

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

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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 (IC₅₀ = 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 incassated and 1.6 g of dried material was obtained for biological and chemical assays. The entire extract was separated by C₁₈ 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-C₁₈, 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

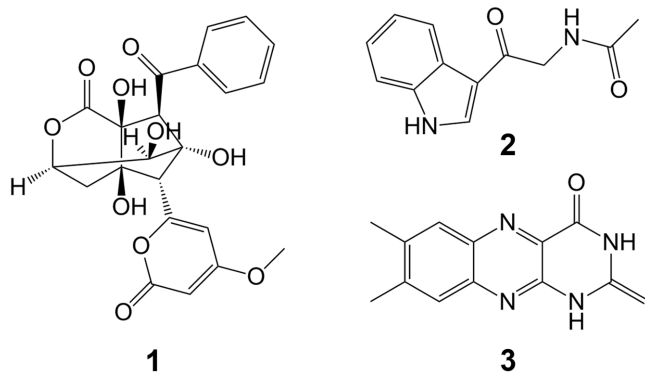


Fig. 1. Structures of compounds 1–3 isolated from marine-derived *Streptomyces* sp. MBTH32: enterocin (1), *N*-acetyl-β-oxotryptamine (2), and lumichrome (3).

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	<i>S. aureus</i> 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. IC₅₀ 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 IC₅₀ 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 IC₅₀ 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

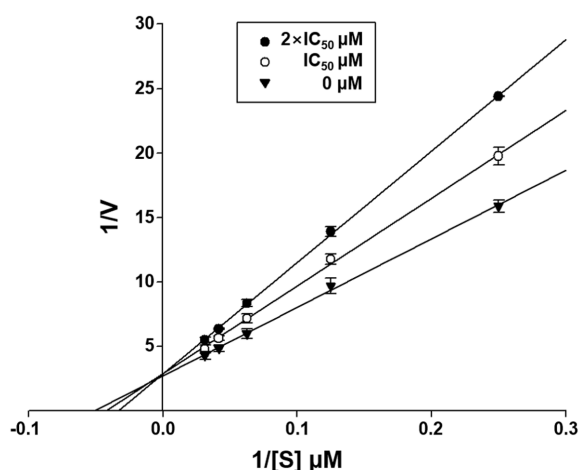


Fig. 2. Lineweaver-Burk plot of SrtA inhibition by compound 3. Compound 3 was applied at IC₅₀ and at 2× IC₅₀ concentrations. [S], reaction substrate concentration; V, reaction velocity (Δfluorescence/min). Each point indicates the mean ± SD of three independent experiments.

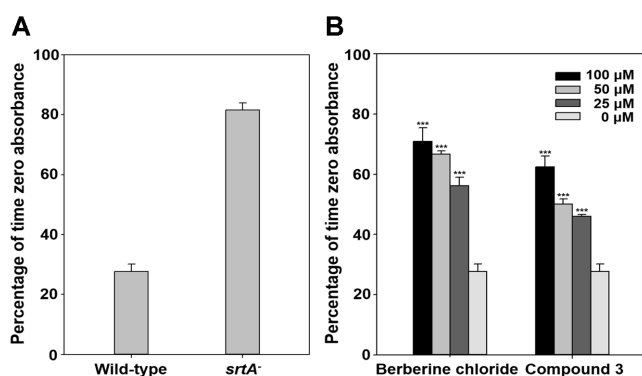


Fig. 3. Effects of *srtA* gene expression and SrtA inhibitors on the clumping of *S. aureus* with fibrinogen.

(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 \pm 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 μ M 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.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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