract was dry loaded onto a column packed with silica gel (particle size  $80$   $\mu\text{m}$ ). The column was eluted at a rate 4-5 x faster than gravity (under  $\text{N}_2$ ) with 9:1 Hexane:EtOAc. The fractions were then analysed by TLC, and pure fractions containing analytes of interest were combined, evaporated to dryness on a rotary evaporator and then re-dissolved and evaporated from  $\text{CH}_2\text{Cl}_2$  several times. Samples were then dried *in vacuo* and weighed. Fusarochromene **1** ( $30$  mg  $\text{L}^{-1}$ ) was isolated as an amorphous yellow solid by flash chromatography over silica gel eluting EtOAc:acetonitrile 80:20,  $R_f = 0.2$ . Fusarochromene **1** ( $30$  mg  $\text{L}^{-1}$ ) was also isolated by preparative HPLC (retention time: 11.3 min; method 20 minute ramp prep: Mobile phase A: Water containing 0.05% v/v Formic Acid Mobile phase B: MeOH containing 0.05% v/v Formic Acid Flow rate:  $4$  mL  $\text{min}^{-1}$  Gradient program: 25% B to 95% B over 13 min. Hold for 2 min. 95% B to 25% B over 2 min, hold for 2 min.).

$[\alpha]_D^{22} - 22$  ( $c$  0.0014,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz  $\text{CDCl}_3$ )  $\delta$  1.44 ppm (6H, s, 2x 1-Me), 1.99 (3H, s, 16-H), 3.14 (1H, dd,  $^2J_{\text{HH}}$  16.1,  $^3J_{\text{HH}}$  6.1, 12-*HH*), 4.28 (1H, m,  $^3J_{\text{HH}}$  6.72,  $^3J_{\text{HH}}$  6.1,  $J_{\text{HH}}$  6.1,  $^3J_{\text{HH}}$  4.4,  $^3J_{\text{HH}}$  4.2, 13-H), 5.91 (1H, d,  $^3J_{\text{HH}}$  10.0, 3-H), 6.20 (1H, d,  $^3J_{\text{HH}}$  8.93, 7-H), 6.35 (1H, d,  $^3J_{\text{HH}}$  9.9, 4-H), 6.42 (1H, d,  $^3J_{\text{HH}}$  6.72, HNAc), 6.64 (2H, br s,  $\text{NH}_2$ ), 7.64 (1H, d,  $^3J_{\text{HH}}$  9.0, 8-H);  $^{13}\text{C}$  NMR (125 MHz  $\text{CDCl}_3$ )  $\delta$  23.5 ppm (16-C), 27.7 (1-C), 39.8 (12-C), 49.7 (13-C), 64.7 (14-C), 76.7 (2-C), 106.3 (5-C), 106.5 (7-C), 112.5 (9-C), 115.3 (3-C), 128.4 (4-C), 133.4 (8-C), 147.9 (10-C), 158.5 (6-C), 170.6 (15-C), 199.6 (11-C); UV/vis  $\lambda_{\text{max}}$  ( $\text{H}_2\text{O}/\text{MeOH}$ ) 265.6, 365.6 nm; IR  $\nu_{\text{max}}$  (neat) 3322, 2926, 1635, 1590, 1549  $\text{cm}^{-1}$ ; MS (ESI<sup>+</sup>)  $m/z$  (%): 242 (90), 301 (100), 319 (30),  $[\text{MH}]^+$ , 341 (10)  $[\text{MNa}]^+$ ; HRMS (ESI<sup>+</sup>) calculated for  $\text{C}_{17}\text{H}_{22}\text{N}_2\text{O}_4\text{Na}$ :  $[\text{MNa}]^+$  341.1471, found 341.1484.

## Synthetic procedures

### ***p*-Bromobenzoyl-fusarochromene**

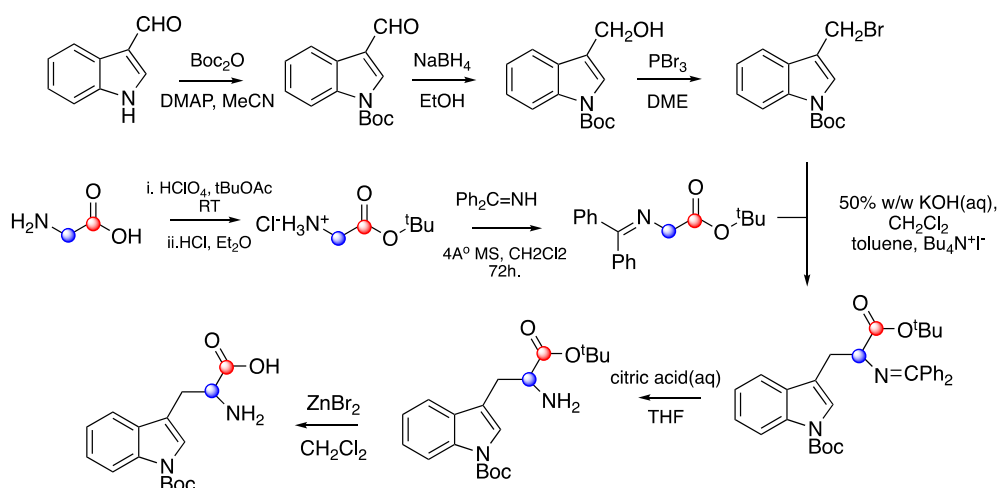
To a vial containing fusarochromene **1** (17.8 mg, 0.056 mmol) was added; pyridine (2 mL) & *p*-bromobenzoylchloride (13.7 mg, 0.067 mmol, 1.2 equiv). The mixture was stirred at RT under  $\text{N}_2$  (g) for 16 h. The reaction solvent was removed by evaporation under a stream of  $\text{N}_2$  (g). *p*-Bromo-benzoyl-fusarochromene **2** (10 mg, 0.02 mmol, 35%) was isolated by preparative HPLC (retention time: 15.8 min; method: 20 minute ramp prep).

$^1\text{H}$  NMR (500 MHz  $\text{CDCl}_3$ )  $\delta$  1.36 ppm (6H, 2 x s, 1-Me), 1.92 ppm (3H, s, 16-H), 3.06 (1H, dd,  $^2J_{\text{HH}}$  16.8,  $^3J_{\text{HH}}$  6.1, 12-*HH*), 3.28 (1H, dd,  $^2J_{\text{HH}}$  16.8,  $^3J_{\text{HH}}$  4.6 12-*HH*), 4.34 (1H, dd,  $^2J_{\text{HH}}$  12.3,  $^3J_{\text{HH}}$  5.5, 14-*HH*), 4.50 (1H, dd,  $^2J_{\text{HH}}$  11.3,  $^3J_{\text{HH}}$  6.4, 14-*HH*), 4.69 (1H, m,  $^3J_{\text{HH}}$  9.2,  $^3J_{\text{HH}}$  6.4,  $J_{\text{HH}}$  5.5,  $^3J_{\text{HH}}$  4.6, 13-H), 5.34 (1H, d,  $^3J_{\text{HH}}$  9.8, 3-H), 6.08 (1H, d,  $^3J_{\text{HH}}$  9.2, 7-H), 6.25 (1H, d,  $^3J_{\text{HH}}$  10.1, 4-H), 6.37 (2H, br s,  $\text{NH}_2$ ), 6.62 (1H, d,  $^3J_{\text{HH}}$  8.9, HNAc), 7.48 (1H, d,  $^3J_{\text{HH}}$  9.2, 8-H), 7.49 (2H, d,  $^3J_{\text{HH}}$  8.6, Ar-H), 7.77 (2H, d,  $^3J_{\text{HH}}$  8.6, Ar-H);  $^{13}\text{C}$

NMR (125 MHz CDCl<sub>3</sub>)  $\delta$  23.5 ppm (16-C), 30.9 (1-C), 38.9 (12-C), 46.2 (13-C), 66.0 (14-C), 76.7 (2-C), 106.4 (7-C), 106.5 (5-C), 106.4 (9-C), 115.3 (4-C), 128.3 (Ar-C), 128.5 (3-C), 128.6 (Ar-C), 131.1 (8-C), 131.7 (Ar-C), 132.8 (Ar-C), 147.6 (10-C), 158.3 (6-C), 169.8 (Ar-CO), 178.2 (15-C), 200.1 (11-C); UV/vis  $\lambda_{\max}$ (H<sub>2</sub>O/MeOH) 268, 363, 383 nm; IR  $\nu_{\max}$  (neat) 3321, 1719, 1635, 1590 1551 cm<sup>-1</sup>; MS (ESI<sup>+</sup>)  $m/z$  (%): 242 (10), 301 (40), 326 (20), 328 (20), 501 (90), [MH]<sup>+</sup> 503 (100) [MH]<sup>+</sup>; HRMS (EI<sup>+</sup>) calculated for C<sub>24</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>Br 500.0962, [M]<sup>+</sup> found 501.0852 [MH]<sup>+</sup>.

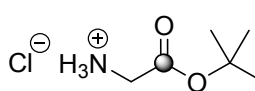
### [1-<sup>13</sup>C] and [2-<sup>13</sup>C]-tryptophan

These were synthesised from [1-<sup>13</sup>C]- and [2-<sup>13</sup>C]-glycine (Sigma Aldrich), respectively.

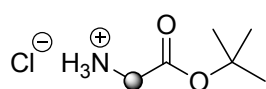


**Scheme S1:** Synthesis of <sup>13</sup>C-labelled tryptophans

### [1-<sup>13</sup>C]-Glycine *tert*-butyl ester hydrochloride

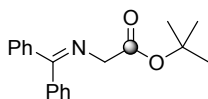
 70% w/w perchloric acid (1.4 ml, 16 mmol) was added dropwise to a suspension of [1-<sup>13</sup>C]-glycine (1.03 g, 13.3 mmol) in *tert*-butyl acetate (18 ml, 133 mmol) behind a blast shield. After 25 h, the solution was diluted with water (20 ml) and poured into a mixture of CH<sub>2</sub>Cl<sub>2</sub> (50 ml) and saturated Na<sub>2</sub>CO<sub>3(aq)</sub> (50 ml). The aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (5 × 50 ml), and the combined organic layers were washed with brine (10 ml), then dried over MgSO<sub>4</sub>. Solvent removal *in vacuo* yielded the free amine of the title compound as a colourless oil (1.26 g, 9.58 mmol). An Et<sub>2</sub>O solution (50 ml) of the free amine was cooled to 0 °C, then 2 M HCl in Et<sub>2</sub>O (5 ml, 10 mmol) was added dropwise to precipitate the title compound as a pale yellow powder (1.13 g, 6.70 mmol, 50%);  $\nu_{\max}$  (neat) / cm<sup>-1</sup> 2977, 2927, 2660 (N–H and C–H stretch), 1707 (C=O stretch); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  ppm 1.49 (9H, br. s., C(CH<sub>3</sub>)<sub>3</sub>), 3.90 (2H, app. br. s., CH<sub>2</sub>), 8.33 (3H, br. s., NH<sub>3</sub>); <sup>13</sup>C NMR (101 MHz, CHCl<sub>3</sub>)  $\delta$  ppm 28.2 (C(CH<sub>3</sub>)<sub>3</sub>), 41.7 (br. d,  $J$ =60.7 Hz, CH<sub>2</sub>), 83.5 (C(CH<sub>3</sub>)<sub>3</sub>), 166.3 (CO<sub>2</sub>-*t*-Bu, enriched); HRMS calculated for C<sub>10</sub><sup>13</sup>C<sub>2</sub>H<sub>27</sub>N<sub>2</sub>O<sub>4</sub> 295.2032 [M + H]<sup>+</sup> found 295.2039.

