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Research paper

Identification of potential antivirulence agents by substitution-oriented screening for inhibitors of *Streptococcus pyogenes* sortase A

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ABSTRACT

Antimicrobial resistance resulting in ineffective treatment of infectious diseases is an increasing global problem, particularly in infections with pathogenic bacteria. In some bacteria, such as *Streptococcus pyogenes*, the pathogenicity is strongly linked to the attachment of virulence factors. Their attachment to the cellular membrane is a transpeptidation reaction, catalyzed by sortase enzymes. As such, sortases pose an interesting target for the development of new antivirulence strategies that could yield novel antimicrobial drugs. Using the substitution-oriented fragment screening (SOS) approach, we discovered a potent and specific inhibitor (**C10**) of sortase A from *S. pyogenes*. The inhibitor **C10** showed high specificity towards *S. pyogenes* sortase A, with an IC₅₀ value of 10 μM and a K_d of 60 μM. We envision that this inhibitor could be employed as a starting point for further exploration of sortase's potential as therapeutic target for antimicrobial drug development.

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1. Introduction

Streptococcus pyogenes is a pathogenic Gram-positive bacterium, harboring the Lancefield group A antigen. *S. pyogenes* can cause infections that are benign and self-healing, e.g. pharyngitis, skin infections and impetigo. However, some *S. pyogenes* strains are equipped with surface virulence molecules that enable the bacteria to invade the organism more deeply and spread to various organs unnoticed. This can lead to life-threatening diseases such as necrotizing fasciitis, septicemia or toxic shock syndrome [1,2]. The worldwide mortality rate caused by severe *S. pyogenes* infections exceeds 500,000 deaths per year [3]. Unsurprisingly, this high mortality is caused by an increasing resistance of this microorganism to currently used antibiotics [4].

Group A streptococci can be classified based on the type of M protein they express. M proteins are virulence factors, and as such they are associated with disease severity and infectivity [5]. In fact, many of the proteins expressed on the surface of *S. pyogenes* are virulence factors, essential for adhesion, colonization, internalization and biofilm formation [6]. Some of these virulence factors are attached to the cell wall through a transpeptidation reaction catalyzed by the enzyme sortase A (SrtA) [7,8].

SrtA belongs to a class of enzymes displayed on the surface of Gram-positive bacteria, where they play a pivotal role in the pathogenicity of the bacterium. All proteins to be processed by SrtA bear a sorting motif of five amino acids at the C-terminus followed by a hydrophobic domain and a positively charged tail. Once the motif is recognized by the SrtA enzyme, it cleaves the peptide bond between the last two residues and forms an acyl-enzyme intermediate linked by a thioester bond between the carboxyl group of the motif and the cysteine located in the active site of the enzyme. Next, lipid II performs a nucleophilic attack on the acyl-enzyme intermediate, which results in the formation of a new peptide bond between the C-terminus of the surface protein and the N-

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terminus of lipid II. As a result, the surface protein is covalently anchored to the cell wall of bacteria [9].

To demonstrate the role of SrtA in the development of infections, a number of studies using Gram-positive *srtA* knockout strains was performed in mouse models for infectious disease. Indeed, mice infected with *Staphylococcus aureus srtA* deletion mutants did not develop infections due to the absence of virulence factors. Interestingly, deletion of *srtA* was not lethal to the bacteria, nor did it lead to growth defects, suggesting that this enzyme is not essential to sustain life processes of *S. aureus* [10]. A study performed with *S. pyogenes* showed that a *srtA* knockout strain had become susceptible to phagocytic killing [11]. Hence, our hypothesis is that a small molecule inhibitor of sortase A from *S. pyogenes* (Sp-SrtA) could function as an anti-infective agent. In contrast to antibiotics, our strategy relies on the inhibition of bacterial virulence thus making pathogens more susceptible to immune clearance by the host [12]. Since Sp-SrtA is not essential for the basic life processes of *S. pyogenes*, inhibition of this enzyme is less likely to lead to the development of antimicrobial resistance [13,14].

