Potent and selective inhibitors of Staphylococcus epidermidis tryptophanyl-tRNA synthetase

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Objectives: The skin commensal and opportunistic pathogen Staphylococcus epidermidis is one of the leading causes of nosocomial and biofilm-associated infections, which urgently requires discovery of new antibiotics. We decided to find new leads that target the S. epidermidis tryptophanyl-tRNA synthetase (SeWRS), which is essential for translation.

Methods: We applied an approach combining structure-based discovery in silico with biochemical and biological experiments in vitro to screen SeWRS inhibitors.

Results: Three compounds have an inhibitory effect on enzymatic activities of SeWRS, of which two show low inhibition of the human tryptophanyl-tRNA synthetase. Binding of these compounds to bacterially expressed SeWRS was demonstrated by surface plasmon resonance technology. These three compounds can also obviously inhibit growth of S. epidermidis in vitro and displayed low cytotoxicity to mammalian cells.

Conclusions: These compounds are good leads to develop new antibiotics.

Keywords: WRS, virtual screening, S. epidermidis, antibacterials

Introduction

As a member of the coagulase-negative staphylococci, Staphylococcus epidermidis is usually regarded as a harmless commensal. In recent years, however, it has increasingly been recognized as one of the most important pathogens of nosocomial infections accompanying the frequent use of surgically implanted materials, such as intravascular catheters, artificial pacemakers, cerebrospinal fluid shunts, artificial organs, etc. With the increasing use of those materials in clinic and the appearance of antibiotic multiresistant clinical isolates, S. epidermidis associated hospital-acquired infection has become a severe problem that urgently requires discovery of new antibiotics. Recently, the complete genome sequences of two S. epidermidis strains, ATCC 12228 (a non-biofilm-forming strain) and ATCC 35984 (a biofilm-forming strain), have been published, making it possible to discover potential antimicrobial targets using in silico genome analyses.

Among essential proteins, prokaryotic aminocyl-tRNA synthetases (ARSs) have attracted attention as potential antibacterial targets. As a group, ARSs are universal enzymes that exist in all living organisms because they catalyse the attachment of amino acids to transfer RNAs (tRNAs), which are the adaptors required for the information flux from the messenger RNA templates to the polypeptide chains. It has been reported that many surrogates of amino acid or aminocyl-adenylate can
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inhibit aminoacyl-tRNA formation by binding to the active sites of the ARSs and blocking their function. Among them, mupirocin, an antibiotic produced naturally by *Pseudomonas fluorescens* that can selectively inhibit prokaryotic isoleucyl-tRNA synthetase, has been widely used as a topical antimicrobial agent against methicillin-resistant *Staphylococcus aureus*. Inhibitors of other ARSs, such as methionyl-tRNA synthetase, tyrosyl-tRNA synthetase, phenylalanyl-tRNA synthetase, glutaminyl-tRNA synthetase and aspartyl-tRNA synthetase, have been proposed as candidates to fight against bacterial infections. In contrast, to our knowledge, there were limited discoveries of tryptophanyl-tRNA synthetase inhibitors acting against Gram-positive bacteria.

Tryptophanyl-tRNA synthetase (WRS) belongs to the class I ARS family, sharing the Rossmann fold containing conserved sequences ‘HIGH’ and ‘KMSKS’. A single *trpS* gene encoding WRS was found in the genomes of *S. epidermidis* or *S. aureus* strains, though two WRS genes were present in the *Deinococcus radiodurans* genome.

To make a good target, a bacterial gene product needs to differ as much as possible from its host counterparts. Bacterial WRS and cytoplasmic human WRS (HWRS) share very low sequence similarity. Furthermore, in contrast to bacterial WRS, the human enzyme is autophosphorylated and has an N-terminal extension of more than 100 residues. Comparison of the crystal structure of *Bacillus stearothermophilus* WRS (BsWRS) with that of HWRS shows that the latter has a more globular shape that can be attributed to its N-terminal extension. The types of residues that interact with tryptophan in the catalytic active site and their interaction modes differ substantially in BsWRS and HWRS, suggesting that eukaryotic WRS employs a different mechanism to recognize and interact with its substrate (tryptophan) that differs considerably from its bacterial counterparts. Furthermore, HWRS has been reported to play additional roles, such as a human autoantigen or one of the regulators of angiogenesis. Those findings, especially the structural differences between bacterial and human WRSs, make the bacterial WRS a promising target for the discovery of species-specific inhibitors.

