Supporting Information

Discovery of penicillic acid as a chemical probe against tau aggregation in Alzheimer's Disease

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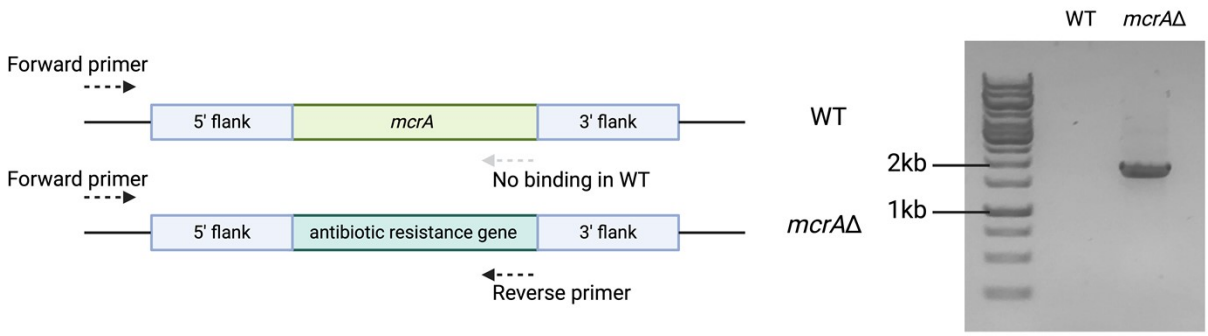

Figure S1. Confirmation of *mcrA*∆ in IMV01140. Nested primers were designed to amplify hygromycin resistance gene since *mcrA* coding region and selectable marker HygB are similar in size. Only strains with resistance gene would have a band (~1.8kb).

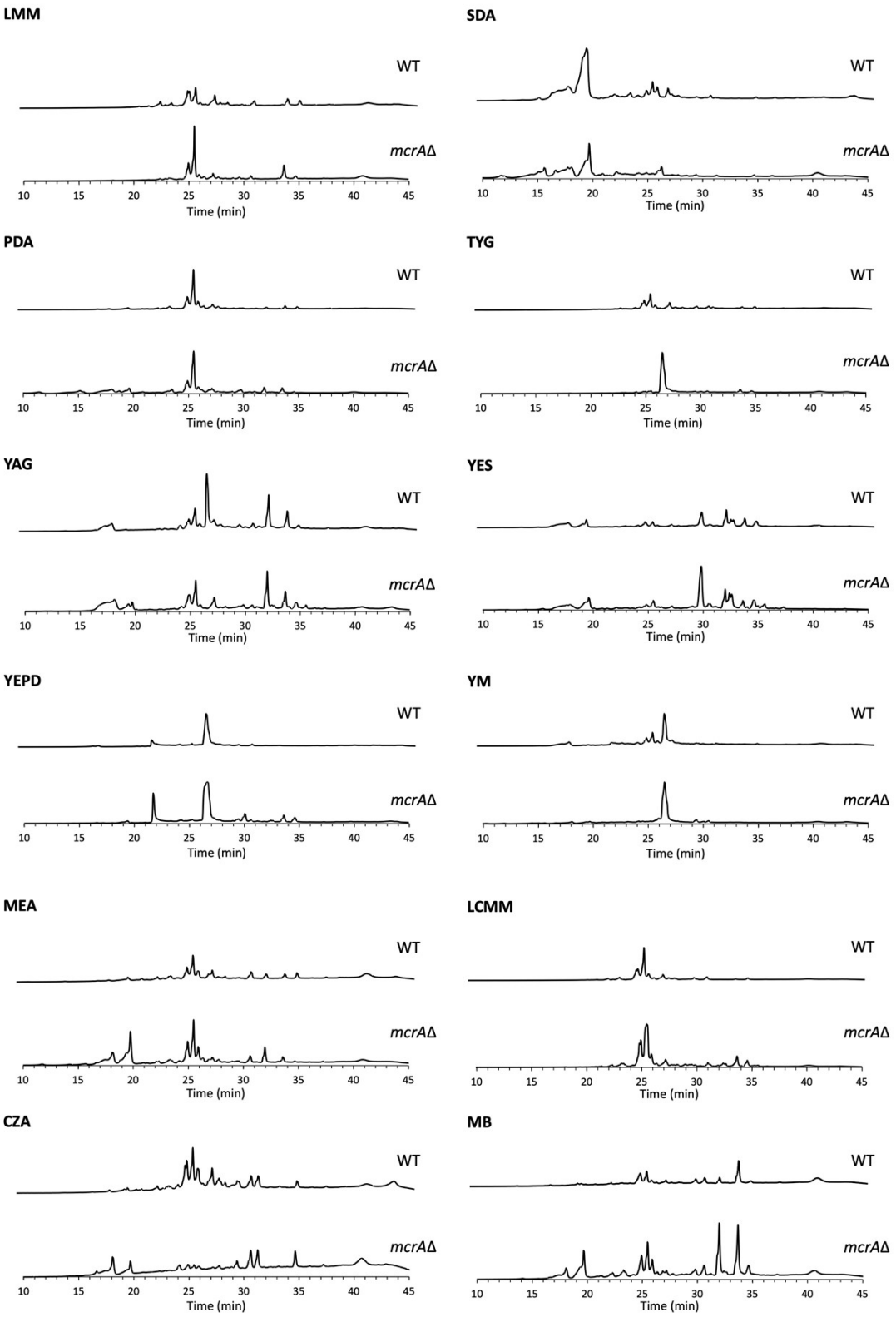

Figure S2. Screening fungal cultivation conditions using HPLC analysis. WT and mutant strains were cultured in 12 different conditions at 28°C for 6 days.

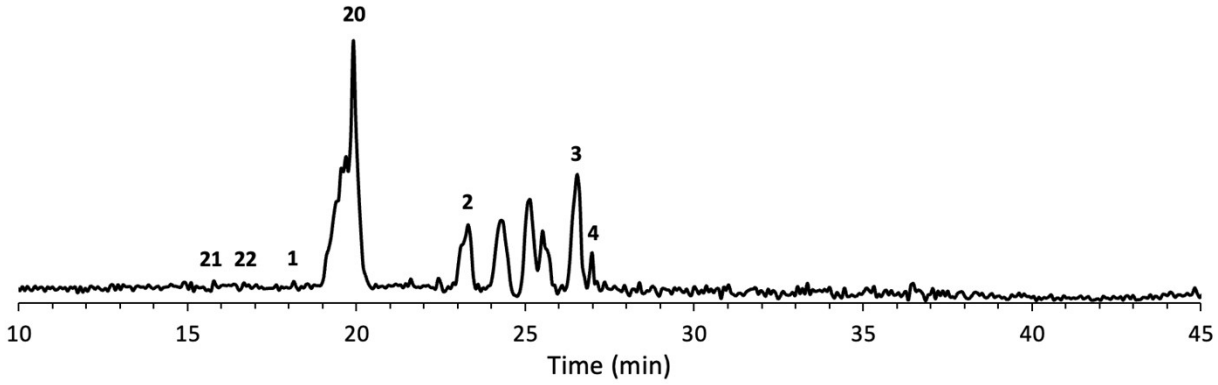

Figure S3. Characterization of select compounds in natural product library. Base peak of TIC in negative mode, range 100.0-500.0 for demonstration of major compounds. The following compounds were identified: (1) Penicillic acid, (2) Mactanamide, (3) Notoamide F, (4) Notoamide R, (20) Orsellinic acid, (21) Orcinol, (22) 2,5-furandimethanol.