### Preparation of <sup>13</sup>C-2 glycine *tert*-butyl ester hydrochloride



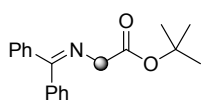
70% w/w perchloric acid (0.6 ml, 6.9 mmol) was added dropwise to a suspension of [2-<sup>13</sup>C]-glycine (478 mg, 6.28 mmol) in *tert*-butyl acetate (9 ml, 67 mmol) behind a blast shield. After 25 h, the solution was diluted with water (20 ml) and poured into a mixture of CH<sub>2</sub>Cl<sub>2</sub> (50 ml) and saturated Na<sub>2</sub>CO<sub>3(aq)</sub> (50 ml). The aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (5 × 50 ml), and the combined organic layers were washed with brine (10 ml), then dried over MgSO<sub>4</sub>. Solvent removal *in vacuo* yielded the free amine of the title compound as a colourless oil (1.26 g, 9.58 mmol). An Et<sub>2</sub>O solution (50 ml) of the free amine was cooled to 0 °C, then 2 M HCl in Et<sub>2</sub>O (5 ml, 10 mmol) was added dropwise to precipitate the title compound as a colourless needles after standing at 0 °C for 1 h; (451 mg, 2.68 mmol, 43%);  $\nu_{\max}$  (neat) / cm<sup>-1</sup> 2977, 2927, 2660 (N–H and C–H stretch), 1707 (C=O stretch); <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  ppm 1.52 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 3.73 (2H, d, *J*=144.9 Hz, CH<sub>2</sub>), 4.85 (3H, br. s., NH<sub>3</sub>); <sup>13</sup>C NMR (101 MHz, MeOD)  $\delta$  ppm 28.3 (C(CH<sub>3</sub>)<sub>3</sub>), 41.7 (CH<sub>2</sub>), 85.0 (C(CH<sub>3</sub>)<sub>3</sub>), 167.7 (d, *J*=62.2 Hz, CO<sub>2</sub>*t*-Bu); HRMS calculated for C<sub>10</sub><sup>13</sup>C<sub>2</sub>H<sub>27</sub>N<sub>2</sub>O<sub>4</sub> 295.2032 [M + H]<sup>+</sup> found 295.2037.

### [1-<sup>13</sup>C]-*tert*-butyl 2-((diphenylmethylene)amino)acetate



Prepared by adaptation of the procedure of Hernández-Toribio *et al.*<sup>1</sup> Benzophenone imine (1.0 ml, 6.0 mmol) was added to a suspension of 4 Å molecular sieves (1.3 g) in a solution of [1-<sup>13</sup>C]-glycine *tert*-butyl ester hydrochloride (1.08 g, 8.28 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (40 ml). After stirring for 24 h, the suspension was filtered through celite and the filtrate washed with water (50 ml), then brine (50 ml). After solvent removal *in vacuo* the title compound was isolated as a pale yellow solid (1.88 g, 6.37 mmol, 88%); m.p. 111-112 °C (EtOAc/hexanes);  $\nu_{\max}$  (neat) / cm<sup>-1</sup> 3067, 2990, 2977, 2886 (C–H stretch), 1691 (C=O stretch), 1621 (N=C stretch); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  ppm 1.47 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 4.13 (2H, d, *J*=7.1 Hz, CH<sub>2</sub>), 7.17 - 7.23 (2H, m, ArCH), 7.29 - 7.53 (7H, m, ArCH), 7.65 - 7.70 (2H, m, ArCH); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  ppm 28.1 (C(CH<sub>3</sub>)<sub>3</sub>), 56.3 (d, *J*=63.0 Hz, CH<sub>2</sub>), 81.0 (C(CH<sub>3</sub>)<sub>3</sub>), 127.7 (ArCH), 128.0 (ArCH), 128.6 (ArCH), 128.7 (ArCH), 130.3 (ArCH), 136.2 (ArCH), 139.4 (ArC), 158.0 (ArC), 168.5 (N=CPh<sub>2</sub>), 169.8 (CO<sub>2</sub>*t*-Bu); *m/z* (ESI<sup>+</sup>) 319.2 [M + Na]<sup>+</sup> (30%), 297.2 [M + H]<sup>+</sup> (84%), 241.1 [M - *t*-Bu]<sup>+</sup> (100%); HRMS calculated for C<sub>18</sub><sup>13</sup>CH<sub>22</sub>NO<sub>2</sub> 297.1679 [M + H]<sup>+</sup> found 297.1682.

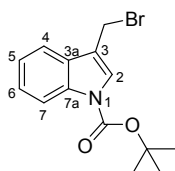
### [2-<sup>13</sup>C]-*tert*-butyl 2-((diphenylmethylene)amino)acetate



Prepared by adaptation of the procedure of Hernández-Toribio *et al.*<sup>1</sup> Benzophenone imine (0.41 ml, 2.4 mmol) was added to a suspension of 4 Å molecular sieves (0.8 g) in a solution of [2-<sup>13</sup>C]-glycine *tert*-butyl ester hydrochloride **9b** (451 mg, 2.68 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (30 ml). After stirring for 24 h, the suspension was filtered through celite and the filtrate washed with water (50 ml), then brine (50 ml). After solvent removal *in vacuo* the title compound was isolated as a colourless prisms (743 mg, 2.51 mmol, 93%); m.p. 111-112 °C (EtOAc/hexanes);  $\nu_{\max}$  (neat) / cm<sup>-1</sup> 3067, 2990, 2977, 2886 (C–H stretch), 1691 (C=O stretch), 1621 (N=C stretch); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$

ppm 1.47 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 4.13 (2H, d,  $J=135.9$  Hz, CH<sub>2</sub>), 7.17 - 7.23 (2H, m, ArCH), 7.30 - 7.50 (6H, m, ArCH), 7.65 - 7.70 (2H, m, ArCH); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  ppm 28.1 (C(CH<sub>3</sub>)<sub>3</sub>), 56.3 (CH<sub>2</sub>), 81.0 (C(CH<sub>3</sub>)<sub>3</sub>), 127.7 (ArCH), 128.0 (ArCH), 128.6 (ArCH), 128.7 (ArCH), 130.3 (ArCH), 136.1 (ArCH), 139.3 (ArC), 139.4 (ArC), 169.8 (d,  $J=63.1$  Hz, CO<sub>2</sub>*t*-Bu), 171.4 (N=CPh<sub>2</sub>);  $m/z$  (ESI<sup>+</sup>) 319.2 [M + Na]<sup>+</sup> (30%), 297.2 [M + H]<sup>+</sup> (84%), 241.1 [M - *t*-Bu]<sup>+</sup> (100%); HRMS calculated for C<sub>18</sub><sup>13</sup>CH<sub>22</sub>NO<sub>2</sub> 297.1679 [M + H]<sup>+</sup> found 297.1684.

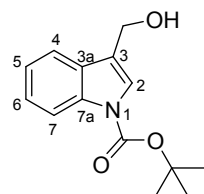
#### *tert*-Butyl 3-formyl-1*H*-indole-1-carboxylate



Prepared according to the procedure of Davies *et al.*<sup>2</sup> DMAP (230 mg, 1.9 mmol) was added to a stirring suspension of indole-3-carboxaldehyde (2.73 g, 19.0 mmol) and di-*tert*-butyl dicarbonate (5.39 g, 24.7 mmol) in acetonitrile (38 ml). After effervescence, the solution became homogeneous, before a white precipitate began to appear. After stirring for 19 h, the solvent was removed *in vacuo* and the residual solid partitioned between CH<sub>2</sub>Cl<sub>2</sub> (100 ml) and NaHCO<sub>3(aq)</sub> (50 ml). The organic layer obtained was washed with saturated NH<sub>4</sub>Cl(aq) (50 ml), then water (50 ml), then brine (50 ml), before being dried over MgSO<sub>4</sub>. Solvent removal *in vacuo* yielded a white solid, which was recrystallised (CH<sub>2</sub>Cl<sub>2</sub> / hexanes) to white prisms (3.84 g, 12.0 mmol, 63%); m.p. 128-129 °C (CH<sub>2</sub>Cl<sub>2</sub>/hexanes); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  ppm 1.72 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 7.39 (1H, app. td,  $J=7.5$ , 1.2 Hz, 5-CH), 7.43 (1H, ddd,  $J=8.2$ , 7.5, 1.5 Hz, 6-CH), 8.16 (1H, br. d,  $J=8.2$  Hz, 7-CH), 8.25 (1H, s, 2-CH), 8.30 (1H, ddd,  $J=7.5$ , 1.5, 0.7 Hz, 4-CH), 10.11 (1H, s, CHO).

