ORGANIC CHEMISTRY
FRONTIERS

RESEARCH ARTICLE

View Article Online
View Journal | View Issue

Cite this: *Org. Chem. Front.*, 2019, **6**, 1194

Optimal design of thiostrepton-derived thiopeptide antibiotics and their potential application against oral pathogens†

Jian Wang, ‡[a] Zhi Lin, ‡[b,c] Xuebing Bai,[a] Jiang Tao*[a] and Wen Liu *[b,d]

Received 7th February 2019,
Accepted 27th February 2019

DOI: 10.1039/c9qo00219g

rsc.li/frontiers-organic

Thiostrepton (TSR), produced by *Streptomyces laurentii*, is a potent archetypal thiopeptide antibiotic that effectively antagonizes a broad spectrum of Gram-positive bacteria and has been used as a safe antimicrobial agent for animals. Siomycin (SIO) is a naturally produced TSR-type antibiotic that has been reported more potent than TSR. Based on a recent understanding regarding the structure–activity relationship of TSR against prokaryotic pathogens, we here constructed an ideal platform to obtain quinaldic acid (QA)-modified SIO analogs and generated a new SIO derivative, 5′-fluoro-SIO, with an unanticipated improvement in water solubility. To investigate whether oral diseases could be developed as novel indications for TSR-type antibiotics, we tested the minimum inhibitory concentrations (MICs) of these antibiotics against common oral pathogenetic microorganisms. Quantitative bioassays indicated that all of the tested TSR-type antibiotics exhibited potent antibacterial activity against the Gram-positive cariogenic microorganisms involved in the development of dental caries, as well as two major Gram-negative periodontal pathogens. Among the tested antibiotics, 5′-fluoro-SIO and SIO exhibited stronger potency than 5′-fluoro-TSR and TSR. These findings suggest that SIO may be more suitable than TSR as a lead compound to develop improved thiopeptide derivatives for clinical use and that TSR-type antibiotics have considerable potential for the prevention and treatment of dental caries and periodontitis.

Introduction

Thiopeptide antibiotics, displaying nanomolar potency toward various drug-resistant strains of Gram-positive pathogens, are a class of ribosomally synthesized and post-translationally modified peptide (RiPP) natural products, and to date, over 100 distinct thiopeptide antibiotics have been identified.[1] These antibiotics are characterized by a macrocyclic core that contains a six-membered nitrogen heterocycle central to multiple azoles (or azolines) and dehydroamino acids. Thiostrepton (TSR, Fig. 1A) is one of the most extensively studied thiopeptide antibiotics, and possesses a quinaldic acid (QA) moiety-containing side ring appended to the core system and has further been found with antitumor, antiplasmodial and immunosuppression activities.[2] TSR exerts its antibacterial effect by binding within a cleft located between the L11 protein and 23S rRNA of the 50S large ribosomal subunit, thereby perturbing translation factor binding and subsequent bacterial protein synthesis.[3] This mode of action is unique and distinct from those of current chemotherapeutics targeting the bacterial ribosome,[3e] making TSR a potent antibiotic for many drug-resistant bacteria in the clinic. Recently, we revealed another novel mode of action of TSR against the intracellular pathogen *Mycobacterium marinum*, which induces endoplasmic reticulum stress-mediated autophagy to enhance host cell defense.[4] Despite the impressive activity, the clinical application of TSR is largely hindered due to its poor water solubility and low bioavailability, which are the drawbacks of thiopeptides in general.[5] Until now, TSR was approved by the US Food and Drug Administration (FDA) only as an external drug for animal skin infections. Thus, the development of new applications, appropriate administration or improved analogs

[a]*Department of General Dentistry, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Key Laboratory of Stomatology, 639 Zhizaoju Road, Shanghai 200011, China. E-mail: taojiang_doctor@hotmail.com; Tel: +86-21-23271699-5202*
[b]*State Key Laboratory of Bioorganic and Natural Products Chemistry, Center for Excellence on Molecular Synthesis, Shanghai Institute of Organic Chemistry, University of Chinese Academy of Sciences, 345 Lingling Road, Shanghai 200032, China. E-mail: wliu@mail.sioc.ac.cn; Fax: +86-21-64166128; Tel: +86-21-54925111*
[c]*State Key Laboratory of Microbial Metabolism, School of Life Science & Biotechnology, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China*
[d]*Huzhou Center of Bio-Synthetic Innovation, 1366 Hongfeng Road, Huzhou 313000, China*

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c9qo00219g

‡These authors equally contributed to this work.

