Supporting Information

Cytotoxic Activities of Aspergillin PZ and Trichoderone B from an Isolate of *Aspergillus flavipes* sp. Against NCI-60 Human Tumor Cell Lines

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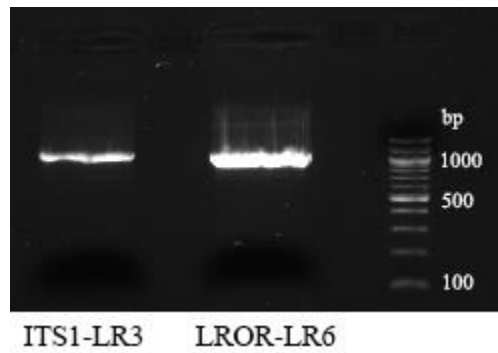
Figure S1: EtBr-stained gel electrophoresis image of two DNA amplicons generated by touchdown PCR with primer pairs ITS1-LR3 and LROR-LR6, respectively.
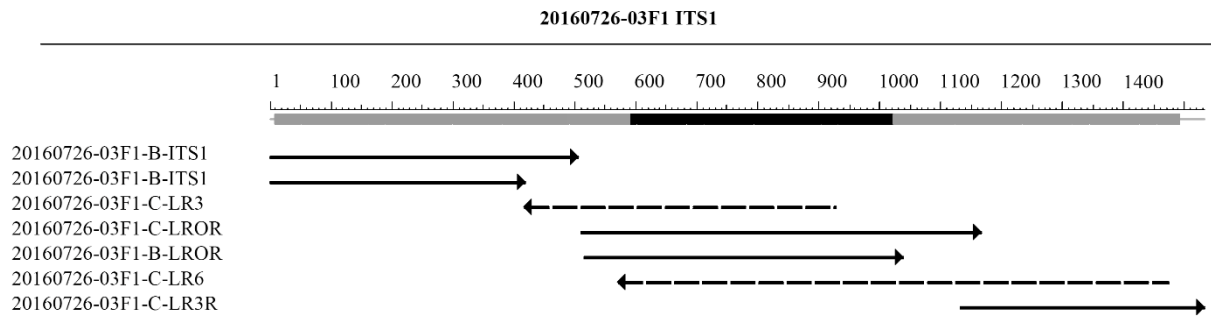

Figure S2: Contig map of the ITS1-28S rDNA region assembled from PCR sequencing reads. Primers used in sequencing reads are ITS1, LR3, LROR, LR6, and LR3R, respectively.

Score		Expect	Identities	Gaps	Strand
2058 bits(1114)		0.0	1118/1120(99%)	0/1120(0%)	Plus/Plus