The development of inhibitors by screening natural products and small molecules or by designing inhibitors *in silico* has so far targeted sortase's catalytic cysteine residue. Until now, several inhibitors of sortase A from *S. aureus* (Sa-SrtA) have been identified, with the best inhibitor displaying a half maximal inhibitory concentration (IC_{50}) of 200 nM [13,15–17]. The focus of the current study, however, is on the inhibition of Sp-SrtA; to our knowledge, only one small molecule inhibitor of this enzyme has been described. The reported triazolo-thiadiazole-based molecule showed a tenfold higher inhibitory effect on Sp-SrtA than on Sa-SrtA [18].

In the current study, we applied substitution-oriented fragment screening (SOS) [19–21], a rational approach, employed by an *in vitro* fluorometric assay. Our approach was to develop an Sp-SrtAΔN81-selective inhibitor that would interact with amino acids located near the catalytic center of the enzyme. Based on the knowledge of currently available inhibitors of Sa-SrtAΔN24 activity and the 3D structure of Sp-SrtAΔN81 (Protein data bank (PDB) IDs: 3FN5 and 3FN7), we designed a SOS library of nitrogen-containing aromatic compounds targeting the active site. After screening, we found an inhibitor for Sp-SrtAΔN81 with an IC_{50} of 10 μ M and a K_d of 60 μ M; this compound is specific, as it does not inhibit Sa-SrtAΔN59.

2. Results and discussion

2.1. Design and screening of the compound library for enzymatic inhibition

In previously reported studies on the homologous Sa-SrtAΔN24, an indole derivative showed promising inhibitory effects on this enzyme [22]. Based on these findings, we designed a compound collection of indole derivatives.

Using the 3D structure of *S. pyogenes* sortase A (PDB ID: 3FN7), we performed molecular modeling studies targeting the active site of the enzyme [23]. By measuring the distances between important active site residues of Sp-SrtAΔN81, we confirmed indoles to be the best candidate scaffold with regard to its structural and in general favorable physicochemical properties. A focused library was assembled, consisting of 92 nitrogen-containing aromatic compounds including 50 indoles and 42 other heterocycles with different substitution patterns. The compounds were selected to have various hydrogen bond donor and acceptor groups targeting the amino acid residues located at the active site (Fig. 1). A full list of the compounds from the library can be found in the supporting PDF file.

A fluorometric assay monitoring the hydrolysis of an internally quenched Abz-LPETA-Dap(Dnp) substrate analogue for sortase A from *S. pyogenes* was used for the inhibitory screening. The screening results for the most active compounds from the SOS library at two different concentrations are presented in Fig. 2.

To calculate the potency of inhibition, an end-point analysis of the formation of fluorescent product was performed. For each run, the positive control (no inhibitor), was determined as 100% residual activity. After the first screening at a concentration of 100 μ M, eight compounds were found to exhibit an inhibition of SrtA activity greater than 50% (Figs. 2 and 3). The residual activity for compound **D9** was set to 0% as the slope of the reaction was negative. For the selection of the most potent inhibitors, the compound library was screened at a concentration of 10 μ M. The most potent inhibitor **C10**, (2-amino-6-chloro-1H-indol-3-yl)(morpholino)methanone, showed a good correlation in inhibition at 100 μ M and 10 μ M. In addition, **C10** presented better solubility properties compared to the other compound hits. After corroborating its purity by LC-MS (Supplementary Fig. S1), **C10** was selected for further characterization and used as a lead compound for a structure-activity relationship (SAR) analysis. The remaining seven compound hits (Fig. 3) were not studied further, though they may form an interesting starting point for future research.

