Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 380–385
Bioorganic & Medicinal Chemistry Letters

Identification of novel inhibitors of bacterial surface enzyme *Staphylococcus aureus* Sortase A

Bala Chandra Chenna,[a] Bidhan A. Shinkre,[a] Jason R. King,[a] Aaron L. Lucius,[a] Sthanam V. L. Narayana[b] and Sadanandan E. Velu[a,*]

[a]*Department of Chemistry, University of Alabama at Birmingham, 901 14th Street South, Birmingham, AL 35294, USA*
[b]*Center for Biophysical Sciences and Engineering, School of Optometry, University of Alabama at Birmingham, 1025 18th Street South, Birmingham, AL 35294, USA*

Received 12 September 2007; revised 12 October 2007; accepted 15 October 2007
Available online 18 October 2007

Abstract—In-silico virtual screening of bacterial surface enzyme *Staphylococcus aureus* Sortase A against commercial compound libraries using FlexX software package has led to the identification of novel inhibitors. Inhibition of enzyme catalytic activity was determined by monitoring the steady state cleavage of a model peptide substrate. Preliminary structure activity relationship studies on the lead compound resulted in the identification of compounds with improved activity. The most active compound has an IC_{50} value of 58 μM against the enzyme.

Many Gram-positive bacteria are human pathogens. *Staphylococci* are responsible for more than one million hospital-acquired bacterial infections every year. Compounding their importance is the recent, steep increase in multi-drug resistance found in bacteria and the potential of 'drug-resistant bacteria' to be used in biowarfare and bioterrorism.[1,2] Hospital strains of *Staphylococcus aureus* are usually resistant to a variety of different antibiotics. A few strains are resistant to all clinically useful antibiotics except vancomycin, and vancomycin-resistant strains are increasingly reported.[3–5] Methicillin-resistant *S. aureus (MRSA)* is widespread, and most methicillin-resistant bacterial strains are also resistant against multiple antibiotics.[6–8] This necessitates the need for identifying new therapeutic agents, which do not promote the emergence of drug-resistant microbes.

New approaches for the prevention and treatment of bacterial infections require greater understanding of the molecular structure and mechanisms of the chosen intervention targets and of the pathogenic role played by the target in the infection process. Bacterial infections are very complex and involve the action of a large, sophisticated arsenal of virulence factors, many of which are surface-bound or secreted. Gram-positive bacteria such as *staphylococci* are endowed with a multitude of cell-wall anchored proteins that serve as an interface between the microbe and its host. Bacterial sortases are cysteine transpeptidases that participate in secretion and anchoring of many cell-wall proteins by a mechanism conserved in almost the entire class of Gram-positive bacteria. Sortases, because of their control over the cellular location of multiple virulence factors, are an attractive potential target for interrupting virulence versus bacterial growth.

Surface proteins can be attached to the bacterial surface in one of several fashions.[9,10] Proteins that are covalently attached to the cell-wall share conserved regions known as the 'sorting signal' or cell-wall anchors.[11,12] The sorting signal includes a conserved amino acid motif, usually LPXTG. Precursor proteins are directed into a secretory pathway by their N-terminal signal peptides. They are translocated across the membrane and the signal peptide is cleaved.[12,13] Then, the C-terminal sorting signal retains the protein in the secretory pathway. The enzyme sortase acts at this point to cleave the protein between the threonine (T) and the glycine (G) of the LPXTG motif.[14] The carboxyl group of the Thr is then amide-linked to the amino group of a 'cross-bridge' peptide in the lipid II precursor for cell-wall synthesis.[15]

Keywords: *Staphylococcus aureus*; Sortase A; Inhibitors; Virtual screening.
* Corresponding author. Tel.: +1 205 975 2478; fax: +1 205 934 2543; e-mail: svelu@uab.edu

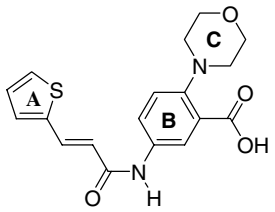
0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.10.051

Sortase-defective strains of various pathogens were shown to be faulty in the display of surface proteins and are less virulent.[16,17] In a number of studies, individual sortase genes have been deleted and the loss of sortase function resulted in less virulence in several animal models of the disease.[16,18–22]