As a structural analogue of L-tryptophan, indolmycin was reported to competitively inhibit bacterial WRS. However, it has been demonstrated that a point mutation (H43N) in the *trpS* gene of *S. aureus* resulted in high-level resistance to indolmycin. For unknown reasons, indolmycin has not as yet been commercially developed.

Computation-based technology plays an important role in drug design and can be used as a first line of exploration (*in silico* experiments) before an experimental set-up is designed to test *in vitro* or *in vivo* for the activity of interesting candidates. Molecular modelling and structure-based virtual screening (SBVS) have been used as efficient techniques for discovery of lead inhibitors. The crystal structure of *B. stearothermophilus* WRS provides a valuable basis for the molecular modelling approaches.

In this work, we apply an approach combining structure-based discovery *in silico* with biochemical and biological experiments *in vitro* to screen *S. epidermidis* WRS inhibitors. A 3D structural model of SeWRS was constructed by using the homologous modelling approach using the *B. stearothermophilus* enzyme as the reference. SBVS resulted in the identification of 111 candidates as potential SeWRS inhibitors using SPECS chemical lead-compound database. Among them, three compounds were potent inhibitors *in vitro*, blocking the activity of SeWRS as well as the growth of *S. epidermidis*, while displaying low cytotoxicity to mammalian cells.

Materials and methods

Bacterial strains and culture media

*S. epidermidis* ATCC 12228 and ATCC 35984 and *S. aureus* ATCC 49230 were purchased from ATCC (American Type Culture Collection, Manassas, USA). *Escherichia coli* ATCC 25922 was provided by Dr Xie Hongmei from Zhongshan Hospital (Shanghai, China). *S. epidermidis* clinical strains were collected from Ruijin Hospital. *Staphylococci* were grown at 37°C in tryptic soy broth (TSB; Oxoid), *E. coli* was grown at 37°C in Luria–Bertani medium (LB; Oxoid). MIC assay was performed in cation-adjusted Mueller–Hinton broth (MHB; Oxoid), according to the method of the CLSI of America. Mueller–Hinton agar (MHA; Oxoid) was used in the time–kill assay.

Antibiotics and chemicals

Ampicillin and chloramphenicol were from AMRESCO Company (Solon, USA). All the compounds screened out by virtual screening were from SPECS Company in the Netherlands. Stock solutions of the compounds were prepared in dimethyl sulfoxide (DMSO; AMRESCO).

3D structure construction

The *S. epidermidis* strain ATCC 12228 WRS sequence was retrieved from UniProt (http://www.expasy.org/uniprot/Q8CT69). The ClustalW program was used for sequence alignment of WRSs from *S. epidermidis*, *S. aureus*, *B. stearothermophilus*, *E. coli*, *Saccharomyces cerevisiae*, *Mus musculus* and human. The 3D model of the *S. epidermidis* WRS was generated by the MODELLER program encoded in InsightII (Accelrys, Inc., USA), using crystal structures of *B. stearothermophilus* WRS (PDB code 1MAW, 1M83, 1MU2 and 1MB2) as templates. The structure of each template protein was used to derive spatial restraints expressed as probability density functions for each of the restrained features of the models. The structure with the lowest violation score and lowest energy score was chosen as the candidate. Refine routine in the Homology module of InsightII was used to adjust the positions of the side chains. Finally, the whole structural models were optimized using Amber force field with the following parameters: a distance-dependent dielectric constant of 4.0, non-bonded cut-off 10 Å and Kollman-all-atom charges. The structures were first minimized by steepest descent, then by conjugate gradient method to the energy gradient root-mean-square <0.05 kcal/mol Å. Several structural analysis programs were adopted to check quality of the simulated structure of *S. epidermidis* WRS. The Prostat module of InsightII was used to analyse the properties of bonds, angles and torsions. The Profile-3D program was used to check the structure and sequence compatibility.

Binding site mapping and virtual screening of potential SeWRS inhibitors

The substrate (ATP and tryptophan) binding site of the SeWRS was analysed *in silico* for the virtual screening. The major residues likely to compose the binding sites of ATP and tryptophan in SeWRS were identified by the sequence alignment with the PDB...
structures 1MAW and 1MB2. The surface structure of the ATP and tryptophan binding pocket was constructed by using the MOLCAD module of Sybyl 6.8 (Tripos, Inc., USA).