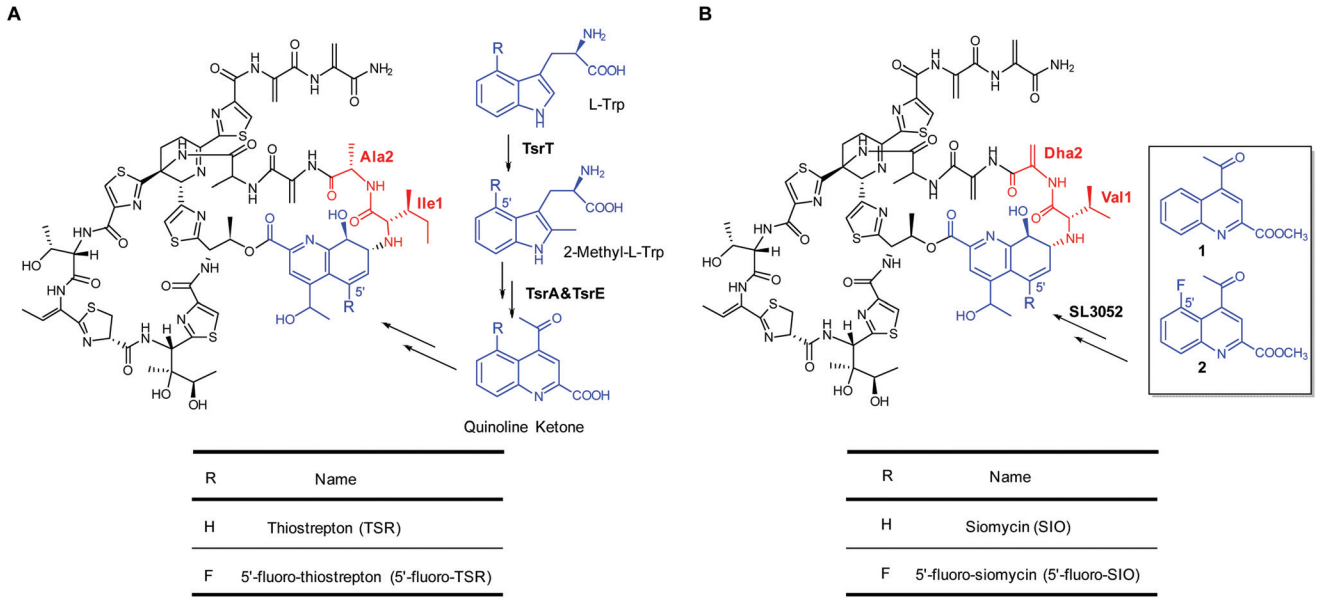

Fig. 1 TSR, 5′-fluoro-TSR, SIO and newly designed 5′-fluoro-SIO derivates. (A) Chemical structures of TSR and 5′-fluoro-TSR, and the pathway of QA incorporation during TSR biosynthesis. The QA moiety is shown in blue. (B) Chemical structures of SIO and 5′-fluoro-SIO, and the biosynthetic strategy for generating these compounds. The differences between TSR and SIO are marked in red.

for TSR would be necessary and effective to overcome the obstacles faced in clinical application.

The oral cavity harbors many microorganisms that together constitute a very complex microecological environment; to date, more than seven hundred bacterial species have been detected in the human mouth.[6] The dynamic balance maintained between the pathogenic bacteria and nonpathogenic bacteria through synergistic and antagonistic action keeps the human mouth healthy, while disruption of this balance causes oral diseases.[6c,7] Dental caries and periodontitis are the two most common oral diseases. The microorganisms related to dental caries are mostly Gram-positive, such as *Lactobacillus* spp., *Streptococcus* spp., and *Actinomyces* spp.[8] At present, the representative drugs for the treatment of dental caries are mainly chlorhexidine (CHX), triclosan, xylitol, cetylpyridinium chloride, and fluoride,[9] but they play only a prophylactic role in the early stage of caries and suffer from some disadvantages, such as discoloration of teeth and the tongue, poor ability to bind to the tooth surface and a short retention time.[10] Periodontitis is caused by a mixture of bacteria, including Gram-negative and Gram-positive bacteria, and is reported to be closely related to root canal infection and cardiovascular disease.[11] Treatment with antimicrobials, such as amoxicillin/metronidazole, tetracycline, levofloxacin (LVX), and macrolides,[12] is the most common method to control periodontal pathogens. However, the use of these drugs caused the emergence of multidrug-resistant microorganisms and has resulted in a series of side effects.[13] Unlike these commonly used antibiotics, TSR shows excellent antibacterial activity. In addition, it is odorless and colorless, making it an ideal potential drug for use as a contact anti-infective medicinal ointment for treating oral diseases.

