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REVIEW

Natural Products and Their Derivatives as tRNA Synthetase Inhibitors and Antimicrobial Agents[1]

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R. V. K. Cochrane,[a] A. K. Norquay[a] and J. C. Vederas[a]

Abstract The tRNA synthetase enzymes are promising targets for development of therapeutic agents against infections by parasitic protozoans (e.g. malaria), fungi and yeast, as well as bacteria resistant to current antibiotics. These ubiquitous enzymes load a particular amino acid onto its cognate tRNA to form an aminoacyl tRNA in preparation for protein synthesis by the ribosome. Although tRNA synthetases for a given amino acid are often similar in different organisms, slight differences in their sequence and structure can permit highly selective interaction with inhibitors. Hence, these enzymes have been successful targets of anti-infective agents for decades. They can also act as anticancer agents by preventing protein synthesis in human cells. Many inhibitors have been isolated from nature or have designs inspired by natural products. This review lists known naturally derived aaRS inhibitors and particularly aims to highlight advances in our understanding of their application as antibacterial, anticancer and antimalarial therapeutics. Advances in structural understanding, overcoming resistance and increasing inhibitor efficacy will also be discussed.

Introduction

The transcription of DNA to RNA and translation of its information into protein sequence has been a fundamental area of research for many decades. The aminoacyl tRNA synthetases (aaRS) that specifically activate and load amino acids onto the correct tRNA molecules are critical players in this process and are essential for cell viability.[1] Although these enzymes are ubiquitous, divergence in their sequence and structure has made it possible for organisms to construct secondary metabolites (natural products) that selectively target the aaRS proteins of their potential competitors or food sources. In addition, differences between prokaryotic and eukaryotic versions make these bacterial enzymes viable targets for antibiotics.[2-5] The threatening increase in antibiotic resistance provide impetus for development of new agents to eliminate pathogens that have become resistant to current therapy.[6] As described below, parasitic protozoans are another major health concern that are being targeted by inhibitors of aaRS. World Health Organization reported 214 million cases of malaria resulting in an estimated 438,000 deaths in 2015, primarily due to *Plasmodium falciparum*.[7] Other protozoan diseases include amoebic dysentary, giardiasis, toxoplasmosis, cryptosporidiosis, trichomoniasis, Chagas disease, leishmaniasis and African trypanosomiasis. Finally fungal and yeast infections may also have an Achilles heel in their aaRS enzymes.

[a] Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2; email john.vederas@ualberta.ca
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[1]The authors declare no competing interests.

Rachel Cochrane obtained her BSc. (Hons.) at Queen's University Belfast, Northern Ireland in 2010. She began her PhD later that year with Professor John C. Vederas at the University of Alberta, where her research focused on the use of biochemical tools to investigate polyketide synthases that produce important biologically active compounds

Rachel V. K. Cochrane

John Vederas is Distinguished University Professor of Chemistry at the University of Alberta. He completed his B.Sc. at Stanford University and his Ph.D. with George Büchi at MIT. His postdoctoral work at the University of Basel (Christoph Tamm) and at Purdue University (Heinz Floss) inspired a continuing interest in application of organic chemistry to understanding of biological mechanisms. He joined University of Alberta in 1977. He received a number of recognitions for his work, including the Chemical Institute of Canada Medal. He is a Fellow of the Royal Society (London) and also of the Royal Society of Canada.

John C. Vederas

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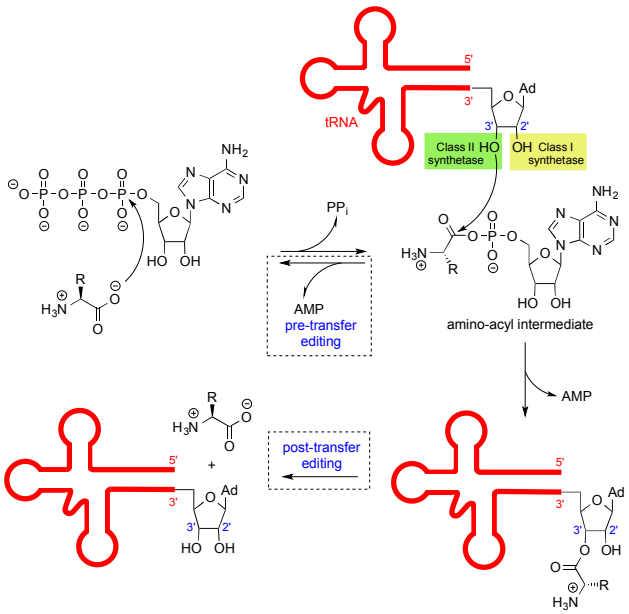

Fig. 1 Mechanism by which tRNA synthetases attach each amino acid to its cognate tRNA molecule. Some aaRS enzymes are also capable of pre or post transfer editing.

Enzyme function and structure

Each tRNA is specific for an amino acid, and tRNA synthetases function to attach each amino acid to its specific tRNA molecule over two steps (Fig 1). The first step involves attack on adenosine triphosphate (ATP) by the amino acid, which releases pyrophosphate and yields the aminoacyl-adenylate intermediate. The amino acid is then transferred to the 2' (class I) or 3' (class II) hydroxyl of the 3' terminal adenosine residue (A^{76}) of the tRNA molecule to furnish the aminoacyl-tRNA complex.[8, 9] If the aminoacyl is initially attached to the nucleotide at 2', the *O*-aminoacyl group will eventually intramolecularly migrate to the 3' position. All characterised aaRSs catalyse an initial two-step activation-acylation reaction, however the two distinct classes of aaRS, also have differences in their catalytic domain structure (summarized in table 1 and represented in Fig 2).[10-12] Class I aaRSs contain a Rossman nucleotide binding fold, which consists of the conserved His-Ile-Gly-His (HIGH) and Lys-Met-Ser-Lys-Ser (KMSKS) amino acid sequences. In some cases this active site is also capable of pre-transfer editing of a non-cognate aa-AMP. Further to this active site, around half of known class I aaRSs contain a separate domain, known as the connective peptide 1 (CP1) domain, which is capable of hydrolysing non-cognate aa-tRNAs.[13-18] This domain is inserted into the Rossman fold, effectively splitting the catalytic centre into two $\beta_3\alpha_2$ subunits. The class II tRNA synthetases have a relatively unique active site architecture in which anti-parallel beta-sheets are flanked by a series of alpha helices. Furthermore, the class II synthetases all contain three short signature sequence motifs: motifs 1; 2; and 3, which either form enzyme homodimer interactions (motif 1) or constitute the active site (motifs 2 and 3).[19-21]

Amy Norquay received her B. Sc. (Hons) in Chemistry from the University of Manitoba in 2011. She subsequently began her Ph.D. training at the University of Alberta under the supervision of John Vederas, studying polyketide biosynthesis by combining chemical and molecular biology methods. In 2015, Amy began her medical training in the joint MD/PhD program (Class of 2019), also at the University of Alberta.

Amy K. Norquay

Table 1 Class I and class II aaRSs and their structural characteristics.

