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Recent development of leucyl-tRNA synthetase inhibitors as antimicrobial agents

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Aminoacyl-tRNA synthetases (aaRSs) widely exist in organisms and mediate protein synthesis. Inhibiting these synthetases can lead to the termination of protein synthesis and subsequently achieve antibacterial and antiparasitic purposes. Moreover, the structures of aaRSs found in eukaryotes have considerable structural differences compared to those in prokaryotes, based on which it is possible to develop highly selective inhibitors. Leucyl-tRNA synthetase (LeuRS) with unique synthesis and editing sites is one of 20 kinds of aaRSs. Many inhibitors targeting LeuRS have been designed and synthesized, some of which have entered clinical use. For example, the benzoxaborole compound AN2690 has been approved by the FDA for the treatment of onychomycosis. AN3365 is suspended in the phase II clinical trial due to the rapid development of AN3365 resistance, but it may be used in combination with other antibiotics. The aaRSs, especially LeuRS, are being considered as targets of new potential anti-infective drugs for the treatment of not only bacterial or fungal infections but also infections by trypanosomes and malaria parasites. This review mainly describes the development of LeuRS inhibitors, focusing on their mechanisms of action, structure-activity relationships (SARs), and *in vitro* and *in vivo* activities.

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United States. Dr. Ma's interests have been focused on the design and synthesis of small molecules against resistant bacteria such as FtsZ, AcrB and QS inhibitors and structural modification and structure-activity relationship of complicated structure antibiotics.

1. Introduction

Aminoacyl-tRNA synthetases (aaRSs) widely exist in all organisms and play an important role in the biosynthesis of proteins. They mainly mediate the condensation of amino acids and homologous tRNAs. There are two main steps (Fig. 1): the first step is to condense the amino acid (aa) with ATP to form an intermediate (aa-AMP) closely linked to the synthetases, and the second step is to transfer the aminoacyl group to the 3' end of the homologous tRNA.^{1–3} Therefore, inhibition of this process will stop the synthesis of proteins and subsequently block bacterial growth. Moreover, the structures of aaRSs found in eukaryotes have considerable structural differences compared to those in prokaryotes, which provide a structural basis for achieving selective inhibitors of bacterial aaRSs.⁴

The aaRSs have a variety of functions.⁵ For example, they not only participate in transcriptional regulation but also play an important role in other aspects such as tRNA mature proofreading and mitochondrial RNA cleavage (Fig. 2). Actually, 20 standard amino acids in most organisms have corresponding tRNA synthetases. The synthetases can be divided

Fig. 1 A schematic diagram of the catalytic process of aminoacyl-tRNA synthetase.

Fig. 2 The cellular activities of aminoacyl-tRNA synthetases.

into two types according to the mutually exclusive sequence motifs which can express distinct active site topologies. The first class has a classical Rossmann dinucleotide-binding domain, including arginyl tRNA synthetase (ArgRS), methionyl tRNA synthetase (MetRS), leucyl tRNA synthetase (LeuRS) *etc.*, and the second class mainly includes histidyl tRNA synthetase (HisRS), glycyl tRNA synthetase (GlyRS) *etc.*, which have new anti-parallel β -sheets.⁶ The aaRSs represent an ideal target for the discovery of new synthetic antimicrobial drugs because of their indispensable role in protein synthesis.⁷ Moreover, since the aaRSs of prokaryotes are structurally conserved, it is possible to develop new broad-spectrum antibiotics.^{8–12} Drug resistance is mainly caused by mutations of key amino acids in the enzyme active site, while aaRSs are not easily mutated.^{13–15} Thus they could not become drug resistant easily. However, only mupirocin and tavaborole are now in clinical applications.¹⁶ Mupirocin is an isoleucyl tRNA synthetase inhibitor which has been used clinically as an antibiotic against methicillin-resistant *Staphylococcus aureus* (MRSA),^{17,18} but it has a narrow antibacterial spectrum and poor pharmacokinetics.¹⁹ Tavaborole, approved by the FDA for the treatment of onychomycosis in 2014,²⁰ is a drug with a boron-containing structure, which primarily inhibits LeuRS that is a proofreading aaRS with unique synthesis and editing active sites separated by more than 30 Å.^{21,22} Therefore, the development of new aaRSs inhibitors, especially LeuRS inhibitors, as antimicrobial drugs has not only great potential²³ but also an important significance for the development of new antimicrobial drugs.

