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Proteins of ANL Family (FACLs and FAALs), Next Anti-TB Drug Targets?

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ABSTRACT

The Adenylating Enzymes (AEs) of the ANL family contribute to lipid synthesis in *Mycobacterium tuberculosis* (MTB). FAALs (Fatty Acyl-AMP Ligase) and FACLs (Fatty Acyl-CoA Ligase) belong to the ANL enzyme family. They play a crucial role in the metabolism of mycobacterial lipids, which are key to the bacteria's ability to cause infections. Therefore, given their role in biosynthesis and breakdown processes within fatty acid metabolism, these two groups of proteins might be potential drug targets. The growing abundance of genetic, biochemical, and structural information on MTB FAAL and FACL could provide a solid foundation for creating next-generation antituberculosis drugs via rational drug design.

Keywords: FACL; FAAL; Lipid metabolism; CoA binding pocket; Inhibitors; Tuberculosis

NOMENCLATURE

MTB	: *Mycobacterium tuberculosis*
NRPS	: Non-Ribosomal Peptide Synthetase
FadD	: Fatty Acyl-CoA Dehydrogenase
FAAL	: Fatty Acyl-AMP Ligase
FACL	: Fatty Acyl-CoA Ligase
PPant	: 4-Phosphopantetheine
AE	: Adenylating Enzymes
PKS	: Polyketide Synthases
PDIM	: Phthiocerol Dimycocerosates
PGL	: Phenolic Glycolipid
ACP	: Acyl Carrier Protein
PheA	: Phenylalanine-activating Domain
ACS	: Acetyl CoA Synthetase

1. INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), is a global health concern due to its infectious nature and the challenges posed by drug resistance[1,2]. MTB primarily targets the lungs but can disseminate to other organs, necessitating a deeper understanding of its pathogenesis and resistance mechanisms[1,3]. It is an acid-fast bacillus with complex, lipid-rich cell envelopes that help in its antibiotic resistance and survival within host cells[4].

The intricate lipid-rich cell envelopes of MTB are responsible for its ability to persist inside host cells and resist antibiotic treatments. These envelopes, made of unique lipids, are essential in host-pathogen interactions. They help MTB evade the immune response and establish infection[5,6]. Central to the biosynthesis of these intricate lipids are enzymes within the ANL family, including Fatty Acyl-AMP Ligases (FAALs) and Fatty Acyl-CoA Ligases (FACLs)[5,7]. In general, enzymes within the ANL family which include luciferase, Acyl-CoA synthetases and the adenylation domains of modular non-ribosomal peptide synthetases (NRPSs), play essential roles in the pathogenesis of MTB through their involvement in the synthesis of intricate lipids which are a distinctive constituent of the mycobacterial cell envelope[7,8,9].

By adenylating fatty acids, FAALs activate them and transfer them to specific positions on Polyketide Synthases (PKSs), generating the unique acyl chains present in mycobacterial lipids[10]. The interaction between FAALs and PKSs is crucial in producing various complex lipids essential for the structural integrity and antigenicity of the mycobacterial cell envelope[11]. Meanwhile, FACLs activate fatty acids into acyl-CoA thioesters that function as a critical intermediate in diverse lipid metabolic processes, such as degradation of fatty acids, biosynthesis of phospholipids and other complex lipids, as well as modulation of gene expression linked to lipid metabolism[12]. Therefore, FAALs and FACLs have significant implications for mycobacterial virulence, survival, and adaptability. These enzymes' involvement in biosynthetic pathways and degradative routes within lipid metabolism suggests they could be potential targets for anti-TB drugs[12].

As discussed above, FACL converts fatty acids to acyl-CoA. Whereas FAALs are structurally distinct

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from FACLs due to a 22 amino acid insertion sequence. This sequence stops the FAAL C-terminal domain's movement, hindering the protein from revealing its CoA pocket for acylation[11]. For a FAAL to function, it has developed an alternative pocket that selectively accepts only 4-phosphopantetheine (PPant) of holo-ACP (Acyl Carrier Protein) to produce various virulence-causing lipidic metabolites[10]. If scientists could develop a novel way to restrict and block this alternative pocket of FAAL proteins, it would block the formation of these infection-causing lipid derivatives.

Mycobacterium produces a repository of lipidic molecules that span from straightforward short-chain fatty acids to intricate mycolic acids. The cell envelope of *Mycobacterium spp.* contains virulence factors like Phenolic Glycolipids (PGL), Phthiocerol Dimycocerosates (PDIMs), mycobactins and sulfolipids[5]. FAAL23 is involved in sulfolipid production, whereas FAAL29, FAAL28, and FAAL26 are pivotal in the PDIM biosynthesis. PDIM is a prominent virulent lipid within the mycobacterial cell wall. FAAL32 activates a long meromycolate chain crucial for mycobacterial growth[13]. Inhibitors, specific to those FAALs, responsible for making virulence factors in the MTB could be used to combat TB[14].

