J. Microbiol. Biotechnol. (2019), 29(10), 1603–1606
https://doi.org/10.4014/jmb.1906.06026

jmb

Inhibitory Effects of *Streptomyces* sp. MBTH32 Metabolites on Sortase A and Sortase A-Mediated Cell Clumping of *Staphylococcus aureus* to Fibrinogen

Beomkoo Chung[1], Oh-Seok Kwon[2], Jongheon Shin[2*], and Ki-Bong Oh[1*]

[1]*Department of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea*
[2]*Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea*

Received: June 13, 2019
Revised: July 23, 2019
Accepted: August 28, 2019

First published online
September 2, 2019

**Corresponding authors*
J.S.
Phone: +82-2-880-2484;
Fax: +82-2-762-8322;
E-mail: shinj@snu.ac.kr
K.-B.O.
Phone: +82-2-880- 4646;
Fax: +82-2-873-3112;
E-mail: ohkibong@snu.ac.kr

pISSN 1017-7825, eISSN 1738-8872

Sortase A (SrtA), a type of transpeptidase responsible for anchoring surface proteins to the peptidoglycan cell wall, is important in the virulence of gram-positive bacteria. Three compounds were isolated from marine-derived *Streptomyces* sp. MBTH32 using various chromatography techniques. The structures of these compounds were determined based on spectroscopic data and comparisons with previously reported data. Among the metabolites tested, lumichrome showed strong inhibitory activity against *Staphylococcus aureus* SrtA without affecting cell viability. The results of cell clumping activity assessment suggest the potential for using this compound to treat *S. aureus* infection by inhibiting SrtA activity.

Keywords: Marine *Streptomyces*, lumichrome, *Staphylococcus aureus*, sortase A, cell clumping

The ocean environment covers 70% of the Earth's surface and has more diverse conditions than the terrestrial environment, such as low oxygen and lack of light [1, 2]. Under these conditions, biosynthesis of secondary metabolites typically involves mechanisms modified for physiological adaptation, which increases the probability that unusual natural products might be present [3, 4]. In the last decade, numerous natural products have been discovered in the marine environment; notably, thousands of those compounds exhibit new structures, and three-fourths of them demonstrate diverse bioactivities [5]. Marine-derived *Streptomyces* species have been identified as major producers of novel antibiotics, such as branimycins B and C [6]; ansalactams B, C, and D [7]; desotamide B [8]; and lobophorin H [9]. Thus, marine-derived *Streptomyces* species could be outstanding sources of novel antimicrobial agents.

Sortases, transpeptidases that anchor surface proteins to the peptidoglycan layer in gram-positive bacteria, have attracted attention as a potential target of novel antibiotics [10]. Surface proteins covalently tethered to the cell wall by sortases allow gram-positive bacteria to adhere to host tissues and to invade epithelial cells [11, 12]. Sortases comprise six isoforms, A–F (SrtA–F). Among them, SrtA has been shown to play an important role in the pathogenesis of *S. aureus* via gene knockout experiments [13, 14]. Indeed, *S. aureus* mutants lacking SrtA were limited in their abilities to make biofilms and infect host cells maintaining cell viability. The effective pharmacophores against SrtA were recently researched and morpholino benzoate, thiazolidine derivatives were identified as promising SrtA inhibitors [15–17].

In our search for SrtA inhibitors in marine-derived *Actinomycetes*, we found that an ethyl acetate extraction of *Streptomyces* strain MBTH32 exhibited moderate activity against *S. aureus* SrtA (IC_{50} = 64.27 μg/ml). Stepwise separation of the crude extract using various chromatography methods yielded three compounds with

SrtA inhibitory activity. The structures of these compounds were determined by extensive spectroscopic analyses. Herein we report the potential of these compounds for inhibition of SrtA and SrtA-mediated cell clumping in *S. aureus*.

