Supporting information for:

The Anti-Tubercular Aminolipopeptide Trichoderin A Displays Selective Toxicity against Human Pancreatic Ductal Adenocarcinoma Cells Cultured under Glucose Starvation

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Materials and Methods

Materials

All reagents purchased from commercial vendors were used without additional purification. *N*,*N*-diisopropylethylamine (DIPEA; ReagentPlus grade), piperidine, propionic acid, butanoic acid, hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid, formic acid (LC-MS grade), acetic anhydride, *N*,*N'*-diisopropylcarbodiimide (DIC), and dimethyl sulfoxide (DMSO, cell culture grade) were supplied by Sigma-Aldrich (St Louis, MO). *N*,*N*-dimethylformamide (DMF), dichloromethane (CH_2Cl_2), and methanol (MeOH) were purchased from ECP Ltd. (Auckland, NZ). Milli-Q water (H_2O) for RP-HPLC and LC-MS was obtained from the Sartorius arium pro ultrapure water production system (Gottingen, Germany). *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate (HATU) and ethyl-2-cyano-2-(hydroxyamino)acetate (Oxyma Pure) were purchased from Novabiochem (Darmstadt, Germany). 1-[(1-(Cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylaminomorpholinomethylene)] methanaminium hexafluorophosphate (COMU) and 6-chloro-1-hydroxybenzotriazole (6-Cl-HOBt) were sourced from Aapptec (Louisville, KY). HPLC and LC-MS grade acetonitrile (CH_3CN) were purchased from Merck (Darmstadt, Germany). Trifluoroacetic acid (TFA) was purchased from Oakwood Chemicals (Estill, SC). 2-Chlorotrityl chloride (2-CTC)

polystyrene resin, Fmoc-α-aminoisobutyric acid (Aib), Fmoc-Ile-OH, Fmoc-Val-OH, and Fmoc-Pro-OH were purchased from CS Bio (Shanghai, China). Boc-Pro-OH was purchased from PolyPeptide Group (Zug, Switzerland). Fmoc-Cha-OH, and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) were purchased from AK Scientific (Union City, CA). Leucinostatin A was purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX). Trichoderin A, (2*R*)-methyldecanoic acid (MDA), (2*S*,4*S*,6*R*)-2-amino-6-hydroxy-4-methyl-8-oxodecanoic acid (AHMOD) and (*S*)-2-(2-aminopropyl(methyl)amino)ethanol (AMAE) were synthesised in house as previously reported [1]. Nunc MicroWell 96-Well Black Optical-Bottom Plates with Polymer Base, PrestoBlue™ Cell Viability Reagent, CyQUANT™ Direct Cell Proliferation Assay, Roswell Park Memorial Institute (RPMI) 1640 medium (ATCC Modification, #A1049101; 25 mM glucose), RPMI 1640 medium (no glucose, #11879020), Dulbecco's Eagle Modified Medium (DMEM; high glucose, pyruvate, #11995040; 25 mM glucose), DMEM (no glucose, #11966025), sodium pyruvate (100 mM), d-glucose solution (200 g/L), antibiotic-antimycotic (A/A), TrypLE™ Express Enzyme (1×) (phenol red, #12605010), and phosphate-buffered saline (PBS, pH 7.4) were purchased from Thermo Fisher Scientific (Waltham, MA). Foetal bovine serum (FBS, heat-inactivated) was purchased from Moregate Biotech (Hamilton, NZ).

General Procedure for Peptide Synthesis

The peptides were assembled manually by 9-fluorenylmethoxycarbonyl/*tert*-butyl solid phase peptide synthesis (Fmoc/*t*Bu SPPS) in a fritted glass reaction vessel.

Method 1) Attachment of Fmoc-Aib-OH on resin

A solution of Fmoc-Aib-OH (5 eq., 162.7 mg, 0.5 mmol) and DIPEA (10 eq., 172 µL, 1 mmol) in CH_2Cl_2 (3 mL) was added to 2-CTC resin (112.4 mg, 0.1 mmol; loading: 0.89 mmol/g) that has been pre-swollen in CH_2Cl_2 (3 mL) for 15 min. The reaction mixture was agitated for 6 h at room temperature, filtered, and repeated with fresh reagents for a further 6 h. Excess solvent was then filtered, and the resin bed treated with a mixture of CH_2Cl_2/MeOH/DIPEA (8:1.5:0.5, *v*/*v*/*v*, 3 mL) for 15 min at room temperature to cap unreacted chlorides on the resin.

Method 2) Spectrophotometric quantitation of first residue loading on resin

Loading of the first residue on resin was quantified on the Shimadzu UV-1280 UV-VIS spectrophotometer (Kyoto, Japan). Resin that has been dried under vacuum was weighed into a Starna Scientific 10-mm matched silica UV spectrophotometric cuvette (Ilford, UK). 20% piperidine/DMF (*v*/*v*, 3 mL) was dispensed into the cuvette, briefly agitated with a pipettor to achieve a uniform suspension, then left for 5 min at room temperature. A reference cuvette, containing only 20% piperidine/DMF (*v*/*v*, 3 mL), was used to zero the spectrophotometer at the wavelength of 290 nm. The absorbance

of the resin-containing cuvette was recorded as an average of three independent measurements, and the loading of the first residue calculated by the following **Equation (1)**:

$$Loading = \frac{Absorbance}{(Resin\ mass\ \times 1.75)}$$

Equation (1). Estimation of first residue attachment on resin. Loading is measured in mmol/g.

Method 3) Removal of Fmoc protecting group

A solution of 20% piperidine/DMF (*v*/*v*, 3 mL) was added to the peptidyl resin to remove the Fmoc protecting group. The reaction mixture was agitated for 5 min at room temperature, filtered, and the reaction repeated with fresh reagents for a further 10 min. Excess solvent was then filtered and the resin bed washed with DMF (3 × 3 mL).

Method 4) Procedure for the difficult sequential Aib-Aib coupling on resin

A solution of Fmoc-Aib-OH (5 eq., 162.7 mg, 0.5 mmol), COMU (5 eq., 214 mg, 0.5 mmol), Oxyma (5 eq., 71 mg, 0.5 mmol) and DIPEA (10 eq., 172 μL, 1 mmol) in DMF (3 mL) was added to the peptidyl resin (0.1 mmol). The reaction mixture was agitated for 2 h at room temperature, filtered, and the reaction repeated with fresh reagents for a further 2 h. Excess solvent was then filtered and the resin bed washed with DMF (3 × 3 mL).

Method 5) Coupling of Fmoc-protected amino acid

A solution of the appropriate Fmoc-Aa-OH (5 eq., 0.5 mmol), HATU (4.9 eq., 186 mg, 0.49 mmol) and DIPEA (10 eq., 172 μL, 1 mmol) in DMF (3 mL) was added to the peptidyl resin (0.1 mmol). The reaction mixture was agitated for 1 h at room temperature, filtered, and the reaction repeated with fresh reagents for a further 1 h. Excess solvent was then filtered and the resin bed washed with DMF (3 × 3 mL).

Method 6) Attachment of N-terminal fatty acid

A solution of the appropriate fatty acid (5 eq., 0.5 mmol), COMU (5 eq., 214 mg, 0.5 mmol), Oxyma (5 eq., 71 mg, 0.5 mmol) and DIPEA (10 eq., 172 μL, 1 mmol) in DMF (3 mL) was added to the peptidyl resin (0.1 mmol). The reaction mixture was agitated for 3 h at room temperature. Excess solvent was then filtered, the resin bed washed with DMF (3 × 3 mL) and CH_2Cl_2 (3 × 3 mL), then dried under vacuum.

Method 7) HFIP-mediated resin cleavage

The resin bed was treated with 20% HFIP/CH_2Cl_2 (*v*/*v*, 5 mL) to release the completed peptide chain. The reaction mixture was agitated for 30 min at room temperature, filtered, and the reaction repeated with fresh reagents for a further 30 min. The collected filtrate was partially concentrated under a gentle stream of nitrogen, reconstituted in CH_3CN/H_2O (*v*/*v*, 10 mL) and lyophilised.

Method 8) Late-stage solution phase C-terminal coupling of AMAE

The *C*-terminal AMAE residue was conjugated to the peptide as a TFA salt. A solution of AMAE.2TFA (3 eq.), DIC (6 eq.), 6-Cl-HOBt (6 eq.), and DIPEA (6 eq.) in DMF was added to the purified peptide from **Method 7**. The reaction mixture was agitated for 12 h at room temperature and completion of the reaction was monitored by analytical RP-HPLC and ESI-MS. The peptide mixture was then purified batch-wise by semi-preparative RP-HPLC.

Synthesis of trichoderin A analogue (12)

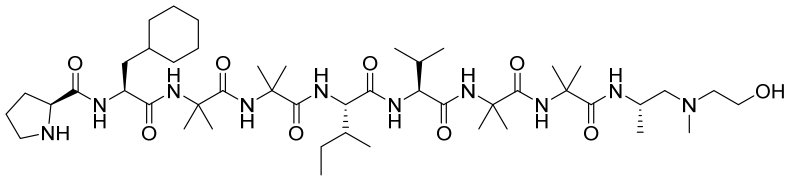
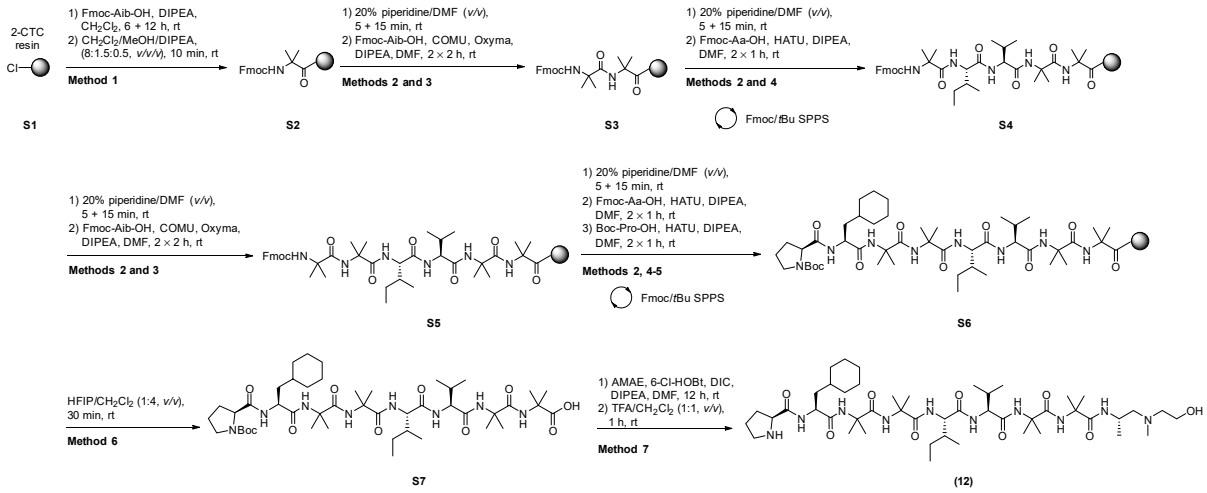


Figure S1. Chemical structure of trichoderin A analogue **(12)**.