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Figure S3: NCBI-BLASTn [1] comparison showed a 99.82% sequence identity of the ITS1-28S rDNA region of a fungal isolate (lab record: 20160726-03F1) to that of *A. flavipes* NRRL 302.
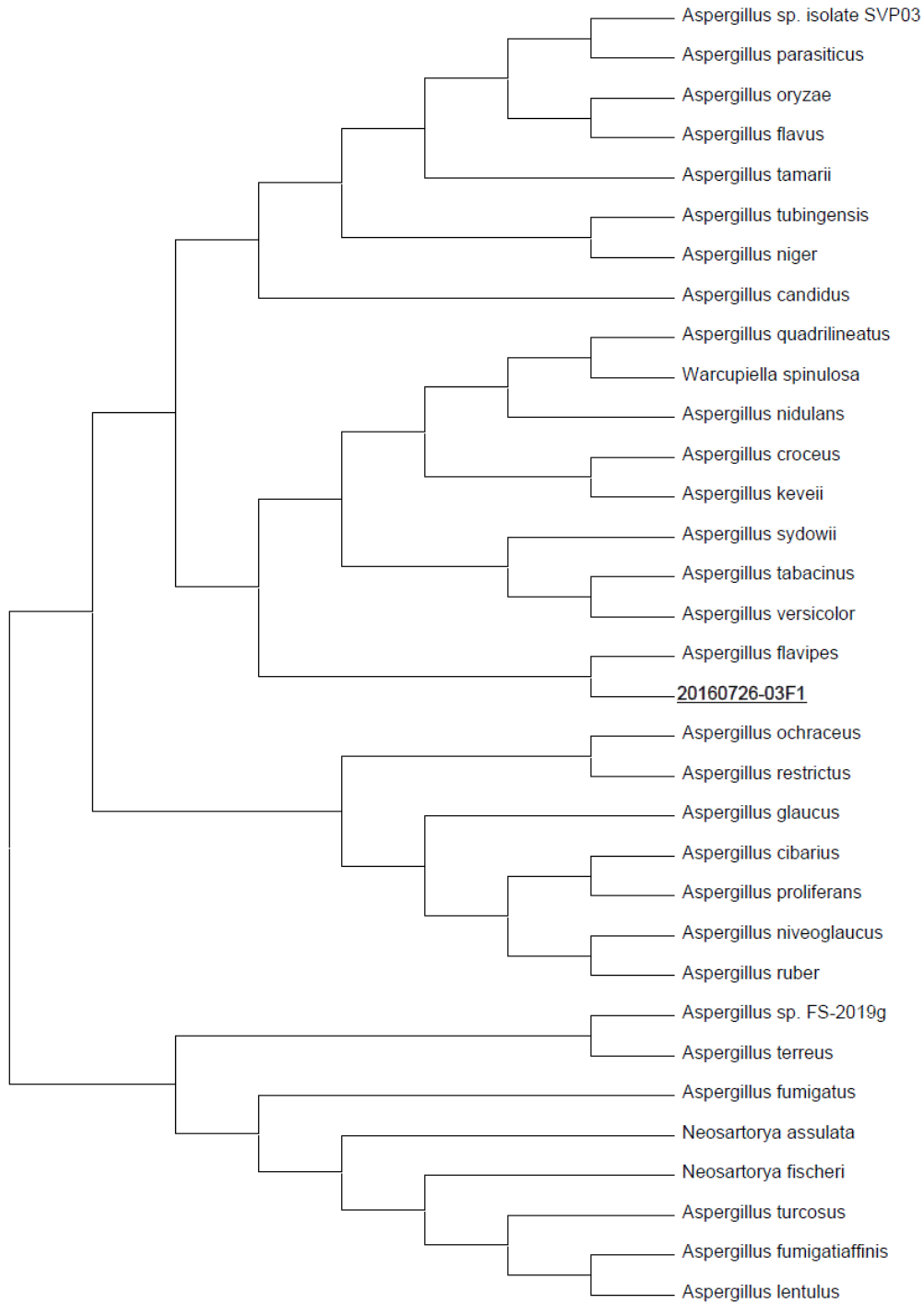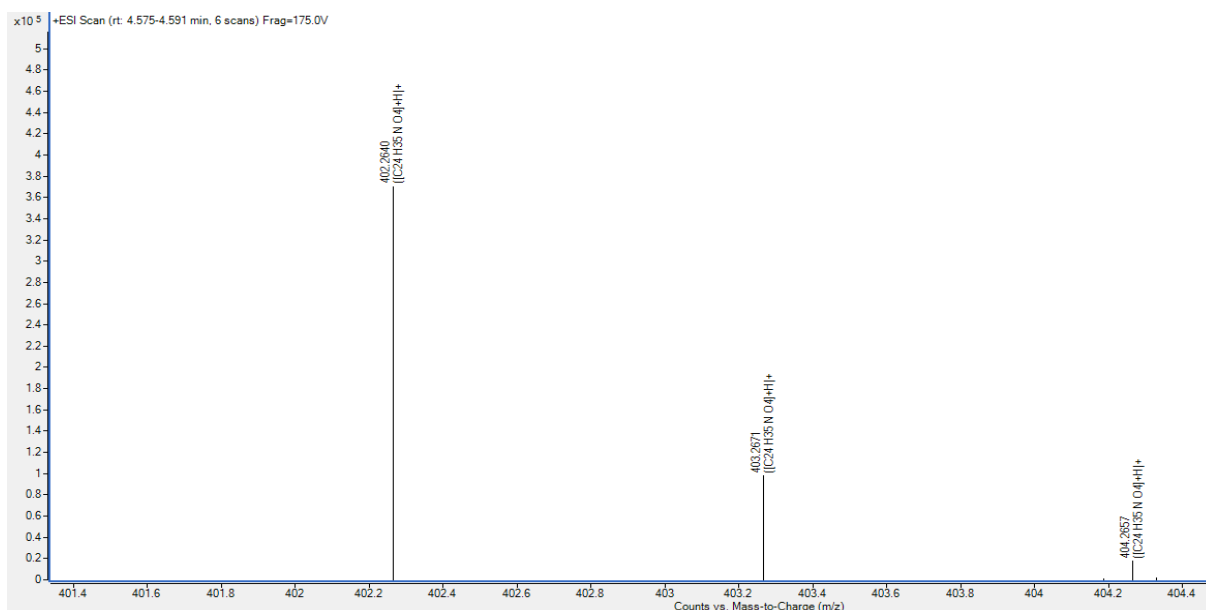
Figure S4: Inferred evolutionary history tree for the ITS-28S rDNA region of a fungal isolate (lab record no. 20160726-03F1) from the Concho County in central Texas, USA, which produced aspergillin PZ (**1**) and trichoderone B (**2**) (MEGA X) [2-4].

