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Late-stage functionalization of platensimycin leading to multiple analogues with improved antibacterial activity *in vitro* and *in vivo*

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Abstract

Bacterial fatty acid synthases are promising antibacterial targets against multi-drug resistant pathogens. Platensimycin (PTM) is a potent FabB/FabF inhibitor, while its poor pharmacokinetics hampers the clinical development. In this study, a focused library of PTM derivatives was prepared through thiolysis of PTM oxirane (**1**), followed by various C–C cross-coupling reactions in high yields. Antibacterial screening of these compounds *in vitro* yielded multiple hits with improved anti-*Staphylococcus* activities over PTM. Among them, compounds **A1**, **A3**, **A17**, and **A28** exhibited improved antibacterial activities over PTM against methicillin-resistant *Staphylococcus*

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Author Contributions

The manuscript was written through the contributions of all authors. All authors have approved the final version of the manuscript.

ASSOCIATED CONTENT

Animal protocols

All animal protocols were approved by the Animal Care and Use Committee of Central South University. All experimental procedures complied with the Guide for the Care and Use of Laboratory Animals (1996).

Supporting Information.

The Supporting Information is available free of charge on the ACS Publications website.

Characterization data of **1**, **A1**, **A3**, **A17**, **A24**, **A25**, **A26**, **A28**, **A36**, **D2** and **D4**, copies of NMR spectra and HRMS of **1**, **A1**, **A3**, **A17**, **A24**, **A25**, **A26**, **A28**, **A36**, **D2** and **D4**, predicted binding modes of **A1**, **A3**, **A17**, **A24**, **A25**, **A26**, **A28**, **A36**, **D2** and **D4** with ecFabF (C163Q), copies of LC-MS spectra of compounds in the library (PDF).

Molecular formula strings. The authors will release the atomic coordinates upon article publication (CSV)

Accession Codes

PDB ID code 2GFX was used. The authors will release the atomic coordinates upon article publication for the homology models between compound **A1**, **A3**, **A17**, **A24**, **A25**, **A26**, **A28**, **A36**, **D2** and **D4** with *E. coli* FabF (C163Q), which was built on 2GFX.

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aureus (MRSA) in a mouse peritonitis model. Compound **A28** was further shown to be effective against MRSA infection in a mouse wound model, in comparison to mupirocin. Therefore, the facile preparation and screening of these PTM derivatives, together with their potent antibacterial activities *in vivo*, suggest a promising strategy to improve the antibacterial activity and pharmacokinetic properties of PTM.

Graphical Abstract

Keywords

platensimycin; antibiotics; cross-coupling reactions

INTRODUCTION

Natural products provide myriad drug leads against cancer, infectious and central nervous system diseases.^{1–2} The advancement of synthetic methodology and medicinal chemistry, such as palladium-catalyzed cross-coupling reactions and C–H bond activation, as well as convenient fluorination and trifluoromethylation, may enable rapid molecular editing of natural products, which results in drug leads with improved pharmacokinetics, efficacy, and safety.^{3–4} Platensimycin (PTM), isolated from soil-dwelling *Streptomyces platensis* by Singh and co-workers, inhibits bacterial fatty acid biosynthesis by strongly binding to the active site of FabF/FabB, which blocks the entry of their native substrate malonyl-acyl carrier protein (Figure 1).⁵ PTM showed potent antibacterial activities against a wide range of Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).⁵ While the undesirable pharmacokinetics of PTM has prevented it into the clinics, its novel molecular scaffold and promising biological activities have inspired the preparation of its analogues to facilitate the structure-activity relationship study.^{5–7} Modification of its 3-amino-2,4-dihydroxybenzoic acid (ADHBA) moiety may lead to reduced antibacterial activity,^{8–11} while morphing the PTM ketolide part generated several analogues, such as 11-methyl-7-phenylplatensimycin (**i**), 7-phenylplatensimycin (**ii**), adamantaplatensimycin (**iii**), carbaplatensimycin (**iv**), PTM sulfa-Michael/aldol adducts (**v**), 6-aryl platensimycin (**vi**) and PTM sulfa-Michael adducts (**vii**), with equal or more potent antibacterial activity *in vitro*.^{9, 12–14}

We have recently been developing a technology platform to facilitate actinomycete natural product drug discovery and development (Figure 1), including (1) The generation of actinomycete strain collection to preserve Nature's biodiversity;^{15–16} (2) Biosynthetic study of the desired natural products;^{17–19} (3) Genome mining of natural products from the strain collection;^{20–22} (4) Engineering high-yield producing strains and pilot-scale production;

^{23–25} (5) Semisynthesis of natural product derivatives;^{6, 10–11, 13–14, 26} (6) Structure-activity relationship study of the natural product analogues *in vitro* and in animal models.¹⁴ This technology platform has thus allowed us to prepare dozens of PTM analogues and identified 6-pyrenylplatensimycin with improved pharmacokinetics in a mouse peritonitis model over its parent compound.¹⁴ However, due to the multiple synthetic steps, the scale-up synthesis of 6-pyrenylplatensimycin remained difficult. Besides, it only saved four out of five MRSA-infected mice, indicating more potent PTM analogues would be required.

