Strategies for identifying new antimicrobial targets

Introduction

The increasing number of human pathogens resistant to existing classes of antibiotics has made the identification of novel antimicrobial agents a matter of urgency. The fact that no new class of broad spectrum antibiotics has been developed since the 1970s highlights the symptom that the majority of current antibiotics have been targeted against a limited number of bacterial cellular processes. These can be generally classed as inhibitors targeted against cell wall biosynthesis, DNA/RNA replication, protein synthesis or specific biochemical bottlenecks (Table 1).

In many cases, the development of these antibiotics was the result of detailed research concerning the target cellular mechanism. In some cases their discovery was serendipitous (e.g. penicillin).

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New strategies reveal new targets

Recent strategies for the identification of antimicrobial targets also rely on a mechanistic understanding of essential biosynthetic pathways in bacteria. However, the explosion in bacterial genomics has revolutionised the search for target mechanisms [1]. For instance, the genomes of pathogenic organisms may be searched for specific genes encoding new target molecules.

A significant advance involves the role of genetic mutants for the identification of 'essential' genes as an important step in confirming the potential antimicrobial target is critical for bacterial survival. The genetic mutants produced can then be used for subsequent biosynthetic pathway characterisation coupled with the overproduction of the target molecule in host strains for subsequent characterisation such as detailed structural analysis of substrate binding by x-ray crystallography [2]. This has then allowed the computer aided design of specific drugs [3, 4].

An early target for antimicrobial development during the 1960s was the pathway of fatty acid biosynthesis in bacteria, essential for cell membrane construction and adaptation to environmental changes. While the mechanism of fatty acid biosynthesis is essentially the same in all organisms, in bacteria it is generally undertaken by a series of monofunctional proteins forming a loosely associated fatty acid synthase (FAS) system (termed type II). In eukaryotes FAS is organised as a multidomain enzyme complex (termed a type I system) [5].

Hence, while functionally similar, type II FAS systems are enzymatically distinct from eukaryotic systems and offered a potential target for broad spectrum antimicrobials. However the characterisation of bacterial fatty acid biosynthesis was complex and proceeded at a modest pace [6]. In addition, the research conducted was almost exclusively on *Escherichia coli* (with limited application to other human pathogens) and very few natural inhibitors of fatty acid biosynthesis were found, for a number of reasons [7]. A small number of FAS antimicrobials have been

Table 1. **Examples of target mechanisms for antibiotic development, representatives of developed drugs for each mechanism and details of their molecular target of action.**

Target mechanism	Example antibiotic type	Molecular target
DNA Synthesis	Quinolones	DNA Gyrase
RNA Synthesis	Rifamycins	RNA Polymerase
Protein Synthesis	Chloramphenol Tetracyclines	50S Ribosome Subunit 30S Ribosome Subunit
Cell Wall	Penicillins	Carboxy- and Trans-peptidases
Biochemical	Trimethoprim	Dihydrofolate reductase
Fatty Acid Synthesis	Isoniazid & Triclosan Rhiolactomycin	Enoyl-ACP Reductase beta-Ketoacyl-ACP Synthase I & II

successful when targeted against unusual fatty acids known to be essential for cell survival.

Isoniazid and tuberculosis

A case in point is the antituberculosis drug isoniazid (INH). While first discovered by random screening in 1952, INH remains an important clinical treatment for tuberculosis [8]. Studies in the early 1970s indicated that the mode of action of INH was related to the inhibition of mycolic acid biosynthesis, an important cell membrane component [9]. However, it was not until the application of recent genomic approaches that the full mechanism of INH inhibition was elucidated [10].

It was demonstrated that INH inhibited the fabI protein (enoyl-ACP reductase; encoded by *fab I)* component of type II FAS (Figure 1), leading to the cessation of mycolic acid synthesis. The significance of this advance has been to open new avenues of understanding regarding the development of INH resistance in *Mycobacterium tuberculosis* and the potential to redesign INH-like antimicrobials to overcome the increasing problems of INH resistance [11].