Benefiting from the elucidation of the biosynthetic pathway of thiopeptides in recent years,[14] we have already developed a few improved TSR analogs *via* synthetic biology strategies that were unattainable by chemical syntheses and semisyntheses due to the complex architecture of TSR and the limited sites available for further functionalization.[15] As with many thiopeptides, TSR is biosynthesized from a ribosomally synthesized precursor peptide (TsrH) with a myriad of post-translational modifications.[14b,16] In our previous studies, we found that *tsrH* may serve as an important regulatory element in the gene cluster *tsr* and that the traditional method of *trans* complementation of a gene deletion cannot restore TSR production. Accordingly, we developed a single "base"-based mutagenesis strategy to modify the TSR precursor peptide and obtained six new TSR-type compounds,[4b,17] among them, siomycin (SIO, Fig. 1B), a naturally occurring compound that was hard to separate from its original strain *Streptomyces sioyaensis*, was obtained *via* simultaneous mutation of Ile1 and Ala2 in the TSR side ring and showed better antibacterial activity than TSR *in vitro*. Furthermore, the QA moiety in the side ring of TSR is biosynthesized independently of the precursor peptide and was biologically relevant, as it approaches A1067 of 23S rRNA, one of the key nucleobases contributing to mutation-induced bacterial resistance.[3d,18] Benefiting from the biosynthetic logic of the QA moiety, we recently designed and biosynthesized three derivatives, 5′-fluoro-TSR, 6′-fluoro-TSR and 12′-methyl-TSR (Fig. 1A and Fig. S1†), *via* the exogenous chemical feeding of synthetic QA analogs into the corresponding mutant strains.[19] Among these derivatives, 5′-fluoro-TSR showed the best antibacterial activity *in vitro*; this phenomenon was attributed to the electron-withdrawing effect of the C5′ fluoro-substitution that decreased the distance between QA and A1067. Given the poten-

tial synergistic effects of improving the antibacterial activity of TSR, we here designed and biosynthesized a new TSR-derived thiopeptide antibiotic, 5′-fluoro-SIO, and explored the activity of TSR and its analogs against common oral pathogens to develop new antibiotics for treating oral diseases in the clinic, as well as novel indications for thiopeptide use.

Results and discussion

We first designed the thiopeptide derivative 5′-fluoro-SIO derived from TSR because our previous work demonstrated that simultaneous mutation of Ile1 and Ala2 of TSR into SIO and introduction of C5′-fluorine into the QA moiety of TSR can increase antibacterial activity.[4b,19] We proposed that combining these beneficial modifications would result in a TSR derivative with better activity than the currently available TSR analogs. Because TSR is structurally complex and these modifications are difficult to attain by chemical syntheses and semi-syntheses, we produced 5′-fluoro-SIO through a biosynthetic method based on the strategies we set before.[14b,19]

The formation of the QA moiety during TSR biosynthesis involves methyl transfer onto and rearrangement of the indole moiety of L-Trp; this process is independent of the precursor peptide and relies on the activities of a SAM-dependent radical methylase, TsrT; a pyridoxal-5′-phosphate-dependent aminotransferase, TsrA; and a flavoprotein, TsrE, to produce a quinoline ketone as the key intermediate (Fig. 1A).[18b,20] In a previous study, we constructed a single "base"-based mutant strain SL2051, in which the GAG codon for Glu-7 of TsrH in the TSR-producing strain *Streptomyces laurentii* was mutated to the stop codon TAG (Fig. 2A), which completely abolished TSR production; moreover, this strain was also capable of restoring TSR production by *trans* complementation of a pSET152-based recombinant plasmid pSL2050, in which a single copy of *tsrH* containing a 530 bp upstream sequence and a 288 bp downstream sequence is under the control of the constitutive promoter of the erythromycin resistance gene *ermE*.[17c] On the basis of this work, we further performed in-frame deletion of the gene encoding TsrT in SL2051, which is responsible for the methyl transfer step for endogenous quinoline ketone generation, resulting in a new mutant strain SL3051 that abolished the production of the QA moiety (Fig. 2B and Fig. S3†). The structure of SIO is strikingly similar to TSR, differing only in the 1st and 2nd residues in the side ring (Val1 and Dha2 in SIO, Ile1 and Ala2 in TSR, Fig. 1). Because Dha2 in SIO is generated from Ser by a glutamate-dependent dehydratase during its biosynthesis,[21] we then constructed the pSL2050 derivative pSL3051, encoding the precursor peptide variant TsrH–Ile1Val/Ala2Ser, by site-specific mutagenesis and introduced it into the host SL3051 to provide the precursor peptide substrate of SIO *in situ* by *trans* expression, resulting in a new strain SL3052 (Fig. 2B). Exogenous feeding of the synthetic ester analog of the quinoline ketone intermediate (**1**) into the strain SL3052 resulted in the robust biosynthesis of SIO (40–66 mg L^{-1}), which is 50–60% of the yields achieved for TSR (80–110 mg L^{-1}) produced by the wild-type strain (Fig. 1B and 3A), demonstrating that the strain SL3052 is an