The optimized 3D model of SeWRS was used as the target for virtual screening with database SPECS (http://www.specs.net/). The virtual screening was performed on the 128-processor SGI Origin 3800 supercomputer at the Drug Discovery and Design Center (http://www.dddc.ac.cn/) and the 32-processor Sunway-I supercomputer in the Shanghai Supercomputer Center (http://www.sscc.net.cn/).

The program DOCK4.028–29 (University of California, San Francisco, USA) was employed for the primary screening. Residues around the catalytic centre (around His) at radius of 6 Å were isolated for constructing the grids of docking screening and the pocket composed by these residues was large enough to include the residues of the binding pocket. During the docking calculations, Kollman-all-atom charges were assigned to SeWRS and Geisterger–Hückel charges were assigned to the small molecules in the small molecular databases due to lack of proper Kollman charges. The conformational flexibility of the compounds from the database was considered in the docking searching.

The orientation of a ligand is evaluated with a shape scoring function and/or a function approximating the ligand–receptor binding energy. After the initial orientation and scoring evaluation, a grid-based rigid body minimization was carried out for the ligand to locate the nearest local energy minimum within the receptor binding site. The position and conformation of each docked molecule were optimized using single anchor search and torsion minimization method of DOCK4.0.

Thirty configurations per ligand building a cycle and 50 maximum anchor orientations were used in the anchor-first docking algorithm. All docked configurations were energy minimized using 100 maximum iterations and 1 minimization cycle. Next, the top 2000 molecules for each database were selected for further analyses. These molecules were re-scored by the scoring function of AutoDock3.040 (The Scripps Research Institute, USA). Based on the second scoring result, 200 molecules were selected from the database according to above comprehensive scorings.

Expression and purification of recombinant SeWRS and HWRS

The 5’ primer (5’-CCCATGAAAACTTTATTCGAGG-3’) was designed to introduce an NcoI restriction site (underlined). The 3’ primer (5’-CGGCTAGTTATTTTCGACTAAGCCC-3’) contains a stop codon (TAA) and an NheI restriction site (underlined). The genomic DNA of S. epidermidis ATCC 12228 was used as template. PCR amplification was run on GeneAMP® PCR system 9700. The target DNA was purified and digested by NcoI and NheI [TaKaRa Biotechnology (Dalian) Co., Ltd, China], then inserted into a prokaryotic expression vector, pET-28a(+) (Novagen, Germany), to obtain the recombinant plasmid pSeWRS. Then the recombinant plasmid was transformed into E. coli BL21 (DE3). The recombinant plasmid pHWRS, constructed by the insertion of human trpS gene into the vector pET-24a(+), was a gift from Professor Youxin Jin (Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, China). The expressed SeWRS and HWRS protein were purified with the ProBond™ Purification System (Invitrogen, CA, USA), according to the manufacturer’s protocol.

Enzymatic activity assay of SeWRS and HWRS

The enzymatic activity assay was performed by using both Kinase-Glo™ Luminescent Kinase Assay (Promega, WI, USA) and Pyrophosphate Reagent (Sigma-Aldrich, MO, USA), according to the manufacturer’s protocols.

In the Kinase-Glo™ Luminescent Kinase assay, reactions were carried out in solid black, flat-bottomed 96-well plates. We optimized the enzymatic reaction conditions with respect to the amount of SeWRS or HWRS substrates (ATP and l-tryptophan). To determine the IC₅₀ values of compounds, we first made 2-fold serial dilutions of each compound with DMSO to final concentrations ranging from 20 to 0.16 mM. Then 1 µL of each compound solution was added into the above reaction mixture. The final reaction mixture contained 2 µg of purified SeWRS or 3 µg of purified HWRS, 16 µM ATP, 10–100 µM compound, 40 mM Tris (pH 8.0), 8 mM MgCl₂, 0.8 mM DTT and 40 µM l-tryptophan. After incubation at 25°C for 20 min, an equal volume of Kinase-Glo™ Reagent was added to each well, mixed and kept at room temperature for 10 min before the final recording of the luminescence on a Fluoroskan Ascent FL machine (Thermo Scientific, USA). The rate of inhibiting the adenylation by the compound was calculated by the following formula:

\[
R_I = \frac{RLU(\text{WRS} + \text{compound} + \text{ATP} + \text{tryptophan} + \text{Reagent})}{RLU(\text{ATP} + \text{tryptophan} + \text{Reagent})} \times 100%
\]

The IC₅₀ value of each compound was determined by Origin 7.0 software (OriginLab, Northampton, USA).