2. Inhibitors of LeuRS

The initial investigation on LeuRS inhibitors is mainly centered on nonhydrolyzable aminoacyl-AMP analogues such as compound 1 (Fig. 3) and **agrocine 84** (2).^{24–26} However, these analogues usually have high inhibitory activity against human LeuRS, which limits their development as antimicrobial agents. Moreover, their antibacterial activity *in vivo* is rather weak due to poor intake of those compounds.^{2,11,27} In addition,

the structure of adenosine analogues is too complex to be rapidly modified for obtaining new inhibitors as needed. It was not until Yu *et al.*²⁸ developed new LeuRS inhibitors 3–5 (Fig. 3) based on an acyl sulfonamide structure by substituting a thiazole ring for the adenine ring that the selectivity problems were overcome. After continuous development, inhibitors with different skeletons have been found through structural optimization, which show good activity against LeuRS. In this review, the LeuRS inhibitors are mainly classified into the following categories: (1) C-3 unsubstituted benzoxaboroles, (2) 1,3,5-triazine compounds, (3) 3-aminomethyl benzoxaboroles, (4) 2-pyrrolinones, (5) sulfonamides, (6) *N*-benzylidene-*N'*-thiazol-2-yl-hydrazines, (7) 5-phenylamino-2*H*-[1,2,4] triazin-3-ones, and others.

2.1. Inhibitors targeting the editing site of LeuRS

The initial development of LeuRS inhibitors was mainly to target the editing sites of LeuRS, which could be divided into boron-containing compounds and boron-free compounds in general.

2.1.1. Boron-based compounds. In recent years, boron-containing compounds targeting LeuRS have been developed for the treatment of not only bacterial or fungal infections^{27,29,30} but also infections by trypanosomes³¹ and malaria parasites.³² What's more, these benzoxaboroles have low biotoxicity and high target specificity.^{33–35} In view of these, the representative compounds, structure-activity relationships (SARs) and mechanism as well as their applications are described below.

2.1.1.1. C-3 unsubstituted benzoxaboroles. In order to develop new antifungal drugs for the treatment of onychomycosis whose local treatment was poor and oral treatment was prone to recurrence, Baker *et al.*³⁶ designed and synthesized a series of benzoxaborole derivatives and studied their SARs (Fig. 4). *Trichophyton rubrum* and *Trichophyton mentagrophytes*, which cause onychomycosis,³⁷ were selected to determine the antifungal activity of the above compounds, and the minimum inhibitory concentration (MIC) against yeast and mold was tested to determine their broad-spectrum

Fig. 3 The structures of compounds 1–5.

activity. The results indicated that 1-hydroxyl substitution both increased the hydrophilicity and had a broader spectrum activity, while fluorine substitution at the 5-position was superior to the same substitution at other positions and was better than other substitutions at the 5-position. This finally resulted in the discovery of AN2690 (6) (Fig. 4) possessing a broad spectrum of antifungal activity.²⁰ In 2007, Rock *et al.*^{10,27} reported that 6 inhibited LeuRS by binding its boron atom to the 2'- and 3'-oxygen of the 3'-end adenosine of tRNA to form a stable tRNA-AN2690 adduct in the editing site of the enzyme and ultimately blocked protein synthesis. Furthermore, pharmacokinetic studies showed that 6 had a slow onset and long residence time in complex, which still bonded to targets after it was cleared by metabolic clearance below the effective concentration.^{38–40} In 2014, 6 was approved by the FDA for the treatment of onychomycosis. Afterward, it was also demonstrated to specifically inhibit *S. cerevisiae* cytoplasmic LeuRS via the oxaborole tRNA capture (OBORT) mechanism and to

inhibit LeuRS of *Leishmania*,⁴¹ which provided new possibilities for the further development of the antiparasitic drug. ZCL039 (7) (Fig. 4), a derivative of 6, was proved to be a LeuRS inhibitor targeting *S. pneumonia*.²⁹ It effectively inhibited the growth of *S. pneumonia* with a MIC value of 5 $\mu\text{g mL}^{-1}$ and has low toxicity to human cells. After kinetics and biochemical analysis combined with the crystal structure of the complex of ZCL039-AMP and the isolated *S. pneumonia* LeuRS editing domain, it was found that 7 united with the editing active sites of LeuRS in the presence of tRNA^{Leu} through a non-competitive inhibition mechanism. These findings suggest that LeuRS has great potential as a new target for the treatment of *S. pneumonia* infection.

Based on the findings on 6, Ding *et al.*⁴² further designed and synthesized other 6-substituted and 7-substituted compounds with a benzoxaborole core structure for potential treatment of parasitic infections such as African trypanosomiasis (HAT) caused by *Trypanosoma brucei*, one of the

Fig. 4 The design strategy of dihydrobenzoxazole and the structures of compounds 6 and 7.

