AN ABSTRACT OF THE THESIS OF

Katherine B. Bateman for the degree of Honors Baccalaureate of Science in Microbiology presented on May 27, 2008. Title: Identification of Small Molecule Inhibitors of the *Staphylococcus aureus* Sortase A Enzyme

Abstract approved: _____
Dennis Hruby

The Sortase A (SrtA) enzyme is a potential target for a new class of anti-infective drugs because it is responsible for anchoring virulence factors to the surface of pathogenic, Gram-positive bacteria such as *Staphylococcus aureus*. High throughput screening yielded low molecular weight compounds that inhibit purified SrtA from *S. aureus*. The efficacy of these compounds at targeting SrtA in live bacteria was successfully determined by developing sensitive biological assays that measure inhibition of sortase-dependent surface protein expression in *S. aureus*. Fibrinogen-clumping and fibrinogen-binding assays were developed to determine the presence of clumping factors (ClfA and B), a fibronectin-binding assay was used to determine the presence of fibrinogen-binding proteins (FnBPA and B), and dot blots were developed to measure protein A on the cell surface. Fifty-six compounds were identified that inhibit surface protein expression in at least one of the biological assays. The effectiveness of these compounds at treating infection will soon be tested *in vivo* using the *S. aureus* septic arthritis model in mice.

Key Words: Sortase, SrtA, *Staphylococcus aureus*, Gram-positive, LPXTG, virulence factor, Anti-infective, Anti-bacterial, Antibiotic resistance

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Identification of Small Molecule Inhibitors of the *Staphylococcus aureus* Sortase A Enzyme

By

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A PROJECT

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Microbiology (Honors Scholar)

Presented May 27, 2008
Commencement June 2008

Honors Baccalaureate of Science in Microbiology project of Katherine B. Bateman presented on May 27, 2008

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I understand that my project will become a part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

Katherine B. Bateman, Author

ACKNOWLEDGEMENTS

Thank you to all those who supported my research and helped me accomplish my goals, especially Dr. Dennis Hruby, Tove Bolken, Dr. Rebecca Wilson, Kira Lebsack, Afang Tang-Christianson, Jennifer Yoder, Cliff Gagnier, Dr. Kevin Ahern, and Dr. Indira Rajagopal, and all the wonderful people at Siga Technologies. I would like to thank all the other Sortase project contributors (past and present), including Kinjal Shah, Rob Jordan, Seva Katritch, Dana Kirkwood-Watts, Dongcheng Dai, Dylan Nelson, Will Weimers, Tracy Tan, Shanthakumar, John McCullough, Dan Rockey, and Kevin Jones. I specifically thank my committee, Dr. Dennis Hruby, Dr. Rebecca Wilson, and Dr. Kevin Ahern for helping me write this thesis. Finally, I thank Ben Hammond and my parents, Steve and Diane Bateman, for supporting me throughout college; especially for those nights they sat patiently in the lab waiting for me to finish experiments.

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Introduction

Pathogenic, Gram-positive bacteria such as *Staphylococcus aureus* are a threat to human health because they have developed resistance to most antibiotics. *S. aureus* is a major cause of hospital-acquired infections (16% in the United States) and can cause severe systemic infections such as endocarditis, osteomyelitis, and septic arthritis [1, 2]. A study by the Centers for Disease Control and Prevention showed that among clinical isolates of *S. aureus*, 95% are penicillin resistant, 50% are methicillin resistant, and there is a rising trend in vancomycin resistance, which is used as the drug of last resort to treat infections [3]. Therefore, identifying new anti-infective agents that reduce the pathogenicity of Gram-positive bacteria and decrease the risk of resistance development is of great importance.

Most bacterial pathogens use some form of surface appendage to adhere to host cells. Many surface proteins of Gram-positive organisms have a common anchoring sequence motif, LPXTG, which is the cleavage site for the cell wall sorting enzyme, sortase. Sortase acts by first recognizing the LPXTG motif, cleaving between threonine and glycine, then acting as an amidase to link the cleaved protein to the peptidoglycan layer of the cell wall [4]. Among the Gram-positive pathogens that use this anchoring pathway are *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*,

Streptococcus gordonii (Group B strep), *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Listeria monocytogenes*, *Enterococcus faecalis*, and *Bacillus anthracis* [4].