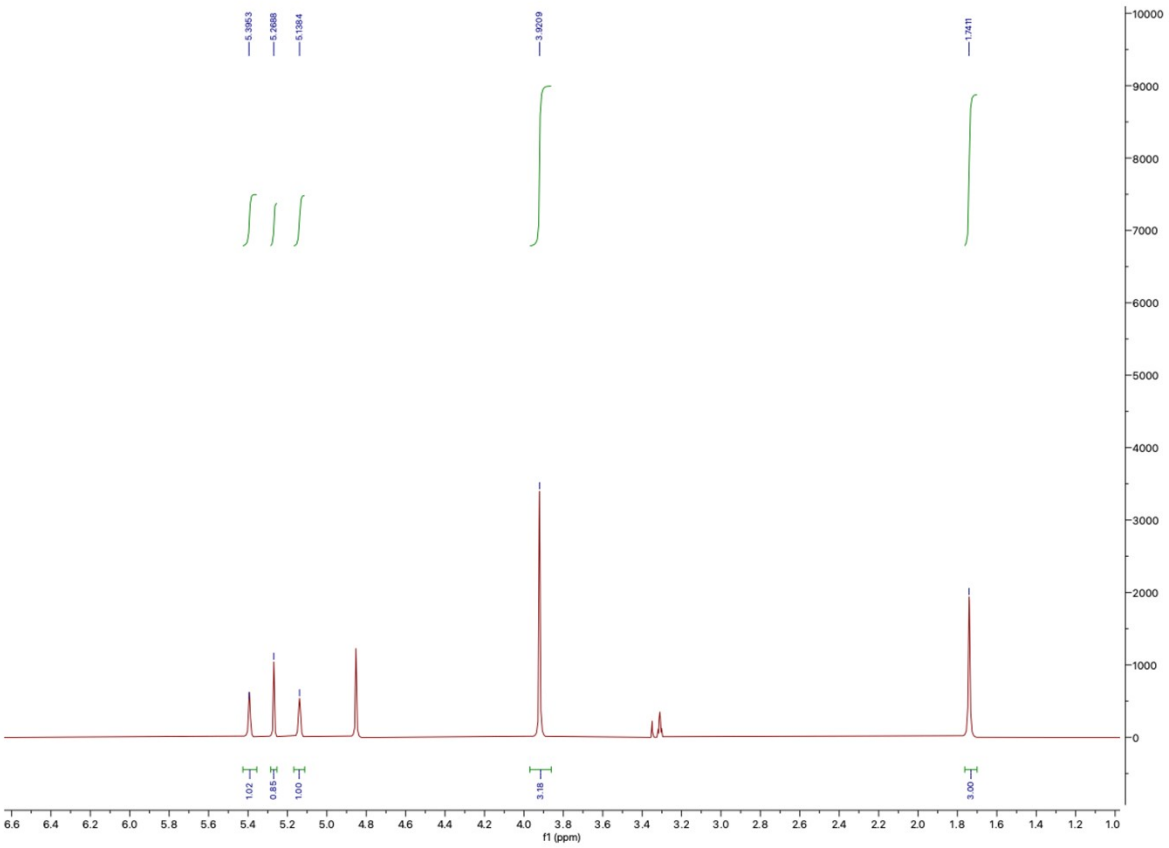

Figure S4. ^{1}H NMR spectrum of penicillic acid in methanol-d4.

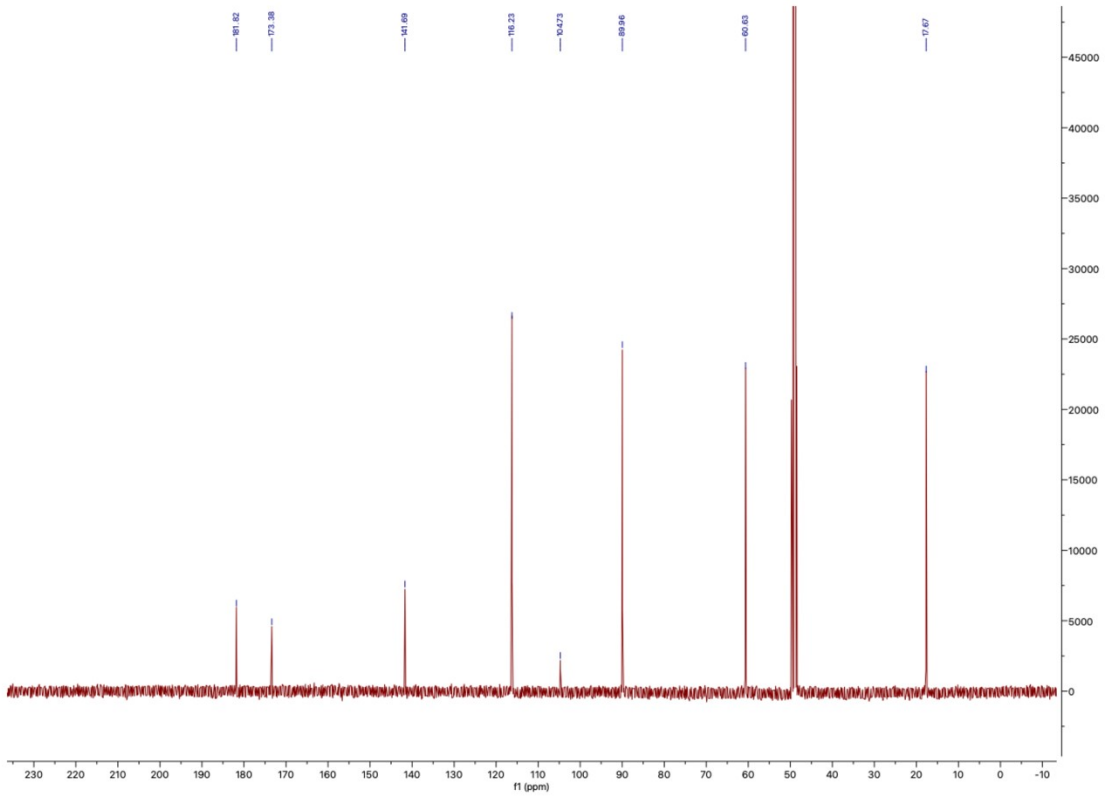

Figure S5. ^{13}C NMR spectrum of penicillic acid in methanol-d4.

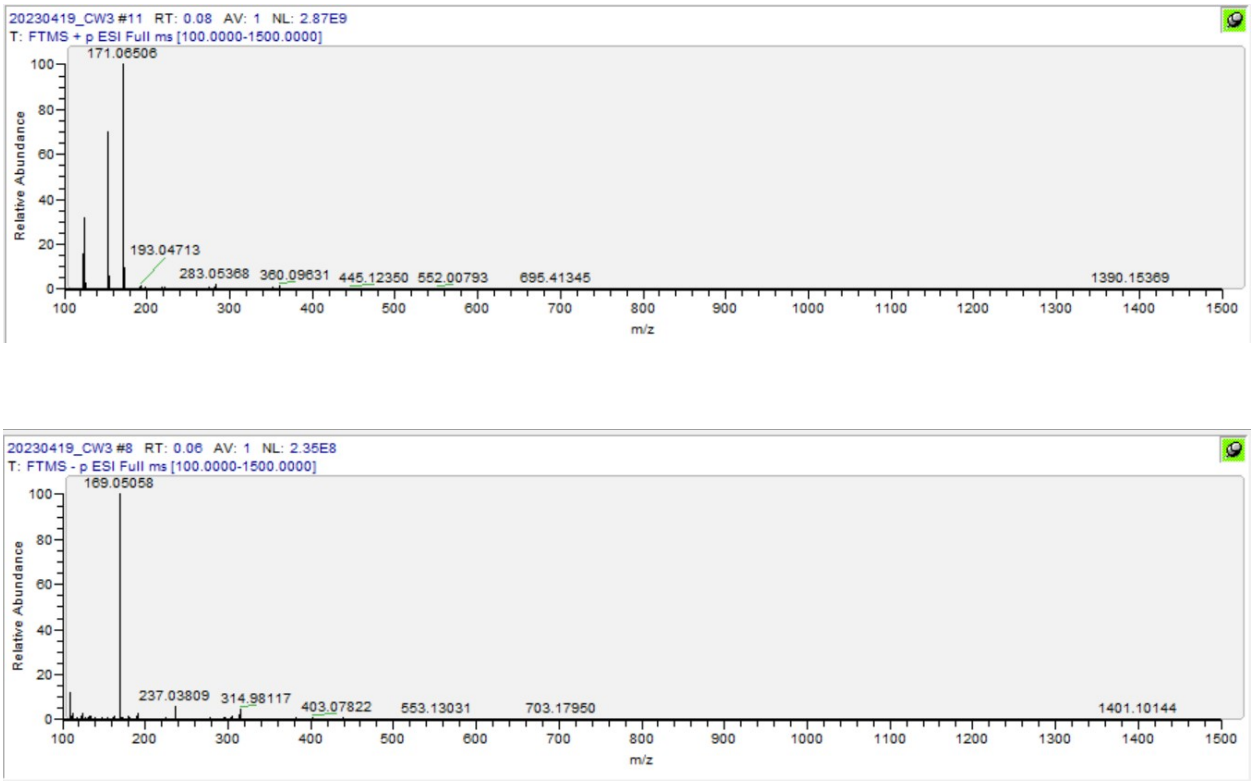

Figure S6. Mass spectrometry data of penicillic acid. Top represents penicillic acid in positive TIC (169 $[M+H]^+$). Bottom represents penicillic acid in negative TIC (171 $[M-H]^-$).