Another thought-provoking aspect of selective inhibitors is that researchers can use them to investigate the functional roles of FACL and FAAL enzymes in lipid metabolism. Among the few reported inhibitors, 5′-O-[N-(dodecanoyl)sulfamoyl] adenosine inhibits FAAL28 and FACL19, and dodecylphosphate-AMP analogue, which inhibits FAAL32. 11-phenoxy undecanoyl-AMS 32 has high biochemical selectivity towards FAAL28[13,15]. With the correct utilisation of inhibitors, it is possible to stop the function of certain Fatty Acyl Dehydrogenases (FACLs and FAALs), which are substantial for the mycobacterium to survive and cause pathogenesis.

In this review article, we study the potential of FAALs and FACLs as novel targets for anti-TB drug development has been studied by elucidating their roles in MTB lipid metabolism and investigating selective inhibitors, we aim to contribute to the ongoing efforts to combat TB and mitigate the threat posed by drug-resistant strains.

2. METHODS

2.1 Search Strategy and Criteria for Data Selection

We conducted this systematic review according to the PRISMA checklist and guidelines. Relevant databases such as PubMed, SCOPUS, and Cochrane Library were considered while curating the publications in English, Portuguese, and Chinese. The studies were included according to the search terms aligning with the scope of our review. The following search terms were used: "Tuberculosis" OR "Leprosy" AND "lipid metabolism" AND "dormancy" OR "latency" AND "FAAL" OR "FACLs". Before curating the final list of publications, the title and abstract of the respective papers were carefully considered. Papers published from 1999 to 2023 were included in the updated literature list, which was imported to Zotero (https://www.zotero.org). After searching the database, a total of 118 papers were found, of which three duplicate papers were excluded. After carefully screening the title and abstract, 104 papers were removed from 115 remaining papers due to irrelevance and unavailability of full text (only one paper). Finally, one paper was excluded from 11 papers due to irrelevance of primary data. The final ten papers were based on the following inclusion criteria: (a) Publications focusing on FACL and FAAL proteins of *M. tuberculosis* and *M. leprae*. (b) Publications dealing with small molecular inhibitors of FACL and FAAL. (c) Publications from 1999 to 2023. (d) Papers in English, Portuguese, and Chinese. The exclusion criteria were: (a) Irrelevant primary focus (e.g., epidemiology, pathophysiology, etc. of *M. tuberculosis* and *M. leprae*). (b) Papers published before 1999, and (c) Unavailability of full text.

We found no relevant information on leprosy in any of the selected papers. Therefore, we restricted the scope of this review to tuberculosis. For a general description of lipid metabolism and TB, 16 more references were included from outside the screening process.

3. FATTY ACYL-AMP LIGASE (FAAL) AND FATTY ACYL-COA LIGASE (FACL)

3.1 General Features

FAALs are a class of enzymes in *Mycobacterium* that synthesise acyl-adenylates (acyl-AMP) from fatty acids. Acyl-AMP is an intermediate in the activation process of fatty acids[7]. Unlike FACLs, FAALs do not complete the reaction to form acyl-CoA. Instead, they transfer the activated fatty acid to an acyl carrier protein associated with PKSs[16]. This route directs the fatty acids toward the biosynthesis of complex lipidic metabolites crucial for the bacterium's virulence and ability to infect the host. On the other hand, FACLs represent the more universally recognised class of enzymes involved in fatty acid metabolism[12]. In two steps, these enzymes activate fatty acids into fatty acyl-CoA derivatives. Initially, FACL activates fatty acids to acyl-adenylates. Then, FACL converts those acyl-adenylates to acyl-CoA by reacting them with Coenzyme A. Fatty acyl-CoAs are then available for numerous metabolic pathways, including lipid synthesis, energy production, and cell membrane formation[11].

3.2 Mechanism for the Activation of Fatty Acids

Fatty acids must be activated before they can be incorporated into various metabolic pathways. FAALs and FACLs exhibit distinct catalytic mechanisms and biological roles, despite both being fatty acid activators. Both FAALs and FACLs function by converting free fatty acids into fatty acyl-adenylates (acyl-AMP). The process is similar to the mechanism of action of the adenylation domains of non-ribosomal peptide synthetases (NRPS)[9,11]. The process occurs in two steps:1. The fatty acid reacts with ATP, forming an enzyme-bound fatty acyl-adenylate intermediate and releasing pyrophosphate. 2. Instead of transferring the acyl

group to CoASH (as is seen with FACLs), FAALs transfer the acyl group to an acyl carrier protein of PKSs, thus channelling the fatty acids into the synthesis of complex lipidic metabolites. Meanwhile, for FACL, in the second step, the enzyme transfers the acyl group to Coenzyme A to form acyl-CoA. This acyl-CoA can enter multiple metabolic pathways such as β-oxidation, individual lipid, and membrane synthesis[11].

3.3 Structural and Functional Aspects of FAAL and FACL

N-Terminal Domains (NTD) of FACLs and FAALs are crucial for their enzymatic action. Three subdomains (A, B and C) make up the N-terminal domain of FACLs. Subdomains A and B adopt an α+β topology, intertwining to form a five-layered αβαβα tertiary structure. In contrast, subdomain C adopts a distorted β-barrel topology[12].

