# Supplementary Information

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

James F. Sanchez,[a] Yi-Ming Chiang,[a,c] Edyta Szewczyk,[d,i] Ashley D. Davidson,[d] Jin Woo Bok,[e,f] Nancy Keller,[e,f,g] Berl R. Oakley,[d,h] Clay C. C. Wang[a,b*]

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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 |
| AN7904.3P2 | CCA TGA TGT CCT TTA CCT CAG C |
| 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 |
| AN7905.3P5 | TCC GTA GAG GTA GTA TGG ACA GG |
| AN7905.3P6 | CCT GCT GAC AAA GAC AAA GC |
| AN7906.3P1 | GGA CAT GCT CTG CTT CTA CC |
| AN7906.3P2 | AGG AGA TGA TGC AGA AGT GG |
| 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 |
| AN7906.3P5 | CAT GGC CAC TGA AAA CAA CC |
| AN7906.3P6 | TCC ACA ACT GTG CTC AAT CC |
| AN7907.3P1 | GCG TCC ACA CAG AAG TTT AGC |
| AN7907.3P2 | TTC CCC TGA GTG ATT TAC GC |
| AN7907.3P3 | CGA AGA GGG TGA AGA GCA TTG AGA TAC AGT GAG CCA GCT TCT ACG |
| AN7907.3P4 | GCA TCA GTG CCT CCT CTC AGA CAG GAA CTT GTC TGA GAC CTG AAC C |
| AN7907.3P5 | CGA CTG ACA TGG TCT TCT AGC |
| AN7907.3P6 | GTC CGT TTC TGA TAG CAT GG |
| AN7908.3P1 | GCT CAT TCC GAC CTC TAT CG |
| AN7908.3P2 | GGT ACC TTT GAG GGC ATT AAC C |
| AN7908.3P3 | CGA AGA GGG TGA AGA GCA TTG TGT AGC GCG TTG GTT CTA GG |
| AN7908.3P4 | GCA TCA GTG CCT CCT CTC AGA CAG AAA GGT GGA GCT ACC CAT GC |
| AN7908.3P5 | CAA CAA AAG CCC AAG TCA GC |
| AN7908.3P6 | CCG TAG CTC TTG TAT GGT CTA TAG GG |
| AN7909.3P1 | CAA AGG AGG AGT GGA TCA CG |
| AN7909.3P2 | CGT TGA CTC GAC CTT GAT GC |
| AN7909.3P3 | CGA AGA GGG TGA AGA GCA TTG CGG CAC TGC TGT TCT ATT GC |
| AN7909.3P4 | GCA TCA GTG CCT CCT CTC AGA CAG CAG GTA TAA TGT GCA CGT CTC C |
| AN7909.3P5 | CAT ACT GGC GCG ATA GAT GC |

| | |
|---|---|
| AN7909.3P6 | GAC AGG ATA ATC TCG GAC TCG |
| AN7911.3P1 | GCA TGT TGA GGA TGA TGT CC |
| AN7911.3P2 | GGT TTG GAG GGA TGT ATT GG |
| AN7911.3P3 | CGA AGA GGG TGA AGA GCA TTG CTA AAT GCT GAC GTG GAT CG |
| AN7911.3P4 | GCA TCA GTG CCT CCT CTC AGA CAG GCC TGC CTA TGC TAC TGT GC |
| AN7911.3P5 | GCA AGT TTC AGT GCG ATA CG |
| AN7911.3P6 | CCA CAC TTC TGC AAG CTT ATC C |
| 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 |

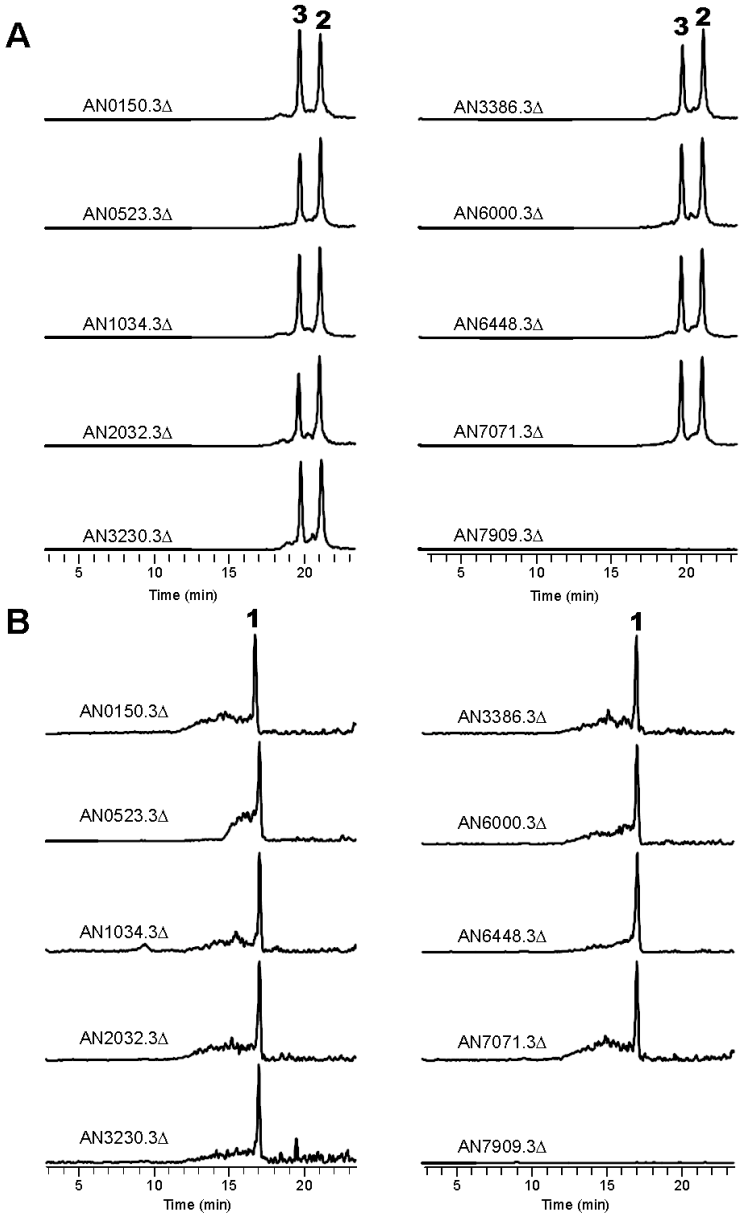


**Figure S1.** (A) Negative mode extracted ion chromatograms (EIC) at *m*/*z* 395, corresponding to F-9775A (**2**) and B (**3**), and (B) Negative mode EIC at *m*/*z* 167, corresponding to orsellinic acid (**1**), for the ten NR-PKS knockout strains studied.

