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Standard Review

New Targets for Antibacterial Agents

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The alarming increase and spread of resistance among emerging and re-emerging bacterial pathogens to all clinically useful antibiotics is one of the most serious public health problems of the last decade. Thus, the search for new antibacterials directed toward new targets is not only a continuous process but also, at this time, an urgent necessity. Recent advances in molecular biological technologies have significantly increased the ability to discover new antibacterial targets and quickly predict their spectrum and selectivity. The most extensively evaluated bacterial targets for drug development are: quorum sensor biosynthesis; the two component signal transduction(TCST) systems; bacteria division machinery; the shikimate pathway; isoprenoid biosynthesis and fatty acid biosynthesis.

Key words: new targets, quorum sensor biosynthesis, TCST systems, bacteria division machinery, shikimate pathway, isoprenoid biosynthesis, fatty acid biosynthesis, antibiotic resistance.

TABLE OF CONTENTS

INTRODUCTION

Bacterial infection is a ubiquitous health hazard. There are a number of very good clinically efficacious antibiotics in use today; however, the development of bacterial resistance has rendered almost all of them less effective. This critical situation necessitates the design of novel antibacterial agents. These agents must target essential bacterial pathways, but may have new modes of action or even interfere with novel bacterial targets. Many essential bacterial proteins have been identified as potential drug targets. However, an ideal target is recognized as that different from existing targets, essential for microbial cell survival, highly conserved in a clinically relevant spectrum of species, absent or radically different in man, easy to assay, and has an understood biochemistry. This review highlights the progress in the search for new antibacterial targets.

IMPACT OF GENOMICS, BIOINFORMATICS AND RELATED TECHNOLOGIES ON THE SEARCH FOR NEW TARGETS

With advancements in genomics and bioinformatics, it is

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now possible to search through a bacterial genome to identify potential antibacterial targets. Selection of an appropriate target begins with bioinformatics to look for open reading frames (ORFs) conserved across the potential bacterial target organisms. Genes or gene products that can actually be used, are chosen by various approaches. These include automated comparisons of bacterial genomes to categorize genes and the encoded proteins. Primary sequence comparison programs, like BLAST (The Basic Local Alignment Search Tool) or PSI-BLAST (Position-Specific Iterated BLAST) determine gene functions by sequence homology, which is also used to determine clusters of orthologous groups (COGs). COGs are groups of genes shared by evolutionarily distant organisms. These orthologous families of genes are prime candidates for broad-spectrum antimicrobial agents (Hood 1999). Another approach is gene expression profiling with cluster analysis which uses microarray technology to analyze gene expression, in order to organize genes into functional groups (Eisen et al. 1998). Unknown genes functions can be estimated based on the general pathways or metabolic functions of nearby clusters. A better method of antibacterial target selection is the structural genomics, which determines the three-dimensional structures of proteins. Function is more directly a consequence of its structure than its sequence (Gerstein and Jansen 2000). Typically, 30% to 50% of the several thousand genes that make up each bacterial genome as yet have no apparent function. Direct comparison of the three-dimensional protein structure to a protein structure database can be used to assign unknown gene function. Advances already made in this area have been described that apply the sequence-structure-function model to function prediction (Fetrow et al. 1998). Moreover, phylogenetic groups that are based on the specific folds shared by organisms can be used. These folds and sequence families in bacterial pathogens can be useful antibiotic targets (Gerstein 2000). Motif analysis is another strategy to identify potential antibiotic targets among genes with unknown functions. Many databases, including PROSITE database, can search for motifs in a sequence (PROSITE is a method of determining what is the function of uncharacterized proteins translated from genomic or cDNA sequences). The motifs may show the approximate biochemical function of the gene. Moreover, gene fusion is a computational method to infer protein interactions from genome sequences. Proteins that interact with each other tend to have homologs in other organisms that are joined into a single protein chain. This method would give additional functional information for target proteins (Hood 1999).

Other tools were also developed in order to identify and study potential targets which although unnecessary *in vitro*, are essential for disease production. Examples include *In Vivo* Expression Technology (IVET), Differential Fluoresence Induction (DFI), and Signature-Tagged Mutagenesis (STM) (Strauss and Falkow 1977), Each of these methods provide a different type of information. IVET identifies genes specifically induced during an active infection and can be used to generate temporal information (Camilli and Mekalanos 1995); DFI uncouples metabolic requirements from selection parameters, thus focusing on infection specific process, while STM identifies genes required for the establishment of an infection (Heithoff et al. 1997).

The drug discovery process

Genes that pass these filters are the best candidates for going to high-throughput screens to generate initial hits (compounds that interact with a target). High-throughput screening can be considered the process in which batches or “libraries” of compounds are tested rapidly and in parallel, for binding activity or biological activity against target molecules. Test compounds act as inhibitors of target enzymes if one has been established, as competitors for binding of a natural ligand to its receptor, as agonists or antagonists for receptor-mediated intracellular processes, and so forth. Then chemical modification of hits are carried out by repeated cycles of synthesis and testing of analogs to produce "leads," which are compounds with improved chemical characteristics, thereby increaseing their suitability as potential drugs. Finally, leads are further optimized by additional repeated modification to produce drug development candidates with optimized characteristics for further pre-clinical and clinical development (Allsop and Illingworth 2002).

