Bioorganic & Medicinal Chemistry Letters 28 (2018) 1864–1869

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Quinone skeleton as a new class of irreversible inhibitors against *Staphylococcus aureus* sortase A

Xiaochen Hou [a,b,f], Meining Wang [c,f], Yi Wen [d,f], Tengfeng Ni [a], Xiangna Guan [e], Lefu Lan [a], Naixia Zhang [d,*], Ao Zhang [c,*], Cai-Guang Yang [a,*]

[a] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 ZuChongZhi Road, Shanghai 201203, China
[b] University of the Chinese Academy of Sciences, 19A Yuquan Road, Beijing 100049, China
[c] CAS Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 ZuChongZhi Road, Shanghai 201203, China
[d] Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 ZuChongZhi Road, Shanghai 201203, China
[e] Ministry of Education, School of Pharmacy, Fudan University, 826 ZhangHeng Road, Shanghai 201203, China

ARTICLE INFO

Article history:
Received 29 January 2018
Revised 30 March 2018
Accepted 3 April 2018
Available online 4 April 2018

Keywords:
Staphylococcus aureus
Anti-virulence
Sortase A
Covalent inhibitor
Quinone skeleton

ABSTRACT

Sortase A (SrtA) anchors surface proteins to the cell wall and aids biofilm formation during infection, which functions as a key virulence factor of important Gram-positive pathogens, such as *Staphylococcus aureus*. At present researchers need a way in which to validate whether or not SrtA is a druggable target alternative to the conventional antibiotic targets in the mechanism. In this study, we performed a high-throughput screening and identified a new class of potential inhibitors of *S. aureus* SrtA, which are derived from natural products and contain the quinone skeleton. Compound **283** functions as an irreversible inhibitor that covalently alkylates the active site Cys184 of SrtA. NMR analysis confirms the direct interaction of the small-molecule inhibitor towards SrtA protein. The anchoring of protein A (SpA) to the cell wall and the biofilm formation are significantly attenuated when the *S. aureus* Newman strain is cultured in the presence of inhibitor. Our study indicates that compound **283** could be a potential hit for the development of new anti-virulence agents against *S. aureus* infections by covalently targeting SrtA.

Antimicrobial resistance of important Gram-positive pathogens, which usually cause severe infections, is a major worldwide health problem.[1] Results of resistance are the reduced efficacy of antimicrobial drugs, thus making the treatment of bacterial infections difficult, costly, or even impossible.[2] Since conventional antibiotics cannot effectively counteract bacterial pathogens and have resulted in increased drug resistance, some researchers have tried to treat infections by way of new strategies focusing on agents that target the virulence of important pathogenic bacteria without inhibiting their growth.[3,4]

Gram-positive pathogenic bacteria interact with host cell and tissues with displayed surface proteins.[5] Many critical steps of bacterial virulence, such as adherence, immune evasion and immunosuppression, are associated with plenty of bacterial cell wall proteins and protein complexes.[6] Sortase A (SrtA) accomplishes the anchoring process of surface proteins.[7] SrtA has been well characterized as an ideal target of anti-virulence.[8] *srtA* knockout mutants have deficits in surface expression of various LPXTG motif proteins and the display of surface proteins, which result in the reduced ability to infect the host.[9] Inhibition of SrtA also causes *S. aureus* to lose bacterial virulence, including binding activity to IgG, fibronectin, and fibrinogen,[10] as well as reduced levels of biofilm formation in some staphylococcal strains.[11] Since SrtA is not necessary for microbial growth and viability, inhibition of SrtA would likely impose less selection pressure for the development and spread of the resistance mechanism.[12]

In the past decade, several S. *aureus* SrtA inhibitors have been identified through High-Throughput Screening (HTS)[4] as well as virtual screening.[13] To our knowledge, however, only a few hit compounds were investigated *in vivo*. Researchers have

Abbreviations: SrtA, sortase A; SpA, protein A; HTS, high-throughput screening; FRET, fluorescence resonance energy transfer; IC_{50}, half maximal inhibitory concentration; MTSET, N,N,N-trimethyl-2-(methylsulfonylthio)ethanaminium chloride; ESI-LC-MS, electrospray ionization liquid chromatograph mass spectrometer; HSQC, heteronuclear single quantum coherence; CSP, chemical shift perturbation.