Strain MBTH32 was isolated from marine sediment from Shinjin Island, Republic of Korea; it showed 98% identity to *Streptomyces longispororuber* and was therefore designated *Streptomyces* sp. MBTH32 (GenBank accession number: MK840992). Strain MBTH32 was cultured in yeast–peptone–mannitol (YPM) medium (2 g yeast extract, 2 g peptone, 4 g mannitol, and 23 g sea salt in 1 L distilled water) at 28°C for 7 days on a rotary shaker. It was then filtered and extracted with an equal volume of ethyl acetate; this was performed twice. The ethyl acetate fraction was incrassated and 1.6 g of dried material was obtained for biological and chemical assays. The entire extract was separated by C_{18} reversed-phase vacuum flash column chromatography using serial dilutions of methanol and water as eluents. Based on the results of the SrtA inhibition assay, the fraction eluted in 20% aqueous methanol was isolated by reversed-phase high-performance liquid chromatography (Agilent Eclipse XDB-C_{18}, 5 μm, 9.4 × 250 mm) to yield compounds **1–3**. A total of 14.6 mg, 3.6 mg, and 3.5 mg of compounds **1**, **2**, and **3** were purified. The structures of these compounds, designated as enterocin (**1**) [18], *N*-acetyl-β-oxotryptamine (**2**) [19], and lumichrome (**3**) [20], were determined based on the results of spectroscopic analyses and comparisons with previously reported data (Fig. 1).

Recombinant SrtA was purified from transformed *Escherichia coli* by nickel-based affinity chromatography [21]. Inhibitory activity against SrtA was determined by

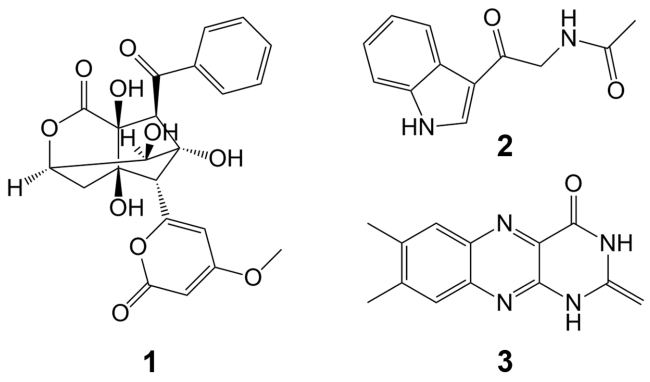

Fig. 1. Structures of compounds **1–3** isolated from marine-derived *Streptomyces* sp. MBTH32: enterocin (**1**), *N*-acetyl-β-oxotryptamine (**2**), and lumichrome (**3**).

Table 1. Inhibitory effects of compounds **1–3** on the activity of SrtA and bacterial growth of *S. aureus* ATCC6538p.

Compound	IC_{50} (μM)	MIC (μM)
	SrtA	*S. aureus* ATCC6538p
1	594.76 ± 3.78	9.00
2	1108.65 ± 7.52	>1185.19
3	198.20 ± 0.94	>528.42
Berberine chloride	106.40 ± 1.36	>344.26

Berberine chloride was used as a reference inhibitor of SrtA. IC_{50} values are the mean ± SD (n = 3).

quantifying the intensity of augmented fluorescence upon cleavage of a synthetic peptide containing LPETG motifs. Fluorescence induced from tested compounds was excluded to avoid interference with substrate [22, 23]. SrtA suppression abilities of isolated compounds and berberine chloride, a known SrtA inhibitor [24], were estimated with IC_{50} values (half maximal inhibitory concentrations) (Table 1). Compounds **1** and **2** exhibited weak SrtA inhibitory activity. In contrast, compound **3** significantly inhibited SrtA, with an IC_{50} value of 198.20 μM. SrtA inhibitors that do not hinder microbial viability are considered to be more valuable therapeutic agents [25]. Therefore, we investigated the efficacies of these three compounds on bacterial growth using the minimum inhibitory concentration assay [26]. As shown in Table 1, compounds **2** and **3** displayed no growth inhibition activity against *S. aureus*. However, the inhibition pattern of

