NOVEL TARGETS FOR ANTIMICROBIALS

Vaishali Ravindra Undale, Suchita Gupta, kedar lakhadive
Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune, Maharashtra

Corresponding Author Information
Vaishali Ravindra Undale
vaishaliundale@dypvp.edu.in
9372435355
orcid.org/0000-0003-3837-7471
18.07.2019
21.04.2020

ABSTRACT:
Antimicrobial resistance (AMR) is the phenomenon developed by microorganism on exposure to antimicrobial agents, making them unresponsive. Development of microbial confrontation is a severe rising risk to global community well-being as treatment in addition, management of such resistant microbial infections is difficult and challenging. The situation requires action across all government sectors and society. The change in the molecular target on which antimicrobial drugs act is one of the key mechanisms behind AMR. One of the approaches to battle with AMR can be exploring newer molecular targets in microbes and discovering new molecules accordingly.

There are various examples of novel targets similar to biosynthesis of cell wall, biosynthesis of aromatic amino acid, cell disunion, biosynthesis of fatty acid, and isoprenoid biosynthesis and tRNA synthetases. Fatty acid biosynthesis (FAB) and their enzymes among all the above is the more appealing target for the advancement of new antimicrobial agents. Number of promising inhibitors have been developed for bacterial fatty acid synthesis and also few of them are clinically used. Some of these potential inhibitors are found to be used in development of new antibacterial as a lead compound and have been discovered from high throughput screening processes like Platencimycin and their analogue, Platencin. The review majorly encompasses bacterial FAB in Type II Fatty acid synthesis (FAS) system and potential inhibitors with respective targets of novel antibacterial.

Keywords: Drug resistance, antibacterial activities, FAS system, thiolactomycin, platencimycin, platencin

INTRODUCTION:
The agents which destroy or prevent the growth of microbes are termed as antimicrobials. Antimicrobials are classified by various ways depending on mechanism or type of action as bacteriostatic and or bactericidal (Mc Dermott PF, et.al, 2003). Antimicrobial have various therapeutic implications like endocarditis, gingivitis, prophylactic or suppressive therapy as presurgical antimicrobials, prophylaxis in immunocompromised patients with HIV/AIDS, traumatic injuries, neutropenia etc. (Kang CI, et.al, 2013). Antimicrobial agents like
antibacterial mark vital element of microbial breakdown, thus limiting the bacteria. For example, the β-lactams, such as penicillin or cephalosporins, inhibit cell-wall synthesis etc. (Kohanski MA, et al, 2010).

Antimicrobial resistance is the capability of micro-organisms to inhibit antimicrobial substances from working against it which results that the standard treatment is ineffective; infections may spread and persist for longer period. (Global Antibiotic Resistance Partnership (GARP), 2011). As we see the current scenario of antibiotic use it will increase steadily in recent years and therefore antibacterial played a crucial part in fatality and mournfulness in the nation (World Health Organization, 2011). Hence resistance is the major concern now days as resistant organism may lead to treatment failure, widespread in the community at increasing rate, undetected low level of resistance, added burden to healthcare cost, selection pressure of right antibiotic, threatening return to pre-antibiotic era. (Ventola CL, 2015).

Amongst various types of molecular mechanisms involved in development of AMR, alteration in the molecular targets on which antimicrobials act is one of key mechanism. The researchers in drug discovery process had tried to develop new antimicrobials by altering the functional molecular groups in parent moiety thereby modulating potency and safety of newly designed molecules. This has helped to overcome the AMR to some extent. But still the problem development of AMR persists as microbes are smart enough to mutate and change the molecular mechanism for survival.

Prevention and control strategies will require overcoming from the situation by feasible way called as the evolution of inhibitors of resistant enzymes. These inhibitors can be delivered as a co-drug with the antibiotics, thereby prevent AMR and sustain the antimicrobial activity of the drugs. Another approach to overcome the resistance is bacterial FAS, which is a necessary goal for antibacterial discovery. The organizational difference of the conserved enzymes and the existence of multiple forms of enzymes mobilizing the similar reactions during the path and create the bacterial FAS which is active against selected target preferably than the established multiple target. Likewise, bacterial FAS inhibitors are found to be narrow-targeting, rather than broad-targeting like old mono-therapeutic and broad-spectrum antibiotics. The narrow-targeting FAS inhibitors found to be fast-developing, resistance is target-based which made it a significant application for antibiotic development (Yao J, et al, 2017). Despite of advanced antimicrobials with novel targets proceed to be recognized and contributed to the continuing struggle against AMR the intimidation to send back humanity to a circumstance commensurate to the pre-antibiotic era. Examples of definite target in the areas of cell wall biosynthesis, aromatic amino acid biosynthesis, cell division, two component signal transduction, fatty acid biosynthesis, isoprenoid biosynthesis and tRNA synthetases demonstrates according to characteristics of the above effectiveness were strike in drug finding and depiction of new antibacterial targets (Maddocks SE, 2016).