Species	m/z (Calc)	m/z (Observed)	Isotope Distribution (Calc)	Isotope Distribution (Observed)
((C24 H35 N O4) + H) +	402.264	402.264	76.2%	76.1%
	403.267	403.267	20.5%	20.2%
	404.27	404.266	3.3%	3.6%

Figure S5: TOF-ESI-MS and HRMS comparison table of calculated to observed isotope abundances and masses of a purified secondary metabolite from *Aspergillus flavipes* sp. collected from the Concho County in central Texas, USA, determined to be aspergillin PZ (**1**) [observed *m/z* 402.2640 (M + H)$^+$; calculated *m/z* 402.264 for (C$_{24}$H$_{35}$NO$_4$ + H)$^+$].
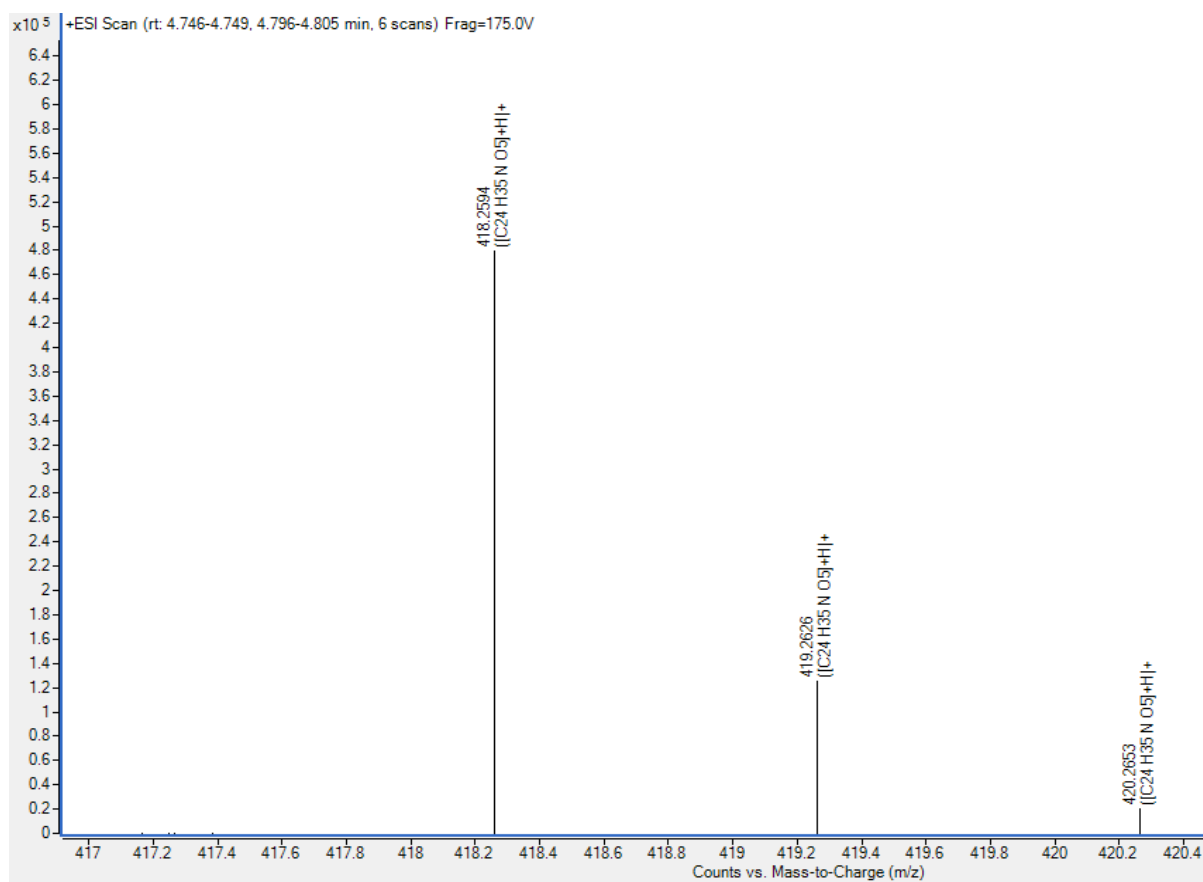
Species	m/z (Calc)	m/z (Observed)	Isotope Distribution (Calc)	Isotope Distribution (Observed)
((C24 H35 N O5) + H)+	418.259	418.259	75.8%	76.3%
	419.262	419.263	20.4%	20.0%
	420.265	420.265	3.4%	3.2%
	421.268	421.267	0.4%	0.5%