Therefore we hypothesized that the preparation of a focused PTM derivative library around its ketolide moiety would be instrumental in discovering more active drug leads for biological evaluation *in vitro* and *in vivo*. In this work, a focused PTM library of 120-member was constructed with highly efficient synthetic routes involving thiolysis of PTM oxirane (**1**), and various cross-coupling reactions. Screening of this PTM derivative library against *S. aureus*, as well as the biological evaluation of the leading hits, revealed several PTM derivatives with improved antibacterial activity *in vitro* and *in vivo*. Our study not only significantly expanded the known active PTM derivatives but showcased that the integration of natural product chemistry and biosynthesis and medicinal chemistry offers a direct route towards antibiotic discovery and development.

RESULTS AND DISCUSSION

Constructing a focused library of PTM derivatives

Although late-stage functionalization of PTM molecular scaffold has resulted in multiple active derivatives against many tested Gram-positive pathogens, the discovery of more active PTM derivatives than the parent compound remained extremely difficult.^{8–9, 14, 27–30} We have envisioned an approach to prepare a focused library of PTM derivatives, followed by screening of the crude semisynthetic products against both MRSA and *S. aureus* ATCC 29213 with the paper disk assay. The potential hits with improved antibacterial activity over PTM would then be re-synthesized, and further characterized *in vitro* and *in vivo*. Central to the construction of the designed library is the semisynthesis of PTM oxirane **1**, which could be opened up by various free thiols under mild basic conditions to afford sulfur-containing PTM derivatives **A** (Scheme 1).³¹ These new ene-thioether derivatives **A** would be more stable than the previously synthesized sulfa-Michael adducts (**vii**), which may undergo facile β -elimination under physiological pH conditions.¹³ Under Suzuki-Miyaura cross-coupling reactions, various organoboronic acids have previously been coupled to platensic acid ethylester with decent yields, catalyzed by the recent reported heterogenous palladium catalyst.¹⁴ Therefore further derivatization of PTM derivatives **A25** and **A26** with 3-iodothiophenol or 4-iodothiophenol moiety may be feasible using Suzuki-Miyaura cross-coupling reactions, as well as Heck and Sonogashira reactions, which would generate compounds **B – G**, with functionalized aromatics, trans-alkenes or alkynes.^{32–34}

LiOH-catalyzed asymmetric epoxidation of PTM in the presence of 30% H₂O₂ and a catalytic amount of TBAF, generated compound **1** in a high yield of over 90%.³¹ The 1D and 2D NMR spectra revealed that **1** was obtained as a single isomer, indicating that this reaction was highly stereoselective and consistent with the previous C-7 substituted PTM adducts (**v**, **vii**), using sulfa-Michael and sulfa-Michael/aldol reactions, or the PTM

cyclopropyl derivative through the Corey-Chaykovsky cyclopropanation (Figure S1).^{13, 35} Also, the relatively high purity of the crude compound **1** could directly proceed to the next thiolysis reaction. PTM derivatives **A1** – **A37** were then synthesized from **1**, using various thiophenol or thiol substrates in basic conditions under room temperature.³¹ When 1.2 – 2.0 eq. of thiophenol or thiol substrates were used, the thiolysis reactions typically led to the total consumption of **1** after two hours, based on ultra-performance liquid chromatography–electrospray ionization mass spectrometry (UPLC-ESI-MS) analysis of the crude reaction products (Figure S16). While most of the reactions were done under this condition, the thiophenols generally exhibited more reactivity than thiols under the unoptimized conditions.

To prepare PTM derivatives **B** – **G** using C–C cross-coupling reactions, compounds **A25** and **A26** with iodothiophenol groups were made in a gram-scale through thiolysis of **1**, with a decent isolation yield of 87% and 91%, respectively. Then more than 50 PTM derivatives **B** and **E** were synthesized by a typical Suzuki reaction condition, using different boric substrates (1.1 – 2.0 eq.), Pd(OAc)₂ (0.1 eq.) and Na₂CO₃ (5.0 eq.) in 1,2-dimethoxyethane (DME) / H₂O at room temperature (Scheme 1).³³ Most of the substituted phenyl boric substrates afforded crude cross-coupling products with good purity, such as compounds **B10** and **E19** (Figures S17 and S20). While some steric boric substrates only had moderate reactivity towards **A25** or **A26**, which yielded less pure products, consistent with previous observations in Suzuki reactions (Figures S17 and S20).^{14, 33} PTM derivatives **C** and **F** were also synthesized via Heck reactions, using various acryl substrates (1.1 – 2.0 eq.) and the palladium catalysts in acetonitrile at 80 – 100 °C.³² For example, PTM derivatives **C5**, **C7**, **F11**, and **F13** of acryl ester substrates could be obtained with relatively higher yields than those of acrylamide substrates, such as **C1**, **C13**, **F1**, and **F8** (Figures S18 and S21). PTM derivatives **D** and **G** were finally prepared by Sonogashira reactions from alkynes (1.1 – 2.0 eq.), PdCl₂(PPh₃)₂ (0.1 eq.), and CuI (0.25 eq.) in degassed tetrahydrofuran (THF) at room temperature.³⁴ Most alkynes could react with **A25** or **A26** to afford **D** and **G** in acceptable yields of 75% to 90%, despite the presence of some uncharacterized side products in the products of **D5**, **D10**, **G10**, and **G15**.