2.2. Selectivity profile and kinetics

The IC_{50} of the **C10** inhibitor against Sp-SrtAΔN81 was determined to be 10 μ M (Fig. 4). Importantly, compound **C10** was found to be specific for Sp-SrtAΔN81 as it did not inhibit the homologous Sa-SrtAΔN59 (Fig. 4A). This finding strengthened our initial modeling studies, as they were specifically directed towards the blocking of the active site of Sp-SrtAΔN81 and not Sa-SrtAΔN59. Although the overall structures of Sp-SrtAΔN81 and Sa-SrtAΔN59 are very similar, there are some important differences in the arrangement of Sp-SrtAΔN81's catalytic triad that lead to distinct activities and substrate specificities for these enzymes [23]. This difference was highlighted in a study by Zhang et al., in which, surprisingly, a small molecule inhibitor identified on Sa-SrtAΔN59 exhibited a 10-fold lower IC_{50} towards Sp-SrtAΔN81 [18]. Lee et al. prepared a series of synthesized analogues of natural products with indole pharmacophores, which showed inhibitory activity towards Sa-SrtA. Here, the indole moiety only interacted with Sa-SrtAΔN59 in the presence of two carbonyl moieties in the molecule, as removal of either group caused a complete loss of inhibitory activity [22]. Our indole-based compound **C10** contains only one carbonyl group, which could be cause for less interaction with the Sa-SrtAΔN59 active site.

Next, steady-state kinetics were determined to evaluate the type of sortase A inhibition in the presence of different concentrations of compound **C10** (Fig. 4B). Using a Michaelis-Menten model and the above mentioned fluorometric assay, the Michaelis constant (K_m) and the maximum rate (V_{max}) were assessed. Both values changed with increasing inhibitor concentration: K_m showed an increase, whereas V_{max} values decreased. This result indicated a mixed type of inhibition, in which the inhibitor can bind to either the free enzyme (E) or the complex of enzyme and substrate (ES).

2.3. Binding affinity studies

In order to confirm the binding and to characterize the interaction of compound **C10** with Sp-SrtAΔN81, we used microscale thermophoresis (MST) [24]. The binding affinity K_d of compound **C10** was determined to be 60 μ M (Fig. 5). Importantly, the MST results demonstrated that the **C10** compound did not show

Fig. 1. Initial modeling of the binding position of inhibitors based on the 3D structure of Sp-SrtAΔN81 (PDB ID: 3FN7).

Fig. 2. Screening the SOS library of SrtA inhibitors at a concentration of 100 μM (white bars) and 10 μM (black bars). The eight depicted compounds triggered a more than 50% decrease in sortase activity compared to the positive (untreated) control (PC).

autofluorescence, nor did Sp-SrtAΔN81 aggregate in the presence of the small molecule. Thus, these results confirm that **C10** specifically interacts with Sp-SrtAΔN81 and therefore minimize the chance that **C10** belongs to the group of pan-assay interference compounds (PAINS) [25].

2.4. Structure-activity relationship (SAR)

To study and verify the importance of the substituents of **C10** on the inhibition of Sp-SrtAΔN81 activity, three **C10** analogues were synthesized (Scheme 1) and analyzed for their inhibitory activity (Fig. 6).

The removal of the chlorine atom from the benzene ring in

Fig. 3. Small molecules that showed moderate inhibition of Sp-SrtAΔN81 activity identified by SOS approach, with the most potent molecule **C10** depicted in the red box. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 4. The potency of sortase A inhibition *in vitro* in the presence of different concentrations of compound **C10**. (A) Inhibition of purified recombinant Sp-SrtAΔN81 with an IC₅₀ of 10 μM. Specificity of sortase A inhibition is shown by the lack of inhibition of Sa-SrtAΔN59. (B) Michaelis-Menten representation of Sp-SrtAΔN81 kinetics in the presence of different concentrations of compound **C10**.

Fig. 5. Binding of compound **C10** to 50 nM purified recombinant Sp-SrtAΔN81 with the dissociation constant of 60 μM.