Another assay was performed to confirm the inhibitory activities of the effective compounds in the previous assay by using the Pyrophosphate Reagent (Sigma-Aldrich). Pyrophosphate is a product in the WRS-catalysed aminoacrylation reaction. The assay related the production of pyrophosphate with the reduction of NADH. The reaction is monitored spectrophotometrically at 340 nm in a 96-well microplate reader (Versamax, Molecular Devices). The total volume of the reaction mixture was 200 µL, with a path length of 0.6 cm. The reaction was initiated by the addition of SeWRS (2 µg) or HWRS (3 µg). The enzyme activity was determined in the presence of various concentrations of compounds 1–3 (0–100 µM) to investigate the dose-dependent inhibition effects. Each measurement was taken in triplicate. IC₅₀ values of compounds 1–3 were obtained by fitting the data to a sigmoid dose–response equation of the Origin 7.0 software (OriginLab).

Binding affinities of compounds to SeWRS

The binding affinity of each compound to SeWRS in vitro was determined using the surface plasmon resonance (SPR) biosensor technology on the dual flow cell Biacore 3000 instrument (Biacore AB, Uppsala, Sweden). The method is similar to that described in our previous study.23 Immobilization of the SeWRS to the hydrophilic carboxymethylated dextran matrix of the sensor chip CM5 (Biacore) was carried out by the standard primary amine coupling reaction. The SeWRS protein was diluted in 10 mM sodium acetate buffer (pH 4.3) to a final concentration of 0.32 mg/mL. Equilibration of the baseline was completed by a continuous flow of HBS-EP running buffer [10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA and 0.005% (v/v) surfactant P20, pH 7.4] through the chip for 1 h. Biacore data were collected at 25°C with HBS-EP as running buffer at a constant flow of 20 µL/min. Sensorgrams were processed by using automatic correction for non-specific bulk refractive index effects. The equilibrium constants (Kₐ) evaluating the protein–ligand binding affinity were determined by the steady-state affinity fitting analysis of the Biacore data.
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**MIC assays**

MIC assays of the compounds were performed following the broth micro-dilution method (in tubes) of the CLSI of America.37

**Effects of antimicrobial agents on culture turbidity and bacterial viability**

The turbidity at 600 nm of cultures growing in MHB was determined by measurement of the absorbance in a CARY 50 Conc UV–Visible spectrophotometer (Varian, Inc., USA). Studies to determine bactericidal activity were performed on exponential-phase cultures of *S. epidermidis* ATCC 12228. Samples were serially diluted in PBS and plated onto MHA. The colonies were counted after incubation at 37°C for 18 h.

**Cytotoxicity assay of the antibacterial compounds**

The cytotoxicity of the antibacterial compounds on cultured Vero cells was investigated by using the Cell Proliferation Kit I (MTT) (Roche, Indianapolis, USA). Pre-incubated Vero cells were seeded at a concentration of 5 × 10^4 cells/well in 100 μL of culture medium into flat-bottomed microplates (Falcon) and cells were incubated for 24 h at 37°C, 5% CO₂. After incubation, the culture medium was removed and 100 μL of fresh medium containing various amounts of compounds (0–200 μM) was added into each well and incubated for 24 h. Then 10 μL of the MTT labelling reagent was added to each well for 4 h, after which 100 μL of solubilization solution was added into each well and incubated overnight in the same conditions. The plates were read at a wavelength of 570 nm. The assay was repeated three times (six replicates each). Comparing with the control (cells treated with 1% DMSO), the absorbance was converted to percentage and the CC_{50} (concentrations that have a 50% cytotoxicity effect on Vero cells) was calculated by the Origin 7.0 software (OriginLab).

**Results**

**Sequence analysis of WRSS and structure-based discovery of SeWRS inhibitors in silico**

We confirmed that there is only one *trpS* gene in the genome of *S. epidermidis* ATCC 12228 (NC_004461, locus tag: SE0685) or ATCC 35984 (NC_002976, locus tag: SERP0575). The protein sequence of the WRS of *S. epidermidis* is highly homologous to those of *S. aureus* and *B. stearothermophilus* (89% and 67% identity, respectively), while it shares low identity with those of *M. musculus* and human (10% and 9% identity, respectively). A detailed multiple sequences alignment is shown in Figure S1 [available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)].

