Supplementary Information

Discovery of the orsellinic acid synthase gene in *Aspergillus nidulans* using a genome mining approach

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Supplemental References.

S1. S. Armitt, W. McCullough and C. F. Roberts, *J Gen Microbiol*, 1976, 92, 263-282.

Table S1. Primers used in this study

primer	Sequence (5′→3′)
AN7901.3P1	ATT GGA AGT CAA GCC GTA CC
AN7901.3P2	TGC TTA CCG AGC TTT TCT GG
AN7901.3P3	CGA AGA GGG TGA AGA GCA TTG CGG TAA ATG GAA CGG AAA CC
AN7901.3P4	GCA TCA GTG CCT CCT CTC AGA CAG CAT ATG CGC TTG CTG AAT CG
AN7901.3P5	GCT GGT CAG TCC TTC TAC AAC C
AN7901.3P6	GGA CGA CTG TAG AGG TAG ATG C
AN7902.3P1	AGT GCG AGT AGC GAT TAT GC
AN7902.3P2	TTG ACA TGT GCT CCA AGA GG
AN7902.3P3	CGA AGA GGG TGA AGA GCA TTG CTC CAG ATG ACA ACC AAT GC
AN7902.3P4	GCA TCA GTG CCT CCT CTC AGA CAG TAG ACT GCG ATT CAG CAA GC
AN7902.3P5	GGG CTC TAC GAG GTA AAT TTC G
AN7902.3P6	GAG CTG CTG GGT TCC TTA GC
AN7903.3P1	TCC ACT TGA CCG AAT ACT CC
AN7903.3P2	CCT TCA GGA GAT CAG TGA GG
AN7903.3P3	CGA AGA GGG TGA AGA GCA TTG TCT CGG AAG ACT GTC TCA AAG C
AN7903.3P4	GCA TCA GTG CCT CCT CTC AGA CAG CAT CCA TTG TCG TCA CTC AAC C
AN7903.3P5	TGA TAC TCG GTT GCT CTC TGG
AN7903.3P6	GCG ACA AAT CTG GAG GTA GG
AN7904.3P1	AAT CTA GGA CCG GTC AGT GC
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AN7904.3P3	CGA AGA GGG TGA AGA GCA TTG TGC CGA TGT CAG TTT TCT GG
AN7904.3P4	GCA TCA GTG CCT CCT CTC AGA CAG CCA CTC AAT ATC GCC ACA CG
AN7904.3P5	AGT GTT TAC CCG CAT TGA GC
AN7904.3P6	TGC TGT ATA GCG GCA AGT CC
AN7905.3P1	CGA ATG CTT GGA TGA CAA CG
AN7905.3P2	CAA AAG CCG CTC AAG ATA GG
AN7905.3P3	CGA AGA GGG TGA AGA GCA TTG TAA GTC TCC GTT GGA TGT CG
AN7905.3P4	GCA TCA GTG CCT CCT CTC AGA CAG TGG GTT GTG GGC TAG TAG AGG
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AN7905.3P6	CCT GCT GAC AAA GAC AAA GC
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AN7906.3P3	CGA AGA GGG TGA AGA GCA TTG TTT TCT CAA CCC CCA AGT GC
AN7906.3P4	GCA TCA GTG CCT CCT CTC AGA CAG CAT TAC TTC GCC TCG TGT GG
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AN7906.3P6	TCC ACA ACT GTG CTC AAT CC
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AN7907.3P3	CGA AGA GGG TGA AGA GCA TTG AGA TAC AGT GAG CCA GCT TCT ACG
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AN7909.3P4	GCA TCA GTG CCT CCT CTC AGA CAG CAG GTA TAA TGT GCA CGT CTC C
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AN7909.3P6	GAC AGG ATA ATC TCG GAC TCG
AN7911.3P1	GCA TGT TGA GGA TGA TGT CC
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AN7912.3P1	GCA TAT AGG GAA GCA TCT CAC C
AN7912.3P2	ACG ATG ATC TTC AGC CTT GG
AN7912.3P3	CGA AGA GGG TGA AGA GCA TTG CGT AGC TAC CCA GAA GAA AAG ACG
AN7912.3P4	GCA TCA GTG CCT CCT CTC AGA CAG GCA ACT CTA AAC GAC ACG ATG G
AN7912.3P5	CAC CAC ACG AAG CTG AAA CG
AN7912.3P6	GCA CCA TTC AGA GTA CTG TTC C
AN7913.3P1	CCA GAT GAC CAG GAC TTT GC
AN7913.3P2	TCG TCA AGA GAG GGA TAC GG
AN7913.3P3	CGA AGA GGG TGA AGA GCA TTG CGT GCA AGA GAC ATG ATG AGC
AN7913.3P4	GCA TCA GTG CCT CCT CTC AGA CAG GTT TCG CTA CAG CCC TTT GC
AN7913.3P5	CCG ATA CCG CTA TCT ATA CTC C
AN7913.3P6	CTC CAG CTT TTC CCA ATT CC
AN7914.3P1	GGT AAT GTC AGG CTC GTT GC
AN7914.3P2	AAG GTC CAC GTT GAC AGA GG
AN7914.3P3	CGA AGA GGG TGA AGA GCA TTG AAG ATG GAC AGA GAC GAG ACT GG
AN7914.3P4	GCA TCA GTG CCT CCT CTC AGA CAG CCA CGC TGA ACT CGT ATT GC
AN7914.3P5	GGT GGC TAT GAA GAT CTG TGG
AN7914.3P6	CGA CTT CGC CTA CTC AAA CC
AN7915.3P1	TTC ACG TCC GCT GTC TAT GC
AN7915.3P2	GTC TAA ACA TCC GCC CAA GG
AN7915.3P3	CGA AGA GGG TGA AGA GCA TTG GCG GTA AAA GGA ACG GAA GG
AN7915.3P4	GCA TCA GTG CCT CCT CTC AGA CAG AAA ACG TCA AGG CGT CAA GG
AN7915.3P5	AGG GTT GAA GAG GTG CAA GG
AN7915.3P6	TTC ACG TCC GCT GTC TAT GC

Blue and red sequences are tails that anneal to the *A. fumigatus pyrG* (*AfpyrG*) during fusion PCR.

Table S2. *A. nidulans* strains used in this study

Strain	secondary metabolite mutations	Genotype	Source
R153	None (WT)	*pyrG89, weA3*	[S1]
LO2026	Δ*stcJ*	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB*	[19]
LO2149	*stcJ*Δ *cclA* AN0150.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB; cclA:AfpyroA;* AN0150*::AfpyrG*	[19]
LO2154	*stcJ*Δ *cclA*Δ AN0523.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB; cclA:AfpyroA;* AN0523*::AfpyrG*	[19]
LO2159	*stcJ*Δ *cclA*Δ AN1034.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB; cclA:AfpyroA;* AN1034*::AfpyrG*	[19]
LO2165	*stcJ*Δ *cclA*Δ AN2032.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB; cclA:AfpyroA;* AN2032*::AfpyrG*	[19]
LO2169	*stcJ*Δ *cclA*Δ AN3230.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB; cclA:AfpyroA;* AN3230*::AfpyrG*	[19]
LO2174	*stcJ*Δ *cclA*Δ AN3386.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB; cclA:AfpyroA;* AN3386*::AfpyrG*	[19]
LO2179	*stcJ*Δ *cclA*Δ AN6000.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB; cclA:AfpyroA;* AN6000*::AfpyrG*	[19]
LO2184	*stcJ*Δ *cclA*Δ AN6448.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB; cclA:AfpyroA;* AN6448*::AfpyrG*	[19]
LO2189	*stcJ*Δ *cclA*Δ AN7071.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB; cclA:AfpyroA;* AN7071*::AfpyrG*	[19]
LO2194	*stcJ*Δ *cclA*Δ AN7909.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB; cclA:AfpyroA;* AN7909*::AfpyrG*	[19]
LO2472 to LO2474	*stcJ*Δ AN7901.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;* AN7901::*AfpyrG*	This study
LO2477 to LO2479	*stcJ*Δ AN7902.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;* AN7902::*AfpyrG*	This study
LO2482 to LO2484	*stcJ*Δ AN7903.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;* AN7903::*AfpyrG*	This study
LO2487 to LO2489	*stcJ*Δ AN7904.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;* AN7904::*AfpyrG*	This study
LO2492 to LO2494	*stcJ*Δ AN7905.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;* AN7905::*AfpyrG*	This study
LO2497 to LO2499	*stcJ*Δ AN7906.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;* AN7906::*AfpyrG*	This study
LO2502 to LO2504	*stcJ*Δ AN7907.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;* AN7907::*AfpyrG*	This study
LO2507 to LO2509	*stcJ*Δ AN7908.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;* AN7908::*AfpyrG*	This study
LO2512 to LO2514	*stcJ*Δ AN7909.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;* AN7909::*AfpyrG*	This study
LO2517 to LO2519	*stcJ*Δ AN7911.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;* AN7911::*AfpyrG*	This study
LO2522 to LO2524	*stcJ*Δ AN7912.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;* AN7912::*AfpyrG*	This study
LO2527 to LO2529	*stcJ*Δ AN7913.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;* AN7913::*AfpyrG*	This study
LO2532 to LO2534	*stcJ*Δ AN7914.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;* AN7914::*AfpyrG*	This study
LO2537 to LO2539	*stcJ*Δ AN7915.3Δ	*pyrG89; pyroA4, nkuA::argB; riboB2, stcJ::AfriboB;* AN7915::*AfpyrG*	This study