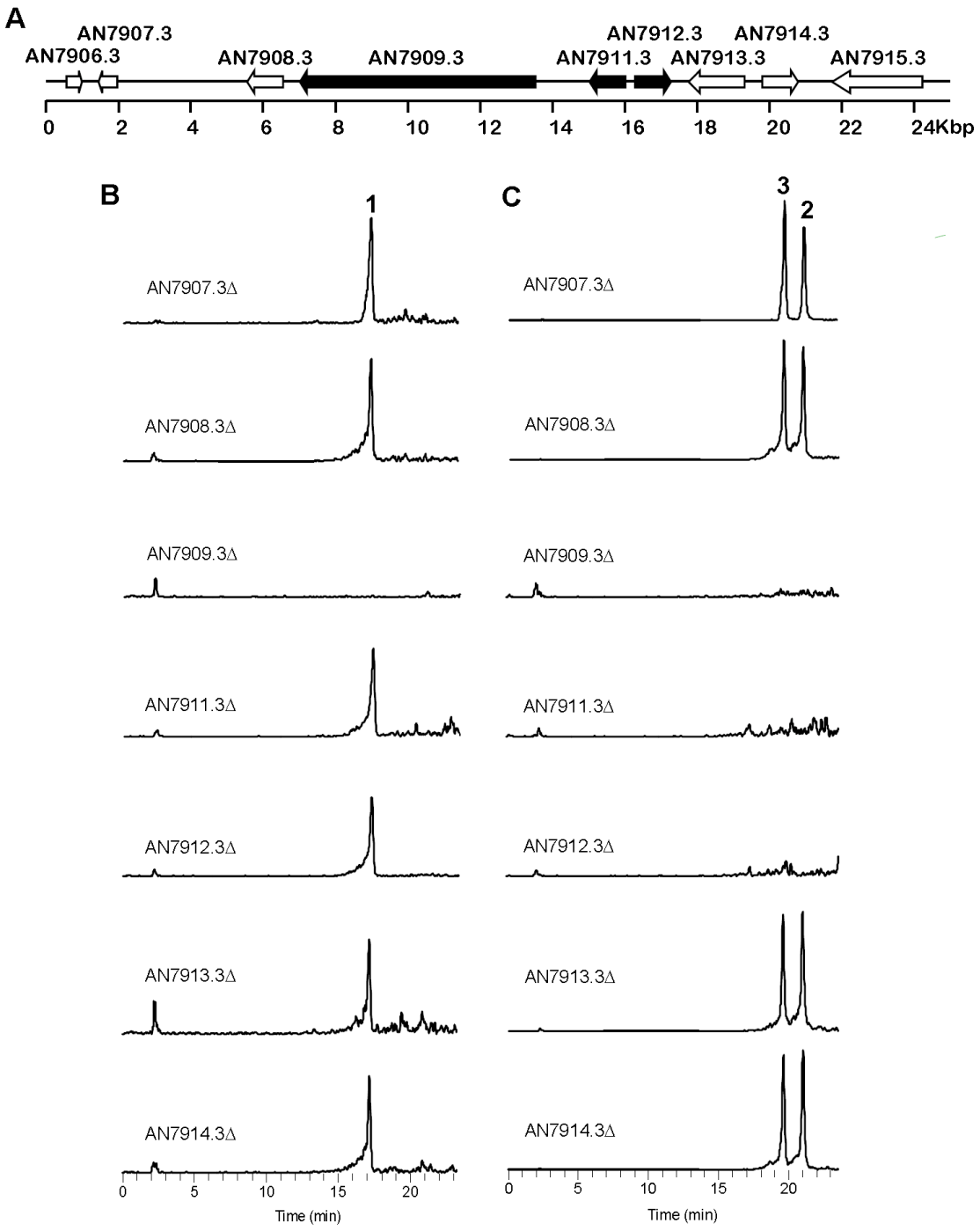


**Figure S2.** (A) Organization of the F-9775 gene cluster in *A. nidulans*. Each arrow indicates the direction of transcription and the relative sizes of the ORFs deduced from analysis of the nucleotide sequences. ORFs AN7909.3 (PKS), AN7911.3 (decarboxylase) and AN7912.3 (tyrosinase) are required for 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.