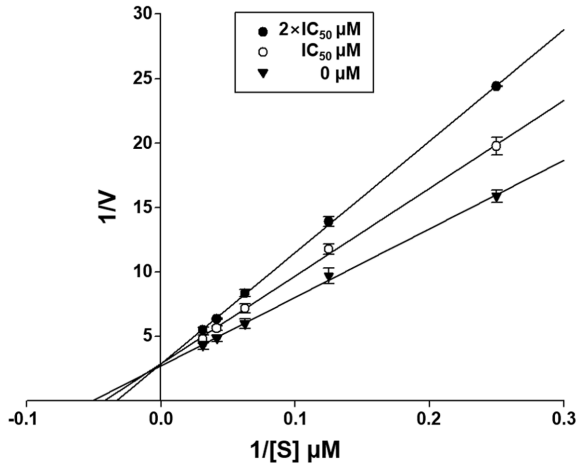

Fig. 2. Lineweaver-Burk plot of SrtA inhibition by compound **3**. Compound **3** was applied at IC_{50} and at 2× IC_{50} concentrations. [S], reaction substrate concentration; V, reaction velocity (Δfluorescence/min). Each point indicates the mean ± SD of three independent experiments.

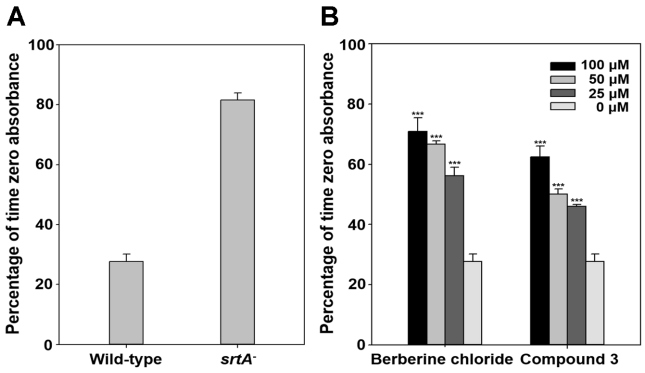

Fig. 3. Effects of *srtA* gene expression and SrtA inhibitors on the clumping of *S. aureus* with fibrinogen.

(**A**) Clumping assay was performed with *S. aureus* Newman (wild-type) and SKM12 (*srtA*-knockout mutant) strains. (**B**) Berberine chloride and compound **3** were applied at the indicated concentrations at 37°C for 2 h. The *t*-test was used for statistical analysis of multiple comparisons. A value of $p < 0.05$ was used as the criterion for statistical significance. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

compound **3,** as determined using the Lineweaver-Burk plot method [27] (K_i = 0.91 mM), indicated that it served as a competitive inhibitor (Fig. 2).

SrtA has been reported to immobilize fibrinogen-binding protein, thus accelerating bacterial adhesion to host tissues and subsequent invasion [28, 29]. We hypothesized that the immobilization of fibrinogen-binding protein may be reduced by suppression of SrtA activity *in vivo*. To confirm our assumption, *S. aureus* Newman (wild-type) and SKM12 (*srtA*$^-$) strains were used in SrtA-mediated cell clumping to fibrinogen [30]. Cells were centrifuged and resuspended with fibrinogen solution. Absorbance at 600 nm was measured for each sample at 0 h and 2 h after resuspension. The data are shown as the absorbance (mean ± SD, three independent experiments) at 2 h, divided by absorbance at time zero, multiplied by 100. Whereas the wild-type strain showed >70% reduction in absorbance at 600 nm after 2 h incubation, the *srtA*-knockout mutant only showed 20% reduction in absorbance after a similar period of incubation; this indicates that SrtA plays a crucial role in anchoring the clumping factor to the cell wall (Fig. 3A). The clumping abilities of the wild-type strain treated with various concentrations of compound **3** were also measured and compared with its clumping ability when treated with berberine chloride. The ability of the wild-type strain to develop SrtA-mediated clumps was reduced in a dose-dependent manner upon treatment with compound **3**. In particular, the absorbance of a sample treated with 100 μM compound **3** for 2 h was estimated to be 60% of the initial value, which was slightly lower than the absorbance when treated with an outstanding inhibitor, berberine chloride (Fig. 3B). These data suggest that compound **3** directly targets SrtA and decreases pathogenicity by inhibiting covalent linkage of surface proteins to the peptidoglycan layer in *S. aureus*.