The *C*-terminal residue Fmoc-Aib-OH was attached to 2-chlorotrityl chloride resin **S1** according to **Method 1** to form peptidyl resin **S2** (**Scheme S1**). Loading was determined to be 0.43 mmol/g by **Method 2**. The N^{α}-Fmoc-protecting group was removed using **Method 3** followed by coupling of Fmoc-Aib-OH via **Method 4** to give **S3**. The N^{α}-Fmoc-protecting group was then removed using **Method 3** followed by coupling of Fmoc-Val-OH, Fmoc-Ile-OH, and Fmoc-Aib-OH via **Method 5** to afford peptidyl resin **S4**. After removal of N^{α}-Fmoc-protecting group using **Method 3**, Fmoc-Aib-OH was coupled via **Method 4** to yield **S5**, followed by coupling of Fmoc-Cha-OH and Boc-Pro-OH by **Method 5** to complete the linear peptide sequence of **S6**. Resin-bound peptide was cleaved using **Method 7**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m*/*z* were combined and lyophilised to afford peptide **S7** as white fluffy flakes (27.9 mg, 30.3% yield). Late-stage solution phase *C*-terminal coupling was employed to introduce the *C*-terminal aminoalcohol moiety on the peptide (27.9 mg, 0.03 mmol) dissolved in 0.5 mL DMF using **Method 8**. The crude peptide solution was purified by semi-preparative RP-HPLC in 5 × 100 μL batches using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m*/*z* were combined, lyophilised, and subjected to N^{α}-Boc deprotection using TFA/CH_2Cl_2 (1:1, *v*/*v*) at room temperature for 1 h to afford trichoderin A analogue **(12)** as a white amorphous solid (7.6 mg, 8.1% overall yield based on 0.1 mmol resin loading, 98.0% purity); t_R 16.0 min; LRMS: *m*/*z* (ESI-MS) 935.2 ([M+H]$^+$ requires 935.3), **Figures S1-S2**. The peptide was subsequently evaluated for its *in vitro* cytotoxicity against BxPC-3 and PANC-1 human PDAC cells under both glucose-replete and glucose-deprived conditions on the same plate (**Figure S3**).



Scheme S1. Fmoc/*t*Bu SPPS of trichoderin A analogue **(12)**.

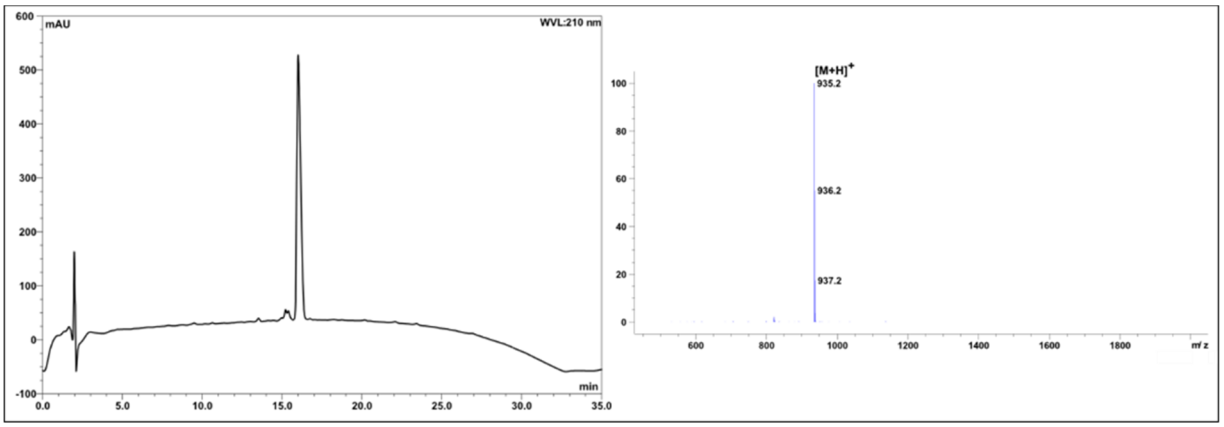


Figure S2. Analytical RP-HPLC profile of pure peptide **(12)** (*ca*. 98% as judged by peak area at 210 nm); xTerra® MS C18, 4.6 × 150 mm, 5 µm, linear gradient of 5% B to 95% B (*ca*. 3% B/min), 1 mL/min. *m*/*z* $[M+H]^+$ calculated: 935.3; found: 935.2.

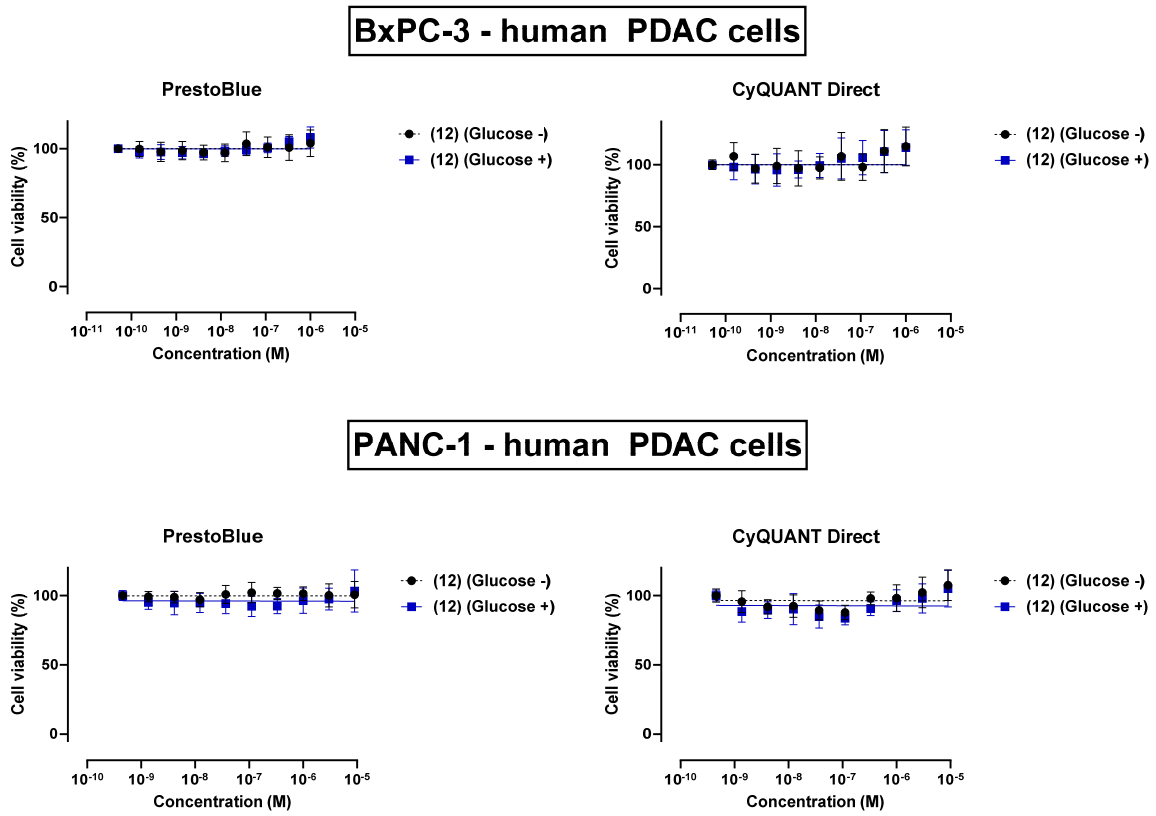


Figure S3. Comparative percent viability of BxPC-3 and PANC-1 human PDAC cells cultured under 'low glucose' (Glucose -) and 'normal glucose' (Glucose +) conditions following a 1-day exposure to trichoderin A analogue **(12)**, as judged by the PrestoBlue™ and CyQUANT™ Direct assays.

Synthesis of trichoderin A analogue (13)

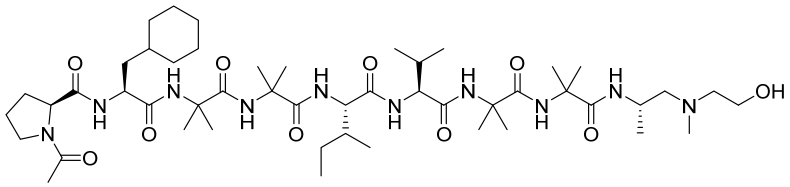
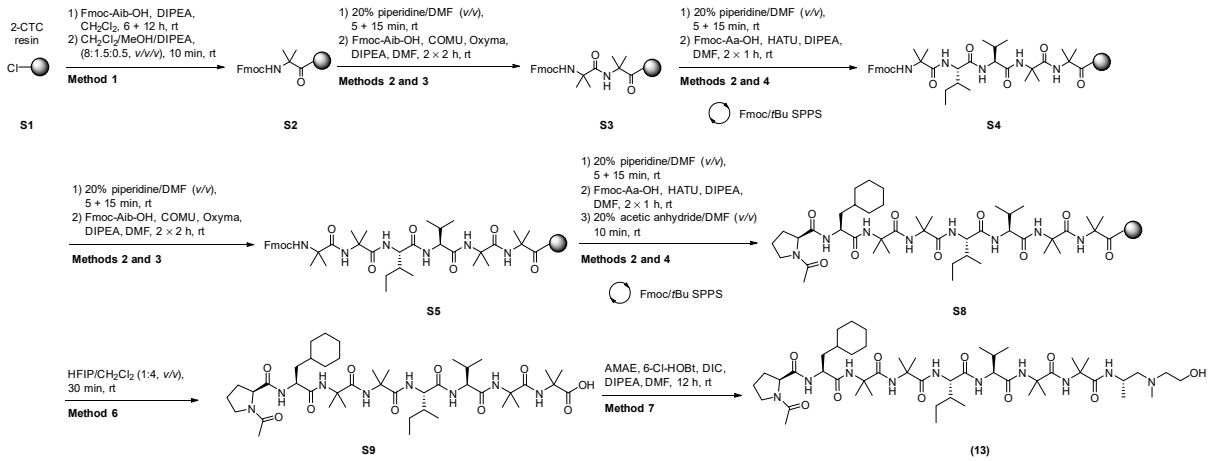


Figure S4. Chemical structure of trichoderin A analogue **(13)**.

The *C*-terminal residue Fmoc-Aib-OH was attached to 2-chlorotrityl chloride resin **S1** according to **Method 1** to form peptidyl resin **S2** (**Scheme S2**). Loading was determined to be 0.43 mmol/g by **Method 2**. The N^{α}-Fmoc-protecting group was removed using **Method 3** followed by coupling of Fmoc-Aib-OH via **Method 4** to give **S3**. The N^{α}-Fmoc-protecting group was then removed using **Method 3** followed by coupling of Fmoc-Val-OH, Fmoc-Ile-OH, and Fmoc-Aib-OH via **Method 5** to afford peptidyl resin **S4**. After removal of N^{α}-Fmoc-protecting group using **Method 3**, Fmoc-Aib-OH was coupled via **Method 4** to yield **S5**, followed by coupling of Fmoc-Cha-OH and Fmoc-Pro-OH by **Method 5**. The N^{α}-Fmoc protecting group was then removed via **Method 3** and the *N*-terminus capped with 20% acetic anhydride/DMF (*v*/*v*) at room temperature for 2 × 5 min to complete the linear peptide sequence of **S8**. Resin-bound peptide was cleaved using **Method 7**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m*/*z* were combined and lyophilised to afford peptide **S9** as white fluffy flakes (7.2 mg, 8.3% yield). Late-stage solution phase *C*-terminal coupling was employed to introduce the *C*-terminal aminoalcohol moiety on the peptide (7.6 mg, 0.009 mmol) dissolved in 0.5 mL DMF using **Method 8**. The crude peptide solution was purified by semi-preparative RP-HPLC in 5 × 100 μL batches using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m*/*z* were combined and lyophilised to afford trichoderin A analogue **(13)** as a white amorphous solid (4.0 mg, 4.1% overall yield based on 0.1 mmol resin loading, >99% purity); t_R 20.7 min; LRMS: *m*/*z* (ESI-MS) 977.2 ($[M+H]^+$ requires 977.3), **Figures S4-S5**. The peptide was subsequently evaluated for its *in vitro* cytotoxicity against BxPC-3 and PANC-1 human PDAC cells under both glucose-replete and glucose-deprived conditions on the same plate (**Figure S6**).