Figure S6: TOF-ESI-MS and HRMS comparison table of calculated to observed isotope abundances and masses of a purified secondary metabolite from *Aspergillus flavipes* sp. collected from the Concho County in central Texas, USA determined to be trichoderone B (**2**) [observed *m/z* 418.2594 (M + H)$^+$; calculated *m/z* 418.259 for (C$_{24}$H$_{35}$NO$_5$ + H)$^+$].
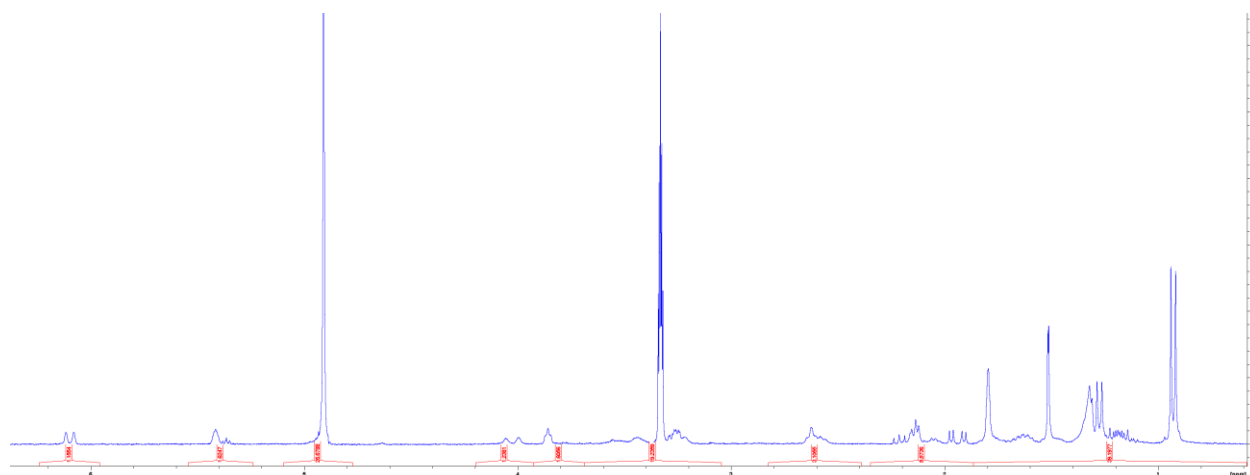
Aspergillin PZ (**1**)
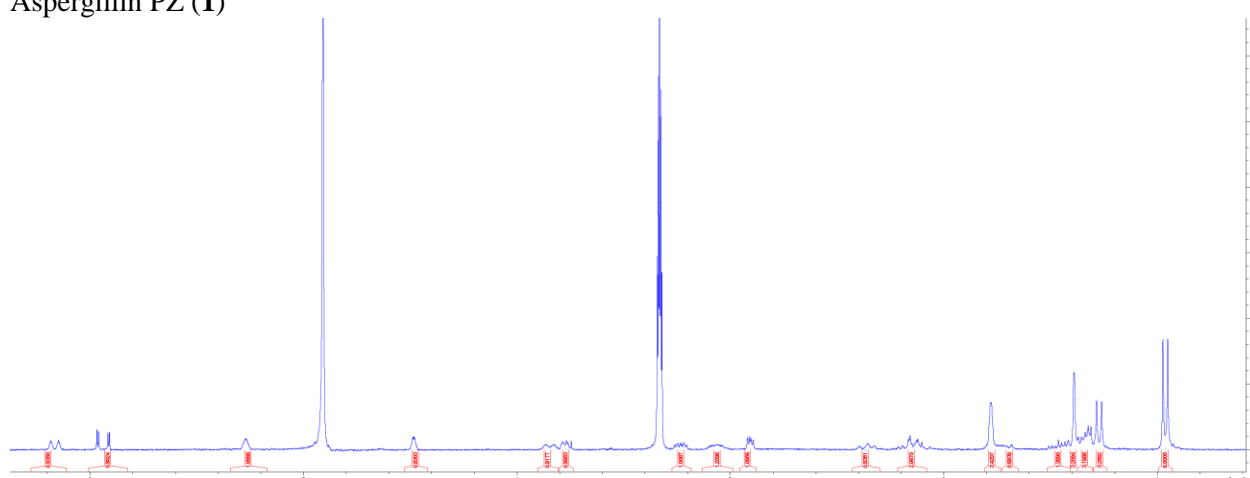
Trichoderone B (**2**)