neglected tropical diseases in the poorest countries^{43,44} in 2011. In general, the 6-substituted compounds have better inhibitory activity against *T. brucei* LeuRS than the 7-substituted ones. This is mainly because the substituent at the 7-position is not conducive to binding to the eukaryotic helix. Next, the effects of various substituents at the 6-position on the activity against *T. brucei* LeuRS were studied (Table 1). It was found that compounds with small non-polar substituents had better activity due to the limited space of the binding pocket. For the effect of different ester substitutions on the activity (Table 1), the results exhibited that a small ester group was better in the activity. Further, a methyl or an ethyl group at the α -position of the ester group was found to increase the activity, while the activity of phenyl substitution at the α -position was the opposite because of the limited space in the pocket. An attempt was made to replace the ester with an amide or a ketone in order to increase the stability of these compounds; consequently, the amide-substituted or ketone-substituted compounds were found to show a remarkable decrease in the activity. However, if a substituent was introduced at the α -site of ketone, it would lead to strong activity equivalent to that of the ester group. Among them, the most active compound **8l** exerted an IC_{50} value of 1.6 μM on *T. brucei* LeuRS. Finally, the assay of *T. brucei* parasite growth inhibition and toxicity to mammalian cells was conducted. Parasite growth inhibition IC_{50} values of between 0.37 and 12.93 μM were observed. In addition, the above compounds were found to have low toxicity characteristics to L929 cells (mouse lung fibroblast cells) with an IC_{50} value of more than 40 μM . Molecular docking results showed that the sp^2 hybridized boron atom of compounds **8** such as compound **8v** formed an adduct (Fig. 5) with the tRNA by accepting electrons from the hydroxyl group of the terminal adenosine, thereby allowing the tRNA to be captured at the editing site of *T. brucei* LeuRS to inhibit the enzyme activity. This is the first successful report on parasite LeuRS inhibitors, some of which not only have good activity against *T. brucei* LeuRS but also inhibit *T. brucei* parasite growth *in vitro* with low cytotoxicity. This indicated that the boron-containing compounds can be further developed as lead compounds of LeuRS inhibitors for the treatment of infections by parasites.

2.1.1.2. 3-Aminomethyl benzoxaboroles. According to World Health Organization statistics, there were approximately 100 million people infected with *M. tuberculosis* (*Mtb*), resulting in 1.7 million deaths in 2016.⁴⁵ Furthermore, the developing widespread drug resistance or complete drug resistance of *Mtb* is becoming more and more serious. Therefore, it is urgent to find new anti-tuberculosis drugs. The amino acid sequences of human and *Mtb* LeuRS active sites are significantly different, which lays a foundation for the selective inhibition of *Mtb*.⁴⁶ Moreover, LeuRS is a target that is less likely to become drug resistant. Based on the above fact, Li *et al.*⁴⁷ initially screened some benzoxaboroles as LeuRS inhibitors for anti-tuberculosis. According to the results of the activity assay, **AN3017** (**9**) (Fig. 6) with a 3-aminomethyl group

Table 1 Inhibitory effect of different 6-substitutions on *T. brucei* LeuRS

Compd		
	R	LeuRS IC_{50} (μM)
8a		>100
8b		63.0
8c		30.6
8d	H	30.9
8e		15.8
8f		43.1
8g		32.3
8h		3.5
8i		3.6
8j		2.1
8k		2.8
8l		1.6
8m		7.4
8n		41.7
8o		>100
8p		77.7
8q		2.5
8r		3.8
8s		2.9
8t		3.5
8u		31.4
8v		5.0
8w		4.5
8x		67.6
8y		>100

Fig. 5 The covalent adduct between compound **8v** and the terminal AMP of tRNA.

displayed significantly increased activity with a MIC value of $1.8 \mu\text{g mL}^{-1}$ against *Mtb* H37Rv and an IC_{50} of $0.64 \mu\text{M}$ against *Mtb* LeuRS than **AN2679 (10)** (Fig. 6) with a MIC value of $7.5 \mu\text{g mL}^{-1}$ and an IC_{50} of $21.3 \mu\text{M}$. Inspired by the results, they further determined the activity of other 3-aminomethyl benzoxaborole derivatives. The results showed that the 4-halogen substituted compound exerted better activity than the 3- or 5-substituted compounds. Moreover, among the active enantiomers, the (*S*)-isomer exhibited better activity than the (*R*)-isomer.

Later, Li *et al.*⁴⁸ obtained three series of compounds **13**, **14**, and **15** (Table 2) by chemical modifications on the C-6 and C-7 positions of compounds **11** and **12** (Fig. 6). Compounds **11** and **12** belong to 3-aminomethyl benzoxaboroles that targeted *Met* LeuRS. These *Met* LeuRS inhibitors used boron atoms in compounds to form bidentate covalent adducts with tRNA terminal nucleotides, and then the covalent adducts captured the 3'-terminal tRNA in the editing sites, thus inhibiting leucylation and finally inhibiting protein synthesis. The preliminary screening results indicated that with the exception of the (*R*)-configuration compounds, all other compounds showed good activity with IC_{50} values of $0.046\text{--}1.11 \mu\text{M}$ for *Met* LeuRS, and the (*S*)-configuration compounds had MIC values between 0.03 and $0.6 \mu\text{M}$ for *Mtb* H37Rv. In general, the activity of the (*S*)-configuration compounds was better than that of their racemates. The anti-tubercular activity decreased by 2- to 5-fold with the increase of 7-alkoxy chains and even lost anti-tuberculosis activity when an aromatic ring was introduced into the 7-position. The 6-substituted compounds have potent activity against *Met* LeuRS, but the anti-tuberculosis activity is significantly reduced compared to that of the corresponding 7-substituted compounds. If a fused ring is introduced at the C-6 and C-7 positions, the five-membered dioxolane compounds will have no anti-tubercular activity, which is due to their unfavorable polarity and/or conformation. In remarkable contrast, the compounds with the six-membered dioxane ring exhibited decent activity. Among them, two (*S*)-isomers, **15d** and **15g**, had excellent activity equivalent to that of compounds **11** and **12**. Then, the *in vitro* selectivity of the initially screened compounds **13e**, **13f**, **13v**, **13y** and **15d** was tested to determine their safety. Fortunately, these compounds possessed varying degrees of increased selectivity for human cytoplasmic LeuRS and HepG2. Finally, the *in vivo* clearance, oral exposure and