Scheme S2. Fmoc/*t*Bu SPPS of trichoderin A analogue **(13)**.

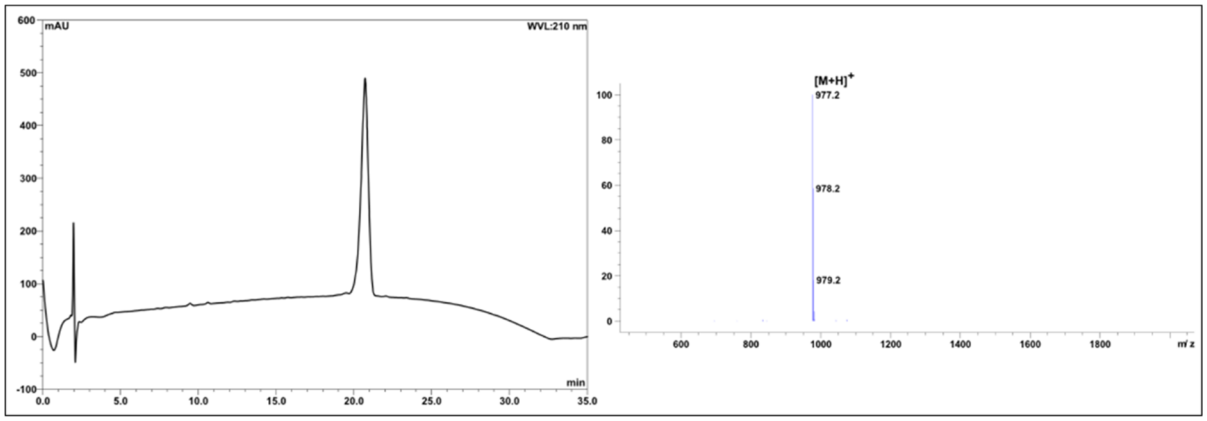


Figure S5. Analytical RP-HPLC profile of pure peptide **(13)** (*ca*. 99% as judged by peak area at 210 nm); xTerra® MS C18, 4.6 × 150 mm, 5 µm, linear gradient of 5% B to 95% B (*ca*. 3% B/min), 1 mL/min. *m*/*z* $[M+H]^+$ calculated: 977.3; found: 977.2.

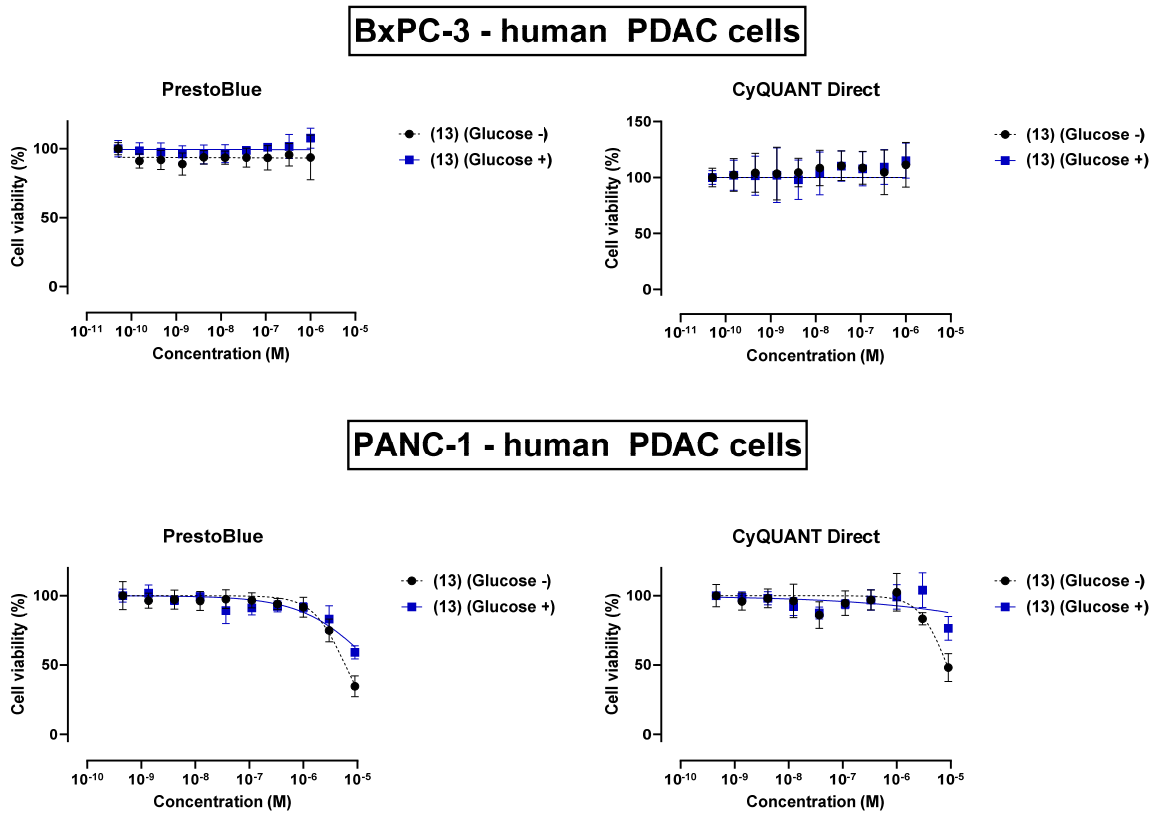


Figure S6. Comparative percent viability of BxPC-3 and PANC-1 human PDAC cells cultured under 'low glucose' (Glucose -) and 'normal glucose' (Glucose +) conditions following a 1-day exposure to trichoderin A analogue **(13)**, as judged by the PrestoBlue™ and CyQUANT™ Direct assays.

Synthesis of trichoderin A analogue (14)

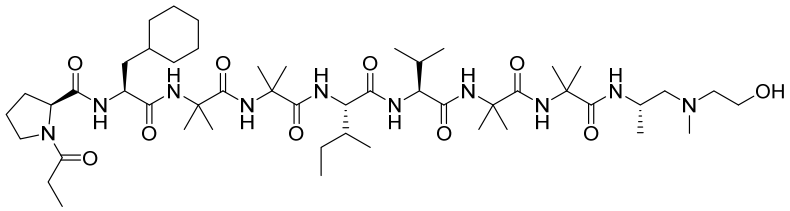
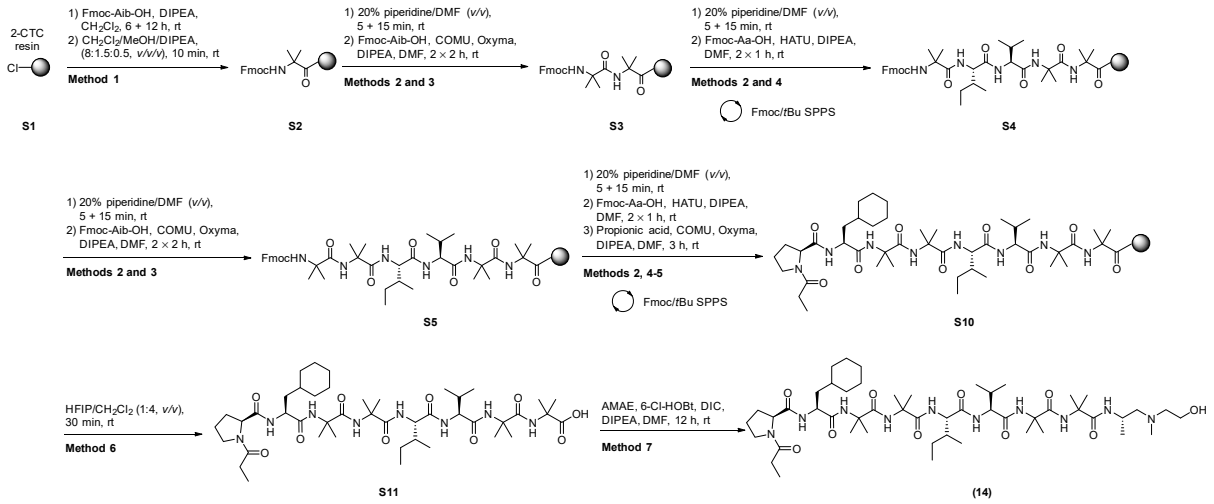


Figure S7. Chemical structure of trichoderin A analogue **(14)**.

The *C*-terminal residue Fmoc-Aib-OH was attached to 2-chlorotrityl chloride resin **S1** according to **Method 1** to form peptidyl resin **S2** (**Scheme S3**). Loading was determined to be 0.43 mmol/g by **Method 2**. The N^{α}-Fmoc-protecting group was removed using **Method 3** followed by coupling of Fmoc-Aib-OH via **Method 4** to give **S3**. The N^{α}-Fmoc-protecting group was then removed using **Method 3** followed by coupling of Fmoc-Val-OH, Fmoc-Ile-OH, and Fmoc-Aib-OH via **Method 5** to afford peptidyl resin **S4**. After removal of N^{α}-Fmoc-protecting group using **Method 3**, Fmoc-Aib-OH was coupled via **Method 4** to yield **S5**, followed by coupling of Fmoc-Cha-OH and Fmoc-Pro-OH by **Method 5**, and propionic acid via **Method 6** to complete the linear peptide sequence of **S10**. Resin-bound peptide was cleaved using **Method 7**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m*/*z* were combined and lyophilised to afford peptide **S11** as white fluffy flakes (27.2 mg, 31.0% yield). Late-stage solution phase *C*-terminal coupling was employed to introduce the *C*-terminal aminoalcohol moiety on the peptide (27.2 mg, 0.03 mmol) dissolved in 0.5 mL DMF using **Method 8**. The crude peptide solution was purified by semi-preparative RP-HPLC in 5 × 100 μL batches using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m*/*z* were combined and lyophilised to afford trichoderin A analogue **(14)** as a white amorphous solid (10.2 mg, 10.3% overall yield based on 0.1 mmol resin loading, 98.9% purity); t_R 21.5 min; LRMS: *m*/*z* (ESI-MS) 991.2 ($[M+H]^+$ requires 991.3), **Figures S7-S8**. The peptide was subsequently evaluated for its *in vitro* cytotoxicity against BxPC-3 and PANC-1 human PDAC cells under both glucose-replete and glucose-deprived conditions on the same plate (**Figure S9**).



Scheme S3. Fmoc/*t*Bu SPPS of trichoderin A analogue **(14)**.