Aspergillin PZ (1): White amorphous powder. ^{1}H NMR (MeOD, 300 MHz) δ 6.10 (d, *J* = 10.76 Hz, 1H), 5.41 (m, 1H), 5.36 (t, *J* = 4.70 Hz, 1H), 4.02 (d, *J* = 18.09 Hz, 1H), 3.86 (m, 1H), 3.30 – 3.18 (m, 1H), 2.67 – 2.53 (m, 2H), 2.24 -2.18 (m, 1H), 2.18 – 2.10 (m, 1H), 1.94 (dd, *J* = 17.73, 5.49 Hz, 1H), 1.79 (s, 4H), 1.63 (m, 2H), 1.51 (d, *J* = 1.32, 3H), 1.32 (m, 4H), 1.27 (d, *J* = 6.92 Hz, 3H), 1.19 (m, 2H), 0.93 (d, *J* = 6.57 Hz, 6H).

Trichoderone B (2): White amorphous powder. ^{1}H NMR (MeOD, 300 MHz) δ 7.27 (dd, *J* = 15.30, 2.40 Hz, 1H), 6.16 (m, 1H), 5.96 (d, *J* = 2.39 Hz, 1H), 5.91 (d, *J* = 2.39 Hz, 1H), 5.27 (m, 1H), 4.28 (m, 1H), 3.85 (m, 1H), 3.80 – 3.75 (m, 1H), 3.23 (m, 1H), 3.06 (m, 1H), 2.93 – 2.88 (m, 1H), 2.36 (m, 1H), 2.23 – 2.08 (m, 2H), 1.78 (m, 4H), 1.50 – 1.42 (m, 2H), 1.39 (s, 3H), 1.37-1.31 (m, 3H), 1.27 (d, *J* = 7.28 Hz, 3H), 0.96 (d, *J* = 6.57 Hz, 6H).