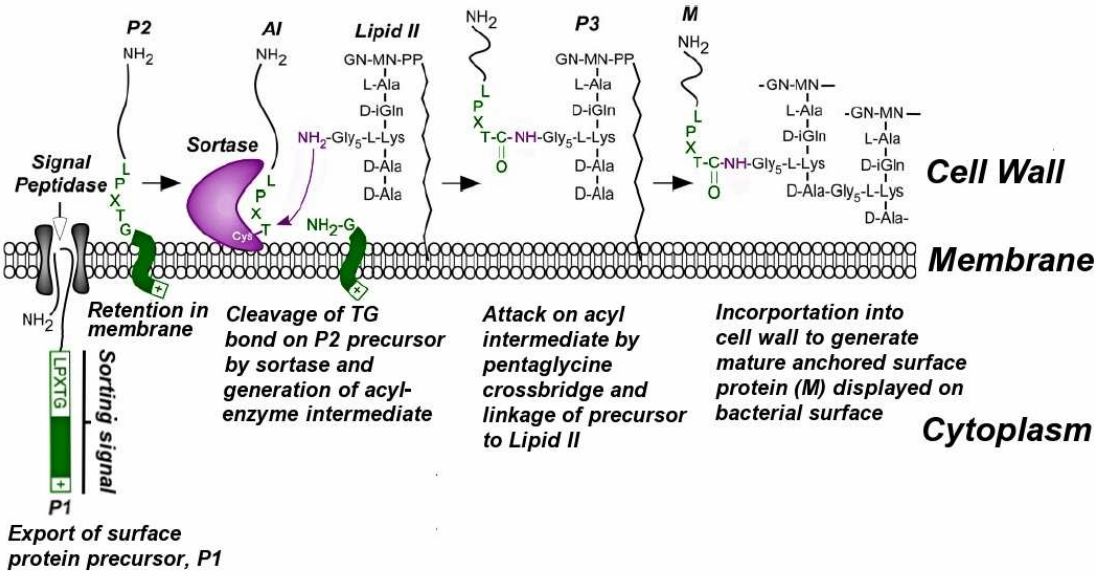

Figure 1. Surface protein sorting mechanism in *S. aureus* and other Gram positives. Sortase cleaves surface protein precursors between the threonine and glycine of the LPXTG motif and anchors them to the cell wall [adapted from Marraffini *et al*].

Some surface-expressed proteins of *S. aureus* covalently anchored by Sortase A include clumping factors (ClfA and B), fibronectin-binding proteins (FnBPA and B), and protein A. The role of these surface proteins in virulence and pathogenesis are as follows: ClfA and B are fibrinogen binding proteins that mediate fibrinogen-dependent adhesion and clumping of *S. aureus* cells on blood clots and damaged tissue. FnBPA and B mediate bacterial adhesion and invasion of epithelial cells, endothelial cells, fibroblasts, and osteoblasts by forming a fibronectin bridge to the fibronectin-binding integrin on the host cell surface [2]. Protein A binds IgG molecules in the wrong orientation (by the Fc region) which disrupts opsonization and phagocytosis [4].

The importance of the enzyme and its cleavage motif with respect to anchoring surface proteins in several organisms has been shown in a number of studies. In *S. aureus*, mutation of the anchoring sequence resulted in improper sorting of protein A to the cell surface. Deletion of the *srtA* gene in *S. aureus* results in a reduced display of surface proteins, as well as a decrease in virulence in mice [4]. A three-log-unit reduction of *S. aureus* growth in multiple organ abscesses is observed for *srtA* mutants in the murine model. In *S. gordonii*, deletion of a *srtA* homologue resulted in reduced surface protein expression and reduced the ability of the mutants to adhere to human pharyngeal epithelium *in vitro* and to colonize mice [4]. Deletion of *srtA* in *S. pneumoniae* also resulted in a virulence defect for murine pneumonia, bacteremia, and nasopharyngeal colonization. In *L. monocytogenes*, which possesses two sortase genes (*srtA* and *srtB*), mutation of the genes caused improper sorting of several surface proteins, including internalin A (InlA). Mutants were defective in entering epithelial cells *in vitro* and for colonizing the liver and spleen in mice. *B. anthracis* sortases are involved in virulence as well, evidenced by the impaired ability of *srtA* and *srtB* mutants to infect J774 macrophages [4].

Traditional antibacterial agents aim to kill or stop the growth of microbes. While these bactericidal compounds are very effective on susceptible organisms, the killing action of these drugs is compromised at low doses, effectively selecting for bacteria that are resistant to their designed action. To avoid drug resistance arising from such selective killing, compounds that act not by killing their intended targets, but rather by inhibiting their ability to place proteins needed for infection on their cell surfaces by targeting

sortase were investigated. Important attributes of these novel compounds are that they should provide no inhibition to the growth of target pathogens (avoiding the selection of resistant cells), but at clinical doses prevent the presentation of specific proteins on cell surfaces. Without these important proteins, the ability of treated cells to infect tissues will be impaired. Thus, these compounds should be anti-infective, but not bactericidal. Treatment with the new compounds should essentially lead to bacteria that will not be pathogenic and will be targets for destruction by the immune system.

The SrtA protein from *S. aureus* has been expressed and purified as a functional enzyme. The enzyme is used in a fluorescence quench biochemical assay for high-throughput screening to find sortase inhibitors. Compounds that appeared to inhibit sortase activity were selected for secondary screening. The IC_{50}s, or concentration at which the enzyme activity is inhibited by one half, and the CC_{50}s, or concentration at which mammalian cell viability is reduced by one half were determined. Compounds that did not inhibit bacterial growth were selected for further biological assays, as bactericidal compounds may lead to selection of drug resistant strains.