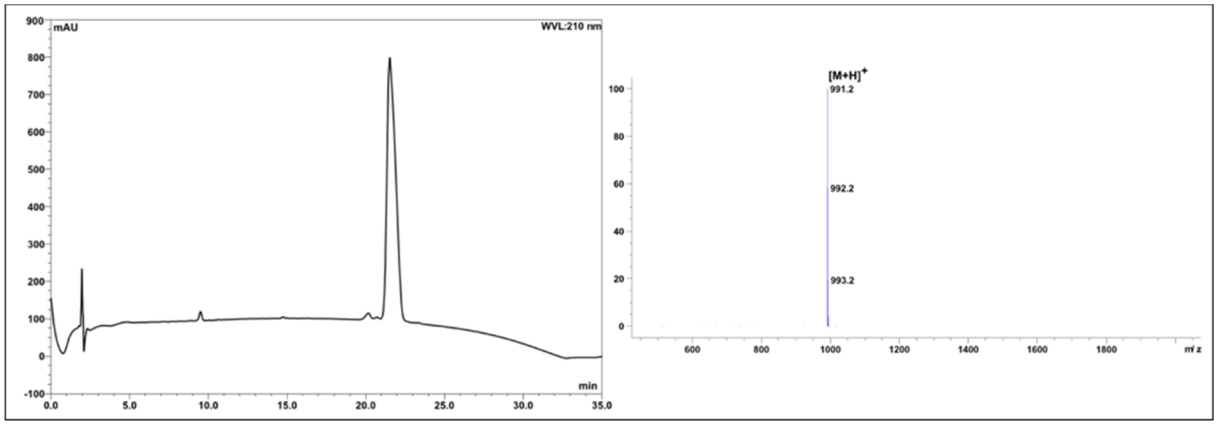


Figure S8. Analytical RP-HPLC profile of pure peptide **(14)** (*ca*. 99% as judged by peak area at 210 nm); xTerra® MS C18, 4.6 × 150 mm, 5 μm, linear gradient of 5% B to 95% B (*ca*. 3% B/min), 1 mL/min. *m*/*z* $[M+H]^+$ calculated: 991.3; found: 991.2.

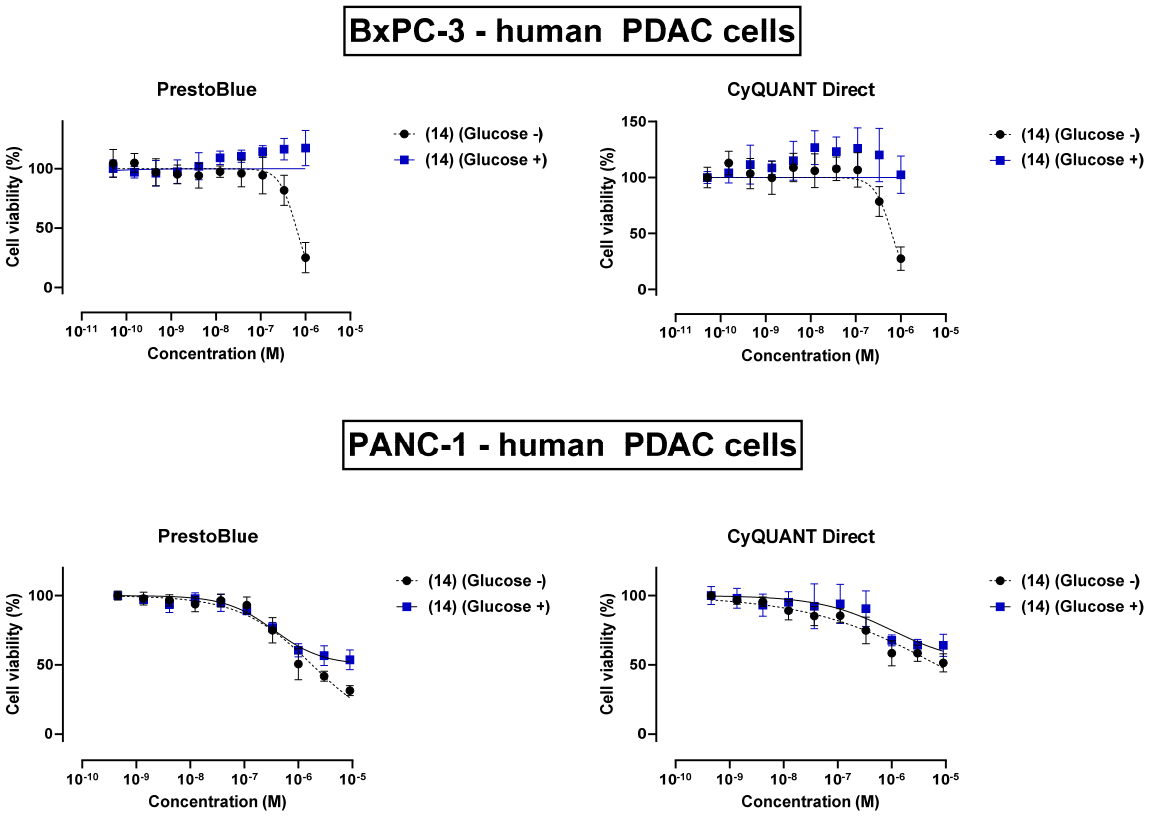


Figure S9. Comparative percent viability of BxPC-3 and PANC-1 human PDAC cells cultured under 'low glucose' (Glucose -) and 'normal glucose' (Glucose +) conditions following a 1-day exposure to trichoderin A analogue **(14)**, as judged by the PrestoBlue™ and CyQUANT™ Direct assays.

Synthesis of trichoderin A analogue (15)

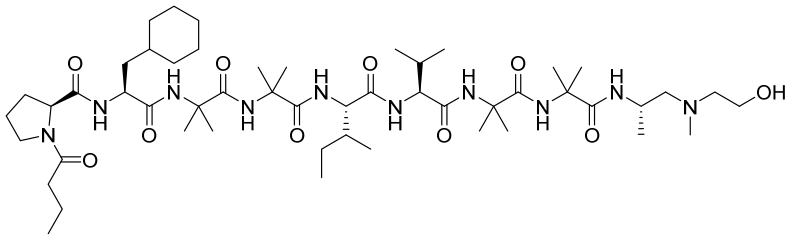
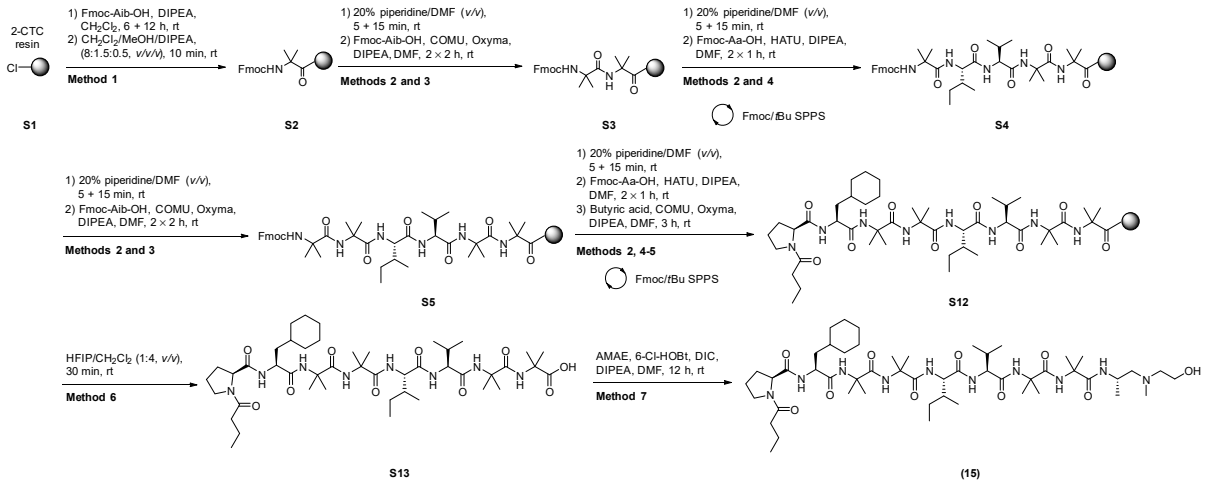


Figure S10. Chemical structure of trichoderin A analogue **(15)**.

The *C*-terminal residue Fmoc-Aib-OH was attached to 2-chlorotrityl chloride resin **S1** according to **Method 1** to form peptidyl resin **S2** (**Scheme S4**). Loading was determined to be 0.43 mmol/g by **Method 2**. The N^{α}-Fmoc-protecting group was removed using **Method 3** followed by coupling of Fmoc-Aib-OH via **Method 4** to give **S3**. The N^{α}-Fmoc-protecting group was then removed using **Method 3** followed by coupling of Fmoc-Val-OH, Fmoc-Ile-OH, and Fmoc-Aib-OH via **Method 5** to afford peptidyl resin **S4**. After removal of N^{α}-Fmoc-protecting group using **Method 3**, Fmoc-Aib-OH was coupled via **Method 4** to yield **S5**, followed by coupling of Fmoc-Cha-OH and Fmoc-Pro-OH by **Method 5**, and butyric acid via **Method 6** to complete the linear peptide sequence of **S12**. Resin-bound peptide was cleaved using **Method 7**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m*/*z* were combined and lyophilised to afford peptide **S13** as white fluffy flakes (16.6 mg, 18.6% yield). Late-stage solution phase *C*-terminal coupling was employed to introduce the *C*-terminal aminoalcohol moiety on the peptide (16.6 mg, 0.02 mmol) dissolved in 0.5 mL DMF using **Method 8**. The crude peptide solution was purified by semi-preparative RP-HPLC in 5 × 100 μL batches using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m*/*z* were combined and lyophilised to afford trichoderin A analogue **(15)** as a white amorphous solid (3.6 mg, 3.6% overall yield based on 0.1 mmol resin loading, 98.9% purity); t_R 22.7 min; LRMS: *m*/*z* (ESI-MS) 1005.2 ($[M+H]^+$ requires 1005.4), **Figures S10-S11**. The peptide was subsequently evaluated for its *in vitro* cytotoxicity against BxPC-3 and PANC-1 human PDAC cells under both glucose-replete and glucose-deprived conditions on the same plate (**Figure S12**).



Scheme S4. Fmoc/*t*Bu SPPS of trichoderin A analogue **(15)**.

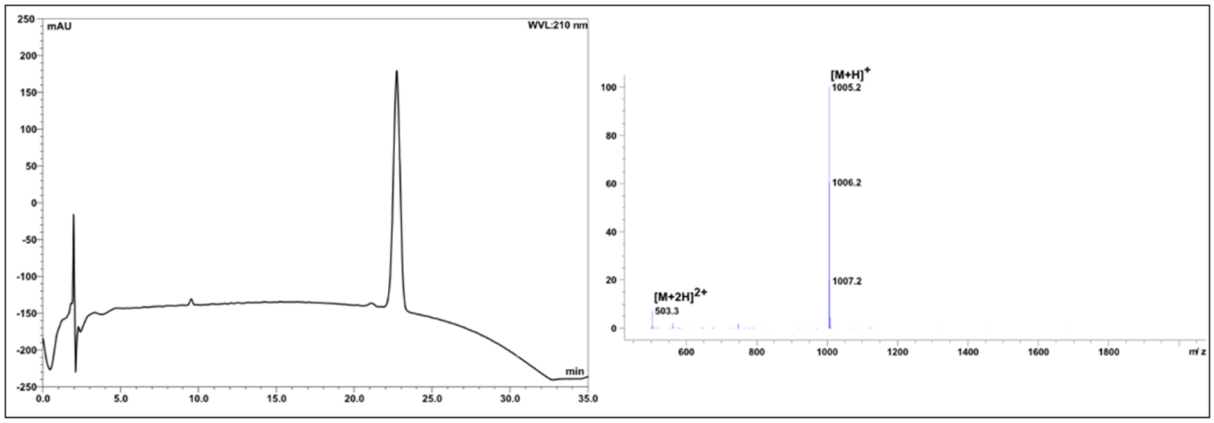


Figure S11. Analytical RP-HPLC profile of pure peptide **(15)** (*ca*. 99% as judged by peak area at 210 nm); xTerra® MS C18, 4.6 × 150 mm, 5 µm, linear gradient of 5% B to 95% B (*ca*. 3% B/min), 1 mL/min. *m*/*z* $[M+H]^+$ calculated: 1005.4; found: 1005.2.