Melting point and <sup>1</sup>H NMR data are consistent with that in the literature.<sup>3</sup>

#### *tert*-butyl 3-(hydroxymethyl)-1*H*-indole-1-carboxylate



Prepared by adaptation of the procedure of Hadley *et al.*<sup>4</sup> Sodium borohydride (0.55 g, 14.5 mmol) was added portionwise to a solution of *tert*-butyl 3-formyl-1*H*-indole-1-carboxylate (1.79 g, 7.30 mmol) in freshly distilled ethanol (40 ml). After stirring for 16 h, the solution was concentrated *in vacuo* and partitioned between Et<sub>2</sub>O (50 ml) and 1 M NaOH<sub>(aq)</sub> (50 ml). The aqueous layer was extracted with Et<sub>2</sub>O (3 × 50 ml) and the combined organic phases were washed with brine (50 ml), dried over MgSO<sub>4</sub>, and concentrated *in vacuo* to a colourless oil. Purification by column chromatography (eluting 20% EtOAc/petroleum ether) yielded the title compound as a colourless oil, slowly solidifying to white prisms (1.79 g, 7.30 mmol, 99%); m.p. 57–58 °C (EtOAc/hexanes);  $R_f$  0.4 (20% EtOAc/petroleum ether);  $\nu_{\max}$  (neat) / cm<sup>-1</sup> 3348 (O–H stretch), 2973, 2932, 2813 (C–H stretch), 1725 (C=O stretch); <sup>1</sup>H NMR (301 MHz, CDCl<sub>3</sub>)  $\delta$  ppm 1.68 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 4.85 (2H, s, CH<sub>2</sub>), 7.25 – 7.29 (1H, m, 5-CH or 6-CH), 7.35 (1H, tt,  $J=7.8$ , 1.0 Hz, 6-CH or 5-CH), 7.60 (1H, s, 2-CH), 7.66 (1H, dq,  $J=7.8$ , 1.0 Hz, 4-CH or 7-CH), 8.15 (1 H, d,  $J=8.6$  Hz, 7-CH or 4-CH).

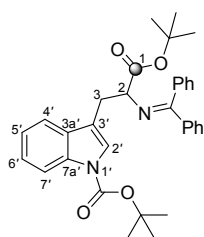
IR<sup>5</sup> and <sup>1</sup>H NMR<sup>6</sup> data are consistent with the literature.

*tert*-butyl 3-(bromomethyl)-1*H*-indole-1-carboxylate

Prepared according to the procedure of Takatori *et al.*<sup>7</sup> Phosphorus tribromide (132  $\mu$ l, 1.41 mmol) in DME (2 ml) was added dropwise over 10 min to a stirring solution of *tert*-butyl 3-(hydroxymethyl)-1*H*-indole-1-carboxylate **13** (453 mg, 1.83 mmol) in DME (6 ml) at 0 °C. This stirring solution was allowed to warm to room temperature slowly over 6 h, then quenched with water (5 ml) and poured into chloroform (50 ml). The resulting aqueous layer was extracted with chloroform (3  $\times$  50 ml) and the combined organic layers were washed with brine (50 ml), dried over MgSO<sub>4</sub> and concentrated *in vacuo* to approx. 3 ml total volume. This concentrated solution was loaded immediately on to a pre-prepared silica column (14% EtOAc/petroleum ether), eluted as rapidly as possible using this solvent mixture and concentrated *in vacuo* to white prisms (587 mg, 1.82 mmol, 99%). These were shielded from light and re-dissolved in the solvent for the following reaction as soon as possible;  $\nu_{\max}$  (neat) / cm<sup>-1</sup> 2977, 2966, 2830 (C–H stretch), 1725 (C=O stretch).

Spectrum is consistent with that in the literature.<sup>5</sup>

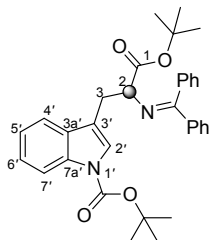
[1-<sup>13</sup>C]-*tert*-butyl 3-(3-(*tert*-butoxy)-2-((diphenylmethylene)amino)-3-oxopropyl)-1*H*-indole-1-carboxylate



Prepared by adaption of the procedure of Ku *et al.*<sup>8</sup> A solution of freshly prepared *tert*-butyl 3-(bromomethyl)-1*H*-indole-1-carboxylate (587 mg, 1.89 mmol), [1-<sup>13</sup>C]-*tert*-butyl 2-((diphenylmethylene)amino)acetate (542 mg, 1.82 mmol) and tetrabutylammonium iodide (150 mg, 406  $\mu$ mol) in CH<sub>2</sub>Cl<sub>2</sub> (6 ml) and toluene (14.5 ml) was cooled to 0 °C. Potassium hydroxide (0.82 ml 50% w/w aqueous solution) was added dropwise and the reaction stirred for 16 h while gradually warming to room temperature. The reaction mixture was poured into Et<sub>2</sub>O (100 ml) and water (100 ml), and the aqueous layer extracted (3  $\times$  100 ml Et<sub>2</sub>O). The combined organic layers were washed with brine (100 ml), dried over MgSO<sub>4</sub>, and concentrated *in vacuo* to a yellow oil (1.03 g), an approximately 1:1 mixture of starting material and product (634  $\mu$ mol starting material with 518  $\mu$ mol product). This mixture was resubmitted to the reaction conditions, stirring at room temperature for 72 h with *tert*-butyl 3-(bromomethyl)-1*H*-indole-1-carboxylate (216 mg, 697  $\mu$ mol), before repeating the aforementioned workup to give a yellow oil (549 mg). Purification by column chromatography (eluting 0.5  $\rightarrow$  10% Et<sub>2</sub>O / petroleum ether with 0.25% NEt<sub>3</sub>) yielded the title compound as a colourless oil (346 mg, 659  $\mu$ mol, 35%);  $R_f$  0.75 (1% Et<sub>2</sub>O/petroleum ether with 0.5% NEt<sub>3</sub>),  $\nu_{\max}$  (neat) / (neat) / cm<sup>-1</sup> 3056, 2978, 2932 (C–H stretch), 1731 (C=O stretch), 1689 (C=N stretch); <sup>1</sup>H NMR (400 MHz, CHCl<sub>3</sub>)  $\delta$  ppm 1.35 (9H, s, one of C(CH<sub>3</sub>)<sub>3</sub>), 1.50 (9H, s, one of C(CH<sub>3</sub>)<sub>3</sub>), 3.05 - 3.14 (1H, ddd,  $J$ =14.0, 8.4, 3.8 Hz, 3-CHH), 3.19 - 3.27 (1H, ddd,  $J$ =14.0, 3.8, 3.1 Hz, 3-CHH), 4.18 (1H, app. dt,  $J$ =8.4, 5.2 Hz, 2-CH), 6.65 (2H, d,  $J$ =7.2 Hz, ArCH), 6.98 (1 H, td,  $J$ =7.5, 0.8 Hz, ArCH), 7.08 - 7.26 (9H, m, ArCH), 7.46 - 7.53 (2H, m, ArCH), 8.02 (1H, d,  $J$ =8.0 Hz, ArCH); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  ppm 28.0 (d,  $J$ =1.6 Hz, ester C(CH<sub>3</sub>)<sub>3</sub>), 28.1 (carbamate C(CH<sub>3</sub>)<sub>3</sub>), 28.7 (d,  $J$ =1.6 Hz, 3-C), 66.0 (d,  $J$ =61.5 Hz, 2-C), 81.1 (d,  $J$ =3.1 Hz, ester C(CH<sub>3</sub>)<sub>3</sub>), 83.1 (carbamate C(CH<sub>3</sub>)<sub>3</sub>), 114.9 (7'-C), 116.9 (d,  $J$ =3.9 Hz, 3'-C), 119.1 (4'-C), 122.1 (5'-C), 124.0 (2'-C or 6'-C), 124.1 (6'-C or 2'-C), 127.5

(ArCH), 127.8 (ArCH), 128.0 (ArCH), 128.2 (ArCH), 128.7 (ArCH), 130.0 (ArCH), 130.6 (3a'-C), 136.0 (7a'-C), 139.4 (ArC × 2), 149.5 (NCO<sub>2</sub>*t*-Bu), 170.8 (1-C, enriched); *m/z* (ESI<sup>+</sup>) 548.3 [M + Na]<sup>+</sup> (5%), 526.3 [M + H]<sup>+</sup> (100%), 428.2 [M + 2 × H – CO<sub>2</sub>*t*-Bu]<sup>+</sup> (3%), 357.9 [M – CPh<sub>2</sub>]<sup>+</sup> (2%); HRMS calculated for C<sub>32</sub><sup>13</sup>CH<sub>37</sub>N<sub>2</sub>O<sub>4</sub> 526.2781 [M + H]<sup>+</sup> found 525.2772.

[2-<sup>13</sup>C]-*tert*-butyl 3-(3-(*tert*-butoxy)-2-((diphenylmethylene)amino)-3-oxopropyl)-1*H*-indole-1-carboxylate



Prepared by adaption of the procedure of Ku *et al.*<sup>8</sup> A solution of freshly prepared *tert*-butyl 3-(bromomethyl)-1*H*-indole-1-carboxylate (1.97 g, 6.35 mmol), [2-<sup>13</sup>C]-*tert*-butyl 2-((diphenylmethylene)amino)acetate (0.941 g, 3.18 mmol) and tetrabutylammonium iodide (150 mg, 406 μmol) in CH<sub>2</sub>Cl<sub>2</sub> (8 ml) and toluene (20 ml) was cooled to 0 °C. Potassium hydroxide (1.3 ml 50% w/w aqueous solution) was added dropwise and the reaction stirred for 16 h while gradually warming to room

temperature. The reaction mixture was poured into Et<sub>2</sub>O (100 ml) and water (100 ml), and the aqueous layer extracted (3 × 100 ml Et<sub>2</sub>O). The combined organic layers were washed with brine (100 ml), dried over MgSO<sub>4</sub>, and concentrated *in vacuo* to a yellow oil. Purification by column chromatography (eluting 0.5 → 10% Et<sub>2</sub>O / petroleum ether with 0.25% NEt<sub>3</sub>) yielded the title compound as a colourless oil (1.644 g, 3.128 mmol, 99%); R<sub>f</sub> 0.75 (1% Et<sub>2</sub>O/petroleum ether with 0.5% NEt<sub>3</sub>), *v*<sub>max</sub> (neat) / (neat) / cm<sup>-1</sup> 3056, 2978, 2932 (C–H stretch), 1731 (C=O stretch), 1689 (C=N stretch); <sup>1</sup>H NMR (400 MHz, CHCl<sub>3</sub>) δ ppm 1.45 (9H, s, one of C(CH<sub>3</sub>)<sub>3</sub>), 1.61 (9H, s, one of C(CH<sub>3</sub>)<sub>3</sub>), 3.19 (1H, ddd, *J*=14.5, 8.3, 5.5 Hz, 3-*CHH*), 3.33 (1H, app. dt, *J*=14.5, 4.9 Hz, 3-*CHH*), 4.27 (1H, ddd, *J*=136.6, 8.3, 5.0 Hz, 2-CH), 6.75 (2H, d, *J*=7.8 Hz, ArCH), 7.04 - 7.11 (1H, m, ArCH), 7.17 - 7.65 (9H, m, ArCH), 7.79 - 7.84 (2H, m, ArCH), 8.11 (1H, d, *J*=7.9 Hz, ArCH); <sup>13</sup>C NMR (126 MHz, CHCl<sub>3</sub>) δ ppm 28.0 (one of C(CH<sub>3</sub>)<sub>3</sub>), 28.2 (one of C(CH<sub>3</sub>)<sub>3</sub>), 28.8 (d, *J*=37.2 Hz, 3-C), 66.0 (2-C, enriched), 81.2 (one of C(CH<sub>3</sub>)<sub>3</sub>), 83.2 (one of C(CH<sub>3</sub>)<sub>3</sub>), 114.95 (7'-C), 116.95 (3'-C), 119.13 (4'-C), 122.19 (5'-C), 124.08 (2'-C or 6'-C), 124.18 (6'-C or 2'-C), 127.8 (ArCH), 128.0 (ArCH), 128.2 (ArCH), 128.7 (ArCH), 130.0 (ArCH), 130.6 (3a'-C), 136.0 (7a'-C), 139.5 (ArC × 2), 149.62 (NCO<sub>2</sub>*t*-Bu), 170.43 (Ph<sub>2</sub>C=N), 170.84 (1-C, d, *J*=62.0 Hz); *m/z* (ESI<sup>+</sup>) 548.3 [M + Na]<sup>+</sup> (8%), 526.3 [M + H]<sup>+</sup> (100%), 428.2 [M + 2 × H – CO<sub>2</sub>*t*-Bu]<sup>+</sup> (5%), 357.9 [M – CPh<sub>2</sub>]<sup>+</sup> (2%); HRMS calculated for C<sub>32</sub><sup>13</sup>CH<sub>37</sub>N<sub>2</sub>O<sub>4</sub> 526.2781 [M + H]<sup>+</sup> found 525.2771.