The three subdomains of FAAL N-terminal domain play a significant role in FAAL's enzymatic activity. The FAAL protein has an overall folding pattern similar to the acetyl-CoA synthetase, with the all-important 22 amino acids inserted between β1 and β2 of the C-subdomain[17]. A study involving FACL13 and FAAL28, two enzymes involved in fatty acid metabolism, have structural similarities despite only 24.4 % sequence similarity. FACL13 has smaller side chains at the end of the substrate-binding pocket, while FAAL28 has bulky residues there. FACL13 lacks the above-mentioned 22 amino acid insertion sequence in the subdomain C, suggesting variations in their function[12,18].

Functionally, the absence or presence of this insertion sequence is not just a structural difference but also influences the enzyme's mechanisms of action and the substrates it can accommodate. Compared to FAAL28,

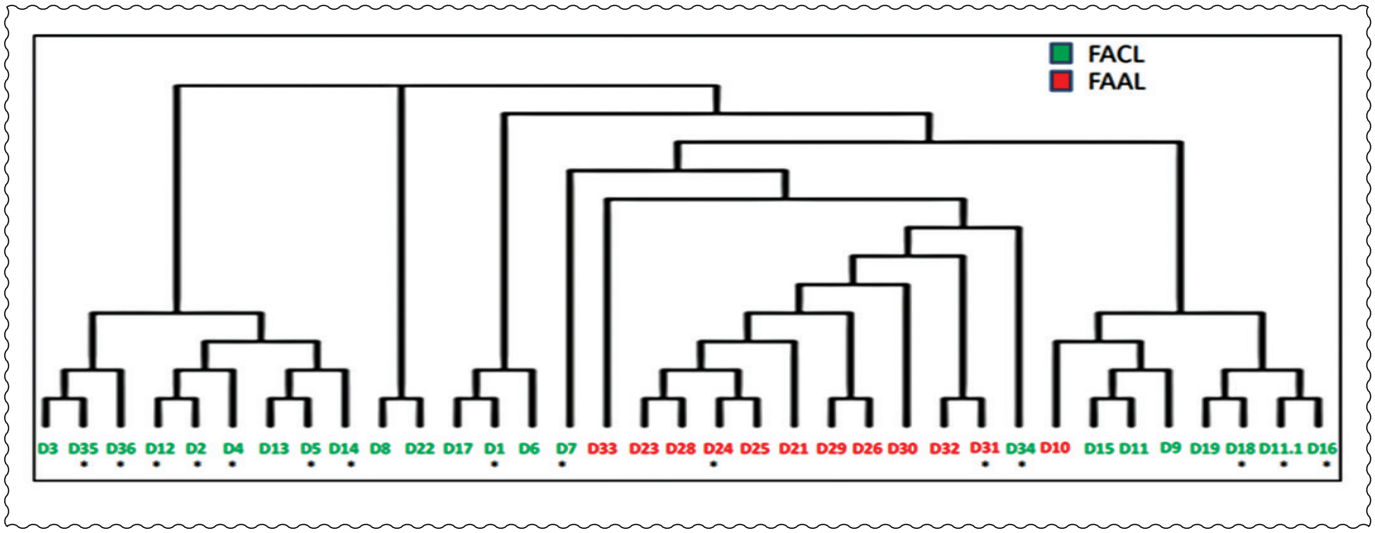

Figure 1. Phylogenetic tree of FadD (a term commonly used to denote both FACL and FAAL) proteins present in MTB (strain H37Rv) constructed using clustal omega.

The red colour code denotes FAALs (Fatty Acyl AMP Ligase) whereas the green colour code denotes FACLs (Fatty Acyl CoA Ligase). An asterisk is used to denote predicted FadD proteins that do not have any evidence at the protein level. FadD7 and FadD11 are inferred from homology. Although belonging to the same group of proteins FACLs and FAALs show some structural and functional dissimilarity. This can be further explained by tracing out their evolutionary paths by analysis of their phylogenetic tree. FadD proteins sharing the same node show homology and thus have similarities. FAALs have 70-80 % sequence similarity, on the other hand, FACLs' sequence similarity is between 20-30 %. This suggests FAALs are more conserved than FACLs.

Meanwhile, FAALs have an additional 22 amino acid insertion tethered to the C-subdomain (of NTD), allowing them to activate fatty acids and link them to complex lipid biosynthesis machinery. The FAAL structure also reveals a functional CoA binding pocket with highly conserved residues in all FAAL proteins[11]. FACL13 has smaller side chains in its substrate-binding pocket. Additionally, the lack of the insertion sequence creates a wider and more open substrate-binding tunnel, allowing FACL13 to potentially handle longer fatty acyl chains than FAAL28[12,18].

Evolutionary Differences: Even though they're evolutionarily linked, FAAL and FACL show divergence in their roles and mechanisms, ultimately leading to functional specialisation during evolution. This functional divergence results in the evolution of the FAAL family of enzymes Fig. 1. Interaction with Substrates: A study mentions possible substrate-induced subtle conformational changes that cannot be ruled out for FAAL and FACL proteins[19].

Role in the Disease Process: Various FAAL and FACL proteins are implicated in the virulence of *Mycobacterium spp.*, indicating that they can have different roles in the disease process[19]. Overall, understanding these structural and functional components offers insights into the role and function of FACL, FAAL and their potential as a target for therapeutic interventions[20].