The robustness of the thiolysis and cross-coupling reactions was witnessed in the successful preparation of 120 out of 142 designed PTM derivatives, based on the LC-MS characterization of the crude products (Figures S19 – S25). Most of the crude products showed over 75% purity by excluding the over-loaded substrates and the catalysts. The total consumption of compound **1** in the thiolysis reaction may also facilitate the following antibacterial assays of PTM derivatives **A1** – **A37**. Similarly, the entire use of compounds **A25** or **A26** in the cross-coupling reactions may also be instrumental to the antibacterial tests of PTM derivatives **B** – **G**.

Predicted physicochemical properties

The physicochemical properties of drugs or drug leads determine their pharmacokinetics behaviors.^{36–37} To understand the physicochemical behavior of the designed PTM derivatives, we predicted several critical parameters of these compounds using SwissADME, such as their lipophilicity and flexibility (Figure 2).³⁸ The molecular weight of most

compounds ranged from 500 to 700 g/mol. Most of these compounds were more lipophilic and flexible, due to the conjugation of various hydrophobic groups.

Screening of the PTM derivatives yielded anti-*Staphylococcus* hits

The synthesized PTM derivative library was screened with paper disk method against MRSA and *S. aureus* ATCC 29213, using PTM and clinically-used linezolid (5 µg/disk) as positive controls (Figure 3 and S14).³⁹ The concentrations of the desired PTM derivatives in the crude reaction products were first estimated based on the complete consumption of the starting materials **1**, **A25**, or **A26**. Then, these derivatives were applied against the tested *S. aureus* strains. Most PTM derivatives showed potent anti-*Staphylococcus* activity, which was comparable to PTM and linezolid (Figure 3A). PTM derivatives **B – G** generally exhibited a relatively smaller inhibition zone than compounds **A**, which indicated that some bulky groups in these PTM derivatives would have attenuated antibacterial activity. Next, the top 70 hits with larger inhibition zone were further screened at lower concentration (2 µg/disk) against MRSA (Figure 3). Encouragingly, even the raw PTM derivatives showed similar inhibitory activity with PTM or linezolid, which were still applied at 5 µg per disk. These results suggested that some compounds, such as **A17** and **A28**, in this PTM derivative library, may be more potent against MRSA than PTM and linezolid.

Based on the size of the inhibition zone against MRSA, we next synthesized and purified 11 PTM derivatives, including PTM oxirane **1**. They were used to determine their minimum inhibitory concentrations (MICs) against *S. aureus* ATCC 29213, as well as MRSA and methicillin-sensitive *S. aureus* (MSSA) strain isolated from local hospitals in central China (Table 1).⁴⁰ Interestingly, compound **1** only had a MIC of 16 – 32 µg/mL against the tested *S. aureus* strains. In contrast, most of the tested PTM derivatives exhibited more potent antibacterial activities than PTM, with their MICs ranging from 0.25 – 0.5 µg/mL, such as compounds **A17**, **A28**, and **D4**. As shown in Figure S15 and S16, the MICs of these purified PTM derivatives were also consistent with the paper disk assay. Note there were only two previously synthesized PTM analogues, the 11-methyl-7-phenylplatensimycin (**i**) and 7-phenylplatensimycin (**ii**), showing improved antibacterial activities against *S. aureus*.³⁰

MRSA infection is a primary global health concern. For example, MRSA accounted for 35.3% of all *S. aureus* strains detected in over 1,400 hospitals in China, based on a recent report from China Antimicrobial Resistance Surveillance System (CARSS) in 2017. We next determined whether one of the most potent PTM derivatives, 6-cyclohexylthioplantensimycin **A28**, might be effective against a panel of 45 MRSA clinical isolates and four methicillin-sensitive *S. aureus* (MSSA) isolates collected by the local CARSS in central China (Table S2). Compound **A28** was active against all the tested isolates, exhibiting MICs of 0.25 µg/mL against 80% of the strains (Figure 4). PTM also maintained its potency against the tested pathogens, showing MICs of 1.0 µg/mL. These data suggested that the tested *S. aureus* strains have not developed drug-resistance against this new type of antibiotic.⁴¹

Docking and Molecular Dynamics Simulations

Since PTM is a potent bacterial FabB/FabF inhibitor, we hypothesized that the drug target of these newly synthesized PTM derivatives might also be bacterial FabB/FabF. Therefore, ten

PTM derivatives, including **A1**, **A3**, **A17**, **A24-A26**, **A28**, **A36**, **D2** and **D4**, were docked to *E. coli* FabF (C163Q) (PDB ID: 2GFX), using Molecular Operating Environment platform (Figures 5 and S15).²⁶ Similar to the co-crystal structure of PTM and ecFabF (C163Q), the ADHBA moiety of **A28** formed hydrogen bonds with His303 and His340 of ecFabF (C163Q).⁵ The hydrophobic interaction between the cyclohexanethiol moiety of **A28** and the amino acid residues located at the edge of FabF was also observed, which suggested that the appended cyclohexanethiol group might contribute to the binding of **A28** to its target (Figure 5A and 5B). The docking results of other PTM derivatives showed similar interactions of their respective ADHBA moiety with His303 and His340 of ecFabF (C163Q) (Figure S15).

