Supporting Information

Total synthesis of Xanthoangelol B and its various fragments: toward inhibition of virulence factor production of *Staphylococcus aureus*

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Supporting Information (ES) available

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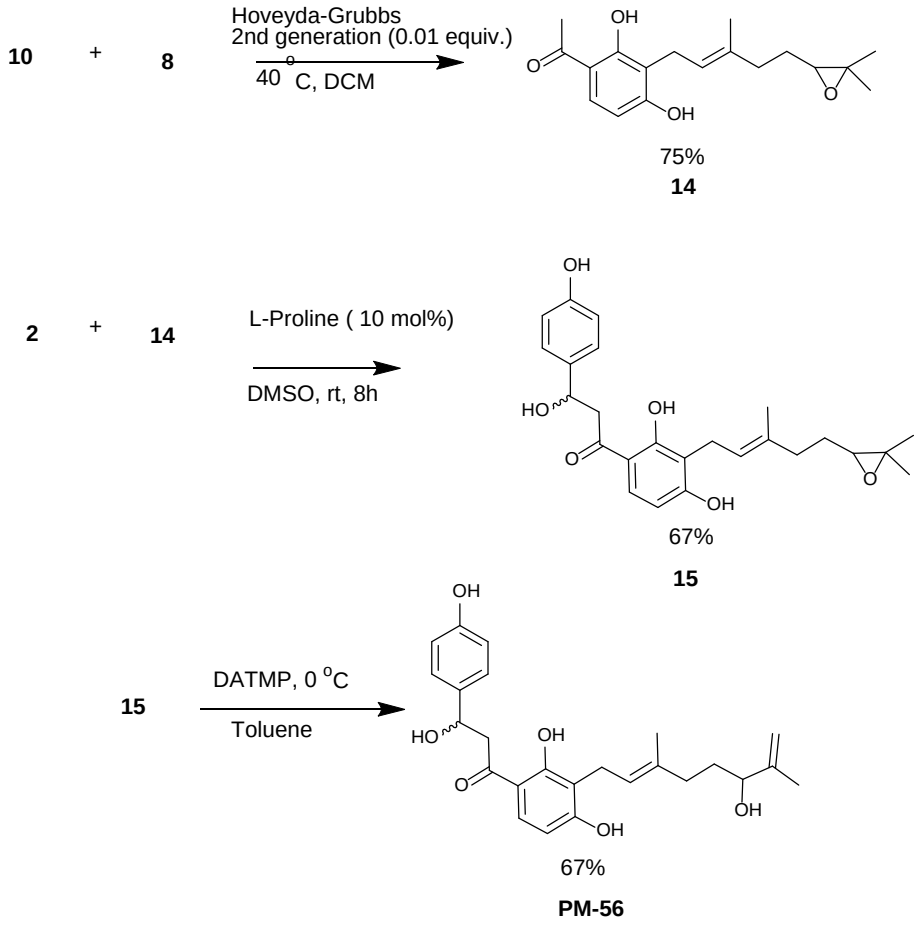
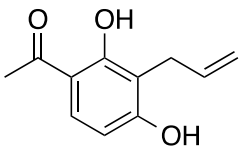


Scheme S1. Synthesis of **PM-56**



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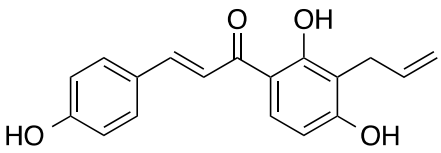

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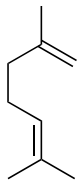

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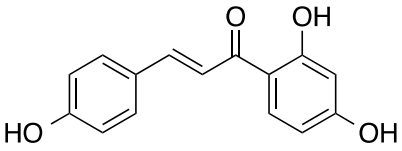

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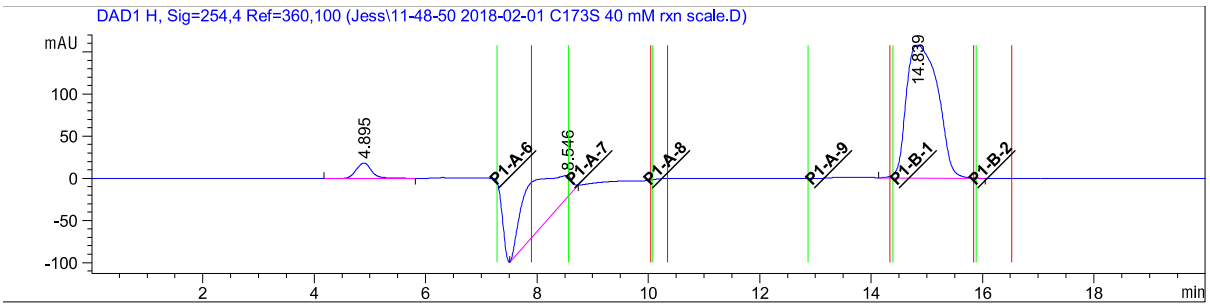
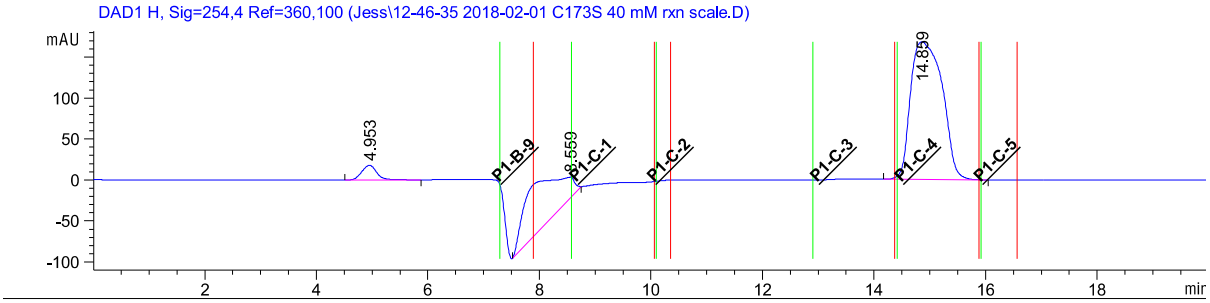
Scheme S2. Fragments of xanthoangelol B tested for inhibition of the SaeRS GFP reporter

(A) Xanthoangelol B: Natural Sources



(B) Xanthoangelo B: racemic



(C) Xanthooangelol B: resolved

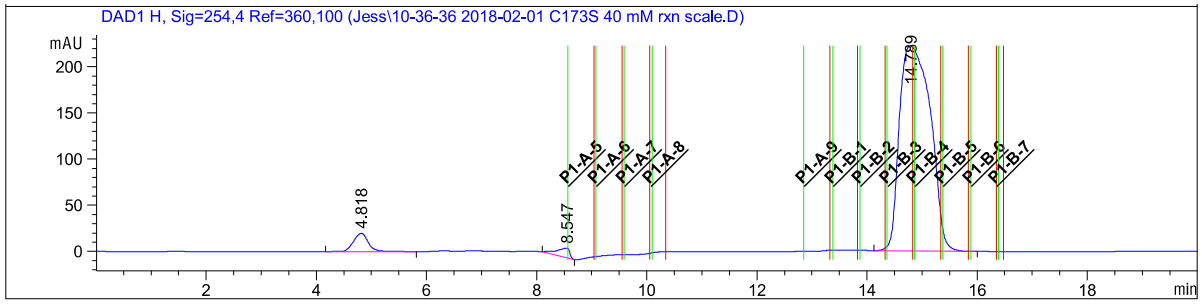


Figure S1. Comparison of HPLC Traces of xanthoangelol B: C18 Column (Kromasil 100-5-C18), Eluent: 15% acetonitrile (Solvent A)/water 85% (Solvent B). (A) xanthoangelol B extracted from natural sources, (B) racemic xanthoangelol B, (C) resolved xanthoangelol B.

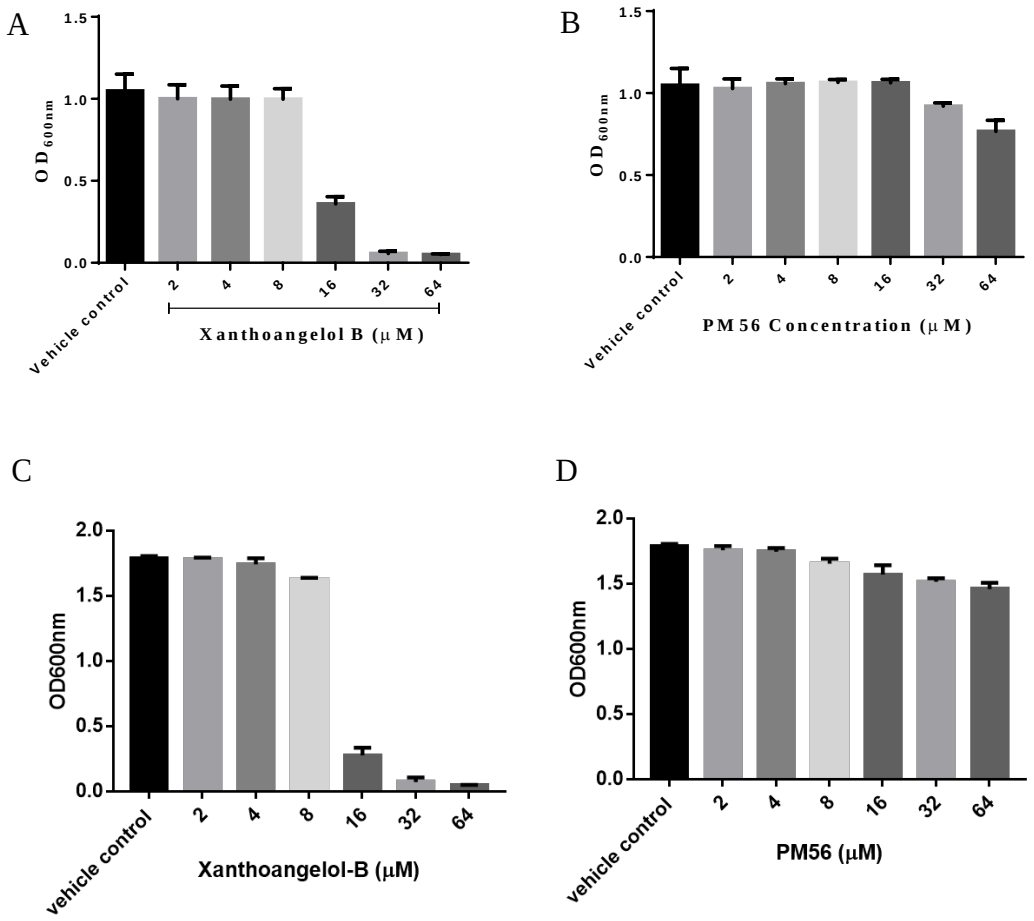


Figure S2. Effects of xanthoangelol B **1** and **PM-56** on the growth of *S. aureus*. The *S. aureus* USA300 was grown to the early exponential phase and treated with xanthoangelol B **1** and **PM-56** at the concentration in the range from 2 μM to 64 μM. The cell density was monitored as the optical density at 600 nm. A, Bacterial growth for 8 h in the presence of xanthoangelol B **1**; B, Bacterial growth for 8 h in the presence of **PM-56**; C, Bacterial growth for 20 h in the presence of xanthoangelol B **1**; D, Bacterial growth for 20 hours in the presence of **PM-56**. All the experiments were performed in triplicates.

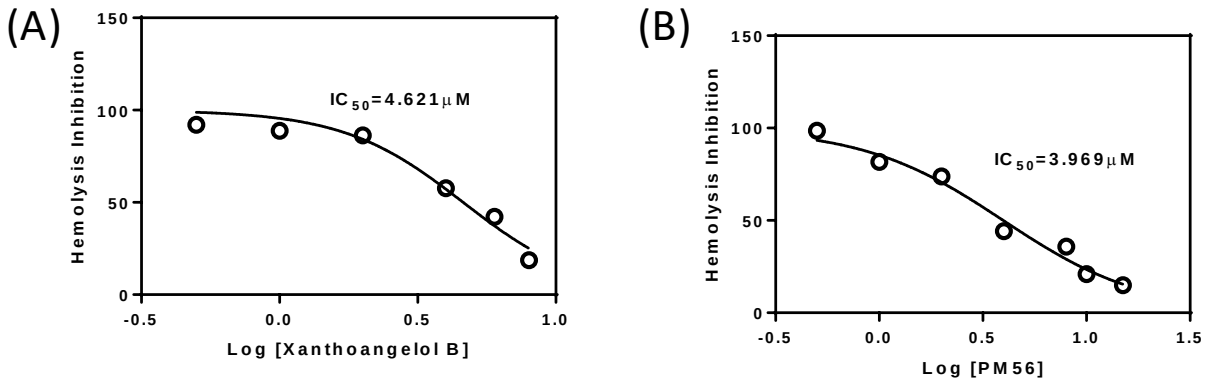


Figure S3. Determination of the IC_{50} of (A) Xanthoangelol B **1** and (B) **PM-56** for an anti-hemolytic activity.