Figure S7: ^{1}H-NMR spectra for **1** and **2** obtained in MeOD at 300MHz.
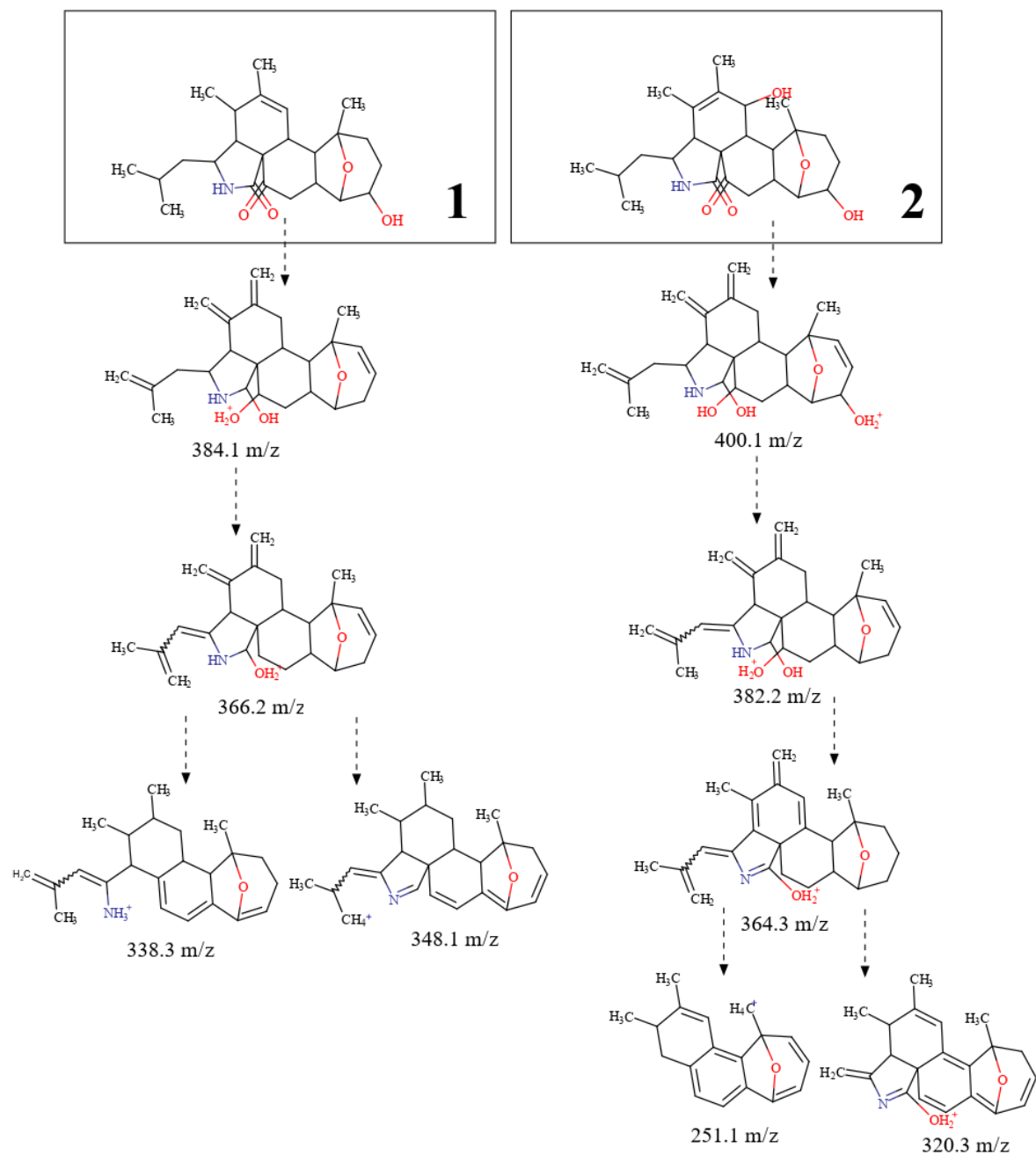
Figure S8: Predicted products generated by MS(n) fragmentation comparison of aspergillin PZ (**1**) and trichoderone B (**2**) with CFM-ID [5].