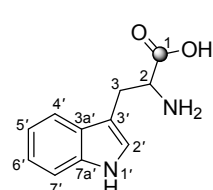
[2-<sup>13</sup>C]-*tert*-Butyl 3-(2-amino-3-(*tert*-butoxy)-3-oxopropyl)-1*H*-indole-1-carboxylate

[2-<sup>13</sup>C]-*tert*butyl 3-(3-(*tert*-butoxy)-2-((diphenylmethylene)amino)-3-oxopropyl)-1*H*-indole-1-carboxylate (1.6442 g, 3.128 mmol) was added to a mixture of THF (48 ml), water (48 ml) and citric acid (0.924 g, 4.81 mmol). The mixture was stirred rapidly for 17 h, then saturated NaHCO<sub>3(aq)</sub> (50 ml) was added. Once effervescence had subsided, the mixture was poured into Et<sub>2</sub>O (250 ml) and water (250 ml). The organic layer was washed with water (3 × 100 ml), brine (100 ml), then dried over MgSO<sub>4</sub>. The colourless oil obtained after removal of solvent *in vacuo* was purified by column chromatography, eluting benzophenone with 14% EtOAc/petroleum ether, and the title compound with 4% MeOH/CH<sub>2</sub>Cl<sub>2</sub> (0.9034 g, 2.50 mmol, 80%); R<sub>f</sub> 0.5 (4% MeOH/CH<sub>2</sub>Cl<sub>2</sub>), *v*<sub>max</sub> (neat) / (neat) / cm<sup>-1</sup> 3383, 2978, 2933 (C–H stretch), 1726



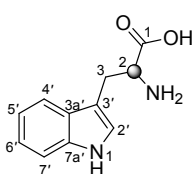
(overlapping C=O stretches);  $\nu_{\max}$  (neat) / (neat) /  $\text{cm}^{-1}$  3383 (N–H stretch), 2978, 2933 (C–H stretch), 1726 (C=O stretch);  $^1\text{H}$  NMR (400 MHz,  $\text{CHCl}_3$ )  $\delta$  ppm 1.45 (9H, s, one of  $\text{C}(\text{CH}_3)$ ), 1.55 (2H, br. s.,  $\text{NH}_2$ ), 1.66 (9H, s, one of  $\text{C}(\text{CH}_3)$ ), 2.92 (1H, dddd,  $J=14.3, 7.8, 5.4, 0.7$  Hz, 3- $\text{CHH}$ ), 3.18 (1H, app. dtd,  $J=14.3, 4.8, 1.0$  Hz, 3- $\text{CHH}$ ), 3.72 (1H, ddd,  $J=139.7, 7.7, 5.4$  Hz, 2- $\text{CH}$ ), 7.25 (1H, ddd,  $J=7.7, 7.2, 1.0$  Hz, 5'- $\text{CH}$  or 6'- $\text{CH}$ ), 7.32 (1H, ddd,  $J=8.3, 7.3, 1.3$  Hz, 6'- $\text{CH}$  or 5'- $\text{CH}$ ), 7.46 (1H, br. s, 2'- $\text{CH}$ ), 7.59 (1H, ddd,  $J=7.8, 1.3, 0.7$  Hz, 4'- $\text{CH}$ ), 8.15 (1 H, d,  $J=6.4$  Hz, 7'- $\text{CH}$ );  $^{13}\text{C}$  NMR (101 MHz,  $\text{CHCl}_3$ )  $\delta$  ppm 28.0 (one of  $\text{C}(\text{CH}_3)_3$ ), 28.2 (one of  $\text{C}(\text{CH}_3)_3$ ), 30.5 (d,  $J=35.0$  Hz, 3-C), 55.1 (2-C, enriched), 81.2 (one of  $\text{C}(\text{CH}_3)_3$ ), 83.4 (one of  $\text{C}(\text{CH}_3)_3$ ), 115.2 (7'-C), 116.4 (3'-C), 119.0 (4'-C), 122.4 (5'-C), 123.9 (2'-C or 6'-C), 124.4 (6'-C or 2'-C), 130.5 (3a'-C), 135.5 (7a'-C), 149.5 ( $\text{NCO}_2t\text{-Bu}$ ), 174.3 (d,  $J=59.2$  Hz, 1-C);  $m/z$  ( $\text{ESI}^+$ ) 384.2 [ $\text{M} + \text{Na}$ ] $^+$  (64%), 362.2 [ $\text{M} + \text{H}$ ] $^+$  (100%), 428.2 [ $\text{M} + 2 \times \text{H} - \text{CO}_2t\text{-Bu}$ ] $^+$  (15%); HRMS calculated for  $\text{C}_{19}^{13}\text{CH}_{29}\text{N}_2\text{O}_4$  362.2155 [ $\text{M} + \text{H}$ ] $^+$  found 362.2155.

### [1- $^{13}\text{C}$ ]-tryptophan



[1- $^{13}\text{C}$ ]-*tert*-butyl 3-(3-(*tert*-butoxy)-2-((diphenylmethylene)amino)-3-oxopropyl)-1*H*-indole-1-carboxylate (269 mg, 512  $\mu\text{mol}$ ) was stirred at 0  $^\circ\text{C}$  for 3 h in trifluoroacetic acid (2 ml). After stirring for a further 16 h at room temperature, the deep brown mixture was poured into  $\text{CH}_2\text{Cl}_2$  (40 ml) and 1 M  $\text{HCl}_{(\text{aq})}$ . The organic layer was extracted with a further 40 ml 1 M  $\text{HCl}_{(\text{aq})}$  and the combined aqueous layers were concentrated *in vacuo* to a purple solid. This was purified to a white solid (39.7 mg, 193  $\mu\text{mol}$ , 38%) by ion-exchange chromatography (Dowex 50WX8 resin). This was impure by  $^1\text{H}$  and  $^{13}\text{C}$  NMR, but the impurity was not identified or removed. Data of title compound obtained from mixture:  $\nu_{\max}$  (neat) /  $\text{cm}^{-1}$  3399, 3122, 3037 (overlapping C–H, N–H and O–H stretches), 1766 (C=O stretch);  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  ppm 3.05 (1H, dd,  $J=14.5, 8.0$  Hz, 3- $\text{CHH}$ ), 3.29 (1H, app. br. d,  $J=14.5$  Hz, 3- $\text{CHH}$ ), 3.53 (1H, app. br. s., 2- $\text{CH}$ ), 6.96 (1H, br. t,  $J=7.3$  Hz, one of 5'- $\text{CH}$  or 6'- $\text{CH}$ ), 7.05 (1H, br. t,  $J=7.3$  Hz, one of 6'- $\text{CH}$  or 5'- $\text{CH}$ ), 7.25 (1H, br. s., one of 4'- $\text{CH}$  or 7'- $\text{CH}$ ), 7.34 (1H, d,  $J=7.3$  Hz, one of 7'- $\text{CH}$  or 4'- $\text{CH}$ ), 11.07 (1H, br. s., indole-NH);  $^{13}\text{C}$  NMR (126 MHz,  $\text{DMSO}-d_6$ )  $\delta$  ppm 26.8 (3-C), 54.4 (d,  $J=51.8$  Hz, 2-C), 111.4 (3'-C), 118.3 (4'-C or 5'-C), 118.4 (5'-C or 4'-C), 120.9 (6'-C), 124.3 (2'-C), 127.3 (3a'-C), 136.3 (7a'-C), 171.0 (1-C, enriched);  $m/z$  ( $\text{ESI}^+$ ) 370.2 [ $2 \times \text{M} - 2 \times \text{H}_2\text{O}$ ] (100%), 206.1 [ $\text{M} + \text{H}$ ] $^+$  (25%); HRMS calculated for  $\text{C}_{10}^{13}\text{CH}_{13}\text{N}_2\text{O}_2$  206.1005 [ $\text{M} + \text{H}$ ] $^+$  found 206.1011.