3.4. OVERALL PICTURE IN THE CONTEXT OF MYCOBACTERIAL LIPID METABOLISM

Lipid metabolism in *Mycobacterium* is complex and involves a variety of enzymes. Among them, FAALs and FACLs prepare acyl-adenylates or acyl-CoAs[7]. FAALs play a pivotal role in channelling fatty acids towards specialised pathways required for synthesising intricate secondary metabolites, such as virulent lipids in mycobacteria and various essential polyketides and lipopeptides in diverse microbial species, including eukaryotes. Recently, scientists have found that FAAL-like domain-containing proteins, including human Disco-Interacting Protein 2 (DIP2) are integral parts of eukaryotic lipid metabolism. Thus, FAAL-like proteins, thought to be exclusive to bacteria, have now been revealed in various non-bacterial genomes, from CMR2 in yeast to DIP2 in humans[20]. Thus, targeting bacterial FAALs with small molecular drugs has become tricky, and one must avoid targeting DIP2-like proteins at the same time.

The specificity of FAALs in acyl-ACP transfer is determined by the precise recognition of the phosphate groups and adenine rings by specific amino acid residues, influencing the rejection of CoA. Additionally, the specificity of FAALs helps bacteria in producing essential natural products. The Fatty Acid-binding pocket of FAALs acts as a "molecular ruler[20]" for determining chain-length specificity, explaining the presence of multiple FAAL paralogs in organisms to expand their metabolic capabilities[21].

FACLs are also crucial for regulating fatty acid pools and distributing fatty acids towards different pathways. They play crucial roles in the dormancy and reactivation of MTB[20]. During dormancy, *Mycobacterium spp* switches from carbohydrate to lipid metabolism, activating fatty acids and producing energy by catabolising them[19,22]. This switching influences various physiological functions, including energy generation, cell membrane construction, and disease progression[22].

Understanding the mechanistic differences between FAALs and FACLs is crucial for drug development. While the adenylation domain of NRPS and luciferases exhibit somewhat nonspecific CoA transfer abilities, FAALs offer a unique and specific ACP binding pocket that can be targeted for designing selective inhibitors[9,20]. By leveraging the structural insights into FAALs and their homologs in eukaryotes, researchers can explore novel strategies for developing drugs targeting these proteins, potentially discovering new therapeutic agents with enhanced specificity and efficacy.

3.5. FACL and FAAL, Contribution in the Biosynthesis of Mycobacterial Glycolipids

FAALs activate fatty acids as fatty acyl-adenylates through ATP hydrolysis, creating a high-energy bond between the carboxylate of the fatty acid and AMP. Following their transfer to PKS, these activated fatty acids undergo several cycles of elongation and modification to generate various complex lipid structures[10].