Fig. 6 The structures of compounds **9–12**.

anti-tuberculosis ability of the above compounds were evaluated in a mouse model. Among them, compound **13v** was found to show the best overall characteristics and good effects on both acute and chronic tuberculosis.

AN3365 (GSK2251025/GSK052) (16) (Fig. 7), a boron-containing compound, which binds to the terminal adenosine ribose (A76) of LeuRS to inhibit protein synthesis,⁵² is dedicated to the treatment of infections of Gram-negative bacteria in hospital intensive care units.^{49–51} **16** can evade the main efflux mechanism and bring down to the clinical treatment of infections by multi-drug-resistant Gram-negative bacteria.³⁰ Unfortunately, due to the rapid development of **AN3365** resistance,⁵³ **16** has been discontinued in the phase II clinical trial.⁵⁴ However, in 2016, Monteferrante *et al.*⁵⁵ found that the combination of **16** with colistin slowed the selection of resistant mutants. **DS86760016 (17)** (Fig. 7) was a new leucyl-tRNA synthetase inhibitor, which reduced more greatly risks of drug resistance than **16** and was able to inhibit multi-drug resistant Gram-negative bacteria such as *Pseudomonas aeruginosa* and *E. coli*. What's more, compared with **16**, **17** not only showed lower mutant prevention concentration against *P. aeruginosa* but also reduced plasma clearance, increased renal excretion and prolonged plasma half-life in mice, rats, monkeys and dogs.⁵⁶ In the same year, Sonoiki *et al.*³² found that **12** had an inhibitory effect on LeuRS of *Plasmodium falciparum* which was resistant to various antimalarials. Moreover, it was further demonstrated to be an inhibitor of LeuRS in the editing active site of *Toxoplasma gondii* and *Cryptosporidium*. It also showed potent efficacy against murine malaria models.⁵⁷ **12** (Fig. 6) was found to exhibit a high activity with an EC_{50} (concentration that gives 50% of maximal effect) value of $2.2 \mu\text{M}$ in Madin-Darby canine kidney (MDCK) cells infected by *C. parvum*, almost equivalent to that of nitazoxanide which was used as the standard of care treatment for *C. parvum*,⁵⁸ 230 times better than paromomycin against *C. parvum*.⁵⁹ The inhibition mechanism of **12** was also found to form a covalent adduct at the editing site of LeuRS to inhibit protein synthesis or block post-transcriptional editing. In general, the boron-containing compounds targeting LeuRS have not only a wide range of antiparasitic and antibacterial activities but also good oral bioavailability³⁰ and low toxicity.⁶⁰

Although the above-described boron-based compounds represent highly promising or clinically used LeuRS inhibitors, it is worth mentioning that some pathogens are naturally resistant to these LeuRS inhibitors. For example,

Table 2 *In vitro* activity of 3-aminomethyl benzoxaborole derivatives

Compd	Structure			IC ₅₀ (μM)		MIC (μM)
	Configuration	R (or n)	X	<i>Mtb</i> LeuRS	<i>Mtb</i> H37Rv	

Class 1						
11	(S)		Br	0.075		0.1
12	(S)		Cl	0.056		0.1
13a	(S)		H	0.13		0.6
13b	(S)		F	0.08		0.2
13c	(S)		Cl	0.31		0.16
13d	(S)		Br	0.24		0.38
13e	(S)		Cl	0.15		0.1
13f	(S)		Br	0.074		0.1
13g	(S)		Cl	0.27		0.6
13h	(S)		Br	0.20		0.45
13i	Racemate		Cl	0.32		5
13j	Racemate		Br	0.47		>5
13k	Racemate		Cl	0.72		5
13l	Racemate		Br	0.47		>5
13m	Racemate		Cl	0.076		0.2
13n	Racemate		Br	0.26		0.2
13o	Racemate		Cl	0.29		0.3
13p	Racemate		Br	0.24		0.31
13q	Racemate		Br	0.69		>5
13r	Racemate		Cl	0.32		5
13s	(S)		Cl	0.47		0.14
13t	(S)		Br	0.16		0.3
13u	Racemate		Cl	0.24		0.63
13v	(S)			0.20		0.08
13w	(R)			10		5
13x	Racemate		Br	0.15		0.16
13y	(S)			0.12		0.08
13z	(R)			14.2		10
Class 2						