### [2- $^{13}\text{C}$ ]-tryptophan



[2- $^{13}\text{C}$ ]-*tert*-Butyl 3-(2-amino-3-(*tert*-butoxy)-3-oxopropyl)-1*H*-indole-1-carboxylate (0.827 g, 2.29 mmol) was stirred with  $\text{ZnBr}_2$  (3.09 g, 13.7 mmol) in  $\text{CH}_2\text{Cl}_2$ . After 6 h, water (70 ml) was added. After stirring for a further 16 h, the layers were separated. The aqueous layer was concentrated *in vacuo* to approx. 10 ml, then purified by ion-exchange chromatography (Dowex 50WX8 resin). The title compound was isolated as off-white prisms (0.1954 g, 952  $\mu\text{mol}$ , 42%), and an analytical sample prepared by recrystallisation from 1:1  $\text{AcOH}:\text{H}_2\text{O}$ ;<sup>9</sup> m.p. 286-

288 °C (dec.) [lit. 289-290 °C, dec.];  $\nu_{\max}$  (neat) /  $\text{cm}^{-1}$  3401, 3124, 3034 (overlapping C–H, N–H and O–H stretches), 1764 (C=O stretch);  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  ppm 2.93 (1H, ddd,  $J=15.0, 8.5, 4.7$  Hz, 3-CHH), 3.22 - 3.40 (4.5 H, m, overlapping 3-CHH, half of 2-CH,  $\text{NH}_2$ , COOH), 3.54 (0.5H, app. dd,  $J=9.1, 3.9$  Hz, half of 2CH), 6.98 (1H, ddd,  $J=8.0, 7.0, 1.0$  Hz, 6'-CH), 7.06 (1H, ddd,  $J=8.0, 7.0, 1.1$  Hz, 5'-CH), 7.19 (1H, d,  $J=2.2$  Hz, 2'-CH), 7.34 (1H, d,  $J=8.0$  Hz, 7'-CH), 7.55 (1H, d,  $J=8.0$  Hz, 4'-CH), 10.86 (1H, s, indole NH);  $^{13}\text{C}$  NMR (126 MHz,  $\text{DMSO-}d_6$ )  $\delta$  ppm 27.2 (d,  $J=35.3$  Hz, 3-C), 54.8 (2-C, enriched), 109.7 (d,  $J=1.9$  Hz, 3'-C), 111.3 (7'-C), 118.2 (4'-C or 5'-C), 118.3 (5'-C or 4'-C), 120.9 (6'-C), 123.9 (2'-C), 127.2 (3a'-C), 136.3 (7a'-C), 172.0 (app. s, 1-C);  $m/z$  (ESI<sup>+</sup>) 228.1 [ $\text{M} + \text{Na}$ ]<sup>+</sup> (100%), 206.1 [ $\text{M} + \text{H}$ ]<sup>+</sup> (99%); HRMS calculated for  $\text{C}_{10}^{13}\text{CH}_{13}\text{N}_2\text{O}_2$  206.1005 [ $\text{M} + \text{H}$ ]<sup>+</sup> found 206.1010.

Feeding of *F. sacchari* cultures with [1,2- $^{13}\text{C}_2$ ]acetate, [U- $^{13}\text{C}_3$ ]glycerol, [4- $^{13}\text{C}$ ]aspartate, and [1- $^{13}\text{C}$ ]- and [2- $^{13}\text{C}$ ]-tryptophans

Using the same procedure for culturing *F. sacchari* as described above, the production of fusarochromene was detected by taking a sample from the liquid culture, centrifuged and the supernatant was analyzed on LCMS.

Sodium [1,2- $^{13}\text{C}_2$ ]acetate, dissolved in sterile water, (100 mg  $\text{mL}^{-1}$ ), was added to *F. sacchari* cultures (1 mL aliquots) two and four days after inoculation to a final concentration of 2 g  $\text{L}^{-1}$  (24 mM). The culture was incubated for 8 days, filtered and then extracted. Fusarochromene (3 mg) was isolated by flash chromatography and analysed by  $^{13}\text{C}$  NMR (Figure S4).

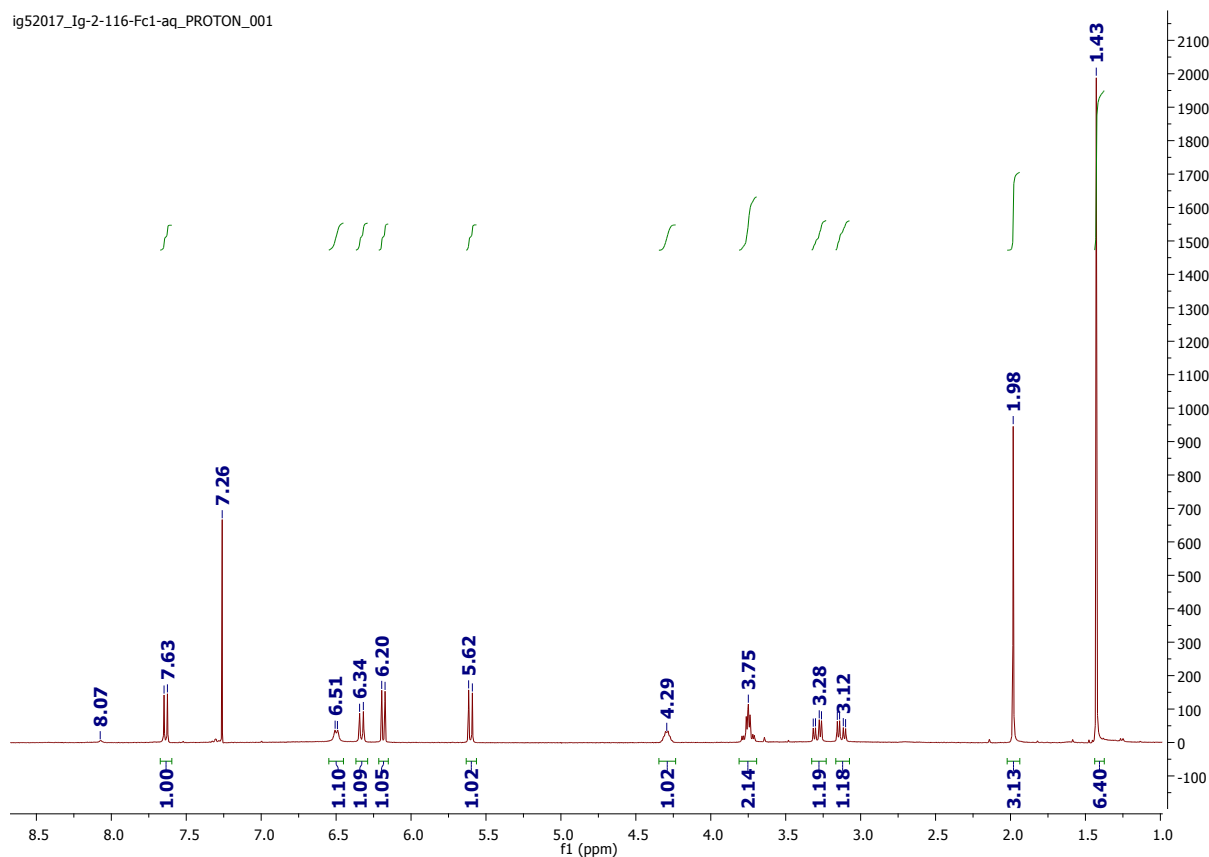
[U- $^{13}\text{C}_3$ ] Glycerol, as a stock solution (200 mg  $\text{mL}^{-1}$  in sterile water) was added to cultures (250  $\mu\text{L}$  aliquots) of *F. sacchari* 1 and 3 days after inoculation to a final culture concentration of 1 g  $\text{L}^{-1}$  (11 mM). The cultures were incubated for 8 days. The resulting crude extract was qualitatively analysed by HPLC and a low titre of fusarochromene was observed, although in this case the low titre was observed in both labelled sample and control sample. Preparative HPLC was used to isolate the fusarochromene (0.8 mg) which was analysed by  $^{13}\text{C}$  NMR. The resulting spectrum (Figure S5) was compared to a reference spectrum and it was found that resonances corresponding to all but two carbons showed evidence of intact incorporation

[4- $^{13}\text{C}$ ] Aspartate was added to cultures of *F. sacchari* 1, 3 and 5 days after inoculation to a final culture concentration of 150 mg  $\text{L}^{-1}$  (1.1 mM). Cultures were incubated for 8 days. Fusarochromene (1 mg), isolated from the culture extract was analysed by  $^{13}\text{C}$  NMR and the resulting spectrum compared to a reference spectrum, however isotopic enrichment of  $^{13}\text{C}$  was not observed suggesting that C4 of aspartate was not the biosynthetic precursor to C11 of fusarochromene.

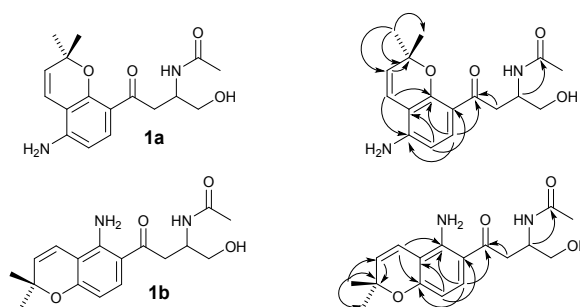
[1- $^{13}\text{C}$ ]-tryptophan (12 mg) was dissolved in 200  $\mu\text{L}$  DMSO and each fed to one flask on day 5 after inoculation. The control and labelled substrate fed flasks were kept shaking for further 7 days at 28 °C and 250 rpm. On day 12, both flasks were extracted and the crude extracts were dried and screened on LCMS. [14- $^{13}\text{C}$ ]-Fusarochromene (0.8mg) was isolated by preparative HPLC and analysed by  $^{13}\text{C}$ -NMR which showed a twenty-five fold  $^{13}\text{C}$  enrichment at C-14 (64.7 ppm) exclusively (Figure S6). Similarly [2- $^{13}\text{C}$ ]-

tryptophan (120 mg) was dissolved in 2 mL DMSO and fed to 5 flasks (each flask 400  $\mu$ L), on day 4 of inoculation. The control and labelled substrate fed flasks were kept shaking for further 8 days at 28  $^{\circ}$ C and 250 rpm. On day 12, all flasks were extracted and [ $^{13}$ C]-fusarochromene (6.3 mg) isolated and analysed as before. A 100 fold enrichment of C-13 (49.7 ppm) exclusively was observed (Figure S7)