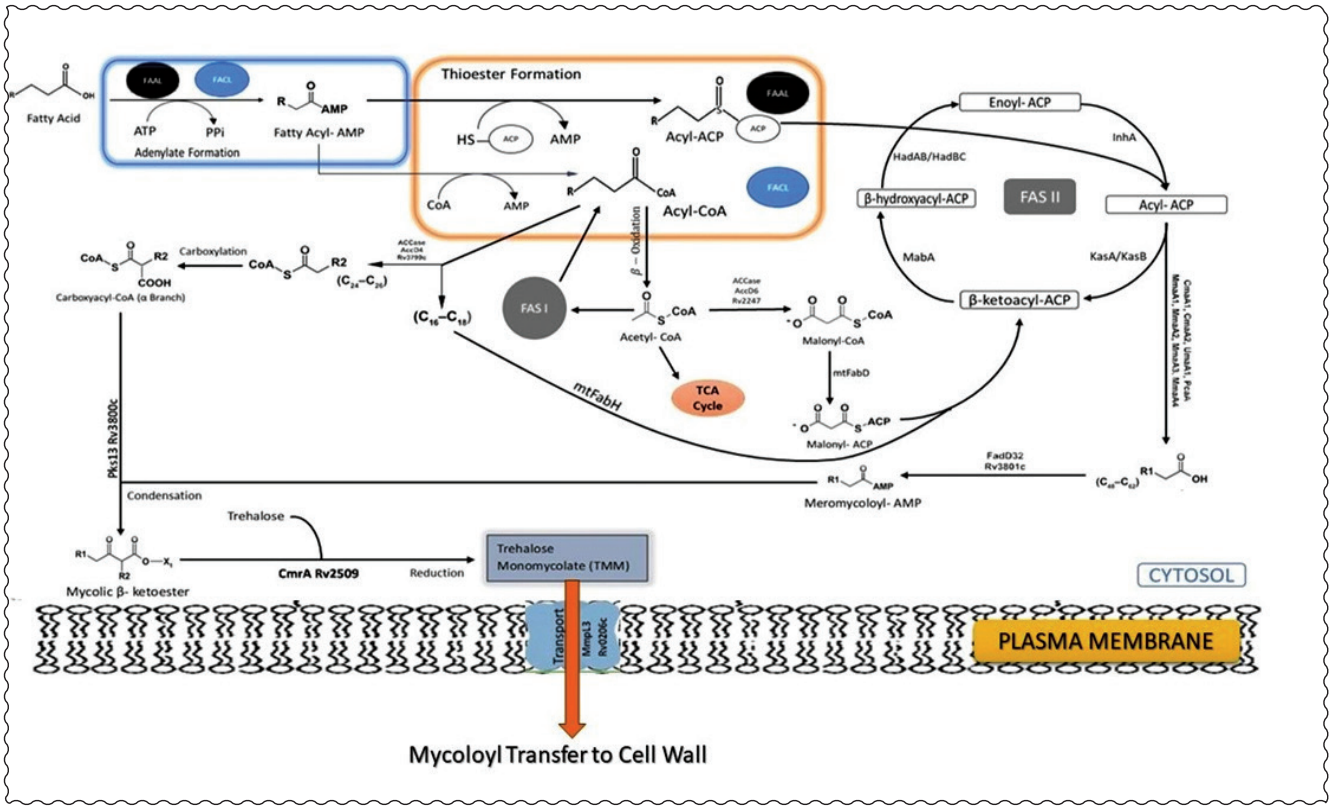

Figure 2. Schematic representation of the mechanism of the ANL-Family enzymes involved in the biosynthesis and regulation of a class of glycolipids in MTB.

The representation includes various steps and components involved in the process, such as adenylate and thioester formation in blue and orange boxes respectively, fatty acid metabolism, fatty acid synthases-I and II network, and probable cellular locations are indicated. It also mentions related molecules and structures for cell wall core in MTB (labelled in the orange box) like acyl glycolipids and mycolic acid.

physical proximity of FAALs and PKSs as neighbouring genes in the *Mycobacterium spp* genome helps efficiently transfer activated fatty acids due to co-regulation[12]. FACLs convert fatty acids into fatty acyl-CoA esters which are essential for synthesising complex lipids and lipoproteins in *Mycobacterium*[7]. As discussed above, mycobacterial lipids are implicated in virulence, pathogenesis, and survival mechanisms, as they shape and preserve unique components of the cell envelope which is crucial for evading the host immune response.[7,17]

The combined action of FAAL and FACL in mycobacterial cells facilitates the synthesis of and maintenance of glycolipids (e.g., phosphatidylinositol manno sides, isoprenoid lipids, glycerophospholipids, mycolic acids, trehalose mycolates and lipoarabinomannan)[5,23]. Their functions demonstrate the intricacy and specificity of the lipid biosynthesis pathways found in mycobacteria, as well as how these pathways are adapted to the structural and pathogenic requirements Figure 2. The disruption of these enzymes' activities, through pharmacological inhibition, might undermine *Mycobacterium*'s ability to maintain its cell envelope integrity and evade the host immune response, providing possible avenues for novel therapeutic interventions[5].

4. COA-BINDING POCKET AND INSERTION SEQUENCE

4.1 General Features

FAAL does not transfer the final product to CoASH. It functions by acylating the activated fatty acid onto acyl carrier proteins of PKSs, which further goes into the biosynthesis of many lipid metabolites[24]. However, FAAL has kept the CoA binding pocket intact, and their novel catalytic function is due to the modified substrate-induced conformational change. This observation suggests that FAAL proteins might have evolved from the omnipresent FACLs[11].

As discussed above, the structural analysis of FAAL28 shows an insertion in the C-subdomain (of NTD). This particular insertion was present in all FAAL homologues but absent in all FACL proteins. To inspect the functional role of the insertion, the C-subdomain of FAAL28 was modelled by using coordinates from phenylalanine-activating domain (PheA) and Acetyl CoA synthetase (ACS) structures. When the C-subdomain is modelled based on PheA, it produces a conformation where it is positioned near the 22 amino acid insertion motif representing the acyl-AMP, directly interacting with the motif. Modelling the C-subdomain based on ACS reveals its relocation away from the insertion motif and towards the active site, facilitating acyl-CoA production. Hydrophobic interactions with the surrounding protein structure stabilise the remaining portion of the insertion motif, limiting its mobility. Based on the observations, it is speculated that the insertion motif can regulate the mobility of the C-subdomain, which disrupts the exposure of active ATP-binding pocket, making them incapable of synthesising acyl-CoA[11].

4.2 Interconversion of FACLs and FAALs

A study suggests that by deleting the FAAL proteins' insertion motif, it could be converted into FACL proteins, as such deletions would expose the hydrophobic patches to the solvent[19]. Deletion mutations were systematically introduced into FAAL28, ensuring the preservation of secondary structural elements within the targeted region. Notably, the mutant FAAL28, with residues 354 to 365 deleted, successfully converts the lauroyl-AMP intermediate to lauroyl-CoA[19]. Remarkably, the catalytic rate constant of this FAAL28 mutant exhibits an 8 5% similarity to that of the wild-type FACL.