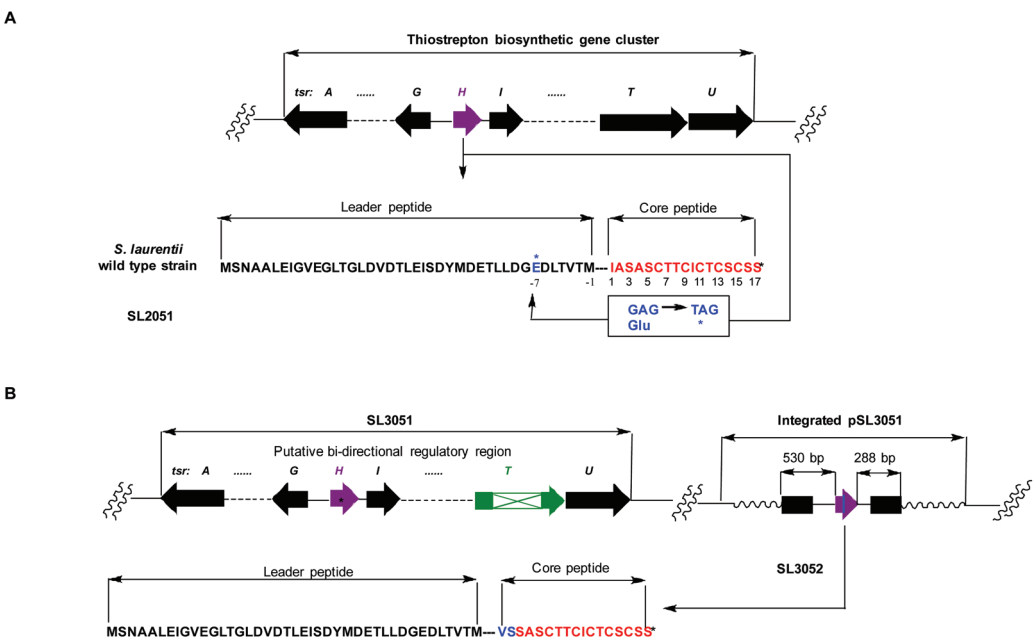

Fig. 2 The single "base"-based strategy of site-directed mutagenesis of *tsrH* and the development of the recombinant strain SL3052. (A) Genotypes of the *S. laurentii* wild-type strain and SL2051 (by mutation of GAG for Glu-7 into the stop codon TAG).[17c] The *tsrH* gene and post-translationally modified genes are marked in purple and black, respectively. The position where the stop codon was introduced *via* an *in situ* single "base"-mutation is indicated by a blue star. (B) Genotypes of the recombinant strain SL3052. The mutational positions of the *tsrH* gene in integrated pSL3051 are marked in blue, and the inactivated *tsrT* gene is shown in green.

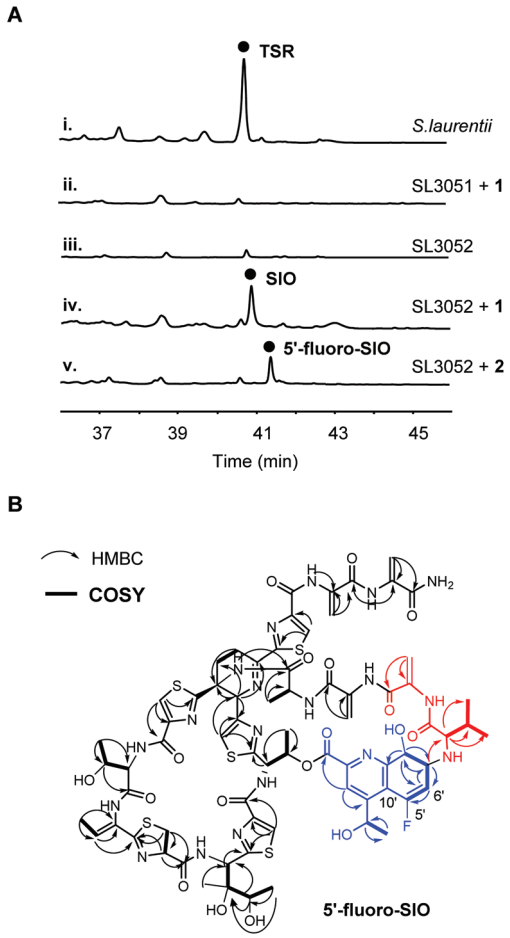

Fig. 3 Production of the TSR-derived thiopeptide derivative 5′-fluoro-SIO and its structural elucidation. (A) HPLC analysis of the fermentation cultures of different strains. i, the wild-type TSR-producing strain; ii, the SL3051 strain fed with **1**; iii, the SL3052 strain; iv, the SL3052 strain fed with **1**; v, the SL3052 strain fed with **2**. (B) HMBC and COSY correlations of 5′-fluoro-SIO. The QA moiety is shown in blue, and the 1st and 2nd residues are shown in red.

ideal host to obtain QA-modified SIO analogs by exogenous feeding of the corresponding ester analogs of the quinoline ketone intermediate.