The review discusses about the novel targets for emerging antimicrobial treatments, featuring important research on which potential to continue to successfully treat bacterial infection relies.

ANTIMICROBIAL: CLASSIFICATION AND ITS TARGETS

According to their application and spectrum of activity antimicrobials are classified as microbicides that kill micro-organisms, whereas bacteriostatic agents inhibit the growth of pathogens and allow the leucocytes and other defense mechanism of the host to confront with immobile invader. The microbicides may show selective toxicity depending on their spectrum of activity. They may act as viricides (killing viruses), bacteriocidic (killing bacteria), algicides (killing algae) or fungicides (killing fungi).
Categorizations of Antimicrobial agents:
They are mainly classified into three categories:-
- Antibiotics and chemically derived chemotherapeutic agents:
  - Beta lactam antibiotics, nucleoside antibiotics, aminoglycosides
- Non-antibiotic chemotherapeutic agents:
  - Disinfectants, antiseptics and preservatives
- Immunological products:
  - Vaccines, polyclonal antibodies.

Antimicrobial targets:
Antimicrobial drugs interfere chemically with the molecular components of microorganisms that play crucial physiological role in them. They may act as a bactericidal or bacteriostatic. There are discrete ways by which these agents exert their antimicrobial activity such as inhibition protein synthesis, DNA gyrase inhibition, affecting cell wall synthesis or cell membrane function and folic acid synthesis etc. Figure 1 shows the targets with drugs acting on the targets.

Figure1: Molecular targets with Drugs action on the targets

Some of the established antimicrobial targets and the drugs with their pathway of action are briefed in table no. 1.

Table 1: Antimicrobial Targets with the drugs.

<table>
<thead>
<tr>
<th>Primary target</th>
<th>Drug</th>
<th>Drug type</th>
<th>Origin</th>
<th>Pathway affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 2 topoiso-merase (DNA gyrase), Type IV topoiso-merase</td>
<td>Nalidixic acid, ciprofloxacin, levofloxacin</td>
<td>DNA synthesis inhibitors</td>
<td>Synthetic</td>
<td>Deoxyribonucleic acid (DNA) replication, division of cell, energy generation, citric acid cycle, ROS formation.</td>
</tr>
<tr>
<td>DNA-dependent RNA polymerase</td>
<td>Rifampicin</td>
<td>RNA synthesis Inhibitor</td>
<td>Natural and semisynthetic</td>
<td>RNA transcription, DNA Replication</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------------</td>
<td>-------------------------</td>
<td>---------------------------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>Penicillin-binding Proteins</td>
<td>Penicillin, cephalosporins</td>
<td>Inhibitors of cell wall synthesis (β-lactams)</td>
<td>Natural and semi-synthetic forms of carbonyl lactam ring-containing azetidinone molecules (from P. notatum)</td>
<td>Synthesis of cell wall, cell division, enzyme that breakdown the biological components called as autolysin activity, ROS formation, and envelope</td>
</tr>
<tr>
<td>Peptidoglycan units (terminal d-Ala-d-Ala dipeptide)</td>
<td>Vancomycin</td>
<td>cell wall synthesis inhibitors (Glycopeptides)</td>
<td>amino sugar-linked peptide chains which is natural and semi-synthetic (for glycopeptides)</td>
<td>Cell wall synthesis, transglycosylation, transpeptidation</td>
</tr>
<tr>
<td>Cell membrane</td>
<td>Daptomycin and Polymyxin B</td>
<td>Disruption of cell membrane function</td>
<td>fatty acid-linked with peptide chains which is natural and semi-synthetic obtained from species S. roseosporus and B. polymyxa</td>
<td>Cell wall synthesis and envelope two-component systems</td>
</tr>
<tr>
<td>30S ribosomes</td>
<td>Tetracycline and aminoglycosides</td>
<td>Protein synthesis inhibitors</td>
<td>It is formed of four-ringed Polyketides and are natural and semi-synthetic originated from species S. aureofaciens</td>
<td>Protein translation occurred through inhibition of aminoacyl tRNA binding to ribosome.</td>
</tr>
</tbody>
</table>
50S ribosomes | Erythromycin and azithromycin | Protein synthesis inhibitors | Natural and semi-synthetic forms of 14- and 16-membered lactone rings (from *S. erythraea* and *S. ambofaciens*) | Inhibition of elongation and translocation process protein translation happened and by depletion of free tRNA.