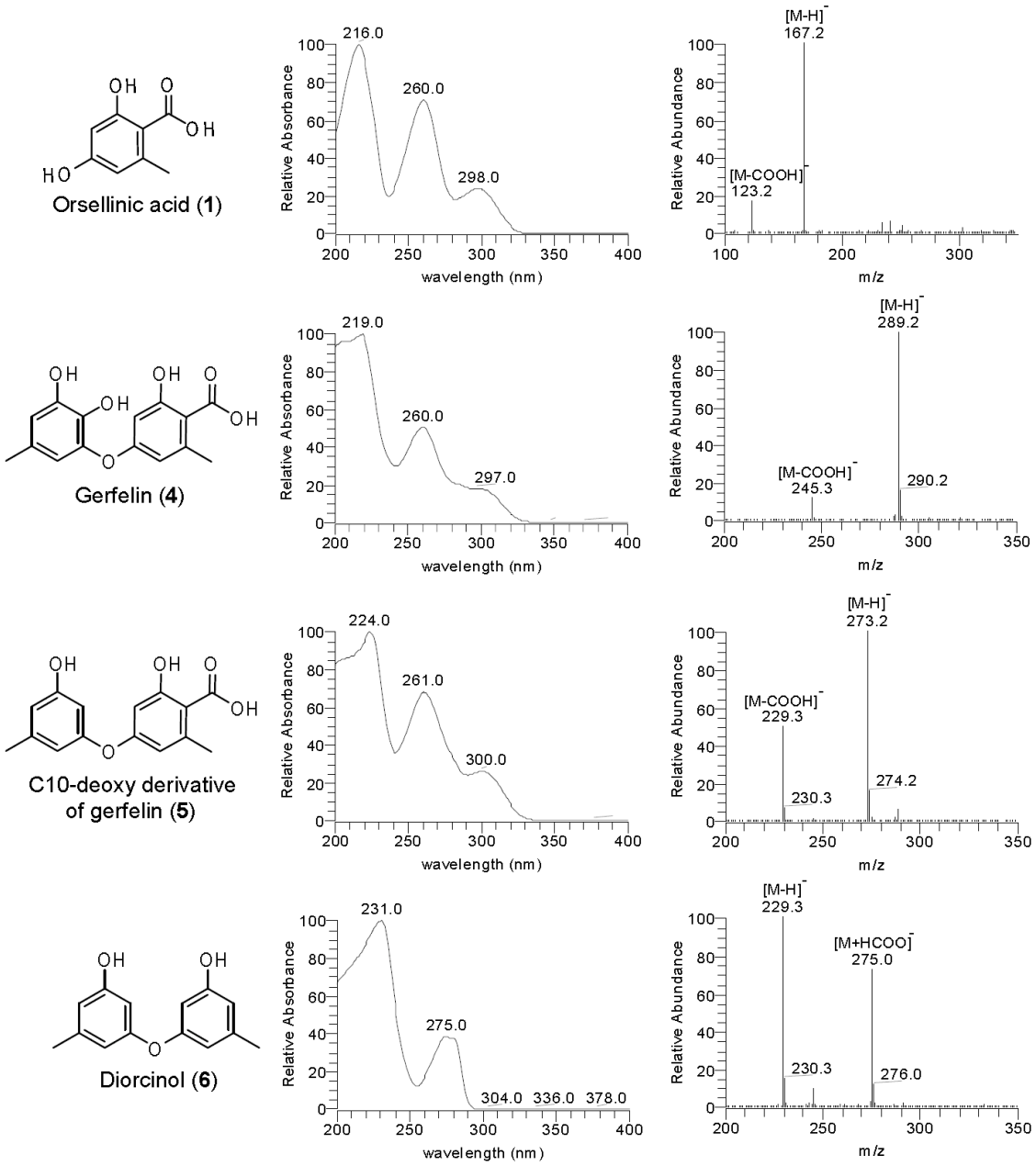


**Figure S3.** UV-Vis and ESIMS spectra (negative mode) of isolated compounds.

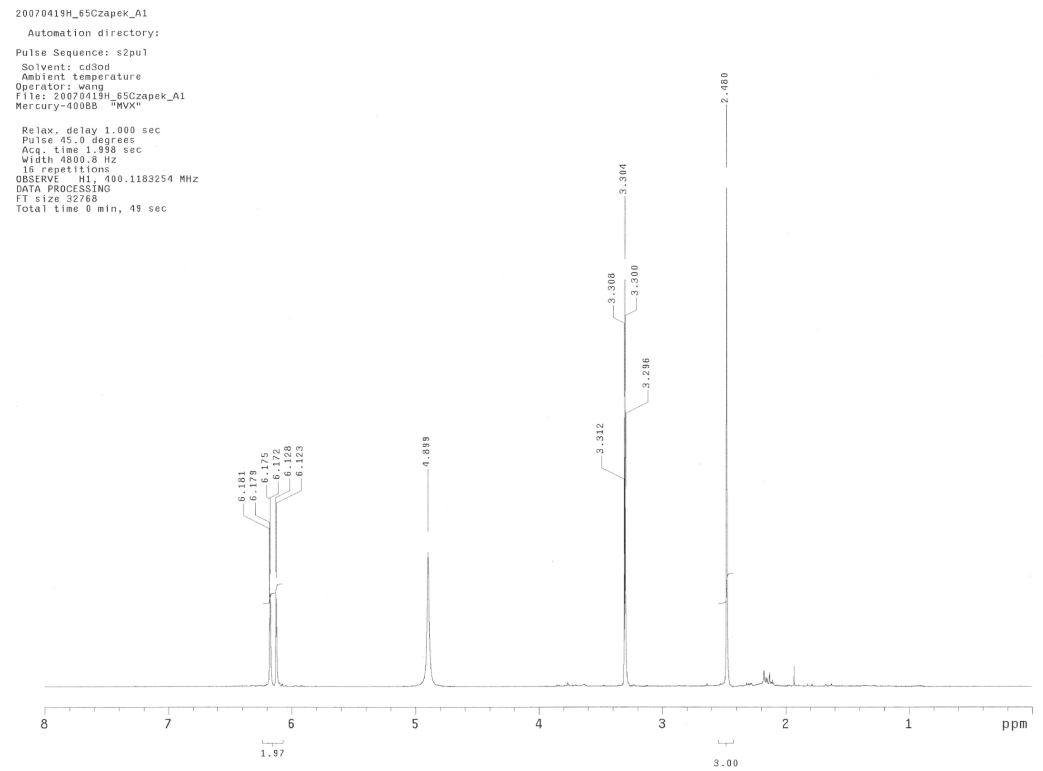


**Figure S4.** $^{1}$H NMR spectrum of orsellinic acid (**1**) in $CD_3OD$

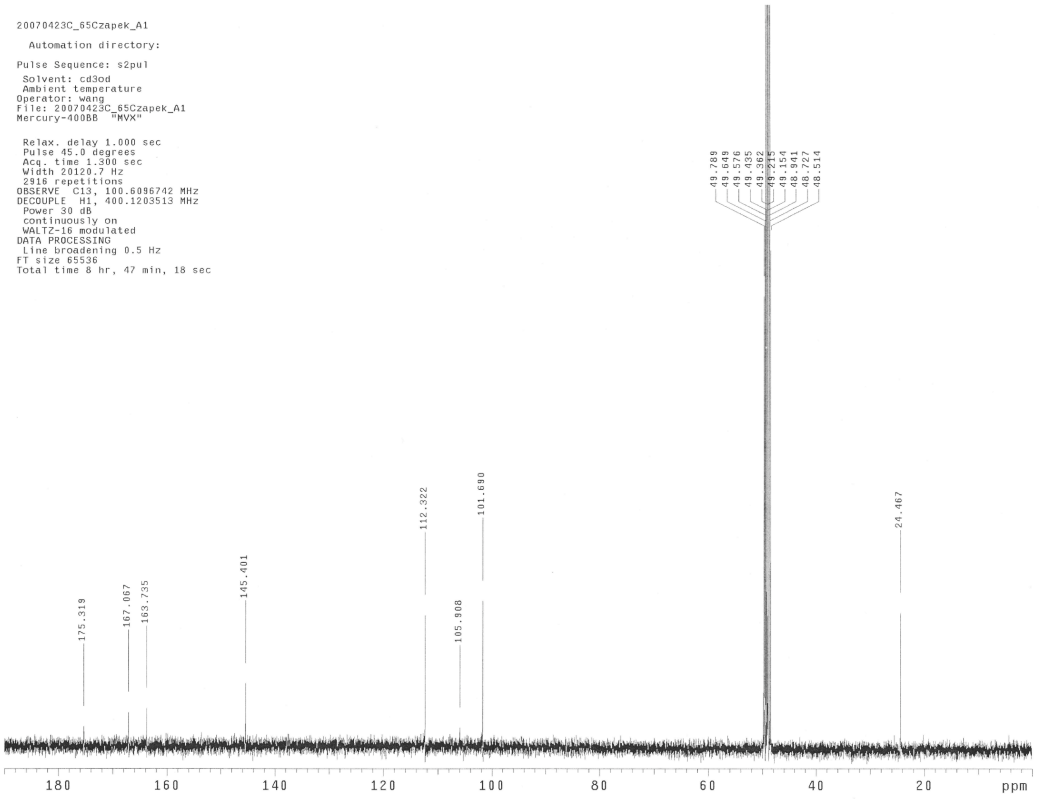


**Figure S5.