To acquire the target compound 5′-fluoro-SIO, we fed the synthetic 5-fluorinated ester analog of the quinoline ketone intermediate (**2**) into the strain SL3052 (Fig. 1B and 3A). As anticipated, 5′-fluoro-SIO was produced with a yield of 10–12 mg L^{-1} ($[M + H]^+$ *m*/*z*: calcd 1666.4589 for $C_{71}H_{80}N_{19}O_{18}S_5F$, found 1666.4574), verifying that the biosynthetic machinery of TSR can tolerate the simultaneous modification of the QA moiety and the 1st and 2nd residues in the side ring. Subsequently, 5′-fluoro-SIO was purified and subjected to comparative NMR spectroscopic analysis with SIO (Fig. 3B, Fig. S5 and Table S3†). Despite the overall similarity in the spectra, distinct signals showed the only difference between these molecules to be the substitution of the QA moiety. The ^{19}F NMR showed a signal at δ_F −110.58 ppm, corresponding to the disappearance of the ^1H NMR signal at δ_H 6.90 ppm found for SIO, and the ^{13}C NMR signals of C-5′, C-6′ and C-10′ of the QA moiety were accordingly shifted. For SIO, the ^{13}C NMR signals of C-5′, C-6′ and C-10′ of the QA moiety were showed at δ_C 123.2 ppm, δ_C 130.3 ppm and δ_C 127.1 ppm, respectively, while the corresponding ^{13}C NMR signals of 5′-fluoro-SIO were shifted to δ_C 170.1 ppm, δ_C 107.0 ppm and δ_C 123.7 ppm, respectively.

The newly obtained thiopeptide derivative, 5′-fluoro-SIO, together with SIO, TSR, and the previously obtained 5′-fluoro-TSR, was subjected to a wide variety of *in vitro* quantitative bioassays against oral pathogens. *Streptococcus* spp., *Lactobacillus* spp. and *Actinomyces* spp. are considered to be the three major pathogens related to dental caries formation, and all of these pathogens are Gram-positive.[18] *Streptococcus* spp. can produce acids and glucans, which are components of a common extracellular matrix of dental plaque biofilms and are considered to be one of the initial colonization that cause dental caries.[22] *Lactobacillus* spp. are later colonizers of the surface of teeth and are often discovered in caries lesions, causing demineralization of teeth,[22b] while *Actinomyces* spp. are a common species in mature dental plaque biofilms, usually found on the surface of root caries.[23] Thus, the resident microflora on the surfaces of teeth have diverse compositions, and the bacterial species will be constantly altered from the initial dental plaque biofilm to the mature dental plaque biofilm. To evaluate the efficacy of thiopeptides in the different phases of dental caries, we selected a representative pathogen from each of these three genera to determine the TSR-type antibiotics' minimum inhibitory concentrations (MICs), namely, *Streptococcus mutans* UA159, *Lactobacillus acidophilus* ATCC 4356, and *Actinomyces viscosus* ATCC 19246 (Table 1). The results indicated that all of the TSR-type antibiotics tested exhibited substantially more potency than the most common anti-caries agents (CHX, sodium fluoride), and 5′-fluoro-SIO, SIO and 5′-fluoro-TSR displayed increased activity compared with TSR, yielding a potency order of 5′-fluoro-SIO (more than 2-fold) ≈ SIO (more than 2-fold) > 5′-fluoro-TSR (2-fold) > TSR, thus demonstrating that TSR-type antibiotics can play a certain

Table 1 MICs (μg mL^{-1}) of TSR and its analogs against Gram-positive cariogenic microorganisms in the development of dental caries. Sodium fluoride (NaF) and chlorhexidine (CHX) were chosen as control drugs

	TSR	5′-F-TSR	SIO	5′-F-SIO	NaF	CHX
Streptococcus mutans UA159	0.064	0.032	0.016	0.016	2000	1.6
Lactobacillus acidophilus ATCC 4356	0.064	0.032	0.016	0.016	>16 000	1.6
Actinomyces viscosus ATCC 19246	0.002	0.001	<0.001	<0.001	4000	0.8

Table 2 MICs (μg mL^{-1}) of TSR and its analogs against two of the major Gram-negative periodontal pathogens. Levofloxacin (LVX) and tinidazole (TNZ) were chosen as control drugs

	TSR	5′-F-TSR	SIO	5′-F-SIO	LVX	TNZ
Fusobacterium nucleatum ATCC 25286	8	4	4	4	0.64	0.32
Porphyromonas gingivalis ATCC 33277	0.064	0.128	0.004	0.016	0.064	1.28

role in the resistance against the Gram-positive cariogenic microorganisms in the early, middle and late phases of dental caries development, and that SIO may be more suitable than TSR as a lead compound to develop anti-caries antibiotics.