	Class I	Class II
tRNA synthetase	ArgRS, CysRS, GlnRS, GluRS, IleRS, LeuRS, LysRS-I, MetRS, TrpRS, TyrRS, ValRS	AlaRS, AsnRS, AspRS, GlyRS, HisRS, LysRS-II, PheRS, ProRS, ThrRS, SerRS
Active site structural motifs	$\beta_6\alpha_4$ Rossman-fold	Anti-parallel β-sheet
Conserved sequence motifs	HIGH, KMSKS	Motifs 1, 2 and 3
tRNA aminoacylation site	3'-OH	2'-OH

Enzyme Inhibitors

Although all aaRSs share similar reaction mechanisms, the divergence between prokaryote and eukaryote enzymes, potentially allows for the selective inhibition of a protein in a pathogen. Numerous natural products and their derivatives are effective inhibitors of these enzymes, with several diverse mechanisms of action. Some aaRS inhibitors bind in the

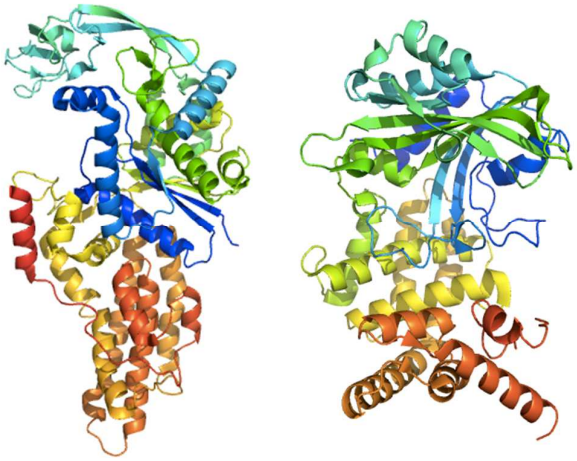

Fig. 2 Structures of the active site fragments of MetRS (PDB: 1QQT),[11] left, and AlaRS (PDB: 1RIQ),[12] right, representing Class I and Class II aaRSs respectively.

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synthetic active site of the enzyme, where they may mimic the amino acid substrate or the reactive aminoacyl intermediate. Others occupy regions outside of the synthetic active site, allosterically affecting the active site itself. Excellent reviews of inhibitors of aaRS as antibacterials and as antiprotozoal agents have been published.[3, 22-25] This review aims to highlight more recent advances in our understanding of naturally derived aaRS inhibitors, and their application as antibacterial, anticancer, and antimalarial therapeutics. Listed in table 2 are the known natural product aaRS inhibitors, some of which will be discussed herein.

Table 2: Natural product inhibitors of tRNA synthetase enzymes

Target enzyme	Compound	Target organism	Ref
AsnRS	Tirandamycins	*Brugia malayi*	26
	WS9326A WS9326C WS9326D WS9326E	*Brugia malayi*	27
	Adipostatins	*Brugia malayi*	28
	Variolin B	*Brugia malayi*	29
	Rishirilide	*Brugia malayi*	29
AspRS	Microcin C	Bacteria	30
IleRS	Mupirocin	*Haemophilus influenza* *Neisseria gonorrheae* *Neisseria meningitides* *Staphylococcus aureus*	31, 32
	Kalimatacin/Batumin	Bacteria, primarily *S. aureus*	33
	Reveromycin A	Eukarya, including human tumour cells and osteoclasts	34
	Furanomycin[a]	Bacteria	35
LeuRS	Granaticin	*Bacillus subtilis*	36
	Agrocin 84	*Agrobacterium tumefaciens*	37
LysRS	Cladosporin	*Plasmodium falciparum*	38
PheRS	Ochratoxin A	Non-selective	39
	Ascamycin[b] , dealanylascamycin	*Xantomonas citri* Broad bactericidal activity when dealanylated	40, 41
ProRS	Febrifugine Isofebrifugine	*Plasmodium falciparum*, and other *Plasmodium spp.*	42-45
	Cispentacin	Fungus, including *Candida albicans*	46
	Phosmidosine[c]	Eukarya	47
SerRS	Albomycin	Bacteria, including *E. coli* and *S. pneumoniae*	48
ThrRS	Borrelidin	Non-selective	49, 50
TrpRS	Indolmycin	Bacteria, including *E. coli*, *Helicobacter pylori, S. aureus*	51-53
	Chuangxinmycin	Bacteria, including *E. coli* and *Shigella dysentariae*	54
TyrRS	SB-219383	*Staphylococcus aureus*	55
tRNA	Purpuromycin[d]	Inhibits all amino-acyl tRNA synthesis	56
tRNA (AspRS)	Tobramycin[e]	Yeast	57
tRNA (PheRS)	Neomycin[e]	*E. coli*	58

[a]Furanomycin is a substrate for IleRS and is incorporated into proteins

[b]Ascamycin analogues are known to inhibit aaRS enzymes, while ascamycin may have other modes of action

[c]Phosmidosine is hypothesized to be an inhibitor of ProRS due to its structure, but this has not been proven

[d]Purpuromycin inhibits the acylation of all tRNAs due to non-selective binding to the tRNA molecule

[e]Tobramycin and Neomycin are known inhibitors of ribosomal function, but have been shown to inhibit aminoacylation *in vitro* through complexation with yeast tRNA(Asp) and *E. coli* tRNA(Phe), respectively.

Alkaloid inhibitors

Indolmycin and Chuangxinmycin

One example of an amino acid mimicking inhibitor is that of the well know tryptophan structural analogue, indolmycin, which can be isolated from *Streptomyces griseus,* and was patented by Pfizer in 1965 (Fig 3). Indolmycin was previously shown to be highly selective for inhibition of prokaryotic tryptophanyl (TrpRS),[52] and has excellent IC_{50} values of 9.25 nM against *E. coli* TrpRS, versus eukaryotic TrpRS (IC_{50} values of 4.04 mM against bovine liver TrpRS).[53] Furthermore, indolmycin has been shown to target several human pathogens, including *Helicobacter pylori* and methicillin-resistant *Staphylococcus aureus* (MRSA).[51, 53]

The bactericidal activity of indolmycin against *H. pylori* was capitalized upon by Takeda Pharmaceuticals (Japan), who patented the metabolite as TAK-083.[59] Their patented formulation can be used in the treatment of *H. pylori* infections and related illnesses such as gastritis and gastric ulcers. The bactericidal activity of indolmycin against *H. pylori* is in contrast to the bacteriostatic activity observed in *S. aureus*, suggesting that it has additional antibacterial activity in *H. pylori*, in addition to simple inhibition of TrpRS. Perhaps of concern in development of any new antibiotic is the development of bacterial resistance. Several non-indolmycin-producing *Streptomyces* species, including *S. coelicolor*, and some *S. griseus* strains that produce indolmycin, harbour an auxiliary indolmycin resistant TrpRS.[60, 61]

Although several synthetic routes to indolmycin and its analogues have been reported, it is still currently made by fermentation. Ryan and co-workers elucidated the complete biosynthetic pathway to indolmycin, including the assembly of the oxazolinone core and the function of an unprecedented PLP-dependent oxidase.[62, 63] Engineering of this pathway may enhance microbial production methods and lead to construction of indolmycin analogues.

A related natural product with an interesting and unique structure is chuangxinmycin, which was first isolated from *Actinoplanes tsinanensis*. This sulfur-containing indole analogue, like indolmycin, is an inhibitor of bacterial TrpRS (IC_{50} value of 30 nM against *S. aureus* TrpRS),[54] showing *in vivo* activity against murine models of *E. coli* and *Shigella dysenteriae* infection.[54] Preliminary clinical studies also showed that chuangxinmycin may be applicable for treatment of septicemia and urinary and biliary infections caused by *E. coli*.[64]

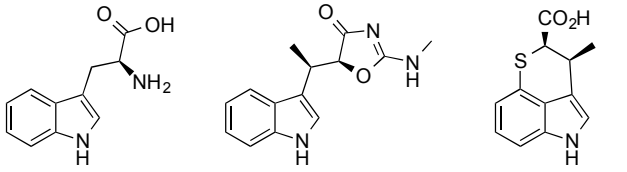

Fig 3 Structure of L-tryptophan, and the structurally related indolmycin and chuangxinmycin.

Febrifugine and Halofuginone

The herb *Dichroa febrifuga* has been used in Chinese herbal medicine to treat malarial for many centuries with the active ingredient isolated as a quinazolinone type alkaloid, named febrifugine.[42-44] Febrifugine, and its isomer isofebrifugine (Fig 4) exhibit *in vitro* activity against both chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum*.[45] Furthermore, febrifugine was found to be effective against several other *Plasmodium* species.[65] The gastrointestinal toxicity associated with febrifugine's use led to the production of less toxic analogues, which still retained their antimalarial properties.