Thus, the activity of FACLs and FAALs can be interconverted by deleting the insertion sequence. Another experiment was carried out using 12 amino acid residues (375-386) from the insertion sequence of FAAL32 and was incorporated into FACL13 between its 322-323 amino acid residues. The engineered FACL13 primarily produces acyl-AMP, yet only half of the acyl-AMP generated undergoes conversion into acyl-CoA. The insertion sequence integrated into the protein structure is positioned distantly from the catalytic core of FACL13, thus not entirely disrupting its structural stability[19].

4.3 Hindrance in FAAL C-terminal Domain Movement by the Insertion Motif

Insertion motifs of the FAALs from different organisms, such as *E. coli* and *L. pneumophila*, adopt a similar structure consisting of two stranded β-sheets with a loop connecting them and a short α helix located in the centre[16]. These configurations give rise to a negatively charged pocket directed towards the C-terminal domain. Furthermore, β sheet structures and the loop region of the N-terminal 22 amino acid insertion motif increase hydrophobic interactions between N- and C-terminal domains. These enhanced interactions between domains caused by the insertion motif predominantly restrict the mobility of the C-terminal domain, hindering the adoption of the CoA binding conformation.

FadDs (FACLs and FAALs combined) have been revealed to show a conserved fold containing a smaller C-terminal domain (green) and a large N-terminal domain (blue) that are linked together by an A8 motif (violet). FACLs go through a 140-degree rotation of its C-terminal

domain around the A8 motif to expose its canonical pocket (cyan) present in the N-terminal domain to accept CoA and activate fatty acyl AMP to fatty acyl CoA. The other variant, FAALs has retained the CoA binding pocket (cyan) however, it is non-functional due to a specific structure unique to FAALs known as insertion sequence (brown) which obstructs the C-terminal domain movement. To transfer the activated fatty acid, FAALs constitute an alternative pocket (pink) that negatively selects CoA and is highly selective for 4'-PPant tethered holo-ACP to convert fatty acyl-AMP into acyl ACP for further reaction to take place. Adapted from[11]. necessary for CoA ligation by FAALs16 (Fig. 3).

4.4 Conserved Nature of FAAL Pockets and Its Universal Rejection Mechanism

FAAL strictly rejects CoA and selectively accepts the 4-PPant of holo-ACP Figure 3. It is interesting to know how FAAL distinguishes holo-ACP, which is chemically identical to the abundant CoA. FAALs operate exclusively with holo-ACP fused with NRPS or PKS, to generate diverse complex lipids found in *Mycobacterium*. FAALs bind holo-ACP using a new cavity in their N-terminal domain since their canonical pocket is blocked. This cavity is not present in FACL crystal structures[10]. The alternative pocket within FAALs exhibits a distinctive architecture, effectively excluding the adenosine 3',5'-bisphosphate and lacking positive selection by Arg/Lys residues. Remarkably, these rejections of CoA are conserved across various other life forms. This unique characteristic of FAALs, steering the destiny of lipid molecules towards specific pathways, suggests an evolutionary origin parallel to that of FACLs [10].

Hence, the accumulated structural insights offer avenues for "combinatorial engineering of PKS/NRPS systems[10]", leveraging FAALs as distinctive modules for fatty acid loading. Alternatively, these findings could be harnessed to develop novel bioactive compounds, capitalising on FAALs' distinctive acceptor-fidelity properties[10].

5. SMALL MOLECULE INHIBITORS OF FADD (FAAL AND FACL COMBINED)

Many FadD proteins have been identified, but only a few structures have been solved. Thus, resulting in only a limited number of published small chemical inhibitors Figure 4. The inhibition of FAAL28 and FACL19 was shown by 5′-O-[N-(dodecanoyl)sulfamoyl] adenosine. Baran and his colleagues conducted a study on the effectiveness of several 5′-O-[N-(alkanoyl)sulfamoyl] adenosine analogues in treating tuberculosis[13]. In the same investigation, they also sought to examine the affinity of these analogues for the FAAL and FACL. They identified several potent molecules with antitubercular properties, such as 12-azido dodecanoyl-AMS, 11-phenoxy undecanoyl-AMS, and non-oxy acetyl-AMS. Using decanoyl-AMS as a model, researchers discovered that 11-phenoxy undecanoyl-AMS has dramatically enhanced anti-mycobacterial activity. To examine the effectiveness of the assessed substances in inhibiting the growth of *M. tuberculosis* H37Rv, two different types of media were used: GAST media, which only contains glycerol as a carbon source, and 7H9 media, which includes both glycerol and glucose as carbon sources. The acyl-AMS analogues exhibited suboptimal performance in the 7H9 medium.