** $^{13}$C NMR spectrum of orsellinic acid (**1**) in $CD_3OD$

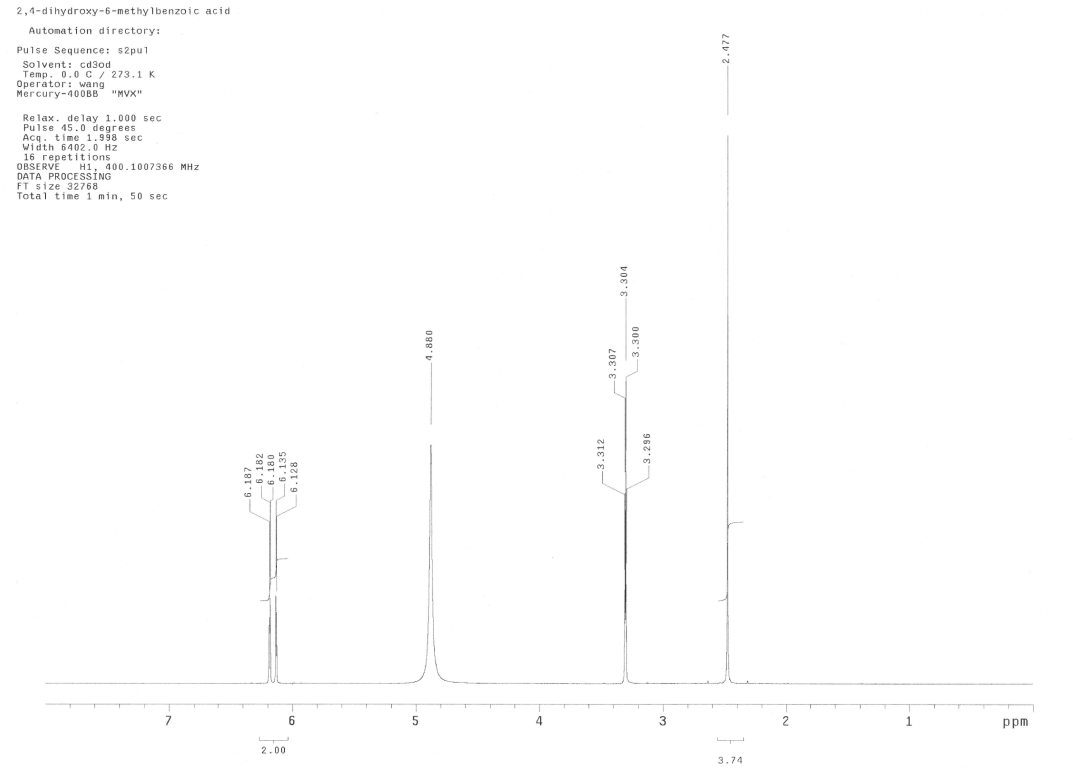


**Figure S6.** $^{1}$H NMR spectrum of orsellinic acid (**1**) purchased from Alfa Aesar in $CD_3OD$

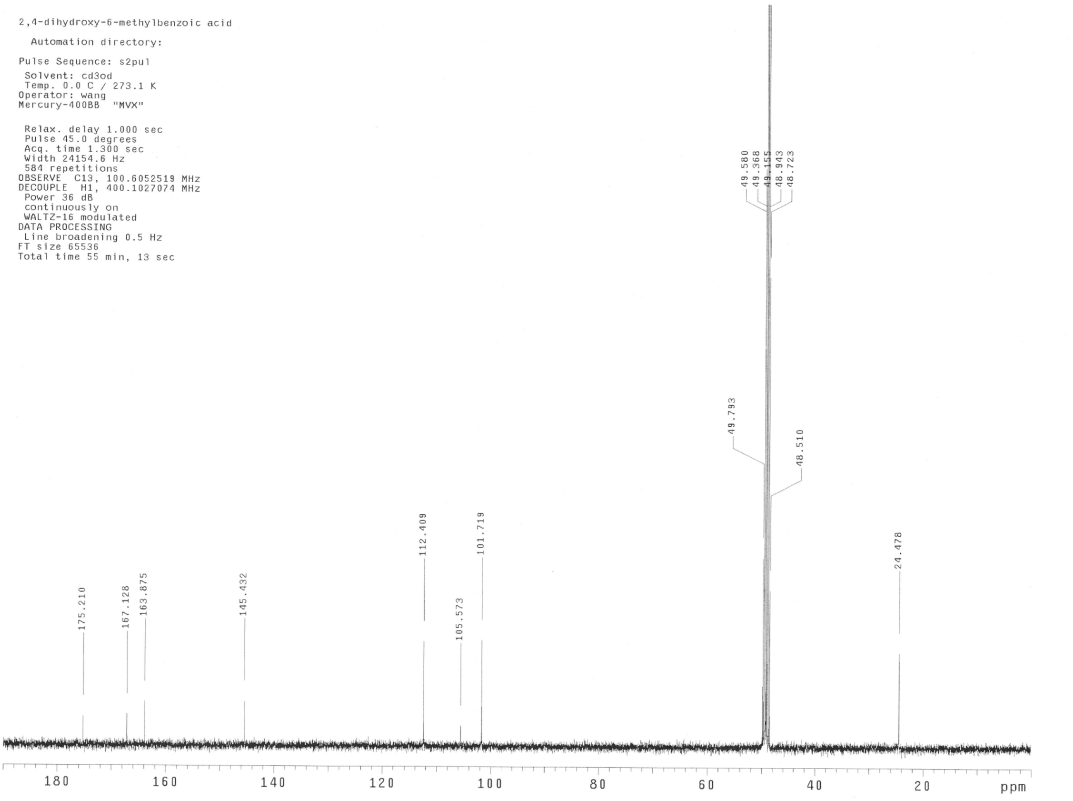


**Figure S7.** $^{13}$C NMR spectrum of orsellinic acid (**1**) purchased from Alfa Aesar in $CD_3OD$

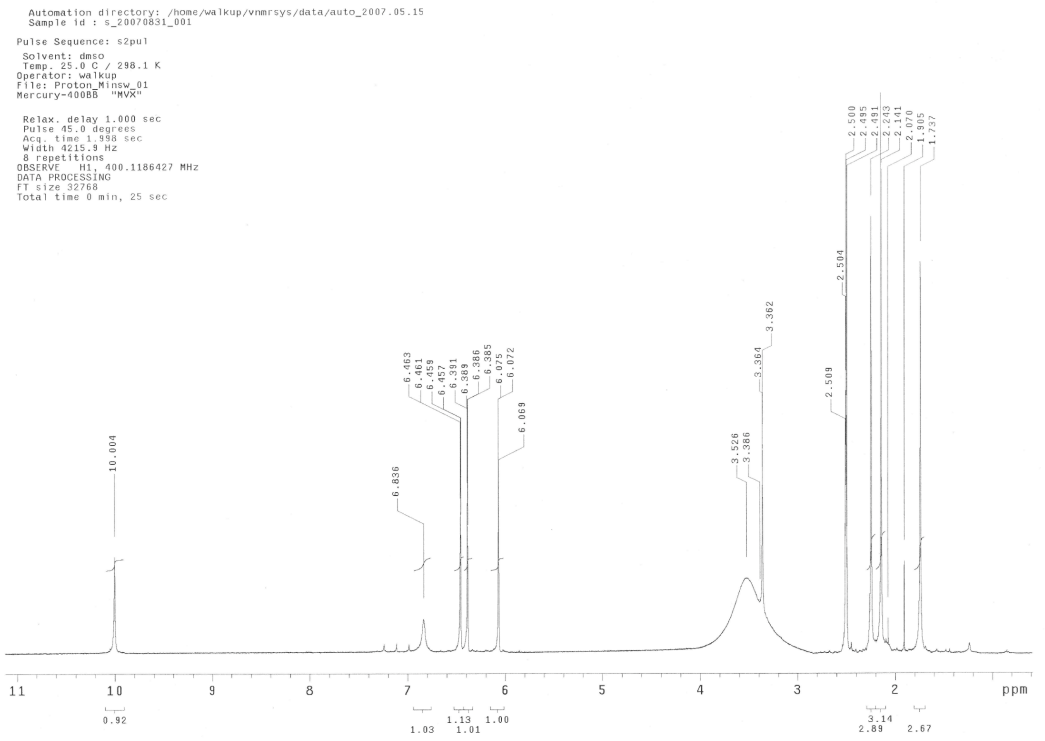