One such analogue is halofuginone, a potent antimalarial active against both the initial asymptotic liver stage of parasitic infection and the liver propagation stage.[66] Halofuginone is currently approved to treat coccidiosis in poultry caused by *Eimeria tenella*,[66, 67] and cryptosporidioisis in cattle caused by *Cryptosporidium parvum*.[68] It was recently shown that halofuginone and other febrifugine derivatives inhibit the prolyl RS (ProRS) activity of glutamyl-prolyl RS (Glu-ProRS) in humans.[69]

Recently, the cocrystal structure of the *P. falciparum* ProRS-halofuginone complex was reported, shedding further light on this interaction (Fig 5).[70] It had previously been proposed that halofuginone binds in the L-proline and adenine-76 (3' end of the tRNA) binding pockets simultaneously, with the assistance of ATP. This was corroborated by the crystal structure, which highlighted areas of the molecule that would be amenable to further derivatization. This report also highlighted the high level of sequence and structural conservation between *Pf*ProRS and other pathogenic PRSs, and also with human (*Homo sapiens*=*Hs*) *Hs*ProRS (Fig 6). This is beneficial in that several human parasitic infections may be targeted by one molecule, but above efficacious doses may also inadvertently affect *Hs*ProRS.

Incidentally, a halofuginone analogue was also reported recently.[71] The authors reasoned that toxic side effects of

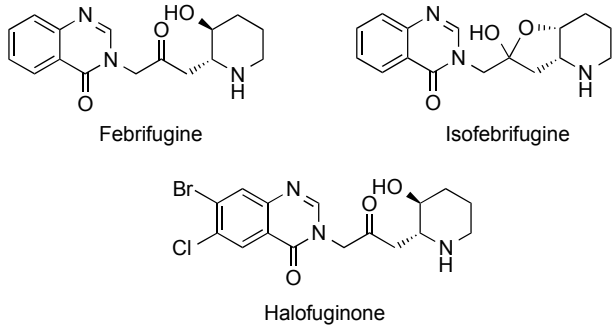

Fig 4 Structure of the natural products febrifugine and isofebrifugine, and the synthetic derivative halofuginone.

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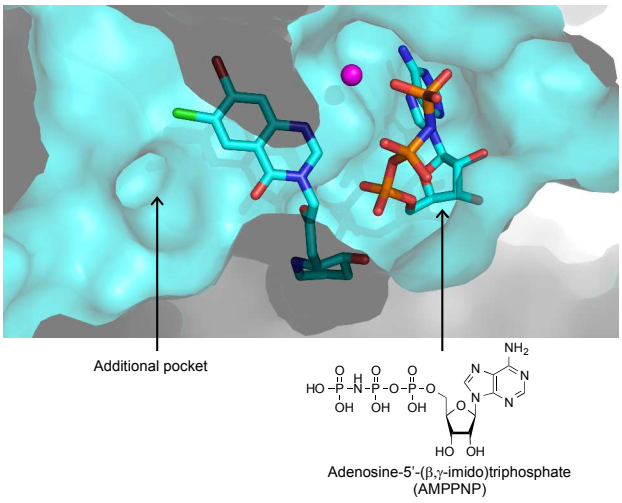

Fig 5 Cocrystal structure of the *P. falciparum* ProRS with halofuginone and an ATP analogue bound, shown in cross section (active site surface show in cyan, transparent cross sectional surface show in grey). The authors highlight an additional binding pocket in the enzyme that may be filled by further derivatization of the quinazolinone ring, thereby improving inhibitor binding.

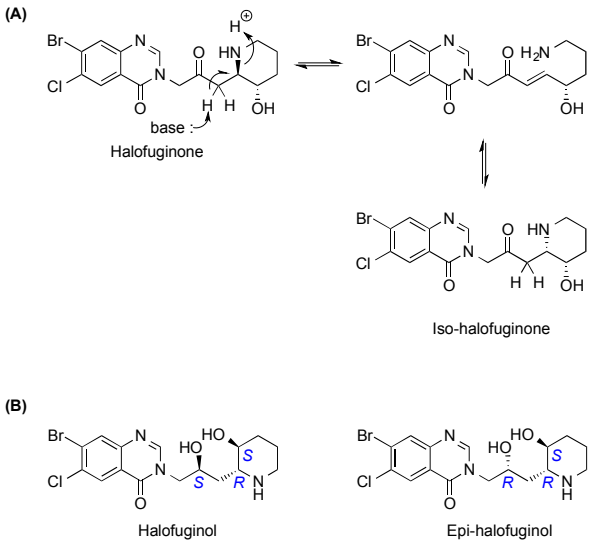

Fig 7 (A) Conversion of halofuginone to iso-halofuginone via a reactive α,β-unsaturated ketone intermediate (B) the two epimers of halofuginol

febrifugine and its analogues such as halofuginone might at least in part be due to HsProRS-independent inhibition of other systems (fig 7). The reactive α,β-unsaturated ketone intermediate may undergo intramolecular attack to generate iso-halofuginone, or attack by other cellular nucleophiles. In hopes of attenuating this off-target activity, the authors synthesised both epimers of the halofuginone derivative halofuginol, which contains a secondary alcohol instead of the ketone functionality. The authors showed that the (*2'S,2R,3S*)-isomer of halofuginol was about 65 times more selective for *Plasmodium falciparum* (*Pf*) *Pf*ProRS, and retained similar *in vitro* activity to halofuginone in their *P. berghei* ANKA liver-stage model (EC_{50} = 14 nM versus 17 nM, respectively). The other epimer however, epi-halofuginol, was 700-fold less active than halofuginone *in vitro*. At therapeutically relevant concentrations, halofuginol was better tolerated than both febrifugine and halofuginone *in vivo*. Additionally, prolonged treatment did not result in any adverse gastrointestinal toxicity or lethality.[71]

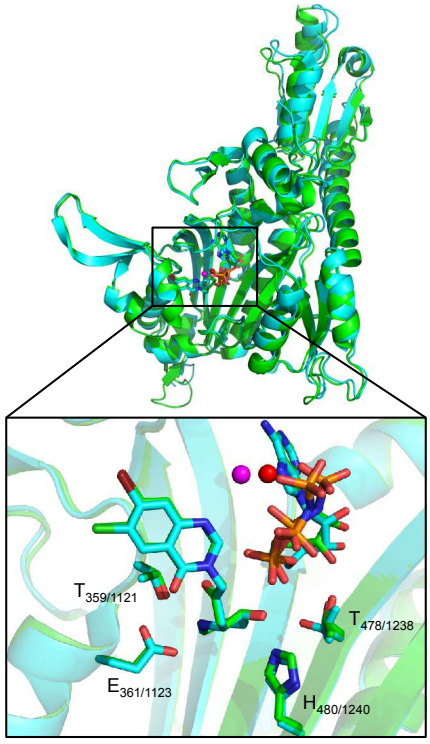

Fig 6 Structural comparison of *Pf*ProRS (cyan) and *Hs*ProRS (green) with halofuginone bound.[45] Conserved residues are indicated.

Amino-acyl adenylate analogues

Microcin C

Trojan Horse aaRS inhibitors are a class of antibiotics whose structure mimics an imported bacterial substrate, thus allowing them to exploit the bacteria's own transport system and gain access to the cell. Once inside the cell, host enzymes process the prodrug to produce the active toxin. This class of aaRS inhibitors is of particular interest for development, as they inherently overcome the problem of low *in vivo* uptake encountered by other antibiotics.