NOVEL ANTIBACTERIAL TARGETS

QUORUM SENSOR BIOSYNTHESIS

Bacteria are sensitive to an increase in population density and respond quickly and coordinately by inducting certain sets of genes. This mode of regulation, known as quorum sensing (QS), is based on the interaction of low-molecular-weight signal molecules called autoinducers (AIs) or pheromones with a sensor kinase and response regulator to activate or repress gene expression. QS systems are considered to be global regulators and play a key role in controlling many metabolic processes in the cell, includeing bacterial virulence. These systems offer attractive targets for a novel class of antibacterial drugs, capable of inducing chemical attenuation of pathogenicity.

Three types of autoinducers have been identified: acylated homoserine lactones, autoinducing peptides and autoinducer-2 compounds (Raffa et al. 2005). Acylated homoserine lactones (AHLs, acyl-HSLs, or HSLs) such as *N*-3-oxohexanoyl-$_L$-homoserine lactones (AI-1), are present in a wide spectrum of Gram-negative organisms. The AHL molecules are produced by LuxI homologues and constitute, in complex with LuxR homologues, transcripttional regulators. On the other hand, autoinducing peptides (AIPs) are amino acids or short peptides synthe-

Table 1. Synthetic quorum-sensing inhibitors (QSIs)

Development of QSI compound(s)	Mode of action	References
Substitutions in the AHL acyl side chain		
- Extended acyl side chains	inhibition of the LuxR homologues	Chhabra et al. 1993 Passador et al. 1996 Schaefer et al. 1996 Zhu et al. 1998
- Introducing ramified alkyl, cycloalkyl or aryl/phenyl substituents at the C-4 position resulting in both inducers (analogues with non-aromatic substitutions) and antagonists (analogues with phenyl substitutions)	Antagonistic activity of the phenyl compounds may result from the interaction between the aryl group and aromatic amino acids of the LuxR receptor, preventing it from adopting the active dimeric form.	Reverchon et al. 2002
- Replacing the C-3 atom with sulphur	inhibition of transcriptional regulators LuxR and LasR*.	Persson et al. 2005
Substitutions and alteration in the AHL lactone ring		
- To C-3	AHL agonist	Olsen et al. 2002
- To C-4	Unable to interact with LuxR homologues.	
- Exchanging the homoserine ring with a 5- or 6- membered alchohol or ketone ring (activators and inhibitors)	Some blocked the two *Ps. aeruginosa* QS systems *in vitro*. Their target specificity for the QS regulon was not verified by transcriptomics.	Smith et al. 2003a, b
Extensive modification in both AHL acyl side chain and lactone ring		
- 4- nitro-pyridine-*N*-oxide (4-NPO)	- Block the *E. coli* established hybrid LuxR QS system. - Target the LasR and RhlR* receptors in *Ps. aeruginosa*.	Rasmussen et al. 2005a Hentzer et al. 2003 Schuster et al. 2003

Adapted from Rasmussen and Givskov (2006).
*LasR and RhlR: transcriptional regulators of *Ps. aeruginosa* QS systems.

sized in Gram-positive bacteria and are processed, modified, and excreted by the ATP-binding cassette export systems. AIPs bind to cell surface-bound histidine protein kinase, which autophosphorylates and in turn phosphorylates a response regulator that activates transcription of one or more target genes. Lastly, autoinducer-2 compounds (AI-2) are common to both Gram-negative and Gram–positive bacteria. They are derived from furanones. LuxS is an enzyme which produces 4,5-dihydroxy-2,3-pentanedione (DPD) acylhomoserine lactones a forerunner of AI-2. This autoinducer binds to LuxP protein (a LuxR homolog). The AI-2/LuxP complex then binds to membrane-bound histidine protein kinase, and a signal transduction occurs by multistep phosphorylation similar to that of AIPs. In other bacteria, extracellular AI-2 is transported back into the cell through a LuxS-regulated (Lsr) transporter.

Based on this information, many QS inhibitor or block-ing strategies can be proposed. Many include the block-ing of signal synthesis. The LuxI family of synthases could be a target. The x-ray structure of the lactone synthase Esa1 from *Ps. stewartii* was solved and could be a starting point for structure-based design of drugs (Watson et al. 2002). If LuxS is the target, inhibitors could well function as broad-spectrum antibiotics. Other strategies are blockade of the autoinducer receptor site of the LuxR homologues, histidine protein kinase, or Lsr transporter. Other strategies include, blocking the formation of active dimers that are required for binding and expression of target genes as well as enhancement of signal molecule degradation. A combination of mechanisms would be expected to be more effective than single mechanism approaches. A number of studies have identified several molecules that function as QS inhibitors (QSI) both *in vitro* and *in vivo*. These included both synthetic and natu-ral QSIs (Tables 1 and 2).

QS systems are also important determinants of morphology and communication when bacteria grow as aggregates in biofilms (Vuong et al. 2003). Biofilm-associated microorganisms appear to contribute to cystic fibrosis, native valve endocarditis, otitis media, period ontitis, and chronic prostatitis. A spectrum of indwelling medical devices and other devices used in the healthcare environment have been shown to harbor biofilms, result-

Table 2. Natural quorum-sensing inhibitors (QSIs).