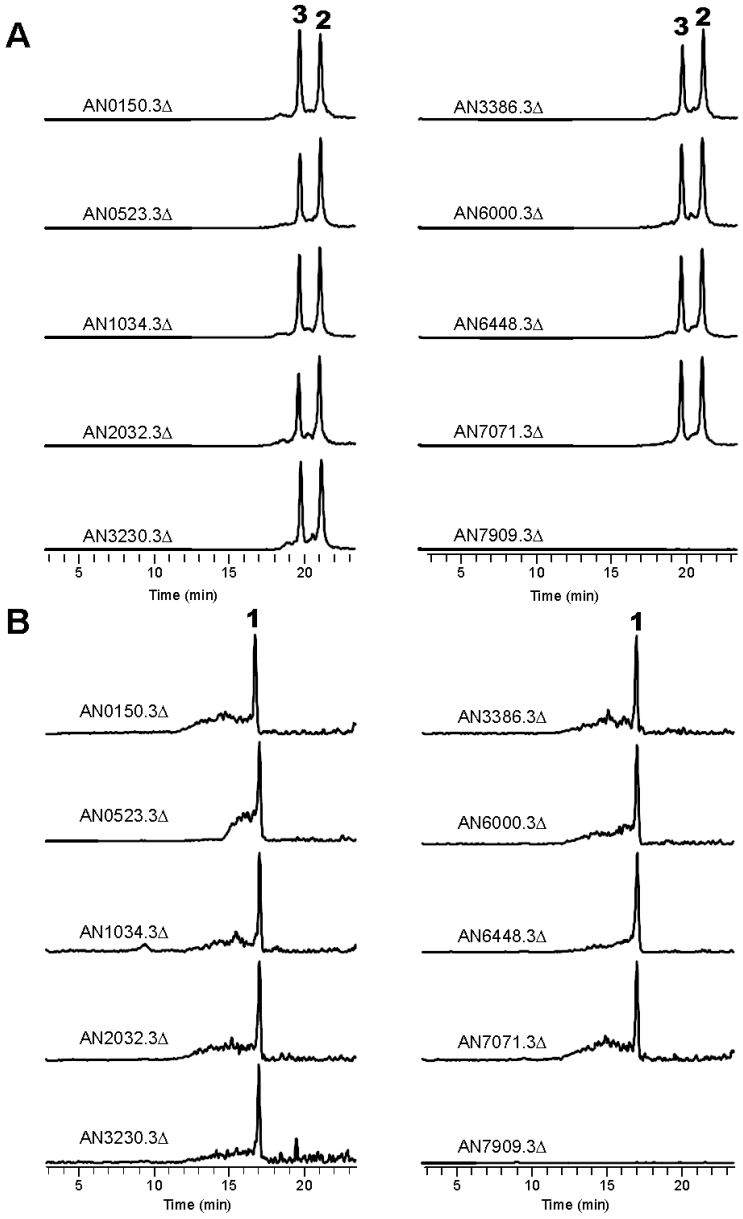

Figure S1. (A) Negative mode extracted ion chromatograms (EIC) at *m*/*z* 395, corresponding to F-9775A (**2**) and B (**3**), and (B) Negative mode EIC at *m*/*z* 167, corresponding to orsellinic acid (**1**), for the ten NR-PKS knockout strains studied.

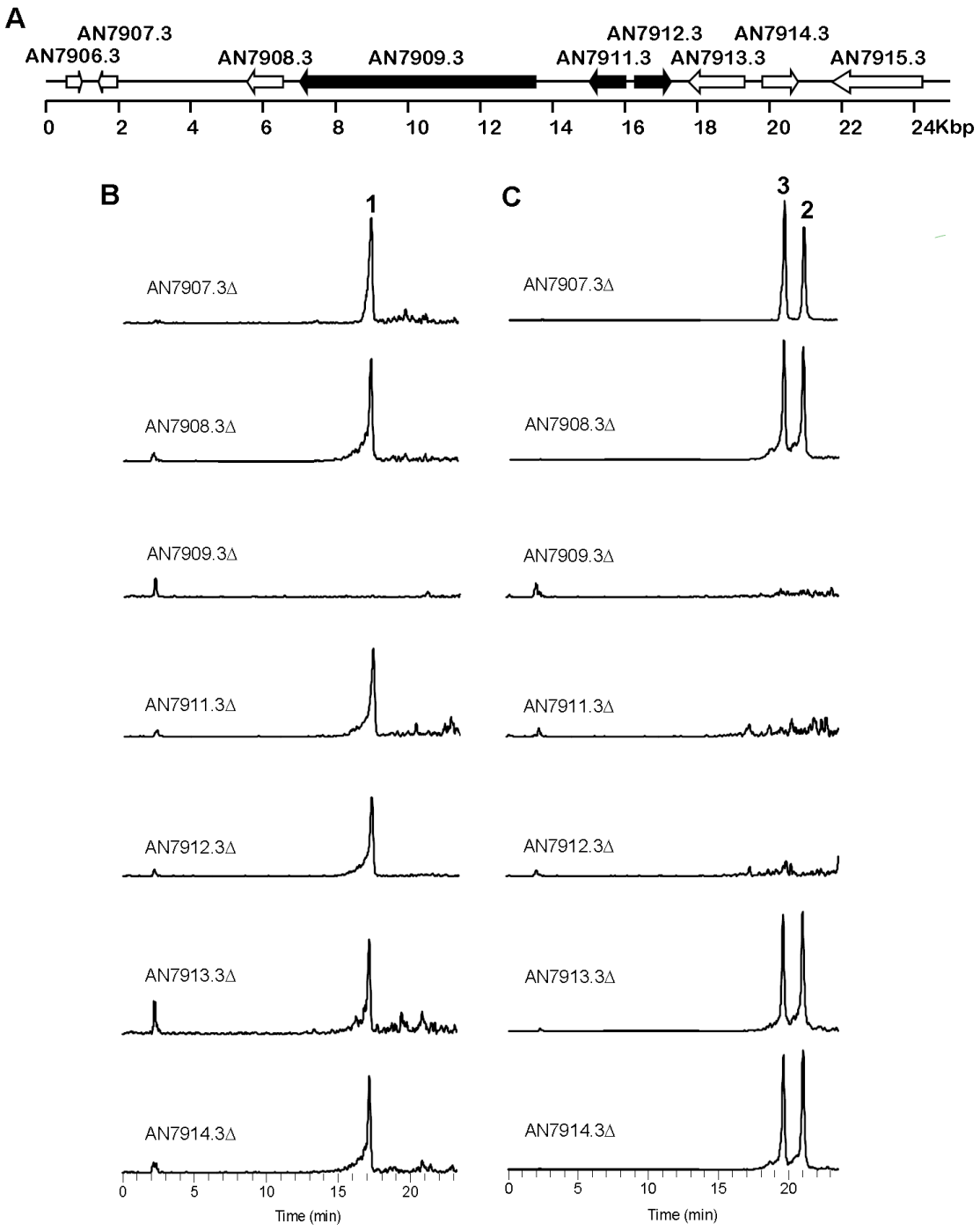

Figure S2. (A) Organization of the F-9775 gene cluster in *A. nidulans*. Each arrow indicates the direction of transcription and the relative sizes of the ORFs deduced from analysis of the nucleotide sequences. ORFs AN7909.3 (PKS), AN7911.3 (decarboxylase) and AN7912.3 (tyrosinase) are required for F-9775 A and B biosynthesis. (B) Negative mode extracted ion chromatograms (EIC) at *m*/*z* 167, corresponding to orsellinic acid, and (C) Negative mode EIC at *m*/*z* 395, corresponding to F-9775 A/B, for the strains AN7907.3Δ to AN7914.3Δ.