NCI-60 µM Single Dose Screening (% Cell Growth)

	Breast					
	MCF7	MDA-MB-231	HS 578T	BT-549	**T-47D**	MDA-MB-468
Compound 1	94.27	103.65	88.52	84.83	98	90.75
Compound 2	76.79	99.3	84.85	77.17	75.49	72.84
Δ	17.48	4.35	3.67	7.66	22.51	17.91

	CNS					
	SF-268	SF-295	SF-539	SNB-19	**SNB-75**	U251
Compound 1	95.85	107.8	96.51	91.89	48.94	96.72
Compound 2	101.48	106.12	91.19	92.81	48.98	95.67
Δ	-5.63	1.68	5.32	-0.92	-0.04	1.05

	Colon					
	COLO 205	HCC-2998	HCT-116	HCT-15	HT29	KM12
Compound 1	110.33	97.48	92.72	93.19	102.12	100.09
Compound 2	96.11	89.75	90.46	81.3	96.93	100.93
Δ	14.22	7.73	2.26	11.89	5.19	-0.84

	Colon	Leukemia				
	SW-620	**CCRF-CEM**	**HL-60(TB)**	K-562	MOLT-4	**RPMI-8226**
Compound 1	94.47	111.65	99.69	107.34	98.77	108.59
Compound 2	87.37	84.7	75.89	95.96	90.81	86.22
Δ	7.1	26.95	23.8	11.38	7.96	22.37

	Non-Small Cell Lung Cancer					
	HOP-92	NCI-H226	NCI-H23	NCI-H322M	NCI-H460	NCI-H522
Compound 1	71.33	97.15	88.82	89.63	99.3	82.32
Compound 2	65.11	92.26	91.16	91.08	94.79	84.8
Δ	6.22	4.89	-2.34	-1.45	4.51	-2.48

	Non-Small Cell Lung Cancer			Melanoma		
	A549/ATCC	EKVX	HOP-62	SK-MEL-5	**UACC-257**	UACC-62
Compound 1	95.67	106.91	98.26	99.1	80.92	96.42
Compound 2	93.84	93.86	101.19	96.01	73.26	89.75
Δ	1.83	13.05	-2.93	3.09	7.66	6.67

	Melanoma					
	LOX IMVI	MALME-3M	M14	MDA-MB-435	SK-MEL-2	SK-MEL-28
Compound 1	95.15	94.06	91.12	98.39	94.25	91.68
Compound 2	96.83	92.44	88.4	97.65	94.22	93.45
Δ	-1.68	1.62	2.72	0.74	0.03	-1.77

	Renal					
	786-0	**A498**	ACHN	CAKI-1	RXF 393	SN12C
Compound 1	99.54	58.49	100.46	89.53	90.87	95.43
Compound 2	96.06	62.06	82.08	85.52	77.84	91.28
Δ	3.48	-3.57	18.38	4.01	13.03	4.15

	Renal		Ovarian			
	TK-10	UO-31	IGROV1	OVCAR-3	OVCAR-4	OVCAR-5
Compound 1	95.27	76.32	101.3	96.91	93.67	93.58
Compound 2	85.45	77.19	87.76	91.69	85.4	95.35
Δ	9.82	-0.87	13.54	5.22	8.27	-1.77