To ascertain the interaction of **A28**-ecFabF (C163Q) complex under dynamic conditions, we conducted a molecular dynamics simulation using PTM as a control.⁴² The stability of the complex from the previous docking experiments was first evaluated by the root-mean-square deviation (RMSD) of ecFabF (C163Q) backbone using the generated trajectory data in a simulation of 50 nanoseconds, in comparison with the experimental PTM-ecFabF (C163Q) complex. The backbone RMSD values of **A28**- and PTM-ecFabF (C163Q) complexes were similar, suggesting that **A28** binds to ecFabF (C163Q) and induces similar structural fluctuations with those of PTM (Figure 5C). The stability of the predicted complexes was further evaluated by the nonbond interaction energies composed of electrostatics and Van der Waals forces (Figure 5D). Both **A28** and PTM had comparable non-bond energies, with values of -128.75 and -232.04 kcal/mol, respectively (Figure 5D). In contrast, PTM oxirane **1** with MICs of 16 – 32 µg/mL against the tested *S. aureus* strains, lost significant hydrogen bond interactions with A309 and T270, when docked to ecFabF (C163Q) (Figure S18). Interestingly, compound **1** showed similar backbone RMSD with PTM and **A28**, while the nonbond interaction energy between ecFabF (C163Q) and **1** was -88.77 kcal/mol, slightly higher than that between the enzyme and **A28**. Taken together, the more potent PTM derivative **A28** could likely bind more tightly to ecFabF (C163Q) than compound **1**.

Therefore, these data suggested that compound **A28** and other active PTM derivatives may similarly bind to FabB/FabF with PTM, while these S-containing moieties on their C-6 position might contribute favorable hydrophobic interactions. These observations were consistent with the previously-reported enhanced antibacterial activities of 11-methyl-7-phenylplatensimycin (**i**) and 7-phenylplatensimycin (**ii**) over PTM by Lee and co-workers.³⁰

Evaluation of the Antibacterial Activities in a Mouse Peritonitis Model

The *in vivo* efficacy of compounds **A1**, **A3**, **A17**, and **A28** were evaluated in a mouse peritonitis model (Figure 6). Six-week-old C57BL/6J male mice were intraperitoneally injected with 2×10^7 colony forming units (CFU) of MRSA strain in saline, containing 5% (w/v) hog gastric mucin.⁴³ The infected mice (n = 5 per group) were then intraperitoneally treated with 10 mg/kg of **A1**, **A3**, **A17** and **A28** at 1 and 5 h after bacterial injection, with PTM (10 mg/kg) and vancomycin (50 mg/kg) as controls. Over 60% of mice in **A3**, **A17**, and **A28** treated groups survived, while none of the mice survived in **A1** and PTM group (Figure 6B and 6C). Encouraged by the improved *in vivo* efficacy of **A3**, **A17**, and **A28** over PTM, they were further evaluated at a dose of 20 mg/kg using the same mouse model (Figures 6D and 6E). Under this dosage, all of the **A28**-treated mice survived, and 80% of

the infected mice in the groups treated by **A3** and **A17** also survived, which suggested that this dosage may be more effective. Finally, there was 80% survival of the infected mice by intraperitoneal treatment of **A28** at 30 mg/kg, suggesting specific toxic issues when applied at a higher dosage, which has been observed for 6-pyrenylplatensimycin (Figures 6F and 6G).¹⁴

Treatment of the infected mice by **A28** at dosages of 30 or 50 mg/kg using oral gavage could only survive the mice for two days, suggesting further improvement of this promising antibiotic for its oral administration would be required. The oral administration of compound **A28** could not survive the infected mice for two reasons. First, we used a deadly mouse peritonitis model, and the mice died within 24 hours without treatment (Figure 6). Second, the bioavailability of **A28** might also be limited, which warrants further study of this promising drug lead.

Evaluation of the Antibacterial Activities in a Skin Infection Model

MRSA-induced skin infection is a serious public health problem. Therefore, the efficacy of **A28** was next evaluated with MRSA-induced skin infection in mice. The MRSA infected female BABL/c mice were treated with **A28** ointment (2 mg per treatment) or mupirocin (2 mg per treatment) twice a day for seven days. **A28** reduced *S. aureus* loads from $(7.9 \pm 0.9) \times 10^8$ CFU/g to $(2.7 \pm 1.8) \times 10^6$ CFU/g of the infected mouse skin, which was comparable to the clinic used drug mupirocin $((6.5 \pm 2.4) \times 10^5$ CFU/g) (Figure 7B). Based on visual inspection and hematoxylin and eosin (HE) staining of the infected skin, a secondary scald and the detachment of the epidermis from the dermis were observed for the MRSA-infected mice (9-days post-injury), while the treated mice by mupirocin or **A28** showed relatively healed skin structures (Figure 7C–7E).⁴⁴ The inset of HE staining in Figure 7D showed regions of mice skins with destroyed skin structure, while those in Figures 7D and 7E showed the retaining of dermis and fat layers.