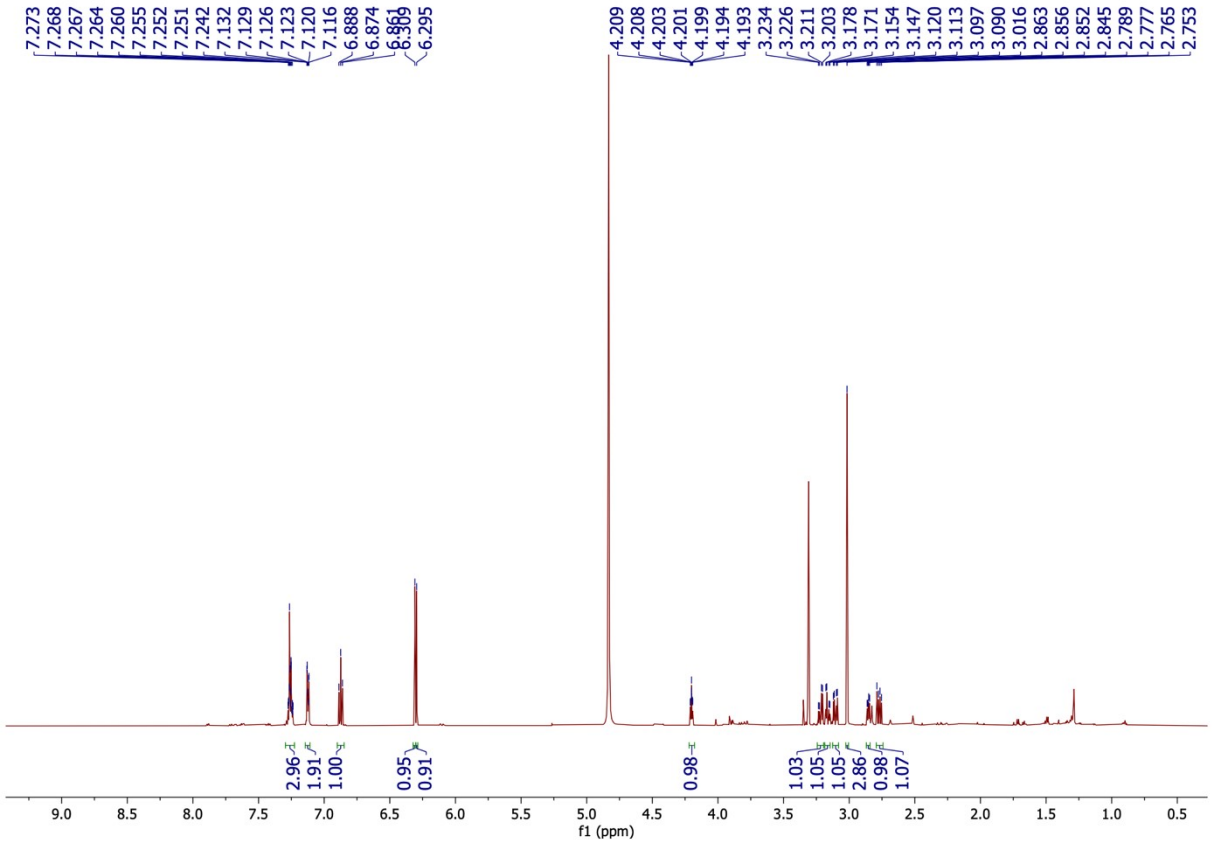

Figure S7. ^{1}H NMR spectrum of mactanamide (2) in methanol-d4.

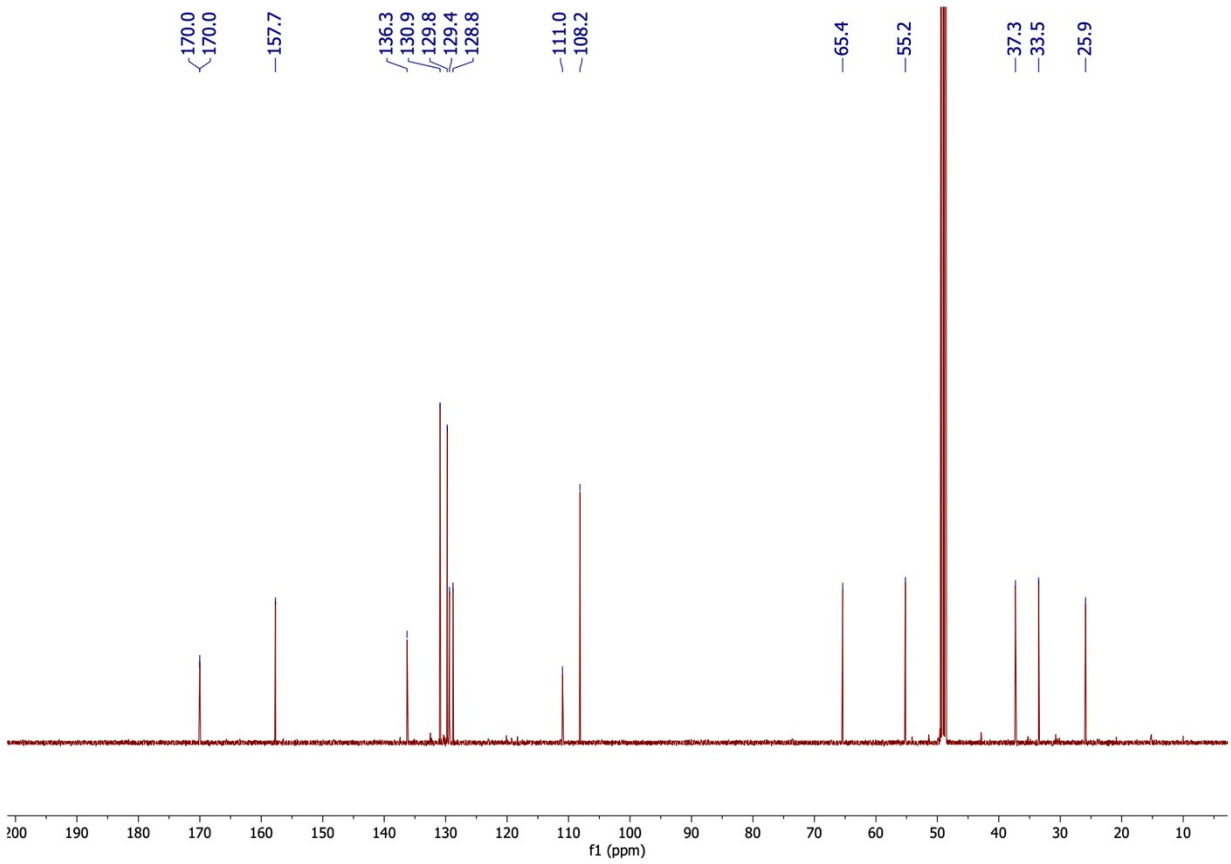

Figure S8. ^{13}C NMR spectrum of mactanamide (2) in methanol-d4.