**Figure S8.** $^{1}$H NMR spectrum of F-9775A (**2**) in DMSO-$d_6$

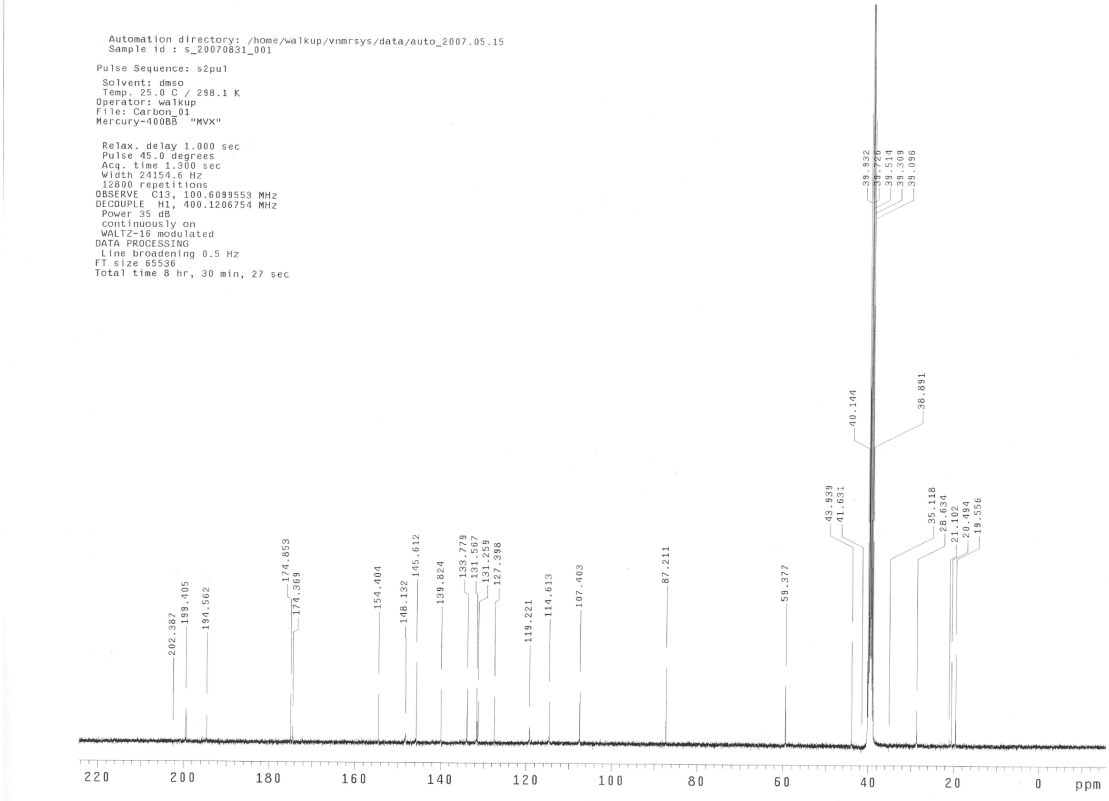


**Figure S9.** $^{13}$C NMR spectrum of F-9775A (**2**) in DMSO-$d_6$

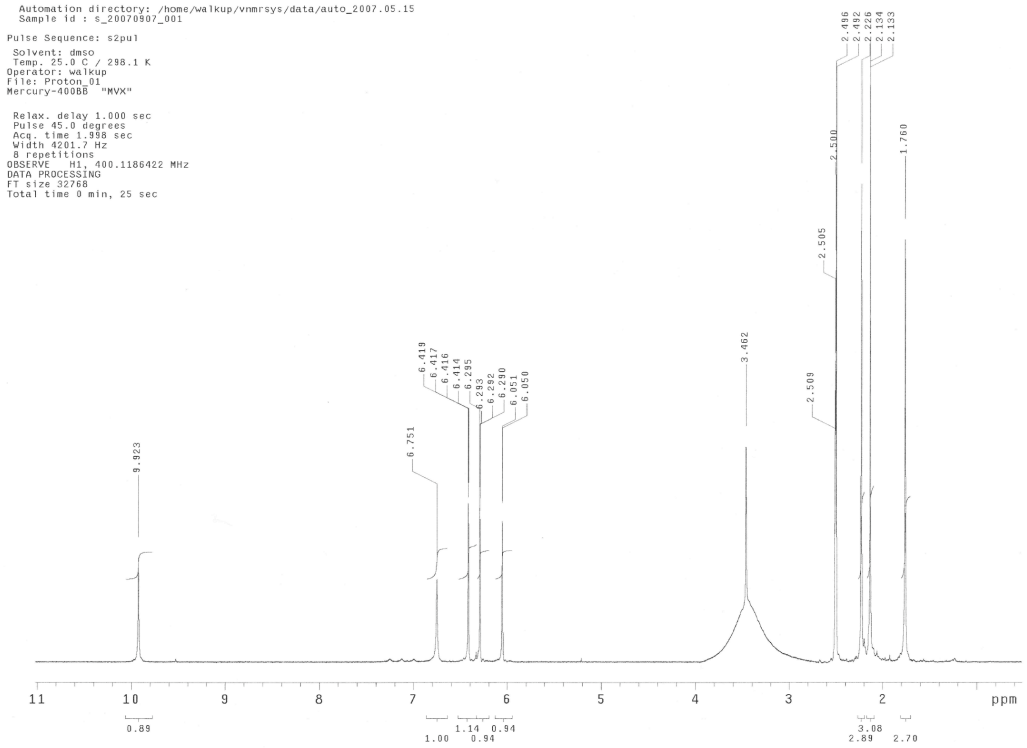


**Figure S10.** $^{1}$H NMR spectrum of F-9775B (**3**) in DMSO-$d_6$

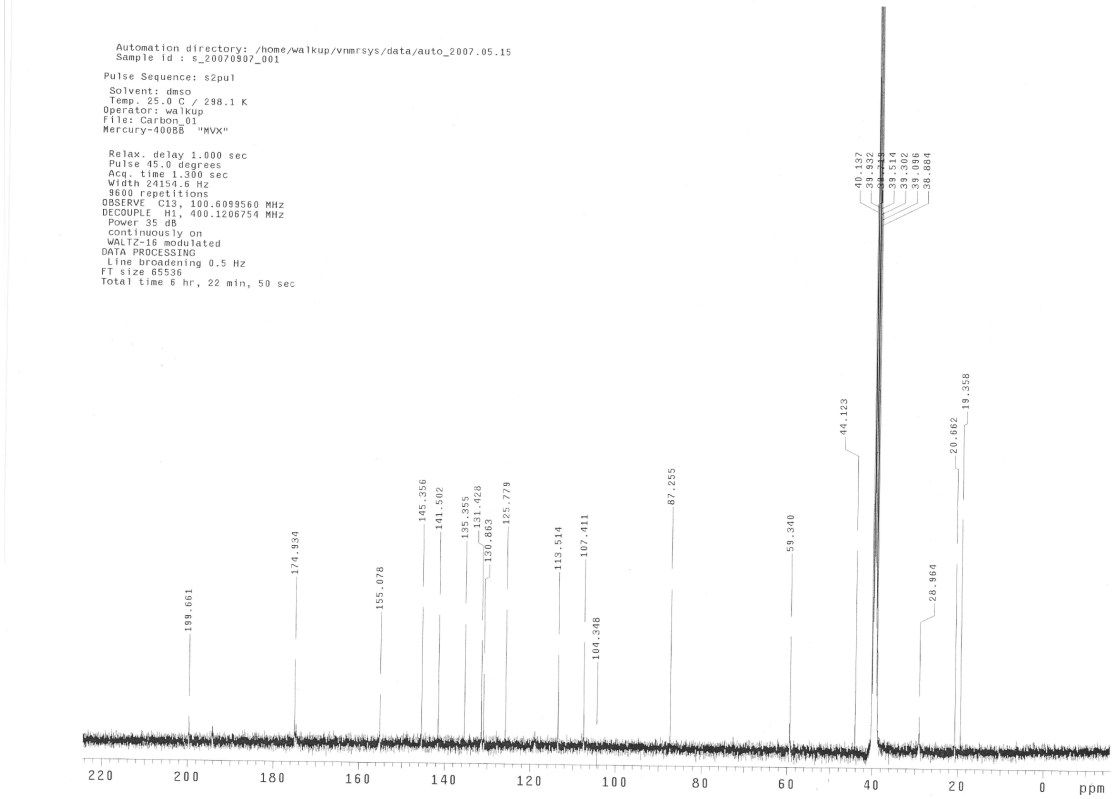