One such example is that of microcin C, a post-translationally modified ribosomally synthesised heptapeptide produced by *Enterobacteriaceae*.[30] Microcin C is a peptide-nucleotide antibacterial, active against several Gram negative bacteria genera including *Escherichia*, *Klebsiella*, *Salmonella*, *Shigella*, and *Proteus* as well as some Gram positives.[72-74] In *E. coli* six genes are responsible for the biosynthesis, maturation and secretion of microcin C, as well as host-immunity, with all genes contained within the same operon.[75] The first gene in the operon, mccA encodes the heptapeptide portion. After

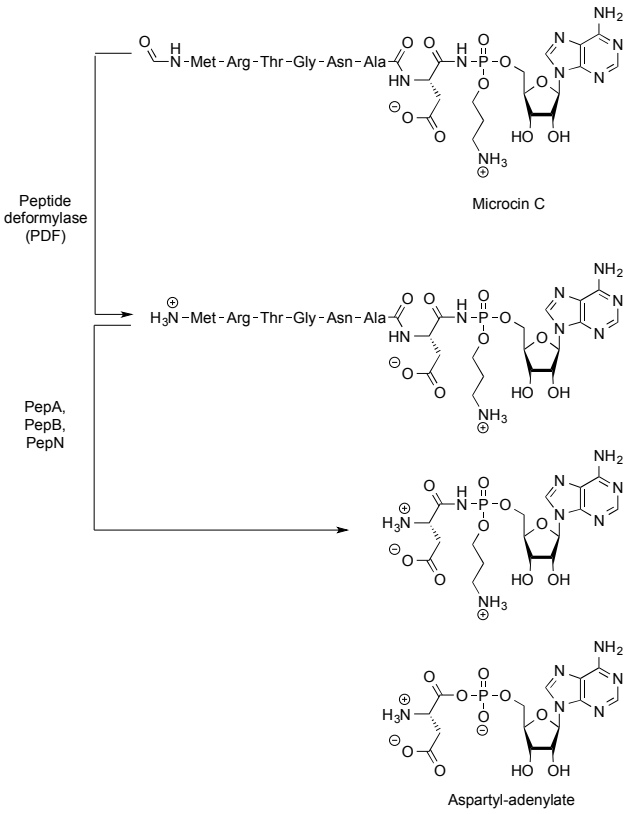

Fig 8 Intracellular processes by which the toxic aspartyl adenylate mimic is liberated.

translation on the ribosome, the heptapeptide is extensively modified to attach an adenosine unit via a N-acyl phosphoramidate linkage to the α-carboxy group of the C-terminal aspartic acid.[76] This intermediate is then further elaborated by addition of a 3-aminopropyl group on the phosphate.[77]

In 2007, it was shown that an inner membrane ABC-transporter, YejABEF, is responsible for uptake of microcin C.[78]As with most ribosomally synthesized bacterial peptides, the N-terminus of the peptide is formylated. Once inside the cell, the peptide is first deformylated, and subsequently processed by peptidases A, B, or N to liberate a hexapeptide and a toxic moiety that was found to be a potent AspRS inhibitor (Fig 8).[79, 80] The inhibitor is a more stable analogue of the reactive aspartyl adenylate intermediate, owing to its nonhydrolyzable N-P bond, and thus it competes with aspartic acid and ATP for binding of AspRS.[80] Further to this, mutations to introduce alternative amino acids to the C-terminus of microcin analogues completely abolishes production.[81] However, aspartate-, glutamate-, or leucine-sufamoyl-adenosine synthetic analogues have been shown to be uptaken and cleaved by the cell, where they inhibit their respective aaRS.[82] Furthermore, recent developments in enzymatic production of microcin C variants highlighted increased bioactivity with some longer microcin c peptides, paving the way for production of new Trojan Horse aaRS inhibitors.[83]

Other naturally occurring Trojan horse aaRS inhibitors include agrocin 84 and albomycin (fig 9). Agrocin 84, produced by *Agrobacterium radiobacter* strain K84, protects crops from crown gall tumours due to the inhibition of the LeuRS of the pathogen *Agrobacterium tumefaciens*.[37] N^6-functionalization of the nucleoside portion with a furanose moiety is required to gain access to the cell. In contrast, albomycin uses siderophore machinery to access the intracellular space.[48] The biosynthetic gene cluster for albomycin biosynthesis in *Streptomyces* sp. ATCC 700974 was identified in 2012, which could open the door to understanding the assembly of siderophore-antibiotic conjugates.[84]

Polyketides and their derivatives

Mupirocin

Mupirocin (pseudomonic acid A) is a polyketide produced by the bacterium *Pseudomonas fluorescens* that represents a clinically approved aaRS inhibitor (trade name Bactroban®) (Fig 10).[31, 32] Mupirocin is widely used for topical treatment of bacterial pathogens such as *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Neisseria meningitides*, and methicillin resistant *S. aureus* (MRSA), where it targets the isoleucyl tRNA synthetase (IleRS) enzyme.[32, 85] One of mupirocin's desirable characteristics is its ability to selectively target bacterial, fungal and archaeal IleRS enzymes and not higher eukarytotic IleRSs.[85] However despite its widespread use, a mupirocin-resistant MRSA strain was discovered, which harbors a plasmid-borne eukaryotic-type IleRS (type-II IleRS).[86-88] Hence, it is clinically desirable to design new drugs for such resistant bacteria.

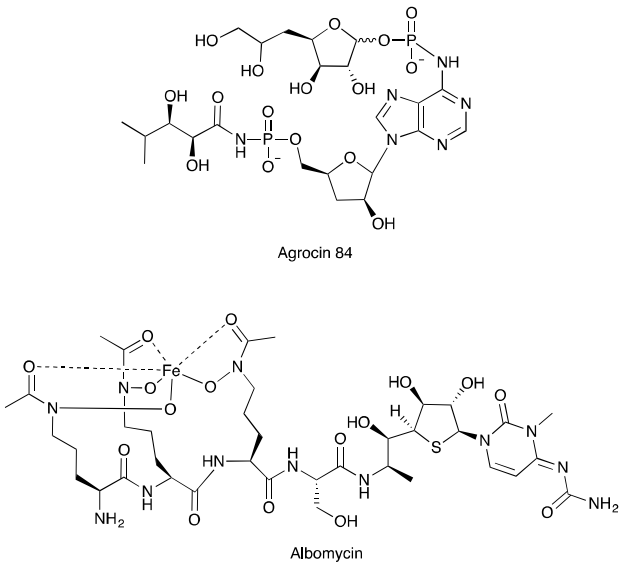

Fig 9: the structures of Trojan horse aaRS inhibitors agrocin 84 and albomycin

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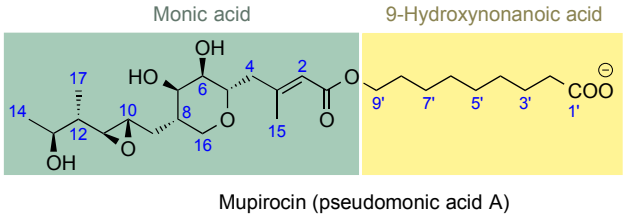


Mupirocin (pseudomonic acid A)

Fig 10 Structure of mupirocin.

Structural studies with IleRS from *Thermus thermophilus* (*Tt*) showed that the C12 – C14 plus the C17 methyl group of mupirocin mimics the side chain of isoleucine, binding in the isoleucine-binding pocket through van der Waals interactions.[89] The pyran ring and C1-C3 bind in a similar orientation to the ribose of ATP, while the long 9-hydroxynonanoic acid lies along a hydrophobic cleft normally occupied by the adenine ring (fig 11). The authors highlighted two key residues thought to be important for sensitivity to mupirocin, namely His_{581} and Leu_{583} of *T. thermophilus* IleRS. They mutated these residues to the analogous residues found in the IleRSs of eukaryotic organisms and also the *S. aureus* mupirocin-resistant IleRS.[89] They observed a one-fold decrease in sensitivity to mupirocin in both mutants (Table 3), confirming the importance of these two residues in sensitivity to mupirocin. Further mutational studies guided by this data, and also the crystal structure of *S. aureus* IleRS in complex with mupirocin and tRNAIle,[13] may improve the activity of mupirocin against resistant organisms.