Due to their universal presence in Gram-positive bacteria, sortases are attractive pharmacotherapeutic targets.[10] Currently there have only been a few reports of specific sortase inhibitors.[23–26] Recently, Oh et al.[27] identified a small-molecule reversible inhibitor with a low micromolar IC_{50} value by structurally modifying a lead compound identified by random screening of a group of small molecules. We have recently determined the high resolution crystal structure of recombinant *S. aureus* $SrtA_{\Delta 59}$,[28] which is a fully active variant of SrtA with 59 residues shorter from the NH-terminus. Utilizing our $SrtA_{\Delta 59}$ crystal structure, we initiated structure based inhibitor design. This manuscript describes the discovery of micromolar inhibitors of Sortase A identified by in-silico virtual screening and preliminary structure activity relationship (SAR) studies.

We have conducted virtual screening of commercial small-molecule libraries from Maybridge and ChemBridge (~150,000 compounds) against *S. aureus* $SrtA_{\Delta 59}$ active site using the FlexX software package integrated in SYBYL 7.0.[29–31] FlexX is a fast, flexible docking program that uses an incremental construction algorithm to place ligands into the active site of the receptor. In FlexX docking, the enzyme active site environment is identified by a receptor description file (RDF). We created the RDF for $SrtA_{\Delta 59}$ using the chain A of $SrtA_{\Delta 59}$ PDB file, the 'active site file' which is constituted of all residues within 6.5 Å distance from the center of the active site and a 'pocket file' which is constituted of a few hand-picked residues that are directly interacting with the substrate peptide. 2D compound data sets from commercial sources were transformed into three-dimensional molecular structures using the Concord program. All compounds were generated in the protonated state that can be assumed under physiological conditions. The following limits were applied for the virtual screening: Molecular weight >150 <500, $C\log P < 5$. In addition, compounds bearing metal ions were omitted from the data set. The structures of top scoring compounds were manually examined for their drug like properties based on Lipinski's rule[32] and for synthetic versatility. 108 best scoring compounds were purchased and screened against $SrtA_{\Delta 59}$ using a previously reported fluorescence resonance energy transfer (FRET) enzymatic assay.[27,33,34] In this assay, the IC_{50} values were determined by monitoring the effect of the compounds on the steady state cleavage of a model substrate peptide, Dabcyl-QALPETGEE-EDANS. Eight out of 108 compounds exhibited in vitro inhibition catalytic activity of $SrtA_{\Delta 59}$ with IC_{50} values ranging from 75 to 400 μM. The most active compound identified from this virtual screening is compound **1** (Fig. 1), which had an IC_{50} value of 75 ± 4.1 μM.

IC_{50} values of compounds **2**–**8** and their structures are given in Table 1.



Inhibitor **1**, IC_{50} = 75 ± 4.1 μM

Figure 1. Structure and IC_{50} value of inhibitor **1**.

A FlexX docking model of inhibitor **1** revealed several critical interactions of the inhibitor within the active site (Fig. 2).

The morpholine ring oxygen atom has a hydrogen bonding interaction with amide backbone NH of Trp194. The cysteine residue (Cys184) is at 2.94 Å distance from the morpholine ring carbon atom next to the oxygen. The carboxylic acid on the middle phenyl ring has a direct salt bridge interaction with the guanidine side chain of Arg197. The amide NH group of the linker has a hydrogen bonding interaction with the carboxylic acid side chain of Glu105, and the amide carbonyl oxygen of the linker has a hydrogen bonding interaction with the OH side chain of Ser116.