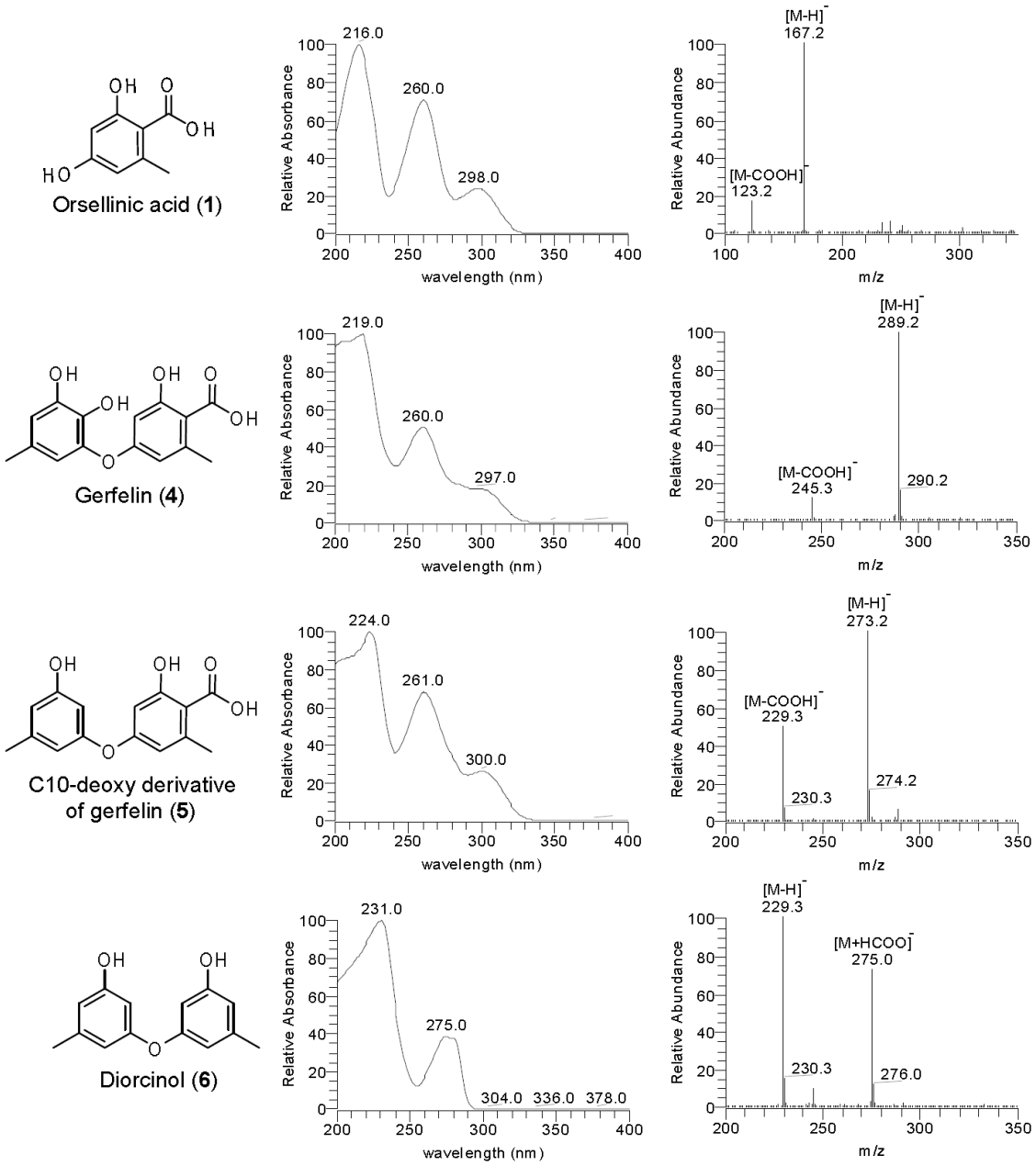

Figure S3. UV-Vis and ESIMS spectra (negative mode) of isolated compounds.