To search for potential inhibitors of SeWRS by high-throughput virtual screening (HTVS), we constructed a 3D model for the SeWRS based on the NMR structure of the homologous WRS of *B. stearothermophilus*. The final structure was checked and validated using several programs such as Prostart and Profile-3D.27 The structure of SeWRS we modelled is similar to the crystal structure of BsWRS, with the root-mean-square deviation for the Co atoms being ~0.382, as shown in Figure S2(a) [available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)]. The solid ribbon representation of the structure model of the SeWRS is depicted in Figure S2(b) [available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)], with a shape of a triangle viewing from the front side of the ATP and tryptophan binding site.

The ATP and tryptophan binding pocket formed by residues within a radius of 5 Å around the ATP site of the SeWRS model of *S. epidermidis* [Figure S3, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)] was used as the target site for HTVS. In a first step, 80,000 potential drug-like molecules, constituting an in-house database (named SPECS_1), were selected from the SPECS database using the drug-selection filter developed by Zheng et al.31 The SPECS_1 database was searched for potential binding molecule structures using the program DOCK4.0,28,29 in a primary screening. The highest scoring 2000 structures were subsequently re-scored by the scoring function of AutoDock3.0.30 Finally, 200 molecules were manually selected as inhibitor candidates, according to molecular diversity, shape complementarity and potential for forming hydrogen bonds in the binding pocket of the SeWRS, and 111 compounds were purchased from the SPECS Company for further bioassays.

**Effect of the compounds on enzymatic activities of SeWRS and HWRS**

In order to investigate the effect of the 111 compounds screened by SBVS on enzymatic activities of SeWRS and HWRS, the recombinant His-tagged SeWRS (~37 kDa) and HWRS (~54 kDa) were overexpressed and purified using the ProBond™ Purification System [both protein purities were above 95%, Figure S4, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)] and the enzymatic activities were measured using the Kinase-Glo™ Luminescent Kinase assay by quantifying the amount of ATP remaining in solution after reaction. A direct relationship between the luminescence measured with the Kinase-Glo™ Reagent and the amount of ATP was observed, as shown in Figure S5(a) [available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)]. The purified SeWRS or HWRS protein were able to catalyse the adenylation of l-tryptophan in vitro in a dose-dependent way [Figure S5b and c, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)]. The final optimal reaction mixture contained 2 μg of SeWRS or 3 μg of HWRS, 16 μM ATP and 40 μM l-tryptophan.

Then we measured the effect of the 111 compounds on enzymatic activities of SeWRS and HWRS. Three compounds (named compounds 1, 2 and 3; chemical structures shown in Figure 1) inhibited SeWRS in a highly significant manner [Figure S6a, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)]. At a concentration of 100 μM, compounds 1, 2 and 3 decreased SeWRS (2 μg) catalysed consumption of ATP by 85.7%, 80% and 91%, respectively, whereas they decreased HWRS (3 μg) catalysed consumption of ATP by 57.2%, 22.6% and 14.6%, respectively (the rate of inhibition was calculated by the formula described in the Materials and methods section). The dose-dependent inhibition was investigated by 2-fold serial dilutions of the compounds and the IC_{50} values of the three compounds were calculated (22.8, 42.2 and 18.3 μM, respectively) and are shown in Table 1.
To confirm the effect of compounds 1, 2 and 3 on the enzymatic activity of WRSs, another assay was performed by using a kit of the Pyrophosphate Reagent (described in the Materials and methods section). SeWRS has a \( K_m \) of 0.168 mM towards ATP and a \( K_m \) of 0.097 mM towards L-tryptophan, whereas HWRS has a \( K_m \) of 0.104 mM towards ATP and a \( K_m \) of 0.053 mM towards L-tryptophan. All of the three compounds showed dose-dependent inhibition of SeWRS [Figure S6b, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)] and the IC\(_{50}\) values obtained by the assay were similar to those determined by the Kinase-Glo™ Luminescent Kinase Assay (Table 1).