Simultaneous binding to bacterial dihydrofolate reductase | Sulfonamide and Trimethoprim | Nucleoside synthesis inhibition | synthetic | Blocks pathways and inhibit folic acid metabolism

**Development of antibiotics:**
In 1930s, the first commercially accessible antibacterial Prontosil (sulfonamide) was introduced by German biochemist Gerhard Domagk. Before this, Alexander Fleming identified the primary antibiotic, Penicillin, in 1928, which is used to control microbial infections. The period after exhibition of penicillin is called as “golden era” of antibiotics. After this, many antibiotics were introduced commercially and /or are useful as medicine even for activities other than the antibiotic activity. Antibiotics are used as enzyme inhibitors, antitumor, immunosuppressive, hypolipidemic and antiparasitic agents, in addition to their applications as antibiotics. [Fair RJ, et.al, 2014].

The research focuses on discovery of new or novel antibiotics and further modifications in existing antibiotics are still in progress. There are several important reasons why the finding and advancement of antibiotics with innovative structural modules are predominantly important, including the expansion of resistant bacteria and other pathogens [Kasanah N, et.al, 2004].

**Development Void:**
Today, very fewer novel antibiotics are seemed to be under development. The consequences are seen worldwide as more and more bacterial infections are becoming hard to treat again. This has led to be unavoidable major health problem today. According to Walsh, “no foremost classes of antimicrobials were presented” between 1962 and 2000 which denotes to a modernization gap. Hence due to this innovation gap majority of pharma companies has been pull back their study from this area [Silver LL, 2011]. The figure 2 illustrates the Gap in the Antibiotic development.

**Figure 2: Era of Antibiotics development**
Newer Targets for Antimicrobial agents:
The fruitful growth of various antimicrobials in the 1930s–1960s (WHO, 2004) established the contented awareness in the therapeutic and methodical societies that microbial infections had been overcome, ensuing in a decreased research in the antimicrobial area. Inappropriately, bacteria altered to their unfavorable novel atmosphere and started surviving even though exposed to the antibiotics. This is a process of gaining resistance that ultimately reduced many of these antimicrobials’ noneffective. Presently, communicable diseases are still increasing and may result into one and only major reasons of death globally (Fischbach MA, et.al, 2009; Walsh C, 2003; Wenzel RP, 2004). To fight against this situation, antimicrobial agents with novel mechanisms of action might be helpful. Though, in the last 7 years out of 11 new antimicrobials that were accepted by the Food and Drug Administration (FDA), out of two of them acts through a different mechanism: linezolid, an oxazolidinone, and daptomycin, a cyclic lipopeptide, mutually they are used to cure the Gram-positive bacterial infections only. Hence there is urgent need of identifying and targeting new targets with novel mechanism that drive a crucial part in the growth of antibiotics dynamic against unaffected pathogens (Lee CS, et.al 2001; Singh U, et.al, 2003; Reck F, et.al, 2005). Table 2 shows difference in single enzyme and multi-enzyme targets on which antibiotics may act.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Single enzyme target</th>
<th>Multi enzyme target</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>MurB to MurF Enzymes as Antibacterial Targets</td>
<td>Single Pharmacophore, Multiple Targets</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a. Dual inhibitors of DNA gyrase and topoisomerase IV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b. Dual inhibitors of Gram-positive DNA polymerases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c. β-Ketoacyl-ACP synthases of FAS II</td>
</tr>
<tr>
<td>2.</td>
<td>Targets of Inhibitors Discovered by Enzyme Screening or Design</td>
<td>Targeting Substrates and Cofactors</td>
</tr>
</tbody>
</table>
3. Single-Enzyme Targets of Novel Inhibitors in Clinical Trials
   a. Peptidyl deformylase
   b. Enoyl-reductases of FAS II
   c. Leucyl tRNA synthetases of Gram-negative organisms
   d. RNA polymerase in *C. difficile*

Hybrid Molecules: Dual Pharmacophore, Multiple Targets

As compared to the sole target approach multi-target concept is thought to provide better resistance inhibition, possibility of generating hybrid molecules that may attack two cellular targets concurrently by fastening distinct inhibitors or pharmacophores together to form a single molecule (Barbachyn MR, et.al, 2008; Bremner J.B, et.al, 2007).

Though there are so many targets as described above, in the review focusses upon β-Ketoacyl-ACP synthases of FAS II which plays a role in microbial fatty acid synthesis. The FAS in microbial cells as following.

**FAS (Fatty acid biosynthesis)**

Beta-ketoacyl-acyl carrier protein synthase is an enzyme that participates in FAS. It take part in the establishment of aceto-acetyl ACP and it is briefed in Figure 3.