**Figure S11.** $^{13}$C NMR spectrum of F-9775B (**3**) in DMSO-$d_6$

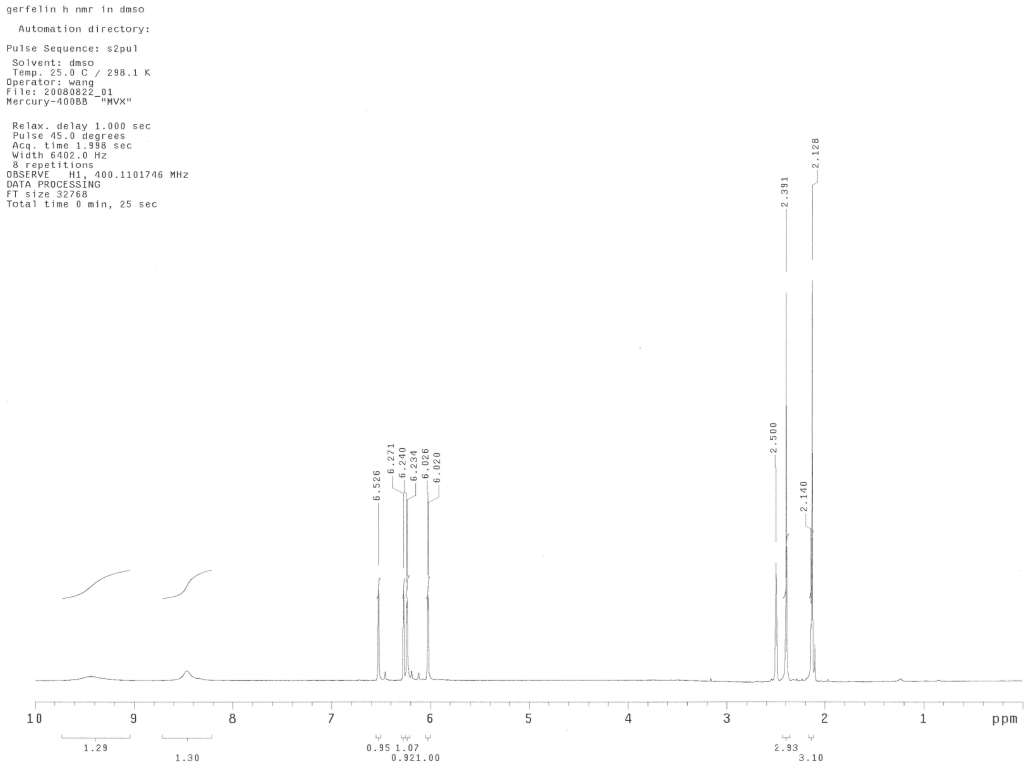


**Figure S12.** $^{1}$H NMR spectrum of gerfelin (**4**) in DMSO-$d_6$

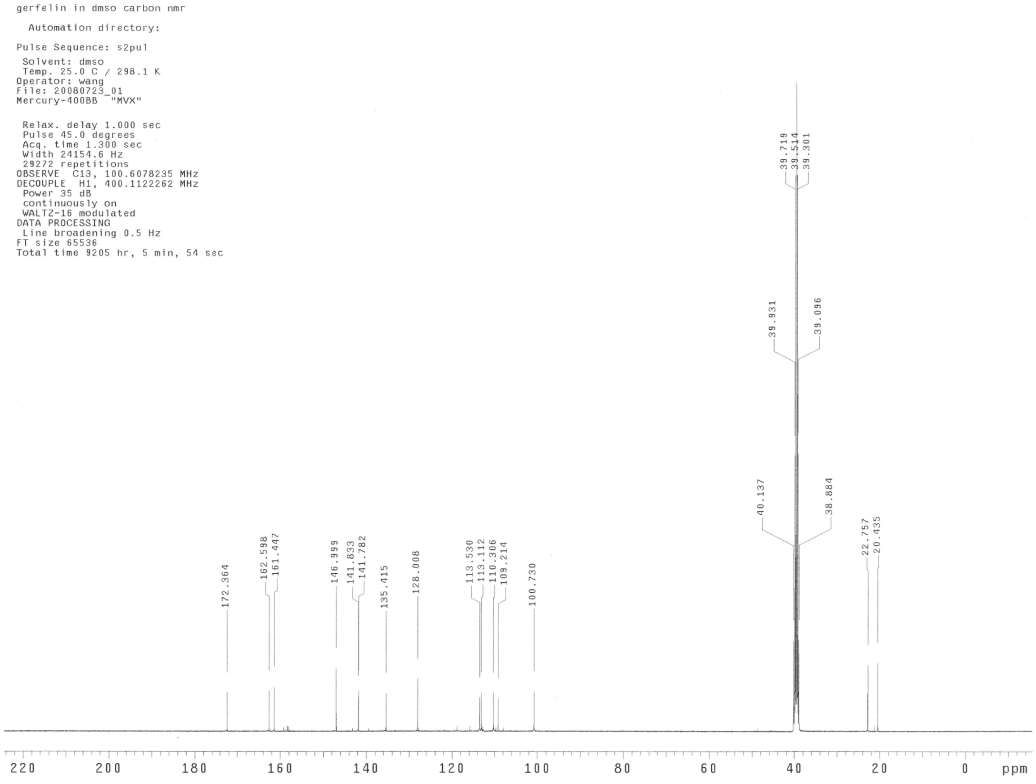


**Figure S13.** $^{13}$C NMR spectrum of gerfelin (**4**) in DMSO-$d_6$

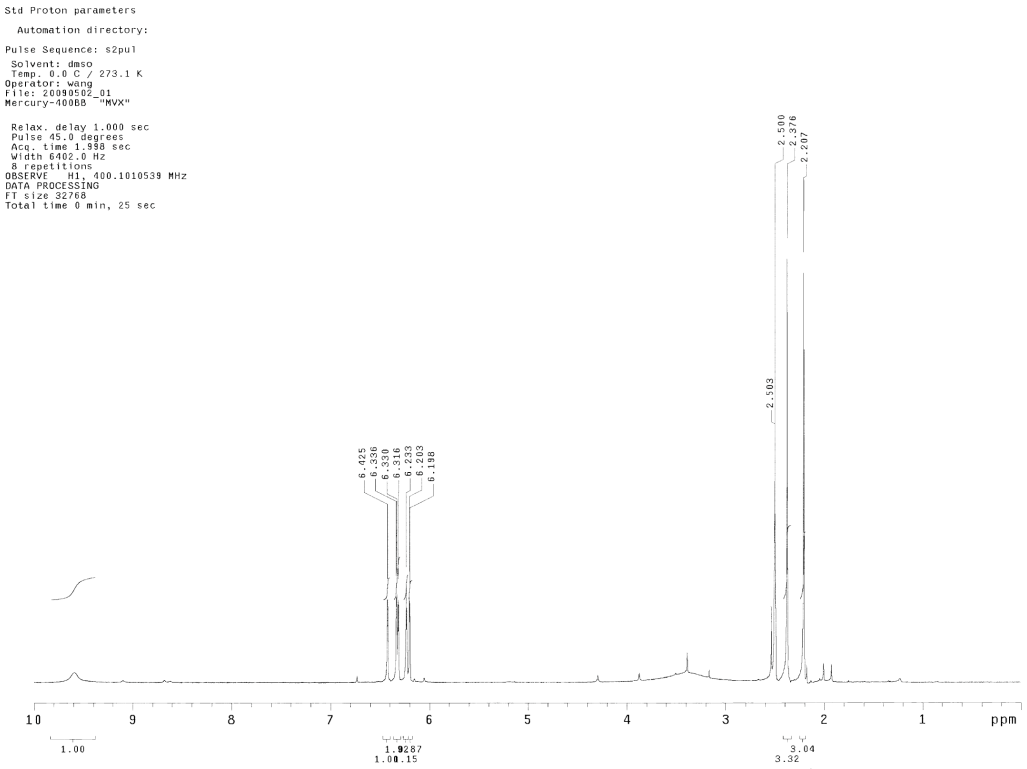