The goal of this research is to develop and use biological assays to determine the effectiveness of compounds that were identified in the high throughput screen at inhibiting surface expression of proteins in *S. aureus*. A fibrinogen-based clumping assay, a fibrinogen binding assay, a fibronectin binding assay, and a whole cell dot blot for protein A were developed to accomplish this objective.

Methods and Materials

Construction of Markerless *S. aureus* RN4220 *srtA* Mutant

A *Staphylococcus aureus* RN4220 *srtA* mutant was constructed to be used as a control in experiments and serve as a comparison for treatments with effective compounds. The gene encoding SrtA was deleted by cloning ~900 base pair regions upstream and downstream of *srtA* into the temperature-sensitive plasmid, pGhost6 (which confers erythromycin resistance). *S. aureus* was transformed with pGhost6:SrtAΔ and grown at 30ºC on brain heart infusion (BHI) plates containing 5 μg/ml erythromycin (BHI + Ery_5). Transformants were grown in BHI + Ery5 broth at 30ºC and the clone was confirmed by PCR. Clones were then grown in BHI + Ery5 at 42ºC (pre-warmed) overnight for three passes. This step forces the plasmid to integrate into the chromosome at the site of homology (upstream of the SrtA gene). Colonies were isolated on BHI + Ery5 plates pre-warmed to 42ºC and integration was confirmed by PCR using a primer upstream of the integration site (not included on pGhost) and another primer within the insert on the plasmid. Isolates were inoculated into 10 ml BHI (no Ery) and grown at 30ºC. In the absence of antibiotic selection the plasmid can resolve from the chromosome in such a way that leaves the *srtA* region deleted, and the plasmid is eventually lost. Cultures were serially passed at 30ºC in BHI. Every few passes, the culture was streaked on BHI (no Ery) plates at 37ºC and isolated colonies were replica-plated on BHI (no Ery) and BHI + Ery5 at 37ºC. Ery-sensitive colonies were confirmed as SrtAΔ mutants by DNA sequencing.

Specifics: *S. aureus* RN4220 genomic DNA was purified with the Qiagen DNeasy Kit and the region upstream of *srtA* was amplified by PCR with the primers KB5 (5'-CCG GGG TAC CTT AAG TAC ACA TTA GCT GTG GC-3') (*KpnI* restriction site is underlined) and KB6 (5'-GCC CAA GCT TAC GTT AAG GCT CCT TTT ATA CAT TTC-3') (*HindIII* restriction site is underlined). The region downstream of *srtA* was amplified with the primer KB7 (5'-GCC CAA GCT TTC TAT TAC GCT AAT GGA TGA ATA TAT TG-3') (*HindIII* restriction site is underlined) and KB8 (5'-CCG GGG ATC CCA ATA AAA TTA TTT ACA CGC TTG TTG-3') (*BamHI* restriction site I underlined). PCR products were purified using the QIAquick PCR Purification Kit. The upstream PCR product was digested sequentially by HindIII and KpnI restriction enzymes, and the downstream product was digested sequentially by HindIII and BamHI. Restricted products were isolated by gel extraction using a QIAquick Gel Extraction Kit. pGhost6 was purified from *E. coli* DH5α with a Qiagen Miniprep Kit then digested sequentially by HindIII and KpnI and isolated by gel extraction. The upstream region was ligated into pGhost6 and the ligation was transformed into *E. coli*. The insertion was confirmed in *E. coli* transformants by PCR with KB5 and KB6. pGhost6 containing the upstream insert was purified from *E. coli*, digested with HindIII and BamHI, and isolated by gel extraction. The BamHI-HindIII digested downstream product was ligated into the vector adjacent to the upstream region insert and *E. coli* transformants were confirmed by PCR using KB7 and KB8, and KB5 and KB8. The resultant plasmid was designated pGhost:SrtAΔ. The pGhost6:SrtAΔ vector was transformed into electrocompetent *S. aureus* RN4220. Transformants with the integrated vector were confirmed by PCR using primer set KB9 (5'-GTA TTT CTT ATG CAT GAG TTT TAC TC-3') and KB10 (5'-

CTC ATA CCA AAC AAA AAA CAG TC-3') to amplify the sequence between the *srtA* upstream region above the insert and the *srtA* downstream region within the insert. After excision and curing of the plasmid, mutants were confirmed by PCR using primers KB13 (5'-AAT GAA TTG CTA TGA GTC ATT TTG-3') and KB14 (5'-ATC AAA AGA AGA AGT CAT AAA TGA TTA C-3') to amplify sequence between the region upstream of *srtA* within the insert and the region downstream of *srtA* below the insert.