**Figure 3. Fatty acid biosynthesis**

The hugely conserved enzyme that is established in practically all lifecycle on earth as

\[
\text{acetyl ACP} + \text{malonyl ACP} \rightarrow \text{aceto-acyl ACP}
\]

Fatty acid synthesis: first step is condensation of acetyl ACP and malonyl ACP.

a domain is fatty acid synthase (FAS) also called as Beta-ketoacyl-ACP synthase [Christensen CE, et.al, 2007]. The enzyme plays a role in the FA elongation series and is tangled in the feedback regulation of the biosynthetic pathway via product inhibition. Two types of FAS enzymes have been identified yet as Type -I and Type -II shown in Figure 4.

**Figure: 4 Types of FAS**
The type I FAS, also called associated form, is present in fungi and animals and is multifunctional protein encrypted by a one gene or two genes. Type II FAS, also called dissociated type, is found in prokaryotes and plant plastids and consists of separate enzymes.

**Structure:**
Beta-ketoacyl synthase (KAS) consists of two protein domains. Between the N- and C-terminal domains, the active site is situated. Most of the structures participating in dimer formation constitute the N-terminal domain and also in the active site cysteine. The substrate restraining and catalysis promoted by residues from both domains [Witkowski A, et al., 2007].

KAS is a domain on type I FAS, which is a wide enzyme compound that has diversified sections to catalyze various distinct reactions [Beld J, et al., 2014]. Further experimentation of KAS I and II of E. coli disclosed that together are homo-dimeric, but KAS II is somewhat bigger. Still, both are tangled in FA metabolism; they have totally different primary structure [Garwin JL, et al., 1980]. In KAS II, every subunit constitutes a penta-stranded β pleated sheet enclosed by several α helices.

The active positions are almost adjacent, which is around 25 Å away, and comprise of a generally aqua phobic pocket [Huang W, et al., 1998]. The occurrence of “fatty acid transport channels” inside the KAS domain that happen into one of many “fatty acid cavities”, which basically acts as the active site [Cui W, et al., 1861].

**Mechanism:**
In the reaction sequence, the first step is organized by the KAS category of enzymes that is involved in a nucleophilic substitution by the active-site cysteine residue on the substrate carbonyl. The complete reaction is mediated by the KAS and it splits into distinct valuable phases:
(a) Beginning with ACP, the acyl moiety is transferred to thioester form (but in situation of KAS III it forms acyl-CoA), to the active-site cysteine residue of the KAS.
(b) To produce a reactive carbanion, binding and replacement of carboxyl group with hydrogen atom of the chain widen moiety is required and
(c) A new C-C bond of the anion in which carbon is trivalent formed by nucleophilic attack on carbonyl carbon of the acyl moiety [Witkowski A, et al., 2002].

Both type I and II FAS system can essentially prolong the small chain-length pioneers to the 14-carbon form, additionally the extension of C16 to C18 ACP, is effectively mobilized by KAS II or FAS II. Hence miscellaneous forms of KAS were identified specifically and plays a crucial part in deciding the structure of the products formed in the particular multi-step’s enzyme catalyzed process [Campbell JW, et al., 2001]. These meaningful characteristics in functioning and arrangement of associated and dissociated form differentiated the type II FAS or
dissociated enzymes, as promising goal in the evolution of innovative antibacterial and antiparasitic agents which shows nominal adverse effects in humans. Considering the limitations, words and pages to be published, only KAS III (FabH) are discussed here in detail [Zhang H, et.al, 201]).

**KASIII (FabH)**

In cells, fatty acids playing a major role in cell membrane formation and fatty acid biosynthetic pathway indicated important targets in drug discovery. FAS called as multifunctional enzyme complex system that helps in regulating and processing of fatty acid synthesis [Lai CY, et.al, 2003]. KAS III is the functional enzymes which initiates the fatty acid synthesis, thus performed a most prominent part in bacterial fatty acid biosynthesis and called as condensing enzyme [Khandekar SS, et.al, 2003; Health RJ et.al, 1996; Health RJ, et.al 2002; Tsay JT, et.al, 1992]. It is used to mobilize the primary elongation reaction called as Claisen-Schmidt condensation of associated type in microorganisms, plant plastids etc. The FabB and FabF other bacterial condensing enzymes that, work in future in the series, actually showed difference from FabH in a way that they use acyl-ACP in spite of acetyl-CoA as the primer for further condensation reactions shown in Figure 5.