Periodontitis is generally caused by a mixture of bacteria, including Gram-negative and Gram-positive bacteria. *Porphyromonas gingivalis* and *Fusobacterium nucleatum* are two of the important Gram-negative periodontal pathogens, especially *P. gingivalis*, which is recognized as being related to cardiovascular diseases.[11a,24] However, the bioactivities of thiopeptides against Gram-negative bacteria have rarely been reported in previous studies. To evaluate the efficacy of thiopeptides in periodontitis, *P. gingivalis* ATCC 33277 and *F. nucleatum* ATCC 25286 were used to determine the TSR-type antibiotics' MICs. Intriguingly, all of the tested TSR-type antibiotics exhibited promising activity, especially the activity against *P. gingivalis* ATCC 33277, which was much higher than that of the commonly used potent antibiotics for periodontitis (levofloxacin and tinidazole, Table 2). It is well known that the antibacterial mechanism of TSR is distinct from those of current chemotherapeutics, indicating that TSR-type antibiotics would not cause drug resistance or chiasmatic resistance when used as preventive and therapeutic drugs for periodontitis, which are the major problems faced by commonly used antibiotics, thus displaying considerable potential for further application in periodontitis. In addition, measurement of the water solubility of these compounds revealed the following order: 5′-fluoro-SIO (15.0 ± 1.1 μg mL^{-1}) > 5′-fluoro-TSR (2.6 ± 1.0 μg mL^{-1}) ≈ TSR (2.5 ± 1.1 μg mL^{-1}) > SIO (1.5 ± 0.1 μg mL^{-1}). Although the newly obtained 5′-fluoro-SIO did not show a significant improvement in the action against the oral pathogens mentioned above, the unanticipated improvement in the solubility, which might be a result of an increase of the molecular dipole moment, makes 5′-fluoro-SIO more potent in clinical applications than the previously available TSR-type antibiotics, highlighting the advantage of modifying the QA moiety in SIO.

Conclusions

The antimicrobial peptide TSR exhibits excellent bioactivities and has been extensively studied over recent decades. Due to its poor water solubility and low bioavailability, TSR has not been developed for human therapy and was approved by FDA only for animal use.[1,13] Considering the biological properties of TSR, we speculated that TSR-type antibiotics might be suitable and effective drugs for common human oral diseases, such as dental caries and periodontitis. In addition, based on the proposed effect of modification of the QA moiety of SIO, a naturally produced TSR-type antibiotic, we constructed a new mutant strain, SL3052, by jointly utilizing a single "base"-based mutagenesis strategy and deletion technology in *S. laurentii*, thus generating an ideal host to obtain QA-modified SIO analogs, such as 5′-fluoro-SIO, by exogenous feeding the corresponding ester analogs of the quinoline ketone intermediate. As anticipated, the newly obtained 5′-fluoro-SIO, along with SIO, TSR and the previously obtained 5′-fluoro-TSR displayed substantially higher antibacterial activity against the Gram-positive cariogenic microorganisms involved in the development of dental caries than did the clinically used control chemotherapeutics (CHX, sodium fluoride). Moreover, these TSR-type antibiotics also displayed promising activity against two major Gram-negative periodontal pathogens, which is hard to predict, suggesting that TSR-type antibiotics have considerable potential to be used as antibacterial agents to prevent and treat both dental caries and periodontitis. During the quantitative bioassays, both 5′-fluoro-SIO and SIO exhibited stronger potency than 5′-fluoro-TSR and TSR, and unexpectedly, the derivative 5′-fluoro-SIO showed approximately 6-fold improvement in water solubility in comparison with TSR. These findings are significant and indicate that SIO may be more suitable than TSR as a lead compound to develop improved thiopeptide derivatives for clinical use; moreover, the mutant strain SL3052 could serve as an effective platform to engineer SIO. A few antimicrobial peptides, such as defensin, histatin, cathelicidin LL-37, lactoferrin, nisin, and pleurocidin, have already received attention in the control of pathogenic microorganisms in the oral cavity and showed good antibacterial activity against a few oral pathogenic bacteria in some studies.[25] However, TSR-type antibiotics are first reported for oral disease, and further application of these antibiotics in the oral cavity requires the support of *in vivo* studies.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported in part by grants from NSFC (31430005, 21750004, 21520102004, 21472231, and 21621002), CAS (QYZDJ-SSW-SLH037 and XDB20020200), STCSM (17JC1405100 and 18ZR1422700), MST (2018ZX091711001-006-010), CPSF (BR0800043), and K. C. Wang Education Foundation.

