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MINI-REVIEW

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Aminoacyl-tRNA synthetases and their inhibitors as a novel family of antibiotics

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Abstract The emergence of multidrug-resistant strains of pathogenic microorganisms and the slow progress in new antibiotic development has led in recent years to a resurgence of infectious diseases that threaten the well-being of humans. The result of many microorganisms becoming immune to major antibiotics means that fighting off infection by these pathogens is more difficult. The best strategy to get around drug resistance is to discover new drug targets, taking advantage of the abundant information that was recently obtained from genomic and proteomic research, and explore them for drug development. In this regard, aminoacyl-tRNA synthetases (ARSs) provide a promising platform to develop novel antibiotics that show no cross-resistance to other classical antibiotics. During the last few years there has been a comprehensive attempt to find the compounds that can specifically target ARSs and inhibit bacterial growth. In this review, the current status in the development of ARS inhibitors will be briefly summarized, based on their chemical structures and working mechanisms.

Introduction

Aminoacyl-tRNA synthetases (ARSs) ligate specific amino acids to their cognate tRNAs, which are subsequently used for protein synthesis. Their role in linking genetic information to the protein world suggests they emerged early in evolution. Since all of these enzymes basically catalyze the same chemical reactions (Arnez and Moras 1997), it was supposed that they originated from a common ancestor with a distinct single domain (Eriani et al. 1990; Schimmel et al. 1993) that contained the active site for adenylate synthesis and the attachment of the activated amino acid to the 3'-end of tRNA. However, it is apparent that these enzymes can be grouped into two distinct classes based on characteristic domain structures and sequence homologies (Table 1). So far no evidence supports the existence of a common ancestor, with the exception of lysyl-tRNA synthetase (Arnez and Moras 1997; Burbaum and Schimmel 1991; Cusack 1997; Ibba et al. 1997). Therefore, the common origin of these enzymes remains an intriguing mystery (Ibba et al. 1997).

The two classes of enzymes showed another contrast in the orientation of the tRNAs that dock to the active sites. The class I enzymes attach the activated amino acids to the 2' hydroxyl of the ribose at the acceptor end whereas the class II enzymes deliver them to the 3' hydroxyl end, with the exception of phenylalanyl-tRNA synthetase (Fraser and Rich 1975). This difference is because each class of enzyme uses the opposite side for

Table 1 Aminoacyl-tRNA synthetases (ARSs) can be divided into two classes, based on their structural features. Each class is further grouped into three subclasses, according to the chemical properties of the substrate amino acids. The two lysyl-tRNA synthetases are found in both of the classes. X of XRSs stands for the substrate amino acid

Subclass	Class I	Class II	Subclass
Ia	IRS (α) LRS (α) VRS (α) CRS (α) MRS (α, α_2) RRS (α)	ARS (α, α_4) PRS (α_2) IIRS (α_2) SRS (α_2) TRS (α_2) GRS (α_2, $\alpha_2\beta_2$)	IIa
Ib	QRS (α) ERS (α) KRS-I (α)	NRS (α_2) DRS (α_2) KRS-II (α_2)	IIb
Ic	YRS (α_2) WRS (α_2)	FRS $(\alpha\beta)_2$	IIc

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their interaction with tRNA. The class I enzymes approach the acceptor stem of tRNA from the minor groove side, with the variable loop facing the solvent, while the class II enzymes approach the major groove side of the acceptor stem and the variable loop faces the enzymes (Ruff et al. 1991).

Structural studies showed that the class I enzymes contain a Rossmann nucleotide-binding fold that is composed of alternating β strands and α helices in their active site. In contrast, the class II enzymes are built around the seven-stranded β structure with three α helices in a barrel-like structure (Leberman et al. 1991; Ruff et al. 1991). The enzymes in each class can be further divided into three subclasses, which are designated a, b, and c (Table 1) (Cusack 1997). The grouping of these subclasses is also consistent to some extent with the structure of the enzymes within each subclass. Each of the subclasses is thought to have its own common ancestor that arose after the emergence of the progenitor for the entire class. The enzymes in each subclass show a tendency to recognize amino acids that are chemically related. For example, the subclasses Ia enzymes recognize amino acids that contain chemically related side chains, such as the nonpolar aliphatic (Ile, Leu, and Val), polar groups containing sulfur (Cys and Met), and Arg. The subclass Ib enzymes recognize charged amino acids (Glu and Lys) and the derivative Gln. The subclass Ic enzymes recognize amino acids with aromatic residues (Tyr and Trp). In addition, analyses of the class II enzymes show a similar pattern to their class I counterparts and these can also be divided into three subclasses (Table 1). The subclass IIa enzymes recognize amino acids that are aliphatic (Ala and Pro), polar (His, Ser, and Thr), and Gly. The enzymes in subclass IIb recognize amino acids (such as charged side chains Asp and Lys, and their derivative Asn), and the subclass IIc synthetase recognizes the amino acid with one aromatic residue, Phe. As mentioned previously, the two classes appear to have symmetry to some extent with respect to their substrate amino acids. In some cases, a given tRNA can be bound and charged by the enzymes from both classes (Frugier et al. 1993; Sissler et al. 1997), although it is still unclear whether or not the enzymes of the different classes can bind tRNA in different modes. Subsequently, the division of the 20 enzymes into two classes with symmetry between the subclasses does not appear to be coincidental. This organization is consistent with the development of paired synthetases, ultimately giving rise to two classes with equal numbers of enzymes (Ribas de Pouplana and Schimmel 2001).

Aminoacylation reactions proceed in two steps. The first stage involves the activation of amino acids by adenylation. The second stage is the transfer of the adenylated amino acids to the acceptor end of the bound tRNAs. The phylogenic relationship, structural features, and reaction mechanism of these enzymes provide the theoretical basis on which to design and synthesize various compounds that could specifically inhibit the targeted ARSs. There are several points that can be explored with the aim of blocking the reaction catalyzed by ARSs. First, the substrate-binding can be interrupted by the substrate analogues of amino acids and ATP. The specific recognition of tRNAs by ARSs suggests that development of specific inhibitors would be an attractive proposition. Secondly, blocking the second stage of the reaction by generating the mimetics of the enzyme-bound reaction intermediate, aminoacyl adenylate, would be another target for the inhibitors. ARSs appear to have some advantage over other proteins as a target for antibiotics because: (1) during the long period of evolution, they accumulated a broad range of structural diversity that can be exploited for designing species-specific enzyme inhibitors; (2) almost all of the organisms contain a set of different ARSs that are in charge of 20 natural amino acids. Each ARS can be used as an independent target for screening inhibitors, basically using the same principle of chemical reaction; (3) there is abundant sequential and structural information that can be used for rational drug design; (4) the ARS inhibitors would not show cross-resistance with other major antibiotics, and (5) there are quite a few natural inhibitors that can be used as the starting structures to derive compounds that improve efficacy, specificity, and pharmacological behavior.

Natural inhibitors of ARSs

A number of natural products have been discovered that inhibit the activities of ARSs. One such product is pseudomonic acid (mupirocin) (Fig. 1) which is produced by *Pseudomonas fluorescens*. This inhibits isoleucyl-tRNA synthetases (IRSs) from gram-positive infectious pathogens including *Staphylococcus aureus* and *Staph. epidermidis*, and from gram-negative bacteria, such as *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and *N. meningitidis* (Class and Deshong 1995). Pseudomonic acid shows about 8,000-fold selectivity for pathogenic IRS over its mammalian counterpart (Hughes and Mellows 1980). However, it is currently used only for topical application because it is rapidly hydrolyzed to inactive monic acid in tissue and blood (Baines et al. 1984).