CONCLUSION

Bacteria fatty acid synthases (FASs) have been considered as promising antibacterial targets, due to their essential role in bacterial lipid metabolism and the structural difference with the human type I FASs.⁴⁵ We have discovered multiple PTM analogues with potent anti-*Staphylococcus* activities based on the screening of a focused PTM derivative library of over 100 members. One of the PTM derivatives, 6-cyclohexylthioplantensimycin (**A28**), showed MICs of 0.125 – 0.5 µg/mL against a panel of 44 MRSA clinical strains. Compound **A28** was further shown to have improved antibacterial activities over PTM in a mouse peritonitis model with the MRSA infection. It was also useful in a skin wound model of MRSA infection in mice. Therefore, the current study not only suggests that the bacterial FabB/FabF enzymes are promising antibacterial drug targets but presents a simple strategy to prepare multiple active PTM analogues with antibacterial activities in two MRSA-infected mouse models. Our study also showcased that late-stage functionalization of natural products may provide a translational route for effective antibiotic development against drug-resistant major pathogens.

EXPERIMENTAL PROCEDURES

General Experimental Procedure

The commercial reagents were used as received. All ^1H and ^{13}C NMR spectra were recorded on a Bruker 500 MHz or 400 MHz spectrometers. Chemical shifts were reported in ppm relative to the internal standard tetramethylsilane ($\delta = 0$ ppm) for ^1H NMR and deuteriochloroform ($\delta = 77.00$ ppm) for ^{13}C NMR spectroscopy. The following abbreviations were used to designate chemical shift multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. HRMS spectra were recorded on an LTQ-ORBITRAP-ETD instrument. Samples were analyzed on a Waters e2695 HPLC system equipped with a PDA detector and a Waters Sunfire C18 column (150×4.6 mm). The mobile phase consisted of buffer A (ultrapure H_2O containing 0.1 % HCOOH and 0.1 % CH_3CN) and buffer B (chromatographic grade CH_3CN containing 0.1 % HCOOH) was applied at a flow rate of 1 mL min^{-1} . All compounds used for their MIC determination and animal experiments possessed a purity of at least 95%, based on HPLC and ^1H NMR analysis.

Chemistry

Synthesis of platensimycin oxirane (1)—To a solution of PTM in DCM (36.0 mL) was added 30% H_2O_2 (12.0 mL), TBAF (100 μL) and 2M LiOH (1.5 mL), the mixture was stirred under room temperature for 8.0 h. Next, the reaction was quenched with 2M HCl (2.0 mL) and washed with water. The organic layer was dried with anhydrous sodium sulfate and concentrated under vacuum. The PTM oxirane (**1**) was obtained in good purity (yields over 90%), which was directly used for the next step reaction without further purification.

General procedure for thiolysis reaction to prepare compounds A1 – A37—To a mixture solution of compound **1** (25.0 mg, 54.7 μmol) in EtOH / H_2O (5.0 mL / 1.0 mL) was added thiophenol or thiol substrates (57.4 μmol). The mixture was basified to pH 11.0 with 2M LiOH and stirred under room temperature for 2.0 h. Then the reaction was neutralized with 2M HCl and concentrated under vacuum. The residue was dissolved in EtOAc, washed with brined and fresh water. The organic phase was concentrated to generate the desired products.

General procedure for Suzuki cross-coupling reaction to prepare compounds B1 – B22 and E1 – E24—To a solution of compound **A25** or **A26** (25.0 mg, 37.0 μmol) in DME / H_2O (5.0 mL / 2.5 mL) was added Na_2CO_3 (20.0 mg, 188.7 μmol), Ar-B(OH) $_2$ (40.7 μmol) and $\text{Pd}(\text{OAc})_2$ (1.0 mg, 4.5 μmol). The mixture was degassed with nitrogen and stirred 12 h at room temperature. At the end of the reaction, the mixture was acidified with 0.1 M HCl and extracted with EtOAc (3×5.0 mL). The organic extracts were dried (Na_2SO_4) and distilled under vacuum to give the desired products.

General procedure for Heck reaction to prepare compounds C1 – C14 and F1 – F14—To a solution of compound **A25** or **A26** (25.0 mg, 37.0 μmol) in MeCN (5.0 mL) was added acrylyl substrate (40.7 μmol), Et_3N (19.0 μL , 129.5 μmol), PPh_3 (3.8 mg, 14.8 μmol) and $\text{Pd}(\text{OAc})_2$ (1.0 mg, 4.5 μmol). The mixture was degassed with nitrogen and

stirred 8.0 h at 80 °C. When the reaction was done, the mixture was acidified with 0.1 M HCl and extracted with EtOAc (3 × 5.0 mL). The organic extracts were dried (Na₂SO₄) and distilled under vacuum to give the desired products.

General procedure for Sonogashira reaction to prepare compounds D1 – D16 and G1 – G15—To a solution of compound **A25** or **A26** (25.0 mg, 37.0 μmol) in THF (5.0 mL) was added alkyne substrate (40.7 μmol), DIPA (13.0 μL, 92.5 μmol), CuI (2.0 mg, 10.5 μmol) and Pd(PPh₃)₂Cl₂ (3.0 mg, 4.3 μmol). The mixture was degassed with nitrogen and stirred 4.0 h at room temperature. When the reaction was finished, the mixture was washed with acidified with 0.1 M HCl and extracted with EtOAc (3 × 5.0 mL). The organic extracts were dried (Na₂SO₄) and distilled under vacuo to give the desired products.