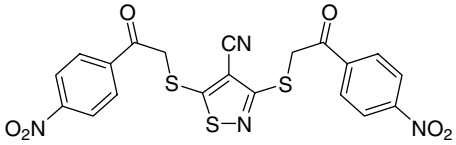
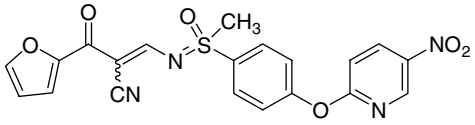
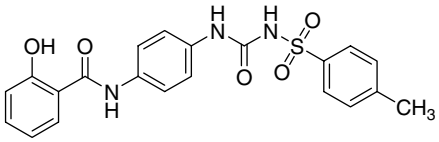
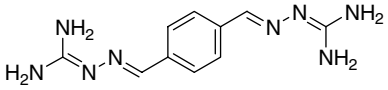
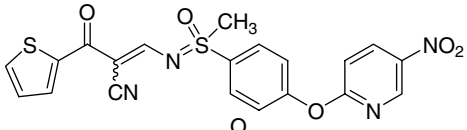
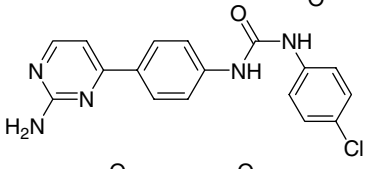
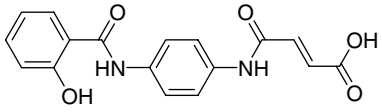
The thiophene ring (ring A) of inhibitor **1** is in close proximity to Gln172 (3.2 Å) and Asn114 (3.1 Å). Appropriate substitution to the thiophene ring may result in additional hydrogen bonding interactions with Gln172 and Asn114. In addition, a wide range of hydrophobic residues (Val201, Phe200, Ile199, Leu169, Ile158, Val168, Val164, Leu179, Ile115, Leu181, Ile182, Ile117, Leu97, and Phe103) surround the middle phenyl ring B on either side.

Inhibitor **1** was initially obtained from a commercial source in milligram quantities. For SAR studies we needed this compound in larger quantities. So, a general method for the synthesis of inhibitor **1** was developed in our laboratory. The synthetic procedures for all analogs were modeled around this synthesis. Chemistry employed for the synthesis of inhibitor **1** is outlined in Scheme 1.

Commercially available methyl 2-(*N*-morpholino)-5-nitrobenzoate (**9**) was reduced using hydrogen in the presence of Pd/C in anhydrous ethyl acetate to afford the corresponding amino compound **10**. The compound **10** was coupled with commercially available *trans*-3-(thiophene-2-yl)acrylic acid (**11a**) in the presence of ethyl(*N*,*N*-dimethylaminopropyl) carbodiimide (EDAC) and *N*,*N*-dimethylaminopyridine (DMAP) in 1,2-dichloroethane to form the amide compound **12a**.[35] Basic hydrolysis of the ester methyl group present in compound **12a** afforded the inhibitor **1** as a white crystalline solid.

We have synthesized a few derivatives of inhibitor **1** in order to derive preliminary structure activity relationship data. Their activities against $SrtA_{\Delta 59}$ were deter-

Table 1. *S. aureus* $SrtA_{\Delta 59}$ inhibitors identified from the FlexX virtual screening and their IC_{50} values

Compound	Structure	IC_{50}[a] (μM)
2		97.3 ± 7.6
3		150 ± 4.8
4		226 ± 16.7
5		275 ± 11.8
6		400 ± 6.5
7		400 ± 41.1
8		125 ± 3.6

[a] IC_{50} values were determined by fluorescence resonance energy transfer (FRET) assay. Measurements were carried out in triplicate and an average value with standard deviation is reported.

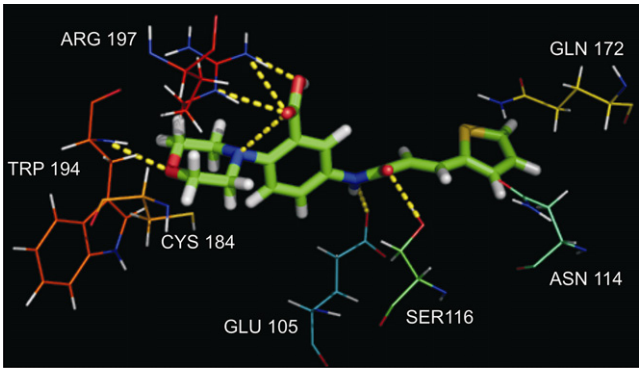
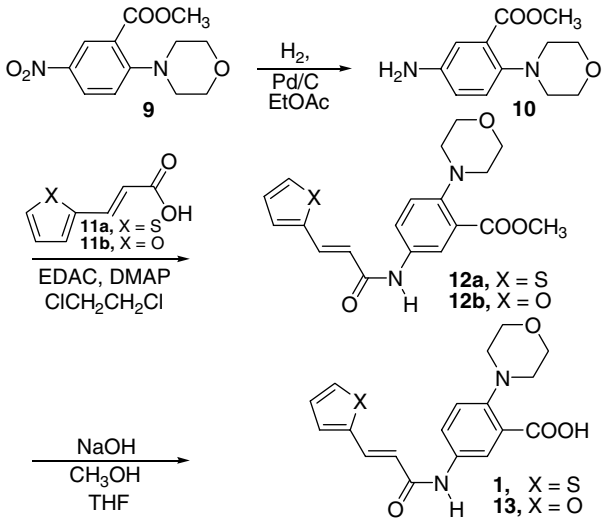