**Binding affinity of the compounds to SeWRS**

By using SPR technology, we determined the binding affinity of compounds 1, 2 and 3 to SeWRS in vitro. The kinetic analysis was performed with the Biacore 3000 instrument. All three compounds showed a significant and dose-dependent increase in the SPR response (Figure 2). The concentration series were fitted to a steady-state affinity model for \( K_d \) determination using the Biacore 3000 evaluation software. The dissociation constants of the compounds 1, 2 and 3 to SeWRS were 13.9, 12 and 3.76 \( \mu \)M, respectively (Table 1).

**Evaluation of antibacterial activities of the three SeWRS inhibitors**

The compounds 1, 2 and 3 were tested for antibacterial activity against *S. epidermidis* ATCC 12228 and ATCC 35984. Compound 1 displayed significant inhibition of the growth of both strains *in vitro* with MIC values of 6.25 \( \mu \)M. Compounds 2 and 3 showed moderate inhibitory activities, with MIC values of 25 and 100 \( \mu \)M, respectively (Table 2). MIC values of each compound are the same for the biofilm-forming and non-biofilm-forming strains. All three compounds have antibacterial activities against six multidrug-resistant clinical isolates of *S. epidermidis*.

We tested antibacterial activities of the three compounds against *S. aureus* strain ATCC 49230. Compounds 1 and 2 displayed moderate inhibitory activities, with MIC values of 25 and 100 \( \mu \)M, respectively. Both MIC values are 3-fold higher than those for *S. epidermidis*. Compound 3 showed no growth inhibition of the *S. aureus* strain even at 200 \( \mu \)M. None of the three compounds had inhibitory activity against *E. coli* strain ATCC 25922.

**Effects of antimicrobial agents on culture turbidity and bacterial viability**

The effects of compounds 1, 2 and 3 on the growth and viability of *S. epidermidis* ATCC 12228 at multiples of the MIC (\( \times 8, \times 16 \)) were evaluated over a 10 h period. All the three compounds displayed bacteriostatic actions, since there was no decline in the absorbance or viability of cultures (Figure 3).
Cytotoxicity of the three SeWRS inhibitors

The cytotoxicity of compounds 1, 2 and 3 was investigated on Vero cells using the MTT assay. The CC$_{50}$ values of all three compounds were higher than 200 µM, which was the highest concentration used in the present study (Table 1). Besides, at the concentration of their respective MIC values, all these compounds displayed very low (<10%) cytotoxicity. As a control, an equal concentration of DMSO (1% DMSO) was added into the well with Vero cells and no obvious effect was observed.

Interaction model of the three inhibitors to SeWRS

As potential SeWRS inhibitors, three compounds selected by in silico screening bound with high affinity to the SeWRS protein and inhibited the adenylation in vitro. To further investigate the sites of interaction between the compounds and the SeWRS protein, and to develop a strategy for designing novel inhibitors, interaction models of the compounds with the SeWRS protein were analysed based on docking simulations. There is a hydrophobic area located at the bottom of the ATP and tryptophan binding pocket of the SeWRS, which is composed of residues Ile-8, Gln-9, Ser-11, Thr-15, Ile-16, Gly-17, Asn-18 and Gly-21, containing the conserved motif ‘TIGN’. The outer part of pocket is composed of residues Val-144, Gly-145, Asp-147, Gln-148, Gly-182, Val-184, Lys-193, Ser-194, Met-195, Ser-196 and Ser-197, containing the conserved motif ‘KMSKS’. The binding conformations of the inhibitors designed in the present study in the binding pocket of the SeWRS are shown in Figure S7 [available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)]. Although their structures are diverse, they adopt similar interactions with the conserved domain.

Discussion

Coagulase-negative S. epidermidis has become a major frequent cause of infections in relation to the use of implanted medical devices, which means that there is in urgent need for the discovery of new antibiotics. In this paper, WRS was investigated as a drug target for the discovery of new inhibitors.

As a class I family member of ARSs, WRS ligates L-tryptophan to the cognate tRNA$^{trp}$, which is subsequently used for protein synthesis. Aminoacylation reactions proceed in two steps. The first stage involves the activation of L-tryptophan by adenylation. The second stage is the transfer of the adenylated tryptophan to the acceptor end of the tRNA$^{trp}$. This reaction mechanism provides the theoretical basis on which to design or
reaction catalysed by ARSs. First, the substrate binding can be
screened various compounds that could specifically inhibit the
WRS. Besides, S. epidermidis WRS shares low protein sequence
identity with either mitochondrial WRS or cytosolic WRS of
human (32.1% and 9%, respectively, in this study), indicating
SeWRS is a potential antibacterial target.