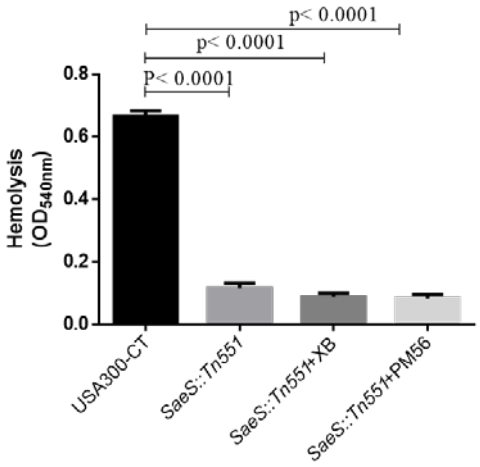


Figure S4. Effects of xanthoangelol B **1** and **PM-56** on RBC hemolysis by the *saeS* knockout mutant of *S. aureus*. The *saeS* mutant strain was treated with **1** and **PM-56,** and RBC hemolysis calculated in percentage. The significance compared to the control (*S. aureus* USA300) in triplicate given as P<0.0001 was calculated by one way ANOVA.

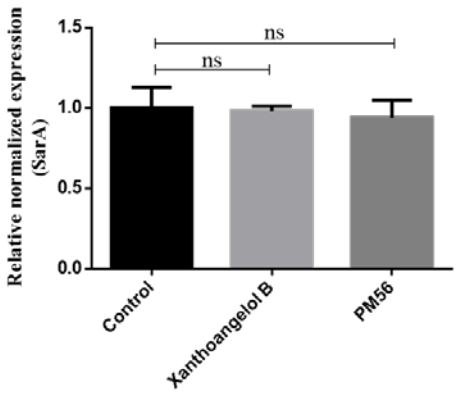


Figure S5. Effects of xanthoangelol B **1** and **PM-56** on *sarA* gene**.** *S. aureus* was grown to the early exponential phase and treated with xanthoangelol B **1** and **PM-56**. The *sarA* gene quantification was determined and compared with 16S rRNA. All experiments were performed in triplicate and the significance compared with control.

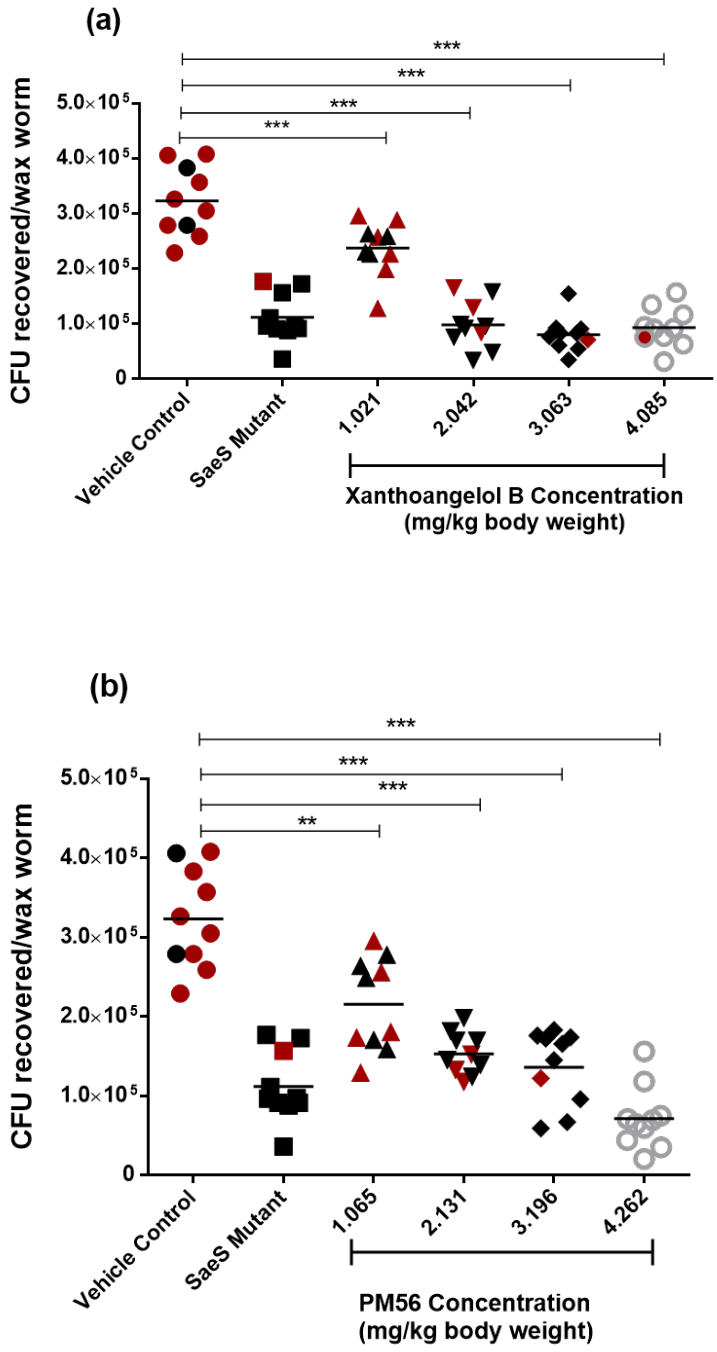


Figure S6. Xanthoangelol B **1** (a) and **PM-56** (b) attenuate *S. aureus* survivals in *Galleria mellonella*. *G. mellonella* was challenged with 5x10^6 cfu of *S. aureus* and treated with **1** and **PM-56**. The *S. aureus* survivals were determined after 72 h. ***P<0.001, **P<0.01, *P<0.05 by one way ANOVA; Each symbol represents the values of individual larvae.

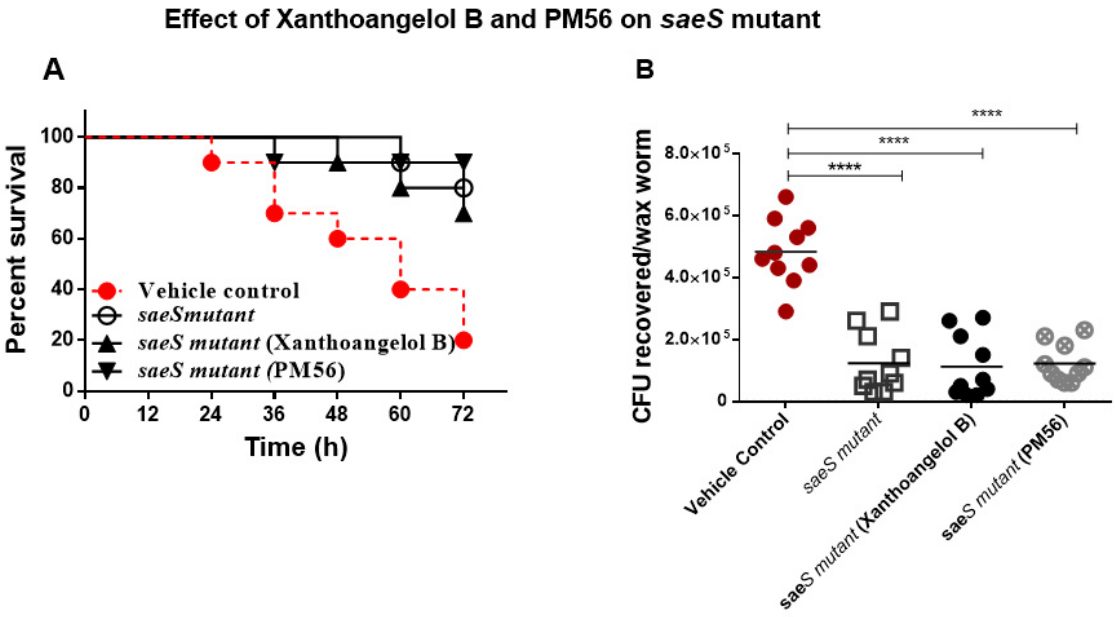
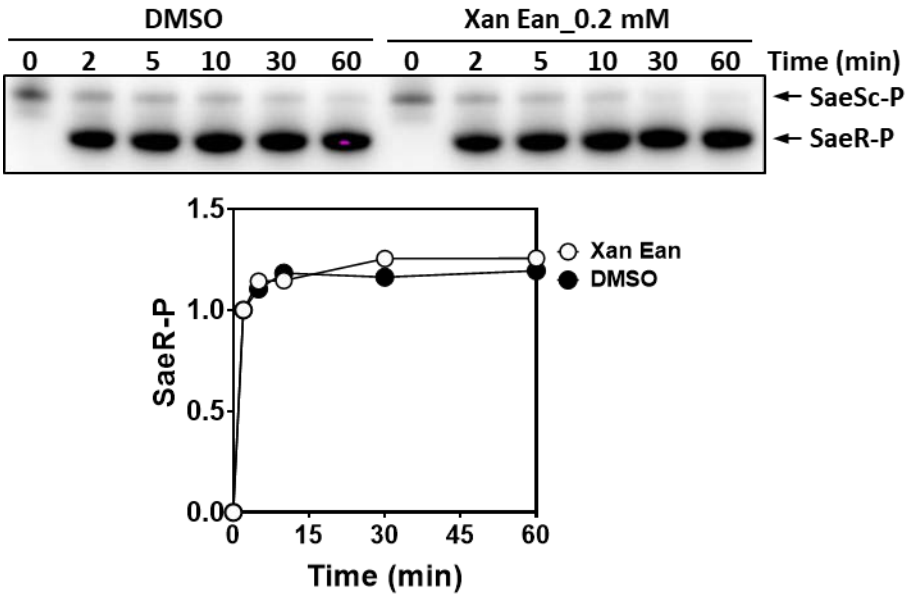


Figure S7. *In vivo* efficacy in *G. mellonella* infection model. (A) Kaplan-Meier survival curves for *G. mellonella* (n=10) challenged with $5x10^6$ cfu of the *S. aureus* wild type and *saeS* knockout mutant. *G. mellonella* infected by the latter was treated with xanthaoangelol B **1** and **PM-56** 2 h post infection. The chemical was injected every 12 h interval for 72 h. (B) *S. aureus* burdens were calculated at 72 h. The experiments were performed in n=10 biological replicate samples. ***P<0.001, **P<0.01, *P<0.05 by student t-test.

(A)



(B)

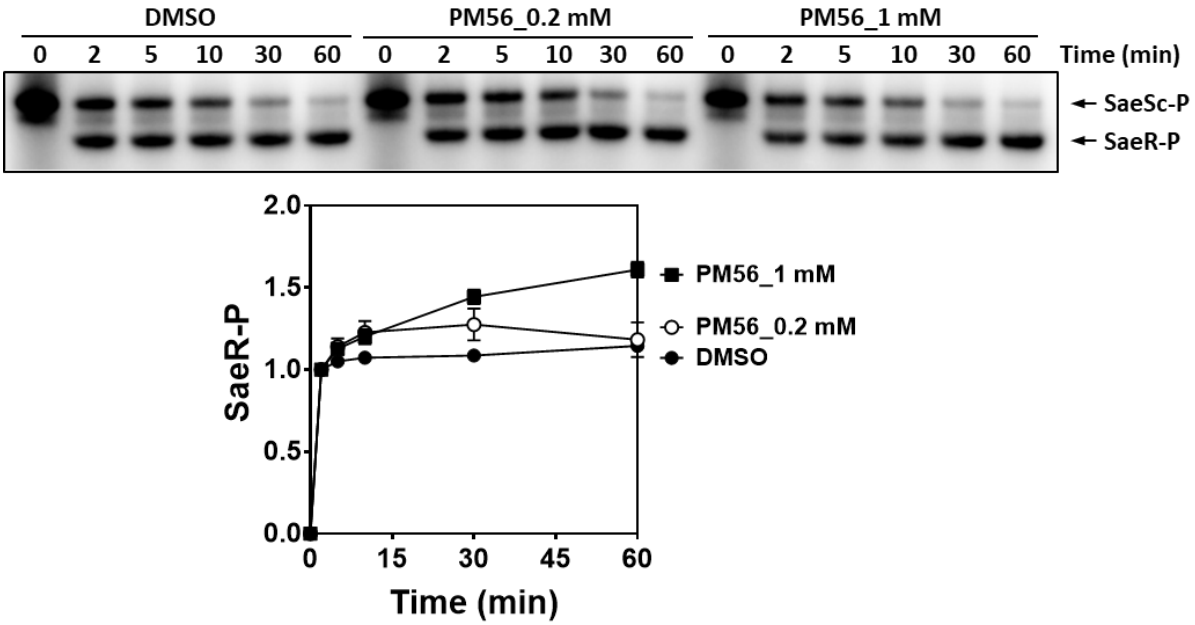


Figure S8. (A) Inhibition test with xanthoangelol B **1** for phosphotransferase activity of SaeS, (B) Inhibition test with **PM-56** for phosphotransferase activity of SaeS.