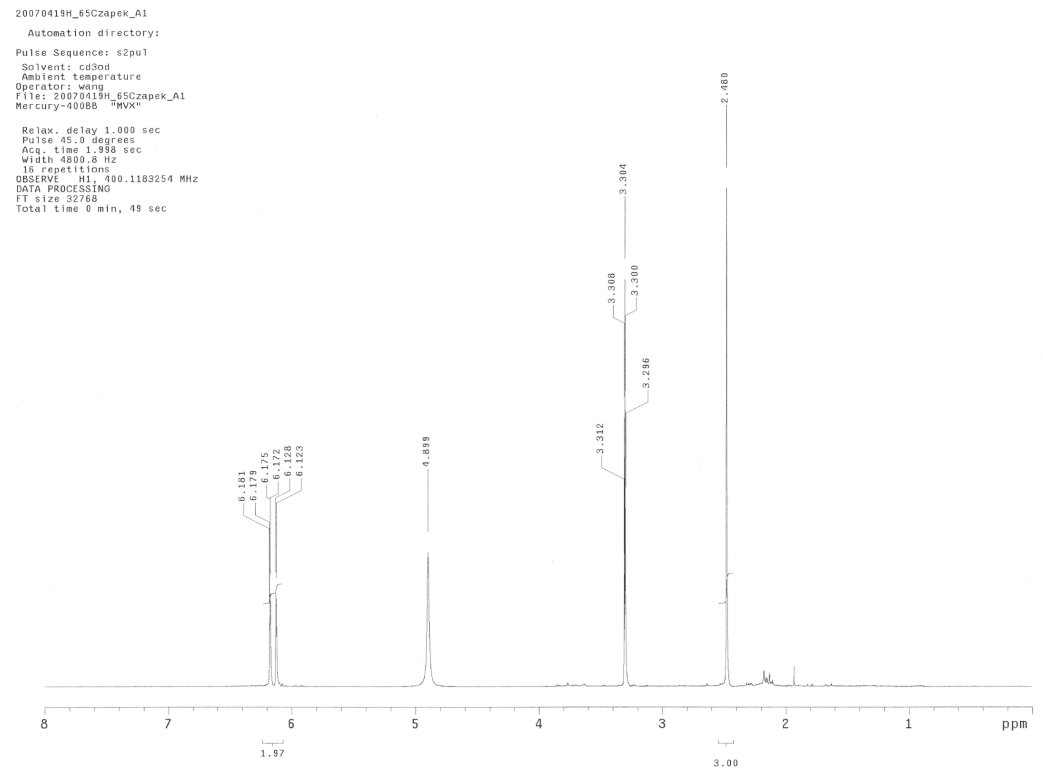

Figure S4. ^{1}H NMR spectrum of orsellinic acid (**1**) in CD_3OD

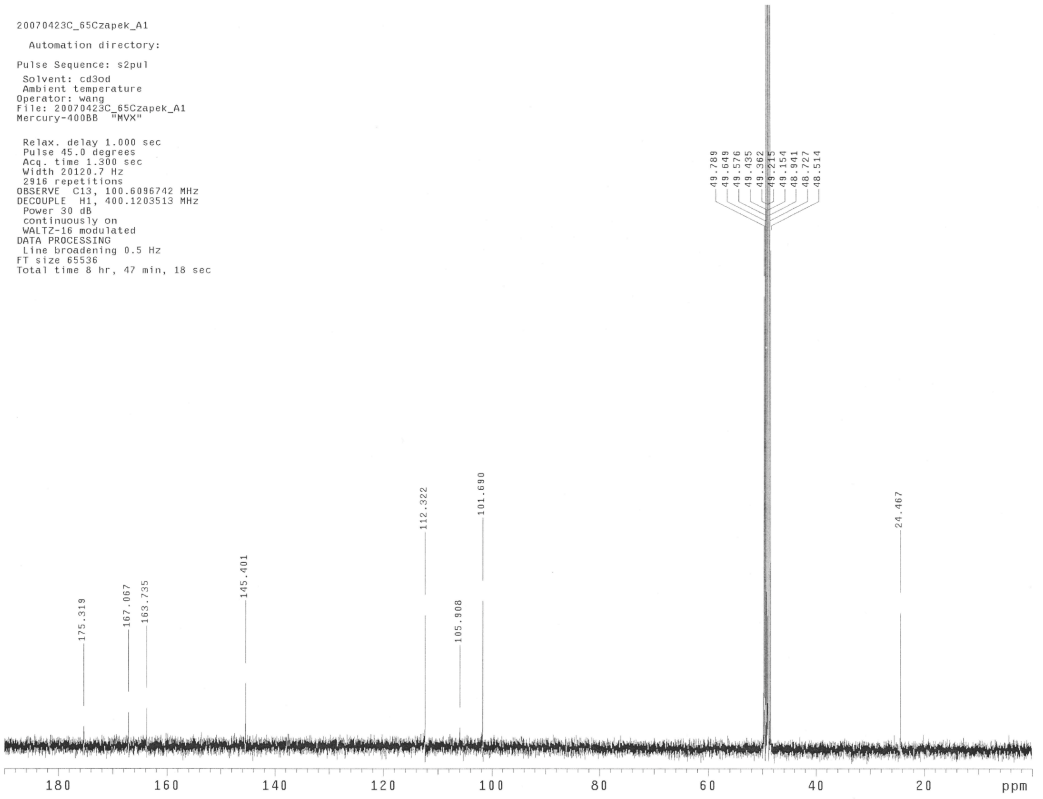

Figure S5. ^{13}C NMR spectrum of orsellinic acid (**1**) in CD_3OD

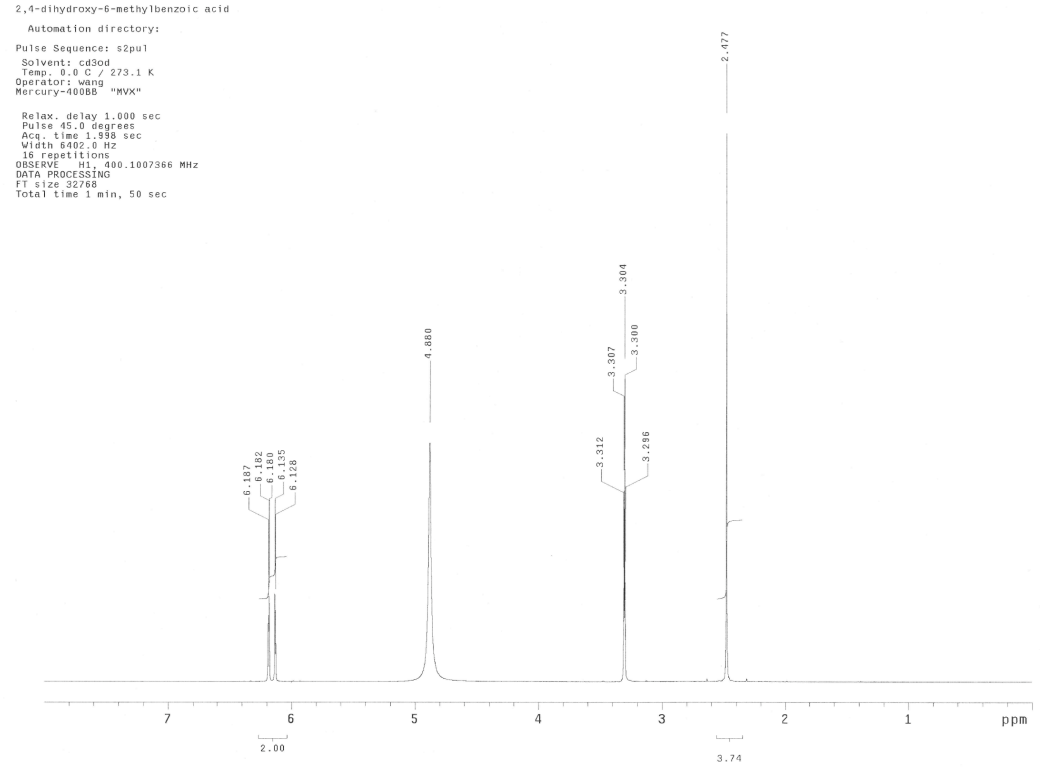

Figure S6. ^{1}H NMR spectrum of orsellinic acid (**1**) purchased from Alfa Aesar in CD_3OD

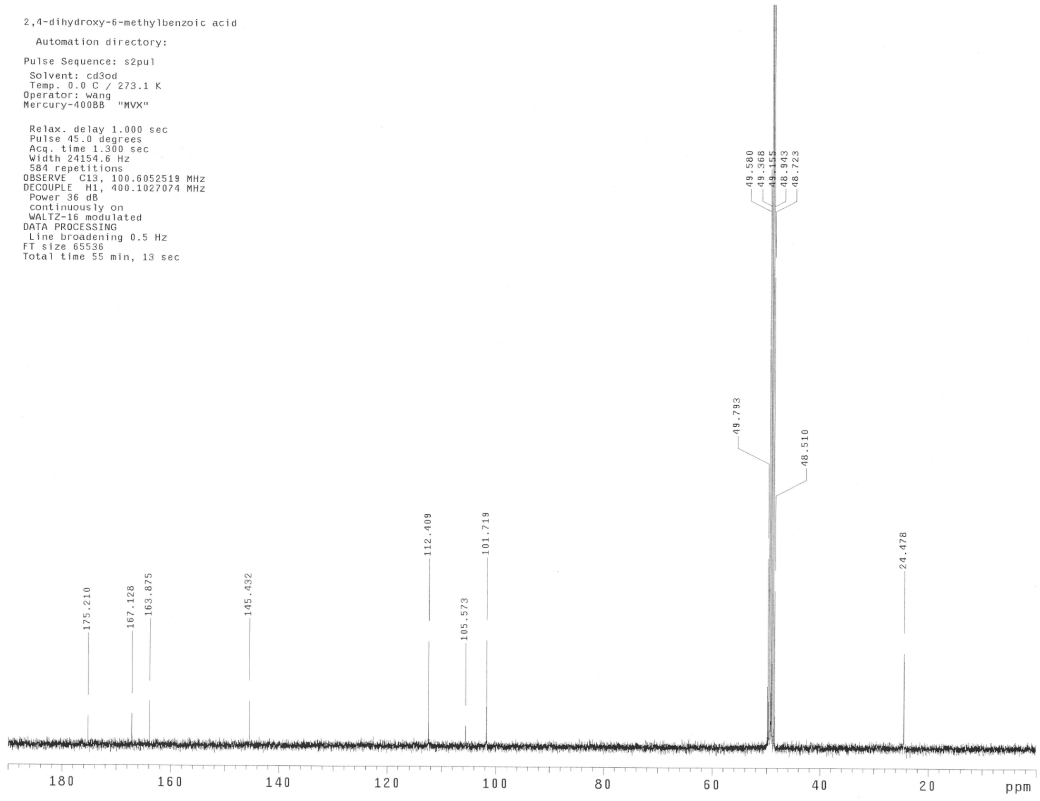

Figure S7. ^{13}C NMR spectrum of orsellinic acid (**1**) purchased from Alfa Aesar in CD_3OD