Cladosporin

Cladosporin is a polyketide natural product that can be isolated from several fungal genera, including Aspergillus,[90] Penicillium,[91] and Cladosporium.[92] It has been shown to have antibacterial, antifungal and plant growth inhibitory properties, and more recently, antimalarial activity. Hoephner *et al*. identified cladosporin as a potent (IC_{50} = 40 nM) inhibitor of *P. falciparum* cytosolic lysyl tRNA synthetase (LysRS), and

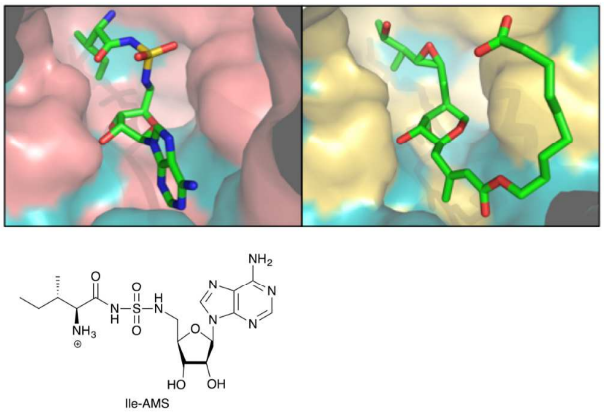


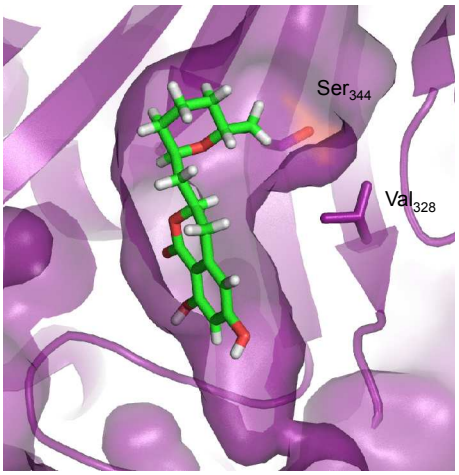
Fig 11 Crystal structure of *Tt*IleRS with Ile-AMS (Ile-AMP mimic) bound (pink) and TtIleRS with mupirocin bound (yellow). Hydrophobic residues in both structures are coloured cyan.

Table 3 Mutational studies. Mutation of *T. thermophilus* IleRS key active site residues to those found in mupirocin-resistant IleRSs decreased mupirocin-binding affinity.

Organism	K_i for mupirocin (uM)	Key active site residues	
		581	583
T. thermophilus	0.25	His	Leu
S. aureus	60 x 10^{-3}	His	Phe
"*S. aureus* II-type mutant"	2.31	Leu	His
S. cerevisae	15	Ser	Ile
"Eukaryotic-type mutant"	2.73	Asn	Phe

showed that cladosporin is >100-fold more selective for the parasitic enzyme than the human version.[38] Through a series of elegant experiments, the authors showed that cladosporin resistance in *S. cerevisae* is related to two key active site residues, namely Gln_{324} and Thr_{340}. Systematic replacement of these two residues with either valine or serine respectively, lead to a 5.7-fold and 10.4-fold increase in cladosporin sensitivity, while the double mutant was 38.7-fold more sensitive to cladosporin.[38] Resistance to cladosporin appears to hinge on the presence of a polar group at position 324 and a more bulky group at position 340.

This is further supported by the recently published co-crystal structure of *P. falciparum* LysRS and cladosporin (Fig 12),[93, 94] wherein the isocoumarin moiety of cladosporin occupies a



Cladosporin

Fig 12 Cocrystal structure of the active site cavity of *Pf*LysRS with cladosporin bound. Cladosporin selectivity for *Pf*LysRS over *Hs*LysRS can be attributed to the highlighted residues Ser344 and Val328.

similar orientation as the adenine of ATP, with its aromatic ring in a hydrophobic interaction with Val_{328}.[94] Additionally, the tetrahydropyran (THP) ring of cladosporin is located directly adjacent to Ser_{344}, where any increase in steric bulk would likely clash with the methyl substituent on the THP ring, and prevent cladosporin binding. The authors also used this data to conduct a sequence-based search for other pathogenic organisms containing LysRS enzymes with cladosporin accommodating residues, which they postulate may be viable targets of cladosporin derivatives. These include *Trypanosoma cruzi*, *Trypanosoma vivax*, *Trypanosoma congolense* (trypanosomiasis causing pathogens), *Schistosoma mansoni* (schistosomiasis causing pathogen) and *Loa loa* (loaiasis causing pathogen). Efforts to produce multitarget specific cladosporin derivatives to capitalise on these potential structural differences are currently underway in our lab.

Further validation that *P. falciparum* tRNA synthetases are druggable targets came from a dual drug inhibition study conducted with cladosporin and the previously mentioned ProRS inhibitor, halofuginone. When cladosporin and halofuginone were co-administered to parasitic cultures at concentrations of 48 nM and 1 nM, respectively, the authors discovered synergistic inhibitory effects on parasite growth.[70] The inevitable development of drug resistant malaria parasites make finding new compounds that can be used alone or in cocktail therapies is a top priority. In both of these respects, the discovery and validation of cladosporin's specific inhibition has been a seminal event. Manipulation of its absorption, distribution, metabolism, and excretion (ADME) properties could make it a broad applicabile anti-infective agent.

Borrelidin

Borrelidin, an 18-membered macrolide polyketide isolated from *Streptomyces* spp,[95-97] is an inhibitor of some bacterial and eukaryotic threonyl tRNA synthetases (ThrRS) (Fig 13).[49, 50] Currently borrelidin is being investigated as an antimalarial agent, due to its efficient inhibition of *P. falciparum* ThrRS. Research has shown that it is a potent inhibitor of parasitic proliferation *in vitro* (IC_{50} values of 1.9 nM against drug-resistant *P. falciparum*), and that it specifically targets the trophozoite stage of parasite growth.[98] Further to this *in vitro* activity, borrelidin has been shown to cure mice of malaria infection,[99, 100] and impart subsequent immunity to further infection by *Plasmodium yoelii*.[100]

Significant cytotoxicity to human cells has so far precluded borrelidin's clinical application. However, several SAR studies have identified borrelidin analogues with decreased mammalian toxicity.[101, 102] Sugawara et al. designed and synthesized a series of borrelidin analogues, with a particular subset of analogues including functionalized triazole units showing particular promise.[102] One analogue in particular that contained a CH_2SPh moiety attached via the triazole linkage, showed reduced mammalian cytotoxicity and increased antimalarial activity.

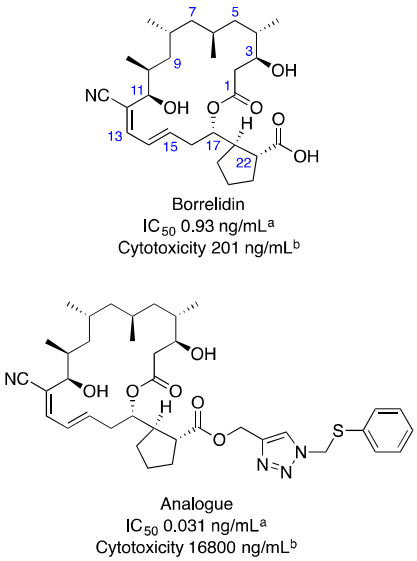


Fig 13 Structure of borrelidin and an analogue containing a CH_2SPh functionalized triazole linker that has increased antimalarial activity and decreased cytotoxicity.

a – tested against drug resistant *P. falciparum* K1 strain.

b – Cytotoxicity for human diploid embryonic cell line MRC-5.