As a result, FabH seems to show a crucial part in the microbial FAB cycle because no additional enzyme in the pathway is found to be capable to carry out valuable reaction [Ali A, et.al, 2006]. Furthermore, the essential part and 3D assembly of the protein is tremendously protective to the several Gram-positive and Gram-negative bacteria and hence, its inhibitors commit to be dynamic antimicrobials with wide ranging action. [Puupponen-Pimiä, R, et.al, 2001]

**Figure 5: KASIII regulation**

**STRUCTURAL EXPLORATION OF β-Ketoacyl-acyl carrier protein (ACP) synthase III**

Fatty acid biosynthesis (FAB) is introduced via KAS [Veyron-Churlet, R, et.al, 2004; Health RJ, 1996]. By various reduction and dehydration steps, FASII cycle helps in prolongation of FA. Between the associated FAS II enzymes, the condensing protein, KAS, is an essential target for novel antibacterial drug design [Veyron-Churlet, R, et.al, 2004; Khandekar SS, et.al, 2003]. The three classes of KAS (I, II, and III) have been established in which KAS I is encrypted by FabB and KAS II by FabF and these are elongation condensing enzymes that prefer acyl-ACP as a primer to abridge with malonyl-ACP [White, S. W, et.al, 2005]. The function and structures of these two enzymes are same, while significant distinct mechanisms are shown by KAS III (encrypt by FabH). KAS III regulates primary condensation of acetyl-CoA with malonyl-ACP, and participates as important part of bacterial FAB [Khandekar SS, et.al, 2003; Health RJ, et.al, 2002]. The crystal structure of KAS I, II & III shows that they all are dimers having α-β-α-β-α pleat and their active sites are distinguished by existence of a Cys-His-His triad, in KAS I and II, and a Cys-
His-Asn catalytic triad in KAS III. [Khandekar SS, et.al, 2003] The concealed active sites of FabH comprised of Cys112, His244 and Asn274. Additionally, Asn343 established near to Asn274, and it was recommended to participate as a major part in the catalysis., Cys112 in the acetyl-CoA/FabH complex structure was found to be acetylated, showing clear proof that, His244 and Asn274 are demanding for decarboxylation and condensation reactions while Cys112 is the catalytical residue [Choi KH, et.al, 2000].

**Type II FAS reaction Pathway: Activation, Elongation & control**

FAB takes place in cytosol not in mitochondria. It usually contains a unit called Acyl-carrier protein (ACP) rather than Coenzyme A (CoA) with reducing agent NADPH instead of NAD/FAD.

It consists of various steps/stages--
- Transport
- Activation & integrating Mechanism
  (i) Acetyl CoA carboxylase (ACC)
  (ii) FAS complex
    - Prolongation of palmitate
  (i)Condensation
  (ii)Reduction
  (iii)Dehydration
  (iv)Reduction
- Desaturation of FA
- Regulation of FAB

**A. From mitochondria to cytosol Passage of acetyl CoA**

In the mitochondria the acetyl CoA is synthesized by two ways shown in Figure 6-
- By β-oxidation of FA
- By united action of pyruvate dehydrogenase and dihydrolipoyl transacetylase

*Figure 6: Synthesis of acetyl CoA in mitochondria*

Acetyl CoA is moved out from the mitochondria to cytosol and initiate the fatty acid synthesis and this process is takes placed by tricarboxylic carrier protein system in the inner mitochondrial membrane which pushed out the citrate is shown in Figure 7.
Sources of NADPH for Fatty acid synthesis

- For each particle of Acetyl CoA, one fragment of NADPH is generated that is relocated from mitochondria to the cytosol.
- NADPH molecules come from the Pentose Phosphate Pathway

B. Activation & synthesis Mechanism

(i) ACC
ACC mediates the first limiting step of FAB and the enzyme biotin which adds CO2, a carboxyl group to methyl end of acetyl CoA. The endothermic reaction described in Figure 8.

(ii) FAS complex
The seven different reactions catalyzed by multifunctional enzyme by which 2 carbon moiety are linked together from malonyl-CoA and finally formed the palmitoyl-CoA. During the complete synthesis process the formation of Palmitate from acetyl CoA it involves total of 7 ATPs and 14 NADPHs.
C. Elongation of Palmitate
The precursors needed for elongation reaction gets loaded via thioester derivatives and included following steps with their respective enzymes like condensation, reduction, dehydration and reduction shown in Figure 9.

- From malonyl-ACP there is an inclusion of an acetyl group between the thioester bond of the acetyl-ACP molecule and this reaction is takes place by KAS and this enzyme known as condensing enzyme.

- And when there is a reduction of the Beta-keto group to a Beta-hydroxyl group in coordination with NADPH then this step is mobilized by Beta-keto-ACP reductase.
- During third step of dehydration the reaction is catalyzed by Beta-hydroxy acyl-ACP dehydrase and result into dehydration between the alpha and Beta carbons.
- And lastly there is a reduction by NADPH of the trans double bond and reaction is accomplished by enoyl-ACP reductase.