Triclosan and malaria

A further example is offered by the drug triclosan which has been widely used for decades as an antibacterial additive in consumer products. Its relegation to usage in this way was based on early studies that reported its mode of action as cell lysis and hence classified it as a

Figure 1. Schematic pathway of the fatty acid biosynthesis cycle in *Escherichia coli* denoting the major fatty acid products. The reactions catalysed by the antibiotic targets fabH and fabI are highlighted in bold.
fabB, product of the *fabB* gene (ß-ketoacyl-ACP synthase I)
fabF, product of the *fabF* gene (ß-ketoacyl-ACP synthase II)
fabH, product of the *fabH* gene (ß-ketoacyl-ACP synthase III)
fabI, product of the *fabI* gene (enoyl-ACP reductase)
fabG, product of the fabG gene (ß-ketoacyl-ACP reductase)
fabA, fabZ, products of the *fabA* and *fabZ* genes respectively (hydroxydecanoyl-ACP dehydrases)
ACP, acyl carrier protein; CoA, coenzyme A.

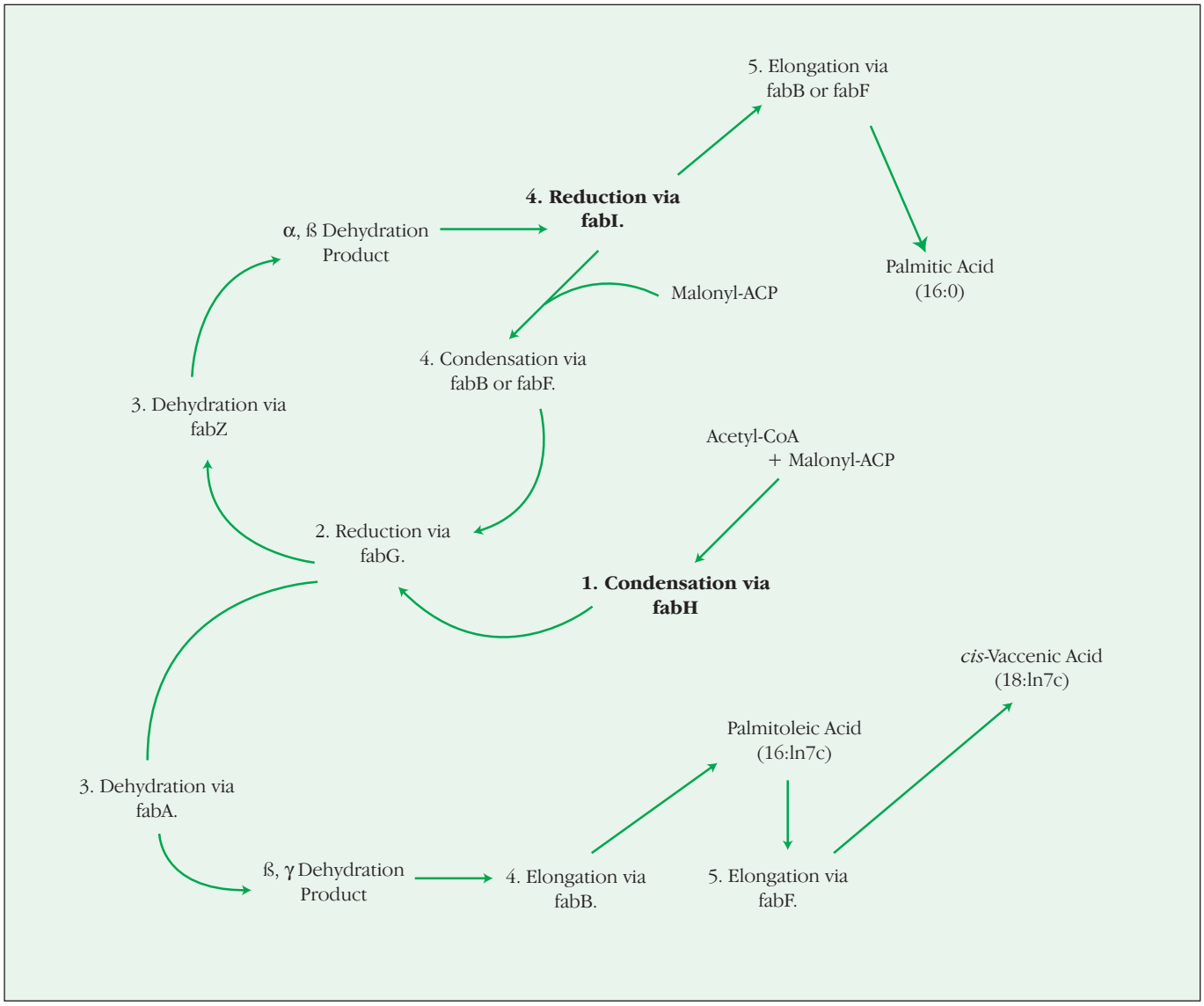
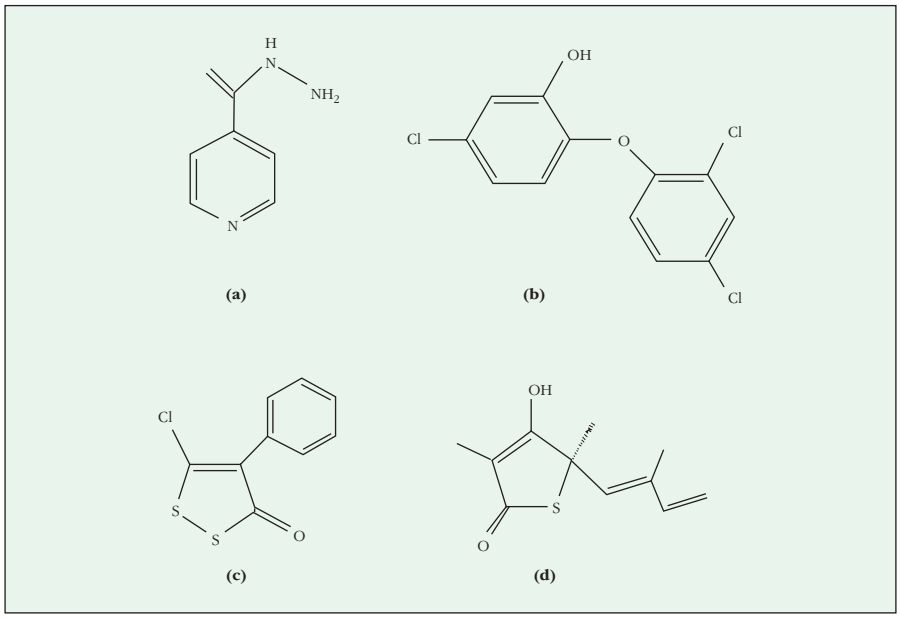