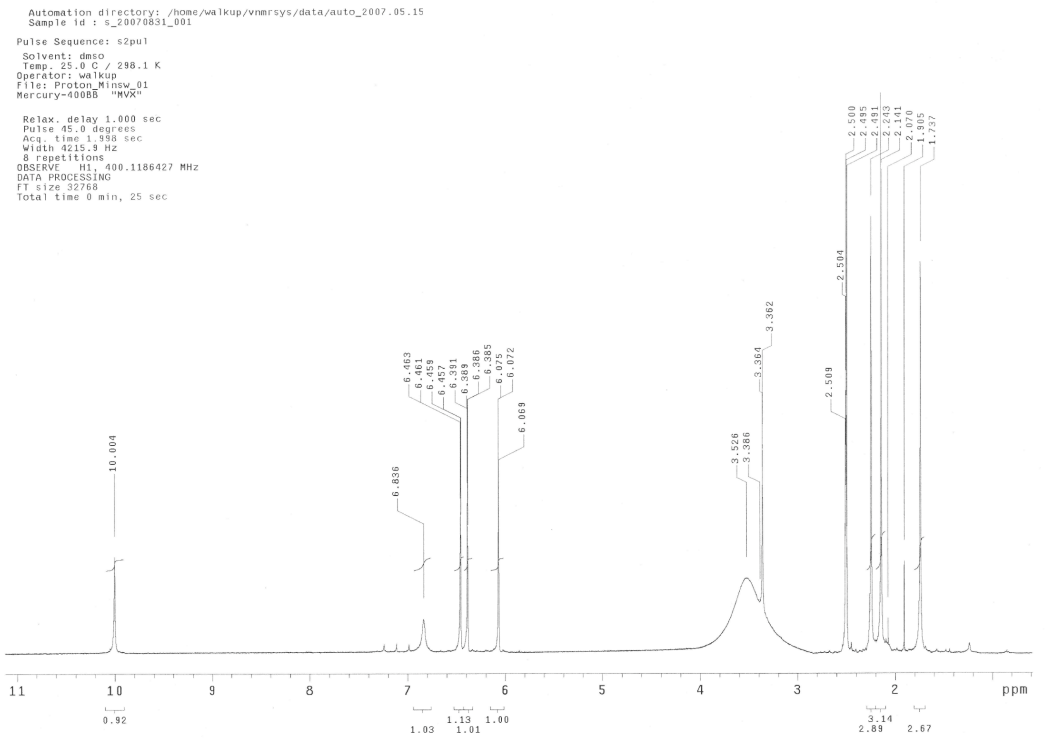

Figure S8. ^{1}H NMR spectrum of F-9775A (**2**) in DMSO-d_6

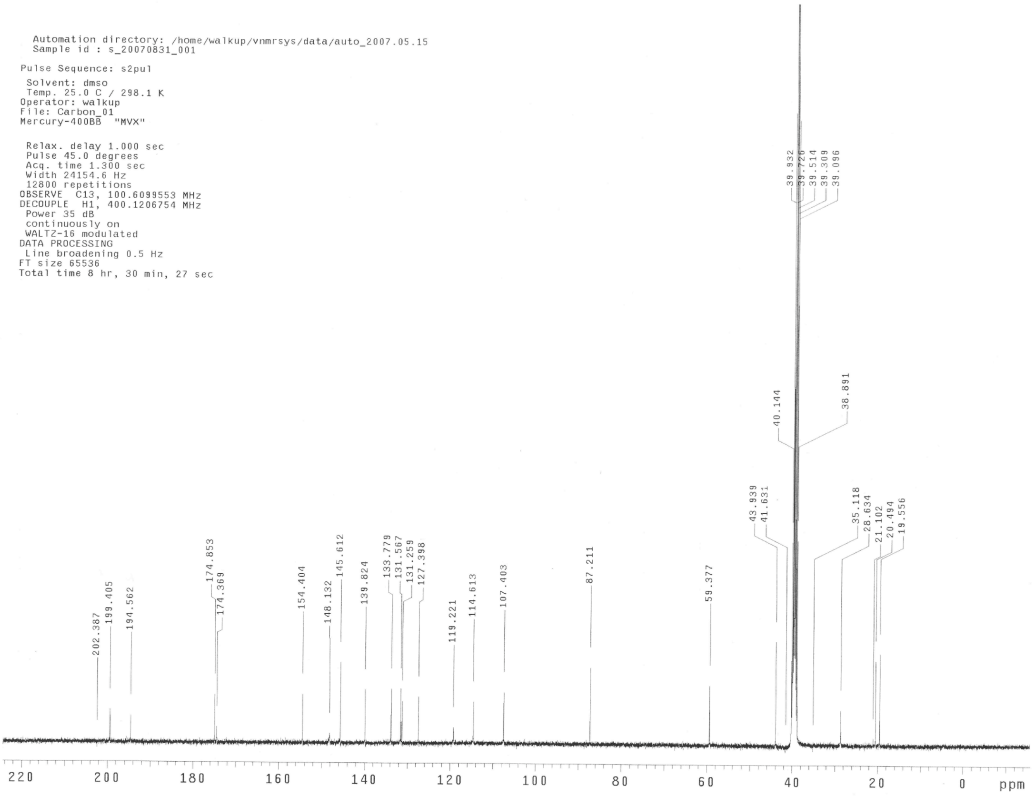

Figure S9. ^{13}C NMR spectrum of F-9775A (**2**) in DMSO-d_6

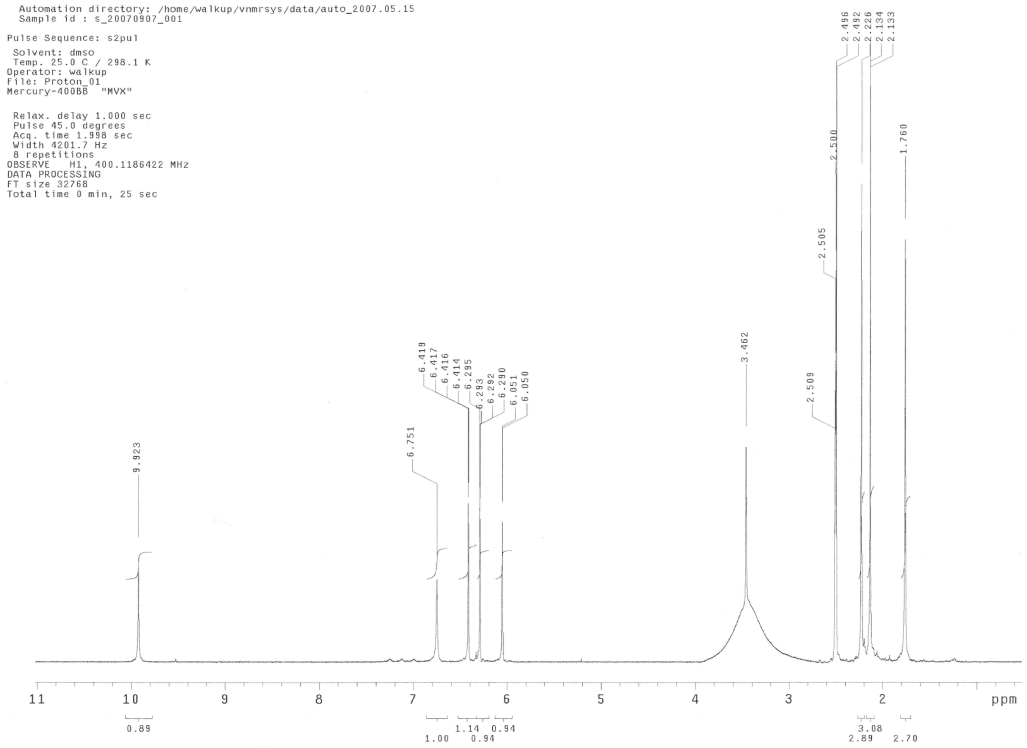

Figure S10. ^{1}H NMR spectrum of F-9775B (**3**) in DMSO-d_6