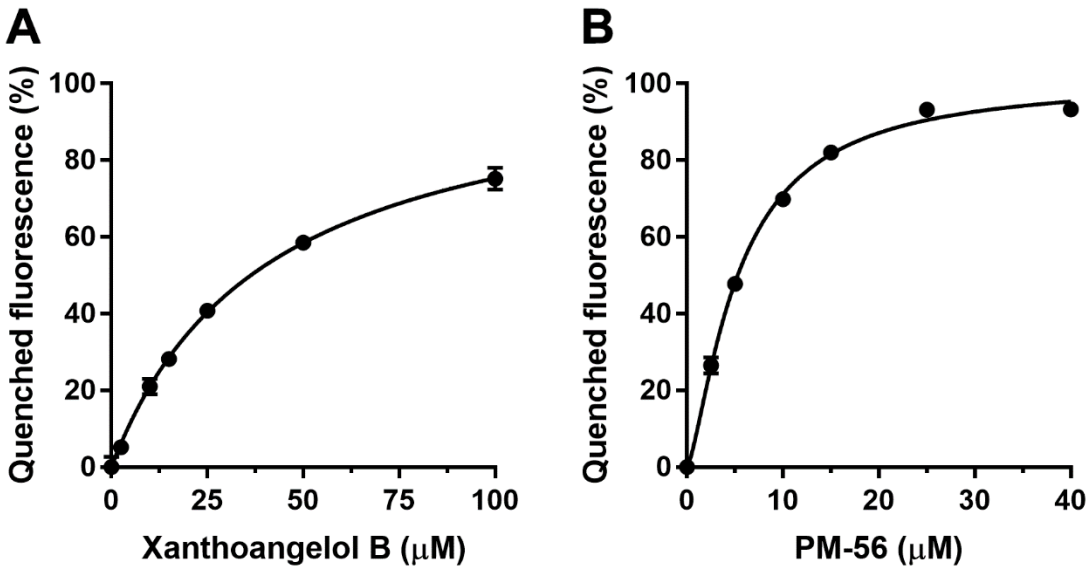


Figure S9. Intrinsic fluorescence quenching of the kinase domain of SaeS by (A) xanthoangelol B **1** and (B) **PM-56**. Purified recombinant SaeS (1 μM) was incubated with various concentrations of either (A) **1** or (B) **PM-56** for 15 min at room temperature. Upon excitation at 276 nm, reductions of SaeS intensities at an emission peak of 306 nm were recorded and plotted against the chemical concentrations. Data points were averaged from three independent measurements and expressed as means (dots) ± standard deviations of means (whiskers). Solid lines represent the fitted curves to calculate the chemical-binding affinities.

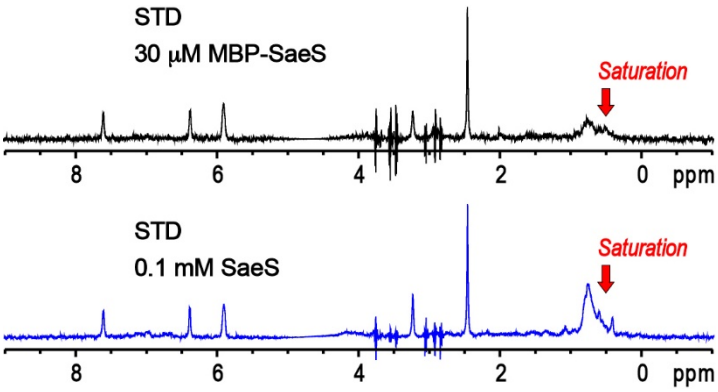
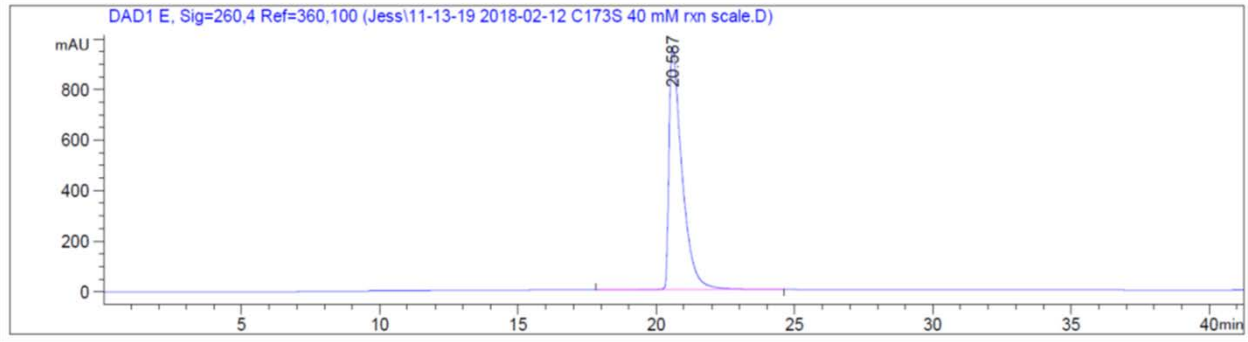


Figure S10. 1D STD spectra of **PM-56** in the presence of 0.03 mM MBP-SaeS (top) and 0.1 mM SaeS (bottom). The **PM-56** peaks are specifically identified in 1D STD spectrum in the presence of 0.03 mM MBP-SaeS and 0.1 mM SaeS protein. Slightly different peak shapes between on- and off-saturation at 0.5 and 30 ppm, respectively, could result in the peak spikes of the remained HEPES molecule with both positive and negative signs.

A. xanthoangelol B **1**



B. **PM-56**

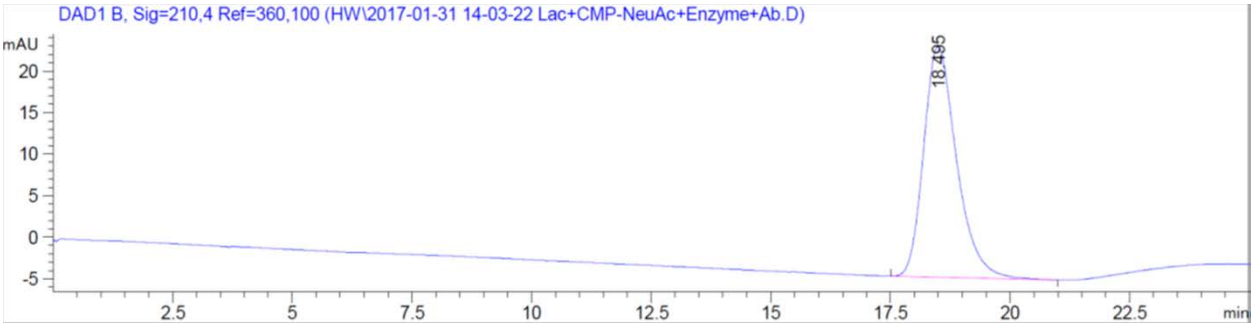


Figure S11. Purity analysis of xanthoangelol B **1** and **PM-56**. Both compounds were analysed by the Agilent Technologies 1260 Infinity HPLC system with a C18 Column (Kromasil 100-5-C18, 5 µm, 21.2 × 250 mm). The column was eluted with 20/80 acetonitrile/water, and the elution monitored with a UV detector at 254 nm. The program was run for 42 minutes for **1** (A) and 25 minutes for **PM-56** (B).

Table S1. Bacterial strains and plasmids used in this study

Strain or Plasmid	**Relevant characteristic**	**Origin**
E. coli		
DH5α	Plasmid-free, restriction deficient	New England Biolabs
BL21(DE3)	Recombinant protein expression strain	New England Biolabs
S. aureus		
USA300	USA300-0114	[1]
USA300-P23	USA300-0114 without plasmid 2 and 3	[2]
Plasmid		
pYJ335	An *E. coli-S. aureus* shuttle vector, Erm^r	[3]
pYJ335-*gfp*	pYJ335 carrying a promoterless *gfp*	[4]
pYJ335-P1-*gfp*	pYJ335 carrying the P1promoter-*gfp* fusion	[5]
pCL-P_{hlam}-*gfp*	pCL55 carrying P_{hlam}-*gfp* fusion	[5]

Table S2. Primers used in the study

Name	**Sequence (5′→ 3′)**	**Target**
P1969	GGGGTACCATTGGAAGTGGATAACATGTCAAAAGGAGAA GAATTATTTAC	*pYJ-gfp*
P1747	ATTGGATTGGAAGTACGGTACCGAGCTCGAATTCACTG	*pYJ-gfp*
P1971	TACTTCCAATCCAATGTCATCATTGGTGGTATTATGTTG	*P1*
P1972	TTATCCACTTCCAATGGCTAACTCCTCATTTCTTCAATT	*P1*
P1992	TTATCCACTTCCAATGATTACAATATAAAAATACAAATATCTTAG	*Phla*
P1993	TACTTCCAATCCAATG TTAATATATAGTTAATTTTTATTTAATAG	*Phla*
P0011	GCG CTG CAT TAG CTA GTT GGT TGG CCG ATC ACC CTC TCA	*16s*
P0012	CAA CAA CAC TAT TGC TAG GTT CCA TAT T CCT GTT TTT ACT GTA GTA TTG CTT CCA	*α-hemolysin*
P0013	TGT CTG CGT GTA CTT TCA CTT C AAG AGT GAT GCG GTC AAA GC	*aureolysin*
P0014	ATC AAT CGG AGG CAG TGG C GCA GAT ACT TGA CCA TTC GGT G	*ϒ-hemolysin A*
P0015	GCT CTG ATA AAT CTG GGA CAA C TGG GCA TTA GAT GCG ACA G	*staphylokinase*
P0016	CAATGGTCACTTATGCTG TCTTTCATCATGCTCATTAC	*sarA*

ADDITIONAL EXPERIMENTAL SECTION

Biology

Materials

All reagents used in this study were purchased from Sigma-Aldrich (St. Louis, USA) without further purification unless otherwise specified.

Ethics Statement

The written consent was obtained from the human subject before taking the blood sample for the hemolysis experiment.

Bacterial strains, plasmid and growth conditions

The description of *S. aureus* strains, plasmids used in the study are provided in Table S1. The USA300 were grown in TSB (Fluka 22092) or tryptic soy agar (TSA) and blood agar plates without glucose supplements. *E. coli* culture were grown in Luria Bertani (LB) broth or on LB agar plates. The reporter strain (P_{hlam}-*gfp*) were grown in the presence of chloramphenicol 5 μg/ml. The cultures were grown at 37°C in a shaker (Orbital Shaker) with 250 rpm of aeration.

Chemical library. An in-house natural product library was used in this study to screen potent natural chemical. The chemicals were dissolved in DMSO (dimethyl sulfoxide) and yielded 10 mM stock solution, respectively. All the chemicals used for the screening experiment had a purity of at least 98%. All the chemicals in the library were dry transferred into a 96-well plate.