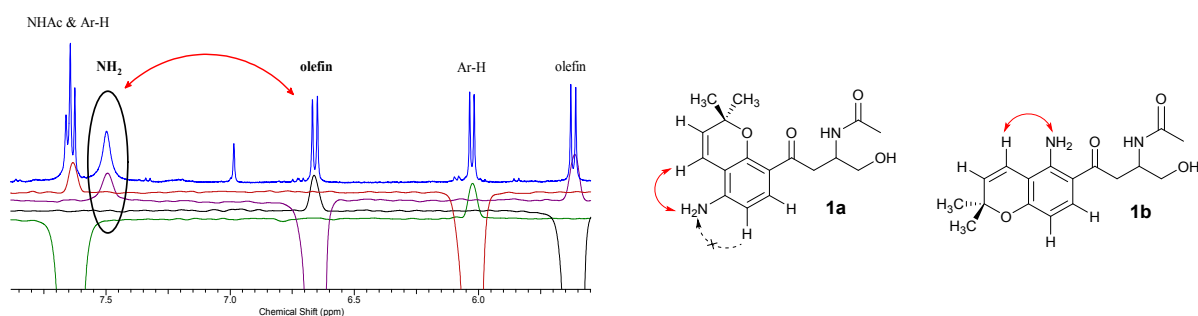
ig52017\_Ig-2-116-Fc1-aq\_PROTON\_001



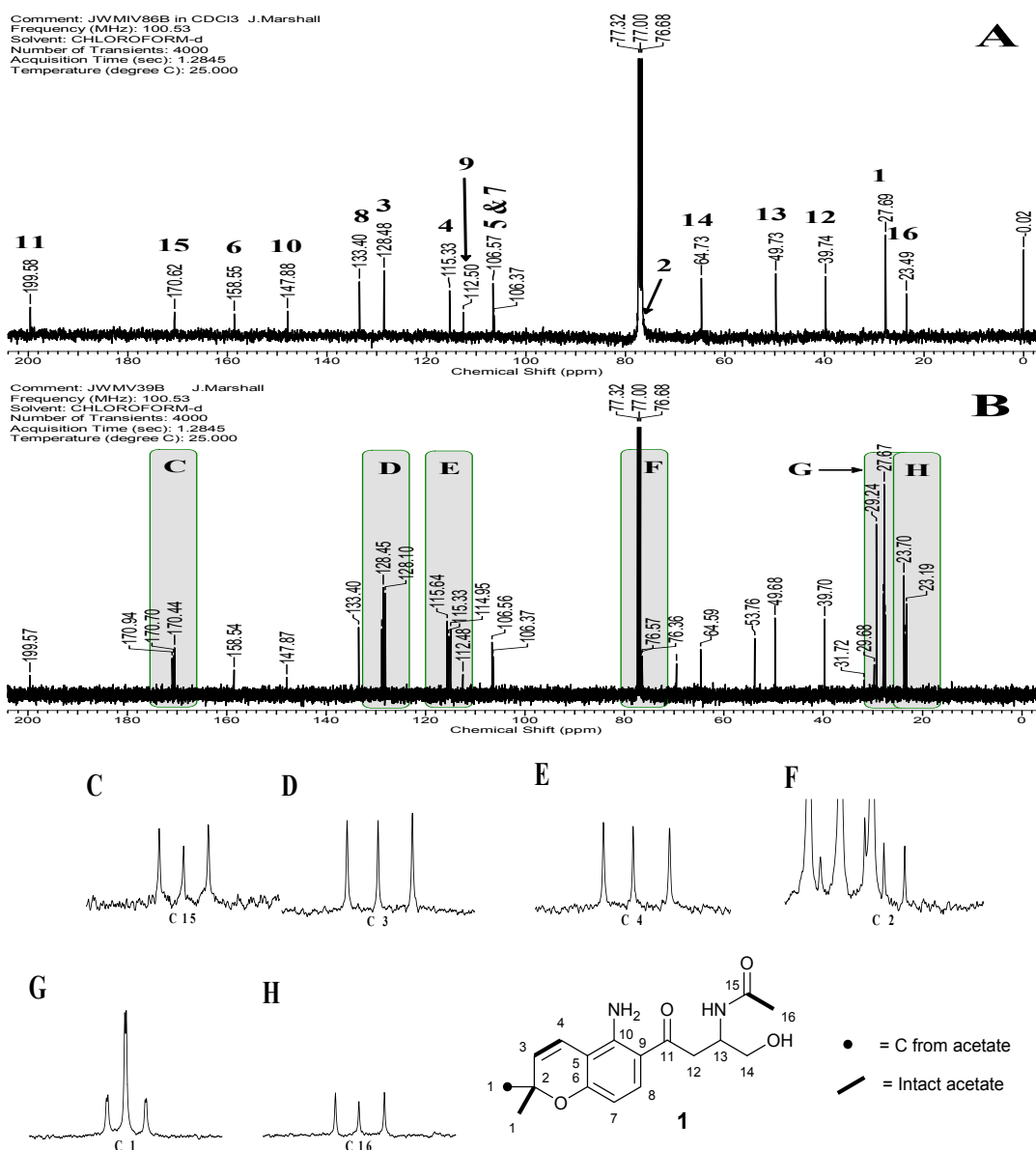
**Figure S1:**  $^1$ H NMR spectrum of fusarochromene **1**



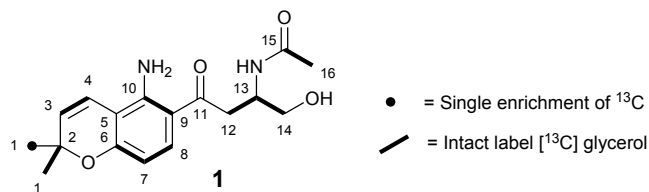
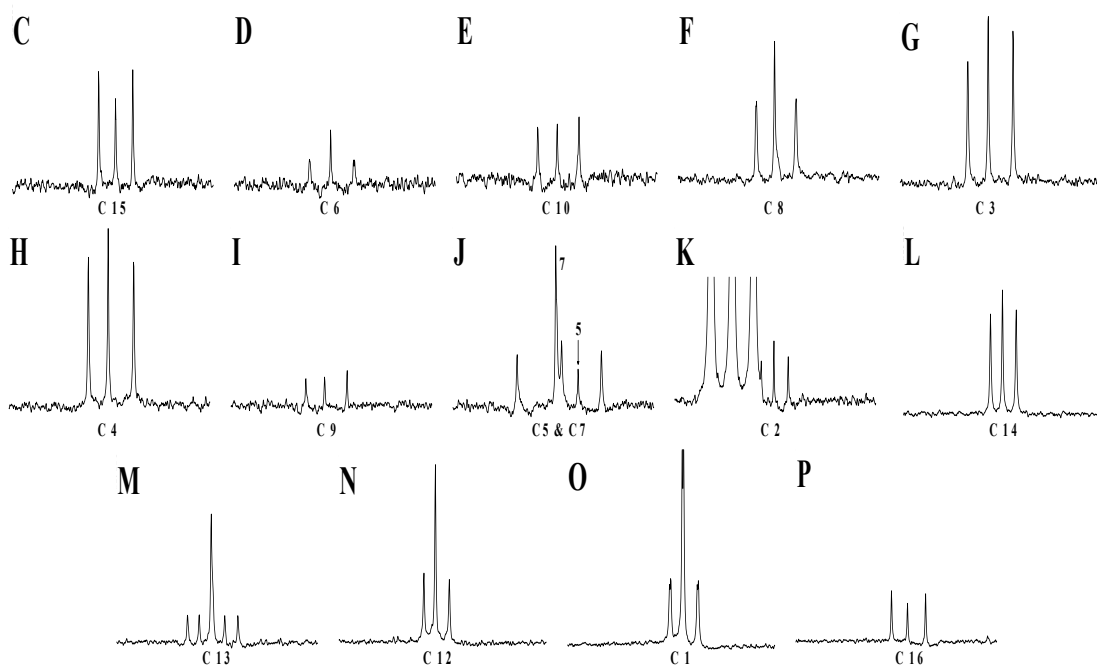
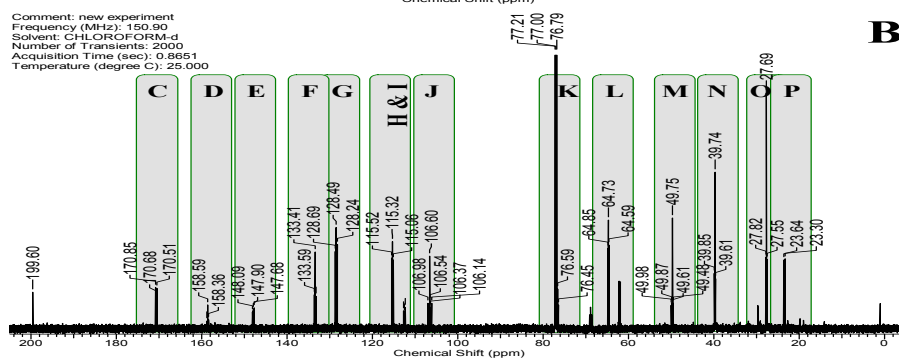
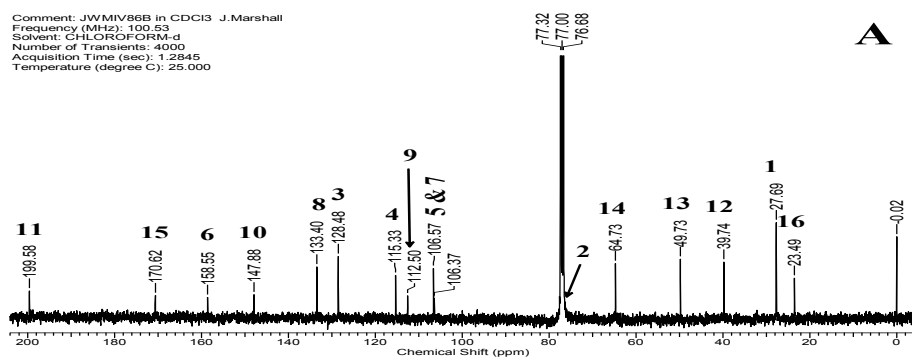
**Figure S2:** Proposed structures of chromene **1a** and **1b** (left) and key HMBC correlations related to each isomer (right).



**Figures S3:** Key nOe between olefin and  $\text{NH}_2$  protons in compound B, irradiations of olefinic and aromatic peaks overlaid with  $^1$ H NMR, irradiated resonance indicated by large negative peak in each case.