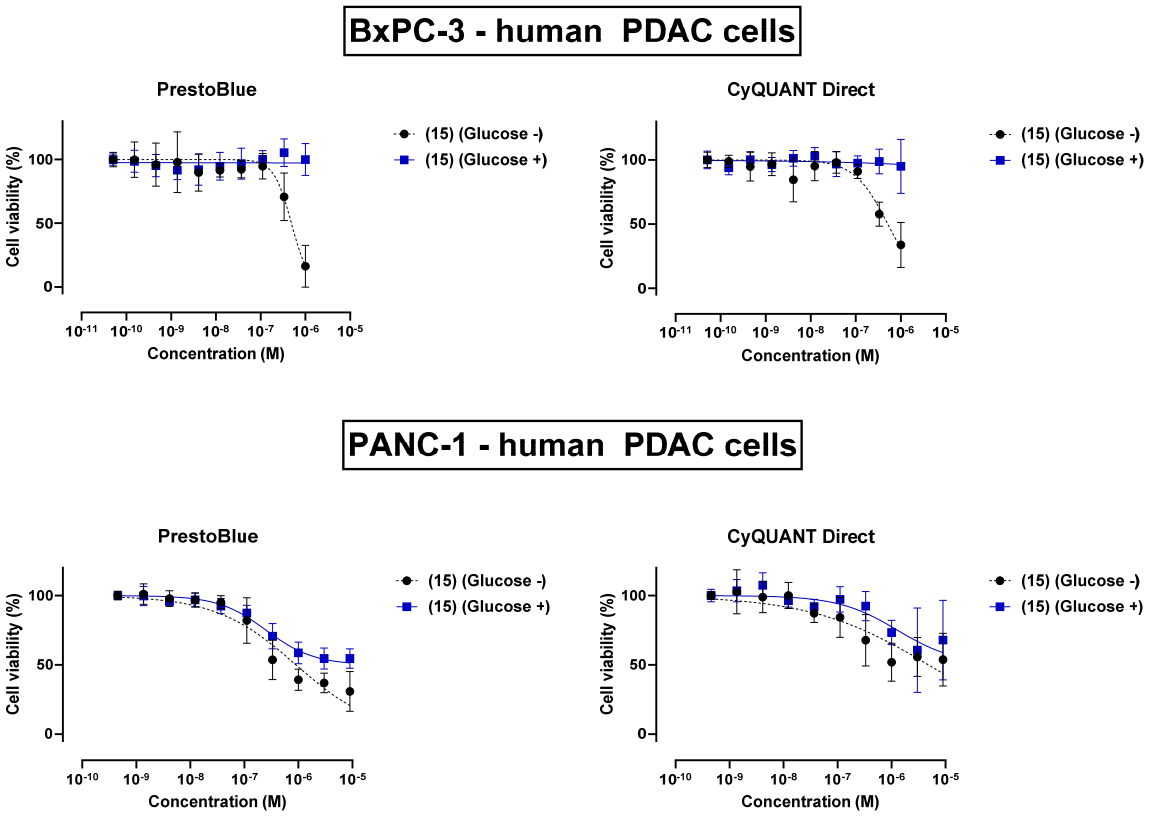


Figure S12. Comparative percent viability of BxPC-3 and PANC-1 human PDAC cells cultured under 'low glucose' (Glucose -) and 'normal glucose' (Glucose +) conditions following a 1-day exposure to trichoderin A analogue **(15)**, as judged by the PrestoBlue™ and CyQUANT™ Direct assays.

Synthesis of trichoderin A analogue (16)

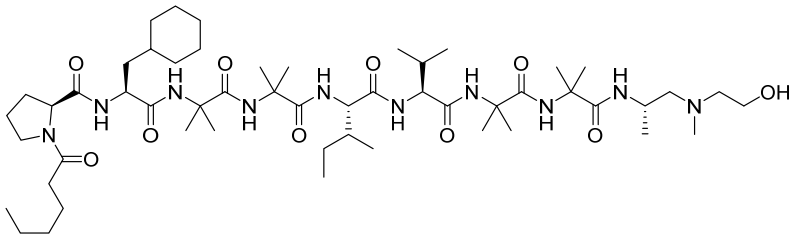
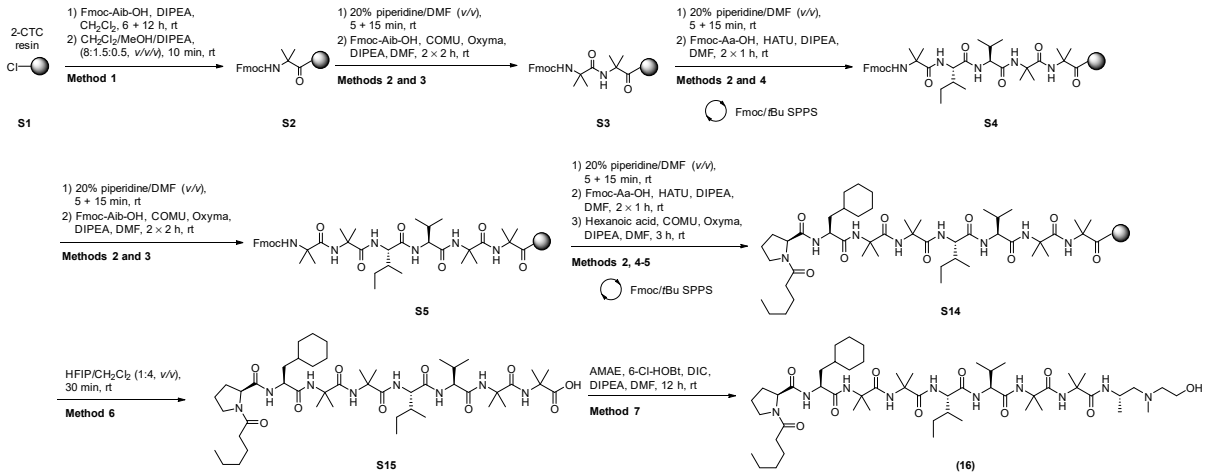


Figure S13. Chemical structure of trichoderin A analogue **(16)**.

The *C*-terminal residue Fmoc-Aib-OH was attached to 2-chlorotrityl chloride resin **S1** according to **Method 1** to form peptidyl resin **S2** (**Scheme S5**). Loading was determined to be 0.43 mmol/g by **Method 2**. The N^{α}-Fmoc-protecting group was removed using **Method 3** followed by coupling of Fmoc-Aib-OH via **Method 4** to give **S3**. The N^{α}-Fmoc-protecting group was then removed using **Method 3** followed by coupling of Fmoc-Val-OH, Fmoc-Ile-OH, and Fmoc-Aib-OH via **Method 5** to afford peptidyl resin **S4**. After removal of N^{α}-Fmoc-protecting group using **Method 3**, Fmoc-Aib-OH was coupled via **Method 4** to yield **S5**, followed by coupling of Fmoc-Cha-OH and Fmoc-Pro-OH by **Method 5**, and hexanoic acid via **Method 6** to complete the linear peptide sequence of **S14**. Resin-bound peptide was cleaved using **Method 7**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m*/*z* were combined and lyophilised to afford peptide A15 as white fluffy flakes (13.4 mg, 14.6% yield). Late-stage solution phase *C*-terminal coupling was employed to introduce the *C*-terminal aminoalcohol moiety on the peptide (13.4 mg, 0.01 mmol) dissolved in 0.5 mL DMF using **Method 8**. The crude peptide solution was purified by semi-preparative RP-HPLC in 5 × 100 μL batches using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m*/*z* were combined and lyophilised to afford trichoderin A analogue **(16)** as a white amorphous solid (4.5 mg, 4.4% overall yield based on 0.1 mmol resin loading, 98.3% purity); t_R 24.5 min; LRMS: *m*/*z* (ESI-MS) 1033.2 ($[M+H]^+$ requires 1033.4), **Figures S13-S14**. The peptide was subsequently evaluated for its *in vitro* cytotoxicity against BxPC-3 and PANC-1 human PDAC cells under both glucose-replete and glucose-deprived conditions on the same plate (**Figure S15**).



Scheme S5. Fmoc/*t*Bu SPPS of trichoderin A analogue **(16)**.

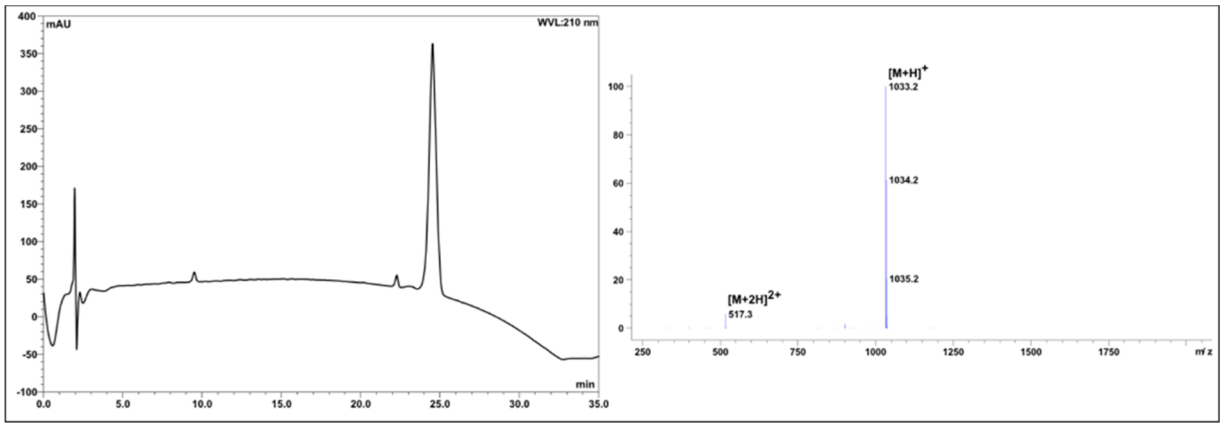


Figure S14. Analytical RP-HPLC profile of pure peptide **(16)** (*ca*. 98% as judged by peak area at 210 nm); xTerra® MS C18, 4.6 × 150 mm, 5 μm, linear gradient of 5% B to 95% B (*ca*. 3% B/min), 1 mL/min. *m*/*z* $[M+H]^+$ calculated: 1033.4; found: 1033.2.