Several strategies can be used with the aim of blocking the
reaction catalysed by ARSs. First, the substrate binding can be
interrupted by the substrate analogues. Secondly, blocking
the second stage of the reaction by generating mimetics of
the enzyme-bound reaction intermediate, aminoacyl-adenylate,
would be another target for inhibitors. Compared with the
substrate analogues, the aminoacyl-adenylate derivatives seem to be
more promising due to the higher affinities for the synthetase,
with dissociation constants typically in the nanomolar range.35
However, chemical synthesis seems to be time consuming and
costly. In this study, another strategy was used combining com-
putational technology in drug design with the bioassay
in vitro.

Figure 3. Effect of the three compounds on growth and survival of S.
epidermidis ATCC 12228. Compound 1 (filled circles), compound 2 (filled
triangles) and compound 3 (open triangles) at 8 (solid lines) and 16 (dashed
lines) times MIC were added to an early logarithmic phase culture in MHB
at time zero. Samples were taken at the times indicated for determination of
culture absorbance at 600 nm (a) and viable bacteria (b). The remainder of
the culture served as a drug-free control (filled squares).

we retained three promising candidates as SeWRS inhibitors.
The three compounds bound to the SeWRS protein in vitro and
inhibited its enzymatic activity. Compared with a strong inhi-
bition of the SeWRS activity, compounds 2 and 3 showed poor
inhibition of HWRS activity. This suggests that their inhibitory
effects towards SeWRS are specific, which is consistent with
their low cytotoxicity to mammalian cells. Although compound
1 displayed moderate inhibition of the enzymatic activity of
HWRS with an IC50 value of ~90 μM, its CC50 value for mam-

malian cells is higher than 200 μM. The three compounds were
active antimicrobials against S. epidermidis and also effective
against S. aureus, because the WRS protein shares high
sequence similarity (87%) in the two bacteria. None of the
compounds displayed inhibition of E. coli, in which the WRS
protein shares 53% sequence similarity with SeWRS. These
results may indicate the three antibacterial compounds are
species-specific. However, another possible reason for these
agents lacking antibacterial activity against E. coli may be extru-
sion from the cell, which needs further investigations. All the
three SeWRS inhibitors exhibited bacteriostatic actions against
S. epidermidis.

The specific recognition of tRNA by WRS suggests that
development of specific inhibitors would be an attractive propo-
sition.32 However, when screening from the same library in silico,
pool scores were obtained for the docking of the SPECS
compounds to the tRNA binding sites of SeWRS. None of the
top 50 compounds selected out showed inhibition of either the
growth of S. epidermidis or the enzymatic activity of SeWRS in vitro (data not shown).

Potential drug-resistant mutants may always be a threat to
new antibacterial agents. To fight against the challenge, two
strategies can be employed. One is to use the agent combined
with other antibiotics. In the present study, we tested the three
compounds against S. epidermidis ATCC 12228 in combination
with ampicillin. Ampicillin at MBC (128 mg/L) displayed very
fast and strong bactericidal effects in the first 6 h, but the anti-
bacterial activity abated from then on. When ampicillin at MBC
was used in combination with compound 1, 2 or 3 at MIC,
respectively, the bactericidal activity of ampicillin was weakened
in the first 8 h. However, the antibacterial effects persisted for
24 h [Figure S8, available as Supplementary data at JAC
Online (http://jac.oxfordjournals.org/)]. Those effects could also be
achieved by combining ampicillin at half MBC (64 mg/L) with
compound 1, 2 or 3 at MIC (data not shown). Thus, the anti-
biotic doses can be reduced to diminish the occurrence of
drug-resistant strains. The other way is the preparation of new
substitutes or novel ARS inhibitors. In this study, we found
three new SeWRS inhibitors. Now we are in the process of
structural optimizations of those lead compounds in order to find
more-effective derivatives. In future work, an appropriate animal
model also needs to be established to investigate the effect of
these compounds in vivo.

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New tryptophanyl-tRNA synthetase inhibitors

Transparency declarations
None to declare.

Supplementary data
Figures S1–S8 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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