Biological Assays

Fibrinogen-based Clumping Assay for *S. aureus* Newman Strain

S. aureus Newman cultures were used for the clumping assays because it is the strain used in mouse models and a sortase mutant was available. *S. aureus* Newman wild-type (wt) and *S. aureus* Newman SrtA- (provided by Dr. Olaf Schneewind) were grown in 5 ml TSB in 50 ml conical tubes, shaking at 200 RPM, 37 ºC for 20 hours. As an untreated control, Newman wt was grown in 0.75% DMSO. The treatments were wild-type Newman + 75 μM compound (or less compound, with correct amount of DMSO added to bring DMSO concentration to 0.75%; compounds are 10 mM in 100% DMSO). As a positive control, the Newman SrtA- was grown in 0.75%DMSO. Cultures were harvested (1.5 ml culture, 14,000 rpm for 3 min at 4°C), the supernatant was discarded, and cells were suspended in 1 ml PBS (Cellgro brand, Dulbecco's Phosphate-buffered Saline 1x, no calcium, no magnesium). Cells were diluted in 3 ml PBS. The optical density at 600 nm (OD_{600}) was adjusted such that a 1:2 dilution in PBS had an absorbance between 1.6-1.7 (Eppendorf Biophotometer).

Fibrinogen solution: A 2X-concentrated fibrinogen solution was prepared by dissolving 2 mg fibrinogen/ml PBS (Sigma: Fraction I, Type I-S, from bovine plasma, 85% clottable protein) and vortexing thoroughly.

Experimental set-up: The OD-adjusted cells were added to 1 ml cuvettes in 500 µl aliquots and 500 µl of the 2X fibrinogen solutions was added at time 0. Readings were blanked against a 1X fibrinogen solution. For the "no fibrinogen" controls, 500 µl of PBS was added to 500 µl of cells and blanked against PBS. The cuvettes were incubated at 37°C and absorbance at 600 nm was read at time 0 and at 90 minutes. The decrease in OD at 90 minutes was graphed as a percent of the OD at time 0 for each treatment [5].

Fibrinogen- and Fibronectin-based Binding Assays for *S. aureus* RN4220

Nunc MaxiSorp 96 well Flat-bottom Immuno plates were coated with 100 µl/well of a fibrinogen or a fibronectin solution: 15 µg/ml PBS of fibrinogen (Sigma: Fraction I, Type I-S, from bovine plasma, 95% clottable protein) or 12.5 µg/ml PBS of fibronectin (Sigma: Fibronectin from human plasma). Plates were kept at 4ºC overnight and washed 2x with 200 µl/well PBS, blocked with 100ul 1% BSA in PBS for 1 hour at RT, and washed 3x with 200 µl PBS. 100 µl of *S. aureus* was added, prepared according to the following:

S. aureus RN4220 wild-type and RN4220 SrtAΔ were grown in 2 ml CDM in 15 ml round bottom tubes. As an untreated control, RN4220 wt was grown in 1% DMSO. The treatments were RN4220 wt + 100 µM compound. As a positive control: RN4220 SrtAΔ

was grown in 1% DMSO. Cells were subcultured from BHI (2 μl RN4220 wt or RN4220 SrtAΔ) into CDM and grown shaking at 225 RPM, 37ºC for 18 hours. Cultures were harvested (1.5 ml culture, 14,000 rpm for 3 min at 4°C), the supernatant was discarded, and cells were suspended in 1 ml PBS (no vortexing). Cells were diluted in 5 ml PBS to an OD_{600} of 1.3. All samples must have the same $OD_{600} \pm 0.05$ to ensure that the difference in binding between the treatments is due to the amount of surface protein per cell and not the number of cells.

Binding and detection of *S. aureus* cells: The fibrinogen- and fibronectin- coated plates were incubated with 100 μl/well of *S. aureus* cells at 37 ºC for 10 minutes. Plates were washed 3X with 200 μl/well PBS and dried. A 440 μM Alamar Blue solution was diluted in BHI 1:10 and 100ul of the BHI + Alamar Blue solution was added to each well. Plates were covered loosely and incubated at 37 ºC for 1.5-2 hours.

Analysis: Once color developed, absorbance of plates were read at 570 and 610 nm (Wallac spectrophotometer) and analyzed with the following formula: $(A_{570}-A_{610}) + X$, where $X = |A_{570}-A_{610}|$ of wells without cells. The values for the treatments were reported as the percent inhibition of binding compared to the wild type control.