Various other enzymes like elongases that help to elongates the palmitate to produce many other fatty acids along the reaction and the origin of this enzyme in mitochondria and endoplasmic reticulum.

**Figure 9: Elongation Chain Reaction**

D. Desaturation of fatty acids
Fatty acids get desaturated with the help of enzymes fatty acyl-CoA desaturase which is terminal desaturases that produce unsaturated fatty acids.

E. Regulator of FAB
During the FA metabolism of cells, like other metabolic pathways there should have an appropriate control mechanism to meet the energy needs. As fatty acid synthesis can be controlled by either of two ways i.e. partly by long term regulatory mechanism in which governing the rate of reaction by which any protein incorporates or separate and hence controlled the quantity of enzymes responsible for this and partly by short term regulatory mechanism like enzymes modification.

**Various Inhibitors of β-KETOACYL ACP SYNTHASES**

The highly conserved, essential and important pathway for survival is Type II bacterial fatty acid synthesis [Magnuson K, et.al, 1993]. Thus, the enzymes required during this procedure are found to be most fruitful goal to the advancement of innovative therapeutics. Additionally, to this there are various inhibitors which are used to treat various untreatable or resistant infections like FabI inhibitors that are recently in preclinical and clinical development against methicillin- resistant S. aureus (MRSA) [Escaich S, 2011; Flamm RK, 2015; Park HS, et.al, 2007]. Along with this there is another appropriate target is FASII β-keto (ACP)synthase enzyme. These enzymes show a foremost part in the beginning and continuation phases of FASII pathway, by mobilizing a decarboxylative Claisen condensation reaction and bacteria has 3 types of KAS enzymes: FabB, FabF and FabH. As FabB and FabF which is also called as KAS I and KAS II which assemble the condensation of malonyl-ACP and acyl-ACP in the elongation cycle and that consist of Cys-His-His catalytic triad, while FabH (KASIII) contains a Cys-His-Asn triad, and is important for the condensation of malonyl-ACP with acetyl-CoA for beginning of the FASII bacterial cycle. [Campbell JW, 2001; Heath RJ, 2002; Davies C et.al, 2000; Jackowski S, 1987]

**Thiolactomycin**

Thiolactomycin (TLM) was first originated from soil samples having a Nocardia species and it is an actinomycetes product having IUPAC name (4S) (2E,5E)-2-4-6-trimethyl-3-hydroxy-2,5,7-octatriene-4-thiolide [Oishi H, et.al, 1982; Sasaki H, et.al, 1982]. Thiolactomycin showed deprived antibacterial activity and found to be dual inhibitor of FabH and FabF. TLM is a chiral compound with +ve optical rotation and competitive reversible inhibitor that attached with the malonyl-ACP of the KAS enzymes. According to earlier studies TLM was established as an inhibitor of Gram +ve and Gram -ve FAS [Borgaro JG, 2011; Hamada S, et.al 1999]. Additionally, efficacious against M. tuberculosis [Choi KH, et.al, 2000] and other unicellular parasites that contain FAS II [Morita YS, et.al, 2000].

According to previous studies it was found that TLM particularly restrict the KAS and acetyl-CoA: ACP trans acylase actions in E. coli. Besides, TLM mimics the malonyl ACP and prevents the syntheses from TLM inhibition on KAS. When done with direct binding studies using fluorescence spectroscopy and provides the confirmation against sensitivity in the given order FabF>FabB>>>FabH [Price AC, et.al 2001]. Majorly two reasons, that showed TLM poor inhibitor of FabH, primary, histidine 298 and 333 in FabB are recouped by His-244 and Asn-274 in FabH, and our FabB-TLM structure shows that the two-histidine made strong H-bonds with the antibacterial but the inhibition activity of TLM against different species varies. The
isoprenoid moiety in TLM makes its advantageous of a particular aqua-phobic gap which exist in the active sites of both FabB and FabF [Jones AL, et al. 2000]. These TLM analogs in plant and mycobacterial fatty-acid synthase systems would be able to explain the results of inhibition in which the isoprenoid moiety regained by different acyl chains. In these organisms, the congeners with lengthy and pliable chains possessed raised activity against FAS II and the lengthy chains might be more thoroughly filled with the possible spaces [Kremer L, et al. 2000]. For example, Mycobacterium tuberculosis FabH was ~3-time more sensitive to TLM than E. coli FabH [Choi KH, et al. 2000]. TLM and the related thiolactones was found to be efficient inhibitors of fatty acid and mycolic acid synthesis in mycobacteria as well as of chloroplast type II FAB [Jones AL, et al. 2000; Slayden RA, et al. 1996]. TLM is found to be in toxic in mice and used to treat UTI and intraperitoneal bacterial infections. [Mc Fadden JM, et al. 2002]. Strangely, TLM found to stop the evolution of various protozoan parasites including T. gondii and P. falciparum [Waller RF, et al. 1998]. Newly, TLM is noted to kill trypanosomes by inhibiting the production of myristate [Morisaki N, et al. 1993]. Myristate is used by the blood-borne form of the parasite to hold the surface glycoproteins that camouflage the parasite from host immune control. This physical awareness shows significant conclusion for the proposal of more effective inhibitors against these enzymes.