QSI compound(s)	Source	Quorum-sensing system affected	References
Agrocinopine B	Crown gall cells of host plants.	Tra system of *A. tumefaciens.*	Oger and Farrand 2001.
Furanone	*D. pulchra.*	Swr system of S. liquefaciens and other Gram-negative bacteria.	Givskov et al. 1996.
Canavanine	*M. sativa*	Sin/ExpR system of *S. meliloti*	Keshavan et al. 2005.
Norepinephrine, epinephrine	Human hormones	AI-3 system of EHEC	Sperandio et al. 2003.
Penicillic acid, Patulin	*Penicillium* spp.	Las and Rhl systems of *Ps. aeruginosa*	Rasmussen et al. 2005b

Modified from González and Keshavan (2006)

Table 3. Microorganisms commonly associated with biofilm on indwelling medical devices

Microorganism	Associated with biofilms on
C. albicans	- Artificial voice prosthesis - Central venous catheters - Intrauterine device
Coagulase negative Staphylococci	- Artificial hip prosthesis - Artificial voice prosthesis - Central venous catheters - Intrauterine device - Prosthetic heart valves - Urinary catheters
Enterococcus spp.	- Artificial hip prosthesis - Central venous catheters - Intrauterine device - Prosthetic heart valves - Urinary catheters
Kl. Pneumoniae	- Central venous catheters - Urinary catheters
Ps. aeruginosa	- Artificial hip prosthesis - Central venous catheters - Urinary catheters
Staph. aureus	- Artificial hip prosthesis - Central venous catheters - Intrauterine device - Prosthetic heart valve

Modified from Donlan (2001).

ing in device-associated infections (Donlan 2001).

Table (3) provides a listing of microorganisms commonly associated with biofilms on indwelling medical device. A very important clinical requirement is to block biosynthesis of the quorum signalers that initiate biofilm development. Calfee et al. (2001), reported that an anthranilate analog (methyl anthranilate) inhibited signal production of the QS system of *Ps. aeruginosa* and decreased the expression of cellular virulence factors in a dose-dependent fashion. Hentzer et al. (2003), showed that the target of a synthetic furanone, which prevented biofilm formation in *Ps. aeruginosa* were the genes of QS system. That the effect was specific to biofilms was shown by the lack of activity against planktonic cultures of the same bacteria.

THE TWO-COMPONENT SIGNAL TRANSDUCTION SYSTEMS

The two-component signal transduction (TCST) systems are the principal means for coordinating responses to environmental changes in bacteria as well as some plants, fungi, protozoa, and archaea. These systems typically consist of a receptor histidine kinase (HK), which reacts to an extracellular signal by phosphorylating a cytoplasmic response regulator, causing a change in cellular behavior. The two-component family presents an excellent novel target for antibacterial drug discovery due to many reasons. First, significant homology is shared among different genera of bacteria, particularly in those amino acid residues located near active sites. Second, pathogenic bacteria use TCST to regulate expression of essential virulence factors required for *in vivo* survival. Moreover, bacteria contain many TCST systems and in several different bacteria, at least one TCST is essential for *in vitro* growth. Lastly, a different mechanism is responsible for signal transduction in mammals.

Antibacterials inhibitors of TCSTs have been identified. The most common inhibitors reported to date are hydrophobic compounds that inhibit HK- autokinase activity, noncompetitively with respect to ATP; however, in the majority of cases these compounds do not appear to be selective for signal transduction pathways and exert their effect by multiple mechanisms of action (Hilliard et al. 1999; Kitayama et al. 2004; Stephenson and Hoch, 2002).

Studies conducted in subsequent years (Stephenson and Hoch 2004), concluded that designing competitive

inhibitors specific to HKs presents a huge challenge, because the Bergerat ATP binding fold in HKs is not unique for prokaryotes. Nevertheless, a novel thienopyridine (TEP) compound has been reported. TEP inhibits bacterial histidine kinases competitively (with ATP) but does not comparably inhibit mammalian serine/threonine kinases. TEP could serve as a starting compound for a new class of histidine kinase inhibitors with antibacterial activity (Gilmour et al. 2005).

BACTERIAL DIVISION MACHINERY

The division machinery of bacteria is an attractive target because it comprises seven or more essential proteins that are conserved almost throughout the bacterial kingdom but are absent from humans.

Most of the effort done to exploit this machinary as a new target has been directed at the FtsZ protein which is a GTPase. That is due to its essential role in prokaryotic cell division, its widespread conservation in the Bacterial Kingdom, its absence in the mitochondria of higher eukaryotes, its evolutionary distance from tubulin, its known biochemical activity and atomic structure and its predominance among all bacterial cell division proteins, totalling 10,000–20,000 copies per single bacterium (Pinho and Errington 2003; Stokes et al. 2005).