Protein A Whole-Cell Dot Blot Assay for *S. aureus* RN4220

The samples from the fibrinogen/fibronectin binding assay protocol above (resuspended in PBS with adjusted OD_{600} 1.3 ± 0.05) were used for this assay. Two microliters of each sample was pipetted onto a 0.45 micron nitrocellulose membrane in replicate and allowed

to dry at room temperature. The membrane was incubated at 37°C for 15 min, then at 75°C for 15 min, and blocked for 1 hr with 5% dry, non-fat milk in tris-buffered saline (TBS) on a rocker. The membrane was washed 2X with TBS + Tween 20 (TTBS) and a 1:5000 dilution of mouse anti-protein A (Sigma: Monoclonal antibody A produced in mouse. Clone SPA-27, ascites fluid) was added (in antibody buffer: TTBS with 5% milk) for 1 hr, RT. The membrane was washed 3X with TTBS and antibody buffer with Goat-anti-mouse AP-conjugated secondary antisera (Bio-Rad: Goat Anti-mouse IgG (H + L)-AP Conjugate, catalog 172-1015) was added at a 1:1000 dilution for 30 minutes, RT. The membrane was washed 3X with TTBS and 2X with TBS and developed with AP developer (BioRad AP color development reagents). The color development was stopped by washing the membrane several times with DI water when the color for the SrtA mutants began to appear. The membrane was dried on Whatman paper. The blot was scanned and the dots were analyzed with the ImageJ program from NIH by measuring the density of the dots for each sample [6]. The value for each treatment was reported as the percent inhibition of Protein A compared to the wild type control.

Results and Discussion

Fibrinogen Clumping Assays

Many sortase inhibitors were identified in the *in vitro* HTS screen. It was important to examine whether these compounds could also inhibit sortase function in live bacteria. To accomplish this, the *in vitro* inhibitors were screened in the fibrinogen-based clumping assay using the *S. aureus* Newman strain. Compounds of interest should yield *S. aureus* cells that do not express fibrinogen clumping factor on their surface, consistent with the inhibition of sortase. Therefore, these treatments should resist fibrinogen-stimulated clumping. When clumping factor on the *S. aureus* surface converts fibrinogen into fibrin, the clumps fall out of solution resulting in a decrease in OD_{600} over time. An ideal sortase inhibitor will have an OD profile similar to the *S. aureus* Newman SrtA- sortase deficient cells (minimum clumping or precipitation) and denser than that of the wild type, untreated cells (significant clumping and precipitation). Figure 2 displays the percent OD at time 0 (after 90 minutes) for several compounds that are considered biological “hits” in the fibrinogen clumping assay.

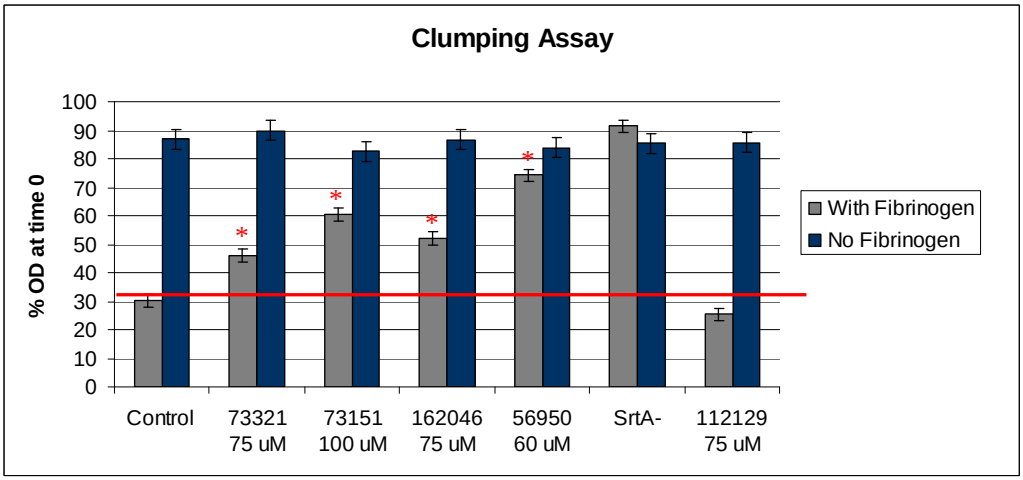

Figure 2. Fibrinogen Clumping Assay. The samples with fibrinogen (gray) show that the wild-type control clumps fibrinogen, resulting in a decrease in OD (30% of the original). Conversely, the *srtA* mutant does not clump fibrinogen, resulting in a constant OD over time. Treatment with compounds 73321, 73151, 162046, and 56950 (at the concentrations shown) result in a decrease in fibrinogen clumping. Compound 112129 is not a biological "hit" because it does not reduce fibrinogen clumping. The samples without fibrinogen (blue) are controls to determine the tendency of cells to fall out of solution in PBS alone over a course of 90 minutes.

The fibrinogen-based clumping assay was instrumental in detecting early biological hits, but it was time-consuming. As more potential sortase A inhibitors were identified in the high throughput screen, it was desirable to have a more high-throughput method to test for surface-sorting inhibition of fibrinogen binding proteins and fibronectin binding proteins. The following fibrinogen- and fibronectin-binding assays were developed with *S. aureus* RN4220 to expedite this search.