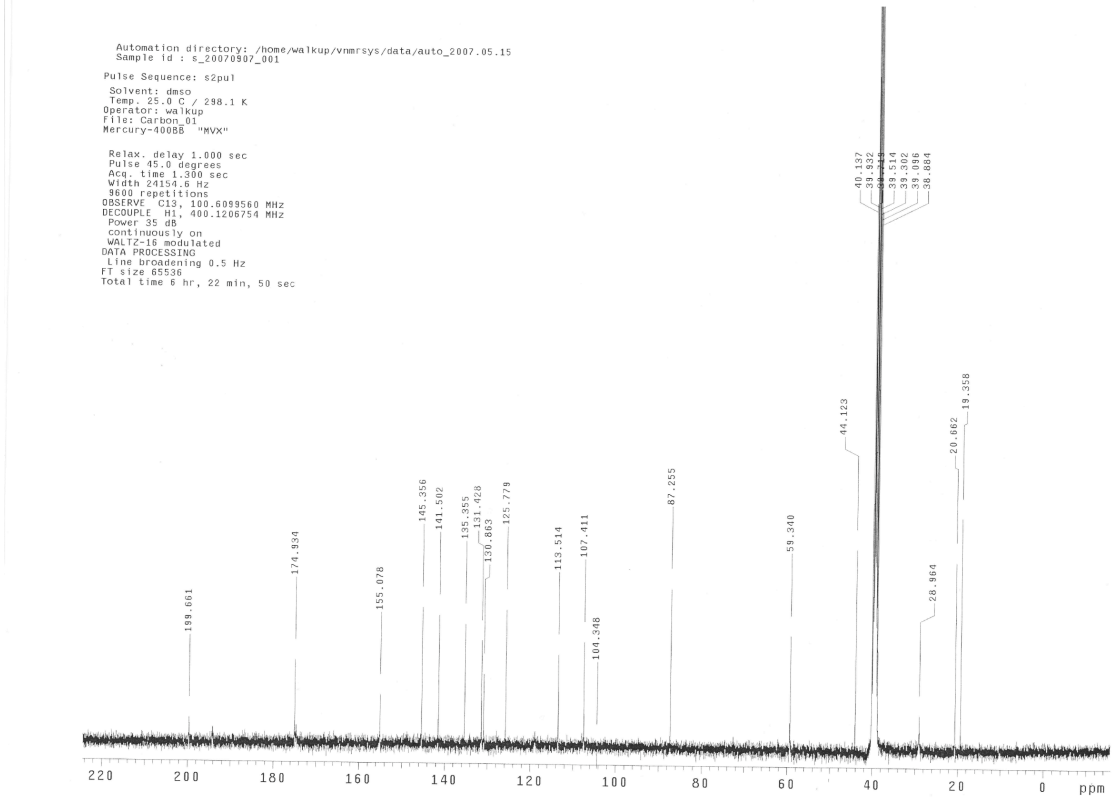

Figure S11. ^{13}C NMR spectrum of F-9775B (**3**) in DMSO-d_6

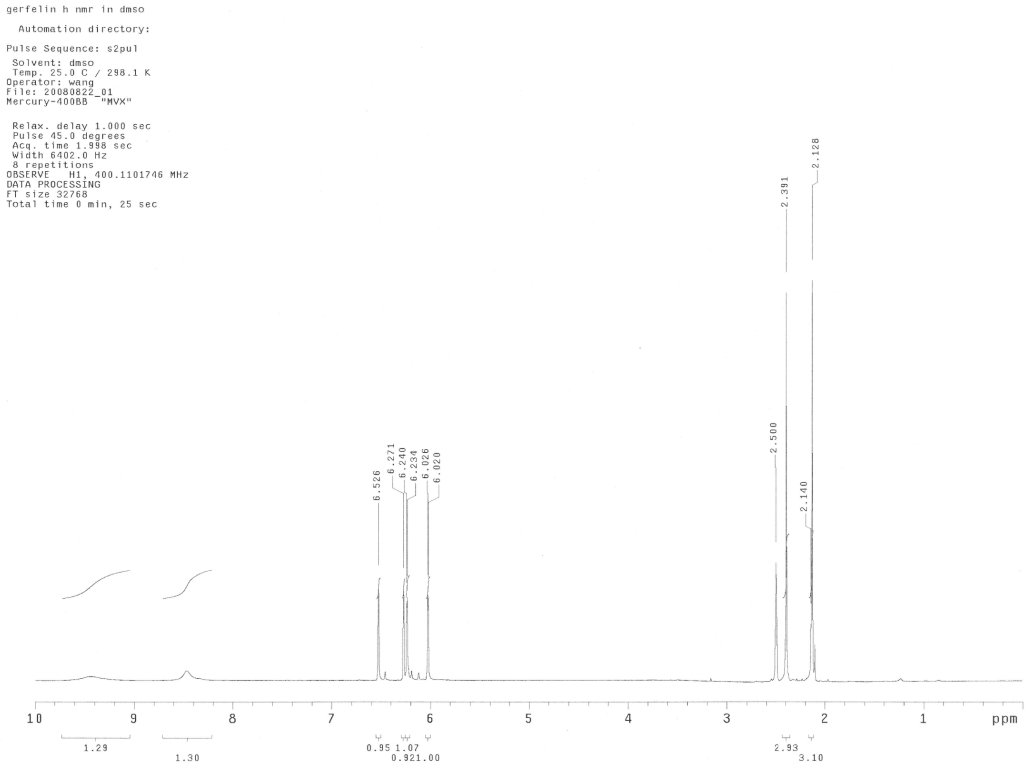

Figure S12. ^{1}H NMR spectrum of gerfelin (**4**) in DMSO-d_6

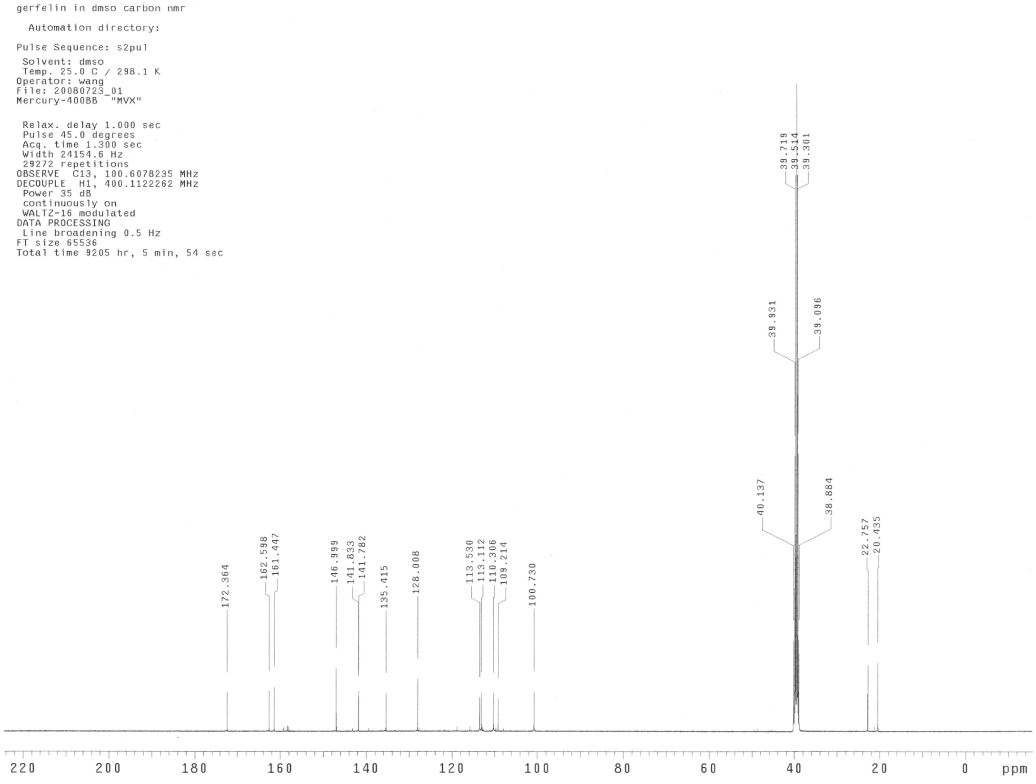