Other known natural-product inhibitors that are directed against synthetases include borrelidin (threonine) (Nass et al. 1969), furanomycin (isoleucine) (Tanaka et al. 1969), granaticin (leucine) (Ogilvie et al. 1975), indolmycin (tryptophan) (Werner et al. 1976), ochratoxin A (phenylalanine) (Konrad and Roschenthaler 1977), and cispentacin (proline) (Konishi et al. 1989) (Fig. 1). Even though, for various reasons, none of these ARSs inhibitors have as yet been commercially developed, they proved that natural products with diverse structural features could interfere with the reaction catalyzed by ARSs. Among them, indolmycin (Fig. 1), produced from *Streptomyces griseus*, is a derivative of tryptophan and shows antibacterial activity against gram-positive and gram-negative microorganisms (Werner et al. 1976). Takeda Pharmaceuticals reported that TAK-083 was potent and had

Fig. 1 Natural ARS inhibitors

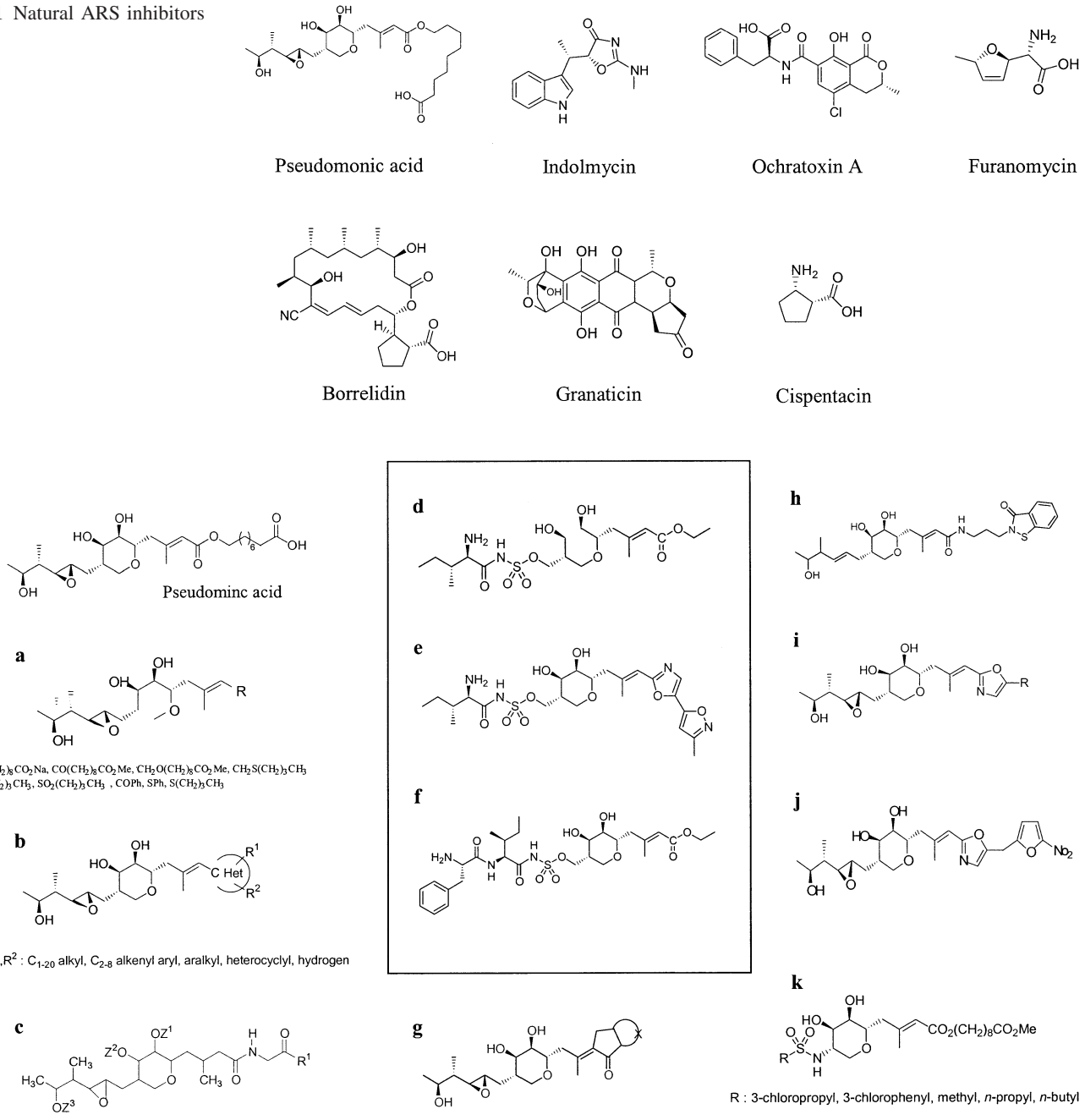

Fig. 2 Derivatives of pseudomonic acid

highly selective activity against *Helicobacter pylori*; however, it was later proven to be indolmycin (Klein et al. 1989). The growth-inhibitory activity of this compound was more potent than that of amoxicillin, clarithromycin, and metronidazole. No significant changes in susceptibility upon repeated exposure to a sub-inhibitory concentration were reported. The compound was efficacious in the treatment of gastric infection caused by *Helicobacter pylori* in Mongolian gerbils.

Mupirocin and its derivatives

Mupirocin (originally known as pseudomonic acid A) belongs to the family of C-glycopyranoside antibiotics and is commercially marketed under the name of Bactroban. Because of its instability in vivo, many attempts have been made to develop the derivatives of mupirocin with the desirable properties for systemic use. Klein et al. (1989) reported several series of mupirocin analogues (Fig. 2a) that have a modification in place of the C1-C3 α,β-unsaturated group. Several derivatives showed enzyme inhibition comparable to mupirocin,

while lacking the hydrolysable ester group at C1. The IC_{50} values of in vitro inhibition against *Staph. aureus* IRS were determined to be in the range of 0.85 to 400 nM. Some of them showed antibacterial activity against *Staph. aureus*, *Staph. epidermidis*, *Strep. pneumoniae*, and *Strep. pyogenes*, with a minimal inhibitory concentration (MIC) in the range 0.39–100 μg/ml. Although several analogues exhibited good in vitro activity, in vivo activity was not significantly improved. Glaxo Smith Kline (GSK), in the US patent 4,861,788 (Beecham Group PLC 1989), reported several derivatives of mupirocin that have a 5-membered heterocyclic group at the C1 position (Fig. 2b). These derivatives are useful in the treatment of mycoplasmal and bacterial pneumonia in animals such as pigs, cattle, and sheep, having antibacterial activity against *Bordetella bronchiseptica*, *Pasteurella multocida*, and *Haemophilus* spp. (MIC range 1.25–80 μg/ml). Another set of mupirocin derivatives (Fig. 2c) were filed by this company (Beecham Group PLC 1991). that were effective in the treatment of enzootic pneumonia caused by the same bacterial species in similar animals (MIC of 0.625–80 μg/ml). They also showed antibacterial activity against *Staph. aureus* (MIC of 0.25–128 μg/ml).

The GSK group filed a series of patents (SmithKline Beecham PLC 1997a, 1997b, 1998a) that claimed novel sulfamate analogues that are selective inhibitors of IRSs from both gram-positive and gram-negative bacteria. They showed that the sulfamate analogues (Fig. 2d) can be used for the treatment of bacterial infections, including staphylococcal infections that are resistant to other antibacterial agents; for instance, methicillin, macrolides, aminoglycosides, and even mupirocin (SmithKline Beecham PLC 1997a). The compounds were also effective in treating fungal infections in humans caused by the genera *Trichophyton*, *Trichosporon*, *Hendersonula*, *Microsporum*, *Candida*, *Cryptococcus*, and *Saccharomyces*. The second consecutive patent (SmithKline Beecham PLC. 1997b) claimed the substituted sulfamate derivatives where the α,β-unsaturated ester moiety of mupirocin was replaced with other moieties, including a 5-membered heteroaryl ring (Fig. 2e). These were most active against *Strep. pneumoniae* (MIC of 1–32 μg/ml), *Haemophilus influenzae* (MIC of 16–32 μg/ml), and *Moraxella catarrhalis* (MIC of 1–16 μg/ml). The compounds also showed antifungal , herbicidal, and antimycoplasmal activity. An additional set of sulfamate derivatives (Fig. 2f) contained an amino acid moiety and showed enhanced whole cell activity (SmithKline Beecham PLC 1998a). These had antibacterial, as well as antimycoplasmal, antifungal, and herbicidal activity. Although the detailed biological data were not mentioned, the compounds were effective against both gram-positive and gram-negative bacteria including *Haemophilus influenzae*, *M. catarrhalis*, *Strep. pyogenes*, *Strep. pneumoniae*, *Staphylococci*, *Escherichia* spp., and *Pseudomonas aeruginosa*.