Synthesis of compounds A1, A3, A17, A24, A25, A26, A28 and A36—To a mixture solution of compound **1** (114.0 mg, 0.25 mmol) in EtOH / H₂O (10.0 mL / 2.0 mL) was added 0.37 mmol of 2-pyridinethiol, benzyl mercaptan, p-toluenethiol, 4-fluorothiophenol, 3-iodobenzenethiol, 4-iodobenzenethiol, cyclohexanethiol and 1-propanethiol respectively. The mixture was basified to pH 11.0 with 2 M LiOH and stirred under room temperature for 2.0 h. Then the reaction was neutralized with 2 M HCl and concentrated under vacuum. The residue was dissolved in EtOAc, washed with brined and fresh water. The organic phase was dried and concentrated, the generated crude products were subjected to column chromatography (eluent: EtOAc/PE/AcOH 40:60:1) to provide compound **A1** (120.0 mg, 87%), **A3** (129.0 mg, 92%), **A17** (128.0 mg, 91%), **A24** (129.0 mg, 91%), **A25** (148.0 mg, 88%), **A26** (152.0 mg, 90%), **A28** (121.0 mg, 87%) and **A36** (111.0 mg, 86%).

Scale-up synthesis of compounds A25 and A26—To a mixture solution of **1** (1142.0 mg, 2.5 mmol) in EtOH / H₂O (30.0 mL / 6.0 mL) was added 3-iodobenzenethiol or 4-iodobenzenethiol (708.0 mg, 3.0 mmol). The mixture was basified to pH 11.0 with 2 M LiOH and stirred under room temperature for 2.0 h. Then the reaction was neutralized with 2 M HCl and concentrated under vacuum. The residue was dissolved in EtOAc, washed with brined and fresh water. The organic phase was dried and concentrated; the generated crude products were subjected to column chromatography (eluent: EtOAc/PE/AcOH 40:60:1) to provide compound **A25** (1468.0 mg, yield 87%) or **A26** (1536.0 mg, yield 91%).

Synthesis of Compounds D2 and D4—To a solution of **A25** (100.0 mg, 0.15 mmol) in THF (10.0 mL) was added 0.163 mmol of 2-ethynylpyridine and 1-ethynylcyclohexanol. Then DIPA (55.0 μL, 0.40 mmol), CuI (8.0 mg, 42.0 μmol) and Pd(PPh₃)₂Cl₂ (9.0 mg, 13.0 μmol) was added to the mixture in sequence. The mixture was degassed with nitrogen and stirred 4.0 h at room temperature. When the reaction was done, the mixture was acidified with 0.1 M HCl and extracted with EtOAc. The organic extracts were dried (Na₂SO₄) and distilled under vacuo, the generated crude products were subjected to column chromatography (eluent: EtOAc/PE/AcOH 40:60:1) to provide **D2** (81.0 mg, yield 83%) and **D4** (82.0 mg, yield 81%).

Evaluation of the inhibition zone of the synthesized raw products—

All antibiotic disks were sterilized before using for inhibition zone evaluation. 10 mL Luria-Bertani broth (LB) agar and 5 mL soft LB agar with 500 μ L *S. aureus* ($OD_{600} = 0.5 \pm 0.1$) were mixed and placed into each plate. The paper disks were added after solidification of LB medium. After the addition of the respective amount of the crude reaction products (**A – G**) (2 or 5 μ g/disk), PTM and linezolid (5 μ g/disk), the plates were incubated at 37 °C for 18 hours and the antibacterial inhibition zones were then measured with a vernier caliper.

The MIC determination using the agar dilution method—

In vitro bioassay for tested compounds were carried out using *S. aureus* ATCC 29213, as well as MSSA and MRSA strains isolated from local hospitals in central China.^{10, 40} All the tested compounds were prepared with methanol. Each purified compound (**1**, **A1**, **A3**, **A17**, **A24 – A26**, **A28**, **A36**, **D2**, and **D4**) (150 μ L) with different concentrations was used to mix with 15 mL melted LB agar in each petri dish, resulting in a final compound concentration of 0.5 – 64 μ g/mL on each plate. Linezolid and PTM were used as controls. The tested bacteria were first cultivated in LB broth and incubated for 16 h at 37 °C and 250 rpm. The bacteria suspensions were diluted till the OD_{600} reaches to 0.25 ± 0.05 , which were further diluted for 10^4 fold. The diluted bacteria (2 μ L) were added to each plate and incubated for 16 h at 37 °C. The MICs were determined to be the lowest compound concentration with no visible growth of bacterial colonies on the plates.

The MIC determination of compound A28 and PTM against 49 clinically

isolated *S. aureus* strains—The MIC determination of compound **A28** and PTM against 49 clinically isolated *S. aureus* strains was the same as the above-described method. The detail information of the isolated strains was listed in Table S2.