Localization of the FtsZ protein at the site of cell division is the first stage in the cell replication process followed by self-polymerization. The polymerized FtsZ recruits other cell division proteins, including FtsA, ZipA, FtsK, FtsQ, FtsL, FtsW, FtsI, and FtsN, leading to the formation of a Z-ring and the initiation of the complex process of septation. All of these cell division proteins are localized at mid-cell, and work in concert to constrict the cell and produce cell division.

Ftsz, and thus represent potential classes of new antibiotics. Ftsz may also be a feasible novel target for antituberculous agents (Reynolds et al. 2004; White et al. 2002).

Another target that has been exploited to screen and analyze inhibitory molecules, is the FtsA. This highly conserved protein presumably constitutes a key bacterial component because of its ATPase enzymatic activity and its essential protein–protein interaction with FtsZ. The FtsZ–FtsA protein–protein interaction and the FtsZ:FtsA ratio are crucial for the progress of bacterial cell division. Accumulating evidence suggests that FtsA plays the role of a motor protein in providing energy for constriction by way of its ATPase activity (Errington et al., 2003). Lately, Paradis-Bleau et al. (2005), reported the identification of peptide sequences which showed specific inhibition of ATPase activity of FtsA. They considered this the first step for the future development of antimicrobial agents via peptidomimetism.

One more interaction which can also be an elegant tool with the aim of developing antibacterials with novel modes of action is the FtsZ-ZipA protein–protein interaction. Briefly these proteins are essential components of the septal ring which forms at the site of cell division. Inhibition of this interaction between the two proteins results in inhibition of cell division, leading to filamenttation and ultimately cell death. Development of inhibitors of FtsZ-ZipA protein-protein interaction has been focused upon only recently (Jennings et al. 2004; Sutherland et al. 2003). It is expected that efforts in this area will not diminish in the future.

THE SHIKIMATE PATHWAY

The shikimic acid pathway (the aromatic biosynthetic pathway), is conserved in bacteria, fungi, plants and apicomplexan parasites but is absent in mammals. Analysis of the genomes of *Strep. pneumoniae* and *Staph. aureus* has illustrated the presence and conservation of the chorismate biosynthetic genes in key Gram-positive pathogens, which can facilitate their characterization and essentiality testing (McDevitt et al. 2002).

This pathway effects the conversion of two simple products of carbohydrate metabolism (phosphoenolpyruvate and erythrose 4- phosphate into the unstable diene chorismate (Figure 1). Chorismate represents a major bifurcation point of the pathway and is a common non-aromatic precursor for the biosynthesis of a range of important aromatic metabolites (Daugherty et al. 2001).

The enzymes of the shikimate pathway constitute an excellent target for the design of new antibacterial agents. Such agents may prove beneficial for immunocom-promised patients who are suffering multiple infection from bacterial and parasitic organisms.

Shikimate kinase represents an attractive target for the development of new antimicrobial agents, herbicides, and antiparasitic agents. The shikimate kinase structure of many bacteria has been determined. These include, *Erw. chrysanthemi* (Krell et al. 2001), *E. coli* (Romanowski and Burley 2002), *Myco. tuberculosis* (Pereira et al. 2004) and *Camp. jejuni* and *H. pylori* (Cheng et al. 2005). These structures provide shikimate-binding information as a rational basis for further investigation towards structure-guided inhibitors.

EPSP synthase can also be an attractive target. Knockout mutations of *aroA*, which encodes EPSP synthase, in both Gram negative and Gram positive bacteria were found to lead to attenuation of bacterial virulence, supporting the utility of this targeting approach (Izhar et al. 1990).

Chorismate Synthase (CS) is the most unusual of the entire pathway and is unique in nature. Studies recently conducted to understanding the structure of *Myco-.tuberculosis* CS together with its cofactor and substrate binding modes compared to *Strep. pneumoniae* reported a degree of similarity (Fernandes et al. 2005). Such studies may facilitate the search for inhibitors of this enzyme as alternative agents to treat tuberculosis.