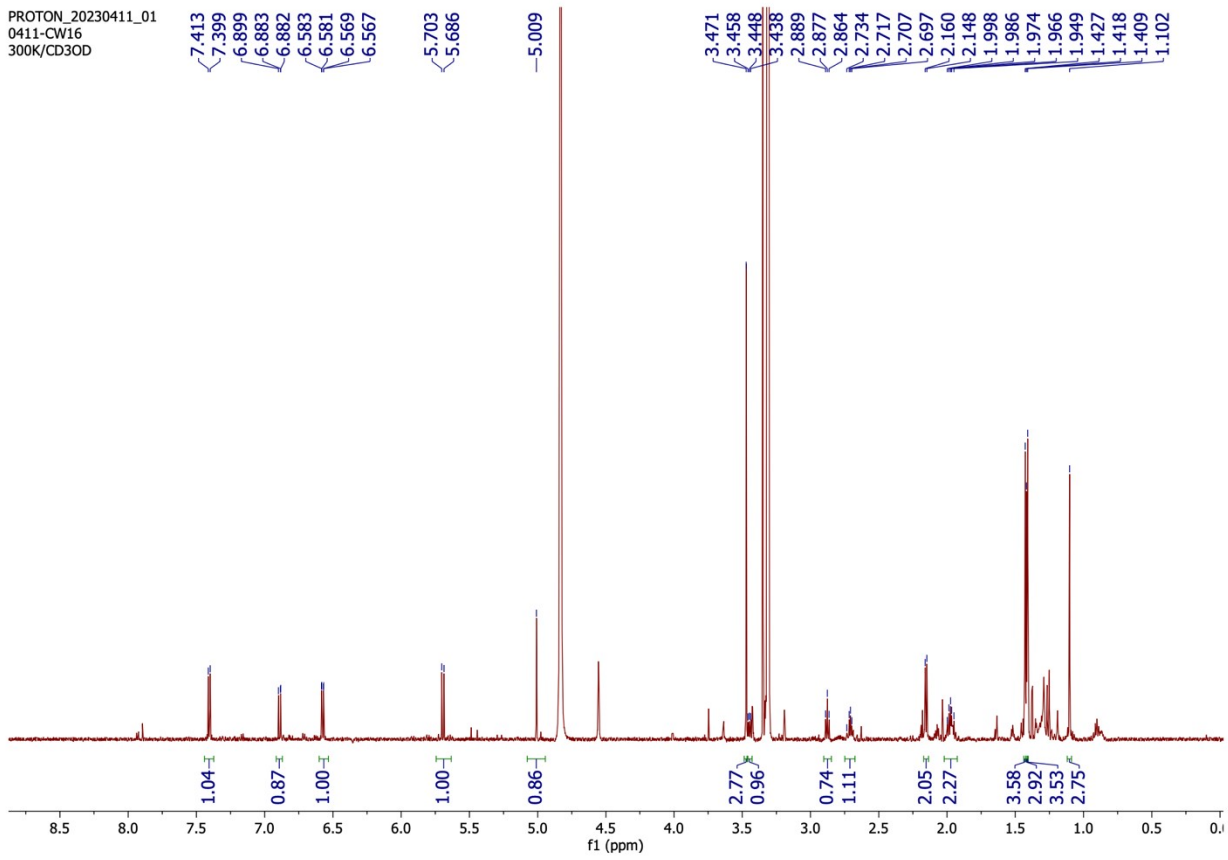

Figure S9. ^{1}H NMR spectrum of notoamide F (3) in methanol-d4.

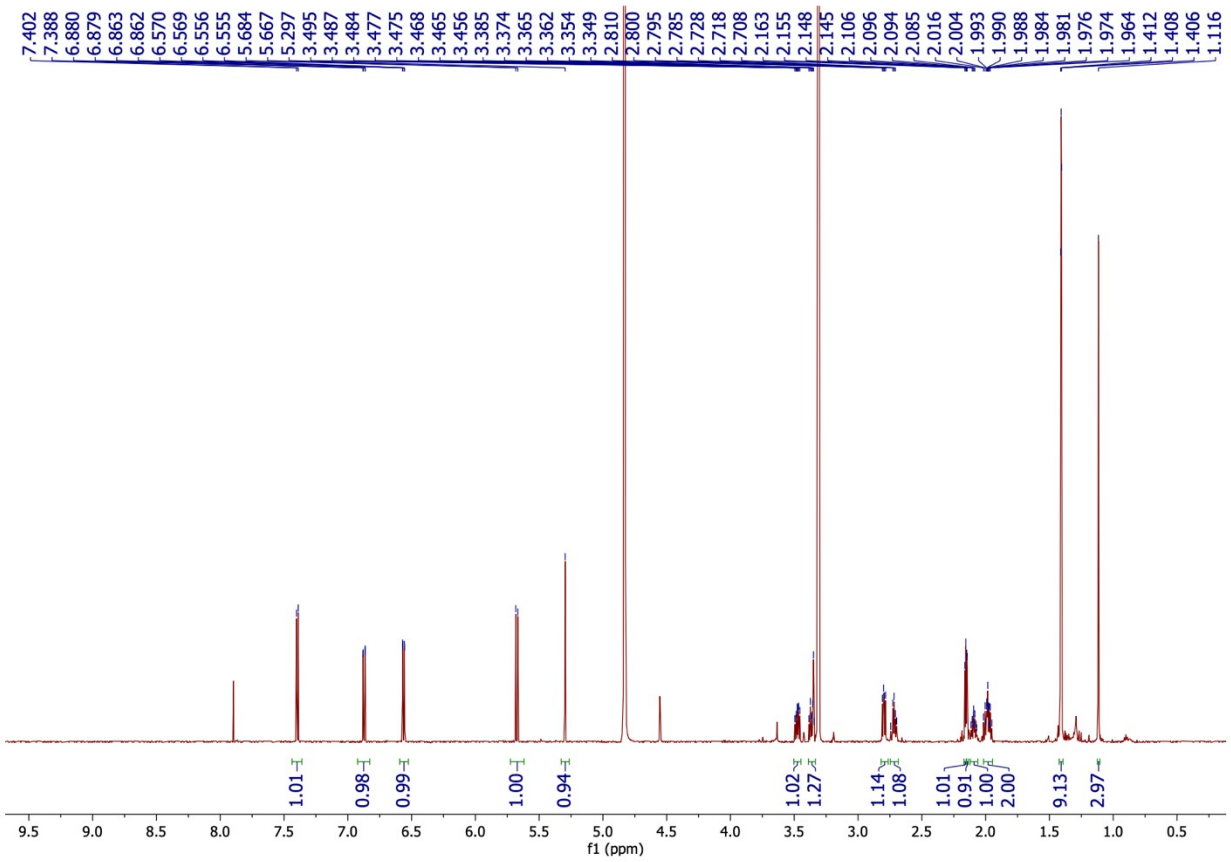

Figure S10. ^{1}H NMR spectrum of notoamide R (4) in methanol-d4.

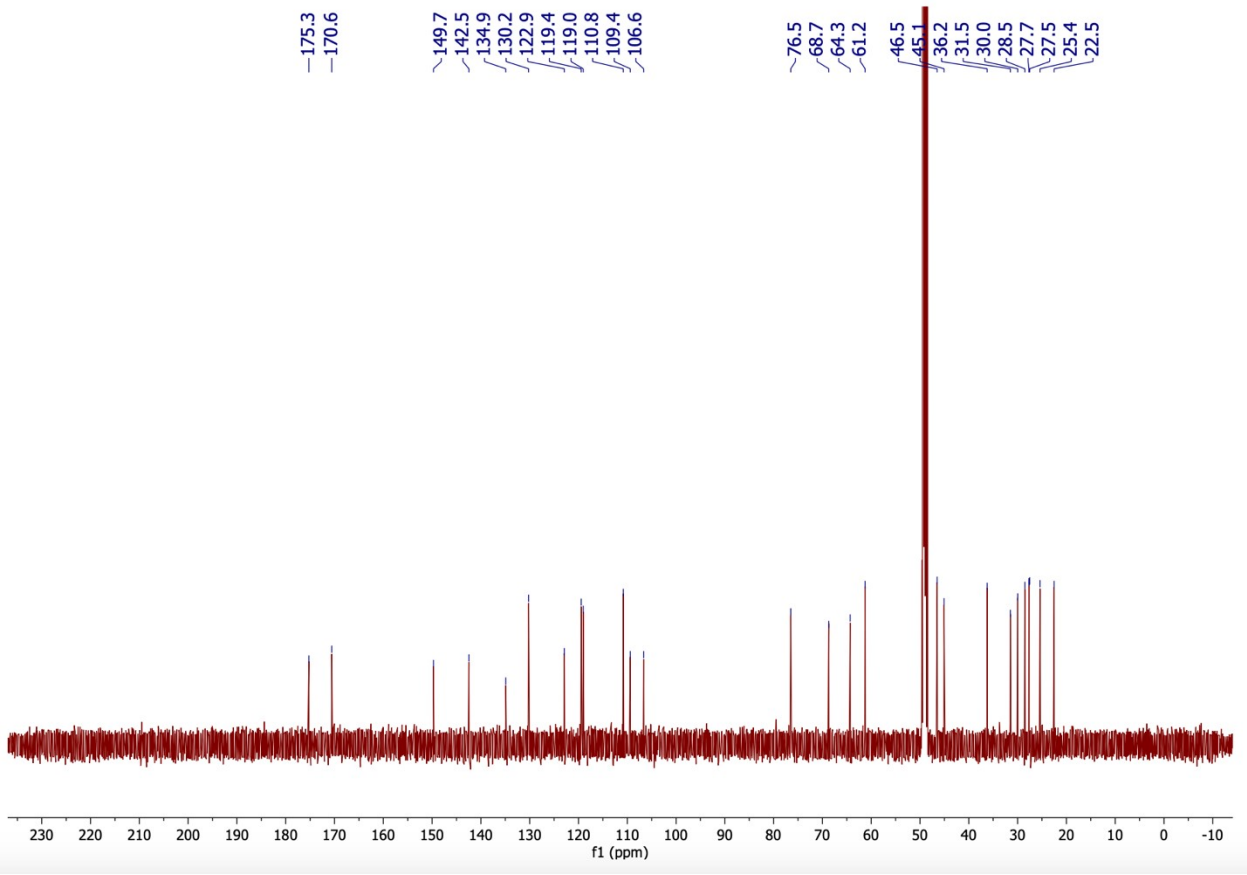

Figure S11. ^{13}C NMR spectrum of notoamide R (4) in methanol-d4.