Fibrinogen- and Fibronectin-Binding Assays

Wild type *S. aureus* is able to bind to immobilized fibrinogen and fibronectin due to sortase-dependent surface expression of ClfA and B and FnbpA and B. A SrtA- mutant, however, has much lower affinity because it does not. If the compounds identified in the

HTS screen inhibit surface expression of sortase-dependent proteins, then wt cells should have decreased ability to bind to the fibrinogen- and fibronectin-coated plates. Alamar Blue yields a colorimeteric change and a fluorescent signal in response to metabolic activity, proportional to the amount of cells bound to the plates. Therefore, the wild-type *S. aureus* control wells turn pink, while cells grown in the presence of a sortase inhibitory compound yield wells with a blue-purple color, similar to the SrtA- control. Figure 3 shows the results of two binding assays.

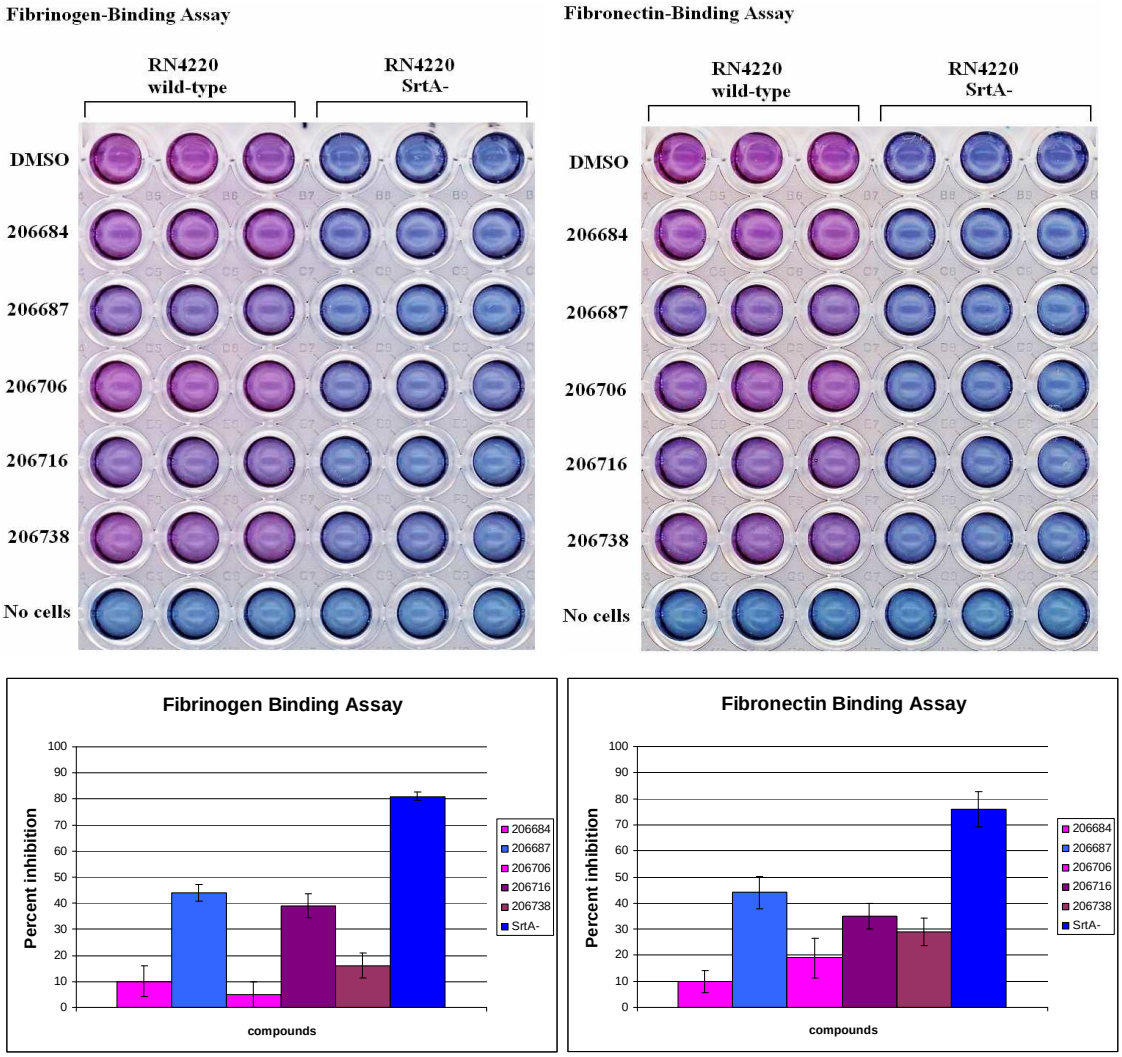

Figure 3. Fibrinogen- and Fibronectin-Binding Assays. The wild-type *S. aureus* DMSO control is pink and the *srtA* mutant control and treatments are blue. Wild-type treatment with compound 206687 (100 μM) inhibits *S. aureus* binding by 45% on fibrinogen- and fibronectin-coated plates. Compounds 206716 and 206738 (100 μM) also noticeably inhibit *S. aureus* binding to both plates.