GSK also reported novel heteroaryl ketone derivatives of mupirocin (Fig. 2g) in US patent 5,536,745 (SmithKline Beecham PLC 1996a). The introduction of conformational rigidity at the carbonyl moiety of a C1 aryl or heteroaryl ketone enhanced the antibacterial activity caused by the improvement of the in vivo and in vitro stability of the derivatives. The derivatives were effective against both gram-positive and gram-negative bacteria, including MRSA. The antibacterial activity of these derivatives showed MIC values against *Haemophilus influenzae*, *M. catarrhalis*, *Strep. pyogenes*, *Strep. pneumoniae*, and *Staph. aureus* in the range of 0.06–64 μg/ml. Sankyo KK reported novel thiomarinol compounds (Fig. 2h) in the JP patent 9,157,269 (SmithKline Beecham PLC 1996b). The derivatives were effective on gram-positive and gram-negative bacteria, and on mycoplasmal infections. Broom et al. (1996) reported a series of C-1 oxazole isosteres (Fig. 2i) of mupirocin bearing a nitroheterocycle. These derivatives showed effective antibacterial activity against *Staph. aureus*, *Haemophilus influenzae*, and *M. catarrhalis* (MIC range 0.06–64 μg/ml). Some members of the series showed potent antibacterial activity against the species that had developed resistance to mupirocin. The potent compound shown in Fig. 2j was transformed by bacterial reductase so another mode of action may arise from its reduction to the species that inhibits cellular targets other than IRS. This compound was more effective than vancomycin when it was evaluated in a *Staph. aureus* intraperitoneal infection model. It is the first report that experimentally demonstrated significant oral efficacy so further data are required to prove the in vivo activity and develop this compound as a clinical candidate. Carcanague (1997) reported mupirocin analogues (Fig. 2k) bearing the replacement for the C9-C14 “left- hand” side chain. It was proposed that the left-hand side chain fitted into the isoleucine-binding pocket in the active site by exchanging the C9-C14 side chain with an amine residue at C8 of the pyran nucleus of mupirocin. The derivatives showed antibacterial activity against *Staph. aureus*. The IC_{50} value for the enzyme was 0.3–3 μM and the MIC value of the most active compound was 25 μg/ml against *Staph. aureus*.

There have been many attempts to develop mupirocin derivatives with desirable pharmacological properties; currently, monic acids are used as a starting material for their production. However, the chemical preparation of monic acid from mupirocin poses a practical problem in the extraction process, hindering the development of the active derivatives of mupirocin. In this regard, it is noteworthy that the enzymatic preparation of monic acid was recently disclosed by SmithKline Beecham PLC (1998b) who claimed that the hydrolases from *Strep. lividans* NCIMB 11416 and *Kitasatosporia* NCIMB 40568 could be used for the enzymatic conversion of pseudomonic acid to monic acid. The yield of monic acid conversion was reported to be 63%. Since several groups reported enhanced antibacterial activity and stability-improvement in mupirocin, it leads us to anticipate further progress in this direction (Broom et al. 1996). While mupirocin was introduced in 1985, resistance to it remains low. Two resistance mechanisms have been

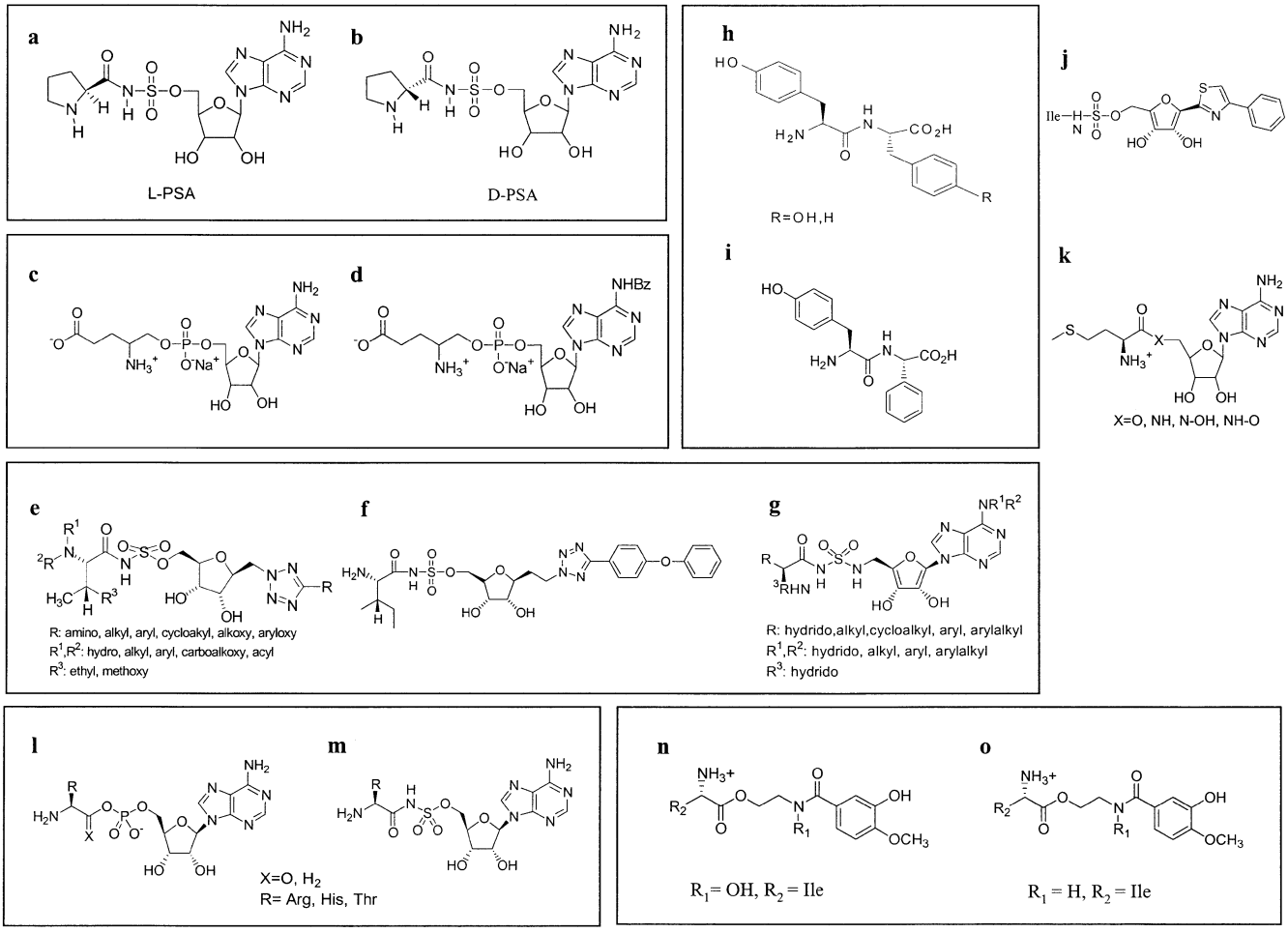

Fig. 3 Analogues of reaction intermediate

reported: (1) high-level (MIC>512 mg/ml), due to the acquisition of a second IRS in the plasmid with a reduced affinity for mupirocin (Morton 1995), and (2) low-level, associated with a single mutation in chromosomal IRS with reduced affinity (Antonio et al. 2002; Farmer et al. 1992). It will be interesting to discover whether the new mupirocin derivatives have inhibitory activity against these two mupirocin-resistant IRSs.