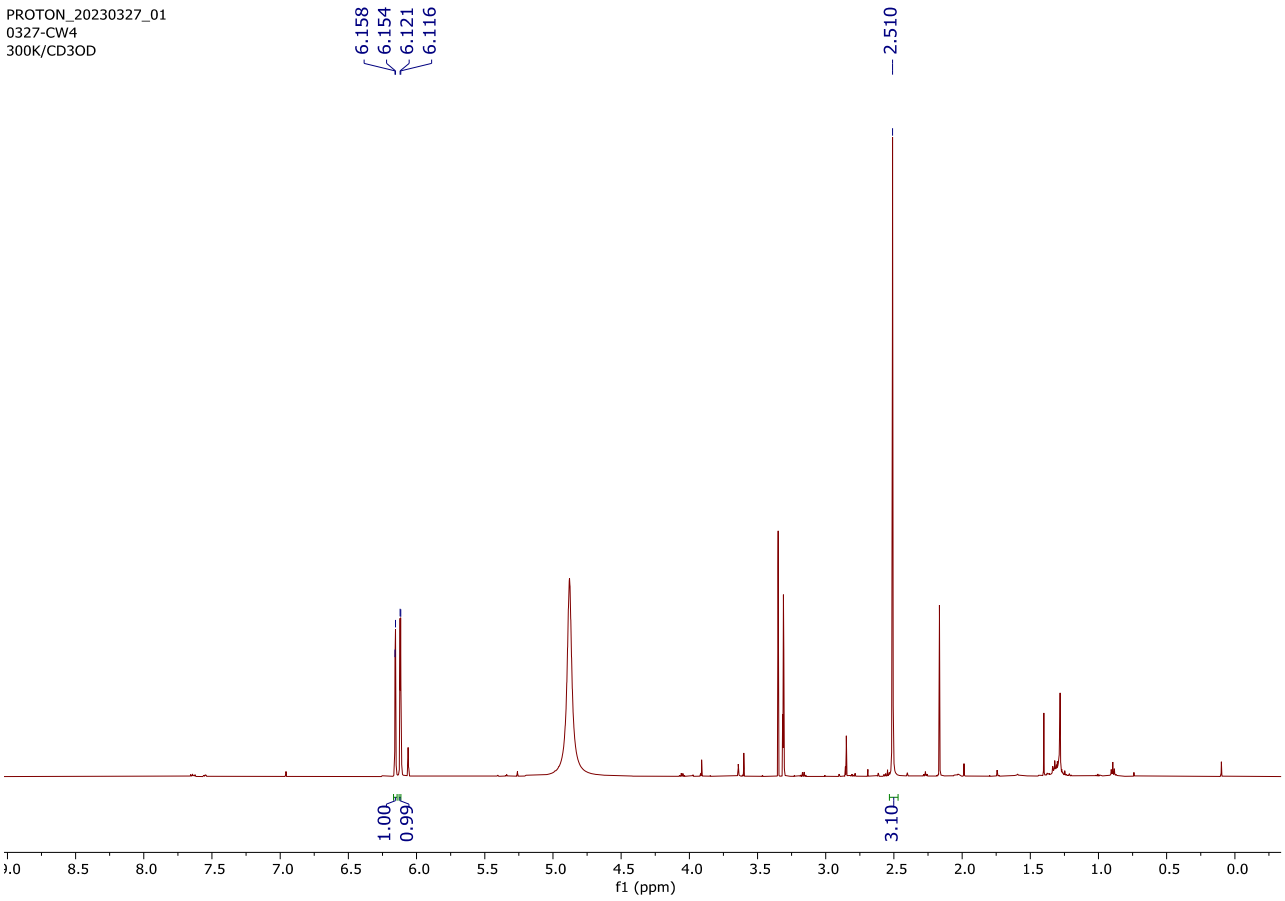

Figure S12. ^{1}H NMR spectrum of orsellinic acid (20) in methanol-d4.

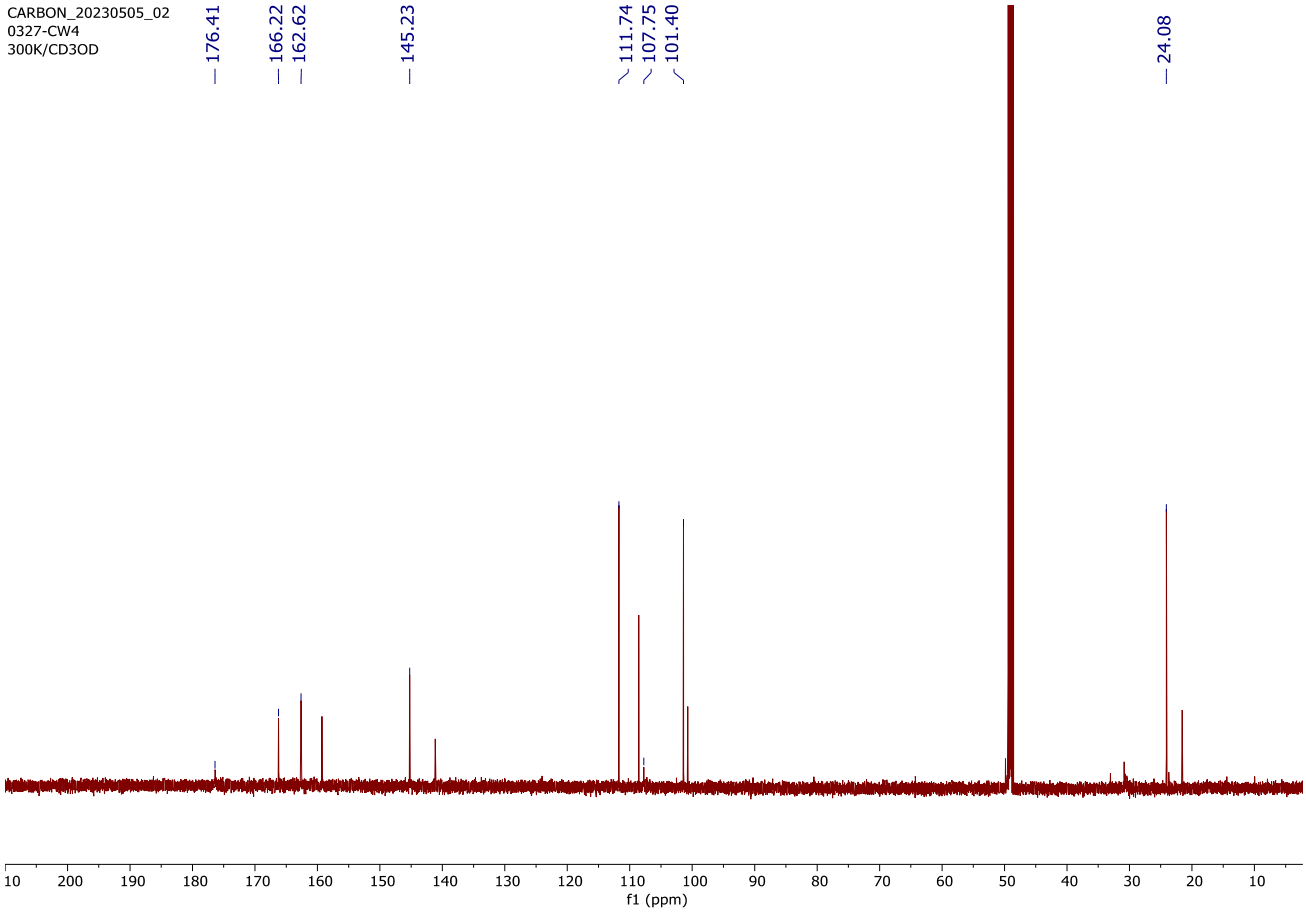

Figure S13. ^{13}C NMR spectrum of orsellinic acid (20) in methanol-d4.