Construction of reporter strain (GFP-promoter fusion)

For *sae* promoter (P1), ligation independent cloning method was utilized to construct the promoter-*gfp* (green fluorescence protein) fusion strain.[6] The pCL-P_{hlam}-*gfp* was used for vector DNA amplification[4] using the primers P1969 and P1747. Then primer pair P1971/P1972 were used to amplify the insert DNA fragment with promoter sequence. The PCR products were treated with

T4 DNA polymerase with the presence of dCTP (vector) or dGTP (insert DNA) and mixed together. The mixed products were used for the transformation in *E. coli* DH5α. All the plasmids were verified and transduced into *S. aureus* USA300 strain with ϕ85.

High-throughput screening using a GFP-promoter fusion

S. aureus harbouring P_{hlam}-*gfp* was used for the screening method. An in-house natural product library of 800 compounds in DMSO was used in the study. Overnight cultures of P_{hlam}*GFP* were grown in tryptic soy broth (TSB) and were diluted 1:100 into fresh TSB and grown again until OD_{600} = 1. Then the cells were diluted to obtain $5x10^5$ cells/ml and transferred into the plate with 10 µM of the chemical. A negative control (DMSO as blank) was used. The initial cell density and GFP fluorescence were measured after the plates were set up, and the plates were incubated for 8 h at 37°C in a shaker-incubator. After 8 h incubation, GFP signal (excitation, 485 nm, emission, 515 nm) and OD_{600nm} were measured using BioTek Gen5 Microplate Reader.

Hemolysin Assay

The assay was performed using human erythrocytes as described.[7-8] The overnight cultures of the wild type USA300 or *saeS* mutant were diluted 1:100 in fresh TSB and grown until OD_{600}=1. Then, $5x10^5$ cells/ml were used along with various concentrations of xanthoangelol B **1** and **PM-56** and allowed to grow for 8 h at 37°C with shaking at 250 rpm. Human blood was washed thrice with DPBS and 5% RBC was prepared for experiment. After an 8 h incubation, the supernatants of chemical treated bacterial cultures were collected and mixed at 1:1 with the 5% RBC, and incubated for 30 minutes. The samples were then centrifuged for 1000 rpm, and hemolysis was determined by measuring the optical density at 540 nm using an ELISA plate reader.

Quantitative RT-PCR analysis

qRT-PCR was performed for the transcripts of interest using an intercalating dye-based assay as described earlier with minor modifications.[9] *S. aureus* cultures were treated with xanthoangelol B and PM56 and after 6h incubation, Total RNA was isolated and purified using the RNA Protect Bacteria Reagent (Qiagen) and RNeasy Mini Kit (Qiagen) using enzymatic disruption method.[10] The cDNA was synthesized using High Capacity cDNA Reverse Transcription Kit (Random hexamer, Clontech). Gene transcription quantification was performed by real-time PCR using SYBR Green PCR Master Mix (Bio-Rad) in a CFX connect real time system (Bio-Rad). The primers used in the experiment to detect transcripts of interest are listed in Table S2. The results were normalized to the levels of 16s RNA.

USA300 infection of *Galleria mellonella* larvae

We used the existing larvae infection model[11-13] and modified the inoculum size to reduce the rapid killing of larvae. The antibiotic free larvae of *Galleria mellonella* were purchased from Sworm suppliers and maintained at room temperature in the incubator with food. The larvae were segregated according to weight (100 to 150 mg) and used for infection experiment.

a) *USA300* infection in *Galleria mellonella*

Ten larvae per group were used in the experiment. Each larva of individual groups was injected with 20 µl inoculum of USA300 $5x10^6$ cfu into the lower left proleg using 6mm insulin syringe. All the groups were incubated at 37 °C in the dark without food supply for up to 2 h.

b) Treatment of *Galleria mellonella* with Xanthoangelol B and PM56

Post infection with USA300, each larva from individual groups was treated with chemicals. Xanthoangelol B **1** was injected at 1.065, 2.042, 3.063 and 4.085 mg/kg body weight. **PM-56** were used at 1.065, 2.131, 3.196 and 4.262 mg/kg of body weight. The repeated doses of **1** and **PM-56** were injected every 12 h interval and the larvae monitored for 72 h.

The larvae were monitored daily for the following parameters: activity, extent of silk production, melanization, and survival. Each parameter was checked properly and scored according to them for overall health index of an individual larva.

c) Analysis of *Galleria mellonella* mortality and *S. aureus* burden

Each group was checked carefully and survivals were plotted in comparison with the non-infected and disease control group. Haemolymph of the individual larva from each group was collected and plated on the blood agar plate to count *S. aureus* burden.

Statistical evaluation

Student's t-tests (two-tailed) were used to compare two data sets. One way ANOVA was used for more than 3 data sets. The log-rank test (Mantel-Cox) was used for survival analysis. The non-parametric Mann-Whitney test (two-tailed) was used to compare two groups of observations. All evaluations were conducted using Graph Pad Prism v. 6.0 and results were considered significantly different with $p<0.05$.

Cloning

To generate a construct expressing a minimal kinase domain of SaeS, a DNA fragment encoding for the DHp and CA domains of SaeS (residues 118–351) was amplified from the genomic DNA of *S. aureus* strain Newman using the polymerase chain reaction (PCR) and the primer pair: 5'-AAA AAA GGA TCC TCC GAA CAA CAA GAA AAA-3' and 5'- AAA AAA CTC GAG TTA TGA CGT AAT GTC TAA TTT-3' (*BamH*I and *Xho*I sites are underlined). The PCR products were then inserted into the *BamH*I/*Xho*I site of the plasmid pVFT1S (Korean Patent No. 1020050051893), from which the minimal kinase domain of SaeS was expressed as a fusion protein with a His_6 tag linked to the N-terminus via a recognition site of the Tobacco Etch Virus (TEV) protease.

The cytoplasmic domain of AgrC was expressed from plasmid pMCSG19-AgrC. Briefly, a DNA fragment encoding for the cytoplasmic domain of AgrC (residues 206–430) was amplified from the genomic DNA of *S. aureus* strain Newman and inserted into the *Ssp*I site of plasmid pMCSG19 by ligation independent cloning method.[6]

Cloning, expressions and purifications of MBP-SaeS was performed as previously described.[14]

Protein expression and purification

The recombinant SaeS and AgrC proteins were expressed and purified by the same procedures as described hereafter. To express the kinase domain of SaeS and the cytoplasmic domain of AgrC, *Escherichia coli* BL21(DE3) (Novagen, USA) harboring pVFT1S-SaeS or pMCSG19-AgrC was cultured in lysogeny broth at 37 °C until the optical density at 600 nm attained 0.8–1.0. After incubation on ice for 30 min, the bacterial culture was supplemented with isopropyl β-D-1-thiogalactopyranoside at a final concentration of 0.25 mM to induce the expressions of target proteins. The bacteria were then continuously cultured at 18 °C for 24 h and harvested by centrifugation at 6,000 x g for 10 min at 4 °C in an Avanti J-25 I centrifuge and a JLA-10.500 rotor (Beckman Coulter, USA). The cell pellets were stored at −20 °C until use.

To purify SaeS and AgrC, the cell pellets were resuspended in buffer A (25 mM Tris-HCl pH 8.0, 0.5 M NaCl, and 20 mM imidazole) supplemented with 1 mM phenylmethylsulfonyl fluoride and lysed by sonication on ice on a Vibra-Cell Ultrasonic Processor VC750 (Sonics, USA) using a 13-mm probe (420 short bursts of 1 s at an amplitude of 60% followed by intervals of 3 s). The cell lysates were clarified by centrifugation at 20,000 x g for 1 h at 4 °C in an Avanti J-26 XPI centrifuge and a JA-25.50 rotor (Beckman Coulter, USA). The supernatants were then loaded onto 5-mL HisTrap HP columns (GE Healthcare, Sweden) precharged with $NiSO_4$ and the columns were washed with 50 mL of buffer A containing 40 mM imidazole to remove non-specifically

bound proteins. The target protein (SaeS or AgrC) was then eluted from the column by 30 mL of buffer A containing 0.5 M imidazole. A homemade His_6-tagged TEV protease was added to the eluted solutions at a protease to target protein ratio of 1:100 (w/w) and the mixtures were dialyzed against a TEV protease working buffer (50 mM Tris-HCl pH 8.0, 50 mM NaCl, 1 mM DTT, and 0.5 mM EDTA) at room temperature for 12 h. To remove the fusion tags and TEV protease, the protein solutions were further purified using the HisTrap HP column. The flow-through fraction containing SaeS or AgrC was dialyzed against a storage buffer (25 mM Tris-HCl pH 8.0 and 100 mM NaCl) at 4 °C for 12 h. The protein samples were then concentrated up to 10 mg/mL and stored at −80 °C until use. Protein concentrations were measured using Bradford assay and bovine serum albumin as a standard.

***In vitro* phosphorylation assay**

The mother stock solutions of xanthoangelol B and **PM-56** (10 mM each) were prepared by dissolving the chemical powders in 100% DMSO. To keep the concentration of DMSO constant at 10% in all phosphorylation reactions, a series of 10× stock solutions (0.25–7.5 mM) of these two chemicals were made by diluting the mother stocks in 100% DMSO. In a 10-μL volume of a standard autophosphorylation reaction, purified protein (5 μM) was incubated with 1× chemical solution or 10% DMSO in a reaction buffer (50 mM Tris-HCl pH 8.0, 50 mM KCl, 0.5 mM DTT, and 5 mM $MgCl_2$) on ice. After 15 min, a mixture of ATP (cold)/[γ-^{32}P] ATP (hot) (cold ATP/hot ATP = 1000:1) was added to the above solution at the final concentration of 130 μM to initiate the autophosphorylation reaction. After a 15-min incubation at room temperature, the reaction was stopped by addition of 10 μL 2× Laemmli sample buffer without boiling. For SDS-PAGE analysis, approximately 0.5 μg protein was loaded into each well of a 12.5% SDS-polyacrylamide gel. After being dried on a 3MM Whatman paper (GE Healthcare, China) in a Model 583 Gel Dryer (Bio-

rad, USA) at 80 °C for 45 min, the gel was exposed to an X-ray film (Agfa HealthCare, Belgium) at −80 °C for 1 h. The film was then developed using a JP-33 automatic X-ray film processor (JPI, Korea) to visualize the ^{32}P-labeled protein bands. Total proteins loaded onto SDS-PAGE were visualized by Coomassie-Brilliant Blue staining.

The densities of ^{32}P-labeled protein bands were quantified using ImageJ software.[15] The autokinase activities were normalized as the percentages of the intensities of phosphorylated proteins in the samples treated with inhibitory chemicals compared to those in the control samples treated with 10% DMSO. Dose-response curves were generated for each kinase and each inhibitory chemical by plotting the chemical concentration against the percent autokinase activity. The IC_{50} values were calculated from non-linear regression curve fitting using GraphPad Prism version 7.03 for Windows (GraphPad Software, USA). Data are presented as the mean ± standard deviation of at least two independent measurements.

***In vitro* phosphotransfer assay**

SaeR was prepared as previously described.[4] The phosphotransfer reaction from SaeS-P to SaeR was determined as described before with modification.[4] Briefly, phosphorylated SaeS was generated by incubating 5 μM of the kinase domain SaeS-His_6 with 0.1 mM of ATP containing 30 μCi of [γ-32P] ATP in TKM (50 mM Tris-HCl [pH 8.0], 50 mM KCl, 1 mM $MgCl_2$) at room temperature for 30 min. Phosphorylated kinase domain SaeS-His_6 protein (SaeS-His6-P) was recovered using a Micro Bio-Spin 6 chromatography column (Bio-Rad) by removing excess ATP. Seven microliters of SaeS-His6-P were kept on ice as a reference. The SaeS-His6-P protein was incubated with the indicated concentration of chemical solution or 10% DMSO in TKM buffer. The phosphotransfer reaction was started by mixing with 10 μM of SaeR-His_6 into the reaction mixture at room temperature. The reaction was stopped at the indicated time points by mixing a

7 µl aliquot with 6x SDS sample buffer. Samples were kept on ice until SDS-PAGE. After electrophoresis, the gel was autoradiographed, and the degree of phosphorylation was determined with a phosphor imaging plate (GE), a Typhoon FLA 7000 imaging system, and Multi Gauge software (Fuji Film).