Scheme 1. Synthesis of compounds 1–3. Reagents (**R1** – **R3**) and conditions: a) NaH, DMF, 24 h, RT, then HCl, FeCl₃ and Zn, 1 h, 100 °C, 30–32% yield; b) benzoylchloride, Et₃N, dry CH₂Cl₂, N₂, 18 h, RT, 50%.

compound **1** (with respect to **C10**) led to a twofold reduction in the effectiveness of Sp-SrtAΔN81 inhibition. In contrast, exchange of the morpholino group of **C10** for a piperidine ring resulted in a total loss of inhibitory properties for compound **2**. A similar effect was observed for compound **3** when the amine group was benzyl-protected. To conclude, the modification of **C10** substituents contributing as donor-acceptor moieties hindered its interaction with Sp-SrtAΔN81.

2.5. Molecular modeling

The docking pose of the **C10** molecule with the highest score

demonstrates a binding in the active site as was initially hypothesized (Fig. 7). In line with that, an overlay of the **C10** binding poses with the highest scores illustrates equivalent docking poses (Supplementary Fig. S5 and Table S2). Notably, two positions that were shown to be crucial for the inhibition of Sp-SrtAΔN81 in the SAR studies were also predicted to be crucial in our model. The morpholino oxygen of the **C10** compound interacts with the two amino acid residues His143 (3.11 Å, −2.1 kcal/mol) and His142 (2.79 Å, −0.9 kcal/mol), whereas the 2-amino functionality interacts with Val206 (2.98 Å, −4.2 kcal/mol) (Fig. 7B, Supplementary Table S3). These residues are located near the active site of the enzyme. This is in line with the mixed type inhibition in which

Fig. 6. SAR study of compound **C10**, illustrating the importance of the chemical substituents indicated with red circles. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 7. (A) Proposed binding mode of inhibitor **C10** in the active site cleft of the enzyme. (B) 2D model of the interaction of the **C10** compound with Sp-SrtAΔN81.

inhibitors can bind close to the enzyme active site and thus affect both K_m and V_{max} constants.

3. Conclusions

It is of key importance to develop new approaches for the treatment of different life-threatening diseases caused by strains of *S. pyogenes*, as resistance to currently used antibiotics is a significant problem [3,26]. In this study, we employed a rational approach based on SOS combined with an *in vitro* fluorometric assay to search for a potent Sp-SrtAΔN81 inhibitor. We identify the compound **C10** with an IC_{50} value of 10 μM and a K_d of 60 μM . Our SAR studies clearly showed that the substituents of compound **C10** are essential for the inhibitory properties towards Sp-SrtAΔN81. These SAR observations were confirmed by our molecular modeling study, which proposed a binding mode of the **C10** compound close to the active site of Sp-SrtAΔN81. The information obtained by SAR and molecular modeling can be used for further improvement of the inhibitory properties of compound **C10**.

The prognosis is that by 2050, infections caused by antibiotic-resistant organisms will lead to the death of up to 10 million people per year [27]. We believe that our indole-based compounds can aid in the exploration of sortase inhibitors, which in turn can lead to the development of new anti-infective agents against *S. pyogenes*.

4. Experimental section

4.1. Chemistry

4.1.1. (2-Amino-1H-indol-3-yl)(morpholino)methanone (**1**)