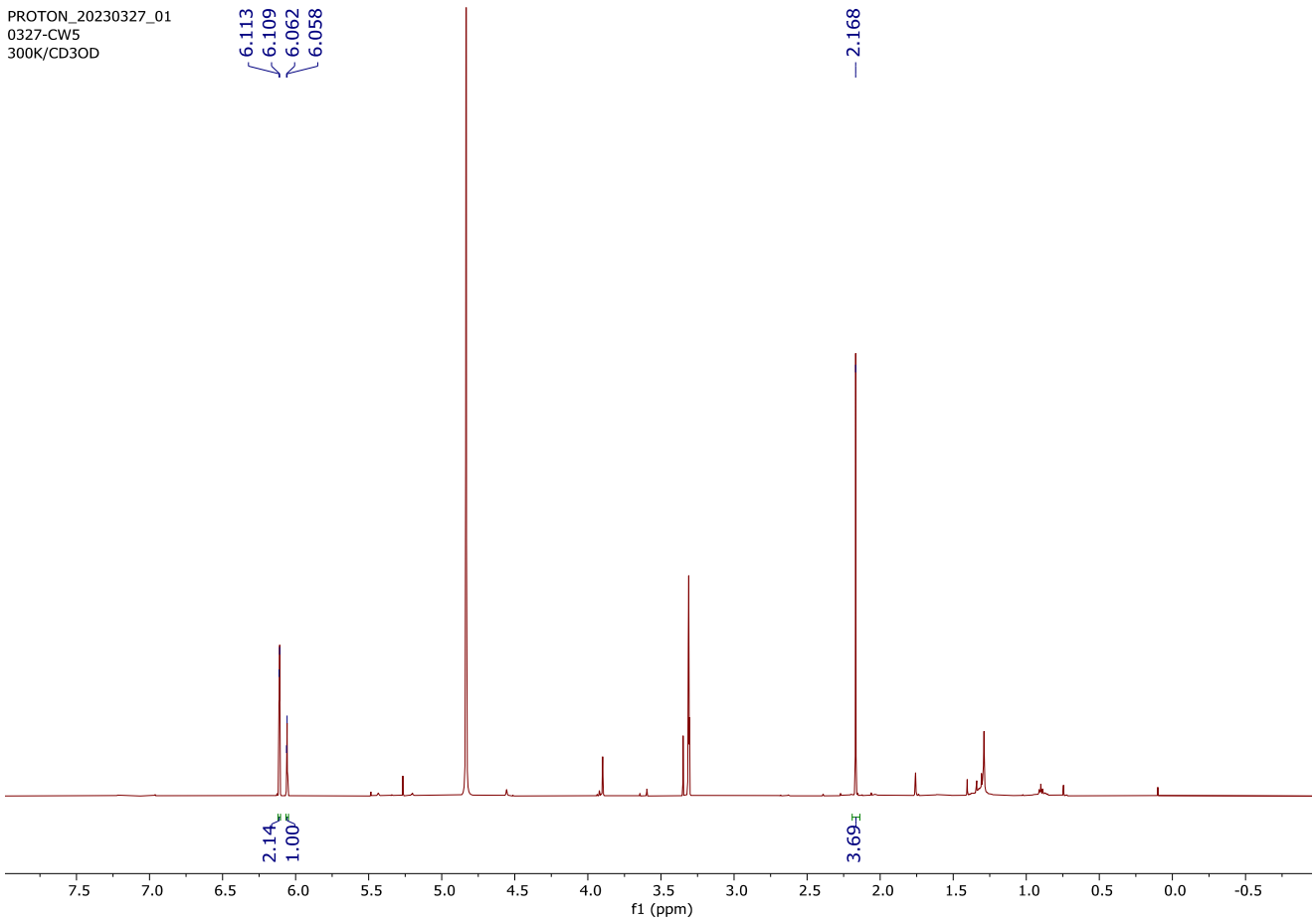

Figure S14. ^{1}H NMR spectrum of orcinol (21) in methanol-d4.

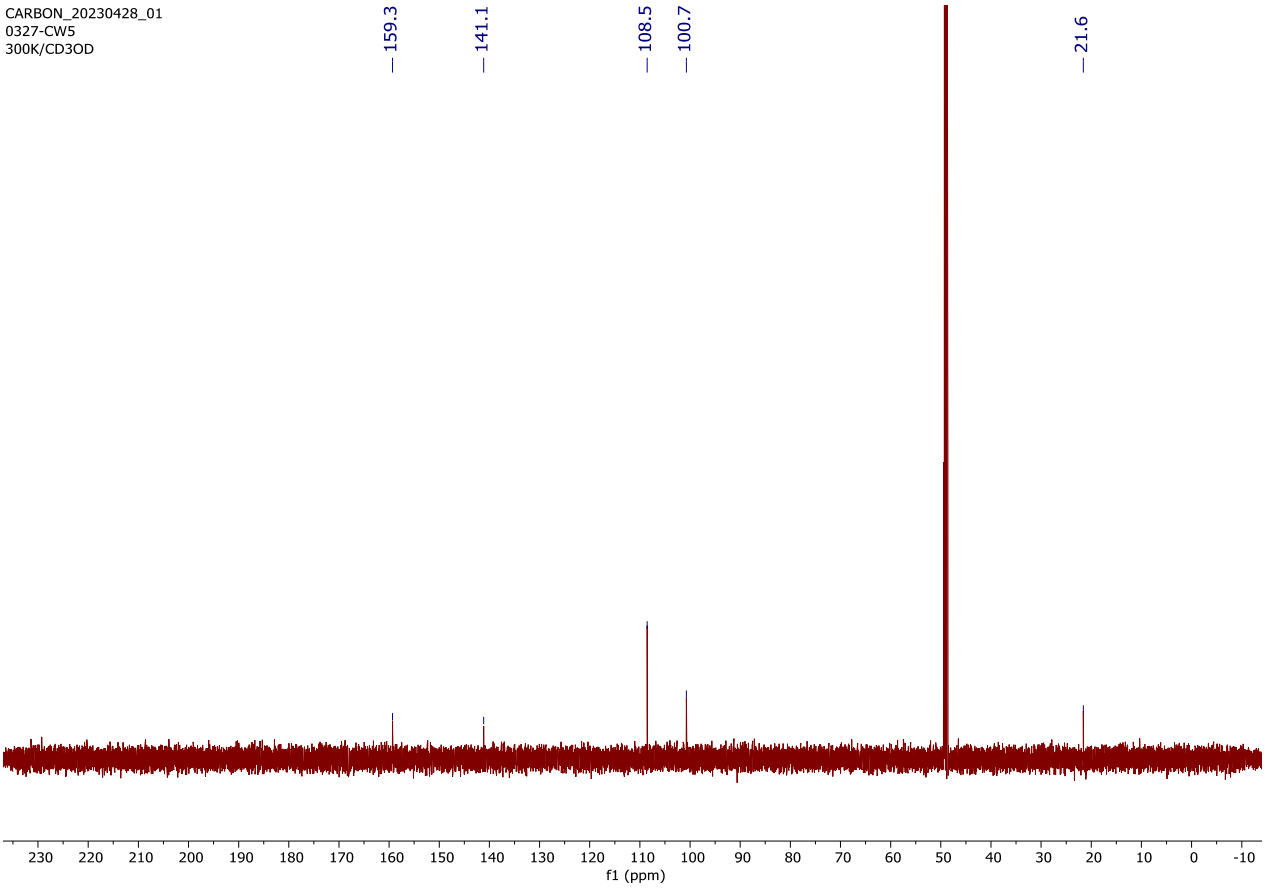

Figure S15. ^{13}C NMR spectrum of orcinol (21) in methanol-d4.