Reaction-intermediate mimics

The structures of the reaction intermediates have been the focus for the development of novel synthetic compounds that target ARSs. The main issue is whether these compounds are sufficiently selective for pathogen enzymes, that is, they do not interfere with their human counterparts. The aminoacyl adenylate intermediate was elucidated and shown to bind tightly to the enzyme with the dissociation constants of the nanomolar range. The dissociation constants for amino acids and ATP are usually higher than those of the reaction intermediates; therefore, the analogues that are based on the adenylate intermediate would be advantageous in the design of novel synthetic compounds with high affinity. Recently, a number of synthetase-substrate complexes have been structurally elucidated. With this structural information, there have been many attempts to design chemically stable intermediates, since the aminoacyl adenylate intermediate is chemically unstable. One example of this approach includes the compounds in which an amino acid carbonyl is replaced with a methylene group, and structures in which the phosphate moiety is replaced with a sulfonamide, phosphonate, or phosphoamide.

Heacock et al. (1996) reported the synthesis of two nonhydrolyzable prolyl adenylate analogues; 5'-*O*-[*N*-(L-prolyl)-sulfamoyl]-adenosine (L-PSA, Fig. 3a) and 5'-O-[*N*-(D-Prolyl)-sulfamoyl]-adenosine (D-PSA, Fig. 3b). Both of these compounds inhibited the in vitro activity of *Escherichia coli* and human prolyl-tRNA synthetase (PRS). The enzyme inhibitory constant (k_i) values of the L-PSA analogues for ATP were quite similar for both synthetases, while those for proline varied approximately seven-fold between the two synthetases (0.6 nM for human and 4.3 nM for *E. coli*). The k_i values for the D-PSA analogues were much higher (51–470 nM) in all

cases. However, the same species-specific differences were observed with respect to the k_i for proline. These results suggest that possible structural differences in or near the active sites of the two enzymes can be exploited for the design of species-specific synthetase inhibitors. Desjardins et al. (1998) reported the synthesis of glutamate adenyl analogues. The compound in Fig. 3c showed competitive inhibition, with a k_i value of 3 μM, and its N^6-benzoyl adenine derivative (Fig. 3d) was a weak inhibitor (k_i of ~60 μM). Replacement of adenine by other bases (purine, cytosine, dihydrocytosine, and uridine) resulted in a more than 1000-fold loss in activity, demonstrating the important contribution of the adenine ring to the enzyme binding.

Hill et al. reported the aminoacyl adenylate mimics that target IRS (Fig. 3e) (Cubist Pharmaceuticals Inc. 1998a). They designed the inhibitors that retain the isoleucyl moiety and replaced the labile acylphosphate linkage of the adenylate with a stable sulfonamide linkage. The compounds showed antibacterial and antifungal activity. The IC_{50} values for the in vitro inhibition of IRS from *E. coli* were determined to be in the range 0.4–135 nM. Biological data showed MIC values of 1–500 μg/ml for *Staph. aureus*, 1–500 μg/ml for *Staph. epidermidis*, and 0.5–16 μg/ml of *Strep. pyogenes*. Among the ones that showed antibacterial activity, the compound in Fig. 3f was chosen for further in vivo study. In a mouse protection study using *Strep. pyogenes*, a subcutaneous injection of this compound increased the number of survivors in a dose-dependent manner. A 50% protective dose (PD_{50}) value was 21.2 mg/kg; however, the dose required for efficacy was too high to be useful for a practical application. A subsequent study revealed that the high dosage was a result of low bioavailability, due to binding to serum albumin. However, the data proved that the inhibitors of IRS could be effective in an animal model. In a subsequent patent (Cubist Pharmaceuticals Inc. 1998b), Hill et al. reported sulfamide derivatives (Fig. 3g) of aminoacyl adenylate. The compounds were evaluated as inhibitors against ARS from HeLa cells. The IC_{50} values of these compounds were 0.9–400 nM, and they showed a ten-fold selectivity between human epidermoid carcinoma cells and normal skin fibroblast. The group claimed the compounds were useful for the treatment of hyperproliferative diseases, such as psoriasis. They also reported the formulation of the pharmaceutical composition and the results of skin penetration data. The claim was made that one of the compounds penetrated target tissue (epidermis) in significant concentrations for 6 h, and the levels were generally increased for 24 h. These data seem to support the suggestion that these compounds would be useful for the topical treatment of hyperproliferative disorders.

Jarvest et al. (1999) published the inhibitory activity of tyrosyl aryl dipeptides (Fig. 3h). Several dipeptides were evaluated for tyrosyl-tRNA synthetase (YRS) from *Staph. aureus*. The L-phenylglycine derivative (Fig. 3i) was a relatively good inhibitor, with an IC_{50} value of 0.9 μM. However, the inhibitors did not show whole cell antibacterial activity against *Staph. aureus*. The inhibition was stereoselective since the D-phenylglycine derivative showed no inhibition. A crystal structure of the enzyme with tyrosyl tyrosine dipeptide suggested that the carbonyl group of the amide function appeared to mimic the phosphoryl moiety of tyrosyl adenylate; the second tyrosine maked the intimate interaction with the HIGH motif in the active site. Yu et al. (1999) reported the synthesis of a series of thiazole aminoacyl adenyl mimics. A wide variety of substituted thiazole analogues were evaluated for tRNA synthetase from human, *E. coli*, and *Staph. aureus*. One of the thiazole adenylate mimics (Fig. 3j) showed good selectivity for the *E. coli* leucyl-tRNA synthetase over its human counterpart. The IC_{50} values for *E. coli* and human were 1.6 nM and 600 nM, respectively. Lee et al. (1999) reported the design of methionyl adenylate analogues (Fig. 3k). The compounds showed moderate inhibitory activity for methionyl-tRNA synthetase (MRS) from *E. coli, Mycobacterium tuberculosis*, *Saccharomyces cerevisiae*, and humans. The ester analogue was the most potent compound in their series with a k_i value of 10.9 μM. This ester analogue showed antibacterial activity against *E. coli* (MIC of 0.5–8 μg/ml). Forrest et al. (2000) reported the aminoalkyl adenylate (Fig. 3l) and aminoacyl sulfamate intermediate analogues (Fig. 3m) that were derived from arginine, histidine, and threonine. The arginyl derivatives were both potent inhibitors of arginyl-tRNA synthetase (RRS). In the derivatives of histidine (HRS) and threonine (TRS), the adenylate analogues showed moderate activity while the acylsulfamates were potent inhibitors for their respective enzymes. The difference in relative affinity of the aminoalkylate and aminosulfamate inhibitors appears to reflect a difference between the class I (RRS) and class II enzymes (HRS, TRS) in the binding of the aminoacyl adenylate intermediate. Recently, Lee et al. (2001) also reported methionyl and isoleucyl phenolic analogues that bear bioisosteric linkers that mimic ribose. Among the synthesized analogues, the isovanillic hydroxamate analogue (Fig. 3n) was reported to be a potent inhibitor of IRS, having an IC_{50} value of 4.5 μM. However, the amide surrogate (Fig. 3o) showed a 24-fold decrease as an inhibitor, suggesting that the hydroxyl group of hydroxamate could act as a mimic of 2'OH in isoleucyl adenylate. In molecular modeling data, the isovanillic hydroxamate analogue aligns itself with a key pharmacophore of isoleucyl adenylate (amino and carbonyl in isoleucine, oxygen and 2'-hydroxyl in ribose, and amine in adenine). This result indicates that isovanillic and hydroxamate moieties can act as surrogates for adenine and ribose, respectively.