Figure 2. FlexX docking model showing the interactions of inhibitor **1** with amino acid residues in $SrtA_{\Delta 59}$ active site.

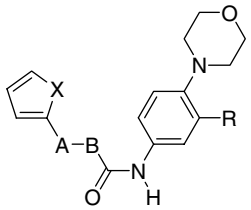
mined using the FRET assay. Structures and IC_{50} values of the newly synthesized derivatives of inhibitor **1** are given in Table 2.

Compound **13** is a furan analog of inhibitor **1**. Design of this structure is inspired by the comparison of activities of thiophene and furan compounds **3** and **6** (Table 1). These two compounds have identical structures except for the fact that one contained a furan ring and the other contained a thiophene ring. Furan compound, **3**, is more than twofold more active than the thiophene compound,



Scheme 1. Synthesis of inhibitor **1** and its furan analog **13**.

Table 2. *S. aureus* $SrtA_{\Delta 59}$ inhibition of synthesized derivatives of inhibitor **1**

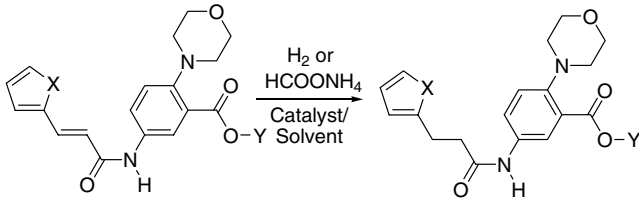


No.	X	A–B	R	IC_{50} (μM)
12a	S	CH=CH	$COOCH_3$	71 ± 2.5
12b	O	CH=CH	$COOCH_3$	58 ± 4.9
13	O	CH=CH	COOH	181 ± 14
14	S	CH_2–CH_2	COOH	>600
15	S	CH_2–CH_2	$COOCH_3$	>600
16	O	CH_2–CH_2	COOH	>600
17	O	CH_2–CH_2	$COOCH_3$	>600
18a	S	CH=CH	CH_2OH	73 ± 2
18b	O	CH=CH	CH_2OH	111 ± 5.7
19a	S	CH=CH	CHO	77 ± 6.5
19b	O	CH=CH	CHO	107 ± 8.5
20	S	CH=CH	$CONH_2$	105 ± 3.7

6. So, we expected that a furan substitution of the thiophene ring of inhibitor **1** may increase its activity. Compound **13** was synthesized by a similar procedure as described for the synthesis of inhibitor **1** (Scheme 1). We have used *trans*-3-(furan-2-yl) acrylic acid (**11b**) instead of *trans*-3-(thiophene-2-yl) acrylic acid (**11a**) in this procedure. Compound **10** was coupled with commercially available *trans*-3-(furan-2-yl) acrylic acid (**11b**) in the presence of EDAC and DMAP in 1,2-dichloroethane to form the amide compound **12b**. Basic hydrolysis of the ester methyl group present in compound **12b** afforded compound **13**. Compound **13** along with the synthetic intermediate methyl esters **12a** and **12b** were evaluated for their enzyme inhibitory activity (Table 2). Compound **13** did not show an enhancement of activity. Instead, it was found to be less active compared to inhibitor **1** in the enzymatic assay (IC_{50} = 181 μM). But, the methyl ester intermediates (**12a** and **12b**) showed improved inhibition as compared to the corresponding acid derivatives **1** and **13**. The methyl ester derivative of the thiophene compound (**12a**) showed an IC_{50} value of 71 μM and methyl ester derivative of the furan compound (**12b**) showed a much improved inhibition at an IC_{50} value of 58 μM. Although this increase in activity of methyl ester analogs is counter intuitive of the FlexX model which shows a possible salt bridge interaction between the carboxylic acid and Arg197 side chain, the increased activity may be explained using the FlexX model of the methyl ester in the active site. This model shows a similar fit of the molecule in the active site with the ester methyl group resting in a small-hydrophobic pocket alongside the central phenyl ring without completely destroying the electrostatic interaction between Arg197 side chain and ester group O atoms of the inhibitor. The increased activity could be a result of these additional hydrophobic interactions.