Furthermore, fluorinated analogues of shikimate, block-

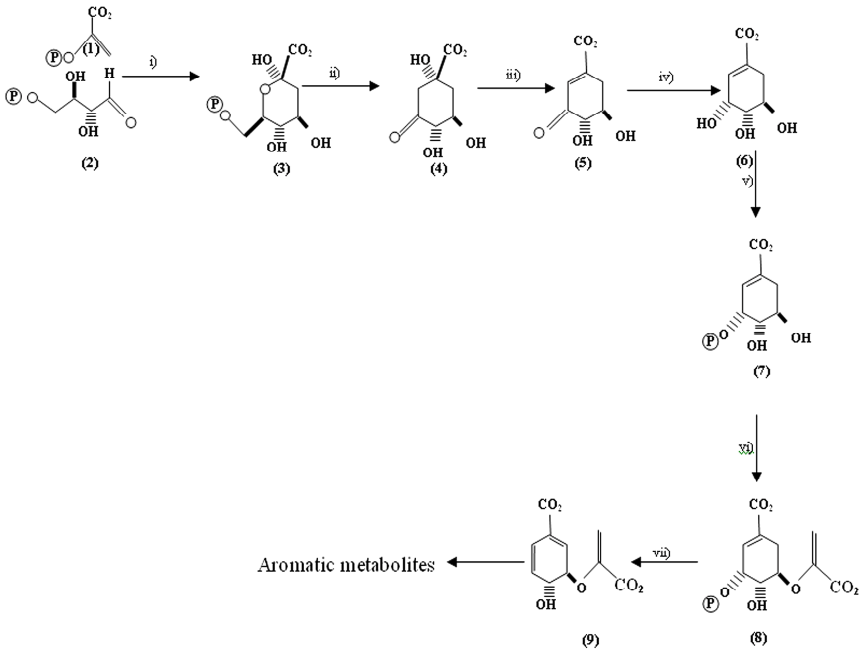

Figure 1. Shikimic acid pathway, modified from Daughery et al. (2001).
(1) Phosphoenolpyruvate (2) Erythrose-4-phosphate i) 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase (3) 3-deoxy-D-arabino-heptulosonate 7-phosphate ii) 3-dehydroquinate synthase (4) 3-dehydroquinate iii) 3-dehydroquinate dehydratase (5) 3-dehydroshikmate iv) Shikimate dehydrogenase (6) Shikimate v) Shikimate kinase (7) Shikimate-3-phosphate vi) 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase (8) EPSP vii) Chorismate synthase (9) Chorismate.

ed the growth of *Plasm. falciparum in vitro*, demonstrating that the shikimate pathway is a valid target for development of new broad-spectrum antimicrobial and antiparasitic agents (McConkey 1999).

ISOPRENIOD BIOSYNTHESIS

Isoprenoids are known in having invaluable role in various biological processes such as cell-wall biosynthesis, electron transport, photosynthetic light harvesting, lipid membrane structure and intracellular signaling. Isopentenyl diphosphate (IPP) and its isomer; dimethylallyl diphosphate (DMAPP), act as a universal precursor in biosynthesis of isoprenoids. The reactions IPP ↔ DMAPP is catalyzed by isopentenyl diphosphate (IPP) isomerase enzyme. These two isomers are produced by either classical (mevalonate) or non-classical (non-mevalonate or deoxyxylulose phosphate) pathway, as shown in Figures 2 A & 2 B, respectively.

The classical `mevalonate' pathway involves the construction of the six-carbon branched chain mevalonate skeleton from three molecules of acetyl-CoA (Figure 2 A). However, the recently identified `non-mevalonate' pathway involves the combination of pyruvate and glyceroldehydes-3-phosphate to make 1-deoxy-D-xylulose-5-phosphate (Figure 2 B). Many Gram-negative bacteria and apicoplast type protozoa including the *Plasmodium* spp. utilize the non-mevalonate pathway. Whereas, eukaryotes, archaea, Gram-positive cocci, *Bor. burgdorferi* and *Cox. burnetii*_employ exclusively the mey-alonate pathway (Hedi and Rodwell, 2004).

The distribution of the new non-mevalonate pathway made it an attractive target for the design of antibiotics against pathogenic bacteria and the malaria parasite *Plasm. falciparum* (Rohmer et al. 2004). Perhaps, any of the enzymes involved in this pathway may be considered as a good antibacterial target. The 1-deoxy-D-xylulose-5-phosphate (DOXP) synthetase is a promising enzyme. The inhibition of this enzyme will block the key step for essential metabolites (Walsh 2003). Another enzyme is the 1-deoxy-D-xylulose 5-phosphate reducto-isomerase (DXR). Fosmidomycin is an active antibiotic against many Gram-negative and some Gram-positive bacteria; speci fically inhibits DXR. Recently, two novel inhibitors of DXR are showing high activity similar to fosmidomycin and are significantly active against *E. coli in vitro* (Kuntz et al. 2005). The genetic code of mevalonate pathway enzymes in Gram-positive cocci are of particular interest in the light of the recent development of multidrug-resistant strains of these group of pathogenic bacteria and conesquently may representing some concern for human health and cost effectiveness. Comprehensive study of the six enzymes of the mevalonate pathway should lead