The adenylate mimics showed species-specificity and antibacterial activity, presenting the possibility of developing the drugs that inhibit pathogen synthetases, but not their human counterparts. However, these compounds generally showed limited whole cell activity due to poor penetration through the cell wall, although some of them showed in vivo efficacy. More trials are needed before bringing them to clinical candidates (Cubist Pharmaceu-

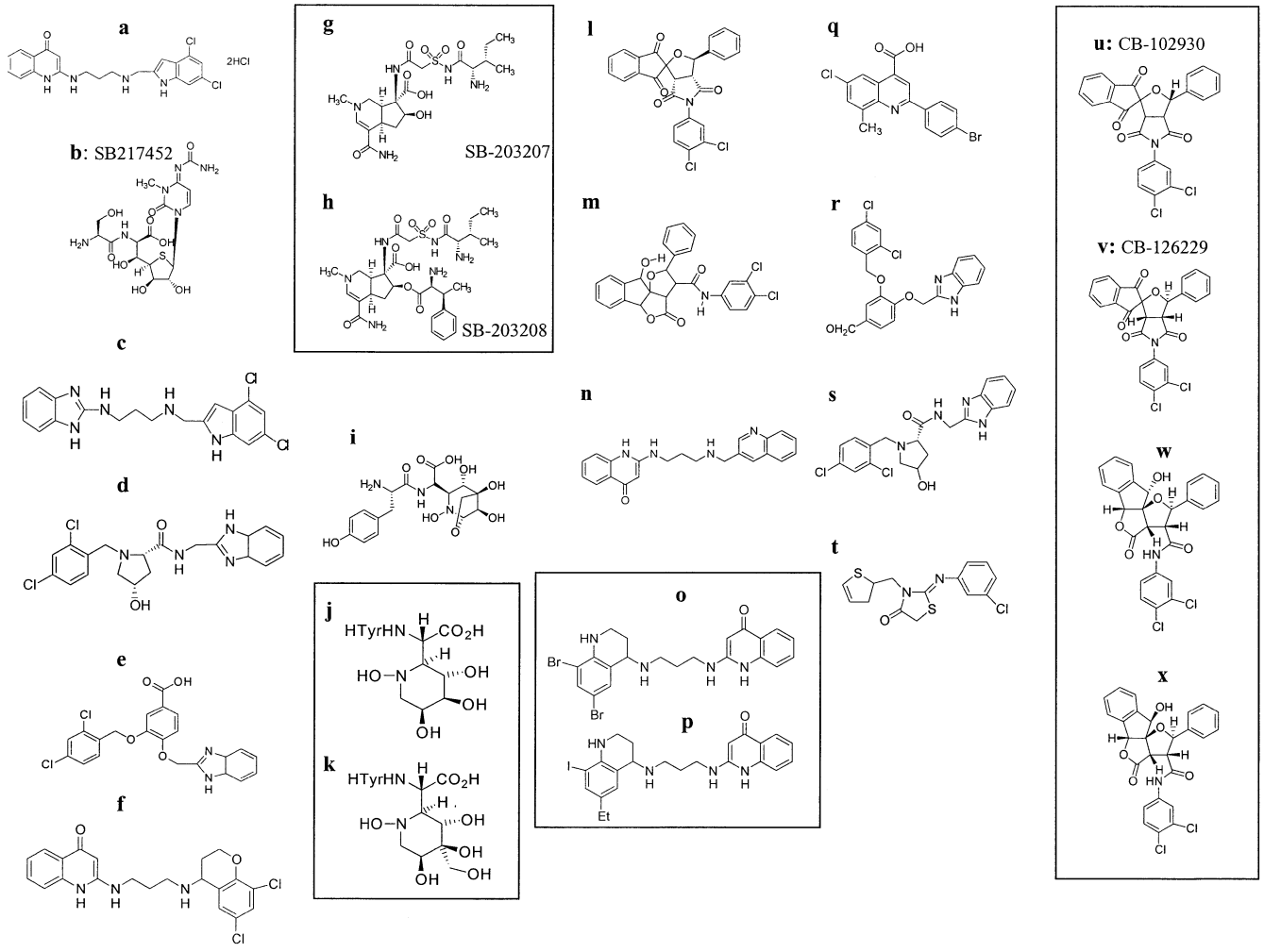

Fig. 4 Novel ARS inhibitors

ticals Inc. 1998a). Considering the close homology in the active site region between different ARSs, it would be worthwhile to develop adenylate mimics that target two or more synthetases. These compounds could greatly slow drug resistance, which would require mutations at multiple targets.

Novel tRNA synthetase inhibitors

Recently, many attempts have been made to discover novel lead structures with properties that are suitable for both enzyme inhibition and antibacterial activity. Typically, a lot of effort has focused on the target-based approach that utilizes high-throughput screening assays (HTS). Various lead structures have been identified from this approach and optimized through the medicinal-chemistry platform to generate multiple series of compounds with good inhibitory activity to target synthetases.

The GSK group has described antibacterial pyridones and pyrimidones that specifically inhibit MRS (SmithKline Beecham PLC 2000a). The compounds showed IC_{50} values in the range 3–800 nM against *Staph. aureus* MRS and were selective against mammalian enzyme. The compound in Fig. 4a showed antibacterial activity against strains of *Staph. aureus*, *Strep. pneumoniae*, *and Enterococcus faecalis* (MIC of <1 μg/ml), and against *M. catarrhalis* (MIC value 2–64 μg/ml). Stefanska et al. (2000a) recently reported the isolation and structural determination of the serine-linked nucleoside moiety of the antibiotic albomycin delta2 as a naturally occurring metabolite from the fermentation of *Streptomyces* spp. ATCC 700974. The compound SB-217452 (Fig. 4b), was a potent inhibitor of both rat and *Staph. aureus* seryl-tRNA synthetase enzymes (IC_{50} of about 8 nM). However, SB-217452 exhibited poor activity against a selected panel of bacteria in a whole-cell assay, possibly due to poor penetration through the cell wall. The GSK group also reported a series of benzimidazole derivatives with antibacterial activity (SmithKline Beecham PLC 2000b). The compounds described in the patent showed inhibitory activity against bacterial MRS with IC_{50} values in the range of 3–700 nM and high specificity (no effect on rat enzyme up to 1 μM). Representative compounds (Fig. 4c)

gave MIC values of 1 μg/ml or less against *Staph. aureus*, *Strep. pneumoniae*, and *E. faecalis*, and 1–32 μg/ml against *M. catarrhalis*.

In a series of patents, Merck & Co. and Cubist Pharmaceuticals claimed proline derivatives that inhibit ARS (Merck & Co. Inc. 2000a). Fifty-three proline derivatives were exemplified in the patent. The representative compounds (Fig. 4d) were evaluated as the inhibitors of undisclosed synthetase targets and had IC_{50} values of 500–10000 nM. The compounds showed MIC values of 100 μg/ml or less against *Staph. aureus*, *Strep. pneumoniae*, *E. faecalis*, and *E. faecium*. In the Merck patent, 91 catechol derivatives were exemplified and evaluated (Merck & Co. Inc. 2000b) with no information on target synthetases. The representative compounds (Fig. 4e) showed IC_{50} values of 500–10,000 nM. The compounds that were tested for antibacterial activity gave relatively high MIC values of 100 μg/ml or less against *Strep. aureus*, *Strep. pneumoniae*, *E. faecalis*, and *E. faecium*.

GSK recently reported novel antibacterial quinolones (SmithKline Beecham PLC 2000c). The compounds gave IC_{50} values in the range of 3–100 nM against *Staph. aureus* MRS with selectivity over the mammalian enzyme. The representative compounds (Fig. 4f) gave MIC values of 1 μg/ml or less against *Staph. aureus*, *Strep. pneumoniae*, and *E. faecalis*, and 8–32 μg/ml against *M. catarrhalis*. Two novel inhibitors of IRS were identified by GSK in a culture of a new *Streptomyces* strain that was designated NCIMB 40513 (Houge-Frydrych et al. 2000a; Stefanska et al. 2000b). SB-203207 (Fig. 4g) and SB-203208 (Fig. 4h) are structurally related to the antitumor compound, altemicidin and act as potent and competitive inhibitors of bacterial and mammalian IRSs. Among them, SB-203207 gave IC_{50} values of 1.7, 401, 1.4, 1.84, and 2 nM against IRS of *Staph. aureus* Oxford, *Streptomyces* spp. NCIMB 40513, *Pseudomonas fluorescens* NCIMB 10586, *Candida albicans* 3153A, and rat liver, respectively. However, both compounds showed only weak and limited antibacterial activity. SB-203208 is a natural product, while SB-203207 appears to be a degradation product of the former.