After potential biological "hits" are detected and verified by the fibrinogen- and fibronectin-binding assays, a whole-cell dot blot is performed.

Whole-Cell Dot Blots for Protein A

The whole-cell dot blot is designed to measure the amount of sortase-sorted protein A on the *S. aureus* cell surface. The wild-type, untreated cells yield a signal for protein A (purple) that is 10 times higher than the signal in the *S. aureus* sortase mutant when measured by densitometry. Compounds that inhibit sortase A are expected to result in a decreased signal for protein A. The following image and graph show a dot blot experiment and the percent of protein A for each treatment relative to the wild-type control.

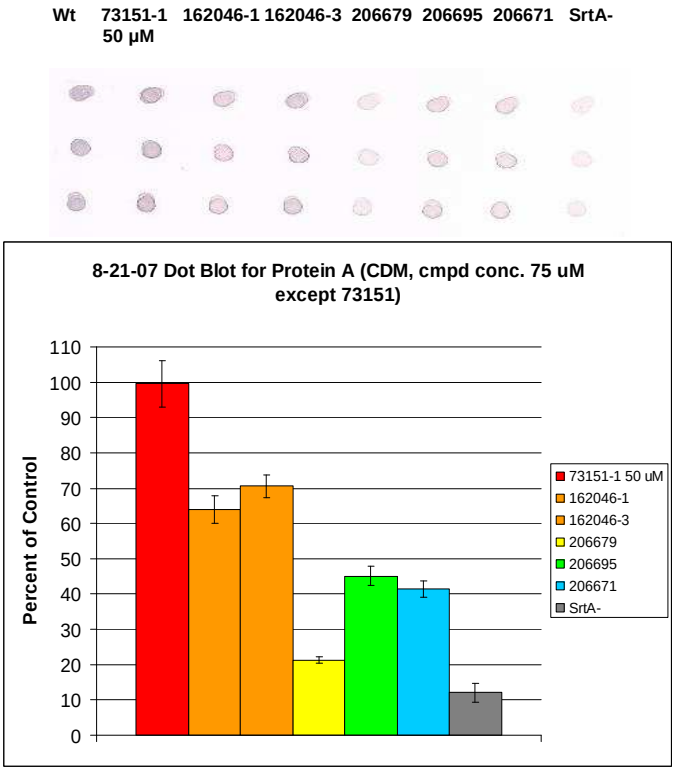

Figure 4. Dot Blot for Protein A. The amount of Protein A on the cell surface is partially reduced by compounds 162046, 206695, and 206671, and greatly reduced by compound 206679.

Data from the fibrinogen- and fibronectin-binding assays, dot blots, and IC_{50}s and CC_{50}s from secondary screening is compiled into a table for compounds that are considered

biological “hits.” It is important to show the compound concentration at which sortase activity is inhibited by 50% and the concentration at which 50% of human embryonic kidney tissue culture cells are killed. A potential anti-infective drug should exhibit a low IC_{50} (in the 1-5 μM range) and a high CC_{50} value (above 25 μM), as well as sortase A inhibition in the biological activity assays. On the following table, the numbers for each biological assay is the percent inhibition of surface protein expression or activity for each compound (a value of 0 indicates no inhibitory activity). The series name is the general chemical group to which the compound was assigned. The concentration of compound that was not inhibitory to *S. aureus* growth is in the far right column.