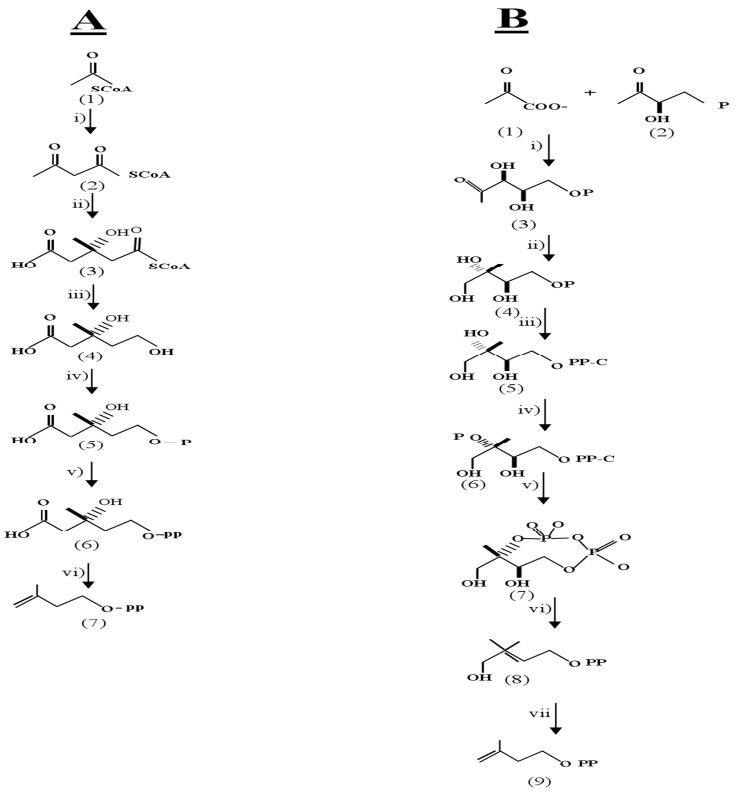

Figure 2. (A) the classical (mevalonate); (B) the non-classical (non-mevalonate) pathways for isoprenoid synthesis in bacteria, modified from Walsh (2003).
(A). (1) Acetyl CoA; i) Acetoacetyl-CoA thiolase; (2) Acetoacetyl-CoA; ii) 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) synthase; (3) HMG-CoA; iii) HMG-CoA reductase (HMGR); (4) Mevalonate; iv) Mevalonate kinase (MK); (5) Mevalonate-5-phosphate; v) Phosphomevalonate kinase (PMK); (6) Mevalonate -5-diphosphate; vi) Diphosphomevalonate decarboxylase (DPMD); (7) Isopentenyl diphosphate (IPP).
(B). (1) Pyruvate; (2) D-glyceraldehyde-3-phosphate; i) 1-deoxy-D-xylulose-5-phosphate synthase (DXS); (3) 1-deoxy-D-xylulose-5-phosphate; ii) 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR); (4) 2C-methyl-D-erythritol-4-phosphate; iii) 4-diphosphocytidyl-2C-methyl-D-erythritol synthase (YgbP, IspD); (5) 4-diphosphocytidyl-2C-methyl-D-erythritol; iv) 4-diphosphocytidyl-2C-methyl-D-erythritol kinase (YchB, IspE); (6) 4-diphosphocytidyl-2C-methyl-D-erythritol-2-phosphate; v) 2C-methyl-D-erythritol 2,4 cyclodiphosphate synthase (YgbB, IspF); (7) 2C-methyl-D-erythritol 2,4 cyclodiphosphate (MEcPP); vi) 4-hydroxy-3-methyl-but-2-enylpyrophosphate (HMB-PP) synthase (GcpE, IspG); (8) = HMB-PP; vii) = HMB-PP reductase (LytB, IspH); (9) = Isopentenyl diphosphate (IPP).

to a synthetic design for inhibitors with therapeutic effect against Gram positive pathogens. It is well known fact that mevalonate pathway is also essential for humans. Therefore, any chemotherapeutic approach targeting this pathway must exploit differences between the bacterial and human enzymes. Comparative genome analysis has identified the complete set of mevalonate pathway enzymes in *Streptococci, Staphylococci* and *Enterococci,* which were found to be heterologous with the coding sequences for comparable enzymes in higher organisms, meaning that there is a difference between isoprenoid metabolism using mevalonate pathway in prokaryotes and in eukaryotes (Voynova et al. 2004). The pathway enzyme HMG-CoA reductase is of particular interest in this context. It exhibits significant differences in the three-dimensional structure and in the sensitivity to inhibition by the active-site inhibitors known as statins (Tabernero et al. 2003). Isopentenyl diphosphate (IPP) isomerase is another important enzyme. Recently, an unrelated IPP isomerase (type II) has been discovered (Laupitz et al.,