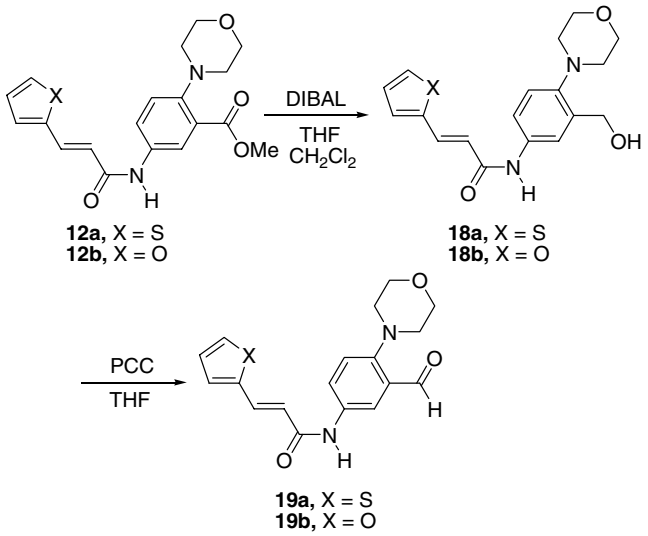
Compounds **14**–**17** were synthesized to evaluate the importance of the double bond present in compounds **1** and **13** for their activity. Compounds **14** and **16** are the saturated analogs of the carboxylic acid derivatives **1** and **13**. Compounds **15** and **17** are the saturated analogs of the methyl ester derivatives **12a** and **12b**. Our assay showed that all four saturated compounds **14**–**17** were inactive up to a concentration of 600 μM, showing that the double bond is necessary for the activity of these inhibitors (Table 2). Synthesis of compounds **14**–**17** is outlined in Scheme 2. Hydrogenation of the thiophene derivatives **1** and **12a** using Pd/C as catalyst did not work, possibly due to the catalyst poisoning effect of the thiophene ring present in these molecules. Use of a stronger catalyst like Pd black in the presence of ammonium formate resulted in the hydrogenation of **1** and **12a** to corresponding hydrogenated compounds **14** and **15**. Hydrogenation of **13** and **12b** was carried out under H_2 in the presence of Pd/C catalyst to afford compounds **16** and **17**.

We have also made a few derivatives of inhibitor **1** by incorporating different substituents such as a $–CH_2OH$

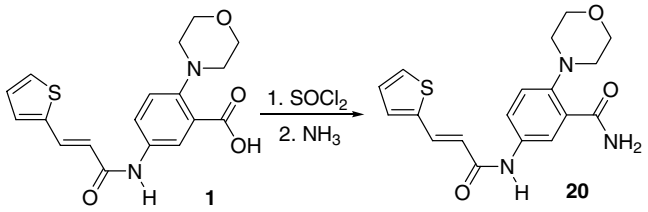


Reactant	X	Y	Catalyst /Solvent	Product	Yield (%)
1	S	H	Pd black/MeOH	**14**	69
12a	S	CH_3	Pd black/MeOH	**15**	82
13	O	H	Pd/C/EtOAc	**16**	88
12b	O	CH_3	Pd/C/EtOAc	**17**	77

Scheme 2. Synthesis of compounds **14**–**17**.