14a	Racemate		Cl	0.63		>5
14b	Racemate		Br	0.26		3.75
14c	Racemate		Cl	0.25		1.3
14d	Racemate		Br	0.37		0.6
14e	Racemate		Cl	1.11		>5
14f	Racemate		Br	0.89		>5
14g	Racemate		Cl	0.44		>5
14h	Racemate		Br	0.37		>5

Table 2 (continued)

Compd	Structure			IC ₅₀ (μM)		MIC (μM)
	Configuration	R (or n)	X	<i>Mtb</i> LeuRS	<i>Mtb</i> H37Rv	

Class 3						
15a	Racemate	n = 1	Cl	0.25		>5
15b	Racemate	n = 1	Br	0.61		>5
15c	Racemate	n = 2	Cl	0.13		0.31
15d	(S)			0.046		0.08
15e	(R)			12.6		80
15f	Racemate	n = 2	Br	0.092		0.5
15g	(S)			0.12		0.03
15h	(R)			4.2		40

pathogens from the genus *Mycoplasma* and the phylum *Microsporidia* were shown to have unusual LeuRS isoforms in which the editing domain is highly reduced or degenerated.^{61,62} Hence, LeuRS isoforms from these organisms are not capable of binding to **6** and are naturally resistant to **6** and, likely, to other boron-based LeuRS inhibitors.^{61,62} Similarly, LeuRS isoforms with altered editing sites and with apparent benzoxaborole resistance were found in more than a hundred bacterial species including representatives of *Rickettsia*, *Yersinia*, *Chlamydia*, *Yersinia*, *Chlamydia*, and other emerging human pathogens.⁶³

2.1.2. Boron-free compounds. Some boron-free compounds have emerged as LeuRS inhibitors, which inject new vitality into the development of LeuRS inhibitors.

Many 1,3,5-triazine compounds have been found to be used as anti-infective drugs.^{64–70} Moreover, 1,3,5-triazine and thiazole compounds play an important role in many other aspects, such as antiviral^{71,72} and anti-cancer^{73,74} activities. In 2013, Singh *et al.* attempted to investigate the inhibitory effect of phenylthiazole-1,3,5-triazine on the editing domain of *Candida albicans* LeuRS by molecular docking. Docking results showed that these compounds acted as inhibitors of

Fig. 7 The structures of compounds **16** and **17**.

C. albicans cytoplasmic LeuRS in stable contact with target proteins through effective hydrogen bonding and pi-cation interactions.

2.2. Inhibitors targeting the synthetic site of LeuRS

2.2.1. 2-Pyrrolinones. At present, drugs such as suramin and eflornithine used for the treatment of HAT all have the disadvantage of inefficiency, serious side effects and insufficient administration. In order to overcome these problems, Zhao *et al.* first investigated some inhibitors targeting the synthetic active site of *T. brucei* LeuRS.⁷⁵ In initial screening for 26 compounds with various structures by a combination of pharmacophore- and docking-based approaches, compound **18a** (Fig. 8) with a 2-pyrrolinone group was found to be active. In order to further study the SARs of the compounds, more 2-pyrrolinone derivatives **18** were tested for *T. brucei* LeuRS. The results showed that any other group substituted for the indole ring at the R³ position (Fig. 8) would reduce the activity. This illustrated that the indole ring played an irreplaceable role in the interaction with LeuRS. Regarding the effect of R¹ substitution (Fig. 8) on the activity of those 2-pyrrolinone derivatives, it can be seen that when the benzene ring at the R¹ position was substituted by a fluorine atom, the activity against *T. brucei* LeuRS would be significantly lowered, while a methoxy group or chlorine atom substitution would retain the activity. On the other hand, the effect of the R² substituent (Fig. 8) on the activity is relatively complicated. A *para*-substituent on the benzene ring generally had better activity than an *ortho*- or a *meta*-substituent. If there are no substituents on the benzene ring, the activity will decrease. If the R² group is replaced by various hydrophobic substituents such as naphthalene and alkylphenyl groups, the resultant compounds will produce certain activity. This is related to the fact that the R² group occupies the hydrophobic pocket in the active sites. The above research results led to the discovery of the most active compound **18b** (IC₅₀ = 31.9 μM) whose R¹ is a *p*-methoxyphenyl group, R² is an isobutylphenyl group, and R³ is an indole group (Fig. 8). Molecular docking of compound **18c** (Fig. 8) with *T. brucei* LeuRS showed that the indolyl moiety inserted into the hydrophobic pocket formed by Pro46, Ser599, Leu596, Leu688, Tyr47 and His692, and the NH group of the indolyl group formed a hydrogen bond with the Tyr47 skeleton carbonyl group. It cannot be ignored that the R² substituent inserted

into a hydrophobic pocket formed by Pro48, Leu62, Phe45 and Leu54 which was not occupied by Leu-AMS. The 2-pyrrolinones discussed above are the first inhibitors targeting the synthetic active site of *T. brucei* LeuRS but their activity needs to be further improved. Their indole ring is essential for the interaction with the leucine-recognition pocket.