References

1 (*a*) M. C. Bagley, J. W. Dale, E. A. Merritt and X. Xiong, *Chem. Rev.*, 2005, **105**, 685–714; (*b*) J. Li, X. Qu, X. He, L. Duan, G. Wu, D. Bi, Z. Deng, W. Liu and H. Y. Ou, *PLoS One*, 2012, **7**, e45878.

2 (*a*) S. K. Radhakrishnan, U. G. Bhat, D. E. Hughes, I. C. Wang, R. H. Costa and A. L. Gartel, *Cancer Res.*, 2006, **66**, 9731–9735; (*b*) M. N. Aminake, S. Schoof, L. Sologub, M. Leubner, M. Kirschner, H. D. Arndt and G. Pradel, *Antimicrob. Agents Chemother.*, 2011, **55**, 1338–1348; (*c*) M. Ueno, S. Furukawa, F. Abe, M. Ushioda, K. Fujine, S. Johki, H. Hatori and H. Ueda, *J. Antibiot.*, 2004, **57**, 590–596.

3 (*a*) Y. Xing and D. E. Draper, *Biochemistry*, 1996, **35**, 1581–1588; (*b*) L. B. Blyn, L. M. Risen, R. H. Griffey and D. E. Draper, *Nucleic Acids Res.*, 2000, **28**, 1778–1784; (*c*) G. Lentzen, R. Klinck, N. Matassova, F. Aboul-ela and A. I. Murchie, *Chem. Biol.*, 2003, **10**, 769–778; (*d*) J. M. Harms, D. N. Wilson, F. Schluenzen, S. R. Connell, T. Stachelhaus, Z. Zaborowska, C. M. Spahn and P. Fucini, *Mol. Cell*, 2008, **30**, 26–38; (*e*) D. N. Wilson, *Nat. Rev. Microbiol.*, 2014, **12**, 35–48.

4 (*a*) Q. Zheng, Q. Wang, S. Wang, J. Wu, Q. Gao and W. Liu, *Chem. Biol.*, 2015, **22**, 1002–1007; (*b*) P. Duan, Q. Zheng, Z. Lin, S. Wang, D. Chen and W. Liu, *Org. Chem. Front.*, 2016, **3**, 1254–1258.

5 X. Just-Baringo, F. Albericio and M. Alvarez, *Angew. Chem., Int. Ed.*, 2014, **53**, 6602–6616.

6 (*a*) H. K. Kuramitsu, X. He, R. Lux, M. H. Anderson and W. Shi, *Microbiol. Mol. Biol. Rev.*, 2007, **71**, 653–670; (*b*) J. A. Aas, B. J. Paster, L. N. Stokes, I. Olsen and F. E. Dewhirst, *J. Clin. Microbiol.*, 2005, **43**, 5721–5732; (*c*) T. Terai, T. Okumura, S. Imai, M. Nakao, K. Yamaji, M. Ito, T. Nagata, K. Kaneko, K. Miyazaki, A. Okada, Y. Nomura and N. Hanada, *PLoS One*, 2015, **10**, e0128657.

7 P. D. Marsh, *Br. Dent. J.*, 1991, **171**, 174–177.

8 (*a*) N. Takahashi and B. Nyvad, *J. Dent. Res.*, 2011, **90**, 294–303; (*b*) E. R. Davenport, *Cell Host Microbe*, 2017, **22**, 251–253.

9 (*a*) J. Autio-Gold, *Oper. Dent.*, 2008, **33**, 710–716; (*b*) S. Zimmer, K. R. Jahn and C. R. Barthel, *Oral Health Prev. Dent.*, 2003, **1**, 45–51.

10 (*a*) O. Fejerskov and E. Kidd, *Dental caries: the disease and its clinical management*, Blackwell Munksgaard, Oxford, 2008; (*b*) J. M. Ten Cate, *J. Dent. Res.*, 2012, **91**, 813–815.

11 (*a*) J. Kim and S. Amar, *Odontology*, 2006, **94**, 10–21; (*b*) F. DeStefano, R. F. Anda, H. S. Kahn, D. F. Williamson and C. M. Russell, *Br. Med. J.*, 1993, **306**, 688–691; (*c*) A. Blaizot, J. N. Vergnes, S. Nuwwareh, J. Amar and M. Sixou, *Int. Dent. J.*, 2009, **59**, 197–209; (*d*) G. S. Sfyroeras, N. Roussas, V. G. Saleptsis, C. Argyriou and A. D. Giannoukas, *J. Vasc. Surg.*, 2012, **55**, 1178–1184; (*e*) E. Nordendahl, A. Gustafsson, A. Norhammar, P. Nasman, L. Ryden and B. Kjellstrom, *J. Dent. Res.*, 2018, **97**, 1114–1121; (*f*) A. Holmlund, E. Lampa and L. Lind, *Atherosclerosis*, 2017, **262**, 101–106.