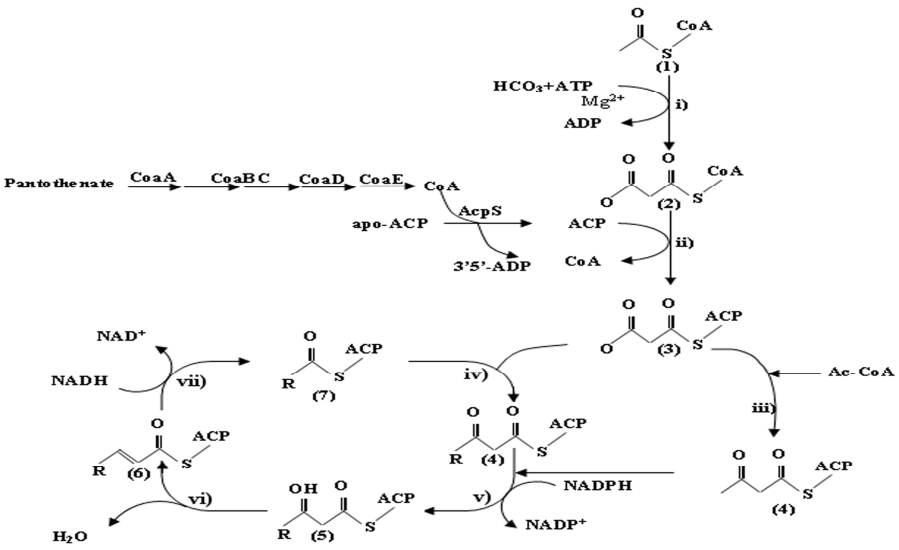

Figure 3. Fatty acid biosynthsis (type II), modified from Zhang et al. (2006).
(1) Acetyl CoA; i) AccABCD (acetyl-CoA carboxylase complex); (2) Malonyl CoA; ii) FabD (Malonyl CoA:ACP transacylase; (3) Malonyl-ACP; iii) FabH (β-ketoacyl-ACP synthase III); (4) β-ketoacyl-ACP; iv) FabB/F (β-ketoacyl-ACP synthase I/II); v) FabG (β-ketoacyl-ACP reductase); (5) β-hydroxyacyl-ACP; vi) FabA/Z (β-hydroxydecanoyl-ACP dehydratase/ β-hydroxyacyl-ACP dehydratase); (6) Trans-2-enoyl-ACP; vii) FabI/K (enoyl-ACP reductase I/II); (7) Acyl-ACP.
CoaA) Pantothenate kinase; CoaBC) Phosphopantothenoylcystein synthetase/decarboxylase complex; CoaD) Phosphopantetheine adenyltransferase; CoaE) Phospho-CoA kinase; AcpS) ACP synthase.

2004).

This type II enzyme invariably accompanies the mevalonate pathway in human pathogens of the Gram-positive coccus group. Since type II isomerase is absent in human, the enzyme appears to be an attractive target for therapy against Gram-positive cocci infection. Currently, the structure of the enzyme is determined by X-ray crystallography this should facilitate the development of cognate inhibitors (Steinbacher et al. 2003). Comparable differences may also recognize other bacterial enzymes of mevalonate pathway from their human counterparts. This will provide a rationale for the potential selectivity of target therapy.

FATTY ACID BIOSYNTHESIS

The fatty acid synthesis (FAS), required for the membrane building phospholipids, in living organisms comprises a repeated cycle of reactions involving the condensation, reduction, dehydration, and subsequent reduction of carbon-carbon bonds. Higher eukaryotes carry out these reactions by a large multifunctional protein (type I pathway). Whereas, in bacteria, plant chloroplasts and *Plasm. falciparum* each reaction is catalyzed by discrete enzymes*;* type II pathway (Figure 3), this allows the prospects of selective inhibition (Zhang et al. 2006). Most of the FAS II enzymes are essential for bacterial viability and consequently they are under investigation as targets for antibacterial drug discovery.

The bacterial acetyl coenzyme A (acetyl-CoA) carboxylase complex (AccABCD) catalyzes the first step in fatty acid synthesis by the interacting AccBC and AccAD complexes and is essential for cell growth. Therefore, it has been strongly proposed as a possible target. The pseudopeptide pyrrolidinedione antibiotics, such as moramide B, have recently been discovered to be specifically targeting the AccAD complex (Freiberg et al. 2004). This natural product together with synthetic analogues shows broad-spectrum antibacterial activity. Further studies of structural variants of the natural product have demonstrated significant *in vivo* effect in a murine model of *Staph. aureus* sepsis, (Freiberg et al. 2006). Nonetheless, *in silico* screening using the AccD5 [(5^{th} subunit of acyl-CoA carboxylases (ACCase)] structure of *Myco. Tuberculosis*, identified one inhibitor (Lin et al. 2006). Such inhibitor may serve as drug lead for the development of new tuberculosis.

An initiation condensing enzyme, FabH, and elongation condensing enzymes, FabF/B, are also essential enzymes in fatty acid (type II pathway) synthesis. They are considered highly attractive new targets for the development of antibacterial and antiparasitic compounds. Two natural products namely cerulenin and thiolactomycin inhibit the condensation enzymes FabH and FabF/B,

with cerulenin showing selectivity for FabF/B, whereas thiolactomycin (TLM) and its analogs inhibit FabH and FabF/B (Dolak et al. 1986). TLM analogs with its increased potency and better pharmacokinetic proper-ties have been sought by a number of groups, but more work is needed ((Douglas et al. 2002; Kim et al. 2006; Kremer et al. 2000; Sakya et al. 2001; Senior et al. 2003; Senior et al. 2004). Many studies were carried out to identify inhibitors of these enzymes, identified compounds with antibacterial activity against MRSA, *B. subtilis, H. influenzae, E. coli* and *Plasm. falciparum* (He et al. 2004; He and Reynolds 2002; Prigge et al. 2003; Young et al. 2006).

The reduction enzyme FabG, is widely expressed and only a single isoform is known in bacteria. These features suggest that drugs targeting this enzyme would have broad spectrum antibacterial activity, but more work is needed (Zhang and Rock 2004).

FabI, is another essential enzyme which is responsible for performing the last step in the fatty acid synthesis, type II pathway. Triclosan (antiseptic) and isoniazid (the anti-*Myco. tuberculosis* agent) are two marketed antibacterial agents that target FabI enzyme and its mode of action is well documented as antibacterial target (Parikh et al. 2002). Moreover, optimization studies in the imidazole series of synthetic 1,4- disubstituted imidazoles reported to be low-micromolar inhibitors of FabI, led to a 16-fold improvement in antibacterial activity and a five fold improvement in potency against the enzyme (Heerding et al. 2001). These clear successes in targeting the FabI component and the determinant role of FabI in completing each cycle of elongation make this enzyme as one of the most attractive antibacterial targets.