2.2.2. Sulfonamides. *N*-(4-Sulfamoylphenyl)thiourea derivatives **19** are a new class of LeuRS inhibitors that target the synthetic site of *T. brucei*^{27,42,76,77} LeuRS. The first compound **19a** with the *N*-(4-sulfamoylphenyl)thiourea core structure (Fig. 9) was screened by Zhang *et al.* It displayed an IC₅₀ value of 174.5 μM for *T. brucei* LeuRS.⁷⁸ The initial structural modifications indicated that acylation of the sulfonamide terminal free-amino group led to a significantly increased activity. Among compounds with different acylation groups, compound **19b** (Fig. 9) with the NHCbz-phenylalanyl substructure presented the most potent activity against *T. brucei* LeuRS. After docking compound **19b** (Fig. 9) with the active sites of *T. brucei* LeuRS, it was found that this compound bonded to the synthetic active site rather than the editing active site, in which the R¹ group extended to the enzyme leucyl-binding pocket and the R² group stretched to the adenylation pocket. Thus, their attempts were made to convert the phenylalanyl analogs to a series of leucyl analogs, which were further tested for their activity against *T. brucei* LeuRS. The NHCbt analog **19c** (Fig. 9) with an IC₅₀ value of 16.7 μM was the best in the activity against *T. brucei* LeuRS, almost equivalent to compound **19b**. In addition, the effect of the R² substitution on the activity was also studied by retaining the NHCbz-phenylalanyl structure of compound **19b**. Unfortunately, all the resultant compounds displayed a reduced activity. Finally, the affinity for *T. brucei* LeuRS was improved by trying to change the 1,4-substitutions of the central benzene ring to 1,3-substitutions. The results showed that leucyl-substituted compounds such as compounds **19d**, **19e**, **19f** and **19g** (Fig. 9) were the strongest in the activity against *T. brucei* LeuRS, which was because the Cbz group was too large to form hydrogen bonds. In order to judge the selectivity for *T. brucei* LeuRS, the four most active compounds **19d**–**19g** were selected to determine their activity against human cytoplasmic LeuRS (HcLeuRS). However, the results indicated that they only had 4.5- to 7.3-fold selectivity for *T. brucei* LeuRS. Moreover, their membrane permeability was found to be poor and their inhibitory activity at 100 μM against *T. brucei* growth was low. The discovery of the compounds

Fig. 8 The design strategy of 2-pyrrolinones and the structures of compounds **18a**, **18b** and **18c**.

Fig. 9 The design strategy of *N*-(4-sulfamoylphenyl)thiourea derivatives and the structures of compounds 19a–19g.

provides a new scaffold for inhibitors targeting *T. brucei* LeuRS, but further improvements in selectivity and membrane permeability are needed.

On the basis of the previously synthesized benzenesulfonamide-based LeuRS or ThrRS inhibitors,^{78,79} Charlton *et al.*⁸⁰ continued to design other *N*-leucinyl benzenesulfonamide derivatives by docking them with the protein model based on the X-ray structure of *E. coli* LeuRS. Finally, compounds 20 (Table 3) obtained by introducing pyrimidine and pyridine with different substituents into the *meta*-position of the benzene ring were found to be positive. Moreover, some of these compounds not only had nanomolar affinity for *E. coli* LeuRS in the isothermal titration calorimetry (ITC) experiment but also showed a potent ability to inhibit *E. coli* LeuRS with high selectivity for human and *S. aureus* LeuRS.⁸¹ Comparing the ITC results, compounds 20a and 20e were found to have similar affinity for *E. coli* LeuRS, indicating that the N1 atom of pyrimidine did not contribute to a binding force. In contrast, compound 20f without an amino group showed a reduced binding enthalpy, demonstrating that the amino group provided an additional hydrogen bond. Finally, it was found that the compound 20j possessed the best binding affinity for *E. coli* LeuRS with a *K_d*

value of 1.4 nM. Then, the standard strain *E. coli* BW25113 was selected to determine their antibacterial sensitivity. Only compound 20j was found to exhibit antibacterial activity and it showed strong activity against *E. coli* ATCC 25922, medium activity against *K. pneumoniae*, *P. aeruginosa* and *Acinetobacter* spp., and no activity against *S. aureus* in a broader assay. Furthermore, compounds 20c and 20j showed no improved activity against *E. coli* lacking the key multidrug efflux transporter components TolC and AcrB,⁸² which demonstrated that mechanisms other than efflux influenced the activity of *N*-leucinyl benzenesulfonamide derivatives.