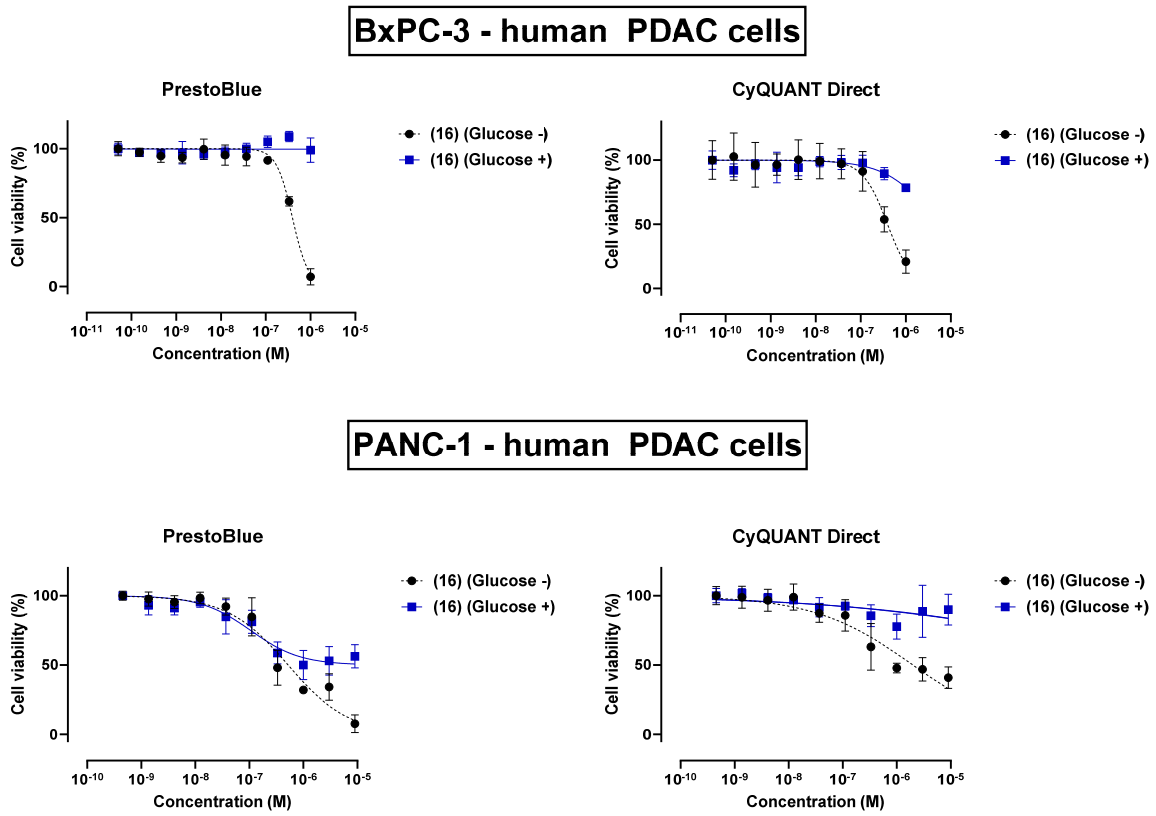


Figure S15. Comparative percent viability of BxPC-3 and PANC-1 human PDAC cells cultured under 'low glucose' (Glucose -) and 'normal glucose' (Glucose +) conditions following a 1-day exposure to trichoderin A analogue **(16)**, as judged by the PrestoBlue™ and CyQUANT™ Direct assays.

Synthesis of trichoderin A analogue (17)

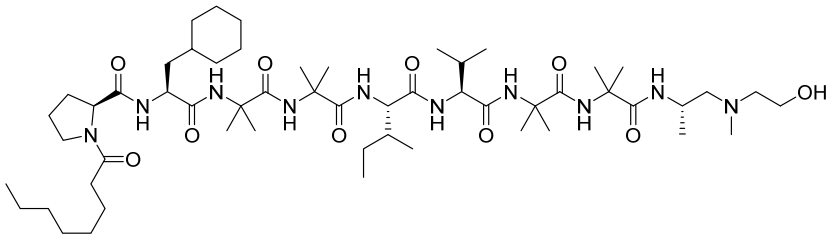
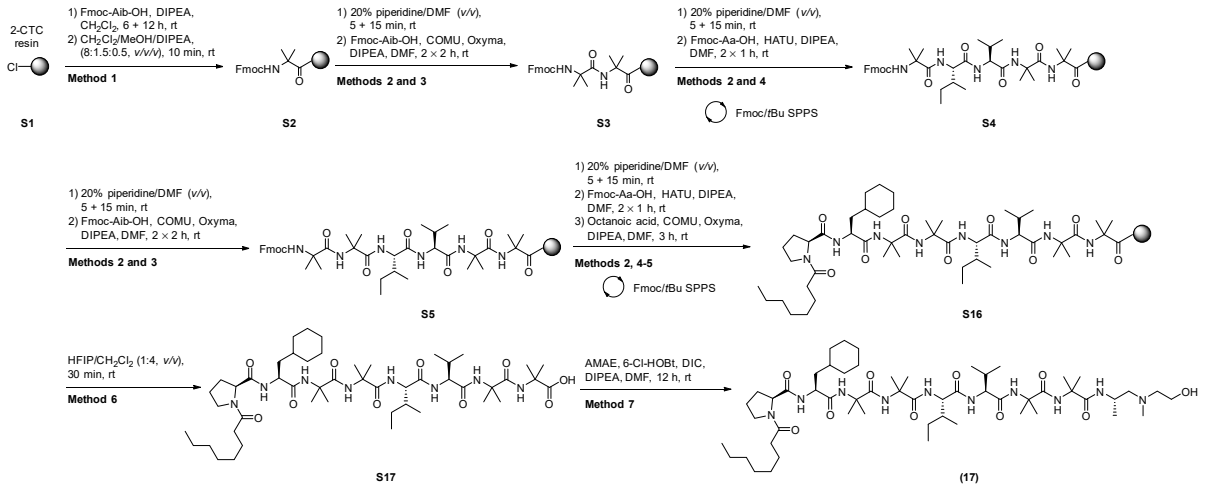


Figure S16. Chemical structure of trichoderin A analogue **(17)**.

The *C*-terminal residue Fmoc-Aib-OH was attached to 2-chlorotrityl chloride resin **S1** according to **Method 1** to form peptidyl resin **S2** (**Scheme S6**). Loading was determined to be 0.43 mmol/g by **Method 2**. The N^{α}-Fmoc-protecting group was removed using **Method 3** followed by coupling of Fmoc-Aib-OH via **Method 4** to give **S3**. The N^{α}-Fmoc-protecting group was then removed using **Method 3** followed by coupling of Fmoc-Val-OH, Fmoc-Ile-OH, and Fmoc-Aib-OH via **Method 5** to afford peptidyl resin **S4**. After removal of N^{α}-Fmoc-protecting group using **Method 3**, Fmoc-Aib-OH was coupled via **Method 4** to yield **S5**, followed by coupling of Fmoc-Cha-OH and Fmoc-Pro-OH by **Method 5**, and octanoic acid via **Method 6** to complete the linear peptide sequence of **S16**. Resin-bound peptide was cleaved using **Method 7**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m*/*z* were combined and lyophilised to afford peptide **S17** as white fluffy flakes (15.8 mg, 16.7% yield). Late-stage solution phase *C*-terminal coupling was employed to introduce the *C*-terminal aminoalcohol moiety on the peptide (15.8 mg, 0.02 mmol) dissolved in 0.5 mL DMF using **Method 8**. The crude peptide solution was purified by semi-preparative RP-HPLC in 5 × 100 μL batches using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m*/*z* were combined and lyophilised to afford trichoderin A analogue **(17)** as a white amorphous solid (6.1 mg, 5.7% overall yield based on 0.1 mmol resin loading, >99% purity); t_R 26.2 min; LRMS: *m*/*z* (ESI-MS) 1061.3 ($[M+H]^+$ requires 1061.5), **Figures S16-S17**. The peptide was subsequently evaluated for its *in vitro* cytotoxicity against BxPC-3 and PANC-1 human PDAC cells under both glucose-replete and glucose-deprived conditions on the same plate (**Figure S18**).



Scheme S6. Fmoc/*t*Bu SPPS of trichoderin A analogue **(17)**.

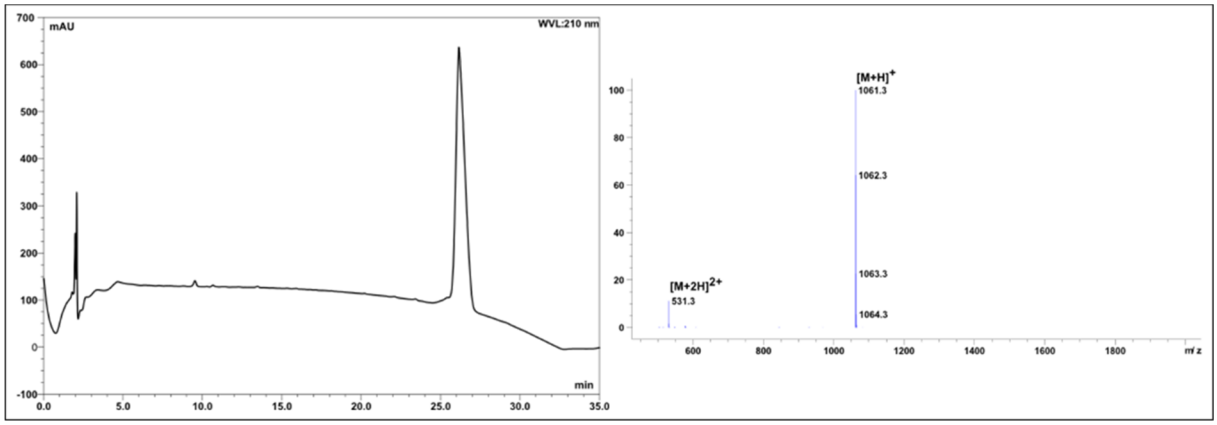


Figure S17. Analytical RP-HPLC profile of pure peptide **(17)** (*ca*. 99% as judged by peak area at 210 nm); xTerra® MS C18, 4.6 × 150 mm, 5 μm, linear gradient of 5% B to 95% B (*ca*. 3% B/min), 1 mL/min. *m*/*z* $[M+H]^+$ calculated: 1061.5; found: 1061.3.

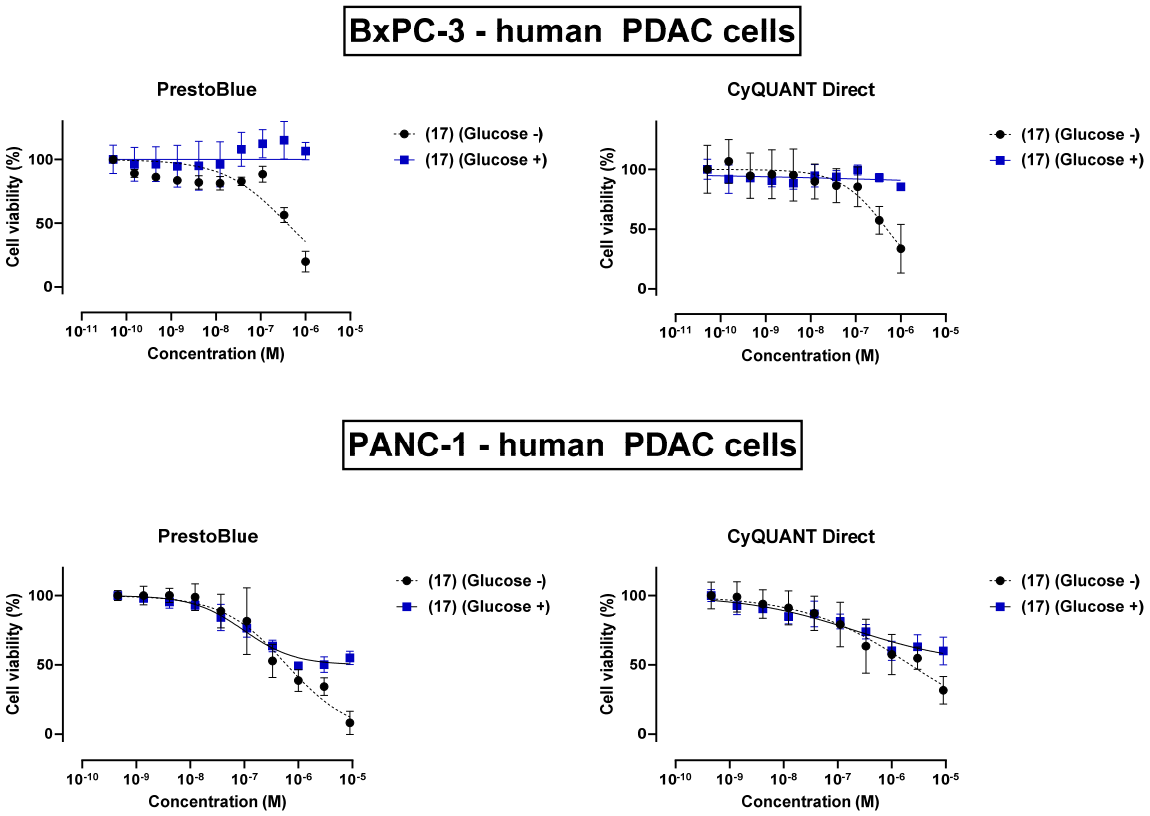


Figure S18. Comparative percent viability of BxPC-3 and PANC-1 human PDAC cells cultured under 'low glucose' (Glucose -) and 'normal glucose' (Glucose +) conditions following a 1-day exposure to trichoderin A analogue **(17)**, as judged by the PrestoBlue™ and CyQUANT™ Direct assays.