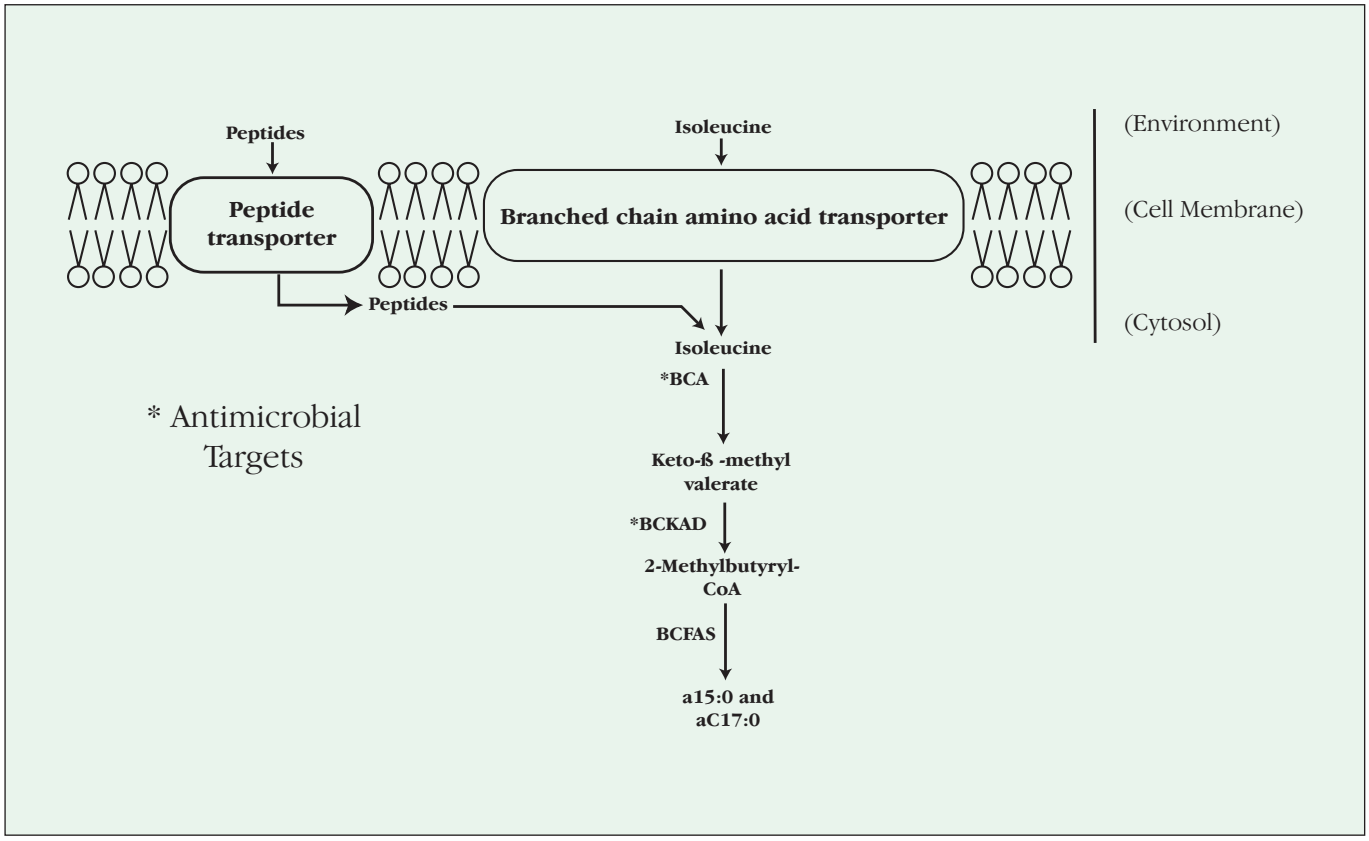
Figure 2. Structures of some fatty acid biosynthetic enzyme inhibitors.
(a) Isoniazid, a front-line antitubercule drug.
(b) Triclosan, formerly a common antibacterial component of consumer products, now recognised as a potent inhibitor of the malaria parasite.
(c) An experimentally designed inhibitor of fabH based on the molecular structure of the purified enzyme.
(d) Thiolactomycin, a well known inhibitor of fabB and fabF enzymes.