Scheme 3. Synthesis of compounds **18a,b** and **19a,b**.



Scheme 4. Synthesis of compound **20**.

(**18a**), –CHO (**19a**), or $–CONH_2$ (**20**) in the place of the carboxylic acid group. Substitution with $–CH_2OH$ and –CHO groups did not result in a major change in the activity (**18a**, IC_{50} = 73 μM and **19a**, IC_{50} = 77 μM), while substitution with $–CONH_2$ group resulted in a decrease in activity as compared to inhibitor **1** (**20**, IC_{50} = 105 μM) (Table 2). Derivatives of Furan compound **13** incorporating substituents such as a $–CH_2OH$ (**18b**) and –CHO (**19b**) in the place of the carboxylic acid group were also made. These compounds showed improved inhibition as compared to the parent furan compound, **13** (Table 2). Compound **18b** showed an IC_{50} value of 111 μM, and compound **19b** showed an IC_{50} value of 107 μM. Synthesis of compounds **18a,b** and **19a,b** is outlined in Scheme 3.

Compounds **12a,b** were reduced using DIBAL in a mixture of anhydrous dichloromethane and THF to afford the alcohol derivatives **18a,b**. Oxidation of alcohols using PCC in anhydrous THF afforded the aldehydes, **19a,b**. Synthesis of amide **20** is outlined in Scheme 4.

Compound **20** was prepared from the inhibitor **1** by treatment with $SOCl_2$, followed by the treatment of the acid chloride produced with ammonia.

Strikingly, the IC_{50} values determined for all of the active compounds are well below the previously measured K_m value of 5.5 mM for SrtA binding to the LPXTG peptide.[33] This suggests that these inhibitors bind ~1–2 orders of magnitude tighter than the LPXTG peptide and thus should be effective at blocking the enzymes' activity in vivo.

In conclusion, we have discovered a novel class of small-molecule inhibitors of *S. aureus* SrtA using in-silico virtual screening. We utilized the software package FlexX integrated in SYBYL 7.0 to carry out the virtual screening of commercial compound libraries against the SrtA active site. Inhibitors were screened for their activity against SrtA using a previously reported FRET assay. Micromolar inhibitors of the enzyme are identified. We have carried out preliminary structure activity relationship studies that have resulted in the identification of inhibitors with improved activity. Further SAR studies and attempts to obtain high resolution inhibitor/SrtA complex co-crystal structures are currently in progress.

Acknowledgments

Authors acknowledge the generous financial support from the Department of Chemistry, University of Alabama at Birmingham, and a faculty development grant from UAB-FDGP to S.E.V.

References and notes

1. Pappas, G.; Panagopoulou, P.; Christou, L.; Akritidis, N. *Infect. Dis. Clin. North Am.* **2006**, *20*, 395.
2. Clarke, S. C. *Br. J. Biomed. Sci.* **2005**, *62*, 40.
3. Mohr, J. F.; Murray, B. E. *Clin. Infect. Dis.* **2007**, *44*, 1536.
4. Webster, D.; Rennie, R. P.; Brosnikoff, C. L.; Chui, L.; Brown, C. *Diagn. Microbiol. Infect. Dis.* **2007**, *57*, 177.
5. Weigel, L. M.; Donlan, R. M.; Shin, D. H.; Jensen, B.; Clark, N. C.; McDougal, L. K.; Zhu, W.; Musser, K. A.; Thompson, J.; Kohlerschmidt, D.; Dumas, N.; Limberger, R. J.; Patel, J. B. *Antimicrob. Agents Chemother.* **2007**, *51*, 231.
6. Baggett, H. C.; Hennessy, T. W.; Rudolph, K.; Bruden, D.; Reasonover, A.; Parkinson, A.; Sparks, R.; Donlan, R. M.; Martinez, P.; Mongkolrattanothai, K.; Butler, J. C. *J. Infect. Dis.* **2004**, *189*, 1565.
7. Deresinski, S. *Clin. Infect. Dis.* **2005**, *40*, 562.
8. Salerno, D.; Vahid, B.; Marik, P. E. *Ann. Thorac. Surg.* **2007**, *83*, 1888.
9. Navarre, W. W.; Schneewind, O. *Microbiol. Mol. Biol. Rev.* **1999**, *63*, 174.
10. Cossart, P.; Jonquieres, R. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5013.
11. Fischetti, V. A.; Pancholi, V.; Schneewind, O. *Mol. Microbiol.* **1990**, *4*, 1603.
12. Schneewind, O.; Model, P.; Fischetti, V. A. *Cell* **1992**, *70*, 267.
13. Schneewind, O.; Mihaylova-Petkov, D.; Model, P. *EMBO J.* **1993**, *12*, 4803.
14. Navarre, W. W.; Schneewind, O. *Mol. Microbiol.* **1994**, *14*, 115.
15. Perry, A. M.; Ton-That, H.; Mazmanian, S. K.; Schneewind, O. *J. Biol. Chem.* **2002**, *277*, 16241.
16. Jonsson, I. M.; Mazmanian, S. K.; Schneewind, O.; Bremell, T.; Tarkowski, A. *Microbes Infect.* **2003**, *5*, 775.