Protein intrinsic fluorescence quenching assay

The intrinsic fluorescence quenching assay to determine the binding affinities of xanthoangelol B **1** and **PM-56** to the kinase domain of SaeS followed the method described by Duong *et al.* [16] with the following modifications. Because the amino acid sequence of kinase domain of SaeS has seven tyrosines, six phenylalanines, and no tryptophan, the intrinsic fluorescence of the kinase domain of SaeS was recorded at an emission peak of 306 nm upon excitation at 276 nm. To keep the concentration of DMSO constant at 1% in all samples, a series of 100× stock solutions (0.25–10 mM) of the two examined chemicals were prepared by diluting the mother stocks in 100% DMSO. In a 200-µL sample solution, purified SaeS (1 µM) was incubated with either 1× chemical solution or 1% DMSO in the protein storage buffer. After a 15-min incubation at room temperature, the protein fluorescence intensities were measured on a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, USA). Samples containing the chemicals but without SaeS were used as blanks for correcting the fluorescence intensities. The corrected emission intensities of the samples treated with the chemicals were then normalized against that of the sample treated with DMSO alone. Subsequently, the graphs were constructed by plotting the reductions of SaeS fluorescence intensity at each chemical treatment against the chemical concentrations. The non-linear regression analyses were done using GraphPad Prism 7 for Windows (www.graphpad.com) to calculate the chemical-binding affinities (K_d) to kinase domain SaeS.

STD NMR experiments.

All samples were prepared in the NMR-buffer (pH 7.0, 50 mM Na-phosphate, 50 mM NaCl, and 5% D2O), and then their 1-dimensional (1D) NMR spectra were recorded using a Bruker 800 MHz cryoprobed spectrometer. 22.2 μM trimethylsilyl propionate was used for the internal reference of the peak intensity. The stock solution of **PM-56** (42.2 mM) was prepared in 99.9% deuterated DMSO (D_6-dimethyl sulfoxide) and the concentration of **PM-56** was fixed to 1.0 mM for all NMR experiments. The binding of **PM-56** to the kinase domain of SaeS was examined by 1D transverse relaxation filtered experiment with a Carr–Purcell–Meiboom–Gill pulse trains (1D CPMG) and 1D saturation transfer difference experiment (1D STD).[17] 0.1 mM of the minimal kinase domain of SaeS and 30 μM minimal kinase domain of SaeS fused to maltose binding protein (MBP-SaeS) were used for 1D CPMG and STD experiments after the dialysis in the NMR-buffer.

REFERENCES

1. Kreiswirth, B. N.; Lofdahl, S.; Betley, M. J.; O'Reilly, M.; Schlievert, P. M.; Bergdoll, M. S.; Novick, R. P., The toxic shock syndrome exotoxin structural gene is not detectably transmitted by a prophage. *Nature* **1983,** *305* (5936), 709-12.
2. Jeong, D. W.; Cho, H.; Lee, H.; Li, C.; Garza, J.; Fried, M.; Bae, T., Identification of P3 promoter and distinct roles of the two promoters of the SaeRS two-component system in Staphylococcus aureus. *J Bacteriol* **2011,** *193* (18), 4672-4684.
3. Ji, Y.; Marra, A.; Rosenberg, M.; Woodnutt, G., Regulated antisense RNA eliminates alpha-toxin virulence in Staphylococcus aureus infection. *J Bacteriol* **1999,** *181* (21), 6585-90.
4. Liu, Q.; Cho, H.; Yeo, W.-S.; Bae, T., The extracytoplasmic linker peptide of the sensor protein SaeS tunes the kinase activity required for Staphylococcal virulence in response to host signals. *PLoS Pathog.* **2015,** *11* (4), e1004799.
5. Yeo, W.-S.; Arya, R.; Kim, K. K.; Jeong, H.; Cho, K. H.; Bae, T., The FDA-approved anti-cancer drugs, streptozotocin and floxuridine, reduce the virulence of Staphylococcus aureus. *Sci. Rep.* **2018,** *8* (1), 2521.
6. Donnelly, M. I.; Zhou, M.; Millard, C. S.; Clancy, S.; Stols, L.; Eschenfeldt, W. H.; Collart, F. R.; Joachimiak, A., An expression vector tailored for large-scale, high-throughput purification of recombinant proteins. *Protein. Expr. Purif.* **2006,** *47* (2), 446-54.
7. Pader, V.; Hakim, S.; Painter, K. L.; Wigneshweraraj, S.; Clarke, T. B.; Edwards, A. M., Staphylococcus aureus inactivates daptomycin by releasing membrane phospholipids. *Nat. Microbiol.* **2016,** *2*, 16194.

8. Ling, L. L.; Schneider, T.; Peoples, A. J.; Spoering, A. L.; Engels, I.; Conlon, B. P.; Mueller, A.; Schäberle, T. F.; Hughes, D. E.; Epstein, S.; Jones, M.; Lazarides, L.; Steadman, V. A.; Cohen, D. R.; Felix, C. R.; Fetterman, K. A.; Millett, W. P.; Nitti, A. G.; Zullo, A. M.; Chen, C.; Lewis, K., A new antibiotic kills pathogens without detectable resistance. *Nature* **2015,** *517*, 455-459.
9. Dastgheyb, S. S.; Villaruz, A. E.; Le, K. Y.; Tan, V. Y.; Duong, A. C.; Chatterjee, S. S.; Cheung, G. Y. C.; Joo, H.-S.; Hickok, N. J.; Otto, M., Role of phenol-soluble modulins in formation of Staphylococcus aureus biofilms in synovial fluid. *Infect. Immun.* **2015,** *83* (7), 2966-2975.
10. Todd, D. A.; Parlet, C. P.; Crosby, H. A.; Malone, C. L.; Heilmann, K. P.; Horswill, A. R.; Cech, N. B., Signal biosynthesis inhibition with ambuic acid as a strategy to target antibiotic-resistant infections. *Antimicrob. Agents Chemother.* **2017,** *61* (8), e00263-17.
11. Pollitt, E. J. G.; West, S. A.; Crusz, S. A.; Burton-Chellew, M. N.; Diggle, S. P., Cooperation, quorum sensing, and evolution of virulence in Staphylococcus aureus. *Infect. Immun.* **2014,** *82* (3), 1045-1051.
12. Haaber, J.; Leisner, J. J.; Cohn, M. T.; Catalan-Moreno, A.; Nielsen, J. B.; Westh, H.; Penadés, J. R.; Ingmer, H., Bacterial viruses enable their host to acquire antibiotic resistance genes from neighbouring cells. *Nat. Commun.* **2016,** *7*, 13333.
13. Kuo, D.; Yu, G.; Hoch, W.; Gabay, D.; Long, L.; Ghannoum, M.; Nagy, N.; Harding, C. V.; Viswanathan, R.; Shoham, M., Novel quorum-quenching agents promote methicillin-resistant Staphylococcus aureus (MRSA) wound healing and sensitize MRSA to β-lactam antibiotics. *Antimicrob. Agents Chemother.* **2015,** *59* (3), 1512-1518.
14. Sun, F.; Li, C.; Jeong, D.; Sohn, C.; He, C.; Bae, T., In the Staphylococcus aureus two-component system sae, the response regulator SaeR binds to a direct repeat sequence and DNA binding requires phosphorylation by the sensor kinase SaeS. *J. Bacteriol.* **2010,** *192* (8), 2111-2127.
15. Schneider, C. A.; Rasband, W. S.; Eliceiri, K. W., NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **2012,** *9* (7), 671-675.
16. Duong, T.; Park, K.; Kim, T.; Kang, S. W.; Hahn, M. J.; Hwang, H.-Y.; Kim, K. K., Structural and functional characterization of an Isd-type haem-degradation enzyme from Listeria monocytogenes. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2014,** *70* (3), 615-626.
17. Stockman, B. J.; Dalvit, C., NMR screening techniques in drug discovery and drug design. *Prog. Nucl. Magn. Reson. Spectrosc.* **2002,** *41* (3), 187-231.

04/05/2018 11:30:13

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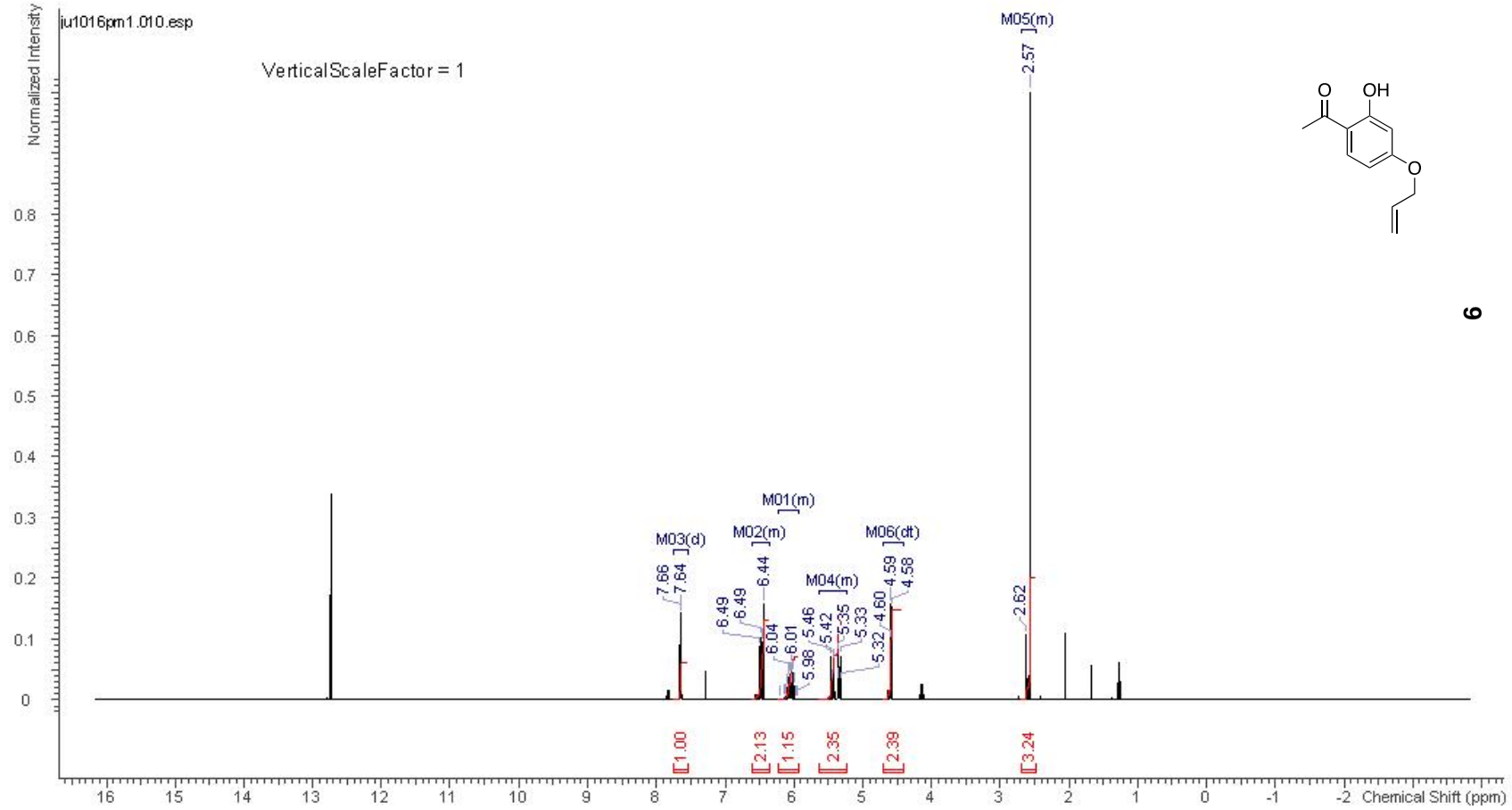