**Cerulenin**

A natural product cerulenin (CER) is originated from Cephalosporium cerulean [Omura S, et al., 1967]. CER is irreversible inhibitor of FAS of bacteria and eukaryotes or cells [D’Agnolo G, et al. 1973] and it also shows therapeutic efficacy in various animal models of yeast infections. [Nomura S, et al. 1972] It shows anticaner activity by inhibiting the FAS in mammals which is highly indicative in neoplastic cells and it also exhibit ability to decrease food intake and body weight in mice [Loftus TM, et al. 2000]. Regardless, of these promising outcomes of CER suggests the root of new therapeutics opportunities, the capability of cerulenin to prevent the mammalian enzymes majorly dabble the interest for further development.

The CER molecule can be differentiate into an aquaphilic head comprising the functional epoxide ring and a 1,4-diene aqua phobic tail [Omura S, et al. 1976]. In case of order of inhibition is FabB >>FabF >>FabH and the reasons of poor inhibition of CER of FabH is because of that, FabH is deficient of the substrate hydrophobic pocket to put up in the acyl chain of the drug. This help our judgement that the His-His active site, is different to the FabH His-Asn active site arrangement, which is critical for optimal cerulenin inhibition. When KAS-CER complexes undergo structural analyses, it proposed that CER effectively mimics the transition state of the condensation reaction and confirm many visible features of the early models of CER enzyme inhibition (Price AC, et al., 2001). The associated type FAS I of eukaryotes also inhibited by CER by binding to the KAS region of the multi-domain FAS protein [Morisaki N, et al., 1993; Kuhajda FP, et al. 2000]. Because of this fact of instability and its toxicity and discouraging pharmacological properties, cerulenin has no practicality as a potential wide-spectrum antibiotic [Parrish NM, et al., 1999].

**Platencimycin and Platencin**

Platencimycin (PTM) and platencin (PTN) were originated from the bacterial soil strain Streptomyces platensis with the help of new antisense differential sensitivity screening
strategy [Wang J, et al 2006; Young K, et al, 2006; Zhang CW, et al 2011]. Fatty acid ACP synthase II (FabF) which is elongation condensing enzyme selectively inhibited by Platencimycin, whereas platencin is a uniformed dual inhibitor of both FabF and fatty acid ACP synthase III (FabH) which is an initiation condensing enzyme [Martens E, et al 2011]. The PTM and PTN shows effective, wide-spectrum Gram +ve in vitro activity, including active against antibiotic-resistant bacteria, such as meticillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococci (VRE) and vancomycin-intermediate S. aureus (VISA), because of their exclusive antibacterial mechanism [Wang J, et al, 2007]. Basically, both PTM and PTN showing no multi drug-resistance to clinically significant pathogens and show in vivo efficacy without toxicity [Peterson RM, et al, 2014]. Platensimycin (C24H27NO7, relative molecular mass 441.47), comprises of two distinct structural elements connected by an amide bond. Platencimycin shown to be inhibition selectivity as it does not inhibit DNA, RNA, protein or cell wall biosynthesis at concentrations up to 500 μg/ml. The selectivity of platencimycin target was first determined within S. aureus strains either showing antisense FabF RNA (AS-fabF) or overexpressing FabF protein. PTM exhibited 200-fold greater potency in comparison to cerulenin and similar FabF target selectivity in both assays, reveal that FabF is the major target of PTM for the inhibition of bacterial growth. PTM found to be potent and discriminating inhibitor of mammalian FAS [Wang J, et al 2007].

Figure 10: Structure of PTM and PTN

PTM and PTN structure shown in Figure 10 having two dissimilar unit attached with a CO-NH bond and both possess an ADHBA moiety. Elastic amide linkage of propanoic acid is common while the aliphatic cages that are linked to ADHBA moiety are dissimilar [Singh SB, et al 2006]. The aliphatic moieties are composed of 17 carbons with cyclohexanone ring [Jayasuriya H, et al, 2007].