17. Mazmanian, S. K.; Liu, G.; Jensen, E. R.; Lenoy, E.; Schneewind, O. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5510.
18. Barnett, T. C.; Scott, J. R. *J. Bacteriol.* **2002**, *184*, 2181.
19. Bierne, H.; Mazmanian, S. K.; Trost, M.; Pucciarelli, M. G.; Liu, G.; Dehoux, P.; Jansch, L.; Garcia-del Portillo, F.; Schneewind, O.; Cossart, P. *Mol. Microbiol.* **2002**, *43*, 869.
20. Bolken, T. C.; Franke, C. A.; Jones, K. F.; Zeller, G. O.; Jones, C. H.; Dutton, E. K.; Hruby, D. E. *Infect. Immun.* **2001**, *69*, 75.
21. Garandeau, C.; Reglier-Poupet, H.; Dubail, I.; Beretti, J. L.; Berche, P.; Charbit, A. *Infect. Immun.* **2002**, *70*, 1382.
22. Jonsson, I. M.; Mazmanian, S. K.; Schneewind, O.; Verdrengh, M.; Bremell, T.; Tarkowski, A. *J. Infect. Dis.* **2002**, *185*, 1417.
23. Kang, S. S.; Kim, J. G.; Lee, T. H.; Oh, K. B. *Biol. Pharm. Bull.* **2006**, *29*, 1751.
24. Kim, S. H.; Shin, D. S.; Oh, M. N.; Chung, S. C.; Lee, J. S.; Chang, I. M.; Oh, K. B. *Biosci., Biotechnol., Biochem.* **2003**, *67*, 2477.
25. Kim, S. W.; Chang, I. M.; Oh, K. B. *Biosci., Biotechnol., Biochem.* **2002**, *66*, 2751.
26. Scott, C. J.; McDowell, A.; Martin, S. L.; Lynas, J. F.; Vandenbroeck, K.; Walker, B. *Biochem. J.* **2002**, *366*, 953.
27. Oh, K. B.; Kim, S. H.; Lee, J.; Cho, W. J.; Lee, T.; Kim, S. *J. Med. Chem.* **2004**, *47*, 2418.
28. Zong, Y.; Bice, T. W.; Ton-That, H.; Schneewind, O.; Narayana, S. V. *J. Biol. Chem.* **2004**, *279*, 31383.
29. Kramer, B.; Rarey, M.; Lengauer, T. *Proteins* **1999**, *37*, 228.
30. Rarey, M.; Kramer, B.; Lengauer, T. *Bioinformatics* **1999**, *15*, 243.
31. Rarey, M.; Kramer, B.; Lengauer, T.; Klebe, G. *J. Mol. Biol.* **1996**, *261*, 470.
32. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3.
33. Kruger, R. G.; Dostal, P.; McCafferty, D. G. *Anal. Biochem.* **2004**, *326*, 42.
34. Ton-That, H.; Mazmanian, S. K.; Faull, K. F.; Schneewind, O. *J. Biol. Chem.* **2000**, *275*, 9876.
35. Skotnicki, J. S.; Kearney, R. M.; Smith, A. L. *Tetrahedron Lett.* **1994**, *35*, 197.