Figure S13. ^{13}C NMR spectrum of gerfelin (**4**) in DMSO-d_6

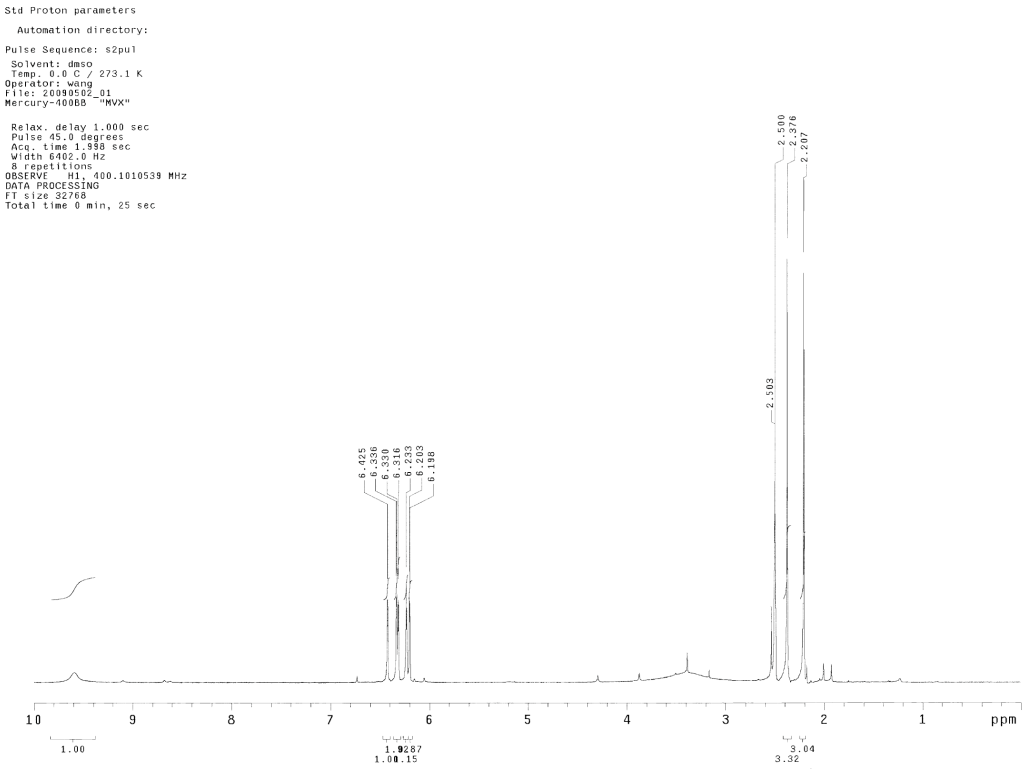

Figure S14. ^{1}H NMR spectrum of C10-deoxy derivative of gerfelin (**5**) in DMSO-d_6

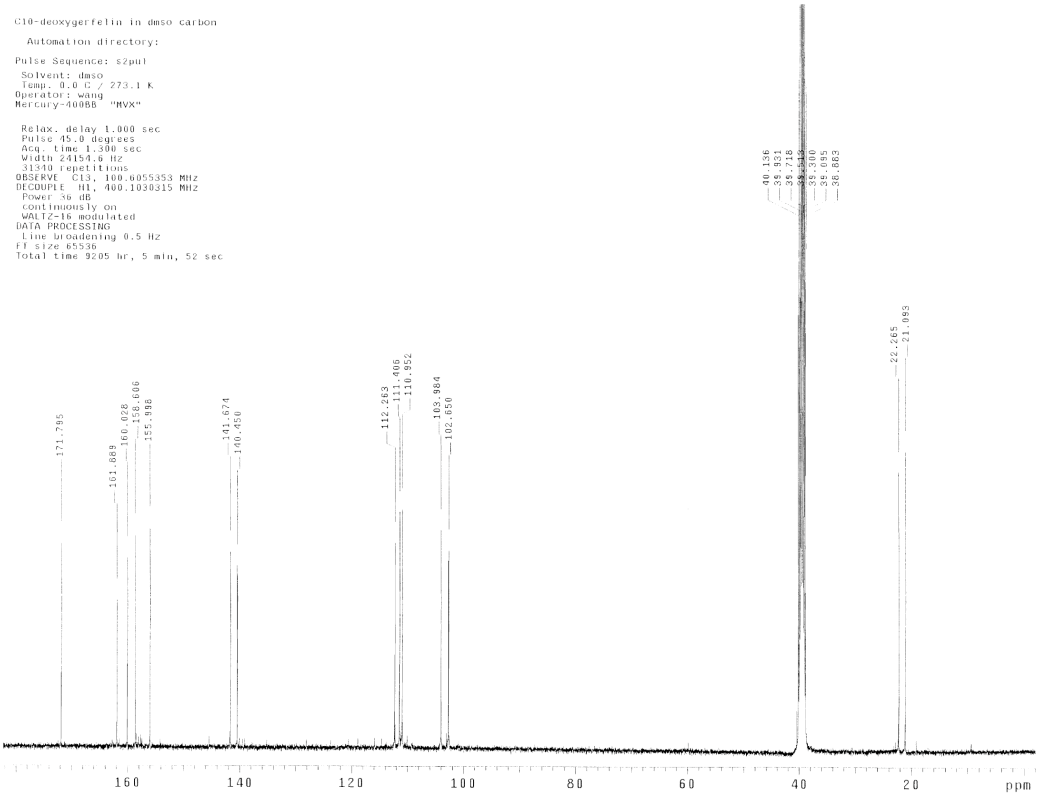

Figure S15. ^{13}C NMR spectrum of C10-deoxy derivative of gerfelin (**5**) in DMSO-d_6