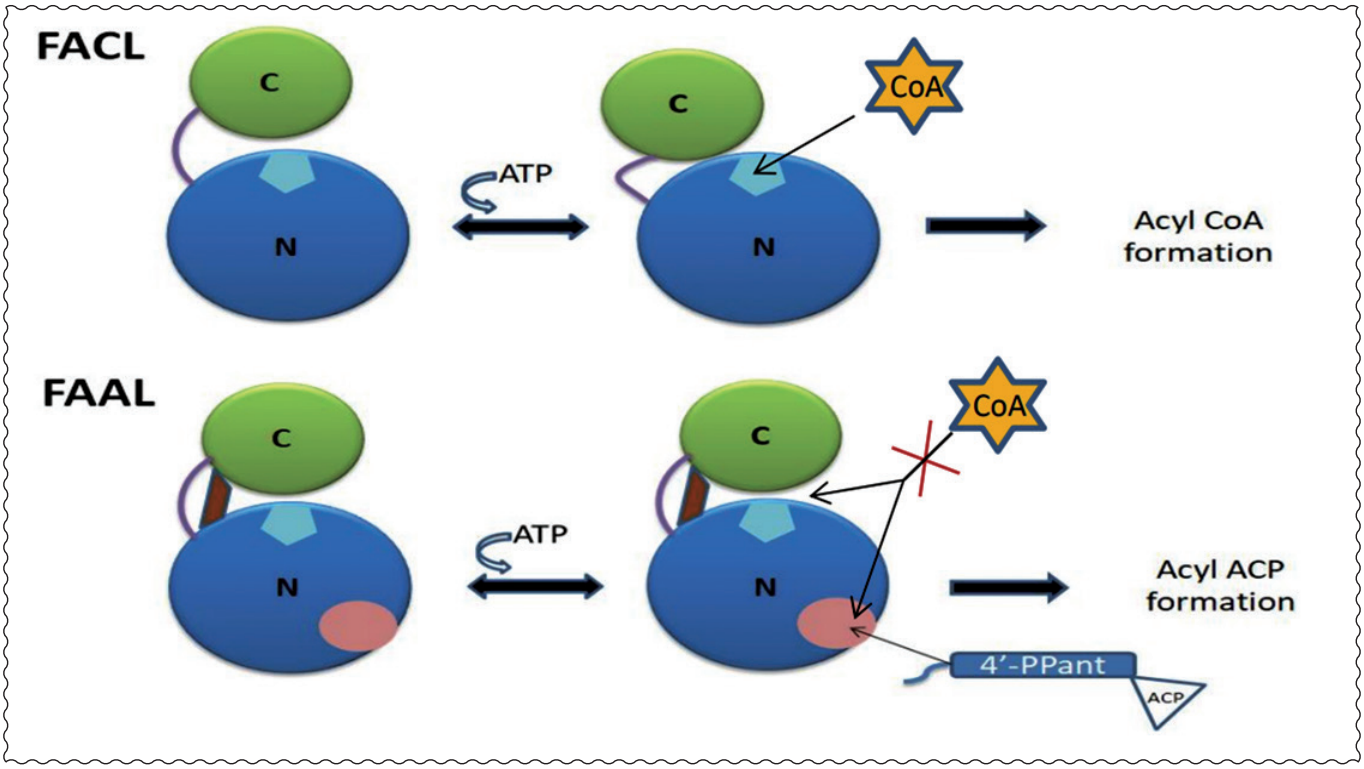

Figure 3. Schematic representation of structural and biochemical differences between FACLs and FAALs.

The 7H9 nutrient-rich medium contains fatty acids and other lipids that can restore the function of a few Fatty Acid Dehydrogenase enzymes. The effect of acyl chain length was investigated using several analogues with even chain lengths. The study revealed a "parabolic relationship[25]" between the antibacterial activity and the acyl chain length, with the highest activity observed at chain lengths C12-C14. The study examined the tolerance of altered lipid chains and evaluated the effects of various nonpolar and polar functional groups at the end of the lipid. The concurrent biochemical assessment of FACL19 and FAAL28 demonstrated that 11-phenoxy undecanoyl-AMS displayed a selectivity of more than 142-fold for FAAL28 over FACL19, exhibiting strong target selectivity[13,15]. It was hypothesized that analogues with selectivity for FAAL enzymes might potentially differentiate antitubercular action from cytotoxicity, as FAAL enzymes are by and large specific to bacteria (excluding the DIP2 group). In contrast, FACL enzymes are found in humans also[20]. Nevertheless, 11-phenoxyundecanoyl-AMS maintained a significant level of cytotoxicity. Therefore, the alkanoyl-AMS scaffold may have inherent cytotoxic properties.

Further investigations showed that substituting the "terminal phenoxy group of 11-phenoxyundecanoyl-AMS[13]" decreased its anti-MTB action and affinity for FAAL28. In the previous series of experiments, researchers discovered that the presence of the carbonyl group is required for the activity. However, the oxygen atom at the 5' position can be substituted with an NH group. The investigation yielded significant insights for further optimising 11-phenoxyundecanoyl-AMS and revealed limits that must be addressed[13].