* Corresponding authors.
E-mail addresses: nxzhang@mail.shcnc.ac.cn (N. Zhang), aozhang@simm.ac.cn (A. Zhang), yangcg@simm.ac.cn (C.-G. Yang).
[f] These authors contributed equally to this article.

https://doi.org/10.1016/j.bmcl.2018.04.005
0960-894X/

demonstrated that (*Z*)-3-(2,5-dimethoxyphenyl)-2-(4-methoxyphenyl) acrylonitrile, a previously reported sortase inhibitor,[14] is able to reduce mortality from *S. aureus* in mouse models of kidney and joint infection,[15] verifying for the first time the therapeutic effect of the small-molecule inhibitor of SrtA on *S. aureus* infections at the animal level. However, the *in vivo* biochemical activity of this inhibitor remains unclear. We have Identified that 3-(4-pyridinyl)-6-(2-sodiumsulfonatephenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole blocks sortase activity both *in vitro* and *in vivo*.[16] Although the compound does not affect *in vitro* staphylococcal growth, the compound protects mice against lethal *S. aureus* bacteremia. Ours is the first study showing that a small-molecule inhibitor indeed suppressing the transpeptidation activity of SrtA *in vivo*. Both compounds act as non-covalent inhibitors of *S. aureus* sortase.

Covalent inhibitors show higher affinity with targets compared with non-covalent inhibitors. Despite controversy over the years regarding the toxicity of covalent binding, covalent drugs have in fact proved successful therapies for diverse indications and have positively impacted on human health in a major way.[17] Indeed, the active site Cys184 provides an ideal targeting site for the development of covalent inhibitors for SrtA. The first synthesis, kinetic evaluation, and application of novel substrate-derived inhibitors against *S. aureus* SrtA were the covalent inhibitors.[18] Subsequently, several classes of covalent hits that show a great inhibitory activity of sortase-catalyzed transpeptidation have been identified, including vinyl sulfones,[19] phosphinic peptidomimetics,[20] (2*R*,3*S*) 3-amino-4-mercapto-2-butanol (a threonine analogue),[21] and aryl (β-amino)ethyl ketones.[22] Most of the inhibitors were only tested on the level of enzymatic biochemistry *in vitro*, however. Some of them are unlikely to be developed further because of their low activity or the lack specificity. Here, we report the identification of new inhibitors showing high affinity with SrtA through an irreversible, covalent binding, which would hopefully provide promising hit compounds for the development of novel anti-virulence agents against *S. aureus*.

HTS of SrtA inhibitor based on FRET assay. In searching new SrtA inhibitors, we performed an HTS of a clinical drug library and a natural product-based library based on fluorescence resonance energy transfer (FRET).[23] SrtA activity is measured by monitoring the cleavage of a fluorogenic substrate (peptide Abz-LPETG-Dnp) between the Thr and Gly residues.[24] In the clinical drug library which contains 2000 drugs of approved or candidates in clinical trials, we found that Shikonin and Alkannin (Fig. 1A), naphthoquinones isolated from the root of the medical herb *Lithospermum erythrorhizon*, display great inhibitory activity against *S. aureus* SrtA with IC_{50} values of about 300 nM (Fig. 1B). The inhibitory activity of both Shikonin and Alkannin on SrtA *in vitro* occurs regardless of their absolute configurations in chiral centers, which is probably due to the mechanism of substitution chemistry as illustrated.[25] We also found that both compounds display strong antibacterial activities against the growth of *S. aureus in vitro* (Fig. 1C),[26] however, which excludes both natural products as inhibitors specifically targeting SrtA, since SrtA is not necessarily required for staphylococcal growth and viability.