The synthesis was adapted from a known procedure [28]. In short, 4 mL dry dimethylformamide (DMF), 3-morpholino-3-oxopropanenitrile (**R3**) [29] (2.0 mmol, 1.0 equiv.) and NaH (60% dispersion in mineral oil, 2.2 mmol, 2.2 equiv.) were added to a 50 mL round-bottom flask equipped with a stirring bar. After 10 min, 2-fluoronitrobenzene (**R1**) (2.0 mmol, 1.0 equiv.) was added, which led to a deep purple reaction mixture. The mixture was left stirring at room temperature for 24 h. Next day, 1.0 N HCl (4.0 mmol, 2.0 equiv.) was added, followed by $FeCl_3$ (6.0 mmol, 3 equiv.) and Zn dust (20 mmol, 10 equiv.). The reaction mixture was heated to 100°C and left stirring for 1 h. The mixture was cooled afterwards and 20 mL of water was added. The crude reaction mixture was filtered and the sticky grey residue was washed with 25 mL of ethyl acetate. The ethyl acetate layer of the resulting

filtrate was separated and the water layer was again extracted with ethyl acetate (2 × 20 mL). The combined organic extracts were washed with a saturated sodium bicarbonate solution (10 mL) and brine (10 mL), dried with anhydrous magnesium sulfate and the solvent was removed under reduced pressure. The resulting crude product was dissolved in ether (10 mL), cooled to 0 °C, and 2 M HCl in ether was added until no more formation of precipitate was observed. The precipitate was filtered and washed with cold ether to afford the product. Beige solid, yield 30%. ¹H NMR (500 MHz, MeOH-d₄) δ 7.40–7.37 (m, 2H), 7.23–7.17 (m, 2H), 3.94–3.85 (m, 4H), 3.77–3.60 (m, 4H). ¹³C NMR (Chloroform-d, 126 MHz) δ 170.42, 165.06, 142.26, 129.49, 126.61, 124.71, 123.52, 112.03, 66.39, 66.12, 44.40, 42.75 ppm. HR-MS, calcd for C₁₃H₁₆N₃O₂ [M+H]⁺ 246.1237, found 246.1236 (Supplementary Fig. S2).

4.1.2. (2-Amino-1H-indol-3-yl)(piperidin-1-yl)methanone (**2**)

The synthesis was adapted from a known procedure [28]. In short, 4 mL dry dimethylformamide (DMF), 3-oxo-3-(piperidin-1-yl)propanenitrile (**R2**) [29] (2.0 mmol, 1.0 equiv.) and NaH (60% dispersion in mineral oil, 2.2 mmol, 2.2 equiv.) were added to a 50 mL round-bottom flask equipped with a stirring bar. After 10 min, 2-fluoronitrobenzene (**R1**) (2.0 mmol, 1.0 equiv.) was added, which led to a deep purple reaction mixture. The mixture was left stirring at room temperature for 24 h. Next day, 1.0 N HCl (4.0 mmol, 2.0 equiv.) was added, followed by FeCl₃ (6.0 mmol, 3 equiv.) and Zn dust (20 mmol, 10 equiv.). The reaction mixture was heated to 100 °C and left stirring for 1 h. The mixture was cooled afterwards and 20 mL of water was added. The crude reaction mixture was filtered and the sticky grey residue was washed with 25 mL of ethyl acetate. The ethyl acetate layer of the resulting filtrate was separated and the water layer was again extracted with ethyl acetate (2 × 20 mL). The combined organic extracts were washed with a saturated sodium bicarbonate solution (10 mL) and brine (10 mL), dried with anhydrous magnesium sulfate and the solvent was removed under reduced pressure. The resulting crude product was dissolved in ether (10 mL), cooled to 0 °C, and 2 M HCl in ether was added until no more formation of precipitate was observed. The precipitate was filtered and washed with cold ether to afford the product. Beige solid, yield 32%. ¹H NMR (500 MHz, DMSO-d₆) δ 10.54 (s, 1H), 7.10 (d, *J* = 8.5 Hz, 2H), 6.92 (t, *J* = 7.7 Hz, 1H), 6.83 (t, *J* = 7.7 Hz, 1H), 6.18 (br s, 2H), 3.39 (t, *J* = 5.5 Hz, 4H), 1.61–1.57 (m, 2H), 1.55–1.50 (m, 4H). ¹³C NMR (126 MHz, DMSO-d₆) δ 169.66, 152.42, 132.27, 126.74, 120.22, 118.57, 117.05, 110.09, 87.66, 46.50 (2C), 26.38 (2C), 24.77. HR-MS, calcd for C₁₄H₁₈N₃O [M+H]⁺ 244.1444, found 244.1443 (Supplementary Fig. S3).