In conclusion, three metabolites isolated from marine-derived *Streptomyces* sp. MBTH32 displayed inhibitory activity against *S. aureus* SrtA. Among them, lumichrome (compound **3**) showed the greatest activity against SrtA without affecting microbial growth. The SrtA-mediated clumping assay demonstrated that SrtA is responsible for covalent linkage of surface proteins to the cell wall. It also indicated that compound **3** may be useful in the treatment of *S. aureus* infections by inhibiting the anchoring ability of SrtA. These findings may be valuable for novel antibiotic research and may facilitate studies of structure-related activities among similar SrtA inhibitors.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation (NRF-2018R1D1A1B07043375) of Korea funded by the Ministry of Education, Science and Technology.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. Tortorella E, Tedesco P, Palma Esposito F, January G, Fani R, Jaspars M, *et al*. 2018. Antibiotics from deep-sea microorganisms: current discoveries and perspectives. *Mar. Drugs* **16:** pii: E355.
2. Tyler PA. 2003. *Ecosystems of the deep oceans,* pp. 1-4. 1st Ed. Elsevier Science, Amsterdam.
3. Wright PC, Westacott RE, Burja AM. 2003. Piezotolerance as a metabolic engineering tool for the biosynthesis of natural products. *Biomol. Eng.* **20:** 325-331.
4. Bull AT, Ward AC, Goodfellow M. 2000. Search and discovery strategies for biotechnology: the paradigm shift. *Microbiol. Mol. Biol. Rev.* **64:** 573-606.
5. Blunt JW, Carroll AR, Copp BR, Davis RA, Keyzers RA, Prinsep MR. 2018. Marine natural products. *Nat. Prod. Rep.* **35:** 8-53.