Synthesis of trichoderin A analogue (18)

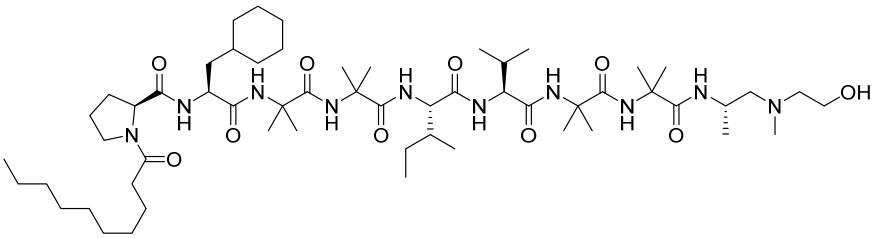
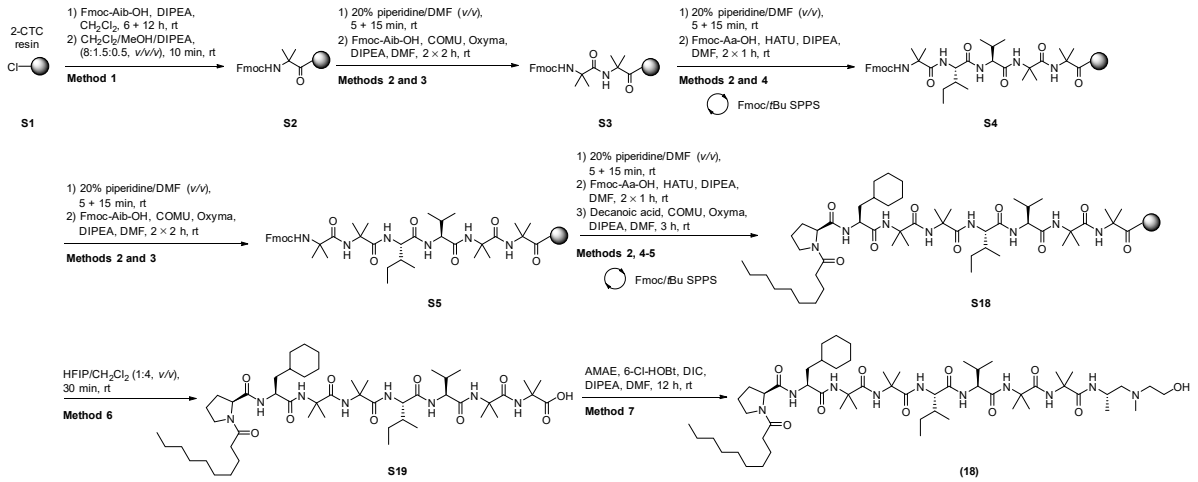


Figure S19. Chemical structure of trichoderin A analogue **(18)**.

The *C*-terminal residue Fmoc-Aib-OH was attached to 2-chlorotrityl chloride resin **S1** according to **Method 1** to form peptidyl resin **S2** (**Scheme S7**). Loading was determined to be 0.43 mmol/g by **Method 2**. The N^{α}-Fmoc-protecting group was removed using **Method 3** followed by coupling of Fmoc-Aib-OH via **Method 4** to give **S3**. The N^{α}-Fmoc-protecting group was then removed using **Method 3** followed by coupling of Fmoc-Val-OH, Fmoc-Ile-OH, and Fmoc-Aib-OH via **Method 5** to afford peptidyl resin **S4**. After removal of N^{α}-Fmoc-protecting group using **Method 3**, Fmoc-Aib-OH was coupled via **Method 4** to yield **S5**, followed by coupling of Fmoc-Cha-OH and Fmoc-Pro-OH by **Method 5**, and decanoic acid via **Method 6** to complete the linear peptide sequence of **S18**. Resin-bound peptide was cleaved using **Method 7**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m*/*z* were combined and lyophilised to afford peptide **S19** as white fluffy flakes (25.9 mg, 26.6% yield). Late-stage solution phase *C*-terminal coupling was employed to introduce the *C*-terminal aminoalcohol moiety on the peptide (25.9 mg, 0.03 mmol) dissolved in 0.5 mL DMF using **Method 8**. The crude peptide solution was purified by semi-preparative RP-HPLC in 5 × 100 μL batches using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m*/*z* were combined and lyophilised to afford trichoderin A analogue **(18)** as a white amorphous solid (9.5 mg, 8.7% overall yield based on 0.1 mmol resin loading, 98.3% purity); t_R 28.2 min; LRMS: *m*/*z* (ESI-MS) 1089.8 ($[M+H]^+$ requires 1089.5), **Figures S19-S20**. The peptide was subsequently evaluated for its *in vitro* cytotoxicity against BxPC-3 and PANC-1 human PDAC cells under both glucose-replete and glucose-deprived conditions on the same plate (**Figure S21**).



Scheme S7. Fmoc/*t*Bu SPPS of trichoderin A analogue **(18)**.

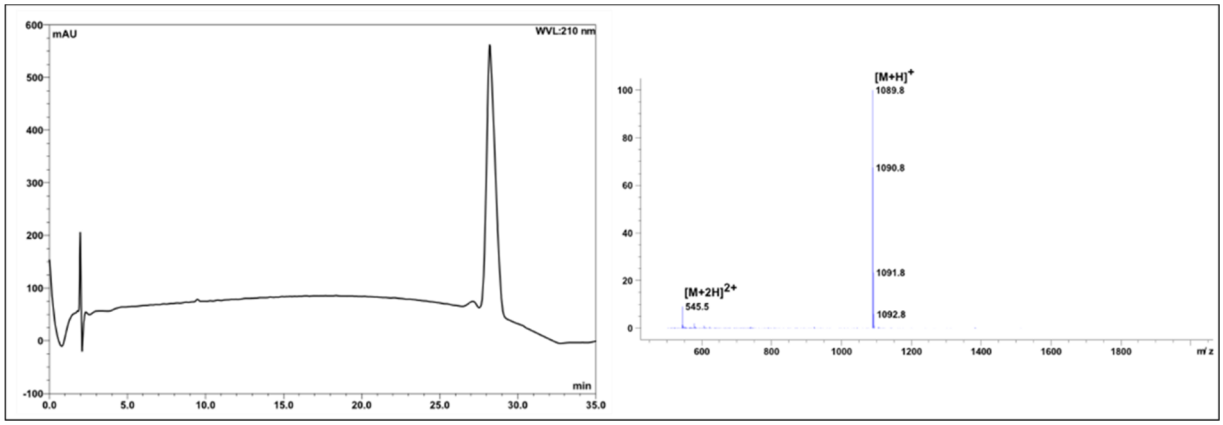


Figure S20. Analytical RP-HPLC profile of pure peptide **(18)** (*ca*. 98% as judged by peak area at 210 nm); xTerra® MS C18, 4.6 × 150 mm, 5 µm, linear gradient of 5% B to 95% B (*ca*. 3% B/min), 1 mL/min. *m*/*z* $[M+H]^+$ calculated: 1089.5; found: 1089.8.

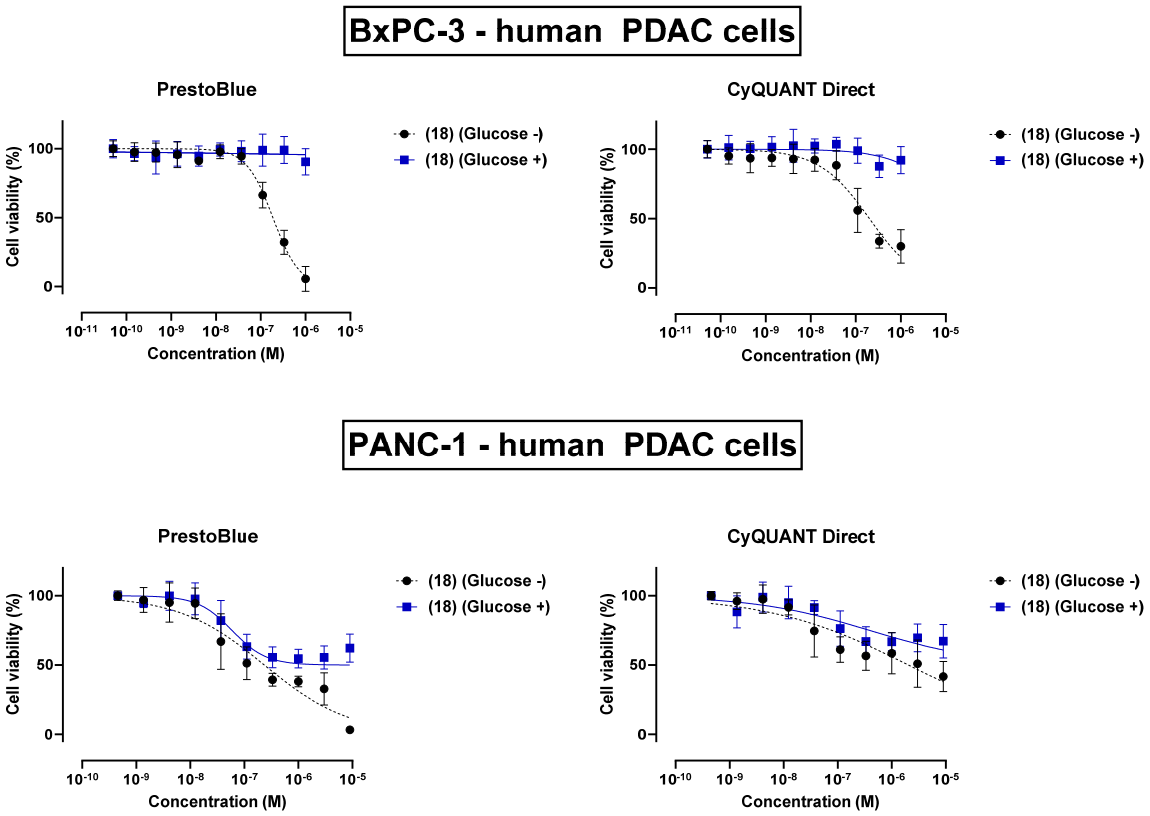


Figure S21. Comparative percent viability of BxPC-3 and PANC-1 human PDAC cells cultured under 'low glucose' (Glucose -) and 'normal glucose' (Glucose +) conditions following a 1-day exposure to trichoderin A analogue **(18)**, as judged by the PrestoBlue™ and CyQUANT™ Direct assays.