4.1.3. N-(3-(morpholine-4-carbonyl)-1H-indol-2-yl)benzamide (**3**)

Benzoylchloride chloride (1.5 mmol, 1.5 equiv.) was slowly added under a nitrogen atmosphere to a cooled (0 °C) solution of compound **1** (1 mmol, 1.0 equiv.) and Et₃N (210 μL, 1.5 mmol, 1.5 equiv.) in dry CH₂Cl₂ (10 mL). After being stirred at room temperature for 18 h, a sat. sol. of NaHCO₃ was added and the mixture was stirred for 15 min to destroy all remaining acyl chloride. Then, more CH₂Cl₂ was added and the layers were separated. The organic layer was washed with 1 N HCl (25 mL), a saturated solution of NaHCO₃ (25 mL) and brine (25 mL), then dried over anhydrous magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified with silica column chromatography with CH₂Cl₂:EtOAc 10:1 (v/v) as eluent. Yellow solid, yield 50%. ¹H NMR (500 MHz, Chloroform-d) δ 7.73–7.69 (m, 2H), 7.69–7.64 (m, 1H), 7.51 (m, 2H), 7.23–7.17 (m, 1H), 7.11 (m, 1H), 6.80 (br s, 2H), 6.72 (m, 1H), 6.31 (m, 1H), 3.83–3.71 (m, 4H), 3.68–3.57 (m, 4H). ¹³C NMR (126 MHz, Chloroform-d) δ 192.49, 168.94, 152.67, 133.41, 131.53, 129.47, 129.27, 129.02, 128.86, 127.13, 123.74, 119.97, 119.83, 117.76, 114.22, 99.98, 67.24 (2C), 46.21 (2C).

HR-MS, calcd for C₂₀H₂₀N₃O₃ [M+H]⁺ 350.1499, found 350.1496 (Supplementary Fig. S4).

4.1.4. Cloning, production and purification of sortases

The DNA sequence encoding soluble, truncated sortase A (Sp-SrtAΔN81), Val82–Thr249, was kindly provided by Dr M.J. Banfield (Newcastle University, UK) and cloned into the *Bam*HI and *Hind*III restriction sites of the expression vector pQIq [30]. The sortase A gene from *S. aureus* (Sa-SrtAΔN59) was cloned into the *Nde*I and *Bam*HI restriction sites of the pET28a vector (Novagen, USA). The *Escherichia coli* BL21(DE3) strain (New England Biolabs, USA) was used for transformation of the plasmids encoding the recombinant sortases and subsequent protein production. The sortase enzymes were each purified via their N-terminal M(R)GSH₆-tags using Ni-NTA resin (Qiagen, Germany). The proteins were further purified by size exclusion chromatography in Tris-HCl buffer (50 mM, pH 7.5) containing 150 mM NaCl and 10% (v/v) glycerol.

4.1.5. Enzyme inhibition studies

A fluorometric assay was used to assess the inhibition of cleavage of the quenched substrate Abz-LPETA-Dap(Dnp) or Abz-LPETG-Dap(Dnp) (Bachem AG, Switzerland). Stock solutions of all inhibitor compounds were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mM and diluted with assay buffer consisting of 50 mM Tris-HCl, pH 7.5, and 150 mM NaCl and 100 mM CaCl₂ in case of Sa-SrtAΔN59, to a final concentration of 100 μM. Sortase enzymes were added to a final concentration of 2 μM and preincubated with the potential inhibitors for 10 min at RT. The reaction was initiated by the addition of internally quenched substrate at a final concentration of 20 μM. The fluorescence intensity was measured in 96-well black plates (Greiner Bio-One, Austria) using a FLUOstar Omega Microplate Reader (BMG Labtech, Germany) at the excitation wavelength of 317 nm and emission wavelength of 420 nm every minute for 2.5 h. The linear increase in fluorescence in the absence of inhibitor was determined as 100% activity of the enzyme and no fluorescence response in the absence of enzyme was observed. The threshold for selection of potent compounds was set to a reduction in enzyme activity of at least 50% compared to untreated controls.