6

04/05/2018 11:22:42

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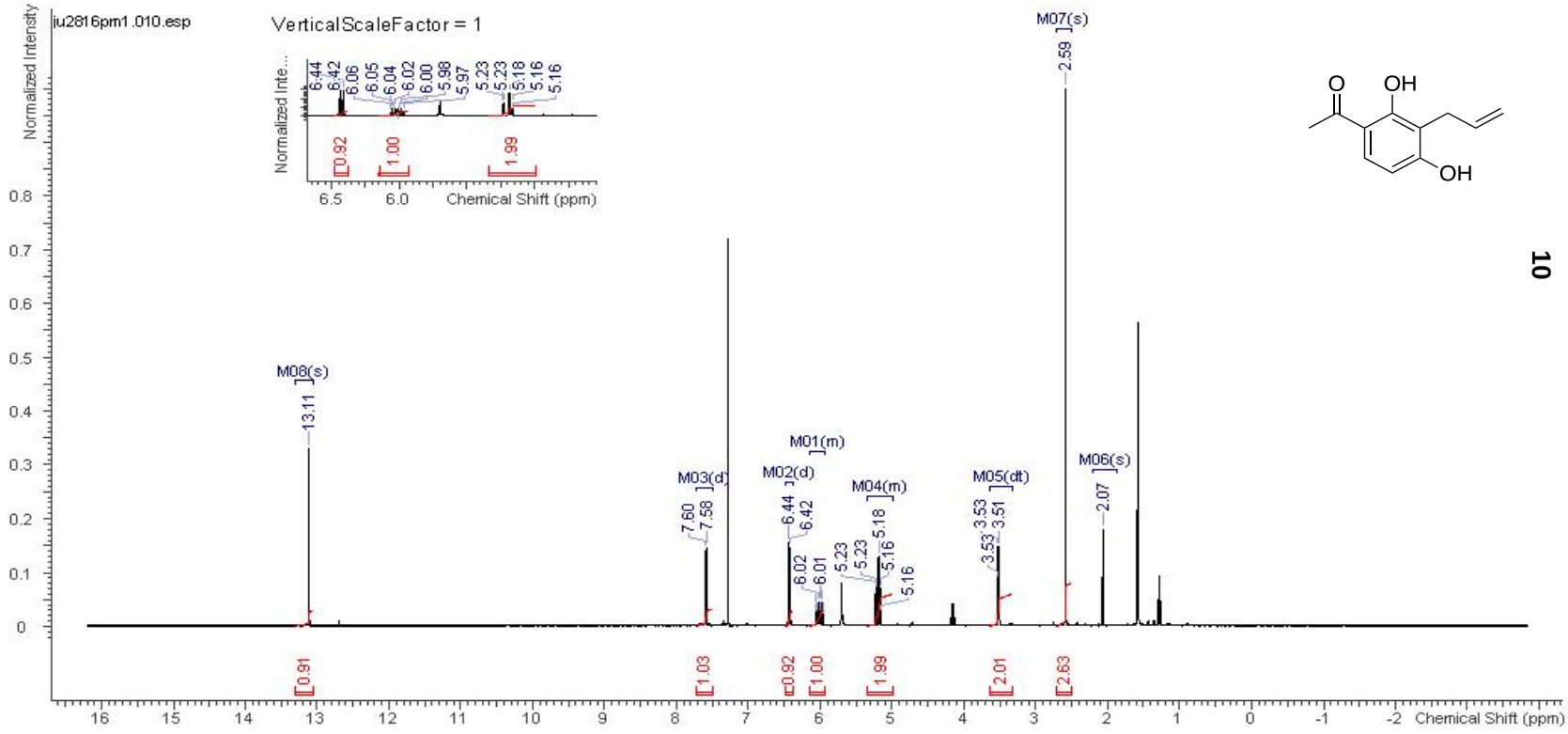

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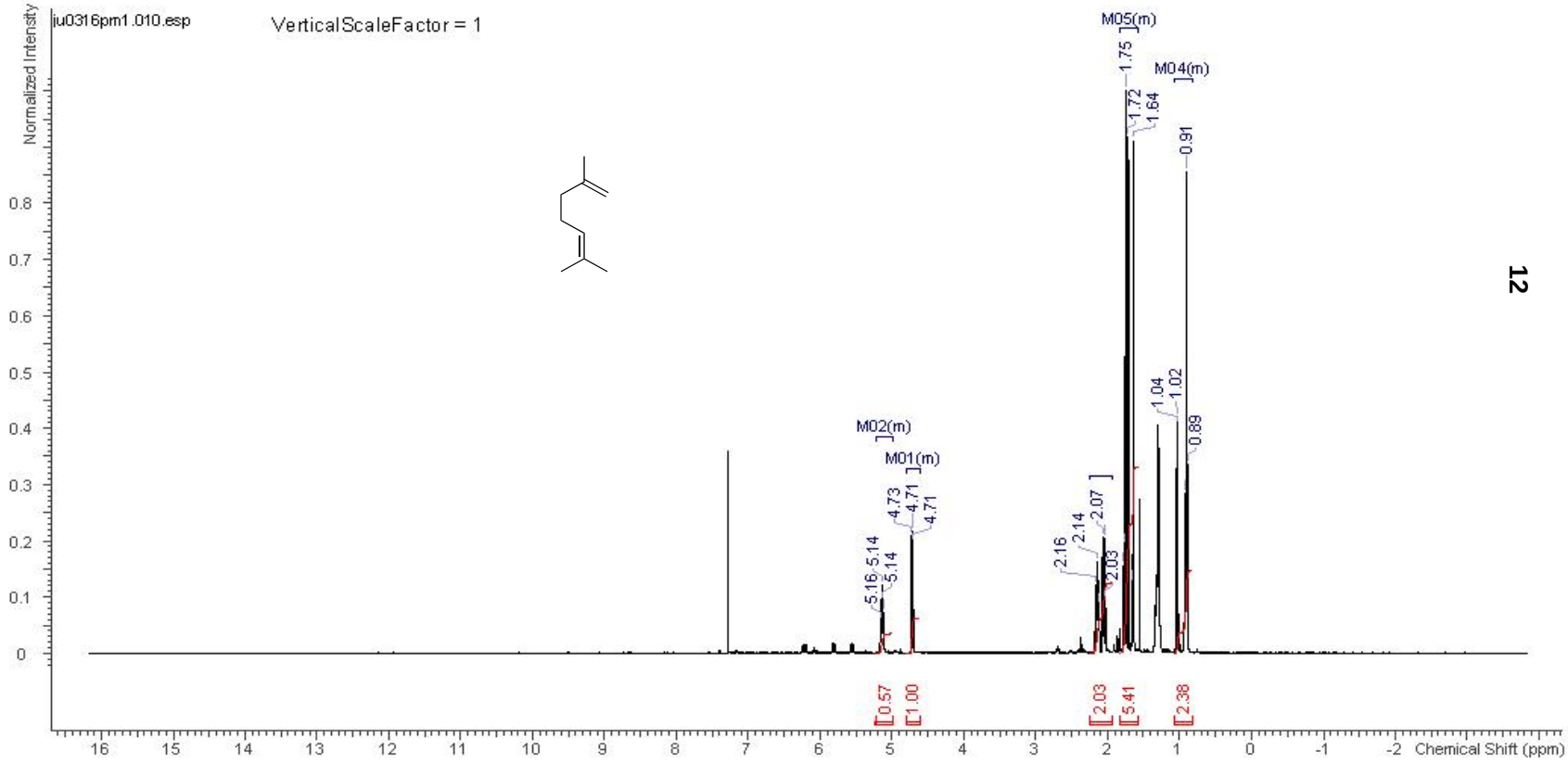

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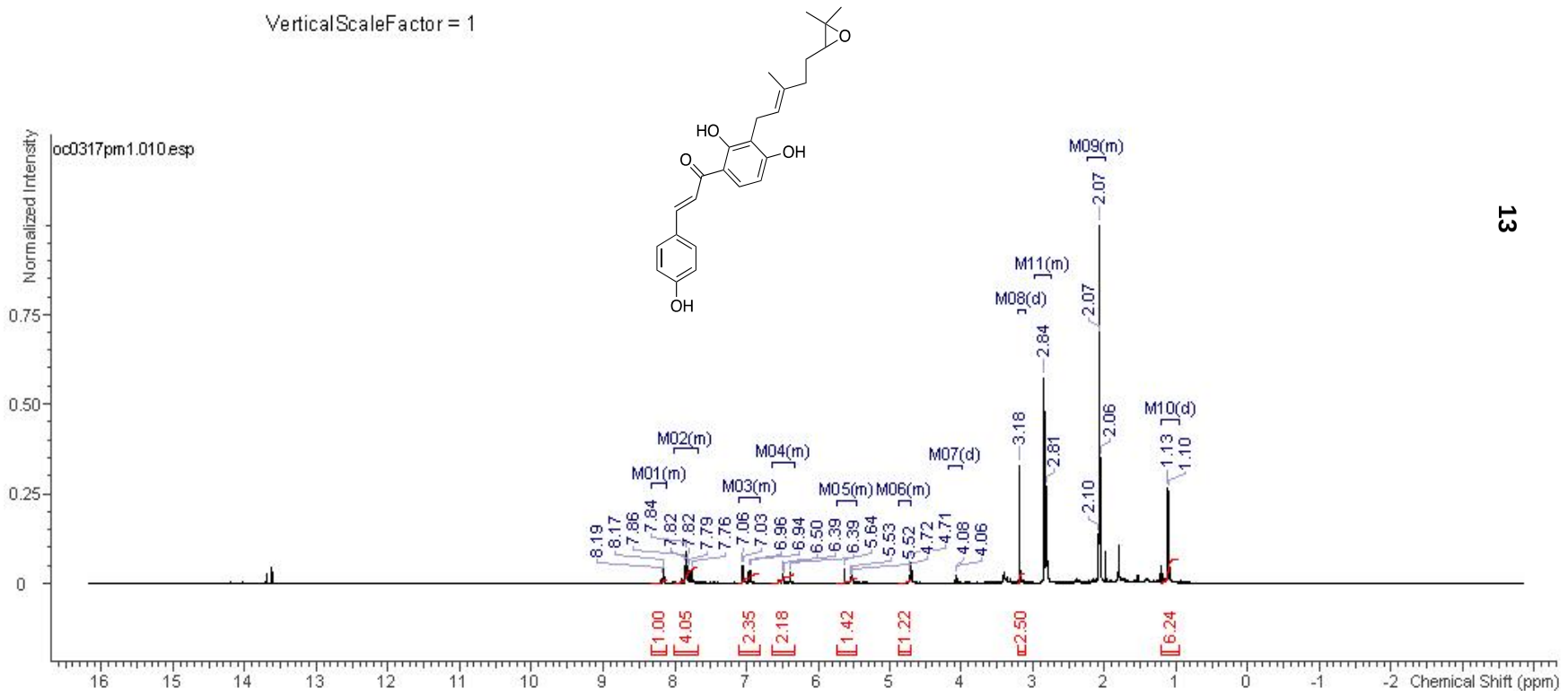

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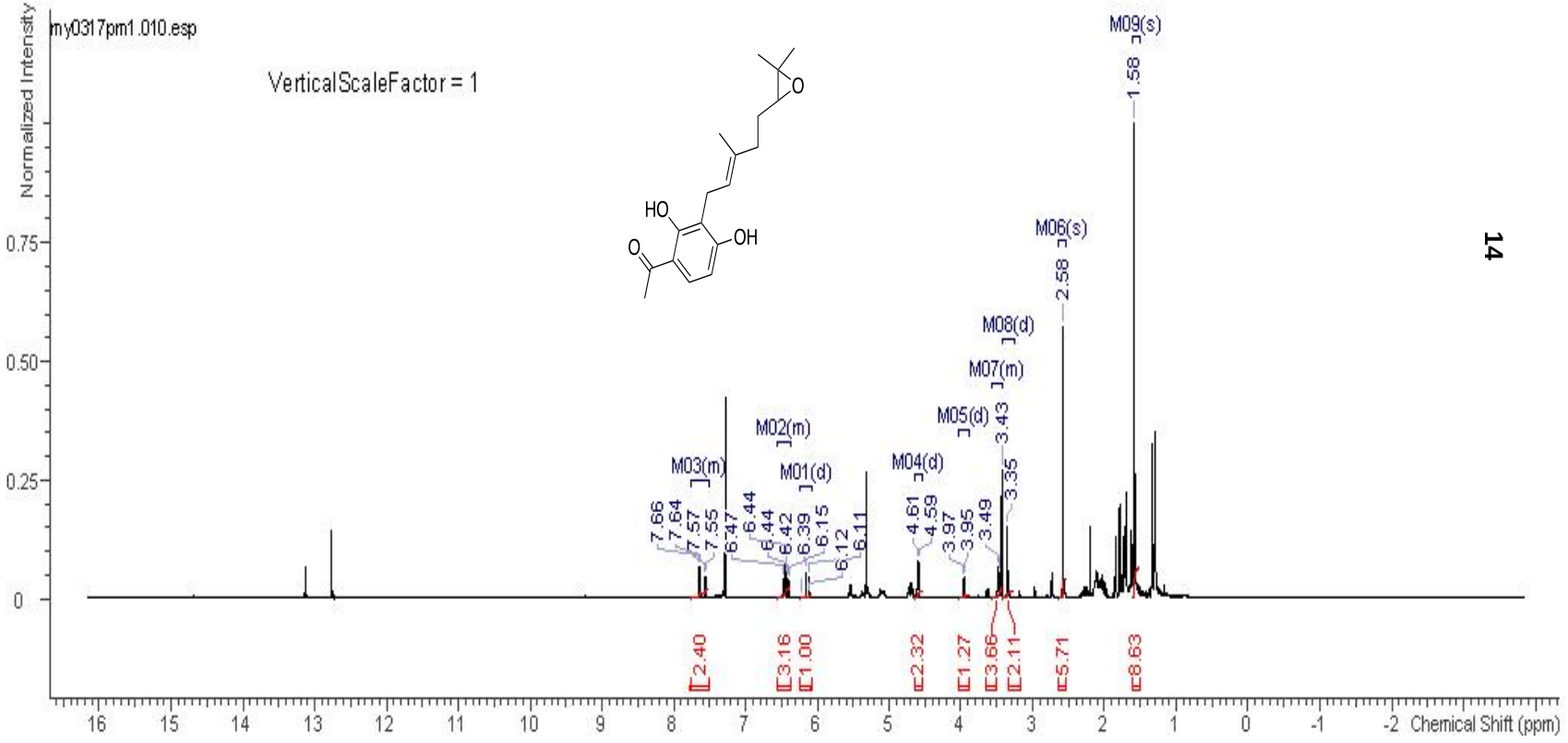