Further to borrelidin's antimalarial activity, it has also been shown to be an anticancer agent in many different cell types and animal models.[103, 104] More recently it was demonstrated by Sidhu et al that borrelidin acts as an efficient inducer of the unfolded protein response (UPR) in a series of oral squamous cell carcinoma (OSCC) cells.[105] The UPR is a conserved cellular response mechanism that enables cell survival under the influence of cell stressors such as unfolded or misfolded protein in the endoplasmic reticulum (ER). Heightened levels of proteins associated with UPR can be found in OSCC cells and is highly indicative of recurrence of OSCC. By overwhelming this adaptive system, borrelidin can selectively target tumor cells, leading to cell apoptosis. This work highlights the feasibility of ThrRS inhibition to induce UPR as a means of selectively target cancer cells.

Borrelidin's mechanism of inhibition has been a subject of much interest since its isolation in 1949. Studies had shown inhibitor binding to be non-competitive with respect to all three substrates, the tRNA, threonine and ATP, instead binding to a unique hydrophobic area near the active site of these enzymes.[106, 107] Interestingly however, Fang et al recently co-crystallised a truncated form of both the *E. coli* and human ThrRS enzymes in complex with borrelidin, and showed that the inhibitor simultaneously occupies all three substrate sites, plus a fourth external site created only upon induced-fit inhibitor binding (Fig 14).[108] Addition of each physiological substrate rescued enzyme activity, indicating that borrelidin is competitive with respect to all three substrates. This data defines borrelidin as an unusual example of a quadrivalent aaRS inhibitor. This, coupled with its broad-spectrum activity in both prokaryotic and eukaryotic systems, make borrelidin a

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unique and attractive candidate for multifunctional clinical usage.

Summary and outlook

With the rapid emergence of multidrug resistance bacteria, there is a constant need for new antimicrobial agents. tRNA synthetase inhibitors represent an attractive class of antimicrobials owing to their often remarkable selectivity against prokaryotic vs. eukaryotic organisms. Over the last number of years, several important advances have been made in this area. Biosynthetic studies on indolmycin and cladosporin have advanced our knowledge of these molecules, and paved the way for improved bioproduction. In the case of the derivatized quinazolinone analogue, halofuginone, crystal structures have illuminated possible areas for further derivitization. Indeed, a less toxic analogue, halofuginol was also reported. Crystallography data on inhibitors such as mupirocin, cladosporin and borrelidin may also lead to enhancements in the efficacy of these aaRS inhibitors. With clinical resistance developing at such an alarming rate, the continued adaption and development of this diverse category of enzyme inhibitors in certainly a worthwhile endeavour.

References

1 M. Ibba, H. Becker, C. Stathopoulos, D. Tumbula and D. Söll, *Trends Biochem. Sci.*, 2000, **25**, 311-316.
2 P. Schimmel, J. Tao and J. Hill, *FASEB J*, 1998, **12**, 1599-1609.
3 J. Tao and P. Schimmel, *Expert Opin. Investig. Drugs*, 2000, **9**, 1767-1775.
4 J. Hurdle, A. O'Neill and I. Chopra, *Antimicrob. Agents Chemother.*, 2005, **49**, 4821-4833.
5 D. Kim, N. Kwon and S. Kim, *Top. Curr. Chem.*, 2014, **344**, 207-245.
6 J. O'Neill, Chair, *Review on Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations*, HM Government, Wellcome Trust, London, 2014.
7 Malaria Fact Sheet, http://www.who.int/mediacentre/factsheets/fs094/en/, (accessed May 11, 2016).
8 X. Zhou, D. Du, M. Tan, H. Lei, L. Ruan, G. Eriani and E. Wang, *Nucleic Acids Res.*, 2011, **39**, 8857-8868.
9 M. Englert, S. Moses, M. Hohn, J. Ling, P. O'Donoghue and D. Söll, *FEBS Lett.*, 2013, **587**, 3360-3364.
10 J. Burbaum and P. Schimmel, *J. Biol. Chem.*, 1991, **266**, 16965-16968.
11 Y. Mechulam, E. Schmitt, L. Maveyraud, C. Zelwer, O. Nureki, S. Yokoyama, M. Konno and S. Blanquet, *J. Mol. Biol*, 1999, **294**, 1287-1297.
12 M. A. Swairjo, F. J. Otero, X.-L. Yang, M. A. Lovato, R. J. Skene, D. E. McRee, L. R. de Pouplana and P. Schimmel, *Mol. Cell*, 2004, **13**, 829-841.
13 L. Silvian, J. Wang and T. Steitz, *Science*, 1999, **285**, 1074-1077.
14 O. Nureki, D. Vassylyev, M. Tateno, A. Shimada, T. Nakama, S. Fukai, M. Konno, T. Hendrickson, P. Schimmel and S. Yokoyama, *Science*, 1998, **280**, 578-582.
15 A. Palencia, T. Crépin, M. Vu, T. Lincecum, S. Martinis and S. Cusack, *Nat. Struct. Mol. Biol.*, 2012, **19**, 677-684.
16 H. Zhao, A. Palencia, E. Seiradake, Z. Ghaemi, S. Cusack, Z. Luthey-Schulten and S. Martinis, *ACS Chem. Biol.*, 2015, **10**, 2277-2285.
17 M. Boniecki, M. Vu, A. Betha and S. Martinis, *Proc. Natl. Acad. Sci. U S A*, 2008, **105**, 19223-19228.
18 S. Fukai, O. Nureki, S. Sekine, A. Shimada, J. Tao, D. Vassylyev and S. Yokoyama, *Cell*, 2000, **103**, 793-803.
19 M. Ruff, S. Krishnaswamy, M. Boeglin, A. Poterszman, A. Mitschler, A. Podjarny, B. Rees, J. Thierry and D. Moras, *Science*, 1991, **252**, 1682-1689.
20 S. Cusack, M. Härtlein and R. Leberman, *Nucleic Acids Res.*, 1991, **19**, 3489-3498.
21 P. O'Donoghue and Z. Luthey-Schulten, *Microbiol. Mol. Biol. Rev.*, 2003, **67**, 550-573.
22 G. H. M. Vondenhoff and A. Van Aerschot, *Eur. J. Med. Chem.*, 2011, **46**, 5227-5236.
23 B. Gadakh and A. Van Aerschot, *Expert Opin. Ther. Patents*, 2012, **22**, 1453-1465.
24 V. Agarwal and S. K. Nair, *MedChemComm*, 2012, **3**, 887-898.
25 J. S. Pham, K. L. Dawson, K. E. Jackson, E. E. Lim, C. F. A. Pasaje, K. E. C. Turner and S. A. Ralph, *Int. J. Parasitol. Drugs Drug Resist.*, 2014, **4**, 1-13.
26 Z. Yu, S. Vodanovic-Jankovic, N. Ledeboer, S.-X. Huang, S. R. Rajski, M. Kron and B. Shen, *Org. Lett.*, 2011, **13**, 2034-2037.
27 Z. Yu, S. Vodanovic-Jankovic, M. Kron and B. Shen, *Org. Lett.*, 2012, **14**, 4946-4949.
28 M. E. Rateb, D. Yang, S. Vodanovic-Jankovic, Z. Yu, M. A. Kron and B. Shen, *J. Antibiot.*, 2015, **68**, 540-542.
29 S. C. K. Sukuru, T. Crepin, Y. Milev, L. C. Marsh, J. B. Hill, R. J. Anderson, J. C. Morris, A. Rohatgi, G. O'Mahony, M. Grotli, F. Danel, M. G. P. Page, M. Hartlein, S. Cusack, M. A. Kron and L. A. Kuhn, *J. Comp. Aid. Mol. Des.*, 2006, **20**, 159-178.
30 F. Moreno, J. Gónzalez-Pastor, M. Baquero and D. Bravo, *Biochimie*, 2002.
31 A. Fuller, G. Mellows, M. Woolford, G. Banks, K. Barrow and E. Chain, *Nature*, 1971, **234**, 416-417.
32 R. Sutherland, R. Boon, K. Griffin, P. Masters, B. Slocombe and A. White, *Antimicrob. Agents Chemother.*, 1985, **27**, 495-498.
33 V. V. Klochko, L. B. Zelena, J. Y. Kim, L. V. Avdeeva and O. N. Reva, *Int. J. Antimicrob. Agents.*, 2016, **47**, 56-61.
34 Y. Miyamoto, K. Machida, M. Mizunuma, Y. Emoto, N. Sato, K. Miyahara, D. Hirata, T. Usui, H. Takahashi, H. Osada and T. Miyakawa, *J. Biol. Chem.*, 2002, **277**, 28810-28814.
35 T. Kohno, D. Kohda, M. Haruki, S. Yokoyama and T. Miyazawa, *J. Biol. Chem.*, 1990, **265**, 6931-6935.
36 A. Ogilvie, K. Wiebauer and W. Kersten, *Biochem. J.*, 1975, **152**, 511-515.
37 J. S. Reader, P. T. Ordoukhanian, J. G. Kim, V. de Crecy-Lagard, I. Hwang, S. Farrand and P. Schimmel, *Science*, 2005, **309**, 1533-1533.
38 D. Hoepfner, C. W. McNamara, C. S. Lim, C. Studer, R. Riedl, T. Aust, S. L. McCormack, D. M. Plouffe, S. Meister and S. Schuierer, *Cell Host Microbe*, 2012, **11**, 654-663.
39 I. Konrad and R. Roschenthaler, *FEBS Lett.*, 1977, **83**, 341-347.
40 K. Isono, M. Uramoto, H. Kusakabe, N. Miyata, T. Koyama, M. Ubukata, S. K. Sethi and J. A. McCloskey, *J. Antibiot.*, 1984, **37**, 670-672.
41 H. Ueda, Y. Shoku, N. Hayashi, J. Mitsunaga, Y. In, M. Doi, M. Inoue and T. Ishida, *Biochim. Biophys. Acta*, 1991, **1080**, 126-134.
42 I. M. Tonkin and T. Work, *Nature*, 1945, **156**, 630.
43 J. Koepfli, J. Mead and J. Brockman, *J. Am. Chem. Soc.*, 1947, **69**, 1837.
44 G. R. Coatney, W. Cooper, W. Culwell, W. White and C. Imboden Jr, *J. National Malaria Society*, 1950, **9**, 183-186.