GSK reported that SB-219383 was isolated from a new strain of *Micromonospora* NCIMB 40684 (Houge-Frydrych et al. 2000b). The compound (Fig. 4i) inhibited *Staph. aureus* tyrosyl-tRNA synthetase (YRS) in a tyrosine-competitive, time-dependent, and reversible manner, with k_i and IC_{50} values of 1 nM. However, no antibacterial activity was detected against *Staph. aureus* or other bacterial strains, except for some activity against *Streptococcus* spp. (MIC of 32 μg/ml), indicating poor penetration through the cell wall. In subsequent papers by the same group (Berge et al. 2000; Brown et al. 2001), the monocyclic compounds shown in Fig. 4j and k were synthesized and evaluated for antibacterial activity. Although these compounds had similar potency to SB-219383, they showed weak antibacterial activity, again due to the poor penetration into the bacterial cell.

Cubist Pharmaceuticals reported a series of condensed imidazolidinones that inhibit ARSs (Cubist Pharmaceuticals Inc. 2000a). The compound shown in Fig. 4l gave an IC_{50} value of 0.17 nM for inhibition of phenylalanyl-tRNA synthetase (FRS) from *E. faecalis,* and exhibited MIC of 3.1 μg/ml against this pathogen. They also reported a series of tetracyclic heterocycles that target ARSs (Cubist Pharmaceuticals Inc. 2000b). The compounds inhibited the growth of gram-positive and gram-negative anaerobic bacteria, *Staphylococci, Streptococci*, *Mycobacterium* spp., and intracellular microbes such as *Chlamydia* and *Rickettsiae*. The compounds were also active against fungal organisms, including *Histoplasma, Paracoccidioides*, *Pneumocystis*, *Trichophyton*, and *Paecilomyces*. The compound shown in Fig. 4m inhibited FRS from *E. faecalis* with IC_{50} of 0.17 μM, and exhibited a MIC value of 3.1 μg/ml against *Staph. aureus*.

The GSK group disclosed a novel class of substituted quinolones that are potent inhibitors of bacterial MRS and which can be used as antibacterial agents (SmithKline Beecham PLC 1999). The compounds gave IC_{50} values in the range of 3–100 nM against *Staph. aureus* MRS with no inhibition of Rat MRS up to 10 μM. The representative compounds (Fig. 4n) gave MIC values of 1 μg/ml or less against *Staph. aureus*, *Strep. pneumoniae*, and *E. faecalis*, and 2–64 μg/ml against *M. catarrhalis*. In a subsequent paper (Jarvest et al. 2002), the same group reported nanomolar inhibitors that were derived from those compounds (Cubist Pharmaceuticals Inc. 2000a). The optimized compounds (Fig. 4o, p) were the potent inhibitors of *Staph. aureus* MRS, with IC_{50} values of 8–12 nM. These inhibitors were highly selective for bacterial MRS showing no inhibition of rat liver MRS up to IC_{50}=1,000 nM. The compounds showed excellent antibacterial activity with a MIC value in the range 0.016 to 1 μg/ml against *Staph. aureus*, *Staph. epidermidis*, *E. faecalis*, and *E. faecium*. Time-kill viability studies with *Staph. aureus* and *E. faecalis* showed no significant reduction in bacterial counts from the test inoculum. This indicates that the inhibitory effect of this compound is bacteriostatic, as was expected for the tRNA synthetase inhibitors. The compound shown in Fig. 4p showed similar in vivo efficacy at a dose of 21 mg/kg to erythromycin dosed at 50 mg/kg. A series of quinoline analogues were designed and evaluated as part of an antifungal agent that targeted ARSs (Yu et al. 2001). The new compounds are potent inhibitors of *C. albicans* PRS, which gave IC_{50} values in the range of 5–500 nM. One of the compounds (Fig. 4q) showed excellent activity against *C. albicans* (IC_{50} of 0.5 μM) with high selectivity over mammalian enzyme (IC_{50}>100 μM). An enzymology study indicated that this compound acts as a noncompetitive inhibitor of proline (k_i=15 nM) and as a competitive inhibitor of ATP (k_i=5.1 nM). However, the compound showed limited whole cell activity against *C. albicans* (MIC>100 μg/ml). Recently, the Cubist group reported other novel catechols and proline derivatives (Finn et al. 2001) (Fig. 4r and s) that were potent inhibitors of *Staph. aureus* MRS. The compound in Fig. 4r inhibited the

enzyme with an IC_{50} value of 20 nM and exhibited MIC values of 3.1 and 1.6 μg/ml against *Staph. aureus* and *E. faecalis*, respectively; the one shown in Fig. 4s gave an IC_{50} value of 210 nM and respective MIC values of 50 and 25 μg/ml against the same bacteria. These compounds also exhibited a good antibacterial spectrum with excellent selectivity over the human MRS enzyme. A series of thiazolidinones were synthesized and evaluated as inhibitors of FRSs (Parr et al. 2001). The compounds showed inhibitory activity against MRSs from *Staph. aureus*, *Enterococcus faecalis*, and *Escherichia coli*, giving IC_{50} values in the range of 10–2000 nM with high selectivity over their mammalian counterparts as well as other ARSs. One compound (Fig. 4t) was described to show the whole cell activity against permeable *E. coli* (MIC of 1.56 μg/ml).

Spirocyclic tetrahydrofurans were developed as inhibitors of FRS (Hill et al. 2001). The compounds, designated CB-102930 (Fig. 4u), were potent inhibitors with an IC_{50} of 0.6 μM, and exhibited MIC of 50 μg/ml against *Staph. aureus*. Although its trans isomer CB-126229 (Fig. 4v) was shown to be more potent, with an IC_{50} of 4 nM, it exhibited only marginal whole cell activity (MIC of 50 μg/ml). The two compounds were shown to be unstable, having half-lives of 101 and 158 min, respectively. A series of their derivatives were synthesized to overcome this instability. The two stable derivatives (Fig. 4w and x) obtained by reduction from CB-126229 were still potent inhibitors, giving IC_{50} values of 0.5–0.8 μM and having selectivity over human FRS (IC_{50}>100 μM). The compounds exhibited improved whole cell activity with a MIC value of 3–6 μg/ml.

Perspectives

Theoretically, the targets that are unique to pathogens and have no human counterparts are ideal for developing antibiotics with a low toxicity profile. For this reason bacterial cell wall synthesis has been the focus of antibiotic research for a long time. In parallel, many natural products (such as aminoglycosides, macrolides, quinolones, and tetracyclines) have been discovered through massive screening for novel antibiotics. Interestingly, the targets of these natural products are the components of the transcription and translation systems, which prove that other essential systems can be the target for antibiotics, even if they have eukaryotic counterparts. Linezolid and Synercid, recently approved by the FDA, also act on a 50S subunit of ribosome, which proves the potential value of the translation apparatus as drug targets. Many pharmaceutical companies are currently developing compounds that fall into this class, such as telithromycin (semisynthetic macrolides) and GAR-936 (tetracycline derivatives).