2.2.3. *N*-Benzylidene-*N'*-thiazol-2-yl-hydrazines. Gudzera *et al.*⁴⁶ preliminarily screened 270 compounds with the best scores from 100 000 organic chemistry library entries using computer modeling methods^{83–86} and finally obtained some inhibitors targeting *Mtb* LeuRS after *in vitro* experiments. It was found that compounds 21–24 (Fig. 10) belonging to *N*-benzylidene-*N'*-thiazol-2-yl-hydrazine derivatives and compounds 25 and 26 (Fig. 10) from 3-[5-(2-methyl-3-phenyl-allidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-*N*-phenyl-propionamide derivatives showed an inhibitory activity against *Mtb* LeuRS, but only the former was further studied since the latter had a higher IC₅₀ value and belonged to the pan-assay interfering

Table 3 ITC binding constants of *N*-leucinyl benzenesulfonamide derivatives to *E. coli* LeuRS and the enzymatic potency against *E. coli*, *S. aureus*, and human LeuRS

Compd	R	<i>E. coli</i> LeuRS ITC, ^a <i>K</i> _d (nM)	ITC, Δ H (kcal mol ⁻¹)	ITC, Δ -T Δ S (kcal mol ⁻¹)	<i>E. coli</i> LeuRS IC ₅₀ ^b (nM)	<i>S. aureus</i> LeuRS IC ₅₀ ^b (nM)	Human LeuRS IC ₅₀ ^b (nM)
20a		14.5 ± 0.3	-9.5 ± 0.5	-1.2 ± 0.5	54	9.2 × 10 ³	3.9 × 10 ³
20b		12.8 ± 1.2	-9.0 ± 0.3	-1.7 ± 0.3	14	650	490
20c		10 ± 3	-12.5 ± 1.2	1.6 ± 1.0	41	4.4 × 10 ³	160
20d		56 ± 17	-7.6 ± 0.3	-2.3 ± 0.4	224	3.6 × 10 ³	33
20e		13.9 ± 1.9	-9.1 ± 0.11	-1.62 ± 0.07	23	1.7 × 10 ³	850
20f		22 ± 4	-7.05 ± 0.05	-3.40 ± 0.13	47	2.7 × 10 ³	2.0 × 10 ³
20g		10.9 ± 1.5	-8.27 ± 0.11	-2.6 ± 0.18	13	920	1.2 × 10 ³
20h		10.2 ± 1.4	-8.1 ± 0.5	-2.8 ± 0.5	32	640	3.4 × 10 ³
20i	H	1.3 ± 0.4	-13.1 ± 0.3	1.1 ± 0.2	35	5.4 × 10 ³	1.1 × 10 ³

^a ITC data for binding to *E. coli* LeuRS enzyme. ^b IC₅₀ values were within 10% of the error range.

compounds. Since the substituted bromine atom at the 4-position of the phenyl ring is capable of forming a hydrophobic interaction with the amino acid residues in the adenine-binding region, compound 21 (IC₅₀ = 6 μM) has better activity

than the 4-nitro-substituted compound 22 (IC₅₀ = 8 μM), and compound 22 with a methoxy group at the 3-position of another benzene ring exerts increased activity than compound 23 (IC₅₀ = 20 μM). This is related to the hydrophobic

Fig. 10 The structures of representative *N*-benzylidene-*N'*-thiazol-2-yl-hydrazines 21–26.

Table 4 *In vitro* inhibitory activity of *N*-benzylidene-*N'*-thiazol-2-ylhydrazine derivatives toward *M. tuberculosis* LeuRS. Inhibitory effect of 2-pyrolinone derivatives on *Tb* LeuRS

Compd	R ¹	R ²	R ³	IC ₅₀ (μM)
27a		H		2.27
27b		H		4.54
27c				11.4
27d		H		15.9
27e		H		19.3
27f		H		>20
27g		H		>20
27h		H		>20
27i		H		>20
27j		H		>20
27k		H		>20
27l				>20
27m				>20
27n				>20
27o		H		>20
27p		H		>20
27q	H	H		>20
27r	H	H		>20
27s	H	H		>20

Table 4 (continued)

Compd	R ¹	R ²	R ³	IC ₅₀ (μM)
27t	H	H		>20
27u		H		>20
27v				>20
27w				>20
27x	H	H		>20
27y				>20
27z				>20

interactions of the methoxy group with amino acid residues in the leucyl-binding region of the active site.