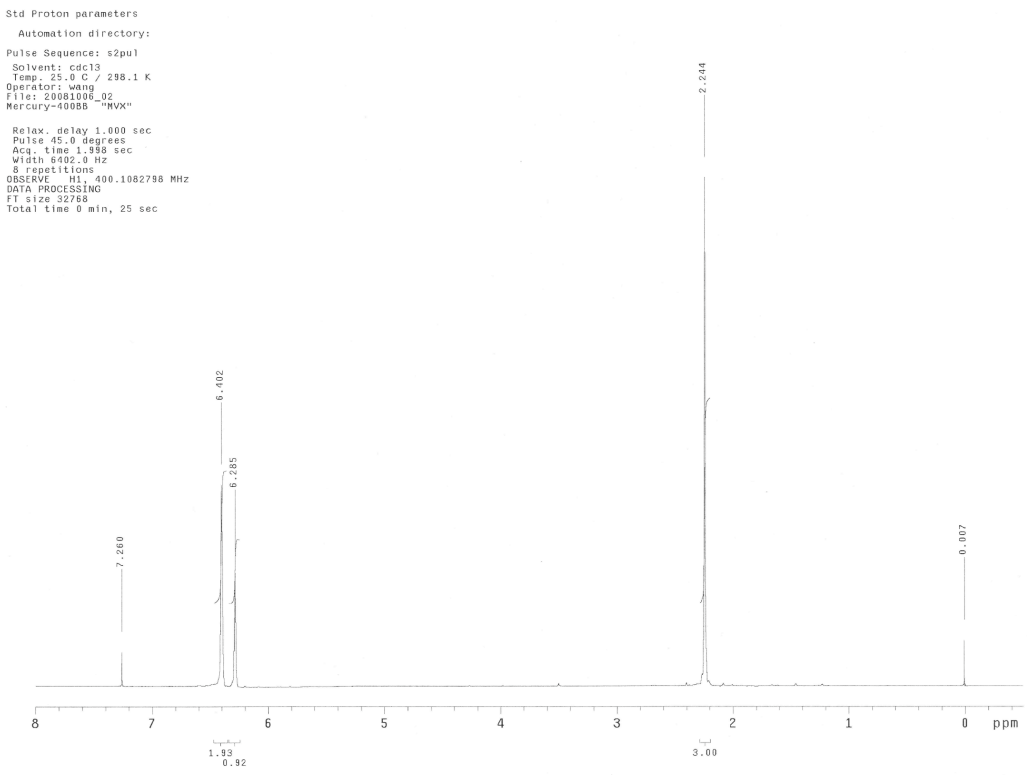

Figure S16. ^{1}H NMR spectrum of diorcinol (**6**) in $CDCl_3$

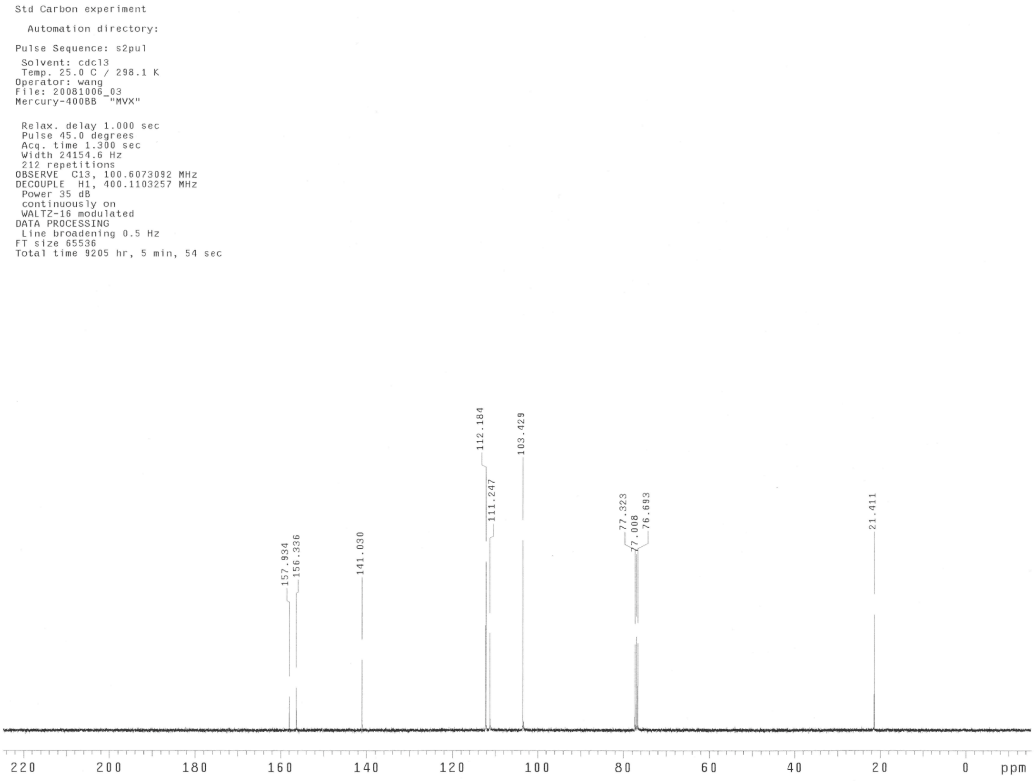

Figure S17. ^{13}C NMR spectrum of diorcinol (**6**) in $CDCl_3$

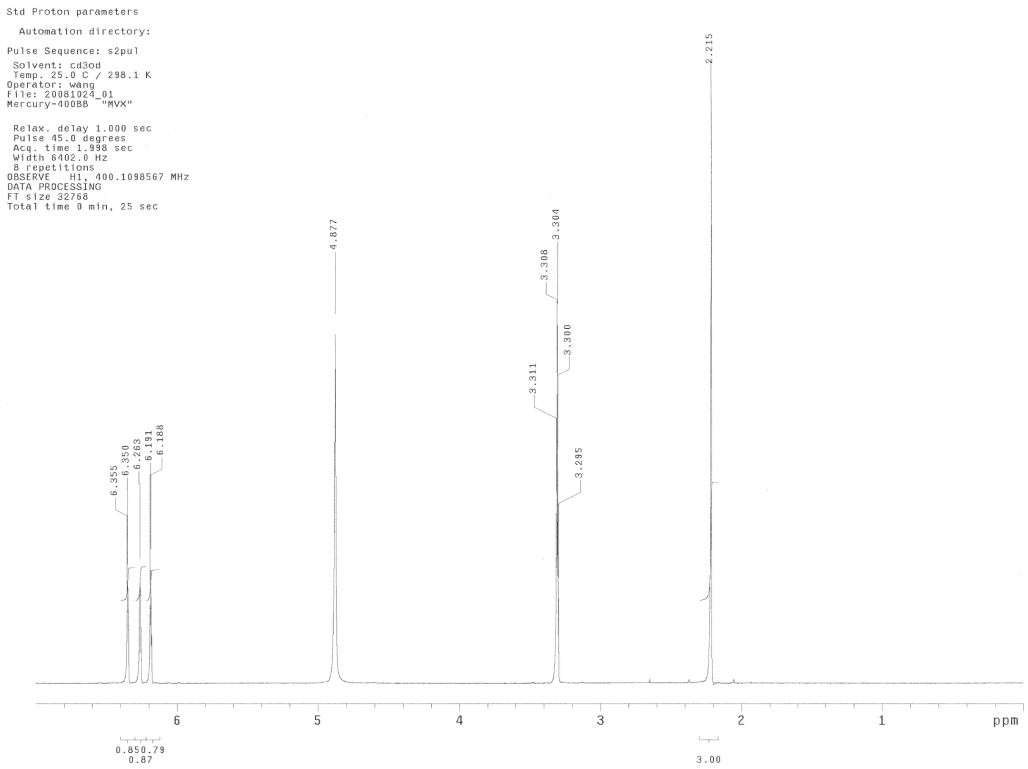

Figure S18. ^{1}H NMR spectrum of diorcinol (**6**) in CD_3OD

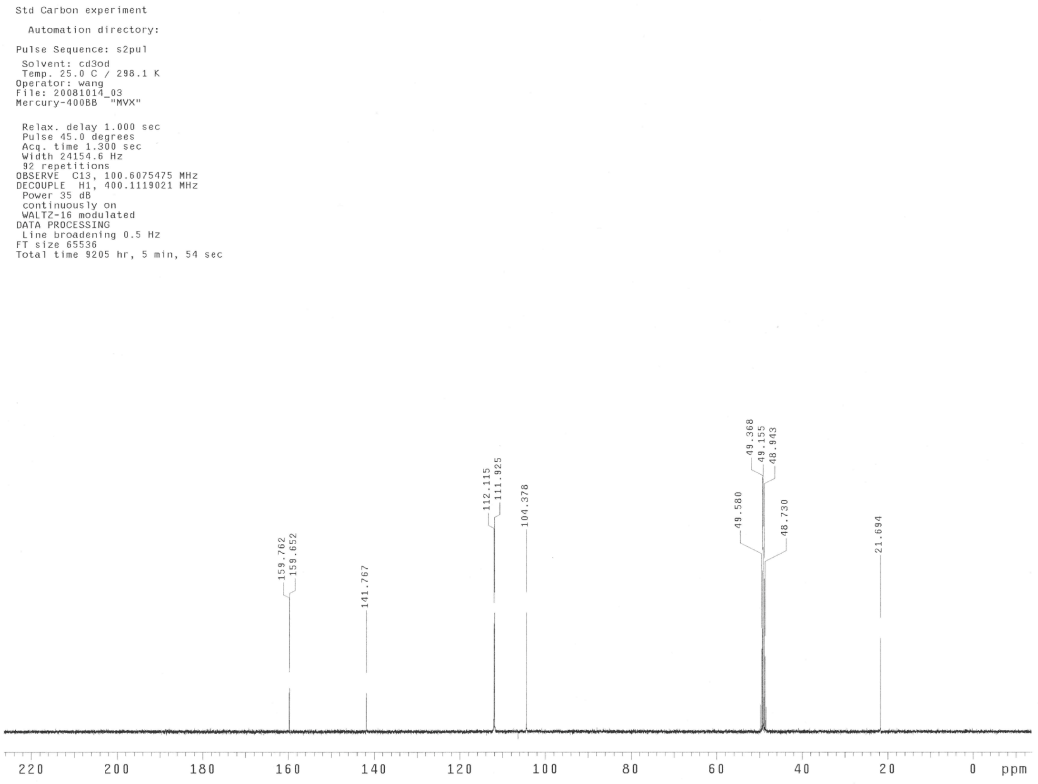

Figure S19. ^{13}C NMR spectrum of diorcinol (**6**) in CD_3OD