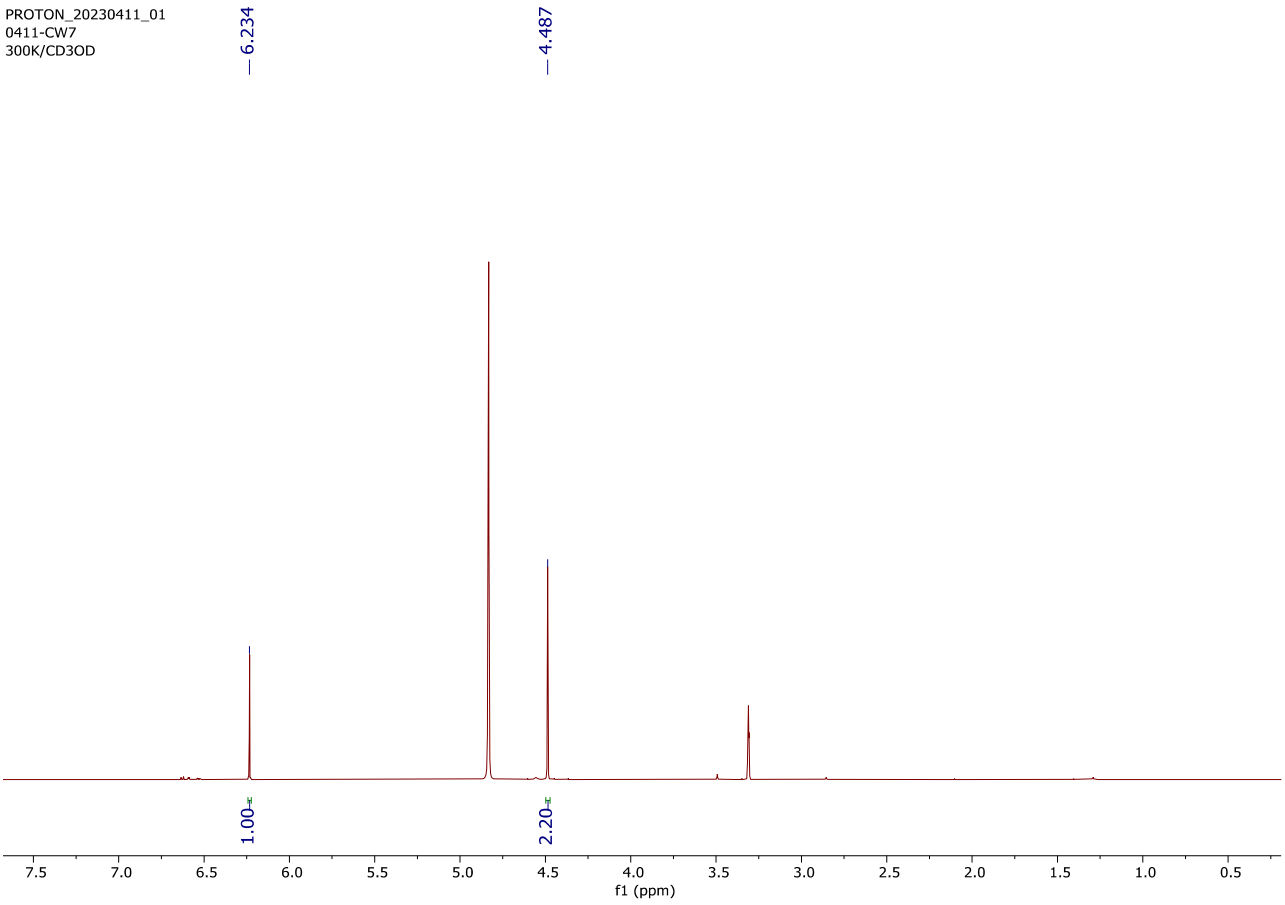

Figure S16. ^{1}H NMR spectrum of 2,5-furandimethanol (22) in methanol-d4.

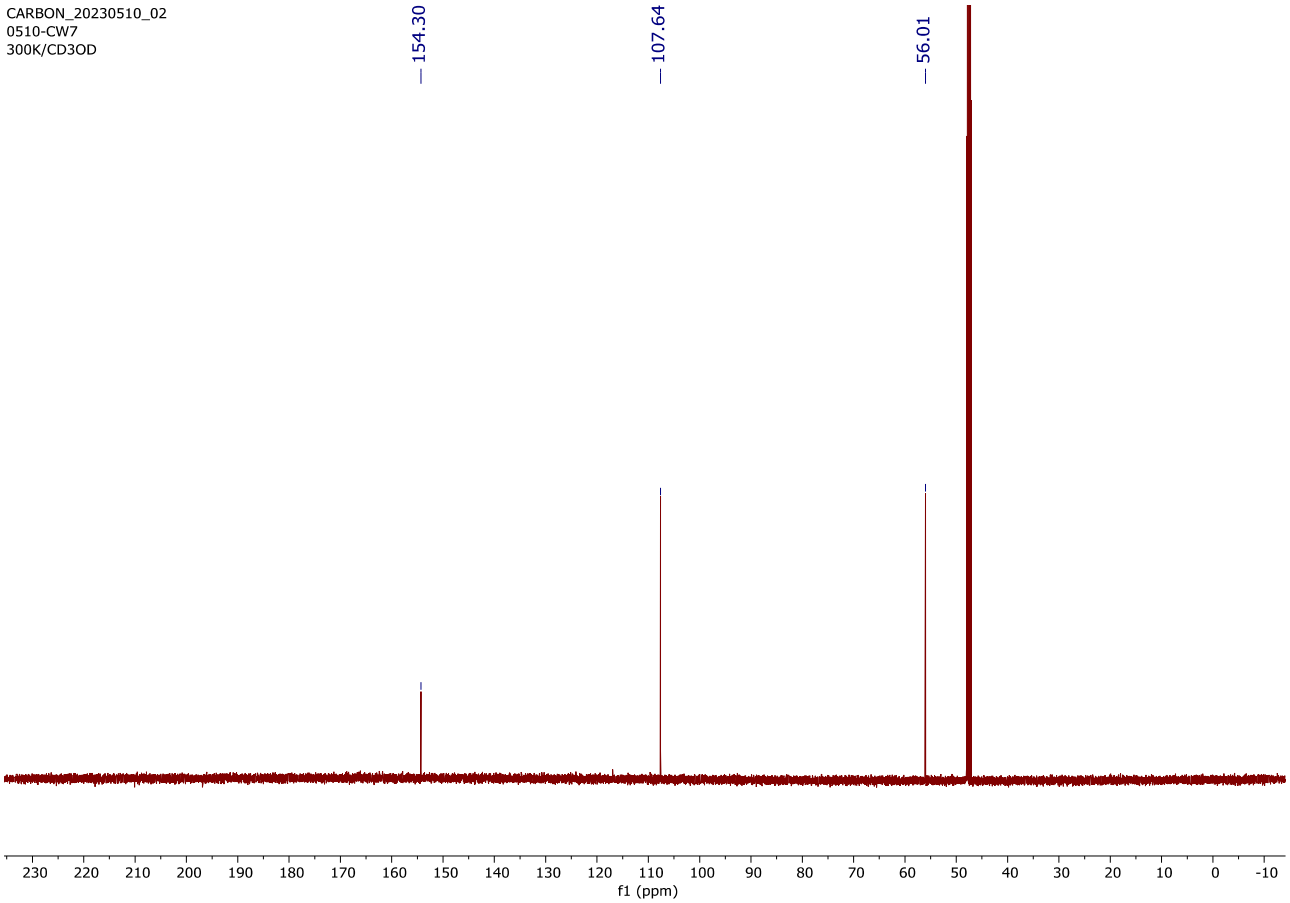

Figure S17. ^{13}C NMR spectrum of 2,5-furandimethanol (22) in methanol-d4.

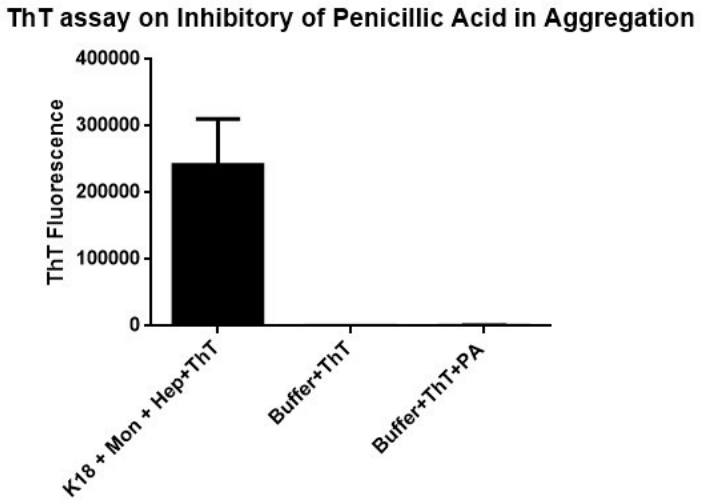

Figure S18. ThT assay on inhibitory of PA in aggregation. Error bars represent standard deviations of triplicate measures.