ARSs are essential enzymes that are present in all living organisms. The inhibition of ARS in the pathogen results in the depletion of the charged tRNA and leads to the eventual shut off of protein synthesis and cell growth. Due to their pivotal role in translation, they have been recognized as a promising target for a new generation of antibiotics with selectivity and specificity. While some of the ARS inhibitors showed encouraging results, the challenge still remains to improve the selectivity. As mentioned above, mupirocin is an encouraging example as it inhibits the bacterial protein synthesis through selective inhibition of isoleucyl-tRNA synthetase and shows excellent selectivity for prokaryote isoleucyl-tRNA synthetase. Computer-aided molecular design will be a good platform to develop inhibitors using the differences between human and bacterial synthetases. However, it has been observed that good in vitro activities are not always correlated to those in vivo, which can be attributed to the high polarity of ARS inhibitors that prevent penetration into the bacterial cell. While aminoacyl adenylate-mimics and other inhibitors have shown whole cell activities, the number of these inhibitors is too limited to predict the permeability and other pharmacological properties. For better pharmacological behavior, other principles have to be taken into account in the design of ARS inhibitors. Given the structural and functional diversity of ARSs, the recent development of selective inhibitors could spur the discovery of more selective inhibitors with suitable pharmacological properties.

While mupirocin has enjoyed its monopolistic position as an effective anti-infective with unique working mechanism, resistant strains began to emerge recently (Cookson 1998; Schmitz et al. 1998; Yun et al. 2003). The mupirocin-resistant strains are classified into two groups depending on the degree of their resistance. While low-level resistance is related to mutation in the host isoleucyl-tRNA synthetase, high-level resistance results from an increase in the copy number due to the introduction of exogenous enzyme. The resistance of the enzyme against mupirocin was endowed by the alteration in the residues that are near the KMSKS conserved motif (Antonio et al. 2002). Efflux mutants have not yet been reported against mupirocin; thus, the preparation of new substitutes for mupirocin or novel ARS inhibitors is an urgent requirement. Considering that mupirocin is a natural product, screening more such products might still be a productive way of searching for ARS inhibitors. Since Mother Nature has created such a smart molecule, there is no reason not to believe that there should be more out there.

References

Antonio M, McFerran N, Pallen MJ (2002) Mutations affecting the Rossman fold of isoleucyl-tRNA synthetase are correlated with low-level mupirocin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 46:438-442

Arnez JG, Moras D (1997) Structural and functional considerations of the aminoacylation reaction. Trends Biochem Sci 22:211–216

Baines PJ, Mellows G, Swaisland AJ, Tasker TC (1984) Mupirocin: its chemistry and metabolism. In: Mupirocin a novel topical antibiotic. Royal Society of Medicine, London, pp 13–22

Beecham Group PLC (1989) Antibacterial 1-normon-2-yl-heterocyclic compounds. US patent 4,861,788

Beecham Group PLC (1991) Antibacterial monic acid derivatives. US patent 5,041,567

Berge JM, Copley RC, Eggleston DS, Hamprecht DW, Jarvest RL, Mensah LM, O'Hanlon PJ, Pope AJ (2000) Inhibitors of bacterial tyrosyl tRNA synthetase: synthesis of four stereoisomeric analogues of the natural product SB-219383. Bioorg Med Chem Lett 10:1811–1814

Broom NJ, Cassels R, Cheng HY, Elder JS, Hannan PC, Masson N, O'Hanlon PJ, Pope A, Wilson JM (1996) The chemistry of pseudomonic acid. 17. Dual-action C-1 oxazole derivatives of pseudomonic acid having an extended spectrum of antibacterial activity. J Med Chem 39:3598–3600

Brown P, Eggleston DS, Haltiwanger RC, Jarvest RL, Mensah L, O'Hanlon PJ, Pope AJ (2001) Synthetic analogues of SB-219383. Novel C-glycosyl peptides as inhibitors of tyrosyl tRNA synthetase. Bioorg Med Chem Lett 11:711–714

Burbaum JJ, Schimmel P (1991) Structural relationships and the classification of aminoacyl-tRNA synthetases. J Biol Chem 266:16965–16968

Carcanague DR (1997) Novel derivatives of pseudomonic acid. Bioorg Med Chem Lett 7:2805–2808

Class YJ, Deshong P (1995) The pseudomonic acid. Chem Rev 95:1483–1857

Cookson BD (1998) The emergence of mupirocin resistance: a challenge to infection control and antibiotic prescribing practice. J Antimicrob Chemother 41:11–18

Cubist Pharmaceuticals Inc (1998a) Aminoacyl adenylate minics as novel antimicrobial and antiparasitic agents. US patent 5,726,195

Cubist Pharmaceuticals Inc (1998b) Aminoacyl sulfamides for the treatment of hyperproliferative disorders. WO 98/41215

Cubist Pharmaceuticals Inc (2000a) Condensed imidazolidinones as tRNA synthetase inhibitors. WO 00/18772

Cubist Pharmaceuticals Inc (2000b) Tetracyclic heterocycles as antimicrobial. WO 00/17206

Cusack S (1997) Aminoacyl-tRNA synthetases. Curr Opin Struct Biol 7:881–889

Desjardins M, Garneau S, Desgagnes J, Lacoste L, Yang F, Lapointe J, Chenevert R (1998) Glutamyl adenylate analogues are inhibitors of glutamyl-tRNA synthetase. Bioorg Chem 26:1–13

Eriani G, Delarue M, Poch O, Gangloff J, Moras D (1990) Partition of tRNA synthetases into two classes based on mutually exclusive sets of sequence motifs. Nature 347:203–206

Farmer TH, Gilbart J, Elson SW (1992) Biochemical basis of mupirocin resistance in strains of *Staphylococcus aureus*. J Antimicrob Chemother 30:587-596

Finn J, Hill J, Ram S, Morytko M, Yu X, Gimi R, Silverman J, Stein R, Lim A, Mak E, Gallant P, Wendler P, Rose S, Stevens A, Keith D (2001) Novel antibacterial agents targeting methionyl-tRNA synthetase: a chemlnformatic approach to convert HTS data into quality medicinal chemistry leads. In: Proceedings of the 41st Annual Interscience Conference on Antimicrobial Agents and Chemotherapy Chicago, Ill.

Forrest AK, Jarvest RL, Mensah LM, O'Hanlon PJ, Pope AJ, Sheppard RJ (2000) Aminoalkyl adenylate and aminoacyl sulfamate intermediate analogues differing greatly in affinity for their cognate *Staphylococcus aureus* aminoacyl tRNA synthetases. Bioorg Med Chem Lett 10:1871–1874

Fraser TH, Rich A (1975) Amino acids are not all initially attached to the same position on transfer RNA molecules. Proc Natl Acad Sci USA 72:3044–3048

Frugier M, Florentz C, Schimmel P, Giege R (1993) Triple aminoacylation specificity of a chimerized transfer RNA. Biochemistry 32:14053–14061

Heacock D, Forsyth CJ, Shiba K, Musiter-Forsyth K (1996) Synthesis and aminoacyl-tRNA synthetase inhibitory of prolyl adenylate analogs. Bioorg Chem 24:273–289

Hill J, Finn J, Wang Z, Silverman J, Oliver N, Gallant P, Wender P, Keith D (2001) Synthesis and activity of spirocyclic tetrahydrofurans as inhibitors of phenylalanine tRNA synthetase. In: Proceedings of the 41st Annual Interscience Conference on Antimicrobial Agents and Chemotherapy Chicago, Ill.