**Figure S14.** $^{1}$H NMR spectrum of C10-deoxy derivative of gerfelin (**5**) in DMSO-$d_6$

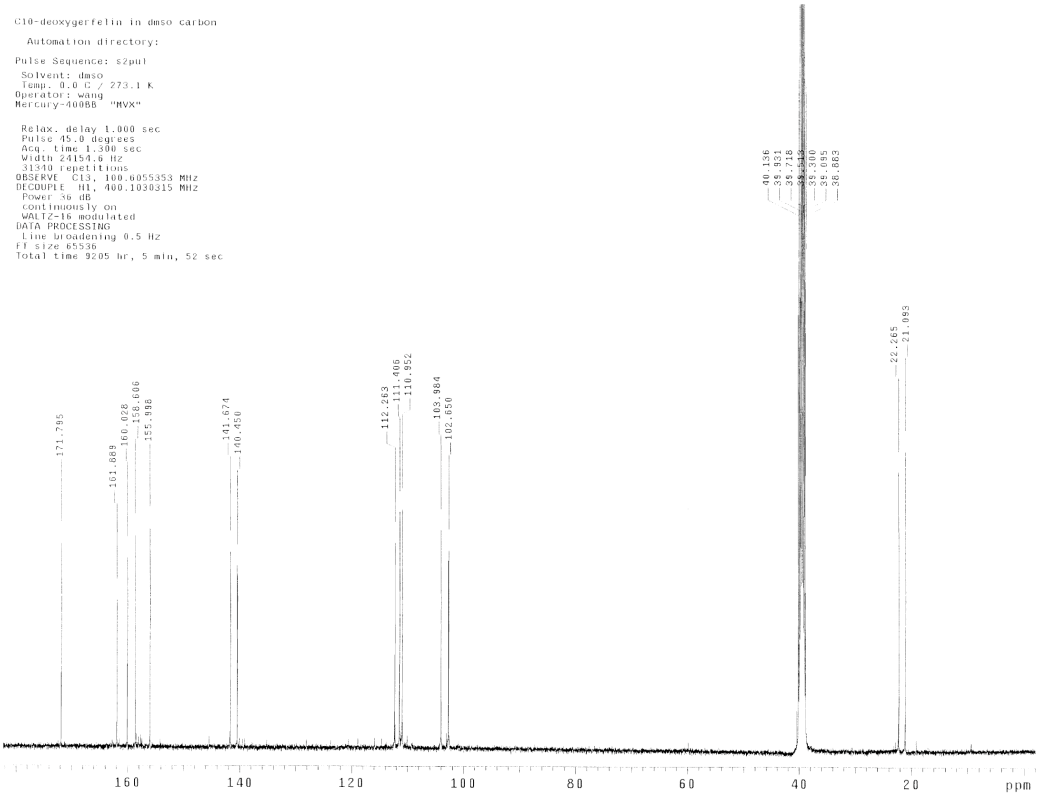


**Figure S15.** $^{13}$C NMR spectrum of C10-deoxy derivative of gerfelin (**5**) in DMSO-$d_6$

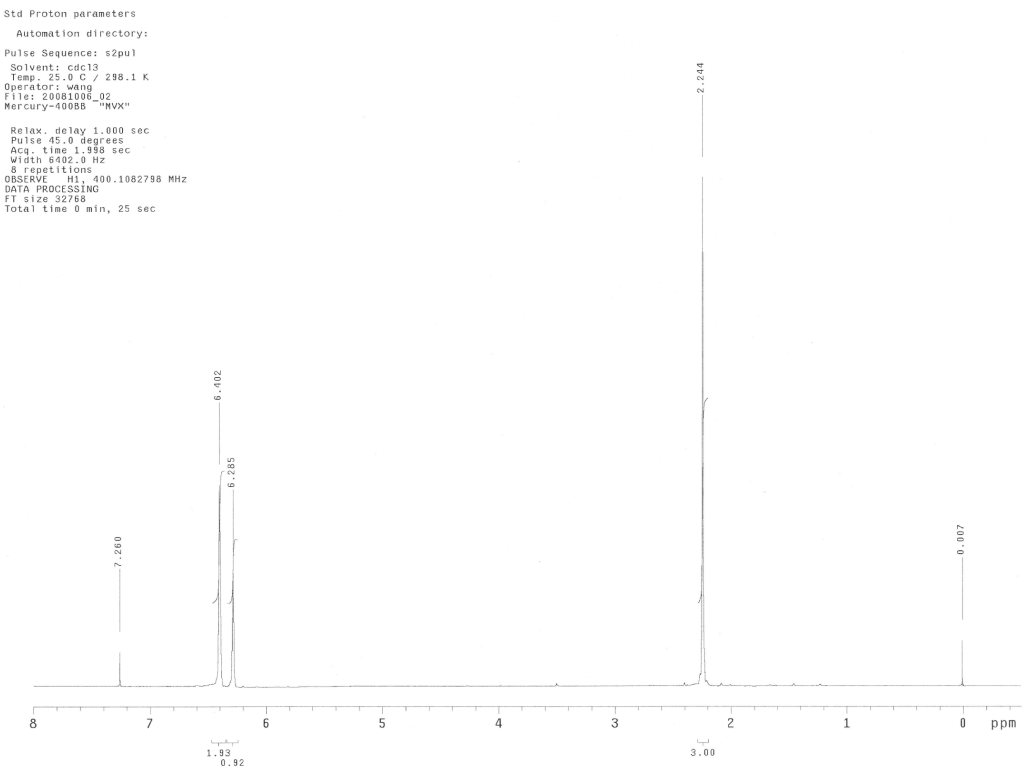


**Figure S16.** $^{1}$H NMR spectrum of diorcinol (**6**) in $CDCl_3$

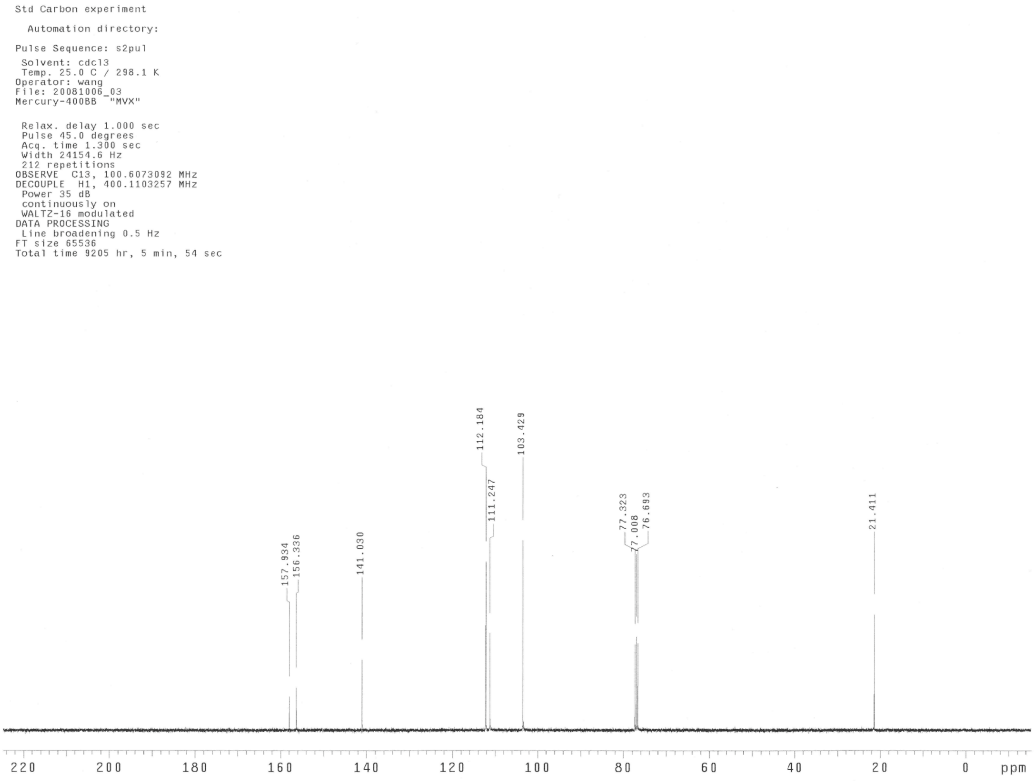