nonspecific membrane permeablising agent [12]. Again, recent genomic investigations have turned this viewpoint on its head, revealing triclosan is also a potent inhibitor of the fabI protein [13,14] (Figure 1). The resultant defects in fatty acid provision for membrane formation lead to the observed cell lysis. The significance of this discovery has been far-reaching, identifying triclosan as a basis for a potential class of broad spectrum antibiotics [14].

Of further impact, and one of those strange convergences, was the realisation that the causative agent of malaria, the sporozoan intracellular parasite *Plasmodium falciparum*, relied upon a type II FAS system for fatty acid biosynthesis which is also potently inhibited by triclosan [15, 16]. Unlike other eukaryotes which utilise a type I FAS, *Plasmodium spp.* house a non-photosynthetic plastid organelle

Figure 3. Schematic pathway of branched-chain amino acid transport and the conversion of isoleucine to the fatty acid *anteiso*-$C_{15:0}$ (a15:0) essential for low temperature survival of *Listeria monocytogenes*. The enzymes branched-chain aminotransferase (BCA; encoded by *ivlE)* and branched-chain ketoacid decarboxylase (BCKAD; encoded by *bmfB)* sequentially deaminate and decarboxylate isoleucine to provide the branched-chain molecule for the branched-chain fatty acid synthase (BCFAS) system. BCA and BCKAD represent potential antimicrobial targets in *L. monocytogenes*.



containing its fatty acid biosynthetic genes which are of bacterial origin; a type II FAS [17]. Triclosan is now set to become a benchmark for the development of new antimalarial drugs [18].

Targets on the horizon

Such examples of the new application of genomic approaches to the study of fatty acid biosynthesis have re-opened international interest in this area as a target for novel antimicrobials [7, 19]. In addition to inhibitors of fabI, a further target in which future interest will expand is the initial condensing enzyme of fatty acid biosynthesis, the fabH protein (ßketoacyl-ACP synthase III; encoded by *fabH)* (Figure 1).

The existence of fabH escaped discovery until the early 1990s as it utilised acetyl-CoA rather than acetyl-ACP as a substrate. Prior to this, *in vitro* investigations of fatty acid biosynthesis had 'traditionally' utilised only an acetyl-ACP substrate, subsequently not detecting the activity of fabH and measuring only the background condensation activity of fabB and fabF (Figure 1).

However, it is now established that fabH is the primary initiator of the first condensation reaction *in vivo* and appears essential for cell survival [7]. This is highlighted by the fact that no viable *fabH*-minus mutants have been constructed, despite great efforts to do so [20]. In particular, fabH inhibitors are considered to have significant promise against multi-drug resistant organisms such as methicillin-resistant *Staphylococcus aureus.* To date, several fabH inhibitors (Figure 2) have been successfully trialed against *S. aureus* [20].

Work in our laboratory has also focussed on the use of targeted mutagenesis to eliminate candidate gene products not essential for the survival of the organism. This work has concentrated on the pathogen *Listeria monocytogenes,* responsible for outbreaks of foodborne disease characterised by high morbidity and mortality.

A key determinant in the cause of disease outbreaks caused by *L. monocytogenes* is its ability to grow to high numbers under refrigeration temperatures during food storage. Early studies using transposon mutagenesis [21] have established that *L. monocytogenes* requires the ability to produce a particular branched-chain fatty acid, *anteiso*-$C_{15:0}$, to survive and grow at low temperature (Figure 3).

Bacteria that biosynthesise branched-chain fatty acids (BCFA) do so by a particular enzymatic adaptation, allowing them to utilise branched-chain-CoA molecules instead of acetyl-CoA (Figure 1). For *L. monocytogenes* the bioinformatics of this process is well advanced (Figure 3). The biosynthesis of the critical component *anteiso*-$C_{15:0}$ occurs from a branched-chain-CoA molecule provided by the catabolism (deamination and decarboxylation) of isoleucine.

As *L. monocytogenes* is auxotrophic for branched-chain amino acids, isoleucine must be provided by transport into the cell. Hence the transport of isoleucine, its deamination and decarboxylation are critical steps in the production of a membrane component essential for *L. monocytogenes* to survive at low temperature and represent potential new targets for drug development. Our existing knowledge of the lipid biochemistry of bacteria, together with advances in bioinformatics and the identification of the genes encoding pathway enzymes, has yielded new potential targets for drug development.

Conclusion

This article has concentrated on examples of new drug targets from our expanding knowledge of bacterial cell membrane function and fatty acid biosynthesis due to the recent advances in this area concerning two important diseases, tuberculosis and malaria. Hopefully the reader has warmed to these exciting stories and potential for future drug discoveries.

However, many further biochemical areas are still to be exploited for drug development and will continue to expand as genomic approaches identify further essential cellular mechanisms. Other areas of particular promise include two component signal transduction systems, cell division targets, aromatic amino acid pathways and isoprenoid biosynthesis [1].

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