**Figure S4:** A:  $^{13}\text{C}$  NMR of fusarochromene **1**. B:  $^{13}\text{C}$  NMR of fusarochromene **1** from [1,2- $^{13}\text{C}_2$ ] acetate labelling study with expansions of resonances corresponding to C15 (C), C3 (D), C4 (E), C2 (F), C1 (G) and C16 (H)



**Figure S5:** A:  $^{13}\text{C}$  NMR of fusarochromene **1**. B:  $^{13}\text{C}$  NMR of fusarochromene **1** from  $[\text{U-}^{13}\text{C}_3]$  glycerol labelling study with expansions of resonances corresponding to C15 (C), C6 (D), C10 (E), C8 (F), C3 (G), C4 (H), C9 (I), C5&7 (J), C2 (K), C14 (L), C13 (M), C12 (N), C1 (O) and C16 (P)

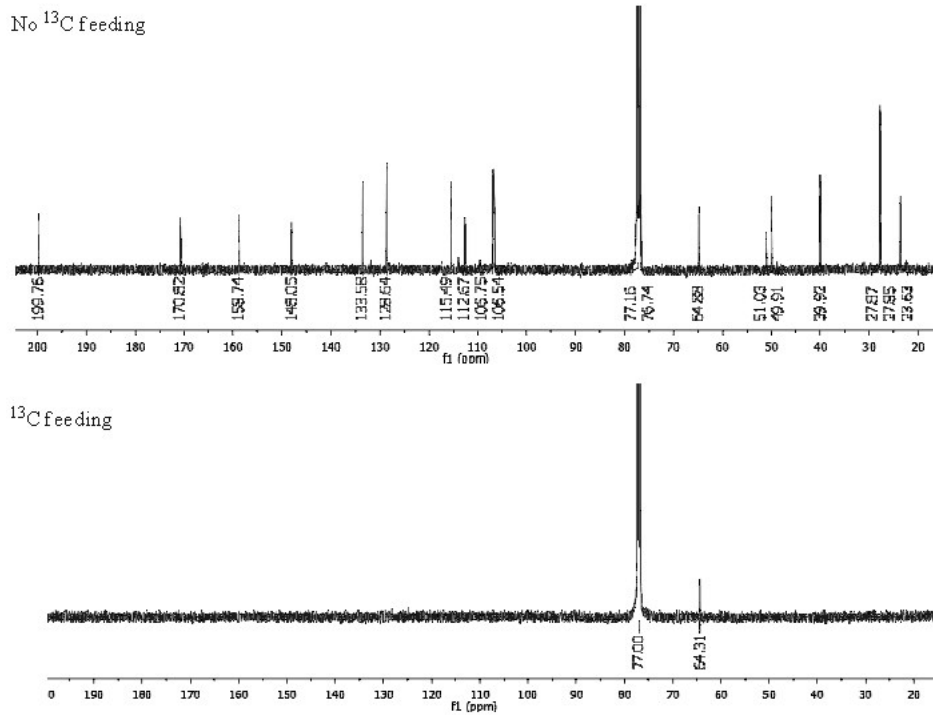


Figure S6: Overlay of  $^{13}\text{C}$ -NMR spectra of fusarochromene **1** after feeding experiment with  $[1-^{13}\text{C}]$ -tryptophan

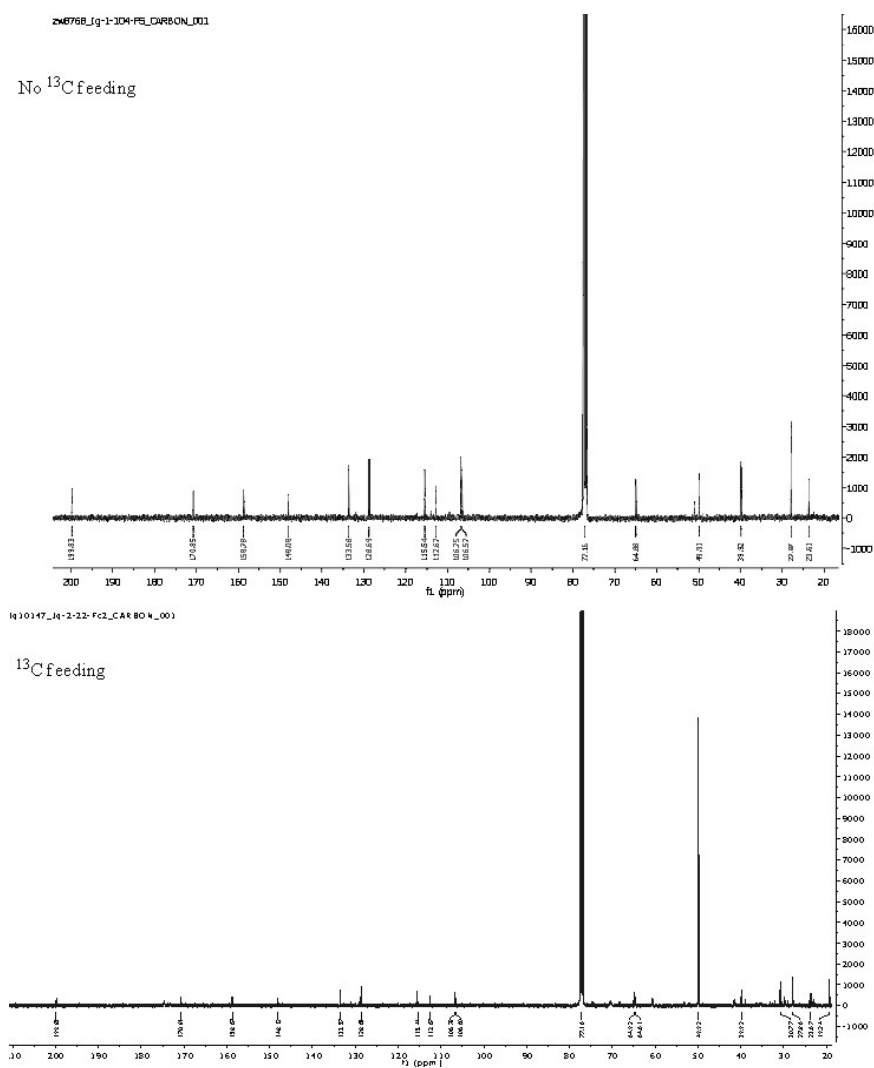


Figure S7: Overlay of  $^{13}\text{C}$ -NMR spectra of fusarochromene **1** after feeding experiment with  $[2-^{13}\text{C}]$ -tryptophan

## Bioinformatics

To search for a putative fusarochromanone biosynthetic gene cluster (BGC), the publicly available genome of *F. equiseti* isolate D25-1 (Assembly ASM331317v1) was downloaded and used to build a Multigeneblast<sup>10</sup> database. The genome was then searched for any region containing homologues to both *cpaD* (a DMATS-type prenyltransferase from the cyclopiazonic acid pathway; accession number F5HN73) and a tryptophan 2,3 dioxygenase (from *Spathaspora passalidarum*: accession number XP\_007377207) within 50 kb.

A locus containing both a putative prenyltransferase and tryptophan 2,3 dioxygenase was identified on scaffold 3 (nt 5400625 – nt 5423586). This region was annotated using a combination of FGeneSH,<sup>11</sup> alignments with homologues from the SwissProt and nr database of NCBI, manual annotation in Artemis<sup>12</sup> and the identification of conserved protein domains using Interpro<sup>13</sup> from EMBL. The boundaries of the putative BGC were predicted based on the presence of conserved housekeeping genes upstream and downstream of the locus. The predicted full BGC is shown in Figure S8. The predicted proteins encoded by the putative fusarochromanone BGC are summarised in Table S1.



**Figure S8:** A putative fusarochromanone BGC identified in the genome of *Fusarium equiseti*. Predicted gene functions shown in Table S1. Red: NRPS-like enzyme. Orange: putative regulator. Yellow: catalytic enzyme.

**Table S1:** Predicted functions for genes located within the putative fusarochromanone BGC. Homologues were identified by searching the Swissprot database using each predicted protein sequence as a query (BLASTp). Domains were identified using InterPro.

Gene	Putative function	Homologue (Accession number)	Identity	E value	Query coverage	Score	Domains
<i>fscA</i>	NRPS-like oxidoreductase	apmA <sup>14</sup> (A0A1W6BT53)	35.19 %	0.0	99 %	590	Adenylation (IPR010071) PCP (IPR009081) NAD(P)-binding (IPR036291)/Thioester reductase (COG3320)*
<i>fscB</i>	Putative transcription factor	AurF <sup>15**</sup> (A0A0M4LAF6)	38.76 %	1e <sup>-32</sup>	36 %	141	-
<i>fscC</i>	NRPS-like Tryptophan epimerase	IvoA <sup>16, 17</sup> (C8V7P4.1)	36.34 %	0.0	98 %	805	Adenylation (IPR010071) ACP (IPR036736) Condensation (IPR001242)/ Epimerisation (cd19534)
<i>fscD</i>	Tryptophan 2,3-dioxygenase	IDO <sup>18</sup> (P47125)	33.10 %	5e <sup>-71</sup>	98 %	232	Indoleamine 2,3-dioxygenase (IPR000898)
<i>fscE</i>	P450 (tryptophan 6-hydroxylase)	IvoC <sup>16</sup> (C8V7P3)	55.17 %	0.0	93 %	545	Cytochrome P450 (IPR001128)
<i>fscF</i>	P450	Apf <sup>19</sup> (S0DPM1)	32.40 %	5e <sup>-80</sup>	98 %	262	Cytochrome P450 (IPR001128)
<i>fscG</i>	DMATS-type prenyltransferase	Xpt <sup>20</sup> (Q5AY46)	36.14 %	2e <sup>-68</sup>	92 %	226	Aromatic prenyltransferase, DMATS-type (IPR017795)
<i>fscH</i>	Kynurenine formamidase-like hydrolase	KFA <sup>21</sup> (Q04066)	29.43 %	8e <sup>-17</sup>	90 %	81.3	Alpha/Beta hydrolase fold (IPR029058)
<i>fscI</i>	Oxidoreductase	TIC32 <sup>22</sup> (A2RVM0)	33.33 %	6e <sup>-31</sup>	94 %	119	SDR (IPR002347) NAD(P) binding (IPR036291)
<i>fscJ</i>	Aromatic peroxidase/ chloroperoxidase	dotB <sup>23</sup> (M2XZY2.1)	35.27 %	2e <sup>-78</sup>	93 %	252	Chloroperoxidase (IPR000028)

\* The domain architecture seen in *FscA* is typical of fungal oxidoreductases such as the well characterised fungal alpha aminoacidate reductases involved in lysine biosynthesis.<sup>24, 25</sup>

\*\* The predicted protein *FscB* does not align particularly well with any characterised proteins, and has no detectable domain, but a portion of the protein does align with *AurF* from the aurovertin gene cluster of *Calcarisporium arbuscula*. *AurF* was annotated as a transcription factor due to a total loss of production upon deletion and a proven downregulation of at least 5 genes in the cluster.<sup>15</sup>

The genome was also searched for any putative *N*-acetyltransferases. There are none apparent near the *fsc* BGC, but multiple predicted proteins containing the *N*-acetyltransferase superfamily domain were located in the genome. One such protein, located on scaffold 7 has the Acyl-CoA *N*-acyltransferase domain (IPR016181) and does demonstrate some homology with Ypa3 (29.3 % protein identity – see Figure S9). Ypa3 (Accession number P39979) is an *N*-acetyltransferase from yeast which specifically acetylates D amino acids, but has very relaxed substrate specificity.<sup>26</sup>