Next, we identified a group of quinone-pyran-lactone tricyclic compounds from the natural product-based library (Fig. 1D), as derived from the natural products kalafungin[27] and medermycin.[28] Although both natural compounds were originally reported to have antibacterial activity against various pathogenic bacteria, the exact mechanism was not clear.[29,30] By using a four-step enantioselective synthetic approach, we successfully prepared the pyranonaphthoquinone framework, the tricyclic pharmacophore of the natural products. Subsequently, a relatively diverse library of 50 analogues was constructed by incorporating various substituents on the naphthoquinone component.[31] Upon screening against *S. aureus* SrtA, we found that the C5-stereochemistry is critical to the inhibition potency, and compounds bearing a C5-substituent with *R*-configuration are generally more potent than an isomer with *S*-configuration. For example, compounds **283** and **284**, a pair of diastereomers differing in the C5-stereochemistry, were previously reported non-toxic against KB, A459, and HL60 cancer cells,[31] but here displayed different potency against *S. aureus* SrtA in the FRET assay with IC_{50} values of 6.2 μM and 19.4 μM (Fig. 1E), respectively. Replacement of the C5-phenylethyl moiety with other alkyl or aryl led to inferior potency (data not shown). Further substitution on the quinone moiety, for example compound **63**, lost the potency with which to inhibit SrtA (Fig. 1D-E), which would be a negative control for further evaluation. Interestingly, the diastereomers **283** and **284** minimally affect the *S. aureus* Newman strain on growth even at high concentrations (Fig. 1F). Both compounds from these two libraries contain the same quinone skeleton, which could be therefore speculated as a new class of SrtA inhibitors.

Inhibition of SrtA-mediated transpeptidation of surface protein. To exclude false positives, we expressed and purified the surface protein $IsdA_{64\text{-}323}$, which is one of the natural substrates of SrtA in *S. aureus*.[32] Incubation of affinity-purified $IsdA_{64\text{-}323}$ with the purified $SrtA_{\Delta N24}$ and NH_2-Gly_3 nucleophile resulted in sorting signal cleavage to yield the transpeptidation product.[16] If compounds can inhibit SrtA, the action would be partly or completely blocked, which can be observed in SDS-PAGE. N,N,N-trimethyl-2-(methylsulfonylthio)ethanaminium chloride (MTSET) was used as the positive control and was reported as a noncompetitive inhibitor of SrtA that can block the transpeptidation.[33] Eventually, we found that compounds block sortase cleavage of sorting signals in $IsdA_{64\text{-}323}$ protein in a dose-dependent manner (Fig. 1G-H), while the negative control compound **63** failed to inhibit the transpeptidation of $IsdA_{64\text{-}323}$ by $SrtA_{\Delta N24}$. The estimated IC_{50} is 5 μM for Alkannin and Shikonin, 10 μM for inhibitor **283**, and 50 μM for **284**, respectively. These data confirm the inhibitory activities of the quinone-derived compounds as SrtA inhibitors *in vitro*.

Irreversible modification of SrtA active site by inhibitor 283. In order to evaluate the reversibility of the binding, $SrtA_{\Delta N24}$ was incubated with compounds **283** and **284** at 10-fold higher concentration of IC_{50}. After dilution, the activity of $SrtA_{\Delta N24}$ was found to be recovered less than 10% compared with mock-treated $SrtA_{\Delta N24}$ (Fig. 2A), which suggests that the two compounds function as irreversible inhibitors. In order to verify our speculation, ESI-LC/MS was performed in order to investigate the modification of SrtA by inhibitors. $SrtA_{\Delta N24}$ was detected at two masses of 22969.2 and 23146.8 Da, respectively (Fig. 2B). After incubation with compound **283**, the m/z of SrtA shifted toward higher masses resulting in two masses of 23290.9 and 23468.8 Da, respectively (Fig. 2B). The mass difference of 322 Da perfectly matched with the relative molecular weight of compound **283**, thus suggesting that **283** modified SrtA in an irreversible way.

In order to investigate the reaction site of SrtA by inhibitor **283**, ESI-LC-MS/MS was performed to identify the modified location in the protein sequence. The peptides were analysed by electrospray ionization tandem MS. Our analysis in ion mode revealed a peptide bearing a mass modification of 321.7 Da, which matched with the relative molecular weight of compound **283**. Fragmentation analysis revealed this peptide to be a 13-mer having the sequence QLTLITC*DDYNEK (Fig. 2C). These data unambiguously show that the binding site of **283** and SrtA is the active site Cys184 residue. As shown in the plausible modification scheme (Scheme S1), the nucleophilic thiol group of Cys184 was deprotonated by certain base in the active pocket, which is ready to add to the conjugated carbon-carbon double bond in compound **283** by Michael addition,[34] then the complex re-aromatized and generated hydroquinone structure.

Next, we measured the initial velocity (V_0) of catalytic transpeptidation of $SrtA_{\Delta N24}$ on the peptide substrate in the