**Figure S17.** $^{13}$C NMR spectrum of diorcinol (**6**) in $CDCl_3$

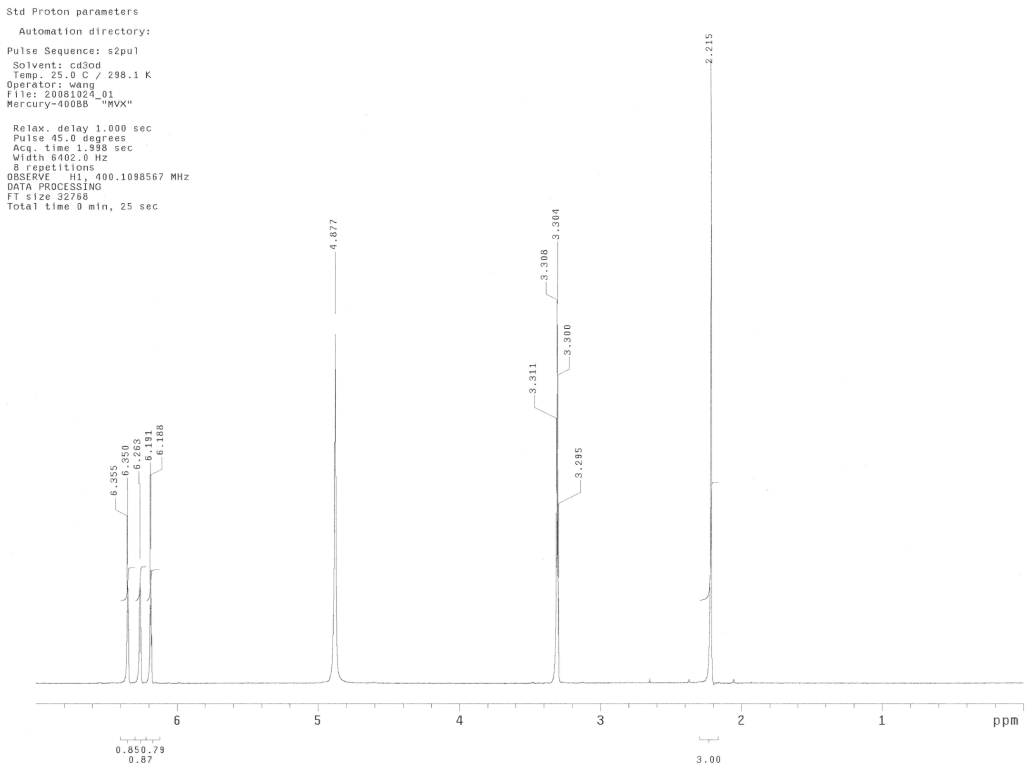


**Figure S18.** $^{1}$H NMR spectrum of diorcinol (**6**) in $CD_3OD$

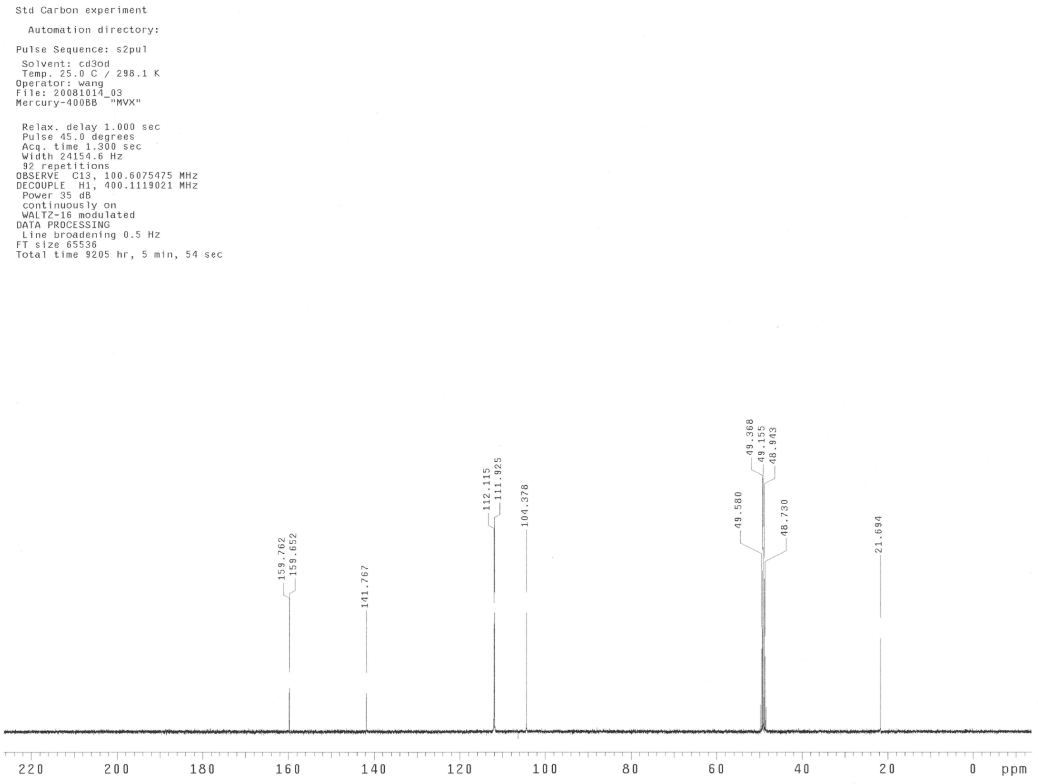


**Figure S19.** $^{13}$C NMR spectrum of diorcinol (**6**) in $CD_3OD$