45 S. Kobayashi, M. Ueno, R. Suzuki, H. Ishitani, H. Kim and Y. Wataya, *J. Org. Chem.*, 1999, **64**, 6833-6841.
46 J. O. Capobianco, D. Zakula, M. L. Coen and R. C. Goldman, *Biochem. Biophys. Res. Commun.*, 1993, **190**, 1037-1044.
47 M. Uramoto, C. J. Kim, S. Y. Kazuo, H. Kusakabe, K. Isono, D. R. Phillips and J. A. McCloskey, *J. Antibiot.*, 1991, **44**, 375-381.
48 A. L. Stefanska, M. Fulston, C. S. V. Houge-Frydrych, J. J. Jones and S. R. Warr, *J. Antibiot*, 2000, **53**, 1346-1353.
49 G. Nass and K. Poralla, *Mol. Gen. Genet.*, 1976, **147**, 39-43.
50 G. Nass and J. Thomale, *FEBS Lett.*, 1974, **39**, 182-186.
51 J. Hurdle, A. O'Neill and I. Chopra, *J. Antimicrob. Chemother.*, 2004, **54(2)**, 549-552.
52 R. Werner, L. Thorpe, W. Reuter and K. Nierhaus, *Eur. J. Biochem.*, 1976, **68**, 1-3.
53 T. Kanamaru, Y. Nakano, Y. Toyoda, K. Miyagawa, M. Tada, T. Kaisho and M. Nakao, *Antimicrob. Agents Chemother.*, 2001, **45**, 2455-2459.
54 M. Brown, P. Carter, A. Fenwick, A. Fosberry, D. Hamprecht, M. Hibbs, R. Jarvest, L. Mensah, P. Milner, P. O'Hanlon, A. Pope, C. Richardson, A. West and D. Witty, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 3171-3174.
55 R. C. Greenwood and D. R. Gentry, *J. Antibiot.*, 2002, **55**, 423-426.
56 S. Kirillov, L. A. Vitali, B. P. Goldstein, F. Monti, Y. Semenkov, V. Makhno, S. Ripa, C. L. Pon and C. O. Gualerzi, *RNA*, 1997, **3**, 905-913.
57 F. Walter, J. Putz, R. Giege and E. Westhof, *EMBO J.*, 2002, **21**, 760-768.
58 N. E. Mikkelsen, K. Johansson, A. Virtanen and L. A. Kirsebom, *Nat. Struct. Biol.*, 2001, **8**, 510-514.
59 *U.S. Pat.,* 6 169 102 B1, 2001.
60 M. Kitabatake, K. Ali, A. Demain, K. Sakamoto, S. Yokoyama and D. Söll, *J. Biol. Chem.*, 2002, **277**, 23882-23887.
61 J. J. Vecchione and J. K. Sello, *Antimicrob. Agents Chemother.*, 2009, **53**, 3972-3980.
62 Y. Du, L. Alkhalaf and K. Ryan, *Proc. Natl. Acad. Sci. U S A*, 2015, **112**, 2717-2722.
63 Y. L. Du, R. Singh, L. M. Alkhalaf, E. Kuatsjah, H. Y. He, L. D. Eltis and K. S. Ryan, *Nat. Chem. Biol.*, 2016, **12**, 194-199.
64 *Scientia Sinica*, 1977, **20**, 106-112.
65 S. Jiang, Q. Zeng, M. Gettayacamin, A. Tungtaeng, S. Wannaying, A. Lim, P. Hansukjariya, C. Okunji, S. Zhu and D. Fang, *Antimicrob. Agents Chemother.*, 2005, **49**, 1169-1176.
66 J. F. Ryley and M. J. Betts, *Adv. Pharmacol. Chemother.*, 1973, **11**, 221-293.
67 J. F. Ryley and R. G. Wilson, *Parasitology*, 1975, **70**, 203-222.
68 M. R. Linder, A. R. Heckeroth, M. Najdrowski, A. Daugschies, D. Schollmeyer and C. Miculka, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 4140-4143.
69 T. Keller, D. Zocco, M. Sundrud, M. Hendrick, M. Edenius, J. Yum, Y. Kim, H. Lee, J. Cortese, D. Wirth, J. Dignam, A. Rao, C. Yeo, R. Mazitschek and M. Whitman, *Nat. Chem. Biol.*, 2012, **8**, 311-317.
70 V. Jain, M. Yogavel, Y. Oshima, H. Kikuchi, B. Touquet, M. Hakimi and A. Sharma, *Structure*, 2015, **23**, 819-829.
71 J. Herman, L. Pepper, J. Cortese, G. Estiu, K. Galinsky, V. Zuzarte-Luis, E. Derbyshire, U. Ribacke, A. Lukens, S. Santos, V. Patel, C. Clish, W. Sullivan, H. Zhou, S. Bopp, P. Schimmel, S. Lindquist, J. Clardy, M. Mota, T. Keller, M. Whitman, O. Wiest, D. Wirth and R. Mazitschek, *Sci. Transl. Med.*, 2015, **7**, 288ra277.
72 J. Garcia-Bustos, N. Pezzi and E. Mendez, *Antimicrob. Agents Chemother.*, 1985, **27**, 791-797.
73 I. Khmel, V. Bondarenko, I. Manokhina, E. Basyuk, A. Metlitskaya, V. Lipasova and Y. Romanova, *FEMS Microbiol. Lett.*, 1993, **111**, 269-274.
74 A. Metlitskaya, G. Katrukha, A. Shashkov, D. Zaitsev, T. Egorov and I. Khmel, *FEBS Lett.*, 1995, **357**, 235-238.
75 J. González-Pastor, J. San Millán and F. Moreno, *Nature*, 1994, **369**, 281.
76 R. Roush, E. Nolan, F. Löhr and C. Walsh, *J. Am. Chem. Soc.*, 2008, **130**, 3603-3609.
77 A. Kulikovsky, M. Serebryakova, O. Bantysh, A. Metlitskaya, S. Borukhov, K. Severinov and S. Dubiley, *J. Am. Chem. Soc.*, 2014, **136**, 11168-11175.
78 M. Novikova, A. Metlitskaya, K. Datsenko, T. Kazakov, A. Kazakov, B. Wanner and K. Severinov, *J. Bacteriol.*, 2007, **189**, 8361-8365.
79 T. Kazakov, G. Vondenhoff, K. Datsenko, M. Novikova, A. Metlitskaya, B. Wanner and K. Severinov, *J. Bacteriol.*, 2008, **190**, 2607-2610.
80 A. Metlitskaya, T. Kazakov, A. Kommer, O. Pavlova, M. Praetorius-Ibba, M. Ibba, I. Krasheninnikov, V. Kolb, I. Khmel and K. Severinov, *J. Biol. Chem.*, 2006, **281**, 18033-18042.
81 P. Van de Vijver, G. Vondenhoff, T. Kazakov, E. Semenova, K. Kuznedelov, A. Metlitskaya, A. Van Aerschot and K. Severinov, *J. Bacteriol.*, 2009, **191**, 6273-6280.
82 G. Vondenhoff, S. Dubiley, K. Severinov, E. Lescrinier, J. Rozenski and A. Van Aerschot, *Bioorg. Med. Chem.*, 2011, **19**, 5462-5467.
83 O. Bantysh, M. Serebryakova, I. Zukher, A. Kulikovsky, D. Tsibulskaya, S. Dubiley and K. Severinov, *J. Bacteriol.*, 2015, **197**, 3133-3141.
84 Y. Zeng, A. Kulkarni, Z. Yang, P. B. Patil, W. Zhou, X. Chi, S. Van Lanen and S. Chen, *ACS Chem. Biol.*, 2012, **7**, 1565-1575.
85 P. Baines, D. Jackson, G. Mellows, A. Swaisland and T. Tasker, in *Mupirocin - A Novel Topical Antibiotic,* eds. J. D. Wilkinson and J. D. Price. Royal Society of Medicine, London, 1984, *Mupirocin: its chemistry and metabolism*, 13-22.
86 M. Rahman, S. Connolly, W. Noble, B. Cookson and I. Phillips, *J. Med. Microbiol.*, 1990, **33**, 97-100.
87 M. Sassanfar, J. Kranz, P. Gallant, P. Schimmel and K. Shiba, *Biochemistry*, 1996, **35**, 9995-10003.
88 C. Thomas, J. Hothersall, C. Willis and T. Simpson, *Nat. Rev. Microbiol.*, 2010, **8**, 281-289.
89 T. Nakama, O. Nureki and S. Yokoyama, *J. Biol. Chem.*, 2001, **276**, 47387-47393.
90 Y. Kimura, N. Shimomura, F. Tanigawa, S. Fujioka and A. Shimada, *Z. Naturforsch. C*, 2012, **67**, 587-593.
91 A. J. Flewelling, J. A. Johnson and C. A. Gray, *Nat. Prod. Commun.*, 2013, **8**, 373-374.
92 R. Cochrane, R. Sanichar, G. Lambkin, B. Reiz, W. Xu, Y. Tang and J. Vederas, *Angew. Chem. Int. Ed. Engl.*, 2016, **55**, 664-668.
93 S. Khan, A. Garg, N. Camacho, J. Van Rooyen, A. Kumar Pole, H. Belrhali, L. Ribas de Pouplana, V. Sharma and A. Sharma, *Acta Crystallogr. D Biol. Crystallogr.*, 2013, **69**, 785-795.
94 S. Khan, A. Sharma, H. Belrhali, M. Yogavel and A. Sharma, *J. Struct. Funct. Genomics*, 2014, **15**, 63-71.
95 L. Dickinson, A. Griffiths, C. Mason and R. Mills, *Nature*, 1965, **206**, 265-268.
96 C. Olano, S. Moss, A. Braña, R. Sheridan, V. Math, A. Weston, C. Méndez, P. Leadlay, B. Wilkinson and J. Salas, *Mol. Microbiol.*, 2004, **52**, 1745-1756.
97 C. Liu, J. Zhang, X. Wang, P. Qian, J. Wang, Y. Gao, Y. Yan, S. Zhang, P. Xu, W. Li and W. Xiang, *J. Agric. Food Chem.*, 2012, **60**, 1251-1257.
98 A. Ishiyama, M. Iwatsuki, M. Namatame, A. Nishihara-Tsukashima, T. Sunazuka, Y. Takahashi, S. Ōmura and K. Otoguro, *J. Antibiot.*, 2011, **64**, 381-384.
99 K. Otoguro, H. Ui, A. Ishiyama, M. Kobayashi, H. Togashi, Y. Takahashi, R. Masuma, H. Tanaka, H. Tomoda, H. Yamada and S. Omura, *J. Antiobiot.*, 2003, **56**, 727-729.