Molecular docking—Docking studies were performed on an Intel Core i5 2.5 GHz processor, 4 GB memory with Windows 10 operating system using Molecular Operating Environment (MOE 2010.06; Chemical Computing Group, Canada) as the computational platform. All the energy minimizations were performed with MOE until an RMSD gradient of $0.05 \text{ Kcal mol}^{-1} \text{ \AA}^{-1}$ with an MMFF94X force field and the partial charges were automatically calculated. PTM-ecFabF (C163Q) complex (PDB ID: 2GFX) was downloaded from the Protein Data Bank. The enzyme was prepared for docking studies according to our previously reported method.²⁶

Molecular dynamics simulation—The molecular dynamics studies in this work were simulated on dual Intel Xeon E5–2678V3 2.5 GHz processors, 32 GB memory, with Ubuntu 16.04 operating system using NAMD (Linux-x86_64-multicore-CUDA edition) as the computational platform. Importantly, dual NVIDIA TITAN Xp graphics cards were used to accelerate the calculations with CUDA 9.1. The PTM-FabF complex (PDB ID, 2GFX) was from the Protein Data Bank. The **A28**-FabF complex used for dynamics simulation was selected from the predicted docking modes. Binding stabilities of the ligand-FabF complex was simulated with CHARMM general force field (protein: <https://www.charmm.org/charmm/>, ligand: <https://cgenff.umaryland.edu/>). An implicit solvent model containing 0.15 M sodium chloride was used for simulation. An equilibration simulation from 60 K to 300 K

for 1.242 ns, was processed before taking 50 ns production simulation at 300 K. The results were saved per 2 fs. The VMD 1.9.3 was used to analyze the generated MD trajectory file, calculate the root-mean-square deviation (RMSD) of the protein backbone and the nonbond interaction energy between the ligand and protein. In RMSD analysis, the actual structural fluctuations were measured by comparing each frame with frame 0.

Mouse peritonitis model—The lethal peritonitis model was based on the reported procedures.^{43, 46} Six-week-old female C57BL/6J mice were intraperitoneally injected with 2×10^7 CFU of MRSA inoculum in 0.5 mL physiological saline containing 5% (wt/v) mucin to the generate lethal peritonitis mice model. After 1 and 5 h post-infection, the mice (n = 5 per group) were injected with 0.2 mL/mouse of each compound. Saline was given to mice in model group, vancomycin (50 mg/kg) and PTM (10 mg/kg) were used for the control groups. Compounds **A1**, **A3**, **A17** and **A28** (10 mg/kg) was formulated in 2.5% DMSO, 30% propanediol, 30% polyethylene glycol 400 and 37.5% physiological saline to 1 mg/mL for injection. Compounds **A1**, **A3**, **A17** and **A28** (20 mg/kg) was formulated in 5.0 % DMSO, 30% propanediol, 30% polyethylene glycol 400 and 35.0% physiological saline to 2 mg/mL for injection. Compound **A28** (30 and 50 mg/kg) was formulated in 18.0 % hydroxypropyl- β -cyclodextrin and 82.0% physiological saline to 3 and 5 mg/mL for injection or oral administration. Vancomycin was dissolved in physiological saline to a final concentration of 5 mg/mL. The ability of the tested compounds to rescue mice from lethal peritonitis was observed twice daily and mice survival was tracked for 7 days.

Mouse Skin Infection with MRSA—Mice induced mice skin infection was handled following the reported methods.^{47–48} Female BABL/c mice (20 – 22 g, 7-week-old) were anesthetized by intraperitoneal injection of 4% chloral hydrate (200 μ L), and the hair of mice back surface was removed and washed with povidone iodine-propanol solution. A gauze (15 $\times$ 15 mm) was preheated in boiling water (90 °C) for 10 min and then placed on the back of each mice for 40 s to generate the burns. Next, the burn was bound with a small dry gauze and infected with 1×10^8 colony-forming units (CFU) of MRSA. An open wound was witnessed at the burns after 48 h post infection. The infected mice then divided into three groups (n = 5 per group). **A28** (200 μ g) was formulated into 2% ointment consisting of 1.4% carbomer, 1.4% triethanolamine, 14% propanediol, 14% glycerinum and 1% azone. Mupirocin (2% w/w) was used as positive control while vehicle used as a negative control. All mice were treated twice a day for seven days. Mice were euthanized after 24 h of the last treatment, and the wound area was slightly swabbed with 70% ethanol. Wound area (1 cm²) was excised, homogenized, and plated onto TSB agar plates to count viable bacteria. Other skin biopsy specimens removed from the wounds were stored at 4% paraformaldehyde solution for HE staining.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

PTM	Platensimycin
FASII	Type II fatty acid synthases
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
MSSA	methicillin-sensitive <i>Staphylococcus aureus</i>
VRE	Vancomycin-resistant <i>Enterococci</i>
ADHBA	3-Amino-2,4-dihydroxybenzoic acid
TMS	Trimethylsilane
TMSE	Trimethylsilylethyl
TBAF	Tetrabutylammonium fluoride
MIC	minimum inhibitory concentration
EtOAc	Ethyl acetate
PE	Petroleum ether
DME	Glycol dimethyl ether
LiOH	Lithium hydrate
THF	Tetrahydrofuran
HCl	Hydrochloric acid
DMF	Dimethylformamide
HATU	2-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate
Et₃N	Triethylamine

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Figure 1.

The developed technology platform to facilitate actinomycete natural product drug discovery and development.

Figure 2.