Houge-Frydrych CS, Readshaw SA, Bell DJ (2000a) SB-219383, a novel tyrosyl tRNA synthetase inhibitor from a *Micromonospora sp*. II. Structure determination. J Antibiot (Tokyo) 53:351–356

Houge-Frydrych CS, Gilpin ML, Skett PW, Tyler JW (2000b) SB-203207 and SB-203208, two novel isoleucyl tRNA synthetase inhibitors from a *Streptomyces sp*. II. Structure determination. J Antibiot (Tokyo) 53:364–372

Hughes J, Mellows G (1980) Interaction of pseudomonic acid A with *Escherichia coli* B isoleucyl-tRNA synthetase. Biochem J 191:209–219

Ibba M, Morgan S, Curnow AW, Pridmore DR, Vothknecht UC, Gardner W, Lin W, Woese CR, Soll D (1997) A euryarchaeal lysyl-tRNA synthetase: resemblance to class I synthetases. Science 278:1119–1122

Jarvest RL, Berge JM, Houge-Frydrych CS, Janson C, Mensah LM, O'Hanlon PJ, Pope A, Saldanha A, Qiu X (1999) Interaction of tyrosyl aryl dipeptides with *S. aureus* tyrosyl tRNA synthetase: inhibition and crystal structure of a complex. Bioorg Med Chem Lett 9:2859–2862

Jarvest RL, Berge JM, Berry V, Boyd HF, Brown MJ, Elder JS, Forrest AK, Fosberry AP, Gentry DR, Hibbs MJ, Jaworski DD, O'Hanlon PJ, Pope AJ, Rittenhouse S, Sheppard RJ, Slater-Radosti C, Worby A (2002) Nanomolar inhibitors of *Staphylococcus aureus* methionyl tRNA synthetase with potent antibacterial activity against gram-positive pathogens. J Med Chem 45:1959–1962

Klein LL, Yeung CM, Kurath P, Mao JC, Fernandes PB, Lartey PA, Pernet AG (1989) Synthesis and activity of nonhydrolyzable pseudomonic acid analogues. J Med Chem 32:151–160

Konishi M, Nishio M, Saitoh K, Miyaki T, Oki T, Kawaguchi H (1989) Cispentacin, a new antifungal antibiotic. I. Production, isolation, physico-chemical properties and structure. J Antibiot (Tokyo) 42:1749–1755

Konrad I, Roschenthaler R (1977) Inhibition of phenylalanine tRNA synthetase from *Bacillus subtilis* by ochratoxin A. FEBS Lett 83:341–347

Leberman R, Hartlein M, Cusack S (1991) *Escherichia coli* seryl-tRNA synthetase: the structure of a class 2 aminoacyl-tRNA synthetase. Biochim Biophys Acta 1089:287–298

Lee J, Kang SU, Kang MK, Chun MW, Jo YJ, Kwak JH, Kim S (1999) Methionyl adenylate analogues as inhibitors of methionyl-tRNA synthetase. Bioorg Med Chem Lett 9:1365–1370

Lee J, Kang SU, Kim SY, Kim SE, Jo YJ, Kim S (2001) Vanilloid and isovanilloid analogues as inhibitors of methionyl-tRNA and isoleucyl-tRNA synthetases. Bioorg Med Chem Lett 11:965–968

Merck & Co Inc (2000a) Novel prolines as antimicrobial agents. WO 00/66119

Merck & Co Inc (2000b) Novel catechols as antimicrobial agents. WO 00/66120

Morton TM, Johnston JL, Patterson J, Archer GL. (1995) Characterization of a conjugative staphylococcal mupirocin resistance plasmid. Antimicrob Agents Chemother 39:1272–1280

Nass G, Poralla K, Zahner H (1969) Effect of the antibiotic Borrelidin on the regulation of threonine biosynthetic enzymes in *E. coli*. Biochem Biophys Res Commun 34:84–91

Ogilvie A, Wiebauer K, Kersten W (1975) Inhibition of leucyl-transfer ribonucleic acid synthetasymol. Biochem J 152:511–515

Parr I, Hill J, Finn J, Yang D, Silverman J, Oliver N, Gallant P, Mak E, Stein R, Lim A, (2001) Synthesis and biological activity of novel di-alkylated thiazolidinone phenylalanyl-tRNA synthetase inhibitors. In: Proceedings of the 41st Annual Interscience Conference on Antimicrobial Agents and Chemotherapy Chicago, Ill.

Ribas de Pouplana L, Schimmel P (2001) Two classes of tRNA synthetases suggested by sterically compatible dockings on tRNA acceptor stem. Cell 104:191–193

Ruff M, Krishnaswamy S, Boeglin M, Poterszman A, Mitschler A, Podjarny A, Rees B, Thierry JC, Moras D (1991) Class II aminoacyl transfer RNA synthetases: crystal structure of yeast aspartyl-tRNA synthetase complexed with tRNA(Asp). Science 52:1682–1689

Schimmel P, Giege R, Moras D, Yokoyama S (1993) An operational RNA code for amino acids and possible relationship to genetic code. Proc Natl Acad Sci USA 90:8763–8768

Schmitz FJ, Lindenlauf E, Hofmann AC, Fluit AC, Verhoef J, Heinz HP, Jones ME (1998) The prevalence of low- and high-level mupirocin resistance in staphylococci from 19 European hospitals. J Antimicrob Chemother 42:489–495

Sissler M, Eriani G, Martin F, Giege R, Florentz C (1997) Mirror image alternative interaction patterns of the same tRNA with either class I arginyl-tRNA synthetase or class II aspartyl-tRNA synthetase. Nucleic Acids Res 25:4899–4906

SmithKline Beecham PLC (1996a) (Hetero)-aryl ketones derivatives with antibacterial properties. US patent 5,536,745

SmithKline Beecham PLC (1996b) (Hetero)-aryl ketones derivatives with antibacterial properties. JP patent 9,157,269

SmithKline Beecham PLC (1997a) Mupirocinsulfamates with antibacterial activity. WO 97/05126

SmithKline Beecham PLC (1997b) Compounds with a sulfamoyl group and pharmaceutical compositions containing them. WO 97/35859

SmithKline Beecham PLC (1998a) Sulfamate derivatives with t-RNA synthetase inhibiting activity. WO 98/32765

SmithKline Beecham PLC (1998b) Enzymatic preparation of monic acids. US patent 5,726,049

SmithKline Beecham PLC (1999) Quinolones used as MRS inhibitors and bactericides. WO 99/55677

SmithKline Beecham PLC (2000a) 2-NH-Pyridones and pyrimidones as MRS inhibitors. WO 00/71524 A.

SmithKline Beecham PLC (2000b) Benzimidazole derivatives and their use as methionyl-tRNA synthetase inhibitors. WO 00/71522 A1

SmithKline Beecham PLC (2000c) Quinolones as t-RNA synthetase inhibitors and antibacterial agents. WO 00/21949

Stefanska AL, Fulston M, Houge-Frydrych CS, Jones JJ, Warr SR (2000a) A potent seryl tRNA synthetase inhibitor SB-217452 isolated from a *Streptomyces* species. J Antibiot (Tokyo) 53:1346–1353

Stefanska AL, Cassels R, Ready SJ, Warr SR (2000b) SB-203207 and SB-203208, two novel isoleucyl tRNA synthetase inhibitors from a *Streptomyces sp*. I. Fermentation, isolation and properties. J Antibiot (Tokyo) 53:357–363

Tanaka K, Tamaki M, Watanabe S (1969) Effect of furanomycin on the synthesis of isoleucyl-tRNA. Biochim Biophys Acta 195:244–245

Werner RG, Thorpe LF, Reuter W, Nierhaus KH (1976) Indolmycin inhibits prokaryotic tryptophanyl-tRNA ligase. Eur J Biochem 68:1–3

Yu XY, Hill JM, Yu G, Wang W, Kluge AF, Wendler P, Gallant P(1999) Synthesis and structure-activity relationships of a series of novel thiazoles as inhibitors of aminoacyl-tRNA synthetases. Bioorg Med Chem Lett 9:375–380

Yu XY, Hill JM, Yu G, Yang Y, Kluge AF, Keith D, Finn J, Gallant P, Silverman J, Lim A (2001) A series of quinoline analogues as potent inhibitors of *C. albicans* prolyl tRNA synthetase. Bioorg Med Chem Lett 11:541–544

Yun HJ, Lee SW, Yoon GM, Kim SY, Choi S, Lee YS, Choi EC, Kim S (2003) Prevalence and mechanisms of low- and high-level mupirocin resistance in staphylococci isolated from a Korean Hospital. J Antimicrob Chemother (in press)