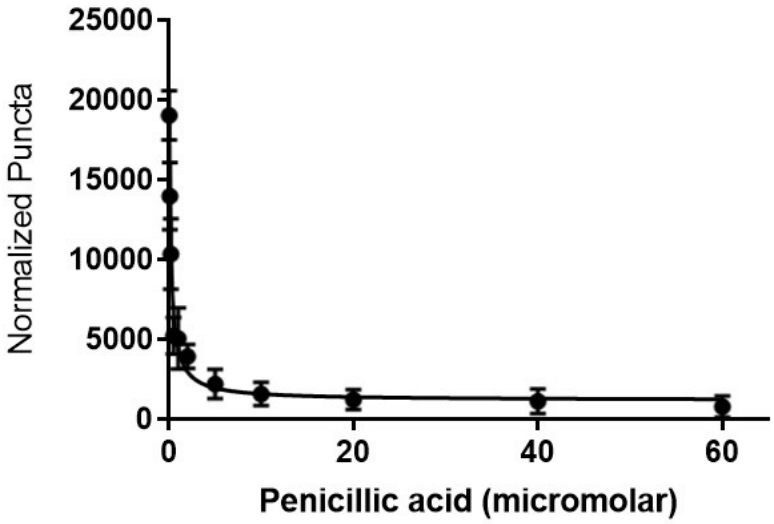

Figure S19. IC_{50} of PA. Using nonlinear curve fitting, we determined the IC_{50} of PA to be 213 nM.

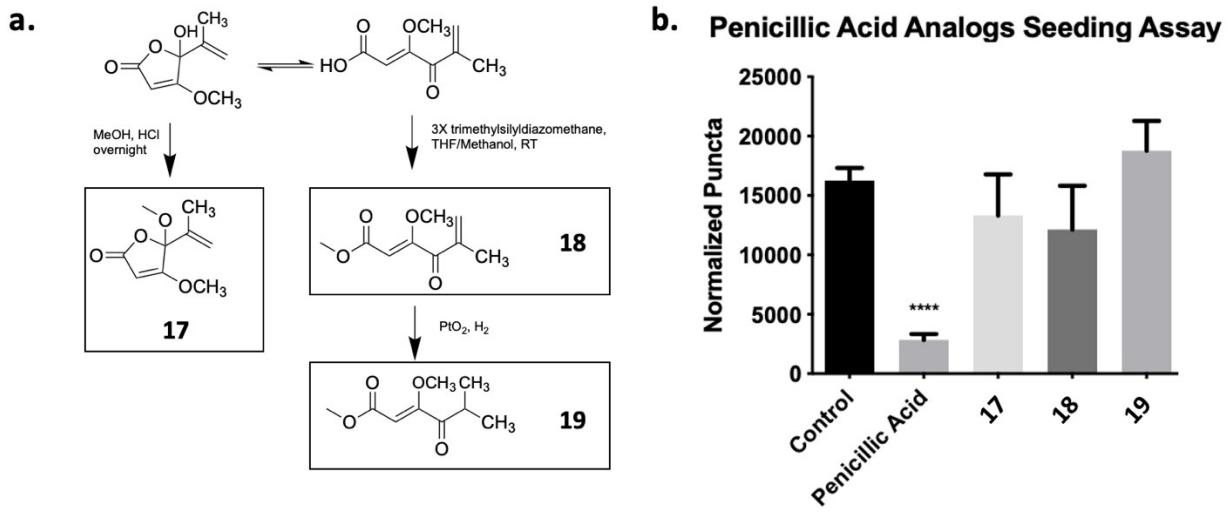

Figure S20. SAR study of penicillic acid using biosensor cell seeding assay as an activity readout. (a) Penicillic acid analogs were synthesized as a series of keto-acid and lactone-stabilized forms (Compounds 17-19). (b) The biosensor seeding tau inhibition assay was used as an activity readout for analogs 17-19.

Table S1. Primers used in this study. (Yuan, et al. 2023)

Sequencing primers used for diagnostic PCR (Confirmation of knockout)	
Primer	Sequence (5’ → 3’)
IMV1140_mcrA_seq_FW	GAACTCCGCATTGCAATCCT
IMV1140_mcrA_seq_REV	GACCGCTTAATGCGGTAGTG

Table S2. Structural characterization of compounds found in natural product library.

Penicillic acid: Molecular formula of $C_8H_{10}O_4$ based on HRESIMS m/z 171.06507 $[M+H]^+$ (cald for $C_8H_{11}O_4$). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm)= 5.39 (brs, 1H), 5.27 (s, 1H), 5.14 (brs, 1H), 3.92 (s, 3H), 1.74 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ (ppm)=181.7, 173.3, 141.6, 116.1, 104.6, 89.9, 60.5, 17.6.

Orsellinic acid: Molecular formula of $C_8H_8O_4$ based on HRESIMS m/z 169.04936 $[M+H]^+$ (cald for $C_8H_9O_4$). ^{1}H NMR (600 MHz, CD_3OD) δ (ppm)= 6.16 (d, *J*=2.4, 1H), 6.12 (d, *J*=2.8, 1H), 2.51 (s, 3H). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm)=176.4, 166.2, 162.6, 145.2, 111.7, 107.8, 101.4, 24.1.

Orcinol: Molecular formula of $C_7H_8O_2$ based on HRESIMS m/z 293.10284 $[2M+HCOO]^-$ (cald for $C_{15}H_{17}O_6$).^{1}H NMR (600 MHz, CD_3OD) δ (ppm)= 6.11 (d, *J*=2.4, 2H), 6.06 (d, *J*=2.4, 1H), 2.17 (s, 4H). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm)=159.3, 141.1, 108.5, 100.7, 21.6.

2,5-furandimethanol: Molecular formula of $C_6H_8O_3$ based on HRESIMS m/z 279.08368 $[2M+ Na]^-$ (cald for $C_{12}H_{16}O_6Na$). ^{1}H NMR (600 MHz, CD_3OD) δ (ppm)= 6.23 (s, 1H), 4.49 (s, 2H). ^{13}C NMR (1150 MHz, CD_3OD) δ (ppm)=154.3, 107.6, 56.0.

Mactanamide: Molecular formula of $C_{19}H_{20}N_2O_4$ based on HRESIMS m/z 339.13501 $[M-H]^-$ (cald for $C_{19}H_{19}N_2O_4$).^{1}H NMR (600 MHz, CD_3OD) δ (ppm) = 7.25 (ovl, 1H), 7.24 (ovl, 2H), 7.12 (m, 2H), 6.87 (t, *J*=8.1, 1H), 6.31 (d, *J*=8.1, 2H), 4.20 (ddd, *J*=4.9, 4.1, 0.9, 1H), 3.22 (dd, *J*=14.1, 4.9, 1H), 3.16 (dd, *J*=14.1, 4.1, 1H), 3.11 (dd, *J*=14.0, 4.2, 1H), 3.02 (s, 3H), 2.85 (dd, *J*=7.1, 4.2, 1H), 2.77 (dd, *J*=14.0, 7.2, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm)= δ 170.0, 170.0, 157.7, 136.3, 130.9, 129.8, 129.4, 128.8, 111.0, 108.2, 65.4, 55.2, 37.3, 33.5, 25.9.

Notoamide R: Molecular formula of $C_{26}H_{29}N_3O_4$ based on HRESIMS m/z 448.22244 $[M+H]^+$ (cald for $C_{26}H_{30}N_3O_4$). ^{1}H NMR (600 MHz, CD_3OD) δ (ppm) = 7.40 (d, *J*=8.4, 1H), 6.87 (d, *J*=9.8, 1H), 6.56 (d, *J*=8.4, 1H), 5.68 (d, *J*=9.8, 1H), 5.30 (s, 1H), 3.48 (m, 1H), 3.35 (m, 1H), 2.80 (dd, *J*=9.0, 5.9, 1H), 2.71 (m, 1H), 2.16 (d, *J*=4.7, 1H), 2.15 (ovl, 2H), 2.09 (m, 1H), 2.00 (m, 1H), 1.96 (m, 1H), 1.41 (s, 3H), 1.41 (s, 3H), 1.41 (s, 3H), 1.12 (s, 3H). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm)= 175.3, 170.6, 149.7, 142.5, 134.9, 130.2, 122.9, 119.4, 119.0, 110.8, 109.4, 106.6, 76.5, 68.7, 64.3, 61.2, 46.5, 45.1, 36.2, 31.5, 30.0, 28.5, 27.7, 27.5, 25.4, 22.5.

Notoamide F: Molecular formula of $C_{27}H_{31}N_3O_4$ based on HRESIMS m/z 462.23737 $[M+H]^+$ (cald for $C_{27}H_{33}N_3O_4$) ^{1}H NMR (600 MHz, CD_3OD) δ (ppm) = 7.40 (d, *J*=8.5, 1H), 6.88 (d, *J*=10.0, 1H), 6.57 (d, *J*=8.5, 1H), 5.69 (d, *J*=10.0, 1H), 3.46 (s, 3H), 1.42 (s, 3H), 1.41 (s, 3H), 1.40 (s, 3H), 1.10 (s, 3H).