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Another putative *N*-acetyltransferase, encoded by a gene on scaffold 5, has the Acyl-CoA *N*-acetyltransferase domain (IPR016181) and, perhaps interestingly, is located next to an oxidoreductase which has a D amino acid deaminating domain (DadA domain: COG0665).

```

Sc07      -----MPAGRLIQELADYEKEPDANKATVETLQATIAFAPSDSPNADASVIPATEPI      52
Ypa3      MSNEEPEKVMVNDRIIVVKAI----EPKDEEAWNKLWKEYQGFQKTVMPPEVAT-TTFARFI      55
          *  .*: :  :      **: :*: :  :  .* : *  *:  :. *

Sc07      SPT-KPARCLLLISPEGQAVGMALYFNYSTWRSRAGIYLEDLYVRESEKGYGKLLS      111
Ypa3      DPTVKLWGALAFDTEGDAIGFAHYLNHLTSHVVEEVYMNLDLYVTERARVKGVGRKLE      115
          .** *  .* : :  *:***: * : : :*: .  :*:***** *  * ** *:**:.

Sc07      TLAQVIAIDGARLDWVVLKWNPEPSIKFYESIG-----AYRMNDWVGMVRVDNEGLNKLA      165
Ypa3      FVYSRADELGTPAVYVWTDHYNHRAQLLYTKVAYKTDKVLKRYG-----          161
          :  :.  :.  :  **: :*: :  : *  :.  :  * : *.:

Sc07      SLTD 169
Ypa3      ---- 161

```

**Figure S9:** An alignment of a putative *N*-acetyltransferase from *F. equiseti* (Sc07) with the D-amino acid *N*-acetyltransferase Ypa3 from *S. cerevisiae* (Accession number P39979)

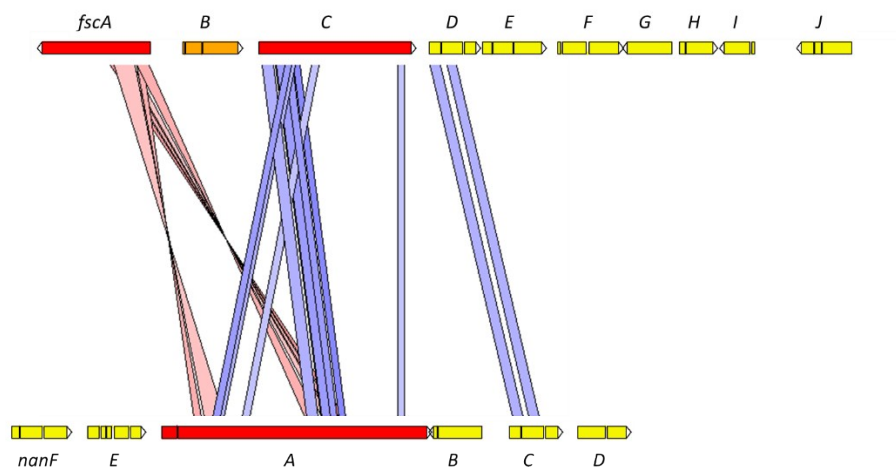
## Comparative Bioinformatics

The putative fusarochromene BGC was compared to the known quinolactacin<sup>28</sup> and nanagelenin<sup>29</sup> gene clusters using ACT (Artemis Comparison Tool)<sup>30</sup> (Figures S10-S11), and this highlighted a relative lack of homology between the clusters. Homology was detected between the encoded NRPS and NRPS-like enzymes, particularly within the A and T domains. However, the level of protein identity (%) was relatively low (Figure S12). Additionally, the domain architecture of these enzymes vary significantly (Figure S13), which reflects their varied catalytic activities; e.g. epimerisation, cyclisation and reduction.

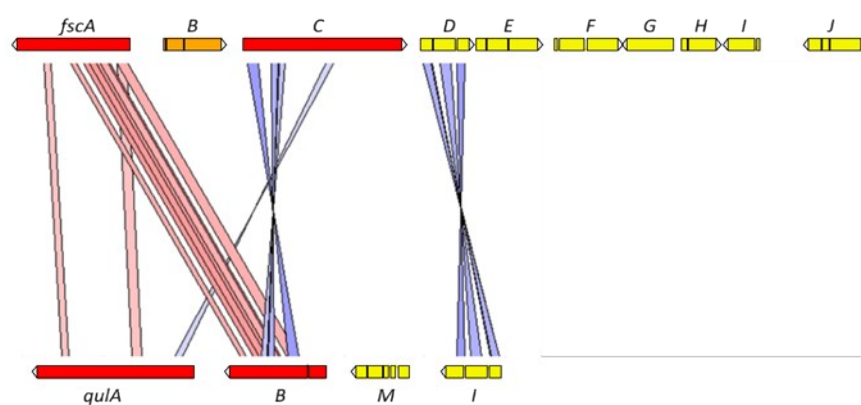
The three clusters all contain an indoleamine 2,3-dioxygenase (IDO), which is consistent with the key oxidative cleavage of the pyrrole ring required for all pathways. An alignment of the three IDOs Qull, NanC and FscD can be seen in Figure S14.

The ACT analysis did not detect homology between the encoded prenyltransferases FscG and NanD. An alignment using Clustal Omega<sup>31</sup> (Figure S15) showed that the two proteins do align, but the protein identity is only 22.8%.





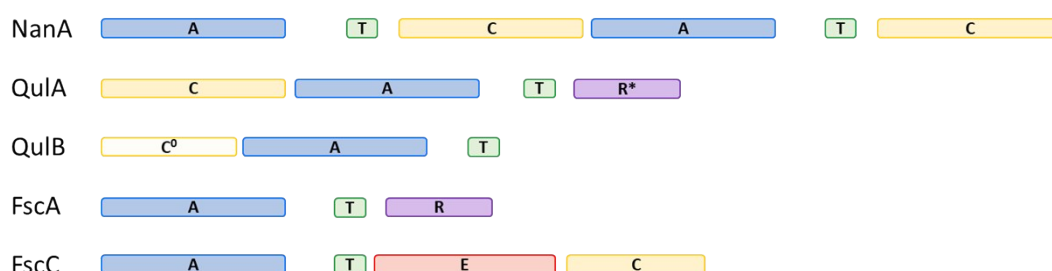
**Figure S10:** An ACT (Artemis Comparison Tool)<sup>30</sup> comparison of the putative fusarochromene (*fsc*) cluster and the nanangelenin (*nan*) cluster, highlights homology between the IDOs FscD and NanC, and the A and T domains of NRPS and NRPS-like proteins (in red). Homology was identified using the tBlastx algorithm.



**Figure S11:** An ACT (Artemis Comparison Tool)<sup>30</sup> comparison of the putative fusarochromene (*fsc*) cluster and the quinolactacin (*qu*) cluster, highlights homology between the IDOs FscD and QuI, and the A and T domains of NRPS and NRPS-like proteins (in red). Homology was identified using the tBlastx algorithm.

	QuIA	FscC	FscA	QuIB	NanA
QuIA	100.00	22.29	22.65	23.27	22.09
FscC	22.29	100.00	25.62	30.61	24.83
FscA	22.65	25.62	100.00	33.49	26.88
QuIB	23.27	30.61	33.49	100.00	32.49
NanA	22.09	24.83	26.88	32.49	100.00

**Figure S12:** A percentage identity matrix for the NRPS and NRPS-like proteins from the *fsc qu* and *nan* clusters, showing relatively low homology. Most similarity is observed within the conserved regions of the A and T domains. Produced using Clustal Omega.<sup>31</sup>



**Figure S13:** Domain architecture of NRPS and NRPS-like enzymes encoded by the *nan*, *qu* and *fus* clusters. C<sup>0</sup>, truncated condensation; C, condensation; A, adenylation; T, thiolation; R\*, Dieckmann cyclization; R, reduction; E, epimerisation.



## X-Ray Crystallography

X-ray diffraction experiments on (*R*)-*p*-bromobenzoyl-fusarochromene were carried out at 100(2) K Bruker Microstar rotating anode diffractometer using Cu-K $\alpha$  ( $\lambda = 1.54178 \text{ \AA}$ ). Intensities were integrated in SAINT<sup>32</sup> and absorption corrections based on equivalent reflections were applied using SADABS.<sup>33</sup> The structure was solved using ShelXS<sup>34</sup> and refined by full matrix least squares against  $F^2$  in ShelXL<sup>34, 35</sup> using Olex2.<sup>36</sup> All of the non-hydrogen atoms were refined anisotropically. While all of the hydrogen atoms were located geometrically and refined using a riding model, apart from the N-H protons which were located in the difference map and refined with isotropic displacement parameters  $U_{iso}(H) = 1.2U_{eq}(N)$ . Crystal structure and refinement data are given in Table 1. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 2022413. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax(+44) 1223 336033, e-mail: deposit@ccdc.cam.ac.uk].

**Table S2: Crystal data and structure refinement for 2.**

Identification code	JWMVI60A
Empirical formula	C <sub>24</sub> H <sub>25</sub> BrN <sub>2</sub> O <sub>5</sub>
Formula weight	501.37
Temperature/K	100(2)
Crystal system	orthorhombic
Space group	<i>P</i> 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
<i>a</i> /Å	4.9355(4)
<i>b</i> /Å	9.9998(8)
<i>c</i> /Å	44.492(3)
$\alpha$ /°	90
$\beta$ /°	90
$\gamma$ /°	90
Volume/Å <sup>3</sup>	2195.8(3)
<i>Z</i>	4
$\rho_{\text{calc}}$ /cm <sup>3</sup>	1.517
$\mu$ /mm <sup>-1</sup>	2.885
<i>F</i> (000)	1032.0
Crystal size/mm <sup>3</sup>	0.08 × 0.06 × 0.01
Radiation	CuK $\alpha$ ( $\lambda = 1.54178$ )
2 $\theta$ range for data collection/°	7.948 to 132.716
Index ranges	-5 ≤ <i>h</i> ≤ 5, -7 ≤ <i>k</i> ≤ 11, -52 ≤ <i>l</i> ≤ 49
Reflections collected	15300
Independent reflections	3778 [ $R_{\text{int}} = 0.0462$ , $R_{\text{sigma}} = 0.0394$ ]
Data/restraints/parameters	3778/2/301
Goodness-of-fit on $F^2$	1.229
Final <i>R</i> indexes [ $I \geq 2\sigma(I)$ ]	$R_1 = 0.0487$ , $wR_2 = 0.1216$
Final <i>R</i> indexes [all data]	$R_1 = 0.0495$ , $wR_2 = 0.1220$
Largest diff. peak/hole / e Å <sup>-3</sup>	1.00/-1.31
Flack parameter	-0.003(14)

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