	Ovarian		Prostate			
	OVCAR-8	NCI/ADR-RES	SK-OV-3	PC-3	DU-145	Mean
Compound 1	90.29	98.66	102.01	94.13	103.31	94.31
Compound 2	87.1	97.7	105.28	80.88	93.96	88.33
Δ	3.19	0.96	-3.27	13.25	9.35	5.98

Figure S9: Percentages of cell growth of 60 cell lines following exposure to a single dosing (10 µM) of aspergillin PZ (**1**) or trichoderone B (**2**). Δ indicates the difference in cell growth percentage between the two tested compounds.
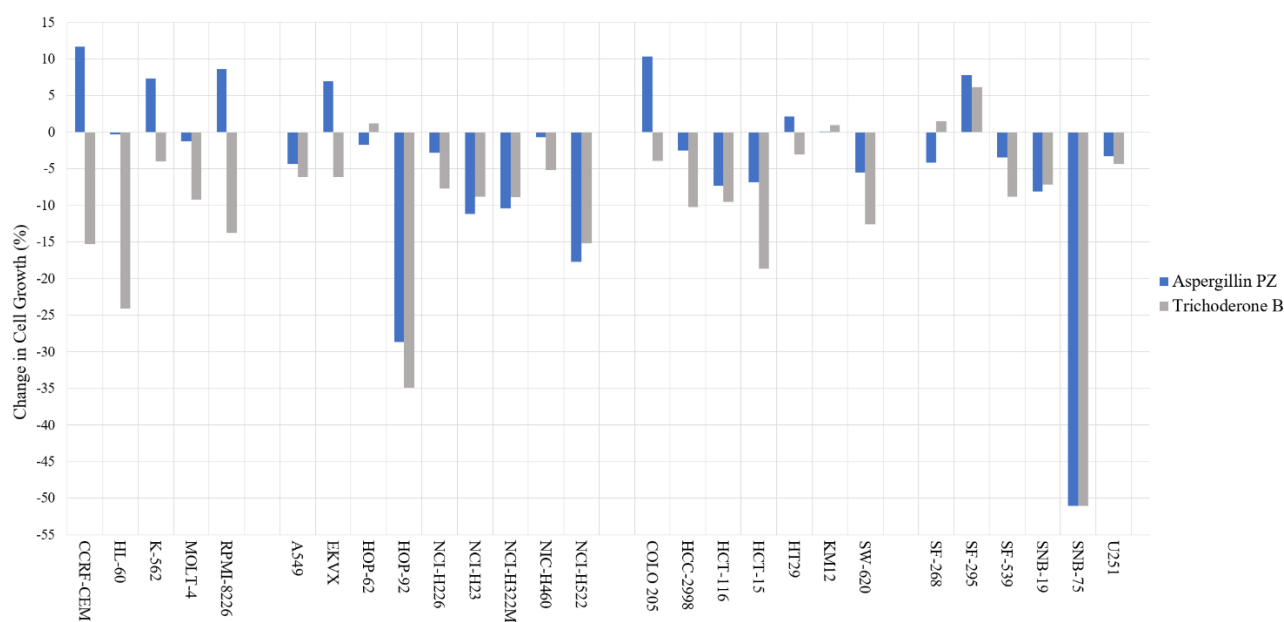
Figure S10: Graph of growth percentages from NCI60 human cell lines screening following single dosing (10 µM) of aspergillin PZ (**1**) or trichoderone B (**2**).

References

[1] Z. Zhang, S. Schwartz, L. Wagner and W. Miller (2000). A greedy algorithm for aligning DNA sequences, *J. Comput. Biol*. **7** (**1-2**), 203-214

[2] S. Kumar, G. Stecher, M. Li, C. Knyaz and K. Tamura (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms, *Mol. Biol. Evol*. **35** (**6**), 1547-1549

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[4] J. Felsenstein (1985). Confidence limits on phylogenies: An approach using the bootstrap, *Evolution* **39** (**4**), 783-791

[5] F. Allen, R. Greiner and D. Wishart (2015). Competitive fragmentation modeling of ESI-MS/MS spectra for putative metabolite identification, *Metabolomics* **11** (**1**), 98-110.