The predicted physicochemical properties of PTM and its derivatives. The properties of PTM were shown with red stars. The compound number was displayed with an increment of two for clarity.

Figure 3.

An anti-*Staphylococcus* screening of the PTM derivatives. (A) The antibacterial activity of all synthesized raw reaction products was tested with the paper disk method against MRSA. PTM derivatives (approximately 5 μ g/disk) were applied. PTM and linezolid (5 μ g/disk) were used as positive controls. (B) The top 70 hits of PTM derivatives were tested at 2 μ g per disk, while PTM and linezolid (5 μ g/disk) were used as positive controls.

Figure 4.
The *in vitro* antibacterial activities of PTM (Purple) and **A28** (Red) against four clinical MSSA (Blue) and 45 clinical MRSA (Black) strains.

Figure 5.

The predicted docking mode of **A28** in ecFabF (C163Q) and molecular dynamics simulation. (A) The docking mode of **A28** (green) was similar to that of PTM (yellow). (B) The binding mode of **A28** with ecFabF (C163Q) was shown in the 2D model, with interactions towards H303, H340, T270, T307 and A309 of the FabF active site. (C) The root-mean-square deviation (RMSD) values of the FabF backbone and (D) the nonbond interaction energies between protein and ligand in the complexes between 2 and 50 ns. **A28** was in red and PTM was in black.

Figure 6.

The *in vivo* antibacterial activities of synthesized compounds in a mouse peritonitis model. (A) The general procedure of the mouse peritonitis model (n = 5 per group). (B) The *in vivo* efficacy of A1, A3, A17, and A28 in a dose of 10 mg/kg. (D) The *in vivo* efficacy of A3, A17, and A28 in a dose of 20 mg/kg. (F) The *in vivo* efficacy of A28 was evaluated with a dose of 30 mg/kg (i.p.), 30 mg/kg (p.o.) and 50 mg/kg (p.o.). (C, E, G) The weights of the survived mice in 7 days. Mean values are presented (n = 5). In each experiment, PTM (10 mg/kg) and vancomycin (50 mg/kg) were used as the positive controls, and physiological saline was used as the negative control.

Figure 7.

The antibacterial activity of **A28** against MRSA skin infection in mice. (A) General procedures for evaluating the therapeutic efficacy of **A28** in a mouse skin infection model. Mupirocin was used as a positive control. The infected mice were treated twice a day for seven days. (B) The total bacterial loads in the skin lesions were determined. Statistics were calculated using the Mann-Whitney test. P values of (**** $P < 0.0001$) is considered as significant. **** $P < 0.0001$ for model versus 2% mupirocin and 2% **A28**. (C) Wounds of BALB/c mice with treated and untreated with compounds after seven days. (D – F) HE staining of *S. aureus*-infected skin lesion on Day 10. The biopsy specimens were taken immediately after the termination of the experiment and then stored in 4% paraformaldehyde solution. They were next stained with HE. Each point represents data from a single mouse. Mean values were presented ($n = 5$).

Scheme 1.

Synthesis of PTM derivatives. (a) 2 – 3 eq. LiOH (2 M), TBAF (catalytic amount), 30% H₂O₂ / DCM (1:3), > 90% from PTM. (b) Thiophenols or thiols (1.2 – 2.0 eq.), methanol (pH = 9). (c) Boric substrates (1.1 – 2.0 eq.), Pd(OAc)₂ (0.1 eq.), Na₂CO₃ (5.0 eq.), DME/H₂O, rt, 65 – 90% from **A25** or **A26**. (d) Acryl substrates (1.1 – 2.0 eq.), Pd(OAc)₂ (0.1 eq.), PPh₃ (0.4 eq.), Et₃N (3.5 eq.), MeCN, 80 – 100 °C, 65 – 90% from **A25** or **A26**. (e) Alkynes (1.1 – 2.0 eq.), PdCl₂(PPh₃)₂ (0.1 eq.), CuI (0.25 eq.), DIPA (2.5 eq.), THF, rt, N₂, Dark, 65 – 90% from **A25** or **A26**. The yields were estimated based on UPLC-ESI-MS analysis of the crude reaction products. The compounds labeled in green were not detected.

Table 1.

The MICs ($\mu\text{g/mL}$) of the selected PTM derivatives against *S. aureus* ATCC 29213, and two clinical MSSA and MRSA strains using the agar dilution method.

The image displays the chemical structures of the PTM derivatives studied. Structure 1 is the parent compound, PTM. Derivatives A1 through A36 and D2 through D4 are shown with various modifications to the PTM core, including changes to the side chain, the pyridine ring, and the thiazolidine ring. The structures are labeled 1, A1, A3, A17, A24, A25, A26, A28, A36, D2, and D4.

Compounds	<i>S. aureus</i> ATCC 29213	MSSA	MRSA
PTM	1	0.5	0.5
Linezolid	1	1	1
1	32	16	16
A1	2	2	2
A3	0.5	0.5	0.5
A17	0.5	0.25	0.25
A24	1	0.5	0.5
A25	1	0.5	0.5
A26	0.5	0.25	0.25
A28	0.5	0.25	0.25
A36	1	0.5	0.5
D2	0.5	0.5	0.5
D4	0.25	0.25	0.25