Mechanism of action of PTM and PTN

In FASII, the two classes of decarboxylating condensing enzymes inhibited by PTM and PTN. During the Claisen–Schmidt condensation the chain initiation mediated by condensing enzyme FabH of acetyl-CoA with malonyl-ACP. The chain elongation is mediated by FabB/FabF when the condensation of malonyl-ACP with the growing fatty acyl-ACP takes place [Health RJ, et al, 2002]. The utilization of two substrates by FabH and FabB/FabF in a three-step double displacement reaction mechanism. The cysteine in the active site is covalently transferred in acetyl-CoA in FabH or acyl-ACP in FabB/FabF that forming an acyl-enzyme intermediate and liberating reduced form of CoA or ACP, respectively. The PTM consists of two moiety that is ADHBA moiety which resides inside the malonate binding pocket of FabF and forms possible interactions with catalytic site residues, and other is ketolide moiety that remains in the opening of the catalytic site, partly showing to solvent [Wang J, et al 2006]. The changes in the organic selectivity’s of PTM and PTN must exist in their definite ketolide moieties. The PTM and PTN have similar ADHBA moieties, and have identical catalytic site interactions that is crucial in preventing FA condensation enzymes. In FabF, PTN is still not
so much active than PTM, but active as much extent than PTM in FabH [Wang J, et al. 2007]. The selectivity of PTM distinguishing lack of the ether ring. In FabF, during the entry of the active site, PTM makes the hydrogen bond to T270 but not with PTN. Vice-versa, in FabH the analogous region is bound with nonpolar residues, allowing PTN, but not PTM, to inhibit FabH [Jayasuriya H, et al. 2007] described in Figure 11.

Figure 11: Diagrammatic representation of Bacterial fatty acid synthesis cycle (FAS II) red emphasized mark are PTM inhibiting FabF/B and PTN dually inhibiting FabH and FabB.

**Pharmaceutical Application**

Most promising antibacterial agents are PTN and PTM because of their particular mechanism of action, efficacious in-vivo activity and no multi drug-resistance with Gram-negative pathogens. But in spite of these promising utilities there are few roadblocks in pharmaceutical advancement such as poor pharmacokinetics and endless discussion on reasonable target for antibacterial therapies of FASII [Wang J, 2007; Wang J, 2006]. Both the natural products found to be unacceptable antibiotics as PTN and PTM both cleared more quickly in-vivo and hence to maintain the efficacy need of continuous delivery. The better differentiation between mammalian and bacterial FAB promoted FAS II as a most interesting and fruitful goal for the growth of antibacterial.

PTM developed as an anticipated or potential drug for the management of diabetes and different metabolic irregularities. As both these natural products PTM and PTN have motivated start up and development in the field of biology, medicine, study of enzymes and off course synthetic chemistry.

The distinct antibiotic effectiveness of platencimycin and platencin might be well-known in clinical trials and their usage in humans for managing with cross-resistance may be acceptable in the upcoming future.
Conclusion:
Increased incidences of antimicrobial resistance and unavailability of potential antimicrobials against these resistant microorganisms is going to be one of the major health challenges in the future. A variety of potential targets have been explored, the discovery of molecules targeting them has not yet succeeded. In spite of it, the efforts to explore these targets and their modulators are still in progress and the future hopes for potential antimicrobials against resistant species of microorganisms remains.

Though the various novel targets like aromatic amino acids, isoprenoids, tRNA synthetases have been identified, fatty acid biosynthesis and the enzymes involved in their biosynthesis are appealing targets in the discovery of Antimicrobial drugs. Though number of the Fatty acid synthesis inhibitors have been developed only few have reached to clinical use.

The review here claims that bacterial fatty acid synthesis offers a sequence of rational and fascinating targets for antibacterial drugs however the question of how to approach these targets is not yet answered completely. Yet based on the structures of FAS intermediates presently available and the action of the inhibitors it can be estimated that the accessibility of purified β-ketoacyl-ACP syntheses from a variety of bacteria, along with the combination of techniques involving combinatorial chemistry, high-throughput screening, and rational drug design based on crystal structures, will undoubtedly support in the future discovery and development of much needed potent and broad-spectrum antibacterial agents.

Platencimycin and their analogue Platencin have been identified as lead compound during the drug development process. They have been characterized as the new classes of potential antibiotics, and their discovery is a success for natural product drug discovery and for new approaches that develop screening sensitivity and specificity. The fact that they inhibit a new target, fatty acid biosynthesis, increases their significance in the combat to stem multidrug resistance against antibiotics that have been in practice for many years.

References:
29. Cui, W; Liang, Y; Tian, W; Ji, M; Ma, X (2016-03-01). "Regulating effect of β-ketoacyl synthase domain of fatty acid synthase on fatty acyl chain length in de novo fatty acid synthesis". Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids. 1861 (3): 149–155.