Synthesis of trichoderin A analogue (19)

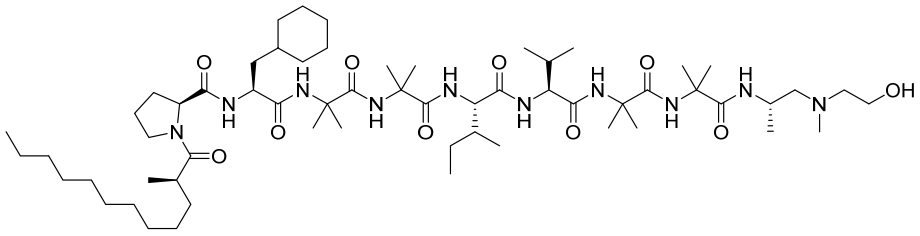
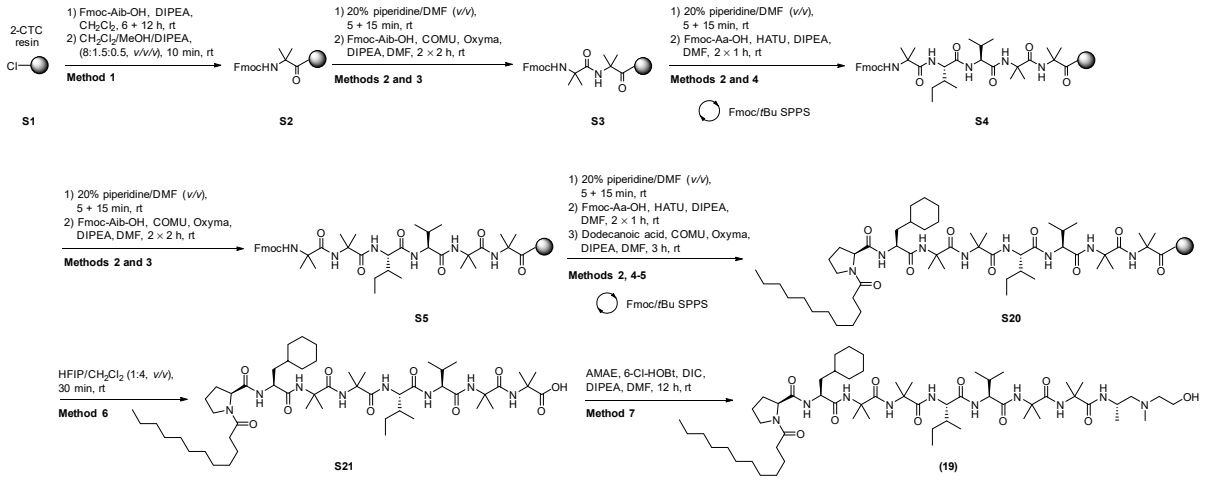


Figure S22. Chemical structure of trichoderin A analogue **(19)**.

The *C*-terminal residue Fmoc-Aib-OH was attached to 2-chlorotrityl chloride resin **S1** according to **Method 1** to form peptidyl resin **S2** (**Scheme S8**). Loading was determined to be 0.43 mmol/g by **Method 2**. The N^{α}-Fmoc-protecting group was removed using **Method 3** followed by coupling of Fmoc-Aib-OH via **Method 4** to give **S3**. The N^{α}-Fmoc-protecting group was then removed using **Method 3** followed by coupling of Fmoc-Val-OH, Fmoc-Ile-OH, and Fmoc-Aib-OH via **Method 5** to afford peptidyl resin **S4**. After removal of N^{α}-Fmoc-protecting group using **Method 3**, Fmoc-Aib-OH was coupled via **Method 4** to yield **S5**, followed by coupling of Fmoc-Cha-OH and Fmoc-Pro-OH by **Method 5**, and dodecanoic acid via **Method 6** to complete the linear peptide sequence of **S20**. Resin-bound peptide was cleaved using **Method 7**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m*/*z* were combined and lyophilised to afford peptide **S21** as white fluffy flakes (23.2 mg, 22.5% yield). Late-stage solution phase *C*-terminal coupling was employed to introduce the *C*-terminal aminoalcohol moiety on the peptide (23.2 mg, 0.02 mmol) dissolved in 0.5 mL DMF using **Method 8**. The crude peptide solution was purified by semi-preparative RP-HPLC in 5 × 100 μL batches using outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m*/*z* were combined and lyophilised to afford trichoderin A analogue **(19)** as a white amorphous solid (12.8 mg, 11.5% overall yield based on 0.1 mmol resin loading, >99% purity); t_R 29.8 min; LRMS: *m*/*z* (ESI-MS) 1117.3 ($[M+H]^+$ requires 1117.6), **Figures S22-S23**. The peptide was subsequently evaluated for its *in vitro* cytotoxicity against BxPC-3 and PANC-1 human PDAC cells under both glucose-replete and glucose-deprived conditions on the same plate (**Figure S24**).



Scheme S8. Fmoc/*t*Bu SPPS of trichoderin A analogue **(19)**.

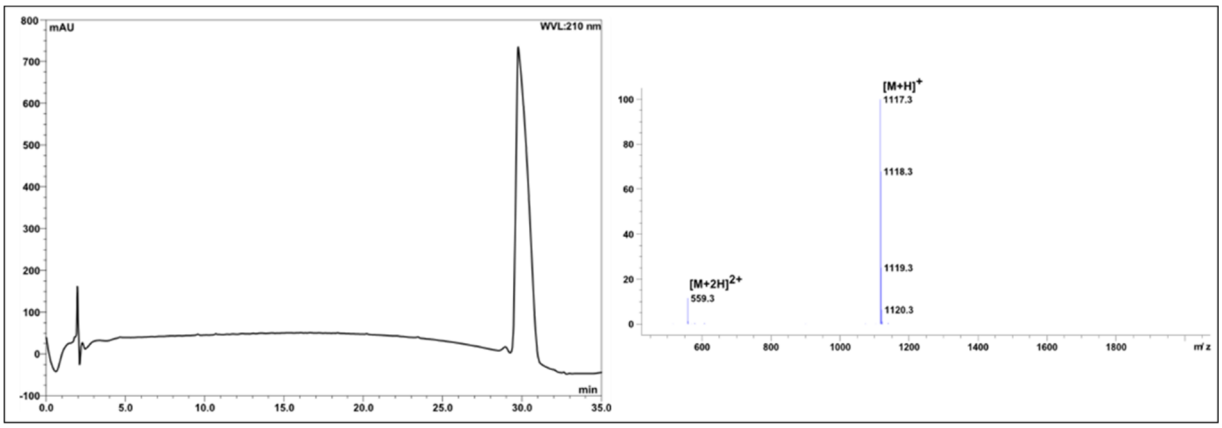


Figure S23. Analytical RP-HPLC profile of pure peptide **(19)** (*ca*. 99% as judged by peak area at 210 nm); xTerra® MS C18, 4.6 × 150 mm, 5 µm, linear gradient of 5% B to 95% B (*ca*. 3% B/min), 1 mL/min. *m*/*z* $[M+H]^+$ calculated: 1117.6; found: 1117.3.

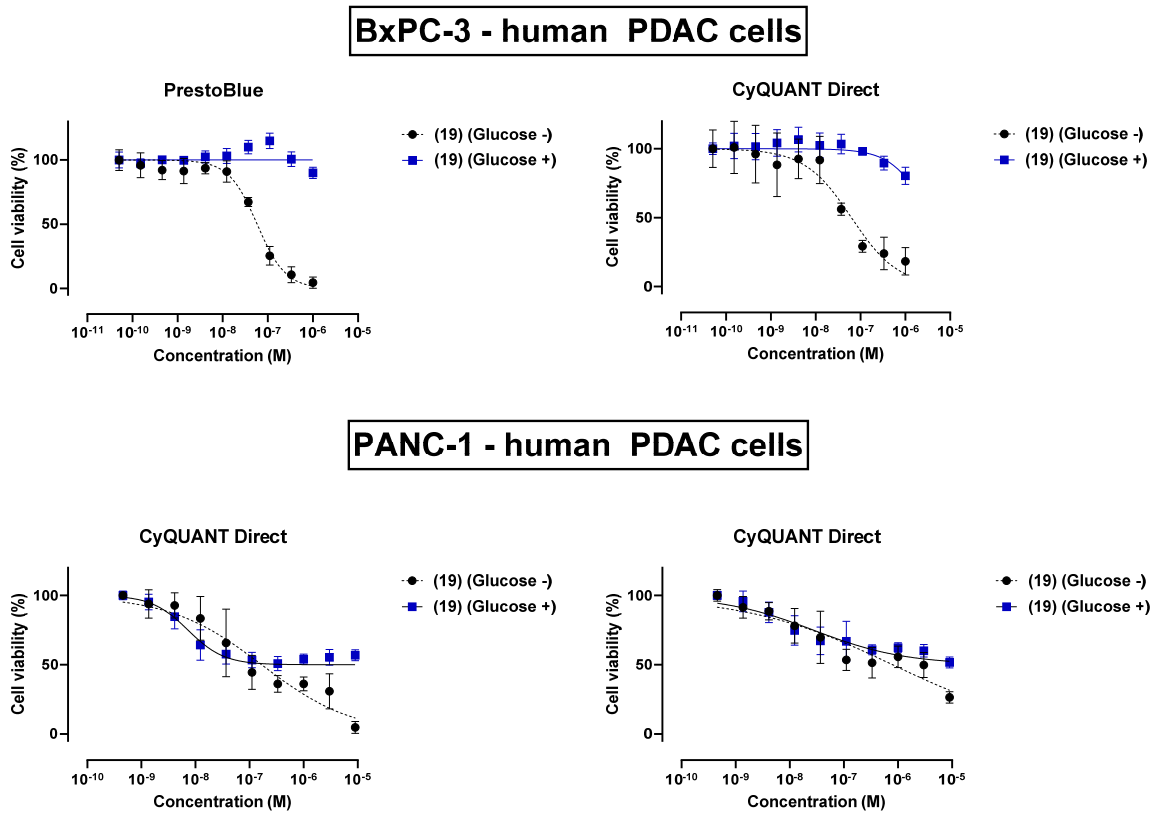


Figure S24. Comparative percent viability of BxPC-3 and PANC-1 human PDAC cells cultured under 'low glucose' (Glucose -) and 'normal glucose' (Glucose +) conditions following a 1-day exposure to trichoderin A analogue **(19)**, as judged by the PrestoBlue™ and CyQUANT™ Direct assays.

References

(1) Kavianinia, I.; Kunalingam, L.; Harris, P. W. R.; Cook, G. M.; Brimble, M. A. Total Synthesis and Stereochemical Revision of the Anti-Tuberculosis Peptaibol Trichoderin A. *Org. Lett.* **2016**, *18* (15), 3878–3881. https://doi.org/10.1021/acs.orglett.6b01886.