The same fluorometric assay was used to determine the half maximal inhibitory concentration (IC₅₀) values of selected compounds. Inhibitors were diluted with the assay buffer using a serial dilution to the concentrations ranging from 0.195 μM to 100 μM. Data were analyzed using GraphPad Prism software; each data point was reported as the average of three measurements and their standard deviations.

4.1.6. Kinetics of sortase A

The fluorometric assay mentioned above was used for the evaluation of enzyme kinetics. The activity assay was performed in 50 mM Tris-HCl, pH 7.5, and 150 mM NaCl. Sp-SrtAΔN81 was diluted with the assay buffer to a final concentration of 2 μM. The Abz-LPETA-Dap(Dnp) concentrations were prepared in the range between 0 μM and 40 μM. The activity of the enzyme was measured in the presence of the inhibitor at the concentrations of 0 μM, 5 μM and 10 μM. The progress of the reaction was monitored every minute for 2 h. The initial reaction velocities (V₀) were plotted against the substrate concentrations. The K_m and the V_{max} values were calculated from the Michaelis-Menten equation using GraphPad Prism software. Each data point was reported as the average of three measurements.

4.1.7. Microscale thermophoresis (MST)

Binding studies between Sp-SrtAΔN81 and the **C10** compound were performed with a Monolith NT.115 instrument using standard

treated capillaries (NanoTemper Technologies, Germany). The His-tag of the target protein was labeled with an NT-647 fluorescence dye (NanoTemper Technologies) according to the manufacturer's instructions and the labeled protein was diluted to a concentration of 100 nM using the assay buffer consisting of phosphate-buffered saline, pH 7.5 (137 mM NaCl, 2.7 mM KCl, 8 mM Na₂HPO₄ and 1.5 mM KH₂PO₄), with the addition of 0.05% (v/v) Tween-20. The conditions for LED and MST power were set to 40% and Medium, respectively. The compound **C10** was diluted in the assay buffer without detergent to a concentration of 4 mM and a DMSO content of 4% (v/v). Equal amounts of labeled Sp-SrtAΔN81 were mixed in a 1:1 ratio with serial dilution of compound **C10** starting from 2 mM. The K_d value was calculated from three replicate studies using NT analysis software (NanoTemper Technologies).

4.1.8. Molecular modeling

A docking study was performed to predict the binding mode of compound **C10**. This study was performed using the Molecular Operating Environment (MOE) software platform and the 3D structure of Sp-SrtAΔN81, PDB 3FN7. The experiments were performed with rescoring model 1 London dG (refinement: forcefield) and rescoring 2: GBVI/WSA dG, followed by minimization energy (forcefield: MMFF94X; eps = r, cutoff {8,10}).

Author contributions

M.W., N.E., F.J.D. and Y.L.B. designed the experiments. M.W. and N.E. performed enzyme inhibition and kinetic studies as well as the molecular modeling. M.R.H.Z. and A.S.S.D. designed and contributed to the synthesis of the compounds. F.J.D. and Y.L.B. supervised the studies. M.W. and Y.L.B. wrote the manuscript with contributions of all authors.

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Abbreviations

Sp-SrtA	<i>Streptococcus pyogenes</i> sortase A
Sa-SrtA	<i>Staphylococcus aureus</i> sortase A
SOS	Substitution-oriented fragment screening
MST	Microscale thermophoresis

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejmech.2018.10.027>.

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