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100 I. Azcárate, P. Marín-García, N. Camacho, S. Pérez-Benavente, A. Puyet, A. Diez, L. Ribas de Pouplana and J. Bautista, *Br. J. Pharmacol.*, 2013, **169**, 645-658.
101 B. Wilkinson, M. Gregory, S. Moss, I. Carletti, R. Sheridan, A. Kaja, M. Ward, C. Olano, C. Mendez, J. Salas, P. Leadlay, R. vanGinckel and M. Zhang, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 5814-5817.
102 A. Sugawara, T. Tanaka, T. Hirose, A. Ishiyama, M. Iwatsuki, Y. Takahashi, K. Otoguro, S. Ōmura and T. Sunazuka, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 2302-2305.
103 T. Kawamura, D. Liu, M. Towle, R. Kageyama, N. Tsukahara, T. Wakabayashi and B. Littlefield, *J. Antibiot.*, 2003, **56**, 709-715.
104 T. Wakabayashi, R. Kageyama, N. Naruse, N. Tsukahara, Y. Funahashi, K. Kitoh and Y. Watanabe, *J. Antibiot.*, 1997, **50**, 671-676.
105 A. Sidhu, J. Miller, A. Tripathi, D. Garshott, A. Brownell, D. Chiego, C. Arevang, Q. Zeng, L. Jackson, S. Bechler, M. Callaghan, G. Yoo, S. Sethi, H. Lin, J. Callaghan, G. Tamayo-Castillo, D. Sherman, R. Kaufman and A. Fribley, *ACS Med. Chem. Lett.*, 2015, **6**, 1122-1127.
106 W. Paetz and G. Nass, *Eur. J. Biochem.*, 1973, **35**, 331-337.
107 B. Ruan, M. Bovee, M. Sacher, C. Stathopoulos, K. Poralla, C. Francklyn and D. Söll, *J. Biol. Chem.*, 2005, **280**, 571-577.
108 P. Fang, X. Yu, S. Jeong, A. Mirando, K. Chen, X. Chen, S. Kim, C. Francklyn and M. Guo, *Nat. Commun.*, 2015, **6**, 6402.

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