It is well known that FabI is not the only enoyl-ACP reductase in bacteria. Access to and analysis of key bacterial genomes demonstrated that an alternative triclosan-resistant enoyl-ACP reductase, FabK, having no similarity to the prototype FabI; is present in several important clinical pathogens. Both FabI and FabK have been found in pathogens such as *Ent. faecalis* and *Ps. aeruginosa* (Heath and Rock 2000). Accordingly, FabI represents a selective antibacterial target for those pathogens in which its essential such as *Staph. aureus* and *E. coli*. It is worth mentioning that, FabI inhibitors are extremely potent against multidrug-resistant *Staph. aureus* and clinically useful drugs are most likely to come up from this line of development in the near future (Ling etal., 2004; Moir, 2005; Payne et al., 2002; Seefeld et al., 2003). Alternatively, a compound that possesses inhibittory potency against both FabK and FabI would be expected to possess a far broader spectrum of antibacterial activity.

The *Myco. tuberculosis* homolog of the *E. coli* enoyl-ACP reductase gene (*fabI* in *E. coli)* is *inhA,* with 36% identity. The enzyme InhA has been validated as an anti-mycobacterial target. High-throughput screening of a structurally diverse library of compounds reported indole-5-amides, 4-aryl-substituted piperazines, and various pyrazole derivatives to provide useful core templates displaying good InhA inhibition. A second more focused library yielded more potent inhibitors with observed good activities versus *Myco. tuberculosis* and *Plasm. Falciparum,* while having no effect against six other common infectious agents (Ballell et al. 2005).

Acyl carrier protein synthase (AcpS) catalyzes the transfer of the 4'-phosphopantetheinyl group from the coenzyme A, to a serine residue in acyl carrier protein (ACP), thereby activating ACP, an important step in cell wall biosynthesis (Joseph-McCarthy et al. 2005). ACP, the acyl group carrier in type II fatty acid synthesis has attracted attention as a target for drug development.

Inhibitors strategies can target AcpS, inactivate ACP, or block CoA biosynthesis.

AcpS is in many cases is essential for bacteria. One of the encouraging findings is the isolation of a natural product that inhibits AcpS with antibacterial activity against *Staph. aureus* (Chu et al. 2003). The high resolution structures of several AcpS proteins are known and structure-based design has been employed to identify a class of anthranilic acid inhibitors. Opportunities for synthetic modification of this group of inhibitors have also been identified (Joseph-McCarthy et al. 2005).

In most bacteria, CoA is synthesized from pantothenic acid (vitamin B_5) in series of 5 steps and phosphorylation of pantothenate by pantothenate kinase (PanK, CoaA) is the first reaction. This pathway also exists in eukaryotes. However, in most cases there is no sequence homology between the prokaryotic and eukaryotic CoA biosynthetic enzymes (Liu et al. 2006). Moreover, these enzymes are essentially required for bacterial survival and/or virulence. Therefore, there is a potential for having highly specific inhibitors of bacterial CoA enzymes. Development of these inhibitors targeting these enzymes is being actively pursued. Among these, the (N-substituted pantothenamides) have shown the greatest promise as growth inhibitors of both *E. coli* and *Staph. aureus*. This is most likely because of their incorporation into multiple acyl carrier proteins (Choudhry et al. 2003; Leonardi et al. 2005; Strauss and Begley 2002; Virga et al. 2006; Zhang et al. 2004; Zhao et al. 2003). Although some problems are encountered regarding the activity of pantothenamides as antibacterial agents, the pantothenamides characterized to date are very effective against drug-resistant *Staph. aureus* and have the potential to be developed further (Zhang et al. 2006).

Furthermore, CoaD, has recently been widely accepted as a unique target for drug discovery. Detailed bioinformatic analysis of the CoA pathway shows that only a single CoaD isozyme is present in bacteria, and its sequence predicts that it is distinctly different from the multifunctional mammalian protein that carries out the same reaction (Gerdes et al. 2002). Up to date, there is only a single publication reporting CoaD inhibitors (Zhao et al. 2003), but the attractiveness of the target and the

availability of high quality x-ray structures (Izard 2002; 2003) suggest that CoaD inhibitors should receive greater attention.

Conclusions

The rising tide of multidrug-resistant pathogens ensures that the search for new antibiotics showing no co-resistance to existing drugs that target well-known pathways, will continue. Many bacterial components appear as promising new targets for progression through the antibiotic drug discovery. Coupled with the increased diversity and number of compounds that have been screened against these validated targets, new antibiotic leads are being identified. Antibacterial activities of such leads include both broad and narrow spectrum mechanisms. The current challenge is to optimize these early-stage discovery leads to make them suitable for clinical evaluation. However, it should be emphasized that the potential of broad spectrum mode of action still carries the risk of inhibiting "beneficial" bacteria and normal flora, which may create some clinical problems. Therefore, it is worth mentioning that all efforts should be directed towards appropriate use of such antibacterial agents.

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