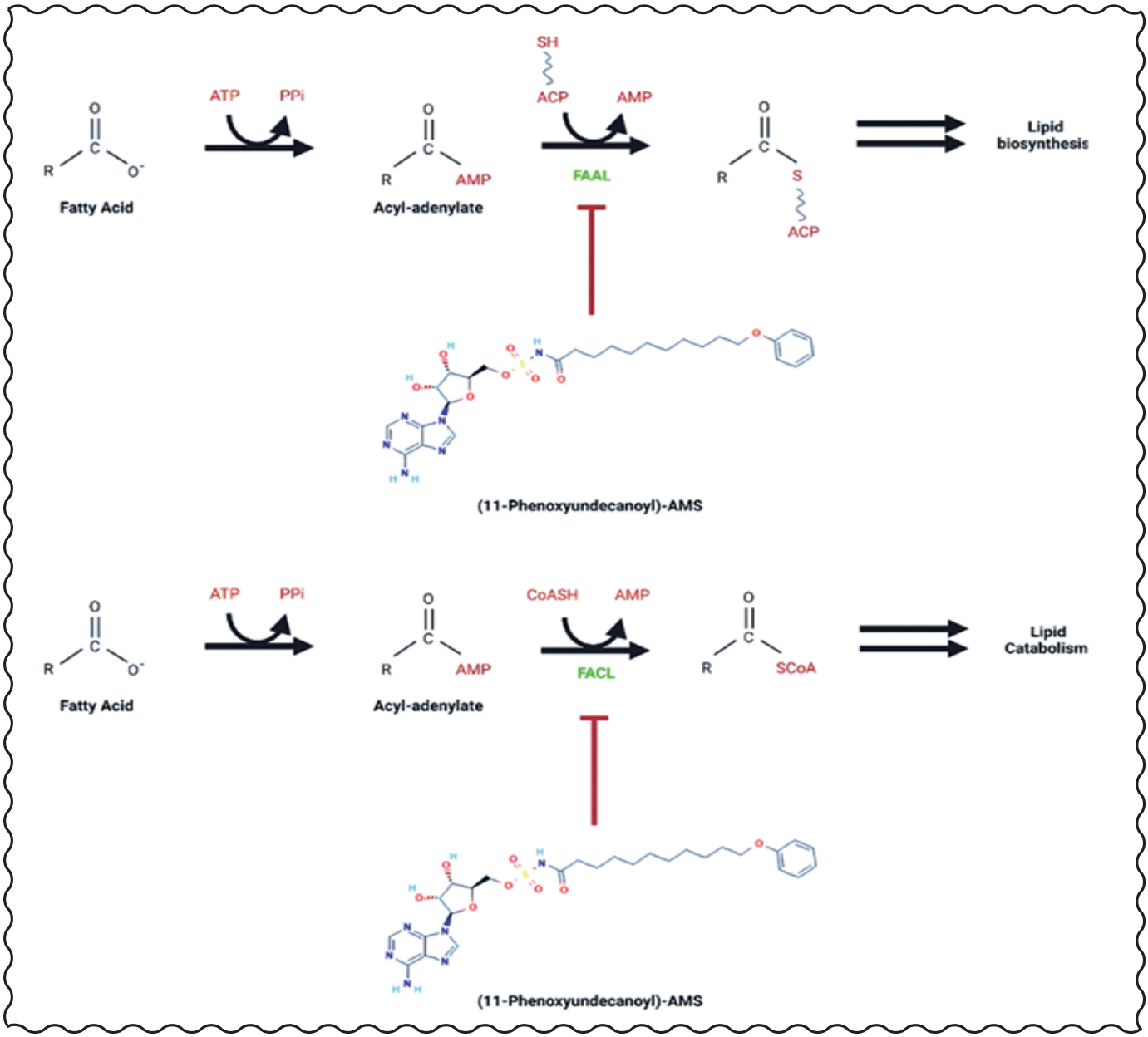

Figure 4. Inhibition of FadD by small-molecule inhibitors [(11- Phenoxyundecanoyl)-AMS in this figure]. Specific inhibitors of MTB FAALs could be future anti-TB drugs.

6. CONCLUSION

TB treatment typically entails a four-drug "first-line" regimen comprising rifampicin, isoniasid, ethambutol and pyrazinamide often lasting six months or longer. However, in instances of drug-resistant TB, treatment durations can extend up to 20 months and second-line drugs are used. MDR TB exhibits a drug efficacy of 56 % and XDR TB shows a drug efficacy of 39% only[26]. Therefore, it is evident how challenging it is to treat drug-resistant Tuberculosis. As part of its End TB Strategy, the World Health Organisation focuses on eradicating drug-resistant TB. Key to this effort is the development of superior therapeutic, diagnostic, and vaccine strategies by the scientific community.

The extensive availability of genetic, biochemical, and structural data on the mycobacterial Adenylating Enzymes (AE) offers a solid footing for developing novel anti-TB medicines through rational drug design. Additional techniques for identifying AE inhibitors include fragment-based screening, high-throughput screening, and finally the synthesis of the bisubstrate inhibitors mimicking the intermediate acyladenylate[12,13,15]. All the techniques above show various degrees of success to date[12,18]. Scientists increasingly favour the use of fragment-based screening for the systematic development of inhibitors[13,15]. However, there is currently just one documented instance of this strategy being applied to a mycobacterial enzyme[12]. Undoubtedly, additional implementation of this potent technique is required. Replacing the adenylate phosphate with a sulfamate is the direct approach, creating potent inhibitors of FACLs and FAALs, known as "bisubstrate inhibitors of the acyladenylate[12]".

The enthusiasm for target-based methods for finding tuberculosis drugs is decreasing, as recent efforts focus more on whole-cell phenotypic tests. However, we believe that many targets previously considered unfavourable could still be good for drug development because they are important and it's easier to create effective and specific inhibitors for them.

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