26/01/2018 13:49:13

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Solvent	Acetone	*Spectrum Offset (Hz)*	2470.9668	*Spectrum Type*	standard	*Sweep Width (Hz)*	8012.58

Original Points Count 32768

SW(cyclical) (Hz) 8012.82

Temperature (degree C) 24.998

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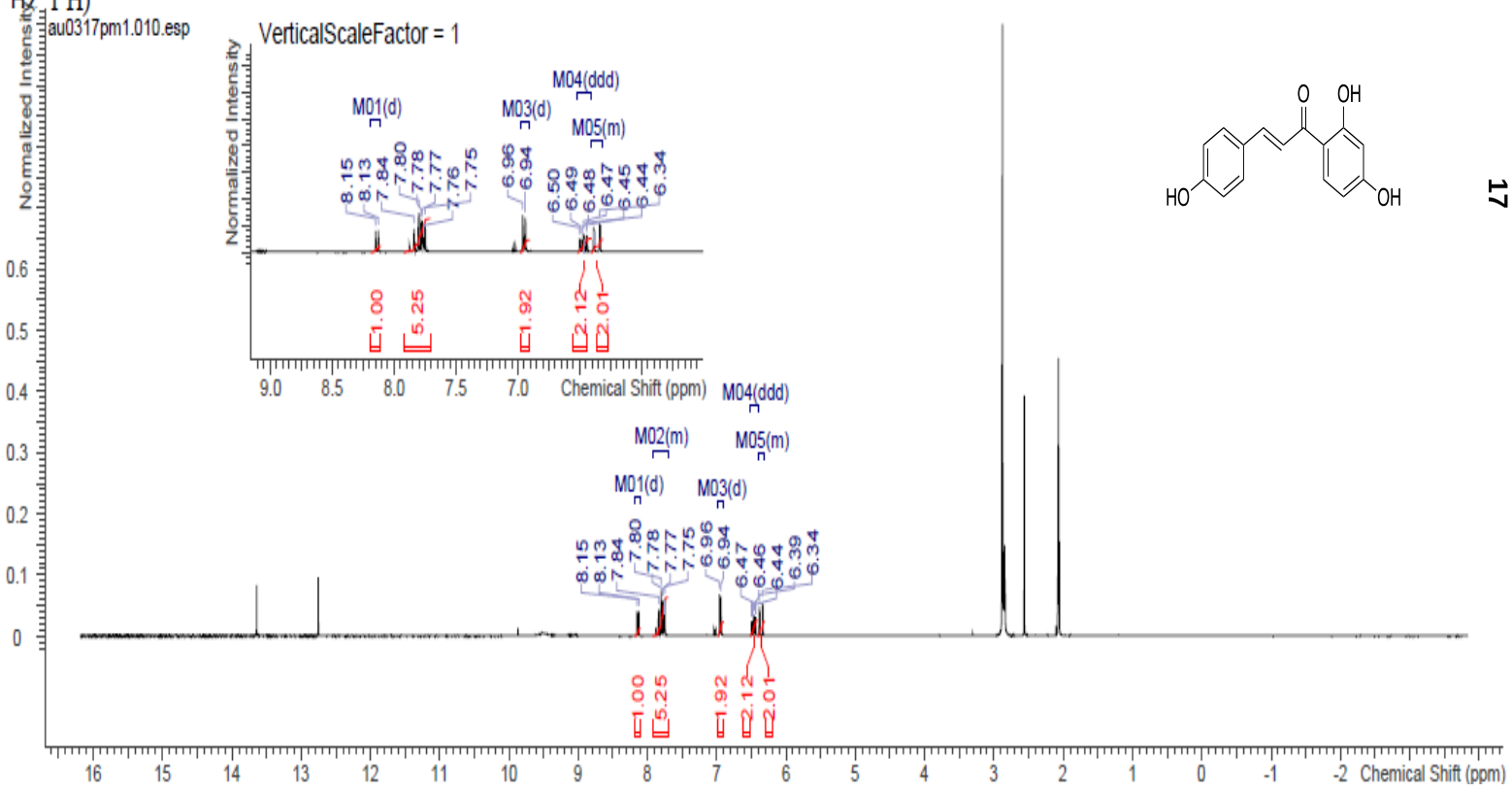

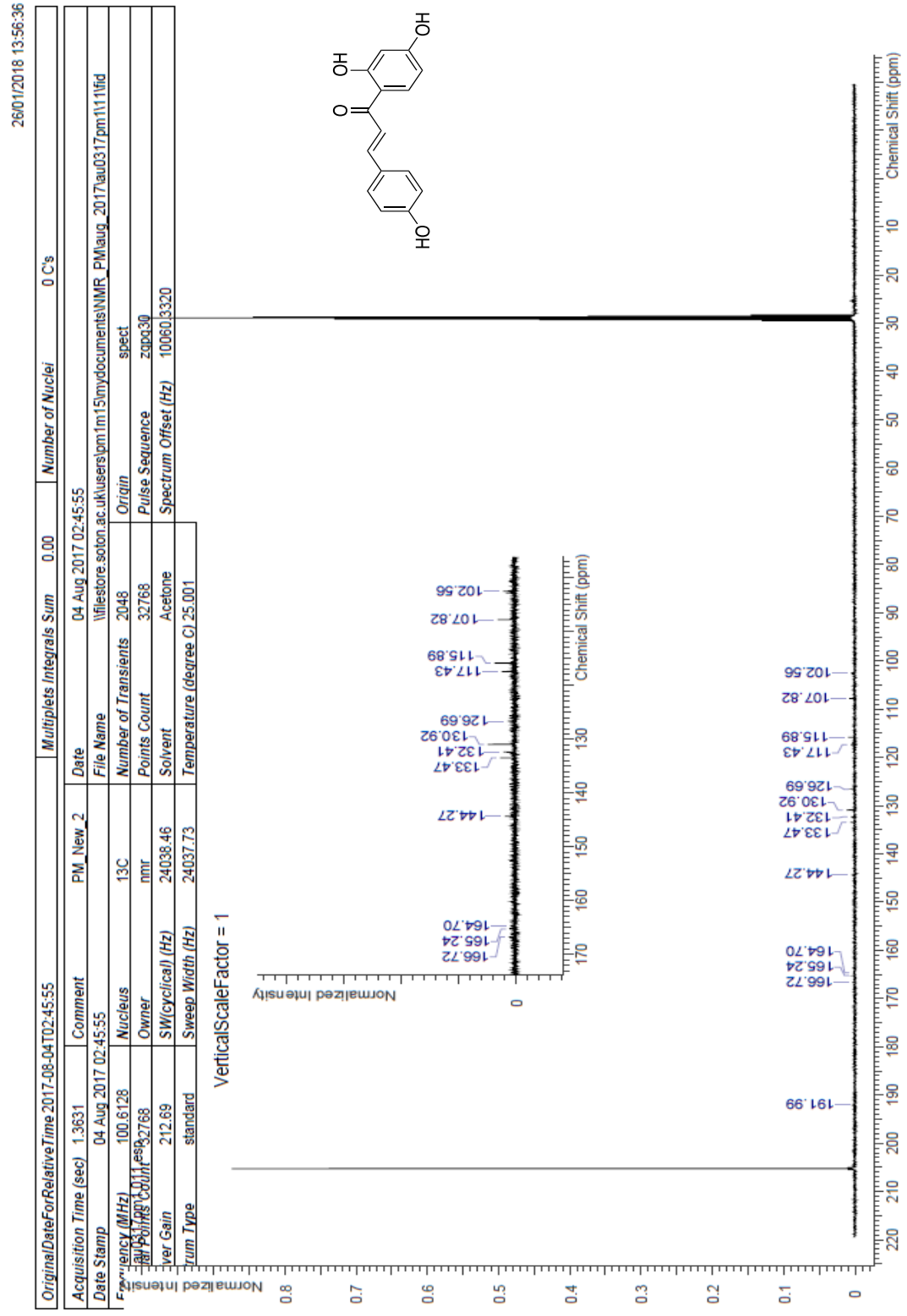
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Owner nmr
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Solvent Acetone
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Spectrum Type standard
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Temperature (degree C) 25.001
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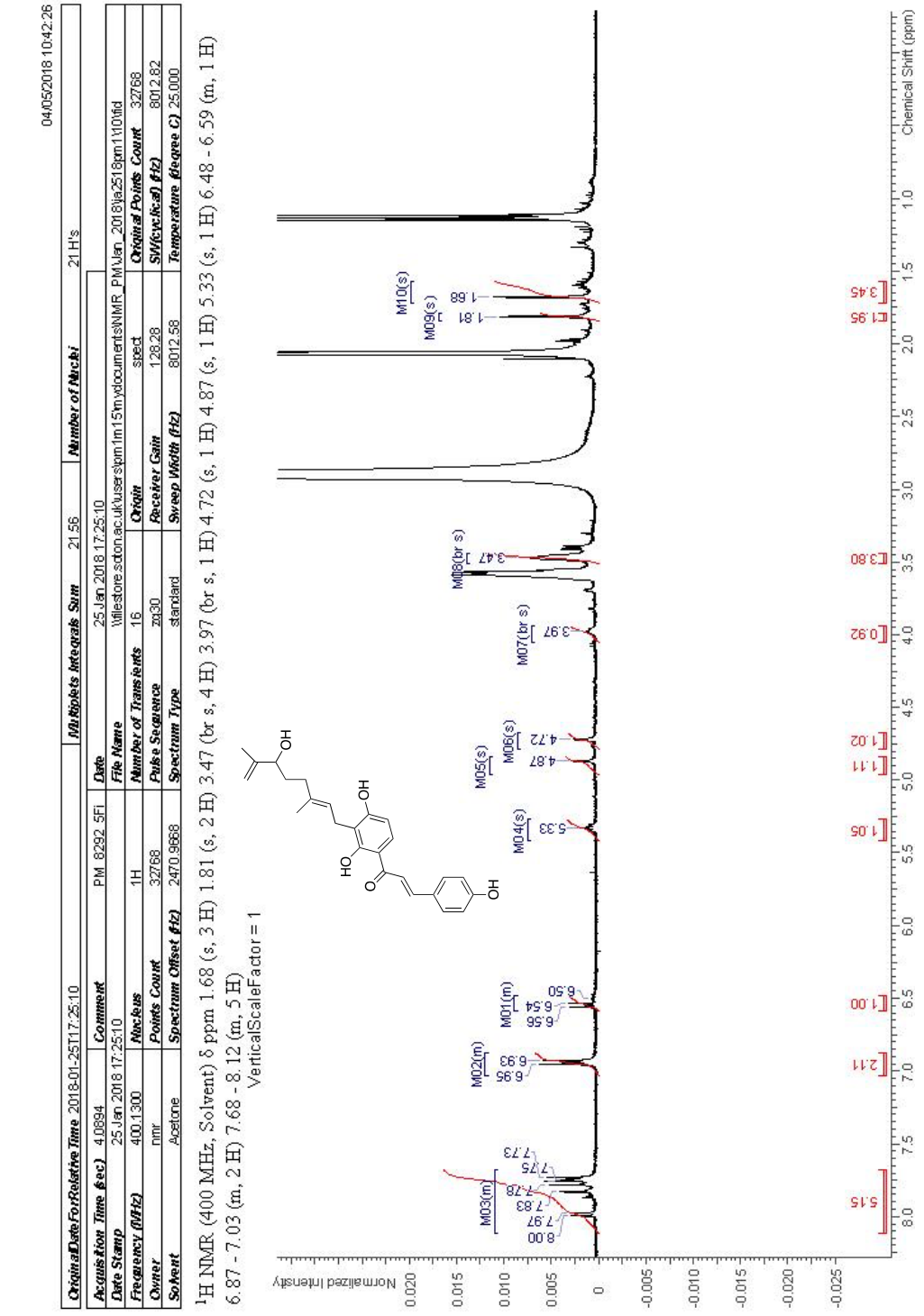
O
OH
HO