Compound ID	IC_{50} (µM)	CC_{50} (nM)	Fibrinogen-Plate	Fibronectin-Plate	Protein A	Series Name	Growth µM
ST-0073151	3	1.E+05	0	77	53	Thiophene series 3	75
ST-0073321	7	5.E+04	48	47	33	Sulfonamide	100
ST-0096222	4	8.E+12	35	36		Sulfonamide	100
ST-0126398	3	8.E+05	0	58	36	Sulfonamide/new thiophene	75
ST-0139762	1	3.E+04	26	0	21	New thiophene	100
ST-0195434	106	3.E+06	0	58	21	Thiophene series 3	100
ST-0195435	21	3.E+06	23	0	0	Thiophene series 3	100
ST-0195436	69	6.E+08	28	64	35	Thiophene series 3	25
ST-0195461	45	5.E+09	0	25	23	Thiophene series 3	50
ST-0195463	11	2.E+06	47	0	21	Thiophene series 3	100
ST-0195464	17	4.E+08	31	0	32	Thiophene series 3	100
ST-0195465	13	3.E+04	26	0	0	Thiophene series 3	100
ST-0195466	27	2.E+04	44	25	17	Thiophene series 3	100
ST-0195467	20	2.E+07	28	0	20	Thiophene series 3	100
ST-0195469	9	6.E+03	44	0	33	Thiophene series 3	100
ST-0195492	73	2.E+06	28	0		Thiophene series 3	100
ST-0206668	52	4.E+06	51	40	28	Thiophene series 3	100
ST-0206671	736	1.E+06	0	37	36	Sulfonamide	100
ST-0206674	68	3.E+10	0	30		Thiophene series 3	75
ST-0206676	72	1.E+05	31	48	19	Thiophene series 3	100
ST-0206679	92	4.E+04	25	40	34	Sulfonamide	100
ST-0206687	1056	2.E+07	25	21	53	Sulfonamide	100
ST-0206691	42	4.E+04	19	65	34	Sulfonamide	100
ST-0206696	90	4.E+10	9	50		Thiophene series 3	100
ST-0206705	35	1.E+06	3	22		Thiodiazol	100
ST-0206709	173	8.E+05	40	38		Sulfonamide/new thiophene	100
ST-0206712	814	9.E+05	23	73	17	Sulfonamide/new thiophene	100
ST-0206713	144	9.E+05	37	27		Thiophene series 3	50
ST-0206716	46	4.E+09	25	28	21	Sulfonamide	100
ST-0206724	23	9.E+05	16	77		Sulfonamide/new thiophene	25
ST-0206725	52	2.E+06	0	64		Sulfonamide/new thiophene	100
ST-0206726	45	9.E+07	42	48		Sulfonamide/new thiophene	100
ST-0206727	45	9.E+05	22	64		Sulfonamide/new thiophene	75
ST-0206728	29	9.E+05	30	63		Sulfonamide/new thiophene	100
ST-0206729	4	4.E+04	19	76	55	Sulfonamide/new thiophene	12
ST-0206731	27	9.E+05	44	67		Sulfonamide/new thiophene	25
ST-0206734	55	2.E+06	20	57		Sulfonamide/new thiophene	100
ST-0206739	91	8.E+05	55	50		Sulfonamide/new thiophene	75
ST-0206740	48	3.E+06	52	59		Sulfonamide/new thiophene	75
ST-0206744	29	3.E+04	31	72	35	Sulfonamide/new thiophene	12
ST-0206746	4	2.E+06	32	61	51	Sulfonamide/new thiophene	100
ST-0206747	31	9.E+05	10	23		Sulfonamide/new thiophene	100
ST-0206748	39	9.E+05	22	32	41	Sulfonamide/new thiophene	100
ST-0206749	49	9.E+05	0	25		Sulfonamide/new thiophene	100
ST-0206750	3	9.E+05	29	36	36	Sulfonamide/new thiophene	100
ST-0045837	14	6.E+05	0	33			100
ST-0092440	4	4.E+04	39	31	46		100
ST-0101604	8	7.E+06	31	10			100
ST-0109618	393	7.E+05	22	26			100
ST-0154260	4	2.E+06	26	12		Thiophene series 3	100
ST-0157880	14	3.E+04	23	7		Thiophene series 3	100
ST-0161348	12	9.E+05	21	32			100
ST-0179986	0	1.E+05	30	0			75
ST-0182042	3	4.E+05	26	18			100
ST-0185118	31	5.E+04	44	50			100
ST-0187206	7	8.E+03	45	9			100

Figure 5. Summary of Biological Data. Compounds that exhibit an IC_{50} below 15 μM are highlighted blue. Compounds that result in inhibition of surface protein or surface protein-associated activity (consistent with sortase inhibition) greater than 50% are highlighted yellow. Some compounds included on this table are structural analogs to compounds identified as sortase inhibitors in high throughput screening. Compounds that do not exhibit inhibition in any of the biological assays are not included on this table.

Conclusion

New classes of drugs are needed to treat infections from Gram-positive bacteria due to the emergence of widespread antibiotic resistance. The sortase enzyme is a target for anti-infective compounds due to its essential role in sorting and anchoring surface proteins that aid in pathogenesis. According to Marresso *et al* (2007), "Evaluation of the *in vivo* inhibition of sortase in *S. aureus* and *B. anthracis* requires the development of assays with improved sensitivity and specificity over those that are currently available." This research shows that fibrinogen clumping assays, fibrinogen- and fibronectin-binding assays, and dot blots for protein A serve as sensitive biological assays that measure inhibition of sortase-dependent surface protein expression in *S. aureus* Newman (clumping assays) and *S. aureus* RN4220. Fifty-six compounds were identified that inhibit surface protein expression in at least one of the biological assays. Compounds that exhibit strong inhibition in biological assays and appropriately low IC_{50}s will be further optimized by examining structure-activity relationships (SAR) and conducting hit-to-lead chemistry modifications. Their efficacy will then be tested *in vivo* using the *S. aureus* septic arthritis model in mice, prior to the initiation of clinical studies.

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