6. Braña AF, Sarmiento-Vizcaíno A, Pérez-Victoria I, Otero L, Fernández J, Palacios JJ, *et al*. 2017. Branimycins B and C, antibiotics produced by the abyssal actinobacterium *Pseudonocardia carboxydivorans* M-227. *J. Nat. Prod.* **80:** 569-573.
7. Le TC, Yang I, Yoon YJ, Nam SJ, Fenical W. 2016. Ansalactams B–D illustrate further biosynthetic plasticity within the ansamycin pathway. *Org. Lett.* **18:** 2256-2259.
8. Song Y, Li Q, Liu X, Chen Y, Zhang Y, Sun A, *et al*. 2014. Cyclic hexapeptides from the deep South China Sea-derived *Streptomyces scopuliridis* SCSIO ZJ46 active against pathogenic gram-positive bacteria. *J. Nat. Prod.* **77:** 1937-1941.
9. Pan HQ, Zhang SY, Wang N, Li ZL, Hua HM, Hu JC, *et al.* 2013. New spirotetronate antibiotics, lobophorins H and I, from a South China Sea-derived *Streptomyces* sp. 12A35. *Mar. Drugs* **11:** 3891-3901.
10. Cossart P, Jonquières R. 2000. Sortase, a universal target for therapeutic agents against Gram-positive bacteria? *Proc. Natl. Acad. Sci. USA* **97:** 5013-5015.
11. Mazmanian SK, Liu G, Jensen ER, Lenoy E, Schneewind O. 2000. *Staphylococcus aureus* sortase mutants defective in the display of surface proteins and in the pathogenesis of animal infections. *Proc. Natl. Acad. Sci. USA* **97:** 5510-5515.
12. Wesson CA, Liou LE, Todd KM, Bohach GA, Trumble WR, Bayles KW. 1998. *Staphylococcus aureus* Agr and Sar global regulators influence internalization and induction of apoptosis. *Infect. Immun.* **66:** 5238-5243.
13. Mazmanian SK, Ton-That H, Su K, Schneewind O. 2002. An iron-regulated sortase anchors a class of surface protein during *Staphylococcus aureus* pathogenesis. *Proc. Natl. Acad. Sci. USA* **99:** 2293-2298.
14. Mazmanian SK, Skaar EP, Gaspar AH, Humayun M, Gornicki P, Jelenska J, *et al.* 2003. Passage of heme-iron across the envelope of *Staphylococcus aureus*. *Science* **299:** 906-909.
15. Frankel BA, Bentley M, Kruger RG, McCafferty DG. 2004. Vinyl sulfones: inhibitors of SrtA, a transpeptidase required for cell wall protein anchoring and virulence in *Staphylococcus aureus*. *J. Am. Chem. Soc.* **126:** 3404-3405.
16. Sibbald MJ, Yang XM, Tsompanidou E, Qu D, Hecker M, Becher D, *et al*. 2012. Partially overlapping substrate specificities of staphylococcal group A sortases. *Proteomics* **12:** 3049-3062.
17. Cascioferro S, Raffa D, Maggio B, Raimondi MV, Schillaci D, Daidone G. 2015. Sortase A inhibitors: recent advances and future perspectives. *J. Med. Chem.* **58:** 9108-9123.
18. Kang H, Jensen PR, Fenical W. 1996. Isolation of microbial antibiotics from a marine ascidian of the genus *Didemnum*. *J. Org. Chem.* **61:** 1543-1546.
19. Martínez-Luis S, Gómez JF, Spadafora C, Guzmán HM, Gutiérrez M. 2012. Antitrypanosomal alkaloids from the marine bacterium *Bacillus pumilus*. *Molecules* **17:** 11146-11155.
20. Andrioli WJ, Lopes AA, Cavalcanti BC, Pessoa C, Nanayakkara NPD, Bastos JK. 2017. Isolation and characterization of 2-pyridone alkaloids and alloxazines from *Beauveria bassiana*. *Nat. Prod. Res.* **31:** 1920-1929.
21. Lee KY, Shin DS, Yoon JM, Kang HJ, Oh KB. 2002. Expression of sortase, a transpeptidase for cell wall sorting reaction, from *Staphylococcus aureus* ATCC 6538p in *Escherichia coli*. *J. Microbiol. Biotechnol.* **12:** 530-533.
22. Oh KB, Kim SH, Lee J, Cho WJ, Lee T, Kim S. 2004. Discovery of diarylacrylonitriles as a novel series of small molecule sortase A inhibitors. *J. Med. Chem.* **47:** 2418-2421.
23. Hu P, Huang P, Chen WM. 2013. Curcumin inhibits the sortase A activity of the *Streptococcus mutans* UA159. *Appl. Biochem. Biotechnol.* **171:** 396-402.
24. Kim SH, Shin DS, Oh MN, Chung SC, Lee JS, Oh KB. 2004. Inhibition of the bacterial surface protein anchoring transpeptidase sortase by isoquinoline alkaloids. *Biosci. Biotechnol. Biochem.* **68:** 421-424.
25. Jonsson M, Mazmanian SK, Schneewind O, Bremell T, Tarkowski A. 2003. The role of *Staphylococcus aureus* sortase A and sortase B in murine arthritis. *Microbes Infect.* **5:** 775-780.
26. Weinstein MP, Limbago B, Patel JB, Mathers AJ, Burnham C, Mazzulli T, *et al*. 2018. *Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow aerobically*, pp. 15-50. 11th Ed. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania.
27. Lineweaver H, Burk D. 1934. The determination of enzyme dissociation constants. *J. Am. Chem. Soc.* **56:** 658-666.
28. Alksne LE, Projan SJ. 2000. Bacterial virulence as a target for antimicrobial chemotherapy. *Curr. Opin. Biotechnol.* **11:** 625-636.
29. Zhang B, Teng Z, Li X, Lu G, Deng X, Niu X, Wang J. 2017. Chalcone attenuates *Staphylococcus aureus* virulence by targeting sortase A and alpha-hemolysin. *Front. Microbiol.* **8:** 1715.
30. Weiss WJ, Lenoy E, Murphy T, Tardio L, Burgio P, Projan SJ, *et al.* 2004. Effect of srtA and srtB gene expression on the virulence of *Staphylococcus aureus* in animal models of infection. *J. Antimicrob. Chemother.* **53:** 480-486.