12 (*a*) M. Kaufmann, P. Lenherr, C. Walter, T. Thurnheer, T. Attin, D. B. Wiedemeier and P. R. Schmidlin, *Dent. J.*, 2018, **6**, 59; (*b*) A. Mombelli, N. Cionca and A. Almaghlouth, *Periodontol.*, 2011, **55**, 205–216; (*c*) A. P. Monteiro, C. M. Rocha, M. F. Oliveira, S. M. Gontijo, R. R. Agudelo, R. D. Sinisterra and M. E. Cortes, *Carbohydr. Polym.*, 2017, **156**, 417–426; (*d*) V. J. O'Rourke, *Aust. Dent. J.*, 2017, **62**, 14–22; (*e*) N. Andere, N. C. Castro Dos Santos, C. F. Araujo, I. F. Mathias, T. Taiete, R. C. V. Casarin, M. A. N. Jardini, L. M. Shaddox and M. P. Santamaria, *J. Periodontol.*, 2017, **88**, 1244–1252; (*f*) T. Nakajima, T. Okui, H. Ito, M. Nakajima, T. Honda, Y. Shimada, K. Tabeta, K. Akazawa and K. Yamazaki, *Antimicrob. Agents Chemother.*, 2016, **60**, 1779–1787; (*g*) G. Khan, S. K. Yadav, R. R. Patel, G. Nath, M. Bansal and B. Mishra, *AAPS PharmSciTech*, 2016, **17**, 1312–1325.

13 (*a*) C. Angebault and A. Andremont, *Eur. J. Clin. Microbiol. Infect. Dis.*, 2013, **32**, 581–595; (*b*) M. L. Cohen, *Science*, 1992, **257**, 1050–1055.

14 (*a*) L. C. Wieland Brown, M. G. Acker, J. Clardy, C. T. Walsh and M. A. Fischbach, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 2549–2553; (*b*) R. Liao, L. Duan, C. Lei, H. Pan, Y. Ding, Q. Zhang, D. Chen, B. Shen, Y. Yu and W. Liu, *Chem. Biol.*, 2009, **16**, 141–147; (*c*) W. L. Kelly, L. Pan and C. Li, *J. Am. Chem. Soc.*, 2009, **131**, 4327–4334.

15 Z. Lin, Q. He and W. Liu, *Curr. Opin. Chem. Biol.*, 2017, **48**, 210–219.

16 S. Wang, S. Zhou and W. Liu, *Curr. Opin. Chem. Biol.*, 2013, **17**, 626–634.

17 (*a*) C. Li, F. Zhang and W. L. Kelly, *Mol. Biosyst.*, 2011, **7**, 82–90; (*b*) C. Li, F. Zhang and W. L. Kelly, *Chem. Commun.*, 2012, **48**, 558–560; (*c*) H. Guo, J. Wang, Y. Li, Y. Yu, Q. Zheng, J. Wu and W. Liu, *Chem. Sci.*, 2014, **5**, 240–246.

18 (*a*) S. Baumann, S. Schoof, M. Bolten, C. Haering, M. Takagi, K. Shin-ya and H. D. Arndt, *J. Am. Chem. Soc.*, 2010, **132**, 6973–6981; (*b*) L. Duan, S. Wang, R. Liao and W. Liu, *Chem. Biol.*, 2012, **19**, 443–448.

19 S. Wang, Q. Zheng, J. Wang, Z. Zhao, Q. Li, Y. Yu, R. Wang and W. Liu, *Org. Chem. Front.*, 2015, **2**, 106–109.

20 Z. Lin, J. Ji, S. Zhou, F. Zhang, J. Wu, Y. Guo and W. Liu, *J. Am. Chem. Soc.*, 2017, **139**, 12105–12108.

21 G. A. Hudson, Z. Zhang, J. I. Tietz, D. A. Mitchell and W. A. van der Donk, *J. Am. Chem. Soc.*, 2015, **137**, 16012–16015.

22 (*a*) B. Rosan and R. J. Lamont, *Microbes Infect.*, 2000, **2**, 1599–1607; (*b*) P. D. Marsh, *Microbiology*, 2003, **149**, 279–294.

23 S. R. Brailsford, E. Lynch and D. Beighton, *Caries Res.*, 1998, **32**, 100–106.

24 (*a*) M. Desvarieux, R. T. Demmer, T. Rundek, B. Boden-Albala, D. R. Jacobs Jr., R. L. Sacco and P. N. Papapanou, *Circulation*, 2005, **111**, 576–582; (*b*) R. J. Genco and T. E. Van Dyke, *Nat. Rev. Cardiol.*, 2010, **7**, 479–480.

25 B. R. da Silva, V. A. de Freitas, L. G. Nascimento-Neto, V. A. Carneiro, F. V. Arruda, A. S. de Aguiar, B. S. Cavada and E. H. Teixeira, *Peptides*, 2012, **36**, 315–321.