In order to further investigate *N*-benzylidene-*N'*-thiazol-2-ylhydrazines as LeuRS inhibitors, an additional 26 derivatives 27 were selected for screening the *in vitro* activity (Table 4), five of which could inhibit *Mtb* LeuRS. In particular, the most active compound 27a (IC₅₀ = 2.27 μM), which was prepared by replacing the nitro and methoxy groups of compound 22 with a bromine atom, even showed greatly improved activity than compound 19. Then, the five most active compounds 21, 22, 27a, 27b and 27c were selected to determine their inhibitory activity against human LeuRS. Their inhibitory ability was significantly lower than that against *Mtb*, meaning that these compounds were selective to *Mtb* LeuRS. In an assay to determine the antimicrobial effect on *Mtb* H37Rv, compound 21 with an IC₅₀ value of 10.01 μM and an IC₉₀ value of 13.35 μM had optimal cell permeability and inhibited pathogen growth. Computer simulation results showed that these compounds interacted with the amino acid residues Gly715, Leu111, Gly108, Gly112, His109, Tyr716, Ile717 and Gly714 in the leucyl-binding region of LeuRS active sites.

2.2.4. 5-Phenylamino-2*H*-[1,2,4] triazin-3-ones. 5-(2-Hydroxy-5-methyl-phenylamino)-6-methyl-2*H*-[1,2,4]triazin-3-one (28) (Fig. 11) that was screened from more than 100 000 different organic compounds not only had the best score in computer simulation but also reduced the aminoacylation activity of *Mtb* LeuRS in *in vitro* experiments. In the presence of this compound at 100 μM, the residual activity of LeuRS of *Mtb* was 46%. Subsequently, 63 analogs of 28 were selected to determine their inhibitory activity against *Mtb* LeuRS. Among them, 5-[5-chloro-2-hydroxy-phenylamino)-6-methyl-2*H*-[1,2,4]-

Fig. 11 The structure of compounds 28–30.

Fig. 12 The structure of TM84.

triazin-3-one (29) and 5-(5-chloro-2-hydroxy-phenylamino)-2H-[1,2,4]triazin-3-one (30) displayed excellent activity with IC_{50} values of 7.6 and 7.2 μM , respectively (Fig. 11). In the assay of LeuRS inhibitory activity in human cytoplasmic LeuRS, the inhibition efficacy of the two compounds decreased by 7- and 9-fold, respectively. Moreover, compared with compound 28, their LeuRS residual activity decreased significantly.⁸⁷ These compounds were found to inhibit the LeuRS mainly by interacting with the amino acid residues such as Gly108, Leu111, Gly115, Tyr 716 and forming a hydrogen bond with Gln714 in the leucyl-binding region.

2.3. Others

Compound 2 is a LeuRS inhibitor produced by the biological control agent *Agrobacterium radiobacter* strain K84.^{88–90} 2 acts as a molecular Trojan horse,^{89,91,92} which mimics plant tumor derivatives to enter *Agrobacterium* cells,^{93,94} where it is processed into the toxin TM84 (31) (Fig. 12). 31 is a potent inhibitor of the *A. tumefaciens* genome-encoded LeuRS. At first, many people thought that the inhibition mechanism of 31 was to mimic other aminonoacylation-AMP analogs to bind the active site, which resulted in inhibiting LeuRS. But later studies showed that 31 possessed a new tRNA-dependent LeuRS inhibition mechanism.^{95,96}

3. Conclusions

Increasing microbial resistance has brought great difficulties to clinical drugs. In this serious situation, new targets are needed to develop new antimicrobial drugs. LeuRS belongs to a class of aaRSs that play an important role in protein synthesis. Inhibition of this enzyme will stop the synthesis of the protein to achieve antimicrobial action. Since the aaRSs of eukaryotes and prokaryotes are structurally different, highly selective antimicrobial drugs can be studied. In addition, the aaRSs of prokaryotes are structurally conserved, thus

it is possible to develop new broad-spectrum antibiotics. The early LeuRS inhibitors were developed mainly based on boron-containing compounds. For instance, the LeuRS inhibitor AN2690 which contains boron atoms has been already approved by the FDA for the treatment of onychomycosis. However, some compounds with novel skeletons like 2-pyrrolinones and *N*-benzylidene-*N'*-thiazol-2-yl-hydrazine derivatives have also been developed as LeuRS inhibitors recently. In particular, some of them have not only good *in vivo* and *in vitro* activity but also high selectivity and cell permeability. For example, compound 27a ($IC_{50} = 2.27\ \mu M$) from *N*-benzylidene-*N'*-thiazol-2-yl-hydrazine not only effectively inhibits *Met* LeuRS but also has low cytotoxicity. These inhibitors of LeuRS are used for the treatment of both bacterial or fungal infections and infections by trypanosomes and malaria parasites. In general, LeuRS as an anti-infection target has aroused widespread concern. Next, various methods should be used to develop new skeleton compounds or to obtain compounds with better activity *in vitro* and *in vivo*. It is believed that LeuRS inhibitors with high selectivity and strong activity as new antimicrobial agents will be discovered and applied in the near future.

Conflicts of interest

There are no conflicts to declare.

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