^{1}H NMR (400 MHz, Solvent) δ ppm 1.68 (s, 3 H) 1.81 (s, 2 H) 3.47 (br s, 4 H) 3.97 (br s, 1 H) 4.72 (s, 1 H) 4.87 (s, 1 H) 6.48 - 6.59 (m, 1 H) 6.87 - 7.03 (m, 2 H) 7.68 - 8.12 (m, 5 H)

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03/05/2018 15:18:57

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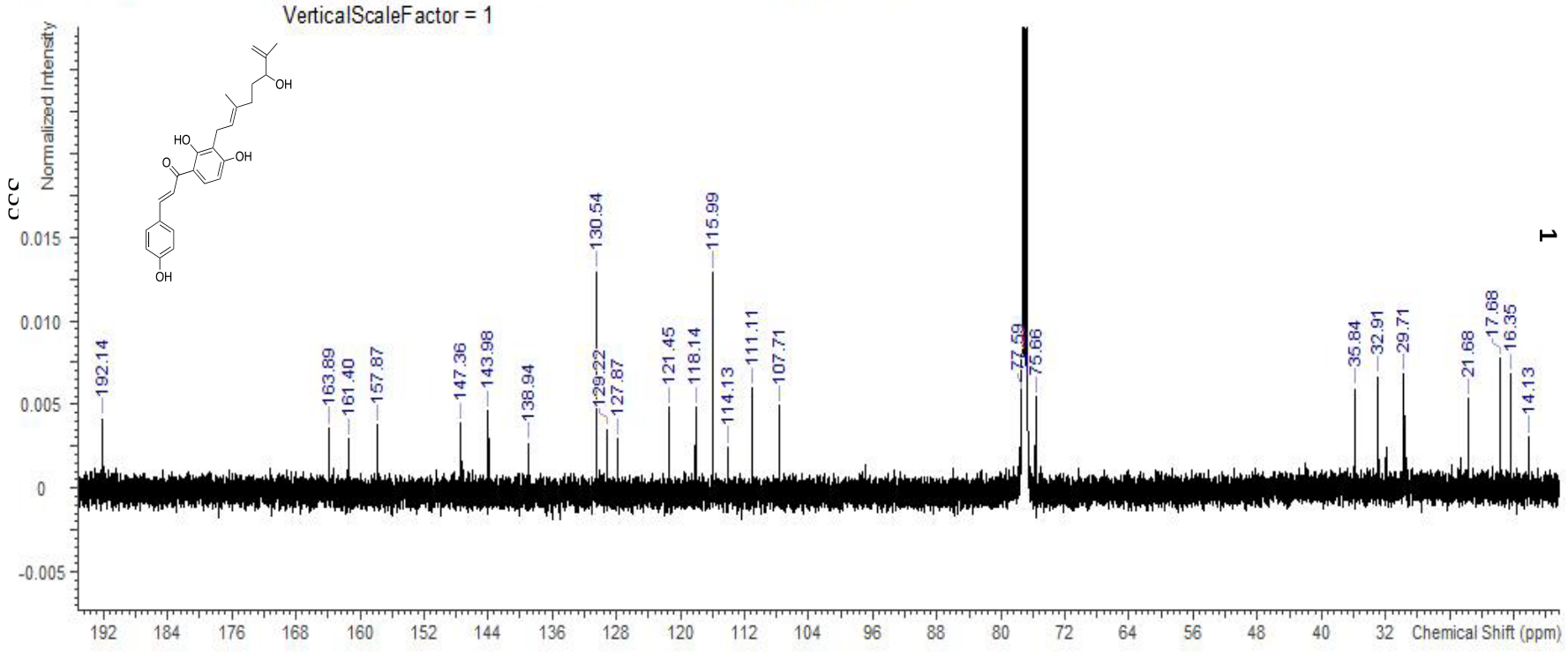

15/05/2018 11:30:39

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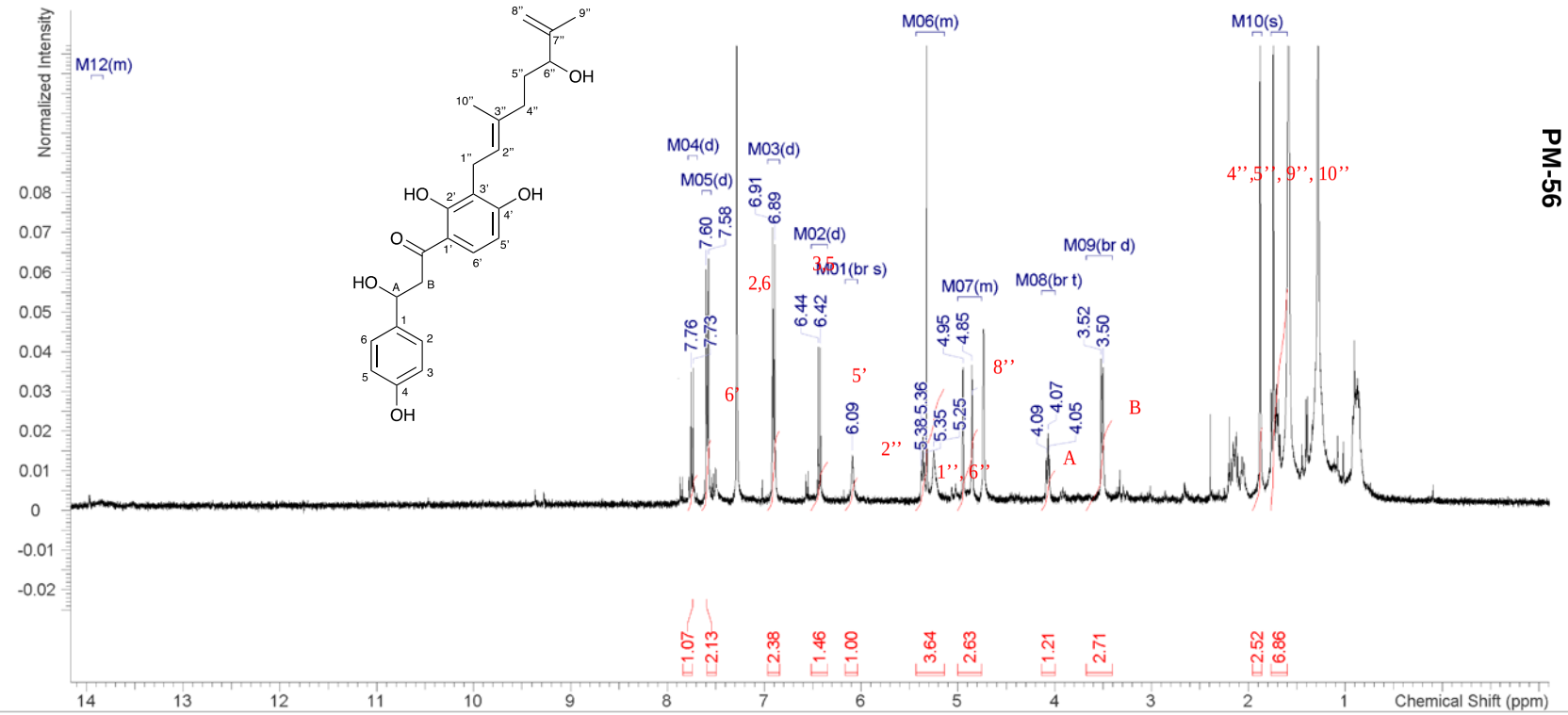

PM-56

04/05/2018 11:03:48

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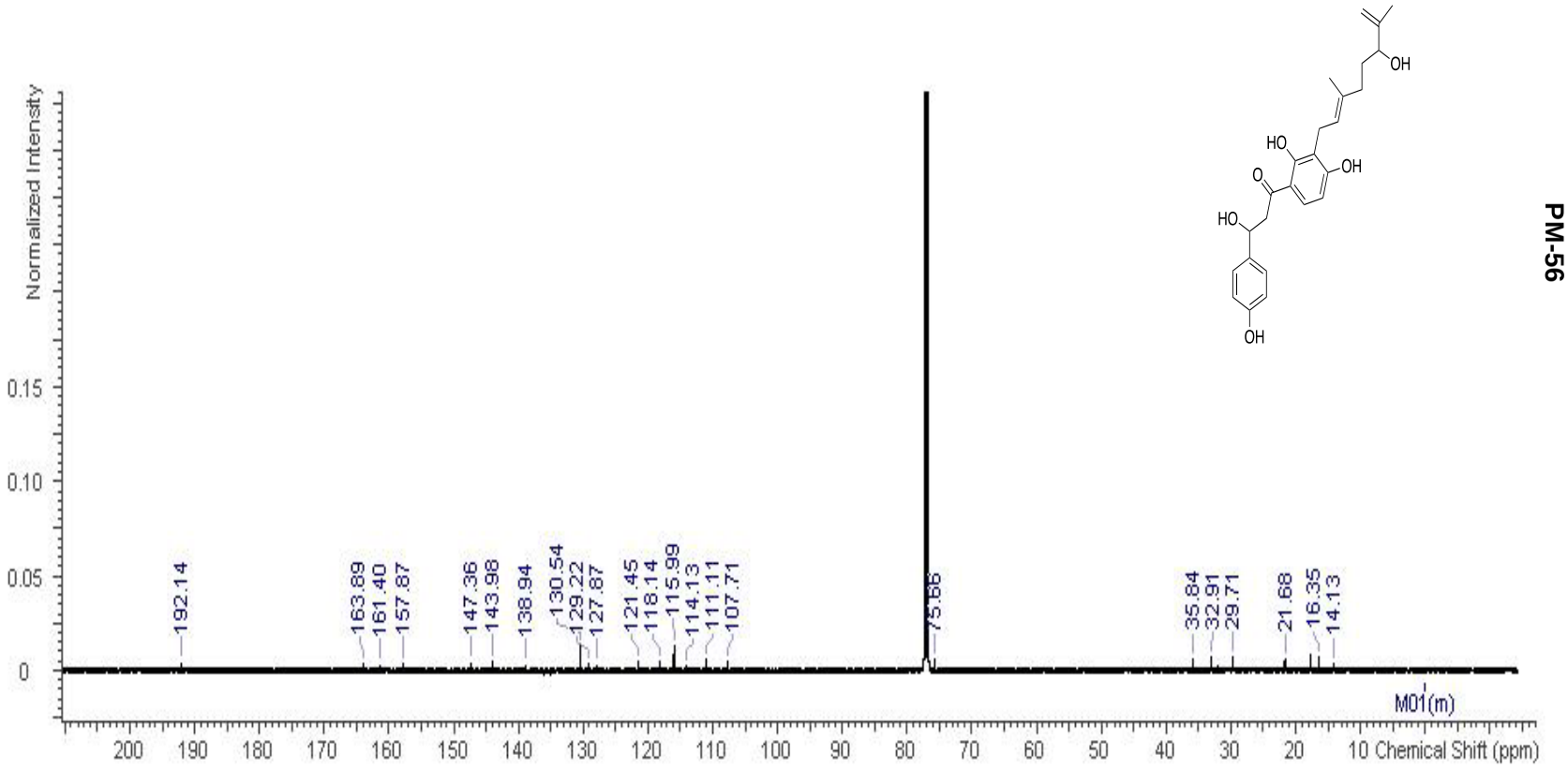

PM-56